Population Dynamics of *Pseudomonas syringae* pv. *syringae* on Spring Wheat

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ABSTRACT

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Resistance of spring wheat genotypes to *Pseudomonas syringae* pv. *syringae* was investigated in growth chamber studies. Population growth and symptom development were monitored after introduction of bacterial suspensions (5×10^6 cfu ml^{-1}) into leaf intercellular spaces and incubation at 18–20 C under light mist. Spray, wound, or vacuum infiltration were inappropriate inoculation methods. *P. s.* pv. *syringae* is a weak pathogen that requires moist conditions during the incubation period for significant infection. Foliar symptoms and $\log_{10}$ bacterial populations 3 days after inoculation were positively correlated ($r^2 = 0.54$). Genotypes Len, Marshall, Nowesta, Red River 68, Bonanza, Bounty 208, and Alex were relatively resistant; Polk, Angus, Chris, Oslo, and Lark were moderately susceptible; and Bounty 309 and L1 were susceptible to *P. s.* pv. *syringae* as measured by symptom development and bacterial populations.

Additional key words: bacterial blight, *Triticum aestivum* L.

Bacterial blight, caused by *Pseudomonas syringae* pv. *syringae* van Hall (8), is a foliar disease of wheat, *Triticum aestivum* L. in the northern United States. The disease was described by McCullock in 1929 as a basal glume rot (17). More recently, concern was generated by a series of epidemics during the late 1960s and 1970s in the spring and winter wheat-growing regions of Minnesota (25), South Dakota (18), and Montana (24). Symptoms included grey-green necrosis and bleaching of leaves, glumes, sheaths, and culms.

Differences in resistance of wheat cultivars to bacterial blight were observed in naturally infected field plots (18,25). These researchers suggested that the pathogen requires fairly specific environmental conditions for disease development, i.e., intense rains before heading. Efforts to repeat these studies and to evaluate resistances to the pathogen in new breeding material have been hampered by the sporadic occurrence of the disease in the field. Sands et al (21) observed at least some resistance to *P. s.* pv.

syringae in winter wheat and barley, but none in spring wheat in greenhouse inoculations. Resistance to *P. s.* pv. *syringae* in other hosts is expressed as retarded bacterial population growth, as well as delayed and subdued symptom expression (7,32).

Various inoculation methods have been used for studying pathogenicity of bacteria on small grains. Otta (18) atomized approximately 10^5 colony-forming units (cfu) ml^{-1} suspensions of *P. s.* pv. *syringae* onto seedlings that had been preconditioned for 24 hr under plastic bags in the greenhouse. Sellam and Wilcoxson (25) sprayed bacterial suspensions of unknown concentrations on seedlings and adult gramineous plants and incubated the plants at 21 C with continuous misting. Peters et al (19) used partial vacuum infiltration or hypodermic needle intromission to inoculate barley seedlings with 10^6 cfu ml^{-1}. Both Hagborg (10) and Bockelman et al (3) used hypodermic syringes fitted with rubber stoppers to force bacterial suspensions into intercellular spaces of barley. The suitabilities of these methods for evaluating resistance of wheat cultivars to bacterial blight have not been studied.

We present evidence for differential susceptibilities of spring wheat genotypes to *P. s.* pv. *syringae* strains using a modified Hagborg inoculation technique. The role of *P. s.* pv. *syringae* as a pathogen on spring wheat was examined by comparative symptomatology and population dynamics studies of numerous bacterial strains. A preliminary report has been published (28).

MATERIALS AND METHODS

Several bacterial strains from various sources were used in these studies (Table 1). All strains were stored under partial vacuum at 5–7 C after lyophilization in single or double strength skim milk. Working cultures were obtained from lyophilized strains that were revived, and stored on nutrient agar (Difco Laboratories, Detroit, MI) slants at 5–7 C for periods of less than 3 wk.

Bacterial strains were classified on the basis of physiological and biochemical tests and host of origin. Bacterial strains defined as *P. syringae* were fluorescent on King's Medium B (13), obligately aerobic, unable to use trehalose, 2-ketogluconate, β-alanine or L-valine as sole carbon sources and negative for gram stain, denitrification, oxidase, and arginine dihydrolase (5). *P. syringae* strains were classified as pathovar *syringae* on the basis of the carbon compound utilization scheme of Sands et al (22).

Tests for potato soft-rotting, nitrate reduction, and levan production were conducted according to Lelliott et al (16). Strains were tested for anaerobic growth potential (1,12), gram reaction (23), and hydrogen sulfide gas production (23). Tests were made for bacterial oxidase (14), arginine dihydrolase (33), and syringomycin (9) activity, and carbon compound utilization (27). A strain unable to grow with DL-β-hydroxybutyrate was presumed as unable to accumulate poly-β-hydroxybutyrate as an intracellular reserve (5).

Bacterial strains were grown on nutrient agar slants at 27 C in the dark. Bacterial suspensions were obtained by flooding 24–48-hr-old cultures with sterile distilled water and adjusting to the desired concentration based on spectrophotometric readings at 425 nm using a calibration curve determined previously. Inoculum concentration and purity were verified by serial dilutions plated on King's Medium B. Bacterial suspensions were kept on ice for up to 1 hr until inoculations could be completed.

All plants were grown in 12.7–15.2-cm-diameter clay pots containing a standard greenhouse mix (soil:sand, 1:1, pH 6.8). Wheat plants were inoculated while in the growth stages from flag leaf to head emergence, decimal growth stages 39–59 (35), to conform to reports that bacterial blight generally appears on wheat in the field during the boot stage (18,25). Bacterial suspensions were infiltrated into intercellular spaces of leaves using a modified 10-ml disposable syringe with the needle replaced by rubber tubing (2 cm long × 0.5 cm inside diameter). The leaf tissue was supported with a finger tip wrapped with Parafilm (American Can Company, Greenwich, CT) during the infiltration process. The youngest three leaves per plant were used, with approximately 11 discrete infiltration sites per leaf. Inoculation sites were identified by small permanent ink marks on the leaf margins. Plants were inoculated only between 10 a.m. to 3 p.m. and on overcast days, under high intensity sodium vapor lamps, ensuring that stomatal apertures remained fully open during the infiltration process. Infiltrated plants were left in place until macroscopic signs of water soaking disappeared (less than 20 min). Plants were then moved to a growth chamber at 18–20 C, 16-hr photoperiod supplied by fluorescent and incandescent lights. The plants were misted, 2 min on and 3 min off, with deionized water during the entire incubation period.

Plants were rated while still in the mist chamber for the percent area of the 0.5-cm-diameter inoculation sites affected and symptom type according to the following rating scale: 0 = no symptoms; 1 = faint chlorosis; 2 = chlorosis < 10%; 3 = chlorosis and grey-green water soaking < 10%; 4 = chlorosis and/or grey-green water soaking in 10–90%; 5 = chlorosis and/or water soaking in > 90%. This rating scale was designed to allow the quick classification of the continuous range of symptoms into discrete categories.

Infiltrated leaves were clipped at the base, placed in 10- × 25-cm polyethylene bags, which were then fastened to prevent tissue dehydration. The samples were stored at 4 C until processing (within 5 hr). Unless otherwise indicated, each sample consisted of 20 6.0-mm-diameter disks cut with a sterile paper punch from two or three leaves from one plant. Each sample was triturated for 20 sec in 100 ml of sterile distilled water in a blender at low speed. The leaf suspension was returned to the original bag and kept at room temperature for 10–30 min to allow bacteria to diffuse from the tissue. Serial dilutions, 1:10, were made from the hand-shaken suspensions and 0.1-ml aliquots of the appropriate dilutions were spread evenly on King's Medium B with sterile bent glass rods. The plates were incubated at 27 C in the dark and examined 48 hr later for fluorescence under near ultraviolet light. Saprophytic bacteria were a minor component of the bacterial populations when plants were incubated at 20 C or less and misting was minimized. Counts were not adjusted for plating efficiency.

Comparisons were made of the relative virulences of *P. syringae* strains obtained from various hosts (Table 1) on Angus spring wheat, which is moderately susceptible. The bacterial strains were tested initially by pressure-infiltration at concentrations of 5×10^7 cfu ml^{-1}. Strains that caused symptoms were tested further by pressure-infiltration at 5×10^6 cfu ml^{-1}. Symptoms and bacterial population development over a 5-day period were monitored as described above. Each treatment was replicated four times, and standard errors of the means were calculated for nontransformed data.

Correlations between symptoms and *P. s.* pv. *syringae* populations following inoculation were determined for crosses between a hard red spring wheat, Len, and a soft red spring wheat, Oligoculm 112-76. Len is moderately resistant to symptom and bacterial population development following infiltration inoculation. Oligoculm 112-76 is an unadapted land race from Israel and supports relatively high *P. s.* pv. *syringae* populations. Single cross, backcross 1 (BC_1) and backcross 2 (BC_2) were made with Len as the recurrent parent. Ten spikes per crossing method were selected from random plants at the F_2 generation. Seeds from each spike, constituting a line, were thinly planted in rows for the F_3 generation advance in Ciudad Obregon, Mexico, during the winter of 1980. Generations F_4 and F_5 were grown at St. Paul, MN, in 1981 and 1982, respectively. No selection was made within lines. F_6 generation plants of the single cross, BC_1, and BC_2 and the parents, Len and Oligoculm, were tested by inoculation with *P. s.* pv. *syringae* strain MSU174 at a concentration of 5×10^6 cfu ml^{-1}. Ten lines per single cross, BC_1, and BC_2 were tested. Each line was represented by a single pot of two plants. The parents were each represented by four pots with two plants. Test conditions were as described above. Symptoms and bacterial populations were measured 3 days after inoculation. A bulk sample of 40 leaf disks was taken from four leaves picked from each pot. The experiment was repeated once, and the results from both experiments were combined. Bacterial population data were subjected to $\log_{10}$ transformation to stabilize the variance (30). The relationship between symptoms and bacterial populations was examined by linear regression.

The relative susceptibilities of spring wheat cultivars and lines (Table 2) were determined by monitoring symptom and bacterial population development for a 6-day period after inoculations with *P. s.* pv. *syringae* strain MSU174 at a concentration of 5×10^6 cfu ml^{-1}. The wheats tested were the cultivars Angus, Butte, Bonanza, Len, Marshall, and Alex, and the lines MT7620 and L1. A completely randomized design was used with four replications per treatment.

Determination of wheat genotype susceptibility to *P. s.* pv. *syringae* was made for 15 wheat cultivars based on the *P. s.* pv. *syringae* bacterial populations (BP, expressed as cfu cm^{-2}) by a single assessment 3 days after inoculation. *P. s.* pv. *syringae* strain MSU174 was inoculated by pressure-infiltration at 5×10^6 cfu ml^{-1}. Rating categories used were moderately resistant (MR) = BP $< 5 \times 10^5$; moderately susceptible (MS) = $5 \times 10^5 <$ BP $< 10^7$; susceptible (S) = BP $> 10^7$. A completely randomized design was used with four replications per treatment.

RESULTS

Numerous inoculation procedures were tried in preliminary tests with *P. s.* pv. *syringae* strains MSU174 and PSMW-1 at 5×10^6 and 5×10^7 cfu ml^{-1} to determine the best method to explore host resistance and virulence of bacterial strains. Excessive moisture accumulation was a problem with the method of Otta (18) in which plastic bags were placed over plants to maintain high humidity before and after inoculation. Only limited and irregular disease symptoms were obtained with spray application of inoculum (25) even though plants were bagged, misted, or placed on a greenhouse bench following inoculation. Symptoms were more pronounced when intercellular spaces were previously infiltrated with water by vacuum or pressure infiltration. However, vacuum infiltration was too cumbersome when inoculating numerous plants. Wounding alone (pinholes in inoculation site) was not sufficient as a preinoculation treatment before spray inoculation due to the lack of appreciable symptoms.

The most satisfactory inoculation method was the use of a rubber-tipped syringe (modified Hagborg method [10]) to infiltrate leaf intercellular spaces with bacterial suspensions, followed by incubation in a mist chamber after macroscopic signs of water soaking disappeared. The delay between infiltration and placement of plants in the mist chamber was necessary to avoid growth of nonpathogenic bacteria (31). This method was used in all subsequent inoculations. Pressure infiltration of wheat leaves with 5×10^6 cfu ml^{-1} suspensions resulted in initial concentrations of approximately 5×10^4 cfu cm^{-2} tissue. The major advantage of the pressure infiltration technique was that inoculum placement and amount could be carefully controlled. Incubation in the mist chamber following infiltration-inoculation was necessary for consistent symptom development—few or no symptoms developed if inoculated plants were not misted.

Pressure infiltration of the saprophyte *P. fluorescens*, and *P. s.* pv. *zizaniae*, PSZ-2, PSZ-6, *P. s.* pv. *glycinea*, 1514, or 1515 at 5×10^7 cfu ml^{-1} caused slight or no symptoms in Angus spring wheat. A total of 30 *P. s.* pv. *syringae* strains from various hosts were pressure-infiltrated at 5×10^7 cfu ml^{-1} into Angus spring wheat. Of these, 17 induced chlorosis and/or grey-green water-soaked tissue and were tested by inoculation at 5×10^6 cfu ml^{-1}. A subset of six strains caused chlorosis and/or necrosis at the lower concentration. Chlorosis symptoms ranged from a faint mottled-flecking to confluent bleaching of the infiltrated tissues, depending on the bacterial strain. Grey-green water-soaked tissue became desiccated tan brown areas once plants were removed from the inoculation chamber.

Symptoms on the moderately susceptible Angus were restricted to the areas initially infiltrated with bacterial suspensions. This was also true for wheat plants that were infiltration-inoculated and then incubated for 3 days outdoors under conditions of rain at 15–25 C (W. W. Shane, *unpublished*).

The strains that caused chlorosis, and with some strains, water soaking and necrosis when inoculated 5×10^6 cfu ml^{-1} into Angus were of two groups—*P. syringae* from barley and a subset of the *P. s.* pv. *syringae* strains from various hosts (Table 1). The strains from barley identified by Peters et al (19) as *P. s.* pv. *syringae* are probably not pathovar *syringae* because, in our tests, the strains 58-81A and 58-81B were unable to use l-lactate or trigonelline as sole carbon sources, whereas most *P. s.* pv. *syringae* strains can use

TABLE 1. Sources of *Pseudomonas* strains tested for pathogenicity to small grains

Strain	Original host	Source[a]
P. syringae pv. *syringae*		
40-82A through 40-82J[b]	Wheat seed (*Triticum aestivum*)	1
19310	Lilac (*Syringa vulgaris*)	2
BS100	Corn (*Zea mays*)	3
50-82C	Wheat	3
B359	Millet (*Panicum miliaceum*)	4
PS35	Wheat	5
PSMW-1	Wheat	6
PS347, PS488	Wheat	7
MSU13, MSU14, MSU16	Bean (*Phaseolus vulgaris*)	8
MSU33, MSU35, MSU39	Sorghum (*Sorghum* spp.)	8
MSU171, MSU187, MSU188, MSU200, MSU202	Wheat	8
MSU174	Barley (*Hordeum vulgare*)	8
P. s. pv. *zizaniae*		
PSZ-2, PSZ-6	Wild rice (*Zizania aquatica*)	9
P. s. pv. *coronafaciens*		
1514, 1515	Oats (*Avena sativa*)	9
P. s. pv. unknown		
58-81A[b]	Barley kernel	10
58-81B[b]	Barley leaf	10
P. s. pv. *glycinea*		
45-83B[b]	Soybean (*Glycine max*)	11
P. fluorescens		
13525	Saprophyte	2

[a] 1. L. Lahman, University of Idaho; 2. American Type Culture Collection, Beltsville, MD; 3. W. W. Shane, University of Minnesota, St. Paul; 4. J. DeVay, University of California, Davis; 5. R. Hosford, Jr., North Dakota State University; 6. D. Fulbright, Michigan State University; 7. J. Otta, South Dakota State University; 8. B. Hemmings and D. Sands, Montana State University. Additional information is available for these strains (11); 9. R. Bowden, University of Minnesota; 10. R. Peters, North Dakota State University; 11. B. Kennedy, University of Minnesota.
[b] Indicates strain designation assigned by authors.

TABLE 2. Spring wheat genotypes identities and sources

Cultivar	Cereal introduction number	Seed source[a]
Alex	17910	1
Angus	17744	1
Bonanza	14077	1
Bounty 208	15078	3
Bounty 309	17315	3
Butte	17681	1
Chris	13751	1
L1	none	4
Lark	17383	3
Len	17790	1
MT7620	none	2
Marshall	17920	1
Nowesta	17390	3
Olaf	15930	1
Oslo	13332	1
Polk	13773	1
Red River 68	14193	3
Sheridan	13586	3

[a] 1, R. H. Busch, University of Minnesota, St. Paul; 2, D. C. Sands, Montana State University, Bozeman; 3, D. H. Smith, Jr., USDA-ARS, NER, Beltsville, MD; 4, R. M. Hosford, Jr., North Dakota State University, Fargo.

both (22). However, only *P. s.* pv. *syringae* strains were able to incite appreciable clorosis and/or water soaking in Angus spring wheat when introduced at the lower concentration of 5×10^6 cfu ml^{-1}. Some *P. s.* pv. *syringae* strains that were pathogenic on spring wheat when introduced at relatively high concentrations were ineffective at lower ones. For example, strain PSMW-1 originating from wheat caused both chlorosis and grey-green water soaking when infiltrated into Angus spring wheat at 5×10^7 cfu ml^{-1} but only chlorosis at 5×10^6 cfu ml^{-1}. No symptoms were detected within 5 days after inoculation with this strain at 2×10^6 cfu ml^{-1}. No strain tested was able to cause symptoms in Angus within 7 days at 18–20 C when introduced at concentrations below 10^6 cfu ml^{-1} by pressure infiltration (W. W. Shane, *unpublished data*).

Differences among strains in the symptoms produced correlated with bacterial multiplication in leaf tissues. No or only very slight symptom development occurred in cases where bacterial populations remained below approximately 5×10^5 cfu cm^{-2} leaf tissue. Chlorosis occurred in leaf tissues when bacterial populations reached approximately 5×10^5–1×10^6 cfu cm^{-2} leaf tissue. Grey-green water soaking followed by light tan necrosis of infiltrated leaf tissue occurred with strains that reached population densities $> 5 \times 10^6$.

A working definition was adopted that a bacterial strain was considered pathogenic to spring wheat if it caused chlorosis and/or necrosis when inoculated by the modified Hagborg method at a concentration of 5×10^6 cfu ml^{-1}. Strains MSU174 and MSU188 were considered pathogenic on Angus spring wheat whereas strains B359, PS347, and PS488 were less virulent on this cultivar (Fig. 1). All *P. s.* pv. *syringae* strains that were pathogenic on the test plants also produced syringomycin in vitro. However, other *P. s.* pv. *syringae* physiological/carbon compound ideotypes, such as PS35, and MSU202 that also produced syringomycin were not pathogenic on Angus. Some of the strains (PS347 and PS488) had been collected more than 5 yr previous to our tests and may have lost virulence in the interim.

The foliar symptom ratings were positively correlated ($r^2 = 0.54$) with the $\log_{10}$ bacterial populations 3 days after inoculation for the Len and Oligoculm crosses when considered as a group (Fig. 2). Populations of 10^6 cfu cm^{-2} of leaf tissue were generally associated with rating scale categories 3, 4, and 5, whereas populations below this amount were found with categories 0, 1, and 2. Category 0, no symptoms, corresponded with populations less than 5.5×10^6 cfu cm^{-2} leaf tissue.

Population dynamics of *P. s.* pv. *syringae* strain MSU174 in leaf tissues differed considerably among genotypes (Fig. 3). Growth in wheat line L1 was consistently high. Butte and MT7620 also supported high populations of this strain. Angus and Bonanza supported moderate populations, whereas Alex, Len, and Marshall supported the lowest populations. Symptoms on each of the wheats corresponded fairly closely with the bacterial population level. As in the above experiments, approximately 10^6 cfu cm^{-2} of leaf tissue were detected 3–5 days after inoculation for the cultivars in symptom class 1 (faint chlorosis). No chlorosis was detected with Marshall, Alex, and Len. Pronounced chlorosis, water soaking, and necrosis occurred on the lines L1 and MT7630. A chlorotic halo was associated with water soaking caused by *P. s.* pv. *syringae* strain MSU174 on these more susceptible genotypes (Fig. 4). The chlorosis and, to a lesser extent, water soaking extended beyond the areas originally infiltrated.

Resistance to *P. s.* pv. *syringae* was measured in a larger group of wheat lines by a single bacterial population determination 3 days after inoculation (Table 3). Again, a considerable range of *P. s.* pv. *syringae* populations were detected in the various wheat genotypes. Some of the apparent susceptibilities contrasted quite sharply with the field observations of Otta and Sellam and Wilcoxson. For example, Bounty 208, Red River 68, and Bonanza were rated as VS-S in the two field studies, whereas in the present studies these cultivars were MR.

DISCUSSION

Spring wheat genotypes relatively resistant to bacterial blight are available to the grower and plant breeder. Len, Marshall, and Alex supported relatively low populations of *P. s.* pv. *syringae*. These cultivars should prove useful to the farming community in years when bacterial blight is a problem. The screening method also indicated Oslo and other cultivars that are likely to be susceptible to bacterial blight in the field.

The stringent environmental conditions required for induction of disease by *P. s.* pv. *syringae* on spring wheat have hindered the evaluation of breeding material for resistance. Uniform misting of inoculated plants was essential for tests. Our most successful incubation chamber consisted of a misting chamber constructed according to a design by Krupinsky and Scharen (15) inside a growth chamber with controlled temperature and light. The strong relationship between symptom development and bacterial

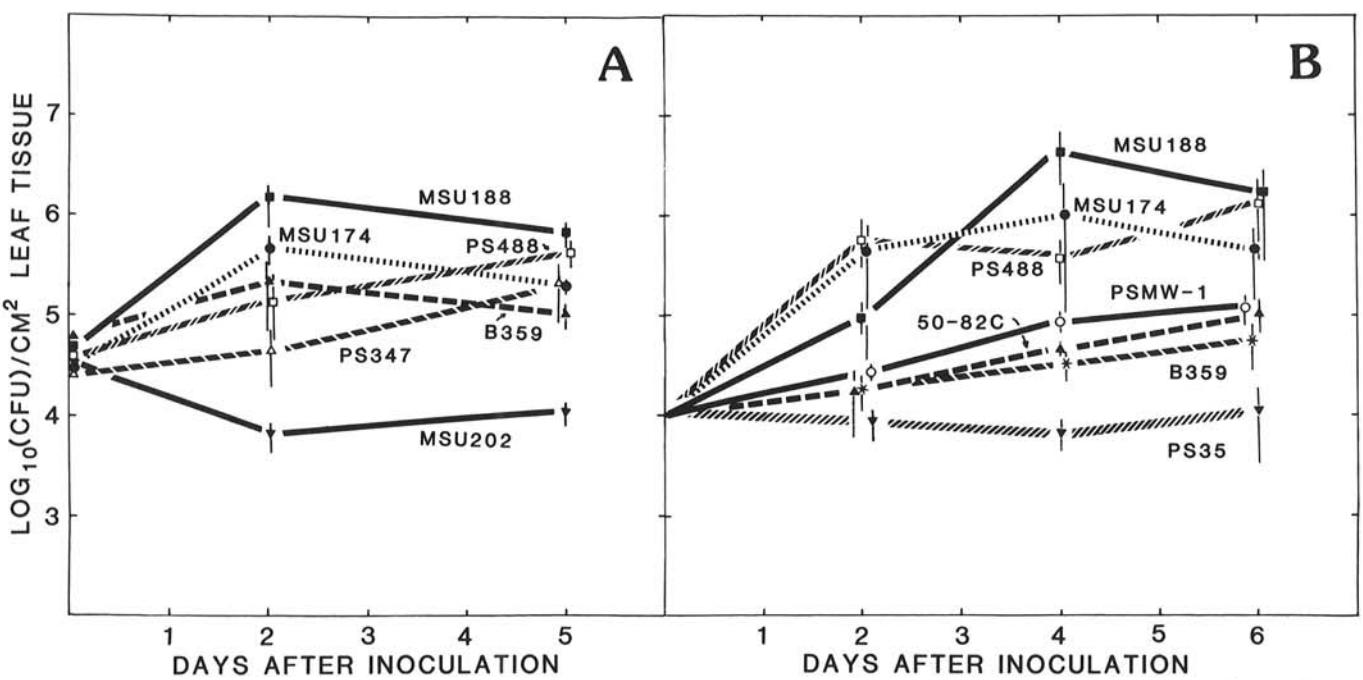

Fig. 1. Population dynamics of *Pseudomonas syringae* pv. *syringae* strains on Angus spring wheat following pressure infiltration with 5×10^6 cfu ml^{-1}. Bars indicate standard errors of the means. **A,** Experiment 1. **B,** Experiment 2.

population indicates that plant breeders can evaluate their material for resistance to the pathogen on the basis of symptoms rather than by laborious bacterial population studies.

Our studies indicate that *P. s.* pv. *syringae* is a weak pathogen on wheat. Criteria for pathogenicity with most bacteria-host combinations (22,29) include the requirements that the prospective pathogen be able to increase to significant concentrations from low initial doses and spread beyond the area of initial inoculations. Even with the most effective inoculation method, pressure-infiltration, symptoms did not spread appreciably beyond the area of inoculation in the moderately susceptible cultivar Angus. However, on more susceptible genotypes such as L1 and Bounty 309, water soaking and chlorosis spread to a limited extent from the point of inoculation with *P. s.* pv. *syringae* strain MSU174. Symptom development was completely halted if misting was stopped shortly before symptom expression. Dosages of inoculum at concentrations below 10^6 cfu ml^{-1} did not cause symptoms within 7 days after pressure infiltration inoculation. This is in contrast to other bacterial pathogen/host combinations where lower inoculum concentrations can induce disease (4,20,29,32). The effect of *P. s.* pv. *syringae* strains on wheat resembled the hypersensitive reaction attributed to a pathogen introduced to a nonhost plant (29,31), i.e., rapid multiplication followed by

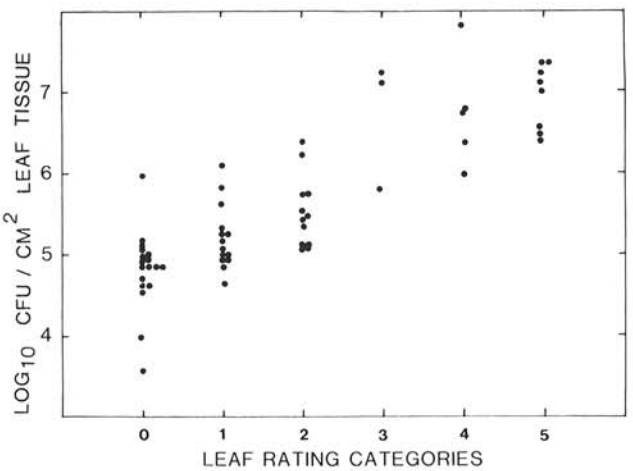

Fig. 2. Correlations between leaf rating categories for bacterial blight symptoms and populations of *Pseudomonas syringae* pv. *syringae* on F_2-derived F_5 lines from Len × Oligoculm crosses 3 days after inoculation with 5×10^6 cfu ml^{-1}. Rating scale used based on symptoms in inoculation site was: 0 = no symptoms; 1 = faint chlorosis; 2 = chlorosis < 10%; 3 = chlorosis and/or grey-green water soaking < 10%; 4 = chlorosis and/or grey-green water soaking in 10–90%; 5 = chlorosis and/or water soaking in >90%.

TABLE 3. Reactions of spring wheat cultivars to *Pseudomonas syringae* pv. *syringae* by three researchers

Cultivar	Log_{10} cfu cm^{-2}[b]	Observer rating[a]		
		Shane[c]	Otta[d]	Sellam[e]
Bounty 208	4.4 a	MR	VS	···
Olaf	4.8 a	MR	R	MR-R
Nowesta	4.8 ab	MR	R	MR
Red River 68	5.0 ab	MR	S	···
Bonanza	5.1 ab	MR	VS	···
Len	5.1 ab	MR	···	···
Sheridan	5.7 bc	MR	R	···
Alex	5.7 bcd	MR	···	···
Polk	6.1 cd	MS	S	MS-MR
Oslo	6.2 cde	MS	···	···
Glenlea	6.2 cde	MR	···	R
Chris	6.3 cde	MS	R	MR
Angus	6.4 cde	MS	···	···
Lark	6.7 de	MS	···	S
Bounty 309	7.2 e	S	···	S

[a] VS = very susceptible, S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.

[b] *P. s.* pv. *syringae* bacterial populations (BP, expressed as cfu cm^{-2}) were measured 3 days after infiltration inoculation with 5×10^6 cfu ml^{-1} *P. s.* pv. *syringae* strain MSU174.

[c] Cultivars were assigned to the following categories according to the bacterial populations 3 days after inoculation: MR = BP < 5×10^5; MS = 5×10^5 < BP < 10^7; S = BP > 10^7.

[d] Field rating of natural epidemic (19).

[e] Field rating of natural epidemic (27).

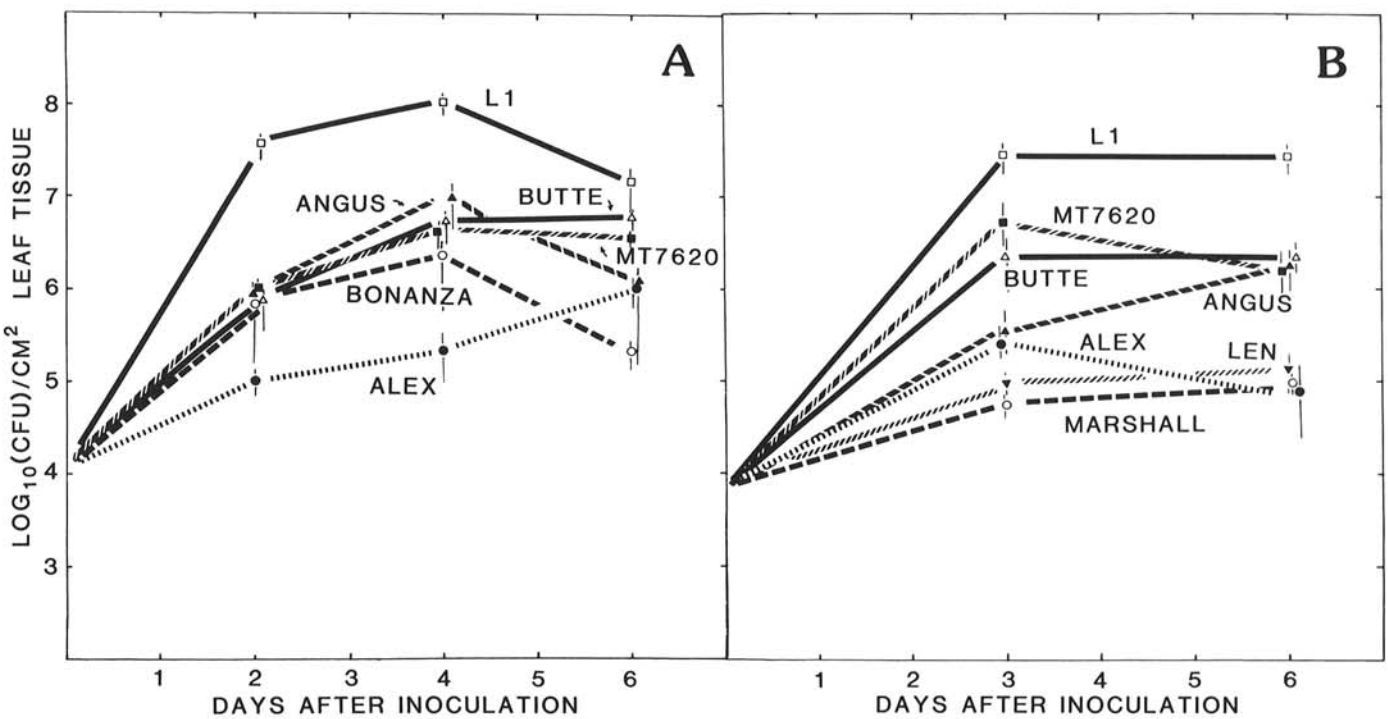

Fig. 3. Population dynamics of *Pseudomonas syringae* pv. *syringae* strain MSU 174 on spring wheat lines and cultivars following pressure infiltration with 5×10^6 cfu ml^{-1}. Bars indicate standard errors of the means. **A,** Experiment 1. **B,** Experiment 2.

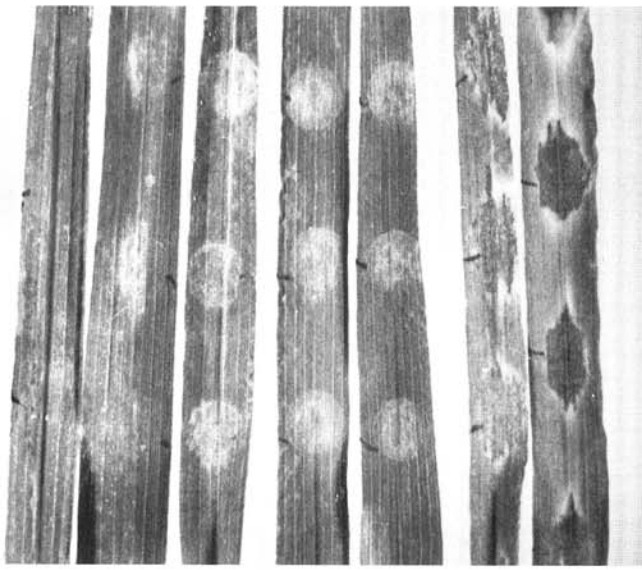

Fig. 4. Symptoms of bacterial blight on spring wheat genotypes 6 days after pressure infiltrations with 5×10^6 cfu ml^{-1} *Pseudomonas syringae* pv. *syringae* strain MSU174. From left to right: Alex, Butte, Bonanza, Angus, MT7620, Bounty 309, and L1.

necrosis from high initial doses and the inability to develop to significant populations from very dilute ($< 10^4$ cfu cm^{-2} of tissue) initial titers. The pathogenicity of *P. s.* pv. *syringae* strains on wheat may be enhanced by environmental conditions not examined in this study. For example, Baca and Moore (2) observed a sharp increase in *P. syringae* populations on sudan grass (*Sorghum sudanense* (Piper) Stapf) after freezing temperatures.

Pressure-infiltration with *P. s.* pv. *syringae* under controlled environmental conditions is the most satisfactory means for evaluation of wheat cultivar resistance to *P. s.* pv. *syringae*. Attempts to evaluate resistance to *P. s.* pv. *syringae* by foliar treatment with the *P. s.* pv. *syringae* toxin, syringomycin, have proved unsuccessful in studies by Shane (26). Cultivars exhibiting a wide range of susceptibility in inoculation studies were essentially equal in sensitivities to the toxin.

Spray-inoculation with *P. s.* pv. *syringae* did not cause appreciable symptoms unless the wheat was preconditioned by a treatment that promoted water congestion in/on leaf tissue. This situation is reminiscent of *P. s.* pv. *tabaci* on tobacco (6) and *P. s.* pv. *lachrymans* on cucurbits (34) where water soaking before spray inoculation is needed for pronounced disease development. Such specific requirements for pathogen development may help to explain the low prevalence of the disease on wheat in Minnesota and North Dakota during 1980–1984, although the bacterial streak organism, *Xanthomonas campestris* pv. *translucens* (Jones, Johnson, and Reddy) Dowson, was relatively common. The latter organism fares better at higher temperatures and requires less moisture for infection and spread.

We have noticed the tendency of certain wheat cultivars (e.g., Angus) to show intercellular water soaking under conditions of prolonged wetting. Such water soaking would likely promote bacterial growth. The pressure-infiltration inoculation method may obscure this possible factor related to susceptibility. The relationships of stomatal opening and prolonged wetting to intercellular water congestion merits further attention.

LITERATURE CITED

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