Races of *Pseudomonas syringae* pv. *glycinea* on Commercial Soybean in Eastern North Dakota

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ABSTRACT

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In a survey conducted from 1991 to 1993 of 170 commercial soybean (*Glycine max*) fields in North Dakota, 80% had plants with obvious symptoms of bacterial blight. Strains ($n = 164$) isolated from field-grown plants and characterized as *Pseudomonas syringae* pv. *glycinea* were inoculated onto wounded, fully expanded unifoliolate leaves of differential cultivars Acme, Lindarin, Harosoy, Chippewa, Merit, Flambeau, and Norchief. Reactions of the differentials showed that five of the eight known races in the United States were present in North Dakota. Race 4 constituted 63%, race 6 was 22%, race 2 was 7%, race 3 was 0.3%, and race 5 was 0.1% of the race profile. Five pathogenic strains could not be characterized as one of the known races.

Bacterial blight, caused by *Pseudomonas syringae* pv. *glycinea*, is one of the most prevalent soybean (*Glycine max* (L.) Merr.) diseases (18,21,24). It is worldwide in distribution and more severe in cooler soybean production regions (16,18). On foliage, bacterial blight lesions initially appear as small, angular, water-soaked areas surrounded by greenish yellow halos. Infected young leaves are often chlorotic and stunted. On older leaves, dark necrotic lesions can expand, coalesce, and shred, giving the leaf a ragged appearance (9,17,23). The pathogen is seedborne, and infected seeds may be discolored and shriveled (21).

North Dakota is situated on the fringe of the major soybean production area in the United States. About 260,000 ha of soybean are grown in North Dakota, where small grains are produced on 70% of the cropland. Over 62% of the soybean acreage is found in two southeastern counties, Cass and Richland (4,8). Diseases are not considered a serious constraint to soybean production in North Dakota (6); however, disease losses have not been well documented. In other locations, measured losses to bacterial blight have ranged from 5 to 18% (18).

The first race delimitation in *P. syringae* pv. *glycinea* was made by Cross et al. (5), who identified seven races based on their reactions on seven soybean differential cultivars. Currently, nine physiological

races have been characterized with standard soybean differentials (7,11,14). Table 1 shows some of the races and their reactions on the differential cultivars.

Specific genes for host resistance have been identified (15,20). Their value in developing resistant cultivars is dependent on the physiological races present in the pathogen population. The distribution of races within the host crop in North Dakota is largely unknown. In this report, we identify the races of *P. syringae* pv. *glycinea* detected on field-grown plants in North Dakota.

MATERIALS AND METHODS

Collections. Leaves showing characteristic bacterial blight symptoms were collected from each of 70 soybean fields located in the eight major soybean-producing counties of eastern North Dakota in 1991. The number of samples from each county were proportional to area of soybean production, and selected fields were 16 to 24 km apart. In 1992 and in 1993, collections were made in July and again in September from 50 soybean fields with known soybean cultivars Dawson, McCall, Evans, Ozzie, Roscoe, Lambert, Hunter, Baker, Proto, and Pioneer 9091. Five fields of each cultivar were sampled, and the fields were located throughout the eight counties. If leaves with symptoms of bacterial blight were present, a sample of 10 infected leaves was collected in pairs from five plants arbitrarily selected along a W-shaped field sampling pattern. Leaves were placed in paper bags and transported to the laboratory in a chilled container. Collections were frozen at -20°C until they were assayed.

Isolation procedure. From each sample collection, 20 to 25 lesions were aseptically excised and placed in a single, sterile

petri dish containing 5 ml of autoclaved distilled water. The tissue was crushed and allowed to soak 5 to 10 min. The suspension was streaked onto King's B medium (KB) (12). To obtain axenic cultures, single colonies that fluoresced upon exposure to ultraviolet light (wavelength 365 nm) were restreaked at least twice. Each culture was checked for purity by plating suspensions from 10-fold serial dilutions. All strains were checked for pathogenicity on greenhouse-grown cv. Acme seedlings. Seventy-five μl of a suspension containing 1×10^8 CFU/ml was applied to just fully expanded unifoliolate leaves that had been freshly wounded with sterile 60-mesh sandpaper lightly pressed against the leaf. Inoculum was spread with a sterile, water-moistened swab, and inoculated plants were immediately placed in a humid chamber (RH $> 90\%$, $25 \pm 5^{\circ}\text{C}$) for 24 h, then returned to the greenhouse where symptom progression was observed. Pathogenic strains were stored either at -20°C in a 40% glycerol solution of 0.01 M phosphate buffer (pH 7.0) or at 4°C on nutrient agar slants covered with sterile mineral oil.

Other sources of bacterial isolates. Reference strains of *P. syringae* pv. *glycinea* races 2, 4, 5, and 6 (provided by N. T. Keen, University of California, Riverside), five known *P. syringae* pv. *glycinea* strains uncharacterized to race (provided by E. Alvarez, Iowa State University), and the pathogenic strains from our field collections were checked for conformity to pathovar phenotype. Those that were levan positive, oxidase and arginine dihydrolase negative, did not rot potato slices, and caused a hypersensitive reaction on tobacco (LOPAT characters of +----+ [12]), were screened for race identity in the greenhouse.

Physiological race determination. Seeds of differential soybean cultivars Acme, Lindarin, Harosoy, Chippewa, Merit, Flambeau, and Norchief were surface-disinfested by immersion in 1% commercial sodium hypochlorite solution for 1 min, rinsed twice in sterile deionized water, and planted, two seeds per 10-cm-diameter clay pot containing 50% sand and 50% Sunshine mix (Fisons Horticulture Inc., Vancouver, British Columbia, Canada). Seedlings were grown in the greenhouse at $24 \pm 5^{\circ}\text{C}$ with supplemental lighting from 1,000-watt high-pressure sodium lamps to provide a 12-h photoperiod.

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Aqueous suspensions of inocula were prepared from cultures grown 24 to 36 h on KB and were adjusted spectrophotometrically to 10^6 to 10^7 CFU/ml. *Pseudomonas fluorescens*, a nonpathogenic strain originally obtained from the Department of Microbiology and Veterinary Science, North Dakota State University, and inoculations with distilled water were included as controls. Inoculum was applied to leaves, and inoculated plants were incubated as previously described. Four unifoliate leaves were tested for each cultivar-race combination. Beginning 3 days after inoculation, plants were examined daily for 5 days and rated by the system of Cross et al. (5), in which leaves with water-soaked lesions were considered susceptible and leaves with no hydrosis were designated resistant. Browning of leaf tissue with or without reddening of veins was considered

a resistant response (7). Each strain was tested twice, on two separate occasions.

RESULTS

Known races 4, 5, and 6 produced expected reactions on the standard differentials (Table 1). Norchief was resistant to race 2 from California. Strains I-140, I-237, and I-239 from Iowa were compatible with the seven soybean cultivars and were designated race 4. Strains ND 4B, ND 11D, I-61, and I-162 were compatible on Harosoy and Merit. Because they also were compatible on Lindarin, they could not be designated race 5.

Over 80% of the North Dakota fields surveyed in 1991, 1992, and 1993 had soybean plants infected by the bacterial blight pathogen. Of the 58 characterized *P. syringae* pv. *glycinea* strains isolated from plants grown in 70 fields in eight counties

of North Dakota in 1991 (Table 2), 40 induced susceptible reactions on all soybean differentials and were designated race 4. Fifteen strains were race 6, two were race 2, and one strain from Richland county was race 3.

In 1992, 44 *P. syringae* pv. *glycinea* strains were obtained from 50 fields with known soybean cultivars (Table 3). Strains collected from Ozzie, Roscoe, Lambert, and Proto were *P. syringae* pv. *glycinea* race 4. Two strains, one from Evans and one from Baker, were race 3. All *P. syringae* pv. *glycinea* strains from McCall were race 6. *P. syringae* pv. *glycinea* was confirmed in only one collection in July of 1992, and that strain was race 5 from Roscoe. In all other fields, bacterial blight was not evident in July, so collections were not made. Furthermore, the failure to obtain *P. syringae* pv. *glycinea* isolate from necrotic

Table 1. Reactions of soybean differential cultivars to strains of *Pseudomonas syringae* pv. *glycinea*

Strain ^a	Race	Soybean differential cultivars						
		Acme	Chippewa	Flambeau	Harosoy	Lindarin	Merit	Norchief
Psg								
R2	2	S	R	S	S	S	S	R
ND 302	2	S	R	S	S	S	S	S
ND 303	3	S	R	R	S	S	S	S
R4	4	S	S	S	S	S	S	S
ND 304	4	S	S	S	S	S	S	S
I-140	4	S	S	S	S	S	S	S
I-237	4	S	S	S	S	S	S	S
I-239	4	S	S	S	S	S	S	S
R5	5	R	R	R	S	R	S	R
ND 305	5	R	R	R	S	R	S	R
R6	6	R	R	S	R	R	R	S
ND 306	6	R	R	S	R	R	R	S
I-61	?	R	R	R	S	S	S	R
I-162	?	R	R	R	S	S	S	R
ND 4B	?	R	R	R	S	S	S	R
ND 11D	?	R	R	R	S	S	S	R
ND 1DF	?	S	R	S	S	R	S	S
ND 9P	?	S	R	R	S	R	S	R
ND1C	?	S	R	R	S	R	S	R
H ₂ O ^b	—	—	—	—	—	—	—	—
Pf ^b	—	—	—	—	—	—	—	—

^a Reference strains R2, R4, R5, and R6 were obtained in 1993 from N. Keen, University of California, Riverside. Strains I-61, I-140, I-162, I-237, and I-239 were from E. Alvarez, Iowa State University. North Dakota Strains are designated ND followed by number or number and letter description.

^b Controls were inoculated with water or with nonpathogenic *Pseudomonas fluorescens*.

Table 2. Distribution of *Pseudomonas syringae* pv. *glycinea* races 2, 3, 4, and 6 in soybean fields from eight counties in North Dakota in 1991

County	Fields surveyed	Total isolates	No. of isolates	Soybean differential cultivar ^a reactions							Race
				A	C	H	L	M	N	F	
Richland	23	21	1	S	R	S	S	S	S	R	3
			14	S	S	S	S	S	S	S	4
			6	R	R	R	R	R	S	S	6
Cass	20	19	2	S	R	S	S	S	S	S	2
			10	S	S	S	S	S	S	S	4
			7	R	R	R	R	R	S	S	6
Grand Forks	5	3	3	S	S	S	S	S	S	S	4
Barnes	4	4	4	S	S	S	S	S	S	S	4
Trail	8	6	4	S	S	S	S	S	S	S	4
			2	R	R	R	R	R	S	S	6
Ransom	4	3	3	S	S	S	S	S	S	S	4
Sargent	4	2	2	S	S	S	S	S	S	S	4
Steele	2	0									

^a Soybean differential cultivar designations A = Acme, C = Chippewa, H = Harosoy, L = Lindarin, M = Merit, N = Norchief, and F = Flambeau.

leaf lesions of some of the samples may be due to severe deterioration or the presence of secondary fast-growing bacteria.

Of the 14 strains collected in July of 1993, four were race 6 and 10 were race 4 (Table 4). By September, additional races were detected. Likewise, all strains obtained from McCall in 1993 were race 6. Five of the strains collected in 1993 gave reactions that did not fit the nine established physiological races of *P. syringae* pv. *glycinea*. Neither *Pseudomonas fluorescens* nor water produced any plant response consistent with a designation of resistant or susceptible.

DISCUSSION

Physiological characterization of *P. syringae* pv. *glycinea* strains from North Dakota showed race 4 to be prevalent. This was expected because Cross et al. (5) noted the dominance of *P. syringae* pv. *glycinea*

race 4 in soybeans grown in neighboring Minnesota. The major soybean production areas of North Dakota are contiguous to soybean production areas of northwestern Minnesota. In some areas, soybean fields in the two states are less than 400 m apart. Not only are seed stocks exchanged, but infested machinery and airborne dispersal (22) allow exchange of inoculum. Fett and Sequeira (7) and Basu (3) also reported the predominance of race 4 in Wisconsin, Iowa, and eastern Ontario. Cross et al. (5) reported that race 3 was the second most prevalent race in Minnesota, but this was not the case in North Dakota. Instead, 22% of all the isolates collected in North Dakota were race 6. This race was obtained only from McCall. McCall matures early and is grown extensively in the state (2).

Races 3 and 5 were found in limited numbers in North Dakota. Strains designated race 2 were compatible with all the

differential cultivars except Chippewa. In our greenhouse tests, the reference strain of race 2 did not produce previously described reactions on soybean differentials. The reference race 2 did produce a characteristic brown pigment on KB (7,13). Race 2 strains from North Dakota did not produce this pigment. Long et al. (13) also were unable to reproduce the original reactions of the differential cultivars inoculated with races 2, 3, and 7. They concluded that these races did not warrant a designated race status. Both Long et al. (13), and Fett and Sequeira (7) found that races 2, 3, and 7 produced plant reactions similar to those produced by race 4, and therefore designated them as race 4.

Some North Dakota strains produced reactions on the differential cultivars that differed from any produced by described races. As a result, no race designation was made. Recently, Abo-Moch et al. (1) des-

Table 3. Distribution of *Pseudomonas syringae* pv. *glycinea* races 2, 3, 4, 5, and 6 in soybean fields planted to 10 cultivars in North Dakota in 1992

Cultivar	Fields surveyed	Total isolates ^a		No. of isolates	Soybean differential cultivars ^b							Race
		July	Sep.		A	C	H	L	M	N	F	
Dawson	5	0	6	5	S	S	S	S	S	S	S	4
				1	R	R	S	S	S	R	R	5
McCall	5	0	7	7	R	R	R	R	R	S	S	6
Evans	5	0	4	1	S	R	S	S	S	S	R	3
				3	S	S	S	S	S	S	S	4
Ozzie	5	0	5	5	S	S	S	S	S	S	S	4
Roscoe	5	1	4	5	S	S	S	S	S	S	S	4
Lambert	5	0	3	3	S	S	S	S	S	S	S	4
Hunter	5	0	3	3	S	R	S	S	S	S	S	2
Baker	5	0	4	1	S	R	S	S	S	S	R	3
				3	S	S	S	S	S	S	S	4
Proto	5	0	4	4	S	S	S	S	S	S	S	4
Pioneer	5	0	3	1	S	R	S	S	S	S	S	2
				2	S	S	S	S	S	S	S	4

^a Fields surveyed in July and again in September.

^b Soybean differential cultivar designations A = Acme, C = Chippewa, H = Harosoy, L = Lindarin, M = Merit, N = Norchief, and F = Flambeau.

Table 4. Distribution of *Pseudomonas syringae* pv. *glycinea* races 2, 3, 4, 5, and 6 in soybean fields from 10 cultivars in North Dakota in 1993

Cultivar	Fields surveyed	Total isolates ^a		No. of isolates	Soybean differential cultivars ^b							Race
		July	Sep.		A	C	H	L	M	N	F	
Dawson	5	3	9	2	S	R	S	S	S	S	R	3
				7	S	S	S	S	S	S	S	4
				1	R	R	S	R	S	R	R	5
				1	R	R	S	S	S	R	R	?
				1	S	R	S	R	S	S	S	?
McCall	5	4	10	14	R	R	R	R	R	S	S	6
Evans	5	1	5	2	S	R	S	S	S	S	S	2
				4	S	S	S	S	S	S	S	4
Ozzie	5	0	3	3	S	S	S	S	S	S	S	4
Roscoe	5	2	4	5	S	S	S	S	S	S	S	4
				1	S	R	S	R	S	R	R	?
Lambert	5	0	2	2	S	S	S	S	S	S	S	4
Hunter	5	1	3	1	S	R	S	S	S	S	S	2
				3	S	S	S	S	S	S	S	4
Baker	5	1	4	1	S	R	S	S	S	S	S	2
				3	S	S	S	S	S	S	S	4
				1	R	R	S	S	S	R	R	?
Proto	5	2	5	6	S	S	S	S	S	S	S	4
				1	S	R	S	R	S	R	R	?
Pioneer	5	0	3	2	S	R	S	S	S	S	S	2
				1	S	S	S	S	S	S	S	4

^a Fields surveyed in July and again in September.

^b Soybean differential cultivar designations A = Acme, C = Chippewa, H = Harosoy, L = Lindarin, M = Merit, N = Norchief, and F = Flambeau.

ignated race 10, which reacted similarly to strains ND 4B, ND 11D, I-61, I-162, and strains described by Basu (3) from eastern Ontario. Therefore, race 10 may be considered present in the state. Three strains remain undesignated. While extrinsic factors can influence plant responses (7,10,19), the consistent and reliable reactions of differentials to undesignated strains indicate that a variant race is probably present in the state.

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