Occurrence and Characteristics of Isolates of Pseudomonas syringae on Winter Wheat

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ABSTRACT

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Pseudomonas spp. pathogenic on wheat were isolated from 21 samples of winter wheat seed from Nebraska, South Dakota, North Dakota, Alberta, and Saskatchewan. Seed infestation ranged from 0.2 to 5.0% for most samples. One Karkhof sample from Lethbridge had 12.8% infestation. Isolates from diseased leaves of wheat, corn, foxtail, and sorghum and from wheat kernels could not be differentiated from known isolates of *P. syringae* in biochemical, serological, or pathological tests. The distribution among *P. syringae* serotypes was different for isolates from wheat, corn, foxtail, and sorghum than from stone and pome fruits.

Additional key words: *Pseudomonas atrofaciens*.

Pseudomonas syringae Van Hall, the incitant of leaf necrosis of spring and winter wheat, has produced annual outbreaks of disase in South Dakota for the past seven years (11, 12). *Pseudomonas syringae* also has been isolated from wheat seed grown in South Dakota (11). One objective of this study was to investigate the occurrence of seed-borne inoculum since it might serve as an important link in the disease cycle.

Leaf necrosis (12) and the leaf spot stage of basal glume rot of wheat incited by *P. atrofaciens* (McCullock) Stevens (10), 12) have a similar symptomatology. Doudoroff and Palleroni (3) include *P. atrofaciens* as a synonym of *P. syringae*. However, Dye et al. (4) pointed out that Doudoroff and Palleroni's treatment of the genus *Pseudomonas* does not provide a system of nomenclature that expresses phytopathogenic individuality, and they recommend that 80 nomenspecies, including *P. syringae* and *P. atrofaciens*, be retained and studied further. A report from New Zealand by Wilkie (18) indicates that *P. atrofaciens* from wheat and *P. syringae* are different species. Schaad et al. (16) reported that little, if any, genetic relatedness existed between *P. atrofaciens and P. syringae* in DNA homology studies. However, the validity of this conclusion should be questioned since the one culture of *P. atrofaciens* studied was reported to be nonfluorescent, which would indicate that it is an atypical culture. In addition, no pathogenicity test was reported for either the *P. atrofaciens* or the *P. syringae* isolate used in the study. The second objective of this study was to compare some of the characteristics of known *P. syringae* isolates with isolates from wheat that I have identified as *P. syringae*.

MATERIALS AND METHODS

Isolations from diseased plant materials and from wheat kernels.—Seed samples (0.5-kg) of three cultivars of winter wheat, Lancer, Froid, and Winoka, were obtained from wheat breeders in Nebraska, South Dakota, North Dakota, Alberta, and Saskatchewan in 1971. Substitutions of seed of locally grown cultivars was requested if seed of the three cultivars was not available. Twenty-one different seed samples representing nine different cultivars were received in response to the request. Upon receipt, each sample was distributed in a uniform layer in a sterilized 12.5×33×6-cm plastic container. One-hundred seeds were selected randomly from the surface layer of seeds with a forceps which was sterilized between each seed selected. The cover was then placed on the plastic container and the entire container was inverted ten times to mix the seeds. A second 100-seed sample was then removed as previously described. The process was repeated until 500 seeds had been selected from each of the seed samples. All isolations of bacteria from seeds was attempted in D-4 broth (6) which has been reported to be selective for *Pseudomonas* spp. Individual seeds were placed with 3 ml of sterile D-4 broth in a 10-ml screw-capped tube and incubated in stationary culture without aeration at 22-24 C for 18 hours. The tubes then were agitated thoroughly and three Pasteur pipette drops of each culture were placed on the agar surface of a separate plate of King's medium B (MB) (7) and spread with an L-shaped glass rod. After incubation for 24, 48, and 72 hours at 22-24 C, possible *Pseudomonas* colonies were detected visually by pigment production and/or by fluorescence under a Model UVL-22, BLAK-RAY® (Ultraviolet Products, Inc., San Gabriel, Calif.) ultraviolet light. Representative colony types were selected and checked for cytochrome oxidase reaction (9). Oxidase-negative colonies were retained, restreaked twice onto MB to check for purity, and one loopful of unwashed cells was placed in 4 ml of sterile distilled water at 7-8 C for long-term storage.

Isolations were made from necrotic lesions on leaves of 11 wheat cultivars, *Triticum aestivum* (L.); corn, *Zea mays* (L.); green foxtail, *Setaria virdis* (L.); and sorghum, *Sorghum vulgare* Pers. var. *subglabrescens* (Steud.) A. F.

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Hill collected from 20 different fields in South Dakota from 1971 to 1974. Diseased tissue was placed in three to four drops of sterile distilled water in a plastic petri dish and triturated with a sterile glass rod. A loopful of the resulting suspension was streaked onto a plate of MB. Plates were observed 48-72 hours after streaking to detect colonies that produced a green-fluorescent, water-soluble pigment. Individual colonies representative of each green-fluorescent colony type present were transferred to MB and checked for oxidase reaction (9). All oxidase-negative cultures were handled as previously described.

Pathogenicity tests.—Results from greenhouse inoculations on wheat and sorghum demonstrated that oxidase-negative isolates of pseudomonads pathogenic on wheat also produced holcus spot, a disease commonly incited by *P. syringae* on sorghum (12). Since sorghum could be used at a younger stage than wheat and gave very distinctive symptoms, it was used to test the pathogenicity of all oxidase-negative isolates. Two-week-old sorghum plants were vacuum-infiltrated with suspensions of 24-hour-old bacteria containing approximately 1×10^5 cells per milliliter, incubated in a mist chamber for 24 hours, and placed on a greenhouse bench at 24-27 C. Symptoms were recorded 1 week later.

Serological tests.—All oxidase-negative isolates were reacted in Ouchterlony (14) double-gel diffusion plates against antisera of the six major *Pseudomonas syringae* serotypes and separated into serotypes on the basis of reaction of the somatic ('O') antigen (13). Antigens were prepared by growing cultures for 2 days on MB plates and suspending all growth from one plate in 8 ml of sterile water.

Physiological and biochemical tests.—All oxidase-negative isolates were tested for inhibition of *Geotrichum candidum* Link ex Fries, a biological indicator for the production of syringomycin by *P. syringae* (2).

Unless otherwise stated, the methods of Cowan and Steel (1) were used to compare selected isolates of *Pseudomonas* from wheat with selected isolates of known *P. syringae* and *P. atrofaciens*. Biochemical comparisons were: utilization of organic acids [on media described by Koser (8)] and carbohydrates; hydrolysis of aesculin, casein, gelatin, starch and urea; production of hydrogen sulfide and indole; reduction of nitrate; and reaction in litmus milk and in the methyl red and Voges-Proskauer tests.

Isolation of syringomycin.—Two cultures of *P. syringae* and two cultures of *Pseudomonas* from wheat (one isolated from a seed and the other from a leaf lesion) which were known to inhibit *G. candidum* were used in toxin isolation studies. Toxin was isolated by the methods of Gross and DeVay(5).

RESULTS

A total of 122 oxidase-negative isolates of *Pseudomonas* from diseased wheat, corn, foxtail, and sorghum and 195 oxidase-negative isolates from wheat seed produced holcus leaf spot when sorghum was

TABLE 1. Occurrence and serotypes of *Pseudomonas syringae* on winter wheat seed collected from eight geographical locations in the United States and Canada

Wheat cultivar	Source of seed[a]	Seeds yielding *P. syringae* (percent of 500 tested)	*P. syringae* isolate (percentage in serotype)					
			I	II	III	IV	V	VI
Sundance	Saskatoon, Saskatchewan	2.2				55	18	27
Winalta	Saskatoon, Saskatchewan	1.2				50	33	17
Kharkof	Saskatoon, Saskatchewan	12.8	1			41	33	25
Winoka	Lethbridge, Alberta	0.2				100		
Froid	Lethbridge, Alberta	0.8				25	25	50
Kharkof	Lethbridge, Alberta	0.6				67		33
Winoka	Dickinson, North Dakota	1.2			17	50	17	17
Froid	Dickinson, North Dakota	0.8				50	50	
Lancer	Dickinson, North Dakota	0.2				50		50
Winoka	Casselton, North Dakota	0.2			100			
Lancer	Casselton, North Dakota	1.4				43	57	
Winoka	Minot, North Dakota	0.6				100		
Froid	Minot, North Dakota	1.2	33			17	50	
Lancer	Minot, North Dakota	1.4				29	42	29
Winoka	Watertown, South Dakota	0.4				100		
Bronze	Watertown, South Dakota	1.2				50		50
Centurk	Watertown, South Dakota	5.0			12	72	4	12
Winoka	Brookings, South Dakota	4.0	20			55	20	5
Lancer	Lincoln, Nebraska	1.2				83		17
Scout 66	Lincoln, Nebraska	1.8				67	33	
Centurk	Lincoln, Nebraska	0.4				100		

[a]Wheat seed kindly furnished by:
V. A. Johnson, Agronomy Department, University of Nebraska, Lincoln, NB.
J. R. Erickson, Agronomy Department, North Dakota State University, Fargo, N. D.
D. R. Knott, Department of Crop Science, University of Saskatchewan, Saskatoon, Saskatchewan.
M. N. Grant, Research Station, Canada Department of Agriculture, Lethbridge, Alberta.
D. G. Wells, Department of Plant Science, South Dakota State University, Brookings, South Dakota.

inoculated and maintained in the greenhouse. Isolations from 21 seed lots representing eight winter wheat cultivars from nine geographical locations resulted in detection of *P. syringae* in all seed lots (Table 1). Contaminated seed varied from a high of 12.8 percent in the seed lot of cultivar Karkhof from Saskatoon, Saskatchewan, to a low of 0.2 percent in those of cultivar Winoka from Casselton, N. D. and Lethbridge, Alberta.

Serological reactions.—The somatic ('O') antigen of each of the 317 isolates reacted in a homologous manner with one of the six *P. syringae* antisera (Tables 1 and 2). More than one serotype occurred in most of the seed lots that were sampled (Table 1). The distribution of serotypes in isolates from wheat did not differ appreciably from that of isolates from corn, sorghum, and foxtail (Tables 1 and 2). However, when the distribution of serotypes from stone and pome fruits (13) was compared to that of serotypes from wheat, corn, sorghum, and foxtail in this study, differences were noted (Fig. 1).

Biochemical characteristics.—None of the cultures reduced nitrate, and all were oxidase-negative. No H_2S or indole was produced; the methyl red test and Voges-Proskauer reaction were negative. The action on carbohydrates was oxidative, and acid was produced from arabinose, galactose, glucose, glycerol, mannose, sucrose, and xylose. Growth on raffinose and rhamnose was variable (Table 3). Growth and an alkaline reaction occurred on citric, formic, lactic, malic, and succinic acid salts, but not on tartaric acid. Aesculin, casein, gelatin, urea, and starch hydrolysis all varied with the cultures tested (Table 3). Litmus milk, after a 2-week incubation period, also gave varied results ranging from reduction and peptonization with an alkaline reaction to just an alkaline reaction. Production of a toxin which inhibits *G. candidum* varied within the groups of isolates studied (Table 3). Syringomycin isolation methods yielded a toxin which inhibited *G. candidum* from each of the four isolates studied.

DISCUSSION

The occurrence of *P. syringae* in every winter wheat seed lot tested from a wide geographical area suggests that seed-borne bacteria may be one of the sources of *P. syringae* in wheat fields. The number of infested seed observed would provide numerous opportunities for the bacterium to become established in a given field.

The predominance of isolates of serotype IV suggests there may be some selection pressure in wheat for bacteria of this serotype, perhaps owing to differences in pathogenic capabilities. Differences in the frequency of occurrence of serotypes in stone and pome fruit isolates compared to wheat, corn, foxtail, and sorghum isolates also may be the result of host selection pressures exerted on a highly variable natural population of bacteria. Also, it is possible that geographical separation is responsible for the serotype differences observed.

I conclude that the organism that incites holcus leaf spot on corn, sorghum, and foxtail is the same as the one that incites wheat leaf necrosis. This organism is *P. syringae*, since no significant differences were detected between known *P. syringae* isolates and wheat isolates. Sellam and Wilcoxson also concluded that a *Pseudomonas* which incited a recent outbreak of wheat leaf blight in Minnesota was *P. syringae* (17). Some host specialization in pathogenic capability of *P. syringae* isolates may exist, as indicated by Perlasca (15), but this slight specialization does not seem to warrant placing them in separate species as Wilkie (18) suggests. Placing *P. syringae* and pathogenic isolates of *Pseudomonas* from

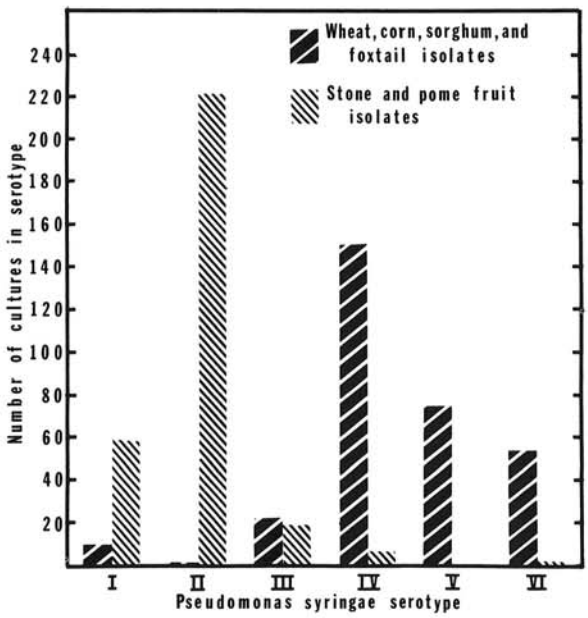

Fig. 1. Comparison of serotype distribution of isolates of *Pseudomonas syringae* from stone and pome fruits with isolates from wheat, corn, sorghum, and foxtail.

TABLE 2. Serotype distribution of *Pseudomonas syringae* isolated from diseased corn, foxtail, sorghum, and wheat in South Dakota

Diseased material	Number of isolates	*P. syringae* isolate (percentage in serotype)					
		I	II	III	IV	V	VI
Corn leaves	10	10		40		50	
Green foxtail leaves	12			33	25	42	
Sorghum leaves	2			100			
Wheat leaves	107	4	1	7	50	19	19
Wheat glumes	2	50			50		

wheat into separate species creates numerous identification problems and would assume that the organism causing holcus leaf spot of corn and sorghum is *P. atrofaciens*. This suggestion seems to be rejected by Gross and DeVay's (5) report that toxin-producing isolates of *P. syringae* produce holcus leaf spot on corn.

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TABLE 3. Some biochemical characteristics of *Pseudomonas syringae*, *Pseudomonas atrofaciens*, and *Pseudomonas* spp. isolates from wheat [a]

Test	Cultures					
	P. syringae (20 cultures)		*P. atrofaciens* (five cultures)		*Pseudomonas* spp. from wheat (21 cultures)	
	Number		Number		Number	
	+	−	+	−	+	−
Aesculin hydrolysis	20		5		16	5
Casein hydrolysis	7	13	5		6	15
Gelatin hydrolysis	11	9	5		7	14
Urea hydrolysis	18	2	5		2	
Starch hydrolysis	3w[b]	17		5	2w[b]	19
Utilization of raffinose	11	9	5		21	
Utilization of rhamnose	18	2	4	1	21	

[a]Sources of isolates:

P. syringae

—National Collection of Plant Pathogenic Bacteria, Harpenden, Hertsfordshire, England. Isolates 649, 1089, 1259.

—International Collection of Phytopathogenic Bacteria, Davis, Calif. Isolates Ps5, Ps137B, Ps144, Ps 173-28, PA-122.

—J. E. DeVay, Department of Plant Pathology, University of California, Davis, Calif. Isolates B-5, B124, B130, B238.

—H. English, Department of Plant Pathology, University of California, Davis, Calif. Isolates 12, 29, 77-A, 89, 857B, 881.

—W. H. Shaffer, Jr., Department of Plant Pathology, University of Missouri, Columbia, Missouri. Isolates M-1 from Lovrekovich in Israel.

—D. W. Dye, Plant Disease Division, Department of Science and Industrial Research Aukland, N.Z. Isolate P171.

P. atrofaciens

—J. Cumming, Research Branch, Chemistry and Biology Research Institute, Canada Department of Agriculture. Isolates P8, P51, P53.

—A. K. Vidaver, Plant Pathology Department, University of Nebraska, Lincoln, NB. Isolates A-1 and S-1.

Pseudomonas spp. from wheat.

All isolated by the author.

[b]The letter "w" indicates a weak reaction.

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