Bacterial Blight of Chickpea Incited by *Pseudomonas andropogonis*

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ABSTRACT

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Stem cankers, leaf spots, and dieback were observed on field-grown chickpeas in Maine in 1981. The causal organism was a white-pigmented, rod-shaped, gram-negative, nonfluorescent bacterium. Twenty strains induced symptoms on alfalfa, corn, soybean, sudangrass, white clover, and chickpea in a host-range study. Of 28 chickpea cultivars tested, all were susceptible to the disease. On the basis of comparisons with type strains, the causal organism was identified as *Pseudomonas andropogonis*. This is believed to be the first report of this bacterium in chickpea in the United States.

Chickpea (*Cicer arietinum* L.) is a large-seeded legume currently grown in India, Pakistan, Spain, Algeria, Mexico, and the United States. Most of the 3,500 tons of chickpeas produced in the United States are grown in California (2). The United States annually imports 10,000 tons of chickpeas from Mexico, but the Mexican government has discouraged production of chickpeas in favor of pinto beans. This preference, combined with decreasing production trends in California, should improve domestic markets for this commodity for several years (2). Consequently, there is increased interest in planting this legume in Maine and elsewhere in the United States.

Several diseases contribute to low or erratic yield and poor quality of the crop. Although Kaiser (12) listed pathogens affecting chickpea, most of our knowledge of chickpea diseases in the United States is from research in California.

In July 1981, blighted plants were observed in a chickpea planting in central Maine. The purpose of this paper is to describe the disease, to characterize and identify the pathogen, and to record its occurrence in Maine. A preliminary report on this disease was published in 1982 (5).

MATERIALS AND METHODS

Isolation of causal organism and studies on pathogenicity. Stem and leaf lesions from several diseased chickpea plants from Albion, ME, were excised and observed for bacterial streaming. Sections were surface-sterilized in 0.5% NaOCl (10% Clorox) for 2 min, washed twice in sterile water, and placed in test tubes containing 5 ml of sterile water for 3 hr. Loopfuls of the suspension were streaked onto nutrient agar plates, which were incubated at 26 C for 48 hr, then single colonies were selected and transfers made to nutrient agar slants.

Twenty white-pigmented bacterial isolates were used in pathogenicity tests. Store-bought chickpea seeds (of an unknown variety) were planted in Pro-Mix BX (Premier Brands, Inc., New Rochelle, NY) and grown in the greenhouse until plants were 5 wk old. Ten plants were inoculated by coating a sterile dissecting needle with cells from each 24-hr slant culture and stabbing four internodes per plant. Ten control plants were stabbed with a sterile dissecting needle. After inoculation, plants were incubated at 100% RH and 28 C for 48 hr, then returned to the greenhouse bench. Symptoms were apparent at 7 days in plants inoculated with the bacterial isolates. Lesions on artificially inoculated plants were sectioned and treated as before and recovered bacterial colonies were also smooth and white. These 20 isolates were used for a more thorough investigation of the disease.

Strain 23060 (from *Zea mays* L.) of *Pseudomonas andropogonis* (Smith) Stapp and strains 12636 (from *Trifolium repens* L.) and 19309 (from *Stizolobium deeringianum* L.) of *P. stizolobii* (Wolf) Staff were obtained from the American Type Culture Collection (ATCC). These strains plus the isolates from Maine-grown chickpeas were used for pathogenicity tests and biochemical characterization.

Ten 8-wk-old plants were sprayed until runoff with 10^8 colony-forming units (cfu) per milliliter of each bacterial strain. At this time, plants were 1 ft tall and had blossoms and young pods. Inocula were prepared by suspending cells from 24-hr nutrient agar slants in sterile water and adjusting the concentration colorimetrically. Plants were incubated as described previously.

Varietal and host-range trials. Chickpea cultivars CA 392, CA 404, IC 323, IC 4882, IC 5899, IC 6832, IC 6837, IC 6895, IC 7519, IC 7520, IC 8129, IC 9026, IC 9419, IC 9455, Lyon, Mission, NEC 1527, NEC 1732, UC 5, 77D-174, 85-21, and 85-22 were obtained from Richard L. Auld, Department of Plant and Soil Science, University of Idaho, Moscow. Cultivars Malawi, Malawi A-80, Red Kenya, Red Kenya A-8, and White Afghanistan were obtained from Johnny's Selected Seeds, Albion, ME. Cultivar CA Musser was obtained from the Vermont Bean Seed Company, Bomoseen.

Alfalfa (*Medicago sativa* L. 'Saranac'), corn (*Z. mays* 'Sugar and Gold'), lespedeza (*Lespedeza capitata* L.), red clover (*Trifolium pratense* L.), soybean (*Glycine max* L.), sudangrass (*Sorghum vulgare* var. *sudanese* 'Sorgo Sudangrass Hybrid'), and white clover (*T. repens*) seeds were obtained from Agway, Inc., Syracuse, NY. Green bean (*Phaseolus vulgaris* L. 'Bountiful') seed was obtained from W. Atlee Burpee Co., Warminster, PA. Hyacinth bean (*Dolichos lablab* L.) seed was obtained from Johnny's Selected Seeds.

Ten 5-wk-old plants were inoculated by stabbing the internodes, and the entire foliage of 10 other plants was sprayed with the bacterial suspension. All plants were incubated as described previously. Twenty test strains and the three type strains were used in these experiments. All inoculations as well as previously described experiments were repeated twice.

Plants were rated visually for disease using a scale of 0–4, where 0 = no disease, 1 = light infection, 2 = moderate infection, 3 = heavy infection, and 4 = plant mortality.

Characterization of causal organism. The following tests (14,16,19–23) were used to characterize the Maine isolates and the three type strains: Gram stain, hanging-drop motility test, flagellar stain (15), poly-β-hydroxybutyrate accumulation, anaerobic growth on nutrient agar, growth in 2, 4, and 6% sodium chloride nutrient broth, and fluorescent pigment production on King's medium B (13). Temperature relationships were observed by growing bacteria in tubes of Difco

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yeast extract (5 g/L) at 6, 15, 20, 25, 30, 37, and 41 C. Biochemical tests included nitrate reduction, hydrogen sulfide, methyl red, Voges-Proskauer, catalase, lysine decarboxylase, oxidase, levan, arginine dihydrolase, aesculin hydrolysis, urease, indole, starch hydrolysis, potato soft rot, ammonia evolution, casein hydrolysis, ornithine fermentation, cetrimide tolerance, citrate utilization, phenylalanine deaminase, Tween 80 hydrolysis, tobacco hypersensitivity (HR), ONPG test for β-galactosidase, and gelatin hydrolysis and liquefication.

Carbon source utilization was determined by adding the sterile filtrate of each carbon source at 0.1% (w/v) final concentration to the autoclaved and cooled mineral salts medium of Ayers et al (3), pH 7.2, which contained 1.2% Ionagar. After addition of the compound, plates were poured. Bacterial strains were streaked onto the media, incubated at 28 C, and read at 3, 7, and 14 days. Growth was compared with plates that contained no added carbon source. Compounds incorporated included acetate, alanine, β-alanine, arabinose, benzoate, cellobiose, fructose, galactose, glucose, glycerol, lactate, lactose, levulinate, malonate, maltose, mannitol, mannose, propanol, pyruvate, raffinose, rhamnose, salicin, sorbitol, succinate, sucrose, D(−)tartrate, *meso*-tartrate, trehalose, and xylose.

Electron microscopy. Five Maine bacterial isolates were grown in low-salt broth until cultures were visibly turbid (10^6 cfu/ml). Bacteria were fixed in 10% formalin for 24 hr, then washed and resuspended in distilled water. Suspensions of cells were placed on a Formvar-coated grid for 2 min, then dried on filter paper. Phosphotungstic acid (1.6%, pH 7) was added to the grid for 2 min. Bacterial cells were viewed with a Phillips 201 transmission electron microscope.

RESULTS

Isolation of causal organism and pathogenicity studies. Field-grown chickpeas showed diverse disease symptoms. Leaflets had small necrotic spots 1 mm in diameter that were irregular in shape with chlorotic margins. Petioles and stems had extensive lesion development that was sometimes present along the entire branch. As a result of this heavy lesion coverage on leaves and stems, some plants were severely defoliated. Few blossoms or pods were observed on the infected plants.

Slow-growing, white-pigmented bacterial isolates reproduced petiole lesions in stab-inoculated greenhouse-grown chickpea plants. Furthermore, spray inoculations induced leaf spots and pod lesions in plants. When these lesions on petioles, leaves, and pods were used for isolations, the same bacterium was always recovered, thus fulfilling Koch's postulates for proof of pathogenicity.

Results of the inoculations of 10 plant species by both chickpea and type strains are presented in Table 1. Alfalfa, chickpea, soybean, sudangrass, and white clover were infected by all strains, and ATTC strain 23060 and the chickpea strains also infected corn plants. Green bean, hyacinth bean, lespedeza, and red clover were not infected by any bacterial strains.

All 28 chickpea cultivars were found susceptible to the chickpea and type strains although disease ratings for the cultivars ranged from 1.4 (moderately resistant) to 2.7 (susceptible).

Identification of causal organism. Bacterial strains from Maine were slow-growing and produced colonies that were smooth, white, and circular on nutrient agar. Growth was initially butyrous but became rubbery after 72 hr, and colonies were difficult to remove with a transfer needle. Bacterial cells were slender rods with rounded ends, occurring singly. Cells measured 0.5–0.7 × 1.2–2.0 μm, were gram-negative, and possessed a single polar flagellum. The sheath reported by Fuerst and Haywood (7) surrounding the flagellum of *P. stizolobii* was not observed in five of the Maine strains subjected to electron microscopy.

Results of selected biochemical tests employed to study chickpea strains and to compare these strains with other previously reported nonfluorescent *Pseudomonas* spp. that attack legumes or grasses (1,4,8,10,11) are presented in Table 2. In addition, the chickpea strains were positive for ammonia evolution, tobacco HR, β-galactosidase, growth at 15, 20, 25, and 30 C, and utilized acetate, alanine, β-alanine, lactate, lactose, pyruvate, and xylose. The strains were negative for anaerobic growth, lysine decarboxylase, indole, potato soft rot, casein hydrolysis, ornithine fermentation, cetrimide tolerance, phenylalanine deaminase, and gelatin liquefication, and did not utilize arabinose, benzoate, cellobiose, levulinate, propanol, raffinose, rhamnose, or trehalose.

Reactions of the three type strains resembled those of the chickpea strains very closely with a few exceptions: 12636 and 19309 did not utilize β-alanine; 12636 did not utilize sorbitol; and 12636, 19309, and 23060 had a very strong citrate test, whereas the chickpea strains had a very weak reaction. On the basis of these results and plant inoculations, the chickpea bacterium was identified as *P. andropogonis*.

DISCUSSION

P. stizolobii was first reported by Wolf (27) to cause a leaf spot on velvet bean (*S. deeringianum*). The bacterium has subsequently been reported to infect white clover (1,4,10,11), red clover (1,4), alfalfa (1), green bean (1), red kidney bean (4), broadbean (1), hyacinth bean (1), lespedeza (4), ladino clover (4), subterranean clover (1), vetch (1), bougainvillea (18), corn (11), and sorghum (11). *P. andropogonis* was found to be the causal agent of bacterial stripe of sorghum and corn (6,11,24–26) but was also reported to infect white clover (8,9). Goto and Starr (10) and Hayward (11) showed that *P. andropogonis* and *P. stizolobii* were very closely related species, and *P. andropogonis* was recommended as the scientific name of the pathogen on all hosts, with *P. stizolobii* as a later synonym.

Chickpea strains were nearly identical to the three type strains (one each from corn, white clover, and velvet bean) in a wide range of biochemical tests. Furthermore, the three type strains differed very little from each other. The chickpea strains compared very closely with strains of *P. andropogonis* isolated from different hosts (Table 2). The host range of the chickpea strains also compared very closely with that of the type strains (Table 1). However, this is the first report of this pathogen causing disease in soybean. Previously, Wolf (27), Burkholder (4), and Allen et al (1) had reported soybean to be a nonhost for the bacterium.

This is the first report of *P. andropogonis* in chickpea in the United

Table 1. Comparisons of mean disease ratings of Maine chickpea isolates and known *Pseudomonas* spp. inoculated on various hosts

	Mean disease ratings[b] of bacterial strains[c]			
Host[a]	ATCC 12636	ATCC 23060	ATCC 19309	Chickpea
Alfalfa[a]	1.0	1.5	2.0	1.2
Chickpea	2.0	2.3	2.2	3.8
Corn	0.0	4.0	0.0	1.7
Green bean	0.0	0.0	0.0	0.0
Hyacinth bean	0.0	0.0	0.0	0.0
Lespedeza	0.0	0.0	0.0	0.0
Red clover	0.0	0.0	0.0	0.0
Soybean	3.0	1.5	0.5	1.0
Sudangrass	1.0	3.5	4.0	2.5
White clover	2.0	0.5	2.5	1.8

[a] Eight plants were inoculated by stabbing the internodes and spraying the foliage until runoff. The experiment was repeated once.
[b] Visual disease ratings: 0 = no disease, 1 = light infection, 2 = moderate infection, 3 = heavy infection, and 4 = plant mortality.
[c] ATCC 12636 and ATCC 19309 = *Pseudomonas stizolobii*, ATCC 23060 = *P. andropogonis*, and 20 strains were isolated from field-grown chickpeas.

Table 2. Comparisons of Maine chickpea isolates with previously reported nonfluorescent *Pseudomonas* spp.

Characteristic	Chickpea strains[a]	Burkholder[b]	Allen et al[b]	Goto and Starr[b]	Hayward[b]	Gitaitis et al[b]
Gram-stain reaction	−[c]	−	−	−	−	−
Viscous growth	+	+	+	+	NT	±
Motility	+	+	+	+	+	+
Fluorescent pigment	−	−	−	−	−	−
Catalase	+	+	+	+	+	+
Oxidase	−	NT	−	−	−	−
Accumulation of						
poly-β-hydroxybutyrate	+	NT	+	+	+	+
Nitrate reduction	−	+	−	−	−	−
Arginine dihydrolase	−	NT	−	−	−	−
Lipase	−	−	−	−	−	−
Urease	+	+	+	NT	+	+
Gelatin hydrolysis	−	−	−	−	−	−
Starch hydrolysis	−	−	−	−	−	+
Aesculin hydrolysis	−	NT	−	NT	−	NT
Levan	−	NT	NT	NT	−	−
Hydrogen sulfide	−	+	NT	−	NT	NT
Methyl red	−	−	NT	−	NT	NT
Voges-Proskauer	−	−	NT	−	NT	NT
Growth in						
2% NaCl	+	+	+	NT	+	NT
4% NaCl	+	−	+	NT	−	NT
6% NaCl	−	NT	−	NT	−	NT
Growth at						
6 C	+	+	+	NT	NT	NT
37 C	+	+	−	NT	NT	NT
41 C	−	−	NT	NT	NT	−
Utilization of						
Citrate	+	−	+	−	+	−
Fructose	+	NT	NT	+	+	+
Galactose	+	NT	NT	+	+	+
Glucose	+	NT	NT	+	+	+
Glycerol	+	NT	NT	+	+	+
Malonate	+	NT	NT	+	+	NT
Maltose	−	NT	NT	−	−	−
Mannitol	+	NT	NT	+	+	+
Mannose	+	NT	NT	+	+	+
Salicin	−	NT	NT	−	−	NT
Sorbitol	+	NT	NT	+	+	+
Succinate	+	NT	NT	+	+	NT
Sucrose	−	NT	−	−	−	−
Tartrate	−	NT	NT	−	−	NT

[a] Twenty strains isolated from field-grown chickpeas.
[b] Burkholder (4) = *P. stizolobii*, Allen et al (1) = *P. stizolobii*, Goto and Starr (10) = *P. stizolobii* and *P. andropogonis*, Hayward (11) = *P. stizolobii* and *P. andropogonis*, and Gitaitis et al (8) = *P. andropogonis*.
[c] + = More than 85% of strains positive, − = more than 85% of strains negative, and NT = not tested.

States. Navaratnam and Hayward (17) reported similar symptoms on chickpea plants in Australia in 1977–1978 and also identified the causal agent as *P. andropogonis*; however, they did not perform a detailed study on the characteristics for their isolate(s).

Although the bacterium never was seedborne when either field-grown or greenhouse-grown seed from infected plants was tested, it may overwinter on such perennial legumes as white or red clover. Both legumes were observed in the vicinity of the infected field-grown chickpea plants. With the increasing acreage devoted to chickpeas in the United States and because isolates tested in this study were pathogenic on soybean, further studies of this bacterium are desirable.

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