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Vidaver, Anne K. and Carlson, Randall R., "LEAF SPOT OF FIELD CORN CAUSED BY *PSEUDOMONAS*
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LEAF SPOT OF FIELD CORN CAUSED BY PSEUDOMONAS ANDROPOGONIS

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The work reported was performed under Projects No. 21-023 and 21-021. Paper No. 5427, Nebraska Agricultural Experiment Station.

We thank R. Lelliott, Curator, National Collection of Phytopathogenic Bacteria, England for reference strains, L. Lane for gel electrophoresis data, N. Smith, B. Douppnik, and D. Wysong for certain specimens, R. Entrekina and B. Stewart for photographic assistance, and C. Gonzalez for electron microscopic assistance.

ABSTRACT

A leaf spot of field corn was shown to be caused by the bacterium Pseudomonas andropogonis. Leaf spot symptoms were observed over 3 years in several States. Inoculation of corn by vacuum infiltration of the bacteria was necessary to reproduce field symptoms.

Plant Dis. Repr. 62: 213-216.

A bacterial leaf spot disease of different cultivars of field corn was observed in early summer of 1973, 1974, and 1975. Leaves with essentially the same symptoms were obtained from South Dakota, Iowa, Kansas, Nebraska, and Michigan. The same symptoms were also seen in Wisconsin. Symptoms consisted of circular to ellipsoidal, tan to brown spots, with irregular margins. The spots were 1 to 4 mm in diameter, with one or more darker brown rings within the lesions. Some spots were surrounded by a chlorotic ring 1 mm wide. All spots tended to have a slightly sunken appearance. Occasionally the spots coalesced into irregular, somewhat elongated blotches. Water-soaking of young lesions was also seen. Bacteria were routinely isolated from the lesions. Because the symptoms did not correspond with previous reports of bacterial diseases of corn, experiments were undertaken to determine the causal bacterium and compare it with known bacterial pathogens of corn.

The pathogen was subsequently identified as Pseudomonas andropogonis, previously reported to cause bacterial stripe disease of sorghum, sudangrass, teosinte, johnsongrass, field corn, broomcorn, and sweet corn (3, 7, 8, 11). As P. stizolobii (syn. P. andropogonis), the bacterium causes leaf spot diseases of Bougainvillea, clover, and other leguminous plants (1, 3, 4).

MATERIALS AND METHODS

Isolations: Bacteria were isolated from lesions by the direct puncture technique (2) onto NBY agar (9). After 3 to 5 days at 24 to 28° C, transfers from single colonies were made twice more to obtain purified clones. Eight strains were obtained in different years from various locations in the Midwest. Cultures were maintained on NBY agar, including reference strains of P. andropogonis NCPPB 933, NCPPB 934, P. stizolobii NCPPB 1024, and NCPPB 1127. Cultures were stored at 2 to 5° C and also lyophilized to serve as reference throughout this study.

Bacteriological observations: Tests for oxidase, arginine dihydrolase, levan production, and fluorescence were carried out by the procedures of Lelliott, et al. (6). Sudanophilic granules were examined by the method of Hugh and Gilardi (5). Flagella of all ten P. andropogonis and two P. stizolobii strains were examined by electron microscopy after negative staining of bacteria with a 3:1 mixture of potassium phosphotungstate and vanadomolybdate (10). The bacteria were treated previously with 4% glutaraldehyde for 10 min. Sodium dodecyl sulfate slab gel electrophoretic patterns of total proteins were obtained for six strains, including all four reference strains; details of this procedure will be reported elsewhere.

Pathogenicity tests: Inoculum for pathogenicity tests was prepared by suspending bacteria from 2- to 3-day-old plate cultures into sterile distilled water. For vacuum infiltration the suspensions were made up in 0.1% Triton X-100 (Rohm & Haas Co.). The A420 nm was adjusted to 0.3 (approximately 4×10^8 CFU/ml) and dilutions were made as necessary.

Sweet corn ('Golden Cross Bantam'), field corn (A619 x A632), and sorghum (RS626) were inoculated initially by needle puncture into the stems. White sweetclover (Melilotus alba) was inoculated by aerosol spray under pressure into the stomates. Corn and sorghum plants were 6 to 8 inches high (3 to 4 leaf stage) and clover plants were about 6 weeks old (4 to 6 leaf stage).

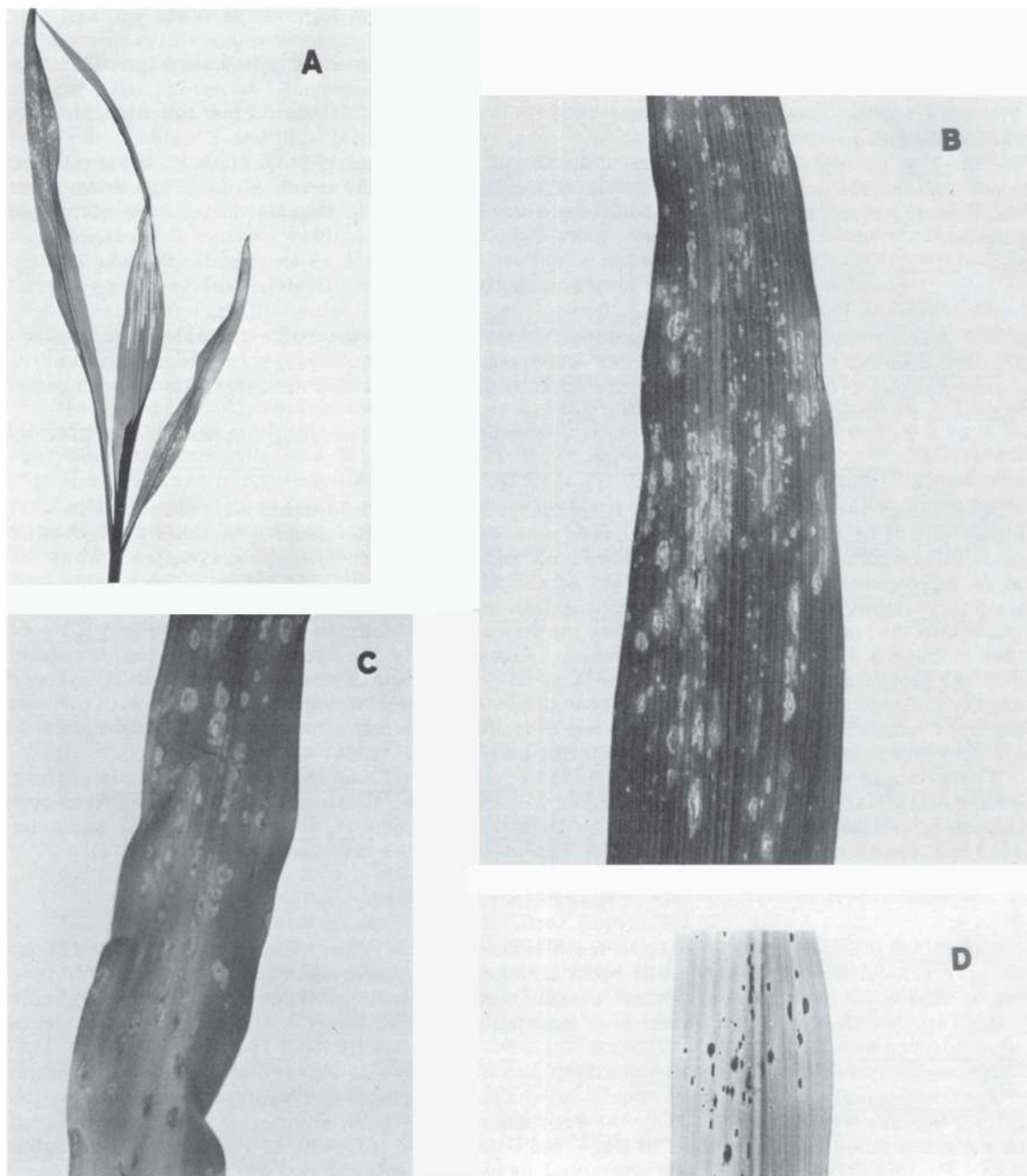


FIGURE 1. Leaf spot symptoms on corn and sorghum following inoculation with *Pseudomonas andropogonis* by vacuum infiltration. Strains GP1 and 3195 were field corn isolates from Nebraska and Iowa, respectively.

- A -- Spots, with limited streaking, 8 days after inoculation of 'Golden Cross Bantam' sweet corn with strain GP1 at 10^6 CFU/ml.
- B -- Close-up of leaf spots from A. (Approx. 2X)
- C -- Spots, with limited chlorosis, 10 days after inoculation of field corn (A619 x A632) with strain 3195 at 10^4 CFU/ml. (Magnified 1.5X)
- D -- Symptoms on sorghum produced by GP1 at 10^6 CFU/ml. (1.5X)

Alternatively, corn and sorghum plants were infiltrated under vacuum at 15 inches of Hg with bacterial suspensions containing 10^4 or 10^6 CFU/ml. The plants were then gently washed with water. All plants were kept in a greenhouse maintained at 24 to 28° C.

RESULTS

Cultural characteristics: After 3 days' incubation at 24 to 26° C on NBY agar, colonies of all isolates of the pathogen were indistinguishable from P. andropogonis and P. stizobii reference strains. The colonies were 1 to 3 mm in diameter, circular with an entire edge, opaque and butyrous in consistency. Upon further incubation, colonies became extremely viscous and adherent to the agar. These observations agree with other reports (1, 3).

Both the new bacterial strains and the reference strains were motile, Gram-negative rods and contained sudanophilic inclusions. The new strains and reference strains were negative for fluorescence, levan production, oxidase and arginine dihydrolase reactions. These results are in agreement with those of Goto and Starr (3) and Allen, et al. (1). The gel electrophoretic patterns of the total proteins of two strains of the new bacterium and reference strains of P. andropogonis and P. stizobii were indistinguishable, but markedly different from those of several other species of Pseudomonas.

The sheathed flagellum of P. stizobii was noted previously by Allen, et al. (1) as a prime distinguishing character. Electron microscopy showed that all new strains and all reference strains of P. andropogonis had a single, polar, and sheathed flagellum indistinguishable from the flagellum of the P. stizobii reference strains.

Pathogenicity tests: Although no typical stripe symptoms were observed in the field, all of the new strains caused typical striping accompanied by severe bleaching, after inoculation into the stem of either sweet or field corn. Field-type symptoms were not observed. Typical bacterial stripe, without chlorosis, was observed on sorghum after stem inoculation. Only strain NCPPB 1127, initially from clover, failed to produce symptoms on corn and sorghum.

Spray inoculation of strains of P. andropogonis gave restricted, initially water-soaked, light brown leaf spot symptoms on clover, as noted previously by Hayward (4) and Goto and Starr (3). Control plants showed no symptoms.

Field-type symptoms of isolated lesions, with limited or no apparent chlorosis, and minimal streaking, could be reproduced in corn only by the vacuum infiltration technique (Fig. 1A-C). Control plants infiltrated with 0.1% Triton in water showed no lesions. The appropriate inoculum concentration to produce isolated lesions varied somewhat among strains but ranged from 10^4 to 10^6 CFU/ml. Higher concentrations produced typical severe chlorosis and elongated streaks. Only one strain of P. stizobii (NCPPB 1024) produced symptoms on corn and sorghum. Both the NCPPB P. andropogonis strains 933 (originally from corn) and 934 (originally from sorghum) produced symptoms on corn and sorghum. The symptoms on sweet corn by strains 933 and 934 were indistinguishable from our strains. The new strains varied in virulence from moderate (few isolated lesions after vacuum infiltration with 1×10^6 CFU/ml or limited streaking after stem inoculation) to severe (numerous lesions and chlorosis after vacuum infiltration with 1×10^6 CFU/ml or extensive streaking after stem inoculation). Infection of sorghum after vacuum infiltration depended on high concentrations of the different strains of bacteria, generally requiring 10^7 CFU/ml to produce typical streaks; lower concentrations produced isolated lesions (Fig. 1D) or no detectable lesions.

DISCUSSION

The bacteriological properties of the newly isolated strains and reference strains of P. andropogonis and P. stizobii were indistinguishable; the latter results agree with those of Goto and Starr (3) and Hayward (4). The gel electrophoresis studies also showed that these two nomenclatures were indistinguishable. Because P. andropogonis has priority over P. stizobii, as already noted by Goto and Starr (3) and Hayward (4), P. andropogonis should be the appropriate nomenclature. Pathovar and serovar designation of strains may eventually be in order because some strains of P. andropogonis show certain host specificities and the species appears to be antigenically heterogeneous (1, 3, 4).

This report distinguishes between the leaf spot symptom caused by P. andropogonis and the striping disease symptom reported previously for corn (8, 11). In nature the former symptom has been seen without the latter; in artificial inoculations both types of symptoms have been observed.

The disease was seen sporadically over a wide geographic region, but is considered to be of little economic importance.

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