Bacterial Leaf Spot of White Clover in Georgia

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ABSTRACT

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Bacterial leaf spot was observed on white clover (*Trifolium repens*) in the middle coastal plain region of Georgia in July 1982. The causal organism was rod-shaped, gram-negative, aerobic, and nonfluorescent. In greenhouse tests, the bacterium caused stripes on leaves of sweet corn and sorghum and leaf spots on velvet bean as well as four other species of clover. These and other criteria fit those described for *Pseudomonas andropogonis*. This is the first report of the disease in Georgia. The economic importance and distribution of the disease within the state has yet to be determined.

White clover (*Trifolium repens* L.) is a cool-season perennial legume used in mixture with grasses in Georgia to increase the carrying capacity of pastures (5). In addition to extending the grazing period and adding total digestible nutrients to livestock diets, white clover in a symbiotic relationship with *Rhizobium trifolii* Dangeard fixes nitrogen and thus improves soil fertility. A number of factors, however, including extreme temperatures, dry weather conditions, improper management, and diseases, limit the survival of white clover in the coastal plain region of Georgia. Consequently, the occurrence of any new disease would be of major concern because of its potential effects on a plant already exposed to several growth-limiting stresses.

In July 1982, a leaf spot disease of unknown origin was observed on white clover in Tift County, GA. The purpose of this report is to describe the disease symptoms, identify the pathogen, and record its occurrence in Georgia. A limited host range study was done to characterize the pathogen and determine its pathogenicity on selected clover species.

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MATERIALS AND METHODS

Leaf spots were observed on 111 of 499 selected plants of white clover in a field nursery. Lesions from representative diseased leaflets were triturated in sterile tap water and the resultant suspensions were streaked on King's medium B (KMB) (4) or nutrient agar. Plates were incubated at 30 C for 48–72 hr and the predominant bacteria on the plates were restreaked for isolation onto nutrient agar. Nutrient-broth cultures started from a single colony were grown overnight and harvested by centrifugation. Inoculum was adjusted in sterile tap water to a concentration of about 10^8 colony-forming units (cfu) per milliliter. White clover plants (4–5 wk old) grown in the greenhouse were inoculated by gently rubbing Carborundum-dusted leaflets with a cotton swab saturated with the bacterial suspension.

In addition to the cultivars Arcadia, Sacramento, Tillman, LA. S-1, and Fla XPL of white clover, the cultivars Amclo, Meechee, and Yuchi of *T. vesiculosum* (arrowleaf clover); Autauga, Chief, Dixie, and Tibbee of *T. incarnatum* (crimson clover); Mt. Baker, Nangeela, Tallarook, and Woogenellup of *T. subterraneum* (subterranean clover); and Arlington, Florie, Kenstar, Lakeland, Nolin's, and Redland II of *T. pratense* (red clover) as well as plants of *Stizolobium deeringianum* (velvet bean); *Sorghum vulgare* (sorghum); and *Zea mays* (sweet corn) were tested to ascertain their susceptibility to the bacterial pathogens. Plants were covered with plastic bags 24 hr before inoculation to create an atmosphere of high humidity. The various clover species and velvet bean were sprayed with a suspension of inoculum using an aerosol chromatography sprayer. Sorghum and sweet corn plants were inoculated by pipetting 1 ml of the bacterial suspension into the whorl. In all cases, inoculum was adjusted to a concentration of 10^8 cfu/ml. Plants remained covered for an additional 24 hr after inoculation to ensure favorable conditions for infection. All other methods used in this study to characterize the biological properties of the bacterium are described in the *Manual of Methods for General Bacteriology* (2).

RESULTS AND DISCUSSION

Lesions on leaflets of white clover were small (1–3 mm diam.), round, dark brown, and often associated with a bright yellow border. The entire leaflet became chlorotic and eventually died. Substantial defoliation occurred on plants severely

Table 1. Characteristics of strains of *Pseudomonas andropogonis* recovered from leaf spots of white clover in Georgia

Characteristic	Response[a]
Gram stain reaction	Gram-negative
Fluorescent pigment on KMB	−
Accumulation of poly-β-OH-butyrate	+
Motility	Single polar flagellum
Arginine dihydrolase	−
Growth at 41 C	−
Nitrate reduction	−
Oxidase	−
Catalase	+
Urease	+
Phenylalanine deaminase	(+)
Starch hydrolysis	((+))
Gelatin hydrolysis	−
Levan production	−
Lecithinase	−
Acid form	
Glucose (aerobic)	+
Glucose (anaerobic)	−
Fructose	+
Sucrose	−
Maltose	−
Cellobiose	−
Lactose	(+)
Xylose	+
Rhamnose	+
Galactose	+
Arabinose	+
Mannose	+
Adonitol	+
Dulcitol	−
Erythritol	−
Inositol	+
Mannitol	+
Sorbitol	+
Glycerol	+

[a] + = Positive, − = negative, (+) indicates weak response, and ((+)) indicates very weak response.

infected in the field. In all cases, a slow-growing white bacterium was associated with the disease. When reinoculated onto white clover plants in the greenhouse, typical leaf spots developed. When extracts from these lesions were cultured, the same bacterium was always reisolated from the leaf spots, thus fulfilling Koch's postulates for proof of pathogenicity.

The bacterium is gram-negative, aerobic, rod-shaped, and does not produce a fluorescent pigment on KMB. Growth on nutrient agar is slow. Colonies are white and circular with an entire margin and a smooth glistening surface. Initially, colony texture was butyrous but later became viscous. The organism is motile with a single polar flagellum, does not grow at 41 C, and contains blue-black granules when stained with sudan black B (a presumptive test for the accumulation of poly-β-hydroxybutyrate). The bacterium does not reduce nitrates to nitrites and is negative for the presence of oxidase and arginine dihydrolase but positive for the presence of catalase and urease and weakly positive for phenylalanine deaminase. Based on these criteria and additional characteristics (Table 1), the pathogen fits the description of *Pseudomonas andropogonis* (Smith) Stapp.

Twenty-two cultivars representing five clover species, including crimson clover (the most widely recommended clover in the coastal plain area of Georgia [5]), were susceptible to the bacterial strains from white clover. Symptoms ranged from leaf spots as described on white clover to small black specks on *T. vesiculosum*, which resembled pepper spot caused by *Pseudoplea trifolii*.

Burkholder (1) originally reported a bacterial leaf spot of white clover in New York caused by *Pseudomonas stizolobii*, a pathogen of *S. deeringianum*. Goto and Starr (3) determined that *P. andropogonis*, which causes bacterial leaf stripe of corn and sorghum, and *P. stizolobii* were identical and recommended that *P. andropogonis* be adopted as the valid name. Typical leaf stripes on sweet corn and sorghum, as well as virulence on velvet bean by the strains found in Georgia, confirmed the identity of the clover bacterium as *P. andropogonis*.

This is the first report of bacterial leaf spot of clover in Georgia. The disease first was observed under conditions of high relative humidity and high temperature at a time in the growing season that is most critical for survival of white clover in southern Georgia. The host range includes several species and cultivars of clover as well as corn and sorghum. Prevalence of the problem and the potential threat to cultivation of clover are not known but should be examined.

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