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Bacterial Wilt of *Zornia* spp. Caused by *Corynebacterium flaccumfaciens*

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Abstract

During 1980 and 1981, young plants and, after cutting, mature plants of the promising tropical forage legumes *Zornia brasiliensis* and *Zornia* sp. (CIAT 7847) became chlorotic, stunted, and wilted and often died at forage evaluation sites in Colombia. The causal bacterium was identified as *Corynebacterium flaccumfaciens*. This is the first report of bacterial wilt in *Zornia* spp. and the first report of the occurrence of the pathogen in tropical Latin America. The seedlings of *Phaseolus vulgaris* were susceptible to this pathogen in inoculation tests. Since the bacterium is seed-borne, care is being taken to prevent its spread from Colombia to other countries.

Introduction

Zornia is a prostrate to erect, herbaceous, perennial, bi- or quadrifoliate legume, native to the tropical savanna regions of South America (6). Over the past 5 years evaluations in Colombia have provided evidence of the high quality and productivity of this species and its potential as a forage legume for the acid infertile soils of the tropics (1, 2).

During 1980 and 1981, young plants and, after cutting, mature plants of promising accessions of *Zornia brasiliensis* Vog. and *Zornia* sp. wilted and often died at forage evaluation sites in Colombia. Cross-sections of lower stems and taproots showed brown coloration of the outer vascular tissue. A bacterium was consistently isolated from affected tissue.

As no previous reports of this disease on *Zornia* spp. could be found in the literature, the following study was made to identify the causal organism and confirm its pathogenicity to *Zornia* spp.

Materials and Methods

Isolates

Sixteen isolates were collected from diseased plants of *Zornia* sp. CIAT 7847 at Santander de Quilichao, Cauca, Colombia. Isolates were grown on yeast extract-dextrose-$CaCO_2$ medium (YDC) at 27°C and maintained on YDC at 15°C (4).

Morphology and Cultural Characters

Cell morphology was observed by using the Hucker modificiation of Gram stain (9) and the Fisher and Conn modification of Bayley's method was used to observe flagella (9). Cultural characters and pigment production were determined on nutrient agar (NA), tetrazolium chloride medium (TZC), and YDC (9).

Biochemical and Physiological Properties

The methods used to determine biochemical and physiological properties of the isolates have been described previously (5).

Pathogenicity

Inocula for pathogenicity tests were grown in Petri plates of YDC for 48 h at 27°C. Cells were suspended in sterile distilled water at a concentration of 10^6 cell/ml. Young plants at the three and four leaf stage of *Zornia* sp. CIAT 7847 and 3-week old plants of *Phaseolus vulgaris* P 635, *P. lunatus*, and *Glycine max* were inoculated by leaf cutting and by needle puncture. The controls were inoculated with sterile distilled water using the same methods. Plants were rated for disease reaction 12 days after inoculation. Reisolations were made from inoculated plants and Koch's postulates were completed.

Seed Tests

The presence of bacteria in seed was tested on nutrient agar with seed of *Zornia* sp. CIAT 7847. Seeds were surface sterilized in 1% sodium hypochlorite solution, washed in sterile distilled water, placed on nutrient agar and incubated at 27°C for 48 h. All bacteria that grew from seeds were compared with isolates from affected plants of *Zornia* sp. CIAT 7847.

Results

Bacteria Characteristics

All isolates were Gram-positive, non-spore forming short rods with rounded ends, with a size range of 0.6 to 3.0 x 0.3 to 0.5 nm. Cells were motile with various flagella. Colonies on nutrient agar after 24 h at 27°C were circular, convex, entire, and butyrous. On YDC, the colonies were colored creamy-yellow (Table 1).

All isolates were catalase-positive, hydrolyzed starch, grew at 37°C, grew on tetrazolium chloride medium, produced yellow colonies on NBY (5), and produced acid from cellobiose, rhamnose, mannose, ribose, mannitol, sorbitol, and inuline (Table 2). In addition, all isolates were oxidase-negative, did not reduce nitrates to nitrites, did not form levan, did not produce a blue pigment in YDC, and did not reduce sucrose (Table 2). Isolates had a variable reaction in the production of acid from melezitose (Table 2).

Table 1. Characters used to differentiate common genera.[a]

Characters	Isolates from *Zornia* sp. CIAT 7847	*Corynebacterium*	*Agrobacterium*	*Erwinia*	*Pseudomonas*	*Xanthomonas*
Growth in common media	+	+	+	+	+	+
Gram stain	+	+	–	–	–	–
Colonies yellow or orange on media YDC, NBY	+	+	–	V-	–	+
Fluorescent pigment on KB	–	–	–	–	V+	–
Anaerobic growth	–	–	–	+	–	–

a From the Laboratory Guide for Identification of Plant Pathogenic Bacteria. Ed. N. W. Schaad.
+ = Result positive; – = result negative; and V = result variable.

Table 2. Determination of four pathogenic species of *Corynebacterium*[a,b] and comparison with an isolate from *Zornia* sp. CIAT 7847.

Characters	*C. michiganense*	*C. ilicis*	*C. fasciens*	*C. flaccumfaciens*	Isolate from *Zornia* sp. CIAT 7847
Motility	–	+	–	+	+
Maximum temperature of growth	29–35	37	34–36	35–37	37
Catalase	+	+	+	+	+
Oxidase	–	–(c)	–	–	–
Nitrates to Nitrites	–	–	–	–	–
Production of acids:					
Rhamnose	–	+	–	+	+
Mannose	V	+	+	+	+
Ribose	–	+	+	+	+
Cellobiose	V	+	–	+	+
Melezitose	–	+	–	+	V
Starch	–	–	–	–	–
Inuline	–	–	–	–	–
Mannitol	V	+	+	+	+
Sorbitol	–	+	+	V	+
Reduction of substances (Sucrose)	V	–	–	–	–
Levan	V	–	–	–	–
Hydrolysis of potato starch	V	–	+	+	+

[a]From Dye and Kemp (1977). [b]All are Gram-positive; strict aerobes. [c]Mandel *et al.* (1961) reported Oxidase positive.
+ = Result positive. – = Result negative; V = Result variable.

Pathogenicity

All isolates caused chlorosis, wilting, dieback and, in some cases, death of young plants of *Zornia* sp. CIAT 7847. The bacterium was readily reisolated from inoculated plants and Koch's postulates were successfully completed with 16 isolates.

In addition, the bacterium caused chlorosis and severe wilting of 3-week old plants of *Phaseolus vulgaris* P 635. *Phaseolus lunatus* and *Glycine max* were not affected when inoculated with isolates of the bacterium.

Seed Tests

The bacterium was readily isolated from seed of *Zornia* sp. CIAT 7847. Levels ranged from 75 to 100% of seed infected.

Discussion

The severe wilting and death of young and mature plants of *Z. brasiliensis* and *Zornia* sp. CIAT 7847 observed at forage evaluation sites in Colombia during 1980 and 1981 were found to be caused by a bacterium. On the basis of its morphological, cultural, biochemical, and physiological properties (Tables 1 and 2), this bacterium was identified as *Corynebacterium flaccumfaciens* Hedges. This is the first report of bacterial wilt of *Zornia* spp. caused by this pathogen.

Corynebacterium flaccumfaciens caused wilting of *Zornia* spp. with similar symptoms to those described in other leguminous hosts (3, 8). In addition, brown coloration of the vascular system was similar to that previously reported in alfalfa (3).

Although *P. lunatus* and *G. max* were not affected by the bacterium, *P. vulgaris* P 635 was most susceptible. In the United States, *C. flaccumfaciens* can cause severe losses in beans (8). As the presence and importance of the pathogen in Latin America is unknown (8), this first report on the bacterium and its pathogenicity in beans should be well noted.

As has been shown in beans (8), the bacterium is seedborne in *Zornia* spp. Importing of seed of infected *Zornia* spp. is being avoided to prevent the spread of this disease.

Further studies are in progress to determine the host range of the pathogen among tropical forage legumes, the survival of the bacterium in soil, and methods of producing clear seed.

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