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BACTERIAL LEAF SPOT AND DIEBACK OF *Centrosema* spp.

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ABSTRACT

A severe leaf spot and dieback of young growth of accessions of the promising tropical forage legumes *Centrosema brasilianum*, *C plumieri*, *C pubescens*, *C virginianum* and *Centrosema* sp was observed at various evaluation sites in Colombia during 1980 and 1981 The causal organism was identified as *Pseudomonas* sp Koch's postulates were sucessfuly completed with 18 isolates of the bacterium Seed of three accessions of *Centrosema* spp were infected with the bacterium at levels of 8-34% Further studies are being made to identify the species, to determine the host range of the bacterium and to select resistant accessions of *Centrosema* spp As this disease has not been reported previously on *Centrosema* spp care must be taken to prevent its introduction to other countries where *Centrosema* spp are promising tropical forage legumes

Centrosema species, especially C pubescens Benth , have shown considerable promise as tropical forage legumes in Australia (3, 7), Central and South America (4) and Southern Florida (5) Lesser known species including C brasilianum (L) Benth , C plumieri (Turp ex Pers) Benth and C. virginianum (L) Benth are presently under evaluation in several tropical areas and their potential as forage legumes is becoming evident (1, 5)

In 1980 and 1981, a previously unreported leaf spot and dieback of young growth was detected on accessions of C brasilianum, C plumieri, C pubescens, C virginianum and Centrosema sp at various evaluation sites in Colombia At the CIAT Research Station, Santander de Quilichao, Cauca, Co lombia, leaf spotting and dieback considerably reduced the yield of promising accessions including CIAT 5112, 5118 and 5278 A bacterium was consi.tently isolated from affected plants The objective of this research was to identify the causal bacterium and to confirm its pathogenicity to Centrosema spp

MATERIALS AND METHODS

Isolates Eighteen isolates were collected from affected plants of Centrosema spp at Santander de Quilichao Colombia Isolates were rown on Difco nutrient agar (NA) and tetrazolium chloride medium (TZC) at 28°C All isolates were maintained on NA + $CaCO_3$ at 24°C (2)

Morphology and cultural characters Cell morphology was observed using the Hucker modification of Gram stain (6) and the Fisher and Conn modification of Bayley's method (6) was used to observe flagella Cultural characters and pigment production were determined on NA, TZC and King's B Medium (6)

Biochemical and physiological properties The methods used to determine biochemical and physiological properties of isolates are described in the Manual of Microbiological Methods (6)

Pathogenicity Inocula for pathogenicity tests were grown in Petri plates of NA for 48 h at 28°C Cells were suspended in sterile distilled water at concentrations of 10^6 cells/ml Four week old plants of Centrosema spp accessions CIAT 438, 5064 and 5118 were used Inoculation methods included (1) wounding by cutting leaves with a blade or by puncture with a needle carrying bacterial suspensions, (2) spraying with bacterial suspensions followed by incubation in moisture chambers for 48 h, and (3) injection of bacterial suspensions into the veins of plants Controls were treated with sterile distilled water using the same methods Plants were rated for disease reaction 8 to 10 days after inoculation Reisolations were made from each inoculated plant and Koch's postulates were completed

Seed tests The presence of bacteria in seed was tested on NA with seed of Centrosema spp accessions CIAT 5112, 5118 and 5278 Seed were surface sterilized in 1% sodium hypochlorite solution, washed in sterile distilled water, placed on NA and incubated at 28°C for 48 h All bacteria that grew from seeds were compared with isolates from affected plants of Centrosema spp

RESULTS

Bacteria characteristics All isolates were Gram negative, non spore forming rods with a size range of 0 5 - 1 0 x 1 5 - 4 0 µm The cells were motile with two flagella Colonies on NA after 24 h at 28°C were convex with regular borders, cream-colored, butyrous, averaged 2-2 5 mm in diameter and produced a florescent pigment

All isolates were aerobic, oxidase-positive, catalase positive, formed levan and were florescent on King's B Medium (Table 1) They did not grow in nutrient broth at 41°C (Table 1)

Symptomatology Symptoms were initially manifest as wilting of young leaves and terminals and chlorotic spotting of mature leaves Young leaves and terminals became partially or completely necrotic and dieback developed On mature leaves, chlorotic spots became necrotic and were of varying size and shape Leaves were often crinkled and distorted

Pathogenicity All isolates caused wilting, dieback and necrotic spotting of four week old plants of Centrosema spp The bacterium was readily reisolated from affected plants and Koch's postulates were successfully completed with eighteen iso ates

Seed tests The bacterium was found in seed of accessions of Centrosema spp CIAT 5112, 5118 and 5278, levels of seed infection ranged from 8-34%

DISCUSSION

The causal organism was identified as a species of Pseudomonas on the basis of its morphological, cultural, biochemical and physiological properties Further tests are in progress to identify the species

Although Centrosema spp have been under forage evaluation in tropical areas for the past 15 years (3, 5, 7), no reports of this disease have been made previously As Centrosema is native to the Americas (5), most probably this bacterial disease evolved with the host species that it presently affects Cross-inoculation studies are in progress to determine the host range of the bacterium and, particularly, whether it is pathogenic to other leguminous crops such as beans

As the bacterium is seed borne, care must be taken to prevent its spread to other areas where Centrosema spp are important tropical forage legumes

Field screening of Centrosema spp for resistance to bacterial leaf spot and dieback is continuing in Colombia To date, the promising C macrocarpum has shown resistance to this disease

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TABLE 1 Characters used to differentiate commonly isolated plant pathogenic bacteria*

CHARACTERS	Isolates from Centrosema spp	Pseudomonas	Corynebac-terium	Agrobac-terium	Erwinia	Xanthomonas
Grow on Common Media	+	+	+	+	+	+
Gram Stain	-	-	+	-	-	-
Yellow or Orange Colonies on NA, YDC or NBY Media	-	-	+	-	V	+
Florescent Pigment on KB	+	V+	-	-	-	-
Anaerobic Growth	-	-	-	-	+	-

* From Laboratory Guide for Identification of Plant Pathogenic Bacteria ed by N W Schaad

V = Variable Result