Bacterial leaf spot of pigeon pea in Puerto Rico[1]

Amelia Cortés-Monllor and Hernán Ruiz[2]

ABSTRACT

A leaf spot bacterium was isolated from diseased leaves of Kaki pigeon pea (*Cajanus cajan*). The lesions were minute, necrotic and surrounded by a yellow halo, eventually coalescing to form larger area approximately 1 to 2 mm. in diameter. The bacterium is a Gram-negative bacillus, motile, forming butter-yellow, mucoid, and shiny colonies. The bacterium hydrolyzes gelatine, starch, fats, casein, and esculin. It also acidifies glucose, sucrose, and maltose but not salicin, and uses citrate and malonate. When artificially inoculated on healthy pigeon pea, bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*), the characteristic lesions were reproduced in their original host only. On the basis of its biochemical characteristics and confirmation by recovery from artificially inoculated diseased pigeon pea leaves, the organism was identified as *Xanthomonas campestris* pv. *cajani*. This is the first record of the disease in Puerto Rico.

INTRODUCTION

Pigeon pea, *Cajanus cajan* (L.) Millsp., is a tropical legume of high quality protein. It is grown in India, Africa, Asia, Australia, Central America, the Caribbean, and other tropical countries.

Because of its great demand as a protein source, pigeon pea breeding, germplasm, seed quality, cultural practices, diseases and pests, and problems affecting yield are being investigated locally and in other institutions.

Yield decline has been blamed on many factors, the primary of which is diseases. More than 50 diseases have been reported to affect pigeon peas, 21 of them from the Caribbean area, but only a few are of economic importance (5).

Fungal leaf spots (20), cankers (1), witches' broom disease (14, 21), virus (14), nematodes (2, 16), and pod borers (6, 7, 22) have been reported in Puerto Rico as pest of pigeon peas.

Most of these diseases have been reported elsewhere (15) but little work has been done on bacterial diseases of pigeon peas. In 1950 Kulkarni et al. (13) gave a detailed description of the bacterial leaf spot. In 1959 and 1969, Sabet (18, 19), reported bacterial leaf blight diseases caused by *Xanthomonas phaseoli* F. sp. *cajani* in Sudan. In 1973, Barnes (3) in

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[2]Associate Bacteriologist and former Extension Agent, respectively, Department of Crop Protection, Agricultural Experiment Station, College of Agricultural Sciences, University of Puerto Rico. Mayagüez Campus, Río Piedras, P. R. The authors thank Mr. Arcángel Rodríguez for the photo published herein.

his report on literature on pigeon pea, and Dahiya (8), in 1977, reported only two diseases caused by bacteria. The 1977-78 ICRISAT report (10) mentions a bacterial blight affecting pigeon peas grown in Panama. In a survey of available literature on serious diseases of pigeon peas in the Caribbean area by Brathwaite in 1980 (5) bacterial diseases are not mentioned. A halo blight disease caused by *Pseudomonas phaseolicola* is reported in the 1981 ICRISAT report (11), and just recently the 1983 International Pigeon Pea Newsletter (12) reported that *Xanthomonas* spp. and *Pseudomonas* spp. in Australia were affecting *Cajanus cajan*. Throughout the past years, bacterial leaf blight has not been reported in pigeon peas grown locally. The purpose of this paper is to report the presence of this disease on pigeon peas in Puerto Rico.

MATERIALS AND METHODS

SYMPTOMS

Diseased pigeon pea leaves of cultivar Kaki were collected at the Fortuna Research and Development Center. The symptoms were first noticed as minute water soaked lesions with dark centers and yellow halos (fig. 1). The lesions coalesced to form blotches approximately 2 mm in diameter. Lower leaves were usually affected first with lesions spreading toward the upper leaves with advancing plant maturity. The main stem and petioles presented longitudinal necrosis occasionally. No symptoms were found on the pods or seeds.

ISOLATION

Diseased leaf tissue was immersed in a 10% solution of sodium hypochlorite for 2 min and rinsed in sterile distilled water. Individual lesions were aseptically teased in a few drops of tryptone glucose broth (TGB) and incubated at 37° C for 2 to 3 hr before streaking on tryptone glucose agar (TGA) plates. Bacterial colonies appeared after 36 hr of incubation at 28° C. Pure cultures of the organism were propagated and maintained in TGA slants.

CHARACTERIZATION

Table 1 presents standard recommended methods for morphological, cultural, and biochemical characterization of the organism.

PATHOGENICITY

We sprayed a bacterial suspension into healthy potted plants of pigeon peas (*Cajanus cajan*), beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*). The aqueous suspension, containing 10^8 cells/ml was prepared from culture grown 48 hr in TGA slants. After inoculation the plants were covered with polyethylene bags for 3 days. Plants sprayed with tap water served as controls.

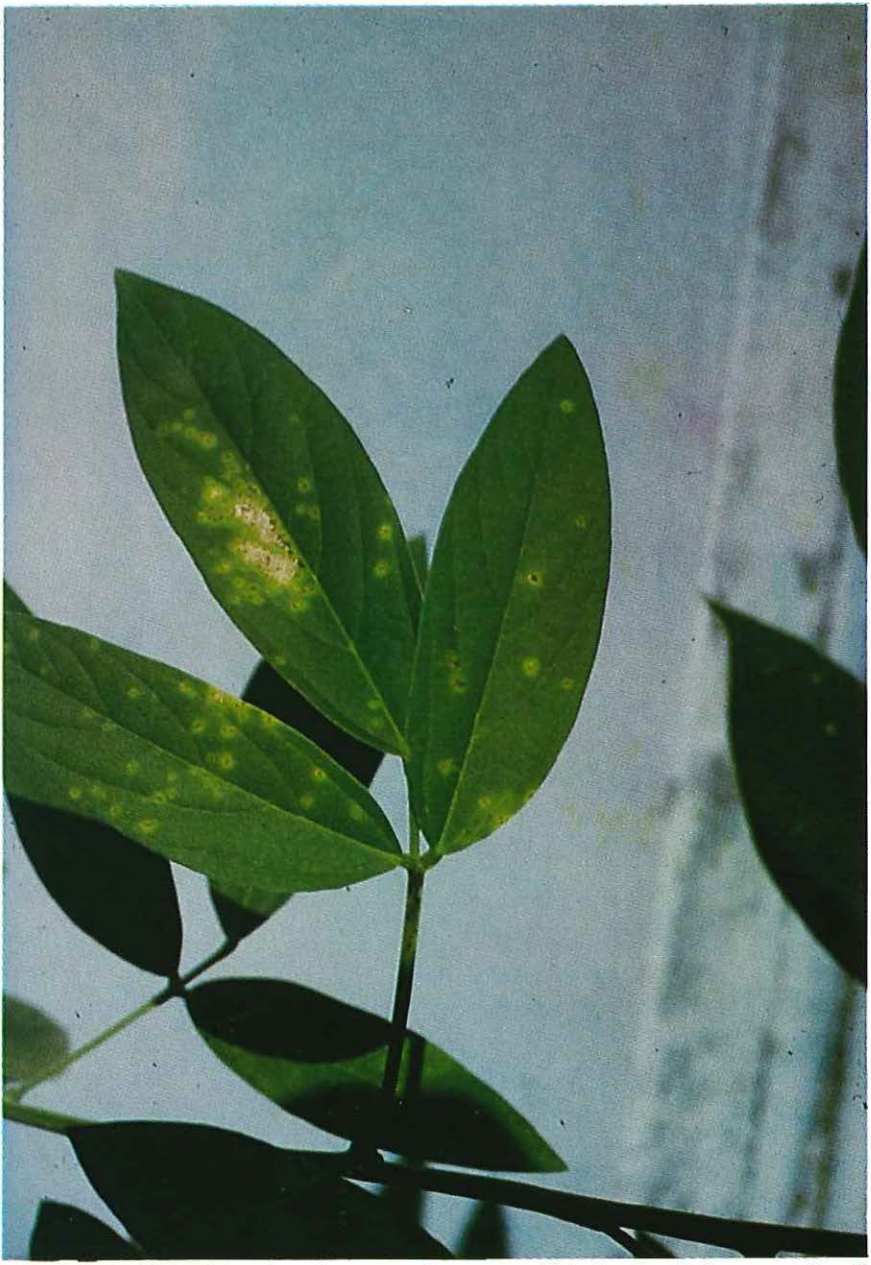

FIG. 1.—Bacterial spots on leaves on pigeon peas. Note the clear halo surrounding the lesions, the blotch and the necrosis on the petiole.

TABLE 1.—*Determinative standard media and tests used in the identification of the causal organism of bacterial leaf spot of pigeon pea*

Test	Manual of information			
	MMM[1]	Difco[2]	Dowson[3]	Others
Gram's Stain	Huckers's Modification			
Motility		Motility Test Medium		
Capsule Stain				LMM[4]
Colony	Tryptone Glucose Agar			
Indole	Kovac's Reagent	1% Tryptone Broth		
Ammonia			Peptone Broth Nessler Reagent	
Methyl-Red, Voges Proskauer (MR-VP)		MR-VP Medium		
Nitrate reduction	Nitrate Peptone Broth Nitrite Reagents			
Litmus Milk		Litmus Milk		
Citrate utilization		Simmon's Citrate Agar		
Malonate utilization		Malonate Broth with 2% Agar		
H_2S			Lead Acetate Paper	
Gelatine liquefaction		12% Gelatine		
Pectate liquefaction				Starr[5]
Esculine hydrolysis				Sneath[6]
Lypolysis			Cabral's Modif.	
Starch hydrolysis	0.2% Soluble Starch and Lugol's Iodine			
Sodium chloride tolerance				LMM
Action on carbohydrates	Synthetic Basal Media and Carbohydrate			

[1]Manual of Microbiological Methods, Committee on Bacteriological Technic, Society of American Bacteriologists, 1957. McGraw-Hill Book Co., Inc., New York, N.Y.

[2]Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures, 1965. 9th ed, Detroit 1, Michigan.

[3]Dowson, W. J., 1957. Plant Diseases Due to Bacteria, 2nd ed, Cambridge University Press, Cambridge, England.

Recovery tests were performed to confirm the presence of the pathogen.

ANTISERUM PREPARATION

Bacterial suspensions used for immunization contained 10^9 cells/ml. Intramuscular injections of 2 ml emulsified in Freund's complete adjuvant was given to a rabbit each week for 3 weeks. Two weeks after the final injection the rabbit was bled and the serum tested for the presence of antibodies. For the agglutination test, a twofold dilution procedure, as described by Pérez and Monllor (17), was used to determine the serum titer.

RESULTS

IDENTIFICATION

The organism is a rod shaped Gram-negative bacterium surrounded by a capsule. On TGA medium, the colonies of the bacterium were of a butter-yellow color, mucoid, shiny and smooth. In its biochemical activities the organism liquefies gelatin, hydrolyzes starch, fat and esculin, digests casein, uses citrate and malonate as carbon sources; liberates H_2S and ammonia, and tolerates up to 3% salt. It does not produce acid from salicin but does so from glucose, sucrose, and maltose. Table 2 shows a detailed description of the reactions.

PATHOGENICITY

Five days after inoculation, pigeon pea plants developed minute dark leaf spots surrounded by a yellow halo. Some spots tended to coalesce forming larger lesions. Recovery tests confirmed the presence of the pathogen on the inoculated pigeon pea plants. No symptoms appeared on the other leguminous plants inoculated, *P. vulgaris* and *Pisum sativum.*

ANTISERUM TITER

The lowest concentration of serum antibody reaction was observed in 1:1280 dilution.

DISCUSSION

The results obtained from the isolation and inoculation experiments on diseased pigeon pea leaves proved that the pathogen involved is *X. campestris* pv. *cajani.* Morphological and biochemical tests are very simi-

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[4]Laboratory Methods in Microbiology, 1966. W. F. Harrigan and M. E. McCance, Academic Press, London and New York.

[5]Starr, M. P., 1947. The causal agent of bacterial root and stem disease of guayule, Phytopathology 37: 296-300.

[6]Sneath, P. H. A., 1956. Cultural and biochemical characteristics of the genus *Chromobacterium,* J. Gen. Microbiol. 15: 70-98.

TABLE 2.—*Results obtained from morphological and biochemical tests of* X. campestris *pv.* cajani *isolated from leaf spot of pigeon pea*

Test	*X. campestris* pv. *cajani*
1. Gram stain and morphology	Negative bacillus
2. Motility	Positive
3. Capsule stain	Positive
4. Colony appearance	Buttery-yellow, smooth, shiny and raised
5. Indole	Negative
6. Ammonia	Positive
7. Methyl-red test (MR)	Negative
8. Voges-Proskauer reaction (VP)	Negative
9. Citrate utilization	Positive
10. Malonate utilization	Positive
11. Nitrite from nitrate	Negative
12. Hydrogen sulfide production (H_2S)	Positive
13. Action on litmus milk	Acid, casein digested
14. Liquefaction of gelatine	Positive
15. Liquefaction of pectate	Negative
16. Starch hydrolysis	Positive
17. Esculin hydrolysis	Positive
18. Lypolysis	Positive
19. Salt tolerance: 1%-5% concentration	Tolerates up to 3%
20. Action on carbohydrates:	
Maltose	Acid
Sucrose	Acid
Glucose	Acid
Salicin	No action

lar to the common characters of the xanthomonads. The production of yellow pigment, the consistency of the colony, and the absence of acid in salicin after 1-month observation are features characteristic of the *Xanthomonas* spp. The pathogenicity of the isolate was confirmed by artificial inoculation into the original host reproduction of characteristic lesions and re-isolation from the lesions, obtained 2 weeks later. Failure to infect other leguminous plants, *P. vulgaris* and *Pisum sativum*, revealed that the pathogen is restricted to its original host. These findings agree with those stated by Sabet (18).

So far we have not observed seeds affected by this organism. Recently, Hepperly and Rodríguez (9) published findings of mycoflora in pigeon pea seeds but no bacterial pathogen was detected.[3] Probably, this disease is not seed-transmitted; therefore, it is not being considered as a serious problem in Puerto Rico since it is the first time it has been noticed affecting local pigeon pea plants.

Control measures to avoid spread of the pathogen that should be taken are discarding crop residues and thoroughly cleaning areas for new plantings.

Under the new nomenclature rules, this organism is listed as a

[3] Dr. P. R. Hepperly, personal communication.

nomenspecies included with the *X. campestris* group, as stated in Bergey's Manual 8th edition (4). It is distinguishable from *X. campestris* only by plant host reaction; *Cajanus cajan* is the only host mentioned.

RESUMEN

Mancha bacteriana del gandul en Puerto Rico

Estudios de laboratorio determinaron que la mancha clorótica que se observa comúnmente en el follaje del gandul la causa la bacteria *Xanthomonas compestris* pv. *cajani*. Las hojas que se usaron para aislar el organismo provenían de plantas de la cultivar Kaki que mostraban manchas necróticas pequeñas (0.5 mm.) rodeadas de un halo amarillo. La coalescencia de las manchas pequeñas forma manchas cloróticas de 1 a 2 mm. Usualmente se encuentran en las hojas adultas, pero gradualmente van apareciendo en las jóvenes. En el fruto no se observan lesiones. En ocasiones en el tallo y pecíolo aparecen estrías pardas. Estos síntomas pueden aparecer en plantas jóvenes de 1 a 2 meses. El organismo aislado se sometió a pruebas bacteriológicas para identificarlo. Es un bacilo gramnegativo móvil que forma colonias amarillas de apariencia suave y cremosa, licúca la gelatina, hidroliza el almidón, las grasas, la caseína y la esculina. Acidula la glucosa, la sucrosa y la maltosa pero no la salicina. Utiliza sales de citrato y malonato en su metabolismo. Las pruebas de patogenicidad *in vivo* demuestran que este organismo está restringido a su huésped original, ya que no afectó las leguminosas *Phaseolus vulgaris* y *Pisum sativum* cuando se las inoculó mecánicamente. Esta es la primera vez que se informa la presencia de este organismo en el gandul Kaki en Puerto Rico.

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