Bacterial Leaf Spot of Cocoyam (*Xanthosoma caracu*), Incited by *Xanthomonas campestris* pv. *dieffenbachiae*, in Florida

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ABSTRACT

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Bacteria isolated from leaf spots of cocoyam fit physiological and biochemical descriptions of *Xanthomonas campestris*. Cocoyam isolates were pathogenic to both cocoyam and *Dieffenbachia maculata*, and disease reactions were identical with those for *X. campestris* pv. *dieffenbachiae*. A 1983 survey of commercial fields in Dade County showed that bacterial leaf spot is widespread, affecting 74–100% of the observed plants.

Xanthosoma caracu Koch & Bouché is an edible aroid cultivated extensively in many parts of the humid tropics. The starchy corms and cormels are a staple part of the human diet. The crop is known by several regional common names including malanga, yautia, tiquisque, tannia, and cocoyam. Since the late 1950s, cocoyam has become a significant cash crop in Dade County, FL. In the 1980–1981 season, 1,620 ha were harvested, with receipts of $17,000,000, making cocoyam second only to tomatoes in farm value among the 17 vegetable crops grown in the county (1).

In 1979, a bacterial leaf spot disease of cocoyam was reported in Florida (22), but the causal organism was not identified. Berniac (2) reported a bacterium very closely resembling *Xanthomonas campestris* pv. *dieffenbachiae* (McCulloch & Pirone) Dye as the cause of bacterial leaf spot of cocoyam in Guadeloupe. He suggested *X. campestris* pv. *aracearum* (Berniac) Dye as the name for the cocoyam strain and all leaf-spotting xanthomonads.

These studies were undertaken to isolate and identify the organism causing bacterial leaf spot of cocoyam in Florida and determine disease incidence.

MATERIALS AND METHODS

Isolation. Leaf portions from affected field-grown cocoyam leaves were surface-sterilized for 10 sec in a 0.5% aqueous solution of sodium hypochlorite. Bacteria were then isolated from 4-mm^2 samples cut from the junction of healthy and diseased leaf tissue. Each sample was placed on a sterile microscope slide, covered with a drop of sterile water, and observed under a dissecting microscope at ×25. Petri plates of nutrient agar amended with 1% sucrose (SNA) were streaked with loopfuls of bacterial suspension seen streaming from the cut tissue surfaces. Plates were incubated for 48 hr at 26 C. Cultures of bacteria were purified by repeated subculturing on SNA. Permanent cultures were maintained in sterile, distilled water at 25–27 and 3 C.

Characterization of pathogen. Initial isolation efforts on SNA consistently yielded yellow colonies of a bacterium resembling a xanthomonad. Three representative isolates, M8, M9, and M10, were chosen for further study. These were compared with known isolates of *X. campestris* pv. *dieffenbachiae* (Xcd), Xcd 2449A-83 from *Dieffenbachia maculata*, and Xcd 2682A-83 from *Philodendron selloum* provided by G. W. Simone, and *X. campestris* pv. *vesicatoria* (Doidge) Dye, isolate Xv 75-1, provided by R. E. Stall.

All isolates were Gram-stained (25) and stained for flagella (17). Growth, colony characteristics, and pigmentation were observed on yeast extract-dextrose-calcium carbonate agar (YDC) (6), MS agar (23), King's medium B (12), SX agar (26), and CVP medium (4) without crystal violet.

Specific physiological and biochemical tests included oxidative and fermentative utilization of glucose (10), catalase (24), oxidase (15), gelatin liquefaction (9), nitrate reduction in Difco nitrate broth (9), hydrogen sulfide production from peptone (18), indole production (9), and urease (27). Aesculin hydrolysis was determined as described by Dye (6), except petri plates of the medium solidified with 15 g of Bacto agar per liter were observed. Proteolysis was studied in tubes of reconstituted Bacto litmus milk.

Use of asparagine as a sole source of carbon and nitrogen was tested according to the method of Dye (5). Growth at 35 C was studied in tubes of yeast-salts broth (6) incubated in a modular Dri-Bath (Thermolyne Corp., Dubuque, IA).

The basal medium of Hugh and Leifson (10) was used to test for oxidative production of acid from various organic compounds. Filter-sterilized (0.45-μm) solutions were added to the autoclaved basal medium for a final concentration of 1%. The following substrates were tested: glucose, arabinose, cellobiose, fructose, galactose, glycerol, lactose, maltose, mannose, mannitol, raffinose, sucrose, trehalose, dulcitol, erythritol, inositol, inulin, salicin, and sorbitol. Hypersensitivity of Burley tobacco was tested by injection of 2×10^8 colony-forming units (cfu) per milliliter of an aqueous suspension of each isolate into leaves (13).

All cultures except Xv 75-1 were examined for production of xanthomonadin pigment, using the thin-layer chromatographic technique described by Irey and Stall (11).

Pathogenicity tests. Cocoyam corms from the field and young *D. maculata* from a commercial nursery were planted in a sterile 1:1 mixture of peat and vermiculite in 21-cm-diameter clay pots. Inocula of M8, M9, M10, Xcd 2449A-83, and Xcd 2682A-83 were prepared by flooding 48-hr-old SNA petri-dish cultures with sterile buffer (16), and the suspension was adjusted spectrophotometrically to about 2×10^6 cfu per milliliter. Volumes of about 0.3 ml of each suspension were then injected into cocoyam and dieffenbachia leaves by a tuberculin syringe fitted with a 27-gauge needle. Eight inoculations were made for each isolate. Control plants were injected with sterile buffer exposed to sterile SNA plates. Treated leaves were covered with plastic bags for 24 hr to ensure a

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postinoculation period of high humidity and kept in a greenhouse with a night/day temperature range of 24–37 C. Plants were subirrigated to prevent cross-contamination among treatments.

Field surveys. In August 1983, a survey was made of the incidence and intensity of bacterial leaf spot of cocoyam in Dade County. Observations were made in six commercial fields (8.1–28.3 ha) at or near harvest. Plantings on both of the locally predominant soil types, Marl and Rockdale, were surveyed. Fields were traversed in a "Z" pattern, with every 10th plant examined for bacterial leaf spot. Disease severity was rated by the following scale: 1 = 0%, 2 = 1–10%, 3 = 10–20%, 4 = 20–40%, and 5 = 40% or more of the leaf surface affected.

RESULTS

Symptoms. Early symptoms of bacterial leaf spot of cocoyam in the field were small, water-soaked spots, usually more evident on the abaxial surface. These enlarged to produce necrotic spots as large as 2 cm in diameter surrounded by prominent chlorotic halos. Pronounced water-soaking continued on the abaxial leaf surface. Although leaf spots were often delimited by veins, infections sometimes progressed into veins and proceeded basipetally, resulting in streaks of infected tissue. Some lesions coalesced, resulting in large dead areas. A cream to light yellow bacterial exudate was often seen on the undersides of young lesions, especially in the morning. Apparently, bacterial leaf spot infections can occur through the hydathodes, resulting in water-soaked and chlorotic leaf margins (Fig. 1A). This reaction is similar to that reported for philodendron (30).

Characterization of pathogen. The isolates were gram-negative rods with single polar flagella. Colonies were yellow and mucoid on SNA and YDC agars. No fluorescent pigment was produced on King's medium B. No isolate grew on MS agar, but all except Xv 75-1 grew well and hydrolyzed starch on SX agar. Isolates M9 and M10 degraded pectate on modified CVP medium.

The isolates were catalase-positive, produced hydrogen sulfide from peptone, liquefied gelatin, hydrolyzed aesculin, and grew at 35 C. Marked proteolysis occurred in litmus milk. The isolates were oxidase negative and did not ferment glucose; indole was not produced and urease activity was not detected. The isolates grew well in the nitrate broth preparation, but there was no reduction of the substrate to nitrite or ammonia.

Asparagine was not utilized as a sole source of carbon and nitrogen. The three cocoyam isolates produced acid from glucose, arabinose, cellobiose, fructose, galactose, lactose, maltose, mannose, raffinose, sucrose, and trehalose. Acid was not produced from glycerol, mannitol, dulcitol, erythritol, inositol, inulin, rhamnose, salicin, or sorbitol. Acid production by the known strains of *X. campestris* was the same, except Xcd 2449A-83 showed a slight positive reaction with inulin, Xv 75-1 and Xcd 2449A-83 did not produce acid from raffinose, and Xcd 2682A-83 did not produce acid from lactose.

All isolates elicited hypersensitivity in Burley tobacco. Production of xanthomadin pigment was variable. Rf values characteristic of this pigment were found for M8 and Xcd 2682A-82 but not for M9, M10, or Xcd 2449A-82.

Pathogenicity tests. The cocoyam isolates induced symptoms in cocoyam and dieffenbachia. Symptoms on cocoyam usually appeared within 3–4 days. After 7–8 days, lesions increased to 1 cm in diameter and were similar to those seen in

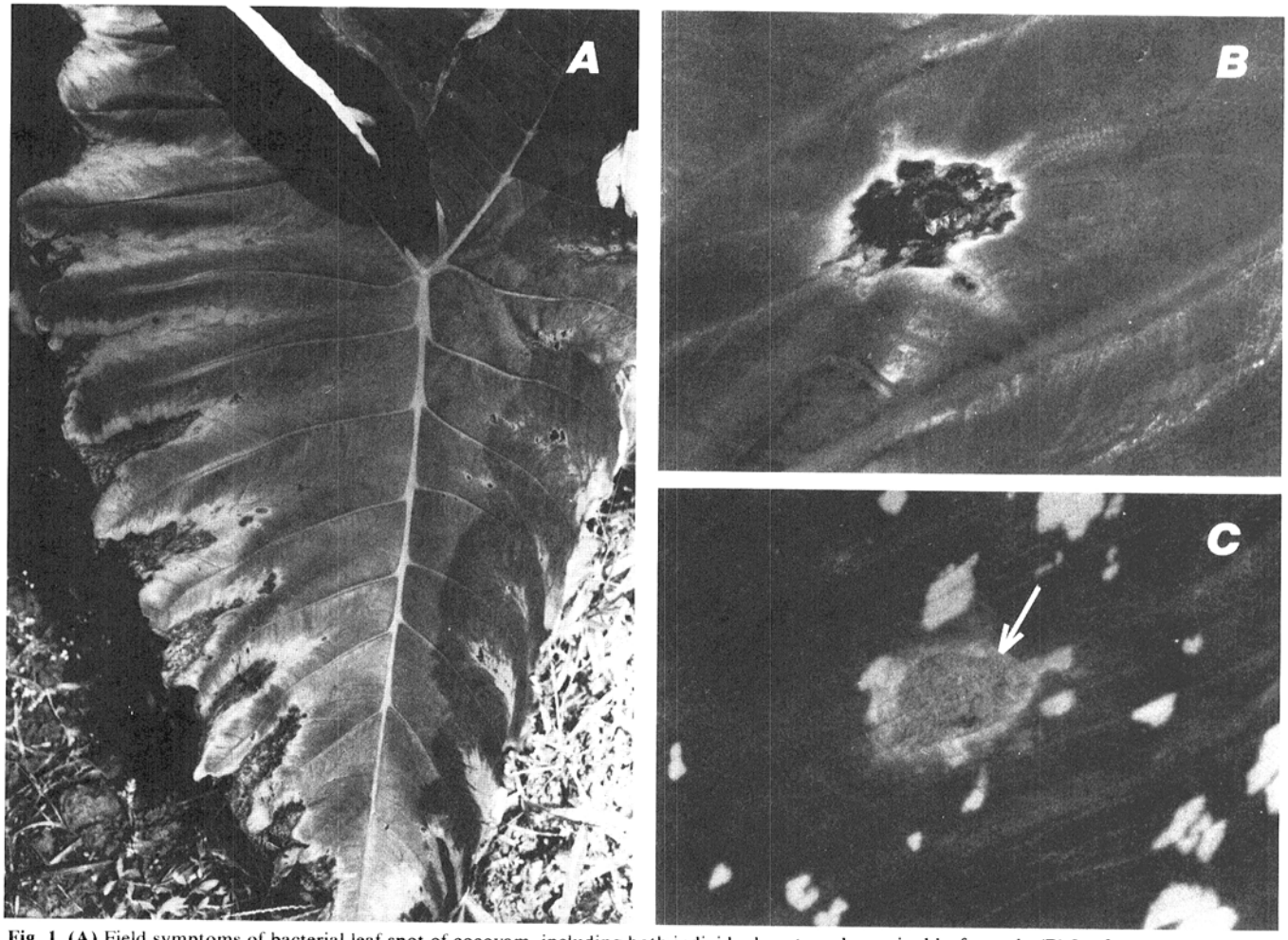

Fig. 1. (A) Field symptoms of bacterial leaf spot of cocoyam, including both individual spots and marginal leaf scorch. **(B)** Leaf spot symptoms on greenhouse-grown cocoyam inoculated with *Xanthomonas campestris* pv. *dieffenbachiae* isolated from diseased cocoyam. **(C)** Symptoms in *Dieffenbachia maculata* induced by isolate of *X. campestris* pv. *dieffenbachiae* obtained from cocoyam.

Table 1. Incidence and severity of bacterial leaf spot of cocoyam (*Xanthosoma caracu*) in six commerical fields in Dade County, FL, in August 1983

Field	No. of plants sampled	Soil type	Disease incidence[a] (%)	Av. severity rating[b]
1	104	Rockdale	86	2.4
2	100	Rockdale	78	2.0
3	83	Rockdale	100	2.5
4	87	Marl	74	1.4
5	73	Marl	81	2.0
6	83	Marl	77	2.0

[a]Percentage of sampled plants with at least one lesion.
[b]Rating system: 1 = 0%, 2 = 10%, 3 = 10–20%, 4 = 20–40%, and 5 = 40% or more of leaf area affected.

the field (Fig. 1B). Prominent chlorotic halos developed around the lesions. The controls showed slight bleaching in the immediate area of the needle puncture but no lesion expansion.

On dieffenbachia, initial symptoms were water-soaked spots on the abaxial leaf surface. Chlorotic lesions appeared on the upper leaf surface within 7–8 days. Within another week, spots on the upper surface were gray-brown with chlorotic halos (Fig. 1C). These symptoms were indistinguishable from those reported for Xcd on dieffenbachia (19). Both known Xcd isolates induced symptoms in dieffenbachia, but only Xcd 2449A-83 did so in cocoyam. Positive results in both cocoyam and dieffenbachia were obtained for an additional Xcd isolate, Xcd 2682C-83.

Field surveys. Bacterial leaf spot was found to be widespread in Dade County cocoyam fields. Incidence of infection ranged from 74 to 100% of surveyed plants (Table 1); however, disease severity generally was not great. Severity ratings for the fields usually averaged 2 or less (Table 1), indicating that less than 10% of the foliar area was damaged by Xcd.

DISCUSSION

On the basis of results of physiological and biochemical tests and pathogenicity trials, we suggest that the bacterium causing leaf spot of cocoyam in Florida is a strain of *X. campestris* pv. *dieffenbachiae* (3,6). Of particular note, the Florida cocoyam strain did not utilize asparagine as a sole source of carbon and nitrogen, had one polar flagellum, hydrolyzed aesculin, was strictly aerobic, digested protein, displayed mucoid growth, and produced acid from arabinose, glucose, and mannose but not from glycerol. The Florida isolates from cocoyam caused disease in both the original host and *D. maculata*. Contrary to the results of Berniac (2), two of the three known Xcd isolates from other aroids grown in Florida induced typical symptoms in cocoyam.

We confirmed the findings of Berniac (2) that the cocoyam strains do not produce acid from glycerol. However, the lack of acid production from glycerol was also noted for the known Florida Xcd isolates from other aroids. The variability in acid production from glycerol may preclude the usefulness of this particular biochemical test in the differentiation of *X. campestris* pathovars. Dye et al (7) recognized a distinct pathovar, *X. campestris* pv. *aracearum*, for the cocoyam pathogen. The suggestion that *X. campestris* pv. *aracearum* be used for all xanthomonads causing leaf spots of aroids (2) has merit; however, we chose to classify the Florida cocoyam strain as *X. campestris* pv. *dieffenbachiae* on the basis of infection of dieffenbachia and the historical use of this pathovar designation for the xanthomonad attacking other aroids (8,19,20,30). Extensive host range studies are needed to resolve relationships and taxonomy of pathovars infecting aroids.

In the survey of commercial fields, bacterial leaf spot was found on large numbers of plants. The original source of inoculum is unknown. Perhaps it came from diseased foliage plants; production of container-grown tropical foliage is extensive in Dade County (21,28) and has included dieffenbachia and other aroids (29). It seems more likely, however, that the pathogen was introduced into Florida in aroid planting stock from the Caribbean. Florida has not established a cocoyam certification program, and current propagation practices are not phytosanitary. Because our observations indicate that the pathogen is systemic in cocoyam and Xcd has been considered systemic in other hosts (14), the pathogen may be carried from crop to crop in contaminated corms and cormels used to establish new plantings.

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