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A Monocot Plant Identified as an Alternate Host of the Causal Agent of Bacterial Spot of Tomato and Pepper

Harold A.A. Gibbs

Department of Biological and Chemical Sciences, Cave Hill Campus, University of the West Indies, P.O. Box 64, Bridgetown, Barbados, W.I.

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***Abstract. Xanthomonas campestris* pv. *vesicatoria* (Xcv) recovered from *Commelina benghalensis* L., caused bacterial spot disease in cultivars of pepper and tomato susceptible to the pathogen. This is the first reported case of a dicot-infecting Xc pathovar infecting a monocot plant, represented here by a member of the Family Commelinaceae. Laboratory strains of the pathogen that included 81-23, 81-23M13, 82:4, 2595, and P6AD4, known to be pathogenic to pepper and tomato, promoted bacterial spot symptoms on leaves of *C. benghalensis* L. Of the 63 field isolates recovered from infected *C. benghalensis* L., 30 gave biochemical and physiological reactions consistent with Xcv pathogens, whereas 10 of the latter promoted bacterial spot disease in the test cultivars resulting in the identification of seven pathogenic races, including P2, P5, P6, P5T1, P5T2, P6T2, and P6T3. Bacterial spot disease symptoms developed on stems only when *C. benghalensis* L. was spray-inoculated with strains 81-23, 81-23M13, and P6AD4. Bacterial concentration increased in planta by as much as 10^3 per lesion of the leaf, whereas growth of the same strains was restricted in the stem of this weed. Growth of these three strains was, however, significantly ($P \leq 0.05$) lower on NYGA amended with *C. benghalensis* L. stem extract than on NYGA amended with leaf extract. The ability of the bacterial spot pathogen to infect the stem of *C. benghalensis* L. has serious implications for management of bacterial spot disease in fields populated with this weed since stems of this plant infected with the pathogen continue to grow vegetatively and disperse throughout all fields in which it is found.**

Pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.) production in tropical areas can be seriously constrained by *Xanthomonas campestris* pv. *vesicatoria* (Diodge) Dye (Xcv), the causal agent of bacterial spot disease of these crops (Kim and Hartman, 1985; Scott et al., 1989). This pathogen causes widespread and severe bacterial spot disease on tomato and pepper crops produced in Barbados (Ward and O'Garro, 1992). Strains of the pathogen designated XcvP, XcvT, and XcvPT are virulent on pepper, tomato, or pepper and tomato, respectively (Higgerd et al., 1987; Minsavage et al., 1990; Reifshneider et al., 1985).

Strains of the bacterium virulent on tomato were isolated from infested pepper fields and strains of the pepper group were recovered from fields cropped with disease tomato (Pohronezny et al., 1992; Ward and O'Garro, 1992). Implications are that, whereas pepper and tomato strains of Xcv are avirulent on tomato and pepper, respectively, the pathogens may use the nonhost plant to enhance the saprophytic phase of their growth cycle. Successful saprophytic survival of the pathogen ensures that there is an adequate quantity of inoculum in the soil to facilitate the infection of freshly planted host cultivars. Increased inoculum density, which favors increased intensity of disease infestation, will be greatly enhanced by the extent to which the pathogen can colonize alternate hosts including weed species that are abundant in crop fields at harvest.

Pepper and tomato are produced in Barbados on highly mechanized farms where weed control is employed only during the early growth stage of the crop. At harvest, therefore, most pepper and tomato fields in Barbados are overrun with weeds, such as pussley (*Portulaca oleracea* L.), wild spinach (*Amaranthus dubius* L.), milk weed (*Chanaesyce hirta* L.), and pond grass (*Commelina benghalensis* L.), which, according to Carrington (1993), are among the most common weeds in Barbados.

There is evidence that suggests that weed hosts of the bacterial spot pathogen have been observed (L.W. O'Garro, unpublished data). The study conducted on the interaction of strains of Xcv with common weeds showed that although members of both monocot and dicot weeds were assessed as alternate hosts, only dicots, namely milk weed and wild spinach, which are both members of the Solanaceae family, showed compatible responses to these pathogens.

Observations of lesions similar to those of bacterial spot disease of tomato and pepper being present on leaves of pond grass growing in two 5-acre plots at Mt. Wilton, Barbados, that were abandoned for more than 2 years, were made for the first time in these fields. This is the first time that such lesions were observed on monocots populating any agricultural field in Barbados and, since these plots have a history of bacterial spot disease of both pepper and tomato (Gibbs, 1998), it warrants investigation into the possibility that the Xcv pathogen might be surviving saprophytically in the leaves of this weed.

Bacterial spot disease affects the stems, leaves, fruits, and floral parts of pepper and tomato (Cook and Stall, 1963; Minsavage et al., 1990; Sowell and Langford, 1963), and in experiments conducted by Gibbs (1998), stems of tomato plants were used in preference to leaves during the assessment of the severity of bacterial spot disease of this crop. This raised concern as to whether stems of the pond grass were restricting the growth of the pathogen that might otherwise destroy the entire weed if bacterial proliferation on the stem is not inhibited.

The fields were last cropped in 1996 to many vegetables, including bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleraceae* L.), corn (*Zea mays* L.), pepper, and tomato, as part of a crop rotation program. This observation raised serious concern because pond grass regenerates primarily via vegetative growth and thrives enormously in all parts of the island. Thus, if the bacterial spot pathogen is able to survive saprophytically on the leaf tissue of this weed, this would increase the inoculum potential of these soils. The rapid vegetative growth and spread of this weed throughout fields cropped to vegetable produce, coupled with the fact that ploughing acts as a major dispersal agent, ensures that pond grass is among the more successful weeds found on the island. Should the bacterial spot disease pathogen be capable of colonizing the leaves of pond grass, then the task of combating bacterial spot disease of pepper and tomato in fields populated by *C. benghalensis* L. can be greatly aggravated.

This study reports for the first time evidence of a monocot-infecting *X. campestris* pathovar capable of infecting *C. benghalensis* L., a member of the Commelinaceae family. The importance of the leaves and stems of pond grass to the saprophytic survival of Xcv was also determined. The pathotype of all *Xanthomonas*-like isolates recovered from infected pond grass leaves was determined using cultivars of tomato and pepper. Finally, the extent to which five laboratory strains of Xcv pathogenic to tomato and pepper caused bacterial spot disease symptoms on leaves and stems of pond grass was determined.

Materials and Methods.

Isolation of the pathogen. The two 5-acre plots selected for this study were overrun by many weeds, including seed-under-leaf (*Phyllanthus amarus* L.), whitehead bush (*Parthenium hysterophorus* L.), milk weed, wild spinach, pussley, nut sedge (*Cyperilus rotundus* L.), Devil's grass (*Cynodon dactylon* L.), and pond grass. Ninety leaves of pond grass having lesions symptomatic of bacterial spot disease were harvested from 45 randomly

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selected locations in each of the two fields. The leaves were surface sterilized by first dipping in 95% ethanol for 2 s, followed by a 10-s immersion in 1.25% sodium hypochlorite after which the sterilized leaves were rinsed in sterile distilled water (SDW). Separate portions of each leaf containing one lesion each were excised and each homogenized in SDW (200 µL) using a sterile mortar and pestle. From each of the 90 bacterial suspensions 40 µL were sampled and streaked onto nutrient agar (Difco Laboratories, Detroit) and incubated at 25–28 °C for 5 to 7 d. Twenty-six xanthomonad-like colonies, appearing yellow and mucoid, were recovered from one field and 37 from the other. The field isolates were designated HG1 to HG63, after the initials of the author, and stored at –20 °C in nutrient yeast-extract glycerol broth (NYGB) (Turner et al., 1984). Each strain represents a single colony grown from the NYGA plates inoculated with extracts from the infected pond grass leaves.

Identification of isolates. Identification of xanthomonad-like characteristics, including gram reaction, cell morphology, xanthomonadin pigment, catalase, nitrate reductase, urease and oxidase, were done using standard bacteriological methods (Schaad and Stall, 1988). The hydrolysis of milk, starch, and gelatin was determined according to methods described by Dye (1968), whereas the utilization of asparagine as the sole source of carbon and nitrogen was studied using the method outlined by Fahy and Hayward (1983).

Pathogenicity and race classification. A differential set of susceptible pepper and tomato cultivars was used to classify xanthomonads isolated from infected pond grass into pepper-tomato races (Minsavage et al., 1990). Near isogenic lines of Early Calwonder pepper, including ECW, ECW 10R, ECW 20R, and ECW30R, and two tomato cultivars, namely Walter and H7998 obtained from J.B. Jones (Gulf Coast Research and Education Center, Univ. of Florida, Bradenton), were used to distinguish races.

Cells of the 10 field isolates were collected from NYGB medium by centrifugation at 15,000 *g*, for 10 min, washed twice with SDW, and resuspended in SDW before the optical absorbance of 0.3 at a wavelength of 600 nm (5×10^8 cells/mL) prepared. A final inoculum containing 10^3 to 10^4 cells/mL was prepared by appropriate dilution. Ten test strains, each recovered from different infected plants, were inoculated into susceptible tomato and pepper plants previously described, by using a syringe without a needle to introduce each suspension of the pathogen into the intercellular spaces of fully expanded leaves (Swanson et al., 1988). Strains of Xcv designated 81-23 and 81-23M13 were two of five bacterial cultures obtained from J.B. Jones (Gulf Coast Research and Education Center), and used as positive controls in this study due to the fact that they are pathogenic to pepper-tomato and pepper, respectively (Gibbs et al., 1997). Sterile distilled water was used to inoculate control plants, and all treated plants were grown in sterile potting compost (Yates, New Zealand) under conditions of 25–2 °C, 90% to 95% relative humidity, and a 12-h photoperiod maintained by fluorescent lamps giving a total light intensity of 1020 $\mu E \cdot s^{-1} \cdot m^{-2}$ in a PGW-108 plant growth chamber (Percival Manufacturing Co., Wis.). Race distinction was made on the basis of the presence of a incompatible or compatible response on tomato and pepper (Minsavage et al., 1990).

Alternate host. Cuttings of *C. benghalensis* L., each 10 cm in length, were planted in pots 15 cm in diameter. Replicates of five plants were then spray-inoculated with suspensions of each of the pepper and tomato strains, including 81-23, 81-23M13, 82:4, 2595, and P6AD4, containing 5×10^6 cells/mL, until runoff. Control plants were sprayed with SDW, and inoculated plants were kept in the growth chamber as before. Symptoms typical of bacterial spot disease were observed 5 to 7 d after to inoculation. All experiments were done in duplicate.

Growth of the pathogen was also assessed in planta. Leaves and stems of *C. benghalensis* L. were pin-prick inoculated using sterile pins dipped into colonies of 81-23, 81-23M13, and P6AD4 that were grown on NYGA for a period of 7 d. The tips of sterile pins were dipped into colonies representative of each strain to be transferred to the experimental plants, and inoculations made in eight positions of both the stems and leaves of the weed at a distance of 1 cm apart. Control plants were inoculated with sterile pins dipped into SDW. One lesion was excised from both the stem and leaf of the plants immediately after inoculation, surface sterilized and homogenized as above before drop-plating 40 µL of the suspension onto NYGA. Subsequent samplings were done 1, 2, 3, 4, 5, 6, and 8 d after inoculation. Bacterial growth in planta was quantified as colony-forming units (cfu) per lesion, and all experiments were repeated once.

A random sample of 10 strains recovered from lesions of pond grass spray and pin-prick inoculated with each of the test strains, as inoculated onto susceptible pepper and tomato plants to ensure that they were pathogenic to the host plants.

Growth of pathogen in plant extract-amended medium. Leaf and stem tissue of pond grass was ground separately in SDW to prepare plant tissue extracts (50 g of tissue to 100 mL SDW). The extracts were then filter sterilized through sterile millipore filters (0.2 µm). NYGA medium (14 g) was homogenized in SDW (900 mL) and autoclaved at 121 °C for 15 min. After the media had cooled to 60 °C, 100 mL of leaf and 100 mL of stem extract were each added to separate batches of the agar to generate leaf extract-amended NYGA and stem extract-amended NYGA, respectively. Plates were inoculated with 100 µL of bacterial suspensions (1.0×10^3 cfu/mL) containing of each of the five laboratory-derived strains. Inoculated plates were incubated at 25–28 °C for 5 d and the cfu/100 mL (1 drop) determined. Statistical differences in viability among strains was assessed using analysis of variance.

Ecological significance of pathogen-weed interaction. Ten pots of 30 cm in diameter were filled with sterile potting mix (Yates, New Zealand) and planted with three 10-cm-long cuttings of pond grass containing two nodes each. Potted plants were kept in growth chamber set as described above and watered until they were fully established. Five of the 10 pots were sprayed with a suspension of the most virulent strains of Xcv, strain 81-23, while the other five plants were sprayed with SDW. Lesions symptomatic of bacterial spot disease, appearing on the leaves of infested pond grass plants were allowed to develop, over a period of 2 weeks, into brown, necrotic spots after which the plants were incorporated into the potting mix of the respective pots. Plants were watered twice daily and regrowth of the weed in the respective pots observed up to 2 months after ploughing plants into the soil mix.

Results and Discussion

All bacterial cells examined were gram-negative rods, measuring 1.0–1.2 × 0.4–0.7 µm with rounded cells. The aerobic cells were motile by a single polar flagellum. Thirty-three of the presumptive pathogens tested for xanthomonadins produced yellow spots with an average Rf value of 0.47 on thin layer chromatography plates, while the other isolates yielded pigments of average Rf values of 0.38–0.42. Thirty of the 53 isolates were nitrate reductase-negative, oxidase-negative, and

Table 1. Race classification of *Xanthomonas campestris* pv. *vesicatoria* obtained from infested *Commelina benghalensis* L. found in derelict pepper and tomato fields in Barbados.

		Pepper cultivars[z]				Tomato cultivars	
Strain	Race	ECW	ECW10R	ECW20R	ECW30R	Walter	H7998
HG2	P2	+[y]	–[x]	–	+	–	–
HG6	P5	+	–	+	+	–	–
HG12	P5	+	–	+	+	–	–
HG4	P6	+	+	+	+	–	–
HG30	P6	+	+	+	+	–	–
HG11	P5T1	+	–	+	+	+	–
HG1	P5T2	+	–	+	+	+	+
HG10	P6T2	+	+	+	+	+	+
HG3	P6T3	+	+	+	+	+	–
HG5	P6T3	+	+	+	+	+	–
81-23	P1T2	+	+	–	–	+	+
81-23M13	P1	+	+	–	–	–	–

[z]ECW10R, ECW20R, and ECW30R possess the Bs1, Bs2, and Bs3 avirulence gene, respectively.
[y]+ indicates compatible response.
[x]– indicates incompatible response.

catalase-positive. They utilized glucose, arabinose and trehalose but failed to use asparagine as a sole source of carbon and nitrogen. The isolates assessed were all positive for starch hydrolysis, gelatin liquification, and proteolysis of milk.

Of the 30 field isolates recovered from this weed, 10 were pathogenic to pepper or pepper and tomato and none were virulent to tomato alone. The isolates were all compatible with pepper cultivars ECW and ECW30R, thus indicating that they overcame the Bs3 avirulence gene in the latter. HG2 was the only strain incompatible with ECW20R, which possesses the Bs2 avirulence gene. Fifty percent of the strains tested were compatible with pepper cultivar ECW10R and tomato cultivar Walters, but two of the 10 strains were compatible with cultivar H7998.

Three pepper and four pepper-tomato race types of the pathogen were identified (see Table 1). The remaining 20 field isolates gave incompatible reactions with all plant cultivars used in this assessment and therefore they were not represented in Table 1. Strains 81-23 and 81-23M13 were confirmed as members of pepper-tomato race 2 and pepper race 1 pathotypes, respectively.

All pepper and pepper-tomato field isolates of the pathogen were found to induce lesions symptomatic of bacterial spot disease between 6 to 8 d after spray inoculation onto leaves of *C. benghalensis* L. Infected leaves deteriorated progressively from the stem tip to the base leaving stems of inoculated plants unaffected (Fig. 1). In contrast, plants serving as controls did not develop symptoms of bacterial spot disease. All bacterial strains recovered from spray and pin-prick inoculations were pathogenic to susceptible pepper and tomato cultivars (data not shown).

Bacterial growth in planta was detected after leaves of the weed were pin-prick inoculated with suspensions of Xcv strains, 81-23, 81-23M13, and P6AD4 (Fig. 2). This evidence indicates that of the test strains used, 81-23 was the most pathogenic, whereas growth of strain P6AD4 in the leaf tissue was the lowest. Growth of these strains in the stem tissue of pond grass was significantly reduced relative to growth in the stem. Strain P6AD4 increased by a factor of 10^2, whereas strains 81-23 and 81-23M13 increased by factors of $\approx 10^4$ and 10^3, respectively, over the 8-d sampling period.

All strains tested were found to grow similarly well on NYGA and leaf extract-amended NYGA. However, growth of these strains was restricted in stem extract-amended NYGA relative to those cultured in NYGA amended with leaf extract (Table 2).

Strain P6AD4 grew less vigorously than the others, but all responded similarly to each treatment. While growth of Xcv seems to be negatively affected by the presence of stem extract, this does not fully support observations made during laboratory experiments which show that the presence of strains pathogenic to tomato, pepper, and pond grass, spray-inoculated onto the stems of this weed, do not cause lesions of bacterial spot disease to de-

Fig. 1. Bacterial spot of *Commelina benghalensis* L. spray-inoculated with *Xanthomonas campestris* pv. *vesicatoria* strain 8-23. Illustrated is (**A**) healthy plant and (**B**) infected plant showing progression of degradation of leaves from stem tip to stem base.

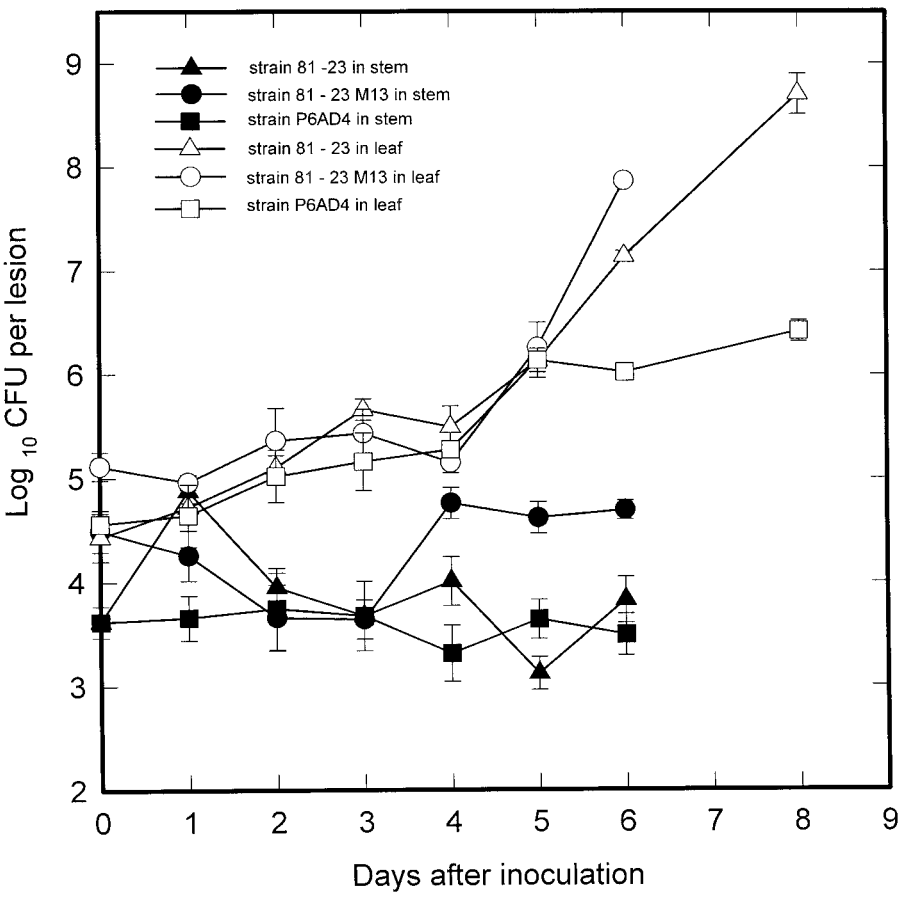

Fig. 2. Growth of *Xanthomonas campestris* pv. *vesicatoria* strains 81-23, 81-23M13, and P6AD4 pin-prick-inoculated into leaf and stem of *Commelina benghalensis* L. points on each plot represent the means and standard errors (error bars) of triplicate determinations as bacterial colony-forming units (cfu).

Table 2. Relative growth of *Xanthomonas campestris* pv. *vesicatoria* on NYGA amended with stem and leaf extract obtained from *Commelina benghalensis* L.

Strain	NYGA	NYGA - Stem extract	NYGA - Leaf extract
		#cfu/100 µL bacterial suspension[z]	
81-23	44 a[y]	na[x]	48 a
81-23M13	42 a	22 b	45 a
82:4	47 a	na	47 a
2595	51 a	24 b	48 a
P6AD4	22 b	6 c	25 b

[z]cfu are the means of 10–100 µL drops done for each of the five strains.
[y]Mean of cfu followed by the same number indicates no significant difference ($P \leq 0.05$).
[x]Indicates that plants were overgrown by nontarget bacteria.

velop on such stems. The mechanism by which this pathogen is excluded from invading the stem of this weed will require further investigation before it is fully understood.

The fact that Xcv was found to infect only the leaves of *C. benghalensis* L. also has ecological significance for both weed and pathogen. After infection by the bacterial spot pathogen, leaves of pond grass become withered, senescent, and eventually abscise. In laboratory experiments where strain 81-23 was used to spray-inoculate the weed, regeneration from the stem of this weed into new plants subsequent to incorporation into the soil, was similar to plants that were similarly treated except that SDW was used instead of the pathogen. This ensures that the pathogen can survive saprophytically in the leaves of this weed while in the soil whereas, the stems, which is unaffected by the pathogen, can regenerate into plants that develop no visible symptoms of bacterial spot disease.

Conclusion

This study shows that strains representative of pepper and pepper-tomato races of *Xanthomonas campestris* pv. *vesicatoria* can infect *C. benghalensis* L. Together with the fact that this weed is ubiquitous and very hardy, the level of threat it poses to these crops can increase. Further studies are needed to determine whether this weed can support saprophytic growth of other species of xanthomonads such as *X. c.* pv. *phaseoli* and *Xanthomonas campestris*, which infect French bean and onion, respectively. Both French bean and onion are economically important crops in Barbados. Since no effort was made during this study to assess the pathogenicity of the 30 xanthomonad-like field isolates obtained from diseased pond grass, on susceptible French bean and *Allium* species, further studies are needed to determine whether xanthomonads recovered from pond grass can infect these and other crops.

Alhough this study was conducted in Barbados, these findings may have serious implications for pepper and tomato production anywhere *C. benghalensis* L. grows competitively with these crops.

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