Pathological Variations Within *Xanthomonas campestris* pv. *mangiferaeindicae* Support Its Separation Into Three Distinct Pathovars that Can Be Distinguished by Amplified Fragment Length Polymorphism

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ABSTRACT

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Bacterial black spot, caused by *Xanthomonas campestris* pv. *mangiferaeindicae*, is an important disease of mango (*Mangifera indica*). Several other plant genera of the family *Anacardiaceae* were described as host species for xanthomonads. We studied pathological variations among strains in a worldwide collection from several *Anacardiaceae* genera. Strains were classified into three pathogenicity groups. Group I strains (from the Old World) multiplied markedly in leaf tissue of mango and cashew (*Anacardium occidentale*). Group II strains (from Brazil) multiplied markedly in cashew leaf tissue, but not in mango. Moreover, mango leaves inoculated with group I and group II strains exhibited lesions with different morphologies, consistent with variations in symptomatology previously reported on mango under field conditions. Group I

strains produced black, raised lesions, consistent with the original description of the pathovar, whereas group II strains produced brownish, flat lesions. Group III strains produced a unique syndrome on ambarella (*Spondias dulcis*) and mombin (*Spondias mombin*). Based on evolutionary genome divergence derived from amplified fragment length polymorphism (AFLP) data, the three groups were genetically distinct and were related to groups 9.5, 9.6, and 9.4 of *X. axonopodis* identified by Rademaker, respectively. As each group was characterized by unique symptomatology and/or host range, we propose that *X. campestris* pv. *mangiferaeindicae* be split into three pathovars of *X. axonopodis*: *X. axonopodis* pv. *mangiferaeindicae*, *X. axonopodis* pv. *anacardii*, and *X. axonopodis* pv. *spondiae*. Within pv. *mangiferaeindicae sensu novo*, AFLP data were consistent with that previously published for restriction fragment length polymorphism groups and suggested long-distance movement of the pathogen, likely through propagative material.

Additional keywords: area under disease progress curve, Brazilian pepper, in planta population sizes, leaf lesion morphology.

Mango (*Mangifera indica* L.) is one of the most cultivated fruits in the world (fifth in worldwide production in 2004 according to the Food and Agriculture Organization of the United Nations [FAO] website). Mango belongs to the family *Anacardiaceae*, which is mostly represented in tropical and subtropical areas. Mango bacterial canker (MBC) (also called mango bacterial black spot [MBBS]) is an important bacterial disease that affects mango in many countries. This disease was first described in 1915 by Doidge (10) in South Africa but may have originated from India, as the disease was observed in herbarium specimens collected in Bihar in 1881 (25). Because no efficient control can be achieved, MBC causes recurrent problems in the major mango producing regions: Asia, southern and eastern Africa, western Oceania, and the Indian Ocean (15). MBC has been reported in Brazil since 1953 (37) and is a major menace for industries in Central America, which have become increasingly important mango exporters (e.g., Mexico, which ranked first in

2004 and represented 23% of the world exports according to the FAO website). Under climatic conditions favoring MBBS, the disease commonly causes fruit losses of up to 85% (27).

On mango, the pathogen infects plants through wounds and natural openings. Leaf and fruit lesions are most commonly observed. Symptoms on leaves are raised, black, angular and necrotic, and those on fruits are star-shaped, erumpent with a gummy exudate. Lesions on twigs or branches occasionally occur, most often after hurricanes, but no twig dieback has been reported (15). The pathogen is disseminated on a smaller scale mostly through climatic events such as wind-driven rains or through cultural practices. Long-distance dissemination is due to international movement of contaminated plant material (15). One report from Brazil (43) indicated a unique symptomatology on mango leaves, twigs, and fruits in this country. Leaf lesions in Brazil were reported as brownish, flat, angular spots. Fruit lesions were described only on a few cultivars as black, depressed and rounded lesions of irregular sizes. Some lesions only display depressions, with no other external changes, but show an internal pulp decay consisting in tissue discoloration sometimes associated with pulp rot. The pathogen in Brazil also induces unique twig symptoms, i.e., yellow to brown lesions, which subsequently longitudinally crack and evolve as a generalized dieback of the youngest vegetative flush.

The causal agent of MBC is a gram-negative bacterium, classified in the genus *Xanthomonas* in the 1970s (11) as *X. campestris* pv. *mangiferaeindicae*. In contrast with most xanthomo-

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* The e-Xtra logo stands for "electronic extra" and indicates that the online version contains a supplemental table not included in the print edition. This table shows the geographic origins and other characteristics of the 338 strains of *X. campestris* pv. *mangiferaeindicae* used in this study. The reference strains used, including pathotype strains of relevant pathovars, are also listed.

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nads, it produces nonpigmented colonies on agar media (15). However, yellow-pigmented cultures were sporadically isolated in Brazil, South Africa, Florida, and Reunion Island (14). These strains are casual pathogens and should not be classified as pathovar *mangiferaeindicae* (15).

Reclassification of the genus *Xanthomonas* by a polyphasic approach including DNA:DNA hybridization did not include the pathovar *mangiferaeindicae* (49).

The pathovar classification established by Dye et al. (11) and reviewed by Young et al. (50) is an infra-subspecific classification applied to bacterial plant pathogens by reference to their host range or to their capacity to cause distinctive symptoms. Pathogenicity tests are an essential part of this classification. The pathovar classification was primarily developed for plant protection regulators and quarantine officers (50) and has no standing in formal nomenclature regulated by the International Code of Nomenclature of Prokaryotes because it is applied at the infra-subspecific level.

Mango is the host species from which the disease was originally described (10), giving its name to the pathovar (11). But early work also indicated that, when inoculated, mango strains from India were pathogenic to other members of the family *Anacardiaceae*, such as cashew (*Anacardium occidentale* L.) and hog plum (syns. ambra or Indian mombin – *Spondias mangifera* Willd., syn. *S. pinnata* [J. G. König ex L. f.] Kurz.) (25). More recently, xanthomonads producing nonpigmented colonies were described as the causal agent of a bacterial dieback of ambarella (*Spondias dulcis* Forst., syn. *Spondias cytherea* Sonn.) in the French West Indies (40) and a black spot of Brazilian pepper (*Schinus terebinthifolius* Raddi) in Réunion Island (28). In Brazil, a disease caused by xanthomonads was described on cashew and mango (24,37), whereas in the Old World no disease on cashew was reported, even in countries where MBC is present (15). In Brazil, nonpigmented xanthomonads were isolated from mango in several states in the north, northeast, and southeast regions (37), where its importance is globally low but depends on climatic conditions and cultivars (3). No report is available of Brazilian pepper infected by an xanthomonad in Brazil.

Phenotypic (29), isozyme (47), and restriction fragment length polymorphism (RFLP) analyses (using an *hrp* gene cluster as probe) (14) suggested that strains isolated in the Old World on mango and Brazilian pepper are closely related. These strains were distantly related to mango strains isolated in Brazil, strains from ambarella, and yellow-pigmented strains from mango. Two RFLP probes, an avirulence (*avr*) gene (*avrXa10*) cloned from *X. oryzae* pv. *oryzae* and belonging to the *avrBs3* gene family (17) and an insertion sequence (IS1595) from *X. campestris* pv. *mangiferaeindicae* were used to further study the genetic variability among *X. campestris* pv. *mangiferaeindicae* strains collected from different geographic regions. The *avr* probe primarily distinguished strains on the basis of the host from which they originated (mango or Brazilian pepper), whereas the IS probe distinguished strains based on the country from which they originated (14).

Because pv. *mangiferaeindicae* is of considerable economic significance worldwide, we examined the genetic diversity (using amplified fragment length polymorphism [AFLP]) and the pathological variations of a worldwide collection of strains from selected genera in the family *Anacardiaceae*. In the present study, we show that *X. campestris* pv. *mangiferaeindicae* is a genetically and pathologically complex pathovar. As recommended by Young et al. (50), we assessed associations between pathogenicity characters and AFLP data, a technique suitable for pathovar identification purpose (33). Some strains had a unique host range and/or differed in terms of symptom morphology and ability to multiply in host tissue. Therefore, we propose to reclassify *X. campestris* pv. *mangiferaeindicae* into three pathovars.

MATERIALS AND METHODS

Bacterial strains and DNA extraction. A total 338 strains of *X. campestris* pv. *mangiferaeindicae* were used in this study.

Strains were stored at –80°C on beads in cryovials (Microbank Prolab Diagnostics, Austin, TX) and also freeze-dried for long-term storage. Cultures were grown routinely on YPGA (7 g of yeast extract, 7 g of peptone, 7 g of glucose, and 15 g of agar liter⁻¹; 20 mg of propiconazole liter⁻¹) at 28°C. Some strains included in this study were deposited in the Collection Française de Bactéries Phytopathogènes (CFBP, INRA, Angers, France), the Belgian Coordinated Collections of Micro-Organisms, (BCCM/LMG, University of Ghent, Belgium), and the National Collection of Plant Pathogenic Bacteria (NCPBB, CSL, York, United Kingdom).

Genomic DNA was extracted from bacteria with the DNeasy tissue kit (Qiagen, Courtaboeuf, France) following the manufacturer's instructions, and DNA concentrations were estimated by fluorometry (TKO 100 Fluorometer, Hoefer, San Francisco).

AFLP analysis. All strains of *X. campestris* pv. *mangiferaeindicae* were submitted to AFLP analysis. To test the reproducibility of the AFLP technique, two independent DNA extractions were used, and strain CFBP 2933 was used as a control in each AFLP experiment. Polymerase chain reaction (PCR) analyses were performed in a GeneAmp PCR system 9700 thermocycler (Applied Biosystems, Courtaboeuf, France). Adaptors and PCR primers were synthesized by Applied Biosystems (Table 1).

AFLP analysis was performed as described previously (18). The selective amplifications were performed using unlabeled *MspI*+1 (A, C, T, or G) primers and 5'-labeled-*SacI*+C primers (fluorochromes used: 6-FAM, VIC, NED, and PET, respectively).

The presence and absence of fragments were scored as a binary matrix. A dissimilarity matrix using Jaccard index was calculated and a weighted neighbor-joining tree (42) was built using DARWIN 5.0 software (26). The robustness of the tree was assessed by bootstrap analysis with the same software (1,000 samplings).

Metric multidimensional scaling (MDS) was used to represent distances between strains, based on the Jaccard dissimilarity matrix. MDS transforms a distance matrix (which cannot be analyzed by eigen-decomposition) into a cross-product matrix and then solves the eigenvector problem to find the coordinates of individuals, so that distortions to the distance matrix are minimized. As in principal component analysis, individuals are projected into *n* dimensions (1). This technique is becoming popular for analyzing ecological data (5,7). MDS was performed using the cmd-scale function in the R software (version 2.3.1; R Development Core Team, Vienna, Austria).

AFLP patterns of strains of *X. campestris* pv. *mangiferaeindicae* were compared with those of the type strain of *X. cam-*

TABLE 1. Amplified fragment length polymorphism primers and adaptors used in this study

Primers/adaptors	Sequence 5' > 3'
Ligation adaptors	
A1MSP	GACGATGAGTCCTGAG
A2MSP	CGCTACGAGCTCATC
A1SAC	CTCGTAGACTGCGTACAAGCT
A2SAC	TGTACGCGAGTCTACG
Preamplification primers	
PMSP	GATGAGTCCTGAGCGG
PSAC	TAGACTGCGTACAAGCTC
Selective amplification primers	
<i>MspI</i> +1 selective nucleotide	GATGAGTCCTGAGCGG + A, C, T, or G
5'-labeled <i>SacI</i> +C	6-FAM - TAGACTGCGTACAAGCTCC VIC - TAGACTGCGTACAAGCTCC NED - TAGACTGCGTACAAGCTCC PET - TAGACTGCGTACAAGCTCC

pestris and *X. axonopodis* (obtained from LMG as LMG 568 and LMG 982, respectively). AFLP patterns of other pathovars of *X. axonopodis* belonging to groups 9.1 to 9.6 *sensu* Rademaker et al. (34) were also compared with strains isolated from the family *Anacardiaceae*. For such comparisons, evolutionary genome divergences (EGD), expressed as the number of nucleotide substitutions per site, were calculated from Dice similarity index and corrected to account for unobserved substitutions by using the standard Jukes-Cantor model, which assumes equal substitution rates between all pairs of bases (22).

Pathogenicity tests on mango, cashew, and Brazilian pepper. Strains used for pathogenicity tests are indicated in Table 2 (comparison of strains identified as *X. axonopodis* groups 9.4, 9.5, and 9.6) and Table 3 (comparison of a larger set of strains identified as *X. axonopodis* group 9.5 from two different host species). Brazilian pepper, mango cvs. Maison Rouge and Haden, and/or cashew were used for inoculations. Bacterial suspensions containing approximately 10^5 CFU ml⁻¹ were obtained by 10-fold dilutions of spectrophotometrically adjusted suspensions (0.06

optical density [OD] at 600 nm $\approx 10^8$ CFU ml⁻¹) prepared from 18-h-old cultures on YPGA. Suspensions were infiltrated (0.3 to 0.4 cm²) into the mesophyll of mature leaves from the youngest vegetative flush. For each strain, 10 inoculation points were performed on each of three leaves on different plants. Each experiment was replicated once. Plants were placed in growth chambers at $30 \pm 1^\circ\text{C}$ day and $26 \pm 1^\circ\text{C}$ night and $95 \pm 5\%$ relative humidity for 35 days with a photoperiod of 12 h. All inoculated leaves were examined daily for 20 days (comparisons of AFLP groups 9.5 and 9.6) or 25 days (comparisons of strains within AFLP group 9.5) for lesion development (i.e., the number of visible lesions per leaf).

X. campestris pv. *mangiferaeindicae* population sizes in lesions from the same host species were estimated 35 days after inoculation (dai) for strains listed in Tables 2 and 3. For each strain–host combination, eight leaf fragments (≈ 1 cm²) collected at inoculation sites were sampled. Leaf fragments were excised from the leaf blade, surface-sterilized with 95% ethanol, and individually homogenized in 4.5 ml of 0.01 M, pH 7.2, Sigma 7-9

TABLE 2. Lesion development based on area under disease progress curve (AUDPC) after inoculation of three host species with xanthomonads originating from *Anacardiaceae* and identified as amplified fragment length polymorphism (AFLP) groups 9.4, 9.5, and 9.6 of *Xanthomonas axonopodis*^a

Strain	Host of origin	Proposed pathovar designation ^v	AFLP group ^w	AUDPC species inoculated		
				Mango	Cashew	Brazilian pepper
CFBP 1716 ^x	Mango	<i>mangiferaeindicae</i> (I)	9.5	101.3 (12.9) ^y c ^z	155.3 (6.6) a	151.5 (12.4) bc
JK147-1	Mango	<i>mangiferaeindicae</i> (I)	9.5	95.8 (12.3) d	151.7 (2.1) a	150.5 (14.1) bc
A5-1	Mango	<i>mangiferaeindicae</i> (I)	9.5	95.0 (15.5) d	144.0 (3.2) b	148.5 (11.9) bc
CFBP 2939	Brazilian pepper	<i>mangiferaeindicae</i> (I)	9.5	119.0 (13.4) bc	150.3 (2.4) a	146.0 (9.4) c
CFBP 2912	Mango	<i>anacardii</i> (II)	9.6	123.8 (4.6) b	145.8 (4.2) b	167.7 (6.7) a
CFBP 2913	Mango	<i>anacardii</i> (II)	9.6	123.3 (3.9) b	152.3 (3.0) a	169.7 (6.4) a
CFBP 2914	Mango	<i>anacardii</i> (II)	9.6	124.0 (4.0) b	154.2 (5.2) a	160.8 (10.5) ab
JY542	Cashew	<i>anacardii</i> (II)	9.6	136.8 (7.3) a	149.8 (0.4) a	160.3 (6.7) ab
LA98	Cashew	<i>anacardii</i> (II)	9.6	138.7 (5.4) a	151.2 (2.5) a	163.7 (11.9) ab
LA99	Cashew	<i>anacardii</i> (II)	9.6	136.2 (4.5) a	150.2 (4.9) a	169.5 (5.7) a
LA100	Cashew	<i>anacardii</i> (II)	9.6	124.8 (2.2) b	152.2 (4.8) a	160.8 (7.9) ab
LA101	Cashew	<i>anacardii</i> (II)	9.6	126.5 (3.9) b	150.3 (5.6) a	161.7 (11.0) ab
LA102	Cashew	<i>anacardii</i> (II)	9.6	124.5 (2.4) b	152.8 (4.3) a	158.7 (12.8) ab
LA103	Cashew	<i>anacardii</i> (II)	9.6	124.7 (5.0) b	150.3 (4.8) a	161.0 (6.7) ab
CFBP 2547	Ambarella	<i>spondiae</i> (III)	9.4	107.3 (13.9) cd	121.2 (4.9) c	97.0 (31.7) d

^a AUDPC values were based on lesion development on 10 inoculation sites per leaf. Six AUDPC values per strain–host combination were used for mean and standard deviation calculation.

^v Pathogenicity groups are given in parenthesis.

^w This study. Group numbers as defined in Rademaker et al. (33,34).

^x Pathotype strain.

^y Values between parentheses are standard deviations, based on two replicated experiments.

^z Strain comparisons based on Mann-Whitney tests. AUDPC values followed by the same letter(s) are not significantly different ($P = 0.05$).

TABLE 3. Pathological variations based on area under disease progress curve (AUDPC) within pathogenicity group I strains (amplified fragment length polymorphism group 9.5) after inoculation of mango and Brazilian pepper^v

Strain	Host of origin	Country of origin	Restriction fragment length polymorphism group ^w	AUDPC species inoculated	
				Mango	Brazilian pepper
CFBP 2935	Mango	Australia	C	165.3 (10.5) ^x b ^y	181.8 (10.1) a
CFBP 1716 ^z	Mango	India	C	151.3 (12.9) bc	181.5 (12.4) a
JK147-1	Mango	Philippines	B	145.8 (12.3) c	180.5 (14.1) ab
A5-1	Mango	Réunion	A	145.5 (18.6) bc	165.8 (2.1) bc
CFBP 2932	Mango	Réunion	A	143.8 (21.2) bc	167.5 (7.9) b
CFBP 2933	Mango	Réunion	C	143.2 (23.1) bc	178.0 (20.7) ab
JV1121	Mango	South Africa	ND	164.0 (14.5) b	181.5 (7.1) a
CFBP 2938	Brazilian pepper	Réunion	D	194.4 (5.9) a	164.7 (4.7) bc
CFBP 2939	Brazilian pepper	Réunion	D	182.5 (7.6) b	149.7 (15.6) d
CFBP 2940	Brazilian pepper	Réunion	D	173.5 (17.1) b	155.5 (19.6) bcd
JP742	Brazilian pepper	Réunion	D	133.3 (18.2) c	160.3 (9.2) cd
JP758	Brazilian pepper	Réunion	D	181.0 (10.5) b	155.3 (12.7) cd

^v AUDPC values were based on lesion development on 10 inoculation sites per leaf. Six AUDPC values per strain–host combination were used for mean and standard deviation calculation.

^w From Gagnevin and Pruvost (14); ND, not determined.

^x Values between parentheses are standard deviations, based on two replicated experiments.

^y Strain comparisons based on Mann-Whitney tests. AUDPC values followed by the same letter(s) are not significantly different ($P = 0.05$).

^z Pathotype strain.

buffer (Sigma, Saint Quentin Fallavier, France) with an Ultraturax T25 homogenizer (Janke & Kunkel, IKA Labortechnik, Staufen, Germany). Aliquots of the homogenized suspensions and of 10-fold dilutions obtained from the suspensions were plated on semiselective media (32) with the Spiral System device (Inter-science, Saint Nom la Bretèche, France). We used three media (KC and NCTM3 semiselective media [32] and modified Wilbrink medium [39]), depending on the strains we compared. Bacterial population sizes were based on enumeration of *X. campestris* pv. *mangiferaeindicae*-like colonies (starch hydrolysis was checked on doubtful colonies).

Data analysis. Area under disease progress curve (AUDPC) values were calculated for each inoculated leaf (8). Variance homogeneity was examined using the Bartlett test. This criterion was not met by some data sets. Therefore, nonparametric tests (Kruskal-Wallis and pairwise Mann-Whitney) were performed using R statistical package (version 2.3.1; R Development Core Team, Vienna, Austria).

Bacterial population sizes were log-transformed to stabilize sample variances. Variance homogeneity was examined using the Bartlett test. This criterion was not met for some data sets, as data derived from strains with a lower in planta growth ability displayed higher sample variance values. Therefore, nonparametric tests (Kruskal-Wallis and Mann-Whitney) were performed using the R statistical software (version 2.3.1; R Development Core Team).

Pathogenicity tests on ambarella and mombin. Strains CFBP 2547, CFBP 2623 (isolated from ambarella in the French West Indies), as well as *X. campestris* pv. *mangiferaeindicae* CFBP 1716 (pathotype strain isolated from mango in India) and CFBP 2913 (isolated from mango in Brazil) were used for inoculation. Bacterial suspensions containing approximately 10^8 CFU ml⁻¹ (0.06 OD at 600 nm $\approx 10^8$ CFU ml⁻¹) prepared from 24-h-old cultures on YPGA were used. Inoculations were performed by wounding a herbaceous part of the main stem with a sterile scalpel and depositing a 10- μ l droplet on the wound. For each strain, three seedlings (3 to 5 months old) of both species were inoculated. Each experiment was replicated once. Plants were placed in growth chambers at $28 \pm 1^\circ\text{C}$ and $95 \pm 5\%$ relative humidity for 30 days with a photoperiod of 12 h. All inoculated plants were examined weekly for lesion development. At the end of each experiment, reisolation of the pathogen from twig symptoms was performed on YPGA or modified Wilbrink medium (39).

RESULTS

Genetic relatedness among pathovar *mangiferaeindicae* strains and outgroups. EGD values were calculated from AFLP data and indicated that xanthomonads associated with the family *Anacardiaceae* were highly heterogeneous and comprised three main groups of strains. The first group consisted of strains isolated from mango and Brazilian pepper and originated from countries in the Old World. The second group consisted of strains isolated from both mango and cashew and originated from Brazil. The third group consisted of strains isolated from ambarella and originated from the French West Indies. Examples of EGD values between selected strains from the three groups and other *Xanthomonas* strains are shown in Figure 1.

EGD values between strains inside a group ranged between 0.001 and 0.014 nucleotide substitutions per site for group I, 0.003 and 0.022 nucleotide substitutions per site for group II, and there was no polymorphism between the two strains that constituted group III. Minimal EGD values between group I versus group II and group III strains were 0.062 and 0.116 nucleotide substitutions per site, respectively. Similarly, the minimal EGD value between group II versus group III strains was 0.103 nucleotide substitutions per site.

Fig. 1. Representation of genetic distances based on evolutionary genome divergence (EGD), expressed as the number of nucleotide substitutions per site and calculated between xanthomonads isolated from *Anacardiaceae*, strains representative of groups 9.1 to 9.6 of *Xanthomonas axonopodis*, and the type strain of *X. campestris*. Symbols are as follows: $\times$, group 9.1; $\star$, group 9.2; +, group 9.3; $\circ$, group 9.4; $\triangleright$, group 9.5; $\diamond$, group 9.6; $\square$, *X. campestris*; and $<$, unknown species. Symbols filled with solid black are for strains isolated from the family *Anacardiaceae*. Strain numbers are as follows: 1, CFBP 1716 (pathotype strain of pv. *mangiferaeindicae* - from mango - 9.5); 2, CFBP 2917 (from mango - 9.5); 3, JR749 (from mango - 9.5); 4, CFBP 2940 (from Brazilian pepper - 9.5); 5, JR606-2 (from Brazilian pepper - 9.5); 6, CFBP 2938 (from Brazilian pepper - 9.5); 7, JR606-1 (from Brazilian pepper - 9.5); 8, JR537-6 (from Brazilian pepper - 9.5); 9, CFBP 2525 (*X. a. pv. citri* - 9.5); 10, LMG 558 (*X. a. pv. cajani* - 9.5); 11, LMG 712 (*X. a. pv. glycines* - 9.5); 12, LMG 761 (*X. a. pv. malvacearum* - 9.5); 13, LMG 9045 (*X. a. pv. clitoriae* - 9.5); 14, LMG 8014 (*X. a. pv. phaseoli* - 9.4); 15, LMG 937 (*X. a. pv. vitians* - 9.5); 16, LA98 (from cashew - 9.6); 17, LMG 867 (*X. a. pv. sesbaniae* - 9.6); 18, CFBP 2913 (from mango - 9.6); 19, LMG 8021 (*X. a. pv. rhynchosiae* - 9.6); 20, LMG 828 (*X. a. pv. vignicola* - 9.6); 21, LMG 936 (*X. a. pv. vignieraediacae* - 9.6); 22, LMG 7511 (*X. a. pv. phaseoli* var. *fuscans* - 9.6); 23, LMG 7455 (*X. a. pv. phaseoli* - 9.4); 24, LMG 695 (*X. a. pv. dieffenbachiae* - 9.4); 25, CFBP 2603 (*X. a. pv. manihotis* - 9.4); 26, CFBP 6369 (*X. a. pv. allii* - 9.2); 27, LMG 811 (*X. a. pv. patellii* - 9.2); 28, CFBP 2547 (from ambarella - 9.4); 29, LMG 899 (*X. a. pv. vasculorum* - 9.3); 30, LMG 568^T (*X. c. pv. campestris* - type strain of *X. campestris*); 31, LMG 551 (*X. a. pv. begoniae* - 9.1); 32, LMG 7303 (*X. a. pv. begoniae* - 9.1); 33, LMG 982^T (*X. a. pv. axonopodis* - type strain of *X. axonopodis* - 9.3); and 34, CFBP 2924 (mango from Brazil, yellow pigmented colonies).

The EGD value between the pathotype strain of *X. campestris* pv. *mangiferaeindicae* (CFBP 1716) and the type strains of *X. axonopodis* (LMG 982) and *X. campestris* (LMG 568) were 0.149 and 0.140 nucleotide substitutions per site, respectively (Fig. 1).

low, with maximal Jaccard dissimilarity values around 0.15 (i.e., EGD values of 0.014 nucleotide substitutions per site). Most branches in the neighbor-joining tree were characterized by low (i.e., $\leq 70\%$) bootstrap values. Seven subgroups were supported by moderately robust bootstrap values. The first subgroup (bootstrap 84%) was composed of mango strains originating from the Comoros. Two subgroups (bootstrap of 78%) were composed of mango strains originating from five Asian countries (Japan, Philippines, and Taiwan, Thailand, and India) (Fig. 2A). Other subgroups with bootstrap values $>70\%$ were composed of mango strains isolated in Réunion Island. Based on the neighbor-joining tree (Fig. 2A) and the representation of axes 1 and 2 of the MDS (Fig. 2B), the population structure derived from AFLP analysis is partly congruent with that previously described by RFLP. Strains from mango or Brazilian pepper were not clearly separated.

Pathogenicity tests on mango, cashew, and Brazilian pepper. All strains listed in Table 2 induced black lesions after inoculation on mango, cashew, and Brazilian pepper, with two distinct morphologies. Mango leaves inoculated with strains originating from mango and identified as group I (which are genetically related to *X. axonopodis* group 9.5) showed raised, black, angular lesions. Brazilian pepper and cashew leaves inoculated with the same strains showed flat, necrotic, angular lesions. All other plant-strain combinations produced necrotic flat lesions. Cashew leaves showed an extensive water soaking (which developed to form black necrotic lesions) only when inoculated with group II strains (which are genetically related to *X. axonopodis* group 9.6). Similarly, Brazilian pepper leaves displayed an extensive water soaking (which developed to form black necrotic

Fig. 2. Genetic diversity among strains of *pv. mangiferaeindicae* based on amplified fragment length polymorphism data using four pairs of primers. **A**, Neighbor-joining tree based on Jaccard similarity index showing the genetic relatedness among the 125 identified haplotypes from several geographic areas. All strains originated from mango, except where stated. **A**, Australia; C, Comoros; I, India, J, Japan; M, Mauritius; NC, New Caledonia; P, Philippines; Rn, Réunion; Ro, Rodrigues; Ta, Taiwan; Th, Thailand; and RB, Reunion from Brazilian pepper. **B**, Multidimensional scaling (MDS) representation of the distances between 125 haplotypes from several geographic areas. The presented two-dimensional MDS has a stress value of 0.045 (1). Corresponding restriction fragment length polymorphism (RFLP) groups (14) are indicated, when available. Three RFLP groups (A, B, and C) contained strains isolated from mango, whereas strains classified in RFLP group D originated from Brazilian pepper.

lesions) only when inoculated with group I strains originating from this host species. AUDPC values summarizing lesion development of selected group I, II, and III strains are presented in Table 2. Except for interactions involving group III strains, large AUDPC values primarily indicated an earlier development of lesions, as maximal incidence values were reached after 5 to 15 dai. Although significant differences among strains were observed, AUDPCs were not very helpful at clarifying distinctive pathogenicity between groups of strains.

On mango cv. Maison Rouge, large population densities (ranging from 5×10^6 to 9×10^6 CFU per lesion) were observed only for group I strains originating from mango (Table 4). Population sizes from all other strains were 1×10^5 CFU per lesion or lower (Table 4). Similar results were obtained from inoculations of mango cv. Haden. On this cultivar, mean population sizes estimated for group II strains from mango (CFBP 2912, 2913, and 2914) ranged from 5×10^4 to 2×10^5 CFU per lesion, whereas the population size calculated for the group I pathotype strain (CFBP 1716) was 1×10^7 CFU per lesion.

In contrast, lesions produced on cashew after inoculation with all group II strains developed large population sizes, ranging from

3×10^7 to 9×10^7 CFU per lesion (Table 4). Similarly, group I strains originating from mango multiplied to high population densities in cashew (2×10^7 to 3×10^7 CFU per lesion) (Table 4), but the group I strain CFBP 2939 originating from Brazilian pepper did not (7×10^4 CFU per lesion) (Table 4).

On Brazilian pepper, the only strains that induced lesions in which large population sizes (7×10^7 to 2×10^8 CFU per lesion) (Tables 4 and 5) were recorded were group I strains originating from Brazilian pepper. All other strains induced lesions in which populations sizes were significantly smaller, but a larger diversity in population size was found on this host species (Tables 4 and 5). Within group I strains, although population sizes obtained for mango strains were significantly lower than those obtained for Brazilian pepper strains, the former strains still reached relatively high levels, ranging from 4×10^5 to 6×10^6 CFU per lesion (Table 5).

Pathogenicity tests on ambarella and mombin. Both strains originating from ambarella induced severe dieback of inoculated ambarella plants. No symptom was observed after inoculation of strain *X. campestris* pv. *mangiferaeindicae* CFBP 1716 or CFBP 2913. On mombin, less severe symptoms were observed. The two strains originating from ambarella induced twig lesions that were

TABLE 4. Population sizes recorded from leaf lesions 35 days after inoculation of three host species with xanthomonads originating from *Anacardiaceae* and identified as amplified fragment length polymorphism (AFLP) groups 9.4, 9.5, and 9.6 of *Xanthomonas axonopodis*^a

Strain	Host of origin	Proposed pathovar designation ^v	AFLP group ^w	Log-transformed CFU ml ⁻¹ species inoculated		
				Mango	Cashew	Brazilian pepper
CFBP 1716 ^x	Mango	<i>mangiferaeindicae</i> (I)	9.5	6.88 (0.62) ^y a ^z	7.51 (0.20) d	6.67 (0.49) b
JK147-1	Mango	<i>mangiferaeindicae</i> (I)	9.5	6.97 (0.48) a	7.34 (0.27) e	5.80 (0.67) c
A5-1	Mango	<i>mangiferaeindicae</i> (I)	9.5	6.70 $\pm$ 0.61) a	7.53 (0.16) d	6.00 (0.84) cd
CFBP 2939	Brazilian pepper	<i>mangiferaeindicae</i> (I)	9.5	4.21 (0.51) cd	4.82 (0.21) g	7.86 (0.29) a
CFBP 2912	Mango	<i>anacardii</i> (II)	9.6	4.68 (0.69) b	7.92 (0.34) ab	3.38 (0.45) e
CFBP 2913	Mango	<i>anacardii</i> (II)	9.6	4.11 (1.16) d	7.92 (0.26) a	5.72 (0.14) c
CFBP 2914	Mango	<i>anacardii</i> (II)	9.6	4.99 (1.33) bc	7.93 (0.22) a	5.82 (0.32) c
JY542	Cashew	<i>anacardii</i> (II)	9.6	4.71 (1.06) bc	7.80 (0.19) abc	3.21 (0.93) ef
LA98	Cashew	<i>anacardii</i> (II)	9.6	3.65 (0.39) de	7.66 (0.25) c	5.27 (0.53) d
LA99	Cashew	<i>anacardii</i> (II)	9.6	3.61 (0.70) de	7.80 (0.22) abc	2.58 (0.39) g
LA100	Cashew	<i>anacardii</i> (II)	9.6	2.90 (0.78) f	7.73 (0.22) b	2.83 (0.83) f
LA101	Cashew	<i>anacardii</i> (II)	9.6	3.53 (0.81) de	7.83 (0.14) ab	2.96 (0.47) f
LA102	Cashew	<i>anacardii</i> (II)	9.6	3.18 (1.43) f	7.70 (0.13) c	2.66 (1.04) f
LA103	Cashew	<i>anacardii</i> (II)	9.6	3.33 (1.22) ef	7.45 (0.27) de	2.57 (0.66) g
CFBP 2547	Ambarella	<i>spondiae</i> (III)	9.4	4.72 (0.86) b	6.01 (0.43) f	2.94 (0.63) ef

^a Population sizes were determined on NCTM3 semiselective medium, except for strain CFBP 2547 (modified Wilbrink's medium). Eight lesions per strain–host combination were used for population size assessment.

^v Pathogenicity groups are given in brackets.

^w This study. Group numbers as defined in Rademaker et al. (33,34).

^x Pathotype strain.

^y Standard deviation, based on two replicated experiments.

^z Strain comparisons based on Mann-Whitney tests. Population size values with the same letter(s) are not significantly different ($P = 0.05$).

TABLE 5. Pathological variations within pathogenicity group I strains (amplified fragment length polymorphism group 9.5) based on population sizes recorded from leaf lesions 35 days after inoculation of two host species^v

Strain	Host of origin	Country of origin	Restriction fragment length polymorphism group ^w	Log-transformed CFU ml ⁻¹ species inoculated	
				Mango	Brazilian pepper
CFBP 2935	Mango	Australia	C	6.68 (0.26) ^x c ^y	6.69 (0.26) cd
CFBP 1716 ^x	Mango	India	C	7.14 (0.23) b	6.61 (0.45) cd
JK147-1	Mango	Philippines	B	7.02 (0.48) b	5.81 (0.66) ef
A5-1	Mango	Réunion	A	7.38 (0.38) a	5.79 (0.64) f
CFBP 2932	Mango	Réunion	A	7.58 (0.33) a	5.56 (0.67) f
CFBP 2933	Mango	Réunion	C	7.38 (0.32) a	6.75 (0.41) c
JV1121	Mango	South Africa	ND	6.69 (0.31) c	6.35 (0.51) de
CFBP 2938	Brazilian pepper	Réunion	D	4.26 (0.50) d	7.97 (0.14) b
CFBP 2939	Brazilian pepper	Réunion	D	3.62 (0.46) f	8.16 (0.22) a
CFBP 2940	Brazilian pepper	Réunion	D	3.85 (0.44) e	7.99 (0.30) b
JP742	Brazilian pepper	Réunion	D	7.10 (0.60) b	8.21 (0.18) a
JP758	Brazilian pepper	Réunion	D	4.19 (0.42) d	8.06 (0.32) ab

^v Population sizes were determined on KC semiselective medium. Eight lesions per strain–host combination were used for population size assessment.

^w From Gagnevin and Pruvost (14); ND, not determined.

^x Standard deviation, based on two replicated experiments.

^y Strain comparisons based on Mann-Whitney tests. Population size values with the same letter(s) are not significantly different ($P = 0.05$).

^z Pathotype strain.

approximately 70 to 80 mm in length 1 month after inoculation, whereas the two strains originating from mango did not cause any lesions. Typical slow-growing nonpigmented colonies were successfully reisolated from assayed lesions.

DISCUSSION

We used AFLP, a technique that has been extensively used to investigate the species classification of the genus *Xanthomonas* (19,33,41), intrapathovar assessment of genetic diversity (6,35), and epidemiology (18). Recently, this technique was given emphasis for species delineation in bacterial taxonomy (48). Based on AFLP data, the pathovar *mangiferaeindicae* was genetically heterogeneous and consisted of three distinct groups of strains. Genetic diversity between strains within group I and group II was low, with maximum EGD values of 0.014 and 0.022 nucleotide substitutions per site, respectively. These values are consistent with those previously published for *X. arboricola* pv. *pruni* (6) and *X. axonopodis* pv. *allii* (41). AFLP results further suggested that the provisional designation of strains of pathovar *mangiferaeindicae* as members of *X. campestris* requires revision, as evolutionary genome divergence of strains of this pathovar with the type strain of *X. campestris* was always higher than 0.130 nucleotide substitutions per site. Likewise, the EGD value between the pathotype strain of *X. campestris* pv. *mangiferaeindicae* and the type strain of *X. axonopodis* was 0.149 nucleotide substitutions per site. Indeed, EGD values between strains of *X. campestris* pv. *mangiferaeindicae* and strains of *X. axonopodis* sensu Vauterin et al. (49) were variable, ranging from approximately 0.03 to 0.15 nucleotide substitutions per site.

AFLP data, which show a good correlation with DNA:DNA hybridization data (33), allowed the allocation of pathovar *mangiferaeindicae* strains within genetic groups of *X. axonopodis*, as defined by Rademaker et al. (34). The pathotype strain of pathovar *mangiferaeindicae*, together with all strains isolated from the family *Anacardiaceae* in the Old World (designated herein as group I), were genetically related to *X. axonopodis* group 9.5 (composed of pathovars *citri*, *malvacearum*, *glycines*...), whereas strains isolated from mango and cashew in Brazil (designated herein as group II) were genetically related to *X. axonopodis* group 9.6 (composed of pathovars *aurantifolii*, *phaseoli* var. *fuscans*, *vignicola*...). Finally, strains isolated from ambarella in the French West Indies (designated herein as group III) were genetically related to *X. axonopodis* group 9.4 (composed of pathovars *dieffenbachiae*, *manihotis*, *phaseoli*...).

One aim of this paper was to clarify the pathovar classification of xanthomonads associated with the family *Anacardiaceae*. Pathovar *mangiferaeindicae* was originally characterized as a pathogen of mango (11). In the original description of this pathogen (25), inoculation of other genera in the family *Anacardiaceae* with mango strains suggested these as putative host species although disease had not been observed on them in the field. In these early reports, pathogenicity was probably assessed solely on the basis of lesion development after inoculation.

AUDPC has been used to quantify pathogenicity tests under controlled environmental conditions. Among xanthomonads, AUDPC was primarily used on vascular pathogens (2,9,20,36), and large AUDPC values were interpreted as successful interactions involving an aggressive pathogen and a susceptible host species. We used AUDPC for characterizing interactions involving foliar pathogens. Interestingly, successful interactions, as determined by population sizes in leaf lesions 35 dai, were characterized by low AUDPC values due to a delayed, rather than an incomplete, lesion development. Conversely, large AUDPC values, due to early lesion development, were correlated with low population sizes 35 dai. This can be interpreted as an early, though moderate, host defense-related necrosis (responsible for the marked population drop observed later during the interaction)

rather than a high susceptibility to the pathogen. These results agree with previous data indicating that lesion development after inoculation with group I strains was faster on a partially resistant (cv. Heidi) than on a susceptible (cv. Haden) mango cultivar (31).

Xanthomonads isolated from mango and cashew in Brazil (designated as group II in this study) were originally included in pathovar *mangiferaeindicae* (38). They formed a homogenous group based on their host range, symptomology, and internal population sizes in leaf lesions on the three *Anacardiaceae* genera used in this study. When two mango cultivars with different genetic backgrounds were inoculated with these strains, the resulting pathogenicity phenotypes were distinct from that caused by group I strains: leaf lesions were flat, larger AUDPC values were attained, and bacterial population sizes were of 1×10^4 to 1×10^5 CFU per lesion, typical of interactions involving nonhost species, resistant cultivars, or interactions involving casual pathogens (4,12,16). Similar population sizes were recovered from black lesions 1 month after inoculation of mango leaves in an incompatible interaction with *X. vesicatoria* CFBP 1545 (O. Pruvost, unpublished data). The atypical symptomology described herein on mango leaves for group II strains is consistent with the markedly different disease expression reported in the field on mango leaves, fruits, and twigs in Brazil versus the Old World (15,43). When cashew was inoculated with group II strains, bacterial population sizes in leaf lesions ranged from 3×10^7 to 9×10^7 CFU per lesion, typical of a compatible interaction (4,12,16). Interestingly, cashew originated from Northeastern Brazil (21), whereas mango originated from Asia (23). Brazil appears to be the only country in which epidemics caused by xanthomonads have been reported on cashew (24). Cashew may therefore be the primary host species of xanthomonads genetically related to *X. axonopodis* group 9.6 and isolated from cashew and mango in Brazil. In contrast, data from our study suggest that the pathogen is casual on mango cvs. Haden and Maison Rouge, although field observations from Brazil suggest that the susceptibility of mango to this pathogen may be cultivar-dependent (J. R. Neto, unpublished data). Further quantitative pathogenicity tests are needed to test this hypothesis. Symptomology differences between group II and group I (including the pathotype strain) strains warrant different pathovar designations, as was previously done for *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* (11). The proposed pathovar designation for the group II strains is *X. axonopodis* pv. *anacardii*, as these strains from Brazil shared a high genetic relatedness with members of *X. axonopodis* group 9.6 by AFLP. The elevation of pathovars of *X. axonopodis* group 9.6 as *X. fuscans* proposed by Schaad et al. (44,45) has not been validated to date in the list of prokaryotic names with standing in nomenclature (the International Committee on the Systematics of Prokaryotes [ICSP]). The proposed common name for the associated disease is cashew bacterial spot. The two available yellow-pigmented xanthomonads isolated from mango in Brazil are not included within *X. axonopodis* pv. *anacardii*, as the EGD values between the nonpigmented and the yellow-pigmented strains differed by more than 0.10 nucleotide substitutions per site (i.e., these strains should likely not be classified as *X. axonopodis*).

Similarly, when mango, cashew, or Brazilian pepper were inoculated with strain CFBP 2547 (group III) originating from ambarella, the resulting lesions were small, flat, black, and necrotic. Population sizes recovered from leave lesions on these three *Anacardiaceae* were 5×10^4 , 1×10^6 , and 9×10^2 CFU per lesion, respectively. Group III strains induced a severe dieback of ambarella, consistent with previous data (40) and induced extensive twig lesions on mombin. Therefore, group III strains, which are genetically related to *X. axonopodis* group 9.4, induced a unique symptomology on *Spondias* spp., a characteristic that warrants their being designated *X. axonopodis* pv. *spondiae*.

Consequently, we propose that the pathovar *mangiferaeindicae* epithet be used for characterizing strains truly pathogenic to mango and strains pathogenic to Brazilian pepper (group I strains). We propose that these strains be classified in a single pathovar, as there was a broad variation of population sizes between strains of the same host origin, resulting in a situation where there was less difference in pathogenicity on a given host between the most aggressive heterologous strain and the least aggressive homologous strain than between the least and the most aggressive strains of one type. Moreover, differences in population sizes measured for homologous and heterologous interactions appeared strain-dependent.

These strains shared a high level of genetic relatedness with several pathovars of *X. axonopodis* group 9.5. Therefore, we propose that these strains be provisionally named *X. axonopodis* pv. *mangiferaeindicae*, as the elevation of pathovars of *X. axonopodis* group 9.5 as *X. citri* recently proposed by Schaad et al. (45) has not yet been validated in the list of prokaryotic names with standing in nomenclature (ICSP). To avoid confusion with the disease caused by *X. axonopodis* pv. *anacardii*, we recommend that the disease caused by *X. axonopodis* pv. *mangiferaeindicae* be referred to as mango bacterial canker (13,46) (consistent with the branch cankers and the raised leaf and fruit lesions induced by this pathogen) rather than mango bacterial black spot.

The three proposed *Xanthomonas* pathovars associated with the family *Anacardiaceae* can be clearly distinguished by means of phenotypic (29), isozyme (47), RFLP (14), and AFLP (this study) analyses.

The genetic diversity of *X. axonopodis* pv. *mangiferaeindicae* was assessed, based on AFLP analysis of 325 strains isolated from mango in different countries and from Brazilian pepper in Réunion Island. The multidimensional scaling representation showed three groups of strains that are largely consistent with those previously described using the RFLP technique with two probes: an avirulence gene of the *avrBs3* family and the insertion sequence IS1595 (14). Both AFLP and RFLP strongly suggested interregional or international migration events. Eleven different haplotypes were identified in more than a single country. This involved South Africa, New Caledonia, Australia, Réunion Island, and Mauritius. Probably more countries were concerned by introduction of new strains from foreign countries, but these events were not detected in our study, primarily because the sample size markedly varied among countries. It is likely that some strains today present in Réunion Island were introduced from South Africa, as there were many mango budwood introductions in Réunion Island from South Africa in the 1970s, for which the quarantine conditions are not known. Similarly, there are propagative material exchanges between Réunion Island and its neighbor Mauritius. This could explain why both islands shared identical haplotypes. *X. axonopodis* pv. *mangiferaeindicae* can survive asymptotically in association with mango buds. It was previously shown, using a marked strain, that populations present in/or mango buds were responsible for subsequent leaf lesions on the neo-formed leaves (30).

Description of *X. axonopodis* pv. *anacardii*. *X. axonopodis* pv. *anacardii* [a.na'car.di.i N.L. adj. *anacardii* from *Anacardium*, the genus of cashew (*Anacardium occidentale* L.).

Based on AFLP, the bacterium is genetically related to members of *X. axonopodis* group 9.6 *sensu* Rademaker (34). Its genome does not contain insertion elements of the IS1595 family. Strains of pv. *anacardii* can also be distinguished from pathovars *mangiferaeindicae* and *spondiae* by isozyme analysis (47).

On cashew, characteristic leaf lesions induced by *X. axonopodis* pv. *anacardii* start as small, angular, water-soaked spots, which extend and eventually coalesce. Lesions turn into necrotic, flat, dark brown spots. No lesions have been reported on other plant organs. On mango, the bacterium produces necrotic, flat, dark brown to black spots, but in contrast with cashew, no initial water-

soaked lesions were observed after inoculation of cvs. Maison Rouge and Haden. One month after inoculation, small population sizes (typically 10^4 CFU per lesion) can be recovered from mango leaf lesions, whereas large population sizes (typically 10^7 CFU per lesion) can be recovered from cashew leaf lesions. Few reports from Brazil mentioned mango fruit lesions (43), and they described black, depressed and rounded lesions of different sizes. Some lesions only display depressions, with no other external changes, but show an internal pulp decay consisting in tissue discoloration sometimes associated with a pulp rot. Therefore, symptomology on mango fruit is also markedly different from that reported for pv. *mangiferaeindicae*, as no internal breakdown was observed for this pathogen (15). Finally, *Xanthomonas axonopodis* pv. *anacardii* induces unique twig symptoms, i.e. yellow to brown lesions, which subsequently longitudinally crack and evolve as a generalized dieback of the youngest vegetative flush (43). Based on pathogenicity tests and field observations, mango (*Mangifera indica*) and Brazilian pepper (*Schinus terebinthifolius*) are not susceptible host species of *Xanthomonas axonopodis* pv. *anacardii*.

The proposed neopathotype strain is ICMP 4088 (International Collection of Micro-organisms from Plants, New Zealand) = CFBP 2913 (Collection Française de Bactéries Phytopathogènes, France).

Description of *X. axonopodis* pv. *mangiferaeindicae*. *X. axonopodis* pv. *mangiferaeindicae* [man'gi.fe.rae'in.di.cae N.L. adj. *mangiferaeindicae* from *Mangifera indica* L., the Latin binomial of mango.

Based on AFLP, the bacterium is genetically related to members of *X. axonopodis* group 9.5 *sensu* Rademaker (34). Its genome contains a large number of insertion elements of the IS1595 family. Strains of pv. *mangiferaeindicae* can also be distinguished from pvs. *anacardii* and *spondiae* by isozyme analysis (47).

Bacterial canker affects all aerial parts of the mango plant. The bacterial pathogen can infect through wounds and natural openings. Leaf and fruit symptoms are most common, but twig and branch cankers may occur when infection is severe (i.e., on highly susceptible cultivars). Leaf symptoms begin as small water-soaked spots delineated by veins, becoming raised, black, sometimes with a chlorotic halo. Individual lesions are usually smaller than 0.5 cm^2 , but they may coalesce to form larger necrotic areas. One month after inoculation, population sizes ranging from 1×10^6 to 1×10^8 CFU per lesion can be recovered from mango leaf lesions on susceptible cultivars. Several months after infection, leaf lesions dry and turn light brown ash-gray. Severe leaf infection may result in early abscission. Fruit symptoms appear as small water-soaked spots on lenticels. These spots later become star-shaped, erumpent, and exude an infectious gum. Often, a "tear stain" infection pattern is observed on the fruit. Severe fruit infections will cause premature fruit drop. Twig cankers are potential sources of inoculum and weaken branch resistance to winds, but such lesions seem to occur only when climatic events (such as tropical storms) are responsible for massive inoculum redistribution and wounds. The disease is not systemic and does not affect the root.

Although inducing leaf lesions after inoculation, *X. axonopodis* pv. *mangiferaeindicae* has never been isolated in the field from *Anacardium* spp. and *Spondias* spp. Brazilian pepper (*Schinus terebinthifolius*) is considered a host species of *X. axonopodis* pv. *mangiferaeindicae*, although a host specialization occurs for most, but not all, strains.

The pathotype strain is ATCC 11637 (American Type Culture Collection, MD) = CFBP 1716 = ICMP 5740 (International Collection of Micro-organisms from Plants, New Zealand) = BCCM-LMG 941 (Belgian Coordinated Collections of Micro-organisms, University of Ghent, Belgium) = NCPPB 490 (National Collection of Plant Pathogenic Bacteria, UK).

Description of *X. axonopodis* pv. *spondiae*. *X. axonopodis* pv. *spondiae* ['spon.di.æ N.L. adj. *spondiae* from *Spondias*, the genus of ambarella (*Spondias cytherea* Sonn.) and mombin (*Spondias mombin* L.).

Based on AFLP, the bacterium is genetically related to members of *X. axonopodis* group 9.4 *sensu* Rademaker (34). Its genome does not contain insertion elements of the IS1595 family. Strains of pv. *spondiae* can also be distinguished from pvs. *anacardii* and *mangiferaeindicae* by isozyme analysis (47).

On ambarella, characteristic leaf lesions induced by *X. axonopodis* pv. *spondiae* start as small, angular, water-soaked spots, which extend and eventually coalesce. Lesions turn into necrotic, flat, dark brown spots, sometimes delineated by the veins. On stems, water-soaked lesions, which turn into extensive cankers, are frequently found. Orange gummy exudates oozing from twig cankers are frequently observed. Numerous twig cankers can induce a severe dieback of ambarella trees. No fruit lesions have been reported. Based on pathogenicity tests, *Spondias mombin* displayed similar symptoms, but appeared less susceptible to the pathogen. In the French West Indies, the only territory where *X. axonopodis* pv. *spondiae* has been reported to date (40), no lesions were observed on mango trees.

The proposed neopathotype strain is CFBP 2547 (Collection Française de Bactéries Phytopathogènes, INRA Angers, France), = LMG 17211 (Belgian Coordinated Collections of Microorganisms, University of Ghent, Belgium).

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