

# Strains of *Xanthomonas campestris* isolated from Ambarella (*Spondias cytherea* Sonn.) in the French West Indies are probably a new pathogenic form of *Xanthomonas campestris* pv. *mangiferaeindicae*.

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**STRAINS OF *XANTHOMONAS CAMPESTRIS* ISOLATED FROM AMBARELLA (*SPONDIAS CYTHEREA* SONN.) IN THE FRENCH WEST INDIES ARE PROBABLY A NEW PATHOGENIC FORM OF *XANTHOMONAS CAMPESTRIS* PV. *MANGIFERAINDICAE*.**

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**ABSTRACT** - Based on their pathogenicity on Mango and Cashew, apigmented *Xanthomonas campestris* strains isolated from Ambarella in the French West Indies were identified as strains close to pv. *mangiferaeindicae*. Mango isolates of that pathovar, however, are avirulent on *Spondias cytherea* Sonn. and on *Spondias mombin*. Thus, these new strains are probably a new pathogenic form of *Xanthomonas campestris* pv. *mangiferaeindicae*. The relationships of the Ambarella strains to Mango under natural conditions in the French West Indies is discussed.

**LES SOUCHES DE *XANTHOMONAS CAMPESTRIS* ISOLEES DU PRUNIER DE CYTHERE (*SPONDIAS CYTHEREA* SONN.) AUX ANTILLES SONT PROBABLEMENT UNE NOUVELLE FORME DE *XANTHOMONAS CAMPESTRIS* PV. *MANGIFERAINDICAE*.**

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**RESUME** - Le pouvoir pathogène sur Manguier et Anacardier de souches apigmentées de *Xanthomonas campestris* isolées à la Martinique à partir de lésions de Prunier de Cythère démontre leur appartenance probable au pv. *mangiferaeindicae*. Les souches de référence isolées de Manguier se sont montrées avirulentes sur *Spondias cytherea* SONN., ainsi que sur *Spondias mombin*. Les souches de Prunier de Cythère sont donc probablement une nouvelle forme de l'agent causal. Une hypothèse pouvant expliquer le fait que ces souches ne semblent pas pathogènes sur Manguier aux Antilles en conditions naturelles est formulée.

## INTRODUCTION

Since 1984, a severe dieback of *Spondias cytherea* Sonn., *Anacardiaceae*, characterized by watersoaked lesions on the leaves, and followed by twig cankers eventually inducing the death of affected trees, was observed in Martinique Island (French West Indies). ROTT and FROSSARD (12), successfully isolated a slow growing, apigmented *Xanthomonas campestris*, thought as the causal agent of the disease. Some similarities with *X. c.* pv. *mangiferaeindicae*, NCPPB 490 (neopathotype strain), based on cytological, physiological, biochemical and serological characteristics were described. However, since no lesions have neither been observed on Mango trees under natural conditions in this island, nor described from ten months old Mango trees (cv. Julie), three months after artificial

inoculation on leaves or wound-inoculation on twigs, it was first admitted, that a bacterial disease of *Spondias cytherea* Sonn. was dealt with, induced by a new *X. campestris* pathovar.

This paper will present a new information concerning the pathogenicity, of these *Spondias* strains, and will give a tentative taxonomic position.

## MATERIAL AND METHODS

### Virulence of Ambarella and Mango strains on *Spondias* spp.

Ambarella and Mango strains inoculated to Ambarella (*Spondias cytherea* Sonn.) and Mombin (*Spondias mombin*) are listed in Table 1. The cultures were grown for 24 hours on YPDA (yeast extract, 7 g; bacto-peptone, 7 g; dextrose, 7 g; agar, 15 g; distilled water, 1 000 ml; pH 7,2). Bacterial suspensions, prepared in sterile distilled water

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TABLE 1 - Characteristics of strains inoculated on *Spondias* spp.

Strain designation	Origin	Host	Color	Inoculated species	
				Ambarella	Mombin
CFBP 2547	Martinique	Ambarella	White	yes	yes
CFBP 2623		Ambarella	White	no	yes
CFBP 1716 = NCPPB 490*	India	Mango	White	yes	no
CFBP 2912 = PDDCC 4089	Brazil	Mango	White	no	yes
CFBP 2913 = PDDCC 4088		Mango	White	no	yes
CFBP 2914 = PDDCC 4087		Mango	White	no	yes
CFBP 2923 = NCPPB 3078		Mango	Yellow	no	yes
CFBP 2924 = NCPPB 3110		Mango	Yellow	no	yes
CFBP 1717 = NCPPB 2885	Reunion Is.	Mango	White	no	yes
CFBP 2920		Mango	Yellow	no	yes

\* - neopathotype strain

and adjusted to approximately  $10^8$  c.f.u./ml, were inoculated to Mombin leaves, as follows. Lamina were punctured with a sterile needle, and 10  $\mu$ l of suspension were deposited on each of ten wounds on each leaf. Each inoculation set was performed on four different plants. The leaves inoculated with sterile distilled water were used as control. The leaves were observed for symptoms, five and seven days after inoculation. Similarly the inoculations were also performed on Ambarella and Mombin twigs which were wounded with a sterile scalpel. The inoculations were made at three wounds per plant as described above, and lesions were recorded after one month. During the study, the temperature fluctuated from 20 to 40°C.

#### Virulence of Ambarella strains on Mango and Cashew.

The characteristics of the strains are given in Table 2. The leaves of Mango (*Mangifera indica* L.) and Cashew (*Anacardium occidentale* L.) trees were inoculated, using the method described above. On Mango (cv. Amélie), bacterial suspensions adjusted to  $10^8$  and  $10^6$  c.f.u./ml were used. Twenty inoculations were made on each leaf on four different plants. As cashew is less susceptible to bacterial black spot disease (PRUVOST, unpublished data), the inoculations were made only with  $10^8$  c.f.u./ml. Twenty inoculations were made on each leaf on three different plants. On both plant species, the inoculations performed with sterile distilled water were used as control.

The percentages of induced lesions and lesions size were estimated five weeks after the inoculations were done.

#### Statistical analysis.

The results were interpreted by Variance analyses. The Duncan's multiple range tests were used in order to compare the means. The Kramer's test was used also when there was a significant interaction between the factors.

## RESULTS

#### Virulence of Ambarella and Mango strains on *Spondias* spp.

*Spondias cytherea* plants inoculated with the homologous strain (CFBP 2547) died after one month. But no lesions occurred on plants inoculated with the Mango strain CFBP 1716 (neopathotype strain).

Watersoaked areas occurred after five days around inoculations performed with CFBP 2547 and 2623 (Ambarella strains) on *Spondias mombin* leaves. The lesion development was associated with defoliation of all inoculated leaves after seven days. No lesions could be identified

TABLE 2 - Characteristics of strains inoculated on Mango and Cashew.

Strain designation	Origin	Host	Color
CFBP 2547	Martinique	Ambarella	White
CFBP 2623		Ambarella	White
CFBP 1716 = NCPPB 490*	India	Mango	White
CFBP 2914 = PDDCC 4087	Brazil	Mango	White
CFBP 2923 = NCPPB 3078		Mango	Yellow
CFBP 2934	S. Africa	Mango	White
CFBP 2922 = PDDCC 6657	Australia	Mango	White
CFBP 1717 = NCPPB 2885	Reunion Is.	Mango	White
CFBP 2920	"	Mango	Yellow

\* - neopathotype strain

on leaves inoculated with the Mango strains, even after one month. On twig-inoculated plants, only CFBP 2547 and 2623 induced extensive lesions. The average canker lengths were 83.3 and 76.7 mm respectively after 1 month (values not statistically different at  $p = 0.05$ ). The Mango strains did not induce lesions on Mombin twigs.

#### Virulence of Ambarella strains on Mango and Cashew.

All the strains were pathogenic on Mango. The lesions size was 3 to 4 mm after 5 weeks for all strains and for both bacterial concentrations. Therefore, it did not appear to be an interesting estimator of strain aggressiveness. The percentages of induced lesions fluctuated from 13.8 to 100% following inoculation with  $10^8$  c.f.u./ml inoculum and from 3.8 to 56.3% following inoculation with  $10^6$  c.f.u./ml inoculum (Table 3).

On cashew, the lesions size was 2 to 3 mm. For the same reason as explained above, the lesion size was not used as an aggressiveness estimator. The percentages of induced lesions fluctuated from 0 to 83% (Table 3).

According to the Duncan's multiple range and Kramer's tests results, these strains were classified in 3 aggressiveness classes (Table 3) :

- A) high aggressiveness
- B) intermediate aggressiveness
- C) low aggressiveness.

The lesions induced on Mango and Cashew leaves with the Ambarella isolates were morphologically indistinguishable from those induced by the Mango strains. Furthermore, very slow growing, apigmented colonies that had exactly the same morphology as Ambarella strains were re-isolated from induced lesions on Mango and Cashew inoculated with CFBP 2547 and 2623.

A very low aggressiveness of pigmented *X. c. pv. mangiferaeindicae* strains from Brazil (11) and Reunion Island (10) on Mango and Cashew has been demonstrated. This is presented in Table 3.

## DISCUSSION

Ambarella strains are highly aggressive to *Spondias cythera*. These strains are also pathogenic to *Spondias mombin*, but they do not seem able to induce plant death. The latter *Spondias* species must be regarded as less susceptible to the pathogen, and the *X. c. pv. mangiferaeindicae* strains are avirulent on these plant species. This is in complete agreement with ROTT and FROSSARD results (12). The avirulence of Mango strains of *X. c. pv. mangiferaeindicae* must however, be checked with a larger number of strains.

The Ambarella strains are also pathogenic to Mango and Cashew, the two primary hosts for *X. c. pv. mangiferaeindicae* (8, 9). On Mango (cv. Amélie), they have an intermediate aggressiveness (class B), as most of other Mango apigmented isolates. On Cashew, they are highly aggressive, giving the higher rates of symptom expression. These results differ strongly from those previously described (12). This can be probably explained by the differences in the inoculation techniques : PAO-JEN (7) reported the high efficiency of wounding leaves to induce some lesions and MANICOM (5) noted the absence of lesion after leaves were sprayed. Similarly, twigs from ten months old mango trees are a very poor material to successfully induce typical lesions, which appears mainly on very tender shoots (10).

We conclude that these Ambarella strains are probably a new pathogenic form of *Xanthomonas campestris* pv. *mangiferaeindicae* with host specialization on *Spondias* spp. However, in order to resolve the taxonomic position of the Ambarella strains and the yellow pigmented strains (10, 11), we think that further work using several different techniques and including DNA: DNA hybridizations (3, 6, 13) polyacrylamide gel electrophoresis (PAGE) of cellular proteins (3, 13) and RFLP analyses on genomic DNA (1, 2, 4) has to be done.

It is not clear why these Ambarella strains are not pathogenic to Mango under natural conditions. This leads to at least two hypothesis: Mango trees grown in Martinique

TABLE 3 - Development of lesion induced by Ambarella and Mango strains on Mango and Cashew (expressed in percent) five weeks after inoculation.

Strain designation	MANGO			CASHEW	
	Percentages of induced lesions		Virulence class	Percentages of induced lesions	Virulence class
	$10^8$ c.f.u./ml	$10^6$ c.f.u./ml			
CFBP 2547	56.3	13.8	B	73.3	A
CFBP 2623	66.3	20.0	B	83.0	A
CFBP 1716*	77.5	50.0	B	61.4	A
CFBP 2914	67.5	30.0	B	43.8	B
CFBP 2923	30.0	10.0	C	4.0	C
CGBP 2934	100.0	56.3	A	43.0	B
CFBP 2922	68.8	25.0	B	16.8	C
CFBP 1717	40.0	23.8	B	47.0	B
CFBP 2920	13.8	3.8	C	0.0	C

\* - neopathotype strain

are highly resistant to *X. c. pv. mangiferaeindicae*, although most cultivars grown in that area are known to be susceptible to bacterial black spot (e.g. Irwin, Eldon, Zill, Haden, Amélie). Alternatively, the biological properties of these strains do not allow the colonization of Mango aerial parts. Epiphytic residence of *X. c. pv. mangiferaeindicae* on strains does not allow them to colonize Mango aerial parts. Epiphytic residence of *X. c. pv. mangiferaeindicae* on Mango leaves has been previously described (5). The epiphytic stage is an important key in the life cycle of *X. c. pv. mangiferaeindicae* and there is a significant correlation between the epiphytic inoculum level and the incidence of the disease (10). The inability of these strains to multiply as epiphytes on Mango must be determined; however, this could explain why the Ambarella strains are not able to induce an epidemic on Mango trees in the French West Indies. Further work is being done to study this phenomenon.

Finally, we were not able to confirm the virulence of *X. c. pv. mangiferaeindicae* on Mombin, as reported in Brazil by ROBBS *et al.* (11), even with strains from that country. These authors did not produce the references of their strains. As only strains from Ambarella were pathogenic to Mombin, this new pathogenic form of *X. c. pv. mangiferaeindicae* might be also present in Brazil, but this has to be confirmed. The virulence of a large collection of *X. c. pv. mangiferaeindicae* strains on Mombin is presently under investigation.

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DIE ISOLIERTEN *XANTHOMONAS CAMPESTRIS*-STÄMME DES CYTHEREA-PRUNUS (*SPONDIA CYTHEREA* SONN.) AUF DEN ANTILLEN SIND WAHRSCHEINLICH EINE NEUE FORM VON *XANTHOMONAS CAMPESTRIS* PV. *MANGIFERAINDICAE*.

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KURZFASSUNG - Das krankheitserregende Potential apigmentierter *Xanthomonas campestris* Stämme, die auf Martinique aus Läsionen des Cytherea-Prunus isoliert worden sind, gegenüber dem Mango- und dem Kaschubaum ist ein Hinweis dafür, dass diese Stämme wahrscheinlich dem pv. *mangiferaeindicae* angehören. Die vom Mangobaum isolierten Referenzstämme erwiesen sich gegenüber *Spondias cytherea* SONN. und *Spondias mombin* als avirulent. Die Cytherea-Prunus-Stämme sind daher aller Wahrscheinlichkeit nach eine neue Form des Verursachers. Diese Annahme erklärt u.a. die Tatsache, dass diese Stämme auf den Antillen unter naturgegebenen Voraussetzungen auf den Mangobaum nicht pathogen wirken.

LAS POBLACIONES DE *XANTHOMONAS CAMPESTRIS* AISLADAS DEL JOBO DE LA INDIA (*SPONDIA CYTHEREA* SONN.) EN LAS ANTILLAS SON PROBABLEMENTE UNA NUEVA FORMA DE *XANTHOMONAS CAMPESTRIS* PV. *MANGIFERAINDICAE*.

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RESUMEN - El poder patógeno sobre Mango y Anacardo de poblaciones apigmentadas de *Xanthomonas campestris* aisladas en Martinica a partir del Jobo de la India muestra su pertenencia probable al pv. *mangiferaeindicae*. Las poblaciones de referencia aisladas de Mango se han mostrado avirulentas sobre *Spondias cytherea* SONN. así como sobre *Spondias mombin*. Las poblaciones de Jobo de la India son, por consiguiente, probablemente una nueva forma del agente causal. Una hipótesis que puede explicar el hecho de que estas poblaciones no parecen patógenas sobre Mango en las Antillas en condiciones naturales se formula en este trabajo.