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Epidemiological evidence for the emergence of a new pathogenic variant of *Ralstonia solanacearum* in Martinique (French West Indies)

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The emergence of a new genotype and pathogenic variant of *Ralstonia solanacearum* in Martinique is described. Bacterial wilt of solanaceous crops caused by phylotype-I and -II strains ('historical strains'), was reported in Martinique in the 1960s. From 1999, *Anthurium* and cucurbit production was strongly affected by strains described as a new pathogenic variant genotyped phylotype IIB/sequevar4NPB (phIIB/4NPB). The following questions concerning these strains were investigated: (i) were they introduced or endemic, (ii) was their distribution widespread in Martinique, and (iii) which factors could explain this emergence? This study examined 221 isolates collected from 1989 to 2003 after several surveys. The main survey (2002–03) included 115 vegetable and ornamental crop farms. From 1999 to 2001, these phIIB/4NPB strains were initially described as the '*Anthurium*-cucurbit' strain. In 2003, they made up one-third of the isolates recovered from solanaceous hosts, particularly tomato. This pathogenic variant of *R. solanacearum* was consistently recovered from wild species and several weeds throughout Martinique, suggesting that these strains were well established in Martinique. Data reported are consistent with the emergence of a new population of *R. solanacearum* in Martinique, which has spread rapidly across the entire island and may overtake the previously established population, particularly on tomatoes. Evidence is presented which suggests that the emergence of these new strains is more frequent on vegetable crops when cucurbitaceous and musaceous plants are grown in succession.

Keywords: Anthurium, bacterial wilt, crop successions, cucurbitaceous crops, *Heliconia*, solanaceous crops

Introduction

Ralstonia solanacearum, the causal agent of bacterial wilt disease, is widespread in tropical and subtropical regions. Its harmfulness, wide host range, persistence and huge genome plasticity have made it one of the world's most important phytopathogenic bacteria and one of the most intensively studied (Denny, 2006). This bacterial species is composed of four monophyletic groups of strains, named phylotypes (Fegan & Prior, 2005), which are correlated with the strains' geographical origins: phylotype I includes strains originating primarily from Asia, phylotype II those from America, phylotype III those from Africa and the Mascarene Islands and phylotype IV those from Indonesia (Fegan & Prior, 2005; Prior & Fegan, 2005b). Strains of phylotypes I and IIA (ex-biovar 3 and biovar 1, respectively) have been known to cause the disease on solanaceous crops in Martinique (French West Indies) since the 1960s (Digat & Escudié, 1967; Prior & Steva, 1990; Fegan & Prior, 2005). From 1999, new bacterial wilt outbreaks were surveyed on *Anthurium* and cucurbitaceous crops. The application of multiplex PCRs (Fegan & Prior, 2005; Prior & Fegan, 2005a) and DNA sequence analysis to characterize the populations of *R. solanacearum* in Martinique revealed that these outbreaks were caused by a previously unidentified group of strains in Martinique, genotyped as phylotype IIB/sequevar 4NPB (phIIB/4NPB) (NPB = non-pathogenic on banana). A previous study (Wicker *et al*., 2007) examined the genetic diversity and pathogenicity of these strains, and found that this pathogenic variant had a wider host range than the previously established 'historical' population of *R. solanacearum* strains genotyped phylotypes I and IIA. In addition, it was more aggressive on Solanaceae and unable to cause Moko disease on Cavendish banana, although it had the ability to invade the vascular tissue of plantain (cooking banana, AAB types). This unique genotype and phenotype, primarily reported from

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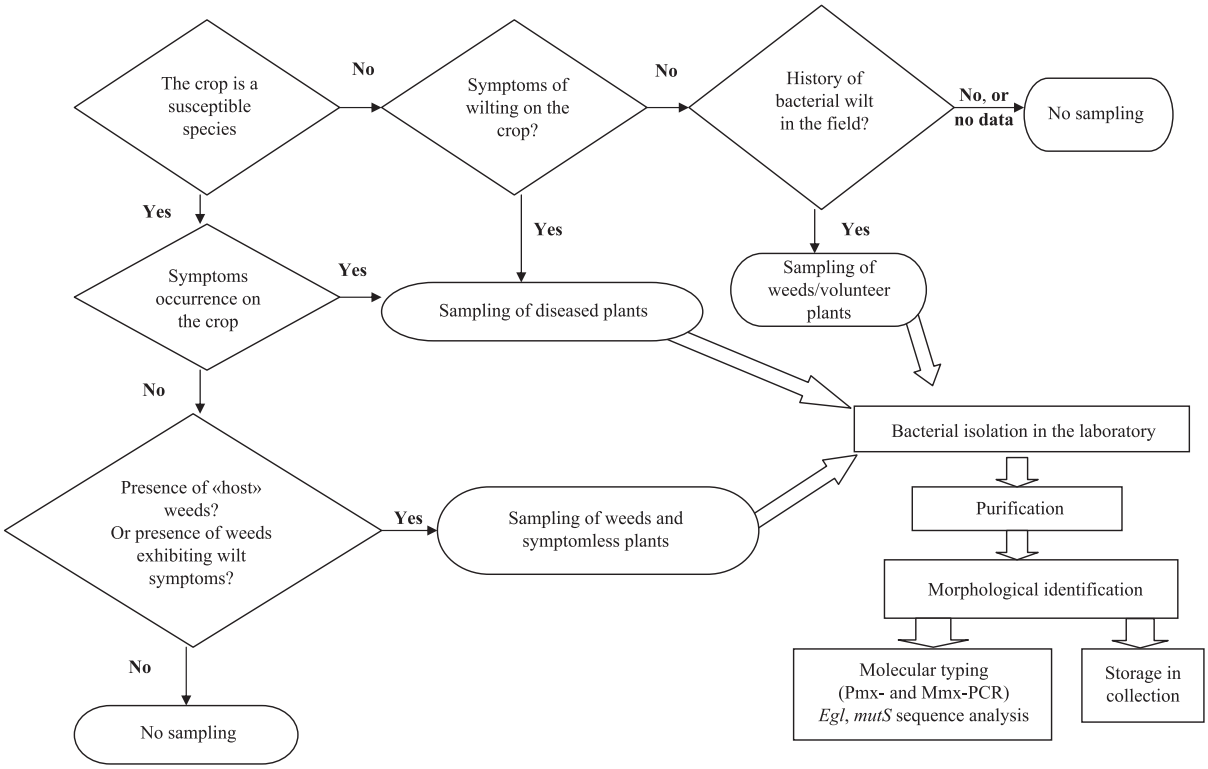

Figure 1 Logical diagram of the decision rules for sampling *Ralstonia solanacearum*-infected plants in the field in Martinique.

Martinique (Prior & Fegan, 2005a), was also identified in collections in Trinidad and Brazil (Wicker *et al*., 2007). However, it has only recently caused significant damage and outbreaks on new hosts and crops in Martinique (Wicker *et al*., 2002, 2005), suggesting that Martinique was experiencing the emergence of a disease.

Emerging diseases or infections were defined by Morse (1995) as diseases that appear in the population for the first time or that existed previously but rapidly increase in incidence or geographic range. Using the data obtained from the surveys conducted to collect the *R. solanacearum* strains in Martinique, the present study examined evolution over recent years of their prevalence, geographical distribution and host range (defined as the diversity of both cultivated and wild hosts from which these new strains were isolated). Efforts were made to identify factors explaining or favouring the emergence of these new strains.

Materials and methods

Bacterial strains and surveys

This research was based on a collection of 221 *R. solanacearum* isolates from Martinique, obtained from plants and genotyped according to Wicker *et al.* (2007), and 531 isolates isolated from waterways during the year 2005.

From 1989 to early 2002, the isolates collected originated from diseased plants during (i) routine bacterial analyses by the Plant Protection Service and (ii) regular surveys throughout the island. Specific surveys focusing on the main production zones of *Anthurium*, the upper and humid central areas of the island, were conducted in 2000 (232 plant samples) and 2001 (432 plant samples).

Bacterial isolates collected in 2002–03 were sampled from 115 farms distributed within the four soil and climatic areas of Martinique and included flower crops (*Anthurium* and *Heliconia*), vegetable crops (Solanaceae and Cucurbitaceae) and weeds. Given that Martinique has highly contrasting climatic zones and soil types (Colmet-Daage, 1989), four soil and climatic areas were distinguished (Louvrier, 1998): (i) north-windward (north-eastern part of Martinique, including the eastern slopes of the volcano Montagne Pelée); (ii) north-leeward (north-western part and western slopes of Montagne Pelée); (iii) centre; and (iv) south. Plant sampling was guided by the sampling scheme explained in Fig. 1. Initially, growers were asked about their farm, the different crops grown and the crops they cultivated previously. A visual survey of crops and weeds was then conducted to determine the presence of typical symptoms of bacterial wilt. Farmers were then asked whether bacterial wilt 'hot spots' had occurred previously. Weeds were sampled from fields with a history of bacterial wilt. As explained in Fig. 1, weeds were sampled and considered for bacterial isolation if (i) they were prevalent in a field with a history of bacterial wilt, and/or (ii) they showed wilting symptoms, and/or (iii) they were already known as 'hosts', according to

Hayward (1994), Janse *et al.* (2005) and Tusiime *et al.* (1998). Finally, all weeds were formally identified according to Fournet & Hammerton (1991).

Isolation and identification of bacterial isolates

Diseased plants (leaf, stem and rhizome) and weeds not showing symptoms (stems and roots) were sampled and cultured to isolate *R. solanacearum*. For the isolations, the plant tissues were first alcohol-sterilized and flamed. Then, a portion of plant tissue was transferred to a sterile Petri dish and macerated in Tris buffer (pH 7·2). An aliquot from this macerate was streaked on semi-selective media and typical *R. solanacearum* colonies were transferred to fresh medium and further purified, as previously described Wicker *et al.* (2007). Hence, each bacterial isolate collected was of endophytic origin, even on the weed samples. Multiplex PCRs were used to identify pure cell cultures of *R. solanacearum* (Fegan & Prior, 2005; Prior & Fegan, 2005a). 'Phylotype'-multiplex PCR (Pmx-PCR) was used to assess the phylotype to which they belonged; '*Musa*'-multiplex PCR (Mmx-PCR) was applied to phylotype II strains (Prior & Fegan, 2005a; Wicker *et al.*, 2007) to determine whether they belonged to the '*Musa*' group (sequevars 3, 4 and 6). PCR reactions, electrophoresis conditions and reagent origins were as described previously (Wicker *et al.*, 2007). Endoglucanase (*egl*) and *mutS* sequence analyses were used to determine the sequevar of each strain (Wicker *et al.*, 2007). The 'historical' phylotype-II strains belonged to subcluster A of phylotype II (sequevar 5) and were thus designated phylotype IIA (phIIA), whereas the new emerging strains belonged to subcluster B and sequevar 4 (phIIB/4NPB).

Detection and monitoring of *R. solanacearum* populations in the waterways

To investigate the occurrence of inoculum transfers from the north to the south of the island via water, bacterial populations transiting from the Lézarde river (centre) to the Manzo lake and at the exit of Manzo lake were monitored. Crops in south-eastern Martinique are indeed irrigated by water stored in Manzo lake. The lake is largely fed (80%) by the Lézarde river's water catchment area. Water samples (two lots of 1 L) were taken once a month from January to December 2005 (i) in the Lézarde river water catchment area, 'Deux-terres' (2T in Fig. 2) and (ii) at the exit of Manzo lake (ML in Fig. 2). Each sample was first vacuum-filtered in a NALGENE filtration device with a 0·2-μm Whatman filter in order to make the bacterial suspension more concentrated. The filter was aseptically cut into pieces, transferred to a tube containing 4 mL Tris buffer (Tris 0·01 M, pH 7·2) and the tube was vortexed for 1 min. Aliquots (100 μL) from this suspension were transferred to a semi-selective medium SMSA in triplicate. Petri dishes were incubated at 28°C for 2–3 days and presumptive *R. solanacearum* colonies were removed and purified on fresh SMSA medium. Each *R. solanacearum*-like colony was then identified by Pmx-PCR and Mmx-PCR.

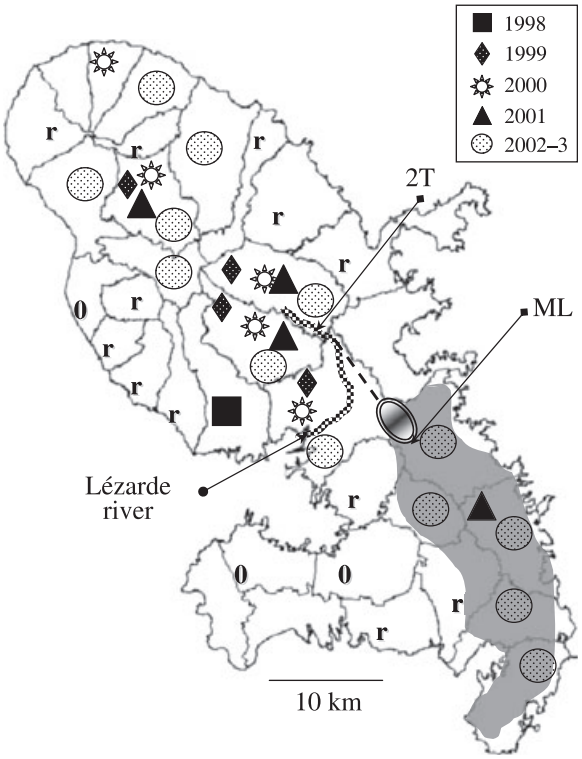

Figure 2 Map of prevalence of phylotype-IIB/4NPB strains of *Ralstonia solanacearum* within the 34 communes (smallest political unit) of Martinique, in 1998, 1999, 2000, 2001 and 2002–03. Symbols indicate the isolation of at least one strain in each commune; **r** indicates sampled commune where no phIIB/4NPB strain was recovered; **0** indicates sampled commune where no *R. solanacearum* strain was isolated. 2T, Deux-terres water sampling site (Lézarde river catchment); ML, Manzo lake water sampling site. The areas irrigated by Manzo lake waters are marked in grey.

Distribution of bacterial strains across the island

The frequencies of each phylotype were reported according to soil and climatic areas, by dividing the numbers of isolates of one phylotype by the total number of strains sampled in each area. The associations between strain genotype and area of origin, period of isolation, host of isolation and preceding crop were assessed using χ^2 goodness-of-fit tests (R software, the R Foundation for Statistical Computing).

Results

Evolution of distribution and host range of phylotype-IIB/4NPB strains over time

To assess whether Martinique was experiencing a disease-emergence, the evolution of the occurrence of phIIB/4NPB

Table 1 Comparison of host isolation frequencies of *Ralstonia solanacearum* in Martinique over the three periods of isolation of the study (before 1999; 1999 to early 2002; 2002–03 survey), between the historical population (phylotype I and phylotype IIA) and the emerging population (phylotype IIB/sequevar4NPB)

Period	Total no. of isolates	No. (%) of isolates from				
		Solanaceae	*Anthurium*	Cucurbitaceae	*Heliconia*	Weeds
Historical population						
Before 1999	19	18 (94·8)[a]	0·0 (0)	0·0 (0)	0·0 (0)	1 (5·3)
1999 to 2002	12	8 (66·7)	1 (8·3)	0	0	3 (25·0)
2002–03	74	47 (63·5)	18 (24·3)	1 (1·3)	0	8 (10·8)
χ^2 0.3298[NSB] overall = 2·998, *P*-value = **0.3298**[Ns][b]						
Emerging population						
Before 1999	1	1 (100)[a]	0 (0)	0 (0)	0 (0)	0 (0)
1999 to 2002	53	1 (1·9)	38 (71·7)	7 (13·2)	1 (1·9)	6 (11·4)
2002–03	62	19 (30·6)	26 (41·9)	13 (21·0)	3 (4·8)	1 (1·6)
χ^{2c}		16·2***	2·25[NS]	1·8[NS]	1·0[NS]	6·4*
χ^2 overall = 27·416, *P*-value = 0·001***[d]						

[a]Numbers in parenthesis are percentages of total number per period (row). Before 1999, mean total analysed samples per year were about 70 for Solanaceae, 53 for Cucurbitaceae, 92 for *Anthurium*, 30 for weeds and 22 for *Heliconia*. From 1999 to 2002, the mean yearly sample numbers were: 211 for Solanaceae, 70 for Cucurbitaceae, 219 for *Anthurium*, 19 for *Heliconia* and 169 for weeds.

[b]Chi-squared test performed on the data corresponding to the 1999–2002 and 2002–03 periods (three columns, two rows). Cucurbitaceae and *Heliconia* columns were not considered.

[c]Chi-squared test performed on the distributions within hosts of origin for the 1999–2002 and 2002–03 periods. *** = very highly significant ($P < 0·001$), ** = highly significant ($P < 0·01$), * = significant ($P < 0·05$), NS = not significant.

[d]χ^2 test performed on the data (five columns, two rows) for the 1999–2002 and 2002–03 periods.

strains within each commune (smallest territorial unit) of Martinique was first monitored from 1998 to 2003 (Fig. 2). Only four out of 34 communes were not sampled. The very first phIIB/4NPB strain was isolated from wilted tomato plants in Fort-de-France in 1998. From 1999, similar strains were consistently isolated on wilted anthuriums grown in the central communes of St Joseph, Gros Morne and Le Lamentin, as well as in two northern communes (Morne Rouge and Macouba). In 2001, the first outbreak on cantaloupe (*Cucumis melo*) was observed in the south-east (Le Vauclin). Finally, the 2002–03 survey revealed the presence of phIIB/4NPB strains throughout the south and south-east of Martinique, in the centre and in the north (leeward and windward sides of the volcano Montagne Pelée).

Secondly, comparisons were made of the hosts of origin of the new strains (phylotype IIB/4NPB) and of historical strains (phylotype I and phylotype IIA) over three periods of isolation: (i) before 1999, (ii) between 1999 and early 2002, and (iii) in the 2002–03 survey. Host distributions were statistically compared to their expected values by Chi-squared tests (Table 1) in order to take into account the differences in sample size between isolation periods. Interestingly, the distributions of historical strains were not significantly different from their expected values, suggesting that no significant host-range shifting occurred over the years (χ^2 test, $P = 0·329$). However, distributions of the emerging strains significantly differed from their expected values (χ^2 test, $P < 0·001$), indicating a clear host-range shift. Indeed, isolation frequencies of emerging strains on Solanaceae and periods of isolation were significantly associated (χ^2 test, $P < 0·001$), suggesting that these strains expanded their host range from *Anthurium*-Cucurbitaceae (1999–2002) to *Anthurium*-Solanaceae-Cucurbitaceae (2002–03). Focusing on the strains isolated from the Solanaceae, it was notable that pepper and aubergine strains were all historical, whereas tomato strains included both historical and emerging strains, with a majority of phIIB/4NPB strains (Fig. 3). The association between solanaceous species of isolation and phylotype composition was highly significant (χ^2 test, $P < 0·001$).

Prevalence on wild species and weeds

To assess the establishment of these emerging strains in the Martinique environment, their prevalence was investigated on weeds and wild plant species. A total of 466 samples covering 82 plant species were considered. *Ralstonia solanacearum* was successfully isolated from 18 species (Table 2), mainly originating from the centre and north-windward areas, whereas 64 species gave no successful isolation. Ten out of these 18 species were infected by phIIB/4NPB strains. Within the three *Heliconia* species sampled, phIIB/4NPB strains infected only *H. caribea*. Within the six Araceae sampled, phIIB4NPB strains were isolated only from *Xanthosoma* sp. Within Solanaceae, only *Solanum americanum* was consistently infected by *R. solanacearum* strains.

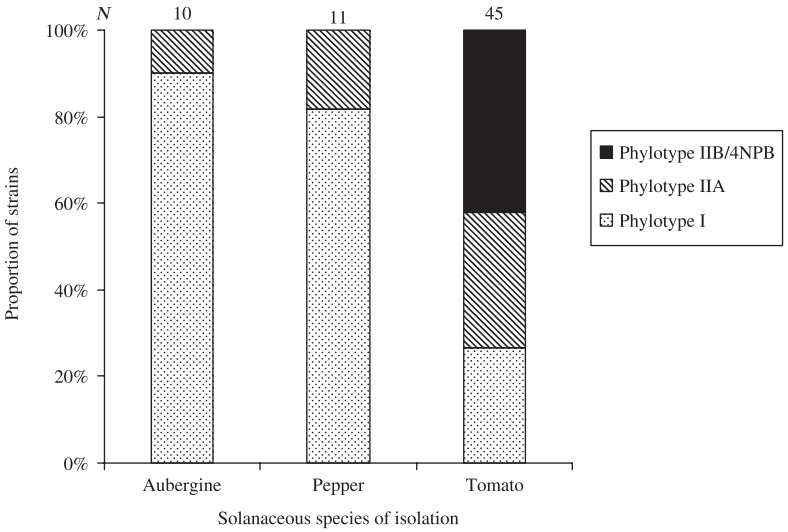

Figure 3 Phylotype distribution within the *Ralstonia solanacearum* isolates recovered from Solanaceae in Martinique during the 2002–03 survey ($N = 66$).

Table 2 Wild species and weeds from which *Ralstonia solanacearum* isolates were successfully recovered in Martinique

Family	Species	Samples		*R. solanacearum*
		Total	Positive[a,b]	Phylotype/sequevar
Araceae	*Xanthosoma* sp.	11	2	IIB/4NPB
Asteraceae	*Ageratum conyzoides*	6	2	ND
Balsaminaceae	*Impatiens balsamina*	19	3	IIB/4NPB
	Impatiens hawkerii	7	3	IIB/4NPB
Capparidaceae	*Cleome viscosa*	19	1	IIB/4NPB
Commelinaceae	*Commelina diffusa*	13	1	I
	Rhoeo sp.	59	1	ND
Euphorbiaceae	*Peperomia pellucida*	7	1	IIB/4NPB
Heliconiacaeae	*Heliconia caribea*	63	4	IIB/4NPB
Liliaceae	*Lilianthus* sp.	1	1	I
Pandanaceae	*Pandanus* sp.	1	1	IIB/4NPB
Piperaceae	*Piper dilatatum*	1	1	IIB/4NPB
Poaceae	*Poa* sp.	1	1	I
Portulacaceae	*Portulaca oleracea*	6	1	IIB/4NPB
Solanaceae	*Solanum americanum*	7	5	I, IIA, IIB/4NPB
	Solanum torvum	7	1	I
Urticaceae	*Laportea* sp.	7	1	IIA
	Urtica dioica	12	1	IIA
Total no.		247	31	

[a]Positive: number of samples from which *Ralstonia solanacearum* was recovered (2000, 2001, 2002–03 surveys).
[b]Two hundred and nineteen plant samples of 64 species gave no positive isolation of *R. solanacearum*, within 32 botanical families: Amaranthaceae: *Achyranthes aspera* (1), *Amaranthus* sp. (9), *Amaranthus viridis* (2); Amaryllidaceae: *Eucharis* sp. (5); Araceae: *Colocasia* sp. (10), *Dieffenbachia seguine* (1), *Scindapsus aureus* (3), *Xanthosoma helleborifolium* (4), *X. sagittifolium* (1); Asteraceae: *Aster* sp. (1), *Emilia* sp. (8), *Erechtites hieracifolia* (2), *Eupatorium* sp. (2), *Felicia* sp. (6), *Galingosa parviflora* (1), *Mikania micrantha* (2), *Vernonia cinerea* (1), *V. parviflora* (1); Begoniaceae: *Begonia* sp. (1); Boraginaceae: *Heliotropium* sp. (1); Caesalpiniaceae: *Senna obtusifolia* (1); Capparidaceae: *Cleome aculeata* (1); Commelinaceae: *Commelina benghalensis* (4), *Commelina* sp. (1); Convolvulaceae: *Ipomea batatas* (1), *Ipomea* sp. (7), *Merremia aegyptia* (1); Cucurbitaceae: *Momordica charantia* (7), *Momordica* sp. (2); Cyperaceae: *Cyperus rotundus* (1), *Cyperus* sp. (2); Euphorbiaceae: *Acalypha* sp. (5), *Euphorbia heterophylla* (12), *Euphorbia* sp. (3), *Phyllanthus amarus* (9), *P. debilis* (1); Fabaceae: *Cajanus cajan* (1), *Glyceridia* sp. (1), unknown species (4); Heliconiaceae: *Heliconia canthasia* (1), *Heliconia* sp. (6); Lauraceae: *Cinnamomum* sp. (4); *Malvaceae*: *Sida acuta* (1), *Urena* sp. (6); Melastomataceae: *Clidemia* sp. (2); Mimosaceae: *Mimosa* sp. (2); Musaceae: *Musa coccinea* (1); Onagraceae: *Ludwigia* sp. (3); Oxalidaceae: *Oxalis* sp. (2); Piperaceae: *Piper* sp. (3); Poaceae: *Brachiaria* sp. (1), *Glyceria* sp. (1), unknown species (22); Pteridophyta: unknown species (11); Rubiaceae: *Rubia* sp. (10); Simaroubaceae: unknown species (2); Solanaceae: *Physallis angulata* (1); Verbenaceae: *Citharexylum* sp. (4), *Stachytapheta* sp. (3), *Vernonia* sp. (2); Vitaceae: *Cissus rhombifolia* (1); Zingiberaceae: *Alpinia purpurata* (1), *Zingiber officinale* (2); Zygophyllaceae: *Zygophyllum* sp. (1).

Table 3 Phylotype distribution of *Ralstonia solanacearum* strains in water samples recovered from the Lézarde river, in the centre of Martinique, and Manzo lake, the source of irrigation for crops in the south-east of the country

	No. (%) of *Ralstonia solanacearum* isolates			
		Phylotype II		
Sampling locations	Phylotype I	A/non-*Musa*	B/4NPB	Total no. of isolates
Deux-terres [2T] (Lézarde catchment)	1	29	4	34
Manzo lake [ML] (lake exit)	3	489	5	497

Table 4 Phylotype distribution of *Ralstonia solanacearum* isolates from all hosts and recovered from tomato only within the four soil and climatic areas of Martinique, according to the 2002–03 survey

	No. (%) of isolates of *Ralstonia solanacearum*			
		Phylotype II		
Climatic and soil areas	Phylotype I	A/non-*Musa*[b]	B/4NPB	Total no. of isolates
Isolates from all hosts				
North-windward	30 (38·0)[a]	17 (21·5)	32 (40·5)	79
North-leeward	1 (6·3)	6 (37·5)	9 (56·3)	16
Centre	6 (25·0)	7 (29·2)	11 (45·8)	24
South	3 (15·0)	5 (25·0)	12 (60·0)	20
χ^2 test: *P*-value = 0.131[NS c]				
Isolates from tomato				
North-windward	6 (33·3)	1 (5·6)	11 (61·1)	18
North-leeward	1 (14·3)	5 (71·4)	1 (14·3)	7
Centre	3 (50·0)	2 (33·3)	1 (16·7)	6
South	2 (28·6)	4 (57·1)	1 (14·3)	7
χ^2 test: *P*-value = 0·016*[d]				

[a]Numbers in parenthesis are percentages of total number per area (row).
[b]The non-*Musa* gave a negative result with Mmx-PCR (Prior & Fegan, 2005a), and thus did not belong to sequevars 3, 4 or 6; analysis of the endoglucanase (*egl*) partial gene sequence revealed that they belonged to subcluster A ('broad host range') of phylotype II (Wicker *et al.*, 2007).
[c]χ^2 test performed for all isolates (four rows, three columns); NS = not significant, * = significant ($P < 0{\cdot}05$).
[d]χ^2 test performed for tomato isolates (four rows, three columns).

Prevalence in the waterways irrigating south-eastern Martinique

Both water sampling and bacterial monitoring were performed for 12 months in 2005. Presumptive *Ralstonia* sp. colonies were successfully identified as *R. solanacearum* by Pmx-PCR (Fegan & Prior, 2005). *Ralstonia solanacearum* isolates were recovered from water on both sites at population levels ranging from 40 to 8000 CFU L^{-1} in the March, June, October and November samples, and in the January sample for the Manzo site only. Pmx- and Mmx-PCR results revealed that these water populations ($N = 34$ and 497 at the Deux-terres and Manzo sites, respectively) were composed of a majority of phIIA strains, with some phIIB/4NPB strains and phI strains (Table 3).

Prevalence within regions

The prevalence of the emerging strains was investigated within the four soil and climatic areas of Martinique (Table 4). During the 2002–03 survey, 363 samples were collected from 115 farms, mainly located in the north-windward and north-leeward areas (40·9% and 29·5%, respectively), as well as in the central and southern areas (14·8% in each). The distribution of farms and total samples were not significantly different from expected values; thus, the farms and total samples could be considered evenly distributed within the regions. However, distribution of positive samples (i.e. samples from which *R. solanacearum* was successfully isolated) within each region was significantly different from expected values (χ^2 test, $P = 0{\cdot}001$): north-windward and north-leeward areas were over- and under-represented, respectively, compared to the other regions.

All phylotypes were found within the four areas (Table 4) and no statistical association was detected between phylotype distribution and region of origin (χ^2 test, $P = 0{\cdot}131$). Thus, phylotype distribution did not seem to have any geographical specificity.

However, phylotype distribution within tomato isolates seemed to be area-specific. In the north-windward area, phIIB/4NPB and phylotype-IIA frequencies were, respectively, significantly higher and lower than expected

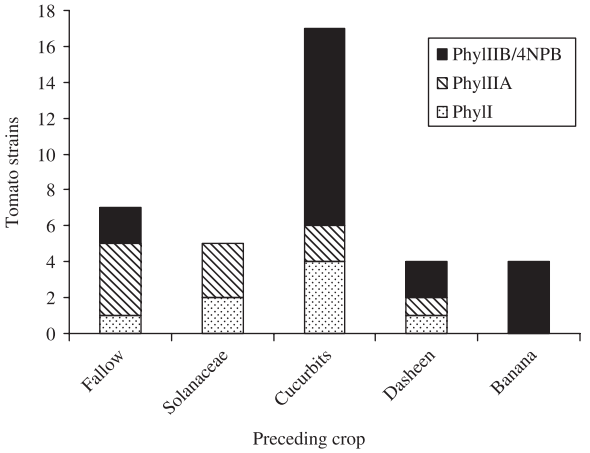

Figure 4 Phylotypes of *Ralstonia solanacearum* isolates recovered from tomato in Martinique, in relation to the preceding crops on the tomato fields from which they were sampled.

(χ^2 test, $P = 0{\cdot}034$). Interestingly, north-windward is the only region in Martinique where banana/vegetable crop rotations are currently practiced.

Effect of preceding crop

To assess the effect of the preceding crop on the occurrence of a particular phylotype in a field, a subsample of surveyed fields was considered where *R. solanacearum* had been recovered from tomato only, because tomato was wilted by all phylotypes. Forty-seven fields were considered initially. However, preceding crops of carrot and bean were under-represented (two and one field, respectively), and the preceding crop was unknown in seven fields. Finally, 37 fields were retained for analysis (Fig. 4). The preceding crop had a significant influence on the phylotype composition of the populations collected on tomato (χ^2 test, $P = 0{\cdot}006$). After a solanaceous crop, only phI and phIIA strains were isolated. When the previous crop was banana, only phII/4NPB strains were isolated from tomato. When fields were left fallow or cropped with dasheen or cucurbits, phIIA, phI and phIIB/4NPB were isolated.

Discussion

The epidemiological data presented here demonstrate that phIIB/4NPB strains constitute an emerging population in Martinique. One major feature of this emergence is the acquisition of new hosts. Up to 2002, these strains were locally considered as '*Anthurium*–cucurbit' strains, since they only caused considerable damage and economic losses on *Anthurium* and cantaloupe farms. In 2002–03, phIIB/4NPB strains represented almost 30% of the *R. solanacearum* isolates pathogenic to solanaceous hosts, indicating a massive spread to tomato crops. Since this new variant was previously described as much more aggressive than the 'historical' strains (Wicker *et al.*, 2007), it may overtake the historical strains on tomato crops.

The second major feature of this emergence was the rapid spread over the island. Emerging strains were first reported in humid north-central areas near the rainforest and progressed over a few years down to the south-east of the island (Fig. 2). Southern vegetable growers have their own nurseries, so infected plant material is not considered to have caused such a rapid dissemination from the north to the south; water transfers seem to be the primary suspect. The south-eastern crops of Martinique are irrigated by a water supply network fed by Manzo lake, whose water comes from the Lézarde river (Fig. 2). The Lézarde river basin covers areas of farmland which are heavily infested with the phIIB/4NPB strains. Moreover, viable and cultivable cells of *R. solanacearum*, including phIIB/4NPB strains, were consistently isolated from water in this waterway. Therefore, it is very likely that phIIB/4NPB strains followed the course of the Lézarde river, contaminating Manzo lake and the entire irrigation network as far as the southern farms.

Throughout Martinique, phylotype-IIB/4NPB strains seemed to be established on several wild species and weeds, including wild Solanaceae and native *Heliconia* (*H. caribea*) located in forest borders. Interestingly, some species, such as *Piper* spp., *Solanum* spp., *Xanthosoma* spp. and a *Dieffenbachia* sp., were also known as weed hosts of the insect-transmitted SFR strains in Honduras (Berg, 1971), Grenada (Frossard, 1987; Hunt, 1987) and Columbia (Belalcazar *et al.*, 1983). Some wild species of *Heliconia* (*H. latispatha*, *H. acuminata* and *H. imbricata*) were reported as natural carriers of strains inducing a 'distortion' syndrome on banana plantations (Sequeira & Averre, 1961).

The data strongly suggest that phylotype-IIB/4NPB strains are well established in the local plant community as well as in the water environment in Martinique. The factors that have favoured this emergence are still unclear, although the results suggest that banana/vegetable rotations have a role to play. Vegetable crops in banana fallows were routinely practiced and were common from 1997 in the north-windward area (C. Chabrier, personal communication). The isolation of phIIB/4NPB strains from wilted tomatoes was only associated with bananas as the previous crop. These strains were significantly more prevalent on tomato in the north-windward area, an area of regular banana/vegetable rotations. Also, isolations of phIIB/4NPB strains from wilted watermelons and zucchinis were only reported on fields with a preceding banana crop (data not shown; Wicker *et al.*, 2002). Thus, banana crops may favour the emergence of phIIB/4NPB strains on tomato. Additional surveys of fields with different cropping histories are underway to test this hypothesis. This is apparently the first study to assess the effect of cropping systems on the distribution and composition of field populations of *R. solanacearum*. Studies dealing with the effect of previous crops on soil populations of *R. solanacearum* (Michel *et al.*, 1996; Stander *et al.*, 2003),

or monitoring the population dynamics of *R. solanacearum* isolates in the rhizosphere of host plants (Melo *et al*., 1999), only considered quantitative changes in bacterial populations, rather than the possible shift of the populations' genetic composition and structure in relation to pathotype and genotype.

The results of this study contribute to the knowledge of the epidemiology of *R. solanacearum*. According to the extensive studies carried out for years in Central and South America on the ecology of the Moko strains and their relatives (Buddenhagen, 1960; French & Sequeira, 1970; Berg, 1971; Woods, 1984), populations of *R. solanacearum* in virgin soils or abandoned banana fields were partitioned into (i) Solanaceae strains (also named 'weed strains'; Buddenhagen, 1960) or T strains (Sequeira & Averre, 1961) found on particular weeds (*Eupatorium odoratum*), pathogenic on solanaceous species only; and (ii) *Musa* strains, maintained by *Heliconia* wild species and displaying a narrow host range towards Musaceae. The present study describes the emergence of a pathogenic variant also found on wild *Heliconia*, which: is genotypically undistinguishable from Moko strains (except by Mmx-PCR); is capable of infecting plantains (Wicker *et al*., 2007); displays an unexpectedly large host range including Solanaceae and Cucurbitaceae; and is possibly favoured by banana/vegetable rotations. Questions remain about the possible threat caused by these strains on *Musa* ABB types, rarely grown in Martinique but widely prevalent in the Caribbean and Central America.

It also remains to be determined whether these phIIB/4NPB strains represent a 'primitive' group of Moko strains similar to or even more primitive than the ecotype 'D' strains described by French & Sequeira (1970) which were found to belong to sequevars 3 and 4 (MLG24 and MLG25) (Fegan, 2005). The phIIB/4NPB strains may never have evolved pathogenicity for cultivated *Musa*; or they may have lost the ability to infect dessert banana, having acquired the ability to infect new hosts. Surveys in virgin areas in the Caribbean or French Guyana, for example, should help to test these two hypotheses. Besides, as more data from molecular genetics and genomics are available (Lavie *et al*., 2004; Guidot *et al*., 2007), it should soon be possible to determine which gene repertoires are common and specific across sequevar-4 'Moko-disease-inducing' strains and sequevar-4NPB strains.

The question of the native or introduced origin of these strains remains unsolved. To date, it is unlikely that these strains were recently introduced to Martinique from Central America or Brazil via infected rhizomes of *Anthurium* and/or *Heliconia*, as in the case of Hawaii (Alvarez *et al*., 1993) and Brazil (Assis *et al*., 2005). Moko-disease-causing strains infecting horticultural species have been reported in Central America, but typed sequevar 3 or 6 (Prior & Fegan, 2005a,b). Pothos cuttings from Central America were found to be infected by sequevar-4 strains in Florida (Prior & Fegan, 2005a), which induced 'mild disease' symptoms on banana but were only slightly pathogenic to tomato, unlike the Martinique strains (Norman & Yuen, 1998). Bacterial wilt caused by phIIB/4NPB strains in Martinique has only been observed on native *Heliconia* and has never been reported on commercial hybrids of this species. In addition, the first cases of anthurium decline were reported on plots of old 'Standard Rose' *Anthurium* (*A. ferrierense*) (Mian *et al*., 2002), and not on recently-introduced *Anthurium* hybrids (*A. andreanum*). Further genetic analyses of the Martinique populations are required, including assessment of their genetic structure, in order to deepen the understanding of the ecology of the emerging strains. Regional surveys of the entire Caribbean basin to search for similar strains are also necessary.

This model of emergence offers a unique opportunity for epidemiologists and genomic experts to collaborate in the investigation of the relationships between cropping practices and genetic rearrangements that govern shifts in pathogenicity. It may also provide new insights into the main driving forces governing speciation in phytopathogenic bacteria.

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