Diversity of *Ralstonia solanacearum* in French Guiana Expands Knowledge of the “Emerging Ecotype”

P. Deberdt, J. Guyot, R. Coranson-Beaudu, J. Launay, M. Noreksal, P. Rivière, F. Vigné, D. Laplace, L. Lebreton, and E. Wicker

First and third authors: CIRAD, UPR HORTSYS, Campus Agro-Environnemental Caraïbe, F-97285 Le Lamentin, Martinique, France; second, fourth, and fifth authors: CIRAD, UPR Biagresseurs, F-97387 Kourou, French Guiana, France; sixth and tenth authors: CIRAD, UMR 53 Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), Pole de Protection des Plantes, 7 chemin de l'IRAT, F-97410 Saint Pierre, Reunion Island, France; seventh author: FREDON, BP 878, F-97333 Cayenne, French Guiana, France; and eighth and ninth authors: DAAF/SALIM, BP 5002, F-97305 Cayenne, French Guiana, France.

Accepted for publication 19 November 2013.

ABSTRACT

Deberdt, P., Guyot, J., Coranson-Beaudu, R., Launay, J., Noreksal, M., Rivière, P., Vigné, F., Laplace, D., Lebreton, L., and Wicker, E. 2014. Diversity of *Ralstonia solanacearum* in French Guiana expands knowledge of the “emerging ecotype.” *Phytopathology* 104:586-596.

Although bacterial wilt remains a major plant disease throughout South America and the Caribbean, the diversity of prevalent *Ralstonia solanacearum* populations is largely unknown. The genetic and phenotypic diversity of *R. solanacearum* strains in French Guiana was assessed using diagnostic polymerase chain reactions and sequence-based (*egl* and *mutS*) genotyping on a 239-strain collection sampled on the families Solanaceae and Cucurbitaceae, revealing an unexpectedly high diversity. Strains were distributed within phylotypes I (46.9%), IIA (26.8%), and IIB (26.3%),

with one new endoglucanase sequence type (*egl* ST) found within each group. Phylotype IIB strains consisted mostly (97%) of strains with the emerging ecotype (IIB/sequence 4NPB). Host range of IIB/4NPB strains from French Guiana matched the original emerging reference strain from Martinique. They were virulent on cucumber; virulent and highly aggressive on tomato, including the resistant reference Hawaii 7996; and only controlled by eggplant SM6 and Surya accessions. The emerging ecotype IIB/4NPB is fully established in French Guiana in both cultivated fields and uncultivated forest, rendering the hypothesis of introduction via ornamental or banana cuttings unlikely. Thus, this ecotype may have originated from the Amazonian region and spread throughout the Caribbean region.

Bacterial wilt caused by the bacterial pathogen *Ralstonia solanacearum* is one of the most destructive bacterial plant diseases in tropical, subtropical, and temperate regions of the world. This bacterial pathogen has an extensive host range and affects >450 plant species and 54 botanical families (13,22). *R. solanacearum* is a highly diversified pathogen, and is considered to be a species complex (i.e., a heterogeneous group of related strains) (19). Historically, the species complex was subdivided into races based on host range (4) and into biovars based on ability to produce acid from a panel of carbohydrates (21). A more phylogenetically meaningful classification scheme, based on sequence analysis of the internal transcribed spacer (ITS) region and the endoglucanase (*egl*), *hrpB*, and *mutS* genes, divided the species complex into four phylotypes (15). Each phylotype can be further subdivided into a variable and additive number of sequevars, which are clusters of strains whose *egl* sequences have at least 99% similarity (15,51). A recent multi-locus sequence analysis (53) established that phylotypes I, IIA, IIB, III, and IV displayed contrasting evolutionary dynamics and identified eight clades. Phylotype I corresponded to clade 1, phylotype IIA was subdivided into clades 2 and 3, phylotype IIB into clades 4 and 5, phylotype III corresponded to clade 6, and phylotype IV was subdivided into clades 7 and 8. *R. solanacearum* includes a wide range of ecotypes (sensu Cohan) (8),

sharing similar hosts and symptomatology and causing disease in similar climatic conditions. Among these, the “Moko” ecotype is relatively specialized and is limited to banana and *Musa* ornamentals, while the “Brown rot” ecotype is specifically able to induce disease on potato and tomato in cool conditions (28); the “emerging” ecotype (51,52) is phylogenetically close to the Moko-inducing clade 4 sequevar 4 of phylotype IIB and has a unique host range, including several species within the families Solanaceae, Araceae, Cucurbitaceae, and ornamental Musaceae. However, the emerging ecotype is unable to cause Moko disease on dessert banana and, consequently, is genotyped as phylotype IIB/sequence 4NPB (NPB = nonpathogenic on banana). Although *R. solanacearum* clades and sequevars sometimes share pathogenic traits or geographic origins, there is no clear phylogenetic basis for several ecotypes. For example, the Moko ecotype is clearly polyphyletic (53). Using this classification, strains were assigned to a specific phylotype, clade, sequevar (30), and *egl* and *mutS* sequence type (ST) (53).

R. solanacearum is considered to be one of the most important bacterial plant pathogens in South America, affecting both vegetable crops (mainly members of Solanaceae), potato, and banana plantations throughout the continent (13). Yet, the diversity of prevalent *R. solanacearum* strains on the continent has rarely been documented, and only limited-scale surveys of specific crops—for example, strains affecting potato in Uruguay (44), Brazil (41), and Venezuela (17) and Moko strains in Colombia (5,6)—have been conducted. However, some ecotypes such as the emerging strains from Martinique, due to their unique ecology (possibly maintained or favored by banana-vegetable rotations) and their huge host range that includes species of both Solanaceae and Cucurbitaceae, and their ability to bypass all

Corresponding author: E. Wicker; E-mail address: wicker@cirad.fr

*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains three supplemental tables. Figures 2, 3, and 4 appear in color online.

<http://dx.doi.org/10.1094/PHYTO-09-13-0264-R>
© 2014 The American Phytopathological Society

resistance factors known to date in tomato (24,50), pose a serious threat to vegetable growers in the Caribbean and South America, particularly when banana and vegetable crops coexist. Yet, by the time emerging IIB/4NPB strains were first described in French West Indies (51), data from collections indicated that this ecotype was already prevalent on the continent (Brazil) and nearby (Trinidad).

In this context, the specific case of French Guiana is of particular interest. The first cases of Moko disease on banana were reported in 1962 in the coastal area of Cayenne (47) but the bacteria were not identified. Outbreaks of bacterial wilt on tomato and pepper were first reported and described in 1967 (12); the strain GMI1000 was isolated in 1978 from one of these outbreaks and was later sequenced in 2002 (40). Routine field analyses indicated that bacterial wilt was prevalent throughout French Guiana from the 1980s on, affecting not only solanaceous crops but also *Anthurium* sp. (31). More recently, in 2006, strains of the emerging ecotype (phylogroup IIB, clade4, sequevar 4NPB) (51) were isolated in water used to irrigate greenhouse tomato (E. Wicker and R. Coranson-Beaudu, *unpublished data*). However, no systematic survey was launched and, consequently, the diversity of *R. solanacearum* strains that currently affect vegetable crops in French Guiana is not known.

Because the assessment of pathogen genetic diversity is a key factor for successful development of efficient sustainable control strategies, and because the emerging ecotype could be a huge threat not only to members of Solanaceae but also to members of Cucurbitaceae, it was considered important to assess the exact

distribution of *R. solanacearum* populations in the main cropping areas in French Guiana, and to investigate their phylogenetic diversity. The specific objectives of this study were to determine (i) whether the emerging ecotype is widespread or only sporadically prevalent, (ii) whether emerging strains from French Guiana share common pathogenicity features with emerging strains from Martinique (high virulence and pathogenicity on Cucurbitaceae), and (iii) which sources of plant resistance have the most potential for controlling *R. solanacearum* populations from French Guiana, as a starting point for building innovative and complementary strategies for the management of this serious disease.

MATERIALS AND METHODS

Bacterial strains. Vegetable cropping areas were surveyed in 2011 and 2012 throughout the main vegetable production areas, which are clustered in three agroecological zones near the Atlantic Coast (Fig. 1; Table 1). In total, 25 farms were chosen based on their location within each agroecological zone, cropping successions, and history of bacterial wilt. Particular attention was paid to fields that had not been cropped for a number of years, and that were cropped with Cucurbitaceae or Solanaceae successions or banana-vegetable rotations or associations. In this way, the first crops after a fallow were sampled on five farms, and samples were collected in fields belonging to six farms that were cropped for the first time after forest clearing.

Wilted plants of the main crops of the families Solanaceae (tomato, eggplant, and pepper) and Cucurbitaceae (squash) were

Fig. 1. Map of French Guiana showing agroecological zones, communes, and farms sampled.

sampled on each farm. In all, 10 to 15 wilted plants were sampled in each field showing symptoms of wilt for bacterial isolation. Samples were taken at the base of the stem of wilted tomato (*Solanum lycopersicon*), eggplant (*S. melongena* and *S. aethiopicum*), sweet pepper (*Capsicum annuum*), and squash (*Cucurbita pepo*) plants.

Plant samples were surface disinfected with 70% ethanol and flamed, then chopped into small pieces and macerated in sterile Tris buffer (0.01 M Trizma, pH 7.1). Macerates (10 µl) were streaked on modified semiselective SMSA medium (11). The plates were then incubated at 28°C for 3 days. Bacterial colonies that were morphologically typical of *R. solanacearum* were then selected and subcultured on casamino acid peptone glucose medium (23) for further purification. Each isolate was given a strain number (indicating the farm on which it was sampled and its host of origin) and was then stored in sterile distilled water at room temperature in the Campus Agro-Environnemental Caraïbe (formerly "Pôle de Recherche Agro-Environnementale de Martinique", PRAM, Martinique, France) and in the DAAF/SALIM (Guyane, France). A collection subset was transferred to the Pôle de Protection des Plantes (CIRAD, Saint-Pierre, Réunion Island). Each isolate was given a RUN accession number and was then stored at -80°C on Cryobank microbeads (COPAN Diagnostics, Marietta, CA).

The reference strains used ($n = 23$) included strains isolated during the first reports in 2006, that were originally purified and stored in Campus Agro- Environnemental Caraïbe; others were obtained from international collections, and stored in the RUN collection of the Pôle de Protection des Plantes (Saint Pierre, Réunion Island).

Molecular typing. Several diagnostic multiplex-polymerase chain reactions (PCRs) were applied to bacterial suspensions ($\approx 10^8$ cells ml⁻¹) of each strain to determine (i) their phylotype (15) and (ii) whether they belonged to the Moko (36), potato brown rot (14), or emerging (51) ecotype. PCR and electrophoresis conditions are detailed by Mahbou Somo Toukam et al. (26) and N'Guessan et al. (30).

DNA sequencing and analysis of *egl* and DNA repair (*mutS*) genes. The genetic diversity and phylogeny of a collection subset ($n = 126$) were characterized using comparative analysis of the partial nucleotide sequences of the *egl* and DNA repair (*mutS*) genes. The primers used and PCR procedures are described by

Wicker et al. (51,53). PCR products were vacuum dehydrated and sent to Beckman-Coulter Genomics (Takeley, UK) for further purification and double-strand sequencing using PCR primers as sequencing primers. Raw sequences from both strands were assembled, checked, and manually edited using Geneious (version 5.5; Biomatters; <http://www.geneious.com/>). All ambiguous sequences were reamplified and resequenced. Consensus sequences were then exported as multifasta files. Alignments were carried out using the ClustalW alignment tool (46) available in Mega 5.0 software (45), along with reference sequences (53). Sequence extremities were trimmed on the basis of the sequence quality and the reading frame, as detailed by N'Guessan et al. (30) and Wicker et al. (53), giving final *egl* and *mutS* sequences of 666 and 651 nucleotides, respectively.

Phylogenetic trees were constructed for each gene using the maximum-likelihood method with determination of the best fit nucleotide substitution model using RDP software (27), with 1,000 bootstrap resamplings. Assignment to *egl*-based sequevars was conducted using reference *egl* sequences (53). Assignment to *egl* and *mutS* sequences types (STs) was conducted using reference sequences (53; unpublished data).

Pathogenicity tests. Seven representative strains chosen according to their phylotype and *egl*-based sequevar, location, and host of origin were assessed for virulence on species of Solanaceae and Cucurbitaceae. The plant accessions tested were chosen according to reference studies on *R. solanacearum*-Solanaceae interactions (24,25). They consisted of (i) one susceptible ('L390', coded T10) and one resistant ('Hawaii 7996', coded T5) tomato (*S. lycopersicum*) accession; and (ii) five eggplant (*S. melongena*) accessions, including four of the main bacterial wilt-resistance sources ('Dingras multiple Purple', 'SM6', 'Surya', and 'AG91-25', coded E1, E2, E4, and E6, respectively) and one susceptible reference ('MM738', coded E8); and (iii) one cucumber commercial variety (*Cucumis sativus*, 'Eureka'; Seminis [Gamm Vert, Le Lamentin, Martinique]).

Bacterial suspensions were prepared in Tris buffer (0.01 M Trizma, pH 7.1) and spectrophotometrically adjusted to optical density at 600 nm = 0.1 ($\approx 1.0 \times 10^8$ cells ml⁻¹). Roots of plantlets with four fully extended leaves, grown in FLORADUR potting mix in 9-by-9-cm pots, were wounded with a scalpel and inoculated by soil drenching (5 ml per plant). Each strain and the negative control (bidistilled water) were inoculated on 10 plants

TABLE 1. Characteristics of the agroecological zones in French Guiana (20), with the locations and farms sampled in 2011 (E codes) and 2012 (E' codes)

Agroecological zone	Characteristics	Rainfall (mm year ⁻¹)	Locations	Farms
Z1	Ferrallitic soils, sandy clay soils	<3,000	Javouhey	E05
				E09
				E15
				E16
			Mana	E06
				E11
			Sinnamary	E13
				E'06
			St. Laurent du Maroni	E'02
				E01
Z2	Sandy soils	3,000–4,000	Macouria	E'04
				E03
			Montsinéry	E04
				E18
				E'01
				E07
			Wayabo	E02
				E12
Z3	Clayish to silky clay soils	>4,000	Cacao	E17
				E'05
				E08
				E14
			Matoury	E19
				E'07
			Régina	E'08

from each solanaceous accession or 12 plants for cucumber. The plants were then kept in a growth chamber at 30 and 25°C day and night temperatures, respectively; 90% relative humidity; and a 14- and 10-h photoperiod. The experiment was repeated once.

The incidence and severity of bacterial wilt were monitored every 2 or 3 days for 28 days (42 days for cucumber) using a 0 to 4 scale, where 0 = no symptoms, 1 = one wilted leaf, 2 = two or three wilted leaves, 3 = all leaves wilted, and 4 = stem collapsed and plant dead (10). Each plant that scored 3 or 4 was considered wilted.

Plants showing no symptoms and scored 1 or 2 at the end of the experiment were harvested and tested for latent infections as reported by Lebeau et al. (24). The percentage of wilted plants and the colonization index (CI) (35) were calculated.

Data analysis. Previous cluster analysis of the CoreTEP-coreRs2 interactions (considering both wilting incidence and CI scores) led to the identification of five phenotypic plant groups, named reference phenotypes, scored as 1 to 5, where 1 = highly resistant, 2 = moderately resistant, 3.1 = partially resistant, 3.2 = latent infection, 4 = moderately susceptible, and 5 = highly susceptible (24). On the basis of the respective final wilting rate and CI, each combination of strain–solanaceous cultivar was assigned a reference phenotype score using the “*k* nearest neighbor” (*knn*) algorithm (package *class* [48], R version 2.15.3).

The effect of strain and plant accession on the incidence of wilt and on the CI was assessed using a generalized linear model and analysis of variance, respectively. The incidence of wilt and the

mean CI were compared using a pairwise comparison test based on Tukey’s method at level 0.05 (package *glm*, R software) (38).

RESULTS

***R. solanacearum* strain collection.** In total, 239 *R. solanacearum* strains were isolated from wilted Solanaceae (tomato, eggplant, and sweet pepper) and wilted Cucurbitaceae (squash) plants. In total, 171 strains were isolated from 18 farms in 2011 and 68 strains were isolated from 7 farms in 2012.

The phylotyping results of the 239 *R. solanacearum* strains in each agroecological zone (Z1, Z2, and Z3) are listed in Table 2. From these 239 strains, a set of 126 representative strains, collected from farms where >10 isolates were taken, was selected for phylogenetic analysis (Table 3), including strains from all phylotypes and from tomato (*S. lycopersicon*), eggplant (*S. melongena* and *S. aethiopicum*), sweet pepper (*Capsicum annuum*), and squash (*Cucurbita pepo*). From these representative strains, a set of seven strains was selected for pathogenicity tests (Table 4).

Molecular typing characterization of *R. solanacearum* strains and phylogenetic analysis. The results of multiplex PCR analyses and gene sequencing showed that two of the four known phylotypes of *R. solanacearum* were prevalent in French Guiana: the Asian phylotype I and the American phylotype II (Table 2; Supplemental Table 1). In total, 46.9% of the collection were phylotype I strains and 53.1% were phylotype II (26.8% phylotype IIA and 26.3% phylotype IIB). Most IIB strains were

TABLE 2. Phylotype distribution of strains in each agroecological zone in French Guiana (2011–12 collection)^a

Zones ^y	Phylotype ^z				Total
	I	IIA	IIB, sequevar 4NPB	IIB, sequevar 27	
Z1 (9)	35 (38.9)	28 (31.1)	27 (30.0)	...	90
Z2 (8)	28 (51)	12 (21.8)	15 (27.2)	...	55
Z3 (8)	49 (52.1)	24 (25.6)	19 (20.2)	2 (2.1)	94
Total (25)	112 (46.9)	64 (26.8)	61 (25.5)	2 (0.8)	239

^a Strains were first genotyped by “phylotype” and “Moko” multiplex polymerase chain reactions.

^y Numbers in parentheses indicate total number of farms in each zone.

^z Numbers in parentheses indicate percentage of the different phylotypes in each agroecological zone.

TABLE 3. Phylotype distribution of strains in each agroecological zone in French Guiana (sequenced collection)^a

Phylotype	I				IIA				IIB		Total
	1/14	1/17	1/18	1/47	2/35	2/38	2/39	2/41	4/4	5/27	
Clade/sequevar											
<i>egl</i> ST	80	30	60	50	27	39	23	75	16	83	81
<i>mutS</i> ST	22				1	4			3	64	
Zone											
Z1	5	5	3	...	15	...	6	3	8	...	45
Z2	...	4	2	5	2	...	...	9	7	1	30
Z3	9	8	...	...	...	24	...	...	8	...	51
Total	14	17	5	5	17	24	6	12	23	1	126

^a Strains were first genotyped by “phylotype” and “Moko” multiplex polymerase chain reactions and a subsample of the collection (*n* = 126) was genotyped by *egl* and *mutS* sequencing to determine sequevars and sequence types (STs). ST as defined on the 666-nucleotide *egl* and 651-nucleotide *mutS* partial sequences (53; unpublished data). New STs are in bold.

TABLE 4. Location, host of isolation, and phylogenetic position of *Ralstonia solanacearum* strains selected for pathogenicity tests

Strain ^y	RUN code	Location (zone)	Host of isolation	Phylotype/sequevar ^z
E02PO3	1947	Cacao (Z3)	<i>Capsicum annuum</i>	IIB/4NPB
E07A01	1966	Wayabo (Z2)	<i>Solanum melongena</i>	IIB/4NPB
E14A06	1976	Régina (Z3)	<i>S. melongena</i>	IIA/38
E16A02	1985	Javouhey (Z1)	<i>S. melongena</i>	I/17
E02CO2	1992	Cacao (Z3)	<i>Cucurbita pepo</i>	IIB/4NPB
E18T02	1994	Montsinéry (Z2)	<i>S. lycopersicum</i>	IIA/41
E19T02	2004	Régina (Z3)	<i>S. lycopersicum</i>	IIB/4NPB

^y Strain code relates to the farm sampled (E02 to -18) and to the host of isolation (PO = sweet pepper, T = tomato, A = eggplant, and CO = squash).

^z Phylotype and *egl*-based sequevar.

Fig. 2. Maximum-likelihood phylogenetic tree based on the partial endoglucanase (*egl*) gene sequences of *Ralstonia solanacearum* strains from French Guiana and reference strains (from clade 4 and from each sequence type [ST]), based on the GTR+I+G4 substitution model. Numbers at each node are bootstrap values (1,000 resamplings). The tree was rooted with the *egl* partial sequence of *Burkholderia multivorans* ATCC 17616 (not shown). Reference strains are identified by their sequevar (SEQ) number, RUN number, and ST (originally from 1 to 60 [53], extended to 83). Clades and STs found in French Guiana were highlighted; “emerging ecotype” strains were clustered within the clade 4.

identified as sequevar 4NPB (61 of 63, corresponding to 96.8%), which were mainly isolated from tomato (*S. lycopersicum*) and sweet pepper (*Capsicum annuum*), then eggplant (*S. melongena*) and squash (*Cucurbita pepo*).

The phylogenetic trees based on *egl* and *mutS* partial sequences produced similar clusters (Figs. 2 and 3), although branching between the different phylotypes differed across genes, as reported by Mahbou Somo Toukam et al. (26). For most of the collection, PCR typing and *egl/mutS* sequence typing gave similar phylogenetic positions, although some discrepancies were observed. Three strains (RUN1976 to -1978), PCR typed as race3, biovar 2 strains (potentially IIB/sequevar 1 and brown rot ecotype), were positioned by *egl-mutS* sequence typing within phylotype IIA, sequevar 38 (*egl* and *mutS* STs 39 and 4, respectively). In addition, 13 strains PCR typed as Moko ecotype, sequevar 6 were positioned either within phylotype IIA, sequevar 35 (bacterial wilt-inducing strains, *egl* and *mutS* STs 27 and 1) or (RUN2221) within phylotype IIB, sequevar 27 (new *egl* and *mutS* STs 81 and 64), only reported in Brazil (39,51) and Peru (32).

The distribution of phylotypes I, IIA, and IIB did not significantly differ across agroecological zones (χ^2 test, $P = 0.242$). Phylotype I strains, prevalent on 14 farms, were distributed within four *egl*-based sequevars (sequevars 14, 17, 18, and 47), each

corresponding to a distinct *egl* ST. The most prevalent sequevar was 17 (ST30), found in all three zones on a total of seven farms. Sequevar 18 (ST60), containing the reference phylotype I strain GMI1000 (40), was only found in zones 1 and 2, on three farms. ST80 within sequevar 14 was reported for the first time, and found in Mana and St. Laurent-du-Maroni (Z1) and Regina (Z3) (Table 3). Phylotype IIA strains, prevalent on 10 farms, were distributed within four sequevars (sequevars 35, 38, 39, and 41), each corresponding to a distinct *egl* ST. One new *egl* ST (ST75) is described here for the first time; it was found in Montsinéry (Z2) in a field in which lettuce was the preceding crop. ST75 was also identified in Mana (Z1) in a tomato field which was previously fallowed. Only sequevar 38 was specifically found in zone 3, at Régina (2011) and Cacao (2012). Phylotype IIB strains were mostly distributed within the emerging ecotype sequevar 4NPB, prevalent in all three zones and on 14 farms, with isolation rates per zone ranging from 20.2% (Z3) to 30% (Z1). These were predominantly isolated from diseased tomato and pepper but also from eggplant and squash. Within sequevar 4NPB, two *egl* STs were identified: (i) the most prevalent, ST16, already identified in French Guiana in 2006 and 2007 as well as in neighboring countries (Brazil, Guyana, Martinique, Honduras, St. Vincent, and Costa Rica); and (ii) the newly described ST83 (RUN1953) isolated from pepper in Montsinéry (Z2), in a field in which the

Fig. 3. Maximum-likelihood phylogenetic tree based on DNA repair gene (*mutS*) sequences of *Ralstonia solanacearum* strains from French Guiana and reference strains, based on the HKY+I+G4 substitution model. Numbers at each node are bootstrap values (1,000 resamplings). Reference strains are identified by their sequence type (ST, originally from 1 to 47 [53], extended to 79) number and RUN number. Clades and STs found in French Guiana were highlighted; “emerging ecotype” strains were clustered within the clade 4. Endoglucanase sequence of one *mutS*-ST04 Guyanese strain could not be amplified.

preceding crop was cantaloupe (*C. moschata*). Two strains (RUN2221 and -2222) belonging to sequevar 27 (already found in Brazil on *Pelargonium* spp. [51] and in Peru on eggplant [32]) but defining a new ST from both *egl* and *mutS* (ST81 and ST64, respectively) were isolated from tomato in a newly cropped field in Cacao (Z3).

Relationships between the preceding crop and the phylotype composition. To investigate the possible impact of the preceding crop on phylotype composition, we used a subset of 199 strains sampled from 22 fields where preceding crops or natural habitats were precisely known (Fig. 4; Supplemental Table 2); strains isolated from fields with a vaguely identified preceding crop (such as “vegetables”) or a very rare preceding crop (such as “bean”) were not used. The preceding crop was shown to have a significant impact on the distribution of phylotype IIA, IIB/4NPB, and I strains (χ^2 test, $P = 8.72 \times 10^{-14}$, 1.03×10^{-7} , and 1.06×10^{-5} , respectively). Preceding crops impacting the phylotype composition (i.e. whose observed phylotype distributions were significantly different from expected distributions) were “forest”, “cabbage”, “fallow”, “Cucurbitaceae”, and “lettuce” (Fig. 4). From our data, phylotype IIA strains appeared to be favored by preceding forest and lettuce (χ^2 test, $P = 1.05 \times 10^{-8}$ and 4.68×10^{-3} , respectively). Emerging IIB/4NPB strains appeared to be

favored by preceding cabbage and Cucurbitaceae (χ^2 test, $P = 2.35 \times 10^{-7}$ and 9.50×10^{-4} respectively). Phylotype I strains appeared to be favored by preceding fallow (χ^2 test, 9.96×10^{-7}). It is important to note that fields that were previously under forest harbored the highest number of phylogenetic groups (I, IIA, IIB/4NPB, and IIB/27).

Pathogenicity on Cucurbitaceae and Solanaceae. On cucumber (‘Eureka’), wilting incidence and the CI did not differ significantly between the two replicates ($P = 0.109$ and 0.154 , respectively) and, thus, subsequent analyses were based on mean results. IIB/4NPB strains ($n = 4$) induced wilting symptoms from the fourth day after inoculation and reached final wilting incidences of 37.5 to 66.7% and colonization rates of 45.8 to 70.8% (Table 5). Phylotype I and phylotype IIA strains induced no symptoms over the 6-week incubation period and, thus, were considered as not pathogenic to cucumber. However, the IIA/41 strain RUN1994 was able to colonize one plant during the first replicate, giving 8.3% of latent infection.

On Solanaceae spp., wilting incidence and the CI did not differ significantly between the two replicates ($P = 0.631$ and 0.303 , respectively) and, thus, subsequent analyses were based on the mean results. According to pathogenicity data (Table 6; Supplemental Table 3), the virulence pattern of the IIB/4NPB strains

Fig. 4. Impact of the preceding crop on the phylogenetic composition of *Ralstonia solanacearum* populations isolated from cropped fields in French Guiana. Only strains ($n = 199$) sampled in fields in which the preceding crop was really known were used. Preceding crops of “tomato” and “sweet pepper” are grouped under “Solanaceae” and “cantaloupe” and “cucumber” under “Cucurbitaceae”; preceding crops denoted “vegetables” (too imprecise) and “bean” (only four strains) were not used for analysis. Preceding crops marked with an asterisk had a significant impact on phylotype composition, as estimated by χ^2 tests.

TABLE 5. Pathogenicity of the *Ralstonia solanacearum* strains on cucumber (‘Eureka’), as estimated by final wilting and colonization indices

RUN code	Strain ^x	Phylotype/sequevar ^y	Wilting ^z	Colonization ^z
1947	E02PO3	IIB/4NPB	66.7 ± 47.1 a	70.8 ± 41.2 a
2004	E19T02	IIB/4NPB	58.3 ± 23.6 a	62.5 ± 29.5 a
1966	E07A01	IIB/4NPB	58.3 ± 47.1 a	58.3 ± 47.1 a
1992	E02CO2	IIB/4NPB	37.5 ± 29.5 ab	45.8 ± 29.5 ab
1976	E14A06	IIA/38	0.0 ± 0.0 b	0.0 ± 0.0 b
1994	E18T02	IIA/41	0.0 ± 0.0 b	8.3 ± 11.8 b
1985	E16A02	I/17	0.0 ± 0.0 b	0.0 ± 0.0 b

^x Strain code relates to the farm sampled (E02 to -18) and to the host of isolation (PO = sweet pepper, T = tomato, A = eggplant, and CO = squash).

^y Phylotype and *egl*-based sequevar.

^z Means ± standard deviations of two replicates (10 plants each). Wilting refers to the mean final percentage of wilted plants, while Colonization refers to rate of wilted and infected asymptomatic plants over total plants (see details in the Materials and Methods). Values followed by the same letter in the same column are not significantly different (Tukey test, $P = 0.05$).

clearly differed from that of strains I and IIA. On tomato, IIB/4NPB strains were highly aggressive on both susceptible L390 and resistant Hawaii 7996 accessions, whereas phylotype I and IIA/41 strains were mildly aggressive on Hawaii 7996 (final wilting indices of 8.3 and 4.2%, respectively); IIA/38 displayed the same virulence phenotype but caused a higher wilting incidence (16.7%). On eggplant, phenotype scores were much more diverse. MM738 (E8) was susceptible to highly susceptible to most strains except RUN1992 and RUN2004. Unexpectedly, the strains that were the least aggressive on this accession were IIB/4NPB. SM6 (E2) and, to a lesser extent, Surya (E4), displayed the highest resistance levels and broadest resistance spectra, controlling all IIB/4NPB, IIA, and I strains; 3.3% of SM6 was wilted only by the strain RUN1976 (IIA/38); 0 to 3.3% of Surya was wilted by I and IIA strains but 6.7 to 23.3% was wilted by emerging (IIB/4NPB) strains. Dingras (E1) controlled only three of the four IIB/4NPB strains, plus I and IIA strains. AG91-25 (E6), the *Ers1*-carrying accession (25), only partially controlled the emerging strains and was susceptible to the phylotype I strain RUN1985.

DISCUSSION

R. solanacearum is a major plant pathogen in South America, where it causes bacterial wilt on vegetables and ornamentals, Moko disease on banana, and brown rot on potato. Priming the development of efficient and regionally sound management strategies in such important crops requires extensive disease surveys designed at country scale, combined with adequate molecular diagnosis tools and epidemiological analyses. This first extensive study showed wide genetic diversity among *R. solanacearum* strains affecting species of Solanaceae and Cucurbitaceae in French Guiana. Because this study was primarily designed to address plant disease management issues, sampling focused on the major vegetable cropping areas, located near the Atlantic coast in French Guiana. However, this first, rather detailed picture should be completed by additional samplings in the inland regions, including Saul, the Maroni river borders of Maripasoula, Grand Santi, where cropping fields are closely interwoven with forest.

In total, 239 strains of *R. solanacearum* were collected from the main Solanaceae and Cucurbitaceae crops, and 126 representative strains were selected for phylogenetic analysis. We identified diagnostic discrepancies between specific PCRs and gene sequencing. Some strains positive to the race 3, biovar 2-specific PCR (14), and thus potential members of IIB, clade 5, sequevar 1, were actually classified as belonging to IIA, clade 2, sequevar 38. Fegan's 630-631 primers (14) target a "putative bacteriophage tail completion protein S" (*GpS*), positioned next to a putative *Mu*-like prophage *FluMu* protein. Thus, this diagnostic fragment may

be mobile, and carried by non-IIB-1 strains. In addition, some strains classified as Moko sequevar 6 strains by the SI28 primer pairs were actually IIA, clade 2, sequevar 35. Such discrepancy has already been identified in *R. solanacearum* populations from Guadeloupe (42). The SI28 primer pairs target a sequevar 6-specific (i.e., only hits in sequevar 6 genomes) conserved protein of unknown function containing three phage-originating domains and located in a region of genomic plasticity. Although phylogenetic positioning (phylotype IIA) is consistent with the two methods, ecotype positioning differs: sequevar 6 strains cause banana Moko disease whereas sequevar 35 strains are only pathogenic to members of the family Solanaceae. Such misleading diagnoses can have serious consequences and lead to erroneous reports of Moko disease. Based on these data, it is highly recommended to confirm any sequevar 6 or sequevar 1 PCR-based identification by *egl* and *mutS* sequencing.

This study expanded the knowledge of the real diversity in *R. solanacearum* prevalent in South America in both phylotype I and phylotype II. Proportions of phylotype I, IIA, and IIB (46.9/26.8/26.3, respectively) in French Guiana differed from those described in Martinique (26.7/22/51.3) (51), Guadeloupe (63/37/0) (42), and Trinidad (38/62/0) (37); sequevar diversity in French Guiana also was higher than in the three other countries. Comparisons with neighboring countries are more difficult because many studies were focused on specific crops, were based on the biovar system, or determined phylotypes but not sequevars. The rare studies dedicated to vegetable crops reported dominance of phylotype IIB sequevar 1 (formerly race 3, biovar 2) on both potato and tomato crops in western Venezuela (17,18), as well as in Uruguay (44). In Brazil, populations were sampled on tobacco, giving only biovar 1 (phylotype IIA or IIB) (49); and potato (41), giving 41.5% biovar 2 (sequevar 1) and 58.5% biovar 2T (sequevars 25 to 28). In the Amazonian region of Brazil, phylotype I (ex-biovar 3) appears to be less prevalent (18.6%) than phylotype II (75.7% biovar 1 and 5.71% biovar 2T, corresponding to IIB, clade 5, sequevars 25 to 28) (9).

Several aspects of *R. solanacearum* populations from French Guiana appear to be original. Sequevars 14 and 18, which have already been reported in the West Indies (37,42,51), Guatemala (39), and Brazil (39), were found within phylotype I; only sequevar 31, reported on *Begonia* spp. in Brazil (39), was absent from French Guiana. However, French Guiana hosted sequevar 47 which, to date, has only been reported in Indonesia and Thailand (RUN collection; unpublished data). Moreover, sequevar 14 from French Guiana consisted of one new ST (ST80). In addition, we observed that the reference GMI1000 lineage (sequevar 18, *egl* ST60), originally isolated in French Guiana, is not the main lineage in the phylotype I. New STs were also identified within phylotype IIA (ST75). IIA, sequevar 41 was previously only reported in Cameroon (26). IIA, sequevar 38 strains, which were

TABLE 6. Virulence phenotypes of *Ralstonia solanacearum* strains from French Guiana on tomato ('L390' [T10] and 'Hawaii7996' [T5]) and eggplant accessions ('Dingras multiple Purple', 'SM6', 'Surya', 'AG91-25', and 'MM738' [E1, E2, E4, E6, and E8, respectively])^y

Strain (RUN code)	Phylotype/sequevar ^z	Plant accessions						
		Eggplant					Tomato	
		E1	E2	E4	E6	E8	T10	T5
1992	IIB/4NPB	2	2	1	3.1	3.1	5	5
1966	IIB/4NPB	2	2	2	3.2	4	5	4
1947	IIB/4NPB	3.1	1	2	3.1	4	5	4
2004	IIB/4NPB	1	2	2	3.2	2	5	4
1985	I/17	1	1	1	4	5	5	2
1976	IIA/38	2	2	2	2	5	4	2
1994	IIA/41	2	1	2	2	4	4	2

^y The number assigned to each strain-accession combination represents the phenotype score, defined by the combination of final wilting incidence and colonization index and calculated as defined by Lebeau et al. (25), where 1 = highly resistant, 2 = moderately resistant, 3.1 = partially resistant, 3.2 = latent infection, 4 = moderately susceptible, and 5 = highly susceptible.

^z Phylotype and *egl*-based sequevar.

found in water courses in French Guiana from 2006, were reported in Florida (3) but also in Martinique (51), Trinidad (37), and Guyana (A. Muhamad and E. Wicker, unpublished data). Within phylotype IIB, clade 4, sequevar 4NPB strains from French Guiana were clustered in the same *egl* and *mutS* ST as strains from Martinique and Brazil (39,51), Guyana, and St. Vincent (A. Muhamad and E. Wicker; unpublished data) but a new *egl* ST (ST83) was identified. Here again, French Guiana is the first South American country in which sequevar 4NPB strains have consistently been isolated on vegetable crops, whereas reports from other countries remain scarce (on eggplant and cucumber in Brazil [39] and on plantain in Colombia [1]). Within clade 5, two IIB, sequevar 27 (RUN2221 and -2222) were isolated on tomato in a field in Cacao cropped for the first time (Z3), constituting a brand new ST in both *egl* and *mutS* genes (ST81 and ST64, respectively). This sequevar is rare and, to date, has only been reported as a brown rot ecotype in Peru (7,32) and Brazil (39,51) (*egl* and *mutS* ST26 and ST32, respectively). Cellier (7) established that sequevar 27 can cause latent infection of banana. This group might have been introduced from Brazil via ornamental or banana cuttings or potato tubers. Thus, this finding may be a phytosanitary issue, and illustrates the urgent need for better control of the movement of plant material between countries.

This study also revealed a significant impact of the preceding crop on the phylotype composition of the populations collected. To our knowledge, this is the first demonstration that solanaceous crops can be affected by bacterial wilt during the first cropping cycle after forest clearing in all three agroecological zones sampled in French Guiana (Z1, Z2, and Z3). These data strongly suggest that all phylotype groups (I, IIA, and IIB) are already well established in the natural environment and that phylotype composition may differ depending on whether the field was previously cultivated or not; further studies should focus on the floristic composition of the natural habitat to identify the link between the botanical families prevalent in the wild and phylotypes that affect the following crop. It is already known that the families Musaceae (different *Heliconia* spp.) (43) and wild Araceae (such as *Xanthosoma* and *Dieffenbachia* spp.) (2,16,34,52) can harbor Moko strains or emerging strains of *R. solanacearum*.

We demonstrate that emerging strains from French Guiana have the same host range and virulence features as the first strains described in Martinique (51,52). They were pathogenic on cucumber, unlike the phylotype I and IIA strains. Their virulence pattern on members of Solanaceae was clearly specific: highly aggressive on both susceptible L390 and resistant Hawaii 7996 tomato accessions, whereas phylotype I and IIA were poorly aggressive on Hawaii 7996. Virulence was controlled by eggplant accessions SM6 (E2) and, to a lesser extent, by Surya (E4), confirming previous results (24). One key result of this study was the first report in America of one phylotype I strain (RUN1985) attacking AG91-25 (E6) and, thus, bypassing the *ErsI* gene (25); whereas, to date, strains virulent on AG91-25 have only been reported in Asia and Africa (25,30).

This study is an important starting point for a regional breeding program on bacterial wilt resistance in French Guiana. In tomato, we confirmed that Hawaii 7996, the most stable tomato resistance genitor, efficiently controlled phylotype I and IIA-41 strains, as did IIA-38, although to a lesser extent. However, this tomato accession is susceptible to the emerging ecotype IIB/4NPB, as reported by Lebeau et al. (24) and Wicker et al. (50), which calls for screening of different resistance sources. For tomato growers, one solution would be the combined use of suppressive crop rotations and partially resistant varieties. In eggplant, accessions SM6 and Surya are the most satisfactory, and should be used as resistance genitors in regional breeding programs.

We confirm previous reports of IIB, clade 4, sequevar 4NPB strains from French Guiana infecting vegetable crops but demon-

strate here that this emerging ecotype is already widespread in French Guiana. It was found to be prevalent in all three agroecological zones and on 13 of the 25 farms sampled. We demonstrated its prevalence on several hosts, including species of Solanaceae (*S. lycopersicon*, *S. melongena*, and *S. aethiopicum*) and Cucurbitaceae (*C. pepo*), thus completing the list of susceptible hosts in natural infestation conditions (52).

It now remains to be determined whether the emerging French Guiana IIB/4NPB strains can establish themselves in xylem vessels of plantain banana, as the original IIB/4NPB strains can (51). Moreover, despite the two extensive surveys, and in spite of a recent report naming *C. maxima* as a new asymptomatic host for Moko strains (34), the link between Moko disease and IIB/4NPB prevalence could not be confirmed. In Columbia, this cultivated crop is frequently used after banana or plantain in crop rotations designed for the control or eradication of Moko disease. Because we were unable to find and sample fields with a recent history of Moko disease, we could not assess whether banana used as a preceding crop facilitates the establishment of IIB/4NPB strains, as was previously demonstrated in Martinique (52), mostly because of the cropping specificities of French Guiana: very few banana-vegetables rotations are practiced, and banana is cropped only in small areas, with no agro-industrial plantations such as those in the French West Indies (Martinique and Guadeloupe) or Central America.

The question that remains to be answered is the origin of the emerging ecotype IIB/4NPB. It was identified in 1998 in Martinique but also in Brazil (51), and our study provides evidence that it is already fully established in French Guiana, including in crop fields and uncultivated forest, rendering the hypothesis of an introduction via ornamental or banana cuttings highly unlikely. Thus, the question of the native or introduced origin of this emerging ecotype in French Guiana is still open. This ecotype may have originated in the Amazon Basin and, from there, spread to the Caribbean. Additional extensive surveys in Northern Brazil and in all countries in the Amazonian region (including Bolivia, Peru, Colombia, and Venezuela) should produce a more accurate map of the distribution of this ecotype and answer these ecological questions. Knowledge of the distribution of this ecotype in Northern Brazil and in all countries in the Amazonian region would be very useful for plant breeding programs or quarantine measures. Understanding the epidemiological and evolutionary relationships across these populations calls for epidemiological typing with more informative markers, such as multilocus VNTR analysis (29,33), which would be very useful to study the epidemiological and evolutionary relationships between clade 4 populations from French Guiana and neighboring countries, with the aim of unraveling the ecology and epidemiology of this important and genetically monomorphic emerging ecotype.

ACKNOWLEDGMENTS

This research was financially supported by the European Union (FEDER project) and the Conseil Régional de la Martinique; the European Union (FEDER project) and the Conseil Régional de la Réunion; the Ministère Français de l'Agriculture (CIOM project) and the DAAF/SALIM de Guyane. M. Noreskal, J. Launay, and P. Rivière participated in the project as Master students of AgroParisTech (Paris Institute of Technology for Life, Food and Environmental Sciences; Paris) and ISTOM (College of International Agro-Development; Cergy-Pontoise, France) and BS student of the University Institute of Technology of Reunion Island (Saint Pierre, Reunion Island), respectively. We thank S. Lebon, J.-M. Baptiste, and F. Maillot for their excellent technical assistance; A. Muhamad and A. Ramsubhag (University of the West Indies, Trinidad), and A. M. Bocsanczy (University of Florida) for providing strains; P. Bouteiller and L. Demade-Pellorce for their technical assistance in collecting strains in the field; and S. Poussier for his critical comments on a previous version of this manuscript.

LITERATURE CITED

- Alvarez, E., and Mejia, J. F. 2006. Activity 1.13. Characterization and identification of phylotypes and sequevars of isolates of *Ralstonia solanacearum* obtained from plantain, banana, and *Heliconia* sp. in Colombia. CIAT Annual Report 2006. Bogota, Colombia.
- Berg, L. A. 1971. Weed hosts of the SFR strain of *Pseudomonas solanacearum*, causal organism of bacterial wilt of bananas. *Phytopathology* 61:1314-1315.
- Bocsanczy, A. M., Achenbach, U. C., Mangravita-Novo, A., Yuen, J., and Norman, D. J. 2012. Comparative effect of low temperature on virulence and twitching motility of *Ralstonia solanacearum* strains present in Florida. *Phytopathology* 102:185-194. Online publication. doi:10.1094/PHYTO-05-11-0145
- Buddenhagen, I. W., Sequeira, L., and Kelman, A. 1962. Designation of races in *Pseudomonas solanacearum*. (Abstr.) *Phytopathology* 52:726.
- Cardozo, C., Rodriguez, P., Cotes, J. M., and Marin, M. 2010. Variabilidad genética de la bacteria *Ralstonia solanacearum* en la zona bananera de Uraba (Colombia) [Genetic variability of the bacterium *Ralstonia solanacearum* (Burkholderiales: Burkholderiaceae) in the banana-growing region of Uraba (Colombia).] *Rev. Biol. Trop.* 58:31-44.
- Cardozo, C., Rodriguez, P., and Marin, M. 2009. Molecular characterization of the *Ralstonia solanacearum* species complex in the banana growing region of Uraba. *Agron. Colomb.* 27:203-210.
- Cellier, G., and Prior, P. 2010. Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. *Phytopathology* 100:1250-1261.
- Cohan, F. M. 2006. Towards a conceptual and operational union of bacterial systematics, ecology, and evolution. *Phil. Trans. R. Soc. Biol.* 361:1985-1996.
- Costa, S. B., Ferreira, M. A. S. V., and Lopes, C. A. 2007. Pathogenic and molecular diversity of *Ralstonia solanacearum* isolates from the Brazilian Amazon. (Diversidade patogénica e molecular de *Ralstonia solanacearum* da região Amazônica Brasileira.) *Fitopatol. Brasil.* 32:285-294.
- Coupat-Goutaland, B., Bernillon, D., Guidot, A., Prior, P., Nesme, X., and Bertolla, F. 2011. *Ralstonia solanacearum* virulence increased following large interstrain gene transfers by natural transformation. *Mol. Plant-Microbe Interact.* 24:497-505.
- Denny, T. P., and Hayward, A. C. 2001. *Ralstonia solanacearum*. Pages 151-173 in: *Laboratory Guide for Identification of Plant Pathogenic Bacteria*, 3rd ed. N. W. Schaad, J. B. Jones, and W. Chun, eds. American Phytopathological Society, St. Paul, MN.
- Digat, B. 1967. Survey of bacterial wilt of solanaceous crops in French West Indies and in French Guyana. Mission Report. INRA, Petit-Bourg, Guadeloupe (FWI).
- Elphinstone, J. G. 2005. The current bacterial wilt situation: a global overview. Pages 9-28 in: *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.
- Fegan, M., Holloway, G., Hayward, A. C., and Timmis, J. 1998. Development of a diagnostic test based on the polymerase chain reaction (PCR) to identify strains of *R. solanacearum* exhibiting the biovar 2 genotype. Pages 34-43 in: *Bacterial Wilt Disease—Molecular and Ecological Aspects*. P. Prior, C. Allen, and J. Elphinstone, eds. Springer-Verlag, INRA Editions, Berlin, Paris.
- Fegan, M., and Prior, P. 2005. How complex is the “*Ralstonia solanacearum* species complex”. Pages 449-462 in: *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.
- Frossard, P. 1987. Moko disease threatens the West Indies. Pages 115-120 in: *Improving Citrus and Banana Production in the Caribbean Through Sanitation*. CTA, Wageningen, The Netherlands.
- Garcia, R., Garcia, A., and Delgado, L. 1999. Distribucion, incidencia y variabilidad de *Ralstonia solanacearum*, agente causal de la marchitez bacteriana de la papa en el estado Mérida. (Distribution, incidence and variability of *Ralstonia solanacearum*, causal agent of bacterial wilt of potato, in Merida state, Venezuela.) *Bioagro* 11:12-23.
- Garcia, R., Garcia, A., and Delgado, L. 1999. Marchitez bacteriana del tomate causada por el biovar 2A, de *Ralstonia solanacearum* en algunas localidades del Estado Merida-Venezuela. (Bacterial disease of tomato caused by the biovar 2A of *Ralstonia solanacearum* in several localities in Merida State-Venezuela.) *Rev. For. Venezol.* 43:183-189.
- Gillings, M. R., and Fahy, P. 1994. Genomic fingerprinting: towards a unified view of the *Pseudomonas solanacearum* species complex. Pages 95-112 in: *Bacterial Wilt: The Disease and Its Causative Agent, Pseudomonas solanacearum*. A. C. Hayward and G. L. Hartman, eds. CAB International, Wallingford, UK.
- Godon, P., and Guillobez, S. 1984. Cartographie numérique en Guyane. DAAF Guyane, Cayenne, Guyane Française.
- Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. *J. Appl. Bacteriol.* 27:265-277.
- Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.* 29:65-87.
- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology* 44:693-695.
- Lebeau, A., Daunay, M. C., Frary, A., Palloix, A., Wang, J. F., Dintinger, J., Chiroleu, F., Wicker, E., and Prior, P. 2011. Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. *Phytopathology* 101:154-165.
- Lebeau, A., Gouy, M., Daunay, M., Wicker, E., Chiroleu, F., Prior, P., Frary, A., and Dintinger, J. 2013. Genetic mapping of a major dominant gene for resistance to *Ralstonia solanacearum* in eggplant. *Theor. Appl. Genet.* 126:143-158.
- Mahbou Somo Toukam, G., Cellier, G., Wicker, E., Guilbaud, C., Kahane, R., Allen, C., and Prior, P. 2009. Broad diversity of *Ralstonia solanacearum* strains in Cameroon. *Plant Dis.* 93:1123-1130.
- Martin, D. P., Lemey, P., Lott, M., Moulton, V., Posada, D., and Lefevre, P. 2010. RDP3: a flexible and fast computer program for analyzing recombination. *Bioinformatics* 26:2462-2463.
- Milling, A., Meng, F. H., Denny, T. P., and Allen, C. 2009. Interactions with hosts at cool temperatures, not cold tolerance, explain the unique epidemiology of *Ralstonia solanacearum* race 3 biovar 2. *Phytopathology* 99:1127-1134.
- N'Guessan, C., Brisse, S., Le Roux-Nio, A., Poussier, S., Kone, D., and Wicker, E. 2013. Development of variable number of tandem repeats typing schemes for *Ralstonia solanacearum*, the agent of bacterial wilt, banana Moko disease and potato brown rot. *J. Microbiol. Methods* 92:366-374.
- N'Guessan, C. A., Abo, K., Fondio, L., Chiroleu, F., Lebeau, A., Poussier, S., Wicker, E., and Kone, D. 2012. So near and yet so far: the specific case of *Ralstonia solanacearum* populations from Cote d'Ivoire in Africa. *Phytopathology* 102:733-740.
- Noreskal, M. 2011. Typologie des pratiques agricoles sur Solanacées et diversité génétique de *Ralstonia solanacearum*, agent causal du flétrissement bactérien en Guyane. SIAFEE Master thesis, AgroParisTech, Paris.
- Nouri, S., Bahar, M., and Fegan, M. 2009. Diversity of *Ralstonia solanacearum* causing potato bacterial wilt in Iran and the first record of phylotype II/biovar 2T strains outside South America. *Plant Pathol.* 58:243-249.
- Parkinson, N., Bryant, R., Bew, J., Conyers, C., Stones, R., Alcock, M., and Elphinstone, J. 2013. Application of variable number tandem repeat (VNTR) typing to discriminate *Ralstonia solanacearum* strains associated with English watercourses and disease outbreaks. *Appl. Environ. Microbiol.* 79:6016-6022.
- Prieto Romo, J., Morales Osorio, J. G., and Salazar Yepes, M. 2012. Identification of new hosts for *Ralstonia solanacearum* (Smith) race 2 from Colombia. *Rev. Prot. Veg.* 27:151-161.
- Prior, P., Bart, S., Leclercq, S., Darrasse, A., and Anaïs, G. 1996. Resistance to bacterial wilt in tomato as discerned by spread of *Pseudomonas (Burkholderia) solanacearum* in the stem tissues. *Plant Pathol.* 45:720-726.
- Prior, P., and Fegan, M. 2005. Diversity and molecular detection of *Ralstonia solanacearum* race 2 strains by multiplex PCR. Pages 405-414 in: *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. C. Allen, P. Prior, and A. C. Hayward, eds. American Phytopathological Society, St. Paul, MN.
- Ramsubhag, A., Lawrence, D., Cassie, D., Fraser, R., Umaharan, P., Prior, P., and Wicker, E. 2012. Wide genetic diversity of *Ralstonia solanacearum* strains affecting tomato in Trinidad, West Indies. *Plant Pathol.* 61:844-857.
- R Development Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Rodrigues, L. M. R., Destefano, S. A. L., Silva, M. J. d., Costa, G. G. L., and Maringoni, A. C. 2012. Characterization of *Ralstonia solanacearum* strains from Brazil using molecular methods and pathogenicity tests. *J. Plant Pathol.* 94:505-516.
- Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J. C., Cattolico, L., Chandler, M., Choisne, N., Claudel Renard, C., Cunnean, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thebault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J., and Boucher, C. A. 2002. Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 415:497-502.
- Santana, B. G., Lopes, C. A., Alvarez, E., Barreto, C. C., Allen, C., and Quirino, B. F. 2012. Diversity of Brazilian biovar 2 strains of *Ralstonia solanacearum*. *J. Gen. Plant Pathol.* 78:190-200.
- Sauboua, P. 2007. Identification des souches de *Ralstonia solanacearum*

- présentes en Guadeloupe et évaluation d'hybrides INRA de tomate pour leur résistance aux maladies à begomovirus TYLCV et PYMV, et au flétrissement bactérien. Master thesis, ENITA Bordeaux, Bordeaux, France.
43. Sequeira, L., and Averre, C. W. 1961. Distribution and pathogenicity of strains of *Pseudomonas solanacearum* from virgin soils in Costa Rica. *Plant Dis. Rep.* 45:435-440.
 44. Siri, M. I., Sanabria, A., and Pianzzola, M. J. 2011. Genetic diversity and aggressiveness of *Ralstonia solanacearum* strains causing bacterial wilt of potato in Uruguay. *Plant Dis.* 95:1292-1301.
 45. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28:2731-2739.
 46. Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res.* 22:4673-4680.
 47. Vallaëys, G. 1962. Base d'un programme d'expérimentation agronomique en Guyane Française. IRAT Report. IRAT, Cayenne, Guyane Française.
 48. Venables, W. N., and Ripley, B. D. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York.
 49. Viana, F., Berger, I., and Duarte, V. 2012. Caracterização de populações de *Ralstonia solanacearum* Smith em tabaco (*Nicotiana tabacum* L.) no Brasil. [Characterization of *Ralstonia solanacearum* Smith populations in tobacco (*Nicotiana tabacum* L.) from Brazil.] *Trop. Plant Pathol.* 37:123-129.
 50. Wicker, E., Coranson-Beaudu, R., Cadasse, S., and William, M. A. 2009. Emerging strains of *Ralstonia solanacearum* in the French West Indies raise new challenges to tomato breeders. *Acta Hort.* 808:279-286.
 51. Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. 2007. *Ralstonia solanacearum* strains from Martinique (French west indies) exhibiting a new pathogenic potential. *Appl. Environ. Microbiol.* 73:6790-6801.
 52. Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., and Prior, P. 2009. Epidemiological evidence for the emergence of a new pathogenic variant of *Ralstonia solanacearum* in Martinique (French West Indies). *Plant Pathol.* 58:853-861.
 53. Wicker, E., Lefeuvre, P., Cambiaire, J. C. d., Lemaire, C., Poussier, S., and Prior, P. 2012. Contrasting recombination patterns and demographic histories of the plant pathogen *Ralstonia solanacearum* inferred from MLSA. *ISME J.* 6:961-974.