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REPORT

Project Grenada-Vitropic

Moko survey in GRENADA 2007

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PRAM - CIRAD
BP 214
97232 Lamentin
Cedex 2
Martinique, F.W.I.

Phone :
+(596) 596 42 30 00
Fax :
+(596) 5 96 42 30 01



Emmanuel WICKER
emmanuel.wicker@cirad.fr
Philippe PRIOR
philippe.prior@cirad.fr

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Emmanuel WICKER¹, Philippe PRIOR²

¹ CIRAD, UPR Horticulture, Pole de Recherche Agro-environnementale de Martinique,
Le Lamentin, MARTINIQUE, F-97285 France.

² INRA-CIRAD, UMRC53, Peuplements Végétaux et Bioagresseurs en Milieu Tropical,
Saint Pierre, La Réunion, F-97410 France.

1. Moko disease in Grenada : brief historical summary

Moko disease was first described in Grenada in 1978, caused by SFR strains (Ambrose 1987). Several surveys were done, in 1979, 1980, 1982-1983, then 1984-1985. The disease was mainly prevalent on cooking-banana “Bluggoe” (ABB), but also on Poyo (AAA). Tezenas du Montcel observed in 1983 that pure stand banana plantations, consisting of AAA varieties (Lacatan, Poyo) were less affected than small farms consisting of Bluggoe (ABB) associated with cocoa or food crops. Despite important eradication efforts done from 1980, the pathogen rapidly spread in the Northern and Center parishes of St Patrick, St Mark, St John, St Andrew (Figure 1).

Thank to regional projects involving WINBAN and funded by FAO (Ambrose 1987) or EEC (see for example IRFA reports from 1983 to 1984 (Tézenas du Montcel 1984), many experts tried to help Grenada banana growers to control Moko disease by adequate diagnosis, trials on chemical destruction of infested mats (glyphosate trials), and trials on sizing of the buffer zones.

Despite these eradication efforts, “intensive but intermittent” as Hunt pointed out in 1987 (Hunt 1987), the disease spread to the Southern parishes of St George and St Davis at the mid-1985 (Ambrose 1987). The main constraints evoked by Hunt to explain the poor control of the disease consisted of

- a lack of reporting of the disease, due to a lack of indemnisation of the growers affected by Moko disease
- the inadequate size of the buffer zones
- the difficulty for eradication teams to manage both large plantations and the very numerous small plantations
- the uncertainties concerning the efficient length of fallow

- the poor availability of suckers guaranteed to be free of Moko disease, for replantation



Figure 1. Map of Grenada showing areas of incidence of Moko disease from June 1978 to August 1980 (from Small, 1980; cited by Ambrose, 1987)

2. The objectives of the survey

The *Ralstonia solanacearum* strains reported in 1983 and 1984 were typed SFR strains (for “small, fluidal, round” colonies). However very little was known about the epidemiological situation twenty years after these surveys. Thus, the objectives of this survey were to have an updated picture of the *R.solanacearum* strains causing Moko disease in Grenada, by using the most recent diagnosis tools developed by Fegan&Prior (Fegan and Prior 2005; Prior and Fegan 2005) and to investigate whether the Maran banana nursery and the water irrigating the facility were free of any *R.solanacearum* cell.

Ralstonia solanacearum (a soilborne beta-proteobacterium) is one of the most destructive phytopathogenic bacteria throughout the world, attacking more than 250 botanical species within 54 families, within monocots and dicots, including, Solanaceous crops, Musaceae, ginger, groundnut, ... This bacterium has been recognized as a species complex composed of four distinct phylogenetic clusters, named phylotypes (Fegan and Prior 2005). Phylotype I consists of strains originating from Asia; phylotype II consists of strains originating from America; phylotype III and IV consist of strains from Africa and Indonesia, respectively. Strains of *R.solanacearum* inducing the Moko disease were previously called “race 2” strains, and subdivided in different ecotypes : B, SFR, A, D, H, AFV (for a review, see (Thwaites, Eden-Green, and Black 2000)). The most recent phylogenetic studies showed that Moko strains were clustered into four genetic groups, called sequevars : (i) sequevar 3 (equal to MultiLocus Genotype 24), originating from Central America; (ii) sequevar 4 (equal to MLG25), originating from Peru, Colombia, Amazonian Brazil ; sequevar 6 (MLG28) originating from Venezuela and Guyana, (iv) sequevar 24, originating from Brazil (Das, Sly, and Fegan 2006; Fegan 2005).SFR strains are found in sequevar 4 and 6.

3. Materials & Methods

3.1. Water sampling and monitoring of the bacterial populations

Two 1L-samples were taken from the tank irrigating the Maran nursery. Then 500 mL-fractions were filtered under vacuum on 0.2µm filter membrane, the filter was cut into fine pieces and macerated in 4 mL Tris buffer. SMSA plates were streaked with 100 µL aliquots of filter suspension (3 plates per filter) and incubated at room temperature for 3 days. Presumptive *R.solanacearum* colonies and other bacterial colonies were counted, and results were expressed in cells.L⁻¹.

3.2. Disease survey

3.2.1. Sites sampled

The surveys were done with the help of Nigel GIBBS (Ministry of Agriculture) and the Moko Eradication Team of the Ministry of Agriculture. The sites sampled were chosen in all the parishes of Grenada, according to the history of Moko disease, the banana varieties cropped (Bluggoe ABB vs. Cavendish AAA), the age and type of plantations (vitroplants vs. suckers).

The position of each site was geospatially referenced with a GPS instrument (Garmin), and mapped on the Grenada map using the software program ArcView (ESRI, Redlands, CA).

3.2.2. Observations and samplings

3.2.2.1. From banana

In each site, samples were taken from diseased banana plants. In the case of insect-mediated contamination, typical external symptoms were observed on the male flower bud (necrosis and narrowing of the raceme) and on the regime (partial yellowing of the fingers). Typical symptoms also consisted punctures within the raceme. Samples were thus taken from the raceme and the fruit fingers, and sometimes within the pseudostem. In the case of soilborne contamination, typical wilt symptoms were observed on the main plant and on the suckers. Orange to red punctuations were observed within the leaf vessels; samples were thus taken from pseudostem cross sections.

3.2.2.2. From alternative hosts and vegetable crops

Several alternative hosts of *R. solanacearum* were reported in the literature, including *Heliconia* spp and weeds species. In Grenada, former surveys identified *Xanthosoma* spp. and *Dieffenbachia* sp. (Araceae), *Colocasia bicolor* as alternative hosts (Frossard 1987). In some sites, *Heliconia* sp, *Xanthosoma* sp. and weeds (*Spermacoce* sp, *Commelina* sp.) were also sampled. An anthurium plantation, in the St Andrew parish, was visited and sampled for detection of possible latent infections.

Some cases of bacterial wilt on Solanaceae (mainly tomato) were observed in the neighbourhood of banana fields affected by Moko disease; samples of typical tomato bacterial wilt symptoms were taken there.

Many former banana estates, that were shifted to vegetable areas due to Moko, were also visited: Paradise estate (St Andrew), La Sagesse estate (St David), Pointzfield estate (St Patrick), Mirabeau estate (St Andrew). Cucurbit and Solanaceous crops were carefully observed, and samples were taken from stem sections.

3.2.3. Bacterial isolations

Isolations were done at the end of each day, after the field tours. Stem and tissue sections were surface-disinfected with ethanol, flamed, then small pieces were chopped in Tris buffer. After being macerated for at least 15 min, suspension aliquots were plated on semi-selective SMSA medium and on Kelman modified medium (K+). Cultures were grown at room temperature for 2-3

days and further plated on K⁺ for purification. Bacterial suspensions were then sealed for transport from Grenada to Réunion Island, and further purified on Sequeira medium.

3.2.4. Molecular analysis

Multiplex-PCRs

Bacterial pure suspensions were used as DNA templates, and submitted to a new version of multiplex-PCRs ((Fegan and Prior 2005; Prior and Fegan 2005); Prior personal communication 2006). Phylotype Mx-PCR was applied to assess the phylotype of each strain; each phylotype II strain was then submitted to “Musa” mx-PCR to assign them to a sequevar. Moko strains are assigned to sequevars 3, 4, 6 and 24 (Fegan & Prior, IBWS2006).

Sequencing (egl and mutS)

This was postponed due to P. Prior’s lab organization. Sequence analysis of Grenadian strains of *R. solanacearum* and their phylogenetic position will be addressed as soon as possible under the form of phylogenetic trees. These analysis will be provided as it was initially planned.

4. Results

4.1. Bacterial monitoring in the Maran nursery's irrigation water (site 1 on Figure 2)

Three samples (two of 500 mL and one of 350 mL) were concentrated by filtration and analysed for monitoring of *R. solanacearum* and total bacteria colonies (CFU = colony-forming unit). Within total bacterial populations, we counted only these which were able to grow on SMSA. These bacteria were about 1.7×10^3 CFU/L in the water, whereas not any single colony presumptive of *R. solanacearum* was found in the water.

Table 1. Mean total bacteria, and *R. solanacearum* concentration in the Maran nursery's water, as estimated by counting on semi-selective medium SMSA

Filtration	Total bacteria (Mean CFU /L)	<i>Ralstonia solanacearum</i> (Mean CFU/L)
Filtration 1	1466,7± 201,3	0±0*
Filtration 2	1946,7± 1103,7	0±0*
Filtration 3	1714,3± 457,1	0±0*
MEAN	1709,2± 640,4	0±0*

* The detection threshold of this technique is 80 CFU/L.

According to these results, irrigation water of the nursery is free of *Ralstonia solanacearum*, or at least its population is below the theoretical threshold level of 80 CFU/L.

4.2. Survey

Twenty-seven sites (n°2 to 28) were sampled (Appendix 1 and 2, and Figure 2), in which 125 plant samples were collected and isolated. *Ralstonia solanacearum* was successfully isolated from 62 samples, mainly from banana (42 samples in 22 sites), tomato (13 samples in 4 sites), unknown vine weed (2), eggplant (1), *Heliconia* sp. (1), and the weeds *Solanum americanum*(1), *Solanum nigrum* (1), *Spermacoce* sp. (1).

In the anthurium production farm of Saint Andrew, no symptom was observed, and no *R. solanacearum* colony was recovered from the sampled plants.

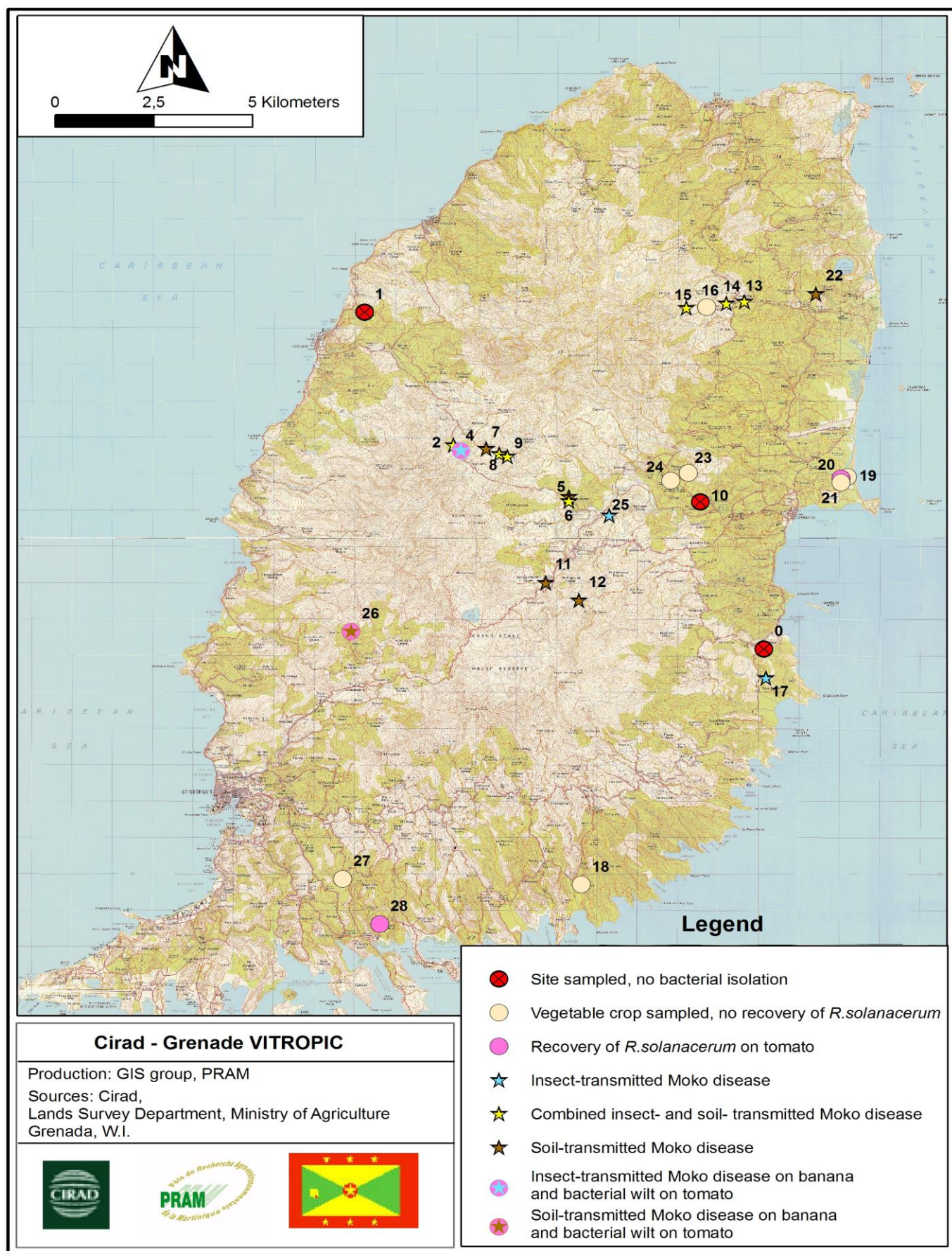


Figure 2. Map of Grenada with the 28 sampling sites of Moko disease, March 2007

During this survey, we thus visited 80-85% of the Moko-infested areas (N. Gibbs, pers. communication); the only unsampled region was St Mark, where very few banana fields remain. We didn't have time either to sample the two Heliconia plantations of Grenada, particularly in Balthazar. The biggest banana plantation of river Antoine (North-East) was not visited but is said to be completely Moko-free; our colleague C. CHABRIER saw no Moko symptom there during his visit. We couldn't sample the most recent Moko disease case, reported in Pomme Rose Estate (St. David parish).

During our survey in the vegetable fields, we noticed several viral symptoms on Solanaceous crops, mainly tomato. We thus sampled tomato leaves, and sent it to the virology lab of the 3P pole in Réunion Island. Viral analyses were done by J.-M. LETT, and the results are presented in the Appendix 3.

4.3. Molecular identification

All strains isolated from tomato were identified as phylotype II-non Musa (IIA/seq5), whereas all Moko strains were genotyped phylotype II /sequevar 6, whatever their mode of infestation.

The correspondence between the classical ecotypes observed (strains B, SFR, A, etc..) and the phylogenetic position of these strains was not assessed, and has to be done.

We could assess the phylotype and sequevar of 4 strains previously isolated by Prior in 1984 that were maintained at -80°C in the LIPM Toulouse. Interestingly, all of these strains were also phylotype II/sequevar 6.

4.4. Epidemiological observations

4.4.1. Insect-transmitted and soil-transmitted Moko disease

In most sites that were sampled, the insect-transmitted Moko disease was clearly distinguishable from the soil-transmitted Moko disease. Symptoms of the insect-transmitted Moko disease consisted of no general wilt, but a necrosis of the male flower bud (Figure 3), with sometimes an uneven yellowing discoloration of the banana fingers (Figure 4). When plants were cut into pieces, red, or yellow-orange in the case of Bluggoe, punctuations were observed in the raceme (Figures 5, 6, 7), as well as browning dry rot within the fingers (Figure 8); the vascular discoloration was found to spread in the raceme within the pseudostem, but not down to the pseudocorm. The young suckers looked healthy at this stage. In all cases, flower male buds were not removed by the farmers, whereas this is known for long as a preventive measure against aerial infestation.



Figure 3



Figure 4



Figure 5: on Cavendish



Figure 6: Cross-section of the pseudostem and raceme



Figure 7: Bluggoe



Figure 8 : symptoms in fruits

Symptoms of soilborne Moko disease consisted of blackening of the young suckers (Figure 9), with some early wilting of the young leaves. Vascular discolorations were found in the leaf vascular tissues of the pseudostem (Figure 10 and 11).



Figure 10

Figure 11



4.4.2. Mode of Moko disease infestation related to banana cultivars

Moko disease symptoms were predominantly found on cv. Williams, then on cv. Bluggoe, then on Cavendish (Table 2). To investigate the possible relationship between banana cultivars and type of infestation, Chi-square tests were applied. No distribution was significantly different of its expected value; however, the proportion of soilborne infestation with the Cavendish cases seemed higher than expected, although not significant ($P=0.058$).

Plantain was affected only by soilborne Moko, but the sample was too small to see an eventual cultivar x infestation-mode interaction.

Table 2. Number of *R.solanacearum* strains isolated from banana, related to banana genome and Moko infection (soilborne/airborne)

Banana genome	Soilborne infestation	Airborne infestation	TOTAL
Williams (AAA)	9	11	20
Bluggoe (ABB)	4	8	12
Cavendish (AAA)	6	1	7
Plantain (AAB)	2	0	2
Figue pomme (AA)	0	1	1
TOTAL	21	21	42

According to these data, there doesn't seem to have a significant influence of the banana type on the susceptibility to one or another mode of infestation, in the Grenada conditions.

4.4.3. Mode of infestation and sites

According to Table 3, seven sites were affected by both soilborne and aerial infestation, whereas six other sites were infested by soilborne Moko only, and four sites were infested by insect-transmitted Moko only.

Table 3. Sites (named by their number) infested by aerial, soilborne infestation, or both modes, related to the banana types

Banana cv.	Aerial infestation	Soilborne infestation	Soilborne and aerial infestation
Bluggoe	Sites 14,17,25	Sites 22, 26	Sites 9,15
Cavendish		Sites 6,7,12,13,14	Site 8
Williams	Sites 3,4	Sites 5,11	Sites 2,13
Plantain	Sites 13,14		
Figue-pomme	Site 6		
For all cv.	Sites 3,4,17,25	Sites 5,7,11,12,22,26	Sites 2, 6, 8, 9, 13, 14,15,

Among these “combined infestation” sites, sites 6 and 13 were particular: insect-transmitted Moko was found either on Figue-Pomme (site 6) or Plantain (site 13), whereas soilborne Moko was found on Cavendish on both sites.

From the map established according to our GPS data (Figure 2), we saw that the combined-infestation sites were distributed in many different areas, in the parishes of St John, St Patrick, St Andrew. Aerial infestations alone were only found in the parish St Andrew, in the main Moko infested area. Soilborne infestations alone were found in the parishes St Andrew, St Patrick, St Georges.

4.4.4. Moko disease related to type of material and history of Moko disease

Sites were ranked in the table 4 according to the type of Moko infection (airborne, soilborne, or both combination), and related to the banana material cropped (vitroplants vs. others) and histories of crop and Moko disease, when available. On crop history, lack of reliable data could not allow us to find out precise correlations. We could only identify trends.

Table 4 : Mode of infestation, type of banana material and history of banana crop and Moko disease.

Infestation	Sites	Parish	Banana material	Banana history	Moko disease history
Aerial	3	St John	vitroplants	5 years	?
	4	St Andrew	Bluggoe	?	?
	17	St Andrew	Bluggoe	?	?
	25	St Andrew	Bluggoe	>15 years	> 15 years
Soilborne	5	St Andrew	vitroplants	>15 years	> 15 years
	7	St Andrew	vitroplants	10 years	4 years
	11	St Andrew	vitroplants	unknown	
	12	St Andrew	vitroplants	unknown	
	22	St Patrick	bluggoe	>15 years	> 15 years
	26	St Georges	bluggoe	>15 years	> 15 years
Both ways of infection	2	St John	Vitroplants	3 years	
	6	St Andrew	Vitroplants and Figue pomme	?	?
	8	St Andrew	Vitroplants	Fallow for 7 years, then replanted	>15 years
	9	St Andrew	Bluggoe	?	?
	13	St Patrick	vitroplants and Plantain	>15 years	6-7 years
	14	St Patrick	plantain and Bluggoe	?	?
	15	St Patrick	plantain and Bluggoe	?	?

Airborne infestations seemed to appear predominantly on Bluggoe, whatever the “age” of the site. Conversely, soilborne infestations were predominant on vitroplants grown on fields with an old Moko history. In one case (site 8), new Moko soilborne cases were reported on a land abandoned for 7 years. It is not known whether the bacterium survived in alternative hosts in the absence of banana, or if it re infested the new banana plots by aerial infestation.

4.4.5. Sites with associated susceptible crops

Several sites were observed where crops susceptible to *R.solanacearum* were associated.

On the site 6, pepper plants were cropped between the banana rows, heavily infested with soilborne Moko disease. Interestingly, no symptom was observed on pepper.

On sampling site #4, tomato bacterial wilt was observed just above an airborne Moko-infested banana field. However, strains affecting tomato (ph II/seq5) were not similar to these infecting banana (ph II/seq6), and no strain that belongs to sequevar 6 was isolated from these tomatoes.

On site 14, pepper and cassava, associated with diseased banana, were sampled. Whereas cassava looked wilting, no bacteria were recovered from it. Pepper looked completely immune, and we found no latent infections within it.

On site 26, where were found soilborne Moko on banana and bacterial wilt on the neighbouring tomato, the sequevars were 6 for banana and 5 for tomato.

5. Discussion and general conclusions

The recent molecular diagnostic tools allowed to update the epidemiology view of *R.solanacearum* populations in Grenada. Surprisingly, Moko strains SFR previously isolated in 1984 were genotyped phylotype II/sequevar6.

From this survey, all strains sampled on banana were typed phylotype II/sequevar 6, whereas some were isolated after an insect-transmitted infection and others were isolated after a soilborne infestations. The expected sequevar 4/SFR strains were not found. This finding implies that phylotype II/sequevar6 strains have a largely underestimated epidemiological importance, as it is thus able to infect banana both by soilborne or mechanical injury, but also by airborne insect transmission.

This finding may be an additional evidence that Moko ecotype classification (strains, A, B, SFR, H, D, AFV) may be not phylogenetically relevant, but rather the result of plant-pathogen interaction, as suggested by Fegan (Fegan 2005). Additional basic studies on the ecology of Moko strains are strongly required to improve the understanding of the actual situation.

Sequevar 6 strains that were studied at the moment originated from Venezuela, Honduras, Belize, Hawaii; this group of strain was also recently quarantined in Australia after being introduced on Heliconia imported from Central Americas. Additional surveys in the Carribean, in particular regarding the Moko populations from Trinidad, are necessary now to investigate on how widely dispersed are these strains in the Carribean countries affected by Moko disease.

At the same time, we were not able to isolate *R.solanacearum* strains from Cucurbits or Araceae or tomato, even within the former banana estates whose banana crops collapsed because of Moko disease (Pointzfield estate, Paradise estate, Mirabeau estate,...). We anticipate that the Grenada situation may be different of the Martinique situation, where strains typed sequevar 4 are prevalent on cucurbits, anthurium, tomato, Heliconia but not on banana.

As observed from the distribution of the Moko cases, Moko disease is still well established in Grenada, and the insect-transmitted strains are still epidemiologically highly active.

Thank to the use of vitroplants, the medium-distance dissemination of the disease by infected suckers is likely to be weak, except for the cultivars that are not grown as vitroplants (plantains, Bluggoe, figue-pomme, etc...). But long distance dissemination by insect transmission can always occur from male bud to male bud. To contain the disease and prevent it to establish within the soil from an insect-transmitted infection, it is very important that the growers learn again the basic prophylactic measures and acts.

Three broad situations were observed, whose management will be specific for each.

Situation 1: No Moko reported (ex: River Antoine)

Here, the most strict quarantine measures must be respected :

- any Bluggoe shoot should be eliminated, to prevent any aerial contamination, since this cultivar is known everywhere to be extremely susceptible to insect-transmitted strains.
- banana plants entering in this area should be only vitroplants
- pruning tools, tractors and car wheels, etc.. should be thoroughly disinfected to prevent any mechanical contamination
- (cf. eradication campaign in 1984).

Situation 2: Moko contamination, with only insect-transmitted disease. This is the early stage of Moko establishment in the field.

The preventive measures should be reactivated, to prevent the bacterium from entering within the soil:

- male buds must be hand cut (not with a tool!),
- any necrosis on the male bud and raceme should be reported to the Moko eradication team
- Buffer zones should be reactivated (8 meters (Ambrose 1987))

Moreover, all Bluggoe in the neighbourhood should be destroyed and any replanting of this cultivar should be prohibited.

Situation 3: Moko soil transmitted, or insect-and soil-transmitted

- Buffer zones should be reactivated
- Innovative control measures should be tested there :

- Varietal screening : although most of the AAA cultivars are susceptible to Moko disease, there are some references concerning the field tolerance of Pelipita (Thwaites, Eden-Green, and Black 2000), as well as F2P2, Babi Yadefana, 1319-01, 1741-01(de Oliveira e Silva et al. 2000). The parents of the new CIRAD banana hybrids may also be evaluated in these situations.
- Cover crops, with the aim of decreasing the inoculum potential to a level acceptable for banana cropping. The few data available concern mainly the vegetable cropping systems , but it may be successfully transferred to banana; examples are the rotation with French marigolds (*Tagetes patula*)(Terblanche 2002; Terblanche and de Villiers 1997), rotations with onion or carrot to control *R.solanacearum* on potato (Lemaga et al. 2005), or association with chive (*Allium tuberosum* and *A. fistulosum*)(Yu 1999).
- Biofumigation: examples exist in the literature with the use of Brassicas (Arthy, Akiew, and Kirkegaard 2005), or the use of lixivado in Colombia

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APPENDIX 1 : Sites analysed during the survey

Site	GPS coordinates		Elevation (m)	Parish	Location	Name of the grower
	North	West				
1	12,17286	61,72195		St John	Maran	Banana Nursery
2	12,13983	61,70175	378	St John	Clozier	CARTAN Curley
3	12,13983	61,70175	378	St John	Clozier	CARTAN Curley
4	12,13845	61,7		St Andrew	Belvedere	
5	12,12698	61,6754	207	St Andrew	Chantilly	
6	12,12698	61,6754	207	St Andrew	Chantilly	
7	12,13888	61,69424	437	St Andrew	Chadeau	
8	12,13763	61,69128	437	St Andrew	Chadeau	
9	12,13699	61,68941	409	St Andrew		
10	12,12573	61,64548	132	St Andrew	Birch Grove ?	SAAB saint-Claude
11	12,10563	61,68074	324	St Andrew	Adelphi	
12	12,10125	61,67313	271	St Andrew	Monplaisir	
13	12,17549	61,6355	125	St Patrick	Hermitage Nord	
14	12,17503	61,63962	190	St Patrick	Hermitage	
15	12,1739	61,64871	278	St Patrick	Peggy's Whim	
16	12,17409	61,64403	220	St Patrick	Hermitage	RICHARD James
0	12,08915	61,63101	98	St Andrew	Hope Estate	
17	12,08195	61,63056	45	St Andrew	Sud de Hope estate	
18	12,0306	61,67257	16	St David	La Sagesse Estate	
19	12,13191	61,61201	22	St Andrew	Paradise	
20	12,13151	61,61344	22	St Andrew	Paradise	CHARLES Benedict
21	12,13151	61,61344	22	St Andrew	Paradise	
22	12,17739	61,61922	40	St Patrick	Poyntzfield Estate	
23	12,13293	61,64825	155	St Andrew	Mirabeau Estate	
24	12,13096	61,65222	191	St Andrew	Mirabeau Farm School	
25	12,12236	61,66622	170	St Andrew	Birch Grove	
26	12,09352	61,72499	204	St Georges	New Hampshire	Granton Estate
27	12,03203	61,72695	88	St Georges	Hope Vale Estate	BEIN Jeffrey
28	12,02081	61,71842	40	St David	Calivigny	WesterHall Agricultural Producers' Company (WAPCO)

Appendix 2 : Samples analysed during the Moko and BW survey

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
2	2,1,1	19/03/2007	Banana	Williams	fruit	Typical symptoms	+	A
	2,1,2	19/03/2007	Banana	Williams	fruit	Typical symptoms	+	A
	2,1,3	19/03/2007	Banana	Williams	raceme	Typical punctures	+	A
	2,2,1	19/03/2007	Banana	Williams	raceme		+	A
	2,2,2	19/03/2007	Banana	Williams	fruit		+	A
	2,3	19/03/2007	Banana	Williams	raceme		+	A
	2,4	19/03/2007	Banana	Williams	pseudostem (centre)	ponctuations in the center (raceme)	+	S?
	2,5	19/03/2007	Banana	Williams	racemes		+	A
	2,6	19/03/2007	Banana	Williams	raceme		+	A
	2,7	19/03/2007	<i>Solanum torvum</i>		Stem	no wilt	-	
	2,8,1	19/03/2007	Weed liane		Stem and roots		+	
	2,8,2	19/03/2007	Weed liane		Stemand roots		+	
3	3,1	19/03/2007	Banana	Williams	raceme		-	
	3,2	19/03/2007	Banana	Williams	raceme		+	
4	4,1	19/03/2007	Banana	Williams	raceme		+	
	4,2,1	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,2	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,3	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,4	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,5	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,6	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,2,7	19/03/2007	Tomato	Heat Master ?	Stem	Wilt	+	
	4,3,1	19/03/2007	<i>Solanum americanum</i>		collar	no wilt	+	

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
	4,3,2	19/03/2007	<i>Solanum americanum</i>		collar		-	
	4,3,3	19/03/2007	<i>Solanum americanum</i>		collar		-	
5	5,1	20/03/2007	Banana	Williams	sucker		+	S
	5,2	20/03/2007	Banana	Williams	sucker		+	S
	5,3	20/03/2007	Banana	Williams	sucker		+	S
	5,4	20/03/2007	Banana	Williams	sucker		+	S
6	6,1,1	20/03/2007	Banana	Bluggoe	pseudostem (leaves)		-	
	6,1,2	20/03/2007	Banana	Bluggoe	pseudostem (leaves)		-	
	6,2	20/03/2007	Heliconia sp.		Stem base		+	
	6,3	20/03/2007	Banana	Figue-pomme	raceme		+	A
7	7,1	20/03/2007	Banana	Cavendish/MA13	pseudostem de sucker		+	S
	7,2	20/03/2007	Banana	Cavendish/MA13	pseudostem de sucker		-	
	7,3	20/03/2007	Banana	Cavendish/MA13	doigts avortés pseudostem de sucker		-	
	7,4	20/03/2007	Banana	Cavendish/MA13	pseudostem de sucker		+	S
	7,5	20/03/2007	<i>Solanum nigrum</i>				+	
8	8,1	20/03/2007	Banana	Cavendish	raceme		+	A
	8,2	20/03/2007	Banana	Cavendish	pseudostem		+	S
	8,3	20/03/2007	<i>Xanthosoma sagittifolia</i>		bulbe	symptomless	-	
9	9,1	20/03/2007	Banana	Bluggoe	raceme		+	A
	9,2	20/03/2007	Banana	Bluggoe	raceme		+	A
	9,3 ^A	20/03/2007	Banana	Bluggoe	raceme		+	A
	9,3 ^B	20/03/2007	Banana	Bluggoe	pseudostem		+	S
	9,4	20/03/2007	<i>Spermacoce</i> sp ?		roots		+	

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
10	10,1,1	20/03/2007	<i>Anthurium ferrierense</i>		rhizome	leaf yellowings	-	
	10,1,2	20/03/2007	<i>Anthurium ferrierense</i>		rhizome	leaf yellowings	-	
	10,2	20/03/2007	<i>Anthurium ferrierense</i>		roots+rhizome	leaf yellowings	-	
11	11,1	20/03/2007	Banana	Williams	pseudostem		+	S
	11,2	20/03/2007	Banana	Williams	sucker		+	S
	11,3	20/03/2007	Banana	Williams	pseudostem		+	S
	11,4	20/03/2007	<i>Xanthosoma sagittifolia</i>				-	
12	12,1	20/03/2007	Banana	Cavendish	pseudostem (leaves)		+	S
	12,2 ^A	20/03/2007	Banana	Cavendish	pseudostem		+	S
	12,2 ^B	20/03/2007	Banana	Cavendish	pseudostem		+	S
13	13,1,1	21/03/2007	Pumpkin		Stem-roots	Leaf Wilting	-	
	13,1,2	21/03/2007	Pumpkin		Stem	Leaf Wilting	-	
	13,2	21/03/2007	Banana	Williams	pseudostem		+	S
	13,3	21/03/2007	Banana	Williams	raceme		+	A
	13,4	21/03/2007	Banana	Plantain	pseudostem		+	S
14	14,1	21/03/2007	Banana	Plantain	pseudostem		+	S
	14,2	21/03/2007	Pepper		Stem	No Wilt	-	
	14,3	21/03/2007	<i>Solanum torvum</i> <i>Manioc (Manihot esculentum)</i>		collar	No Wilt	-	
	14,4	21/03/2007			Stem	Wilt	-	
	14,5	21/03/2007	Banana	Bluggoe	raceme in pseudostem	aerial infection	+	A
15	15,1	21/03/2007	Banana	Bluggoe	raceme		+	A
	15,2	21/03/2007	Banana	Bluggoe	pseudostem		+	S
16	16,1	21/03/2007	Tomato		Stem	Wilt	-	
17	17,1	21/03/2007	Banana	Bluggoe	raceme		+	A

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
18	17,2	21/03/2007	Banana	Bluggoe	raceme		+	A
	18,1	21/03/2007	Honeydew		Stem	Wilt	-	
	18,2	21/03/2007	Pumpkin		Stem	Partial Wilt	-	
	18,3	21/03/2007	Pepper		Stem		-	
	18,4	21/03/2007	Honeydew		Stem	Wilt	-	
	18,5,1	21/03/2007	Courgette		Leaf	Leaf Wilting	-	
	18,5,2	21/03/2007	Courgette		Stem	Leaf Wilting	-	
	18,6	21/03/2007	Sweet pepper		leaf	Xanthomonas	+	
19	18,7	21/03/2007	Sweet pepper		leaf	Xanthomonas	+	
	19,1	22/03/2007	Water melon		Stem	Leaf Wilting	-	
	19,2	22/03/2007	Water melon		Stem	Leaf Wilting	-	
	19,3	22/03/2007	Banana	Cavendish	raceme		-	
	19,4	22/03/2007	Tomato	Cherry	Stem		-	
	19,5	22/03/2007	<i>Portulaca oleracea</i>		roots	in tomato field	-	
	19,6	22/03/2007	Tomato	Heat Master	Stem		-	
	19,7	22/03/2007	Spermacoce sp ?		roots	in banana field	-	
	19,7	22/03/2007	Tomato		fruit	Xanthomonas	+	
20	19,8	22/03/2007	Tomato	Heat Master	leaf	Xanthomonas	-	
	20,1	22/03/2007	Tomato	Heat Master	Stem		-	
	20,2	22/03/2007	Tomato	Heat Master	Stem		-	
	20,3	22/03/2007	Tomato	Heat Master	Stem		-	
	20,4	22/03/2007	<i>Cleome aculeata</i>		roots		-	
	20,5	22/03/2007	Tomato		Stem	Wilt	+	
	20,6	22/03/2007	Tomato		Stem		-	
21	21,1	22/03/2007	Pumpkin		Stem		-	

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
22	22,1	22/03/2007	Tomato		Stem		-	
	22,2	22/03/2007	Tomato		leaf	Xanthomonas	+	
	22,3	22/03/2007	Banana	Bluggoe	pseudostem	Moko + Panama disease	+	S
23	23,1	22/03/2007	Pepper		leaf	Xanthomonas	+	
24	24,1	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,2	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,3	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,4	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,5	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,6	22/03/2007	Eggplant		Stem	Wilt, holes in the stem	-	
	24,7 ^A	22/03/2007	Tomato		leaf	Xanthomonas	+	
	24,7 ^B	22/03/2007	Tomato		leaf	Xanthomonas	+	
25	25,1	22/03/2007	Banana	Bluggoe	raceme	Yellow male bud	-	
	25,2	22/03/2007	Banana	Bluggoe	raceme	near 25.1	+	A
	25,3	22/03/2007	Banana	Bluggoe	pseudostem	Same plant as 25.2	-	
26	26,1	22/03/2007	Tomato	Heat Master	Stem	Wilt	+	
	26,2	22/03/2007	Tomato	Heat Master	Stem	Wilt	+	
	26,3	22/03/2007	Tomato	Heat Master	Stem	Wilt	+	
	26,5	22/03/2007	Tomato	Heat Master	Stem	Wilt	+	
	26,6	22/03/2007	Banana	Bluggoe	raceme in pseudostem		-	
	26,7	22/03/2007	Banana	Bluggoe	pseudostem		+	S
	26,8	22/03/2007	Sygin		rhizome	near 26.6	-	
	26,8	22/03/2007	Sygin		rhizome	near 26.6	-	
27	27,1	22/03/2007	Tomato		fruit mûr		-	
	27,2	22/03/2007	Tomato		fruit vert	Xanthomonas	+	

Site	N°	Isolation date	Species	Cultivar	Organ	Observations	Isolation of <i>R.solanacearum</i>	Aerial/ Soilborne Moko
28	27,3	22/03/2007	<i>Cleome aculeata</i>		roots	near tomato	-	
	28,1	22/03/2007	Tomato		Stem		-	
	28,2	22/03/2007	Tomato		Stem		-	
	28,3	22/03/2007	Tomato		Stem		+?	
	28,4	22/03/2007	Eggplant		Stem		+	
	28,5	22/03/2007	Sweet pepper		leaf	Xanthomonas	+	

APPENDIX 3 : Characterisation of the viruses affecting tomato crops in Grenada.

(J-M. LETT, UMR PVBMT, Réunion Island)

Ref. Labo	Location	Host plant	Storage	Detection Begomo PCR		Sequencing result ADN-B	Sequencing result ADN-A	Comments
				ADN-A	ADN-B			
308_GBel0307	Belvedere	Tomato	Bos	-	-			
309_GBel0307	Belvedere	Tomato	Bos	-	-			
310_GHe0307	Hermitage	Tomato	Bos	+++	-	99% TYLCV-Martinique		
311_GHe0307	Hermitage	Tomato	Bos	++	-			
312_GHe0307	Hermitage	Tomato	Bos	+++	+++	ADN-A 99% TYLCV-Martinique	Probleme resequençage !?	ADN-A PYMV si confirmation ADN-B PYMV?
313_GSE0307	La sagesse estate	Pepper	Bos	-	-			
314_GSE0307	La sagesse estate	Sweet pepper	Bos	-	-			
315_GPa0307	Paradise	Tomato	Bos	+++	-	99% TYLCV-Martinique		
316_GPa0307	Paradise	Tomato	Bos	+++	+++	ADN-A 99% TYLCV-Martinique	ADN-B 95% PYMTrinidadV-Trinidad&Tobago	ADN-A PYMV ?
317_GME0307	Mirabeau estate	Pepper	Bos	-	-			
318_GMF0307	Mirabeau farm school	Tomato	Bos	+++	-	99% TYLCV-Martinique		
319_GSG0307	St-Georges	Pepper	Bos	-	-			

320_GSG0307	St-Georges	Tomato	Bos	+++	-	99% TYLCV-Martinique		
321_GSG0307	St-Georges	Tomato	Bos	++++	++++	ADN-A 99% TYLCV-Martinique	ADN-B 96% PYMV-Puerto Rico et PYMTrinidadV-Trinidad and Tobago	ADN-A PYMV ?
322_GSD0307	St-David	Tomato	Bos	++++	-	99% TYLCV-Martinique		
323_GSD0307	St-David	Sweet pepper	Bos	+	-			
324_G0304	?	? Tomato	Bos	++++	-	99% TYLCV-Martinique		

Conclusion : Viral diseases of tomato in Grenada are caused by the Tomato Yellow Leaf Curl Virus (TYLCV), invasive from the Old World, and by a bipartite begomovirus, the Potato Yellow Mosaic Virus, from the New World, strain Trinidad.