Cucumis melo, *Cucumis sativus*, *Cucurbita moschata*, and *Anthurium* spp, New Hosts of *Ralstonia solanacearum* in Martinique (French West Indies)

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In the French West Indies (FWI), endemic bacterial wilt caused by lowland tropical strains of *Ralstonia solanacearum* is known to be a devastating disease to major solanaceous food and cash-crops like potato, tomato, eggplant and pepper (Prior & Steva, 1990). Since 1999, hybrid and local anthurium (*Anthurium sp*.) productions which have developed in the wetter and elevated areas of Martinique (Centre-North Atlantic) were dramatically affected by an unknown bacterial disease. The disease was not bacterial spot (*Acidovorax anthurii*) nor bacterial blight (*Xanthomonas axonopodis* pv. *dieffenbachiae*), but bacterial wilt due to novel and unexpected strains of *R. solanacearum*. Late in 2001 *R. solanacearum* was isolated for the first time from wilted native heliconia (*Heliconia caribaea*, Strelitziaceae) established in the same area. Simultaneously, massive wilt damage due to bacterial wilt was also noted for the first time on cantaloupe (*Cucumis melo*), zucchini (*Cucurbita pepo*), pumpkin (*Cucurbita moschata*) and cucumber (*Cucumis sativus*). These outbreaks were caused by the same new pathotypes of *R. solanacearum.*

Symptoms (*colour plate, back-cover*).

Bacterial wilt on anthurium resulted in a definitive decline of many plantation and the disease extended rapidly in Martinique. Characteristic symptoms consist of greasy, water-soaked lesions (lower face) which turn necrotic with greasy margins (upper face). When the disease becomes systemic, these lesions, generally originating from the insertion point of the leaf on the petiole, develop following the main and secondary veins in a full or partial glove-shape. External infection (disseminated by water) may developed from any natural opening such as hydatodes. Regardless of the way the infection occurs, R. solanacearum establishes itself by flooding into parenchyma tissue, resulting in colonization of the vascular system until most of the leaves or the whole plant collapse. Leaves may turn yellow or not depending on the severity of the systemic invasion, and the stem, a rhizome structure (formally named 'caudex') may rot with abundant bacterial ooze. Major characteristic bacterial wilt symptoms on native heliconia, surrounding heavily infested and abandoned anthurium plantations, as browning and rotting of the cigar, which is the youngest rolled-up emerging leaf. Fully expanded leaves may also wilt. A section of the pseudo-stem showed brown-reddish discoloration within and along vascular elements. Bacterial wilt on cucurbit plot began in foci. At that stage, symptoms developed quite late (fruit setting) on cantaloupe and pumpkin. When established in the plot, bacterial wilt may destroy the entire crop only 2-3 weeks after planting. Disease is typically soilborne and develops rapidly from older to youngest leaves which may wilt or not. Leaves turn yellow with necrotic lesions between or along major veins (same as for anthurium). The whole plant collapses and dies, while there are no apparent symptoms on mature fruits. Such bacterial wilt development is very fast on zucchini compared to cantaloupe and pumpkin.

Characterization.

Bacteria were isolated from oozing stems of rotted rhizomes and surface-disinfected leaves of anthurium, and from stems of wilted cucurbits. The culture medium used was Kelman's triphenyltetrazolium chloride medium (TZC). Individual bacterial colonies were selected and assessed for purity after 48 h incubation at 28±1°C. Representative isolates were mucoid, with pink-reddish swirling egg-shaped pigmentation pattern characteristic of *R. solanacearum* (Kelman, 1954). All strains tested were oxidase positive, strictly aerobic, nitrate and citrate positive, and induced a hypersensitive reaction on tobacco leaves (cv. Xanthi) within 24 h. Strains from anthurium, pumpkin, zucchini and cantaloupe were tested for biovar determination using a modified version of the procedures of Hayward (1964), and were metabolically classified as biovar 1. PCR amplification, using the *R. solanacearum* species-specific primers set 759 and 760 (Opina *et al*. 1997) generated the expected 280bp amplicon (internal control for the following multiplex-PCR) with all isolates tested. In terms of genetic diversity, typing was assayed using multiplex PCRs for subspecies: phylotype- and Musa-specific primers (Fegan and Prior 2002, Prior and Fegan 2002). Our results clearly demonstrate that these anthurium and cucurbit strains are not distinguishable from reference strains ANT307 and 11212 (Prior and Fegan 2002), falling in the phylotype II, sequevar 4 (or MLG25) cluster, unifying strains (ecotype SFR/A) that induce the insect-transmitted form of the Moko disease (banana bacterial wilt). Pathogenicity tests were performed on cantaloupe (*Cucumis melo,* cv. Charentais), cucumber (*C. sativus*, cv. Gemini), pumpkin (*Cucurbita moschata*, cv. Phoenix), *Anthurium andreanum* (cv. Amigo), and Cavendish banana (*Musa acuminata*) cv. Grande naine. Inoculation was by infiltrating a bacterial suspension (10^7 cfu.ml^{-1}) into the stem or pseudo-stem, or by pouring 10 ml of inoculum (10^8 cfu.ml^{-1}) onto the pot surface after wounding of roots. One or two weeks after inoculation, these tests reproduced symptoms observed in natural conditions : wilting and necrosis to cucurbit leaves, water-soaking and blackening to anthurium leaves and *R. solanacearum* was successfully recovered from these symptoms. All strains were avirulent on banana (encoded NPB) as no symptom were observed 45 days after inoculations. However, *R. solanacearum* was consistently recovered from these asymptomatic plants.

Discussion

From our knowledge, this is the first record of cantaloupe and pumpkin as hosts of *R. solanacearum*. In the literature, bacterial wilt caused by biovar 1 strains was reported on summer squash, zucchini summer squash and cucumber from Brazil (Oliveira and Moura, 1994; Sinigaglia *et al.,* 2001). Natural occurrence of bacterial wilt on anthurium was reported to be caused by biovar 3 strains in Singapore (Yik *et al*., 1994), Reunion Island (Poussier *et al.*, 2000) and Mauritius Island (Dookun *et al.,* 2001), or by biovar 4 strains in Taïwan (Su and Leu, 1995). Unlike regular biovar 3 (phylotype I) strains pathogenic to anthurium which developed as focus under the shade-house, behaviour of strains falling in phylotype II seems to be dramatically active with regard to major epidemiological traits like fast spreading, rapid disease onset and acquisition of new hosts. This is the first time that biovar 1 strains are found to cause so much damage in anthurium fields. These novel strains had also the unique feature of being genotypically indistinguishable from Moko strains, while being not pathogenic to banana. Recently, biovar 1 strains infecting anthurium pots were reported from Florida, but these strains were reported pathogenic to banana (Norman and Yuen, 1999). At present we don't know if they also belong to phylotype II, sequevar 4.

Using the hierarchical classification from Fegan and Prior (2002) and relevant molecular tests, these cucurbitaceous and anthurium NPB strains fall in phylotype II, sequevar 4 and more specifically in the NPB sub-group of Musa's sequevar 4. Whereas, they are pathogenic to different botanical families and were simultaneously isolated from distinct geographic and climatic areas (North and Center hills for anthurium strains, Windward South border for cucurbitaceous strains). In the FWI, bacterial wilt has only been known to affect solanaceous crops, being caused by biovar 1 (phylotype II, sequevar 5) and biovar 3 (phylotype I) strains (Prior and Steva 1990; Fegan and Prior, 2002). In Martinique, the phylogenetic position of a large collection of *R. solanacearum* strains was predicted using multiplex-PCRs tools developed by Fegan and Prior. This collection includes strains isolated from 1982 to 2002. Molecular typing indicated all phylotype II, NPB-sequevar 4 isolates of *R. solanacearum* were collected after 1998, the first one (strain 98-1537) being isolated on tomato. This raises questions about the origin of these new outbreaks and epidemiological features of these particular NPB sequevar 4 strains of *R. solanacearum,* especially their potential for dissemination as the "Moko" phylogenetic groups are the only ones known to be insect transmitted. It may be possible that NPB strains still share this phenotypic traits with historical Moko strains pathogenic to banana. In addition, following that survey, other isolates of *R. solanacearum* belonging to the same group of NPB sequevar 4 strains were baited from New-Guinea impatiens (*Impatiens hawkerii*), wild heliconia (*Heliconia caribaea*) and weeds *Portulaca olaracea, Cleome viscose* and *Solanum americanum*. This indicates the establishment of these strains in Martinique. We hypothesised NPB sequevar 4 strains emerged as an original and endemic pathogenic population of *R. solanacearum*. It is anticipated that resistance to bacterial wilt selected for in solanaceous species attacked by common biovar 1 or biovar 3 strains may be by-passed by these strains. From an agronomical point of view, these strains remain a major threat for banana industry and provide a unique model to explore genetic determinism for host specificity within *R. solanacearum* complex species.

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Occurrence of Bacterial Wilt of Bittergourd and Ridgegourd in Kerala

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Bacterial wilt caused by *Ralstonia solanacearum* is a devastating soilborne disease in the tropics. It is one of the major constraints for cultivation of solanaceous vegetables in Kerala. Bacterial wilt of cucurbits caused by this bacterium has become a problem in Kerala in recent years. Mathew *et al.* (1994) reported the incidence of bacterial wilt in pumpkin and snake gourd from Kerala. Serious wilt symptom was noticed in some of the bittergourd (*Momordica charantia* L.) and ridgegourd (*Luffa acutangula* L.) plants grown in vegetable research plots of College of Horticulture, Vellanikkara,Thrissur, Kerala. The characteristic symptoms of the disease was drooping of the leaves followed by sudden wilting of the entire plant. Brown vascular discolouration was noticed in stem and root tissues. The diseased plants subjected to laboratory studies showed, profuse bacterial ooze from the cut end of the stem. Isolation of the pathogen on TZC medium showed smooth, raised, creamy white fluidal colonies having pinkish center after 48h incubation at 28 ± 2^0C. Pathogenicity test on respective hosts and other cucurbits such as pumpkin, snakegourd, ashgourd and solanaceous crops viz. Tomato, eggplant and chilli showed typical wilt symptoms within 5-15 days after inoculation.

Characterization tests carried out using standard techniques showed that, both bittergourd and ridgegourd isolates were gram negative, aerobic, positive for oxidative reaction, hydrolysis of Tween-80, nitrate reduction and negative for arginine dihydrolase reaction. Identification of race based on host range and their ability to induce hypersensitive response on bell-pepper (Capsicum) leaves showed that both isolates belonged to race-1. Determination of biovars according to their ability to oxidize disaccharides or utilize hexose alcohols indicated that, the isolates of bittergourd and ridgegourd belonged to biovar 3.

Thus based on morphological physiological, biochemical and pathogenicity tests, this pathogen was identified as *R. solanacearum* race 1, biovar 3. Pan *et al* (1996) reported bacterial wilt of loofah (*Luffa cylindrica*) caused by *R. solanacearum* belonging to race 1 and biovar 3. However, this is the first report on this bacterial wilt pathogen of cucurbits from India.

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Bacterial wilt symptoms
due to phylotype II, sequevar 4 strains
non pathogenic to banana (NPB)

Wicker *et al*, this issue

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[1] Bacterial wilt on lower face of an anthurium leaf after development of a typical soilborne and vascular infection by *R. solanacearum*, which results in water soaked lesions along the main vein. **[2]** Symptom include yellowing of the upper face of the leaf and a partial glove-shaped aspect due to necrotic lesions originating from the insertion point of the leaf on the petiole. **[3a]** Greasy, water-soaked lesions following main and secondary veins allowing bacteria to oozefrom stomata **[3b]** as better seen after magnification of the orange frame. **[4]**. Full glove-shaped necrotic aspect of the leaf which will progressively die. **[5]**.Typical greasy, water-soaked and necrotic symptoms observed on an anthurium leaf after an airborne contamination allowing the bacterium to invade parenchyma tissues, probably from hydatodes. **[6]**.Typical symptoms of bacterial wilt on cantaloupe result in necrotic lesions along and between veins, as well as on the lamina edge **[7]**. Systemic invasion progressively wilts the entire plant from older to younger leaves, which remains hanging onto petioles. There are no external symptoms on fruits. **[8]**. Bacteria oozing from the stem of a wilted cantaloupe 36 h after artificial soil inoculation. Such ooze is observable under natural field infestation. **[9]**. Bacterial wilt on zucchini leaves showing water-soaked and necrotic symptoms similar to those described on anthurium and cantaloupe. **[10]** Typical wilt symptoms on cucumber.