Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Wilt Caused by *Ralstonia solanacearum* on *Plectranthus amboinicus* in Martinique

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Plant Dis. 105:2239, 2021; published online as <https://doi.org/10.1094/PDIS-12-20-2622-PDN>. Accepted for publication 19 February 2021.

Plectranthus amboinicus, commonly known as Gwo ten in the French West Indies (Martinique), is a semisucculent perennial plant of the Lamiaceae family. This aromatic plant, which is widespread naturally throughout the tropics, is of economic importance because of the therapeutic and nutritional properties attributed to its natural phytochemical compounds, which are highly valued in the pharmaceutical industry. In March 2019, wilted *P. amboinicus* plants intercropped with tomato plants (cv. Heatmaster) in order to reduce the insect-pest damages on tomato were observed in a field located at the CIRAD experimental station in Lamentin, Martinique (14.663194° N, -60.999167° W). Average disease incidence of 65.74% was recorded on *P. amboinicus*, in three plots with an area of 22.04 m². The initial symptoms observed were irregular, black, necrotic lesions on leaves. After 10 days, plants wilted and black stripes were observed on stems. Within 4 weeks, >50% of plants were fully wilted. Longitudinal stem sections of the wilted plants showed brown vascular discoloration. The cut stems of the wilted plants released a whitish bacterial ooze in water. In all, 108 stem sections were collected and surface disinfected with 70% ethanol; each was crushed in 2 ml of Tris buffer and then processed for bacterial isolation by plating on modified semiselective medium from South Africa (SMSA) (Engelbrecht 1994). Typical *Ralstonia solanacearum* colonies grew on SMSA for 100 of the 108 samples after incubation for 48 h at 28°C and were identified as *R. solanacearum* using diagnostic PCR with 759/760 primers (Opina et al. 1997). A phylotype-specific multiplex PCR (Fegan and Prior 2005) classified all the strains in *R. solanacearum* phylotype IIA. A subset of 11 strains was selected for sequevar identification. All the strains were identified as sequevar

I-39 (100% nucleotide identity with strain ANT92, GenBank no. EF371828), by partial *egl* sequencing (Fegan and Prior 2005) (GenBank nos. MT314067 to MT314077). This sequevar has been reported to be widespread in the Caribbean and tropical America on vegetable crops (particularly on tomato) but not on *P. amboinicus* (Deberdt et al. 2014; Ramsubhag et al. 2012; Wicker et al. 2007). To fulfill Koch's postulates, a reference strain, isolated from diseased *P. amboinicus* (CFBP 8733, phylotype IIA/sequevar 39), was inoculated on 30 healthy *P. amboinicus* plants. A common tomato cultivar grown in Martinique (cv. Heatmaster) was also inoculated on 30 plants with the same bacterial suspension. Three-week-old plants of both crops grown in sterilized field soil were inoculated by soil drenching with 20 ml of a calibrated suspension (10⁸ CFU/ml). *P. amboinicus* and tomato plants drenched with sterile water served as negative controls. Plants were grown in a fully controlled environment at day/night temperatures of 30 and 26 ± 2°C under high relative humidity (80%). The *P. amboinicus* plants started wilting 9 days after inoculation, and within 4 weeks 60% of the *P. amboinicus* plants had wilted. The tomato plants started wilting 5 days after inoculation with 62% of plants wilting within 4 weeks. *R. solanacearum* was recovered from all symptomatic plants on modified SMSA. No symptoms were observed and no *R. solanacearum* strains were isolated from negative control plants. To our knowledge, this is the first report of *R. solanacearum* causing bacterial wilt on Gwo ten (*P. amboinicus*) in Martinique. The importance of this discovery lies in the reporting of an additional host for *R. solanacearum* that can be associated with other crops such as the tomato crop in order to reduce the abundance of insect pests. Further studies need to be conducted to assess the precise distribution of bacterial wilt disease on *P. amboinicus* in Martinique and to develop a plan of action avoiding its association with *R. solanacearum* host crops such as tomato for reducing epidemic risk.

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The author(s) declare no conflict of interest.

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Keywords: prokaryotes, epidemiology, disease development and spread, pathogen survival

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