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# plant disease

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## DISEASE NOTES

## First Report of Copper-resistant *Xanthomonas citri* pv. *citri* Pathotype A Causing Asiatic Citrus Canker in Réunion, France

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## ABSTRACT

*Xanthomonas citri* pv. *citri* causes Asiatic citrus canker (ACC), which induces erumpent, callus-like lesions on all aerial organs, and consequently defoliation, premature fruit drop, and twig dieback under high inoculum pressure. Present in most tropical and subtropical citrus producing regions, the disease can lead to important decreases in yield and limitations to export markets due to the quarantine status of the pathogen in some countries ([Graham et al. 2004](#)). Strains assigned to genetic lineage 1 within pathotype A, which has the widest host range comprising most citrus cultivars and citrus relatives, were shown to be primarily involved in the worldwide expansion of the bacterium over the 20th century ([Pruvost et al. 2014](#)). ACC has been considered endemic in Réunion Island since the 1970s, but was likely present earlier and was not subjected to any eradication attempt. Most citrus growers on the island frequently apply copper-based pesticides as part of integrated pest management programs. A recurring lack of ACC control in several citrus groves was investigated in 2014 using our lab collection of authenticated *X. citri* pv. *citri* strains. A total of 501 strains from active epidemics as well as 123 historical strains isolated from 1978 onward were tested for copper resistance using PCR assays targeting *copL* ([Behlau et al. 2013](#)). A total of 115 strains produced the expected amplicon whereas others (including all historical strains) and the negative control (H<sub>2</sub>O) did not. Phenotypic tests were carried out on low-complexing mineral salts casitone-yeast extract-glycerol-agar (CYE) plates ([Zevenhuizen et al. 1979](#)) flooded with bacterial suspensions ( $\approx 5 \times 10^6$  cfu ml<sup>-1</sup>) in sterile tris buffer (pH 7.2), allowed to dry for 15 min., inoculated with a sterile

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CuSO<sub>4</sub>, 5H<sub>2</sub>O solution (10 mg ml<sup>-1</sup>) with an easySpiral plater used to create a gradient of log-increasing copper concentrations toward the plate center and incubated for 2 days at 28°C. Using this assay, *copL*<sup>+</sup> strains grew over a radius (measured from the plate periphery where the concentration of copper are lowest) >23 mm whereas *copL*<sup>-</sup> strains growth radius were constantly lower. Copper-resistant strains (CRS) had minimum inhibitory concentrations for copper sulfate ranging from 128 to 256 µl ml<sup>-1</sup> (Pruvost et al. 1998). A subset of 20 CRS and 40 copper-susceptible strains (CSS) (including historical strains) were all assigned to genetic lineage 1 by minisatellite typing (performed as reported by Pruvost et al. 2014) and split into two multilocus haplotypes shared also with CSS. CRS were widespread in most citrus production areas of the island where they coexisted with CSS. The oldest CRS dated from 2010. Because no extreme hurricane hit the island between 2010 and 2014, the fast spread of CRS over the island was very likely related to their presence in a major citrus nursery, as confirmed by our data. In Réunion, the European *Conformitas Agraria Communitatis* (CAC) standards (directive 92/34/EEC) certify the disease-free status of citrus nursery plants (assessed by visual inspections for ACC). CAC is presently recommended but not compulsory. This report suggests that a strict implementation of this procedure would have helped limiting the spread of CRS in Réunion.

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