

DISEASE NOTES OR NEW RECORDS

Detection of Huanglongbing (citrus greening disease) in Timor-Leste (East Timor) and in Papua New Guinea

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Abstract. Huanglongbing (greening disease) of citrus has been detected for the first time in Timor-Leste (East Timor) and Papua New Guinea. Identifications were confirmed using a polymerase chain reaction-restriction enzyme digest procedure.

Huanglongbing (HLB), formerly known as citrus greening, is a lethal disease of citrus caused by a phloem-limited, uncultivable bacterium. In Asia, HLB is caused by ‘*Candidatus Liberibacter asiaticus*’ (Garnier *et al.* 2000). With an increasing distribution, HLB is a major problem for Asian citrus growers, having been the principal constraint to orange and mandarin production in Indonesia for many years (Aubert *et al.* 1985). The disease is vectored by the Asian citrus psyllid, *Diaphorina citri* and can also be moved in infected plant material. No verified instances of seed transmission have been reported. As there is no successful treatment, removal and destruction of infected trees are the only methods to stop disease spread. Symptoms of the disease include erect, yellow new leaves, mottling and blotchy yellowing of leaves and development of interveinal chlorosis. These symptoms are the same as, and can be confused with, those of nutrient deficiencies or other disorders. Characteristic of HLB, however, is that one branch or part of the tree first shows symptoms, which then spread throughout the tree canopy.

The Northern Australia Quarantine Strategy (NAQS), a program of the Australian Quarantine and Inspection Service (AQIS) conducts plant health surveys of the northern Australian coastline from Cairns to Broome and the islands of New Guinea and Timor in collaboration with their respective governments. Lists of exotic species, considered as threats to agriculture and the environment in Australia, are used to focus surveys. HLB and *D. citri* are included on these lists. *D. citri* has been recorded in West Timor, in several districts of Timor-Leste and in the north of the Indonesian

province of Papua (formerly Irian Jaya) on the island of New Guinea. HLB was recently found at Sorong and Jayapura in northern Papua (Davis *et al.* 2000).

Surveys of Timor-Leste were conducted in July 2000, May 2002 and April 2003, to assess the risk posed to Australia by increased civilian and military traffic from Timor-Leste. HLB and *D. citri* were detected in 2000 in the districts of Dili and Liquica and in 2002 in the districts of Aileu, Ainaro, Bobonaro, Cova Lima and Liquica. In September 2002, HLB and *D. citri* were detected at Vanimo, PNG, during a regular, biennial survey of the PNG / Papua border by plant health scientists from Australia, PNG and Papua. Subsequently, in November 2002, a delimiting survey was conducted in and near the towns of Wewak and Vanimo in north-west PNG by scientists from PNG and the Secretariat of the Pacific Community (SPC).

Each sample corresponded to a single tree and consisted of a representative sample, approximately 12 leaves, collected from symptomatic areas of the canopy. Petioles and midribs were removed from surface sterilised leaves, finely chopped and desiccated in sealed vials over anhydrous calcium chloride. Samples were returned to Australia for testing under AQIS permits 200007018 and 200203642. Testing for HLB was conducted as per Davis *et al.* (2000). Duplicate reactions of each extract were spiked with a positive control to eliminate the possibility of false negatives due to PCR inhibition. A selection of positive and negative samples from East Timor and the first PNG survey was tested by Dr D. Hailstones, Elizabeth Macarthur Agricultural Institute, NSW Agriculture, and some samples from the

second PNG survey were also tested by Dr M. Garnier, INRA, Bordeaux, France, using similar methods. *L. asiaticus* positives were confirmed by all three laboratories.

Surveys for psyllids were conducted by visual examination, sticky yellow traps and sampling trees with a sweep net. Where psyllids were in high numbers, they were aspirated from leaves. Nucleic acid extractions from psyllids, either dried over calcium chloride or stored in 70% ethanol, were performed using the method of Schneider *et al.* (1999). Both storage methods gave positive PCR results. Psyllid specimens are held in the NAQS insect collection at Mareeba and the Northern Territory Entomology Economic Insect Reference Collection in Darwin.

HLB was found to be widespread in the western half of Timor-Leste. A total of 36 samples from the surveys was tested. Twenty HLB-positive samples were collected from the towns of Dili, Ermera, Liquica, Ailieu, Maubisse, Suai, Hera and Maliana. The vector was present in each of these locations. Sixteen samples collected from symptomatic trees from the locations of Mehara, Lospalos, Lautem, Baucau, and Manatuto in the east of the country tested negative. The vector was not detected in these locations. No published data are available on the distribution of the disease in West Timor.

In PNG, HLB was found to be restricted to a small area in the Sandaun Province in the far north-west of the country. This region of PNG lies ~100 km from Jayapura, the closest location in Papua where the disease and vector are established (Davis *et al.* 2000). The disease was confirmed in five trees growing in two locations in Vanimo and in a nearby village. All positive samples were collected from species of citrus. Samples from 69 other trees from the second survey (11 at Wewak and 58 in/near Vanimo) tested negative. The citrus psyllid was found throughout Vanimo and in nearby villages in a strip along ~50 km of the coast. Thirty-four samples, collected since 1997 from elsewhere in the Sandaun and Western provinces of PNG, also tested negative. The large number of healthy trees, the widespread distribution of the vector and the spatial separation of the disease foci suggest that the introduction is recent and may have occurred on more than one occasion.

All positive results were obtained from species of citrus. One citrus relative, *Aegle marmelos*, from a region in Timor-Leste, where both the disease and vector are present, tested negative to HLB and the vector was not present on this tree. In PNG, potential hosts sampled for psyllids included a

Murraya paniculata, on which psyllids were detected, and several *Micromelum minutum* trees, on which no psyllids were detected. None of these trees was symptomatic and were not tested for HLB. No other citrus relatives were encountered during the surveys. Barbara Waterhouse and Andrew Mitchell provided botanical identifications in Timor-Leste and PNG, respectively.

The detection of the disease in Timor-Leste is a significant threat to citriculture there and on the island of Timor as a whole. Citrus is an important, although minor, crop in Timor-Leste providing nutritional requirements and generating some income for subsistence farmers. Citrus in PNG is also of nutritional importance to back yard and subsistence growers. With the detection of the disease in PNG, citrus trees there and in the South Pacific now face a major new quarantine concern. A campaign to confine the disease to the Vanimo area is being implemented by the PNG government with assistance from the SPC.

HLB is considered a serious threat to Australian citriculture by AQIS, particularly from cuttings or budwood illegally introduced into the country. Since 1999, a total of 74 symptomatic citrus trees across northern Australia, 56 from north Queensland and the Torres Strait islands, 13 from the Northern Territory and 6 from far north Western Australia, have been indexed for HLB as part of the NAQS program. None of these trees has tested positive for HLB.

References

- Aubert B, Garnier M, Guillaumin D, Herbagyandono B, Setiobudi L, Nurhadi F (1985) Greening, a serious threat for the citrus productions of the Indonesian archipelago. Future prospects of integrated control. *Fruits* **40**, 549–563.
- Davis RI, Jacobson SJ, Rahamma S, Gunua TG (2000) Surveillance for citrus Huanglongbing (greening) disease in New Guinea and north Queensland. *Australasian Plant Pathology* **29**, 226.
- Garnier M, Jagoueix-Eveillard S, Cronje PR, Le Roux HF, Bové JM (2000) Genomic characterization of a liberibacter present in an ornamental Rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal of a '*Candidatus Liberibacter africanus* subsp. *capensis*.' *International Journal of Systematic and Evolutionary Microbiology* **50**, 2119–2125.
- Schneider B, Padovan A, De La Rue S, Eichner R, Davis RI, Bernuetz A, Gibb K (1999) Detection and differentiation of phytoplasmas in Australia: an update. *Australian Journal of Agricultural Research* **50**, 333–342.

Accepted 3 October 2003