# DETERMINING THE POSSIBLE EVOLUTIONARY PATHWAY OF '*CANDIDATUS* LIBERIBACTER AFRICANUS' THROUGH GRAFT TRANSMISSION

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South Africa was ranked globally as the primary exporter of oranges and grapefruit during the 2013/2014 production season, generating an income of R9.3 billion in 2013. This economically important industry is however, under constant threat from various debilitating diseases including citrus greening disease. In 1929, citrus farmers around Rustenburg and Tzaneen noted that trees which showed mottling symptoms similar to zinc deficiencies produced fruits which were lopsided and failed to ripen, giving rise to the name citrus greening disease. In this period, citrus farmers from Guangdong province, China, reported symptoms similar to those seen in South Africa and the name citrus Huanglongbing (HLB) was used by Chinese farmers to describe the yellowing of leaves, characteristic of this disease. Due to the similarities in symptom expression, it was suggested that citrus greening and HLB were the same disease.

Through grafting experiments conducted in the late 1950s, it was demonstrated that both citrus greening and HLB were caused by a biological agent, thought at that time to be a virus. Further studies showed that the yet unknown agent of both citrus greening and HLB was transmitted by the triozid, *Trioza erytreae* Del Guercio and *Diaphorina citri* Kuwayama. It wasn't until 1983 when the causal agents of both citrus greening and HLB were identified as a phloem limited, gram negative bacterium and it was only in 1994 when 16S sequence information revealed that Citrus greening was associated with '*Candidatus* Liberibacter africanus' (Laf) whereas HLB was associated with a closely related bacterium, '*Candidatus* Liberibacter asiaticus (Las).

Since resolving the two Liberibacter species, Las has been identified from citrus orchards across Asia and the Americas whereas Laf has thus far only been identified from citrus in Africa and the Mascarene islands. As citrus is not indigenous to the African continent, the sole occurrence of Laf on commercial citrus from Africa has long puzzled researchers and it was speculated that Laf may have made a host jump from an indigenous citrus species to commercial citrus. During an outbreak of citrus greening in the Western Cape in 2000, researchers identified a novel Liberibacter from a Cape Chestnut (*Calodendrum capense*) tree which is more homologous to Laf than Las and is now known as '*Candidatus* Liberibacter

africanus subsp. capensis' (LafC). However, there is no evidence to suggest that LafC plays any role in the epidemiology of Laf.

To further address the possible origin of Laf, a recent study focused on the occurrence of Laf from native citrus hosts of *T. erytreae* Del Guercio. The three species studies included Horsewood (*Clausena anisata*), White Ironwood (*Vepris lanceolata*), and Forest Knobwood (*Zanthoxylum capense*). Typical citrus infecting Laf was not identified from any of these tree hosts, however, novel Liberibacter subspecies related to both Laf and LafC were described per host species. These are: '*Candidatus* Liberibacter africanus subsp. clausenae' (LafCl), '*Candidatus* Liberibacter africanus subsp. vepridis' (LafV) and '*Candidatus* Liberibacter africanus subsp. zanthoxyli' (LafZ).

The existence of these various Liberibacter subspecies from South Africa, lead to the hypothesis that the preferential feeding of *T. erytreae* Del Guercio on commercial citrus could have assisted in multiple transmission of a Liberibacter subspecies from its indigenous host to citrus, indirectly placing selective pressure on the Liberibacter subspecies to evolve and adapt to a new host. In order to test this hypothesis, bark strips from Liberibacter infected Horsewood, White Ironwood and Forest Knobwood were grafted onto Madam Vinous seedlings. At one year post-inoculation, 40%, 47% and 17% of the citrus trees inoculated with LafCl, LafV and LafZ respectively were dead. Any attempts to identify the various Liberibacter subspecies from the remaining citrus trees failed. In an attempt to establish sources of the various Liberibacter subspecies, 12 Madam Vinous seedlings were once again grafted with LafCl material, and once again 29% of the recipient citrus died suddenly. A generic Liberibacter real-time PCR performed on DNA extracted from the dead trees tested positive for a Liberibacter, however, in subsequent conventional PCR testing, the dead trees tested negative for LafCl. As LafCl could not be detected from any of the dead citrus trees from both transmission studies by conventional PCR, it is not possible to comment on whether LafCl plays any role in the sudden death of the citrus trees.

Further work is still required to determine whether any of the Liberibacter subspecies are capable of being transmitted to, and multiplying within citrus hosts. It is also vital to establish whether Laf can in turn be transmitted to any of the indigenous citrus species and if this bacterium cause disease in our indigenous trees. The answers to these question will in turn lead to a better understanding of how Laf came to be.


**Plant Virology Group in persuit of novel Liberibacter subspecies in: 1) Northern Natal, 2) Oribi Gorge, 3) Sabie and 4) Schoemans Kloof**