



## ■ THE PROBLEM ADDRESSED

### Risk of the spread of the '*Candidatus Liberibacter africanus*'

Very little is known about the alternate host plants and insect vectors of the important pathogen of citrus, '*Candidatus Liberibacter africanus*' which is associated with the citrus greening disease. This is an important knowledge gap, as import of these alternate hosts plants or accidental introduction of an insect vector, could serve as a means of spread of the pathogen to not yet infected parts of the world, including Europe. A survey of a number of samples of potential alternate plant hosts was performed focussing mainly on plants occurring in the Western Cape province of South Africa. It has been shown that tree and shrub specimens of the Rutaceae, indigenous to South Africa, are commonly infected by these bacteria, with each plant genus having a unique subspecies of it. It is important to ascertain if alternate hosts to citrus exist in South Africa. Morphology and barcoding were used to confirm the identity of the plant hosts. The monitoring of psyllid species observed on these plants and the barcoding of all morphogroups observed, as well as attempts at more classical identification by morphology were carried out.



- Top figures: symptoms of citrus greening. Lower left figure: the psyllid vector, *Trioza erytreae*. Lower right figure: pockmarks of *T. erytreae* nymphs on leaves of *Vepris lanceolata*.



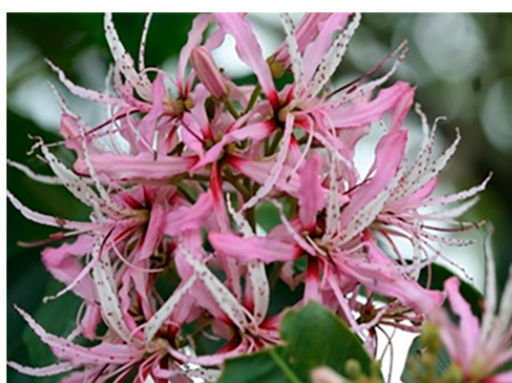
## ■ LATEST RESEARCH RESULTS

# Alternate host plants of '*Candidatus Liberibacter africanus*' *sensu lato* in South Africa

Studies to determine whether indigenous members of the Rutaceae (*Calodendrum capense*, *Clausena anisata*, *Oricia* spp., *Teclea* spp., *Vepris* spp. and *Zanthoxylum* spp.) serve as reservoirs for '*Ca. L. africanus*', associated to the citrus greening disease in commercial citrus orchards are in progress. Each indigenous plant genus contained a unique lineage of '*Ca. L. africanus*' (Roberts *et al.*, 2015). A relatively large percentage of the samples collected of each plant genus was positive for presence of the pathogen, suggesting the common and widespread occurrence of this bacterium in South Africa. These studies were expanded to include the relatively large group of Western Cape fynbos members of the Rutaceae, but not to non Rutaceae hosts. The genus-wide '*Ca. Liberibacter*' PCR assay (Roberts *et al.*, 2015) was used for these studies. The psyllid *Trioza erythrae* is known as a vector of '*Ca. L. africanus*' and the *Diaphorina citri*, can also transmit this bacterium experimentally. The insect vectors of the diverse '*Ca. L. africanus*' strains detected are still unknown.



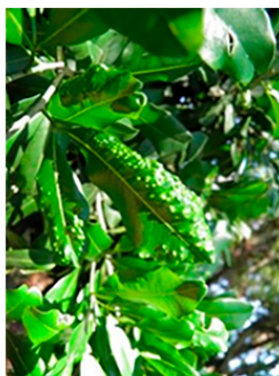
*Vepris lanceolata*



*Calodendrum capense*



*Zanthoxylum capense*



*Teclea* spp



*Oricia bachmannii*



*Clausena anisata*

- Rutaceous species hosts of '*Candidatus Liberibacter africanus*' *sensu lato* identified in South Africa.



## ■ THE TROPICSAFE RESEARCH AND DEVELOPMENT ACTIVITY

## Survey for identification of alternate host plants of '*Candidatus Liberibacter africanus*' sensu lato

Under the framework of TROPICSAFE, a survey and characterization of '*Ca. L. africanus*' subspecies, and their potential insect vectors in alternative host plants to citrus, was conducted. Weed and indigenous plant samples were collected at several localities in the Western Cape, South Africa during different seasons in 2017 and 2018, often around citrus orchards or vineyards. The leaf material collected was stored at -80°C until DNA extractions could be performed. Quantitative PCR tests specific for '*Ca. Liberibacter*' detection were conducted. PCR was also conducted on samples positive by qPCR, and the amplicons obtained were sequenced. The barcoding of alternative host plants was performed for their taxonomic identification.

The same areas were also surveyed for the presence of potential insect vectors (psyllids) prior to sampling them for the tests to detect the bacteria. This was done by vacuum sampling (DVAC machine) the insects and storing them in absolute ethanol for subsequent species identification and molecular analysis. Gross insect identification was made by studying morphological characters and relevant insects were subjected to DNA extraction using a non-destructive TNES buffer-based extraction method that maintain the insects intact for a following identification and for deposition of voucher specimens in museums.



- Using a suction device to collect insects from fynbos plants in South Africa.



## ■ SCIENTIFIC DATA AND FIRST RESULTS

# Identification of alternate host plants for 'Candidatus Liberibacter africanus' and its subspecies

Three field trips were conducted, in September 2017, January and August, 2018. The first two were to the Worcester/Robertson/Slanghoek areas of the Breederiver valley, Western Cape, while the last one was to the Vredendal/Lutzville, on the Cape west coast. Twelve collection sites were utilised, of which two were in the vicinity of citrus orchards. Using the DVAC device, insects were collected from 1,001 plant samples. Leaf and twig materials of each of these plants were also collected. DNA was extracted from all plant samples. Between 5 and 100 specimens of each of 42 species of plants were collected (with a maximum of 20 samples of a specific species per site). These plants were identified by morphological characters and amplifying the *rbcl* gene of representative plant samples. All samples were tested for 'Ca. Liberibacter' spp. presence by qPCR. Samples with Ct values lower than 30 (143) were also tested for 'Ca. Liberibacter' presence by PCR targeting the *rplJ* and the *omp* genes. Seventy-eight of the samples with Ct values less than 30 were from one of the three *Atriplex* species collected, while 14 were from *Lycium* species, 15 from *Rapistrum rugosum*, while fewer samples were from a number of other species. None of the samples yielded amplicons in PCR. No psyllids were observed amongst numerous other insects collected from these samples. One sample of *Atriplex*, positive in qPCR was submitted to next-generation sequencing.



- Stands of *Atriplex lindleyii*, a plant species with a large number of samples yielding low Ct values for 'Candidatus Liberibacter' detection by specific qPCR tests.

### KEY WORDS

'Candidatus Liberibacter africanus', citrus greening, alternate hosts, *Trioza erytreae*

### FURTHER INFORMATION

Roberts R., Steenkamp E.T., Pietersen G. 2015. Three novel lineages of 'Candidatus Liberibacter africanus' associated with native *Rutaceae* hosts of *Trioza erytreae* in South Africa. *International Journal of Systematic and Evolutionary Microbiology* 65, 723–731.

### AUTORSHIP

**Gerhard Pietersen** University of Pretoria, Pretoria, South Africa [gerhard.pietersen@up.ac.za](mailto:gerhard.pietersen@up.ac.za) / [gpietersen@sun.ac.za](mailto:gpietersen@sun.ac.za)

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