# BACKGROUND, CURRENT SITUATION AND MANAGEMENT OF HLB AND ITS VECTOR IN SOUTH AFRICA.

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## Abstract

*"Candidatus* Liberibacter asiaticus" (Las), the fastidious bacteria associated with a severe debilitating disease of citrus known as Huanglongbing (HLB), and its natural vector *Diaphorina citri* are absent from South Africa. Instead, a similar, but heat sensitive disease, known locally as Citrus Greening since the late 1920's, is associated with the presence of "*Candidatus* Liberibacter africanus" (Laf) and spread by the pyslla vector *Trioza erytreae* in this country. A significant amount of research was conducted locally on the vector and the disease during the 1970'S to the late 1990's. The disease has been controlled through the use of healthy planting material from a certification scheme, chemical control of the vector and reduction of disease inoculum through removal of infected branches and trees. Following recent surveys to confirm that Citrus greening in South Africa is associated solely with infection by Laf, current research is focusing on determining alternate hosts to citrus of this bacterium as there is some circumstantial evidence that the persistence of the disease may be due to such hosts of Laf. No naturally infected hosts of Laf have been found thus far. A second Liberibacter,subspecies, "*Ca.* L. africanus ssp. capensis" (LafC) previously detected infecting an indigenous Rutaceous species, *Calodendrum capense* (Cape Chestnut) in the Western Cape, was shown to occur on this host in most localities tested, including natural settings. Studies are currently proceeding to determine whether an overlap in the epidemiology of Laf and LafC occurs.

## Introduction

Huanglongbing (HLB) (Bové, 2006) is a serious, debilitating, insect-transmissible citrus disease, initially described from China. Since 2004, the disease has spread to citrus orchards in a number of New World countries such as Brazil (Coletta-Filho *et al.*, 2004; Teixeira *et al.*, 2005b), Cuba (Martinez *et al.*, 2009), the Dominican Republic (Matos *et al.*, 2009) and Florida, USA (Gottwald *et al.*,2006) where it was not previously found. Spread to these new areas has generally been due to accidental introduction and spread of the HLB vector, a psylla *Diaphorina citri* (Kuwayama) followed by introduction and spread of the pathogen *"Candidatus* Liberibacter asiaticus" (Las). In Sao Paulo State, Brazil, a new Liberibacter species, "*Ca.* L. americanus" (Lam) was found in symptomatic citrus trees concurrent to the Las introduction (Texeira *et al.*, 2005a)

A very similar, but heat sensitive (Schwarz and Green, 1970) and somewhat less severe citrus disease occurs in South Africa. It has been known in South Africa since the late 1920's and was initially called either "yellow branch disease" or "greening" (da Graça, 1991), but later became established in the literature as greening disease of citrus (Oberholzer *et al.*, 1965). Citrus greening diseased plants appear to recover from symptoms at 32°C but not at 27°C (Bové *et al.*, 1974), and the trees seldom die (Van Vuuren, personal communication). Greening disease in South Africa is associated with the presence of "*Candidatus* Liberibacter africanus" (Laf), a heat sensitive, phloem-limited, non-cultured alpha-Proteobacterium (Jagoueix *et al.*, 1994; Korsten *et al.*, 1996). Specific detection of Laf was first accomplished in the early 1990's with monoclonal

antibodies (Garnier *et al.*, 1991; Korsten *et al.*, 1993), and then later with DNA probes (Planet *et al.*, 1995). A PCR specific for Laf (Jagoueix *et al.*, 1996), was used, along with DNA hybridization which was able to detect Laf and Las, to confirm the presence of Laf in South Africa between 1993 and 1996 (Korsten *et al.*, 1996). During 1998, 82 samples from Nelspruit, Rustenburg, the Eastern and Western Cape were tested by Laf specific PCR's and resulted in the first detection of Laf in the Western Cape (Garnier *et al*., 2000a). A recent survey on 249 trees collected throughout citrus growing regions of South Africa has confirmed the association of Laf with greening in South Africa and the absence of Las or Lam (Pietersen *et al.,* 2010). Geographically representative samples which tested negative for Liberibacter also tested negative for phytoplasmas based on real-time PCR results. Analysis of the unidirectional sequence of the outer membrane protein gene (*omp*) of 45 samples infected with Laf showed no variability in this region (Pietersen, *unpublished*) and appears to represent a homogenous population as the omp gene was shown to be variable in the case of Las (Bastianel *et al*., 2005).

*D. citri* is not found in South Africa and Laf is transmitted locally by a psylla, *Trioza erytreae* (Del Guercio) (McClean & Oberholzer, 1965a; 1965b; van den Berg, 1990), recorded in South Africa already in 1897 (Lounsbury, 1987). As in the case of the bacteria Laf, *T. erytreae* is heat sensitive and high temperatures and low humidity were reported to be extremely detrimental to the psylla's population (Catling & Annecke, 1968). Survival rates of up to 100% of the egg to first instar of the *T. erytreae* were observed when the mean daily maximum temperatures and relative humidity were 23.7$^{o}$C and 54.5% respectively. However temperatures of 32$^{o}$C for approximately 8 hours a day are sufficient to prevent eggs from hatching (Moran and Blowers, 1967). Green and Catling (1971) combined the mean of three vapour pressure values and the three saturation deficits coinciding with the three highest maximum temperatures daily maximum temperatures in a -5 or -10 day period as a saturation deficit index (SDI), which correlated well with egg mortality. Samways (1987), confirmed that an increasingly high SDI has a negative long term impact on adult psylla numbers but that weekly fluctuations in SDI could not be correlated with adult psylla population number. As a consequence of the vector and bacteria's heat sensitivity the disease is mainly a problem in the cooler citrus production areas of South Africa.

Nevertheless, citrus greening has been responsible in South Africa for major production and economic losses, fluctuating with climatic conditions. Serious outbreaks occurred in 1932 to 1936 and again in 1939 to 1946 in the high lying cooler citrus production areas of the Eastern Transvaal (Oberholzer *et al*., 1965). This was followed, after greening had existed at low levels for many years, in a general outbreak commencing in 1958 (Oberholzer *et al*., 1965) and resulting in 30% to 100% fruit losses in individual orchards of both the Eastern Transvaal and the Western Transvaal, around Rustenburg by 1965 (Schwarz, 1967). In the mid 1970's an estimated 4 million citrus plants were infected with the disease (Buitendag & Von Broembsen, 1993; le Roux *et al*., 2006) while in the 1980's an annual estimated loss of R35 million in production was considered due to the disease (van den Berg *et al*., 1991-1992). In more recent times, the incidence and distribution, based on symptoms severity, were reported by Pretorius and van Vuuren (2006). The highest incidence includes the Karino-White River, Hazyview, Brondal, Nelspruit, Tzaneen, Rustenburg, Mokopane, Zebediela areas and certain magisterial districts of the Western Cape Province. Moderate incidences occur in Letsitele, Letaba Valley, Lydenburg-Ohrigstad, Muden-Pietermarizburg-Richmond areas. Low incidences are found in Kaapmuiden-Malelane-Hectorspruit, Groblersdal-Marble Hall and Northern KwaZulu Natal including the Nkwaleni Valley. The disease was reported absent from the major citrus production areas of Citrusdal and the Eastern Cape, as well as from Northern Cape citrus growing areas (Pretorius and & van Vuuren, 2006).

Citrus greening disease incidence, spread and economic impact have been reduced to lower levels in South Africa through planting of healthy plant material obtained through an efficient certification scheme, legislation limiting the movement of planting material from greening affected and greening free areas, stringent chemical control of the vector and to a lesser extent

removal of Laf inoculum in the form of infected branches and trees in greening endemic regions, but remains a persistent problem in cooler citrus production areas of South Africa. Studies being conducted currently on control strategies involve looking for resistance to Laf amongst embryo-rescued seeds from healthy looking chimaeric sectors of fruit from greening affected branches (van Vuuren, *personal communication*).

The perpetuation of the disease in regions where control strategies are meticulously followed may be due to the presence of hosts other than citrus. Current research efforts in South Africa focus on determining potential alternate hosts to citrus of Laf amongst the indigenous citrus family (*Rutaceae*) as a means making disease pressure reduction more efficient. One such alternate host to citrus of greening was found by van den Berg *et al*., 1991-2. These authors side grafted citrus indicator plants, protected against psylla infection, to naturally occurring *Clausena anisata* (Wild) Hook. f. ex Benth., trees located close to an infected citrus orchard. Symptoms were detected on one of the citrus indicator plants and it tested positive for "greening" by thin layer chromatography. Korsten *et al* (1996) used dot hybridization probes as well as PCR to detect the Laf from a *V*epris *lanceolata* (Lam) G. Don. plant cited by its previous name *Toddalia lanceolata* Lam. During a recent survey of 193 plants, incorporating 14 genera and 35 species, primarily of the *Rutaceae*, none of the plants were found infected with Laf by PCR tests (Phahladira, 2010)

The development from egg to adult of *T*. *erytreae* has been recorded on a number of rutaceous plants indigenous to Southern Africa including *Clausena anisata*, *Vepris lanceolata*, *Zanthoxylum capense* (Thumb) Harv., *Oricia* and *Fagara* species (van den Berg, 1990; Halbert & Manjunath, 2004). Moran (1968a, b) reported that *V*. *lanceolata* was the preferred host plant and also supports nymphal development of the citrus psylla when compared to *C*. *anisata*, *Z*. *capense* and *C. capense*. The *C. capense* leaves attract the adult citrus psyllid for feeding but are not suitable for nymphal development. In another study, Moran and Buchan (1975) concluded that leaf hardness characteristics could not be a factor in host plant discrimination by *T*. *erytreae*, between *Citrus limon* and indigenous hosts, but that the larger soft flush leaves of lemon which flush regularly thus provided copious, soft flush for oviposition and nymphal development.

Van den Berg *et al* (1987) monitored indigenous plants near a citrus orchard and found citrus psylla *Trioza erytreae* on fifty *C*. *anisata*, twenty *Z*. *capense* and ten *V*. *lanceolata* plants. Adult citrus psylla were also found, in a feeding position, on *Casimiroa edulis* however it was uncertain as to whether the psylla could feed on the plant (van den Berg & Deacon, 1989). It is not mentioned whether these plants were monitored for greening symptoms or the presence of the bacteria. *T. erytreae* was observed to be feeding on the prevalent *C. anisata* trees in the highlands of Cameroon and Ethiopia (Aubert *et al*., 1988).

The continuous movement of citrus psylla between unsprayed citrus orchard and indigenous host plants surrounding orchards has been observed (van den Berg *et al*., 1991; van den Berg *et al*., 1991-2). Possible vector transmission of the greening pathogen from infected citrus to other Rutaceous plants could lead to a sustained cycle of transmission of the pathogen. This possibility still needs to be determined experimentally to identify new hosts of the fastidious bacterium.

A second Liberibacter species, "*Ca.* L. africanus ssp. capensis" (LafC) was detected infecting an indigenous Rutaceous species, *Calodendrum capense* (Cape Chestnut) in the Western Cape in 1998 (Garnier *et al*., 2000b). No instance of LafC infection was found on Citrus during a recent survey for Liberibacters in South Africa (Pietersen *et al*., 2010).

In recent, thus far unpublished studies a real-time PCR, capable of specific detection of LafC was developed, based in part on the system of Li *et al*., 2006. Using this, a total of 263 Cape

Chestnut samples, collected from Mpumalanga, Limpopo, Gauteng, KwaZulu-Natal, Western Cape and Eastern Cape were tested. The widespread infection of *C. capense* by LafC has been confirmed including areas in South Africa where citrus trees are free of Laf (eg. The Eastern Cape). These results are extremely interesting and possibly support the hypothesis that LafC may represent be the "parent lineage" from which Laf on citrus emerged through host species jumping and selection. This is supported by the fact that the two bacteria have very similar nucleotide sequences within those regions already determined. Furthermore, in the origin of species of Citrus (Central Asia/China) Laf is not known to occur, but occurs in Africa to which Citrus was introduced. The relatively mild symptoms of the LafC infection on *C. capense* also suggest that the bacterium has co-evolved with this host over a very long period of time. Furthermore the widespread occurrence of the bacteria in *C. capense* specimens in widely separated and often isolated natural locations also suggests an ancient association. It is important that further studies are conducted to determine whether the LafC/*C. capense* epidemiology overlaps with that of Laf/Citrus with regards reciprocal vector transmission. Sites in the Eastern Cape, in which LafC infected *C. capensis* trees were identified in close proximity to Citrus groves are ideal sites to assess if transmission of LafC occurs amongst the two Rutaceous species naturally, and are the focus of a study by one of the co-authors, R. Viljoen. Controlled reciprocal transmission experiments with *T. erytreae* between citrus and *C. capense* are however essential to proving this but have been hampered by an inability to establish and maintain a healthy colony of this insect. Of 10 *C. capense* plants graft inoculated with Laf and maintained in insect-free greenhouses for three years one tested weakly positive for Laf by real-time PCR suggesting that *C. capensis* does support replication of Laf (Phahladira, 2010, *unpublished results*). The plant however died two years post-inoculation, prior to confirmation of the positive result and before the possibility of inherent LafC infection could be discounted, and while it is possible that the plant death may be due to the Laf infection this cannot be reported with certainty.

## Conclusions

Greening is South Africa, while not as debilitating as the Las associated HLB, and, in spite of relatively successful control of spread by insecticide application and inoculum removal, it remains a persistent problem for the local citrus industry. The current state of the disease and research in South Africa are discussed.

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