Several Liberibacter and Phytoplasma Species are Individually Associated with HLB: towards a common denominator.

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Several sieve tube-restricted bacteria are associated with HLB, worldwide. In Africa, only *Candidatus* Liberibacter africanus (Laf) has been found to be associated with HLB, and the insect vector is the African citrus psyllid, *Trioza erytreae* (see 2 for references on discovery, characterization, and detection of rutaceous liberibacters). Both the African liberibacter and the psyllid-vector are heat sensitive, as temperatures of 32°C and above are detrimental. In addition to being heat sensitive, the vector is also affected by dry conditions. These reasons explain why the disease in Africa is encountered most severely in cool areas, namely at altitudes at or above 600 m and where relative humidity rarely falls below 25%, while at lower altitudes the disease is less severe, and practically absent below 200m. Only in the Cape Town region, at the most southern tip of Africa, the disease occurs at sea level temperatures because there, the southern latitude compensates for lack of altitude. In the Cape Town province, an arborescent *Rutaceae*, *Calodendron capensis*, or Cape chestnut tree, showed leaves with blotchy mottle and was found to be infected with *Candidatus* Liberibacter africanus subsp. capensis. The subspecies has not (yet) been found in citrus. It has also been detected in apparently symptomless chestnut trees (14).

Until recently, *Candidatus* Liberibacter asiaticus (Las) was the only liberibacter found to be associated with HLB in Asia. In 2008, *Candidatus* Liberibacter americanus (Lam) has been reported in one of 97 citrus leaf samples from eight provinces of southern China, Las being present in the 96 other samples (13). If this report is confirmed, it would be the first time that Lam is detected out of Brazil. The insect vector in Asia is *Diaphorina citri*, the Asian citrus psyllid, an insect more heat tolerant than *T.erytreae*. Asian HLB is heat tolerant, and involves Las, not yet affected at 35°C, while Lam, like Laf, is heat sensitive (11). The disease occurs in hot, dry oases of south-western Saudi Arabia.

In Florida and Cuba, HLB was recognized in 2005 and 2006, respectively, and is of the classic Asian type, with Las as the liberibacter and *D. citri* as the psyllid vector.

A third liberibacter species has been identified in 2004 in São Paulo State (SPS), Brazil: *Candidatus* Liberibacter americanus (Lam). However, Las is also present. In 2004, Lam was widely predominant, Las being detected only in very few sweet orange trees. By 2008, the situation has reversed, as newly affected trees are now found to be more frequently infected with Las than with Lam. Both Lam and Las are transmitted by the Asian psyllid, present in SPS since the 1940s. Lam in SPS, like Laf in South Africa, is heat sensitive, while Las is heat tolerant. Also, the titres of Lam in sweet orange trees are lower than those of Las, and might result in lower insect-transmission efficiency (12). The differences between Lam and Las may explain the decrease of Lam in favour of Las in SPS.

Surprisingly, in 2007, a phytoplasma of group 16SrIX (*Candidatus* Phytoplasma phoenicium group), closely related to the pigeon pea witches' broom phytoplasma (99% 16SrDNA sequence identity) has been found associated with HLB in SPS (16). Phytoplasmas are wall-less, sieve tube-restrited bacteria (class Mollicutes). The HLB associated phytoplasma is probably transmitted to citrus by an insect vector becoming infected on an external, non-citrus source. Several *Crotalaria junceae* plants, grown in between citrus rows for soil improvement, and showing typical witches' broom symptoms, have been found to be infected with the HLB phytoplasma (17) Thus, the presence of the HLB phytoplasma in citrus from central, northern and southern SPS, is probably the result of cultural practices, which have become widely used throughout SPS. Transmission of the phytoplasma from citrus to citrus has not yet been observed. Interestingly, in southern China, a phytoplasma of group 16Sr1 (*Candidatus* Phytoplasma asteris group) associated with HLB has been reported recently (3). The SPS phytoplasma of group 16SrIX and the China phytoplasma of group 16Sr1 are associated with characteristic HLB leaf and fruit symptoms. Even *Spiroplasma citri*, the helical mollicute responsible for citrus stubborn disease, causes HLB-like symptoms on sweet orange fruits (small size, color inversion, lopsidedness, and seed abortion); on *S. citri*-infected leaves, some blotchy mottle can be seen, but it is less conspicuous than HLB blotchy mottle . However, *Candidatus* Phytoplasma aurantifolia, the phytoplasma associated with witches'broom disease of lime (WBDL), is not associated with HLB-like fruit and leaf symptoms, and the WBDL symptoms on lime are very different from those of HLB. Thus, only some phytoplasmas are associated with HLB symptoms.

Finally, a new liberibacter species (*Candidatus* Liberibacter solanacearum) has been shown to be associated with affected solanaceous plants (9, 10), and is transmitted by the potato psyllid, *Bactericera cockerelli.* The liberibacter from the psyllid vector has also been characterized and named *Candidatus* Liberibacter psyllaurous (8). *Ca*. L. solanacearum, isolated from solanaceous plants and *Ca*., L. psyllaurous, isolated from the psyllid vector, are probably one and the same organism.

At this moment, no relationship has been established between huanglongbing and the liberibacter from solanaceous plants. In 1992, a Philippines' strain of Las was transmitted by dodder from citrus to tobacco (*Nicotiana tabacum, "xanthi*") plants, which became severely affected, but had liberibacter titers much lower than those in periwinkle (*Catharanthus roseus*) plants (Fig. 1) (6). Las has also been dodder-transmitted to tomato (*Lycopersicon esculentum*) plants, which became symptomatic (4). Hence, the Asian citrus liberibacter is associated with symptoms in solanaceous plants. Inversely, the solanaceous liberibacter might very well be associated with symptoms in citrus.

Why would three different liberibacter species, two different phytoplasma species, and even one spiroplasma species be associated with similar, if not identical, HLB symptoms on leaves and/or fruits? These different bacteria have one feature in common: they are strictly restricted to the phloem sieve tubes. They might also have similar mechanisms of pathogenicity! As *S. citri* is available in axenic culture since 1970 (15), transpositional mutants could be produced. A non-pathogenic mutant, GMT553, was obtained from wild type *S. citri* strain GII-3 and used to elucidate the pathogenicity mechanism of the citrus spiroplasma (5). In the presence of both fructose and glucose, GII-3 uses preferentially fructose. In GMT553, the transposon was found to be inserted into the fructose operon, rendering the mutant unable to use fructose, the utilisation of glucose, however, being unaffected (7). In healthy plants (Fig. 2A), the vacuola invertase in the companion cell produces normal amounts of fructose and glucose from sucrose. In the *S. citri*-infected plants (Fig. 2B), fructose is used by the sieve tube-restricted spiroplasmas and, as a consequence, fructose concentration decreases with a concomitant increase in invertase activity, resulting in the production of more fructose and glucose. The concentration of fructose remains low, invertase activity remains high, but glucose concentration increases. It could be shown that the concentration of glucose in leaves infected with the wild type *S. citri* strain reached levels 20 times higher than those in healthy leaves or leaves infected with non-pathogenic mutant GMT553 (1). Also, fructose utilization by the spiroplasmas could impair sucrose loading into the sieve tubes by the companion cells and result in accumulation of carbohydrates in source leaves and depletion of carbon sources in sink leaves (7). Such mechanisms of pathogenicity are not based on specific genes, such as genes for toxins, but on deviations of sugar metabolism. Experiments are underway to examine whether such mechanism could apply to liberibacters and phytoplasmas involved in HLB.

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