ETIOLOGY, HISTORY AND WORLD SITUATION OF CITRUS HUANGLONGBING

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Abstract

Citrus huanglongbing (HLB)(greening disease) is now considered to be the most serious disease of citrus, causing serious crop and tree losses in much of Asia and Africa, and is now spreading through the Americas. The disease is caused by a phloem-limited, psyllid-vectored, unculturable (thus far) bacterium, *Candidatus* Liberibacter. Three species have this far been identified in HLB-affected citrus; *Ca*. L. asiaticus, *Ca.* L. africanus and *Ca.* L. americanus. HLB symptoms have also been reported in Brazil and China in trees infected with *Ca*. Phytoplasma spp. The origins of the disease remain unclear. Citrus is not considered an original host since the disease has only recently been, and no natural tolerance or resistance has been found in this 4,000-yr-old crop. The first description of symptoms was published in India in 1927, and ascribed to psyllid damage. Although the name Huanglongbing originated in China in the late 19th century, this may have referred to nutritional and/or root problems; scientific description of clear HLB symptoms were only observed in 1938. It has been suggested that Asian HLB originated in citrus in India from unidentified native rutaceae, and that infected plants may have been moved to other Asian countries. It is known that citrus was moved from the 1940s on from China to several south-east Asian countries, where HLB appeared a few years later. *Ca.* L. asiaticus has been identified in all the affected American countries, and most likely originated in Asia. *Ca.* L. americanus was also identified in Brazil, but may also have an Asian origin. The African form probably first infected citrus in the 1920s, after transmission by psyllids from native rutaceae. In the last few years, spread has continued in Asia (west into Iran and east into Papua New Guinea), and since its confirmation in Florida in 2005, it has appeared in several Caribbean nations, Belize and Mexico. It is likely to continue its spread since the psyllid vectors continue to appear in new areas.

Introduction

Citrus tristeza virus (CTV), the cause of quick decline of trees on sour orange rootstock, and of stem pitting disease, has been long considered to be the most serious disease of citrus, with 100 million trees reportedly lost to it (Román et al., 2004). However, citrus huanglongbing (HLB)(greening disease), which has been causing serious losses for several decades in parts of Asia and Africa (Aubert, 1993), has now spread to the Americas, and the total losses are now most likely overtaking those caused by CTV. Since the confirmation of HLB in the largest citrus producing areas of the world, the states of São Paulo (Brazil) and Florida (USA), large amounts of money have been directed towards surveys, psyllid control and management strategies and research into long-term solutions for this disease. This overview summarizes the identification of the causal organisms, their possible origins and the extent of their spread.

Etiology of HLB

The classic symptoms of HLB (blotchy mottle on leaves, vein thickening, fruit drop, the development of misshapen, improperly colored and bitter taste) are all indicative of phloem disruption resulting in starch accumulation in parts of the leaves, and could be caused by one of several pathogens. For example, citrus stubborn, caused by *Spiroplasma citri*, causes similar symptoms (Bové & Garnier, 2000), and phytoplasmas have been associated with HLB-like symptoms in Brazil (Teixeira et al., 2008) and China (Chen et al., 2009). The causal organism of HLB is now accepted as *Candidatus* Liberibacter spp. which belong the α-protobacteria (Bové, 2006). Three species have been identified, each causing the same symptoms, but with some different properties – *Ca.* L. asiaticus occurs throughout Asia where the disease exists, in some Indian Ocean islands and has most recently been introduced into the Americas. It is transmitted by the Asian citrus psyllid, *Diaphorina citri,* and is a heat-tolerant bacterium. In Africa, *Ca.* L.africanus is associated with HLB and is transmitted by the African citrus psyllid, *Trioza erytreae*. It is a heat-sensitive species, and African HLB is therefore found in elevations above 700 m where temperatures over 30C are not sustained for long periods. When HLB was first discovered in Brazil in 2004, a previously unknown species, which was given the name *Ca.* L. americanus, was identified from most samples, although *Ca.* L. asiaticus was present in a few (Teixeira et al., 2005). It is transmitted by *D. citri*, but it is intolerant of high temperatures, and is now being supplanted by *Ca.* L. asiaticus (Lopes et al., 2009). One report of *Ca*. L. americanus in China has since appeared (Bove, pers.comm.).

Many attempts to isolate liberibacters over the years have been made. Claims of success in South Africa in the 1980s were not repeated (Garnier & Bové, 1993), and for many years no further attempts were reported. Recently, two laboratories in the USA have reported renewed attempts – one reported the co-cultivation of liberibacter with an actinomycete (Davis et al., 2008), while a second reported pure cultures of all three species, but they were not maintained (Sechler et al., 2009). Although Koch's have not been fulfilled, there is now widespread acceptance of *Ca.* Liberibacter as the casual organism. Its genome has been sequenced and is reported to be approx. 1.22 Mb pairs (Duan et al., 2009). It appears to have a limited ability for aerobic respiration, and is auxotrophic for some amino acids, which may contribute to its fastidiousness.

History and Origins

For many years, the origin of HLB was considered to be China (Zhao, 1981). Farmers in southern China in the late 1800s observed yellowing of their citrus trees, and coined the term 'huanglongbing' (yellow shoot disease) for it. When Lin (1956) conducted his survey in the late 1940s, he referred to these anecdotes, and several reviewers perpetuated this theory (da Graça, 1990; da Graça & Korsten, 2004; Bové, 2006).

However, Beattie et al. (2008) dispute this, and provide convincing evidence. In India Husain and Nath (1927) described *D. citri* and tree damage that they said was caused by the insect – however, it reads like a description of HLB. *D. citri* was described in China in 1936 (Hoffman, 1936), without mention of these symptoms, and clear HLB

disease symptoms were only observed in 1938 (Chen, 1943). The experience of everywhere HLB has invaded is that soon after its discovery, it spreads rapidly. Had HLB been present in China the 19th century, it would most likely have become widespread before the 1940s. In India, the term 'citrus die-back' has been used since the 18th century (Capoor, 1963). In 1969, it was shown that die-back was the same as greening (HLB) (Raychaudhuri et al.,1969), but since die-backs can be caused by a number of causes, one cannot be sure that HLB was in citrus there 250 years ago.

In South Africa, symptoms were observed by farmers in the north west and the north west of the country in 1928/29 and were called 'yellow branch' or 'greening'; initially it was thought that a mineral deficiency or toxicity was responsible (van der Merwe & Andersen, 1937)

Before infecting citrus, where were the liberibacter? – the bacteria have obviously been in existence for millions of years. Citrus cultivation began in China 4,000 years ago, and in India not long after (Singh et al., 2002). Yet, HLB only appeared during the past century – clearly citrus is not its original hosts. The severity of symptoms in citrus, and the apparent absence of tolerance or resistance in any citrus species also support this theory.

In Africa, *Ca.* L. africanus has been detected in two native rutaceaous trees, *Vepris undulata* and *Clausena anisata* (Korsten et al., 1996). Both of these have been identified as likely original hosts for *T. erytreae*. In addition, a subspecies of the bacterium, Ca. L. africanus ssp. capensis, has been detected in a third indigenous species, *Calodendrum capense*, (Garnier et al., 2000) but thus far, it has not been found in citrus, nor has *Ca*. L. africanus been found in *C. capense* (Pietersen et al., 2010).

Asian rutaceae which have been reported as hosts of *Ca.* L. asiaticus are orange jasmine (*Murraya paniculata; M. exotica*), wood apple (*Limonia acidissma*), Chinese box orange (*Severinia buxifolia*) (Hung et al., 2001) and wampee (*Clausena lansium*) (Ding et al., 2005), but as yet there is no evidence to support any as the original host(s) – *C. lansium* may even be of African origin (Beattie et al., 2008).

Any of the rutaceae identified as hosts could act as reservoirs of disease for citrus plantings. Studies are now underway in the USA to determine if any native North American rutaceae could act as hosts for *D. citri* and liberibacters (Sandoval et al., 2010). Two *Choisya* spp and *Helietta parvifolia* have been shown to support *D. citri* feeding and reproduction.

World Situation

From the first records of HLB in citrus in India in 1927 and South Africa in 1928/29, the disease has spread into all the major citrus producing areas except the Mediterranean countries and Australia. Movement has been through initial human transportation of HLB-infected and psyllid-infested citrus or its ornamental relatives to new areas, followed by further spread by humans and psyllids. In Africa it is not clear if HLB spread in citrus from South Africa to East Africa. It has been suggested that this is how CTV was spread to Anglophone countries (Mendel, 1968), but since CTV is in both East and West Africa, and HLB has not been reported in West Africa, it is possible that *Ca.* L. africanus was

endemic in indigenous plants in both the eastern and southern areas of Africa prior to infecting citrus.

In Asia, there are records of the movement of citrus plants between different countries after the 1930s (Beattie et al. 2008). Both India and China have been mentioned, and it is possible that this was how HLB was spread throughout south east Asia. *D. citri* has moved eastwards into more recent years into Papua New Guinea, with HLB detection occurring soon after (Weinert et al., 2004) – this poses a threat to Australia. To the west, the psyllid has moved into eastern Iran and Oman; Iran has since reported the detection of HLB (Faghihi et al., 2008). The rest of the Middle East and Mediterranean countries are now threatened by this disease. *Ca.* L. asiaticus has also recently been detected in the north of Ethiopia where temperatures are higher (Saponari et al., 2010); this is the first record for Africa, and it may have been introduced from nearby Yemen.

*D. citr*i was first detected in Brazil in 1942 (Lima, 1942). No HLB was reported until 2004 (Coletta-Filho et al., 2004). This suggests that no indigenous rutaceae were infected, and that HLB was introduced from Asia. *Ca.* L. americanus has since been detected in China (), and it may have been introduced first and was therefore found initially in most infected citrus trees. When *Ca.* L. asiaticus arrived, it took time to be spread and overtake Lam (Coletta-Filho et al., 2010), possibly because if its ability to survive the higher summer temperatures in São Paulo.

Guadeloupe and Florida reported the presence of *D. citri* in 1998 (Halbert & Nuñez, 2004). The source is not known for sure, but possibly directly from Asia. HLB was confirmed in 2005, and was soon found to be widespread in south eastern Florida (Halbert, 2005). It is possible that HLB-infected plant material; was introduced before the psyllid, and movement was by humans moving infected citrus and orange jasmine plants.

The spread of the psyllid and HLB through the Caribbean, Central and North America has been rapid. HLB has now been detected in Puerto Rico, Belize, Cuba, Mexico, Jamaica, US Virgin Is., and three southern US states. The psyllid is also in Texas, California and Arizona, but so far no HLB has been detected.

Short term and Long term actions

For the short to middle term, since there is no resistance to HLB in *Citrus* spp., introduction of the disease into those areas still apparently without the disease, state laws must be enforced as far as possible. Surveys must be intensified with real-time PCR testing of psyllids and plant tissues. Budwood sources need to be covered with insect-resistant screens, and psyllid control in open nurseries, pending future possible requirement of screening as is in place in Florida, must be stringent. For orchards, area wide management and dormant sprays are likely to play an important role in slowing down any spread.

In the long term, resistance is the best option. There is some evidence of possible resistance obtained by embryo recovery from chimeric fruit in South Africa (van Vuuren & Manicom, 2009), seedlings in Florida (Brlansky & Castle, 2010), and transgenic plants with anti-bacterial genes (Stover et al., 2008). Broad spectrum resistance obtained by manipulating citrus genes is another option (Kunta et al., 2008). It will be a number of years before any of these are commercially available.

References Cited

Aubert, B. 1993. Proc. 4th Congr. Int. Soc. Citrus Nurserymen, pp.134-142.
Beattie, G. A. C. 2008. Proc. Int. Res. Conf. Huanglongbing, Orlando FL, pp. 25-57.
Bové, J. M. 2006. J. Phytopathol. 88: 7-37.
Bové, J. M. & M. Garnier, 2000. Compendium of Citrus Diseases (2nd ed.), pp. 48-50.
Brlansky, R. H. & W. S. Castle, 2010. Proc. Fla. St. Hort. Soc. (in press)
Capoor, S. P. 1963. Bull. Nat. Inst. Sci. India 24: 48-64.
Chen, J. C. 2009. Phytopathology 99: 236-242.
Chen, Q. 1943. New Agric. Quart. Bull. 3: 142-175.
Coletta-Filho, H. D. et al., 2004. Plant Dis. 88: 1382.
Coletta-Filho et al., 2010. Proc. 17th Conf. Int. Org. Citrus Virol. (in press)
Da Graça, J. V. 1990. Annu. Rev. Phytopathol.
Da Graça, J. V. & L. Korsten. 2004.
Davis, M, J., et al. 2008. Plant Dis. 92: 1547-1450.
Ding et al., 2005. J. Plant Pathol. 87 : 207-212.
Duan, Y., et al. 2009. Mol. Plant Microbe Interact. 22: 1011-1020.
Faghihi, M. M. et al., 2008. New Dis. Repts. 18: 42.
Garnier, M. & J. M. Bové. 1993. Proc. 12th Conf. Int. Org. Citrus Virol., pp 212-219.
Garnier et al., 2000. Proc. 14th Conf. Int. Org. Citrus Virol., 369-372.
Halbert, S. 2005. Proc. 2nd Int. Citrus Canker & HLB Workshop, p. 50.
Halbert, S. E. & C. A. Nuñez, 2004. Fla. Entomol. 87: 401-402.
Hoffman, W. E. 1936. Lingan Sci J. 15: 127-132.
Hung, T.-H. et al., 2001. Eur. J. Plant Pathol. 107 : 183-189.
Husain, M. A., & D. Nath. 1927. Mem. Dept. Agric. India, Entomol. Ser. 10(2): 5-27.
Korsten, L., et al. 1996. Proc. 13th Conf. Int. Org. Citrus Virol. Pp. 395-398.
Kunta, M. et al., 2008. Phytopathology 98: S85.
Lima, A. da C. 1942. Insectos do Brasil. Vol 3. 327 pp.
Lin, K.-H. 1956. Acta Phytopathol. Sin. 2 : 1-42.
Lopes, S. A., et al. 2009. Plant Dis. 93 : 275-262.
Mendel, K. 1968. Proc. 4th Comf. Int. Org. Citrus Virol., pp 35-37.
Pietersen, G. et al. 2010. Plant Dis. 94 : 244-249.
Raychaudhuri, S. P., et al. 1969. Proc. 1st Int. Citrus Symp. Vol.3 : 1433-1437.
Román, M. P., et al., 2004. Plant Disease 88: 453-467.
Sandoval, J. L., et al., 2010. Subtrop. Plant Sci. 62 : Abstr. p. 10.
Saponari et al., 2010. Plant Dis. 94 : 482.
Sechler, A., et al. 2009. Phytopathology 99 : 480-486.
Singh, A. et al., 2002. Citrus Germplasm. Cultivars and Rootstocks. Kalyani Publishers.
Stover, E. et al. 2008. Proc. Int. Res. Conf. Huanglongbing, Orlando FL, p. 327.
Teixeira, D. C., et al., 2005. Proc. 16th Conf. Int. Org. Citrus Virol., pp. 432-438.
Teixeira, D. C. et al., 2008. Phytopathology 98: 977-984.
van der Merwe, A. J., & F. G. Andersen, 1937. Fmg. S. Afr. 12: 439-440.
van Vuuren & Manicom, 2009. S. Afr. J. Plant Soil 26: 220-224.
Weinert, M. P., et al., 2004. Austral. Plant Pathol. 33 : 135-136.
Zhao, X.-Y. 1981. Proc. Int. Soc. Citricult., pp. 466-469.