Citrus Huanglongbing : Review, Present status and Future Strategies

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Abstract: Citrus huanglongbing (HLB), formerly known as greening, is a highly destructive disease of citrus, especially on sweet orange and mandarin varieties. A range of primary and secondary leaf symptoms are associated with HLB, making field diagnosis difficult, unless the typical lop-sided greened fruit are present. To date, the causal organism has not been cultured on artificial media and a diagnostic polymerase chain reaction technique has been developed to confirm the presence of the pathogen. The disease is caused by two closely related phloem-limited bacterial species, "*Candidatus* Liberibacter asiaticus" and "*Candidatus* L. africanus". The former, which causes the more severe Asian form, is found in Asia from southern Japan in the east through southeast and south Asia to eastern Iran in the west, as well as Saudi Arabia, Mauritius and Reunion. It is transmitted by the psylla, *Diaphorina citri*. The milder, yet still serious, African form is less heat tolerant, and is transmitted by another psylla species, *Trioza erytreae*. This form is found in Yemen, throughout eastern and southern Africa, as well as in Mauritius and Reunion. Of concern is the fact that both psylla species are capable of transmitting both bacterial species under experimental conditions. In Reunion, propagating healthy trees and releasing hyperparasites for vector control achieved almost complete control. Elsewhere, HLB is best controlled through integrated disease management involving the use of healthy nursery material, removal of infected trees or branches, and integrated vector control. The recent arrival of the vectors in citrus producing areas previously regarded as HLB-free highlights the potential threat of one of the most serious diseases of citrus, thus emphasizing the need for effective quarantine services.

1. Introduction

Citrus huanglongbing (yellow shoot disease) was first noted by farmers in southern China in the late nineteenth century as a problem of unknown cause (Zhao, 1981). By the 1920's similar diseases were recorded in Taiwan, known there as likubin (drooping disease) (Ôtake, 1990), Philippines (mottle leaf disease) (Lee, 1921) and India (citrus die-back) (Raychaudhuri *et al.*, 1974). In the late 1920's, a similar malady of citrus was observed in South Africa, called yellow branch or greening, depending on the production region (van der Merwe and Andersen, 1937). In Indonesia, the disease was first recorded in the 1940's and was described as citrus phloem degeneration (Aubert *et al.*, 1985). During the 1960's, the connection was made between the diseases described under these different names (McClean and Schwarz, 1970).

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For many years, the disease was known outside China as "greening", probably because the name was more commonly used in South Africa where extensive research was done from the 1950's. Although the disease was first described in English in 1919 by Reinking as a yellowing and leaf mottle of citrus occurring in China, no reference was made to earlier descriptions from China (da Graça, 1991). According to the international nomenclature rules, the first official description of the disease should have priority over subsequent names used. Therefore, because of the earlier recognition of the disease in China and the pioneering work done by K.H. Lin (Lin Kungxiang), citrus pathologists at the 13th conference of the International Organization of Citrus Virologists in China adopted "huanglongbing" (HLB) as the official name (Moreno *et al.*, 1996). According to Zhao (1981), "huanglong" means the yellowing of some new shoots in the green canopy, and "bing" means disease. Specifically, "huanglong" means "yellow dragon" because as symptoms progress they appear "draped" over the tree almost like a "yellow dragon" (CAB International, 2000).

The identity of the causal organism followed a similar treacherous path with the causal agent being first attributed to poor drainage (Raychaudhuri *et al.*, 1974), nematode damage (Ôtake, 1990) and later mineral deficiency or toxicity (Hector, 1944, van der Merwe and Andersen, 1937). The demonstrations of graft- and insect-transmissibility suggested that the causal organism was a virus (McClean and Oberholzer, 1965). In 1970, Laflèche and Bové, reported mycoplasma-type bodies in sieve tubes of sweet orange infected with HLB. Soon thereafter, Moll and Martin (1973) observed bacterium-like organisms in HLB-infected citrus plants similar to that observed in the insect vector *Trioza erytreae* (Del Guercio). The effective suppression of symptoms and the disappearance of the organisms after penicillin G treatment of HLB-infected trees proved the Gram negative nature of the unculturable bacterium (Bové *et al.*, 1980). Based on 16S rDNA comparative studies, the phloem-limited bacterium was identified as belonging to the alpha subdivision of the *Proteobacteriaceae* (Jagoueix *et al.*, 1994).

Two distinct forms of greening are recognised based on the wider geographical spread of the more severe, lower elevated (360 m) and higher temperature (30-35° C) disease with its psylla vector *Diaphorina citri* (Kuwayama) (Capoor *et al.*, 1967). The more restricted, less severe, temperature sensitive (27° C) African form of HLB is normally found at higher elevations (900 m above sea level) and is transmitted by *T. erytreae*. Comparative 16S rDNA studies (Jagoueix *et al.*, 1994), led to the proposed classification of the causal agent "*Candidatus*" with the generic name Liberobacter (meaning, bacteria of the phloem) (Jagoueix *et al.*, 1996). Initially, the proposed names were "*Candidatus* Liberobacter africanum" and "*Candidatus* L. asiaticum" (Jagoueix *et al.*, 1994), but were soon changed to "*Candidatus* Liberibacter africanus" and "*Candidatus* L. asiaticus", to comply with the rules of the *International Code of Nomenclature of Bacteria* (Garnier *et al.*, 2000b).

Da Graça (1991) published an extensive review of greening, but this was just prior to any significant molecular understanding of the causal organism, while Garnier and Bové (1993) covered the initial DNA studies in their review. Since then, much has been elucidated in terms of the identity of the causal agent and its control. This review attempts to give an overall picture of the disease and the causal organism up to the

present, and to discuss present and possible future management strategies.

Geographical distribution and economic impact

Da Graça (1991) lists 24 countries and territories in east, south-east, south and western Asia and in eastern and southern Africa, where HLB had been reported. Since then, its presence has been confirmed in four additional south-east Asian nations, namely Vietnam (Garnier and Bové, 1996), Myanmar, Laos and Cambodia (Garnier and Bové, 2000).

For other major citrus production regions such as North and South America, Australia and the Mediterranean countries, HLB remains a major threat if introduced. Globally, HLB has been regarded as one of the most important threats to commercial and sustainable citrus production. For instance, HLB has resulted in the destruction of 30 million trees in Indonesia (Tirtawidjaja, 1980). On the Indonesian island of Bali four million trees were eradicated during 1986-88, although these trees were replaced with mandarins in 1991, 40% were infected by 1993, and 90 % by 1996 (Aubert, 1993). In the early 1960's, nearly 25,000 ha were planted to citrus, but 10 years later five million (*i.e.*, 60% of the plantings) were lost to HLB. In Thailand, many trees are dying and going out of production five to six years after planting. Such losses are significant, since profits are only attainable 10 years after planting resulting in losses of over US$8,000/ha (Roistacher, 1996). In Bali, four million trees were eradicated during 1986-88 and replaced with mandarins in 1991. However, by 1993, 40% of these trees were infected and in 1996 more than 90% showed HLB symptoms (Aubert, 1993). In south-western Saudi Arabia, all sweet orange and mandarin trees had declined by 1986 leaving only limes (Aubert, 1993, Bové, 1986).

Crop losses of 30-100% have been reported in South Africa during the 1932-1936 and 1939-1946 periods (Oberholzer *et al.*, 1965, Schwarz, 1967). By 1958, the disease affected 100 000 sweet orange trees (Oberholzer *et al.*, 1965) and by the mid 1970's, it was estimated that four of the eleven million trees planted in South Africa (36%) were affected with HLB (Buitendag and von Broembsen, 1993). By then, major citrus production areas, which represented 20% of the industry, were eliminated, making it the most serious disease in South Africa. Of even greater concern was that areas previously regarded as HLB-free were showing tree symptoms (Green and Catling, 1971). By the mid 1990's the disease were reported in the Cape which, with its Mediterranean type climate, was regarded as "not likely" to get HLB. The use of a national quarantine barrier (McClean *et al.*, 1969) and restriction on sales of citrus trees from the northern regions were the disease is endemic to the coastal areas where psylla are endemic (McClean *et al.*, 1969) and not controlled, proved ineffective.

Although losses resulting from HLB has been more extensively documented in Asia than in Africa, it is estimated that globally more than 60 million trees had been destroyed by the disease by the early 1990's (Aubert, 1993).

3. Disease symptoms

Depending on the age of a tree and time and stage of infection, the first symptoms of

HLB usually start with the appearance of a yellow shoot. If infection occurs soon after propagation, yellowing progresses over the entire canopy. However, if infection occurs at a later stage of growth, the symptoms and the causal organism remain confined to the sector initially infected. If a sector of a tree is affected, then only those parts will show typical symptoms (Fig. 1), while the rest of the tree exhibits normal growth and produces normal healthy fruit of good quality (Oberholzer *et al.,* 1965).

A range of symptoms can be observed on infected trees and branches, which include heavy leaf and fruit drop, followed by out of season flushing and blossoming (Catling, 1969, Martinez, 1972, Oberholzer *et al.*, 1965). Severely infected trees often appear stunted, usually are sparsely foliated and can die back. Chronically infected trees are sparsely foliated and show extensive twig die-back symptoms. Infected trees produce reduced crops of low quality fruit (Oberholzer *et al.,* 1965).

Figure 1: Citrus tree with HLB infected segment showing appearance of a dragon draped over the tree.

Initial foliar symptoms of African HLB are vein yellowing and a variegated type of chlorosis (blotchy mottle), which appear on fully mature leaves (Schneider, 1968, Manicom and van Vuuren, 1980). Secondary symptoms include small, upright leaves ("rabbit's ears") with a variety of chlorotic patterns resembling those induced by zinc, iron, manganese, calcium, sulphur and/or boron deficiencies (Oberholzer *et al.,* 1965, Schneider, 1968, McClean and Schwarz, 1970). Many of the latter may be almost entirely devoid of chlorophyll, except for occasional circular green spots ("green islands") distributed at random on the leaves (Fig. 2) (Oberholzer *et al.,* 1965).

The Asian form of the disease induces similar symptoms, but with more exten-

sive yellowing, die-back and decline (Martinez and Wallace, 1968, Zhao, 1981), and in me cases death of small trees (1-2 years) (Lin, 1963). It is also more tolerant to heat, and thus is found in lower lying, hotter areas. In South Africa, leaf symptoms are more pronounced in the cool areas, compared to the lower lying hotter areas, and are more pronounced in winter (Schwarz, 1968b). African HLB can also be eliminated by exposure to extended periods of heat (Labuschagne and Kotzé, 1984). Both forms of greening have only been found in Reunion and Mauritius, usually separated by the temperature preferences, although both forms were detected in some trees using molecular probes (Garnier *et al.*, 1996).

The most reliable diagnostic symptom of HLB represent the fruits which when infected, are small, lopsided with a curved columella and seed, if present, is mostly aborted. A bitter, salty taste is also characteristic of affected fruit. With infected trees there is a continuous and premature shedding of greened fruit while those remaining on

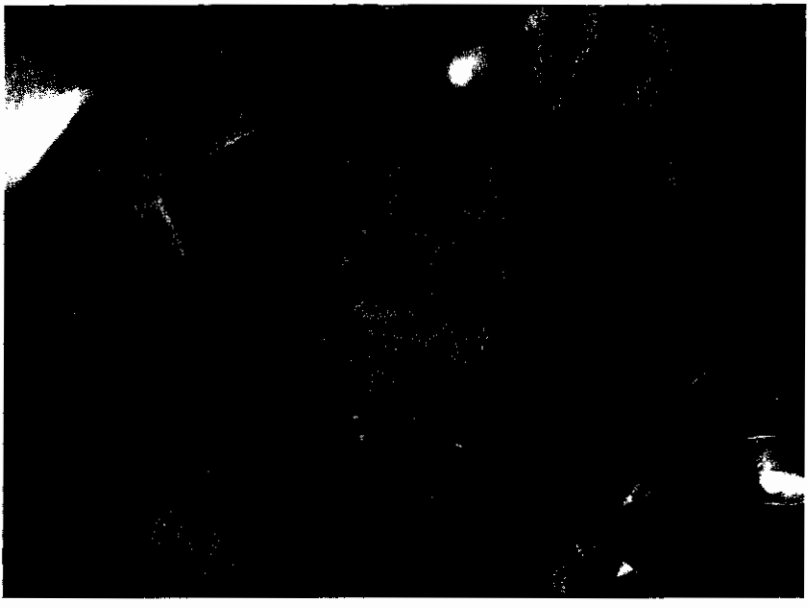

Figure 2: Leaf mottle symptoms of HLB with green islands.

the tree do not color properly (McClean and Schwarz, 1970), hence the former name "greening disease".

Symptoms can be exacerbated by the presence of other pathogens. Co-infection with *Citrus tristeza virus* (CTV) is common, and there are reports from several Asian countries that such trees have more severe symptoms (Martinez, 1972, Bhagabati and Nariani, 1980, Huang *et al.*, 1980). Of interest is that some isolates of CTV apparently protect trees from HLB infection (van Vuuren *et al.*, 2000). Blotchy mottle, the most characteristic leaf symptom, can be confused with other diseases such as stubborn (*Spiroplasma citri*), severe forms of CTV, Phytophthora root rot and water logging

(Calavan, 1968, McClean and Oberholzer, 1965, Schneider, 1968). Due to the non specific nature of leaf symptoms, HLB can often be confused with mineral deficiency or other stress related leaf symptoms (Korsten *et al.,* 1993). Symptoms of zinc deficiency are also associated with the early stages of citrus blight (Brlansky, 2000).

Root systems are usually poorly developed in severely affected trees, with relatively few fibrous roots (Oberholzer *et al.,* 1965), possibly due to root starvation. New root growth is suppressed and the roots often start decaying from the rootlets (Zhao, 1981).

4. Transmission

HLB was first transmitted experimentally by grafting (Chen, 1943), thereby establishing the causal agent as a pathogen. Natural spread was demonstrated by exposing healthy seedlings in an infected citrus orchard (Schwarz, 1964), and the vector in Africa was identified shortly thereafter as the citrus psylla, *T. erytreae* (McClean and Oberholzer, 1965). The vector of the disease in Asia was then identified as another species of psylla, *D. citri* (Tirtawidjaja *et al.*, 1965, Salibe and Cortez, 1966, Martinez and Wallace, 1967, Capoor *et al.*, 1967).

T. erytreae exists in Africa from the Red Sea coast through east and central Africa to South Africa, as well as in Cameroon. It is also found in Yemen, Madagascar, Mauritius, and, before bio-eradication, Reunion. More recently it has also been described in Madeira Island (Jagouiex *et al.,* 1996). It is sensitive to excessive heat, and thrives in cooler, higher areas 500 m and more above sea level. *D. citri*, on the other hand, is found in hotter, lower lying areas throughout south and south-east Asia, as far west as eastern Iran and Saudi Arabia, in Reunion, Mauritius, St Helena, Guadeloupe, Brazil, Florida (Knapp *et al.*, 1998) and, most recently, Venezuela (Cermeli *et al.*, 2000), Texas (French *et al.*, 2001), and Mexico (D.Thomas, pers.comm. 2002). Samples have also been collected in Argentina, Bahamas, Cuba, Dominican Republic and Puerto Rico, and there are unconfirmed reports from Costa Rica and Honduras (S.Halbert, pers.comm). The heat preferences of the two species correspond to that of the two forms of HLB, although it has been shown experimentally that both species can transmit both forms (Massonie *et al.*, 1976, Lallemand *et al.*, 1986). Recently *D. citri* was reported from northern Irian Jaya (West Papua) province in Indonesia near Papua New Guinea (PNG) (Davis *et al.*, 2000). During a survey of northern Australia, PNG and adjacent regions by the Australian Quarantine and Inspection Service (AQIS), it was found that the eradication campaign near Sorong failed and that HLB established more than 1000 km to the east (Davis *et al.*, 2000). This raised concerns of movement of planting material or ornamental hosts of *D. citri* that can result in further spread of the disease. Thus far PNG and north Queensland remain psylla and HLB-free.

Under experimental conditions, HLB can be transmitted by some species of dodder (Raychaudhuri *et al.*, 1974, Garnier and Bové, 1983, Ke *et al.*, 1988).

5. Causal organism

The demonstrations that greening is a graft- and insect-transmissible disease led to the

conclusion that a virus was responsible (McClean and Oberholzer, 1965, da Graça, 91). In 1970, Laflèche and Bové reported mycoplasma-type bodies in sieve tubes of sweet orange infected with HLB. The observation of cellular organisms in the phloem of HLB-infected citrus and their absence in healthy material indicated that a procaryotic organism was responsible (Laflèche and Bové, 1970). Similar organisms were observed in psylla (Chen *et al.*, 1973, Moll and Martin, 1973). On the basis of electron microscope studies it was suggested that the organism was a true bacterium, belonging to the Gracilicute division (Garnier and Bové, 1978). All attempts to isolate and culture the organism on artificial medium and proof Koch's postulates have been unsuccessful (Garnier and Bové, 1993). The development of monoclonal antibodies (MA) using extracts of infected plants (Martin-Gros *et al.*, 1987) enabled researchers to show that there is considerable serological diversity (Garnier *et al.*, 1987, 1991). One MA (MA 1A5) was able to recognize all non-Chinese Asian strains, but not the African strain (Gao *et al.*, 1993).

The next step in the study of the HLB bacterium was the use of DNA probes. DNA digests from infected plants were inserted into a bacteriophage; one of the inserts, In 2.6, hybridizes with all Asian strains tested, but not the African form, at high stringency (Villechanoux *et al.*, 1992). This insert contains genes for conserved ribosomal proteins (Villechanoux *et al.*, 1993). In 1994, Jagoueix *et al.*, proposed, on the basis of the sequence of the 16S rDNA and the ß operon, that the bacterium responsible for HLB is a member of the subdivision of the *Proteobacteriacea*, Subsequently the Asian species was designated "*Candidatus* Liberobacter asiaticum", and the African species "*Candidatus* L. africanum". These names have since been corrected to "*Candidatus* Liberibacter asiaticus" and "*Candidatus* L. africanus" (Garnier *et al.*, 2000b).

Host range

All species of citrus appear to be susceptible, irrespective of the rootstock used (Aubert, 1993, da Graça, 1991). However, symptoms are often severe on sweet orange, mandarins and their hybrids; moderate on grapefruit, lemon and sour orange; while lime, pummelo and trifoliate orange are regarded as being more "tolerant" (Manicom and van Vuuren, 1990). Both species of liberibacter have been transmitted to periwinkle (*Catharanthus roseus*) via dodder inducing marked foliar yellowing (Garnier and Bové, 1983, Ke *et al.*, 1988), the dodder itself also appears to support HLB multiplication (Ghosh *et al.*, 1977).

The psylla species which transmit HLB from citrus to citrus, feed on many other rutaceous species. *D. citri* has a preference for *Murraya* spp. (Chakraborty *et al.*, 1976), and it has been suggested that *T. erytreae*'s original hosts include *Vepris undulata*, *Clausena anisata* and *Zanthoxylum capense* (Moran, 1978). Su *et al.*, (1995) has reported the detection of Asian HLB by DNA-hybridization in *Severinia buxifolia* and *Limonia acidissima*, and African HLB was detected in *Toddalia lanceolata* (= *Vepris undulata*) (Korsten *et al.*, 1996). The Cape chestnut (*Calodendrum capense*), an ornamental rutaceous tree in South Africa, has been shown to be infected with HLB (Garnier *et al.*, 2000a), subsequently this organism was shown to be a subspecies of the African

form of greening (Garnier *et al.*, 2000b). This third Liberibacter was classified as '*Candidatus* Liberibacter africanus subsp. capensis' (Garnier *et al.*, 2000b). Phylogenetic analysis demonstrated that the bacterium belongs to the genus '*Candidatus* Liberibacter' and further 16S rDNA sequencing together with serological studies, classified the *C. capense* Liberibacter as being more closely related to '*Candidatus* L. africanus' than to '*Candidatus* L. asiaticus' (Garnier *et al.*, 2000b).

7. Detection

Field diagnosis of HLB is difficult because of the non-specific nature of foliar symptoms. Since it is easy to confuse HLB leaf symptoms with nutrient deficiencies, other diseases or stress related factors, positive confirmation with fruit symptoms is often required in the field. Prior to the more recent positive identification of the causal agent with molecular techniques, the only other method for confirmation was inoculation of biological indicators such as sweet orange, Orlando tangelo (Schwarz, 1968a) or Ponkan mandarin (Matsumoto *et al.*, 1968). Following the identification of a fluorescent phenolic compound, gentiosyl-ß-glucoside from fruit albedo or bark extracts, a diagnostic technique for confirmation of HLB was developed (Schwarz, 1968b, van Vuuren and da Graça, 1977). However, this method soon proved non-specific since stressed trees contained the same marker. In addition other similar diseases such as stubborn diseased also contained the same marker (Schwarz, 1970). Alternative detection techniques were subsequently developed for rapid identification of the disease. These included, immunofluorescence microscopy (Korsten *et al.*, 1993, 1996), ELISA using monoclonal antibodies (MAs) (Garnier *et al.*, 1987, 1991, Gao *et al.*, 1993, Korsten *et al.*, 1993, 1996) and DNA hybridization (Korsten *et al.*, 1996) or PCR (Jagoueix *et al.*, 1996, Korsten *et al.*, 1996).

The development of monoclonal antibodies allowed for more rapid and sensitive detection, but the specificities of the MAs and the strain diversity of the bacterium made their use for general detection impractical (Villechanoux *et al.*, 1992, 1993, Korsten *et al.*, 1993). At the time the use of DNA probes proved more suitable for detection of HLB in infected citrus material (Korsten *et al.*, 1993, Su *et al.*, 1992, Villechanoux, *et al.*, 1992). Two DNA probes, In-2.6 (Villechanoux *et al.*, 1992) and AS-1.7 (Planet *et al.*, 1995) containing genes for ribosomal proteins (ß operon), were developed for L. asiaticus and L. africanus respectively. The necessity of using two different probes for detection of L. africanum and L. asiaticus, and the fact that DNA extraction for dot-blot hybridisation is very time consuming, led to the development of additional detection procedures (Jagoueix *et al.*, 1996). The polymerase chain reaction (PCR), first described in the mid-1980s has since become a powerful technique for the selective amplification of DNA or RNA sequences. In the detection of HLB it is necessary to identify the causal agent of the disease unambiguously, rapidly and at a level of infection that is not visually apparent. Ribosomal genes are particularly appropriate targets for PCR-directed identification, as the genes occur in high copy numbers, are highly conserved and are flanked by spacer regions that contain comparatively variable sequences. DNA sequence data on ribosomal genes can be obtained by PCR with broad-range primers (universal primers) that anneal to the highly

conserved ribosomal gene sequences and amplify across regions that contain nucleotide variation. Planet *et al.*, (1995) developed a PCR technique whereby a fragment of the *plKAJL-rpoBC* operon (b operon) (Jagoeix *et al.*, 1994) of the Asian Liberibacter strain from Poona (India) and the African Liberibacter was amplified. This section of the Liberibacter genome represents a conserved region of the 16SrDNA of the Liberibacter spp. Three primers have been developed and are currently commercially used during PCR detection of HLB (Jagoeix 1996, Hocquellet, *et al.*, 1999). Currently the PCR technique is ISO 17025 accredited and is being used for commercial detection of HLB in suspect plant material by Plant Pathology Laboratories, University of Pretoria, South Africa.

8. Control and management

The evidence that a procaryote was the causal organism led to research on the use of tree injections with antibiotics to eliminate the bacteria. Tetracycline hydrochloride had some beneficial effects (Schwarz and van Vuuren, 1970, van Vuuren, 1977, van Vuuren *et al.*, 1977), but proved to be phytotoxic (van Vuuren, 1977), and attention turned to a more soluble less toxic derivative N-pyrrolidinomethyl tetracycline (Buitendag and Bronkhorst, 1983). Due to the potential for re-infection, and high costs, attention turned to vector control (Buitendag and von Broembsen, 1993). Several insecticides against psylla are available, and the development of a trunk application technique has proved effective (Buitendag, 1988). To assist in determining the optimum timing of insecticides, Samways *et al.*, (1986) proposed the placing of sticky yellow traps in orchards which could detect a population threshold; this method has not been widely adopted and scouting is often used.

Several parasitic wasp species attack citrus psylla. *D. citri* is a host for *Tamarixia radiata*, and *T. erytreae* is attacked by *Tetrastichus dryi*. However, the efficacy of these parasites is limited by the existence of hyperparasitic wasps. Only in the Indian Ocean islands of Reunion and Mauritius was success achieved where *T. radiata* and *T. dryi* were introduced without the hyperparasites (Aubert *et al.*, 1984, Quilici, 1988). In Reunion, the use of these parasites, combined with the establishment of disease-free foundation blocks and nurseries, resulted in a dramatic reduction in the incidence of HLB - in 1995, 20 years after the launching of this strategy, only 0.5% of trees surveyed had symptoms (Aubert *et al.*, 1996). *T. radiata*, and another wasp species, *Diaphorencyrtus aligarhensis*, are currently being evaluated in Florida for potential use there (Hoy and Nguyen, 2000). In addition, *D. citri* appears to be an excellent food source for several ladybeetle species (Michaud *et al.*, 2002).

The only way to grow citrus productively in countries where the disease has become endemic such as in South Africa and Asia, is by managing the disease using sound integrated pest management strategies (Aubert, 1990,1993, Aubert and Quilici, 1984, Aubert and Xia, 1990, Buitendag, 1991, Buitendag and von Broembsen, 1993, Samways, 1990). In South Africa, Buitendag and von Broembsen (1993) recommend a strategy of providing growers with disease-free nursery trees, focussing on reducing the inoculum by removing infected trees or branches, and following an effective psylla control program. In China, there are reports of successful management by eradicating

infected trees and non-citrus psylla hosts, planting HLB-free trees, and controlling psylla populations (Ke and Xu, 1990, Xu *et al.*, 1991). Bové *et al.*, (2000) conducted a program in Indonesia, and showed that if citrus is eradicated before replanting, only HLB-free budwood is used for replanting and the control *D. citri* using insecticide sprays is effective, rehabilitation of a citrus industry could be possible. Eradication of alternate hosts in close proximity (5 km) to nurseries or commercial plantings of citrus, have been suggested and shown to be effective in Asia where *Murraya* spp are the principal alternate hosts (Aubert 1990, 1993, Aubert and Xia 1990). However, this approach is impractical in the African continent as natural forest species are hosts to both the HLB organism and the psylla vector.

In cases where the disease is not present, effective quarantine measures are essential to prevent the introduction of the HLB organism or the vector. Since numerous alternative hosts have been reported, it is essential to include them in any preventative quarantine strategy. Risk assessment studies have become essential for Sanitary and Phytosanitary issues in international trade. In addition perceived risks in terms of biological warfare, has become a reality and HLB has been listed as a national biological threat for the USA even before the September 11th, 2001 terrorist attacks.

Despite the fact that *D. citri* has been present in Brazil for several decades, HLB has not been detected there. Furthermore, *D. citri* was recently discovered in Florida (Knapp *et al.*, 1998) and Texas (French *et al.*, 2001), and *T. erytreae* in Madeira Island (Jagoueix *et al.*, 1996). This constitutes a major risk for the respective citrus industries if the pathogen should ever be introduced. Preventative measures require a thorough risk assessment study and effective quarantine regulations. The possibility furthermore exist that the vector could be introduced "naturally" or through alternate hosts such as *Murraya* spp., which has recently been identified by the plant quarantine regulatory authorities in the USDA APHIS/PPQ data base records show 40 interceptions of live *D. citri* at ports between 1985 and 1998. One interception contained 46 live *D. citri* from India. The majority of these interceptions were on *Murraya* spp., especially *M. koenigii* (Halbert, 1998). This poses a potential threat for the local industry since the adult *D. citri* can transmit the disease since it can persist in the vector for up to three months, thus highlighting the importance of an effective risk management plan.

9. Conclusion and future prospects

HLB can be considered one of the classic diseases in the history of plant pathology. Its checkered history, riddled with incorrect assumptions, cultural and social differences and economic impact, provides valuable insight into human-insect-plant disease ecology. The global spread of the disease in its two forms with different vectors provides a challenge to countries that are currently HLB-free, such as Australia, Mediterranean countries and the Americas, to retain their status through effective quarantine. For countries that have the less severe African form, prevention of acquiring the more severe Asian form is crucial. The spread of the vectors across international boundaries is especially difficult, C. N. Roistacher (pers.comm.), in reference to tristeza and its aphid vectors, commented that laws can be enacted forbidding moving pathogens and infected plants across borders, but "someone forgot to tell the insects".

The successful biocontrol program in Reunion has raised hopes that the vectors can be controlled in other areas (Hoy and Nguyen, 2000), provided no hyperparasites are present. The recent reports of native predators feeding off the recently introduced psylla in Florida (Michaud *et al.*, 2002) are encouraging, even though predators were not found to be effective in controlling HLB in South Africa (van den Berg and Deacon, 1987). Another possibility is the introduction of resistance genes. De Lange *et al.*, (1985) began a breeding program in South Africa, but thus far no commercial varieties have appeared. Recently, the gene for bovine lysozyme, an enzyme with anti-bacterial properties, was cloned and introduced into citrus where it was expressed (Yang *et al.*, 2001). It may prove to play a future role in controlling bacterial diseases such as HLB and canker in the future.

Risk assessment models used to predict the likelihood of disease introductions into disease-free areas or countries, and Geographical Information Systems, which can provide predictive global maps of how diseases can spread, provide valuable tools to study plant diseases. So far the HLB organism has defied culture in artificial media, but future research groups will periodically re-attempt culturing as new technologies become available. There is still much to be learned about this disease, its vectors, the causal organism, and the control or management of the disease.

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