Huanglongbing Disease of Citrus:

1 Introduction:

Huanglongbing (HLB) disease of citrus is one of the most destructive disease of citrus worldwide representing an alarming threat to the world citrus industry, and is slowly invading new citrus-growing areas. The term “huang long bing” was given by farmers of china where “bing” stands for disease, “huang” stands for yellow and “long” meaning dragon. So, the term “huanglongbing” means yellow dragon disease. Before the disease got its name, similar set of symptoms were recognized by several different names in different parts of the world such as yellow shoot disease (huanglungbin) in China, likubin (decline) in Taiwan, dieback in India, leaf mottle yellow in the Philippines, vein phloem degeneration in Indonesia and yellow branch, blotchy-mottle or citrus greening in S. Africa. Originally thought to be caused by a virus, it is now known to be caused by endogenous, phloem sieve tube-restricted gram negative bacteria, named liberibacters, vectored by psyllids. There are three forms of HLB that have been described: the Asian form, the African form and the American form. The Asian form of disease originating from China produces symptoms under warmer conditions and is transmitted by the Asian citrus psyllid *Diaphorina citri,* while the African form produces symptoms only under cool conditions and is transmitted by the African citrus psyllid *Trioza erytrea*. Recently, a third American form transmitted by the Asian citrus psyllid was discovered in Brazil in 2004. This American form of the disease apparently originated in China.

The disease HLB is widespread in most citrus growing areas of Asia, Africa and the US causing serious devastation in citrus production. Wherever the disease has appeared, production of citrus has declined rapidly rendering them unproductive. Importantly, all commercial citrus species and scion cultivars are susceptible to HLB infection regardless of rootstock.

In North America, the disease was discovered in the south florida region of homestead and florida city in August 2005. Since that time, HLB has been found in commercial and residential sites in all counties with commercial citrus. The spread of HLB through the Caribbean, Central and North America has been rapid. HLB is now known to occur in Florida, Louisiana, South Carolina, Georgia, Cuba, Puerto Rico, Belize, Mexico, Jamaica and US virgin islands.

2 History and Geographical Distribution

Citrus dieback was documented in India in the eighteenth century. However, it was first accurately described in 1929 and attributed to poor drainage. Citrus HLB was first observed in the coastal Chaoshan Plain of Guangdong Province, China, in the late 19th century based on description of yellow shoot symptoms. Likubin, a disease with similar symptoms, was identified in Taiwan 60 years ago and described as a nematode associated problem.

A disease similar to HLB was observed in 1928/29 in the western Transvaal province of South Africa and named “yellow branch/shoot”, while in the eastern Transvaal province, the name “citrus greening” was more prevalent. In the first description of disease “greening” in 1937, it was yet assumed to be a result of mineral toxicity.

In the Philippines, the disease was described in 1921 as “mottle leaf”, however, thought to be related to zinc deficiency.

India is another country with its long association to the disease. Pertinent symptoms of HLB have been attributed to “dieback”, a disease that was first observed by Roghoji Bhonsale (Capoor, 1963) in the 18th century in the central provinces, soon after the introduction of citrus in India. In early periods, dieback was merely a symptom picture, and many factors such as soil disorders, nutrient deficiency or toxicity, pathogens (fungi, virus) were thought to be the cause of it. In some cases, *Tristeza* virus was assumed to be a major cause of “dieback”.

The disease has now spread into all the major citrus producing areas of the world except the Mediterranean countries and Australia. In Asia, movement of citrus plants between different countries after 1930’s have been recorded. This is the probable reason for spread of the disease throughout south-east Asia. The Asian vector *Diaphorina citri* has been found to be moving eastwards into Papua New Guinea, with HLB detection occurring soon after. To the west, the psyllid has moved into eastern Iran and Oman. The rest of the Middle East and Mediterranean countries are now threatened by this disease.

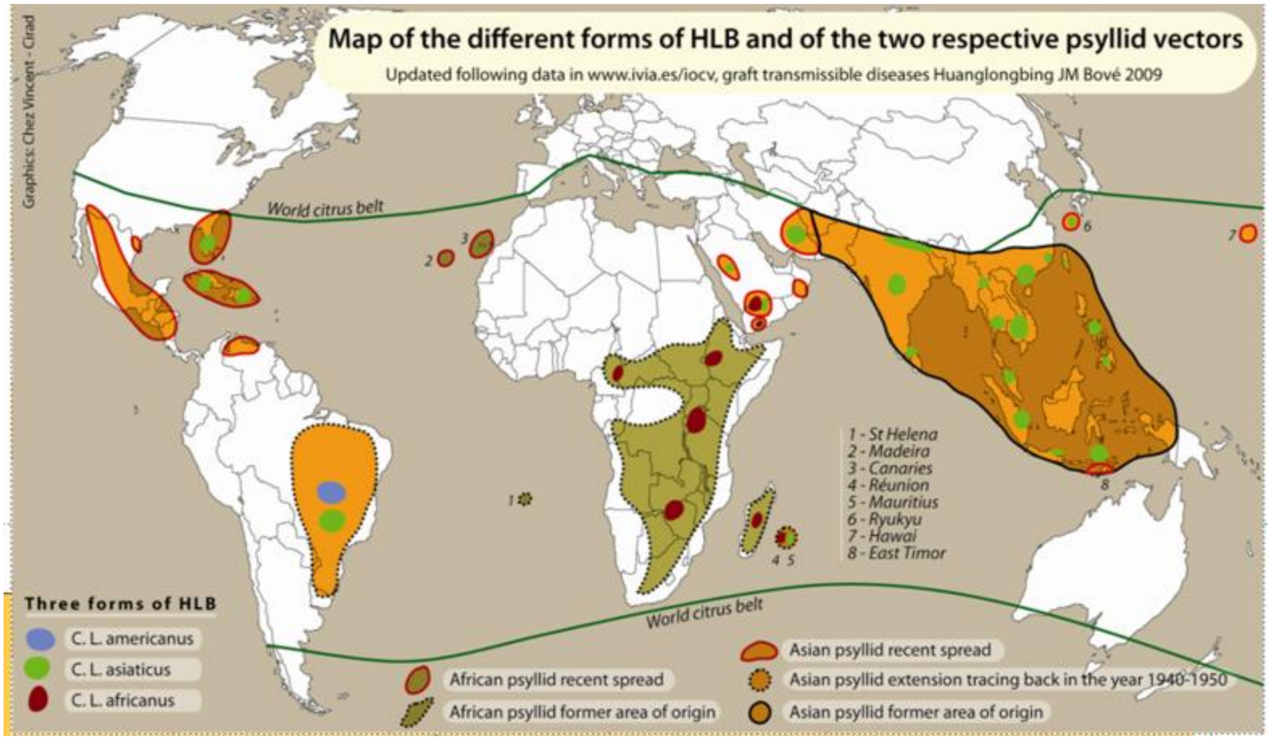


Fig1: Distribution of HLB over several regions of the world together with the vector.

Diaphorina citri was detected in Brazil in 1942 but HLB was not reported until 2004. Guadeloupe and Florida reported presence of the vector *Diaphorina citri* in 1998, however, the disease HLB was confirmed in 2005. The major concern is the rapid spread of HLB and psyllid vectors towards new citrus production areas. The Asian citrus psyllid has been found in Florida, Texas, California, Arizona, Hawaii, Louisiana, Georgia, and Alabama in the United States, as well as in parts of south and central America, Mexico, and the Caribbean. In lieu of this, HLB has been spotted in Louisiana (2008), South Carolina (2009), Georgia (2009), and most recently in Texas and California (2012) of the United States. HLB has also been detected in Belize, Cuba, Jamaica, Mexico and other countries in the Caribbean.

3 Economic Impact of HLB

HLB economically affects citrus in three potential ways: i. reduction of yield, ii. Production of poor quality fruits, iii. Increased cost of production.

HLB causes blocking of phloem tissue that is responsible for translocation of nutrients from leaf to different parts of plants thus limiting ability of a plant to translocate nutrients. With the infection of citrus trees by HLB, symptom may or may not appear for a while. As soon as the symptoms are visible, the disease gains prevalence throughout the tree canopy. Severe symptoms in trees are observed one to five years after onset of first symptoms, depending on the age of tree at the time of infection. Trees in the orchards usually die in about 3-8 years after becoming infected and require removal and replanting.

As disease severity increases, the yield is reduced and also the fruit quality is degraded. The reduction of yield can be attributed to early abortion of fruits from infected branches. Although some fruits can be harvested from infected trees, asymptomatic fruits are smaller, lighter and acidic and have a reduced brix percentage. Besides this, the infected fruits are misshaped with decreased juice percentage and quality, bitter taste and are generally unsuitable for sale as fresh fruit or juice. When reduction in production is coupled with production of poor quality fruits, total yield loss can be estimated as 30-100%.

Citrus fruits are produced all over the world. Around 70% of the world's total citrus production is grown in the Northern Hemisphere, in particular countries around the Mediterranean and the United States.

In the United States, citrus fruits for consumption as fresh fruit are mainly grown in California, Arizona and Texas, while most orange juice and grapefruit is produced in Florida.

Spreen and Baldwin (2013) have explored that the presence of HLB has reduced the willingness of Florida growers to invest in new plantings. A study conducted to find out the economic impact of HLB in Florida through 2006/07 season to 2010/11 season indicated profound impact of HLB over citrus production in the state, and significant employment impacts on the agricultural sector.

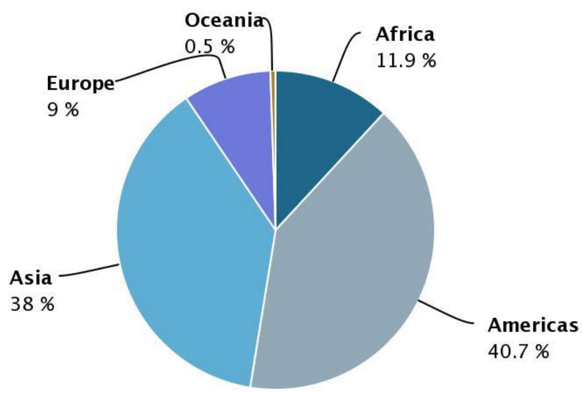


Fig 2: Production of Citrus (total) by region. Average (2000-2013).

Source: Food and Agriculture Organization of the United Nations, Statistics Division (http://faostat3.fao.org/browse/Q/QC/E)

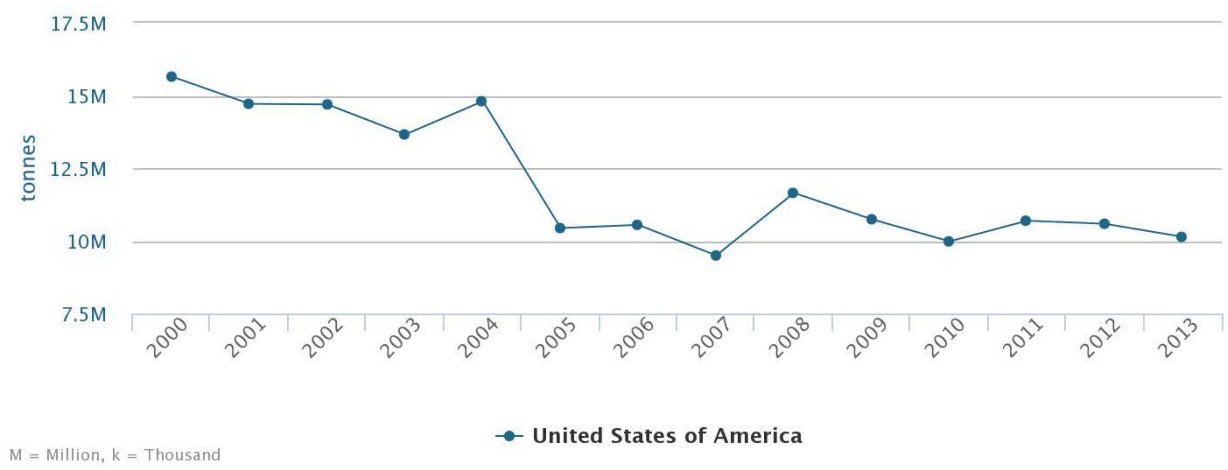


Fig 3. Trend in Citrus Production in the USA. (2000-2013)

Source: Food and Agriculture Organization of the United Nations, Statistics Division (http://faostat3.fao.org/browse/Q/QC/E)

4 Disease Symptoms

Citrus disease HLB cannot be diagnosed with symptoms alone as similar symptoms are a result of many plant pathogens or cultural conditions. Identification of HLB can be successful only when symptoms are thoroughly monitored.

Symptoms of HLB can be visible year round, but are more readily seen from September through March. Symptoms can occur throughout the tree, especially if the infection occurs at or soon after propagation, however, if infection occurs at a later stage of growth, the symptoms and causal organism are often confined to the sector initially infected. When infection is limited to a sector, rest of the tree shows normal growth producing fruits of fair quality.

The first sign of infection is yellowing of young shoots from where the disease gets its name “yellow shoot disease”.

Fig 4: Yellow disease symptom observed in Pummelo.

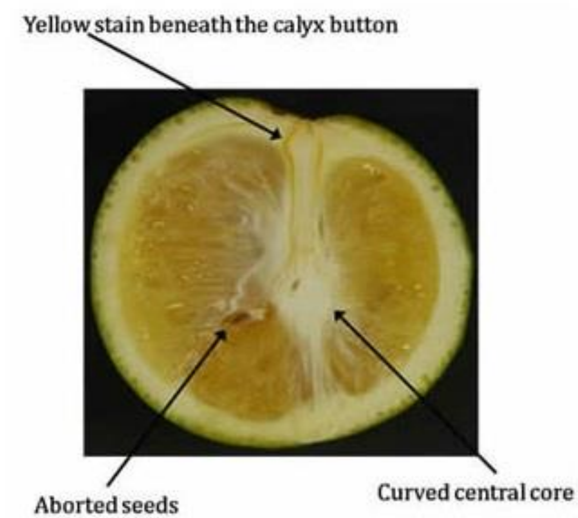
In leaf, characteristic blotchy mottle symptoms and vein yellowing are observed. The blotchy mottle symptom in leaf, a random pattern of yellowing on leaves that is not the same on right and left side of leaves, is often confused with the nutrient deficiency symptoms (such as zinc deficiency) however, mottle symptom with HLB is vivid. In addition, the mottling incited by HLB usually crosses leaf veins while nutrition related mottling are usually restricted between or along leaf veins. Also, the nutrient deficiency symptoms are exhibited uniformly throughout the canopy while mottling due to HLB causes yellow shoots on one or many branches randomly arranged in the canopy. The blotchy mottle is frequently found on newly mature leaves but mottling fades with leaf age. This causes the formation of yellow and green patches on both sides of midrib and is often referred to as “green islands”.

Fig 5. Differences between symptoms caused by nutrient deficiency and HLB on citrus leaves.

Infected leaves are small, upright and erect with chlorotic patterns and are often referred as “rabbit’s ears” symptoms.

HLB also causes infection in fruit which is characterized externally by small, lopsided, misshapen and green color of ripened fruits. Green color of the fruit even after ripening gives disease a common name “greening”. Symptomatic fruits in comparison to healthy fruits are small, light, and more acidic with lower juice percentage, brix, total soluble solids (TSS). Infected fruit would taste salty and bitter which probably may be due to higher acidity and lower sugars. Many fruits fall off prematurely from infected trees. Internally, the fruits have curved axis, aborted seeds and orange-yellow stain on vascular columella. Fruit ripening may occur backwards with the stylar ends remaining green as the fruit colors.

Lopsided fruit



http://www.crec.ifas.ufl.edu/

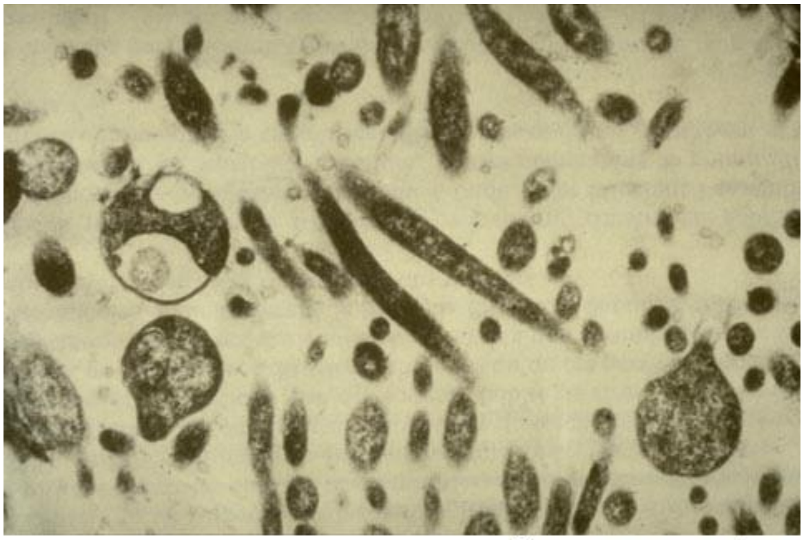
Fig 6. Symptoms of HLB on citrus fruits.

As the disease progresses, the infected trees become sparsely foliated and tree suffers dieback. Trees are stunted and declining in production. Out of season flushing and blossoming Root systems are poorly developed in case of severe infection. No new root grows while existing roots start decaying from the rootlets.

5 Causal Organism

At around 1967, the causal organism of HLB of citrus was supposed to be a virus as the disease transmission was known to occur through graft inoculation and through vectors i.e. citrus psylla. The only agent known then to transmit disease through this process were viruses. Later, Mycoplasma like organisms were found to be associated with some plant diseases of “yellows” type and symptoms of such diseases also resembled the HLB disease. Mycoplasmas are a genus of bacteria that lacks a cell wall around their cell membrane. So, Electron Microscopy (EM) came into action to find out any MLO’s associated with greening. Through EMs, micro-organisms were detected in the phloem sieve-tubes of symptomatic leaves but not on healthy leaves. Thus found micro-organism could not be obtained in culture. Furthermore, the organism which was supposed to be a mycoplasma like organism was found to be enclosed in a 25nm thick envelope which was different than the properties of a mycoplasma which possessed a 7-10 nm thick cell membrane. This property suggested that the HLB organism possessed a cell wall in addition to its cell membrane. This made the bacteria known as the causal organism for HLB. The gram negative nature of the HLB was finally demonstrated in 1984 (Garnier and Bove, 1984a, 1984b).

Since the bacteria could not be cultured on artificial media, the term *Candidatus,* which indicates an organism that has not been cultured and is characterized on the basis of DNA properties, is used. The bacteria is classified under genus *Candidatus Liberibacter.*



Source: www.apsnet.org

Fig 4. TEM micrograph of Candidatus Liberibacter asiaticus bacteria, causal agent of HLB, within citrus phloem sieve tube.

5.1 Forms of HLB: Asian, African and American

The disease HLB of citrus is caused by a gram-negative bacteria with a double-membrane cell envelope in the genus *Candidatus Liberibacter*. Three forms or species of this genus have been identified in different regions of the world: *Candidatus Liberibacter asiaticus, Candidatus Liberibacter africanus and Candidatus Liberibacter americanus.*

These three species of *Liberibacter* cause the same symptoms wherever HLB occurs. Although HLB can be detected through Electron Microscopy (EM) technique, the causal organism cannot be differentiated through this process as there are no morphological differences to distinguish between the *Liberibacter* species causing HLB.

The African and Asian form can be distinguished on the basis of serological methods or by temperature sensitivity. The Asian form; *Candidatus Liberibacter asiaticus* is heat tolerant while the African form; *Candidatus Liberibacter africanus* is heat sensitive. The American form; *Candidatus Liberibacter americanus* is also heat tolerant (Das et al. 2007).

The symptoms produced by all forms of HLB are generally the same but the Asian form is considered severe than the African form as the Asian form causes die-back which can lead to tree death.

The influence of temperature on HLB symptoms caused by Asian and African forms were studied in the frame of international cooperation experiment started in 1969 under two temperature controlled chambers. Sweet orange plants graft inoculated with African HLB form produced severe disease symptoms under cool chamber (24^{0}C with a 16-hr light period and 22^0C with a 8-hr dark period) but recovered soon when transferred to warm chamber (32^0C with a 16-hr light period and 27^0C with a 8-hr dark period). On the contrary, the Asian form produced pronounced symptoms under both warm and cool chamber conditions. This study together with other field condition study revealed that the African form of HLB is heat-sensitive and occurs only in cool areas with temperatures remaining below 30-32^0C.

Sweet orange seedlings graft-inoculated with American form of HLB (originated in Brazil) showed severe HLB leaf mottle at both cool (22-24^0C) and warm (27-32^0C) conditions (Teixeira et al., 2005c). This observation suggests that the American form of HLB is also heat tolerant.

Since African HLB form are heat sensitive, the symptoms produced by African form of HLB are suppressed by long exposure to temperatures above 30^0C and are dominant in citrus at elevations above 700m. In contrast, Asian forms of HLB, originated in china, is dominant in low lying hotter areas.

6 Spread / Transmission

The disease HLB is graft transmissible. Graft transmissibility of all forms of HLB has been confirmed in various time frames in the history by different people (by Lin in China, by McClean & Oberholzer in S. Africa). Long distance spread of HLB occurs b grafting with diseased budwood.

Natural spread of Asian and American form of HLB occurs by the Asian citrus psylla, *Diaphorina citri* whereas *Trioza erytrea* is the primary vector for transmission of African form of HLB. The temperature tolerances and sensitivities of these two species match those of HLB type; *D. citri* is heat tolerant, while *T. erytrea* is sensitive to high temperatures.

The Asian citrus psyllid *D. citri* is the principal insect vector of the disease in Asian countries, Brazil and USA. Studies reveal that fourth to fifth instar nymphs and adults can acquire *Candidatus Liberibacter asiaticus* by feeding on infected citrus trees while can transmit the pathogen to citrus plants during their entire life. The pathogen is acquired by *D. citri* in 15-30 min with a latent period of 8-12 days while one or more hour is required for 100% transmission. On the other hand, *T. erytrea* acquires the organism after one day of feeding and is able to transmit the pathogen 7 days later within 1 hour of exposure to the host plant. The longer the insect overwinters in the infected old leaves, the more infective the insect becomes on young flush in spring. In addition, the psylla are strongly attracted to yellow green light thus making diseased trees attractive targets and thereby increasing the proportion of disease-carrying insects.

Other species of psyllids have also been noted on citrus such as *T. eastopi, Mesohomatoma lutheri* in Reunion, *D. communis* in India, *T. citroimpura, Psylla citrisuga and P. citricola* in China, *D. auberti and D. amoena* in Comoros, and *D. punctulata* and *D. zebrana* in Swaziland, but no evidence has been found of transmitting greening.

Dodder plant (*Cuscuta campestris*) is another potential vector of HLB bacterium to transmit the disease from citrus to periwinkle (Garnier & Bove, 1983).

7 Host Range

Citrus has been identified as the primary host for HLB. Almost all citrus cultivars have been found to be affected by HLB with varying degree of responses (Folimonova et al. 2009). Results from graft transmission of different citrus genotypes with buds from *Candidatus Liberibacter asiaticus*- infected trees were grouped into different categories based on the symptoms developed and the ability of the plants to continue growth; Severe (Sweet orange, tangelo and mandarin), Moderate (grapefruit, lemon, sour orange) and Tolerant (lime, pummelo and trifoliate orange). Symptoms have also been observed in *Microcitrus australasica, Swinglea glutinosa, Atlantia missionis, Clausena indica, Limonia acidisimma, Balsamocitrus dawei, Aeglopsis chevalieria, Severinia buxifolia, Murraya paniculata. Catharanthus roseus* (Periwinkle) and *Nicotiana xanthi* (Tobacco) are the only reported non-rutaceous hosts. These non-rutaceous hosts have been shown to be infected only under laboratory conditions and acted as indicator plants.

8 Detection

8.1 Biological Indexing:

Development of disease symptoms; yellow shoots, blotchy mottle leaves etc. in the indicator plants was the first diagnostic test developed ever. Indicator plants are Valencia sweet orange or Orlando tangelo in South Africa, Ponkan mandarin in Taiwan and Darjeeling orange or Mosambi sweet orange in India. This method of detection used graft sticks which were grafted in indicator stems and the symptoms appeared in 3-4 months in the greenhouse maintained at 21-23^{0}C (R. E. Schwarz, 1968). This method was greatly dependent upon the transmission percentages that varied from 54.7

to 88 % for *Candidatus L. asiaticus* and 10.0 to 45.2% for *Candidatus L. americanus* even by the very efficient “seedling inoculation method” for citrus disease.

As the use of indicator plants for detection of HLB was time consuming and not feasible, a quicker laboratory test was developed by Ralph E. Schwarz which was based on biochemical changes occurring in infected plants. A fluorescent substance was found to be associated with reproductive and vegetative tissues of sweet orange infected with HLB when the fruits were about 20-30mm long or even earlier. This substance was not found in fruits of trees free of HLB.

Later in 1987, fluorescence microscopy method was found to detect the HLB. Bright yellow fluorescence was observed only at the phloem of infected petioles but never on healthy plants or other kinds of diseased plants. However, this method soon proved non-specific since stressed trees contained the same marker. At the same time, Serological identification using monoclonal antibodies against the Indian forms of citrus greening bacterium has been successfully used to detect the bacterium in greenhouse-grown citrus and *Catharanthus roseus* by immunofluorescence and ELISA (Garnier et al. 1987). Until 1992, electron microscopy visualization of the HLB organisms in the phloem sieve tubes of HLB infected citrus leaves was the only reliable method of detection. However, this technique was unable to distinguish between the African and Asian Liberibacters.

8.2 DNA Hybridisation:

Two DNA probes, In2.6 and AS1.7, containing genes for ribosomal proteins have been produced for Asian liberibacter strains and African liberibacter strains respectively. Used in dot-blot hybridization assays, they allow detection of the respective greening liberibacter species in citrus leaf samples collected from infected orchards. The necessity of using two different probes for detection of two liberibacter species and time consuming nature are two drawbacks.

8.3 Conventional PCR:

Two PCR systems had been used for detection and identification of pathogen. First is based on amplification of a 1160bp fragment of liberibacter 16S rDNA. The primer pair, OI1/OI2c is able to amplify the rDNA of both liberibacter species, while the pair OI2c/OA1 preferentially amplifies the *L. africanum*. Thus, the use of three primers OI2c/OI1/OA1 was recommended in countries with both species suspected or present. Moreover, in order to distinguish the two liberibacter species in a given tree, a time consuming enzyme digestion of the PCR products with XbaI is needed (Jagoueix et al., 1996).

Several other primer sets have been developed based on the β-operon ribosomal protein gene which allow identification of the two liberibacter species directly by the PCR amplicon size.

8.3: Real time PCR (QPCR/ RTPCR):

Due to its improved speed, sensitivity, reproducibility, robustness and the reduced risk of carry-over contamination as compared to standard format PCR, Real-time quantitative PCR has gained acceptance. With RTPCR, diagnosis can be performed with DNA extracts in the field in less than 1 hour.

In 2005, species specific TaqMan probe-primer sets, HLBaspr, HLBafpr and HLBampr were developed for detection and identification of three known species of Liberibacter in complex PCR with the positive internal control of host plant cytochrome oxidase gene. The low detection limits of these three HLB probe-primer sets are down to 1 to 10 copies of Liberibacter's 16S rDNA per reaction and their PCR efficiency is up to 99.90%. In addition, the low detection limit and the PCR efficiency of the positive internal control probe-primer set COXfpr are almost the same as those of the HLB probe-primer sets, which allows accurate estimation of the ratio of Liberibacter DNA to the host plant DNA in total DNA extracts obtained from infected plants. The HLBaspr set has been successfully applied in detection, identification and quantification of '*Candidatus Liberibacter asiaticus'* in host plants of citrus (Tatineni et al. 2008; Li et al. 2008a) and in vector psyllids (Manjunath et al. 2007).

9 Control and Management:

Following the introduction of HLB into the United States and Brazil, three tiered management program for HLB has been recommended by most scientists: (1) maintain low psyllid infestation levels to reduce transmission, (2) regularly identify and remove trees infected by HLB to remove inoculum sources, and (3) only plant nursery stock known to be free of HLB (Gottwald, 2007). Implementation of these three tiered program for management of HLB has not been effective since many growers are reluctant to remove the infected trees that are still productive. To mitigate the disease without removing the infected trees, researchers have now investigated into the benefits of increasing tree nutrition for sustaining productivity of infected trees. Tree nutrition is being evaluated as a fourth tier of HLB management program.

9.1 Managing the Pathogen:

As soon as the disease was known to be caused by a prokaryote, use of antibiotics for tree injections to eliminate the bacteria was adapted. Tetracycline hydrochloride put some control over the disease however was proved to be phytotoxic. This turned the attention to a more soluble less toxic derivative N-pyrrolidinomethyl tetracycline. Due to high costs and potential for re-infection, strategies were turned to control the vector.

9.2 Managing the Vector:

There are examples where low vector populations have limited the spread of pathogen. In china, HLB disease has severely limited citrus production in the lowland areas where *D. citri* is abundant. In contrast, in the highland areas of China, disease is not so severe. This is because the vector has lower survival rates in the colder areas and thus spread is limited. But, limiting the vector in lowland areas is impractical as reducing the vector population to undetectable levels would require unsustainable amount of insecticides. Eventhough several insecticides to control citrus psylla were used and trunk application technique was proved effective. Timing for the application of insecticide is another aspect that cannot be overlooked. To assist in determining optimum timing of insecticides, placing of sticky yellow traps in orchards are proposed however, till date, no studies have been conducted to show the level of psyllid control needed to be "effective" in HLB disease management.

Also, biological control of vectors using parasitic wasp are sought. The complex of natural enemies attacking Asian citrus psylla, *Diaphorina citri,* includes various species of ladybeetles, syrphid flies, lacewings, spiders and entomopathogens such as *Hirsutella citriformis* and *Isaria fumosorosea.* In Asia, *D. citri* is a host for *Tamarixia* *radiata & Diaphorencyrtus aligarhensis. T. radiata is considered more effective.* On the other hand, *Trioaza erytreae* is attacked by *Tetrastichus dryi.* Efficacy of these parasites is limited by the existence of hyperparasitic wasps. Also, the biological control of vectors by insect predators and parasitoids in commercial citrus is difficult in areas where intensive insecticide programs have been operated.

9.3 Scouting for reducing Inoculum levels in an orchard

Reduction of inoculum is another potential strategy that can be utilized to manage the disease severity of HLB without limiting the citrus production. Removing trees infected by HLB makes sense from a disease epidemiology standpoint because this reduces inoculum levels. Nowadays, a team of orchard workers have been institutionalized by many large commercial citrus companies to survey tree-to-tree, scouting for symptoms of HLB. Trees suspected of being infected are tagged & evaluated and ultimately diseased trees are removed. Early detection and confirmation of disease can be accomplished by using the most sensitive real-time quantitative PCR techniques.

9.4 Planting Disease-Free Nursery Stock

Growing new trees devoid of pathogen and maintaining their health throughout the growing period is the most important aspect for sustainable production of citrus. For maintaining a disease free nursery stock, source trees for budwood and rootstock are grown in large screened protected enclosures and guarded against psyllid infestations. Such kind of protection is also important for young seedlings that have been grafted.

9.5 Foliar Nutrition Program

With a relatively low impact of vector control over minimizing HLB disease, many growers in Florida opted to not removing their infected trees. Instead, they used various foliar nutritional products, primarily micronutrients, to maintain tree health and productivity. Until now, the success of foliar nutrition program has not been certain. However, foliar nutrition program does not appear to prevent or correct fruit symptom but significantly slows disease development within a tree (Tim Spann, 2010).

A study conducted by Gottwald et al. (2012) states that the application of many of the standard essential micronutrients augmented with putative resistance promoting agents of phosphite and salicylic acid is not a viable substitute for an effective disease management program, but rather a last resort in bad situation. For foliar nutrition programs to be viable, vector management should be carried out effectively over a wide region together with application of micronutrients for better results.

10 Future Prognosis

It is of no doubt that HLB is one of the most serious disease of citrus. The commercial citrus industries in Florida and Brazil are facing a huge loss through the introduction of this disease while other citrus producing areas have become sensitized. So, several strategies are being implemented in exclusion as well as eradication of this disease. The outcome of such activities being undertaken have been the implementation of tree-to-tree survey, detection of insidious infections and limit the spread.

The effect of spread of HLB infection over new additional areas would be severe. So, for the short to middle term, since there is no resistance to HLB yet discovered, state laws must be enforced in areas with no apparent disease. Improved early detection, removal of symptomatic plants, better vector control coupled with novel new management strategies should lead to planting longevity and viability.

In the long term, resistance development in plants against the pathogen would be the best option. Some evidences of possible resistance through embryo recovery have been found (van Vuuren & Manicom, 2009, Stover et al. 2008). Broad spectrum resistance obtained by manipulating citrus genes would be another option (Kunta et al., 2008).

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