Research Article
Open Access

# Citrus huanglongbing disease in Southern Vietnam and its management stratergy

## Abstract

In the Mekong Delta, Vietnam, Citrus Huanglongbing (HLB) was officially announced in 1994 and its causal organism was described by Bové et. al.,[1] in 1996 to be *Candidatus Liberibacter* asiaticus, which is transmitted by citrus psyllid, *Diaphorina citri* Kuwayama, is a destructive citrus disease. Throughout the years, intensive works have been carried out for the establishment of HLB management under small scale orchards and the achievements are discussed, the model for effective control of HLB under small scale which could elongate the life cycle of citrus tree for better and longer harvesting. The management is mainly consisted of: 1) planting free-diseased citrus young trees through shoot tip grafting (STG) when the population density of the vector insect is low, and 2) controlling the vector by application mineral oil, soft and systemic insecticides either every ten to seven days before planting or every two months for the first two years after the planting. An optimal management is interpolating of guavas, *Psidium guajava*, between citrus trees. These techniques are included in a new integrated pest management (IPM) protocol for HLB in Southern Vietnam. Following the establishment of the above IPM, the Japan International Cooperation Agency (JICA) has performed a project transferring appropriate cultivation techniques of King mandarin to citrus farmers in five provinces of Southern Vietnam. Recently, we succeeded in lowering HLB symptoms on infected leaves and turning them into healthy leaves by applying iron (Fe) with some micro nutrients: foliar application of the solution alleviated HLB impacts on infected trees both in greenhouse and in field experiments. We further examined 130 rutaceae related accessions for their HLB tolerance, screening four tolerant cultivars, Hanh, Tac and Quat from *Citrus microcarpa* and Long Co Co pummelo from *Citrus maxima*. Furthermore, no symptoms appeared on local varieties, QuytDang, Quytrung, Camrung, BuoiRung, BuoiDang, BuoiBung, MacRun, MacMat, CanThang, Quach, NguyetQue, Truc, Cari, Datubien and GioiLom, in all of which no pathogens were detected by PCR analyses . In a molecular study, 38 primers have been designed and used for screening of HLB tolerance, obtaining 49 citrus varieties/clones. The preliminary results showed that the tolerant varieties were grouped into Group D, which somehow matched with the tolerant group screened under greenhouse conditions.

**Keywords:** huanglongbing, HLB tolerant, *candidatus liberibacter* asiaticus, *diaphorina citri*, interplanting guavas, *psidium guajava*, rutaceae, Iron (Fe)

Volume 10 Issue 1 - 2023

Nguyen Van Hoa,[1] Dang Thi Kim Uyen,[1] Nguyen Thi Kim Thoa,[1] Hiroyashi Kikuchi,[2] Katsuya Ichinose[3]

[1]Southern Horticultural Research Institute (SOFRI), Vietnam
[2]Smart Business Management Division, Aichi Steel Corporation, Japan
[3]Technical adviser, Aichi Steel Corporation, Japan

**Correspondence:** Nguyen Van Hoa, Deputy Director General, Southern Horticultural Research Institute (SOFRI), Vietnam, Tel +842733834680, Email hoav2003@gmail.com

**Received:** March 03, 2023 | **Published:** March 27, 2023

**Abbreviations:** HLB, huanglongbing; SOFRI, southern horticultural research institute; ACIAR, Australian centre for international agricultural research

## Introduction

Citrus is an important fruit crop and gives a very high yield for growers in Vietnam, especially in the South. However, there are many pests, diseases, which cause severe damages, Huanglongbing (HLB) is one of the most dangerous and destructive disease in Vietnam. South China was regarded as the first place of the disease source in Asia,[2] but other descriptions suggested that the disease was present first in India.[3] Beattie et al.,[4] hypothesized that the disease was originated in Africa, possibly from the host plant Verpris lanceolata without the symptoms. In Vietnam, HLB is also called as a golden leaf disease, which was first reported in the early 1960s. Throughout the years, intensive works have been carried out for HLB management under many different projects; many results have been summarized and revealed in this paper.

## Review the research result

### HLB occurrence in citrus at the Mekong Delta, Vietnam

HLB is caused by Gram-negative, fastidious bacteria *Candidatus Liberibacter*. At least three species are known in this genus in the world. *Ca.* L. asiaticus is adapted to high temperatures above 30°C. *Ca.* L. africanus[5] is restricted to milder climate and proliferated in areas where dominant temperatures are within the range between 16-22°C, while making no symptoms at 27-30°C. *Ca.* L. americanus prefers similar cool temperatures as *Ca.* L. africanus. All species are transmitted by the hemipteran insect, *Diaphorina citri* Kuwayama,[2,6] while *Trioza erytreae* Del Guercio is the dominant vector of the pathogens in Africa.[7] Only *Ca.* L. asiaticus has been reported from Vietnam as the causal organism of HLB, vectored by the insect.[1]

In Vietnam, once HLB invades an orchard, it spreads there very quickly, leading trees to be low and terminating the orchard within some years eventually. Many farmers have removed and re-planted with other crops, while others still continue citriculture, replanting citrus trees. These continued farms inevitably fail in reducing the risk of re-invasion of the disease, resulting in destroying the orchard by the pathogen again. The history of citrus cultivation in Vietnam distinctly tells us the seriousness of this disease. Orchards affected by this disease in Vietnam were only 1 to 5% in 1960s, soaring to 60% by 1975, among which 30 to 40% produced no economic yields. Soon after that, most of citrus orchards including Northern Regions were invaded by the disease. In 1995, the Southern Fruit Research Institute (SOFRI), now named as Southern Horticultural Research Institute, surveyed the HLB infection in the Tam Binh district of the Vinh Long province with the agricultural office of the province over 300 citrus orchards of sweet orange, finding 288 infected orchards

Submit Manuscript | http://medcraveonline.com



©2023 Hoa et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and build upon your work non-commercially.

Copyright: ©2023 Hoa et al.


that accounted for 96% of all. Among the infected orchards, 18.4% suffered from more than 50% the infected trees, 64.8% e orchards with infected 5 to 50% of the infected plants, 13.4% with less than 5% of the infected plants. Only 3.3% were without any symptomatic trees. The age of the orchards were:

a. Trees 4-5 years old in 409 hectares, 80% infected trees;

b. Trees 5-8 years old in 276 hectares, varying 20-50%; and

c. Tree 8 years old, 49 hectares, 1-5%.

Phan Thanh Tri and Nguyen Van Hoa carried out in 2004 a survey of the occurrences of HLB in citrus orchards in Tien Giang, Vinh Long, Can Tho and Dong Thap Provinces (personnel communication). In two districts, Cai Be and Chau Thanh of Tien Giang where pomelos and King Mandarin were mainly cultivated, the disease was very serious to level, revealing HLB infection on all trees in 32.4% of the orchards. In Tam Binh, Vinh Long, King Mandarin and sweet orange were dominantly cultivated, and 55% trees were found infected in HLB-invaded orchards. In Binh Minh of the province, mostly pummelos were planted and no symptoms were observed in 31.5% orchards, less than 10% trees were infected in 60.1% orchard, but no seriously infected orchards were seen. In Tra Vinh province, 53% orchards were subjected to disease-invasion, where 28.5% trees were infected. In Long Tuyen commune of Can Tho, posing lemon as the major cultivar, no symptoms were observed in 6.5% orchards and 77.1% orchards with slightly to moderately infected trees. In Dong Thap, tangerine as the major cultivar, 75% orchards were without any symptomatic trees and 25.0% orchards were slightly to moderately invaded by the disease.

## HLB management in the South of Vietnam

SOFRI has worked with many different international organizations for the establishment of HLB management to restore the citrus industry. The first survey was conducted with Aubert and Bourdeaut,[8] 1994 (CIRAD-FLHOR) in the citrus growing areas of Can Tho and Tien Giang provinces and the disease was officially confirmed at the first conference held at Long Dinh Fruit Research Center, the former organization of SOFRI, on 22 November 1994, leading to the next survey by Prof. Bové and Dr. Garnier from the Institute National des Recherches Agricultura de France (INRA). In the course of these projects, it was reconfirmed that the disease was caused by Gram negative bacteria Candidatus Liberibacter asiaticus and transmitted by *Diaphorina citri* from plant to plant. Then, receiving helps from CIRAD-FLHOR, the Southern California Institute of Crop Research has constructed a system for the production of disease-free trees by heat treatment and later all the STG for free-diseased seedlings were provided. Thus, disease-free trees produced on this system were first provided to local authority as the mother trees, from which further disease-free trees were produced and distributed to local communities. In these decades, SOFRI found out in the cooperation with international organizations the most effective management measures like using STG free-diseased seedling, horticultural oil, systemic and soft contact insecticides for controlling of psyllids, guava- citrus intercropping, including CIRAD-FLHOR, ACIAR, TNU, FFTC, JIRCAS and JICA. Besides that, the national project was carried out to screen the tolerant citrus related varieties as mentioned above.

## Removing diseased plants and replant with free-diseased seedlings

Removal of infected trees is apparently effective in a given time period, but the pathogen is not present only in the symptomatic leaves of the tree but also in the root system. Infected trees can be visually addressed with symptoms on them, helping the decision of clear-off. Growers are often hesitant to remove symptomatic trees with yield fruits, while knowing the importance of the removal of all infected trees. Thus, the recommendation by SOFRI that all infected trees should be completely removed by one month before replanting at late is not always followed by farmers, leading the replanted orchard continued shorter than expected.

Another effective management component for HLB is the use of disease-free seedlings, achieving longer-lasting orchards in Vietnam, Taiwan and China. In Vietnam, especially in the southern provinces, disease free seedlings have been produced through the grafting of disease-free new shoots on the top of scions around 30 cm grafted in greenhouses protected from the invasion of vector insects since 1996 at the Southern Fruit Research Institute. Prof. Hong Ji Su of Taiwan National University (TNU) helped us to free-diseased seedling produced through STG as mother free-diseased trees to nurseries of Tien Giang, Ben Tre and Vinh Long Provinces, where more disease-free trees are produced by the propagation with using the mother trees in 26 different varieties. In Vietnam, the Ordinance on Plant Varieties has been issued, however, the application in the production and management of citrus seedlings is not fully implemented. Thus, citriculture that does not follow the issue exists in unignorable proportions lately and the disease problems still remain very serious.

## Vector control by parasitic organisms

Psyllid nymphs are attacked c by parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligarhesis*. An entomopathogenic fungus, suspected as *Isaria fumosorosea*, was examined for its lethal effects on the vector under laboratory conditions, showing very high pathogenicity until seven months after spraying. It still remains, however, to test its effects in field for the evaluation of its efficacy as a biocontrol agent (JICA project after planting).

## Vector control by predatory organisms

The arboreal weaver ant, *Oecophylla smaragdina*, effectively protects tropical tree crops as workers of the ant actively forage canopies and predate small arthropods as food for their immature in their colony made of woven leaves. Recent research showed that Vietnamese citrus farmers who maintain weaver ants in their farms, on average, can save half the amount of cost on agrochemicals compared to those who depend only on synthetic produces for similar yields.[9] Up to 20% of the citrus growers produced their crop entirely without pesticide by appropriately using the weaver ant.

Although the weaver ant is known as one of the most ancient biocontrol agents in human history, scientific research on ants in smallholder citrus took off only gradually, starting in China after problems of insecticide resistance and in Vietnam during the 1990s.[9,10] Weaver ants were reported to control a range of pests, including the citrus stinkbug *Rhynchocoris humeralis* (Thnb.) (Hemiptera: Pentatomidae), aphids of *Toxoptera* spp., leaf-feeding caterpillars of *Papilio* spp., inflorescence eaters, Coleoptera and various other pests.[11] By contrast to the above examples, research on the weaver ant in citrus was driven by natural scientists working closely with farmers, their culture and their knowledge system. Due to pressure from the pesticide industry, the use of weaver ants in citrus orchards decreased during the 1990s. However, various small-scale financial injections performed from the mid-1990s onwards, media campaigns and farmer associations were rather contrary to this trend.[11] In addition, researchers of SOFRI revealed that near or within growing tall trees like mango, durian, high longan trees close to or around citrus

**Citation:** Hoa NV, Uyen DTK, Thoa NTK, et al. Citrus huanglongbing disease in Southern Vietnam and its management stratergy. *Int Clin Pathol J.* 2023;10(1):24–29. DOI: 10.15406/icpjl.2023.10.00213

Copyright: ©2023 Hoa et al.


orchards led weaver ants to make nests and form big colonies from which workers forage insect pests for their food. Bridging trees with bamboo sticks or nylong strips linking between citrus trees enhanced their foraging efficiency and signifncantly the psyllid populations.

Although competition among dominant ant species proved a key challenge to researchers in Africa, experienced Vietnamese citrus farmers can raise ingenious measures to optimize the performance of weaver ants. For example, they trap the black ant *Dolichoderus thoracicus* (Smith) and avoid the intercropping of sapodilla fruit trees, because black ants favor these trees as nesting habitat.[11] Less experienced farmers without learning from skilled farmers, wrongly introduce sapodilla as an intercropping plant to diversify their source of income and care this fruit tree very little. This apparently worthwhile attempt to combine two valuable crops has misfired. The competition with the black ant will drive away weaver ants from the citrus orchards, resulting in less protecting citrus from stink-bugs and leaf-feeding caterpillars. The ecological conditions that are realised in traditionally sustained natural pest control by the weaver ant in citrus are thus perturbed by mismatching crops and predatory species and, consequently, the fail in control efficiency with the ant heads the unskilled farmers more likely to use the pesticide treadmill.[11]

## Chemical measures

There are no practical pesticides for this disease, except for antibiotics. Nguyen Thanh Hieu et al.,[13] reported the efficacy of Penicillin and Tetracyline injection to the citrus trunk for the control of HLB on the green grapefruit and did not found detectable residues of antibiotic from leaves at 8 months after treatment. In addition, another measure of antibiotics is soil dripping of Streptomycin into the soil or trees, also allowing the control of fungal pathogens that cause root rot diseases. The last method increases symbiotic microbes with more chances to survive in roots, increasing the resistance to HLB (JICA). Most of synthetic pesticides are used to control vectors, reducing the transmission potential of psyllids only. Mineral oil is effective only moderately, preventing the transmittions only within a few hours after the application.

## Intercropping with other crops

In December 2006, the HLB International Conference in Ishigaki, Japan. The JIRCAS cooperation project between Japan and Vietnam has reported the effectiveness of citrus intercropping to reduce HLB in the field.[14] Intercropping of citrus orchards can reduce psyllid numbers in the orchard through the evaporation of psyllid from the turf. In Indonesia, Andrew G etal.,[15] reported guava fruit effective three years after planting. In terms of Vietnam, Gottwald TR et al.,[16] argue that guava can effectively banish psyllid 1 to 1.5 years after planting. Other studies in Florida and Brazil are not effective because they grow very thin, tall citrus and citrus are planted on very large areas (several thousand hectares), more than half of guava cannot live in cold conditions in these areas.

Neuman studied the use of volatile compounds for biological control of psyllid. In theory they are highly effective, but it is difficult to apply in practice and the evaporation effect of synthetic compounds is not long, spraying must be applied continuously and on large scale, so the cost is very high. More research is needed to identify the solution. Therefore, the intercropping in the garden is the natural solution, continuous, long-term and most economical. In Thailand, their use of intercropped logs on large orchards is also good.

In the study of Andrew G etal.,[15] the olfactometer results revealed that the repellent action of guava against citrus psylla is dose-dependent, with very low doses having little effect on citrus psylla. This result indicates that to control citrus HLB by interplanting guava trees in citrus groves, sufficient numbers of guava trees are needed to keep the dosage of volatile compounds emitted from guava at an effective level in the entire grove. In Vietnam, it was suggested that guava trees are interplanted prior to citrus at a ratio of one guava tree to one citrus tree.[3] Besides, guava interplanting may increase severity of fruit fly damage to citrus. Guava is a widely planted tropical and subtropical fruit. Guava leaf is traditionally used as an antidiarrheal drug. The oral acute toxicology of leaf extract to mice is low with a $LD_{50}$ of more than 20.0g/kg.[17] This suggests that use of guava chemicals as insecticides will be safe to mammals.

Since long, famers from the Mekong Delta of Southern Vietnam have been practicing interplanting of citrus with guava and those citrus orchards planted with guava showed much lower psyllid infestation levels and low incidence of HLB compared to citrus orchards lacking guava.[14] This practice adopted by Vietnamese farmers drew the attention of scientific communities of many countries during 1990s and various scientific studies were initiated to understand the scientific reasons. The first of this, kind of research was a collaborative research projects involving Vietnamese, Japanese and Australian scientists.[3] The findings of this research which was presented in a meeting held during December 2006 in Japanese International Research Center for Agricultural Science showed that interplanting citrus with guava negated infestations of Asian citrus psyllid and consequently HLB.[3] This study collected the scientific information that young citrus interplanted with guava remained disease-free for a year whereas sole citrus crop showed signs of the disease within four months of planting and reached over 30% trees infected within a year (Figure 1).[14]

**Figure 1** King mandarin interplanting with Guava.

A team of American scientists who also made an observation tour to Vietnam in April 2004 noted that the normal life of sole citrus plantings in the Mekong region of Vietnam was 2 to 4years, but those interplanted with white guava were surviving for up to 15 years.[16] Although raising guava as an intercrop reduced psyllid population in citrus orchards,[14] the mechanism by which this occurs was unknown. It was postulated that the effects of guava on citrus psylla could be due to mechanical/physical disruption on host recognition, repellent effect of volatile compounds from guava or chemical alteration of the volatile compounds emitted by citrus reacting with guava compounds. From various studies conducted in Florida, USA to determine, if guava has a repellent effect on adults of Asiatic citrus psyllids. Hall DG et al.,[14] noted high adult mortality rates occurred when psyllids were confined to guava in no choice situations, with 95% mortality occurring within 6-9 days. They postulated that the effect may be due to volatile compounds produced by guava that are deleterious to psyllids. In another study, Andrew G et al.,[15] evaluated the repellent

Copyright: ©2023 Hoa et al.


effect of guava leaf and factors attributed to this activity, response of adult psyllids to guava leaves and its odor in cage test and Y-tube alfactometer test. The alfactometer response of adult psyllids to guava leaf odor was dose-dependent and both male and female psyllids responded similarly to the guava leaf odor.

## Proper nutrition prolongs the life of citrus

Xia and Sequeira[18] report on the application of nutrient solutions in the management of HLB. Utilizing a variety of micronutrients as part of Florida's disease management solution, prolongs the life of the plant. This solution has long been applied in China. Summing up the results of more than 60 years of experiments in the field and the field shows that nutrients contribute greatly to increasing resistance to the plant and prolonging harvesting time from diseased plants. Under optimum nutrition conditions combined with rational irrigation. A 10-year-old honey will last for 3-5 years, while pomelos will last longer than half. However, the quality of fruit juice, fruit quality is much worse. Increasing organic and inorganic fertilizers with good effect in orange trees in Vietnam.[14] Weishou Shen et al.,[19] define the role of nutrition and pesticides in reducing HLB. Pesticides are the key to eliminating psyllid, mediating the disease, reducing the incidence of HLB. Spraying of micronutrients is considered the current solution that increases resistance to plants and produces yield. Nutrition is mixed and sprayed 3 times on the main leaves of young trees in combination with pesticide spraying for high effectiveness. Hoa, Uyen, Thoa, Ichinose (personnel information-on going research, 2020) show that using of iron-plus solution on HLB infected citrus trees could help the tree much recover after 2 to 3 application (weekly internal application).

Application of the Fe and other mineral nutrients results: in the greenhouse experiement, all the treatments with Fe or Zn solutions could help to increase number of shoots after treatment and also could reduce number of leaves showing HLB symptom. In field experiment, the Fe solution has good effective to recover of the leaf of citrus after spraying event it was infected by HLB.

## Change planting time

Historically farmers in the western provinces have grown citrus at the time of rain (from May to July), but recent studies of Gottwald TR. et al.,[15] showed that citrus's psylla were usually very high during this period, but the numbers are low in the period during from October to December. The authors also experimented with citrus planting in the period of October to December, at this stage is significantly lower than that in May-July period as before. In the Mekong Delta region also earlier we suggested the farmers to plant citrus during May to July, since it is the rainy season time, the tree will grow better and no need of making irrigation for trees, however, with research and demonstrating plots we could see that the best time for planting citrus in this region is from September to January next year since of less spylla population leading to less spreading of HLB inoculum/ infection Figure 2.[19]

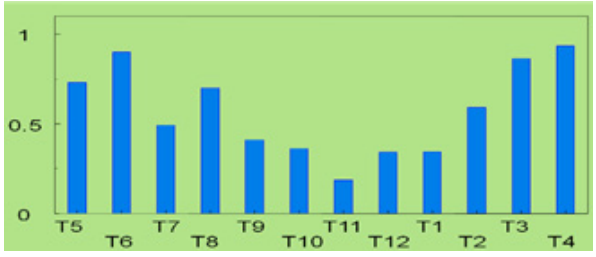


**Figure 2** The population of psylla/tree in a year.

## Thinning method

In the old way, the distance between trees was 2.5m or less. Studies in the SOFRI - JIRCAS project showed that the success of the planting of oranges at a distance of 3-4m per tree combined with pruning and scattering results in better orchard management reduce the number of seedlings per unit area.

## Pesticide treatment on seedlings is free of disease before planting

Research results from Gottwald TR. et al.,[14] showed that treatment of citrus seedlings with pesticides in the Imidacloprid group or Thiamethoxam or Clothianidin, prior to planting for about 10 days, very good seedling protection and effective insect management to the time after planting.

Study by Yuasa. et al.,[19] on both using guava interplanting and shoft chemical showed that the expansion of appropriate cultivation techniques of King mandarin (*Citrus nobilis* Loureiro) to growers who have average-sized farms of 1,000 to 10,000m$^2$ in southern Vietnam. In this region, most citrus trees are infected by Huanglongbing (HLB) within two years of planting. The HLB management techniques implemented consisted principally of Nippa palm or Banana (*Musa* spp.) windbreaks on all sides of the orchards, planting of guavas (*Psidium guajava*) one to two months prior to planting disease-free King mandarin trees, and insecticide applications. Insecticide applications were scheduled as follows: 1) soil-drenching with neonicotinoid around King mandarin trees 10 days before planting and every two months for one year thereafter; and 2) leaf-spraying every month from the second year on. In seven of the eleven orchards included in the project, 0.3 to 2.6% of King mandarin trees were infected by HLB in the first year and 3.0 to 21.9% in the second year. However, the infection rate in the first year reached at 21.1 to 39.3% in four orchards where the insecticide was not correctly applied. The results indicate that the appropriate use of neonicotinoid curtailed the infection of King mandarin trees by HLB. The result show that the success thus depends on how precisely the techniques are transferred and incorporated by the growers.

## Screening for HLB tolerant variety

Binh et al.,[13] studied on the ability of 130 citrus and citrus related varieties to HLB under glass house conditions and confirmed by PCR showed that the orange, mandarin group is most suceptable to HLB with the serverity symptoms of yellow motling on leaves, dry branches and tree die very fast; The medium one is the group of lemon and pummelo with the mild symptoms of vein corking, smaller leaf of young leaves, they are Daxanh, Duonglacam, Namroi, Longcoco pumelo varieties; The important citrus group is negative PCR result and no symptom after HLB bacterial artificial inoculation, they are Quytdang, Quytrung, Camrung, Buoirung, Buoibung, Matrun, Matmat, Canthang, Quach, Nguyetquoi, Kimquyt, Truc, Comruou, Daudaulacam, Cari, Datubien and Goilom. They also designed 54 primer pairs for screening the HLB tolerant purpose, the result showed that there are 32 primer pairs showing diversify of the tolerant ability and only 5 pairs show no amplify on tolerant varieties.

Albrecht U and KD Bowman[21] studied under the net house conditions and suggested that the hybrids of Citrus x Poncitrus were resistant to Candidatus, especially US 897 (*Citrus reticulata* Blanco, *Poncirus trifoliata* L. Rat) Graft root results in delayed onset of symptom on grafted eyes when surveyed by Binh et al.,[13] in Vietnam there are some varieties of rutacease family that are resistant to HLB. Especially, there are two varieties of Tac and Long Co Co poummelo

**Citation:** Hoa NV, Uyen DTK, Thoa NTK, et al. Citrus huanglongbing disease in Southern Vietnam and its management stratergy. *Int Clin Pathol J.* 2023;10(1):24–29. DOI: 10.15406/icpjl.2023.10.00213

Copyright: ©2023 Hoa et al.


in the group of citrus trees which have good resistance to disease. This is a valuable source of genes for the breeding of resistance/tolerance in the future.

### Gene transfer

Genes studied in citrus to combat HLB and insect infesions are being implemented: (i) for disease: Transmission of genes that control resistance to pathogens such as Anti-microbial peptide, Attacin gene, STX 1A gene. (ii) For RCC: RNA - interference (RNAi).

Zhang MQ et al.,[22] Nineteen AMPs were used to purify HLB resistance under in vitro conditions. These include Tachyplesin 1 from horse, SMAP-29 from sheep and synthetic AMP - D4E1. These AMPs have the potential to inhibit the growth of bacteria at a concentration of 1μM. With this result will select the AMP that is suitable for the gene in the future. Benyon argue that because Candidatus Liberibacter is a gram-negative bacterium, mainly infected with plant tissue, gene expression is mainly expressed in lib or tissue involved. In the study, "the authors attempted to find the promoter, the B-glucuronidase gene (GUS) reporter or D4E1 AMP could work on the Trifoliate-US-802 rootstock. According to Felipe and colleagues U11) Genes that synthesize peptides resistant to microorganisms used to transfer crops for bacterial resistance have been investigated. The results of this study suggest that Cam Hamlin's reaction was to transfer Attacin A gene (attA) against Candidatus Liberibacter asiaticus. Two groups of genes showed very little symptom after 8 months of disease and very low qPCR results.[23]

### RNA-induced genes (RNAi)

Have been successfully used in the killing of insects through the injection of ds DNA from RNAi.[24] This use of genes (RNAi) to inhibit aspirin RCC injection of dsDNA resin from RNAi causes them to die rapidly afterwards. Today, the gene can survive for up to 33 weeks. Borovsky developed RNAi for psylla control, successfully demonstrating this gene modeling through art-induced extraction, using 3 dsRNAs for three RCC specific genes and resulting in high death.

### The causes of failure to prevent HLB in Southern Vietnam

Despite the availability of disease-free trees, the supply of seedlings to farmers is too low compared to the high demand of production. The system of producing and supplying disease-free seedlings has not been organized and operated effectively due to various factors such as high capital infrastructure, high cost of seedlings, owners of private nurseries too many and produce many different types of fruit seedlings, of which citrus is not clean quality at a discounted price. As a result, the number of seedlings floating higher than that of disease-free seedlings has been circulated in the market where the government does not have a uniform policy to manage this.

Extension activities are many and widespread but not effective, disease prevention knowledge of farmers is low, spontaneous, lost confidence. Farmers have not boldly planted the disease before planting the diseased plant due to the high price of seedlings, farmers inadvertently re-planted the floating plant with the ability to carry too high disease, creating more imbalance in nature and facilitates the identification of germs. Farmers are not aware of the harmful effects of gillnets, so the application of preventive measures is too poor, sometimes without any measures.

Due to the fact that the trees are diseased and dying gradually, they are still harvested for the first time, and farmers therefore apply the thickening and fast-growing method, which is costly and profitable for 2-3 years. The system of windbreaks around the garden and in the wind, direction is not available or has ineffective, not enough to prevent the spread of the disease transmission. The amount of manure and chemical is applied purely and fully exploits the reproductive power of the plant, but it does not compensate for the amount of plant and organic fertilizer and the activities of the animals are less interested in farmers.

## Future aspect and direction

With all the results from research work and the fulairs in management of HLB in the South Vietnam, we plan to make more actions on research and application on HLB management in the future for better life of the crop and farmers:

Using the genetic resources from our collection for more studies on what happen with that and use of that tolerant gene for future development of new citrus varieties for commercialization or rootstock for commercial varieties.

Keep continue to do research on enhance the citrus tree ability to tolerant/resistance to HLB under field conditions for better yield/production, etc.

We need more support from the Government to control or give the rule to the nursery man provide only free-diseased seedlings to growers – Strick regulation for citrus nursery.

## Acknowledgements

None.

## Conflicts of interest

The author declared that there are no conflicts of interest.

## Funding

None.

## References

1. Reinking OA. Diseases of economic plants in southern China. *Philippine Agricultural*. 1919;8(4):109–135.
2. Capoor SP, Rao DG, Viswanath SM. *Diaphorina citri* Kuwary – a vector of the greening disease of citrus in India. *Ind J Agric Sc*. 1967;37:572–576.
3. Beattie GAC, P Holford, DJ Mabberley, et al. Aspects and insights of Australia–Asia collaborative research on huanglongbing. In: Proc. Intl. Workshop for Prevention of Citrus Greening Diseases in Severely Infested Areas, 7–9 December 2006, Ishigaki, Japan: Japanese Ministry of Agriculture, Forestry and Fisheries; 2006: Pp.47–64.
4. Bové JM, Chau NM, Trung HM, et al. Huanglongbing (greening) in Vietnam: Detection of Liberobacter asiaticum by DNAhybridization with probe in 2.6 and PCR–amplification of 16S ribosomal DNA. In: JV da Graça P Moreno, Yokomi RK, editors. Proceedings of the 13th International Organisation Citrus Virology Conference; 1996: p.258–266; IOCV, USA.
5. Garnier M, Eveillard SJ, Cronje PR Le, et al. 2000. Genomic characterization of a liberibacter present in an ornamental rutaceous tree, *Calodendrum capense*, in the Western Cape Province of South Africa. Proposal of 'Candidatus Liberibacter africanus subsp. capensis'. *Int J Syst Evol Microbiol*. 2000;50 Pt 6:2119–2125.
6. Martinez AL, Wallace JM. Citrus leaf–mottle–yellows disease in the Philippines and transmission of the causal virus by a psyllid. *Diaphorina citri*. *Pl Dis Reptr*. 1967;51:692–695.

7. Oberholzer PCJ, von Standen DFA, Basson WJ. 1965. Greening disease of sweet orange in South Africa. International Organization of Citrus Virologists Conference Proceedings (1957–2010); USA: 1965.
8. Aubert A, Bourdeaut. Citrus greening disease a serious limiting factor for citriculture in Asia and Africa. *Proc Int Soc Citriculture*. 1992;2:817–820.
9. Van Mele P, N. T.T. Cuc. 2000. Evolution and status of *Oecophylla smaragdina* (Fabricius) as a pest control agent in citrus in the Mekong Delta, Vietnam. *International journal of pest management*. 2000;46(4):295–301.
10. Barzman MS, Nick J Mills, Nguyen Thi Thu Cuc. Traditional knowledge and ration for weaver ant husbandry in the Mekong Delta of Vietnam. ResearchGate. *Agriculture and Human Values*. 1996;13:2–9.
11. Van Mele P, Cuc NTT, Vanhuis A. Direct and indirect influences of the weaver ant Oecophylla smaragdina on citrus farmers' pest perceptions and management practices in the Mekong Delta, Vietnam. *International Journal of Pest Managemen*t. 2002;48(2):225–232.
12. Paul Van Mele, HoVan Chien. Farmers, biodiversity and plant protection: developing a learning environment for sustainable tree cropping systems. International Journal of Agricultural sustainability. 2011;2(1)67–76.
13. Binh NT, Ngoc Lan BT, Bich Ngoc NT, et al. Result on study the genetic diversity of Citrus relative species to HLB tolerant using molecular tools. 2010.
14. www.itfnet.org/fruit/Slides/Session204/FES_Guava_Presentation_2007_v3%5B1.5D.
15. Andrew G, Zaka SM, Xin–Nian Zeng, et al. Repellent effect of guava leaf volatiles on settlement of adults of citrus psylla, Diaphorina citri Kuwayama on citrus. *Insect Sci*. 2010;17(1):39–45.
16. Gottwald TR, DG Hall, GAC Beattie, et al. Investigations of the effect of guava as a possible tool in the control/management of huanglongbing. International Organization of Citrus Virologists Conference Proceedings (1957–2010); USA: 2010.
17. Aimmanas AP, Chavalittumrong PR, Pranee C. Toxicity study of Psidium guajava Linn. leaves. *Bulletin of the Department of Medical Sciences*. 1995;37(4):289–305.
18. Weishou Shen & Juan M. Cevallos–Cevallos & Ulisses Nunes da Rocha, et al. Relation between plant nutrition, hormones, insecticide applications, bacterial endophytes, and Candidatus Liberibacter Ct values in citrus trees infected with Huanglongbing. *Eur J Plant Pathol*. L. 2013; 137:727–742
19. Xia Y, R Sequeira, Gecheng Ouyang, et al. A Review of Huanglongbing (Citrus Greening) Management in Citrus Using Nutritional Approaches in China. *Plant Health Progress*. 2011;12(1):8–10.
20. Kazuyoshi Yuasa, Hoa Nguyen Van, Katsuya Ichinose. Reduction of Huanglongbing Due to the 'Appropriate Techniques Expansion for the Cultivation of King Mandarin' Project in Southern Vietnam. *International Society for Horticultural Science*. 2015;925–930.
21. Albrecht U, Bowman KD. Tolerance of trifoliate citrus hybrids to *Candidatus Liberibacter asiaticus*. *Scientia Horticulturae*. 2012;147:71–80.
22. Zhang MQ, CA Powell, LJ Zhou, et al. Chemical compounds effective against the citrus Huanglongbing bacterium, *Candidatus Liberibacter* asiaticus in planta. *Phytopathology*. 2011;101(9):1097–1103.
23. Huang HT, Pei Yang. The ancient cultured citrus. *Bioscience*. 1987;37(9):665–671.
24. Hunter W, James Ellis, Dennis vanEngelsdorp et al. Large–scale field application of RNAi technology reducing Israeli acute paralysis virus disease in honey bees (*Apis mellifera*, Hymenoptera: Apidae). *Plos pathohens*. 2010;23;6(12):e1001160.

**Citation:** Hoa NV, Uyen DTK, Thoa NTK, et al. Citrus huanglongbing disease in Southern Vietnam and its management stratergy. *Int Clin Pathol J.* 2023;10(1):24–29. DOI: 10.15406/icpjl.2023.10.00213