

Review

## Control of Huanglongbing (HLB) disease with reference to its occurrence in Malaysia

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The center of diversity for citrus was originally found on the northeastern India, eastward through the Malay Archipelago and south to Australia. Today citrus is produced in 140 countries, mainly between the north and south 40° latitudes. Citrus ranked first among fruit crops in the international trade based on value. Citrus production of the world is around 105 million tons per year. Orange (*Citrus sinensis*) accounts for almost two thirds of the total citrus production (65%), followed by tangerine (*Citrus reticulata*) (21%), lemon (*C. limon*) (6%) and grapefruit (*C. paradisi*) (5.5%). Other significant commercially grown species are lime (*C. aurantifolia*), pummelo (*C. grandis*) and citron (*C. medica*). The largest citrus producers are Brazil (20%), United States (14%), China (12%), Mexico (6%) and the countries of the mediterranean basin (15%). Humidity and day-to-night temperature fluctuations influence which varieties are best adapted to an area. Most citrus fruits are produced for fresh market consumption and only around 30% is processed. Fresh fruits are rich in vitamin C which plays a vital role in prevention of scurvy. After extraction of the juice, the skin and fruit pulp can be used as livestock feed or making compost. The rind acid (oil) of the citrus is considered an expensive commodity in international trade (F.A.O 2003). Unfortunately, the citrus industry is threatened by 2 destructive diseases namely Witches' Broom disease of lime (WBDL) and Huanglongbing (HLB) disease. WBDL has been known to be caused by *Candidatus phytoplasma aurantifolia*. It is one of the destructive diseases on citrus industry in the Middle East, India and Pakistan. It was reported in 1970 for the first time from lime orchard of Oman, united Arabian emirate (UAE) (1988), Iran (1998) and India and Pakistan (1999). WBDL is a phloem limited phytoplasma disease of lime. HLB disease causing citrus greening (*Candidatus liberibacter* spp.) is the second most severe disease on citrus industry all over the world. HLB has destroyed an estimated 60 million trees in Africa and Asia. More than 40 countries were infected by HLB in Africa, Asia and USA (Chau et al., 1996; Bove, 2006; Roux et al., 2006; Batool et al., 2007). The HLB pathogens are highly fastidious phloem-inhabiting bacteria in the genus *Candidatus liberibacter*. The bacteria have not been cultured yet in laboratory media and do not survive outside the host cells. Three types of phloem limited bacteria causing HLB disease have been described and identified (Bove, 2006). The isolate from South Africa (*Candidatus liberibacter africanus*) is considered heat-sensitive and found in Africa. It is vectored by African citrus psyllid (*Trioza erytrae*) and was described by Guercio in 1918. The isolate from Asia (*Candidatus liberibacter asiaticus*) is more severe and widespread; it is vectored by Asian psyllid (*Diaphorina citri* Kuwayama) (Garnier et al., 2000). This type of HLB is heat-tolerant (Garnier et al., 2000). It can show the symptom on humid, cool and hot temperature, up to 35°C (Garnier et al., 2000; Bove 2006; Le Roux et al., 2006). The isolate from America has been named *Candidatus liberibacter americanus*; it was detected in Brazil and Florida (Coletta-Filho et al., 2005; Texeira et al., 2005).

**Key words:** Citrus, HLB, greening disease.

## HOST RANGE OF HLB PATHOGEN

HLB causing citrus greening disease can affect almost all citrus cultivars; relatives like sweet orange, tangelo and mandarin are the most susceptible, while lime, sour orange and trifoliate orange are the least susceptible (Knapp et al., 2004). Infected lemons, grapefruits and sour oranges remained non-productive, whereas Mexican limes, trifoliate oranges and some trifoliate orange hybrids showed only leaf symptoms (Chung and Brlansky, 2005). Symptoms have also been observed in *Microcitrus australasica*, *Swinglea glutinosa*, *Atalantia missionis*, *Clausena indica*, *Limonia acidissima*, *Balsamocitrus dawei*, *Aeglopsis chevalieria*, *Severinia buxifolia*, *Murraya paniculata*. *Catharanthus roseus* (Periwinkle) and *Nicotiana xanthii* (Tobacco) are the only reported non-*Rutaceae* hosts. Both of the latter hosts were infected only under laboratory conditions and acted as indicator plants (Knapp et al., 2004). In order to declare a species a host, the positive presence of pathogen needs to be established. Some species (*Citrus indica* Tan and *Citrus macroptera* Montr.) remained symptom-free under heavy inoculum pressure (Bhagabati, 1993). *Citrus limetta* remained symptom-free after a laboratory inoculation. *Citrus* species such as *Murraya paniculata* L. (orange Jasmine), *C. jambhiri* (Rough lemon), *C. aurantium* (*Sour orange*) and *C. paradisi* (Grapefruit) were screened against HLB in laboratory. Grape fruit was the most susceptible host, followed by the other species (Halbert and Manjunath, 2004). Only a few lemon cultivars and a few other species like *Citrus indica* and *Citrus macroptera* reportedly exhibited some tolerance or possible resistance to the bacterium. However most of the citrus and citrus relatives are potential hosts for the citrus greening pathogen. The most susceptible hosts are sweet oranges, tangelos, and mandarins. Moderately susceptible hosts include grapefruit, sour orange, lemons and Rangpur lime. Mexican limes and trifoliate oranges are the most tolerant (Halbert and Manjunath, 2004).

## WHAT TYPE OF THE CONTROL STRATEGY CAN WE FOLLOW?

HLB disease is spread by insect vectors and the pathogen is limited in the phloem. Control of the HLB vectors and the pathogen of citrus greening disease are involving all aspects of an integrated pest and disease management program. The following is a review of the HLB vectors and HLB disease management. This review focused on chemical, biological and cultural controls which were found to reduce the vector population and HLB disease severity. HLB free planting materials should

be used in order to reduce disease severity and incidence in the area to establish a citrus orchard.

## Chemical control

### Insecticide to control the vector

When the insect and the pathogen of HLB are present, conceivably, the easiest HLB control strategy is chemical control with the use of pesticides. The use of pesticide will reduce the population of HLB psyllid vectors (Aubert, 1990; Graça and Korsten, 2004). Spraying pesticides is expensive; so doing it would depend on the economy of individual producers. Gottwald et al. (2007) found that all commercial citrus production has access to the machinery or commercial grove care companies to accomplish chemical sprays for psyllid control, however the necessity of additional sprays is both costly and depending upon the economics of individual producers can be marginally feasible. Although there is much information on the effect of chemical control of various insecticides and programs on psyllid vectors, the effect of this practice on HLB increase and spread remains largely anecdotal and undocumented. An even greater challenge is the large population of HLB positive residential citrus trees where regulated chemical control is not an option.

In the study conducted in China, 10-13 sprays per year was required during flush periods to rehabilitate citrus production in a HLB infected area (Roistacher, 1996). *D. citri* was controlled using systemic insecticides like dimethoate and monocrotophos (Graca, 1991). Methomyl or melathion sprays on citrus trees between 10 - 12 days from March to May were not effective against citrus greening. Sometimes 44% dimethoate EC, 50% melathion EC and 40.64% carbofuran FP showed economically good control of the psyllid (Chen, 1998). Two patent applicators are available in South Africa that calibrate the dose based on the diameter of the tree (Buitendag and Broembsen, 1993). Supriyanto and Whittle (1991) and Shivankar et al. (2000) also had success with trunk applications. The best time to apply the trunk application is just prior to spring flush (Rao et al., 2000). Synchronized chemical control is very important for farmers in a citrus production area affected by HLB. Also the use of sticky cards are recommended for monitoring *D. citri* in order to time control action (Aubert, 1988; Aubert, 1990). In Asia, a range of insecticides, mostly organophosphates and pyrethroids, are used in very intensive spray programs to kill nymphs and eggs of the HLB vector on flush growth. In Southeast Asia control has not been achieved with 35-52 applications of synthetic pesticides a year. These sprays often comprise four or more active ingredients (some of which are highly toxic and banned). Fruit quality is not improved by heavy use of pesticides; heavy pesticide use destroys natural

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enemies and results in serious outbreaks of minor pests such as citrus red mite, *Panonychus citri*. In South Africa, applying systemic insecticides aimed specifically at psyllids to tree trunks has been the most effective (Davis, 2005).

### Using antibiotics to control the HLB pathogen

Antibiotics were also used to control the greening disease pathogen. Different types of antibiotics were injected into infected citrus trees, such that the injection of antibiotics temporarily relieved the crop plant of symptoms (Su and Chang, 1976; Shamsudin et al., 1990; Buitendag and Broembsen, 1993; Tonhasca and Byrn, 1994; Shelton and Badenes, 2006). Injecting antibiotics recommended as a part of an integrated management program in India (Nariani, 1981).

Leaf symptoms of HLB were reduced when tetracycline hydrochloride was used and obtained complete control of HLB using penicillin carbendazim (Cheema et al., 1986). Tetracycline can also be used to treat budwood and the budwood was deeply absorbed in 1,000 µg/ml tetracycline hydrochloride for 2 h, or 500 µg/ml for 3 h (Zhao, 1981).

The best results of the tetracycline were observed when it using through the injections to control the HLB and the spring season is the best time for injection (Aubert and Bove, 1980; Graça and Korsten, 2004). Trunk injections of tetracycline hydrochloride have also been successful in Taiwan, China, Reunion and the Philippines (Graca, 1991). In South Africa, tetracycline used up to 20 g/adult tree with high capacity compressors working at 10 kg/cm<sup>2</sup> on citrus trees which were infected by greening disease. Trees were also treated by rolitetracycline and the symptoms of HLB were reduced. However, control in South Africa is based on the use of healthy nursery trees and effective systemic insecticides (against the vector *T. erytrae*) (Buitendag, 1991).

### Using mineral oils to reduce disease severity

Horticultural mineral oils (HMOs) have been developed as alternative treatments to control some vectors and reduce the disease. Using mineral oils have some advantages. It is less damaging to the environment and less disruptive to the biocontrol of other pests. In citrus using mineral oil is effective in the control of the citrus psyllid which is a vector of the HLB. Mineral oils are also effective in controlling other pests such as citrus red mite, aphids, scale insects and fungus diseases such as greasy spot, algae, thus leaving trees and fruits clean. It can also reduce the severity of virus which is transmitted by aphids. HMO and machine oil were as effective as omethoate and diflubenzuron. The oils suppressed egg laying (oviposition) by adult female psyllids. In the study conducted by Rae et al. (1997), it was found that the use

of HMO as a control measure was observed to have no phytotoxicity. The foliage and fruit was clean and free of pest compared to the foliage on trees that were treated by synthetic chemicals. The use of mineral oils also has some disadvantages. For example, the heavy mineral oils may injure the tree and phytotoxicity may occur. However, using mineral oils do not allow female leaf miner and psyllid to lay eggs on oil deposit because the oil moves into the spiracles of the insects. Mineral oils are used from 2 to 1% concentration (Rae et al., 1997). In Sarawak, as low as 5% concentration of mineral oils used and did not induce phytotoxicity (Andrew and Holford, 2007).

### Biological control of HLB vector

There are different approaches to controlling vectors of HLB such as, cultural or biological control measures. In biological control, the parasites *Tamarixia radiata* and *Tamarixia dryi* were found effective against HLB disease vectors. Nymphs of both psyllid species are parasitized by hymenopterous ectoparasites *Tamarixia dryi* Waterston and *T. radiatus* Waterston, a fact that has been used to accomplish biological control of vector populations (Catling, 1969; Etienne and Aubert, 1980; Aubert and Quilici, 1984; Chiu et al., 1988). The parasites significantly reduced the psyllid populations and damage of HLB (Chiu et al., 1988). *Diaphorencyrtus aligarhensis* has also been found in parasitoid *D. citri* (Tang, 1989). Field observation in Florida where *T. radiatus* was introduced, indicate that the effect of biological control can range broadly from 4 to 70% reduction in psyllid populations (da Graça et al., 2007).

In Mauritius, biological control of *T. erytrae* was much more effective than biological control of *D. citri*. There was found several reasons for this. First, the initial population of *T. erytrae* was much lower than that of *D. citri*. *T. erytrae* reproduces principally on citrus, which is regularly treated with pesticide, whereas *D. citri* utilizes *M. paniculata*, which is unsprayed and is ubiquitous as an ornamental throughout the island. The climate on much of the island also is more suitable to *D. citri* than to *T. erytrae*. Second, the parasite of *T. erytrae* (*Tamarixia dryi* Waterston) has an alternate host in the common psyllid *T. litseae*, whereas *T. radiata*, the parasite of *D. citri*, has no alternate host (Halbert and Manjunath, 2004). Biological control of the pathogen has not been well studied and both Asian and African types of greening disease are able to infect the citrus plants (Garnier and Bove, 1996).

### Eliminating the source of inoculums

The effectiveness of the removal of diseased trees is directly related to the latency of infection. The first occurrence of visual symptoms can be dramatic in some

**Table 1.** HLB management as practiced in South African orchards to minimize psyllid populations (Buitendag and Broembsen, 1993).

Age of tree	Amount of canopy with symptoms	Treatment
< 5 years	Any symptoms	Destroy tree
6 - 10 years	< 75%	Remove only affected branches
6 - 10 years	> 75%	Destroy tree
> 10 years	< 40%	Remove only affected branches
> 10 years	> 40%	Destroy tree

trees yet subtle in others. It is generally recommended that diseased trees should be removed but multiple asymptomatic and potentially infected trees or trees with subclinical symptoms must be recognized because it may probably exist in the vicinity. It is unknown how much inoculums these early stage infected trees, including asymptomatic and trees with limited symptoms, contribute to inoculums dispersal. Depending on the diligence and speed with which the individual grove manager removes trees after discovery, these early stage infections may contribute to more or less inoculums to an epidemic. Removal of the disease trees may be more effective if we take this subclinical portion of the population of infected trees into account and develop a threshold of tolerance that we will accept. Entire blocks of commercial citrus are removed when the acceptable level of disease severity is surpassed since the potential infection capability exist (Graça et al., 2007).

There are 3 main aspects to managing citrus greening disease: propagation of clean nursery stock, psyllid control and removal of potential inoculums sources (Su et al., 1986; Wu et al., 2000). In South Africa, control measures were done by removing the infected plants and applying chemical control measures to keep psyllid population at a minimum (Baniqued, 1998; Roux et al., 2006). HLB inoculum reduction on individual branches affected by HLB will not produce good fruit and usually do not recover. As the bacterium spreads slowly through infected trees, removing parts of the tree showing symptoms can be useful depending on the age of the tree and the infection level. Young trees which are less than 4 years and those not bearing fruits but showing symptoms should be eradicated and replaced, whereas trees with fruit should only be pruned. Trees infected up to 50-70% should be eradicated. Tree owners are encouraged to remove these trees from the site if possible. The following recommendations for African greening disease (Table 1) were also made in 1993; however tree destruction or pruning must be done when psyllid populations are at a minimum, otherwise the disturbance will increase tree to tree spread of both psyllids and disease (Buitendag and Broembsen, 1993).

In many Pacific Islands, it may be possible to carry out activities to reduce inoculums when psyllid populations are naturally low, simply by timing the work carefully. This

would avoid the environmental impact and costs of killing psyllids prior to working on trees. If a distinct wet season occurs, psyllid numbers will be at their lowest towards the end of the season, making this the ideal time to get rid of HLB infected branches and trees. Replacement trees must be known as HLB free and should be obtained either from disease indexed nursery stock, or from disease free areas.

#### Using resistant citrus rootstocks to reduce the HLB severity

Using HLB free seedlings is the most effective way to reduce the severity of greening disease. After planting these disease free seedlings, proper maintenance and monitoring should follow in order to keep it healthy. Good orchard management plays a very big role in controlling the pathogens. The emphasis is on improving the orchard environment. The layout and design should ensure good ventilation and exposure to sunlight, good soil management including efficient fertilization program and having grassy cover in orchards where rainfall is heavy. While these practices may not keep the trees free of greening and virus diseases indefinitely, they will prolong their useful lifespan and keep the trees productive for some years even after they have been infected (F.F.T.C., 2003).

Citrus plant is normally propagated through grafting. It is done by taking a scion from a disease free citrus plant and placing it into the disease free rootstock. Selection of healthy plant source material is very important so as not to spread HLB disease. Pathogen free rootstocks are obtained through a shoot tip grafting (STG) system. The main thing to do is to keep the orchard free from any pests, especially HLB vectors. However, infected trees inside an orchard must be immediately eradicated, including its alternative host plants.

Rootstock selection in citrus is chosen primarily on criteria including cold tolerance and disease resistance (particularly resistance to virus disease) in the growing area (Ferguson, 2003). In some cases, the rootstock can affect the occurrence of the HLB disease symptoms (Kapur et al., 1982). In South Africa the percentage of greening in Valencia oranges was higher on trifoliolate orange rootstock than on empress Mandarin or *Troyer*

*citrangue*. The trifoliolate rootstock caused an extension of the growth flesh period thereby extending the feeding time for the virus (Vuuren and Moll, 1985).

However, no differences were found in a Chinese study on the effects of 13 rootstocks on symptoms in Ponkan mandarin (Lin, 1963). Unfortunately, no readily available source of resistant citrus has yet been identified either for conventional breeding or transgenic improvement systems. It is hoped that the efforts in pathogen and host sequencing and bioinformatics will point to resistant genes or pathogen vulnerabilities thus paving the way for future incorporation of disease resistance mechanisms. At the present time, the citrus industry is composed of numerous commercial cultivars and species, a vast majority of which are moderately to highly susceptible to HLB. Thus when sources of resistance are identified, this resistance will have to be incorporated, perhaps using transgenic technology, into a large number of cultivars and species to meet with current marketing demand (F.F.T.C., 2003).

Other than this, there are 23 rough lemon strains, 8 of them namely 'Australian', 'Florida', 'Helseth', 'Milam', 'Miri', 'South Africa-I', 'South Africa-II' and 'Volkamar' lemon, remained free from infection. In 'Jambiri', 'Jullundri Khatti' and local rough lemon, 100% infection was obtained. The infection in other strains varied from 20 to 80%. The tolerant rough lemon strains were re-evaluated by re-inoculation and re-indexing. 5 strains, namely 'Milam', 'Miri', 'South Africa-I', 'South Africa-II' and 'Volkamar', showed no infection. Of the remaining 3 strains, Australian rough lemon showed visual symptoms of greening on 'Pineapple' sweet orange indicator. The other 2, that is, 'Florida' and 'Helseth', although not showing any visual symptoms in the indicator bud shoots, were found positive for greening marker substance, thus showing their susceptibility to greening. As for the 10 other rootstocks, none was found free from infection. 100% infection was observed in Chinese box orange. In others, the infection varied from 60 to 80% (Kapoor et al., 1982).

### Reducing HLB severity by inter cropping

In Japan, the severities of HLB was controlled by inter planting citrus with guava which apparently inhibits the psyllid vector. It was reported that this inhibition could be in 2 ways. One is the vector directly attracting to the guava and kill it when it feeds presumably by some toxin and the other is the vector indirectly repelling or confusing by some released volatile substance. The exact effect is unknown but preliminary data are encouraging (Beattie et al., 2006).

The effect of interplanting crops or mixed crops to deter insect pests and diseases via a "push-pull" strategy is not new, but rarely used in western agriculture (Zeyaur et al., 2007; Tonhasca and Byrn, 1994; Isman, 2006; Shelton,

2006). The potential of this strategy and its integration with more traditional management tools such as chemical vector control and rouging are being examined in commercial citrus plantings in Florida but are not recommended at this time due to insufficient research data (Graça and Korsten, 2004).

Hirohisa et al. (2008) conducted a study on different model of infected HLB disease of new citrus orchards in Tan Phu Thanh village (Chau Thanh district, Can Tho) in Japan. The models were:

- A) Supplemental mixed planting,
- B) Intercropping Cam Sanh and mango,
- C) Quit Duong solitary planting with *Murraya* traps of psylla,
- D) Intercropping of Quit Duong and mango.

Trees with HLB symptoms were 76.3% in orchard A, 19.7% in orchard B, 5.1% in orchard C and 3.3% in orchard D. The study showed that the situations were different among model orchard. In orchard B, the ratio of infected plants in bed set intercropping showed decrease tendency from near to far side from Chanh Num spreader bed, while a non-intercropping bed shows no influence of the distance. In orchard C, infected trees are mainly located near a Chanh Giay bed and efficacy of *Murraya* traps was not clear. The results suggest the Chanh Num and Chanh Giay were dangerous spreader and distance from them is an important factor for early HLB transmission into new orchards (Hirohisa et al., 2008).

### CITRUS INDUSTRY IN MALAYSIA

Fruits and vegetable production in the world has been recorded 1,383,649 thousand tons. Production of fruits and vegetable in Malaysia is 1,818 thousand tons in 2004. Malaysia's fruit and vegetable percentage production in the world was recorded 0.2% from 1997-1981 and 0.13% in 2004. Citrus fruit production in the world has been recorded 94 million ton in 2005. Average import of citrus in Malaysia was from 12.3 thousand tons in 1970 - 71 and 1978 - 79 to 150 thousand tons in 2004-2005. Orange has been imported in Malaysia more than any other type of citrus, while some species of citrus originated from Malaysia (Table 2.). Citrus species are grown in industry orchards, backyard orchards and some collection in Malaysia. Some citrus collections are also observed in areas such as the Taman Negara national park in Pahang and the Danum Valley in Sabah (The Citrus and Date Crop Germplasm Committee, 2004). Citrus species such as Sour orange (*C. aurantium* L.), Sweet orange (*C. sinensis* L.), Shaddock (*C. grandis* L.) originated from southeast Asia. Shaddock (*C. grandis* L.), was found to have originated from Malaysia. In Malaysia citrus plants are grown in Johor, Kedah, Kelantan, Melaka, Negri Sembilan, Pahang, Perak, Perlis, Pulau

**Table 2.** Production of fruits and vegetable and share in the world and Malaysia.

Area	Production of fruits and vegetable (1000 ton)					Share in world (%)				
	1997 - 81	1989 - 91	1999 - 01	2003	2004	1997 - 81	1989 - 91	1999 - 01	2003	2004
World	629744	812733	1207588	1345056	1383649	100	100	100	100	100
Malaysia	1244	1459	1672	1797	1818	0.20	0.18	0.14	0.13	0.13
<b>Production of citrus in the world and Malaysia (1000 tons)</b>										
Area	1970 - 71 1978 - 79 average	1980 - 81 1988 - 89 Average	1990 - 91 1998 - 99 average	2000 - 01 Average	2001 - 02 average	2002 - 03 average	2003 - 04 average	2004 - 05 average		
World	47 133.3	57 774.6	81 120.8	89 706.5	98357.5	94 084.4	100 852.3	94 793.1		
Malaysia	-	-	-	-	87018	-	-	-		
<b>Importing of citrus in Malaysia (1000 tons)</b>										
Total import	12.3	30.4	81.5	126.4	128.0	132.2	132.4	150.0		
Orange	12.3	30.1	49.4	78.3	69.1	70.6	78.2	85.8		
Tangerines and mandarins	-	-	31.1	45.7	54.9	58.8	50.5	59.2		

Source: www.fao.org.

Pinang, Selangor, Terengganu, Sabah and Sarawak. In Sarawak, Limau Manis makes up most of the production, with Limau Madu becoming increasingly more popular. Pummelo and Limau Kesturi are also grown in certain areas. Neck orange is grown only by a small number of farmers, in addition to either Limau Manis or Limau Madu. The major producing areas are in the following divisions: Samarahan, Sarikei, Bintulu and Sibul (Shamsudin et al., 1990). Based on area planting, the important area of citrus planting are Johor, Kedah, Kelantan, Pahang, Terengganu, Sabah and Sarawak.

In 2005, the estimated total acreage of citrus planting in Sarawak was 3,249 ha, with 2,191 ha planted with Limau Manis (Langkat etc.), 762 ha planted with Limau Madu and 296 ha with pummelo (Agric. Statistics of Sarawak, 2005). In 2005, Sarawak exported an estimated 28.35 metric tonnes of fresh orange and at the same time imported 5,245.38 metric tonnes of fresh, chilled or dried citrus fruits. By 1991, the disease managed to destroy a total of 1,143 ha of citrus trees in the Samarahan Division, resulting in an estimated economic loss of RM 6.5 million (Wee, 1996).

### HLB disease is a series disease of citrus in Malaysia

*Phytophthora* sp., citrus tristeza virus (CTV), canker, HLB and micronutrient deficiency are causing citrus decline in Malaysia. HLB vector has been present since 1970s but the disease was overlooked. According to the Lime (1990), HLB symptom was observed by Tanaka and Euno (1978), in Ringlet areas in Cameron highland and Ulu Tiram in Johor (Shamsudin et al., 1990). Huang-longbing' was detected in Sarawak in the Samarahan division in 1988. The occurrence of HLB disease in Malaysia was confirmed by field symptoms and trans-

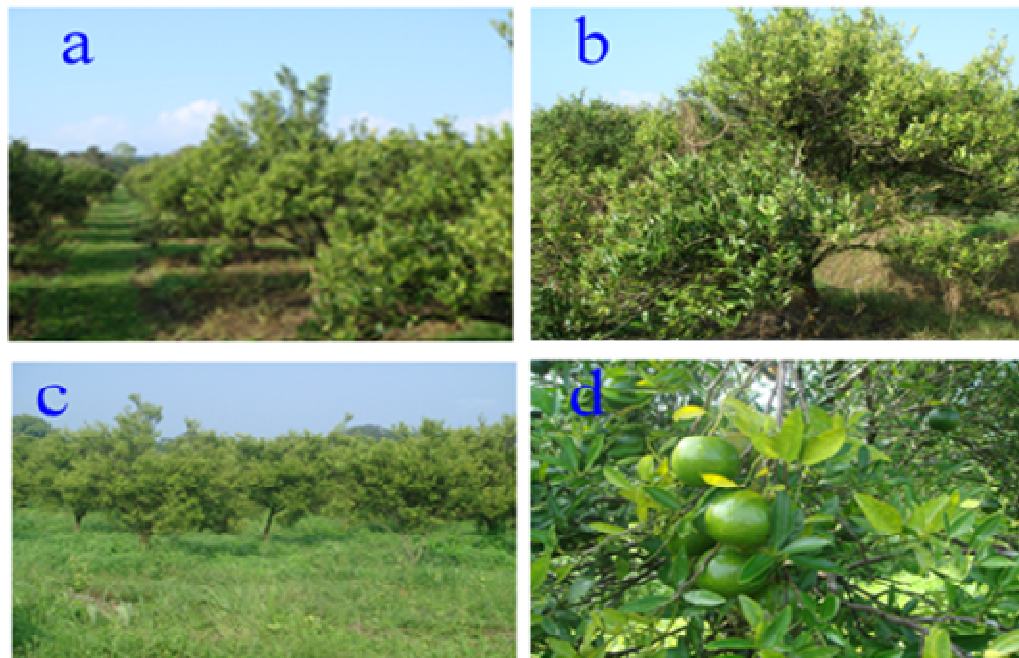
mission electron microscope (TEM) in 1989. HLB disease is known as 'penyakit greening limau' in Malaysia (Ko, 1991).

Garnier and Bove (1996) reported the observation of high population of HLB vector, *Diaphorina citri* on mandarin trees and trees showing HLB symptoms in Kuala Terla, Cameron highland in 1987 (Garnier and Bove, 1996). The department of agriculture in Sarawak started producing disease-free planting material in 1996 to re-planting. It was produced using budwood which was obtained through shoot-tip grafting (STG). Reduction of the source of inoculum was also recommended. However, only an estimated 40% of growers followed the recommendations. Many of the citrus growers were propagated by marcot from their existing trees, which were infected with HLB disease. HLB became widespread again in the Samarahan division around 2001-2002, destroying many orchards of citrus (Teo, 1996).

Zizah and Zazali (2005) reported that all over Peninsular Malaysia including Sabah and Sarawak these areas have been infected by HLB disease. This report showed that most of the cultivated citrus varieties have been infected. About 2,458 ha out of 3,526 ha of cultivation areas (69.7%) have been found to be infected with the HLB disease (Figure 2). This disease was distributed widely in Terengganu, increasing from 641 ha in 2001 to 1262 ha in 2004 (Azizah and Zazali, 2005). A new research was conducted by Khairulmazmi Ahmad in university Putra Malaysia. He reported in 2008 that all the major citrus growing areas in Peninsular Malaysia such as Terengganu, Pahang, Selangor and Kelantan have been infected with HLB disease (Figure 1). The highest mean percentage of disease incidence (80%) was recorded in Kelantan amongst the surveyed areas followed by Terengganu (63%), Selangor (46.67%) and Pahang (42%) (Sijam et al., 2008).



**Figure 1.** Map of Peninsular Malaysia showing the infected areas i.e. Selangor, Pahang, Terengganu and Terengganu (Sijam et al., 2008).



**Figure 2.** Symptom of HLB disease in citrus Mandarin orchard in Peninsular Malaysia; (a) HLB symptom in Selangor, (b) close up of HLB symptom on citrus mandarin in Selangor, (c) HLB symptom in Terengganu, (d) infected fruits in Terengganu showing lopsided fruits.

### Research recommendations for Huanglongbing (HLB) in Malaysia

#### a. Hosts reaction and breeding

1. Determine the different alternative hosts of HLB and vectors (especially, collect and evaluate citrus and citrus relative species on HLB in Malaysia) for breeding and assessing the resistance or susceptibility.

2. HLB quantities on different host using biotechnology methods
3. Possibility of harm estimate on different host.

#### b. Diagnostics of pathogen and vector

1. Detection and annihilation of infected sources of nursery and field
2. Improving HLB detection methods
3. Sample collection study - eradicate the susceptible and introduce the tolerant species

#### c. HLB characterization and taxonomy

1. Discrimination of species and strains of HLB in different geographical
2. Genetic diversity

#### d. Culturing HLB (find a way to HLB culturing in media)

#### e. Reduce the severity and control of HLB

1. Control the vector population using different insecticide to reduce the severity and spread
2. Urban Homeowner control of Psyllids
3. Herbal or natural chemicals
4. Chemicals to reduce the stunting and improve the fruits quantity and quality

## Conclusion

Control of HLB is difficult if inoculum sources are widespread and the psyllid vector is well established. There are no HLB tolerant mandarin, orange or grapefruit cultivars to replace declining healthy trees. The general control strategy has been to eradicate all existing sources of HLB within an area, then replant with HLB-free trees grown from clean budwood. Psyllid populations must also be reduced as much as possible. Biological control of the psyllid vector is only possible in locations that do not favour build-up of psyllid populations and is often compromised when hyper-parasites are present. HLB is a vector-borne disease. Preventing HLB from entering to healthy area is much easier than trying to eradicate or control it. It is important to avoid bringing propagation materials from HLB-infected areas to non infected area. Any citrus materials from overseas must be inspected and tested to ensure they are free of HLB. Growers are also encouraged to keep vigilant on the conditions of trees, particularly a sudden change in vigorously growing trees.

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