BACKGROUND, CURRENT SITUATION AND MANAGEMENT OF THE HLB AND ITS VECTOR IN CHINA

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Abstract: Huanglongbing(HLB), yellow shoot disease, is a destructive disease prevailing in the southern part of citrus producing area in China. The history of its occurrence has more than 100 years. “Yellow shoot” and ”mottling yellow leaf” are characteristic symptoms of HLB. Graft transmissibility and virus nature of HLB were experimentally confirmed in the mid 1950’. In 1970’, the pathogen was identified as rickettsia-like organism or a new type of mycoplasma-like organism. In 1990’, it was classified as *Candidatus* Liberibacter asiaticus. Recently, a phytoplasma related to *Candidatus* Phytoplasma asteri was discovered in some leaf samples collected in Guangdong Province. The results of heat sensitivity tests revealed that diseased budlings could be cured by the treatment for 50 minutes in 49□ or 50□ moist air, and the budwoods collected from diseased trees could be sterilized by immersing in 47□ water for 10 minutes and repeat twice with the interval of 24 hours. The transmission of HLB by Asian citrus psyllid, *Diaphorina citri* Kuw. was experimentally confirmed in 1970’. Extensive surveys in different localities showed that the psyllid is not occurred in the localities wih high latitude or high altitude, and the northwards expansion of the distribution of psyllid is followed by HLB prevalence. HLB could be transmitted by 4^{th}-5^{th} instar nymphs of the psyllid and by just emerged adults but not by 1^{st}-3^{rd} instar nymphs. In the graft transmission tests, as the bud or piece of stem was used as inoculum, the transmission rate was much higher than that as a piece of stem bark was used. As the budwoods were collected from the same diseased tree, and grafting conducted in different season of one year, HLB incidence of the progeny grafted in May-July was apparently lower than that grafted in other seasons. The paper on the exclusion of HLB by shoot-tip grafting was published in 1987. All of the varieties of genus *Citrus* and *Fortunella* are susceptible to HLB, whereas trifoliate orange is tolerant. *Murraya paniculata* and *Clausena lansium* have been identified to be susceptible to *Ca*. L. asiatics. As the graft transmissibility of HLB was confirmed in the mid 1950’, HLB was defined as a target of plant quarantine by the Department of Agriculture. Since late 1950’, mottling yellow leaf has been commonly used as main criterion for HLB diagnosis in the field, and since late 1990’, PCR has been used for exact diagnosis. Producing HLB free budlings has been recommended. In the past, mother block and nursery should be established in a separated area, 1.5-3 Km apart from existed citrus orchards. In recent years, the establishment of mother block and nursery in screen house was practiced in some localities. The budwoods were collected from healthy trees standing in the orchards with low HLB incidence, and treated by tetracycline immersing or intermittent heat treatment. Accompanying with other diseases control, shoot-tip grafting has been used in preparing HLB free mother plants. Strictly control psyllid in a large area by insecticides is the most important procedure in HLB control. In general, there are 1-2 sprays in the period after picking and before spring sprout, in spring growth, in summer growth, and in autumn growth, respectively. Removing diseased trees promptly is also an important procedure in HLB control. Usually, the diseased trees are removed after picking.

The experience of Yangcun Citrus Farm with the acreage of about 2000 ha demonstrated that the annual HLB incidence could be kept less than 1% by strict control of psyllid and prompt removal of diseased tree. Since the early 2000', much extension works for HLB control have been done by the Agricultural Department of Provincial Government in Zhejiang, Guangxi, Fujian (Yongchun) and Guizhou (Congjiang), and the quick spread of HLB in most areas has been effectively checked.

Introduction: Huanglongbing (HLB), yellow shoot disease, is a destructive disease prevailing in the southern part of citrus producing area in China. According to old farmer's memory, the history of its occurrence is about 120 years, and the record history goes back to 1919. The graft transmissibility and virus nature of HLB was confirmed in 1956, and it was determined as a quarantine target in 1957. Since 1960', a group of isolated citrus nurseries have been established for propagating HLB free budings. After the late 1970', the strict control of citrus psyllid has been emphasized in HLB control. In recent years, the HLB incidence was kept in low level in most citrus orchards, but it usually caused serious damage in others.

1. Distribution and importance

In the Mainland of China, HLB and Asian citrus psyllid are distributed in Guangdong, Fujian, Guangxi, Yunnan, Southern Zhejiang, Southern Jiangxi, Southern Hunan, Southern Guizhou and in a limited area of Southern Sichuan.

In 1980', there was an apparent northward movement of HLB prevailing area occurred in Guangxi. Before 1980', HLB prevalence had not been found in Northern Guangxi, but in 1980', the distribution of citrus psyllid was found and followed by HLB epidemic. Since the early 2000', the northward movements of HLB prevailing area have also occurred in Zhejiang, Hunan and Jiangxi Provinces.

In the area where citrus had not been planted, the young citrus orchards usually have few trees shown HLB symptoms in 2-3 years after planting, if the psyllid was not controlled, the incidence of HLB would quickly increase, and the orchard would be destroyed in about 8 years after planting. If the new orchard established close to the orchards with high HLB incidence, it would be destroyed by HLB in 1-3 years after planting.

2. Symptoms

"Yellow shoot" and "mottling yellow leaf" are characteristic symptoms of HLB. "Yellow shoot" is shown in the initial stage of the disease, new shoots of a part of the branches become yellowing, in summer or autumn, the yellow shoots usually appear first on the top of the tree. "Mottling yellow leaf " is shown as the leaf mature, the yellowing starts near the midrib, the lateral veins and leaf base. As the yellowing spreads, the leaf shows a mottled pattern.

Most fruits of the diseased tree dropped earlier, the left fruits are small, poorly colored, shown "red nose" in some mandarins and being lopsided in some varieties.

New root growth is suppressed and the roots usually start decaying from the rootlets.

3. Causal agent

Several causes of HLB have been suggested, i.e. (1) water injury, (2) virus infection, (3) root rot caused by Fusarium sp., (4) nutrient stress, and (5) micro-elemental deficiency.

The graft transmissibility and virus nature of HLB was confirmed in the mid 1950' (Lin, 1956). In the early 1960', there was a dispute about whether the HLB virus is the same as tristeza virus or not. Chen *et al.* conducted an indicator test and found that all of the HLB diseased trees were infected with tristeza virus, suggesting that HLB should be caused by tristeza virus (Chen et al., 1965). But according to the difference in host susceptibility, Lin suggested that HLB should not be caused by tristea virus (Lin, 1977). There after, indexing results revealed that some young seedlings in the field shown characteristic HLB symptoms were free of tristeza. Therefore, tristeza is an agent which usually infects citrus simultaneously with HLB, but it is not the actual cause of HLB (Zhao et al.,1979).

Tetracycline sensitivity of the causal agent of HLB was revealed by budwood dipping and trunk injection in the mid 1970' (Kwangxi Citrus Yellow Shoot Study Group, 1975). The tetracycline sensitivity of HLB pathogen indirectly proved that HLB is caused by mycoplasma-like organism but not by virus. After the electron microscopic examinations of the phloem tissues of diseased leaf veins collected in Guangdong, Guangxi and Fujian, Ke *et al.* suggested that the causal agent of HLB may belong in the rickettsia-like organism (Ke *et al.*, 1979) and Chen *et al.* suggested that the causal agent of HLB may be a new type of mycoplasma-like organism (Chen et al., 1979).

Afterwards, the results of PCR test revealed that HLB is caused by *Candidatus* Liberibacter asiaticus.

Recently, Chen *et al.* discovered a phytoplasma related to *Candidatus* Phytoplasma asteri in some leaf samples shown HLB symptoms collected in Guangdong (Chen *et al.*, 2009).

4. Some characteristics of HLB in graft transmission

Incubation period after graft inoculation. In general, young seedlings graft-inoculated in September-October, usually shown HLB symptoms in next May-June, and the seedlings graft -inoculated in February-March, shown symptoms also in May-June. In above cases, the incubation period is 2-9 months. As 1-16 years old mandarin trees were graft-inoculated in September, most of the trees shown symptoms on the inoculated branch in 7-9 month after inoculation (Lin, 1963).

The graft transmissibility and the HLB incidence of the progeny plants of a diseased tree is variable and never reaches 100% (Lin, 1956; Kao et al.,1963; Zhao et al., 1982). It shows that the distribution of the pathogen in host plant might be uneven.

Graft transmissibility influenced by the tissue used for inoculum. As different tissues from the same diseased budwood were used as inoculum, the transmissibility (number of diseased plants/number of inoculated plants) is different as follows: single bud, 22/26 (85%); piece of stem without bud, 29/43 (67%); piece of stem bark, 2/43 (5%). Above data indicated that the graft transmissibility by bark as inoculum is much lower than that by the other tissues (Zhao *et al.* 1982).

As the budwoods collected from the same diseased tree are propagated in different seasons, the HLB incidence of the progeny plants is quite different as shown in table 1 (Zhao *et al.*, 1982).

Above data indicate that HLB incidence of the progeny plants propagated in hot seasons is much lower than that in other seasons.

5. Heat sensitivity

Lin et al. (1965) reported that 3-year-old Tankan mandarin affected with HLB recovered normal growth after treatments for 45, 50, 55, 60 or 65 min in 48, 49 or 50℃ moist air, and for 35, 40, 45, 50 or 55 min in 51℃ moist air.

Lo et al. (1981) further proved that diseased budlings recovered normal growth after moist air treatment for 50 min at 49℃ or 50℃.

Lo (1983) reported that budwoods collected from diseased tree could be sterilized by immersing in 47℃ water for 10 min and repeat twice with the interval of 24 hours.

6. Vector

The transmission of HLB by citrus psyllid (*Diaphorina citri* Kuw.) was experimentally confirmed in the mid 1970' (Kwangxi Citrus Yellow Shoot Study Group, 1977; Plant Pathological Teaching and Research Section of South China Agricultural College, 1977; Wu, 1977).

Under experimental conditions, the transmissibility of HLB by psyllid was relatively low. The tests carried out in Liuzhou, Guangxi in 1973-78, healthy caged seedlings were exposed to the psyllid collected from diseased trees in the field, 50-200 adults were released per caged plant. Thirty two of 398 plants (8.0 %) developed HLB symptoms(Chao et al.,1979). The tests carried out in Fuzhou, Fujian in 1978-1984, healthy caged seedlings were exposed to the psyllid collected from diseased trees in the field, or to the psyllid feeding on diseased seedlings in the lab, 20-300 adults were released per caged plant. Forty of 329 plants (12.2%) developed HLB symptoms. Similar tests carried in Raoping, Guangdong in 1982-1984, 10-40 adults were released per caged plant, 33 of 70 plants (47.1%) developed HLB symptoms (Xu et al.1985).

Young seedlings could be infected by HLB after feeding by one psyllid adult (Xu, 1988). The minimum incubation period of young seedlings after psyllid inoculation is 2 months and 5-8 months in general.

HLB could be transmitted by 4^{th}-5^{th} instar numph of the psyllid and by just emerged adult, but not by 1^{st}-3^{rd} instar nymph (Xu, 1988).

7. The relation between HLB prevalence and environmental conditions

In the early 1960', Kao et al. surveyed the occurrence of HLB in Fujian and indicated that suppressive conditions for HLB may exist in the districts with high latitude or high altitude (Kao et al. 1963).

A survey of 27 counties in Guangxi was made in 1973-1977 to correlate the occurrence of citrus psyllid and HLB. The data obtained showed that there was no natural spread of HLB in the northern part of Guangxi where the psyllid was not found, and usually there was a high population

of psyllids in the southern part where HLB was epidemic (Chao et al., 1979)

The Sichuan Citrus Huanglongbing Survey Group (1977) reported that the incidence of HLB was different in the orchards with different altitudes near Jule commune in Ningnan County (Table 2).

A survey conducted in 1977 in the farm of Red Flag commune in Wuzhou, Guangxi revealed that in the orchard located in a valley, no psyllid was found among the budlings planted in 1965 and 3 of 80 trees showed HLB symptoms; on the other hand, in budlings of the orchards outside of the valley from the same source and also planted in 1965, high population of psyllids was found and nearly all of the trees were destroyed by HLB (Chao et al., 1979).

Above situation indicates that the environmental conditions can directly influence the distribution of psyllids as well as influence the prevalence of HLB.

8. Host

In the general surveys, all of the varieties of genus *Citrus* and *Fortunella* are susceptible to HLB, trifoliate orange is tolerant. *Murraya paniculata* and *Clausena lansium* have been identified to be infected in the field by *Candidatus* Liberibacter asiaticus (Li et al.,2002;Ding et al.,2005; Deng et al.,2007).

9. Control

9.1 Quarantine

HLB was defined as a target of plant quarantine by the Ministry of Agriculture, China in 1957.

A national standard Pant Quarantine Rules for Producing Areas of Citrus Nursery Stocks was issued in 1985. It is a guideline for producing HLB free and citrus canker free budlings.

9.2 Diagnosis

Since the late 1950', mottling yellow leaf has been commonly used as main criterion for HLB diagnosis in the field (Kao et al., 1958; Kao et al., 1959), since 1990', PCR has been used for the exact diagnosis of HLB (Tian et al.,1996). In general, HLB diagnosis is completed in the field by finding mottling yellow leaf, and in the new prevailing area, PCR is also practiced.

9.3 Producing and planting HLB free budlings

Since 1960', a group of HLB free nurseries have been established in different areas.

In the past, the site of mother block and nursery should be established in a separated area, 1.5-3 km apart from exist citrus orchards. In recent years, a part of mother block and nursery are established in screen house.

The budwoods are collected from HLB free area, or from healthy trees standing in the orchards with low HLB incidence, and treated by tetracycline immersing or intermittent heat treatment.

The exclusion of HLB by shoot-tip grafting was experimentally confirmed in 1987 (Jiang et al., 1987; Chen et al., 1987). Accompanied with other diseases control, shoot-tip grafting is used for preparing HLB free mother plants.

9.4 Psyllid control.

Strictly control psyllids in a large area by insecticides is the most important procedure for HLB control. In general, there are 1-2 insecticide sprays in the period between after picking and before spring sprout, and each 1-2 sprays in spring, summer and autumn growth period. Organic phosphorus insecticides are usually used, in recent years, other than chlorpyrifos, phoxim etc., imidacloprid and thiamethoxam (Actara) are also used.

9.5 Remove diseased plants

Remove diseased plants promptly is also an important procedure for HLB control. In general, the diseased plants are removed after picking. In some farms, removing the diseased plants 3 times per year is practiced.

Serious damage caused by HLB and the management of HLB accelerated the development of citrus production in the Yangcun Citrus farm, Guangdong is a representative example of HLB control. Yangcun Citrus Farm was the biggest national citrus farm in last century with the acreage of 1300-2000 ha.

Citrus planting was began in 1953. In 1953-1972, for the control of leaf rollers, leaf minor, geometrid and red mite, organic chloride and organic phosphorus insecticides were sprayed several times per year, hence psyllid was very difficult to be found, the annual incidence of HLB was not more than 1%, and the citrus production developed successfully in that period.

Since 1974, leaf rollers and geometrid have been controlled and organic chlorides have been eliminated to be used, due to limitation of insecticides effective for psyllid, the psyllid population significantly increased and followed by high incidence of HLB in 1978-1982. Up to 1982, all of 960 thousand trees planted before 1972 had been destroyed.

Since December, 1978, psyllid control was enhanced, 2 sprays of organic phosphorus after fruit picking and before spring sprouting, each 1 or 2 sprays in spring, summer and autumn growth period. Other than psyllid control, all of the trees showing HLB symptoms were removed 3 times in each year. There after, the psyllid population significantly decreased, the incidence of HLB was 6.8% in 1978, 1.3-3.8% in 1979-1982, and less than 1% in 1983-1992. The citrus production developed smoothly in 1983 -1992.

Since 1993, the management system of the farm changed, technical procedures were decided by producer themselves, but not by farm manager and technicians, and less amount of insecticides was applied. Hence, the psyllid population and HLB incidence increased again.

The situation similar with “Yangcun Experience” has appeared in different localities. Since the early 2000’ much extension work for HLB control has been done by the Agricultural Department of Provincial Government in Zhejiang, Guangxi, Fujian (Yongchun) and Guizhou (Congjiang), and the quick spread of HLB in those areas was checked.

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Tab.1 Percentage of progeny plants showing HLB symptoms from propagations made in different seasons

Grafting season	No. plants diseased/ No. plants grafted	Percentage
Test 1		
1976-Nov.	21/30	70.0
1977-Jan	6/14	42.9
1977-Mar.	6/8	75.0
1977-May	1/21	4.8
1977-Jul.	0/22	0.0
1977-Sept.	2/15	13.3
Test 2		
1979-Feb	29/30	96.7
1979-Apr.	14/20	70.0
1979-Jun.	1/9	11.1
1979-Aug.	6/9	66.7
1979-Oct.	18/19	94.7
1979-Dec.	18/22	81.8
1980-Feb.	24/25	96.0
1980-Jun.	4/27	14.8

Tab. 2 A survey for the incidence of HLB and psyllid population in the orchards with different altitudes.

Orchard NO.	Altitude (m)	Psyllid population	HLB incidence
1	1090	abundant	nearly 100 %
2	1200	abundant	nearly 100 %
3	1210	exist	40 %
4	1385	not found	3.6 %
5	1420	not found	3.0 %
6	1500	not found	3.0 %
7	1620	not found	3.0 %