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Background, Current situation and management of the HLB and its vector in China

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Huanglongbing (HLB) means 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 the mid 1950'.

Since 1960', a group of isolated citrus nurseries were established for propagating HLB free budings.

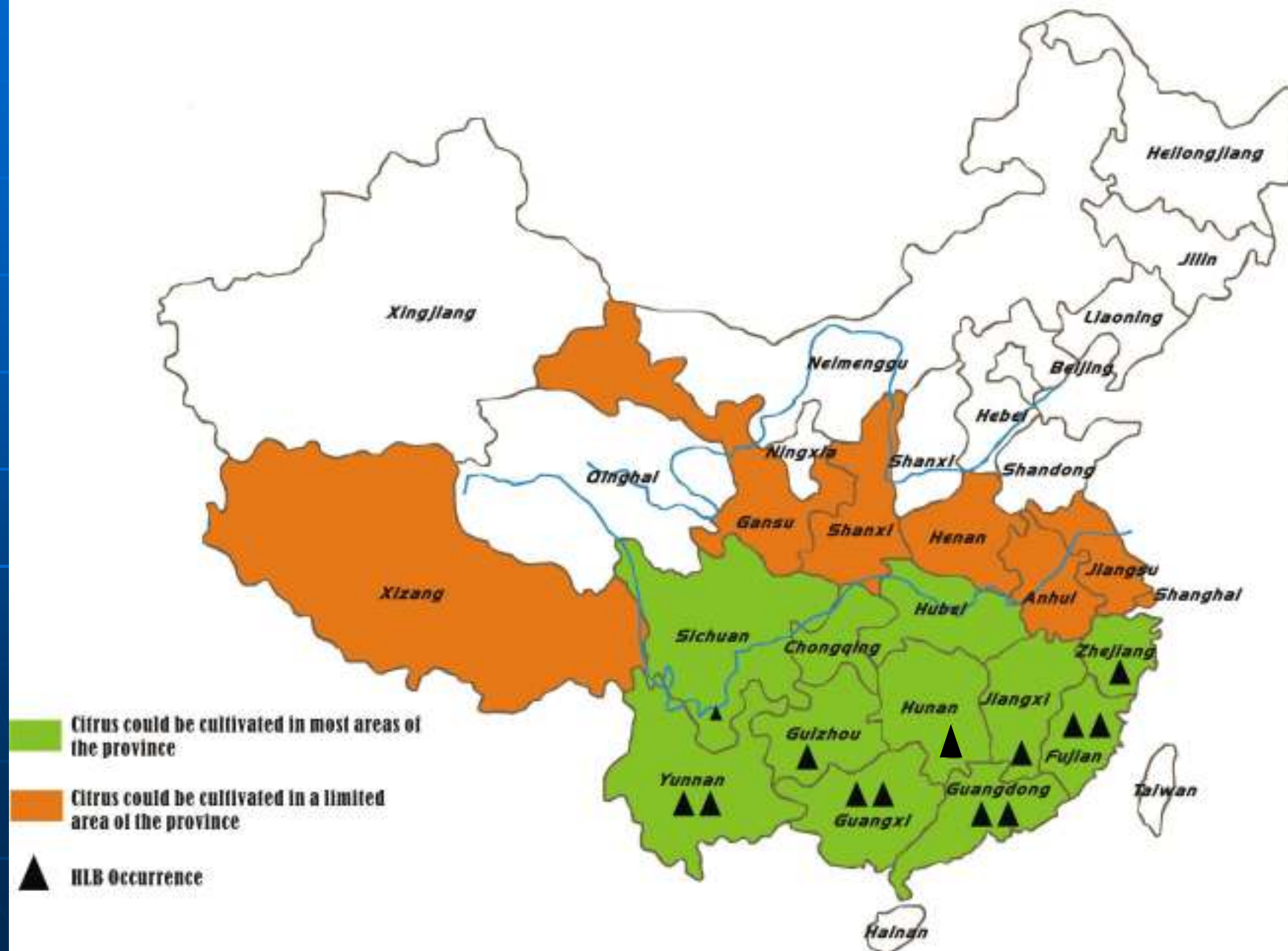
After the late 1970', the strict control of citrus psyllid was emphasized in HLB control.

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
2. Symptom
3. Causal agent
4. Some characteristics of the pathogen in graft transmission
5. Heat sensitivity
6. Vector
7. The relation between HLB prevalence and environmental conditions
8. Host
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Distribution and importance

Citrus Cultivation and HLB in China



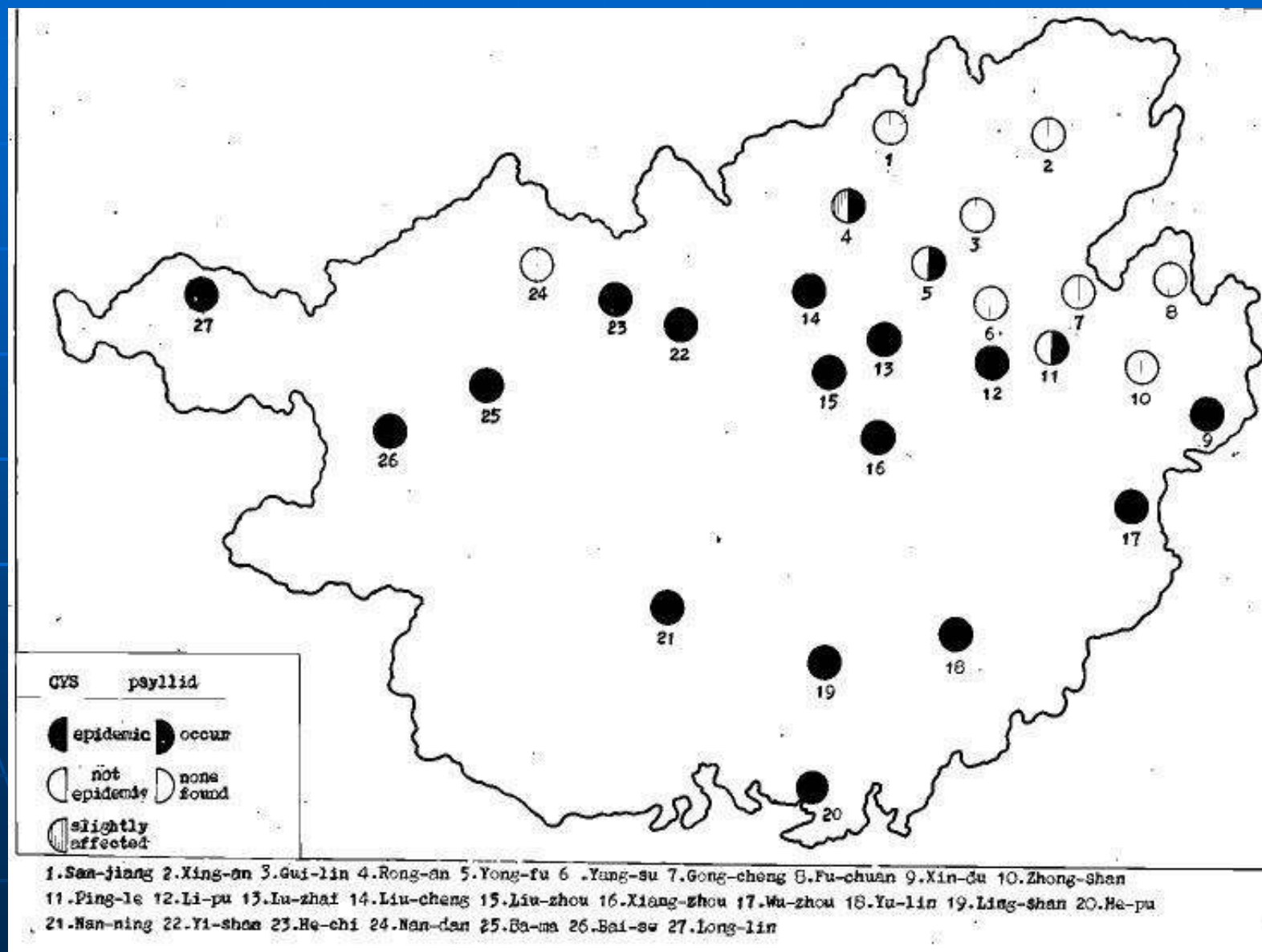
HLB prevailing area in China
has a **northern “border”**.

The “border” is not stable.

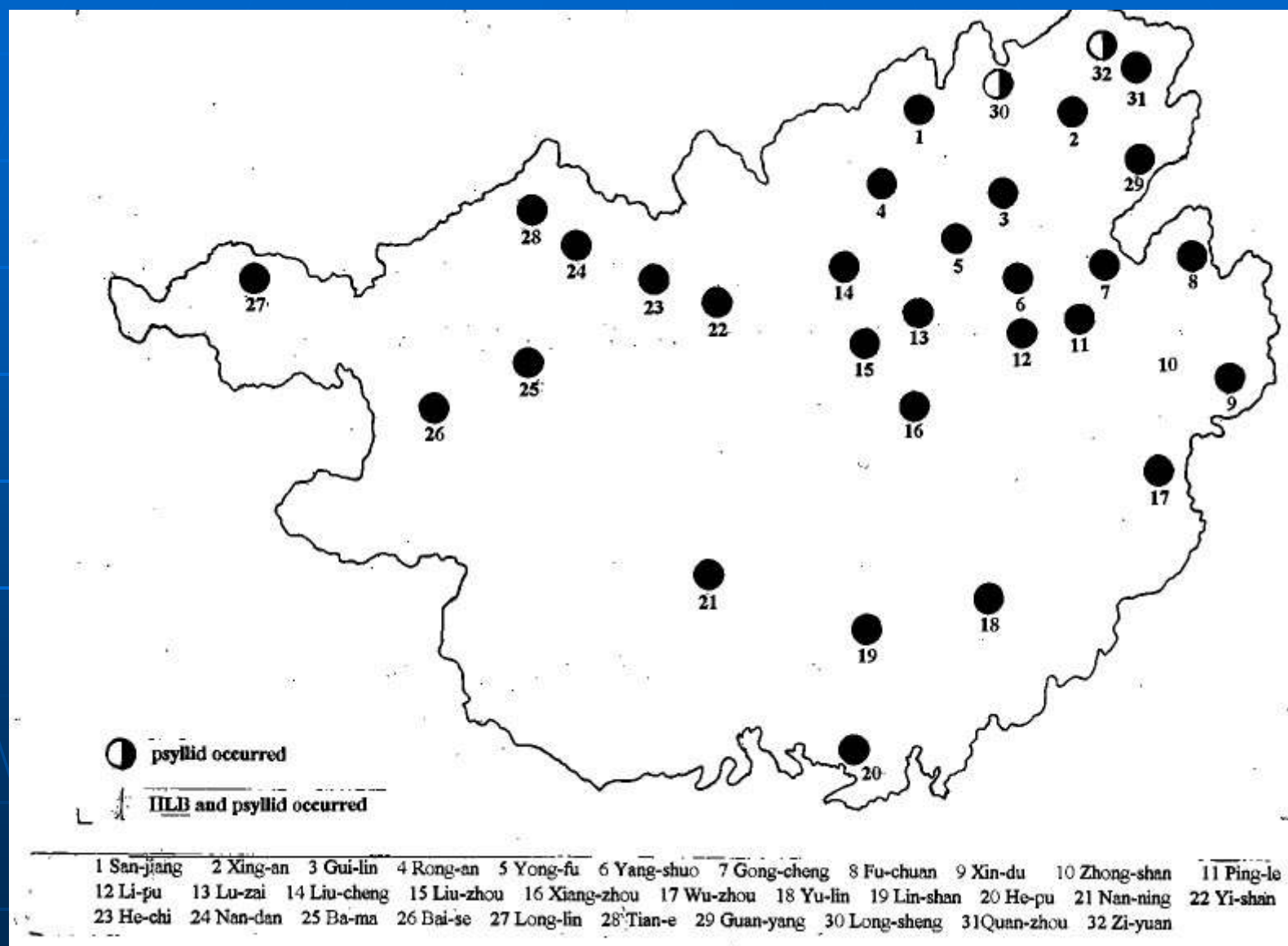
In 1980', there was an apparent northward movement of HLB prevailing area occurred in Guangxi.

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 (Zhou et al., 1989).

HLB Occurrence and Psyllid Distribution of Guangxi in Late 1970'



HLB Occurrence and Psyllid Distribution of Guangxi in Late 1980'



Since the early 2000', the northward movements of HLB prevailing area have also occurred in Zhejiang, Hunan and Jiangxi Provinces.

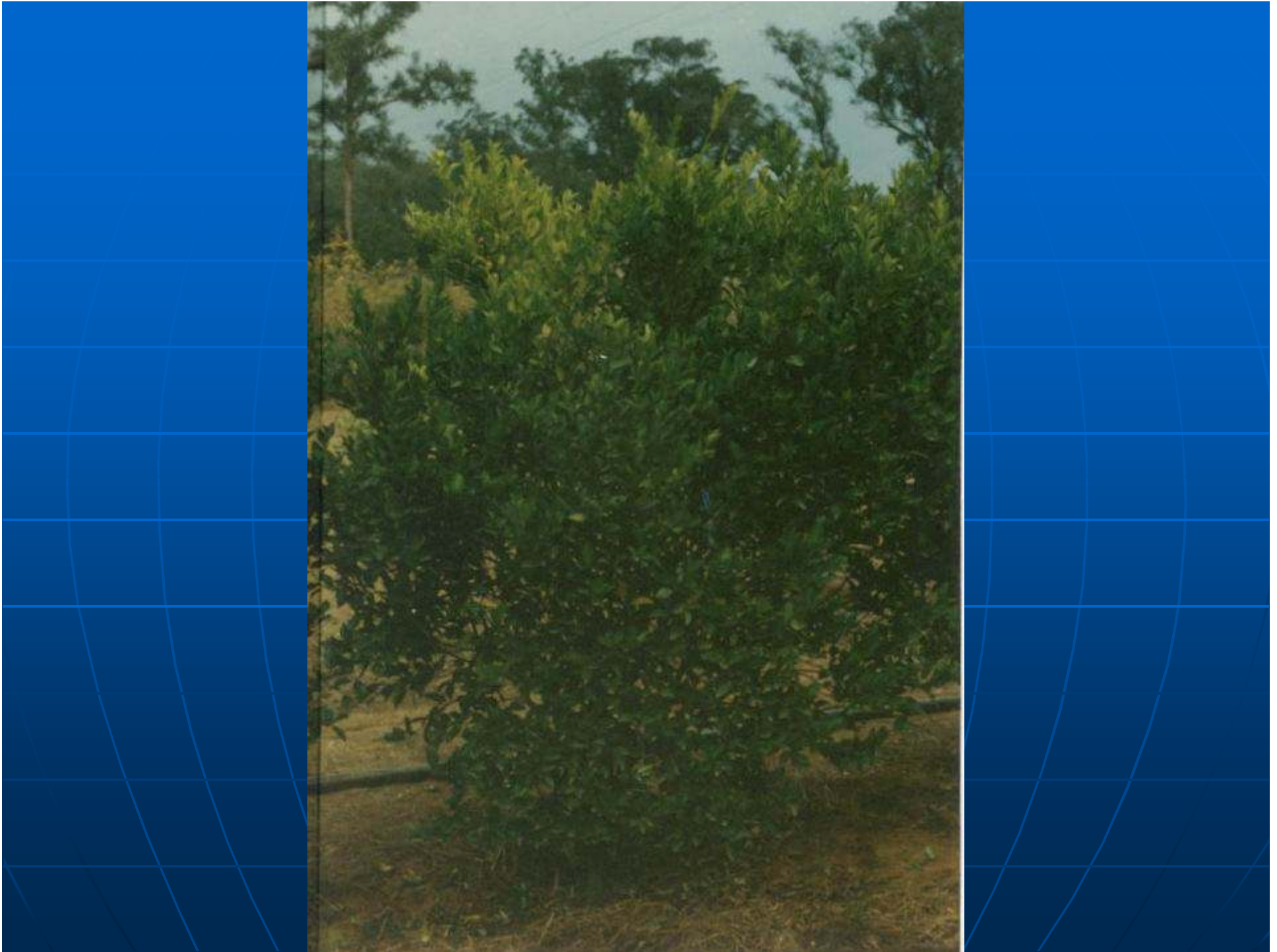
In the area where citrus had not been planted, the young citrus orchards usually have a few trees shown HLB symptoms in 2-3 years after planting, if the psyllid was not controlled, the incidence of HLB would quick increase, and the orchard would be destroyed in about 8 years after planting.

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.

Symptom

“Yellow shoot” and “mottling
yellow leaf” are characteristic
symptoms of HLB.

“Yellow shoot” 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.

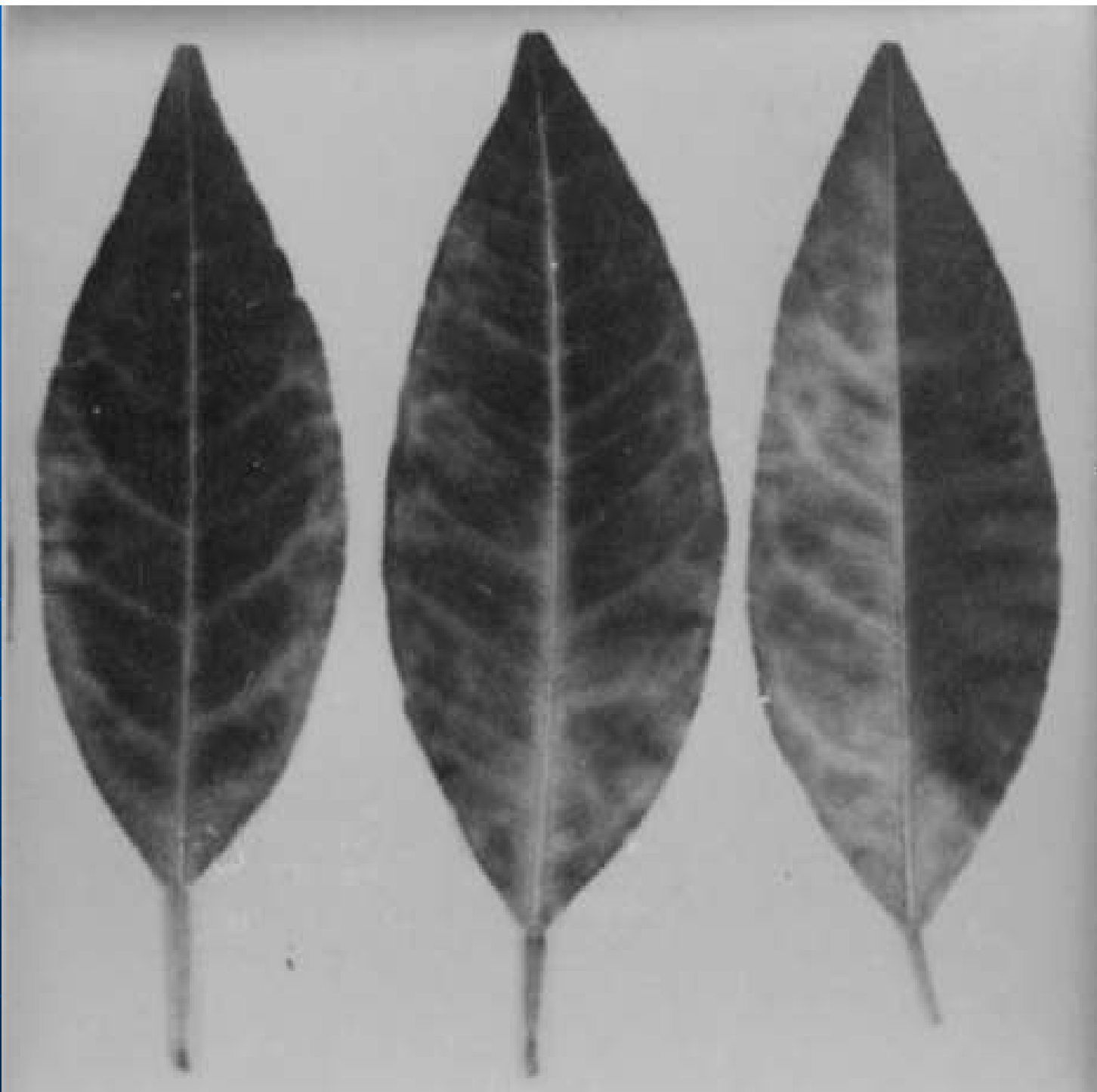


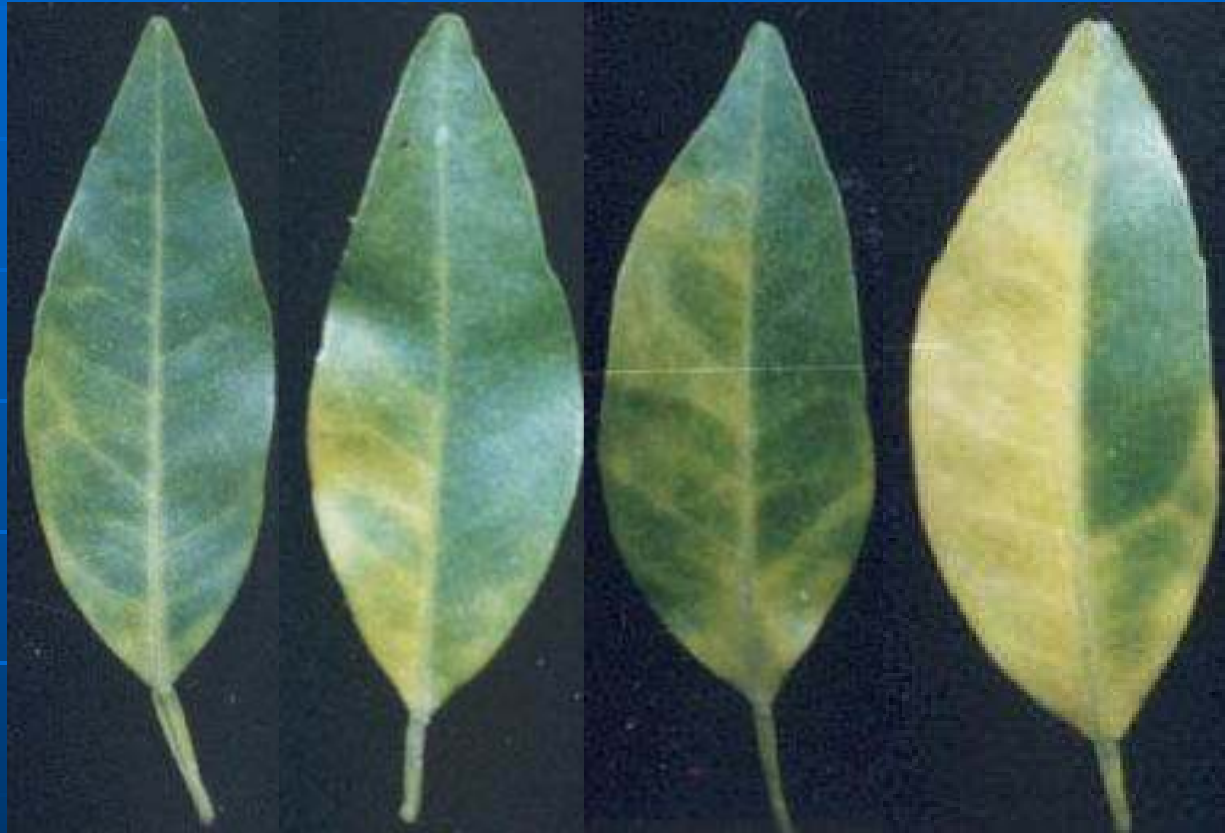






“Mottling yellow leaf ” is shown as the leaf mature, the yellowing starts near the midrib, the lateral veins and leaf base. As the yellowing spreads, the leaf shows a mottled pattern.







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.







“Red nose fruit”



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

Causal agent

Several causes of HLB have been suggested:

- (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 tristeza 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).

Tetracyclin 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, 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., 1979a; Chen et al., 1979b).

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).

Some characteristics of HLB in graft transmission

Incubation period after graft
inoculation in young seedlings 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 months 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 tissue from the same diseased budwood were used as inoculum, the transmissibility 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.

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

Above data indicated that
HLB incidence of the progeny
plants propagated in hot
seasons is much lower than
that propagated in other
seasons (Zhao *et al.*, 1982)

Heat sensitivity

Lin *et al.* (1965) reported that 3-yr.-old Tankan mandarin affected with HLB recovered normal growth after treatments for 45-65 min in 48-50°C moist air, and for 35-55 min in 51°C moist air.

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

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

Vector



- The transmission of HLB by citrus psyllid (*Diaphorina citri* Kuw.) was experimentally confirmed in the mid 1970'.

Under experimental condition, the transmissibility of HLB by psyllid was relative 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. 40 of 329 plants (12.2%) developed HLB symptoms. Similar tests carried in Raoping, Guangdong in 1982-1984, 33 of 70 plants (47.1%) developed HLB symptoms (Xu et al.1985).

Young seedlings could be infected by HLB after feeding by only 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 4th-5th instar nymph of the psyllid and by just emerged adult, but not by 1st-3rd instar nymph (Xu, 1988).

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, and found 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 psyllid 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 altitude near Jule commune in Ningnan county.

Orchard	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 %

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

Above situation indicated that the environmental conditions directly influenced the distribution of psyllid, as well as inference the HLB prevalence.

Before 1980', in order to increase HLB tolerance, experiments had been conducted to improve the nutrient situation of citrus plant by fertilization, irrigation and soil improvement.

No positive results had been obtained.

Host

In the general survey, all of the varieties of genus *Citrus* and *Fortunella* are susceptible to HLB, trifoliolate 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).

Control

1. Quarantine.

HLB was defined as a target of plant quarantine by the Department of Agriculture in 1957.

A national standard <Plant 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.

2. **Diagnosis.**

Since the late 1950', **mottling yellow leaf** is 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.

3. Producing and planting HLB free budlings.

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.



4. Psyllid control.

Strictly control psyllid 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.

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 (Zhuang, 1987).

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, psyllid was very difficult to be found, the annual incidence of HLB was not more than 1%, the citrus production developed successfully in that period.

Since 1974, leaf rollers and geometrid were controlled and organic chlorides were eliminated to be used, insecticides effective for psyllid were less sprayed, 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 shown HLB symptoms 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 were 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 works 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.

Thank you for your attention!



张为平摄影作品精选 群峰倒影山浮水 (Peaks cast their reflections on the waters)