THE CITRUS HUANG LUNG BIN (GREENING) DISEASE IN CHINA

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Original document published in 1956 in Acta Phytopathologica Sinica, Vol. II, Part 1, No. 1, pp. 1-11 and Part 2, pp. 14-38

FOREWORDS

*Born in CUNXIA, a village located in the Fujianese citrus belt, **Professor LIN Kung Hsiang** obtained his PHD at **CORNELL** University, USA in the late 1930's. After returning to China he joined the **Christian University of LINGNAN** in **Guangzhou (Canton)**, which eventually became the **South China Agricultural University** where he worked at the **Plant Protection Department**, and dedicated his life to understand and solve the difficult problem of **Huanglungbin (HLB)**. As early as 1953 he produced a clear evidence that HLB was graft transmissible and should neither be attributed to physiological disorders such as mineral deficiencies or water logging, nor be considered as a soil borne debilitating disease. He confirmed his demonstrations by adequate and precise experimental work combined with frequent visits to infected citrus areas and numerous discussions with farmers. This inclined him to predict that besides being graft transmissible, HLB was probably also vector-borne.*

*In spite of fierce criticism from some of his Plant Pathology colleagues, but also from other disciplines, he published a first document on HLB in 1956 which is being presented here in its English version. More than fourty years later, Pr. **LIN Kun Hsiang**'s writings are still enlightening the ticklish problem of citrus graft transmissible diseases in general and **HLB - Greening** in particular - With the techniques available in those days, Pr. **LIN Kun Hsiang** examined the three main components of the disease: its symptomatology, aetiology and epidemiology. Needless to say that the most important part of his work was carried out during the uneasy period of the Chinese Revolution.*

*We express our deep appreciation to Pr. **LIN Kun Hsun**, who was directly involved for so many years in the research work of his brother. He accepted the task of translating this document, and provided the original negatives of the plates. One should not be misled by the term "adapted", which appears in the subtitle. We strictly followed the original manuscript, with its sequence of demonstrations. But in agreement with Pr. LIN KUN HSUN we reduced the non essential parts and decided to incorporate various mappings which were not included in the Chinese text.*

This document, far in advance of his contemporaries writings or considerations on the topic, is answering many questions. Even now it is not outdated.

*Professor LIN Kun Hsiang passed away in June 1986 and was buried in his original CUNXIA village. His grave is located in a mountain area near the **MINHOU** foundation block orchard, as a sort of post mortem warrant that disease-free material will be delivered to Fujianese Citrus farmers.*

*But for greater safety, Professor **LIN Kun Hsiang** published an extension service pamphlet in 1980, presenting a list of 10 essential instructions to Chinese citrus nurserymen operating in **HLB** contaminated areas. These recommendations are presented at the end of this document.*

B. AUBERT

Part 1

PRELIMINARY OBSERVATIONS ON THE DISEASE (SYMPTOMATOLOGY)

Citriculture has a long history in southern China, but over the last 10 years, a destructive disease known as **Yellow Shoot Disease** has spread in most citrus areas of Guangdong and Fujian Provinces. This disease is inflicting severe losses and threatens the development of the citrus industry. It has also been found to some extent in Guangxi Province. Reinking (1919) mentionned the **yellow shoot disease** for the first time but thought it was a benign problem. However several citrus surveys, made between the 1930's and 1950's by Tuzhi (1932), He Wei Ren (1937), Huang Sian (1943) and Lin Kung Shiang, mentioned the occurence of the yellow shoot disease in Guangdong Province; Huang Quan Yiao made a general agronomic survey of Guandong Province and reported some preliminary observations on "citrus dieback". Between 1938 and 1941, Chen Chi Bao published an accurate account of the citrus dieback in the Chaozhou/Shantou area and started the first experimental inoculations in order to explain the origin of the **citrus yellow shoot disease**.

We began the present research work in autumn 1941 with a survey in Northern Guangdong, then in Nanfeng of Jianxi Province, and finally Rongxi and Fuzhou in Fujian Province. In 1943, we visited Zhaojiazhen and Chengdu in Shichuan Province. In 1948 a survey took place in Xinzhu and Yunlin of Taiwan Province, and in 1951 a general investigation was implemented in citrus orchards, focusing on the occurence of **yellow shoot disease** and its agronomic impact.

During the regular field visits, between 1947 and 1955, we had an opportunity to question many farmers and technical personnel. Our investigations included the development of the condition of the disease, its historical background, the susceptibility of seedlings and scions, the distribution, spreading, and effect on yield.

Finally our first experiments of graft transmission of the **yellow shoot disease** were initiated in 1949.

The first part of this document deals with the general description of the disease. In a second part, we will explain the results of our etiological and epidemiological studies.

SYNONYMS OR COMMON NAMES

The **yellow shoot disease** is called **Huang lung**, because in the Chaozhou district the new flush on a tree is called **Huang Lung** (yellow shoot or yellow dragon) which describes the first aspect of the disease. Although this common name has finally prevailed, there were many other names given in other districts like **Wei Huang** (withering yellow), **Ji Tou Huang** (yellow chicken head), **Huang Pi Tou** (yellow surface disease), **Shuo Ding** (withering head), **Huang Qi Bin** (yellow crab disease) or **Jin Ban Yie** (golden board leaf) and **La Jiao Yie** (pepper leaf), these names refering to the general visual aspect of the canopy of affected trees. The other denominations are considering more specific symptoms: **Gen Fu** or **Gen Xiu** which means rotten roots, **Diao Huang** (withering yellow) (Reinking 1919, Tu 1932), and finally **Decline** (Lin 1947).

Rotten rootlets are not a major symptom of the disease, since many varieties are not affected by rootlet decay in the initial stages of the disease. We consider therefore that the names Gen Fu or Gen Siu are not appropriate. Similarly **Diao Huang** and **Huang Gu** often used by farmers for describing twig death and decline do not express the visual character of the disease. Consequently the name **Yellow Shoot Disease (Huang Lung Bin)**, given by the farmers of Chaozhou/Shantou area is the most appropriate because it refers to the specific early symptoms of the disease.

HISTORICAL BACKGROUND

From the discussion we had with the farmers of Guanchao, Silin and Qianrong villages in Chaozhou county, the **yellow shoot disease** has been known for 70 or 80 years there. At that time the disease was not serious and nobody paid much attention to it. But in the last 30 years, it spread quickly and became a serious problem, especially in 1936. The farmers of this area cut down their citrus trees, and returned to rice cultivation, owing to the shortage of rice during the war against Japan. After recovering from the war, the newly planted citrus orchards were healthy but 7 to 8 years later the disease appeared again.

★ *places visited by LIN KUNG HSIANG between 1941-55 and found affected by Huanglungbin*
○ *No clear evidence of Huanglungbin*

Fig. 1. Early geographical mapping of the citrus greening (Huang Lung Bin) in China

In the past several years, the citrus industry in Yitan, Zhuchuan, Jishan, Lade area of Guangzhou city was very prosperous, but all these areas have been recently affected by the **yellow shoot disease**.

There is no proper record relating the exact area where the **yellow shoot disease** might have originated. Although the disease was found in the suburbs of Guangzhou city, it is well known that farmers from Chaozhou were selling citrus seedlings on the Guangzhou market as well as other markets for many years. In 1949, during our research work at Lingnan University, in Guangzhou we bought 200 young plants of **Ponkan** and 200 of **Jiokan** from Mr. Zhang Mu Rom, a citrus farmer who brought this planting material from Chaozhou. Now all these citrus trees display the symptoms of **yellow shoot disease**.

Zhangzhou in Fujian Province is another important citrus area. It was also seriously affected by the disease, as the **Lukan** and **Jiokan** mandarines had been imported from Chaozhou. Therefore we assume the disease was also imported there from Chaozhou/Shantou area through planting material. This can be proved from Li Lai Rong's survey. He said: "In Jiaowei, the seventh district of Zhangzhou, the citrus seedlings in which the greening disease occur are all from Chaozhou/Shantou area". During the war against Japan, some farmers in Fuzhou transplanted citrus trees from Chaozhou/Shantou area, and these trees showed the symptoms of yellow shoot within a few years. Mr Wan Yi Ping a citrus farmer from Wenzhou in Zhejiang Province, reported in his letter to us: "We planted sweet orange trees which were from Guangdong, but after three or four years, these trees were quickly yellowing and declining."

Based on these facts, we believe that the yellow shoot disease originated from Chaozhou/Shantou area in the North Eastern part of Guangdong province, and spread gradually from this area to other citrus growing areas.

HOST RANGE AND CULTIVAR SUSCEPTIBILITY

According to the current survey, the host plants of the yellow shoot disease were found on the following citrus plants: **Citrus reticulata**, **Citrus sinensis**, **Citrus Limon**, **Citrus grandis** and **Citrus mitis**", irrespective of the scion rootstock combinations.

The trees obtained from air-layering propagation are equally susceptible. A preliminary range of susceptibility based on our field observations is given in Table 1.

In our investigations, we found that **Ponkan**, **Jiokan** and **Cha Zhi Gan** were seriously injured. We didn't find any disease on **Hong Tu**, **Jiu** and **Chen** (**Guan Gan**). These citrus cultivars are reproduced by seedlings.

DISEASE SYMPTOMS

Citrus trees can be affected at any stage, from the nursery to the age of ten years, but susceptibility differs with age. Generally young bearing trees below 7-8 years of age are easily affected, but nursery trees, non bearing young trees, and older trees above the age of 15 years are less easily affected. Trees affected at an old age generally decline less rapidly.

Early symptoms of the **yellow shoot disease** on citrus are yellowing of the leaves, defoliasion and rooting of rootlets. One year later, affected trees bloom earlier and heavily. The fruits are often lobsided, easily drop, and are small in size and tasteless. Within several months or years after they have been affected, these trees will die. The symptoms are more or less similar and independent of variety. A typical list of symptoms on **Jiokan** mandarine is presented.

PRIMARY SYMPTOMS ON THE YOUNG BRANCHES AND LEAVES

As new leaves begin to expand and before they become dark green, they may turn yellow, harden and loose color. Another feature is the sectorial attack of the disease on the canopy: one shoot is affected but the symptoms do not occur on other new shoots. The yellowing of new leaves is even, and usually occurs from July to September in the Chaozhou/Shantou area. Sometimes the disease occurs in autumn, and very seldom in spring time. After the first period of yellowing, the midrib and lateral veins of new leaves show nitrogen deficiency. The interveinal area is mottled, slightly reminiscent of iron deficiency, and there are irregular lumps with under edging. These kinds of leaves drop early. The yellow pattern can also extend on large parts of the leaves, the latter being sometimes undeveloped.

With time, the old or green leaves on the lower part of the branches begin to turn yellow, progressively from the midrids and lateral veins. The surface of these older yellow leaves are coarse, with swollen veins and corking, similar to boron deficiency. However, before these old leaves turn completely yellow, the younger ones located toward the branch tip would have dropped. During the following winter the affected branches shed their leaves.

BRANCHES AND LEAVES SYMPTOMS

Secondary symptoms refer to the syndromes shown by new shoots coming from previously affected branches. Whether these branches shed their leaves or not, they produce a new flush showing another set of symptoms. At the early stage of this secondary flush, some leaves have a small size with upright orientation on the branches. When the leaves reach their final size, an interveinal decoloration takes place. Normal size leaves exhibite manganese deficiency while the small yellow upright leaves show zinc deficiency pattern. In addition, some leaves become leathery and rolled. All affected leaves will drop, and twig dieback occurs at a later stage. The yellowing process and leaf abortion is more rapid on the secondary flush.

ROOT SYMPTOMS

Rootlets do not decay until the leaves are completely yellow and start to drop. Decay normally starts at the apex of the rootlets. The rootlet cortex is separated from the central cylinder, peels off and eventually roots rotten completely and turn dark.

FLOWER SYMPTOMS

Flowering of affected trees is earlier and flowers are generally more plentiful. The average size of the flower is smaller, with petal malformations and a slight yellowish color. Flower set is very poor and off-season blooming occurs on severely affected trees.

FRUIT SYMPTOMS

During the early stages of the disease, the fruits do not exhibit symptoms. However, with increasing symptoms of yellow shoot in the canopy, the fruits become smaller, drop very easily, and partially break color near the pedoncular area. They are tasteless and juiceless, with a very tight and solid central columella. Some fruits are lobsided and their seeds, brown in color, and aborted.

OTHER VARIETIES

The above descriptions refer to young bearing **Jiokan** trees. This cultivar may exhibit intense yellowing at the early stages of infection. But secondary stage symptoms are quite similar to that of other sensitive cultivars, although old leaves of **Jiokan** react slowly, compared to other cultivars. **Ponkan** mandarines, for instance, decline quickly, and the rootlets are already rotten at the beginning of leaf yellowing.

The first reaction on sweet oranges is a dense canopy with shorter internodes and upright orientation of the leaves so that the general architecture is slightly disturbed. This phenomenon occurs prior to yellowing. The lâtter reaction is of the same pattern and sequence as for **Jiokan**.

Lemons react with a leaf mottle symptom of the foliage, their fruits becoming thick skined and greyish, and having a low juice content.

YELLOW SHOOT DISEASE COMPARED TO ABIOTIC PROBLEMS

It is clear that the decolorations induced by yellow shoot disease are quite different from those induced by floods or drought. In 1955 a serious drought occurred in the Chaiozhou/Shantou area, and adult trees suffered badly. The leaves reacted by turning yellow at the tip of the lamina, without a clear demarcation line down to the petiole. Leaf tips occasionally dryed and the basal part turned yellow, but those leaves did not drop, and the damaged trees recovered quickly.

Waterlogging is characterized by two types of symptoms. One is apoplexy or sudden dieback which affects young trees recently fertilized and then flooded by summer typhon rainfall. The whole canopy collapses after being flooded for 3 days or more.

Chronic waterlogging affects citrus trees planted in low-land areas. Yellowing generally begins at the lower part of the canopy and extends towards the top. The yellowed leaves soon drop, while the extremity of the canopy is still green. The decoloration of the leaves starts from the midvein and spreads towards interveinal area. This type of yellowing is exactly opposite to that of

yellow shoot from the point of view of canopy symptoms and leaf symptoms. The trees recover after improving the drainage conditions, which is not the case for the **Huanglungbin** disease.

In conclusion we think the early stage yellow shoot disease symptoms are quite distinctive, while secondary symptoms of the disease can be easely mistaken with different kinds of degenerating diseases.

Table 1: Yellow Shoot (greening) Sensitive Citrus Lines Observed in Guangdong and Fujian Provinces

Species	Varieties	Type of propagation	Area	Sensitivity
Citrus reticulata	Ponkan	Grafted on Sunki Grafted on Nienchu Grafted on Citrus mitis Grafted on Citrus limonia	Chaozhou/Shantou district Chaozhou/Shantou district Chaozhou/Shantou district Chaozhou/Shantou district	+ + + + + + + + + + + +
Citrus reticulata	Tankan	Grafted on Sunki Grafted on Nienchu Grafted on Citrus mitis Grafted on Citrus limonia	Chaozhou/Shantou district Chaozhou/Shantou district Chaozhou/Shantou district Chaozhou/Shantou district	+ + + + + + + + + + + +
Citrus reticulata	Lukan + Tankan	Grafted on San Hu Hong Chu (mand) Marcots	Zhangzhou, Fujian Zhangzhou, Fujian	+ + + +
Citrus reticulata	Xa Zhe Gan Nienchu Hong Chu	Marcots Grafted on Citrus limonia Grafted on Sunki Marcots Seedlings	Xinhui, Guangdong Guangzhou suburbs Guangzhou suburbs Guangzhou suburbs Zhangzhou, Fujian	+ + + + + + + + + - (?)
Citrus sinensis	Xin Hui orange Snow orange An Liu orange Xian Shui orange In Zhe Gan Guang Gan	Marcots Grafted on Sunki Grafted on Citrus limonia Marcots Grafted on Citrus limonia Seedlings	Xinhui, Guangdong Chaozhou Shantou Guangzhou suburbs Guangzhou suburbs Zhangzhou, Fujian Fuzhou, Fujian	+ + + + + + + + + + + (-1?)
Citrus grandis	Ping Shan Shaddock Sangma pomelo	Seedlings Marcots	Zhangzhou, Fujian Guangzhou suburbs	- ? +
Citrus lemonia	Hong lemon	Marcots	Guangzhou suburbs	+ +
Citrus mitis	Calamondin	Unknown	Chaozhou/Shantou	+ +

\+ *very slightly affected*
\+ + *mildly affected*
\+ + + *seriously affected*

Part 2

INVESTIGATION ON THE CAUSE OF HUANGLUNGBIN (ETIOLOGY)

The nature of the **Huanglungbin Disease** (**HLBD**) has given rise to fierce controversy among plant pathologists and horticulturists. Originally thought to be the result of water logging (Reinking 1919 and Tu 1931), the **HLBD** was later considered a soil born fungus disease. Ho (1937), for instance successfully isolated *Fusarium* sp. from damaged rootlets of affected trees and quickly concluded it was the causal agent of **HLBD** without further reinoculation for confirmation. Mixed deficiencies of micronutrients were also suggested as a possible cause, but repeated sprayings of mineral solutions were unable to offset the yellowing symptoms. In 1955 Reinfeierg suggested again that **HLBD** was due to high water table in lowland areas, or eventual poor drainage in upland areas thus producing anaerobic conditions linked with deficient mineral nutrition. Reinfeierg suggested that resettling Citrus plantations on hilly areas with good drainage would solve the problem and citrus growers would soon forget the **HLBD**. Several Chinese scientists supported this point of view viz Zeng Xian Po, Zhang Wen Cai, Wang Jiaen. However Chen (1943) published the results of several experimental inoculations combined eventually with chemical treatments. His conclusions were that **HLBD** was neither induced by *Fusarium* infection, micronutrient deficiency, nor water injury, but rather by a virus infection.

Since unanimous consensus on the disease was not reached, no proper strategy for preventing the spread of **HLBD** could be developed.

In 1949, we initiated a series of experiments to elucidate the cause of HLBD and admitted the necessity of conducting a clear demonstration of water injury hypothesis, versus a possible virus-like systemic disease. It was thought that such a demonstration would be very helpful to develop, at last, a proper management of this dangerous expanding disease.

1. RELATIONSHIP BETWEEN WATER INJURY AND HLBD

FIELD EXPERIMENTS:

These studies compared different Cultural management schemes of citrus orchards, and their possible relation with HLBD.

Materials and Methods:

Three localities were selected: 1) **Kanle** near the Lingnan University campus in **Guangzhou**, 2) **Tainlu** a village of the Xinhui county, 3) and **Guangchao** in the **Chaoan county**. In each of them we established field trials with the best planting techniques locally available for farmers.

<u>GUANGZHOU experiment</u>

The citrus farm of **Kanle** which was affiliated to the Lingnan University was divided into 7 blocks: The blocks A, B, C and G were established on a lowland area formerly planted with rice, while blocks D, E and F were located in an adjacent sloped area. The blocks C and G were planted according to the traditional **Fuzhou** technique by placing the trees on individual mounts of soil 0.6m high and 2m wide, prepared with mounded soil. Blocks A, B and C were on raised beds separated by ditches 60cm deep and 90cm wide and were divided into two subplots. In the **high subplots** the beds of soil were 30cm higher than in the **low subplots**, and the water table was maintained 30cm lower as well, so that an additional 60cm of topsoil was available for the former subplot. A general description of these plots is given in Table 2.

The methods of cultivation and fertilizer applications were basically the same in every block. During the first and second years, fertilizers were applied once a month, then every two months during the third year, and only 4 times a year afterwards; in January, March, August and October. The nature of fertilizers were pig manure, soybean cakes, ammonium sulphate and ammonium phosphate.

The conditions of citrus growth in each block were examined every month. Soil profile inspections were made several days after heavy rainfall in order to check the water table and root development.

Tab. 2 The Relation of HLBD to Water Injury: Topography, Soiltypes and Cultivation Methods (Kanle, Guangzhou, 1949)

Procedure							Results	
Block No.	Topography	Soil types	Water table below top soil (cm)	Distance between rows (m)	Distance between plants (m)	Varieties* and No. trees/plot	No of trees affected with dieback 1953	1955
A	Flat land	Brown sandy loam	68	2.6	2.6	50 C. p. 50 C. t.	11/50 6/50	50/50 50/50
B	Flat land	Brown loam	57	2.0	2.6	50 C. p. 50 C. t.	7/50 5/50	50/50 50/50
C	Flat land	Brown clay loam	100	2.6	2.4	50 C. p. 50 C. t.	24/50 15/50	50/50 50/50
D	Slopping highland	Brown sandy loam	150	2.6	2.6	30 C. p. 30 C. t.	4/30 2/30	30/30 30/30
E	Do	Brown sandy loam	100	2.6 2.6	2.6 2.6	40 C. p. 40 C. t.	31/40 30/40	40/40 40/40
F	High mound on slopping land	Brown sandy loam	100	2.6	2.6	20 C. p. 20 C. t.	3/20 3/20	20/20 20/20
G	High mound on flat land	Brown clay loam	100	2.6	2.6	30 C. p. 30 C. t.	16/30 21/30	30/30 30/30

See discussion of results p. 8

* *C. p.* = *Citrus ponkan*; *C. t.* = *Citrus tankan*

XINHUI experiment

This second field trial was located in the Citrus Experimental Farm of **Tianlu** and included 4 main blocks A, B and C compared with a standard **low plot** D. Block A was planted following the **Fuzhou** technique of mounding soil, Block B was planted on raised bed, where the average water table was 70 cm below topsoil, as compared with 45 cm below topsoil for standard **low plots** of block D. Block C was established in a separate narrow stretch of highland 40m width. Tab. 3 recapitulates the

Tab. 3 The Relation of HLBD to Water Injury: Topography, Soiltypes of Land and Cultivation Methods (Xinhui)

Procedure							Results
Block	No. rows	Water table below top soil (cm)	Root* stock varieties	Fertilizer **	No. plants ***	Date of planting	Trees affected with dieback in Nov. 1954
A high mound	4	60	C.r.t.n.	Ammon.	50	June 21, 1950	68/100
				Comp.	50		
			M	Ammon.	50	June 4, 1951	67/100
				Comp.	50		
B high plot	2	70	C.r.t.n.	Ammon.	51	June 4, 1951	90/100
				Comp.	51		
			M	Ammon.	51	June 4, 1951	88/100
				Comp.	51		
C high-land		90	C.r.t.n.	Ammon.	100	June 4, 1951	87/100
			M	Comp.	50		74/90
D low plot (check)	4	35	C.r.t.n.	Ammon.	50	June 21, 1950	75/100
				Comp.	50		
			M	Ammon.	50	June 4, 1951	82/100
				Comp.	50		

See discussion of results p. 8

* C.r.t.n. = *C. reticulata tangerina* Var. Nienji
M = Marcott
** Ammon. = Ammonium sulfate; Comp. = Compound fertilizer
*** Variety: *C. reticulata* Var. Chachiensis

treatments of the 4 blocks which were subdivided into subplots comparing different rootstocks and different types of fertilizer application. In this experiment the soil was brown sandy loam.

CHAO AN experiment

This experiment conducted in the Eastern part of **Guangdong** Province (County of **Chaoan**), was established on brown loam soil, and was divided into 4 blocks A, B, C and D. As for the other experiments, each main block was again divided into subplots of high planting and low planting as shown on Table 4. All together, 1063 trees were planted in May 1951 with the same distance of plantation and fertilizing program as formerly described.

Tab. 4 The Relation of Yellow Shoot of Citrus to Water Injury: Methods of Land Preparation and Planting (Guangchao, Chao-an)*

Procedure						Results	
Block No.	Soil preparation	Group No.	Plot No.	Varieties	No. of plant	No. of trees affected with dieback in 1955	
A	High plot	1	6	C. ponkan	122	95/122	78%
	Low plot (check)	1	6	C. ponkan	122	73/122	60%
B	High plot	1	4	C. ponkan	84	77/84	91%
		2	4	C. ponkan	86	82/86	95%
	Low plot (check)	1	5	C. ponkan	106	91/106	86%
C	High plot	1	3	C. tankan	56	39/56	69%
		2	3	C. tankan	56	45/56	80%
		3	3	C. tankan	56	50/56	89%
	Low plot (check)	1	9	C. tankan	174	133/174	76%
D	High plot	1	9	C. tankan	100	67/100	67%
	Low plot (check)	1	6	C. tankan	101	66/100	66%

* Planted in August, 1951 to next March.

Results:

The three field experiments of **Kanle**, **Xinhui** and **Chaoan** included a total of 2397 trees and the results have been summarized in Table 2, 3 and 4. Concerning the experiment of **Kanle**, the trees established with more top soil available above the water table started to grow significantly faster than those on lower plots, but the difference decreased after the second year. In 1950, i.e. 12 months after plantation, 3% of the Tankan trees established on the sloping area exhibited the first symptoms of yellow shoot.

In 1951, similar symptoms were noticed on the **Ponkan** of Block C, and gradually the disease spread in the experiment. In 1955, six years after the planting, the 7 blocks were 100% affected, and the dieback had spread evenly, irrespective of the treatments. As early as 1953, i.e. four years after the planting there were no differences in the number of affected trees whatever the treatment. The only exception was Block C where the **Ponkan** plot had double the infected trees of the **Tankan** plot (Tab. 2 results).

The **Xinhui** experiment which was planted partly in 1950 and partly in 1951 produced slight differences of growth between the treatments. However in 1954 the 4 main blocks and subtreatements were equally affected with typical symptoms of yellow shoot, and the percentages of affected trees ranged from 74 to 90.

In **Chaoan** all the treatments reached the level of 70% of affected trees 4 years after plantation except for the **Ponkan** of Block A (low plot), and the **Tankan** of Block D.

This first set of experiments clearly demonstrated that by using the standard **Guangdong** citrus nursery procedure we obtained uniform infection of what we considered **Huanglungbin**, irrespective of the amount of top soil available, and of the type of fertilizer program, rootstocks or scions selected. These results indicated that the traditional soil management in South **China** was not involved in the spread of the disease, even on low areas. However, we initiated another type of trial with potted plants in order to obtain artificially induced water logging symptoms.

EXPERIMENTS CONDUCTED WITH PLANTS GROWING IN CONTAINERS:

Materials and Methods:

Young healthy trees of **Ponkan** and **Tankan** mandarines grafted on **Citrus sunki** were imported from Hsingchu (Taiwan). The roots of these trees were carefully washed in soapy water before planting. Previous experience of root disinfection by dipping in a 0.1% mercuric chloride solution for 5 minutes had resulted in severe damage of the rootlets, therefore we decided to use soapy water which gave excellent results.

On October 24th 1949, we planted a total of 48 trees in gasoline type drums filled with citrus soil. These containers 70 cm, high and 41 cm in diameter, were divided into three blocks of 16 each (one tree per drum). For each of these blocks, the water table was adjusted at a certain level below top soil by automatic regulation.

In the Block A the water level was maintained at only 13 to 18 cm below soil level. Since water injury appeared at an early stage, this block was subdivided into two subtreatments of water management. Half of the trees (8) were constantly cultivated for 4 years with only 13 to 18 cm of available top soil. The other half (8 trees) were equally treated, but for only 15 months, since after January 1952 their water level was lowered down to 31 to 36 cm below soil level.

The Block B received a constant water level of 31 to 36 cm below soil level and the Block C was permanently adjusted to 49 to 54 cm below top soil.

Two types of soil were chosen for filling the drums: 1) a brown fertile paddy loam soil, and 2) a paddy sandy loam soil. But in each case half of the drums were filled with steam treated soil for two hours, and half with non-treated soil.

The general procedure and the results are summarized on Table 5.

Results:

<u>Block A</u>

All the trees of Block A started to show leaf yellowing two months after the plantation, but these symptoms were more serious on non-treated soil. Eight months after plantation (summer season of June 1950), the trees of Block A on non-treated soil had very few and very weak new shoots. The trees of the same block planted on steam-treated soil produced many more shoots but the leaves turned yellow and dropped while becoming older.

There were no difference between the two types of soil during this experiment.

The symptoms of water injury were seen on mature leaves, with a yellow decoloration initiating from the main veins at the basal part of the lamina. We didn't notice any decoloration of the young leaves, with reduced size and upright aspect as usually seen for **Huanglungbin** symptoms.

Twenty months after planting, the trees of Block A were very weak and root examination showed root decay from which we isolated *Fusarium* sp.

Thirty months after planting most of the trees of Block A with non-treated soil had died while the trees established in steam-treated soil were still surviving. It was then decided to lower the water table as for Block B in half the trees of Block A. This resulted in the recovery of surviving trees with subsequent luxuriant flush up to the end of the treatment in early 1954.

Block B and C

All the mandarine trees of these two blocks grew normally without significant difference between the subtreatments.

Conclusion:

In this experiment it was possible to observe the exact symptoms of water logging on a group of trees which was originally healthy. By manipulating the level of water table we could artificially obtain a clear recovery of water damage affected trees. Not only the foliar symptoms were different from those of **Huanglungbin**, but the damage was reversible as unlike that observed for **HLBD**.

Tab. 5 Water logging induced experimentally on mandarine trees growing in containers

Procedure				Results	
Level of water table below top soil (cm)	Treatments		Trees	Observation Jan. 1952 (15 months)	observation July 1953 (34 months)
13/18 cm constant	Steamed soil	Loam soil	1 C. Tankan	Water logging injury	Very serious damage
			1 C. Ponkan	Water logging injury	Very serious damage
		Sandy loam soil	1 C. Tankan	Water logging injury	Very serious damage
			1 C. Ponkan	Water logging injury	Very serious damage
	Non steamed soil	Loam soil	1 C. Tankan	Very serious injury	Many trees died
			1 C. Ponkan	Very serious injury	Many trees died
		Sandy loam soil	1 C. Tankan	Very serious injury	Many trees died
			1 C. Ponkan	Very serious injury	Many trees died
13/18 cm from Oct. 1949 to Jan. 1952 and 31/36 cm after Jan. 1952	Steamed soil	Loam soil	1 C. Tankan	Water logging injury	Recovery of normal growth
			1 C. Ponkan	Water logging injury	Recovery of normal growth
		Sandy loam soil	1 C. Tankan	Water logging injury	Recovery of normal growth
			1 C. Ponkan	Water logging injury	Recovery of normal growth
	Non steamed soil	Loam soil	1 C. Tankan	Very serious injury	Recovery of normal growth
			1 C. Ponkan	Very serious injury	Recovery of normal growth
		Sandy loam soil	1 C. Tankan	Very serious injury	Recovery or normal growth
			1 C. Ponkan	Very serious injury	Recovery of normal growth
31/36 cm constant for 4 years	Steamed soil	Loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponakn	Normal growth	Normal growth
		Sandy loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
	Non steamed soil	Loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
		Sandy loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
49/54 cm constant for 4 years	Steamed soil	Loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
		Sandy loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
	Non steamed soil	Loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth
		Sandy loam soil	2 C. Tankan	Normal growth	Normal growth
			2 C. Ponkan	Normal growth	Normal growth

Total trees 48

Fig. 2: Effect of water logging on young citrus trees. Rows 1 to 4 show the trees cultivated with a water table maintained at only 13 to 16 cm below top soil. Rows 5 to 12 show the trees cultivated with a water table maintained at 31 to 36 cm below top soil.

Fig. 3

Fig. 4

Fig. 3 and 4:

Two examples of root damage on Ponkan induced by ***Huanglungbin disease*** *(**Kanle** orchard established with deep drainage). Note the absence of feeding roots.*

FURTHER FIELD OBSERVATIONS COMPARING WATER INJURY AND HUANGLUNGBIN

Between 1949 and 1953 we visited many citrus areas occupying several thousand square kilometers, from Northern **Fujian** down to Southern **Guangdong** Province, for comparative field observation of water injury and **Huanglungbin**. Special attention was paid to disease similarity in lowland and upland areas. Several cases will be described below.

A) JINGDONG village

This village, located in the Eastern part of **Guangdong** Province, belongs to the **Riaoping** county, bordering **Fujian**. The Tankan mandarine groves are located on terraces near the foothills of the inland mountains. Local farmers had paid great attention to establishing efficient drainage on the terraces. Nevertheless a large proportion of trees, both young and old, were suffering from **Huanglungbin** symptoms.

B) ZHUCHUN village

This village, established on a hilly area not far from **Guangzhou**, had the reputation of growing sweet oranges of good quality. The groves were traditionally planted on raised beds, 5 to 6 meters wide and separated by ditches of 1 meter width, thus providing excellent drainage. Flooding was thus avoided even during the high rainfall associated with typhoons. In the late 1920's the citrus industry of this village was very prosperous and the orange groves covered an area of 670 ha.

We visited **Zhuchun** on May 8th 1953, and found only moribund groves. The young trees which were reestablished for replacing previous affected trees were all contaminated by **Huanglungbin**, three to four years after planting. At the time of our visit all productive groves had disappeared from this village.

C) CHIPAI orchard

This grove established on a sandy loam soil in the suburb of **Guangzhou** was comprised of different blocks, separated by drainage ditches 60 cm deep to avoid water stagnation even during the rainy season. But in this grove the Ponkan and Tankan mandarins were infected with **Huanglungbin** as well as the oranges and lemons blocks.

D) Suburbs of GUANGZHOU

The suburbs of **Guangzhou** were traditionally planted with many citrus groves, especially in the villages of **Longtu** and **Lade**. Citrus farmers of these villages had, for a long time, established their groves on raised beds separated regularly by deep drainage outlets directly connecting to the **Zhujiang** river. When this river was at high tide, the water table could reach 40 to 50 cm below the top soil, while at low tide this level was lowered down to 100 or 120 cm below the soil surface.

Mr Li Qi Jing, farmer of **Longtu** explained to us that citrus plantations were providing most of the income of this village in the 1930's. However, when we visited **Longtu** in January 1950 we realized that Huanglungbin had destroyed a great majority of the groves. The Ponkan grove of Mr. Li Qi Jing which was 3 years old was entirely infected, and an adjacent 4-year-old grove of **citrus limonia** showed 90% of the trees affected with **Huanglungbin**.

E) SHANTOU prefecture

This prefecture located in the Eastern part of **Guangdong** Province has long been seriously affected by the **Huanglungbin** disease although citrus plantations were traditionally established with appropriate drainage systems.

In Puning county, for instance, all the plantations were on narrow beds of soil 1.8 meter wide, separated by deep drainage ditches. This was also a common practice in **Chaoan** and **Chaoyang** Counties, and similar planting techniques were also applied in the disease free areas of **Fujian** and **Jiangxi** provinces.

In **Shantou** prefecture, citrus orchards were often planted near the river bank, but the level of water table was always maintained 60-80 cm below soil surface at high tide, and could be lowered to 100 cm at low tide. The soil in these groves was generally alluvial with no underlying hardpan and had good drainage.

There are many other observations confirming the presence of **Huanglungbin** in a wide range of situations, both in lowland areas and upland areas, on brown loam or sandy loam soils and even red porous soils in hilly regions. One of the most striking characteristics was the high frequency of contamination at an early stage: 2 to 3 years after planting.

Conclusions:

The results of our field experiments and field surveys led to the conclusion that **Huanglungbin** was spreading in South East China, regardless of topography, soil texture and drainage techniques.

Furthermore, our experiments conducted on individual trees planted in containers could clearly reproduce the physiological disorder of water logging by maintaining the water table to only 13 cm below top soil. In the orchards we visited, the water table was always deeper than 13 cm. The container experiment showed also clearly that citrus trees could recover from water injury if the water table was lowered. We never observed recovery of **HLBD** affected trees even by lowering significantly the water table. Yellowing symptoms induced by water logging were quite different from those of **HLBD**, and demonstrated without doubt that both disorders were due to different causes.

2. HYPOTHESIS OF NEMATODE INFESTATION

S. Kanekichi (1937) in **Taiwan**, mentioned that citrus **Likubin** was a nematode problem linked with infestations of *Tylenchulus semipenetrans* Cobb. However we did express reservations about Kanekichi's point of view, since the initial symptoms of *T. semipenetrans* did no show the characteristic **young shoot yellowing** of **HLBD**, but rather a sparse foliage with occasional yellowing of adult leaves.

At any rate, it was necessary to clarify if *T. semipenetrans* infestations could be considered a causal agent of **HLBD** symptoms. For this reason, we collected several samples of feeding roots on **HLBD** affected trees belonging to mandarine and orange orchards known to have heavy **HLBD** infestation. A group of 28 mandarine trees exhibiting yellow shoot symptoms were selected, from which we sampled a total of 155 rootlets. A similar sample was taken from 24 orange trees from which we collected 120 rootlets.

The two root samplings were washed and rinsed in clean water. We selected newly rotten feeding roots in the front of a rotten area on the one hand, and advanced rotten segments on the other. Both types of segments (5 mm long) were finely dilascerated with dissecting needles. These samples were then mounted on slides and observed under a microscope.

The results of the examination produced only 2 positive segments from the mandarine orchard which yielded 5 and 29 nematodes respectively. All the other samples were negative.

According to Thomas (1913), Byars (1921) and Suit (1948), the symptom seen on *T. semipenetrans* infecting roots is a swelling of fibrous roots, which appear coarse, but not necessarily rotten. In addition, the canopy of the trees whose roots are attacked by *T. semipenetrans* exhibits a sparse foliage, with occasional yellowing of adult leaves. According to the available literature, *T* *semipenetrans* infestation induces slow **decline** but does not kill the trees, and no yellowing of the young shoots, similar to that induced by **HLBD**, has been so far attributed to nematode attacks.

Based on these results it was concluded that **Huanglungbin** can not be related with nematode infestation.

3. HYPOTHESIS OF FUSARIUM INFECTION

The trees affected with **Huanglungbin** have deficient feeding roots, with physical damage appearing several centimeters from the tip (Lin 1956).

Ho (1937) and Chen Qibao (1943) isolated separately *Fusarium* sp. from rotten roots of **HLBD** infected trees. Ho concluded that *Fusarium* was the causal agent of the disease. Therefore it was necessary to initiate new experiments to elucidate the possible relation between Fusarium and **Huanglungbin**.

Materials and Methods:

Experiment No. 1

In the **Kanle** grove, 80 Citrus sunki seedlings showing normal growth of uniform size, and 25 **Ponkan** and **Tankan** mandarines were inoculated by mixing a culture of *Fusarium* with the soil of the nursery plot where they were planted.

Experiment No. 2

In **Chaoan** county we collected several kilogrammes of decayed feeding roots under **HLBD** affected trees. These roots were chopped into fine pieces and mixed with the root system of one year old healthy **Ponkan** and **Tankan** mandarins. One hundred trees of both varieties were inoculated with 100g per tree of "root inoculum".

Experiment No. 3

In a third experiment a group of 14 **Ponkan** mandarines (5-year-old) growing in metallic drums were inoculated by mixing *Fusarium* pure culture growing on liquid wheat media, at a rate of 200 cc solution per tree poured on the feeding roots.

Experiment No. 4:

Finally the last experiment was conducted in the experimental farm of **Kanle**, where we selected two groups of mandarine trees: one group of healthy vigourous trees and the other of weakened **HLBD** affected trees.

For each group of plants, we selected several 0.5 cm roots and made a cut where we inserted a pure culture of 3 different strains of *Fusarium*. A total of 115 roots were inoculated and observed respectively 14 days and 30 days after inoculation.

Results:

Experiment No. 1: Two years after the soil inoculation, all the seedling trees were healthy.

Experiment No. 2: None of the trees which received 100 g of root inoculum exhibited any disease symptoms.

Experiment No. 3:

Ten months after the soil inoculation, we found the root system of two trees seriously injured. The leaves turned yellow and dropped, and the twigs dried but the typical symptoms of yellow shoot and small leaves could not be observed at the onset of symptoms.

Experiment No. 4:

Root necrosis were observed on inoculated roots, but their severity varied with *Fusarium* strains. It was also noticed a greater host susceptibility for a given strain, with weakened trees affected by **Huanglungbin**. The *Fusarium* strains of low virulence could invade only weakened trees, especially those affected by **Huanglungbin** or other debilitating diseases. The strains of high virulence such as *Fusarium oxysporum* were able to induce root rot on healthy and weakened trees.

Conclusion:

In conclusion, Fusarium seems unable to induce citrus wilt under natural field conditions and therefore cannot be recognized as the causal agent of **Huanglungbin** disease. But evidently, the fungus can invade the root system of **HLBD** affected trees and speed up the deterioration of the weakened trees.

4. HYPOTHESIS OF VIRUS LIKE INFECTION

Several viruses have been reported to be pathogenic to citrus (Fawcett and Klotz 1948). One of the most dangerous is **Citrus Tristeza** Disease reported in **South America** and in other citrus areas of the world.

In 1943 Chen Qibao expressed the idea that **Huanglungbin** was probably of viral origin, based on preliminary results of graft inoculations. In March 1939 he had collected **HLBD** affected spring shoots in a diseased orchard, to use as budwood on healthy Sunki rootstocks. Seven months after grafting he observed 58% of graft recovery from which 20% were showing **HLBD** symptoms. In a control test using healthy looking budwood, no **HLBD** symptom was noticed after one year.

A similar experiment was conducted in February 1940 with **Ponkan** and **Tankan** **Huanglunbin** infected shoots from different origins, grafted onto 2-yr-old healthy Sunki seedlings. By examining the progeny of these trees it was found that

- individuals grafted with shoots originating from **HLBD** sectorially infected mother trees, became contaminated at a rate of 10 to 16% for grafts originating from symptomless mother twigs, and 40% for grafts originating from mother twigs exhibiting obvious **HLBD** symptoms.
- symptoms in progeny trees appeared 3 to 9 months after grafting.
- none of the individuals originated from healthy looking mother trees subsequently exhibited **HLBD** symptoms.

The results of this experiment are presented on Table 6.

Tab. 6 Disease incidence in the progeny of nursery trees grafted with scions twigs obtained from HUANGLUNGBIN-infected mother trees or from healthy mother trees

(From CHEN QIBAO 1940)

Type of budstick	Geographic origin of budsticks	Number of plant suriving after grafting	% of HLBD infection
Healthy twigs from healthy Tankan trees (check)	Chao yang Lugan	42 68	0 0
Healthy looking twigs from sectorially infected Tankan	Yanting	32	15.6
Same as above from Ponkan trees	Sitou	29	10.3
Diseased twigs from diseased Tankan trees	Yanting	68	26.5
Diseased twigs from diseased Ponkan trees	Puning	42	40.5

In another experiment conducted in a nursery at **Chaoyang,** Chen Qibao confirmed his previous results. There were no infection of progeny when budwood was taken from healthy trees, but the rate of infection was 18% when symptomless budwood was taken from diseased trees, and 30 to 35% when the budwood was taken from diseased twigs sampled on infected mother trees.

However, Chen's demonstrations indicated possible transmission of the disease from the source plant by ordinary grafting but he did not demonstrate any evidence of systemic infectious nature for **HLBD**. For this reason, we initiated a series of three experiments in an attempt to demonstrate the systemic graft transmissibity of **HLBD**.

Experiment 1:

Materials and Methods

This experiment was conducted under field conditions (in the open), in a farm affiliated with our University. A selection of 60 Ponkan and 62 Tankan healthy mandarin trees was made in the nursery. These trees were grafted on **Citrus sunki** the previous year and had produced vigourous growth without yellow shoot symptoms. These were used as indicator plants.

A separate grove, heavily infected with HLBD, was chosen as a source of inoculum. One year old twigs were sampled on affected branches, to be graft-inoculated on the healthy indicator **Ponkan** and **Tankan** mandarin plants.

The graft inoculation consisted of opening an inverted T cut on the main stem of the indicator plant and inserting and fixing under the bark a beveled piece of twig coming from the source tree. Two inverted T inoculations were made on each indicator plant. This operation was repeated 2 and 4 weeks later if inoculum piecies dried. The symptoms of HLBD were later observed on the new shoots produced by the inoculated plants.

Results:

The trees were examined 11 months, 24 months and 27 months post graft inoculation and the percent infections presented in Tab. 7.

Tab. 7 Percentage of HLBD infection after side-graft experimental inoculation

Time after inoculation	Rate of infection, %			
	Ponkan		Tankan	
	29 inoculated trees	31 control trees	26 inoculated trees	36 control trees
11 months	41	3	50	0
24 months	59	18	56	17
27 months	70	29	60	28

Symptoms observed on contaminated trees were typical of Huanglungbin, i.e. yellow shoot symptoms at an early stage combined with the decay of feeding roots, and followed by a premature off-season blooming during the second year. On subsequent new shoots, zinc, manganese or boron deficiencies appeared combined with twig dieback.

It should be noted that the adjacent control trees also became infected but at a slower rate. This prompted us to consider a possible vector transmission of HLBD.

Experiment 2:

Materials and Methods

This experiment was conducted in **Chaoan** County on two blocks of mandarines: one block comprised of 276 **Ponkan** grafted on **Sunki**, and the other block comprised of 290 **Tankan** also grafted on Sunki. These trees were obtained from a healthy nursery of Riaoping, which propagated its citrus material from disease-free mother trees. Immediately after planting, each block was again divided into two subplots. One subplot was graft-inoculated with **HLBD** affected twigs by using the same inverted T technique described in Experiment 1, with two inoculations per tree. The other subplot was kept as a control. In addition, to reducing a possible ingress of arthropod vectors, we encompassed each subplot within guard rows. The guard row trees were from the same origin and did not receive graft inoculations. As described in Fig. 5 and Fig. 6 we ended up with a total of 94 graft-inoculated **Ponkan** versus 188 non inoculated **Ponkans**, and 130 graft-inoculated **Tankan** versus 160 non-inoculated **Tankans**.

Since not all inoculations were successful on the first grafting, we repeated the graft inoculation for those which had dried too quickly.

The **Tankan** block was assigned to farmer Yan Qiru who was in charge of its maintenance, while the **Ponkan** block was assigned to another farmer group: **Yang Musui**.

Fig. 5: Tankan experimental block

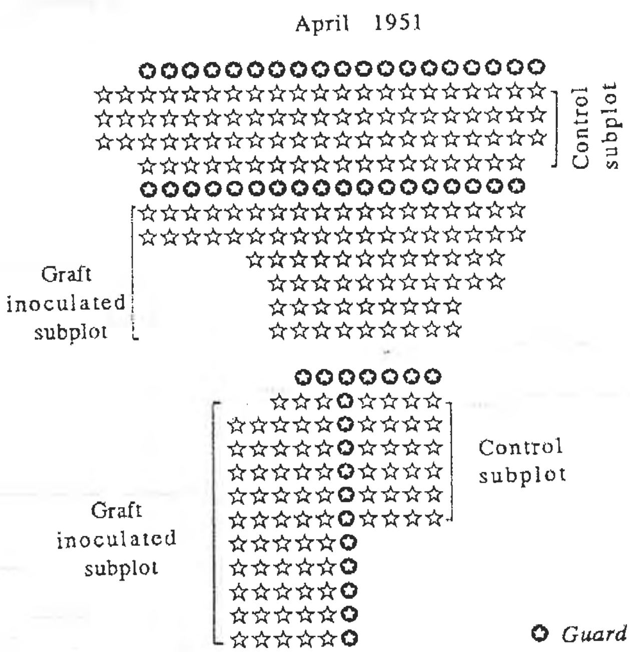
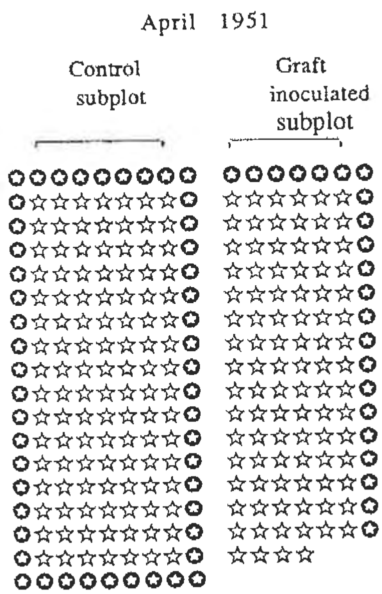


Fig. 6: Ponkan experimental block



✪ *Guard row trees.*

☆ *Trial block trees.*

Results

1) Status of grafted inoculations

In November 1951, seven months after the beginning of this experiment, and also in December of the same year, we inspected all the inoculated trees and found that **HLBD** inoculum twigs on 58 of the **Tankan** trees were still alive i.e. 45%. This number was 57 for **Ponkan** i.e. 61%. We graft-inoculated again all the **Tankan** and **Ponkan** which exhibited dried **HLBD** inoculum twigs using the same source of infected trees. This operation was repeated again in October 1953 for those trees showing graft inoculation failure, but at this time most of the early successfully inoculated trees were showing advanced **HLBD** symptoms.

2) Percentage of **HLBD** transmission, and symptoms appearance

The percentage of successful **Huanglungbin** transmission observed in 1952 and 1953 is given on Table 8. The first symptoms appeared in 1952, and again on the spring flush of 1953. During autumn of 1953 a great number of trees showed **HLBD** symptoms on the summer flush. But most trees of the control plot and of the guard rows were healthy. During the spring of 1954, infected trees produced numerous small flowers, and small new leaves with strong manganese and zinc pattern deficiencies. Heavy defoliation occurred during the following autumn. All the trees successfully inoculated up to 1952 were showing symptoms, as were most of the trees reinoculated in 1953. However, a small proportion of the control and guard row trees also exhibited **HLBD** symptoms but with a lower intensity than inoculated trees. In August 1955 the **Ponkan** and **Tankan** which were successfully graft inoculated at an early stage were dying. More severe symptoms were noticed on the trees which had received two successful graft inoculations, followed by the trees where only one graft inoculation took. The regrafted trees developed symptoms at a later stage.

Tab. 8 Results of graft inoculation of HLBD of experiment No.2

Treatments	Rate of infection					
	Citrus Tankan			Citrus Ponkan		
	No. of trees observed	% trees showing HLBD symptoms in 1952	% trees showing HLBD symptoms in 1953	% trees observed	% trees showing HLBD symptoms in 1952	% trees showing HLBD symptoms in 1953
Non inoculated control	105	0	6.6	112	0	0
Non inoculated guardrows	58	0	31.0	73	0	5.4
Graft inoculated trees	127			93		
From which inoculum alive	58	48.3	82.0	57	57.9	84.0
inoculum dried and regrafted	69	7.2	63.6	36	22.2	81.0

a. Tankan mandarins:

As shown in Tab. 8 in 1952, the proportion of early symptoms was significantly higher on plants with live **HLBD** inoculum twigs than with dried twigs. Since the latter were subsequently regrafted, the difference in percent affected trees decreased in 1953. We recorded only 6% infection on control plots in 1953 while infected guard rows reached an impressive proportion of 31%. However, at that time, 82% infection was already determined on inoculated trees with an immediate successful graft inoculation.

b. Ponkan mandarins:

The percentage of infected trees in 1953 among the control plot was zero, and only 5.4% in the guard rows. A difference was also noticed in the percentages of infected trees between initial successful inoculation and late successful inoculation.

3) Spatial distribution of the infected trees

The spatial distribution of infection at the end of 1954 is presented on Figs. 7 and 8 which are homologous to Figs. 5 and 6 in 1951.

The **Ponkan** block, which was efficiently managed by the farmers group **Yang / Musui**, clearly demonstrated that we used healthy nursery trees to plant this trial, since there were not a single affected tree in the control block, including the guard rows. After 4 years we found only 4 trees infected all in one guardrow (Fig. 8).

The **Tankan** block, which was entrusted to another group, was also properly managed but exhibited a higher percentage of infection of control block and guard row trees (Fig. 7).

Fig. 7: Tankan Experimental block at the end of 1954

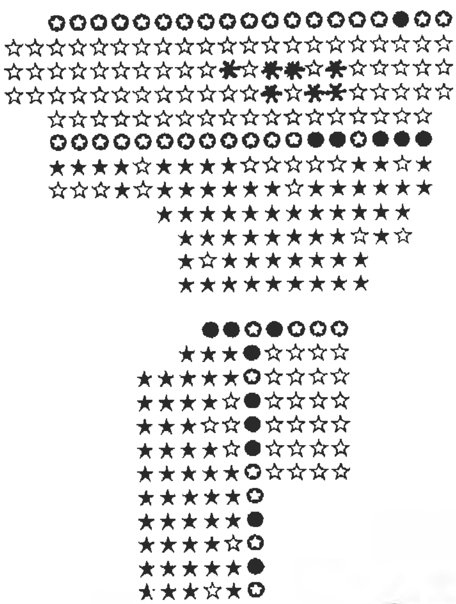
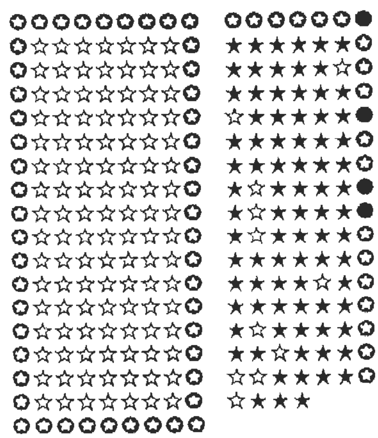


Fig. 8: Ponkan Experimental block at the end of 1954



✪ *Symptomless guard row trees.*
● *Naturally infected guard row trees showing **HLBD** symptoms.*
☆ *Symptomless trees.*
★ *Graft inoculated trees showing advanced stage of **HLBD** symptoms.*
✱ *Control trees naturally infected.*

*Fig. 9: Symptom expression in a grove where experimental transmission of **HLBD** was attempted.*

Conclusion:

Based on our inoculation trials we consider citrus **Huanglungbin** to be a graft transmissible disease. The early symptoms of **HLBD** are distinct and cannot be mistaken with physiological disorders. Natural spread of the disease exists, possibly transmitted by an insect vector, but the rate was significantly lower compared to graft inoculation.

Part 3

NATURAL TRANSMISSION AND SPREAD OF HUANGLUNGBIN (EPIDEMIOLOGY)

To assess natural transmission and spread of **Huang Lung Bin (Yellow shoot)**, we decided to: i) monitor the procedures implemented in selected nurseries and observe the history of citrus plants delivered by these nurseries in experimental orchards, ii) check the "infectiousness" of the disease itself following an accidental ingress of inoculum into citrus orchards.

Monitoring different origins of Nursery trees

Grafted trees:

Two different commercial nurseries were selected. One was located in **Baojinyuan, Puning** county in **Chaoshan** Prefecture, a well known area, where **Yellow shoot disease** was prevalent. The other nursery was selected in **Hsiang Chu - Taiwan**, a disease free area by then.

A group of 66 young trees was selected at random in each nursery. They included one year old **Tankan (Jiokan)** and **Ponkan** mandarines, grafted on **Citrus Sunki**. Due to slightly different nursery management, the trees of the former nursery were comparatively larger. But after being established in our experimental farm, under different blocks and replicates, these trees recovered well. Both lots of trees were healthy looking and delivered as presumably **disease-free**.

The trees were transported into the University affiliated farm in **Kanle**, a suburb of **Guangzhou**. We divided them in 8 different plots, each containing 28 trees, half from **Chaoshan** nursery and half from **Hsiang Chu** nursery.

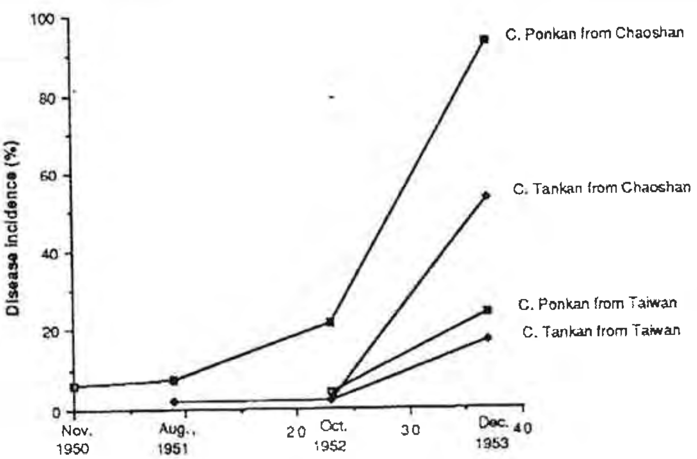


Fig. 10. *History of Huanglungbin Yellow shoot infection rate of two groups of trees originated from two different nurseries*

In November 1950, i.e. 20 months after plantating, 3 **Ponkan** trees from **Chaoshan** nursery showed the first symptoms of **Yellow shoot disease**. They became defoliated, suffered root rot, and by January 1952 had died. In December 1952, a total of 11 **Chaoshan Ponkan** and 1 **Chaoshan Jiokan** were found with yellow shoot symptoms, against only 1 **Ponkan** and 1 **Jiokan** from Taiwanese origin. In May 1953, 100% of the **Ponkan** and of the 50% **Tankan** of **Chaoshan** origin were affected by yellow shoot disease irrespective of plot, while for trees from Taiwan, the figure did not exceed 20% infection. However, in January 1955, i.e. 6 years after the planting, both groups of trees, regardless source of origin, were affected. The history of these two groups of trees is summarized in Fig. 10.

These results can be interpreted as follows: the **Chaoshan** trees were most likely disease-carriers, and provided the initial inoculum with greater infection of **Ponkan** than **Tankan**. Another explanation would be that **Chaoshan** trees were more susceptible to the disease, compared to **Taiwanese** trees. But such an interpretation is in contradiction with the fact that all **Taiwanese** trees also became infected by 1955. In fact we observed an infection pattern starting from a small group of **Chaoshan** trees with a gradual spread towards adjacent trees: this conforms with the usual spread of an infectious disease. The influence of nursery techniques does not explain the difference in susceptibility to **Huang lung** bin. Such a difference might only be taken into account if we compare **Ponkan** versus **Tankan**, the latter exhibiting symptoms at a lower rate of incidence.

Citrus propagated by air-layering and farmers comments

The author also investigated several nurseries producing citrus trees by air-layering. For instance in the county of **Xinhui**, a group of 50 air-layering saplings were observed after being planted in an experimental farm. The young trees were obtained from partially infected trees, but the nurserymen generally practiced the air-layering technique from the apparent healthy side of the mother trees. Although most of them were symptomless when planted, these trees showed 84% infection within 11 to 14 months. During the same period at the same farm, another group of similar trees obtained from another nursery propagating apparently healthy mother trees became infected at a rate of only 20%.

These kinds of observations were made several times in citrus orchards affiliated with the South China Agricultural College.

During April 1954, we decided to make an enquiry near three well-known senior citrus growers of **Chaoan** county MMr Zeng Lai Rong 76, Zheng Ren Xian 65, and Zeng Mu 60. The three growers confirmed that the **Yellow Shoot Disease** was brought into their village via **Xilin's nursery** trees. These farmers had noticed that a high percentage of trees became infected if they originated from **Xilin,** where air layering was the traditional method of propagation. But conversely, the trees originating from Riaoping nurseries exhibited much lower percentages of infection, where budding from healthy mother trees was performed. It must be stressed that nurserymen operating contaminated nurseries have greatly increased the spread of **yellow shoot**, not only within their immediate vicinity, but also occasionally to areas long distances away. For instance, one hectare of **Jiokan** mandarine planted in Fujian Province (250 km North of **Chaoshan**) was totally infected. This grove was established with nursery trees imported from **Chaoshan** Prefecture.

Preliminary epidemiological surveys

We have seen in the first part of this document, that Chen Chi Bao (1943) made an extensive survey between 1937 and 1941 of Huanglungbin in the **Chaoshan** Prefecture. He concluded that pattern of spreads of "**Yellow shoot disease**" was different from that of an "ordinary disease". Further evidence of the infectious nature of the disease is given in the second part of the present paper where we present the results of our experimental inoculations.

In order to confirm these results with additional field information, we initiated an extensive survey in 6 villages in **Puning** county, 3 villages in **Chaoan** county, and two villages in **Riaoping** county. These three counties are located in the **Shantou** citrus belt.

Various data were collected during this survey, which substantiated the infectious character of the **Yellow shoot disease**. For instance, in some localities, we clearly observed a tree-to-tree spreading pattern i.e. around an initial small disease area exhibiting severe symptoms, adjacent trees were showing milder symptoms in relation to their distance with the main focus. This gradual decreasing from one principal focus was not only observed within a given orchard, but also from one orchard to another. Several examples of contaminated orchards infecting adjacent plantating were found. One of the most striking examples was seen in 1949 in **Puning** county where 4 different farms were planted with **Jiokan** mandarines, in 3 separated blocks of land, but connected by a narrow bridges of trees. Within three years we observed the appearance of **Yellow shoot disease** in one orchard and its gradual spread into these bridges, through the lateral band. By 1953 all these orchards were completely devastated by the disease.

A typical infection gradient is presented in Fig. 11. This observation was made in a 200 **Jiokan** grove in **Chaoan** county in May 1951.

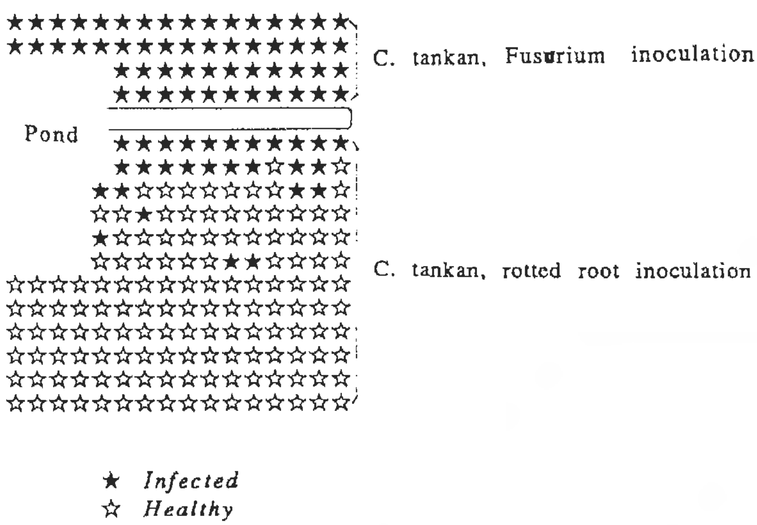


Fig. 11 *Example of a* ***Yellow shoot disease*** *gradient observed in an experimental orchard of* ***Citrus tankan*** *in May 1951. This observation was made during a* ***Fusarium*** *inoculation experiment. In fact* ***Yellow shoot disease*** *predominated over expected* ***Fusarium*** *infection.*

During our survey, we had an opportunity to talk with 47 farmers. Among them, 42 considered **Huanglungbin** as "an infectious disease, often appearing in small foci and spreading gradually to adjacent trees of the same orchard, and from orchard to orchard". Many of them were pessimistic about the possibility of efficient control, even by eradication and replanting.

In some villages, like the **Zhu cun** village, the history of citrus could be traced back more than one century, and the **Yellow shoot disease** was known to have appeared only during the last 20 years. The disease started from the lowland area and gradually reached the groves established on the slopes of upland areas. In spite of several tentative programmes of eradication, the newly planted trees became infected within 3 to 4 years. In the past, citriculture in this village was simple. But this situation was completely reversed with the appearance of **Huanglungbin**.

Part 4

GENERAL CONCLUSION

Our field observations and the results of our experimental work have clearly indicated that

i) The **Yellow Shoot (HLB) Disease** can appears in any kind of grove irrespective of its location in lowlands or uplands, on light or heavy soils, in well drained or water clogged areas.

ii) The disease has dramatically expanded in **South East China** over the last decade, especially through infected planting material from contaminated nurseries.

iii) **HLB** is a graft transmissible infectious disease which is apparently not connected with soil-borne debilitating problems such as water logging, **Fusarium** or nematodes.

iv) **HLB** exhibits a distinct symptomatogy on new shoots, which turn yellow and show mottling with upright leaves. This typical symptomatology is shared by most citrus cultivars/species: *C. sinensis, C. tankan, C. ponkan, C. limonia, C. grandis* ...

v) The disease is spreading naturally from original foci **with the pattern of an infectious disease**. Generally the source of inoculum can be traced back to contaminated nurseries which have not strictly propagated citrus material from healthy mother trees.

There have been considerable discussions concerning the exact origin of the **Yellow Shoot HLB Disease**. Our **inverted T grafting** technique demonstrates that when budwood is taken from infected trees it is easy to contaminate healthy challenged trees. Transmission is systemic, and is much like that of a virus disease.

We believed that several essential measures should be taken urgently to control the disease. First, it is important to determine the location of disease-prevalent areas, especially in Guangdong, Fujian, and Guangxi provinces. The survey should concentrate on young groves. Suspected symptomless trees should be tested by graft inoculation of indicator plants, to check their actual sanitary condition.

Thermotherapy could eventually be used as a treatment for contaminated budwood. For instance, if a hot water treatment can eliminate the virus it would be of extreme aid to producing disease-free material.

However, using healthy material is only one aspect of the control strategy which has to be developed. Much information is needed on the natural spread of the disease especially if an insect-vector is involved in transmission. Besides insect transmission, is there a possibility of mechanical, seed, and even soil transmission?

An important measure for controlling this virus-like disease, is to select resistant varieties. Such an approach should not be overlooked and should examine rootstocks and scions, separately or in combination.

Early diagnosis is another important aspect. For instance a symptomless tree cannot be detected presently although it carrys the disease. It is likely that adequate techniques such as histology combined with biochemical reagents would provide reliable diagnostic procedures. Such techniques would be important for investigating host range susceptibility as well as the different biological avenues for disease spread.

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TEN BASIC INSTRUCTIONS FOR PRODUCING DISEASE-FREE CITRUS MATERIAL

(after LIN Kun Shiang 1979)

1) *Establish disease free nurseries or orchards within a minimum distance of 5-8 km from contaminated areas (eliminate all existing citrus or citroid plants within that radius).*

2) *Use hot water treated rootstock seeds (56° C for 50 minutes).*

3) *Use disinfected budwood (steam treatment 49° C for 50 minutes) or budwood selected from disease free nucellar lines.*

4) *Apply quarantine measures for protecting nurseries and multiplication blocks by strictly avoiding the introduction of Citrus and Citroids plants or fruits, within the perimeter of propagation.*

5) *Protect nurseries and multiplication blocks by fences and windbreaks and set up double entry door. Between the two doors organize enough space for disinfecting shoes and changing clothes and hats. This will avoid transport of microorganisms and vectors through human movements.*

6) *Avoid entry of visitors unless they disinfect their shoes and wear special clothes.*

7) *Disinfect pruning tools and knives in formaline and check quality of water for irrigation.*

8) *Always separate old and new blocks of nurseries or foundation blocks.*

9) *Make frequent inspections and eradicate immediately accidental contaminated individuals (Greening, Canker Phytophthora ...).*

10) *Deliver disease-free material with special tags indicating the names of cultivars and rootstock lines.*

N.B. The strict application of LIN Kun Shiang's recommendations in FUJIAN, GUANGDONG and GUANGXI Provinces has secured Hanglungbin-free, canker-free and Phytophthora-free citrus material in the municipal and regional nurseries of these Provinces.