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Epidemiological Aspects of Citrus Huanglongbing (Greening) Disease in Thailand

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Abstract

Field investigation on citrus huanglongbing (HGB, greening) disease caused by insect-borne liberobacter was carried out in Thailand from 1992 to 1993. The disease and its vector *Diaphorina citri* were present in all the citrus orchards surveyed in 12 districts throughout Thailand. Electron microscopic examination confirmed the presence of causal organisms in sieve cells of leaves with symptoms. Many viruliferous psyllas that transmitted the pathogen on young citrus seedlings were collected in HGB-infected fields. Many orchards had been destroyed due to the disease, while some pummelo orchards were still productive. HGB disease spread from infected trees to healthy pummelo trees. Sour lime, *Citrus aurantifolia*, was the major HGB-carrier and inoculum source. Circulation of infected nurseries or bud-sticks through growers promoted the inter-field transmission of the disease. The disease was more prevalent in flat or plain fields than in those surrounded by hills or wind breakers, due to the enhancement of vector dissemination by the wind. Rough lemon, Calamondin, Som-pan and Ladu mandarins were tolerant, while large numbers of sweet orange, mandarin and tangelo trees were susceptible. The tree growth and yields were markedly reduced by the disease when the trees were infected at young ages. There were 2 types of citrus trees in the relation of yield to canopy volume after infection, those with high and low yields. Grapefruit, some tangelos and Som-pan mandarin trees were highly productive, while large numbers of mandarin and sweet orange trees showed a low productivity.

Discipline: Plant disease/Horticulture
Additional key words: liberobacter, vector transmission, *Diaphorina citri*, host resistance

Introduction

Citrus huanglongbing (HGB, greening) disease caused by insect-borne liberobacter is the most serious disease in tropical and subtropical Asia. The following integrated methods of control have been proposed[1)]: eradication of infected trees, propagation of disease-free trees and control of vector psylla by biological or chemical method. These measures, however, depend on the incidence of the disease, susceptibility of citrus cultivars, vector population and environmental factors related to the transmission or disease development. Therefore, we investigated the epidemiology of HGB in fields and identified some key factors to control the disease in the country. We previously reported on field evaluation of citrus cultivars for greening disease resistance[5)] and the host range of the vector psylla among various Rutaceae plants[6)]. This is the third report, in which emphasis was placed on epidemiological aspects.

The study was carried out under a collaborative project between the Japan International Research Center for Agricultural Sciences (JIRCAS, formerly TARC) and Department of Agriculture (DOA), Thailand from June 1992 to January 1994.
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Materials and methods

1) Field investigation on disease occurrence

Citrus orchards in 12 districts in Thailand were surveyed from June 1992 to November 1993. Observed trees included local mandarin (*Citrus reticulata* Blanco), pummelo (*C. grandis* Osbeck), sweet orange (*C. sinensis* Osbeck), sour lime (*C. aurantifolia* Christm.) and introduced varieties such as grapefruit (*C. paradisi* Macf.), tangelo, tangor, tangerine and mandarin. Huanglongbing (HGB) disease was diagnosed based on the following symptoms: leaf mottling or yellow leaf with irregular green spots (Plate 1), small leaves, dieback, greening at the stylar-end of fruit, abortive seeds and decline of the tree. These symptoms were usually associated. Bud woods collected from trees with symptoms were grafted on nucellar 1–2 year old seedlings of rough lemon kept in an insect-proof screenhouse. The grafted plants were used for further experiments.

2) Relation between growth and yield of affected trees

A total of 64 cultivars of citrus, with 10 trees each planted in the fields of Nan Horticultural Research Station were investigated. All the trees were propagated by grafting on rootstock Cleopatra mandarin, *C. reshni* Hort. ex Tanaka and planted in the fields in 1986. Tree size (height (H) and diameter (W)) was measured once a year from December to February from 1989 to 1993. Fruit yield was recorded at the time of harvest and the average yield per tree or per canopy volume was calculated. The canopy volume (V) was calculated as follows:

$$V = W^2 \cdot H \cdot 0.7.$$

3) Transmission by field-collected vector

Approximately 20-day-old seedlings of Som-keowan, *C. reticulata* Blanco that grew under vector-free conditions were used as receptors. Twenty-five seedlings in a plastic container were exposed to vector feeding. Adult psyllas, *Diaphorina citri* Kuwayama that fed on citrus trees with symptoms at Nan Horticultural Research Station were collected in July 1992 and immediately transferred to the receptor at the rate of 50 adults per container. The plants were covered with a screen to allow psyllas to feed for 2 days. After killing of the psyllas by spraying insecticides, the plants were kept in a growth chamber at 26–28°C under illumination of 5,000 lux for 14 h per day for 3 weeks. The receptors were individually planted into a small plastic pot and moved to an insect-proof screenhouse.

4) Identification of pathogen by electron microscopy

Leaves with symptoms were collected from plants maintained in a screenhouse, followed by cutting the mid-vein into small pieces, 1–2 mm in diameter. The pieces were immediately fixed with 2.5% glutaraldehyde and post-fixed with 2% osmium, followed by embedding in Spur resin. Ultra-thin sections were prepared with a diamond knife and Sorvall MT-B microtome and stained with uranium acetate and lead nitrate. They were observed under an electron microscope Hitachi H-300.

Results

1) Field investigation on disease occurrence

(1) Central part of Thailand

An orchard of pummelo Khaw-nam-phung in Suan Phung district, Ratchaburi province was surveyed in June 1992. It was located between hills and isolated from other citrus orchards. The trees were propagated by layering 13 years ago. A large number of trees were healthy, but some trees showed mild symptoms. The latter were located close to Special mandarin trees showing symptoms that had been introduced from the southern part of Thailand in recent years. Similar observations were made in Muang district, Chai Nat province, where 6-year-old pummelo Khaw Tang Kua trees were grown in a flat field surrounded by tall windbreak trees. Sour lime trees of the same age showed typical HGB symptoms and dieback. A pummelo tree close to sour lime was severely infected (Plate 2). Some trees displayed partial dieback and others were apparently healthy but with mild symptoms on a few branches. These facts suggested that the disease was transmitted from infected sour lime or mandarin trees to pummelo.

In Sam Phran district, Nakhon Pathom province, a large orchard of pummelo trees had been destroyed by the disease a few years ago. Four to 5-year-old sour lime trees in Damnoen-Saduak district, Ratchaburi province showed decline and dieback. There were many Som-keowan orchards in paddy fields in Pathum Thani province. A large number of 5 to 6-year-old trees were healthy, but some trees showed decline. Psyllas were easily observed. In other orchards, young trees also showed similar symptoms.

They had been propagated by layering from the local trees.

At Phichit Horticultural Research Center, 10 cultivars of pummelo originating from different districts had been planted with 16 replications 6 years before. Among them, all the trees of Sai-nam-phung were severely infected, while 25 out of 27 trees of Thakoy from Phichit were healthy. The infected trees of Thakoy were located in windy areas of the field. Several trees of other cultivars showed symptoms. These observations suggested that Sai-nam-phung had been previously infected and that the other trees were infected recently after planting in the field. In the vicinity, there were many commercial orchards with Thakoy trees that grew vigorously but some trees showed mild symptoms. This cultivar seems to be more resistant than other pummelo cultivars based on the slow development of HGB symptoms.

(2) Northern part of Thailand

At Nan Horticultural Research Station, more than 64 varieties or cultivars were planted since 1986 in flat fields located between lower hills. In a preliminary investigation carried out in December 1989, local mandarin Som-keowan had experienced symptoms of decline with yellow foliage. Some sweet orange trees also developed yellow shoots. Detailed investigations were conducted 3 times, in July 1992, January and October 1993. Although HGB disease was more prevalent, there was a large variation in disease development among the varieties. Murcott, Fremont, Ponkan, Queen mandarin, some other mandarin, sweet orange and tangor trees were severely infected and exhibited symptoms of decline. In contrast, rough lemon, Avon-ever-bearing (probably Calamondin), Ladu and Som-pan mandarins showed mild symptoms but grew vigorously. Among the introduced varieties, a large number of sweet orange trees developed yellow shoots, while tangelo and grapefruit trees were healthy until July 1992. However, the latter 2 developed symptoms in some parts of the branches in October 1993. Psyllas were easily observed. Tahiti lime and sour lime in the peripheral fields showed typical symptoms but grew vigorously. Som-keowan in commercial orchards 1–2 km apart from the station were also severely infected and exhibited decline.

HGB disease was observed at Khao Kho Highland Agricultural Station located at an elevation of ca. 600–700 m and at Chiang Rai Horticultural Research Center. The occurrence of the disease seemed to be related to the nursery source.

A commercial orchard of mandarin, about 160 ha, was located in a mountain area in Fang district, Chiang Mai province. In January 1993, 7 to 8-year-old trees of Honey mandarin and Som-keowan were free of the disease. However, several 3-year-old trees of Murcott that were planted on a slope and in a windy area showed typical yellow-shoots. The distribution of the infected trees suggested that the disease had recently spread in the leeward direction. Other orchards about 1,000 ha in area had been recently established on hills with a gentle slope. One to 2-year-old mandarin trees that propagated from the trees in the mountain field by grafting were planted. Large numbers of trees were healthy but a few showed symptoms.

(3) Southern part of Thailand

Eight-year-old local mandarin trees Shogun and Som-keowan planted in a flat and 16 ha field in Muang district, Yala province were investigated. In March 1993, a large number of trees were severely infected and many trees had died (Plate 3). Similar observations were made on Neck orange, probably sweet orange in Chana district, Songkhla province. There had been many citrus orchards, but most of them had disappeared in recent years because of

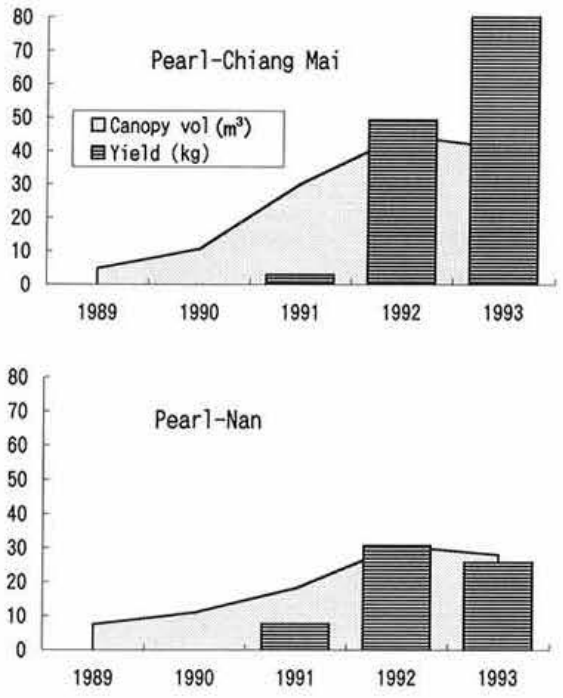

Fig. 1. Annual changes in canopy volume and yield of Pearl tangelo trees planted in a HGB-infected field
Chiang Mai line was free of HGB disease until 1992 and then infected, while Nan line had been infected before 1992. Data were means of 10 trees.

lower production. These facts suggest the prevalence of HGB disease in the area.

2) Relation between growth and yield of affected trees

There were 2 budlines of Pearl tangelo, that is, Chiang Mai and Nan sources. The former which had been free of HGB symptoms until 1992 started showing symptoms in some branches in 1993. The Nan budline showed the symptoms throughout the trees in 1992. The canopy volume of the Chiang Mai budline increased to 40 m^3 or more and the yield reached almost 80 kg per tree, whereas the canopy volume of the Nan budline remained at 30 m^3 and the yield was about 30 kg per tree (Fig. 1). These facts indicate that earlier infection suppressed both tree growth and yield markedly.

Similar observations were made in grapefruit. Miami was free of HGB until 1992 and developed symptoms in 1993. Both canopy volume and yield increased to ca. 90 m^3 and 115 kg, respectively, up to 1992, but they decreased in 1993. Red Mexican was infected earlier than the former showing severe dieback with yellow foliage. Although the canopy volume and yield were lower than those of Miami, the yield per m^3 canopy was more than 1 kg as high as that of Miami (Fig. 2).

Murcott mandarin and Washington Navel orange trees were severely infected since the early stages with poor foliage and severe dieback. Their yield remained very low and not proportional to the canopy volume (Fig. 3), about 0.5 kg or lower. In contrast, the yield of Som-pan mandarin increased in proportion to the canopy volume, more than 1 kg per m^3. The cultivar showed mild mottling of leaves but little dieback and few abortive seeds.

There are 2 types of cultivars in terms of relative yield to canopy volume, those with higher

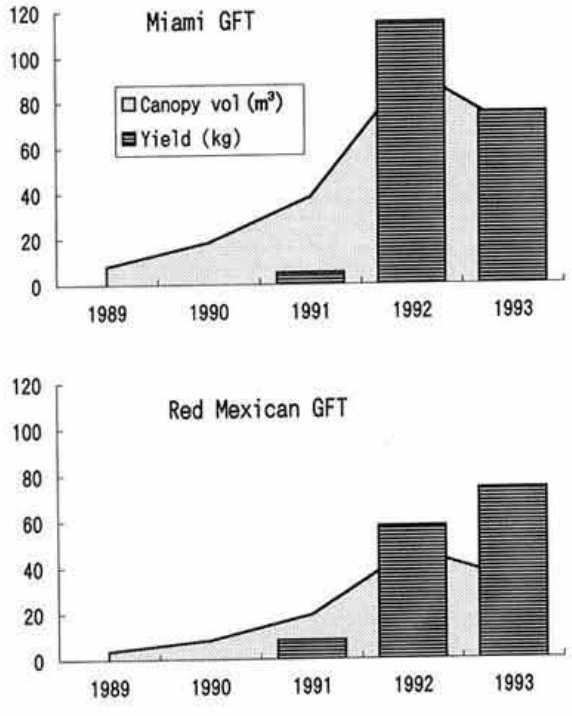

Fig. 2. Annual changes in canopy volume and yield of grapefruit trees planted in a HGB-infected field
Miami was free of HGB disease before 1992 and partially infected in the year, while Red Mexican was severely infected before 1992.
Data were means of 10 trees.

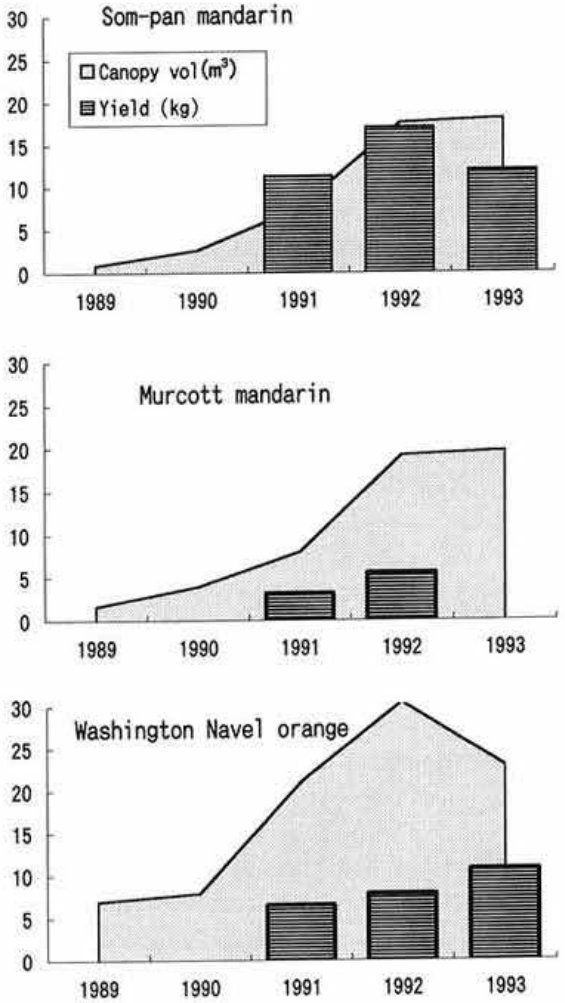

Fig. 3. Annual changes in canopy volume and yield of sweet orange and mandarin trees planted in a HGB-infected field
All the trees showed HGB symptoms, but in Murcott and Washington the symptoms were severe while in Som-pan mild.
Data were means of 10 trees.

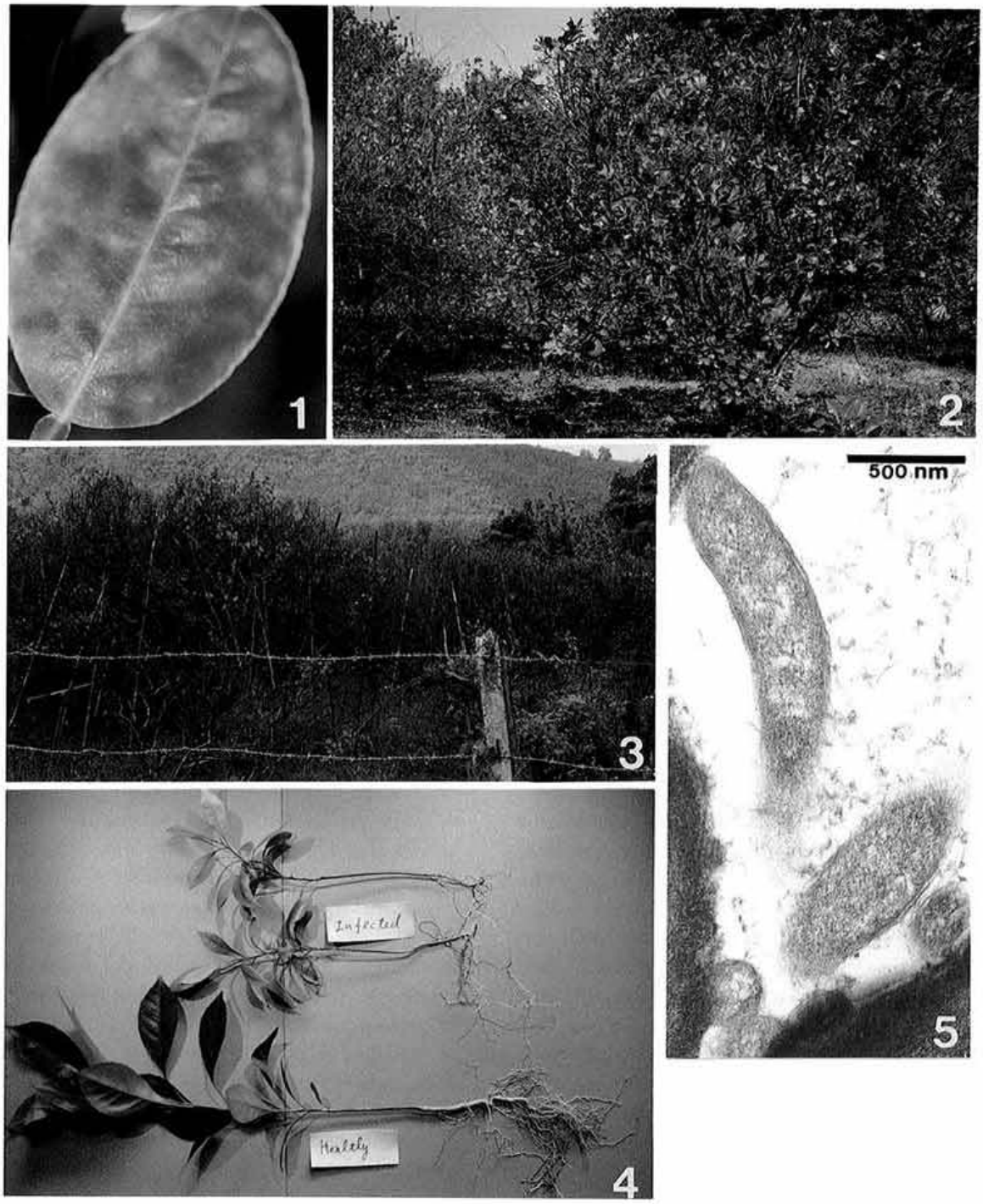

Plate 1. Leaf mottling of sour lime, a typical symptom of HGB disease
2. Severely infected pummelo (right) and sour lime (left) trees in Chai Nat province, central Thailand
3. Destruction of mandarin orchard by HGB disease in Yala province, southern Thailand
4. Seedlings with HGB symptoms (upper 2) caused by feeding of wild psyllas collected in the HGB-infected field of Nan Horticultural Research Station
Note poorly developed root systems, dwarfing and yellow foliage of infected plants.
5. Bacterial structures detected in a sieve cell of the mid-vein of Madam Vinous orange which had been inoculated by grafting with diseased pummelo collected from Chai Nat and showed symptoms

and lower yields, after infection with HGB disease. The following cultivars were productive: Som-pan mandarin, tangelos Orlando, Pearl and Nova, a large number of grapefruits except for Old Sprite and Robinson, and local sweet orange Som-kleang. Other sweet orange and mandarin trees showed a low productivity.

3) Transmission by field-collected vector

Most of the psyllas sucked the main stems when transferred to young seedlings. Because of their feeding, 16 out of 50 seedlings wilted and died within a few days. After transfer to a screenhouse, some plants showed leaf mottling, yellowing and they finally died. Until March 1993, 11 out of 34 plants had died, 4 showed mottling and stunting, and 19 were healthy. The root systems of the infected plants were very poorly developed (Plate 4).

The results showed the presence of many viruliferous psyllas at Nan Horticultural Research Station and psyllas were able to transmit HGB disease at a rate of at least 41% (15 out of 34) after 2-day-feeding. If the receptor had not died because of sucking, the transmission rate would have been higher.

4) Identification of pathogen by electron microscopy

In the tissues of plants with symptoms, there were many necrotic cells in the sieve parenchyma characterized by an electron-density in the cytoplasm and disintegrated organelles with a thick cell wall. Prokaryotic, elongated or spherical structures were found in the sieve cells close to the necrotic cells (Plate 5). Their envelope consisted of a bilayered membrane about 25 nm thick, similar to that described previously[4]. Such a structure was not observed in healthy plants. In the following 4 samples, this structure was present: Special mandarin from Suan Phung district, Ratchaburi, Hiroshima sweet orange and Som-keowan from Nan Horticultural Research Station, and Khaw Tang Kua from Muang district, Chai Nat.

Discussion

Huanglongbing (greening) disease and its vector were prevalent in citrus orchards throughout Thailand. Many orchards with local mandarin and pummelo trees had been destroyed by the disease. There was a wide variation in the occurrence in fields. We observed some orchards of pummelo (Ratchaburi, Chai Nat and Phichit provinces) and Som-keowan (Chiang Mai and Pathum Thani provinces) with mostly healthy trees, where the disease had been transmitted from sour lime or mandarin trees with symptoms to the pummelo or Som-keowan trees. These infected budlines had been recently introduced from other districts. Of the 2 major routes of dissemination of HGB, within field and among fields, the former is due to the vector and the latter to circulation of infected nurseries or bud-sticks by growers.

In Thailand, sour lime grows commonly in private yards as well as in orchards. Almost all of these trees developed typical HGB symptoms but with mild damage probably due to tolerance to the disease. They had been propagated by layering for a long time. Thus, sour lime appears to be the major inoculum source in the country. The senior author recorded similar findings in Calamondin trees in Malaysia.

We collected many viruliferous psyllas in the fields and demonstrated that they transmitted the HGB agent to citrus seedlings. DNA hybridization revealed the presence of 5–39% viruliferous psyllas in Malaysia[2]. It was shown that a single adult of *D. citri* can transmit the HGB agent and the 4th and 5th instar nymphs perform the transmission. The minimum latent period of psylla is 1–2 days after acquisition and the infected adult can continue to transmit the disease throughout its life[8]. It is well known that *D. citri* breeds on *Murraya paniculata, M. koenigii* and many citrus plants. Recently, we have confirmed that *Limonia acidissima* (= *Feronia limonia*) is a suitable host for the psylla[6]. On the other hand, the host range of the HGB agent includes the genus *Citrus* and some relatives but not *M. paniculata*[3,7]. These facts suggest that viruliferous psyllas occur in fields where HGB disease has been endemic and that complete elimination of the pathogen is difficult.

Among the environmental factors, strong wind seems most important. *D. citri* can fly but within a short range from leaf to leaf or twig to twig when observed in an incubator. A strong wind may disseminate the viruliferous psyllas from the donor to the receptor in a leeward direction, which enhances the disease transmission. The distance of dissemination probably depends on the population of psyllas, wind velocity and the survival time in the air. The senior author observed a dissemination over a distance of more than 500 m in a flat field located among rice fields in Malaysia.

Although almost all the citrus plants could be

infected with HGB agents and developed symptoms, the damage varied markedly among the cultivars[5]. The study revealed that both tree growth and yield were markedly reduced by early infection with HGB and that the yield of tolerant cultivars was proportional to the canopy volume even when the tree was infected. Therefore, it is very important to protect citrus trees from HGB infection at their young ages. Planting of tolerant cultivars is also recommended. Som-pan mandarin is tolerant, but the fruit quality must be improved because of the small size and large number of seeds. The results obtained suggest that it is important to breed HGB-tolerant cultivars in future.

In conclusion, to improve the epidemiological condition of the disease in Thailand, the following measures are proposed: production of HGB-free stocks for propagation and propagation under vector-free conditions, construction of wind-breakers around the citrus fields, selection of field without strong wind if possible, elimination of all citrus and its relatives from the field before new trees are planted, planting of HGB-free trees in avoiding to mix different varieties, covering the trees with an insect-proof net for the first 1–2 years after planting, periodical sprays of insecticides, and rapid elimination of infected trees if new foci appear.

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