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### Authors

Koizumi, M.  
Prommintara, M.  
Linwattana, G.  
et al.

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# Field Evaluation of Citrus Cultivars for Greening Disease Resistance in Thailand

M. Koizumi, M. Prommintara, G. Linwattana, and T. Kaisuwan

**ABSTRACT.** Forty-three cultivars of citrus planted in the field at Nan Horticultural Research Station were evaluated for their resistance to greening disease. All the trees were propagated at the Station using Cleopatra mandarin rootstock, and were established in the field in 1986. Most of the scions were free from greening disease when propagated. Field observations in July 1992 revealed that a large number of trees had declined, showing typical symptoms. The cultivars Wilking, Ellendale, Pet-yala, Beauty, Onesco, Pong-chieng-ga (Tankan), Nian-ju, and sweet orange were most susceptible. Many mandarins including Fairchild, Murcott, Kinnow, Clementine, Fremont, Ponkan, King, and Som-keo-wan were next. Queen mandarin, Avon Ever Bearing (calamondin) and rough lemon grew well with mild symptoms. Ladu mandarin and Som-pan mandarin showed the most resistance, with good growth, few symptoms and yielding healthy fruit.

Citrus greening is the most serious disease in the southern part of Asia, including Thailand. Many attempts have been made to control the disease, namely production of disease-free budwood by heat therapy, antibiotic treatments, shoot-tip grafting, propagation of healthy plants under restricted conditions, elimination of infected trees from fields, control of vector by insecticide sprays or release of natural enemies. However, citrus production is still limited by the disease.

Sweet orange and Bearrs lime were reported to be somewhat tolerant to greening in the Philippines (1, 2, 3). In India, Nariani (4) analyzed the reaction of different species and cultivars of citrus to greening under glasshouse conditions. He reported that sweet lime seedlings and trees of Mosambi orange on sweet lime, Eureka lemon or Italian lemon rootstock developed few symptoms. However, the use of tolerant rootstock and scion cultivars has not been practiced.

The objectives of this paper were to analyze the field performance of various citrus varieties in Thailand where greening disease is prevalent. Attempts were made to evaluate their resistance to the disease.

## MATERIALS AND METHODS

**Citrus varieties and cultivars.** Forty-three citrus cultivars or budlines were planted in the field at Nan

Horticultural Research Station. All the trees were propagated at the Station by grafting on Cleopatra mandarin rootstock. A large number of scions had been introduced from foreign countries and others were propagated from nucellar seedlings originating from introduced seeds. The trees were assumed to be greening-free when propagated. However, one of the two budlines of Clementine, Fremont and Som-keaw and all Som-keowan lines originated from infected mother trees planted at the Station.

Ten trees of each cultivar or variety were planted in the field with a 6 x 8 m spacing in 1986. Local mandarin Som-keowan which were propagated by layering and showed typical greening symptoms surrounded the field at a distance of 50-100 m. Insecticides were occasionally sprayed to control the vector.

**Observation.** All the trees were inspected on July 28-29, 1992. Each tree was rated as -, + or ++ based on the tree vigor; also "few", +, ++ or +++ referred to the intensity of greening disease symptoms such as yellow shoots, zinc deficiency, leaf mottling, and blotching. In addition, die-back, dwarfing, small leaves and feeding of psylla were noted. Other serious diseases were also noted. Data on the horticultural characteristics of the trees such as sizes of canopy, yields, fruit quality and number of seeds in fruit were collected at the Station.

**Detection of pathogen.** Scion material of Hiroshima sweet orange and Som-keowan from the field were grafted onto virus-free seedlings of rough lemon in a glasshouse. Small samples from young stems of these varieties were fixed and embedded in resin for electron microscopy. Ultrathin sections were cut, stained and observed with a Hitachi H-300 electron microscope.

## RESULTS

By December 1989, local mandarin Som-keowan trees had already declined, showing yellowing foliar symptoms. Some sweet orange trees developed yellow shoots with typical leaf symptoms such as mottling and blotching. However, most of the other trees were still healthy and were growing well.

The second observation in July 1992 revealed that most of the trees of the following cultivars had declined and had developed severe symptoms: Wilking mandarin (MD), Ellendale MD, Julong-ka MD, Som-keowan MD, Pet-yala MD, Beauty MD, Onesco, Ponchieng-ga (Tankan), unknown cultivars of sweet orange and Nian-ju (Table 1). Half or more of the trees of each cultivar were severely infected. The trees of the cultivars Wilking, Ellendale, Julong-ga, Beauty, Tankan, and Nian-ju showed severe dieback while Pet-yala, Beauty, Onesco, and sweet orange seedlings were dwarfed.

The trees of the following cultivars lost their vigor and developed typical symptoms on the whole canopy, or partially in about half of the tree: Ortanique, Fairchild, Murcott, Kinnow, Bower, Clementine, Fremont, Ponkan, King MD, Valencia orange, Hiroshia sweet orange, Hybrid Fairchild, Hybrid Som-keowan, and Bayue-ju. Leaf sizes of these trees were reduced. Fremont mandarin originated from two different scion stocks, one from Chieng Mai and the other from a tree planted at the Station. The trees originated from the Station were severely infected, while the Chieng Mai

budline trees showed diversity in the symptoms.

Satsuma mandarin and Necked orange (Natsudaidai) showed few or mild symptoms, but the trees lost their vigor and the attached leaves were small and scattered. Among satsuma cultivars the early maturing ones such as Miyakawa and Sugiyama were more susceptible than the late maturing ones such as Hayashi, Ishikawa, Owari, and Silver Hill.

Although Avon Ever Bearing (calamondin) and rough lemon trees grew well, their foliage was yellow with mild symptoms such as mottling. Most of the Sripri, Queen mandarin and Som-keaw trees were vigorous with few or mild symptoms, but one or two trees declined with typical symptoms.

All the trees of Ladu mandarin and Som-pan grew very well with few or very mild symptoms. Their foliage was still dark-green. All the trees set normal fruit without any abortive seeds. However, the canopy of Ladu mandarin was flat.

In another field, 23 cultivars including sweet orange and grapefruit were planted. Most of the sweet orange trees were infected with greening disease and declined. These trees developed yellow shoots with typical symptoms. In contrast, all the grapefruit trees were still vigorous and grew well. However, some trees developed one or two yellow shoots on the outer canopy.

The psylla vector of greening disease, *Diaphorina citri* was frequently observed in the fields. Foot rot occurred on a few trees. Pink disease caused by *Corticium salmonicolor* was observed, but it did not cause serious damages to the trees. Tristeza virus and brown aphids also occurred in the fields. However, severe stem-pitting was not observed on the twigs of either sweet orange or grapefruit, suggesting that severe strains of tristeza were not common at the Station.

An electron microscopy showed many prokaryotic organisms in the degenerated cytoplasm of sieve parenchyma cells of Hiroshima and Som-keowan (Fig. 1).

TABLE 1  
PERFORMANCE OF CITRUS CULTIVARS GROWN IN A FIELD WHERE GREENING DISEASE WAS PREVALENT IN THAILAND

Citrus cultivar <sup>z</sup>	Total no. of trees	No. of trees in each group							Remark <sup>w</sup>
		Tree vigor <sup>y</sup>			Intensity of symptoms <sup>x</sup>				
		++	+	-	Few	+	++	+++	
Ortanique MD	10	1	7	2	1	4	3	2	
Wilking MD	9	0	2	7	0	1	8	0	DB
Fairchild MD	9	0	9	1	0	3	5	1	
Murcott MD	10	0	5	5	0	8	2	0	
Kinnow MD	10	1	8	1	1	7	2	0	
Bower MD	10	0	9	1	0	6	3	1	SL
Ellendale MD	10	1	3	6	0	3	7	0	DB
Ladu MD	10	10	0	0	8	2	0	0	DW
Clementine	19	0	8	11	0	15	4	0	DB,SL
Fremont MD <sup>u</sup>	10	2	5	3	1	8	1	0	
Fremont MD <sup>v</sup>	10	0	3	7	0	7	3	0	DB
Ju-long-ka	10	0	2	8	1	5	4	0	DB,SL
Ponkan	10	3	4	3	5	3	2	0	
Som-pan MD <sup>u</sup>	10	10	0	0	2	8	0	0	
King MD	9	0	1	8	1	5	2	0	SL
Som-keowan MD <sup>v</sup>	19	0	2	17	0	10	9	0	DB,DW
Pet-yala MD	9	0	0	9	0	2	7	0	DB,DW
Beauty MD	10	0	1	9	0	4	6	0	DB,DW
Satsuma MD <sup>t</sup>	35	0	3	32	35	0	0	0	SL
Satsuma MD <sup>s</sup>	10	0	0	10	0	10	0	0	SL
Early satsuma MD	9	0	2	7	4	4	1	0	SL
Sripri <sup>u</sup>	10	10	0	0	8	0	2	0	
Queen MD	10	8	0	2	3	7	0	0	
Onesco	10	0	0	10	0	0	10	0	DW
Neck orange (Natsudaikai)	3	0	3	0	0	3	0	0	
Valencia SW	7	0	5	2	0	6	1	0	
Hiroshima SW	10	0	7	3	0	9	1	0	
Po-chieng-ga (Tankan)	10	0	2	8	4	4	2	0	DB
Sweet orange <sup>r</sup>	19	0	8	11	1	6	12	0	DW

Avon Ever Beraing (Calamondin)	10	0	10	0	0	10	0	0	
Som-keaw	16	0	15	1	0	14	2	0	
Hybrid Fairchild	10	0	5	5	0	8	2	0	DB,SL
Hybr. Som-keowan	10	0	8	2	0	9	1	0	
Nian-ju	9	0	1	8	0	0	9	0	DB
Ba-yue-ju	10	0	8	2	0	8	2	0	SL
Rough lemon <sup>q</sup>	10	0	10	0	0	10	0	0	

<sup>a</sup>MD: mandarin, SW: Sweet orange. All the trees were propagated by grafting on Cleopatra mandarin rootstock and planted in the field in 1986.

<sup>t</sup>++ = vigorous, + = intermediate, - = decline.

<sup>b</sup>Based on leaf symptoms such as yellow shoots, zinc deficiency, mottling and blotching.

<sup>w</sup>DB = dieback, SL = small leaves, DW = dwarf.

<sup>v</sup>Propagated from a tree planted in Nan Station.

<sup>u</sup>Propagated from scions introduced from Chang Mai.

<sup>l</sup>Late maturing varieties including Ishikawa, Hayashi, Silverhill and Owari.

<sup>m</sup>Mid-maturing Sugiyama.

<sup>n</sup>Nucellar seedlings of unknown cultivar.

<sup>q</sup>Self rooted.

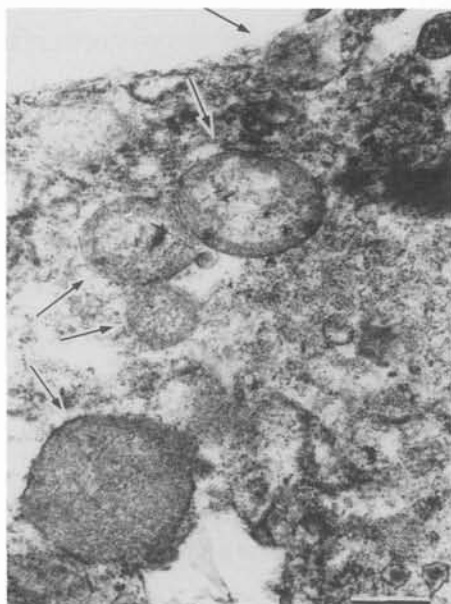


Fig. 1. Greening organisms (arrows) in the degenerated sieve tube of Som-keowan young shoot. Som-keowan was collected at Nan Horticultural Research Station. The bar represents 500 nm.

## DISCUSSION

On the basis of our observations, greening disease and its vector were prevalent in the field, and greening disease was the major cause of decline. The tree decline was correlated mainly with the intensity of the foliar greening symptoms. Most of the citrus cultivars grew uniformly and developed similar symptoms in the same budline. However, some cultivars or budlines markedly differed in tree vigor and symptoms, possibly due to different infection times. For example, local mandarin Som-keowan or Fremont trees propagated from budlines infected with the greening disease declined uniformly while in the others that were propagated from disease-free scions the symptoms varied; some of the trees were healthy while others showed severe symptoms. This observation suggests that a lower percentage of diseased tree is not correlated with the degree of resistance. In contrast the

intensity of tree decline or symptoms is a more suitable index for the evaluation of disease resistance.

Satsuma mandarin trees uniformly declined in spite of showing few or mild mottle or zinc-deficiency. However, the attached leaves were small and scattered, presumably due to greening disease.

The citrus cultivars were classified into four groups according to the disease resistance. The most susceptible cultivars were Ortanique, Wilking, Ellendale, Pet-yala, Beauty, Onesco, Pon-chieng-ga (Tankan), Nian-ju, and sweet orange. The second group included the cultivars Fairchild, Murcott, Kinnow, Clementine, Fremont, Ponkan, King, Som-keowan, satsuma mandarin, Som-keaw and Ba-yue-ju. The third group of the following cultivars was more resistant than the second group: Queen, Avon Ever Bearing (calamondin) and rough lemon. Grapefruit also belonged to the third group. Ladu mandarin and Som-pan mandarin that were the most resistant belonged to the fourth group.

Field observations in the Philippines showed that Szinkom-Ladu mandarin hybrid, calamondin, lime and lemon trees grew well in areas where the greening disease was prevalent (1). However, subsequently it was reported that many mandarin trees including Ladu selection, Ladu hybrid selections, Szinkom hybrid selection, Szinkom and Ponkan were removed from the field due to the severity of the disorder (3). We observed that the canopy of Ladu mandarin was flat and the branches hanged. These would result into over fruit-bearing and decline of trees in future. However, the difference in Ladu mandarin between Philippines and Thailand should be further studied, considering pathogenic strain or other destructive causal factors. We are carrying out to detect resistance to greening disease by analysis of the host reaction after artificial inoculation under controlled conditions and population trends of the pathogen in host tissues.

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