

Presence of Citrus Greening and its Psylla Vector in Thailand

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Greening disease of citrus was first described in South Africa in the early 1930s. Since then, it has been reported from various countries in South Asia: West Pakistan (3, 12), India (10, 15, 16), Nepal (11, 13), Indonesia (22), and the Philippines (14, 18). On the basis of symptoms, the presence of greening was suspected in Thailand by Thrower (21) and a few specimens of the vector *Diaphorina citri* Kuway were collected here by Catling (1, 2).

This report presents symptomatological, chromatographical and transmission evidence to confirm the presence of the greening pathogen in Thailand. In addition, observations are recorded on distribution of the disease and its vector, on economic importance, and on symptoms not previously mentioned as being the result of greening.

The survey discussed here was conducted in 1971-73 under the auspices of the UNDP/FAO project, Strengthening Plant Protection Services of Thailand. Coverage was spotty owing to manpower shortage and to the widespread and inaccessible distribution of citrus in the country. According to a recent census, the average number of citrus trees per holding exceeds 100 only in 2 provinces, lies between 71-100 in another 2 provinces, 41-70 in 1 province, 21-41 in 9 provinces and 1-20 in 58 provinces. Very small dooryard plantings of one or more trees are very common and widely distributed.

Survey for the disease

Criteria for establishing the presence of citrus greening were: (a) macroscopic (i.e. external) symptoms, (b) the bark fluorescence test (19, 20), (c) the midvein phloem necrosis test (22),

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(d) the graft transmission test, and (e) the psylla transmission test.

MACROSCOPIC SYMPTOMS

Because of the variability of symptoms among citrus varieties, tree symptoms are discussed in relation to varieties encountered.

1. Som Keowan (mandarin, willowleaf type, *Citrus reticulata* Blanco). The symptoms of greening differed considerably in the various orchards inspected. Such observed variations might have been due to host differences, to various strains of the greening pathogen, or to modifications brought about by environmental factors. Typical yellow-vein leaf symptoms were found only on Ponkan trees, whereas practically the only symptoms shown by mandarin varieties in Thailand are zinc-deficiency-like patterns: small upright leaves, yellow areas between the veins, and only a small band of green along the veins. Sometimes (especially in the Chanthaburi area) even these symptoms are not found regularly; the leaves simply remain small, dull, and rolled — symptoms that more nearly resemble boron deficiency. This variability of greening symptoms in mandarins has also been reported by Salibe and Cortez (18) from the Philippines. They found that in some mandarin varieties, symptoms resembling zinc deficiency and in others those of boron deficiency predominate. In Thailand, affected trees show a progressive decline. At first, growing points and later whole branches die back from the tips. Fruit of affected trees often drop prematurely and those that remain hanging are markedly undersized. Apart from size reduction and the small dark seed, few fruit symptoms can be observed that are useful for diagnosis. Although affected tops are often sectored during early stages (i.e., normal and diseased branches are found on the same tree), involvement takes place so rapidly,

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spreading within the tree and from one tree to another, that two years after a severe outbreak of psylla, hardly a normal branch or tree can be found within an orchard.

2. Som Kleang [sweet orange, *Citrus sinensis* (L.) Osbeck]. Symptoms on sweet orange are clearly typical and easy to diagnose. Patterns of zinc deficiency, yellow vein and blotchy mottling of the foliage are prominent. Affected trees show a rapid and severe dieback. Fruits are also undersized but in addition often show more specific symptoms such as lopsidedness.

3. Som O [pummelo, shaddock, *Citrus grandis* (L.) Osbeck]. This species appears rather tolerant of greening. Nevertheless, affected leaves show zinc-deficiency-like patterns, fruits are reduced in size and some fruits drop prematurely.

4. Manao [small acid seedy lime, West Indian types, *Citrus aurantifolia* (Christm.) Swing.]. Greening is often masked by the more severe symptoms of tristeza. Symptoms of vein clearing, decline and dieback in manao are usually caused by the universally present tristeza disease and not by greening.

BARK FLUORESCENCE TEST

This test is a modification of the one described earlier by Schwarz (19, 20). Three 4-cm twig sections, each from two- to three-year-old branches, were collected per tree. Each of the three samples was taken from a different side of the tree. The bark was stripped and cut in 2- to 3-cm wide pieces. The bark portions were shaken for half an hour with 5 cc of water in a small vial. The water extract was decanted into a round plastic disc fitted over a watchglass and dried in an incubator at about 50°C. The concentrate was taken up with some drops of water and spotted in quantities of x, 2x and 4x on silica gel TLC plates that had been previously activated by heating at 110°C for half an hour. The plates were then developed with a chloroform/methanol mixture (9:1). After drying, plates were sprayed with a saturated aqueous Na-borate solution and inspected under a 360-nm UV lamp. It is important that the 360-nm light

be nearly pure, emitting not more than 5 percent visible components. The samples from greening-affected trees show a bright purple spot at an Rf of 0.5 to 1.0, i.e., just next to the spotting point. As a control, a sample from a citrus tree of the same species known to be infected with greening is run with each batch of about 50 samples. Depending on the concentration of the marker substance in the sample, the spot is better visible in the profile of either the low concentration (x) or the high concentration (4x). Citrus species and varieties show different fluorescent nonmarker spots. Occasionally the sample contains fluorescent nonmarker substances that interfere with the reading of the true marker. In such cases, the mobile phase can be modified by increasing the methanol content from 10 to 20 percent and decreasing the chloroform. This increases the Rf of the gentisic acid glucoside marker substance (6) and facilitates reading of the results. The test works reliably with mandarin, sweet orange and tangelos but not assuredly with such species as lemon, pummelo and lime (20).

MIDVEIN PHLOEM NECROSIS TEST

Soelaeman Tirtawidjaja's test, developed for the diagnosis of greening in Indonesia (22), was also utilized. Thin hand sections of citrus leaf midribs were examined under a dissecting microscope at a magnification of about 15x. The phloem of greening-affected leaf midribs showed groups of necrotic cells not present in the phloem of healthy leaves.

GRAFT TRANSMISSION TEST

Sweet orange or mandarin seedlings were side grafted with material from trees to be tested for greening. If the donor material was infected, the young growth of the inoculated seedlings showed leaf symptoms of greening about three months after grafting. Six seedlings were each inoculated with material from the Chanthaburi and Phetchabun areas.

PSYLLA TRANSMISSION TEST

Batches of ten adult citrus psylla, each collected from severely affected trees, were transferred to young mandarin seedlings and allowed to feed for one week. After this period, adults and eggs were killed with an insecticide and the seedlings were kept under observation for the appearance of symptoms. Five seedlings each were used per collection of psylla from Chiang Mai and Phetchabun.

Macroscopic symptom and bark fluorescence tests were used in all areas, the midvein phloem necrosis test in the Chanthaburi, Amphoe Fang (Chiang Mai) and Phetchabun areas, the graft transmission test in the Chanthaburi and Phetchabun areas, and the psylla transmission test in the Chiang Mai and Phetchabun areas.

Survey for the vector

As the Asian citrus psylla (*Diaphorina citri*) develops only on the new flush, and as the flushing periods vary considerably in different parts of Thailand, trees sampled could not always be visited during the best time for psylla development. Obviously, the absence of psylla during the inspection of a particular orchard does not mean that they have never previously been present.

In each orchard sampled, ten buds on each of ten trees just starting to flush were inspected with a hand lens for the presence of eggs and nymphs. In addition, the same number of trees were swept ten times each and the catch inspected for the presence of psylla and their possible parasites and predators.

For facilitating the identification of *D. citri*, the following description of the Asian citrus psylla in subtropical India, as given by D.B. Reddy (17), is reproduced:

"The adults are very active and jump on slight disturbance. They are small in size, yellowish-brown in colour and measure about 2.5 mm in length. The wings are transparent with white spots. About 400-1 900 almond-shaped orange eggs are laid

singly in the leaf axils of tender shoots, on tender leaves and stems. Eggs hatch in 4-5 days in summer and 23 days in winter. The nymphs are light yellow and bear well-developed wing pods and moult 4 times in about 11-25 days. The total life cycle is completed in 15-47 days. Adults may live for about 6 months. There are 9 generations in a year. The pest is most active during spring and early summer and again during and after the rains. The winter is passed in adult stage."

Results of the greening and psylla surveys

The results of the bark fluorescent test are as follows:² Chanthaburi and Trat Provinces: Mandarin (52/37),³ Ponkan (9/7), Sweet orange (3/2); Phetchabun Province: Mandarin (23/15); Phichit Province: Mandarin (18/14), Sweet orange (5/3); Bangkok: Mandarin (12/6); Chiang Mai Province: Mandarin (18/14); Sweet orange (7/7); Nan Province: Mandarin (18/15); Southern Provinces (13/1). The test was applied selectively; therefore, the ratios between positive and negative readings are not representative of any particular orchard or area. In severely affected orchards, samples were taken mainly from trees obviously infected as well as from a few trees appearing normal. In orchards apparently nonaffected, samples were taken from a few questionable trees.

There was close agreement between the mid-rib test and the fluorescence test as determined in material gathered at Chanthaburi, Amphoe Fang (Chiang Mai) and Phetchabun. Though still in progress, experiments with grafting and psylla already show positive transmissions in a few indicator seedlings.

Based on the various diagnostic techniques used, the incidence of greening in Thailand may be summarized as in Figure 1 and under the following headings.

² A detailed account of the results will be given in a paper on the same subject to be published in *Kasikom*, a Thai journal reporting on agricultural research.

³ (Trees tested/trees positive).



Figure 1. Pro with relation

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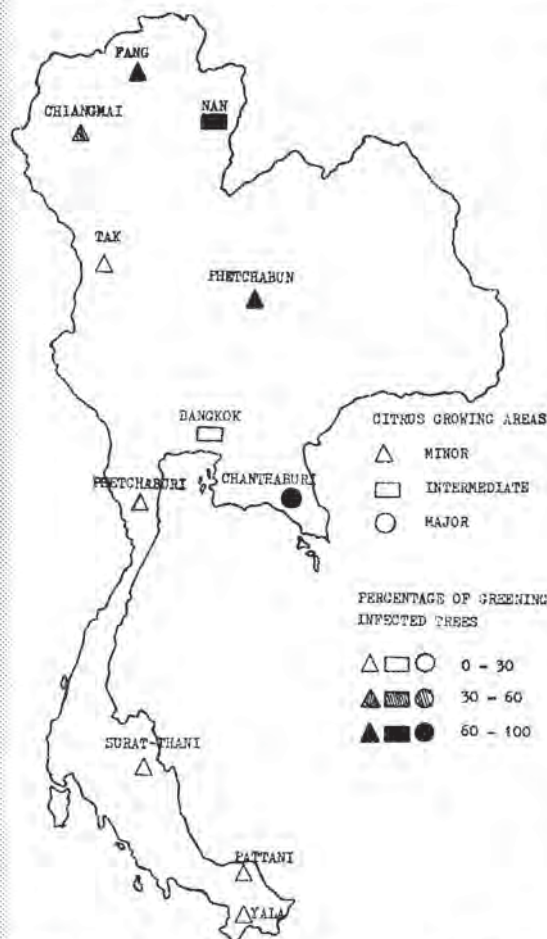


Figure 1. Principal citrus-growing areas of Thailand with relation to incidences of greening-affected trees.

AREAS WITH HIGH INCIDENCE OF GREENING

Chanthaburi area

Orchards in this area, consisting mostly of mandarin trees, were severely affected by greening. Decline was obvious in orchards along the road from Rayong to within 50 km of Chanthaburi, also in orchards from Chanthaburi to Amphoe Pong Nam Ron (Chanthaburi). Unusual in the area was an orchard along the Rayong-Chanthaburi road with little or no evidence of the disease, a result possibly of intensive spraying against miscellaneous pests. Mandarin trees at the P.R.E.W. experiment station (on the road between Chanthaburi and Trat) were also severely infected.

At the times the area was visited no psylla were in evidence except at the P.R.E.W. station where populations were low, suggesting that in previous years infestations must have been heavy.

Phetchabun area

Greening in mandarin orchards was extremely severe and many plantings have been abandoned. The 30 000-tree orchard of the Nai Jun Kunwong family at Wangchompoo — at one time Thailand's largest — has been ripped out and is now replanted to mulberry. Because of the productiveness of that former orchard, it seems likely that the cause of destruction was greening.

Extremely high populations of psylla were observed during two visits in all orchards at Chayopruk Farm, near Dong Kui Village.

Nan area

In plantings at the Nan experiment station, as well as in surrounding locations, greening was very severe. Only in a single orchard of about 80 trees was no greening to be seen, presumably because the planting was surrounded by hedges of bamboo.

At the times of the visits to the Nan station, psylla were found in low numbers.

As a commentary on varietal susceptibility, it is interesting to note the severe effects of greening in trees of mandarin, satsuma and sweet orange and, in contrast, the mild effects in trees of pummelo, lemon and small acid seedy lime. As a matter of fact, the latter varieties continue to be grown with profit despite infection with the greening agent.

AREAS WITH VARIABLE INCIDENCES OF GREENING

Chiang Mai — Amphoe Fang (Chiang Mai) area

On the one hand, at Fang experiment station and in nearby orchards, the incidence of greening was very high. Mandarins showed severe symptoms but pummelos, despite infection, continued to bear good crops of normal-sized fruits.

On the other hand, at a large citrus estate

just 32 km south of Amphoe Fang (Chiang Mai), the percentage of affected trees was very low - out of 6 000 trees, only 100 aged trees were infected. The orchard had been sprayed twice weekly with miscellaneous insecticides.

At Raming tea station, Amphoe Chiang Dao (Chiang Mai), and at the Agricultural training centre, Chiang Mai University, mandarin trees were severely affected. *Psylla* infestations were high at both sites, especially at the training centre.

At another estate 30 km southwest of Chiang Mai, about 15 percent of trees were affected. Judging from the presence of infection in two- to three-year-old trees, inoculation must have occurred in the past few years.

Trat area

Orchards near Tusai Village and near Wangtakean Village were found to be free of greening. There was no indication of *psylla* activity - possibly because of the isolation from infected orchards provided by intervening rubber plantations. In one orchard near Wangtakean Village, about 15 percent of eight-year-old mandarins were infected with greening. Most probably these trees had become infected in the nursery. There was no indication of *psyllid* dissemination from tree to tree in the orchard.

AREAS WITH LOW OR NO INCIDENCE OF GREENING

Southern Thailand

Mandarin orchards around Ranong, Surat Thani, Satun, Amphoe Hat Yai (Songkhla), and Yala were found to be free of greening, though the validity of this generalization is limited owing to the occurrence of many small plantings that have not yet been sampled. Furthermore, many orchards in the Satun and Amphoe Hat Yai (Songkhla) areas were suffering from drought, making diagnosis of greening difficult. On the whole, however, southern Thailand appeared to be relatively free of greening. *Psylla* were not encountered and, according to reports, little citrus nursery stock is brought into the area from the north.

Bang Mot and Tung Kru areas (Amphoe Rat Burana, Bangkok)

These areas south and west of Bangkok have long been famous for high-quality mandarins. Trees are usually planted on raised beds with ditches seasonally filled with water. Because roots come in contact with the fluctuating water table after trees are more than three years old, root rot abounds and tops decline by the time trees are four to six years old. Despite the short longevity, plantings are maintained at a profitable level by removing dead trees and replanting. Some greening-affected trees were found at Tung Kru but, in general, plantings were unaffected and *psylla* were not found - perhaps because of the use of insecticides every 7 to 14 days.

Phetchaburi area

About 6 000 hectares of mandarins and acidless sweet oranges (resembling the Mosambi of India) are grown in Amphoe Khao Yoi (Phetchaburi) near Nonyangplong Village. Trees were in excellent condition, showing no symptoms of greening. *Psylla* were not encountered.

Phichit area

This locality has long been known for its high-quality pummelos. It appeared to be free of greening-affected trees except for two orchards around Mu Hah, Katmang Village, where pummelo and sweet-orange trees showed greening symptoms and the presence of *psylla*.

Discussions and conclusions

This report provides several new insights into the nature, behaviour and possible control of greening.

In areas where the disease is reported to be pandemic and citrus culture, therefore, seems to be precluded, successful cultivation may yet be possible. In this connection, it is interesting to compare the results of the present survey with those carried out in the Philippines (5)

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and Indonesia (4).¹ In these countries, green-
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the Philippines, it is destructive only in the
regions of Batangas, Laguno, Quezon, Rizal
and Cavite, whereas it is either benign or non-
existent in the Bicol, Mindanao, Mindoro and
Mountain Provinces. In Indonesia, greening
is present in Java and some parts of Sumatra
but is not known in west and south Borneo,
south Celebes or the islands of Salayar, Bali
and Madura. Unlike tristeza, which appears
to be present throughout eastern Asia, the dis-
tribution of greening is remarkably discontin-
uous. This discontinuity strongly suggests
that much can be done to limit the spread of
greening by the exclusionary approaches of
plant quarantine.

The erection of natural barriers may offer
the possibility of limiting the spread of psylla
from adjacent areas. In the present survey,
two separate orchards in the otherwise severely
affected Chanthaburi area were found to be
free of greening. These orchards were sur-
rounded by rubber plantings. In the severely
affected Nan area, similar protection to one
planting appeared to be afforded by bamboo
hedges. From these observations, it seems
worthwhile to investigate the hypothesis that
the citrus psylla — in contrast to the aphid vec-
tor of tristeza — has a low diffusion rate. The
scattered distribution of citrus together with
the apparent inability of psylla to disseminate
widely appear to be the main reasons why
greening has not become pandemic in Thailand.

Though it was early feared that greening
might one day make citrus growing untenable
in South Asia (7, 9), the pall of pessimism
seems in part to be dispelled by new evidence
of varietal resistance. On the basis of obser-
vations in India, Fraser and Singh (8) classified
certain varieties as rather tolerant, and Knorr

and Moin Shah (11), reporting on reactions
seen in a varietal trial in Nepal, found a tangor
(Fewtrell's Early) to be remarkably resistant.
In the present survey, it was also found that
certain species, such as pummelos, lemons, and
small acid seedy limes continue to be grown
profitably despite infection. Unfortunately, no
indications of tolerance were found in the man-
darin group during this survey.

The symptomatological picture of greening
needs to be enlarged to include an expression
which is found particularly in the Chanthaburi
area. The condition can best be characterized
by a lack of specific leaf symptoms, a sudden
decline of entire orchards and a sudden re-
duction in fruit size. Leaves do not show the
characteristic blotchiness and zinc-deficiency-
like pattern, and there may be no indication
of sectorial infection. The Chanthaburi mani-
festation is in fact so unspecific for greening
that the cause of the decline was only realized
after positive results were obtained from vari-
ous tests. A typical symptom is the generalized
and severe dieback that eventually involves
mandarins in Thailand. The tips become nec-
rotic and are later invaded by secondary fungi
similar to the condition reported from India.

There is increasing evidence that greening
is not indigenous to Southeast Asia. As simi-
larly reported from Nepal (11), the citrus in-
dustry of Thailand has long flourished. Ac-
cording to farmers and citrus specialists, this
type of decline was not seen until 1965 in areas
surrounding Nan and Phetchabun and not until
1970 in the Chanthaburi area. As pointed out
above, other areas of Thailand are still not
affected.

In summary, it is concluded from these ob-
servations that for any projected expansion of
citrus in Thailand consideration must be given
to the past history of a particular area and to
a thorough study of psylla distribution, natural
barriers, exclusionary domestic quarantines and
tolerant varieties.

¹ Tirtawidjaja, S. 1973. Personal communication.

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