Distribution of the Psyllid Vectors of Citrus Greening Disease, with Notes on the Biology and Bionomics of Diaphorina citri

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In 1968, the author, under the auspices of FAO, assisted the Philippine Bureau of Plant Industry in initiating research on the Oriental insect vector of citrus greening disease. Surveys were carried out in the Philippines on the distribution of the vector, and observations on symptoms in declining citrus groves were made during short visits to Hong Kong, Thailand, India and Nepal.

Greening diseases

In the last three decades a number of closely related citrus virus diseases have appeared in most regions of the world where citrus is grown. A number of workers now believe that the greening disease of South Africa, leaf mottling of the Philippines, and much of the citrus dieback in India are caused by one and the same virus. It is also becoming increasingly clear that the stubborn disease of America, vein-phloem degeneration of Indonesia, yellow shoot of China (Mainland), and likubin of China (Taiwan) are very similar to greening. Results described in this paper tend to support this view.

Excellent descriptions of the symptomology of greening, leaf mottling and stubborn disease may be found in recent papers (5, 21, 23, 28). In the field, however, particularly in Asia and the Far East, recognition of the disease from symptoms alone is often difficult. Very similar leaf symptoms may be caused by a wide variety of factors varying from nutritional disorders to the presence of other diseases such as root rots and gummosis, and even other virus diseases such as tristeza and exocortis. Hence, tissue graft and insect transmission studies are often necessary before a reliable diagnosis can be made.

The two main vectors responsible for greening disease are Trioza erytreae (Del G.) and Diaphorina citri (Kuw.).

Distribution of Trioza erytreae

In 1965, McClean and Oberholzer (22) proved that greening in South Africa is transmitted by the psyllid T. erytreae. This vector is restricted to Africa south of the Sahara (see Figure 1), and has also been recorded in Madagascar (3), Mauritius (26), Réunion (4, 26) and Saint Helena (15). It is suspected to occur in Angola,1 and has been observed by the author in Rhodesia, and in Swaziland (10).

Distribution of Diaphorina citri

It has been shown conclusively that the psyllid D. citri, is the main vector of greening disease in Asia and the Far East. In 1965 Tirtawidjaja et al. (34) demonstrated that the vein-phloem degeneration disease of Indonesia is transmitted by D. citri. Salibe and Cortez (28) published preliminary evidence that D. citri is the vector of Philippine leaf mottling and this was later confirmed by Celino et al. (14) and Martinez and Wallace (21). In India, Capoor et al. (6) have shown convincingly that D. citri is an efficient vector of greening.

Figure 1 and Table 1 show that D. citri is widely distributed in the Orient and is present in Brazil. The vector occurs in the Ryukyu archipelago (24) but as far as can be ascertained it has not been recorded in Japan. Greening-like diseases have been reported from 11 out of the 15 countries where this vector is known to occur on citrus. Furthermore,

the disease is suspected in Malaysia and Hong Kong and there is a strong possibility that it is present in Burma and Pakistan — especially in West Pakistan bordering the Indian Punjab.

**Table 1. - Distribution of D. citri and the presence of greening-like diseases of citrus**

<table>
<thead>
<tr>
<th>Country</th>
<th>Greening-like diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burma (16)</td>
<td>Greening (26)</td>
</tr>
<tr>
<td>Ceylon (35)</td>
<td>Likubin (5, 31)</td>
</tr>
<tr>
<td>China (Taiwan) (19)</td>
<td>Greening (17)</td>
</tr>
<tr>
<td>India (16, 19)</td>
<td>Decline (32), vein-phloem degeneration (34)</td>
</tr>
<tr>
<td>Indonesia (16, 19)</td>
<td>Probable greening (8)</td>
</tr>
<tr>
<td>East and West Pakistan 1 (19)</td>
<td>Possible greening 2</td>
</tr>
<tr>
<td>Hong Kong (30)</td>
<td>Greening (9)</td>
</tr>
<tr>
<td>Malaysia (16)</td>
<td>Greening (leaf mottling) (21, 28)</td>
</tr>
<tr>
<td>Nepal 1</td>
<td>Greening (26)</td>
</tr>
<tr>
<td>Philippines (16, 19)</td>
<td>Greening (4, 26)</td>
</tr>
<tr>
<td>Mauritius 1 (26)</td>
<td>Stubborn (5), probable greening (27)</td>
</tr>
<tr>
<td>Réunion (4)</td>
<td></td>
</tr>
<tr>
<td>Brazil 1 (20)</td>
<td></td>
</tr>
</tbody>
</table>


*D. citri* was collected by the author in the Philippines, China (Taiwan), Hong Kong, Thailand and Nepal, and he has since received specimens from Brazil and Réunion. Both *D. citri* and *T. erytreae* occur in Réunion and Mauritius.

**Surveys for D. citri and greening in the Philippines**

Surveys were carried out by two methods, depending mainly on the size of the grove selected. In most large groves, 50 to 200 flushes were examined for eggs and young stages of the insect with a hand lens either *in situ* or after removal from the trees (surveys). In small or backyard groves, or where trees were semidormant, a rapid search was made, lasting 10 to 20 minutes or until the vector was discovered (spot checks). The rate of discovery was taken as a measure of prevalence.

Greening was identified mainly by leaf and fruit symptoms. In addition, a small number of fruit and bark samples were taken from diseased trees for indexing by the fluorescent test of Schwarz (29).

The results are summarized in Table 2.
Table 2. - Surveys and spot checks for D. citri in citrus groves in the Philippines during July and August 1968

<table>
<thead>
<tr>
<th>Region</th>
<th>Province</th>
<th>Surveys</th>
<th></th>
<th>Spot checks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of groves surveyed</td>
<td>Number of groves infested</td>
<td>Number of flushes examined</td>
</tr>
<tr>
<td>Southern Tagalog</td>
<td>Batangas</td>
<td>11</td>
<td>11</td>
<td>1 100</td>
</tr>
<tr>
<td></td>
<td>Cavite</td>
<td>22</td>
<td>22</td>
<td>2 00</td>
</tr>
<tr>
<td></td>
<td>Laguna</td>
<td>1</td>
<td>1</td>
<td>8 0</td>
</tr>
<tr>
<td></td>
<td>Quezon</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Bicol</td>
<td>Albay</td>
<td>5</td>
<td>4</td>
<td>3 50</td>
</tr>
<tr>
<td></td>
<td>Camarines Norte</td>
<td>4</td>
<td>0</td>
<td>2 30</td>
</tr>
<tr>
<td></td>
<td>Camarines Sur</td>
<td>4</td>
<td>3</td>
<td>2 50</td>
</tr>
<tr>
<td></td>
<td>Sorsogon</td>
<td>1</td>
<td>1</td>
<td>5 0</td>
</tr>
<tr>
<td>Mindoro</td>
<td>Oriental</td>
<td>4</td>
<td>0</td>
<td>6 00</td>
</tr>
<tr>
<td>Mindanao</td>
<td>Cotabato</td>
<td>4</td>
<td>2</td>
<td>4 00</td>
</tr>
<tr>
<td></td>
<td>Davao de Sur</td>
<td>2</td>
<td>1</td>
<td>1 50</td>
</tr>
<tr>
<td></td>
<td>Davao Oriental</td>
<td>3</td>
<td>1</td>
<td>2 50</td>
</tr>
<tr>
<td>Northern Luzon</td>
<td>Benguet</td>
<td>1</td>
<td>0</td>
<td>1 00</td>
</tr>
<tr>
<td></td>
<td>La Union</td>
<td>--</td>
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</tr>
</tbody>
</table>

Southern Tagalog. With one exception, D. citri was fairly abundant in all groves searched in this region. Centered in Batangas province, this is the oldest and formerly the most important citrus-growing area in the Philippines. Since the rampant spread of the disease in the late 1950s, however, more than a million trees have died and the 1968 crop was estimated at about one tenth of that harvested in 1960. Since much of the propagative material used in other citrus areas originated from Batangas, the disease has probably been inadvertently introduced into most of the recognized citrus areas of the Philippines.

Disease symptoms were found in every grove, most trees showing severe decline, with the exception of the Rosario/Padre Garcia area, where trees continue to bear and grow well. Apart from the absence of vein-yellowing, there was a striking resemblance between leaf symptoms and general tree appearance of mandarins in Batangas (mainly Ladu and Szinkom varieties), with greening-affected sweet orange trees in South Africa. It was too early in the season for a valid comparison of fruit symptoms, but it would appear that they are not reliable in mandarins. No definite marker substance was found in two fruit samples from diseased Ladu mandarins, but a bark sample from the same trees was clearly positive for greening. Greening symptoms were also observed in several neglected Valencia trees in Batangas, three fruit samples showing low concentrations of marker substance and a single bark sample again being clearly positive.

Bicol and Mindoro. Table 2 shows that the vector was common in the Bicol region, with the exception of Camarines Norte, but was not found in seven groves in Mindoro Oriental. It is possible, however, that further search would reveal its presence in these areas. Scattered leaf symptoms were observed in both regions but in only three groves could this be associated with greening. In one grove in Mindoro there was evidence that the disease had been introduced with propagative material from Batangas.

Mindanao and northern Luzon. The vector was present in most groves examined in Min-
danao and in one in northern Luzon. Very few symptoms were observed in either region. The vector is thus widely distributed in the Philippines and the potential exists for greening to spread to most of the citrus-growing areas. Fortunately, diseased groves outside of the severely affected Batangas areas appear to be relatively few and are isolated.

**Surveys in other countries**

*Hong Kong.* *D. citri* was found in one of the three groves visited in 1968. During another visit in 1969 fairly conclusive greening symptoms were observed in sweet orange, mandarins and pomelos.

*Thailand.* Low populations of *D. citri* were found in two out of six groves in the Chantaburi district. Specimens in the insect collection of the Plant Industry Division indicate that the vector is widespread in Thailand. No greening symptoms were evident in the young citrus area of Chantaburi but the disease has been observed in other parts of the country (33).

*India.* *D. citri* was not found in any of the three groves visited but greened trees were seen at the Indian Agricultural Research Institute, New Delhi. At Poona Agricultural College and in a nearby grove, severely affected sweet orange trees were observed with the full syndrome of the disease. One fruit sample was found to give a strong positive response to the albedo-fluorescence test.

*Nepal.* The vector was not observed in Katmandu valley, and all trees appeared free from greening. In the Pokhara valley, where a virulent strain of greening has caused a severe decline of citrus, three out of six sites were infested with *D. citri*. At the Pokhara Research Substation, the complete syndrome of the disease was observed. Sweet orange trees showed abundant leaf mottling or "frenching," vein-yellowing, and lopsided fruit with dark, aborted seeds, and two fruit samples yielded high concentrations of the greening marker substance. Mandarins showed identical leaf symptoms and general tree appearance to trees examined in the Philippines. As in South Africa and the Philippines, lemons and limes at Pokhara were only slightly affected by the disease and were still growing and bearing well. The vector is also reported to be present in the eastern terai (belt of marshy jungle between the foothills and the plains) from where leaf symptoms have been noted. There is evidence that greening-infected material has been introduced into Nepal from Uttar Pradesh, India.

**Notes on the biology of *D. citri***

From the literature, it appears that there has been little or no further study on the biology or ecology of *D. citri* since the work of Husain and Nath in India in 1927 (19), who also included a detailed description of all stages of the insect. The following notes, mainly from observations in the Philippines, are intended to supplement the above paper and are in general agreement with it.

Figure 2, drawn from specimens freshly preserved in alcohol, shows the egg, five nymphal instars, and an adult female of *D. citri*. In the insectary, in July/August, at a mean temperature of 25-26°C, there was a daily egg production of 8.0 eggs, a mean incubation period of 3 days, and nymphal development was completed in 11 to 15 days (based on 32 reared adults). The mean preoviposition period of 5 females was 12 days. In Batangas, the mean colony size per flush point was 7.8 individuals comprising 4.5 eggs and 3.3 nymphs. Although larger colonies were observed on some occasions, and in Camarines Sur one isolated colony was found with 160 fourth and fifth-instar nymphs and 25 adults, in general, colony size was small at this time of the year. Fecundity and colony size are reported to be far greater on the first flush cycle at the start of the rainy season. In a group of 518 adults investigated, 44.8 percent were males.

*D. citri* was observed on sweet orange, mandarins, lemons, limes, pomelos and calamondins (*Citrus × Fortunella* hybrid), and heavy populations may cause blossom and fruitlet
Figure 2. Egg, five nymphal instars and adult female of Diaphorina citri.
The insect also attacks a wide range of rutaceous plants and has been recorded on five different genera of this family in India and southeast Asia. The Philippine flora is rich in Rutaceae and there is evidence that in some areas the insect has spread to citrus from alternate hosts.

**Bionomics of D. citri**

Psyllids are known to undergo violent fluctuations in numbers. Owing to their high fecundity and short life cycle they are able to multiply very fast to exploit their environment when limiting factors are relaxed. From field studies in South Africa, Catling and Annecke (7) found that the main factors regulating populations of *T. erytreae* were flushing rhythm of citrus, flush quality, extremes of weather, and natural enemies. The possible importance of such limiting factors in the population dynamics of *D. citri* will be discussed briefly in the light of preliminary observations in the Philippines.

*Flushing rhythm.* The population fluctuations of psyllids breeding on citrus are closely correlated with flushing rhythm (10), because eggs are laid exclusively on young flush points and nymphs develop on immature leaves. Populations of *D. citri* in Batangas are at their lowest on semidormant trees during the dry season from December to April. The main upsurge in numbers takes place during the main flush cycle in May/June, which is stimulated by the first rains. Moderate populations were observed by the author during July and August 1968.

The heavy and prolonged flushing of young trees makes them very attractive to the vector; this partly explains the rapid spread of greening in replanted groves. At Lipa (Batangas) and Pokhara, healthy replants had become infected in three to four months. Pruning should be carefully timed so as not to stimulate out-of-season flushes which may be rapidly colonized by the vector. Differences in the inherent flushing rhythm of various citrus species and varieties also affect population dynamics (10).

**Natural enemies.** Several species of internal parasites, at least two of which are probably hyperparasites, were reared from *D. citri* nymphs collected in the Philippines. The three main species were: (a) *Psyllaephagus* sp. (Encyrtidae) females, from Batangas, Bicol and Mindanao; (b) Marietta nr exitiosa Compere (Aphelinidae) from Batangas; (c) *Aphidencyrtus* sp. (Encyrtidae) from Batangas.

Of 147 parasites reared, 51.7 percent were *Psyllaephagus* sp., which appears to be the most common primary parasite in the Philippines. This has been recorded from China (Taiwan) by Dr. B.D. Burks 4 of the U.S. National Museum, Washington, D.C. 25.2 percent were Marietta nr exitiosa, which is probably the main hyperparasite, and 17.0 percent were *Aphidencyrtus* sp.

The external eulophid parasite *Tetrastichus radiatus* Waterston was not found during the field surveys, nor has it been recorded in the Philippines (2). This species has been reported as an effective parasite of *D. citri* in India (18) and attacks *T. erytreae* in South Africa (11). It is, therefore, a candidate for introduction into the Philippines.

In addition, syrphid larvae, a neuropteran nymph and a coccinellid larva were found associated with *D. citri* nymphs on several occasions.

**Extremes of weather.** Extremes of weather were found to play a dominant role in regulating the numbers of *T. erytreae* (12); further evidence of this is provided by the laboratory studies of Moran and Blowers (25). Mortality of the highly sensitive egg and first-instar nymph stages was closely associated with high temperature and low relative humidity. However, it appears that *D. citri* is more resistant to extremes of weather than *T. erytreae*. In Réunion, fairly high populations of *D. citri* were observed in the hot coastal zone, whereas *T. erytreae* was confined mainly to cooler areas above 500 to 600 metres (4). In equa-

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3 Celino, C.S. 1968. Personal communication.
torial regions, such combinations of tempera­
ture and humidity are rare. An examination
of meteorological data for the driest citrus area
of the Philippines, Batangas, revealed that the
daily maximum saturation deficits for the hot­
test days during the 1968 dry season were
considerably below the critical level of 26 mil­
limetres Hg (34.6 mbars), a value used in
predicting population densities of *T. eryt­
treae* (12).

Control of *D. citri*

The insect is widely distributed and well
adapted in the profusion of small plantings
and backyard trees which characterize most
citrus areas of the Orient. It has been collect­
ed on a wide range of alternate host plants
from which it could reinfest sprayed groves.
It would therefore seem that there is little
prospect of eradicating *D. citri* in these coun­
tries.

There is, however, evidence that fairly high
vector populations are needed before signifi­
cant transmission of the disease will occur.
In South Africa it is also believed that adults
colonizing the spring flush cycle, which have
previously fed for long periods on mature
leaves containing high concentrations of virus,
are particularly infective (13). This means that
the prevention of high populations --- espe­
cially on the first flush cycle at the start of the
season --- may be extremely important in check­
ing the spread of the disease.

Many workers have shown that *D. citri* can
be effectively controlled with a wide range of
modern insecticides. This is also true of *T.
erytreae*, concentrations of dimethoate as low
as 0.0025 percent active ingredient having prov­
ed effective against eggs and nymphs (13).
In a small field test in the Philippines, dime­
thoate at 0.0075 percent active ingredient gave
a 79 percent kill of *D. citri* nymphs despite
the occurrence of heavy rain two hours after
spraying. It is important that the natural
balance of beneficial insects is not disturbed
when chemical measures are used. Low con­
centrations of fairly safe systemics, such as
dimethoate or demeton methyl, should be ap­
plied at the first signs of a buildup of nymphs
on a new growth cycle.

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