

Citrus Virus Research In Indonesia

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Citrus fruit is very important to the Indonesian farmer, since it can give him more income than other crops. According to some surveys (Widjono and Pekerti, 1977), he can get at least five to six times as much income from a 5-6-year-old citrus planting as from groundnut and four to five times as much as from rice cultivated on equal acreages. Because of good yields and prices for certain mandarin varieties, more and more acreage is being planted. Although no exact statistics are available, it is estimated that in West Java alone, at least 10,000 ha are planted with citrus (Anon., 1978).

Except in some places, i.e., the southern part of West Java and the northern part of West Borneo, mandarin trees are not planted as special citrus orchards, but are usually mixed together with other crops. Citrus plantings are scattered throughout the larger islands of Sumatra, Java, Madura, Borneo, Sulawesi, Bali, Lombok, etc. There are varieties that can be grown well at lower altitudes, like the Siam, but there are also good varieties that can grow and give good quality fruit at altitudes of 700 to 900 m like the well-known Garut variety. Therefore, the farmer has the opportunity to choose varieties suited for his situation.

The gradual, but steady extension of citrus plantings carries with it some problems that are becoming more and more serious. Nearly every citrus grower knows that in some places the original good quality mandarin trees which were many years old declined and were replaced by small, chlorotic citrus trees with marble-sized fruits. The farmer could not realize that introductions of new stock material from certain other areas could endanger the already-established citrus trees, and instead, soil and climatic factors were blamed for the disease situation. The farmers tried to

eliminate the symptoms by putting on more manure and fertilizers, and even by spraying the trees with pesticides. Presently, most of the citrus growers know that these efforts are useless.

The magnitude of destruction is not small. In West Java alone, it was estimated that from 1960 onward, not less than 3 million trees were destroyed (Anon., 1970), and we know that this destruction is still taking place. Research on this problem was begun in 1954 and research workers of the Horticultural Research Institute, the Bogor Institute of Agriculture, and the Padjadjaran University have made contributions.

EFFORTS TO DETERMINE THE PRIMARY CAUSE

Since deficiency symptoms of some macro- and microelements were found on leaves of declining trees nearly everywhere, much was done to correct these symptoms by applying fertilizers containing these elements to the soil or by foliar sprays. Reitsma *et al.* (1954) were optimistic that some carbamates such as Zn-dithiocarbamate, Fe-dithiocarbamate, and Mn-dithiocarbamate could remedy these respective deficiencies. Soil applications of NPK fertilization trials together with microelements were made by some other workers at the Bogor Institute of Agriculture. Some of these trials yielded a more-or-less positive result, but generally it was temporary. After the applications had ceased for some months, the deficiency symptoms reappeared as before.

The idea that there was not only a shortage of some elements, but that there could be other factors causing the deficiency symptoms was suggested by some workers. Reitsma and Hadiwidjaja (1955) stated, "Though as a rule poor drainage and hardpan are the main fac-

tors which lower the vitality of the trees, giving rise to injury of the rootlets by secondary parasites, malnutrition, on the other hand, resulting from insufficient or inadequate fertilization may indirectly intensify the severity of the disease." Indeed, this statement was not totally incorrect, i.e., for trees not affected by the disease which will be discussed later.

Tylenchulus semipenetrans. Cobb, a citrus nematode that causes some problems in other citrus regions of the world, was found by Thrower (1956) on citrus roots in several places in Java. Also he found *Xiphinema* sp. He applied Nemagon to correct the general vigor of the trees, but no positive results were obtained. Inoculation trials with *T. semipenetrans* also failed to produce chlorotic symptoms on the inoculated seedlings, although the nematode was established on the roots (Muchtadji, 1959; Liang, 1959; Fachrudin, 1960; and Siregar, 1960).

It was Terra (1951) who first expressed the opinion that the probable cause of the bad vigor of citrus trees in general, and the decline of some varieties on sour orange, in particular, was tristeza virus. Inoculation trials done by Thrower (1959) on lime seedlings indeed proved that tristeza virus was present in the declining mandarin trees tested. For the moment, it seemed certain that tristeza was to blame for the destruction of so many trees. However, if one considers the problem more closely, symptoms manifested on indicator plants such as vein clearing, vein flecking, and stem pitting merely indicate the presence of tristeza, and these symptoms are by no means generally found on declining mandarin or other citrus trees, except perhaps on limes. So these results did not yet prove that the decline was caused by tristeza. Proof that the decline was caused by a virus or a complex of viruses required that certain symptoms generally present on declining trees could be reproduced constantly on inoculated seedlings of the same variety.

Tirtawidjaja (1964) showed that

certain symptoms could be reproduced consistently on graft-inoculated seedlings of several species and varieties, including the variety of mandarin declining in the field. At that time, there was no knowledge about mycoplasmas causing plant disease, so it was concluded that the cause of the constant and persistent symptoms was a virus or a complex of viruses (Tirtawidjaja *et al.*, 1965).

Owing to the absence of similarities with symptoms and other properties of known virus diseases at that time, the authors concluded that this was a new virus disease. It was named "Citrus vein phloem degeneration disease," for the most specific symptom, the collapse of certain cells of the phloem in leaf veins.

SYMPTOMS AND DISTRIBUTION OF CITRUS VEIN PHLOEM DEGENERATION DISEASE (CVPD)

The constant features of the disease, discovered during and after the inoculation trials at Bogor are described below.

External Symptoms. Leaves turn yellow on a part or on the whole crown. The yellow leaves are more or less stiff, leathery, and upright on twigs and branches. Blotching may be present, but it is not a constant symptom. Small veinlets (not mid- or main veins) of the yellow leaves are usually contrasted against the yellow or light-green color of the leaf blade. These are mature leaf symptoms and one has to be careful not to confuse them with yellow leaves on girdled twigs or branches.

Internal Symptoms. The phloem of the mature, yellow or chlorotic leaves is much thicker than normal (not so the phloem on young leaves). Sieve tubes and companion cells are collapsed, and form white bands extending from the sclerenchyma to the xylem. Ray cells remain intact, but are filled with abnormal quantities of starch granules. These symptoms are readily observed microscopically on transverse sections of midveins.

The external and the internal symptoms were used for diagnosis of CVPD,

first in Java and later in other islands of western and central Indonesia. In 1964, the disease was found widespread in several places in west Java (Bogor, Jakarta, Bandung, and others) and was also found in central and southern parts of central Java (Jogyakarta, Cilacap) and in certain areas of east Java (Malang).

Further surveys in recent years revealed that CVPD is not confined to the island of Java, but is also present in several places in Sumatra. Nearly all provinces of the island have citrus areas with declining trees which show the characteristic symptoms. On the other hand, no indication of the disease was encountered in the citrus areas of west Borneo, south Borneo, Sulawesi (Celebes), Madura, Lombok, and others, except on limited numbers of trees in some yards of Pontianak and Ujungpandang (Makassar).

So it is Sumatra and Java that suffer the most from CVPD. This is probably due to the fact that Pasarminggu (Jakarta) was and still is functioning as a center of distribution for citrus nursery stock, and Pasarminggu is blamed for the distribution of CVPD in Java and Sumatra. A certain mandarin variety was once known only in Pasarminggu, but now it is widely grown throughout Java and Sumatra.

The spread of CVPD was further enhanced by the acquired ability of nurserymen to produce budlings. In certain places, which are CVPD-endemic areas, hundreds of thousands of mandarin budlings are being produced, sold and distributed to new and old citrus centers. The Garut area is the most important source of CVPD-affected nursery stock. With past experience that one could obtain more income from citrus than from other crops, but without the new knowledge that CVPD can wipe out whole areas of citrus, this nursery material is being used extensively, especially in Java and Sumatra.

FURTHER STUDIES ON CVPD

In 1967, some studies were resumed with the main purpose of finding means of control. In this respect, some proper-

ties of the disease were revealed. Almost all of the following findings are written in reports to the Horticultural Research Institute (Tirtawidjaja, 1968-1977) in the Indonesian language. Some of them are presented here:

- (1) Pruning and removal of symptom-bearing twigs and branches did not prevent the spread of the disease in a tree.
- (2) Using small mandarin seedlings as test plants, it was found that *Diaphorina citri* alone could not transmit the CVPD symptoms. Inoculations with *Toxoptera citricida* and *D. citri* reproduced CVPD symptoms.
- (3) Electron microscopic observations indicated that, in addition to slender, flexuous viruslike particles, there were also mycoplasma-like bodies in the phloem of midveins from chlorotic CVPD-affected leaves.
- (4) CVPD symptoms are usually accompanied by stem pitting in the rootstock.
- (5) Inoculation trials with tissues from different parts of a CVPD-affected tree revealed that the most infective inocula were from ends of small, yellow twigs and from midveins of chlorotic leaves.
- (6) No resistant species or varieties of citrus are available in Indonesia as marcots, budlings, or seedlings.
- (7) Although CVPD is widespread in Java and Sumatra, CVPD is not endemic in all citrus localities.
- (8) Isolated citrus orchards and nurseries owned by the government could be freed from CVPD by eradication, and by spraying the replants with insecticides.
- (9) Sprays of tetracycline antibiotics did not improve conditions of inoculated seedlings substantially.

Based on some of these findings, we have come to a conclusion that, at least in theory, CVPD could be controlled by the following: (1) Enforcement of domestic quarantine to exclude the disease from islands not yet affected, i.e., Borneo, Sulawesi, Madura, Lombok, Salayar, etc. (2) Prohibition of private nurseries in endemic areas. (3) Roguing of CVPD-affected trees in nonendemic citrus areas. (4) Total eradication of citrus trees in endemic areas and prohibiting replants until at least 2 years after the eradication of the last trees.

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