Presence of Seedling Yellows Complex in the Citrus of South India

D_{ECLINING} CITRUS TREES in commercial groves in Coorg (Mysore State), in Ahmednagar district (Maharashtra State), and in Andhra Pradesh showed a variety of symptoms. Most of the trees were affected by tristeza virus (2), which was recorded in India on Mosambi sweet orange [*Citrus sinensis* (L.) Osbeck] for the first time in 1958 (8). The presence of tristeza virus was demonstrated by its characteristic reactions on inoculated seedlings of sweet orange on sour orange (*C. aurantium* L.) rootstock or on seedlings of Key lime [*C. aurantifolia* (Christm.) Swing.]. Later, the Indian Kagzi lime (*C. aurantifolia*) was used as a test plant (1).

McClean (5) in 1960 made a distinction between *tristeza*, *seedling* yellows, and *lime reaction* in order to avoid confusion in describing symptoms or host reactions of the so-called tristeza virus complex. Thus, the *lime reaction* indicates only the presence of the tristeza virus complex but does not identify the various components of that complex. Since the relationship between stem pitting of grapefruit, seedling yellows, and tristeza has been reviewed by Wallace and Drake (7), the literature dealing with this relationship will not be summarized here; pertinent references are cited by Wallace and Drake.

The results of investigations carried out at Poona for the past eight years have conclusively shown that seedling yellows virus exists in citrus groves in peninsular and southern India. The experimental results obtained are described briefly and the relationship of seedling yellows is discussed in the light of the observations made.

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Experimental Results

Indication of the presence of the tristeza seedling yellows complex was first obtained in 1955-56 in some of the Mosambi sweet orange trees growing on rough lemon (C. *jambhiri* Lushington) rootstock in orchards in the Deccan (8). These trees, about 12 years old, showed severe symptoms of decline and when indexed on seedlings of Standard sour lemon [C. *limon* (L.) Burm. f.] produced in them typical seedling yellows symptoms as first described by Fraser (3). Symptoms consisted of chlorotic patches followed by vein yellowing on subsequently developed leaves after about four months. Further growth of affected seedlings completely ceased and their leaves gradually turned yellow, and after nearly 13 months of deterioration they collapsed suddenly. Similarly, sour orange seedlings developed typical seedling yellows symptoms within three months of inoculation by budding. The lime reaction was given on inoculated seedlings of Key lime (8).

Following these observations, citrus trees in groves in different localities were indexed on seedlings of sour orange, grapefruit (*C. paradisi* Macf.), and Eureka lemon for the seedling yellows reaction. Several Mosambi sweet orange, mandarin (*C. reticulata* Blanco), and Kagzi lime trees in Coorg and Andhra Pradesh, and Mosambi sweet orange trees in the Deccan were found to carry the seedling yellows virus.

Sources of Seedling Yellows Virus

MANDARIN ORANGE.-The mandarin orange in Coorg is mostly grown as a seedling on its own roots. Although this species is susceptible to tristeza virus (2), it is comparatively tolerant and trees 30-40 years old, though infected, produce a fairly good crop. Some trees, however, showed severe symptoms of decline, including leaf rolling and yellowing, twig decline, heavy leaf fall, and severe stem pitting (Fig. 1, c). A tendency to develop stem pitting as a result of tristeza virus infection is usually absent in the mandarin group, though a very few pits were seen in 1 or 2 per cent of mandarin trees in Brazil (4). Material from these trees when grafted onto seedlings of Eureka lemon, sour orange, and grapefruit gave the typical seedling vellows reaction and, on seedlings of sweet orange on sour orange roots, a typical tristeza reaction. When seedlings of Key lime and Kagzi lime (1) were budded with this source, they developed the lime reaction. As reported by McClean (5), no recovery has yet been observed either in the budded grapefruit seedlings, which have remained in a severe state of decline for more than 21/2

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FIGURE 1. a. Severe yellows and decline in a grapefruit seedling that was budded with buds from a seedling yellows source (photograph taken 2½ years after budding). b. Mosambi sweet orange seedling with distorted young shoots following infection with seedling yellows virus. c. Trunk of Coorg mandarin infected with seedling yellows virus, decorticated to reveal stem pitting. d. Trunk of seedling-yellows-infected calamondin on rough lemon rootstock, decorticated to reveal bud-union necrosis.

years (Fig. 1, a), in Eureka lemon, or in sour orange. The Key lime developed a severe decline, deep stem pitting, which heavily corroded the wood, and unusually thick bark, and the leaves showed corking of the veins.

Yellowing of veins and veinlets in leaves of Key lime budded with a source of seedling yellows virus is usually of a very severe type and is accompanied by general chlorosis of leaves and chlorotic splashes between veins. On the other hand, the seedlings that were budded with material from trees showing typical tristeza but free of the seedling yellows virus developed a mild yellowing of veins and veinlets and mild stem pitting, and were not much dwarfed.

Budlings of calamondin (C. madurensis Loureiro) on rough lemon rootstock when budded with the mandarin orange budwood carrying seedling yellows virus grew well and produced a heavy flush of fruit one year after infection. When the second flush of fruit was developing the plant showed signs of wilting, but it recovered from this sudden shock. It then made no apparent growth and symptoms of decline gradually became prominent. Finally the plant collapsed two years after infection. The trunk when examined after removing the bark showed a brown ring at the bud-union (Fig. 1, d), which suggested that wood necrosis

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of the bud-union was perhaps the cause of death of the calamondin top. On the other hand, budlings infected by budding from another source of virus known to be free of the seedling yellows virus developed typical decline symptoms and remained extremely stunted, but continued to grow for six years. This plant consistently gave the characteristic lime reaction in Key lime and the tristeza reaction in budded sweet orange on sour orange root, but not the seedling yellows reaction in Eureka lemon or grapefruit seedlings.

In contrast, nucellar seedlings of calamondin graft-inoculated with seedling yellows virus reacted differently from those inoculated with tristeza virus alone. Those infected with the seedling yellows virus were much dwarfed and had very little growth as compared with the others.

KAGZI LIME.—Most of the trees of Kagzi lime in Coorg were showing severe decline symptoms and usually were extremely stunted. The entire tree appeared palish green, its twigs and branches had restricted growth, and the wood of stem and branches was heavily pitted. Even 1-year-old twigs had numerous stem pits. All these Kagzi lime trees gave a positive reaction in Key lime and the tristeza reaction in budlings of sweet orange on sour root. On indexing in seedlings of Eureka lemon, sour orange, and grapefruit, they showed the presence of seedling yellows virus. Kagzi lime growing in a grove in Poona also showed severe decline symptoms and gave positive lime, tristeza, and seedling yellows reactions.

MOSAMBI SWEET ORANGE.—Declining Mosambi sweet orange trees in the Deccan are usually infected by tristeza virus, which on indexing give positive lime and tristeza reactions. But a few trees on rough lemon rootstock in a plantation at Anandnagar, Andhra State, and at Rahata, Bombay State, showed severe symptoms of decline. These trees, when indexed in seedlings of lemon, sour orange, and grapefruit were also found to carry the seedling vellows virus.

EUREKA LEMON.—One nucellar seedling of Eureka lemon was planted near a Coorg mandarin budling in the orchard of the Station. The Coorg orange budling was prepared by budding from a mandarin tree in Coorg known to carry the seedling yellows virus. The Eureka lemon was originally raised from seed inside a glasshouse and evidently did not carry any virus. After about four years of growth the lemon tree produced its first flush of fruit and showed symptoms of leaf roll, mild vein clearing, and leaf fall, and pitting of the wood of the stem. On indexing it gave a positive lime reaction and also the tristeza reaction. It was then indexed on seedlings of sour orange, grapefruit, and Eureka

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lemon, which developed typical seedling yellows symptoms. Evidently, this plant must have been infected with the seedling yellows virus by the aphid vector, *Toxoptera citricidus* (Kirk.), which is very active during spring and winter seasons in Poona.

OTHERS.—Seedlings of Rangpur lime (C. limonia Osbeck), rough lemon, Mosambi sweet orange, and trifoliate orange [Poncirus trifoliata (L.) Raf.] were inoculated by budding from the Eureka lemon plant mentioned above. Rangpur lime developed patchy chlorotic mottling of leaves, rough lemon showed appreciable retardation in growth, sweet orange developed distortion and curling of young leaves and very severe reduction of growing twigs (Fig. 1, b), and trifoliate orange first showed faint vein clearing and later chlorosis of younger leaves. These symptoms are considered to be distinct from those induced by the typical tristeza virus in these species. The disease syndrome in Rangpur lime, rough lemon, trifoliate orange, and Mosambi sweet orange induced by the seedling yellows virus has not been previously recorded. Fraser (3) reported that seedlings of most sweet orange and mandarin varieties, rough lemon, and Rangpur lime are insensitive to seedling yellows and rarely show symptoms.

Discussion

The data presented prove the existence of seedling yellows virus in certain declining trees of Coorg mandarin, Kagzi lime, Mosambi sweet orange, and Eureka lemon in groves in peninsular and southern India. Results of preliminary tests also establish new and previously unrecorded reactions to the seedling yellows virus. As has already been reported earlier (3), the stem pitting disease of grapefruit of the type prevailing in South Africa, Argentina, Brazil, and Australia does not occur in India. Tristeza virus causes typical decline of grapefruit trees on rough lemon rootstock, and a mild type of stem pitting, or none at all, in the wood of affected grapefruit trunks. On no occasion were grapefruit trees found to be affected by stem pitting of the type originally described by Oberholzer et al. (6). It is evident, therefore, that the seedling yellows virus in peninsular and southern India is free of the stem pitting virus and that it can by itself cause a serious decline of citrus trees. The virus induces the characteristic tristeza reaction (decline of budded sweet orange on sour orange roots), the lime reaction (veinal flecking and wood pitting in Key lime seedlings), and seedling yellows in Eureka lemon, grapefruit, and sour orange seedlings.

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The data presented also conclusively show that seedling yellows is caused by a severe strain of tristeza virus. Another strain of the same virus that has been obtained does not produce seedling yellows but gives the lime reaction in Key lime as well as the tristeza reaction in budded indicator trees. In citrus groves in peninsular and southern India the seedling yellows strain prevails in certain regions, whereas in other regions, the citrus species are affected mostly by a strain of tristeza virus. This is additional evidence of relationship between tristeza and seedling yellows viruses.

The above observations as well as the mass of evidence put forward by other workers suggest that tristeza is not a complex disease but is caused by a single virus that exists in nature in the form of numerous strains. Accordingly, tristeza, stem pitting, seedling yellows, and lime dieback are induced by different strains of tristeza virus. These strains are widely distributed in the world and one or another is prevalent in certain zones and predominates on certain species or variety of citrus.

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