CITRUS VIRUS DISEASE RESEARCH IN EGYPT

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INTRODUCTION

In 1955, J. F. L. Childs *et al.* (*3*), under the auspices of the International Cooperation Administration and with the cooperation of the Plant Pathology Section and the Department of Horticulture of the Egyptian Ministry of Agriculture, conducted a survey of citrus diseases in Egypt. This survey covered the most important centers of citrus production in the Nile River Valley. The search for citrus virus diseases and viruslike disorders was given special attention during examination of the citrus orchards.

This paper is presented to give an account of the survey and of the citrus virus disease research now under way in Egypt. It covers mostly the diseases which we have been able to transmit, and other disorders which we are inclined to consider as viruslike diseases. It also includes a discussion of the citrus budwood certification program and its progress.

VIRUS AND VIRUSLIKE DISEASES

Psorosis. The first report of the presence of a citrus disease in Egypt (later proved to be due to a virus) was by Fawcett (*4*), who found psorosis-affected trees there in 1931. Later, in 1936, R. W. Hodgson, in a report to the Egyptian Ministry of Agriculture (unpublished), mentioned the same disease.

Only during the past nine years have we begun to recognize citrus diseases as a danger to the citrus industry in Egypt. In 1948, psorosis was reported by M. Abdel Salam and A. Omar (unpublished) as a disease present in sweet orange orchards. Since then some surveys have been made in our citrus orchards for the presence of the psorosis disease. From 1949 to 1953 the surveys were conducted to determine the percentage of psorosis-infected trees in the governmental orchards and in privately owned estates throughout Egypt, with the objective of finding psorosis-free trees to be used as sources of psorosis-free budwood (A. Omar *et al.*, unpublished reports). The surveys were made during the spring by examining the new growth of trees that showed no bark scaling. Such surveys revealed that most of the trees examined displayed typical young-leaf symptoms of psorosis. Only 13 trees out of more than 5,000 examined did not show young-leaf symptoms. The symptomless trees were tested in a greenhouse for the presence of psorosis by bud-inoculation from them to sour orange seedlings cut back to stimulate new growth. Periodical examinations of new growth were made. This test revealed the freedom of these trees from the psorosis disease. These psorosis-free trees were considered as parent trees and were used as the start of a budwood certification program. Propagations were made from these psorosis-free trees on rootstocks of sour orange seedlings from seeds taken from psorosis-free sour orange trees. These progeny trees (future parent trees) were planted in four government nurseries, one in upper Egypt and three in lower Egypt (in the Nile River Delta).

Later, in 1955, Nour-Eldin *et al.* (unpublished reports) found, on examining these psorosis-free parent trees, that they exhibited phloem discoloration, stem pitting, and

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splitting of the bark of the scions. Although these disorders have not yet been proved to be due to viruses, this remains a possibility. It was therefore decided to discontinue the attempt to establish sources of budwood from existing orchard trees. It seemed preferable to use mature nucellar seedling trees of sweet orange and mandarin as the sources of budwood for new plantings. This new approach to the budwood certification program will be discussed later.

From this and the previous surveys, it was found that more than 90 per cent of Egypt's budded citrus trees are infected with psorosis virus. Three types—psorosis A, concave gum, and blind pocket—were noted. It should be mentioned that more than 60 per cent of the old sweet orange trees do not show bark symptoms of psorosis A. Psorosis examinations were therefore based on young-leaf symptoms. It can be said that psorosis is the most widespread citrus disease in Egypt.

Besides psorosis, several other diseases were discovered in this later survey. Before discussing these diseases, I should like to call attention to the fact that we have not yet obtained evidence of transmission of these disorders. We began our transmission experiments only about two years ago. Although our transmission tests have not yet given any results, we are inclined to believe that these newly found disorders are caused by viruses. Some of them resemble, on a symptom basis, other diseases which have been transmitted successfully in other countries and reported to be caused by virus infection. So, for the sake of simplicity, I shall take the liberty of calling these diseases by the name of similar diseases which have been proved to be bud-transmitted.

Cachexia. In Egypt, Beladi mandarin trees were found with symptoms which appear to be identical with those described in 1950 by Childs (*1*) in Florida on Orlando tangelo trees affected by cachexia. By scraping the bark across the bud union, the phloem tissue of the Beladi mandarin scion was found to be impregnated with gum. On the other hand, no phloem tissue of the sour orange rootstock was discolored. This disease is also characterized by conoid pits in the cambial face of the wood of the Beladi mandarin scion due to the suppression of xylem tissue formation at these points. Coinciding with these pits, there are pegs on the bark. Only slight stem pitting is exhibited by the sour orange rootstock (fig. 1, *A*). It has been found that more than 60 per cent of the Beladi mandarin trees budded on sour orange are affected by this disease.

Phloem Discoloration of Sweet Orange. This disease was first noted in 1954 on Beladi sweet orange trees budded on sour orange rootstock (*5*). Later, Childs *et al.* (*3*) found it on navel, Valencia, Sukkary, Khalily White, Beladi Blood, and Sanguinoval sweet orange varieties. About 40 per cent of the trees examined exhibited the phloem discoloration symptom. This is sometimes accompanied with stem pitting of either a conoid or a channeling type (fig. 1, *B*, *C*). Neither phloem discoloration nor stem pitting was ever seen on the sour orange rootstock.

I have used this name "phloem discoloration" to describe the actual symptom characterizing this disease. We felt at first that this disease might be due to the same causal agent that produces cachexia symptoms on Orlando tangelo, but in other studies by Childs (*2*) cachexia disease failed to induce any symptoms on several sweet orange varieties. This gave us the idea that phloem discoloration might be caused by another agent rather than by the one that causes cachexia. At any rate, we cannot confirm this difference until our transmission and host-range tests have been completed.

Xyloporosis. This disease, first reported by Reichert and Perlberger (*6*) in Palestine, is widespread wherever sweet lime grows, either as a tree budded on sour orange or as a rootstock for the Shamouti sweet orange variety. In Egypt, more than 50 per cent of the sweet lime trees budded on sour orange rootstock exhibit stem pitting of the scion just above the bud union but not below, on the sour orange rootstock. Sometimes the cambial face of the wood of the sweet lime scion just above the bud union

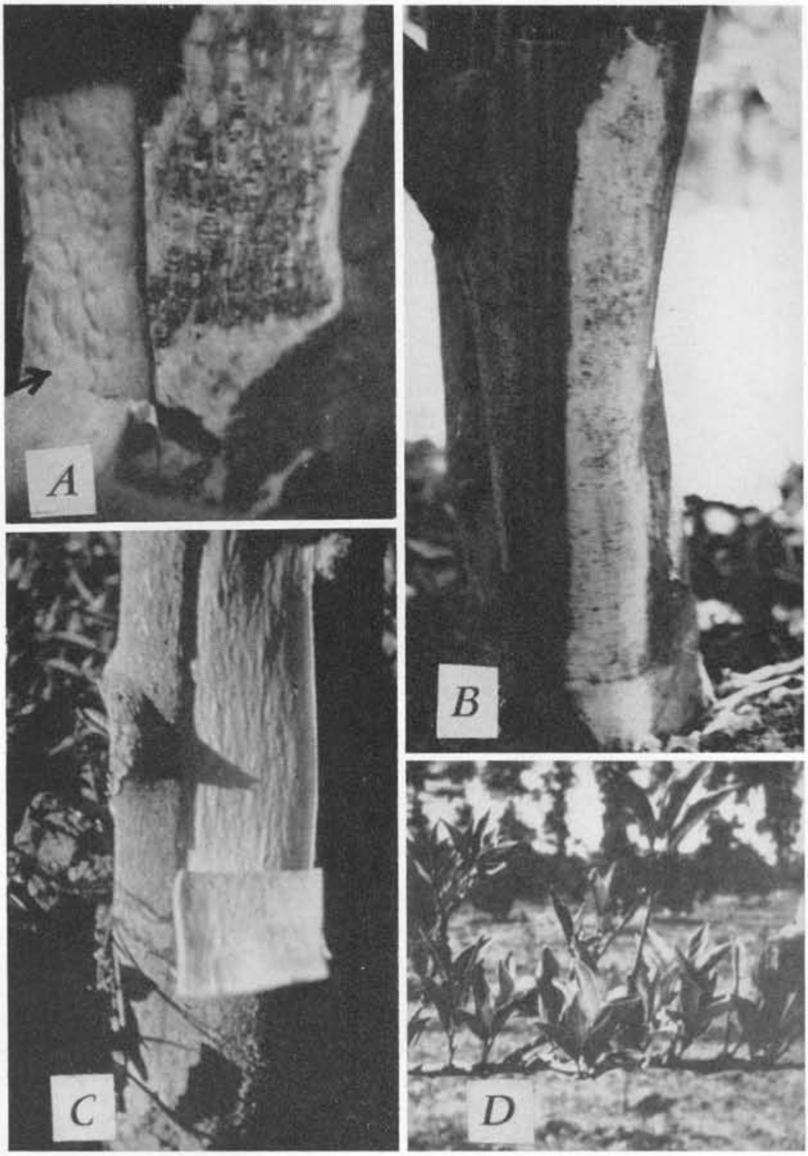

Fig. 1. A) cachexia-like symptoms on mandarin. Note phloem discoloration where outer bark is removed at right, and heavy pitting of wood in peeled area at left, with less pitting and no cachexia discoloration on sour orange below bud union (arrow). B) local sweet orange variety on sour orange rootstock, showing cachexia symptoms in sweet orange bark. C) pitting in wood of local sweet orange on sour orange rootstock. D) small, stiff, upright leaves of Safargali sweet orange affected with stubborn disease.

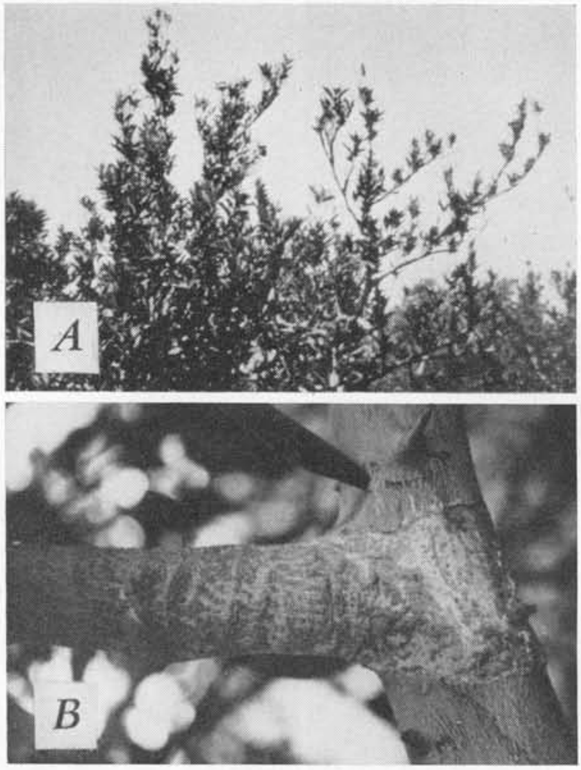

Fig. 2. A) "crazy-top" symptoms on a sweet orange tree affected with stubborn disease. B) "rusty bark sloughing" on sweet lime tree grown from a cutting.

is marked with projections that coincide with and fit into pits in the cambial face of the bark. Moreover, the bark tissues are always impregnated with gum, and splitting of the bark of the sweet lime scion is very characteristic of this disease.

Most of the Shamouti sweet orange trees are budded on sweet lime rootstock, and more than 60 per cent of them are affected with this disease, which causes them to be unthrifty and unproductive. The sweet lime rootstock exhibits the same symptoms mentioned above, whereas the Shamouti sweet orange scion usually exhibits bark pitting but no stem pitting above the bud union.

Stubborn Disease. This disease was first recognized in Egypt on declining trees of a variety of sweet orange called "Safargali." Propagation of this variety is now prohibited by governmental law because of its poor characteristics. The trees of this variety have a bushy type of growth and are flat-topped. Some of the limbs tend to grow in a horizontal manner, producing stunted upright twigs that have rather short internodes and small, stiff, rolled leaves (fig. 1, *D*). One of the most characteristic types of growth of this variety is that described as "crazy top," with some of the top branches growing upright and giving the tree a very irregular shape (fig. 2, *A*). Such trees are also characterized by low production of small fruits in which creasing of the rind is common. The blue albedo symptom of fruits has not been found on the trees thought to have stubborn disease.

Rusty Bark Sloughing. A disorder which has been given the name "rusty bark sloughing" was noticed on sweet lime branches and sour lemon limbs. The outer layers of the bark slough off as minute reddish scales. It begins as areas of a few square centimeters on small branches and then spreads upward and around the entire circumference of the branch (fig. 2, *B*). Usually, there is some bark splitting which exposes localized areas of wood.

OTHER DISORDERS OBSERVED ON TREES IN A ROOTSTOCK EXPERIMENT

More bud-union disorders have been observed on examining a 20-year-old rootstock experiment in an area of about 10 acres at Sids. It was interesting to study the reaction of the different rootstocks to the diseases present, because our previous studies for the most part had been limited to trees on sour orange or sweet lime rootstocks.

These bud-union abnormalities cannot be said to be caused by viruses until transmission tests are completed. The most important abnormalities noted in this experiment are described below.

Bud-union Creasing of Sweet Orange on Rough Lemon Rootstock. This bud-union disorder is exhibited on more than 90 per cent of the examined trees of Beladi sweet orange variety budded on Rough lemon rootstock. This creasing is exhibited at the bud-union line and can be noticed when bark is removed from across the bud union (fig. 3, *A*). An irregular and almost continuous ring of projections is present on the cambial face of the bark at the bud-union line, which fits into the crease in the wood (fig. 3, *B*). The length of these projections varies from 2 to 8 mm, and gum deposits are present in the phloem tissue of these projections. Stem pitting and phloem discoloration have been observed occasionally on the Rough lemon rootstock, but not on the sweet orange scion.

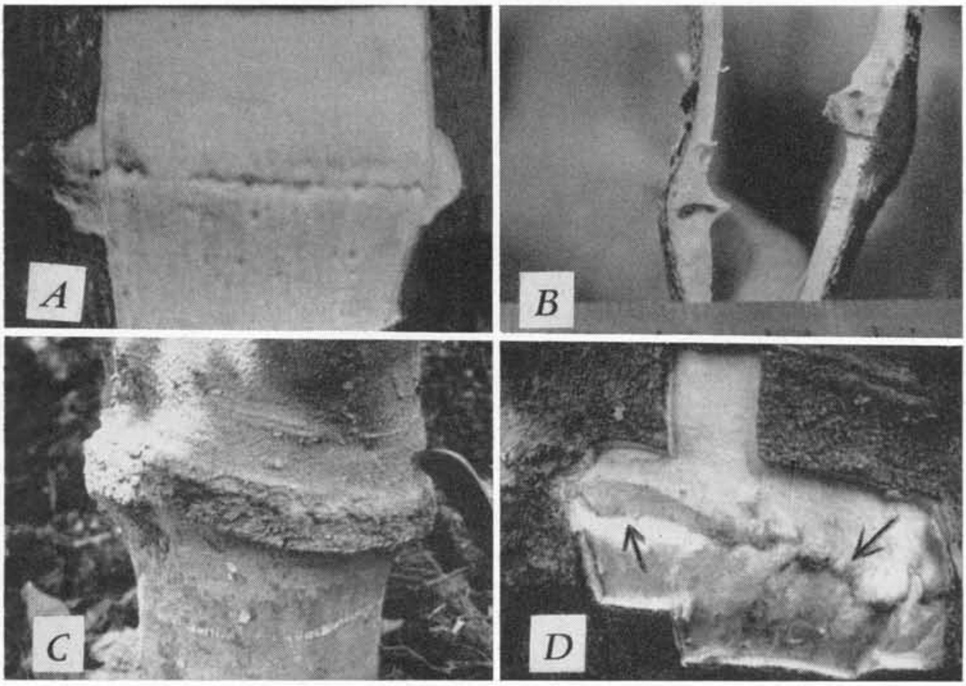

Fig. 3. Bud-union disorders on citrus in Egypt: A) creasing in wood at bud union of sweet orange on Rough lemon rootstock; B) bark strips removed from across bud union of sweet orange on Rough lemon rootstock, showing gum-impregnated projections at union; C) bud-union abnormality of blood orange on Rough lemon rootstock; D) bark removed from bud union of blood orange on Rough lemon rootstock, showing overgrowth of scion wood and a layer of gum at junction of scion and stock (indicated by arrows).

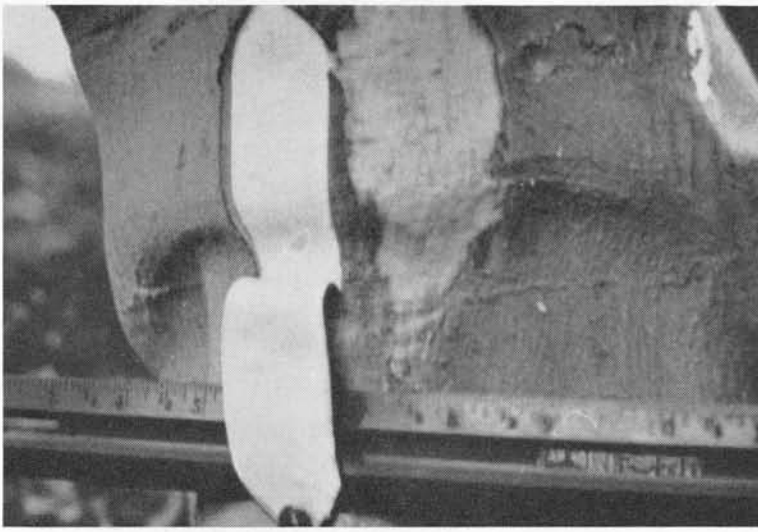

Fig. 4. Abnormal overgrowth at bud union of sweet orange on sweet orange rootstock.

Bud-union Creasing of Blood Orange on Rough Lemon Rootstock. This abnormality differs in external and internal symptoms from the creasing of Beladi sweet orange trees budded on Rough lemon rootstock described above. In this disorder the location of the bud union is usually determined by a very pronounced bulge with a very rough surface (fig. 3, *C*). The diameter of the scion is larger than that of the rootstock. Removal of the bark across the bud union exposes an overgrowth or folding of the wood of the scion onto the stock, more or less embedding the bud union. On cutting through the wood across the bud union, a layer of discolored material (presumably gum) can be seen between the scion and rootstock (fig. 3, *D*). Application of iodine solution to the wood tissue across the bud union resulted in a positive reaction by the scion wood, but the wood of the rootstock was free of starch. These symptoms have been found in more than 80 per cent of the trees of blood orange budded on Rough lemon rootstock.

Bud-union Overgrowth of Sweet Orange on Sweet Orange Rootstock. In this disorder, the scion bulges out above the bud union, giving an idea of incompatibility (fig. 4), but surprisingly it is present in Beladi sweet orange trees budded on Beladi sweet orange rootstock. More than 40 per cent of the trees examined of this scion-rootstock combination showed this abnormality. On examining these trees, some were found to exhibit discoloration of phloem, stem pitting, or both. Other trees did not show any symptoms that we could detect. On applying iodine solution for the detection of starch in the wood tissue across the bud union, it was demonstrated that the wood of both scion and rootstock had starch in equal amounts.

Stem Pitting of Grapefruit Rootstock. In this disorder, conoid stem pitting was noticed on Marsh Seedless grapefruit rootstock budded with navel orange. The scion exhibited the same pitting. Moreover, the scion and rootstock exhibited phloem discoloration. On testing such trees for the presence of tristeza on Mexican lime seedlings, we did not get any positive results.

THE STATUS OF TRISTEZA IN EGYPT

In a variety collection maintained by the Ministry of Agriculture at the Barrage Experiment Station, four declining trees showing the honeycomb type of pitting below the bud unions were found in October 1957. Two of these were Bergamot orange on sour lime rootstock, one was Tanarif sweet orange on sour lime rootstock, and the fourth was Valencia on what was thought to be sour orange rootstock. These four trees were indexed to Mexican lime and Beledy lime. All inoculated lime seedlings later developed vein-clearing symptoms typical of tristeza (fig. 5). Inoculations from four normal trees in this planting gave negative results.

This is the first indication we have had that tristeza virus is present in any citrus in Egypt. It has not been found in any commercial plantings and is apparently not being spread to any detectable extent by aphid vectors. The details of this discovery of tristeza will be published elsewhere.

THE BUDWOOD CERTIFICATION PROGRAM

As mentioned before, the budwood certification program was dependent at the beginning on budded orchard trees. Later, the parent trees and progenies which were found by test to be psorosis-free exhibited several disorders suggesting the presence of other virus diseases, such as phloem discoloration, stem pitting, and splitting of the bark of sweet orange and mandarin scions. Such findings called for another approach to the problem of locating virus-free citrus material.

In Upper Egypt, in Assiut, Gerga, and Quena Provinces, it is customary to grow sweet orange and mandarin trees from seeds. About 2,000 acres of such seedling trees are growing in these three provinces. Some of these orchards are more than fifty years old. These seedling trees are now considered to be the solution to the budwood certification program. The methods we are using in this program can be summarized as follows.

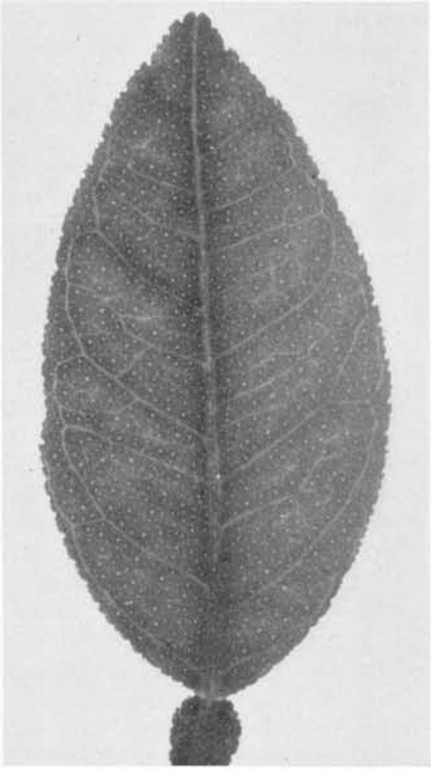

Fig. 5. Tristeza vein-clearing symptoms on leaf of Mexican lime seedling. These symptoms are typical of those obtained by inoculations from four declining trees in a variety collection at the Barrage Experimental Station, Egypt.

Prospective parent seedling trees should be in an orchard in which no budded trees are grown. Such trees are examined first from the horticultural point of view. If a tree is found to have desirable characters and is in good condition, it is then inspected from the disease aspect. Such a prospective parent tree should be free from foot rot, gumming diseases, and bark disorders. Because of the great effort needed to examine these huge, thorny seedling trees, we do not examine them for young-leaf symptoms of psorosis, but depend on the tests we conduct in the greenhouses. From trees that pass this preliminary inspection, budwood is taken separately and budded on the following eight citrus varieties to test for the presence of the virus diseases: Orlando tangelo and Beladi mandarin, for cachexia and cachekia-like symptoms that appear on Beladi mandarin; sweet lime, for xyloporosis; Rough lemon, for the bud-union creasing of Rough lemon; trifoliate orange, for exocortis; Rangpur lime, for the Rangpur lime disease; Key lime, for tristeza; and Beladi sweet orange, for psorosis, phloem discoloration of sweet orange, and creasing of sweet orange rootstock. In this test, two seedlings of every test plant are budded with at least four buds from each prospective parent tree. These test plants are grown in 14-inch clay pots.

The use of so many test plants makes it difficult for us because of the space needed and the work involved in budding and keeping records, but we feel that we cannot afford to make any mistakes in such a long-term program. Until we know the relation between all the citrus virus and viruslike diseases, we think it is worthwhile to be on the safe side and to consider every disorder a virus disease.

In the meantime, we cut budwood from these prospective parent trees and bud it on sour orange rootstock in a government nursery. These sour orange seedlings are raised from seeds taken from trees that are free from psorosis. One year after budding, the budlings are removed and planted in government orchards located in the centers of citrus-growing areas. These trees are planted 7 meters apart and are left for further inspections and until horticultural and disease records of the parent trees are completed.

This approach to the problem began in June 1955, when Dr. Childs and the author obtained budwood from three especially desirable seedling trees (Beladi sweet orange, Sukkary sweet orange, and Beladi mandarin) growing at Assiut. The buds were propagated on sour orange rootstock. From this, we had a total of 187 trees budded from nucellar seedlings.

Later, about 200 nucellar seedling trees were selected for preliminary horticultural and pathological inspection. During 1956 and 1957, about 15,000 progeny trees were propagated from these 200 prospective parent trees. These progeny trees are now planted in three different orchards located at Sids in Upper Egypt, at Wadi El Natroun on the western side of the Nile River Delta, and at the Barrage Experiment Station at the head of the Delta.

These progeny trees are of the following varieties: Beladi orange, Sukkary orange, blood orange, and Beladi mandarin. We now have about 100 progeny trees originating from a navel nucellar seedling tree. Test plants of 40 parent trees were examined and found to be free of psorosis. Additional examinations are made periodically.

All these progeny trees will be left until they bear fruit. This will take about five to six years. The progenies of every prospective parent tree that passes all the horticultural and pathological tests during a five-year period will be subjected to other horticultural examination for two successive years. Any progeny tree that has horticultural defects or abnormalities will be discarded. This will be the end of the first step of our budwood certification program. From there on it will be a matter of organizing the distribution of this virus-free budwood.

DISCUSSION

With this miscellany of diseases, abnormalities, and inconsistencies of symptom appearance, we feel that we are dealing mostly with different virus complexes rather than with single virus diseases. Budding and rebudding of the rootstock seedlings in the nursery, and top-working of old infected trees with scion wood infected with one or more viruses, have brought us to this situation of having a mixture of viruses in most of our citrus.

It appears that the solution of our virus disease problems of citrus in Egypt will come chiefly through the use of virus-free nucellar lines. These must now be started from seeds and carried through the required years to bring them to the condition of growth and productivity desired for commercial use. Some progress is being made by selection and testing of old seedling trees in established plantings. However, it is necessary to index these latter trees to determine that they are free of viruses. This can be done over a period of time for such known diseases as psorosis, tristeza, exocortis, and xyloporosis; but for some of the other possible virus diseases described in this paper, indexing techniques have not been developed.

Some studies are now being undertaken to learn if viruses can be detected in citrus by paper chromatography techniques. A chemical or colorimetric test for these viruses would prove most useful, but until such tests become available, the usual inoculation methods of indexing must be relied upon to find sources of citrus propagative material that is free of the disease-causing viruses.

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