# AN INDEXING PROGRAM TO AVOID VIRUSES IN CITRUS INTRODUCED INTO THE UNITED STATES[1]

**J. M. Wallace and R. J. Drake**[2]
University of California Citrus Experiment Station,
Riverside, California

## INTRODUCTION

Citrus is not native to any of the countries of the world which today are leading producers of citrus fruits. In these countries the citrus industry has been established and to a large extent maintained by the importation of citrus varieties and selections from other parts of the world. The improvement of citrus or the production of new selections or types by the usual plant-breeding techniques is slow and uncertain. Naturally occurring changes arising by bud mutation, and to some extent by natural hybridization, have provided valuable citrus types much oftener than planned experimental procedures.

Until about thirty years ago, there was free exchange of citrus seeds and plants or propagative material between citrus-producing countries. This permitted the collection in a given country of a wide assortment of citrus species, varieties, and selections for trial as to their value as fruit types, rootstocks, or use in breeding programs. However, as citrus moved from its natural habitat in the Far East to other parts of the world, it carried with it certain fungus and virus diseases and insect pests. Some of these no doubt originated in the native home of citrus, while others were perhaps acquired as citrus moved from one country to another.

It is unnecessary to review the reasons why most citrus-producing countries eventually set up certain quarantine restrictions to prevent the indiscriminate introduction of citrus propagative material from other parts of the world. Such restrictions were originally designed to prevent the introduction of bacterial and fungal diseases and insect pests. As knowledge and understanding of virus diseases developed and after it was learned that some viruses can be carried by certain host species in a latent form, quarantine regulations were made more strict. Because of the recognized danger of virus diseases, the importation of new citrus varieties into California during the past twenty-five years has been restricted to seed importation. This was considered a safe procedure because studies of some of the known citrus viruses had shown that even though the mother plant is infected, the viruses are seldom transmitted to its seedling progeny.

There are several disadvantages in establishing a new citrus variety from a distant country by seed, even though most varieties produce nucellar seedlings which are considered to be genetically like the mother plant. The nucellar seedlings are commonly very thorny, with many long, sharp spines. Additionally, the seedlings selected cannot be compared with the parent source to determine their trueness to type. There is usually a delay of ten years or more before the seedlings approach normal fruiting, and it is sometimes even longer before budling trees can be grown from them which will not be objectionably thorny. Furthermore, some citrus types do not produce nucellar seed-

[1] Paper No. 1114, University of California Citrus Experiment Station, Riverside, California.

[2] Plant Pathologist and Principal Laboratory Technician, respectively, Citrus Experiment Station, Riverside.

lings. Finally, some desirable selections are seedless or practically so. None of these disadvantages are encountered when a citrus selection can be established from imported budwood.

The fungi and viruses that cause diseases of citrus have become widely distributed. It is expected that they also undergo changes and that new types or strains will continue to appear from time to time. This requires a constant search for new kinds of citrus to replace those which are proving to be susceptible to certain disease-causing agents. The widespread occurrence of such virus diseases as tristeza, exocortis, and xyloporosis necessitates a revaluation of the rootstocks currently in use. New fruit types for improved quality and production should be constantly sought after. Finally, it is desirable that a wide collection of material be available at citrus research centers for use in breeding programs and for trial when some special need or emergency arises.

In California, such problems as tristeza disease, certain rootstock-scion abnormalities (possibly of virus nature), citrus nematode, citrus replanting, small-sized Valencia fruit, and lemon decline have made it essential that new citrus material be made available for experimental study if the citrus industry is to be successfully maintained. This need is now recognized by citrus growers and citrus research workers, as well as by quarantine and regulatory officials of both the California State Department of Agriculture and the United States Department of Agriculture. Consequently, a cooperative program between the two above-named departments and the University of California is now in progress and permits the introduction of citrus budwood from other parts of the world. It is the purpose of this paper to describe the procedures of this program and the safeguards being taken to avoid the introduction of dangerous diseases.

## EXPERIMENTAL PROCEDURES

The avoidance of fungal and bacterial diseases and of insect pests on imported citrus material does not present any special problem. In the first place, the collector of the citrus budwood to be sent to the United States is usually familiar with these dangers and can select material that is free of infection or infestation. The material is sent under permit to the Plant Introduction Section, U. S. Department of Agriculture, and upon arrival at the quarantine greenhouses in Glenn Dale, Maryland, it is routinely fumigated against insects. Propagations are then made, and the resulting trees are grown there under quarantine and inspection over a period of about two years, to determine that they are free of insect pests, bacterial or fungal diseases, known virus diseases, or any symptoms resembling those of virus infection.

After passing the initial inspection, one or more small budded plants of each introduction are sent to the University of California at Riverside, where further increases of each are made in insect-proof greenhouses to provide sufficient material for the virus-indexing procedures that are to follow. Because of the possibility that the original collector of the budwood may have cut budsticks from more than one tree, increases are made at Riverside from only one budling tree sent from the Plant Introduction greenhouses at Glenn Dale, Maryland. Thus, a single clone of each introduction is indexed and reserved for release if it successfully passes the screening tests. The increases are made by propagating each introduction on seedlings of Rough lemon which have been grown under quarantine conditions.

Two different procedures are used in the indexing:

1) Seedlings of selected species or varieties are grown in the quarantine greenhouses from seeds collected from field trees that have been indexed and shown to be free of the viruses of tristeza, psorosis, and vein enation, and that are believed to be free of other known viruses. When ready for transplanting, the seedlings are selected for uniformity and trueness to type and planted in 1-gallon cans, four seedlings to each can. After these are large enough for inoculation, buds or small scions from the im-

ported selections are grafted to two seedlings of each can, leaving the remaining pair of seedlings as controls. Four seedlings of each index-variety are "inoculated," and four noninoculated seedlings are used for comparison. At the time of inoculation, all seedlings are decapitated at a uniform height, as described for seedling inoculation with the psorosis virus.[3] The varieties used in this test were selected because of their reaction to known viruses, and, although it could not be assured, it was hoped that at least one of the index-varieties would reveal the presence of any new or unknown virus that might be carried in the introductions. The seedling varieties used and the citrus viruses that can be identified on them are listed in table 1.

Table 1. CITRUS VARIETIES USED FOR SEEDLING-INDEX TESTS OF IMPORTED CITRUS SELECTIONS AND THE VIRUSES OF WHICH THEY ARE INDICATORS

| Citrus variety | Viruses indicated | Minimum time required for symptom development |
|---|---|---|
| Mexican lime | Tristeza, psorosis, vein enation | 3–4 weeks |
| Thornton tangelo | Xyloporosis (cachexia) | 2–3 years |
| King mandarin | Satsuma dwarf | 2–3 months (?)* |
| Eureka lemon | Seedling yellows, psorosis | 3–4 weeks |
| Calamondin | Psorosis, tristeza | 3–4 weeks |
| *Citrus excelsa* | Psorosis, tristeza | 3–4 weeks |

* Exact minimum time for symptom development not found in literature.

Following inoculation of sets of the index seedlings from the imported selections, periodic examinations are made for leaf symptoms or other indications of virus infection. After about four months, if there have been no symptoms of disease, the seedlings are pruned back to a point above the "inoculation site." The new growth that develops is examined as before for symptom effects. If all seedlings of one test still appear to be normal and disease-free after one year, they are cut off near the base and the bark is peeled off to determine if any wood pitting is present. If at any time positive, identifiable virus symptoms appear on the test seedlings, the corresponding introduction is declared to be diseased and the original material is destroyed. When there are only questionable disease symptoms on the test seedlings, additional transfers and, if necessary, further tests of the imported variety are made.

When the seedling test is judged to be negative, the seedlings are discarded, with the exception of the Thornton tangelo. The seedlings of this variety are used as indicators of xyloporosis (cachexia) and, on the basis of the experience of other workers, may require two years or longer to express symptoms.

2) It was clearly recognized that the seedling test would not prove with certainty that some unknown latent virus might not be carried in some of the introductions which would not cause symptoms on the seedling varieties used. It seemed necessary, therefore, to make further tests as a precautionary measure to determine that no hidden virus would escape detection. Additional tests were designed to assure that no such virus be carried in any one of the imported varieties released for field trials and thus be in a position, provided an insect vector were present, to spread to and cause damage to existing commercial citrus. To avoid such a possibility, each introduction is indexed to budded trees of the most important scion variety-rootstock combinations now being grown commercially in California. Three trees, each, of 19 different top-rootstock combinations are required for each introduction, two trees being inoculated and one being

[3] Wallace, J. M. Technique for hastening foliage symptoms of psorosis of citrus. Phytopathology **35**: 535–541. 1945.

Fig. 1. Outside view of screenhouse in which budded trees of various scion and rootstock variety combinations, used as indicator plants, are held under quarantine conditions following "inoculation" from citrus selections introduced from foreign countries.

grown as a noninoculated control. Included was navel orange on various rootstocks and these trees should eventually reveal whether or not an imported variety is a carrier of the virus of stubborn disease. Among the rootstocks used, trifoliate orange will serve to show if the exocortis virus is present in any of the introductions. The incubation period of both of these diseases is long, and there is no short-term seedling index test for them. The budded scion-rootstock combinations used are the following:

A) Navel orange on sweet orange, sour orange, Cleopatra mandarin, Rough lemon, trifoliate orange, Troyer citrange, and grapefruit.

B) Valencia orange on sweet orange and Cleopatra mandarin.

C) Lemon on sweet orange, sour orange, Rough lemon, and Cleopatra mandarin.

D) Grapefruit on Rough lemon and Cleopatra mandarin.

E) Mandarin on sweet orange and Cleopatra mandarin.

F) Lime (sour) on Rough lemon and Cleopatra mandarin.

The seedlings for the rootstocks of the above combinations are grown under quarantine and are selected for uniformity in size and trueness to type. When large enough, they are transplanted singly to 1-gallon cans. When they have reached the size for budding, the scion-variety buds are collected from field-grown nucellar trees of the varieties indicated. The budwood parent trees are indexed periodically to determine that they are free of psorosis and tristeza. Inasmuch as the source trees are of nucellar origin, they are believed also to be free of other viruses.

When the budlings caliper 0.5 cm or larger at a point 5 cm above the bud union, two trees of each combination are "inoculated" from each introduction by means of two buds. The "inoculum" buds are later checked for survival but are not forced or allowed to develop. At the time of inoculation, or shortly thereafter, the budlings are cut back to a uniform height and transplanted singly to containers of approximately 4-gallon capacity. Because of the differences in rate of growth, as well as sometimes a difference in age, the three budlings of one combination may be smaller than those of another. For this reason not all groups are cut back to the same height, but always the three of the same combination are treated uniformly. If complete sets of the 19 scion-rootstock combinations (57 trees total) are not available for inoculation at the same time, the available sets of three trees are inoculated and the remaining sets are inoculated when they become available.

Because of the number of plants involved, the inoculated budlings are transferred to a screenhouse such as that shown in figure 1. This structure is provided with a double entrance and is covered by 32-mesh plastic screen. This house and the quarantine glass-houses are locked at all times so that only authorized personnel can enter. The floor of the screenhouse is kept free of weeds, and regular inspections are made for the presence of insects. Occasionally one or more plants have been found to be infested with small citrus mite which is apparently able to pass through the screen. When these mites are found, the entire house is sprayed with kelthane and/or malathion.

Each budded tree is given a permanent record tag on which are recorded the scion and rootstock varieties, date of inoculation, and the Plant Introduction number of the imported variety from which the inoculum is derived. The trees involved in the indexing of a single introduction are grouped, the containers being placed on low wooden racks resting on cement blocks. When complete sets of 57 trees are not inoculated at the same time, spaces are left for those which will be filled in later. An inside view of the quarantine screenhouse is shown in figure 2.

Complete records of seeding and source of rootstock seeds, transplanting, budding, inoculation sources and dates, and survival of inoculation buds are kept in loose-leaf record books. Each tree is calipered 5 cm below and 15 cm above the bud union at the time of inoculation and is measured annually thereafter. Inspections are made regularly for evidence of virus infection, such as foliage symptoms, growth retardation, or other abnormalities.

Fig. 2. Two three-tree sets of budling index trees in quarantine screenhouse.

## DISCUSSION

The length of time the "inoculated" budded trees should be held under quarantine is a matter of conjecture. One could postulate the existence of unknown viruses that might be present in the test citrus trees ten years or more before revealing their presence. However, the work involved, as well as the space required, for such an extensive program as we are now engaged in necessitates that the tests be completed in much less time than ten years. Consequently, the personnel of both the University of California and the California State Department of Agriculture have agreed that a period of three years is sufficient for this test. Thus if the seedling indexing is negative and the "inoculated" budded combinations show no indication of having been infected from the respective introductions, the introduced variety may then be released for field planting.

After such testing, chances seem to be very good that the new varieties or selections will not be carriers of new or unknown viruses that would present any danger to the existing commercial citrus plantings. Support for this can be reasoned from the fact that, at least in many of the countries from which the introductions come, sufficiently wide selections of citrus varieties on different rootstocks have been growing without showing evidence of the presence of unknown serious virus diseases. Argument against this, of course, is the fact that a virus which is relatively unimportant in one region could become important elsewhere because of a different insect-vector relationship.

As brought out at the beginning of this presentation, citrus moved from its native home in the Orient to other parts of the world and carried with it many or most of the maladies to which it is subject. Now it seems that the known virus diseases of citrus are, for the most part, widely distributed in the citrus-growing areas. In spite of this, the industry has survived and in many countries it has prospered. However, it is well established that citrus varieties which have been vegetatively propagated through many generations for a long period of time have acquired many disease-causing viruses. They have also developed other weaknesses, possibly of genetic nature, that have been perpetuated by continuous vegetative propagation. When these things occur, fruit production, quality, and size can be affected. Fungi, viruses, and nematodes sometimes become such important factors in citrus production as to make it necessary to change from a long-used rootstock, for example, to others less subject to injury from these agents. It is therefore essential to have available the widest possible assortment of citrus species, varieties, and selections for experimental use if we are to solve the problems that most certainly will continue to arise in the field of citriculture.