Frequency of Virus Infection in Citrus Budwood Introduced into the United States

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A PROGRAM of citrus budwood importation and testing was developed at the University of California Citrus Research Center and Agricultural Experiment Station, Riverside, in cooperation with the United States Department of Agriculture. The program was designed to introduce, for experimental study and possible commercial use, citrus scion and rootstock selections from other parts of the world, and to develop further the techniques necessary to accomplish this end. The program was started in 1954; it has been outlined in detail by Wallace and Drake (9), and preliminary reports have been published (4, 7). This paper presents recent results of indexing tests and discusses the implications of the findings.

Materials and Methods

Citrus budwood entering the United States is first sent to the Plant Introduction Quarantine Facility at Glenn Dale, Maryland, where it is fumigated against insect pests, propagated, and observed for 1 year. About 200 citrus importations were being maintained at Glenn Dale at the start of the indexing program. Some of these were introduced as far back as 1915. Many of the introductions still being held at Glenn Dale had been distributed before state quarantine regulations were imposed

and are now established in California varietal collections. A few older introductions, and many of the more recent acquisitions by staff members of the Citrus Research Center, were chosen for indexing. These were shipped to the University quarantine facility at Riverside and propagated on Rough lemon (*Citrus jambhiri* Lush.) rootstock. In certain cases, preliminary indexing on West Indian lime [*C. aurantifolia* (Christm.) Swing.] seedlings revealed tristeza virus, whereupon the candidate was discarded. In other cases, leaf symptoms of concave-gum or psorosis viruses were found in the young leaf flushes of the candidate, and some of these were discarded.

Procedures for the short-term indexing of seedlings and the 3-year indexing of budded trees have been described in detail elsewhere (9). The short-term index was designed to test introductions for tristeza, seedling-yellows, psorosis, concave-gum, vein-enation, tatter-leaf, and Satsuma dwarf viruses. The long-term budded tree index was designed for testing for exocortis and xyloporosis plus any bud-union abnormalities or other viruses. Selections found to be infected with tristeza or seedling-yellows viruses were discarded, and all increases and index plants were destroyed. Certain importations found to have psorosis or xyloporosis viruses were held in a quarantine screenhouse for fruiting or seed.

Poncirus trifoliata (L.) Raf. was used as a rootstock to detect exocortis virus in the early years of the program. After discovery of the rapid index for exocortis (1), many candidates being held in quarantine were indexed on Etrog citron.

Of 106 selections given the full seedling index test, 77 were also tested for exocortis virus by the Etrog citron test. Since many introductions were discarded because of virus infection or for other reasons, only 37 selections were indexed 3 or more years for xyloporosis virus. No direct index for stubborn disease virus was available. However, an indirect test was utilized in which 38 inoculated trees of the various stock-scion combinations for each candidate were observed during the 3-year budded tree index.

Results

From 1954 to 1966, a total of 138 introductions were partially or completely indexed; 128 of these entered the United States as budwood and 10 were of seed origin. Excluding the ten introductions that entered the United States as seed, 70 per cent of the budwood selections tested contained one or more viruses; many selections carried more than one

virus. Viruses found and country of origin were as follows: Tristeza—Brazil, China, India, Italy, Japan, New Zealand, Okinawa, Pakistan, and Taiwan; seedling yellows—China, India, Japan, and Pakistan; psorosis—Brazil, China, Israel, Italy, Morocco, Sicily, and Spain; concave gum—France, Japan, Sicily, and Spain; exocortis—Brazil, China, India, Israel, Japan, Morocco, Philippine Islands, Sicily, and Spain; xyloporosis—Sicily and Spain; vein enation—China; impietratura—Spain.

With the exception of a Bergamot orange (*C. bergamia* Risso et Poit.) P.I. (Plant Introduction) 189558 introduced from Italy, tristeza or seedling yellows were not found in selections from the Mediterranean area. Of 77 candidates indexed on Etrog citron for exocortis, 45 were found to be infected. Eleven selections from Sicily, nine from Spain, and one from Japan, all containing psorosis or concave-gum viruses, were also found to contain exocortis virus when indexed on Etrog citron. Exocortis virus was found in the citrus relative *Feroniella oblata* Swingle (P.I. 127853) from China, demonstrating that it is a host of this virus.

Psorosis and concave-gum viruses were prevalent in introductions from the Mediterranean area. Of 46 selections from Sicily and Spain, all but five contained these viruses.

Vein-enation virus was found in Paak Ling Mung (*C. sinensis*) P.I. 93379, from Paakshan, China. In addition to vein enation, this selection also contained seedling-yellows virus which produced a severe yellows reaction on Eureka lemon [*C. limon* (L.) Burm. f.], *C. excelsa* Wester, and Thornton tangelo [*C. sinensis* (L.) Osb. x *C. paradisi* Macf.].

Gum pockets, suggesting symptoms of impietratura, were found in the albedo of fruit of Sanguina Murteras (*C. sinensis*) from Spain (P.I. 209532) on trees in the quarantine screenhouse. This selection also contained exocortis and a mild strain of concave-gum virus.

Viruses indexed for, but not found in any of the importations, were Satsuma dwarf, infectious variegation, yellow vein, and tatter leaf. Bud-union crease was not found on any of the stock-scion combinations observed over a three-year period under screen. Leaf curl as described by Salibe (8) was not found. Stubborn disease was not detected with the procedures used.

Nineteen candidates were found negative in all indexing tests; 13 of these were released from quarantine at Riverside and established in the field at the Lindcove Field Station in the San Joaquin Valley of central California, an area relatively free of tristeza virus. These selections, listed in Table 1, remain under observation for xyloporosis and will be indexed for stubborn disease by recently developed techniques (2). The other

six candidates not established at Lindcove were discarded after they produced fruit. Most were found to be varieties already established at Riverside and were useful only at rootstocks.

Discussion and Conclusions

The objective of this study was to introduce and establish virus-free citrus selections into California while improving index techniques necessary to accomplish this end. Although the number of importations tested

TABLE 1. CITRUS IMPORTATIONS FOUND FREE OF VIRUS IN BOTH SEEDLING INDEX AND BUDDED TREE INDEX PROGRAMS AND ESTABLISHED IN A HOLDING BLOCK IN CENTRAL CALIFORNIA

Species	Variety name	Country of origin	P.I. No.[a]	Year introduced
Citrus grandis	Sarawak	Tahiti	223642	1955
C. nobilis unshiu	Asahikan (Satsuma)	Japan	71234	1927
C. paradisi	Atuani	India	214012	1954
C. reticulata	Ladi 2	India	214016	1954
C. sinensis	Nagpur orange	India	49851	1920
" "	Pitcairn Island orange	New Zealand	76017	1928
" "	Comuna	Spain	209529	1953
" "	D. Joao	Portugal	210341	1953
" "	Sweet Orange	Iran	225124	1955
" "	Shamouti #1	Israel	227929	1955
" "	Shamouti #2	Israel	227930	1955
Eromocitrus glauca	Australian desert kumquat	Australia	144056	1942
Paramygnia monophylla		India	109758	?

a. Plant Introduction Number, United States Department of Agriculture, Glenn Dale, Md.

was not very large, the results of the tests are of interest and have important implications.

The high incidence of virus infection in the imported selections, and especially those collected by experienced personnel from apparently healthy trees, demonstrates that visual survey of a tree is unreliable in determining the viruses present in that tree and that a great many trees over the world are carriers of one or more viruses even though they show no external evidence of infection. The spread of citrus viruses from country to country by what were presumed to be healthy plants or propagative parts has been discussed by Olson (5). The danger of indiscriminately introducing citrus budwood has likewise been known. However, the experience gained from this budwood importation program further emphasizes the extreme difficulty of finding old-line citrus trees free of all known viruses. This is in agreement with the findings in Florida as

reported by Childs and Knorr (3) who state that less than 0.5 per cent of their old-line trees when indexed were found free of tristeza, psorosis, exocortis, and cachexia. The results of this and other studies point up the danger of visual observation as a basis for budwood selection. The visual survey of trees in the Mediterranean area for citrus viruses by Reichert (6), in 1957, failed to show or suggest the extent of concave-gum and exocortis virus present in the countries visited.

In this study 70 per cent of budwood importations were found to be virus-infected. With more thorough indexing for xyloporosis, stubborn disease, and perhaps impietratura and other viruses, the incidence would certainly be higher. Failure to find more virus-free material indicates that the labor and expense involved are too great to justify the operation of this type of program for indexing random introductions of citrus budwood. However, with the advent of indexing programs and the establishment of nucellar clones in other countries, occasions may arise when introduction of certain citrus varieties into the United States will be feasible. Valuable experience has been gained in the difficult problem of detecting viruses in citrus, and the quarantine facilities at Riverside are available when that occasion arises.

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