SYMPTOMLESS CARRIERS AND THEIR ROLE IN THE SPREAD OF CITRUS VIRUSES

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SUMMARY

Many individuals and organizations have unwittingly introduced citrus viruses into new areas or spread them around the world, usually in budwood from or in apparently healthy plants. Viruses spread in this manner include psorosis, tristeza, cachexia, xyloporosis, and exocortis. Virus-infected but apparently healthy plants have become reservoirs of infection from which the citrus viruses have been spread by man from one country to another and from one citrus variety to another. When a virus like tristeza is once introduced, insects may spread it from tree to tree and orchard to orchard. Virus spread is not evident in symptomless plants. It is detected only in virus-sensitive plants when there are recognizable symptoms on leaves, stems, or bark, and when stunting or death occurs. Symptomless carriers of one virus or virus strain are not necessarily symptomless when infected by a different virus or virus strain.

MOVEMENT OF CITRUS PLANTS FROM COUNTRY TO COUNTRY

Citrus fruits as a class are native to southeastern Asia—eastern India, Indochina, southern China, and the Philippines—where they were first brought into cultivation. From the Orient the various types and varieties were carried to other parts of the world along the trade routes. The first distribution was by seed or plants carried by explorers, sea captains, traders, monks, colonists, and other travelers. As interest in citrus production increased, varieties or types from specific regions became known and their propagation increased. The propagation of varieties by planting seedlings was gradually discontinued, as it was realized that budded trees on some rootstocks were better adapted to local soils, climates, and hazards. The selection of rootstocks was on a trial-and-error basis. As the speed of transportation increased and knowledge of budding procedures became more common, citrus varieties were introduced as budwood to permit propagation on locally adapted rootstocks. In each case, the objective of introducing a variety was to obtain a special kind of citrus and grow it in a new region.

Sometimes new insect and fungus pests were carried to new regions with plants or budwood and attacked existing citrus. This led to quarantine restrictions designed to prevent introduction of such insect and fungus pests from other regions. Prior to 1933 there was no concern with citrus viruses, since none were known.

Individuals and organizations in some countries brought together variety collections of the major citrus types grown in other countries. Probably every citrus region in the world has commercial varieties introduced as plants or budwood from distant citrus regions. In countries where citrus is not native—and they comprise the world's

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leading producers of citrus—citrus was established and then improved by selection and breeding or by importation of budwood from other parts of the world. This process of budwood distribution and exchange between distant citrus areas still continues, as each region needs and searches for improved varieties and rootstocks better adapted to local conditions.

SOME ROOTSTOCK FAILURES DUE TO VIRUS INFECTION

Horticulturists became interested in the effect of rootstocks on fruit production and quality and set up rootstock tests. Some rootstocks proved to be worthless with some tops; rootstocks successful with one top were not necessarily successful with the same top variety from another source. In India, Malta blood-orange trees grew well on Rough lemon but were stunted on sweet lime and sour orange rootstocks; the Sangtara mandarin grew well on sweet lime and sour orange, but not on Rough lemon rootstock (*4*). Sweet orange trees on sweet lime rootstock in Palestine showed xyloporosis disease (*21*). Sweet orange trees on rootstocks of *Poncirus trifoliata* were sometimes vigorous; at other times those with exocortis disease were stunted and commercially worthless (*3*). As a result of tristeza (*2*), sweet orange trees on sour orange rootstock in Africa, Java, Australia, and later in South America and the United States (California) declined and died. In Argentina and Brazil it soon became evident that trees on rootstocks of sweet orange, Rangpur lime, some tangerines, Rough lemon, and *P. trifoliata* were not visibly affected by tristeza, while those on sour orange rootstock died. New rootstocks used to replace rootstocks that had become unsatisfactory also came down with diseases previously unknown in the area. In South America, Rangpur lime was used to replace the sour orange rootstock, but some sweet orange trees on this rootstock declined and died; others made vigorous growth (*15*).

The diseases were recognized first; and unfavorable weather, improper nutrition, and fungi were suggested as the causal agents. Later, scientists demonstrated that these diseases were due to systemic infection by viruses, some of which affected one or more rootstocks but had no visible effect on others.

VIRUSES CARRIED IN CITRUS BUDWOOD AND PLANTS

Psorosis was the first citrus disease shown to be caused by a virus (*8*). Following this discovery, other workers demonstrated that many other citrus diseases are caused by viruses. Tristeza was shown to be transmitted by aphids (*14*) and by grafts (*9*). Citrus dwarf virus affecting Satsuma orange is likewise graft-transmitted, and an insect vector has been reported (*27*). Other bud-transmitted virus diseases include exocortis on *P. trifoliata* (*3*) and on Rangpur lime (*15*); cachexia on Orlando tangelo (*5*); and vein enation (*24*). The causal agent of xyloporosis of sweet lime rootstocks (*21*) was considered similar to or identical with cachexia (*6*). McClean (*13*) recently reviewed other published reports on the viruses infecting citrus.

APPARENTLY HEALTHY TREES OFTEN VIRUS CARRIERS

Beginning with the studies on psorosis, it became evident that some trees apparently healthy for most of the year could still be virus carriers. With tristeza it was soon learned that many rootstock-top combinations would tolerate tristeza virus and show no symptoms (*10, 11*). As studies progressed, it was shown that mild strains could cause less-evident symptoms than severe strains and that detection of the causal virus necessitated specific methods. It has also been demonstrated that cachexia, xyloporosis, and exocortis viruses were present in numerous citrus varieties without causing specific symptoms (*3, 7, 18, 19*). Tissue grafts from apparently healthy trees caused recognizable disease symptoms only on specific varieties. Disease symptoms on leaves, stems, and bark, and sometimes stunting and death of the trees, were associated with specific viruses and virus strains.

Thus the absence of viruslike symptoms in plants is not proof that they are virus-free. Citrus pathologists now recognize the impossibility of selecting a virus-free tree on the basis of its apparently healthy condition. Virus-affected trees in many citrus rootstock plantings in different countries are evidence that the trained horticulturists who planted them—only a few years ago—could not consistently select virus-free budwood from apparently healthy trees. Likewise, the plant quarantine officials who examined critically the plants and budwood brought into the United States failed to detect viruses in such plant materials.

In recent years restrictions on importations of citrus have been tightened in many countries. However, the free exchange of plants and budwood continued so long that, according to McClean (*13*), it would be surprising if any viruses remain to be introduced.

Virus Spread from One Region to Another. The spread of tristeza virus can serve as an example of virus spread from country to country by shipments of apparently healthy plants and budwood. It is impossible to untangle the multiplicity of possibilities in the many exchanges of plant material, but some specific information is available. All indications are that tristeza virus was introduced into Argentina from South Africa by shipments of sweet orange trees on Rough lemon rootstock (*25*).

The question then arose: Had tristeza been unwittingly introduced into the United States in budwood from apparently healthy trees selected for propagation of varieties popular in other regions of the world? Since tristeza virus is transmitted by certain species of aphids, data on the first infected tree could not be readily obtained in areas where the introductions were repeatedly exposed to natural infection. In Texas, tristeza was shown to be almost completely absent in commercial orange and grapefruit plantings on sour orange rootstock, and the few plants found carrying tristeza virus were thought to have been infected before their introduction into Texas.

Many Texas-grown Meyer lemon trees have been shown by the writer (*16*) to be infected by tristeza virus. Other workers have since found tristeza-infected Meyer lemon trees in other areas in the United States and foreign countries. It now seems probable that tristeza virus moved in Meyer lemons from China to the United States and subsequently spread in infected cuttings and budwood to Israel (*26*), Sicily (*22*), Algeria and Morocco (*1*), and probably to other countries. Such spread was possible because Meyer lemon trees on their own roots show few if any symptoms of tristeza.

It also seems probable that tristeza was introduced repeatedly into the United States before 1900 in Satsuma orange selections from Japan and in subsequent introductions of mandarin orange selections from Australia and oranges from India (*16*). The same pattern of results has been found in Israel, where tristeza occurred in symptomless introductions (*20*). Thus tristeza virus was spread from one region to another in symptomless plants or budwood. The spread preceded the identification of tristeza as a virus-caused disease.

The impetus to citrus virus research given by the studies on tristeza did not stop with its identification as a virus-caused disease. Citrus trees on tristeza-tolerant rootstocks were planted, and other virus-caused diseases developed on some experimental rootstock varieties. Buds taken from tristeza-free sweet orange trees on sour orange rootstock and supposedly healthy grapefruit trees caused exocortis, xyloporosis, and cachexia on susceptible rootstocks. Wherever rootstock experiments are made, there is evidence of exocortis and xyloporosis in the local varieties (*13*). Evidence accumulated that many grapefruit, sweet orange, mandarin, and lemon trees were symptomless carriers of one or more of these viruses. It became apparent that scion-rootstock combinations which are symptomless carriers of one virus are not necessarily symptomless when infected by a different virus or virus strain. Likewise, trees free of one specific virus are not necessarily free of another virus. Similarly, trees with symptoms of one virus-caused disease are not necessarily free of other viruses.

Grapefruit, sweet orange, mandarin, and lemon budwood have been distributed from their points of origin to almost every citrus area of the world. There can be little doubt that other viruses have been spread with them in the same manner that tristeza virus has been spread.

Recently, virus strains and mixtures have been recognized, as in tristeza (*12*). New viruses, as well as new virus strains and new host responses to old and new viruses, are being found. Some or all of these strains and mixtures likewise have been distributed around the world. A severe strain of tristeza virus, apparently identical with that found in South Africa, Brazil, and Australia, was found in a Texas-grown Satsuma introduction from Japan (*17*). Wallace (*23*) discovered that tristeza-carrying introductions from widely scattered areas of the world included both mild strains and a strain causing "seedling yellows" of Eureka lemon seedlings. The "seedling-yellows" strain was apparently absent in citrus infected by aphids in California. Thus budwood introductions from foreign areas may bring in strains more virulent than those already present in the locality.

The significant fact is that many people and organizations have unwittingly introduced or spread citrus viruses around the world, usually in budwood from or in apparently healthy plants.

Virus Spread Within a Region. The spread of citrus viruses between regions is generally traceable to distribution of infected but apparently healthy nursery stock. Spread within a region may be by man or, as in the case of tristeza, by insects. Spread from infected trees to trees of the same or other varieties may be accomplished by insect vectors if they are present, or by bud transmission in the nursery, or in top-working of grove trees. Insect vectors of a virus greatly increase the rate of spread from a symptomless carrier to other citrus trees in the area.

Virus-disease outbreaks within an area depend upon three factors: 1) the presence of a virulent strain of virus, 2) a disease-susceptible host population, and 3) an effective means of spread. All three factors must be present before virus spread is noticeable. An infected symptomless host or an infected susceptible tree may provide the virus source. Where the virus is spread by an insect vector, a symptomless host may grow normally, while susceptible scion-rootstock combinations make comparatively little growth. Where spread begins by insects feeding on young foliage, as in aphid transmission of tristeza, the normal-growing, infected, symptomless host is a reservoir of infection, a "Typhoid Mary" among citrus. The hazard of virus transfer by insects from such plants is far greater than that from dying susceptible plants. The spread of the well-established virus-caused diseases has probably reached the limits of their insect vectors. New epidemics are the result of new introductions of virus, or of introductions of new vectors, or of increased use of disease-susceptible plants.

Virus spread from symptomless carriers may have different effects depending upon circumstances. Virus spread to other symptomless carriers increases the number of infected virus carriers, but the effect may not be noticeable. If the budwood source is an unsuspected carrier of a virus, the virus will injure trees on rootstocks or scions susceptible to it. If the budwood source is known to carry a virus or viruses, the use of susceptible but otherwise desirable scion-rootstock combinations is avoided.

The effect of virus spread from symptomless carriers may depend on the strain of the virus. Strains of great virulence may cause injury to rootstocks tolerant to milder strains of the same virus.

The injury to the trees may also be affected by their age. The results of virus infection may sometimes be seen in a few years. In other instances, as with psorosis, the virus may be harbored, apparently harmlessly, in a tree for many years and yet be responsible for serious decline of mature trees. It is possible that other viruses in symptomless hosts also may have a delayed effect not recognized at present.

Virus-infected but apparently healthy plants have become reservoirs of infection, from which the citrus viruses have been unwittingly spread by man from country to country and by man and insects within citrus-growing regions. Fortunately, symptomless hosts infected with certain viruses can be detected by use of suitable virus-sensitive plants in indexing procedures (*3, 5, 6, 7, 10, 15, 16, 17, 18, 19, 20, 22, 23, 24, 26, 27*). Thus visual inspection of budwood-source trees is not adequate precaution against the propagation of virus-infected trees, since apparently healthy citrus trees are not necessarily virus-free.

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