TRISTEZA and RELATED DISEASES

The Tristeza Virus Complex

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The present paper is based largely on results published in an earlier paper (15), following an extensive study of tristeza virus in South African citrus trees. It became obvious in the preliminary phase of indexing for tristeza virus that using small acid lime (West Indian or Key lime) alone as an indicator was not sufficient. Although lime was infallible in recording whether the virus was present or absent, its behavior failed to record all differences that exist in tristeza virus present in different citrus trees. A better understanding of properties of the virus in different trees became available by using two other indicators, sour orange and composite trees of sweet orange on sour orange stock.

The tendency in many parts of the world is to use only lime as an indicator. This paper, written at the request of the Chairman of IOCV, is to encourage the use of all three indicators in studying tristeza virus, particularly when assessing properties of the virus prevailing in a region.

DISEASES CAUSED BY TRISTEZA VIRUS

Tristeza is the name of the causal virus of such diseases in citrus as stem-pitting (lime dieback, Hassaku dwarf), seedling-yellows, and tristeza (quick decline). The virus is not a simple one, existing as mild and severe strains, but is best considered as a complex of strains and components. The type of disease depends on source and composition of the complex, kind of citrus tree into which the complex is introduced and, in the case of composite trees, kinds of citrus used as scion and stock.

The expression “tristeza disease,” to describe all diseases produced by the virus complex, is confusing. It is better to distinguish among such reactions as stem-pitting, seedling yellows, and tristeza.

In the southern hemisphere, the main vector of tristeza virus is Toxoptera citricidus Kirk. This efficient vector transmits all forms of the tristeza complex. Three aphid vectors, Aphis gossypii Glover, A. spiraecola Patch, and T. aurantii B. de Fonsc, are reported in Florida and California (4, 18, 19), and all are less efficient than T. citricidus. Difference in efficiency of the vectors may be partly responsible for differences in tristeza virus that prevail in the two regions. This applies especially to virus that is actually being spread within the region by insects.

Stem-pitting, lime dieback, and Hassaku dwarf. These diseases result from a direct reaction of citrus species to tristeza virus (12, 21, 25). Two important symptoms are vein clearings in the young leaves and pit lesions in the vascular tissues. Extent and severity of symptoms depend partly on whether the strain of virus is severe or mild, and partly on sensitivity of the citrus species concerned.

Most, if not all, citrus species are susceptible to tristeza virus, in the sense that the virus is readily transmitted to them by insect vectors or by tissue grafts. Following is a rough classification of some species and varieties in re-
lation to their behavior to severe strains of stem-pitting:

**Very sensitive.** Small acid lime, citron, Mauritius papeda, and probably Marumera (26). They react with severe and extensive pitting, and the whole tree shows harmful effects at an early stage.

**Moderately tolerant.** Grapefruit, pummelo, *C. hassaku* Y. Tanaka [probably a hybrid of pummelo and a mandarin (11)], and some varieties of sweet orange [Pera (22), Verna, Mediterranean-Sweet and Bailidge Early (a South African midseason)]. They react with fairly extensive and sometimes severe pitting, but are more tolerant of its effects than are very sensitive species. Although infected trees grow reasonably well in the beginning and may remain productive for a number of years, they tend to decline prematurely.

Grapefruit proved very sensitive to a severe strain isolated from a tree of Marsh Seedless grapefruit in South Africa (21). This isolate caused severe pitting and stunting in experimentally-infected trees.

**Tolerant.** Most varieties of sweet orange, tangerine and mandarin, tangarles, sour orange, lemon (Eureka and Lisbon types), Rough lemon, Rangpur lime, and probably trifoliate orange and certain citranges. Most of them develop pits in the trunks, but such pits are usually mild and few in number. Occasional vein clearings are often seen in young leaves. Infected trees do not appear to be harmed, and remain in good condition for many years.

To include sour oranges and lemons in this category may seem contradictory. Both species, however, are tolerant to the tristeza virus they acquire in the field, when they are grown on their own roots or on the roots of other species. It is only when they are rootstocks under scions of certain species that they suffer injury, but that is not injury from stem-pitting.

Very little information is available on the reaction of trifoliate orange and citranges to stem-pitting, but probably both are very tolerant. Curiously enough, tristeza virus is always difficult to recover from inoculated seedlings of trifoliate orange and even from trifoliate stock shoots under infected scions. This was the case in work done in South Africa. A similar result is recorded from Japan (17). However, symptoms of pitting in trifoliate stocks have been observed by the writer in South Africa. Two examples of this occurred in trees (with seedling grapefruit scions) that picked up infection naturally after being planted in the open.

**Seedling yellows.** This disease is induced in young seedlings of sour orange and lemon by inoculating them with grafts from certain sources of tristeza virus, especially naturally infected sweet oranges and tangerines. This is the case in most countries in the southern hemisphere (6, 14). Seedling yellows is essentially an artificially induced, hypersensitive reaction. It is rarely seen in the field as a naturally occurring disease. Larger seedling trees growing in the orchard, however, can be made to develop the disease by graft-inoculating trees and then cutting them back severely. This was done both in South Africa (14) and in California (28).

The disease is characterized by severe stunting and formation of small, pale, and often distorted leaves. When the disease is severe, growth almost ceases; in milder cases, it may resume, with an improvement in vigor and appearance. Secondary effects include a poor root system, thickening of bark, proliferation of medullary rays and, sometimes, corky eruptions along the veins of some leaves.

Studies (15) in South Africa have shown that experimentally-infected seedlings, especially those with the mild
reaction, often recover and thereafter grow well, usually without again developing symptoms of yellows. Nor do symptoms reappear when recovered plants are reinoculated from a source of seedling yellows. Healthy sour orange or lemon plants inoculated from recovered plants never develop yellows. Similar results are reported by Wallace and Drake (28).

Grapefruit seedlings are probably also sensitive to seedling yellows, but usually the symptoms in young seedlings are masked by effects of severe stem-pitting strains. A reaction to seedling yellows is more obvious, however, when the virus complex in the inoculum source produces only a mild stem-pitting reaction in addition to that of yellows. In such cases, seedlings react to yellows with stunting, small, discolored leaves and, sometimes, with a mild proliferation of medullary rays.

Tristeza. Originally described from Brazil (1), tristeza is a disease of citrus trees (mainly with sweet orange scions) on rootstocks of sour orange and some other stocks. Quick decline, described from California (5), is the same kind of disease. When it infects adult trees it causes a sudden and severe decline. Effects are reflected in the scion by discoloration of foliage, dieback of branches, and retarded growth. Schneider (23, 24) found the primary cause of the decline in tristeza disease to be due to sieve-tube necrosis in the sour orange stock in the region just below the union. The necrosis girdles the tree and prevents downward passage of carbohydrates, which then accumulate above the union and become depleted in the stock. Other disturbances at the union are an abnormal thickening of bark and proliferation of medullary rays.

Tristeza disease is best defined as a specific reaction by trees of certain stock-scion combinations to a particular type of tristeza virus complex. Symptoms shown by scions of such trees are not due to susceptibility of the scions. They are secondary effects following damage to phloem cells of the rootstock just below the union. Two factors are thus necessary to induce tristeza disease—a susceptible tree, and an appropriate virus complex. As we shall see later, not all sources of a tristeza virus complex induce tristeza disease. The disease is essentially one that affects composite trees, causing an incompatibility to develop between species forming the scion and stock.

In general, susceptible trees are ones with a tolerant species forming the scion and a sensitive species, the rootstock. They include trees with scions of sweet orange, tangerine (or mandarin), and grapefruit topworked on rootstock such as sour orange, lemon, citradia, and citremon. Species such as sour orange, lemon, and some of their hybrids are sensitive to the tristeza virus complex normally picked up and carried by so-called tolerant scions. This complex induces sieve-tube necrosis in sensitive species when they are stocks under such infected scions. However, sensitive species are tolerant of the type of tristeza virus that they pick up naturally. For this reason, sour oranges and lemons can be grown safely on their own roots or as scions on any tolerant rootstock.

The important factor in creating a tree tolerant to tristeza is to use as stocks only species that tolerate whatever virus complex is carried by scions. The best examples of tolerant stocks are Rough lemon, sweet orange, some tangerines and mandarins, Rangpur lime, trifoliate orange, and citranges.

TRISTEZA VIRUS COMPLEX IN SOUTH AFRICAN CITRUS TREES

Extensive tests for tristeza virus in orchard trees in South Africa (13, 15) revealed that virus is present in most if not all trees. This is understandable, because Toxoptera citricidus is common on citrus and related species in South
Africa, and because the virus, once established in the tree, is readily perpetuated in all the vegetative progeny of the tree.

Young seedlings of three indicators were used in the tests: (a) lime, as an indicator for stem-pitting; (b) sour orange, as an indicator for seedling yellows; (c) composite trees with sweet orange scions (tops cut from young Valencia seedlings) on sour orange stocks, as indicators for tristeza. Grapefruit seedlings were sometimes used as a supplementary indicator.

Results, as determined by the behavior of the indicators, showed that tristeza virus varied in South African citrus trees. It sometimes varied in trees of the same species, but in trees of different species the differences in its properties were more important. Orchard trees tested included trees of old clonal lines and new seedling lines. The latter were ones originally planted in the open as healthy trees and allowed to become infected with tristeza virus naturally by vectors. Results from the two types of trees were much the same. There was no evidence of any influence on results by viruses such as exocortis and xyloporosis, known to be present in trees of some of the old clonal lines. Nor was there any influence by vein-enation virus, which occurred in many trees, both old clonal and seedling lines. Detailed results of these tests have already been published (15). The important facts are summarized in table 1.

With respect to naturally-occurring tristeza virus, species of citrus fall into three main groups: one contains the virus that causes stem-pitting, seedling yellows, and tristeza; the second contains the virus causing stem-pitting and tristeza; the third contains the virus causing only stem-pitting. Results for different trees of each species or variety were remarkably consistent in the case of older trees. Exceptions, however, were found among young trees. Seedling yellows, although never found in the isolates from older trees of grapefruit, lemon, and sour orange, was sometimes found in young seedling trees of all three species, and in young scions of

<table>
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<tr>
<th>Orchard trees tested</th>
<th>Reaction of indicators</th>
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<tr>
<td></td>
<td>Lime</td>
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<tr>
<td>Sweet oranges, tangerines, mandarins, some tangelos (Sampson, Orlando, Minneola) and Rough lemons</td>
<td>Severe stem-pitting</td>
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<tr>
<td>Grapefruits (Marsh, Cecily, Ruby), some tangelos (off-type seedling lines of Minneola and seedling line of Umatilla)</td>
<td>Stem-pitting (severe, intermediate, mild)</td>
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<tr>
<td>Sour oranges and lemons</td>
<td>Stem-pitting (severe, intermediate, mild)</td>
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new-line grapefruits and lemons budded on stocks of Rough lemon and Rangpur lime (14). In the latter examples, evidence indicated that seedling yellows was due to virus picked up naturally by stock seedlings.

Young trees of grapefruit, lemon, and sour orange in which seedling yellows was present showed symptoms typical of that disease. Affected trees mostly outgrew the symptoms, however, and the virus in them tended to lose the property of causing yellows. In some older trees of lemon and sour orange on Rough lemon stocks, with shoots growing from the stocks, the virus in the two parts of the trees was found to have different properties. That from the scions caused only stem-pitting, whereas that from stock shoots caused, in addition, yellows and tristeza. Occasionally, in older trees of grapefruit, a virus was recovered that caused only very mild stem-pitting and no tristeza.

**TRISTEZA VIRUS COMPLEX AND ITS VARIABILITY**

The difference in severity of stem-pitting disease produced in limes inoculated from different sources indicates that the virus responsible for the stem-pitting reaction exists as more than one strain. Some isolates cause a severe disease, others a mild one. This is a simple difference shown by the virus, irrespective of whether its source is sweet orange, grapefruit, lemon, or any other kind of citrus. The virus in most old-line trees, especially that from sweet oranges, tends to be severe in lime. Milder strains are more common in young seedling trees and in young new-line trees—that is, trees with recent exposure to natural infection. But mild strains can be found in almost any citrus tree. When the virus complex from a tree is passed through a series of citrus seedlings either by grafts or by aphids, milder segregates appear in some of the seedlings. In studies in Florida (9, 10), a similar segregation was obtained by passing a more severe strain of tristeza virus from one plant to another.

But more complex differences in the tristeza complex are present in naturally infected trees of different species of citrus in South Africa, Australia (6, 7), and probably South America as well. Differences in the properties of the virus complex are demonstrated only when sour oranges and sweet oranges on sour orange stocks are used as indicators. In the field, tristeza virus as a complex is apparently ordinarily taken up as a whole by certain species of trees, such as the sweet orange, tangerine, and Rough lemon, as a result of aphid transmission, but only in part by others, such as grapefruit, sour orange, and lemon (16).

The whole complex can be experimentally transmitted to seedlings of grapefruit, sour orange, and lemons by grafts. The seedlings react unfavorably at the start, but after an interval of weeks or months they may recover, and the virus in them may change, with the result that it is no longer capable of inducing seedling yellows or even tristeza disease. These citrus species seem to have the property of modifying and changing a tristeza virus complex. In fact, in experiments by the writer, this has been done repeatedly, simply by passing isolates recovered from naturally infected sweet oranges through seedlings of grapefruit, sour orange, and lemons.

Convincing proof of the influence of the host tissues on the tristeza virus complex is that, in trees made up of two or more species, the properties of the virus may be different in each species of the tree. As noted above, this was the case in some trees with lemon or sour orange scions on Rough lemon stocks. In Australia, Fraser (7) found the same thing in some field trees with limbs of both grapefruit and sweet orange or mandarin. Virus from the grapefruit limbs gave a stem-pitting reaction but
not one for yellows. Virus from the sweet orange or mandarin limbs gave both stem-pitting and a yellows reaction in indicators.

Species such as sour orange and lemon, that are hypersensitive to seedling yellows, have the property of rejecting that part of the complex that causes yellows when the complex is being introduced into them by aphids. Thus, while these species on their own roots, or while they form the scion on the roots of other species, are safe from severe injury. It is when they are stocks under scions that acquire a tristeza-inducing virus naturally that tristeza disease develops. Grapefruits do not seem to go as far as lemons in changing the virus complex brought in by aphids. The virus present in most trees does not cause yellows, but retains the power of causing tristeza disease when transferred to a sweet orange on sour orange stock. This is thought to mean that a mild form of seedling yellows remains as a part of the complex associated with grapefruit. There is an obvious association between seedling yellows and tristeza disease. The two yellows-sensitive species (lemon and sour orange) are ones that, when used as stocks under sweet orange and other scions, make the trees sensitive to tristeza. It would be surprising if different components in the complex were involved in causing these two diseases.

TRISTEZA VIRUS IN OTHER COUNTRIES

The properties of tristeza virus prevalent in citrus trees and its variability in different citrus species are probably much the same in most countries of the southern hemisphere, where *Toxoptera citricidus* is the vector.

Tristeza virus prevailing in Florida and California causes a stem-pitting reaction in limes and is also responsible, in both states, for the natural occurrence of tristeza disease in trees on sour orange stock. In California, Wallace (27) reported that up to 1957, no seedling yellows virus had been encountered in sweet oranges or other citrus known to be naturally infected with tristeza virus. This virus, however, was found in Meyer lemon, Satsuma, and other citrus varieties known to be infected with tristeza virus and believed to have been carriers of tristeza when originally imported to the United States. In Florida, Grant (9) and Grant and Higgins (10) reported that, in their mild isolates of tristeza, there was no indication that stem-pitting was combined with a seed-
ling yellows component. However, their severe T3 strain (isolated from a naturally infected lime in Florida) caused initial shock symptoms consisting of stunting and yellowing in some plants of sour orange, lemon, and grapefruit. This reaction answers the description of seedling yellows. Grant noted that the reaction was not so severe as the one produced in sour orange by severe tristeza prevailing in Brazil.

Severity of stem-pitting produced by tristeza that occurs naturally in citrus trees in Florida seems to be milder than that produced by the virus prevalent in the southern hemisphere (10, 20). In Florida, and possibly in California as well, grapefruits seem to be much less prone to stem-pitting disease. Reports indicate very little evidence of field spread of tristeza in Florida grapefruit (2), and in infected trees, extensive symptoms of stem-pitting are rare (10).

There is evidence, however, that tristeza virus present in many sweet oranges and other citrus species on sour stock in Florida remains latent in trees without causing tristeza disease although still detectable by the lime indicator (20). The number of trees declining from tristeza in Florida is extremely small in relation to the number of known infected, nontolerant trees (3). This suggests that in Florida two types of tristeza may be present in infected trees, both able to cause stem-pitting in the lime, but only one able to cause tristeza disease. The reason for this may be partly due to inefficiency of vectors in Florida, and their transferring only a part of the tristeza complex to some trees, excluding the component associated with the development of tristeza. There is a possibility that the tristeza fraction in infected trees on sour stocks may be protecting those trees against the full complex that causes tristeza disease.

Fraser (8) commented on the distribution of tristeza virus to components: “The tristeza complex appears to have been introduced at some time to all citrus-growing countries. However, the rate of spread has varied considerably in different countries, and in some areas, it has not spread at all. Moreover, the virus strain components and disease expression vary from country to country. This behaviour is probably a function of the vector or vectors in the different areas. The distribution of the virus complex comprising seedling-yellows strains and strains causing stem-pitting disease of grapefruit is coincident with the range of T. citricidus, the most efficient vector. When this complex is introduced into an area where T. citricidus does not occur the strain assemblage transmitted will depend on the indigenous aphid population.”

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