TRISTEZA DISEASE INVESTIGATIONS, AN EXAMPLE OF PROGRESS THROUGH COOPERATIVE INTERNATIONAL RESEARCH[1]

James M. Wallace[2]
University of California Citrus Experiment Station,
Riverside, California

INTRODUCTION

It is now known that the effects of the tristeza disease were recognized prior to 1900. In the early 1890's, when sour orange, *Citrus aurantium* Linn., was first used as a rootstock in South Africa, it was observed that sweet orange, *C. sinensis* (Linn.) Osbeck, and mandarin orange, *C. reticulata* Blanco, trees budded on sour orange rootstock usually died or declined severely within two or three years. Inasmuch as these two scion varieties, and also lemon, *C. limon* (Linn.) Burm., grew satisfactorily on Rough lemon, *C. jambhiri* Lushington, this last-named variety was used almost exclusively thereafter as a rootstock in South Africa.

For many years the failure of sweet orange trees on sour orange rootstock in South Africa was attributed to incompatibility between sour orange and certain scion varieties. After a study of the citrus industry of South Africa in 1924–25, Webber (*19*) rejected the use of uncongenial strains of sour orange as a cause of the tree failures. His studies led him to the conclusion that the disorder was an infectious disease. In a later publication (*20*) Webber discussed the tristeza disease of Argentina and Brazil as the same as the disease in South Africa and suggested a virus as its cause.

As early as 1928, Toxopeus (*15*), in Java, began studies of what appeared to be the same disease as that in South Africa. He also eliminated physiological and environmental factors as causes of the disorder. The disease was next observed in the early 1930's in Argentina, but it was not until it had begun to cause serious damage in Brazil (about 1937) and in California (1939) that concerted efforts were made to determine its nature and to find means of control. The disastrous losses suffered by the citrus industries in Argentina and Brazil in a relatively short time clearly indicated the seriousness of the disease and stimulated research investigations in nearly all of the world's citrus-growing areas. The joint efforts of citrus scientists all over the world have resulted in rapid progress toward a comprehensive knowledge of tristeza. Published papers and reports on this disease perhaps number more than three hundred.

The writer can think of no other plant virus disease that has been studied so intensely on an international scale, nor of any other instance in which exchanges of knowledge and ideas between workers have been made more fully and more cooperatively. On this occasion, therefore, it seems fitting to emphasize this feature of tristeza research rather than to review information already available in the literature. For a detailed summary on the subject of tristeza, the reader is referred to *FAO Plant Protection Bulletin No. 4* ("Symposium on Tristeza Disease of Citrus"), published in 1956 (*5, 10, 11, 16*).

[1] Paper No. 1103, University of California Citrus Experiment Station, Riverside, California.
[2] Plant Pathologist, Citrus Experiment Station, Riverside.

EARLY OBSERVATIONS ON VARIETAL SUSCEPTIBILITY, DISTRIBUTION, AND PROBABLE CAUSE OF TRISTEZA

The visit of H. J. Webber to South Africa in 1924–25 was probably the first instance of international cooperation in the study of the disease now known as tristeza. In addition to stressing the unlikelihood that the disorder was due to incompatibility between citrus varieties, Webber (*20*) made the following observations:

1. Trees of sour or Seville orange grown as seedlings or budded on Rough lemon grew normally.
2. Trees of sweet orange and of mandarin on Rough lemon rootstock were not subject to the disease.
3. Lemon varieties worked on sour orange rootstock were unaffected.
4. Sweet orange and mandarin trees grew normally when budded on sweet orange rootstock.
5. No trees of either grapefruit or mandarin propagated on sour orange root were found, but growers reported these combinations to be unsatisfactory.

These early observations and Webber's insistence that the tree failure was due to a disease and not to varietal incompatibility might have led to investigations by South African workers and an earlier knowledge of the nature of the disease if sour orange had been of importance there as a rootstock. With the existing citrus plantings consisting largely of the tolerant Rough lemon rootstock, there was no great economic need for further investigation of the problem at that time.

In experiments begun in 1928 in Java, Toxopeus (*15*) observed that sweet orange varieties budded on sour orange rootstock grew well at first but soon showed symptoms of decline and usually died within 8 to 12 months. The "Japanese citron," *C. nobilis* X *C. medica?*, which grew fairly well on sour stock, was used as an interstock between the sour stock and sweet scion variety but had no effect on prolonging the life of the trees. All possible combinations of sour, sweet, and "Japanese citron" were used as stock, interstock, or scion; failure resulted only when the top was sweet orange and the interstock was sour orange. Toxopeus postulated that sweet orange produced some substance that was injurious to sour orange but he gave no indication as to the nature of that substance.

The first published account of the presence of tristeza in South America is believed to be that of Carrera (*4*) in 1933. This disease, which became known in Argentina as *podredumbre de las raicillas*, is thought to have made its appearance in 1930 or 1931 in citrus plantings in the Province of Corrientes. The disease spread at an alarming rate, and many theories were advanced as to its cause.

TWO DECADES OF PROGRESS IN TRISTEZA INVESTIGATIONS

In 1937, at the invitation of the Argentine Government, Drs. A. A. Bitancourt and H. S. Fawcett visited the Province of Corrientes to study the threatening disease. Their observations suggested that the disease was caused by an infectious agent, possibly a virus (*3*). In 1940 Bitancourt (*2*) reported that the disease was present in the Paraiba Valley of Brazil, where it had first appeared in about 1937.

A disease originally given the name "quick decline" was observed in California in 1939. At first the increase or spread was slow, and it was not until 1942 that its possible relationship to tristeza was given serious consideration. During this period close contact was maintained between workers in California and in the tristeza-affected areas of Brazil and Argentina, and exchange of information was continued. In 1944 a comprehensive research program was initiated by the University of California Citrus Experiment Station in an effort to determine the nature of "quick decline," its means

ERRATUM:
On page 30 the sentence beginning on line 25 should read: All possible combinations of sour, sweet, and “Japanese citron” were used as stock, interstock, or scion; failure resulted only when the top was sweet orange and the interstock *or stock* was sour orange.

of spread, and its control or prevention. By that time research on tristeza was being advanced in South America, and from the free exchange of information between workers in the two continents, many similarities between tristeza and quick decline had been established.

In 1946 the U. S. Department of Agriculture initiated cooperative investigations on tristeza with the Brazilian Government, with headquarters at the Instituto Agronomico at Campinas. At approximately the same time, the Florida Citrus Experiment Station began cooperative study with the Argentine Ministry of Agriculture. Plant pathologists from the United States were stationed in both countries to collaborate with scientists there. At this time the nature of the disease was still unknown, and at all locations of study experiments were begun to determine the cause. At the same time extensive rootstock trials were established. Because the rapid spread of the disease indicated that it was due to an insect-transmitted virus, studies on insect vectors were also begun in Brazil and California.

The progress made in the study of tristeza at the various stations is so well known that it need not be reviewed in detail. In 1946 Fawcett and Wallace (*7*) reported that the causal agent of quick decline was a virus transmissible by tissue-grafts. Simultaneously, Meneghini (*12*) demonstrated that tristeza, also, was caused by a virus and, further, Meneghini reported that the causal virus was transmitted by an aphid, *Toxoptera citricidus* (Kirk.) (*Aphis tavaresi* Del Guercio). Bennett and Costa (*1*) later confirmed the findings of Meneghini and, using the aphid vector, added much information concerning the transmission and host range of the tristeza virus. *Toxoptera citricidus* does not occur in the United States, but Dickson *et al.* (*6*) have found that the melon aphid, *Aphis gossypii* Glover, is the principal vector in California. Throughout the period of study of insect vectors there has been cooperation between workers in Brazil and California, particularly in the exchange of aphid specimens for identification.

Early in the investigations of quick decline in California, Schneider (*14*) studied the anatomical effects of the disease on the bud-union tissues of diseased trees and developed a diagnostic test. By comparing bark from tristeza-affected trees with that from trees having quick decline, he demonstrated that the two diseases induced the same anatomical changes in the phloem tissues of the bark. Schneider diagnosed numerous bark samples from trees suspected by workers in several citrus-growing countries of having tristeza, and supplied details of this test so that the workers could make the diagnosis themselves.

One of the most valuable contributions to the study of tristeza came from the Gold Coast of West Africa and to the best knowledge of the writer this came before any work on the subject had been published there, and before few if any investigators in other countries knew that the disease existed in the Gold Coast.

In the early years of study, transmission and infection tests had to be made by using budded trees of susceptible combinations of top and rootstock, such as sweet orange on sour orange. After infection of such trees, symptoms did not develop for several months. The need for a quick test was urgent, and workers in both California and Brazil were attempting to find such a test. Hughes and Lister (*8*) described veinal fleckings and wood-pitting of seedling lime plants in November 1949, but approximately a year before their publication, workers in the Union of South Africa, Brazil, and the United States had been informed by correspondence that limes were useful as an indicator host of tristeza virus. The release of this information prior to publication provided a much-needed inoculation technique for studies of tristeza in the Americas and made it possible for workers in the Union of South Africa to demonstrate that the so-called stem-pitting disease of grapefruit is caused by the tristeza virus (*9, 13*).

The linking of stem-pitting disease with tristeza provided information that was used immediately by workers conducting extensive rootstock trials in Argentina, Brazil, and California. The discovery that tristeza virus caused wood pitting and subsequent decline and unproductivity on top and rootstock combinations that were not subject to the previously discovered bud-union phloem necrosis made it clear that resistance of a given combination could be determined only after several years' study.

Other instances of international cooperation in the study of this disastrous disease will be mentioned only briefly. The cooperative studies between workers in California and South Africa provided information regarding the origin of the virus and the means by which it could have been introduced to other parts of the world, particularly South America (*17*).

While serving as consultant for International Cooperation Administration in Israel in 1955, the writer assisted other workers in demonstrating that Meyer lemon trees growing there were carriers of tristeza virus (*18*). Other suspected selections in the variety orchard of the Agricultural Research Station at Rehovot were located, and later testing revealed some of them to be carriers. This made it possible to remove sources of infection in a citrus region where there is no known tristeza infection in commercial plantings.

Similar inspections were made of plantings at the Experiment Station in Acireale, Sicily, and certain "suspect" trees were indicated. Transmission tests made later by Dr. Franco Russo, who had been trained in indexing procedures at the University of California Citrus Experiment Station, demonstrated that some of the Sicilian trees were infected.

The University of California, the California State Department of Agriculture, and the U. S. Department of Agriculture are at present engaged cooperatively in introducing citrus varieties and selections to the United States from foreign countries. Each importation is tested under strict quarantine conditions to determine that it is free of virus infection before it is released for field testing. Of 56 importations now under test, 7 have been found to be infected with the tristeza virus. These tests have already revealed that tristeza virus is present in three countries in which it has not been reported, and workers in these countries are being informed of this fact.

Finally, the contacts made by students and visiting scientists have provided further opportunities for exchange of ideas, information, and research techniques, and have no doubt helped to advance our knowledge of the tristeza disease.

During the decade of 1940–1950, rapid progress was made in the study of tristeza, a disease which threatened to destroy more than half of the world's citrus. The disease still remains a threat to vast acreages of citrus in the Mediterranean and other regions where its causal virus is apparently not yet present in commercial plantings. Should conditions change to favor the spread of the disease, damage would be lessened to some extent because of the information now available. Already, in some of these regions new plantings consist of trees on resistant rootstocks. Preventive measures and/or procedures for salvaging infected trees, such as inarching to resistant rootstocks, top-working to lemons, et cetera, are fully understood and can be made use of if deemed practicable.

With intensive studies of tristeza proceeding simultaneously in several countries, it might be expected that needed information would develop rapidly. It is evident, however, that progress is faster when there is close cooperation and free exchange of information between research workers in many parts of the world who join their efforts to meet a common threat.

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