

# A PRELIMINARY REPORT OF WORK AT CAMPINAS, BRAZIL, ON TRISTEZA DISEASE OF CITRUS

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In September, 1946, work was started at Campinas, Brazil, on the disease of citrus known as tristeza, with the hope of contributing information on the nature of the disease, its method of spread, its host range including susceptible scion-stock combinations, and on control. This work was initiated, and is being continued, as a joint cooperative project between the Instituto Agronomico of the State of Sao Paulo, Brazil, and the Division of Fruit and Vegetable Crops and Diseases of the United States Department of Agriculture. This paper reports the progress of the work during the first year.

## ECONOMIC IMPORTANCE AND SYMPTOMS OF THE DISEASE

In South America tristeza appeared first in Argentina about 1932. In Brazil it was found first in the Paraiba Valley in the State of Sao Paulo in 1937 and within a period of 10 years it spread to all of the major citrus-producing areas of the country and has killed or rendered unprofitable a high percentage of the trees of the standard varieties of sweet orange on sour orange stock.

On bearing trees first signs of the disease

often appear on one side of the tree and soon spread to all parts of the top. Leaves are slightly bronzed, or otherwise discolored, and more brittle and leathery than those of healthy trees. In some cases there is a distinct yellowing of the midrib, the midrib and lateral veins, or even the entire leaf. Soon many of the older leaves fall. Lateral buds start growth and produce short, weak shoots with small leaves. Usually the first year after the disease appears the tree blossoms heavily and sets a large crop of fruit which is very conspicuous when ripe, partly because of the sparseness of the foliage. Trees decline rapidly in vigor. Twigs die back from the tips and sprouts are produced from the main limbs. Yields are drastically reduced after the first or second year and the trees are of little value.

## TRANSMISSION TESTS

Experiments have been made to transmit the tristeza disease from affected plants to healthy plants of known susceptible scion-stock combinations, both in the field and in the greenhouse, by various methods available for such transmission.

## FIELD TESTS

In the first test for transmission of tristeza to nursery trees, 20 trees of the variety Bahianinha on sour orange stock were selected from a nursery near Limeira and planted in plots near Campinas, October 31, 1946. Ten of these trees were inoculated November 20, 1946, by placing 2 buds from diseased trees into each plant. The trees were reinoculated December 18, 1946. The 10 trees remaining were held as checks and were not inoculated. All of these trees came from a nursery in an area where tristeza was prevalent and it was recognized that

some of the trees may have been infected before inoculation. One of the check trees began to produce yellow leaves by January 15, 1947, and showed other symptoms characteristic of tristeza. By June 1 all of the 10 inoculated trees and 3 of the check trees showed rather marked symptoms of tristeza. All of these diseased trees blossomed and 9 of the 10 inoculated trees set fruit which matured in September. The 7 healthy-appearing trees on October 1 were consider-

should be free of the disease. All of the trees were on sour orange stock. One-half, or more, of the trees of each variety was inoculated with buds from diseased trees January 18, 1947, and reinoculated April 9, 1947.

By the first of May, or approximately three and one-half months after the first inoculation, some of the inoculated trees of the variety Barao began to produce symptoms characteristic of tristeza. About a

TABLE 1—RESULTS OF INOCULATION OF NURSERY TREES IN PLOTS AT FAZENDA SANTA ELIZA, CAMPINAS, WITH BUDS FROM DISEASED TREES

Variety Tested	Number of trees used in test	Trees inoculated		Trees used as checks	
		Number	Number diseased	Number	Number diseased
Bahianinha*	20	10	10	10	3
Barao	25	15	10	10	0
Seleta	25	15	13	10	0
Pera do Rio	20	10	9	10	0
Abacaxi	10	5	0	10	0
Parnasia	10	5	5	5	0
Serrana	10	5	4	5	0
Campista	10	5	5	5	0
Lima	10	6	4	5	0
Coronel	10	5	5	5	1
Mangaratiba	10	5	3	5	0
Bahianinha	5	5	5	—	—

\* These trees were planted October 31, 1946, and inoculated November 20, 1946, with buds from diseased trees; they were reinoculated with buds from the same source December 18, 1946. All other trees were planted the first week in December, 1946, and inoculated January 18, 1947, and reinoculated April 9, 1947. The above records were taken October 1, 1947.

ably larger than the inoculated trees, had normal foliage, had blossomed sparsely, and had set no fruit.

In a second field test trees of the varieties Barao, Seleta, Pera do Rio, Abacaxi, Parnasia, Serrana, Campista, Lima, Coronel, Mangaratiba, and Bahianinha were obtained from a nursery near Santa Rita the early part of December, 1946, and planted at Campinas. At the time the trees were selected tristeza was only just beginning to appear in the area around Santa Rita and it was thought that most of the younger trees

month later some of the inoculated trees of the variety Seleta began to show abnormal coloration of the foliage. Symptoms characteristic of tristeza appeared on other varieties until by October 1, as indicated in table 1, most of the inoculated trees of all of the varieties except Abacaxi, were obviously abnormal. These abnormalities consisted chiefly of stunting of the trees, production of various shades, types and degrees of yellowing of the foliage, dropping of leaves, and profuse blossoming. Leaf cast was severe on some of the trees of the

varieties Barao and Mangaratiba and was especially severe in the case of the inoculated trees of the variety Coronel. Leaf dropping was followed by the production of many weak axillary shoots. With the exception of one tree of the variety Coronel which lost most of its leaves, blossomed heavily, and was obviously diseased, none of the check trees of any of the varieties have shown symptoms characteristic of tristeza.

#### GREENHOUSE TESTS

An extensive series of transmission tests has been made in greenhouses screened to give a considerable degree of protection against insects. Most of these experiments were made with small plants 4 to 24 inches tall growing in pots. Each of the small plants used for inoculation was produced by grafting a sweet orange top on a sour orange root when the plants of sweet and sour orange were only about 3 inches tall.

*Inoculation with Twigs and Buds.* Since all virus diseases are transmissible by graft, the first attempts to transmit the tristeza disease were made by grafting twigs and buds from diseased plants onto healthy plants. In one of these tests, small healthy plants were approach-cleft-grafted to twigs of diseased trees growing in barrels in the greenhouse. After a contact period of about 30 days the diseased twigs were severed just below the point of union with the healthy trees and allowed to continue to grow on the inoculated plant. Of 28 trees inoculated in this manner, 21 have shown definite symptoms of tristeza. Of 10 trees grafted with twigs of healthy seedling orange plants, none has shown symptoms of disease.

In a modification of the method of inoculation just described, twigs were taken from diseased trees and approach-cleft-grafted into the stems of healthy trees. The cut ends of the twigs from the diseased trees were kept in vials of water until union was complete and then severed just below the area of contact with the inoculated tree. This method of inoculation has given a

relatively high percentage of infection with twigs from known disease sources, and little or no infection from certain other sources.

Several experiments have been made in which buds from diseased trees were placed in the stems of healthy plants. So far this method of inoculation has given a very low percentage of infection when used with small potted plants.

In all cases of infection in these experiments in which inoculations were made by the grafting of diseased tissue to healthy plants, symptoms have begun to appear in periods varying from 30 to 90 days. Usually first signs of the disease appear in the youngest leaves. These are somewhat more yellow than normal and soon growth is retarded and the older leaves of the plant become pale, pale yellow, or yellow. The degree of yellowing varies greatly with different plants, but the degree of stunting is more or less uniform. Some plants have remained yellow and stunted for more than four months; others have produced some additional growth with more or less normally colored leaves that are smaller than those of healthy plants. Leaves have fallen from some of the more severely affected plants.

*Inoculation with Insects.* Small citrus plants composed of sweet orange tops on sour orange roots have been inoculated with most of the species of insects with sucking mouthparts that have been found feeding naturally on citrus plants in the plots at Campinas, and with certain other species of insects that have been induced to feed on citrus plants. These insects have consisted of 5 species of leafhoppers, one species of whitefly, and 6 species of aphids, including the black citrus aphid of Brazil, *Aphis citricidus* Kirkaldy.

Only plants that were inoculated with the black citrus aphid have shown symptoms of yellowing and dwarfing characteristic of tristeza. In all experiments in which this aphid was transferred from diseased to healthy plants in large numbers, a high percentage of the inoculated plants has later

shown symptoms of the disease. Usually the inoculated plants began to produce yellow leaves at the growing tips about 30 to 60 days after inoculation. Growth was much retarded and the plants showed various degrees of yellowing. In some cases the older leaves turned bright yellow in color and some leaves dropped. In other plants yellowing was less marked and in some cases the leaves were only slightly paler than those of normal plants. A few plants of this latter type have produced new shoots with small leaves more or less normal in color. Check plants infested with approximately the same number of aphids from healthy plants have remained normal in all cases. In some of the earlier experiments the check plants are now 3 to 4 feet tall, whereas the inoculated plants are pale to very yellow in color and average only about 12 inches tall.

These results with the black citrus aphid of Brazil are similar to those obtained by Meneghini<sup>1</sup> in tests with this insect. The evidence now available seems sufficient to justify the conclusion that the black citrus aphid, *Aphis citricidus*, is an agent of transmission of the tristeza disease in Brazil.

*Inoculation with Sap from Diseased Plants.* In tests of transmissibility of tristeza by means of juice, sap was pressed from young succulent shoots of orange trees that had been diseased for more than two years and which showed marked symptoms of tristeza. The juice was then used immediately to inoculate small rapidly-growing orange trees. The inoculations were made by rubbing the juice over the surface of the leaves that had been sprinkled with an abrasive before inoculation. Forty trees were inoculated in this manner and 10 trees were retained as checks. Five months after inoculation no symptoms characteristic of tristeza were evident on any of the inoculated or check trees.

Attempts have been made also to transmit tristeza to annual plants by means of juice inoculation. Obviously, if a type of annual plant susceptible to infection and producing marked symptoms, could be found, such a plant would be extremely useful in further studies on the tristeza disease, particularly in the determination of the presence of virus in different species and varieties of citrus on which no evidence of infection has so far been recognized and in detection of early stages of infection in trees of sweet orange on sour orange stock. However, although some 50 species and varieties of annual plants have been tested, none has shown abnormalities that have been attributed to the tristeza virus.

*Tests for Seed Transmission.* All of the seedling plants of both sweet and sour orange used thus far in the work on tristeza at Campinas have been produced from seeds from diseased trees or from trees that have been exposed to infection over a considerable period. A total of over 1100 plants composed of sweet orange seedlings grafted on sour orange seedlings has been prepared. Some of these have been inoculated by grafts or by the black citrus aphid and have shown the disease, but only after a reasonable period of time following inoculation. More than 325 plants, however, have been held as checks or have been inoculated by juice, by dodder, or by means of insects that appear not to be vectors of the virus. Only one of these plants has shown symptoms characteristic of the tristeza disease. This plant was not inoculated. Symptoms have persisted for more than four months but it has not been determined by inoculation tests that the plant carries the virus. It should be emphasized, however, even if this plant proves to be infected, that this cannot be accepted as conclusive proof that the virus was transmitted through the seed since possibility of accidental infection by aphids is difficult to avoid in tests of this type, and more evidence must be available before definite conclusions may be reached. From the results obtained thus far it seems prob-

<sup>1</sup>MENEGHINI, M. Sobre a natureza e transmissibilidade de doença "tristeza" dos citrus. *O. Biologico* 12:285-286. 1946.

able, however, that the virus is either not transmitted through the seeds of sweet and sour oranges tested or, at most, it is transmitted through a very low percentage of such seeds.

Additional and more extensive tests are now under way to obtain further evidence on the problem of seed transmission. For these tests seeds were collected from trees of the variety Pera in advanced stage of disease. Sour orange seeds were harvested from trees on their own roots showing no obvious symptoms of tristeza but surrounded by badly diseased trees of sweet orange. The seeds of the two varieties of oranges were planted in flats. When the seedlings attain sufficient size the sweet orange seedlings will be grafted onto the sour orange seedlings and the resulting plants will be watched for appearance of symptoms of disease. Symptoms of tristeza should appear in plants of this combination if the virus is carried through the seeds of either the sweet or sour orange which give rise, respectively, to the scion and stock of the test plant.

#### SCION-STOCK COMBINATION SUSCEPTIBLE TO TRISTEZA<sup>2</sup>

All trees of the varieties Pera, Bahia, Bahianinha, Barao, and other sweet varieties of orange, when grafted on sour orange stock, seem to be susceptible to tristeza. The disease has been reported also on mandarin on sour orange stock. In tests of different types of stocks being conducted at the Citrus Experiment Station at Limeira, Brazil, by Senhor Silvio Moreira, trees of the variety Pera on grapefruit stock are dwarfed and show symptoms more or less characteristic of tristeza. The trees, however, have not declined so rapidly as trees of this variety on sour orange stock. Certain varieties of

tangelo on sour orange stock also appear to be susceptible to the disease. However, a strain of the variety Sampson appears to be resistant. Trees of Marsh Seedless grapefruit on sour orange stock also show symptoms characteristic of those produced by tristeza.

The common varieties of sweet orange in Brazil when grafted to sweet orange, rough lemon, Rangpur lime, sweet lime, ponderosa lemon, "cravo" tangerine, citron, and trifoliolate orange have been resistant. However, citron and trifoliolate orange have proved to be of little value as stocks.

Although certain scion-stock combinations seem to be required in order to have trees show symptoms so far recognized as characteristic of tristeza disease, it has seemed reasonable to suspect that certain types of citrus trees, both on their own roots and on various types of so-called immune stocks, might be capable of harboring the virus which causes the disease, and thus might serve as sources of infection without showing symptoms of the disease.

To obtain information on the possible host range of the virus an extensive test is being conducted in which as many as possible of the different varieties and types of citrus trees growing in areas where they have been exposed to infection over a period of years, are being tested for presence of the virus. Inoculations by buds, by twig grafts, and by aphids are being made from each source. Thus far transmission of the disease has been obtained from an unidentified citrus tree on its own roots, from the "cravo" tangerine on its own roots, and from the Pera variety of sweet orange on Rangpur lime. None of these three types of trees showed the severe types of symptoms recognized as characteristic of tristeza. Transmission has been obtained also from grapefruit trees on their own roots that showed marked yellowing of veins and yellowing of the entire leaf in the case of some of the twigs produced during periods of rapid growth. It seems evident from these results, therefore, that the casual virus

<sup>2</sup>The authors are indebted to Silvio Moreira, Chief of the Department of Horticulture of the Instituto Agronomico, Campinas, Brazil, for information on varietal susceptibility, and for the privilege of making observations on the effects of tristeza on trees on different stocks in the extensive stock tests at Limeira.

is not limited to trees that show symptoms, such as those that occur on trees of sweet orange on sour orange roots, but may occur in at least certain trees on their own roots or on stocks other than sour orange, in which no symptoms or only questionable symptoms of the disease have been recognized.

#### TESTS OF STOCKS FOR RESISTANCE

Experiments are being made at Campinas in which all of the available varieties and types of citrus and citrus relatives are being tested for their reaction to tristeza disease when used as stocks for sweet orange. Thus far seeds of 125 varieties and types of citrus and citrus relatives have been forwarded to Brazil by F. E. Gardner of the U. S. Subtropical Fruit Station at Orlando, Fla. These seeds were germinated in flats and at present plants of 119 types are growing in nursery rows and will be budded to sweet orange, probably in January. After the sweet orange buds have grown into shoots they will be inoculated with tristeza. Later the trees will be planted out 3 1/4 feet apart in rows 6 1/2 feet apart in locations exposed to further infection by tristeza and watched for appearance of symptoms of the disease. This test in the course of a few years should furnish valuable information on the degree of resistance to tristeza of a large number of citrus types when used as stocks for standard varieties of sweet orange.

#### SUMMARY AND CONCLUSIONS

The evidence now available seems ample to justify the conclusion that tristeza disease of citrus in Brazil is caused by a virus

which can be transmitted from diseased to healthy plants by means of buds or twigs and by means of the black citrus aphid, *Aphis citricidus*. The virus probably is not juice transmissible and as yet there is no conclusive evidence that it is transmissible through seeds of diseased plants.

Probably the chief natural agent of transmission of the disease in Brazil is the black citrus aphid. This insect occurs abundantly on the new growth in the spring and, despite the fact that it appears to be a very inefficient agent of transmission when compared with vectors of certain other virus diseases, it probably has been responsible for most of the spread of the disease in Brazil.

Tristeza causes severe damage to all varieties of sweet orange, to mandarin, and to certain varieties of tangelo when these types are on sour orange stock. There is evidence also that it causes severe injury to sweet orange on grapefruit stock and to grapefruit on sour orange stock.

In addition to these types of trees on which definite injury is produced, the virus occurs in certain kinds of citrus trees in which no symptoms have been detected. It has been recovered from an unidentified citrus seedling on its own roots, the "cravo" tangerine on its own roots, and from the Pera variety of sweet orange on Rangpur lime stock, none of which showed symptoms of the disease. It was recovered also from a seedling grapefruit tree on its own roots that showed a type of yellowing of leaves of rapidly growing twigs. These preliminary results indicate that the virus may be rather generally distributed in the various kinds of citrus trees in Brazil.