

Fig. 3. Comparison of purple mite and six-spotted mite infestations, 1951.

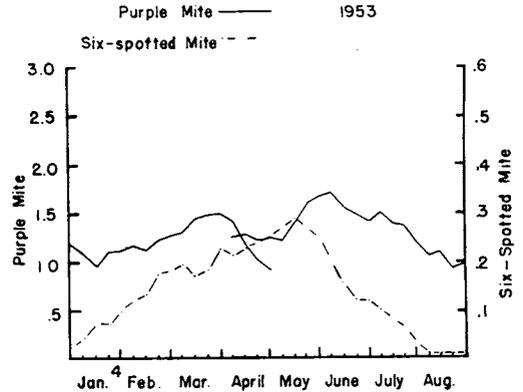


Fig. 5. Comparison of purple mite and six-spotted mite infestations, 1953.

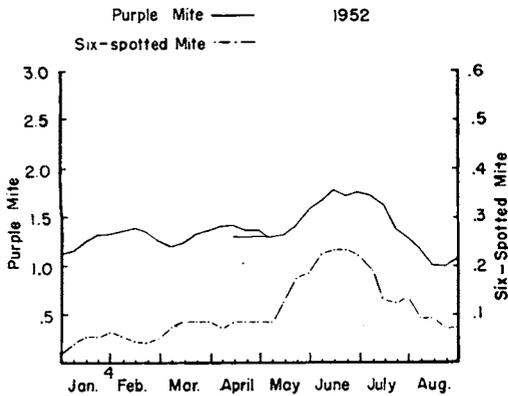


Fig. 4. Comparison of purple mite and six-spotted mite infestations, 1952.

other closely during the past three seasons. This correlation is significant at the 99:1 level.

This suggests that populations of both of these closely related mites may be influenced by the same climatic factors. It is possible that the winter temperature effect described for six-spotted mite also applies to purple mites, but the period of the record is too short for analysis. Since purple mite occurs every year as an economic problem, there are no old records of sporadic infestations available.

While purple mite appears to be much more sensitive to moisture conditions, this factor cannot be overlooked in analysing population trends for six-spotted mite.

#### SUMMARY

Over a period of three years, it has been found that six-spotted mites tend to recur in the same groves. The abundance of mites in these groves is influenced by location, spray program, and variety.

Severe spring infestations of six-spotted mites occur in seasons when the winter temperatures, particularly those of December, are low.

Moisture conditions influence purple mite infestations more strongly than those of other major citrus pests, and an increase in rainfall is usually followed by a reduction in mite activity.

Peak infestations of six-spotted mite and purple mite tend to occur at the same time and to be of similar magnitude.

#### LITERATURE CITED

1. Pratt, Robert M. New field work in citrus research. *The Citrus Industry* 31: 11:8. 1950.
2. Pratt, Robert M. Forecasting Citrus Insect Infestations. *Florida Grower* 10: 11:21. 1952.
3. Pratt, Robert M. Seasonal and Geographical Distribution of Some Citrus Insects and Mites in Florida. *Proc. Florida State Hort. Soc.* 65: 50-55. 1953.

## AIDS IN THE DETECTION OF TRISTEZA IN FLORIDA CITRUS

THEODORE J. GRANT<sup>1</sup>

In view of the fact that the causal agent of a virus disease can not be isolated and studied

<sup>1</sup>/Principal Plant Pathologist, U.S. Horticultural Station, Orlando, Florida.

in culture media, as is the case with many bacterial and fungus diseases, it is necessary to employ other methods of study. The first general observations on tristeza, or quick decline, made under field conditions in South Africa

lead to the knowledge that sweet orange tops on sour orange rootstocks showed decline symptoms while tops of the same sweet orange variety on some other rootstocks did not show disease symptoms. This then was the first method of recognizing the disease in the field. As a result of anatomical studies by Schneider et al. (9) in California of bark at the bud union of infected sweet orange on sour orange rootstock, there is not only a better understanding of the effects of tristeza, or quick decline, on the phloem tissues but there has developed an anatomical method of identifying this disease in the field.

The discoveries that tristeza, or quick decline could be transmitted by buds (10) and by the *Aphis citricidus* (Kirk) (1, 7), furnished evidence that the disease is caused by a virus. The knowledge of transmissibility of quick decline virus by means of buds led to the establishment of rootstock tests in California (2), where buds from diseased trees were used as sources of inoculum. The knowledge that tristeza virus could be transmitted by an aphid in Brazil was followed by extensive rootstock tests carried out cooperatively by the Instituto Agronomico of Sao Paulo and the United States Department of Agriculture (5) in which scion-rootstock combinations were inoculated by means of the aphid vector. This cooperative work resulted in classification of the rootstocks as tolerant or non-tolerant to the tristeza virus. Inoculated seedlings of some 50 different citrus varieties were found to express disease symptoms. The cooperative investigations also led to the knowledge that there are strains of the tristeza virus and that some strains cause severe disease while others cause milder disease symptoms (6).

The inoculation of citrus seedlings in Brazil by means of viruliferous aphids led also to the finding of vein clearing symptoms on *Aeglopsis chevalieri* Swingle and on West Indian type limes such as Key and Beledy (*Citrus aurantiifolia* (Christm.) Swingle) (4).

The combination of vein clearing and stem pitting symptoms on West Indian type limes such as the Key lime inoculated with tristeza, or quick decline, virus has been observed in tests carried out in South America (4), South Africa (8), and California (11). This combination of vein clearing and stem pitting of inoculated Key lime plants was the initial means of detection of a mild strain of the tris-

teza virus in Florida. Later a combination of Key lime plant inoculations, and the bark-sampling technique worked out by Schneider was employed by the Florida State Plant Board and cooperating organizations (3) to determine the distribution of the tristeza virus in this State.

In the latter part of August 1952, Dr. J. F. L. Childs of the U. S. Horticultural Station found, in Florida, some Persian, or Tahiti, limes with distinct vein clearing. Collection of branch material from these vein-cleared Persian lime plants and bottle-grafting to Key lime test plants showed that they were carrying the tristeza virus. This initial test for presence of the tristeza virus in vein-cleared Persian limes was repeated. When positive vein clearing and stem pitting were obtained on the inoculated Key lime plants, the State Plant Board officials were advised. They then gave the information to their grove inspectors, who proceeded to look for these symptoms on Persian limes. It was thought that in this way further information as to tristeza virus distribution might be obtained. Each grove inspector, upon finding what he thought to be vein-cleared Persian lime leaves, collected scionwood and forwarded it to the U. S. Horticultural Station at Orlando. The budwood thus received was bottle-grafted to Key lime test plants. In tests of 35 samples budwood of 7 samples failed to make positive unions; of the remaining 28 samples 14 from Persian limes transmitted the tristeza virus to Key lime plants.

The State Plant Board grove inspectors also found and collected samples of what they considered to be vein clearing and stem pitting on 8 limequat trees. However, only one of these proved to be carrying the tristeza virus. Likewise the inspectors collected budwood from a few lemon trees showing vein clearing symptoms and one of these, a Meyer lemon, proved to be carrying the tristeza virus.

The proof that the tristeza virus was the cause of vein clearing would require that healthy Persian limes, limequats, and Meyer lemon be inoculated with a pure strain of the tristeza virus. The development of vein clearing on these inoculated plants would then be evidence that the tristeza virus alone was the cause. However, the fact that the virus was recovered from 14 of the 28 Persian limes constitutes good evidence of a definite association.

On the other hand, the finding of only one limequat with positive tristeza virus out of 8 samples tested is considered very poor evidence of a definite association of tristeza virus and stem pitting on limequat. Likewise, the finding of one Meyer lemon with vein clearing that gave positive transmission of tristeza virus to Key lime is of considerable interest but is inconclusive until samples from other similar trees can be tested and found infected.

#### VEIN DASHES ON SOUR ORANGE AND GRAPEFRUIT

Study of the tristeza disease of Florida citrus is being carried out under controlled conditions in the greenhouse and screenhouses at Orlando. Healthy seedlings of several citrus varieties as well as Key limes have been inoculated. Three sources of tristeza virus have been used. The origin of two of the virus sources was a mandarin lime, and an Orlando tangelo. Scionwood from these field trees was bottle-grafted to Key lime plants, and the presence of tristeza virus was judged by the development of typical vein clearing and stem pitting symptoms. These infected Key lime plants were used as two of the sources of tristeza virus. The third source of inoculum was a potted Key lime seedling exposed for a ten day period to natural infection by placing it under a known infected tree in the field. This seedling lime was naturally infected, presumably by an insect vector, and was therefore considered to be free of psorosis-type viruses, which are not known to be insect-transmitted.

Tristeza virus from all three of these sources produced vein clearing and stem pitting on Key limes. The insect-inoculated virus source produced the mildest symptoms. When sour orange seedlings were inoculated by means of scionwood bottle grafts, it was noted that in some cases the young top leaves of the sour orange became yellow but that subsequently new normal growth appeared. All inoculated sour orange plants were somewhat stunted, but they continued to live and produce new growth. Transfers by bottle grafts to Key lime showed that the tristeza virus had lived and multiplied in the sour orange without killing the plants. Careful examination of the new young sour orange leaves revealed the presence of occasional tiny vein-cleared dashes in the lateral veins. This symptom has been called vein dashes as distinct from the more conspicuous and general vein clearing charac-

teristic of tristeza-virus-infected Key lime. Similar vein dashes were noted on young leaves of Duncan grapefruit seedlings inoculated with scionwood from the same three sources of tristeza virus.

These vein dashes tended to disappear as the sour orange and grapefruit leaves matured. Although this symptom was transitory and could be found only by careful examination of the young leaves in strong sunlight, it was considered important to determine whether it could be used under field conditions to identify tristeza-infected trees. Two State Plant Board inspectors, A. C. Crews and H. M. Van Pelt, were shown the diagnostic vein dashes and asked if they would bring in from the field material that showed this symptom. Within a short time these men brought in four samples of grapefruit and one sample of sour orange. Bottle-grafts of branches from these samples to Key lime test plants all showed that the tristeza virus was present in the samples collected.

The value and practical importance of this work have yet to be demonstrated, but it does open up the possibility of detection of tristeza in grapefruit trees by inspection of the young foliage. The application of this knowledge may be especially helpful in determining distribution of tristeza in the Florida Ridge section, where grapefruit is grown on Rough lemon rootstock and where the bark-sampling identification technique is not yet applicable.

#### DISCUSSION OF PRACTICAL APPLICATIONS

The citrus grower may question the practical value of information concerning vein clearing on limes and vein dashes on grapefruit. However, as a means of detecting presence and distribution of tristeza virus in his grove they give the trained State Plant Board inspector and the scientist, additional methods of determining the presence of the disease. Increase in our knowledge of this disease has been arrived at slowly, but each step has led to greater accuracy and to reduction in the number of plants that have to be tested in detail in order to be sure that the tristeza virus is present.

In the survey (3) for presence and distribution of tristeza in Florida many tests of virus transmission have been made from diseased field trees to Key lime plants. Results indicate that in many instances not only tristeza virus but also psorosis and other virus-like dis-

ease agents were present in the scionwood from the declining field trees. This information, combined with field observations of declining sweet orange on tristeza-tolerant rootstocks such as Rough lemon with no evidence of recognized cause, indicates that other virus or virus-like diseases in Florida present a complex picture. The obtaining of a detailed understanding of these diseases and the action or interaction of their causal agents will require appreciable time and study. In the meantime the grove owner wants to know what can be done. The answer at this time must of necessity be based on a knowledge of general behavior of virus diseases and on precautionary measures of sanitation.

In plant disease as in human disease outbreaks one of the first steps to be taken is that of practical common-sense sanitation. If the source of a human disease infection is the water or milk supply these should be cleaned up. In the case of virus diseases of citrus, budwood can be a definite carrier of the infectious agents. The grower's active cooperation in the State Plant Board's bud-certification program is an important first step toward freedom from virus diseases. This program for a supply of healthy budwood can not be accomplished overnight. As knowledge increases, the methods of detecting the virus diseases can be improved so that eventually the State Plant Board may be testing for more viruses than are now recognized and thus may be able to eliminate them or reduce their occurrence in budwood supplies. In the future it may also be advantageous to establish certified sources of seed as well as of budwood. The advantages of certified seed sources would be to insure, for example, that the particular Rough lemon is one known to have the desirable horticultural characters and tolerance or resistance to disease.

A sanitation measure that also can be taken is that of removing declining citrus trees from the groves. When a grove owner has citrus trees in a state of decline that do not respond to fertilizer or spray treatments it would be well to get rid of them. This is especially true where these trees are observed to occur singly or in scattered small groups. The fact that these trees are scattered might well indicate that some infectious agent is being spread. Certainly these trees are of little economic value, and they are a potential hazard for further disease spread. Replanting of spots,

where declining trees have been removed, with healthy scions on tristeza-tolerant rootstocks is recommended wherever possible.

The nurseryman and the grove owner can also take effective precautions with respect to multiplication of budwood. In the past a common method for rapid multiplication of budwood was topworking it into old trees. Such a procedure is a sure way of introducing into the budwood any viruses that may be present in the stock tree. The subsequent use of buds from such sources can result in a grand-scale distribution of the viruses that are the causal agents of disease. The safest procedure for establishing a source of budwood is to bud it into young seedling stocks which have never had a bud inserted in them.

Topworking of old groves for the purpose of changing varieties similarly offers an opportunity for virus infections from the old stock to spread into the new variety. The subject is mentioned because of the current interest in topworking grapefruit and other old citrus trees to lemons and Persian limes. From a virus-disease standpoint topworking is a hazardous practice especially when the trees selected for topworking are obviously diseased or are in a poor state of growth suggestive of the presence of disease.

The outstanding exception is tristeza-infected trees topworked with acid lemons. In Brazil it was found that topworking sweet orange on sour orange rootstock with acid lemons such as Eureka and Lisbon could be successful even when the trees were showing definite tristeza symptoms. Similar experience in California (2) showed that topworking of quick-decline-infected Valencia orange on sour orange rootstock was successful when the topworking to lemon was complete but that partial topworking to lemon was not satisfactory. The presence of the diseased sweet orange top on a part of the tree greatly inhibited the growth of the lemon scions on the other part.

How this procedure of topworking of old field trees to acid lemon varieties will work under Florida conditions remains to be demonstrated. The strain of the tristeza virus is mild and there are indications that many of the visibly diseased field trees are carrying more than just the tristeza virus.

The vein clearing reactions of the Persian lime in association with the mild strain of the tristeza virus in Florida have been mentioned.

Infected trees of Persian lime on sour orange rootstocks observed under field conditions may not have been quite as vigorous as the surrounding trees in some instances. At the same time these infected trees were not in a serious state of decline. The exact effects of tristeza virus on the acid Persian lime and of other possible virus combinations that may be encountered in any topworking operations have yet to be determined.

It is not expected that this warning as to the virus hazards will stop the practice of topworking citrus, but when the grower does decide to topwork he should take every possible precaution to insure that his source of budwood is healthy and the trees he topworks are as healthy as possible.

#### LITERATURE CITED

1. Bennett, C. W., and A. S. Costa. Tristeza disease of citrus. *Jour. Agr. Res. (U. S.)* **78**: 207-237. 1949.
2. Bitters, W. P., and E. R. Parker. Quick decline

of citrus as influenced by top-root relationships. *Calif. Agr. Exp. Sta. Bull.* **733**: 1-35. 1953.

3. Busby, Joe N. Tristeza in Florida. *Citrus Industry* **34**:(8) 5-7. 1953.

4. Costa, A. S., T. J. Grant, and S. Moreira. On a possible relation between tristeza and the stem pitting disease of grapefruit in Africa. *Calif. Citrog.* **35**: 526-528. 1950.

5. Grant, T. J., A. S. Costa, and S. Moreira. Studies of tristeza disease of citrus in Brazil. III. Further results on the behavior of citrus varieties as rootstocks, scions and seedlings when inoculated with the tristeza virus. *Proc. Fla. State Hort. Soc.* (1949) **62**: 72-79. 1950.

6. Grant, T. J., and A. S. Costa. A mild strain of the tristeza virus of citrus. *Phytopath.* **41**: 114-122. 1951.

7. Meneghini, M. Sobre a natureza e transmissibilidade da doenca 'Tristeza' dos citros. *Biologico* **12**: 285-287. 1946.

8. McClean, A. P. D. Virus infections of citrus in South Africa. *Farm. in South Africa* **25**: (293) 262, 25: (294) 289. 1950.

9. Schneider, Henry, J. M. Wallace, and J. E. Dimittan. The pathological anatomy of bud union tissues of orange trees and its value in diagnosis of quick decline. *Phytopath.* **40**: 24. 1950.

10. Wallace, J. M., and Fawcett, H. S. Evidence of the virus nature of citrus quick decline. *Calif. Citrog.* **32**: 50, 88-89. 1946.

11. Wallace, J. M. Recent developments in studies of quick decline and related diseases. *Phytopath.* **41**: (9) 785-793. 1951.

## RELATION OF pH AND SOIL TYPE TO TOXICITY OF COPPER TO CITRUS SEEDLINGS

WALTER REUTHER, PAUL F. SMITH AND  
GEO. K. SCUDDER, JR.  
*U. S. Horticultural Station*  
Orlando

In a previous study (5) it was shown that the topsoils in most bearing citrus groves in the sandy soils of central Florida have accumulated between 100 and 500 pounds of total copper (calculated as CuO) per acre-six-inches. Natively such soils usually contain between 5 and 20 pounds of CuO per acre in the topsoil. This accumulation results largely from the fixation of copper added in mixed fertilizers and to a lesser extent from that in residues of fungicidal or nutritional sprays containing copper. For the past decade or more it has been common practice to apply 10 to 25 pounds of CuO (from copper sulfate) per acre in the regular fertilizer program each year and in some cases an additional 5 to 25 pounds per acre per year from foliage sprays. In other studies (6) it was shown that under acid soil conditions, copper concentrations well within the ranges commonly found in mature grove soils caused stunted, abnormal root development, and under certain conditions, so-called iron chlorosis of the foliage of young citrus seedlings in pots.

This paper summarizes the results obtained from additional pot studies designed to clarify further the relation of type, cultural history, acidity and zinc and manganese contents of soils to toxicity of copper to citrus seedlings.

#### MATERIALS AND METHODS

Soil number 1 described in table 1 was obtained in Lake County from land newly cleared in anticipation of planting to citrus.

Table 1. Description and composition before treatment of soils used in pot studies.

Soil No.	Type	Depth (inches)	Cultural history	Exch. cap. (me./100gms.)	Total CuO (lbs./A)	Total P <sub>2</sub> O <sub>5</sub> (lbs./A)
1	Lakeland sand	0 - 4	Virgin	1.51	5.7	690
2	Lakeland sand	6 - 12	Virgin	1.15	1.8	690
3	Orlando fine sand	0 - 6	Old grove	7.22	262.0	7,690
4	Orlando fine sand	0 - 6	Virgin	6.15	3.5	2,640

Similar soil is common in the rolling hills of central Florida, and is extensively used for citrus culture. Soil number 1 is typical of the lighter, sandier phase of the Lakeland series known as "blackjack" land. Soil number 2 is subsoil of a type similar to soil 1, but obtained from a different location.

Soil number 3 described in table 1 was obtained in a 30-year-old Valencia orange grove affected periodically in the past four to five