CHAPTER 2

Tristeza and Related Diseases

Recent Advances in the Study of Tristeza and Seedling Yellows

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SINCE THE DEMONSTRATION that failure of sweet orange on sour orange stock (tristeza disease) was caused by an aphid-transmitted virus, numerous symptoms have been ascribed to the virus. These include reactions of sweet orange on sour orange stock ranging from negligible to lethal; reactions of other susceptible stock/scion combinations; wood pitting, vein flecking, and decline of acid lime; wood pitting and fruit distortion in grapefruit; and mild pitting of orange and other species. Also, claims have been advanced that many strains and perhaps more than one virus are concerned.

In recent years, significant contributions have been made relative to host reaction, electron microscopy, virus strain interaction, vector transmission, and epidemiology.

HOST REACTION.—Information is now available on the reaction of a wide range of species and combinations of scion and stock. Moreira and co-workers (7) reported the poor tristeza tolerance of sweet lime (*Citrus limettioides* Tanaka), sweet lemon (*C. limetta* Risso), and others as rootstocks. They also noted the occurrence of wood pitting in sweet orange [*C. sinensis* (L.) Osb.], tangelo (*C. reticulata* x *C. paradisi*), and citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis*] stocks under appar-

ently normal orange trees. Occasional mild pitting, apparently of a harmless nature, has been rather generally noted in many countries on tristeza-tolerant varieties. However, Giacometti and Araujo (3) were unable to induce pitting in the Pera orange using a particularly severe strain of tristeza in an experiment lasting ten months. This does not preclude the possibility that pitting of Pera orange is caused by this strain of tristeza virus since the disease symptoms ordinarily appear when trees are five years old or older. Other suggested explanations of Pera orange pitting were that it is due to re-combined mixtures of the tristeza virus complex or to an entirely different strain of tristeza. The relationship of this severe type of pitting, occurring on Pera orange in Brazil, to tristeza is important because if it can be related to the tristeza virus complex the range of symptoms would be materially extended.

Capoor (1) described seedling yellows from declining sweet orange trees on Rough lemon stock in India and attributed the decline to tristeza, although Rough lemon is elsewhere a tolerant stock. The symptoms described on sour orange and other indicators were of a type and severity which suggest the presence of another virus in addition to seedling yellows-tristeza. This second virus is probably greening virus (2), to which sour orange is susceptible. Published descriptions indicate that a similar situation may exist in other countries in Southeast Asia. This situation emphasizes the importance of confirming symptoms by vector transmission whenever a new symptom is attributed to an already well-known virus.

STRUCTURE OF TRISTEZA VIRUS.—At the Brazil Conference in 1963, Kitajima and co-workers (4) announced the partial purification of tristeza virus and exhibited electron micrographs showing thread-like particles 10 to 12 mμ x 12,000 mμ in partially purified preparations and in leaf dip preparations. This work was taken further by Price (8), who confirmed the presence of flexuous rods in partially purified virus preparations and in leaf dip preparations. He demonstrated by electron microscopy of ultrathin sections the presence of these flexuous rods in the veins of leaves of West Indian lime infected with the T_3 strain of tristeza of Grant and Higgins. The particles were restricted to one or a few phloem cells, but were present in enormous numbers. Other phloem cells in the veins of infected leaves were necrotic, suggesting that the protoplast had been killed by the virus. These may be the chromatic cells described earlier by Schneider (9). Price was working with a single virus strain of the mild Florida virus purified by aphid transfer, whereas Kitajima and associates worked with naturally infected and greenhouse-

infected plants of many species, which would certainly have a variety of mild and severe strains including seedling yellows.

CROSS-PROTECTION AND STRAIN ANALYSIS.—Cross-protection methods have been used by several investigators to study strain relationships. In general, cross-protection has been short-term or incomplete, which suggests that once a challenging strain gains entry to a host already infected with a strain of the same virus, some multiplication and spread of the challenging strain generally occurs. Differences in the protection obtained in different countries may be the result of the different methods of testing and the different strains of viruses present. Giacometti and Araujo (3) reported the results of cross-protection trials using a very mild strain and a severe strain. The mild strain was one which caused little pitting or reduction in growth in seedling sour orange or Eureka lemon, no growth reduction and few pits in grapefruit, and little or no stunting and little pitting in acid lime, whereas the severe strain produced the seedling-yellows reaction. When the severe strain was introduced by budding into plants inoculated with the mild strain 4 months previously, no seedling-yellows reaction occurred nor was the growth of sour orange or Eureka lemon reduced over a period of 10 months. In grapefruit and lime, however, the double inoculation caused growth reduction intermediate between that caused by mild and severe strains, but no pitting.

Stubbs (12) in Australia, claimed that inoculum introduced by a vector would not be as concentrated or permanent as when introduced by a tissue graft, and that, "strains having the greatest affinity for the host could be expected to multiply and move ahead fastest and impede or prevent the multiplication of less adapted strains." Stubbs worked with an isolate from Lisbon lemon which gave good protection against tristeza during the first few years following inoculation. However, this protection was not complete, and later the size of the inoculated trees was somewhat reduced as compared with trees carrying only the Lisbon isolate. Isolates from grapefruit and Meyer lemon were less effective as protectants. Stubbs concluded that, "the tristeza virus consists of a wide spectrum of co-existing strains with pure strains rarely if ever existing in nature. The less closely related strains co-exist with little mutual interference. Others exhibit considerable mutual antagonism which is reflected by the variable symptoms expressed by their hosts and the order of strain co-existence is influenced by a degree of host virus selectivity."

Wallace and co-workers (14, 15) found that for lemon plants that had recovered following inoculation with seedling-yellows virus (SYV), the

degree of protection against reinfection varied according to the strain of SYV with which they were originally infected. Protection ranged from complete to very little. The strain (SY-T) in recovered lemon did not cause yellowing of lemon seedlings, but usually stunted them more than did the California strain (T) of tristeza. It was also demonstrated that *Aphis gossypii* Glover could transmit the SY-T component from plants carrying SYV complex.

Wallace (14) found that SY-T, California tristeza (T), and SYV could cause typical tristeza decline of sweet orange on sour orange stock, and in one case found that SY-T caused a more severe decline than did its source, SY. They suspect that two distinct, unrelated viruses are involved in the seedling-yellows reaction, a virus closely related to tristeza with which the other reacts synergistically to give the seedling-yellows reaction. Stubbs (12) preferred to regard the symptom variability of the whole complex as being best explained by strain differences within a single virus, by differences in host reaction, and by host selection of strains. Capoor (1) also regards tristeza as a single virus with numerous strains.

In South Africa, McClean (6) found isolates from field trees of lemon and sour orange that caused flecking and pitting on acid lime, but did not cause tristeza on sweet orange on sour orange rootstock. He also found that isolates from grapefruit generally, though not always, caused a milder type of tristeza in sweet orange on sour orange rootstock than did isolates from mandarin and sweet orange containing the full SY plus T complex. One strain from a very severely stem-pitted grapefruit produced only very mild tristeza in sweet orange on sour orange stock, and sometimes no tristeza reaction was produced by grapefruit isolates.

McClean (6) considered there is some evidence that, "two distinct viruses are associated with the tristeza virus complex. The main objection (to that hypothesis) is that no separate component has been isolated which will induce only yellows disease. It (yellows) is always accompanied by the component causing stem pitting in limes." He preferred the expression "tristeza virus complex," and to think of the complex as consisting of two main components, stem pitting and yellows, and concludes: "To use the expression tristeza disease to describe all the diseases produced by the virus complex is confusing. It is better to distinguish between such reactions as stem pitting, seedling yellows and tristeza."

No investigator has so far been able to separate seedling-yellows virus or SY-T in pure form, nor can the properties of such strains be foretold. Possibly, as Wallace suggested, they are unable to exist alone.

Proof of the existence of two or more distinct viruses, each with a range of strains, or of one multistrain virus must await further analysis. Possibly a relationship such as exists in carrot motley dwarf virus, where its two components have linked transmission (16) is involved.

VECTORS.—The number of species which can transmit viruses of the tristeza complex has been extended by Varma *et al.* (13) in India, who added *A. craccivora* (Koch.) and *Dactynotus jaceae* L. *Aphis craccivora* has been used unsuccessfully by other workers, and this, and the lack of success in Israel with *A. gossypii*, *Myzus persicae*, and *Toxoptera aurantii* as vectors suggest the occurrence of strains of aphids of different transmitting ability.

EPIDEMIOLOGY.—Schwarz (10) reported studies on the epidemiology of the tristeza virus complex in South Africa in which he used yellow water traps and bait seedlings to assess aphid movements and the virus strains carried. Two aphids, *T. citricida* and *A. gossypii*, were examined. The number and type of virus strains trapped in two areas varied considerably. Severe and very severe strains (including seedling-yellows virus) were most prevalent. The trapping of tristeza virus on bait seedlings was correlated with movements of *T. citricida*, but not of *A. gossypii*. Year to year differences in relative abundance of the two species suggest that ecological factors may limit the presence of the vectors in some areas. Similar observations are required in other countries to complete the picture of aphid behavior in relation to environment. The results could be of value in forecasting spread of the disease.

TOLERANCE.—A degree of tolerance to tristeza has been reported by Stubbs (11) in two types of sour orange when used as rootstocks. A flat sour orange has been used successfully by a grower in Mildura, Victoria, as a stock for grapefruit carrying a mild stem-pitting strain and for oranges that carry the full SY-T complex. Stubbs also found that a sour orange clone from Israel is tolerant when used as a stock for infected sweet orange scions. He has pointed out that in Israel the commonly used sour orange is tristeza-susceptible. Trials with the Mildura flat sour orange in New South Wales have shown that young seedlings are sensitive to seedling yellows and also give a tristeza reaction when used as stocks for infected budwood. Further investigation of this tolerance and the circumstances under which it is effective is necessary.

DISTRIBUTION OF TRISTEZA VIRUS 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, such as Israel, it has not spread at all.

Moreover, the virus strain components and disease expression vary from country to country. This behavior is probably a function of the vector or vectors in the different areas. There is evidence (10, 14) of differences in the ability of aphid species to extract and transmit strains from a complex. 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. citricida*, the most efficient vector. When this complex is introduced into an area where *T. citricida* does not occur, the strain assemblage transmitted will depend on the indigenous aphid population.

The analysis of strains by Wallace and co-workers (15) offers some prospect of explaining the different results obtained by different investigators in different countries. Their derivation, under experimental conditions, of SY-T strains with properties widely different from the source SY indicates that this must also occur in the field.

Considerable evidence has accumulated to indicate that the host species exerts a sorting-out effect on virus strain mixtures (11, 14, 16). This is a function of the sensitivity or tolerance of the host species, and deserves further study.

The problems of virus strains, strain interactions, and rate of virus spread in relation to the vector and of the mechanism of sorting out of strains by host varieties require further elucidation. This is of immediate importance in areas where the disease is not spreading, though introduced in the past, or where only a limited range of strains occurs.

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