

***Anatomical Aspects of Tristeza-Diseased
Citrus. Aeglonis, and Afraegle***

THE FOLLOWING HYPOTHESES have been proposed (5). Tristeza virus moves through sieve tubes and multiplies in parenchyma cells adjacent to them. Cells in which multiplication occurs become altered in a distinctive manner and are called chromatic cells. Chromatic cells are the earliest known symptom of tristeza and are found in all hosts whether external symptoms are present or absent. Species fall into two distinct types with respect to their response to the presence of chromatic cells. In one type, the chromatic condition spreads from the phloem parenchyma cells into meristems, disrupts them, and interferes with normal initiation of tissues. Localized lesions are thus formed which result in such macroscopic symptoms as wood pitting, stem pitting, and vein clearing. Sour lime [*Citrus aurantifolia* (Christm.) Swing.], sweet orange [*C. sinensis* (Linn.) Osbeck], and grapefruit, (*C. paradisi* Macf.) show meristem disruption. In the other type, represented by lemon [*C. limon* (Linn.) Burm.] and sour orange [*C. aurantium* (Linn.)], the chromatic condition does not spread to meristems but sieve-tube necrosis occurs. In lemon seedlings infected with a virus from Meyer lemon, leaf and stem growth are interfered with, resulting in the well-known yellows symptoms. As yet no satisfactory anatomical explanation of this growth interference and yellowing of shoots has been observed. In elongating roots, severe sieve-tube necrosis was found, the feeder roots deteriorated, and the root system was deficient (1, 5).

Evidence has been presented which indicates that seedling yellows is caused by a severe strain of tristeza virus (4, 5). A mild strain of tristeza virus from commercial orchards of California produced chro-

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matic cells and slight amounts of sieve-tube necrosis in newly formed roots of lemon seedlings. The condition was similar to that which occurs so abundantly and severely in roots of seedlings which develop yellows symptoms following inoculation with virus from Meyer lemon. This suggested that two strains of one virus having differences in severity were involved and not two distinct viruses.

It was further hypothesized that the decline and wilt which occur in sweet orange trees on sour orange rootstocks result from an incompatibility induced by tristeza virus (5). Perhaps a substance moving from the chromatic cells of the sweet orange into the translocation stream kills the sour orange sieve tubes immediately below the bud union but does not affect the sweet orange sieve tubes.

Studies have been made (a) to determine whether the yellows symptoms in lemon shoots result from the malformed and deficient feeder root system, (b) to determine whether seedling yellows symptoms in shoots result from an anatomical disarrangement, and (c) to determine why tristeza virus is not graft-transmissible from *Citrus* to *Aeglopsis* and *Afraegle*.

Materials and Methods

The strain of virus used, obtained originally from Meyer lemon, produced vein clearing and wood pitting in lime and yellows in lemon seedlings. The virus was passed through *Aphis gossypii* Glover, to lime seedlings, and therefore the lime plants from which inoculum was obtained were probably free of viruses other than that of tristeza. Bradbury Lisbon seedlings were used for experimental plants. Procedures in microtechnique were those used previously (5, 6). The normal anatomy of growing shoots is already known from previous studies (3).

Results

POSSIBLE EFFECT OF MALFORMED ROOTS IN INDUCING YELLOWS.—Sieve tubes become necrotic, excessive phloem forms, and there is a severe depression of xylem formation and the feeder roots deteriorate in newly formed roots of tristeza-diseased lemon seedlings (4, 5). To determine whether yellows will develop in the absence of defective roots, scions from yellows-affected Frost Eureka lemon plants were grafted into healthy sweet orange seedlings. New shoots began to grow on scions in 3 of the plants, but when the new shoots were several mm

ong, they dropped off. Although the scions remained alive for a year or more, it was not possible to force their growth. Scions of a fourth plant grew, but the growth was severely affected by yellows. Apparently root failure is not responsible for yellows symptoms because sweet orange shoots on the same plants grew normally.

Another experiment was devised to determine how lemon seedlings are affected when the yellows strain of virus infects only the basal part of the plant. It was found previously that the severe strain of virus did not invade long leafy lemon shoots, nor was it possible to separate out distinct strains of viruses by grafting segments of the branches into lime and lemon seedlings. The seedlings remained symptomless. In the current experiment, plants were chosen with one main branch about a meter tall and with smaller branches near their base. Three lime grafts containing the yellows strain of virus were placed in the small basal branches of each test plant. The main branch was cut to a uniform height of 1 meter. Included in the experiment were 6 check trees, 6 trees in which inoculating scions were removed after the lower branches developed symptoms, and 6 trees in which the scions were not removed. All the inoculated plants developed symptoms in the basal branches, and 7 of them developed symptoms in upper branches which were induced to form by the pruning. Yellows symptoms appeared quickly, and the root system present at the time of inoculation was no doubt still adequate when symptoms appeared. For several months the main branches of the other 5 plants appeared to grow as well as the checks. However, a suppression of growth but not yellows of some of the leafy shoots was evident by 6 months after inoculation; apparently the root system gradually became inadequate. The reason the long main leaders of 5 of the plants remained free of typical yellows symptoms was probably that the virus failed to enter a leafy shoot. These experiments indicate that virus activity induces symptoms directly in growing shoots and not indirectly by causing degeneration of the root system.

ANATOMICAL STUDIES.—It was previously determined that chromatic cells and sieve-tube necrosis are present in slight to moderate numbers in the secondary phloem of stems and in the leaf midribs of Lisbon lemon seedlings inoculated with the yellows strain of virus (4, 5). However, these anatomical abnormalities were not severe enough to interfere with translocation and cause the yellows symptoms.

In the current studies, the primary tissues of the stem and expanding leaves were examined at 17 days and 31 days after graft inoculation.

Sections began with the apical meristem and progressed downward through the primary tissues. Several of the internodes below the tips were also sectioned. Chromatic cells were found throughout the phloem of the stem tip, including the protophloem, but they were not present in striking numbers, and tended to be localized. Some necrotic sieve tubes were present, but the ones most recently formed appeared to be functional. Partially crushed chromatic cells were conspicuous between parenchyma cells and were often observed even in the smallest veinlets.

The chromatic condition did not spread from protophloem sieve tubes into meristematic tissues such as the apical meristem, procambial strands, and ground meristem. There appeared to be no interference with normal vascular development such as was found in the roots. It could not be visualized how the slight to moderate occurrence of chromatic cells and sieve-tube necrosis could interfere with translocation and cause growth stoppage and distortion of stem tips. Possibly chromatic cells produce a substance which interferes with cell division.

RECIPROCAL GRAFTS BETWEEN CITRUS, AEGLOPSIS, AND AFRAEGLE.—Dr. A. P. D. McClean attempted to transmit tristeza to *Afraegle paniculata* (Schumn.) Engl. and to *Aeglopsis chevalieri* Swing. by grafting them with stems from infected citrus (2). Although the disease was readily transmitted by the insect vector *Toxoptera citricidis* (Kirk.), graft transmission failed. Dr. McClean supplied us with graft unions from plants which he had attempted to infect by grafting. Included were 7 acid limes and 7 sour orange seedlings with *Afraegle* side grafts, and 1 lime and 2 sour orange seedlings with *Aeglopsis* grafts. The grafts had remained alive for many months, but they did not grow, nor did they transmit tristeza virus. Also studied were top grafts of healthy Frost Eureka, Valencia orange, and Marsh grapefruit on healthy *Afraegle*. The grafts grew out several inches and formed 6 or more leaves and then stopped growing. The scions just above the bud union were greatly overgrown, but the rootstocks hardly grew at all.

It was not easy to detect whether sieve tubes were continuous between nongrowing side grafts and the receptor plant because phloem formation was meager. There was also the problem of getting a radial section, suitable for study, through the vascular tissues of some plants because the bark of the grafts was inserted far down into the wood of the host, and the cambium and phloem tissues of the two parts did not match up in radial sections. However, in 4 lime plants and 2 sour orange plants with *Afraegle* grafts, radial sections were obtained through the

union of the vascular tissues. In the xylem, there appeared to be a fusion of tracheary elements and a definite interlocking of fibers. On the other hand, in the phloem, hypertrophied parenchyma and crushed cells were often present where sieve tubes should have been. Where elongated cells were present, they were identified as parenchyma.

In plants formed by grafting healthy Frost Eureka, Valencia orange, and Marsh grapefruit onto Afraegle seedlings, sieve tubes were not observed in sections of vascular tissue at the graft union. Starch accumulated above the bud unions, and was absent below. This evidence that there is not continuity of sieve tubes between scion and stock, along with McClean's failure to transmit tristeza by grafting, substantiates the hypothesis that tristeza virus moves through the sieve tubes.

Chromatic cells and wood pits in infected Afraegle and Aeglopsis resembled those previously described in citrus.

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