

THE ANATOMY OF TRISTEZA-VIRUS-INFECTED CITRUS¹

Henry Schneider²

University of California Citrus Experiment Station,
Riverside, California

INTRODUCTION

In this paper previous anatomical work on tristeza of citrus is reviewed, and some new observations are presented. In particular, the author wishes to show that 1) the vein flecking and stem pitting in seedlings of Mexican lime, *Citrus aurantifolia* (Christm.) Swing., 2) the yellows in lemon seedlings, *C. limon* (Linn.) Burm., and 3) the symptomless external condition in infected mature trees of sweet orange, *C. sinensis* (Linn.) Osbeck, are all preceded by the same primary cytological symptom. He wishes to show also that sieve-tube necrosis immediately below the bud union in sour orange rootstocks, *C. aurantium* Linn., is a symptom of incompatibility with infected sweet orange scions.

Terms used here follow the definitions of the Committee on Technical Words of the American Phytopathological Society (24). The terms *hypersensitivity*, *immunity*, *resistance*, *sensitivity*, *susceptibility*, and *tolerance* are used in a technical sense in describing the reaction of a host to a parasite. The reactions of a composite budded plant of two species to a virus are very complicated, and the words *satisfactory* and *unsatisfactory* are sometimes used in describing them, but without technical implications.

Five types of diseases of budded trees are referred to in this paper. A *bud-union disease* will be thought of as one in which a continuous interaction between the virus, the scion, and the rootstock is needed to produce the disease. The injury must occur most intensively at the bud union. A *scion* or a *rootstock disease* occurs when one sensitive species (part) of the composite plant becomes infected. The affected part might depend on the other species (counterpart) of the tree for its initial inoculation, but once inoculated the disease would become independently systemic in the sensitive part. A modification of a scion or a rootstock disease would be one in which a resistant scion or rootstock would depend for symptom expression on its counterpart for continuous or intermittent inoculation, but in which the causal agent of the disease would be systemic in the resistant part and in which no important bud-union injury would occur. Such situations will be referred to as *rootstock-dependent scion diseases* and *scion-dependent rootstock diseases*. The scion or rootstock might also change the susceptibility of its counterpart.

The word *complex* will denote a group of tristeza-virus strains. Each tristeza-virus strain will be characterized by the intensity of symptoms it can produce in each of three host types. These host types are characterized by the nature of the injury they suffer when infected. The injury types are as follows: 1) bud-union, 2) wood pitting and vein flecking, and 3) yellows. A virus strain, for instance, may be mild for the bud-union disease, severe for pitting, and so extremely mild for yellows that the yellows would not be apparent.

¹ Paper No. 1104, University of California Citrus Experiment Station, Riverside, California.

² Associate Plant Pathologist, Citrus Experiment Station, Riverside.

The age and size of the host affects susceptibility, and a description of age groups follows. Upon germination, seedlings first produce leaves without distinct petioles; in some species there are two such leaves with an opposite arrangement. Seedlings in which petioleless leaves are the dominant foliage will be referred to as in the *two-leaf stage*. The term *potted plant* will designate plants of various sizes beyond the two-leaf stage. *Nursery-sized trees* are headed trees about 1 meter in height and recently transplanted from the nursery to the field. *Mature trees* are those which are several years old and have reached a maximum volume.

REVIEW OF LITERATURE

In the 1940's, extensive research was conducted on a mysterious wilt and decline disease of sweet orange trees on sour orange rootstock (8, 9, 26). External symptoms are of two types in field-planted trees. Trees which decline gradually are characterized by heavy sets of fruit, suppression of new growth, bronzing and yellowing of leaves, defoliation, and dieback of twigs. New growth subsequent to decline is weak and confined to the main branches. External symptoms of another type are exemplified by trees which suddenly wilt and die without losing their leaves.

Tristeza causes a utilization of reserve starch in roots, followed by rotting (8). Early in 1946, Schneider described a necrosis of sieve tubes immediately below the bud union of affected trees (26). He pointed out that this symptom was similar to the anatomical symptoms of buckskin-virus-infected trees of sweet cherry, *Prunus avium*, on rootstocks of mahaleb cherry, *P. mahaleb* (25). The nature of the pathological anatomy indicated that some material injurious to the rootstock sieve tubes but not injurious to the scion sieve tubes was moving from the scion to the rootstocks in both diseases. The anatomical symptoms at the bud union were more fully described later and used in diagnosing tristeza in sweet orange trees on sour orange rootstocks (1, 28, 32, 36). Fawcett and Wallace (9), in California, reproduced the tristeza disease in young trees of sweet orange on sour orange rootstock by bud-inoculation from diseased sources. Shortly thereafter, Meneghini transmitted the causal agent of the disease by means of aphids in Brazil (20). He produced not only decline but starch depletion in the rootstock (21). The nature of the disease and its viral cause were thus established.

This syndrome of developmental symptoms has not yet been worked out for bud and rootstock combinations other than sweet orange on sour orange rootstock. In many cases, it is not known which of the five types of diseases described above are associated with unsatisfactory combinations. Since sweet orange on sour orange rootstock is the host for which tristeza was described, important diagnoses of tristeza should be made in this host.

Evidence that it is a bud-union disease and not a systemic rootstock or interstock disease which causes the injury responsible for decline and collapse of nursery-sized and larger trees is overwhelming. Schneider *et al.* (28, 35) showed that sieve-tube necrosis is most extensive immediately below the bud union, and that it becomes less extensive as distance from the bud union increases. That some phloem remains functional in the sour orange rootstock at points removed from the bud union is substantiated by the occurrence of starch depletion in the rootstock. Functioning sieve tubes would be required to transport carbohydrate stored as starch out of the rootstock portion of the trunk to functioning growing roots. Furthermore, trees affected by tristeza react like girdled trees (30). Thus starch depletion in the xylem of the rootstock is a significant symptom of older, tristeza-diseased sweet orange trees on sour orange rootstock.

Concurrently with the work on the bud-union disease in the Americas, a stem- and wood-pitting disease of grapefruit, *Citrus paradisi* Macf., (17, 22), and a wood-pitting and vein-flecking disease of limes (15, 16) were described and investigated in Africa.

Grapefruit stem pitting and lime dieback were found to be of a common virus nature, with a common insect vector, *Toxoptera citricidus* (Kirk.). In Africa, sweet orange trees on sour orange rootstocks have never grown satisfactorily. After the outbreak of tristeza in the Americas, it was surmised that the tristeza virus was the cause of this incompatibility in Africa. Discovery of stem pitting in seedlings of grapefruit and limes which had been inoculated by aphids from trees with tristeza pointed to the possibility that the stem-pitting disease of grapefruit and the lime-dieback disease were caused by the tristeza virus (6, 13, 39).

A third and equally important symptom type was described by Fraser (10). She reported a *Toxoptera citricidus*-transmitted virus disease which produced "seedling yellows" in seedlings of lemon, Seville sour orange, grapefruit, and other *Citrus* species. There is some question as to whether grapefruit belongs in this group of seedling plants exhibiting the yellows type of injury or with plants developing stem-pitting and vein-flecking symptoms. The yellows-producing inoculum that Fraser used was obtained from commercial mandarin, *Citrus reticulata* Blanco, and sweet orange trees in New South Wales and invariably also produced stem pitting and vein clearing in Mexican lime. Inoculum from commercial grapefruit, sour orange, and lemon trees produced stem pitting in Mexican limes, but not yellows in lemon seedlings. Seedling-yellows infection in Eureka lemons resulted in severe stunting and cessation of growth. The leaves were reduced in size and were yellow, at first marginally and finally the whole blade. There was no vein clearing or flecking or mosaic pattern. Seedlings remained in a shock phase. Similar symptoms were described for sour orange seedlings. Passing the stem pitting—seedling yellows complex through grapefruit and sour orange seedlings did not screen out the seedling yellows. Following Fraser's (10) description of seedling yellows, other workers presented evidence that seedling yellows was already known and that it was caused by the tristeza virus.

Possibly the first transmission of seedling yellows was by Bennett and Costa (3). Working with small grafted plants of sweet orange on sour orange in the greenhouse, they produced top symptoms which were not similar to those described for decline, but more closely resembled symptoms of seedling yellows subsequently described by Fraser (10). That the rootstocks of these trees were systemically infected by seedling yellows virus is further indicated by an incubation period of about 30 days, which is shorter than that required for decline. There is further indication that Bennett and Costa's small field-inoculated trees were also suffering in part from seedling yellows of the rootstock, because they were unable to reproduce the symptom of starch depletion in rootstocks which was described by Fawcett and Wallace (9) and by Meneghini (21).

Prior to Fraser's report of seedling yellows (10), Costa, Grant, and Moreira (5) had reported that citrus seedlings in the two-leaf stage, including Key lime, lemon, and sour orange, inoculated from tristeza-diseased trees developed symptoms. Later they reported (7) that the symptoms in their sour orange seedlings in Brazil did not differ from the seedling yellows of Fraser. In 1953 Hughes and Lister (16) reported that sour orange seedlings inoculated with lime-dieback virus developed a yellowing type of symptom, but lemon seedlings were not affected. It was not stated whether different inoculum was used.

McClellan and van der Plank (19), on the basis of their experience in Africa, agreed with Fraser that inoculum from field-infected trees of sweet orange and mandarin caused both pitting and yellows, and that inoculum from grapefruit trees produced only pitting. McClellan (18) succeeded in screening out the causal agent of seedling yellows by passing the inoculum from sweet orange and mandarin through grapefruit. McClellan and van der Plank (19) thought the symptoms produced by the seedling-yellows virus and the tristeza bud-union virus had to be identical. They proposed a

hypothetical plant to demonstrate their point. An infected sweet orange bud would be grafted onto the top of a decapitated sour orange seedling. For this plant they proposed two options: 1) Only the bud would be allowed to grow and no shoots from the sour orange, and 2) that the sour orange axillary bud would grow and not the sweet orange bud. They state that the same virus would have to cause the resulting bud-union disorder of the composite plant in case one, and the yellows of the seedling in case two.

McClellan and van der Plank (19) were not in agreement with Fraser regarding the occurrence of seedling yellows in grapefruit seedlings. They found in Africa that grapefruit seedlings develop pitting. McClellan (18) suggested that very old Eureka lemon trees were susceptible to tristeza. However, he did not determine whether they were suffering from "lemon-sieve-tube necrosis" which is associated with the Eureka variety (37).

In California, Wallace (40) reported that the strains of tristeza virus spread by insects through commercial sweet orange orchards did not cause yellows in seedling lemons. He found that some *Citrus* species which had been imported into the United States by budwood, carried virus which produced seedling yellows in lemon seedlings as well as the vein-flecking and xylem-pitting symptoms in limes. None of the literature indicates whether the causal agents of seedling yellows and stem pitting can cause the bud-union disease of sweet orange on sour orange rootstock, which is tristeza as originally described.³

From reports in the literature it is difficult to determine the degree of susceptibility of various *Citrus* species and to group the species into injury types. This is because it is not clear whether researchers are writing about susceptibility of a plant to a virus or of a rootstock to a virus-infected top. The matter is further complicated because the host plants that have been studied have ranged in size from seedlings not much beyond the embryo stage to large trees. Inoculum used in testing hosts in various countries has been of numerous strains. Some workers have used inoculum containing several viruses without being sure which of the effects produced in trees should be ascribed to tristeza.

The term *nontolerant* was introduced into the literature, and new meanings were given to the long-established term *tolerant*. Grant and Costa (11) used the term *nontolerant* interchangeably with *unsatisfactory* when referring to such rootstocks as sour orange and lemon. Costa, Grant, and Moreira (5) used *nontolerant* to describe 1) "graft and intergraft combinations," 2) stock-scion combinations, and 3) rootstocks. Later, two classes for rootstocks—*nontolerant* and *tolerant*—were proposed (12). *Nontolerant* was used in describing rootstocks which are unsatisfactory under sweet orange tops regardless of whether the rootstock is injured by the virus or by toxins produced by the virus in the sweet orange. Rootstocks which are satisfactory under infected sweet orange were described as being *tolerant*. In the series of papers by these workers, the term *tolerant* takes on several meanings: It is used in describing 1) a host which is susceptible to tristeza, and which allows virus multiplication without injury, as in sweet orange; and 2) a rootstock which is satisfactory under sweet orange top regardless of whether it, as a seedling, is *tolerant*, *resistant*, or *immune*. The word *nontolerant* has

³ Wallace, J. M., R. L. McClain, and R. J. Drake (unpublished), in California, inoculated field-grown nursery-sized trees of Valencia and navel on sour orange rootstock from seven different Meyer lemon sources. These Meyer lemons had previously been shown to carry a virus or a virus complex that induced seedling yellows in Eureka lemon and vein clearing and xylem pitting in Mexican lime. The sweet on sour trees inoculated from each of these sources developed typical tristeza (quick decline) symptoms. Inoculations of sweet orange on sour orange trees with virus from naturally infected sweet orange trees which did not cause seedling yellows on Eureka lemon also resulted in quick-decline symptoms.

been used in describing 1) a grafted plant which does not grow properly, and 2) a rootstock which is not satisfactory under a certain top regardless of whether it, as a seedling, is hypersensitive, sensitive, resistant, or immune.

METHODS AND MATERIALS

Citrus plants were inoculated by side grafts from lime plants. The lime plants used for inoculum were tissue-inoculated from other lime plants inoculated by *Aphis gossypii* Glover. Periodically after inoculation, plants were sectioned and stained according to procedures described earlier (27).

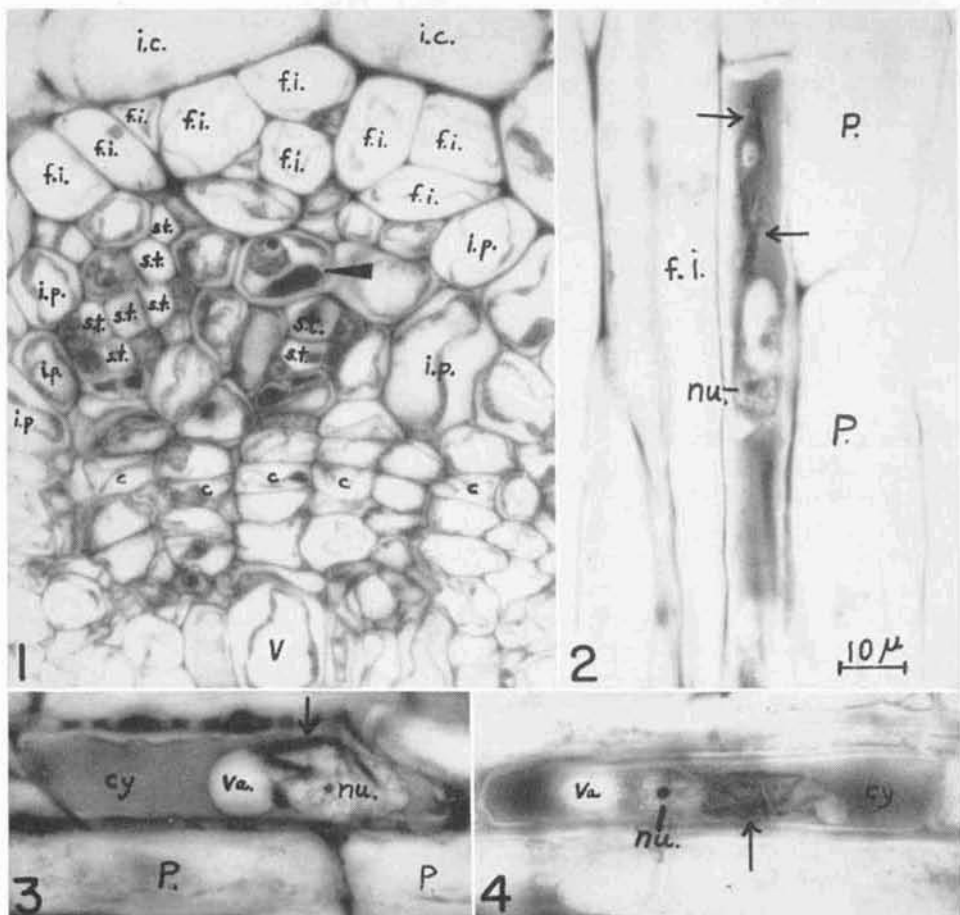
Concepts of the anatomy of healthy plants in this report were based on anatomical studies of Hayward and Long (14); Scott, Schroeder, and Turrell (38); and Schneider (27, 29, 31).

PATHOLOGICAL ANATOMY

Chromatic Cells. There is evidence that the tristeza virus moves through the sieve tubes and infects parenchyma or parenchyma-like cells adjacent to them (33). These infected cells were described as *chromatic cells* because their protoplasts become darkly stained in microscopic preparations (33, 34). Formation of chromatic cells begins with a thickening of the cytoplasm on the side of the cell adjacent to a sieve tube (fig. 1). In fully developed chromatic cells, the abnormally thickened cytoplasm typically consists of a lightly staining clear material (figs. 2, 3, 4). Thick cytoplasm occurs in other normal kinds of cells in various plants, but in contrast to the clear cytoplasm in the diseased material, healthy cells with thick cytoplasm contain granules and small vesicles. In fully developed chromatic cells the formerly large central vacuole is reduced to one or several smaller ones by the increase in thickness of the cytoplasm. Within the clear cytoplasmic material of chromatic cells darkly staining masses develop and subsequently seem to break up into needles and strands (figs. 2, 3, 4). Such masses, needles, and strands in some stages of development become purple when stained according to Bald's (2) procedure with Giemsa stain, and assume an old-rose color in the Sakaguchi test for arginine as modified by Rawlins, Weierich, and Schlegel (23). These color reactions indicate that the darkly staining masses, needles, and strands may be virus. Thus chromatic cells may be thought of as virus-containing cells, and their effects on surrounding cells vary with the host in which they occur.

Wood Pitting and Vein Clearing. Mexican lime tissue reacts to the presence of chromatic cells in the following way. In mature tissues, the chromatic cells have no effect on adjacent tissues and eventually degenerate (fig. 5). However, when chromatic cells are located adjacent to meristems (cambium, procambium, ground meristem), the chromatic condition spreads from cell to cell through the meristem. Later, in these affected areas, necrosis of some chromatic cells occurs. Partially chromatic cells, parenchyma cells, and meristem cells adjacent to the chromatic cells hypertrophy. The result is a destruction of the meristem in localized areas (34).

In the formation of wood pits, the cambium is affected first by this chromatic condition (fig. 6); hypertrophy and necrosis develop later (fig. 7). Because of this disruption of the cambium, normal xylem and phloem are not laid down. The soft, disorganized tissue of the lesion may remain attached to the bark when the bark is removed, and thus leave a pit in the wood. Sometimes there is regeneration or partial regeneration of the cambium, followed by an orderly production of xylem and phloem. In these cases the disorganized xylem tissue is buried in normal wood, and the disorganized phloem is pushed outward with the nonfunctioning phloem. Remnants of hypertrophic cells and of necrotic chromatic cells, and other characteristics to be described in a later paper, should serve to distinguish tristeza pits from pits induced by other causes.



Figs. 1-4. Chromatic cells in phloem of various *Citrus* species (all of equal magnification). Arrows point to darkly staining needle-like structures in lightly staining clear cytoplasm.—Fig. 1. Cross section of primary phloem from stem of Mexican lime 15 days after inoculation. In one parenchyma cell (see black indicator) the cytoplasm is thickened on one side (the initial step in the formation of chromatic cells).—Fig. 2. Radial section of chromatic cell in metaphloem of stem of Mexican lime 19 days after inoculation.—Fig. 3. Longitudinal section of chromatic cell in secondary phloem of long-diseased Meyer lemon stem. Needle-like masses are clustered around the nucleus.—Fig. 4. Longitudinal section of chromatic cell in long-diseased sweet orange secondary phloem at a point above the bud union of a tree on sour orange rootstock. Explanation of symbols: (c) cambium, (cy) cytoplasm, (f.i.) fiber initial, (i.c.) inner cortex, (i.p.) interfascicular parenchyma, (nu.) nucleus, (p.) parenchyma cell, (s.t.) sieve tube, (v) vessel, (va) vacuole.

In some feeder roots the cambium was found to be affected in its entirety rather than in localized areas; therefore, no normal xylem or phloem was produced after the infection.

In the formation of cleared veins, chromatic cells develop adjacent to the protophloem sieve tubes of young veinlets. The chromatic condition spreads from the chromatic cells adjacent to the sieve tubes into surrounding tissues which are still meristematic. Primary phloem fiber initials and bundle sheath cells hypertrophy, and normal differentiation fails to occur. As a result veins are formed without caps of primary phloem fibers and without sheath cells (figs. 8, 9). Air spaces normally found around the veins are

