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HENRY SCHNEIDER

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

ANATOMY OF BARK OF BUD UNION, TRUNK, AND ROOTS . . .

This paper concerns the sequence of anatomical changes occurring in sweet orange trees on sour orange rootstock after infection by quick decline. The primary symptom is necrosis of sieve tubes below the bud union. This symptom is followed by degeneration of the older sieve tubes above the union, accelerated cambial activity, and other reactions of a secondary nature.

CONDITION OF PHLOEM OF SOUR ORANGE TREE TRUNK IN WINTER

Since the sieve tubes of the functioning phloem of sour orange stock of sweet orange trees become necrotic when the tree is affected by quick decline, the condition of the functioning phloem of the trunk of the healthy sour orange, both as a rootstock and as a seedling tree, especially in winter, was investigated.

Throughout the winter months trunks of healthy sour orange trees, and of sour orange rootstock under sweet orange tops, maintained a band of functioning phloem averaging about 500 microns in width. A ring of degenerating phloem external to this was either absent or as much as 100 microns wide. Occasionally, there were abnormally wide bands of degenerating phloem or bands of necrotic sieve tubes within the functioning phloem.

EFFECT OF TRUNK GIRDLING . . .

. . . on phloem of trunk of sweet orange trees on sour orange rootstock was studied to discover whether the phloem above the removed bark reacts in the same way as that above the necrotic sieve tubes of the sour orange rootstock of quick-decline-affected trees. Sieve-tube degeneration above artificial girdling seems to be identical with that induced by girdling brought about naturally by necrosis of sieve tubes immediately below the union of a quick-decline tree.

EXPLANATION OF TERMS

Normal deterioration of sieve tubes after they have functioned for a year or two is termed degeneration. Abnormal deterioration of sieve tubes in diseased plants is termed necrosis. Which term should be used for some anomalous types of deterioration reported in these papers is open to question. The term induced degeneration has been applied to deterioration resulting from artificial or disease-produced girdling. For further clarification of terms, you are referred to the diagram presented on the inside of the back cover of this issue of Hilgardia.

CONDITION OF PHLOEM OF SOUR ORANGE TREE TRUNK IN WINTER[1]

HENRY SCHNEIDER[2]

INTRODUCTION

THE PURPOSE of the present study was to determine whether or not the sour orange tree, *Citrus aurantium* Linn., maintains throughout the winter a band of functioning phloem which is equal in width to that found in the summer. This work was undertaken in connection with a study of quick-decline-affected sweet orange trees on sour orange rootstock. Sieve tubes of the sour orange stock of such trees become necrotic for a short distance below the bud union (Schneider, 1954). Because of the similarity of necrosis and normal degeneration of sieve tubes, it was important to determine whether normal seasonal degeneration, which might be confused with sieve-tube necrosis, occurs in extensive amounts in trunks of sour orange trees and in sour orange stocks of sweet orange trees.

The various zones of the trunk phloem of the sour orange at different seasons of the year are here compared with those of the sweet orange, *Citrus sinensis* (Linn.) Osbeck, described earlier (Schneider, 1952). At some seasons of the year there is present in typical sweet orange bark a narrow zone of developing phloem (phloem that is differentiating from cambial derivatives into functioning phloem). Throughout the year there is a zone of functioning phloem about 500 to 1,000μ wide, a relatively narrow zone of degenerating phloem (phloem in which sieve tubes and parenchyma are degenerating), and a vast area of nonfunctioning phloem (phloem in which the sieve tubes and some parenchyma cells have degenerated) .

In deciduous trees it is characteristic for all but possibly a few small sieve tubes near the cambium to undergo degeneration after leaf fall and then be crushed by radial growth the following year (Esau, 1950).

MATERIALS AND METHODS

Bark samples from trunks of sour orange trees were collected in test plots at the Citrus Experiment Station at Riverside. Most of the samples were taken from five 11-year-old seedling trees. Five 12-year-old sweet orange seedling trees were sampled at the same time for comparison.

Trees of sour orange rootstock under sweet orange tops were sampled in two orchards at the Citrus Experiment Station at Riverside and in one orchard near Covina. Bark samples were taken 1 inch and 18 inches above and below the bud union. Since the bud union is located a few inches above the ground, bark from 18 inches below the bud union was from roots.

The rootstock in one orchard at the Citrus Experiment Station was of the

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[2] Associate Plant Pathologist in the Experiment Station, Riverside.

Paraguay variety of sour orange, which belongs in the Bittersweet group. Data and evidence available indicated that rootstocks in the other orchards belonged to the normal group of sour orange varieties. (See Webber and Batchelor, 1946, for descriptions of these groups.)

Methods of collecting samples and of fixing and staining sections were the same as those described previously (Schneider, 1952). Bark samples were fixed in Randolph's modified Navashin's solution, sectioned on a freezing microtome, stained progressively with Heidenhain's hematoxylin, counterstained with lacmoid, and mounted in Canada balsam after dehydration in an ethyl alcohol series. (For further details, see Schneider, 1952.)

OBSERVATIONS

Commercially grown citrus varieties are usually budded onto a seedling rootstock several inches above the ground level. After the bud has grown out and the stem has reached a height of several feet, the young tree is topped about 30 inches above the ground and lateral (scaffold) branches are encouraged to grow out. This process is called "heading." As the tree grows, the trunk, especially the upper part, may develop furrows. The base of the trunk is usually more rounded than the part immediately below the scaffold branches, but the occurrence of large lateral roots near the ground level may result in furrowing at the base of the trunk. Therefore, part of the bark of the trunk may be in concavities and the rest on ridges.

Enlargement of the tree trunk by divisions of the vascular cambium results in stresses of different kinds in the concave and convex portions of the bark. On the ridges or convex portions there are tangential stresses and radial compression, which cause the degenerated sieve tubes and parenchyma cells of the nonfunctioning phloem to be stretched tangentially and flattened (fig. 1). The living rays may become twisted or bent in the nonfunctioning phloem, and the ray cells may become compressed in such a way that their radial walls become accordionlike. Cells of other rays become stretched tangentially, and divide repeatedly to form blocks of living parenchyma in which groups of sclereids may form (fig. 2, *A*). In the bark on the ridges, functioning and nonfunctioning phloem stand out in bold contrast to each other because the cells of the nonfunctioning phloem are flattened almost as soon as they have degenerated.

In the furrows or concave portions of bark, on the other hand, there may be radial tensions. Under these conditions the rays remain straight in the nonfunctioning phloem; the ray cells are not stretched tangentially, nor do they divide. In the blocks of nonfunctioning phloem between the rays in these areas, there is less tendency for the parenchyma cells to degenerate, and the nonfunctioning sieve tubes are crushed less completely or not at all.

Bark samples for this and other work by this writer have, wherever possible, been taken from convex portions of the trunk. Only occasionally has it been necessary to sample flat portions, where cambial growth may not have been so active, and where radial compression may not have been so great as on convex portions. The trunks of trees sampled in this study were fairly rounded and free of fluting.

It is presumed for the purposes of the present study that the radial width

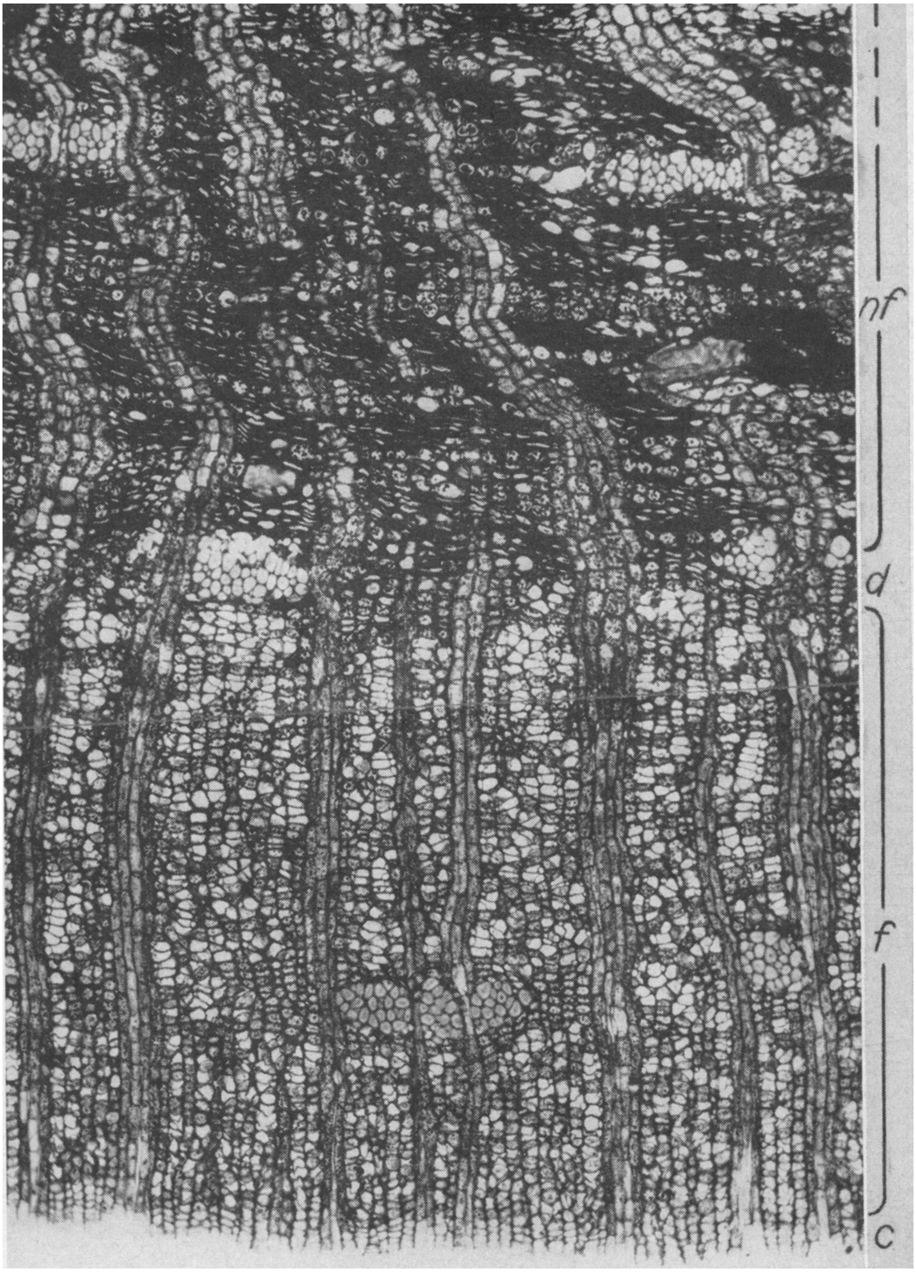
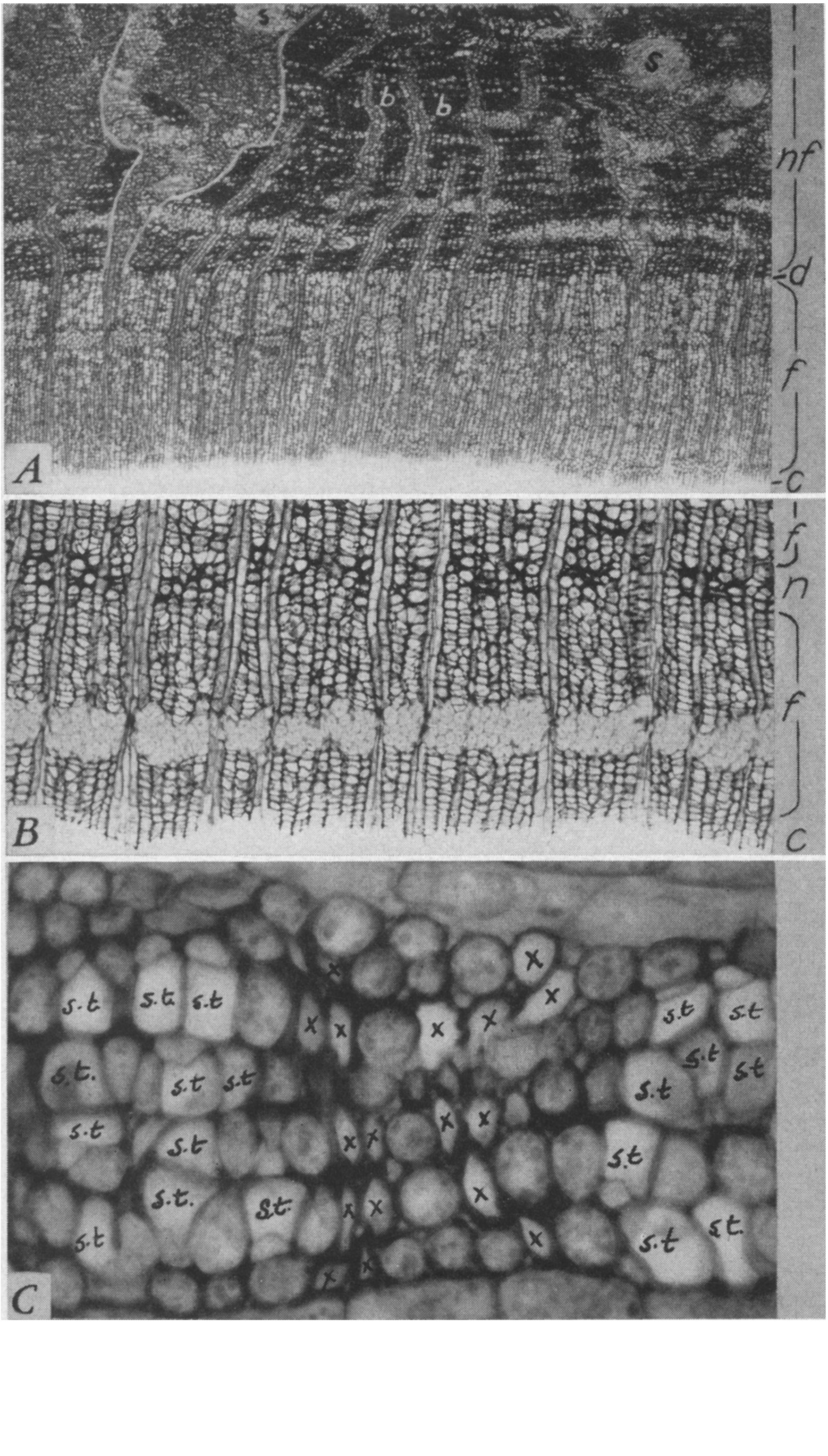


Fig. 1. Cross section of inner bark of sour orange tree trunk, showing cambium (*c*), functioning phloem (*f*), degenerating phloem (*d*), and nonfunctioning phloem (*nf*).(× 120.)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of blocks of functioning phloem between rays is uniform in any one section (fig. 2, *A*), but that there is some variation in width on different sides or in different parts of the trunk. It is also presumed that the ring of degenerating phloem should not normally be more than 50 to 100μ wide at any time. Sieve-tube degeneration supposedly occurs only on the outer margin of the functioning phloem, and degenerating sieve tubes are therefore not scattered throughout the functioning phloem.

When necrotic sieve tubes occur abnormally in the functioning phloem, they are, in their final stages of necrosis, folded masses of walls between expanded parenchyma cells. Under the low power of the microscope such dead cells appear to be masses of structureless, heavily staining material, but the crushed, folded walls may be seen under higher power. At times it is impossible to determine whether such masses of material are sieve tubes, parenchyma cells, or a combination of both. In some instances, the origin of the material may be recognized as sieve tubes by the sieve-plate callus, if it has not yet been eroded away.

Other heavily staining masses may occur in the functioning phloem. Clusters of dead parenchyma cells have been observed in the phloem of both the sweet and the sour orange. That these are parenchyma cells is deduced from the fact that clusters of cells which have died, but which have not yet been crushed, have no companion cells (fig. 2, *C*). Sieve-plate callus has not been observed on such cells. Crystal idioblasts are also heavily staining masses, but an idioblast is easily recognized by the rhomboidal calcium oxalate crystal embedded in the heavily staining matrix. Although crystal idioblasts are usually found around fibers, those dispersed throughout the phloem vary from few in most sections to many in others. Other heavily staining masses appear at times when cells of the outer functioning phloem are pulled apart by the tangential stresses set up by radial growth. The pectic middle lamella increases in amount under these conditions. Such intercellular masses of heavily staining materials may be distinguished from dead cells by the absence of folded walls.

Trunk Phloem of Sour and Sweet Orange Trees Grown from Seed. In two sour orange trees sampled at Riverside March 1, 1946, before cambial awakening from winter dormancy, bands of functioning phloem about 500μ in width were separated only by occasional degenerating sieve tubes from the tangentially flattened nonfunctioning phloem (fig. 1).

Five other sour orange seedlings were sampled on five dates between November 18, 1946, and April 17, 1947. By January 22, 1947, winds had caused some defoliation on the north sides of the trees; thereafter, bark samples were taken from both the north and the south sides of the trees. Normal

Fig. 2. Transverse sections (*A, B, C*) of phloem of trunks of sour orange trees grown from seed. *A*, section showing cambium (*c*); uniformly thick ring of functioning phloem (*f*); degenerating phloem (*d*) consisting of only occasional degenerating cells; and some of the nonfunctioning phloem (*nf*) within which are blocks of folded phloem cell walls (*b*) between the rays, some of which have become stretched tangentially and divided (outlined with white ink) to form blocks of parenchymatous tissue in which sclereids (*s*) form. *B*, part of a band of functioning phloem with a band of necrotic sieve tubes (*n*) within the functioning phloem. *C*, a cluster of dead parenchyma cells (*x*) within the functioning phloem (*s.t.* = sieve tube). (*A*, × 45; *B*, × 120; *C*, × 630.)

bands of functioning phloem were present in 32 of 40 of these samples. Degenerating phloem was either present in narrow bands or absent. Unusually wide bands of degenerating phloem 130, 150, and 200μ wide were present in 3 of the other 8 samples. Narrow bands of phloem with necrotic sieve tubes and hypertrophied parenchyma were located tangentially through the functioning phloem of 4 of the samples (fig. 2, *B*). In one sample, necrotic sieve tubes were scattered through the functioning phloem.

TABLE 1

CHARACTERISTICS OF TRUNK PHLOEM OF SWEET AND SOUR ORANGE TREES GROWN FROM SEED, AND OF SWEET ORANGE SCIONS AND SOUR ORANGE ROOTSTOCKS

Phloem samples		Average radial width (μ) of fiber bundles*		Average number of rows of fiber bundles in functioning phloem†		Average width (μ) of band of functioning phloem		Calculated number of fibers in band of functioning phloem 500μ wide	
Source	Total number	Sweet orange	Sour orange	Sweet orange	Sour orange	Sweet orange	Sour orange	Sweet orange	Sour orange
Valencia orange seedlings	20	43	..	2.1	...	761	...	1.4	...
Sour orange seedlings‡	20	..	53	...	0.4	...	488	...	0.4
{Valencia orange scion	20	35	..	1.6	...	586	...	1.4	...
Paraguay sour stock	20	..	52	...	0.6	...	560	...	0.5
{Valencia orange scion	18	38	..	1.4	...	479	...	1.5	...
Griffith's sour stock‡	18	..	68	...	0.7	...	592	...	0.6
{Navel orange scion	20	41	..	2.7	...	679	...	2.0	...
CES field-12 sour stock‡	20	..	55	...	0.2	...	561	...	0.2
{Navel orange sandwich (Valencia orange top)	20	51	..	3.6	...	842	...	2.1	...
Griffith's sour stock‡	20	..	71	...	0.7	...	695	...	0.5
Average	..	42	60	2.3	0.5	669	579	1.7	0.4

* Measurements made on bundle appearing to be of average thickness in each section.
† Based on sum of rows or partial rows of fibers in functioning phloem of a cross section. If a row of fibers was between the zones of degenerating and functioning phloem, only half of its length was recorded.
‡ All indications were that these belonged to the normal or true sour orange group.

Thirty-nine of 40 samples from five 12-year-old sweet orange seedlings collected on the same dates as the sour orange material exhibited no abnormalities of functioning and degenerating phloem. The functioning phloem of one section had a band of degenerating sieve tubes through it.

In summary it may be stated that in the bark of sour orange seedling trees a wide band of functioning phloem is generally present throughout the winter, together with a narrow band of degenerating phloem which shows no seasonal variation in width. Variations from this pattern in the functioning phloem were observed in 8 of 40 samples, however.

Anatomically, the functioning phloem of the sour orange is much like that of the sweet orange (fig. 1). Bundles of fibers are usually present, as in the sweet orange, but the bundles are wider radially and there are generally fewer of them than in the sweet orange (table 1). Fiber bundles are occasionally isolated rather than in bands (fig. 1).

Phloem of Sour Orange Rootstock of Sweet Orange Trees. In the budded tree, the scion and rootstock of different species may affect the anatomy of each other. For this reason, the phloem of the sour orange stock of sweet orange trees is here considered independently of that of the sour orange seedlings described above.

Rings of functioning phloem about 500μ in width, with only narrow zones of degenerating phloem, were generally present throughout the year in the sour orange rootstocks of these studies. In some samples from the Azusa strain of Valencia orange trees on Paraguay sour stock, however, there was what appeared to be an abnormal degeneration of sieve tubes below the bud union.

Callusing of the sieve plates and degeneration of the older sieve tubes of the ring of functioning phloem immediately below the union were observed in some of the Azusa strain of Valencia orange trees on Paraguay sour orange rootstock. From each tree, pairs of samples were taken, one sample 1½ inches above the bud union and the other 1½ inches below the bud union. Of nine trees sampled, only three were consistently free of such callusing. Fourteen pairs of samples from one of the healthy trees were free of the degeneration on seven different collection dates throughout the year. Six pairs of samples from another healthy tree were free of the degeneration on three collection dates in winter, spring, and summer, as were also two pairs of samples from a third tree on one collection date in July. In contrast to these three trees, six other trees showed callusing of sieve plates and collapse of the older, outer sieve-tube elements on some samplings. Of 23 pairs of samples from these six trees, 10 showed necrosis below the union but not above. Three sample pairs showed necrosis both above and below the union, but the necrosis was more extensive below. In one sample pair there was a wide band of degenerating phloem above the union but not below. (This sample could have been from a different file of cells.) Nine pairs of samples from these trees showed no abnormalities. In roots 18 inches below the union only 1 section out of 23 showed callusing of the outer sieve tubes.

Twenty-three samples from the California normal sour orange rootstocks of six navel orange trees growing in the Citrus Experiment Station orchards showed normal rings of functioning phloem and narrow bands of degenerating phloem, as did also the navel orange scions.

Many samples collected at various times of the year from trees in an orchard near Covina, in which quick decline was spreading, showed no abnormal degeneration of the rootstock or scion phloem except when from trees affected by quick decline.

As was found in the comparison of bark samples from sweet orange and sour orange seedlings, the fiber bundles of the sour orange rootstock are wider than those of the sweet orange scion, and there are generally fewer fiber bundles in the sour orange rootstock (table 1). The band of functioning phloem in the sour orange rootstock tended to be narrower than that in the sweet orange scion.

Normally, bark of the sour orange rootstock under sweet orange trees apparently has a band of functioning phloem about 500μ in width, with only a narrow zone of degenerating phloem on its outer margin. Deviations from this proposed normal were found, however, in part of the group of Valencia orange trees on Paraguay sour stock. Observations on trees in orchards not considered in this study have shown that this type of injury can become sufficiently extensive and prolonged to restrict translocation seriously and cause trees to go into chronic decline.

DISCUSSION

Materials present in the scion of the tree, if they move across the bud union, may have a toxic or stimulating effect on cells or sieve-tube elements of the rootstock, and vice versa. In commercial practice different varieties of citrus scions and rootstocks are budded together. Some of these combinations may be inherently incompatible, or some scions may carry viruses which cause incompatibility.

In these studies the Azusa strain of Valencia orange on Paraguay sour orange rootstock showed tendencies toward incompatibility, in that the older sieve tubes of the band of functioning phloem were necrotic in some samples from below the bud union. In quick-decline-affected trees, the younger sieve tubes are the first to become necrotic, and a number of secondary reactions follow the initial necrosis. No decline was observed in trees of the Azusa strain of Valencia orange on sour stock, but a decline in which necrosis of sieve tubes involves not only the oldest, but also the middle-aged and occasionally even young sieve tubes has been observed in some commercial Valencia orchards. This disease, which is tentatively designated "chronic decline," will be discussed in a later paper.

The normal anatomy of the sour orange rootstock under sweet orange is thought to include a uniformly wide band of functioning phloem throughout the year. Two types of apparently abnormal phenomena have been observed, however, in the functioning phloem of the trunk of the sour orange when grown as a seedling or as a rootstock for sweet orange: (1) In some samples from seedling sour orange trees, there were bands of phloem containing necrotic sieve tubes in and concentric with the functioning phloem. Similar bands are frequently found as an initial symptom in the sour orange stock of sweet orange trees affected by quick decline. In the quick-decline trees the sieve-tube necrosis eventually affects the entire phloem, however, whereas in the seedlings no other sieve tubes became involved. (2) The other apparently abnormal phenomenon was a degeneration of unusually large numbers of old sieve tubes on the outer portion of the functioning phloem. In quick-decline-affected trees, necrosis first occurs in the young sieve tubes at the inner portion of the functioning phloem.

SUMMARY

Since sieve tubes of the functioning phloem of sour orange stock of sweet orange trees become necrotic when the tree is affected by the quick-decline virus, the condition of the functioning phloem of the trunk of the healthy sour orange, both as a rootstock and as a seedling tree, is of interest. The functioning phloem of such trunks, especially during the winter months, has been investigated.

Throughout the winter months the trunks of healthy sour orange trees

maintained a band of functioning phloem averaging about 500μ in width. A ring of degenerating phloem external to this was either absent or as much as 100μ wide. Occasionally, there were unusually wide bands of degenerating phloem or bands of necrotic sieve tubes within the functioning phloem.

Sour orange rootstocks under sweet orange tops, also, usually had a band of functioning phloem averaging 500 or 600μ in width. In one group of trees (Azusa strain of Valencia orange on Paraguay sour orange stock), however, there was an abnormal amount of degeneration in the outer part of the functioning phloem.

The trunk phloem of the sour orange, either as seedling trees or as rootstock for sweet orange trees, had fiber bundles which averaged wider radially than those of the sweet orange but were fewer in number. The width of the band of functioning phloem of the sour orange was, on the average, about 100μ less than that of the sweet orange.

No alterations in anatomy of the trunk phloem of the sour orange were noted as a result of its use as a rootstock, except in the Paraguay sour orange rootstock under the Azusa strain of Valencia orange.

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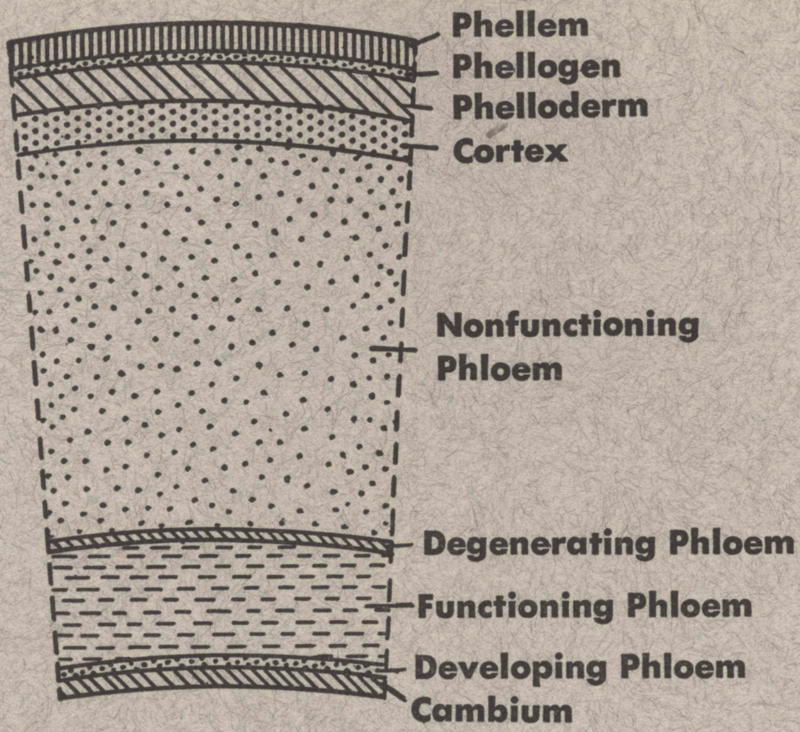

The normal phloem of the orange tree trunk is a complex tissue. Its vertical system consists of sieve tubes, companion cells, parenchyma cells, fibers, and crystal idioblasts. The four zones of the phloem may be described as follows: (1) the developing phloem is the ring of phloem adjacent to the cambium, in which cells derived from the cambium divide further and then differentiate into mature phloem tissue. (2) The functioning phloem is the ring of phloem located just outside the developing phloem and containing mature sieve tubes. (3) The degenerating phloem is the ring of phloem located at the outer margin of the functioning phloem. In this tissue the sieve plates of the sieve tubes become callused, the sieve tubes lose their turgor and then collapse, and the contents are lysed. Some parenchyma cells also degenerate, lose their contents, and are crushed. (4) The vertical system of the nonfunctioning phloem contains blocks of degenerated sieve tubes and living and dead parenchyma cells.

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