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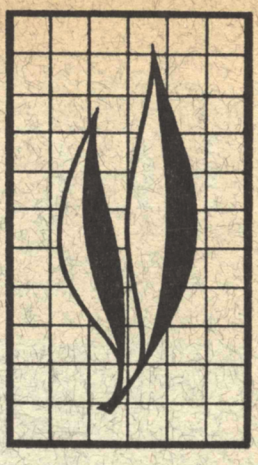

This paper reports on a study of the causes behind the drastic effects of corky bark disease on LN-33. An anatomical study revealed that corky bark virus probably influences the functions of the vascular and cork cambia. The first symptoms of corky bark appear in the vascular cambial zone. Certain derivatives of the vascular cambium do not differentiate into cells which become lignified, either to the phloem or xylem side. The production of cells toward the xylem side is drastically reduced, while an abnormal amount of secondary phloem is produced. The phloem contains sieve-tube-like cells, but these cells occur in narrow bands between abnormally wide rays.

No normal cork is formed, but there is a stimulation of cork-like cells in the region of the phloem where the cork-cambium usually arises.

A secondary effect of corky bark is the formation of proliferative tissue in the vascular rays of the cane. The cells of this tissue are irregular in shape and have large nuclei; they divide irregularly and are tumorlike in appearance. These findings indicate that corky bark is probably closely related to wound-tumor virus.

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INTRODUCTION

CORKY BARK, a graft-transmissible virus disease of grapes, was first described as a virus-like disease under the name "rough bark" by Hewitt (1954). The disease was observed in the varieties Palomino and Petite Sirah. The virus was first graft-transmitted from Carignane, French Colombard, and Grenach vines, which did not show obvious corky bark symptoms, to the selection LN-33 at Davis (Hewitt *et al.* 1962).

Corky bark may go undetected in some varieties (e.g. Carignane), show mild symptoms in others (e.g. Mondeuse), or display severe symptoms (e.g. LN-33 on which the disease may kill the vine in three years or less). In LN-33, corky bark is characterized by severe swelling and cracking of the one-year-old canes.

Hewitt *et al.* (1962) observed that, in current-season canes of infected LN-33, the bark is thick, spongy-soft, and often splits into longitudinal cracks that heal from the margins to form fissures as the canes mature. The canes on diseased vines are greenish, poorly matured, and are limber or rubbery. In older canes the bark becomes rough and the leaves often become pinkish, tend to droop, and do not stand out normally from the canes.

The disease is first observed in the basal parts of the plant. Symptoms may consist either of rolling of the basal leaves, or of swelling of the basal internodes and cracking of the cane. Reddening of the leaves spreads from the base to the apical end of the cane. The basal parts of the cane may have deep cracks, while the tip of the cane may show no symptoms. In indexing work, the establishment of the bud on the indicator is often recognized by a severe dwarfing of the LN-33 vine. Often the one-year-old cane will die during the winter, and the next spring a new shoot will orginate below it. This observation was made on index host plants.

VIRUSES IN RELATION TO PLANTS

Plant viruses, with a few exceptions, are regarded as systemic, and therefore it is assumed that all the plant parts are being invaded (Bennett, 1940). The tissues, however, differ in the amount of resistance offered. This resistance varies depending on the plant and on the virus involved. The effectiveness of the virus in its ability to invade probably depends upon the hereditary makeup of the host. According to Braun (1960) a virus may (1) fail to multiply in the host, (2) multiply, but fail to produce symptoms or (3) multiply and produce symptoms. Esau (1961) divides plant viruses into three classes: (1) histologically nonlimited viruses, distributed throughout the parenchyma, including that of the conducting tissues; (2) viruses limited to the phloem and (3) viruses limited to the xylem. These differences in tissue relationships are reflected in the methods of virus transmission, by anatomic changes in the host plant, or by translocation of viruses in the plant.

[1] Submitted for publication February 3, 1969.

ANATOMICAL CHANGES INDUCED BY VIRUSES

General response to viruses.—Plants respond with certain fundamental changes in structure to a variety of injurious, or noninjurious stimuli. In virus-diseased plants the following basic pathological symptoms may be found (Esau, 1956): hypertrophy, an excessive enlargement of a cell or part of a cell; hyperplasia, an excessive multiplication of cells; hypoplasia, inhibition of development; and necrosis, death of cells and tissues. Other results of virus infection in plants are the formation of trabeculae (Schneiders, 1937; Maier, 1939; Gifford *et al.*, 1956); starch accumulation in leaves (Esau, 1956; Bawden, 1964; Hoefert and Gifford, 1965); and starch accumulation in the pith (Samuel *et al.*, 1933). In the mosaic group of virus diseases, however, starch accumulation decreases (Bawden, 1964). Giant nuclei are common in buds affected by curly top disease of sugar beet (Artschwager and Starrett, 1936).

Inclusion bodies are regarded as reaction products of the cell to a viral irritant (Smith, 1930). The inclusion bodies probably indicate the presence of virus in the host cells because mosaic-infected tobacco cells containing inclusions showed the highest concentration of virus (Livingstone and Duggar, according to Esau, 1938). Except for the apical meristem, inclusions have been described in most kinds of plant tissues, including the abnormal tumorous tissue produced by plants infected with wound-tumor virus (Bawden, 1964). However, in some virus diseases such as "big bud" of tomatoes, no inclusion bodies have been noted (Samuel *et al.*, 1933).

The changes in virus infected plants may be divided, on the basis of cytology, into two categories: (1) the production in the cytoplasm (sometimes the nucleus) of microscopically visible bodies that differ from any that occur in uninfected plants and (2) the destruction or modification of normal cells or cell contents (Bawden, 1964). Viruses in some instances may constitute a new and potent factor in plant morphogenesis, and as a rule such viruses cause a systemic infection and induce definite morphological changes without necrotic lesions (Shapovalov, 1939).

Responses in phloem to virus invasion.—All evidence points to the phloem as the tissue through which most viruses move rapidly, and in some plants this is the tissue where the greatest concentration of virus occurs (Samuel *et al.*, 1933). Characteristic of infection by the yellows-type viruses is for the phloem to be deranged, usually for the sieve tubes and companion cells to die (Bawden, 1964). The virus may have a necrotic effect only in the phloem as in the case of the dwarf virus of barley (Esau, 1957b) and the yellow leafroll virus of peach in celery (Esau, 1958). On the other hand, the virus may produce abnormal phloem the sieve tubes of which may become necrotic as in the buckskin disease of peach (Schneider, 1945). In curly top disease of sugar beet the phloem becomes hyperplastic, but necrotic obliteration is found only in the older parts of the phloem (Esau, 1957a). Hyperplasia occurs both before and after necrosis in the phloem of tomato infected with curly top disease (Rasa and Esau, 1961). Such divisions result in the production of numerous short, haphazardly arranged cells which were interpreted as abnormal sieve elements. Aster yellows also induces degenerative changes in the phloem of tomato (Rasa and Esau, 1961).

In curly top, some hypertrophy, but mainly hyperplasia is initiated in the vicinity of the first sieve tubes, and the new tissue deviates strikingly from the normal phloem by the overabundance of sieve tube elements, usually of abnormal

shape (Esau, 1948b). Fibers usually fail to develop in the diseased phloem. The occurrence of hyperplasia in the secondary phloem of tobacco infected with curly top virus indicates that the stimulus for abnormal growth continues to be present in the phloem tissue as primary growth is supplanted by secondary growth (Esau, 1948b).

Responses in xylem.—Usually the xylem appears to respond to virus infection with a gummous degeneration, as in Pierce's disease of the grapevine and in alfalfa dwarf disease (Esau, 1948a). Necrosis of the xylem also occurred in pepper when infected with *Nicotiana* Virus (McKinney and Hills, 1941). The occurrence of trabeculae in the vessels of fanleaf-diseased grapevines has been used as a tool for diagnosing the disease (Schneiders, 1937; Maier, 1939; Gifford *et al.*, 1956).

Responses in Meristematic cells.—Bawden (1959) believes that meristematic tissues preclude virus infection or that they support virus multiplication poorly. He cites as evidence for this phenomenon the fact that viruses do not penetrate into the apical meristems, or if they penetrate they fail to survive there. This view is shared by Bennett (1940, 1956) who states that cambial cells appear to remain uninjured in most infected plants.

In contrast to the preceding statement, relatively high concentrations of virus were found in the cambium near the lower ends of stems of tobacco plants inoculated with curly top virus (Lackey, 1946).

Proliferation of the cambium was reported in Fiji disease of sugar cane (Wood, 1937), curly top in sugar beet (Artschwager and Starrett, 1936), wound-tumor virus in the roots of *Hyocyamus,* and wound-tumor virus in *Lychnis* (Kelly and Black, 1949).

The stimulus of abnormal growth is carried over to secondary growth in tobacco infected with curly top virus, which may indicate that the cambium is influenced by the virus (Esau, 1948b). Excessive amounts of phloem and xylem are produced in the case of cacao swollen shoot virus, but the tissues are abnormal (Bawden, 1964). In the roots of *Rumex* infected with wound-tumor virus, small superficial overgrowths originated as proliferations from the cork cambium (Kelly and Black, 1949).

Formation of tumors.—The standard dictionary definition of a tumor is: "An abnormal mass of tissue that is not inflammatory, arises without obvious cause from cells of pre-existent tissue, and possesses no physiological function." The growth of tumors is by cell multiplication rather than cell enlargement (Kelly and Black, 1949). A tumor cell can be regarded as an altered cell, and as a result of its alteration, has acquired the capacity to direct its own activities largely irrespective of the laws that govern growth of cells within an organism (Braun, 1960).

Tumors caused by the wound-tumor virus that developed in both the roots and stem of sweet clover, *Melilotus alba* Derr., originated in the pericycle (probably phloem parenchyma in the stem) by abnormal cell multiplication (Kelly and Black, 1949). The internal phloem produced in tomato plants infected with the "big bud" disease is unlike normal phloem in that the great bulk of tissue consists of small cells with prominent nuclei very similar to companion cells; sieve tubes are rare, and isolated tracheids are also present. (Samuel *et al.*, 1933).

In the roots of sweet clover infected with wound-tumor virus several centers of growth may be found; their margins are marked by meristematic cells which stain heavily and which have prominent nuclei (Black, 1946). The xylem of this tumor

tissue is highly disorganized; it may be arranged in whorls, or it may be so disorganized that two adjacent xylem elements are disoriented with regard to each other.

During the early development of the tumor in the roots of sweet clover, tissues neighboring on the pericycle—namely the cortex, endodermis and vascular cambium—were unaffected (Kelly and Black, 1949). The first tissue to become differentiated in the tumor was abnormal phloemlike tissue and there was no evidence of sieve plates or other characteristics of advanced differentiation. Xylem cells became differentiated when the tumors were about ten cells in diameter. The xylem elements were short with complete end walls and reticulate thickenings. No xylem vessels were observed. The xylem cells in the tumor had no spiral or annular thickenings, and except for occasional connections to the stele, the xylem consisted primarily of distorted tracheids. In the phloem cells no sieve plates or slime plugs could be detected. Kelly and Black (1949) commented on tumor cells as follows: "Tumor cells are apparently unable to differentiate into the most specialized cell types found in normal xylem, the vessel or its counterpart in the phloem, the sieve tube."

THE MECHANISM OF SYMPTOM DEVELOPMENT IN VIRUS-DISEASED PLANTS

In pepper, McKinney and Hills (1941) found that *Nicotiana* Virus was most concentrated in or near to the necrotic cortex or cambium, but no virus was detected in the necrotic xylem of the stem. They also observed that necrosis occurred considerably in advance of the presence of the virus in the xylem of inoculated plants, but not in the cortex. They concluded that secondary chlorosis, mosaic mottling, and xylem necrosis were induced directly by translocation of the diffused products of a deranged metabolism, which in turn was induced by a relative small amount of virus in "remote zones." This view is shared by Schneider (1945) who stated that the products of metabolism rather than the virus itself may be the immediate cause of some of the symptoms seen in infected plants.

It has been shown that viruses often constitute a potent factor in plant morphogenesis and, as a rule, such viruses cause systemic infection and induce definite morphological and physiological changes without apparent necrotic lesions (Shapovalov, 1939). A parallel exists between the effects of viruses and growth-regulating substances on plants (Esau, 1957b). Viruses may cause disturbances in different cells by interfering with the biochemical gradients of the differentiation process (Esau, 1956), and in virus-induced tumors the host cells may have acquired new genetic information as a result of the presence of the virus (Braun, 1960).

ANATOMICAL RESPONSES OF *VITIS* TO SPECIFIC VIRUS INFECTIONS

Pierce's disease.—The development of gum and the plugging of the xylem vessels and other xylem cells are caused by the virus of Pierce's disease (Esau, 1948a). These symptoms are usually accompanied by the excessive development

of tyloses in the wood. As a secondary effect Esau (1948a) mentions the occurrence of irregular cork formation and an excessive accumulation of nonfunctioning phloem.

Leafroll.—The principal internal symptom is phloem degeneration—necrosis and obliteration of sieve tube elements, companion cells, and phloem parenchyma cells (Hoefert and Gifford, 1965). Secondary symptoms are (1) hypertrophy and hyperplasia of phloem parenchyma cells, and (2) the formation of trabeculae in stems and leaves.

Flavescense dorée.—According to Caudwell (1964), an excess of phloem is formed at the expense of the xylem, and the phloem fibers are irregular, if present at all. Phloem necrosis is also associated with the development of abundant abnormal phloem. Suberized cork cells are absent and the walls of cells in the region where cork is normally formed become thinner, or the formation of a layer of cork occurs nearer to the wood than usual.

Legno ricco (rugose wood).—The internal symptoms are abnormal development of bark, phloem necrosis, and wood pitting (Graniti, 1964). Hyperplastic and anomalous phloem is formed and the growth of the xylem is much reduced. The hyperplastic medullary rays may extend as fingers into the wood.

MATERIALS AND METHODS

The indicator variety LN-33 was used primarily for the present anatomical studies. Healthy LN-33 vines came from the Foundation Vineyard of the University of California at Davis, or were check vines from the indexing nursery of the University's Department of Plant Pathology. Diseased vines also came from the indexing nurseries of the Plant Pathology Department. Material from both diseased and healthy vines were collected at three different times in the growing season. The first collection was made on October 4, 1963 from a vine [Plant Pathology (P.P.) No. 2-469-3] which showed symptoms of corky bark. This vine had been rooted as a cutting in 1962 and a bud from a corky bark source (a Carignane vine from Napa Valley) had been grafted onto it in the Spring of 1962. The first collection also included healthy material from vine P.P. No. 2-502-2.

On July 11, 1964, material was collected from vine P.P. No. 2-457-3. This vine had been rooted and grafted in 1962 with a corky bark source, a Grenache vine from Santa Clara Valley. On November 12, 1964, material was collected from a vine infected with corky bark, P.P. No. 3-385-2. This vine had been rooted and grafted in 1963. The source of the bud had been a Mondeuse vine (number 14S13W) from the Napa Valley. Healthy material was collected from vine P.P. No. 3-383-1. Material of an infected Carignane vine from Napa Valley was collected on November 4, 1964.

Canes of the current-year growth were collected and transported in plastic bags to the laboratory. (A few two-year-old canes and roots were also collected.) Portions of internodes, petioles and leaves were selected, numbered and then killed in either F.A.A. (90 ml 70 per cent ethanol, 5 ml formaldehyde, and 5 ml glacial acetic acid) or Craf III (Sass. 1958). The containers with the material and the killing solution were placed under vacuum for two hours to aid penetration of the killing solutions.

The specimens killed in F.A.A. were stored in F.A.A. Those killed in Craf III were washed in running water for 12 hours after they had been in the fixative

for about five days. The tissue was then dehydrated in alcohol and stored in 70 per cent alcohol.

Further dehydration was done according to the tertiary-butyl-alcohol method of Johansen (1940). Because of the hardness and the size of the specimens the interval between each step was at least 12 hours, and in some cases even 24 hours. Aerated parowax was used for the initial infiltration. Finally, the tissue was embedded in Tissuemat, melting point 56.5°C.

Sections were cut on a rotary microtome with a microtome knife. The thickness that gave the best results with this material was 13μ. In the case of very hard material, especially in the healthy tissue where lignification of wood was normal, the embedded material was softened by soaking it in a solution of 15 ml glycerol and 85 ml of 70 per cent alcohol for from one to four weeks, after the tissue had been exposed.

Serial sections were mounted with Haupt's fixative. After the removal of the paraffin with xylene, the sections were either stained with the progressive hematoxylin-safranin-fast green schedule (modified from Esau's hematoxylin-safranin schedule, 1941) or the lacmoid schedule (Cheadle *et al.*, 1953).

The photomicrographs were taken with an "Orthophot" using a micro tessar lens for low magnification and a microscope for the high magnifications.

The internodes on the canes were numbered from the tip to the base of the cane. The internode above the first expanded leaf was taken as the first internode. In this way the number of the internode had a relationship with the age of the internode. In comparing healthy and diseased material the numbers of the internodes usually, but not necessarily, indicated that they were of comparable development.

ANATOMY OF THE NORMAL STEM (CANE) OF THE GRAPEVINE

In describing the gross anatomy of the cane at the end of primary growth, Esau (1948a) lists the regions from the center of the stem to the outside as: a parenchymatous pith, xylem, vascular cambium, phloem, cortex, and epidermis. She further points out that the xylem and phloem are arranged in the form of collateral strands separated from each other by wide parenchymatous rays. During secondary growth new vascular tissues are formed, and the epidermis, cortex, and some phloem are cut off by a cork cambium which originates in the phloem. The ray parenchyma cells are produced by the interfascicular cambium. Rays may also arise within the vascular strands from ray initials which are formed from converted fascicular cambial initials. New cork cambia arise year after year in successively deeper layers of the phloem and the latter tissue is periodically sloughed off. Esau (1948a) further defines a "mature cane" as a current-year shoot in which cork is formed at the end of the season.

The secondary phloem is described by Esau (1948a) as being composed of two kinds of cell masses occurring in radial blocks; the rays (transverse system) and the longitudinal system. The latter consists of alternate tangential tissue bands: (1) The sieve tubes, companion cells, and phloem parenchyma, (2) Fibers. Most of the fibers are septate, and all remain alive until they are separated from the stem by the cork. Starch storage occurs in the phloem parenchyma and fibers.

According to Esau (1948a) secondary xylem consists of radial alternating

blocks of transverse (rays) and longitudinal systems. The latter is composed primarily of vessel members, fibers, and xylem parenchyma. Vessels occur singly or in groups. Many xylem and parenchyma cells show dark contents because tannins are present. Starch storage occurs in the xylem parenchyma, ray parenchyma, and fibers.

PATHOLOGICAL ANATOMY OF LN-33

Diseased and healthy material of comparable age or development were compared in the present studies of the virus effects on the various plant tissues. The abnormalities of the diseased material were much more obvious when studied in a comparative way.

Pith.—No abnormality of the pith tissue was found (figures 1, 3), indicating that there are no primary or secondary symptoms in the pith tissue of LN-33 canes infected with corky bark.

Xylem.—The amount of secondary xylem is less where symptoms are apparent in a corky bark infected cane of LN-33 (figures 1, 2). The difference in xylem production is even more pronounced at a later stage, for example, when the 18th internode of a diseased cane is compared with the 20th internode of a healthy cane (figures 3, 4). Corky bark virus infection reduces the production of xylem elements but the xylem elements produced are normal.

As described earlier, the tip of the cane from an infected vine may be free from symptoms of corky bark, especially early in the season (July). A comparison of sections made in October (end of the growing season) through the 6th internode of a mature healthy cane (figure 5) and the 4th internode of a diseased cane (figure 6) shows that both are symptomless. It can be observed in the section of the diseased vine that the xylem elements are fully matured up to the vascular cambium and no abnormality can be seen in the xylem.

In a healthy cane at the 16th internode the xylem elements are well formed and mature up to the cambial area (figure 8), and all xylem elements are arranged in precise radial rows. In the diseased cane at the 9th internode (figure 7) the last formed vessel members are partly collapsed and the walls are only partially thickened. The last few cells formed are thin-walled and the cells are arranged irregularly, not in definite radial rows as those of the healthy cane (figure 8).

Another section from the 9th internode of a diseased cane shows secondary xylem protruding into the tissue made up of irregular cells with thin walls and prominent nuclei (proliferative tissue) (figure 9). The last formed vessel members of this protrusion are near the cambium. A definite interruption can be observed in the area between the healthy xylem and the xylem associated with this protrusion.

In a two-year-old cane the first year's (1962) increment of wood (xylem) appears perfectly normal (figure 11). The second year's (1963) wood formed early in the season is normal, but the wood formed later consists of thin proliferative cells.

Phloem.—The primary phloem fibers are present regardless of the stage of severity of the corky bark symptoms in the canes (figures 1 and 3). With the advent of symptoms, proliferative growth appears in a part of the otherwise orderly phloem with its differentiated cell types.

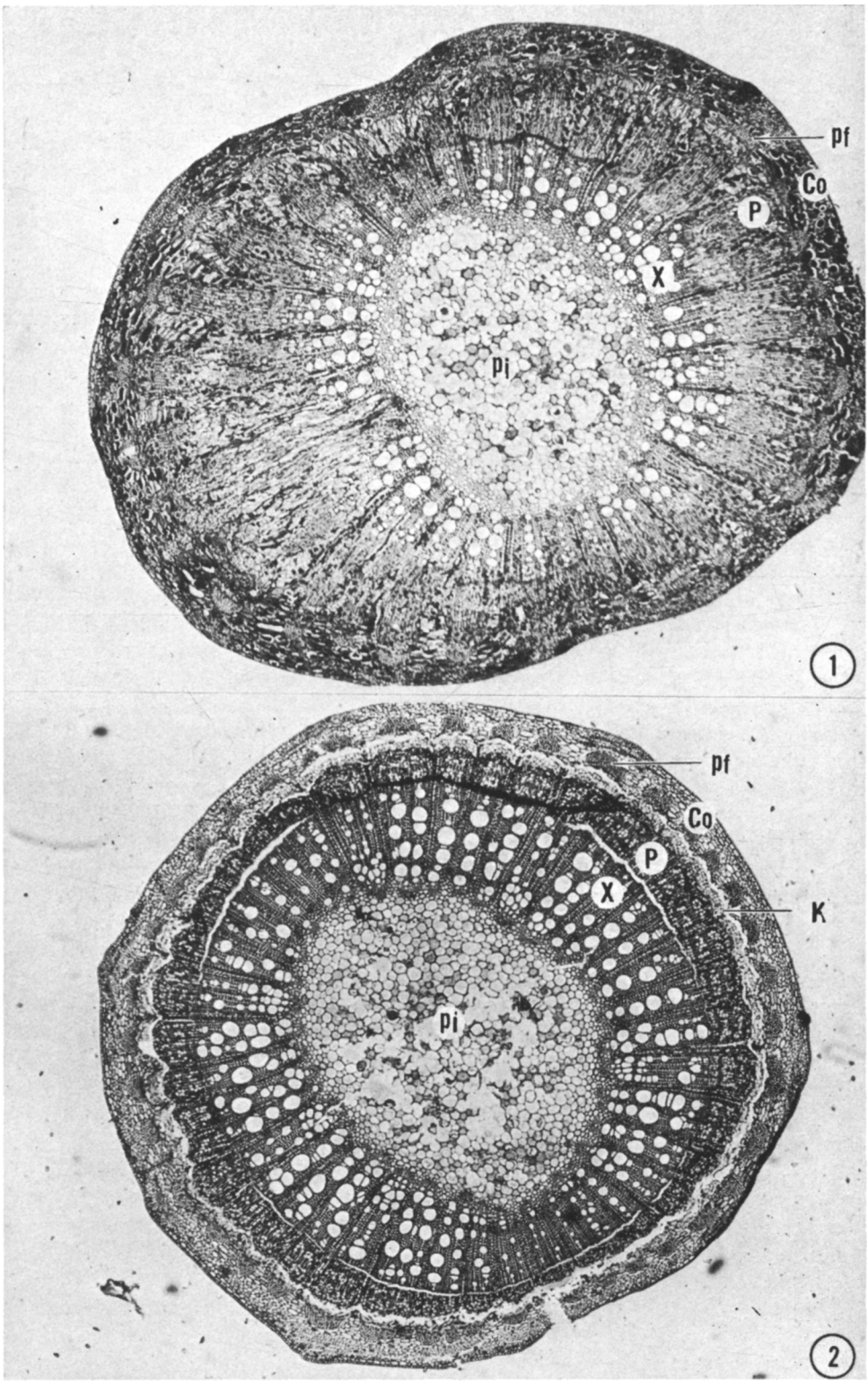

Fig. 1. Transverse section of the 11th internode of a diseased LN-33 cane collected in October 1963. Symptoms of corky bark are reduction in the amount of xylem produced, presence of proliferative tissue, and the absence of a cork cambium. × 27.

Fig. 2. Transverse section of the 16th internode on a healthy LN-33 cane collected in October 1963. The xylem and the phloem are normal and cork is present. (The cracks in the section are due to sectioning of the cane). × 27. Details Co, cortex; K, cork; P, phloem; Pf, primary phloem fibers; pi, pith; X, xylem.

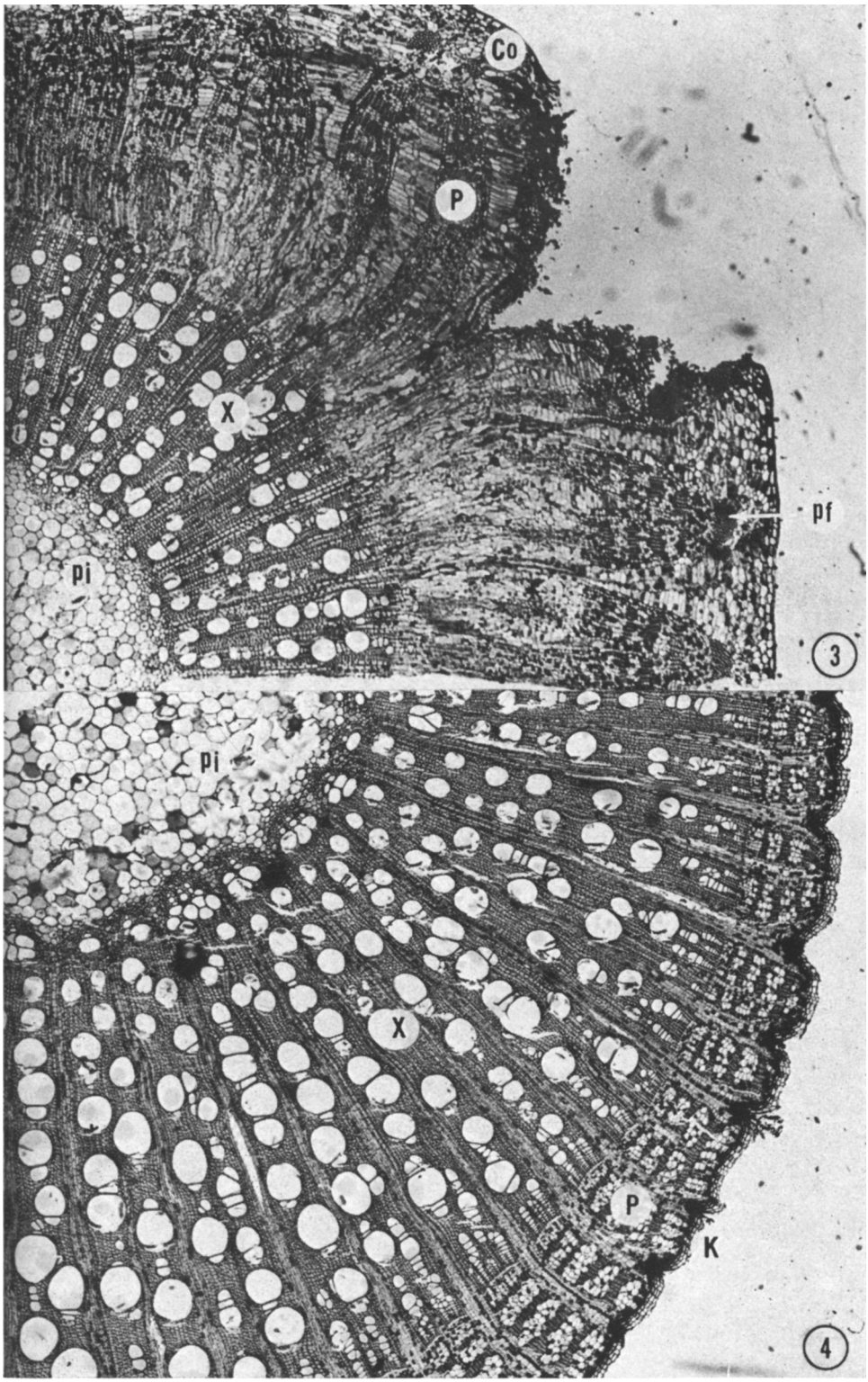

Fig. 3. Transverse section of the 18th internode of a diseased LN-33 cane collected in October 1963. Note the considerable reduction in the amount of xylem produced. The phloem, on the other hand, is in excess of the normal amount present in a healthy cane. Very distinct in this section is the proliferation of tissue between the xylem and phloem. The phloem rays are dilated and no cork is present. As a result of the extensive proliferation of cells and the lack of a sloughing-off mechanism, a crack developed in the cortex to accommodate the expanding tissues. × 32.

Fig. 4. Transverse section of the 20th internode of a healthy LN-33 cane collected in October 1963. The amount of xylem present is far in excess of the amount of phloem present. The cortex and primary phloem fibers have been sloughed off by the formation of cork. × 32. Details: Co, cortex; K, cork; P, phloem; pf, primary phloem fibers; pi, pith; X, xylem.

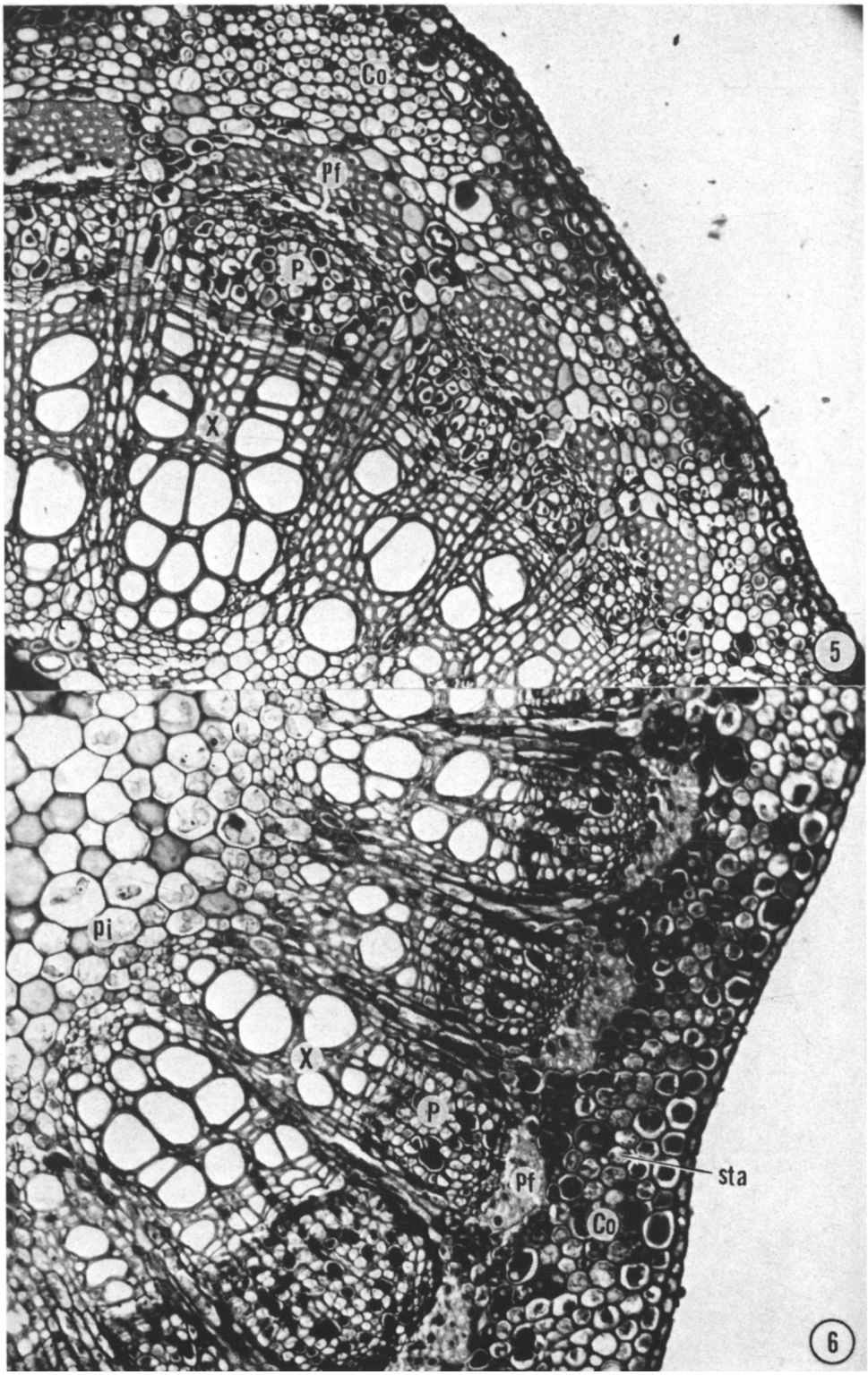

Fig. 5. Transverse section of the 6th internode of a healthy LN-33 cane collected in October 1963. In this stage of development the primary phloem fibers are present but no secondary phloem fibers have been formed. No sign of cork can be detected between the phloem and primary phloem fibers at this level. × 170.

Fig. 6. Transverse section of the 4th internode of a diseased LN-33 cane collected in October 1963. This stem was in the same stage of development as the healthy one above. There is, however, an accumulation of starch grains in the cortical cells not evident in figure 5. × 170. Details: Co, cortex; P, phloem; pf, primary phloem fibers; pi, pith; sta, starch; X, xylem.

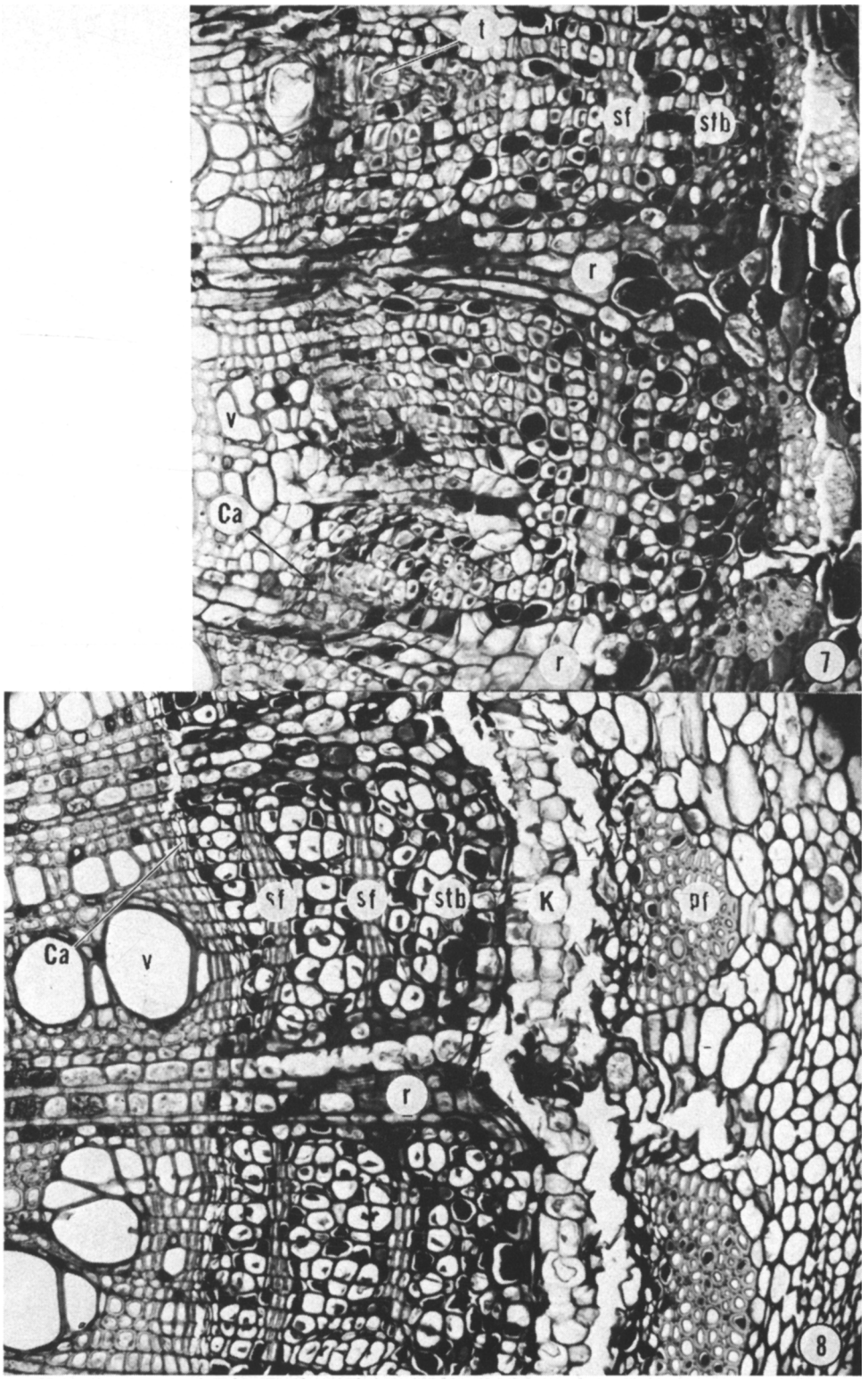

Fig. 7. Transverse section of the 9th internode of a diseased LN-33 cane collected in October 1963. Early signs of corky bark symptoms. Some of the last formed vessel members are partly collapsed; only one band of secondary phloem fibers; the phloem rays are dilated; and some proliferation is present. × 170.

Fig. 8. Transverse section of the 16th internode of a healthy LN-33 cane collected in October 1963. The xylem and phloem rays are normal. In the phloem there are two bands of secondary phloem fibers. The presence of a cork layer between the phloem and the primary phloem fibers is evident. × 170. Details: Ca, cambium; K, cork; pf, primary phloem fibers; r, ray; sf, secondary phloem fibers; stb, sieve tube band; t, proliferated cells; v, vessel.

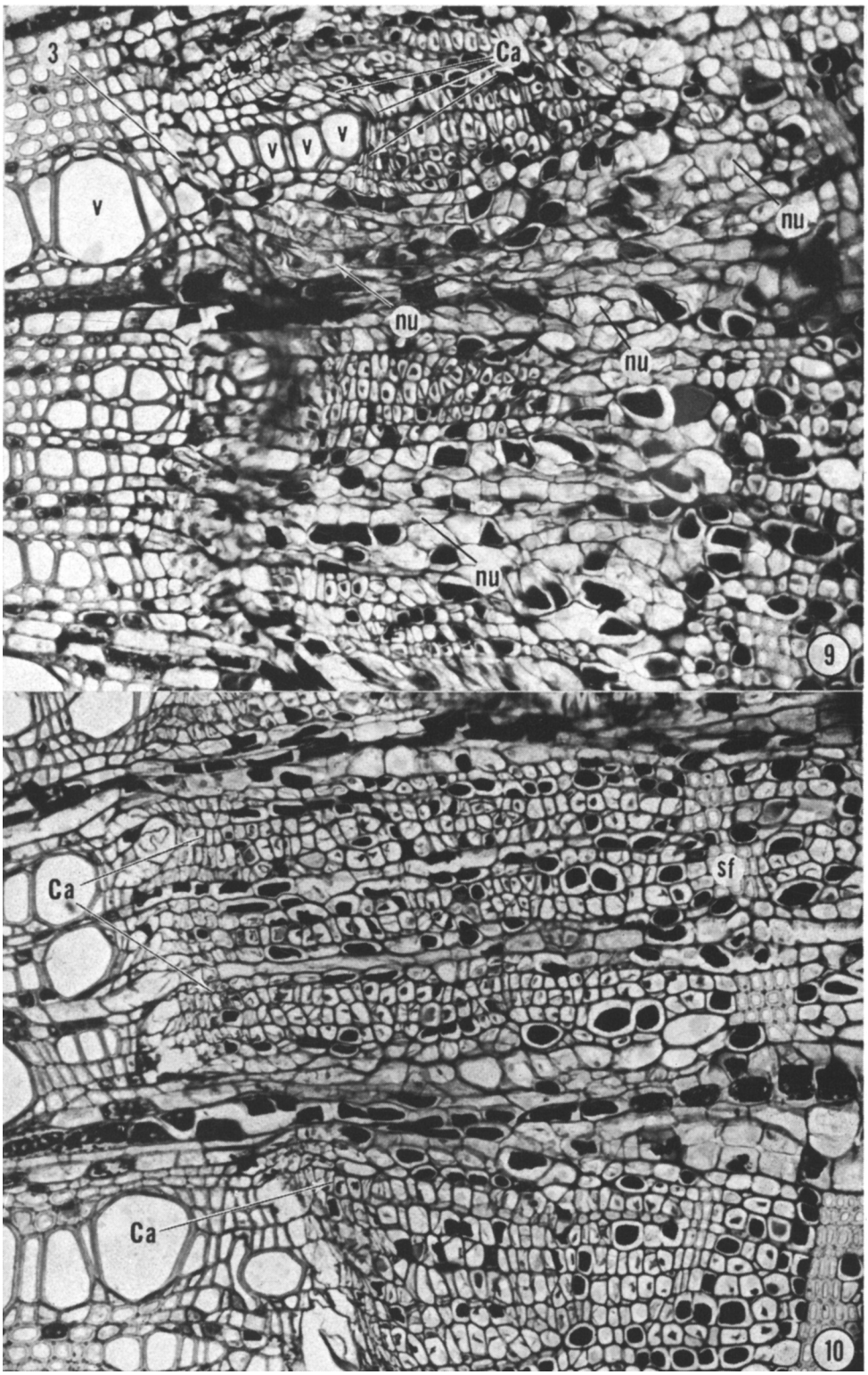

Fig. 9. Transverse section of the 9th internode of a diseased LN-33 cane collected in October 1963. An interruption in the formation of vessel members can be seen (number 3). The vascular cambium is located next to the last-formed vessel member of this isolated group of vessel members. Note numerous large, proliferated ray cells with prominent nuclei. × 170.

Fig. 10. Transverse section of the 16th internode of a diseased LN-33 cane collected in October 1963. Undifferentiated cells are present in the cambial zone. Furthermore, the cells of the cambial zone show signs of disorganization. × 170. Details: Ca, cambial zone; nu, nucleus; sf, secondary phloem fibers; v, vessel member; 3, interruption in radial series of vessel members.

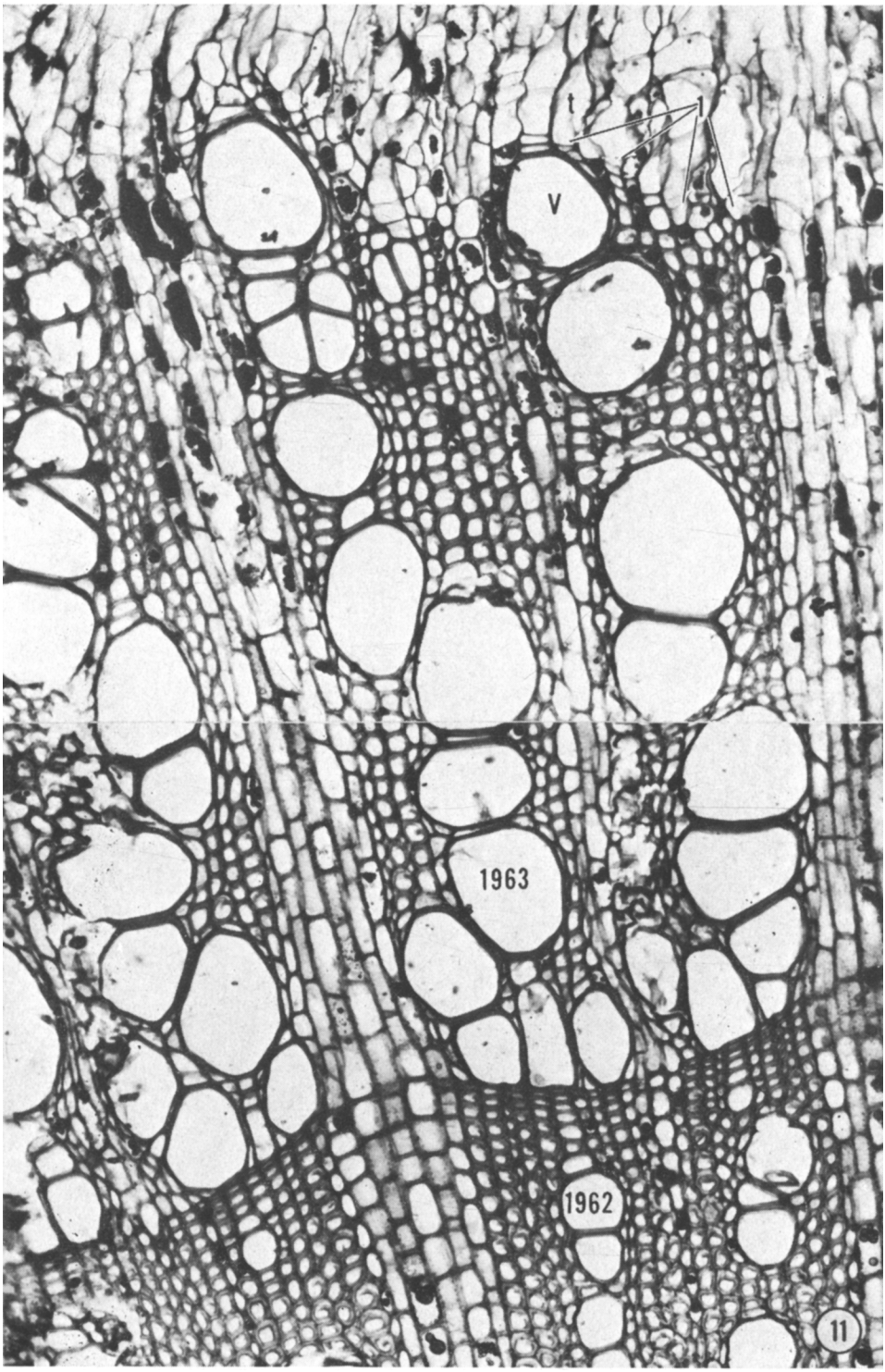

Fig. 11. Transverse section of a two-year-old cane of a diseased LN-33 vine collected in October 1963. The xylem formed in 1962 is normal. The xylem formed early in 1963 is normal also. Note the suddent change from thick-walled, regularly-arranged vessel members to thin walled proliferated cells (see lines at 1 at the upper right). × 170. Details: t, proliferated cell; V, vessel member; 1962: year in which first xylem was formed; 1, transition from healthy to proliferated cells.

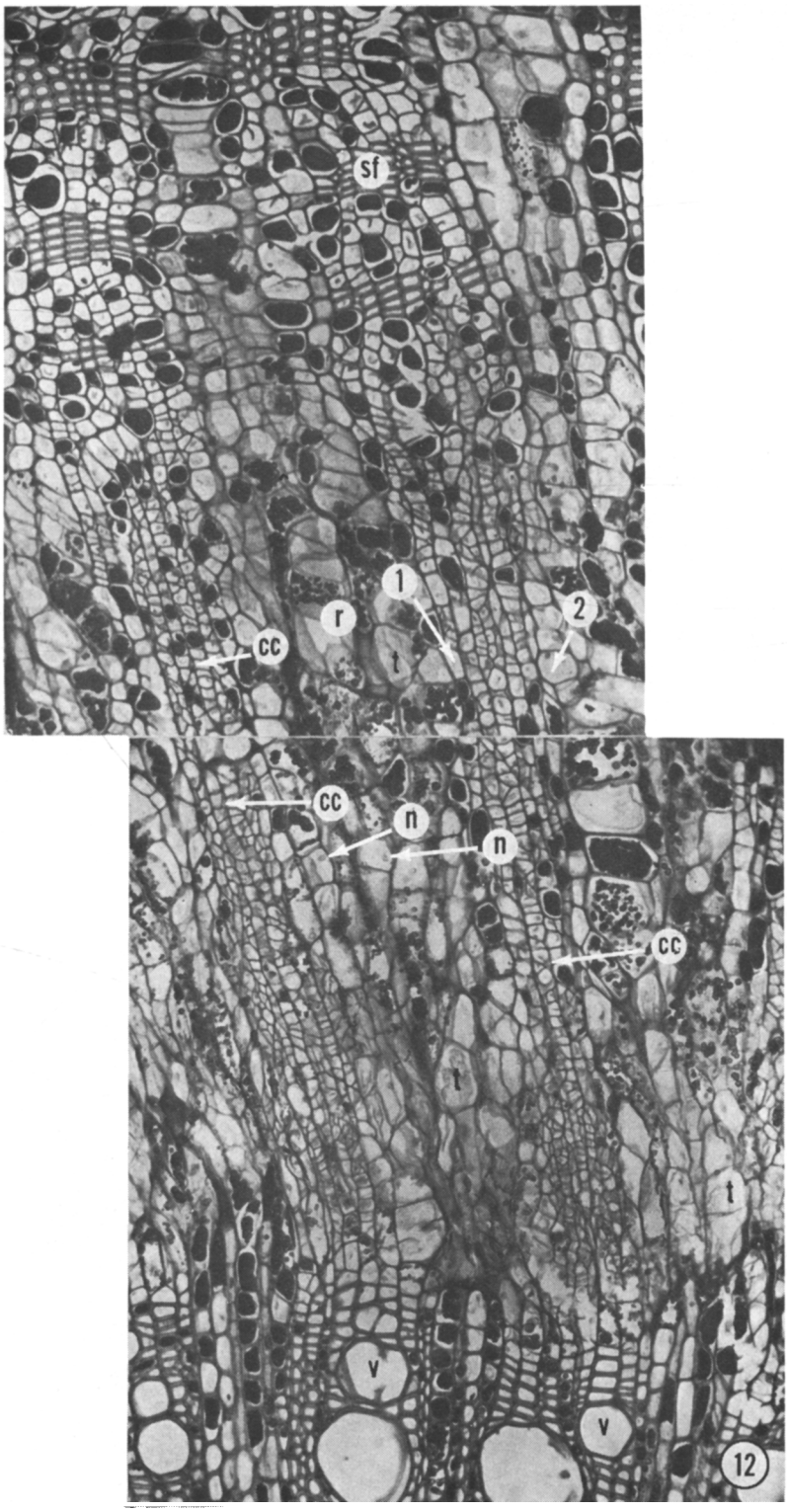

Fig. 12. Transverse section of the 10th internode of a diseased LN-33 cane collected in October 1963. The first-formed secondary phloem is normal and is located toward the upper edge of the figure. There is considerable distance between the last-formed band of secondary phloem fibers and the normal xylem. This space is occupied by narrow strands of phloem or "phloem-like" tissue. The wide rays contain proliferated cells with prominent nuclei. × 170. Details: cc, companion cells; n, nucleus; r, rays; sf, secondary phloem fibers; t, proliferated cells; v, vessel members; between 1 and 2, a narrow strand of phloem.

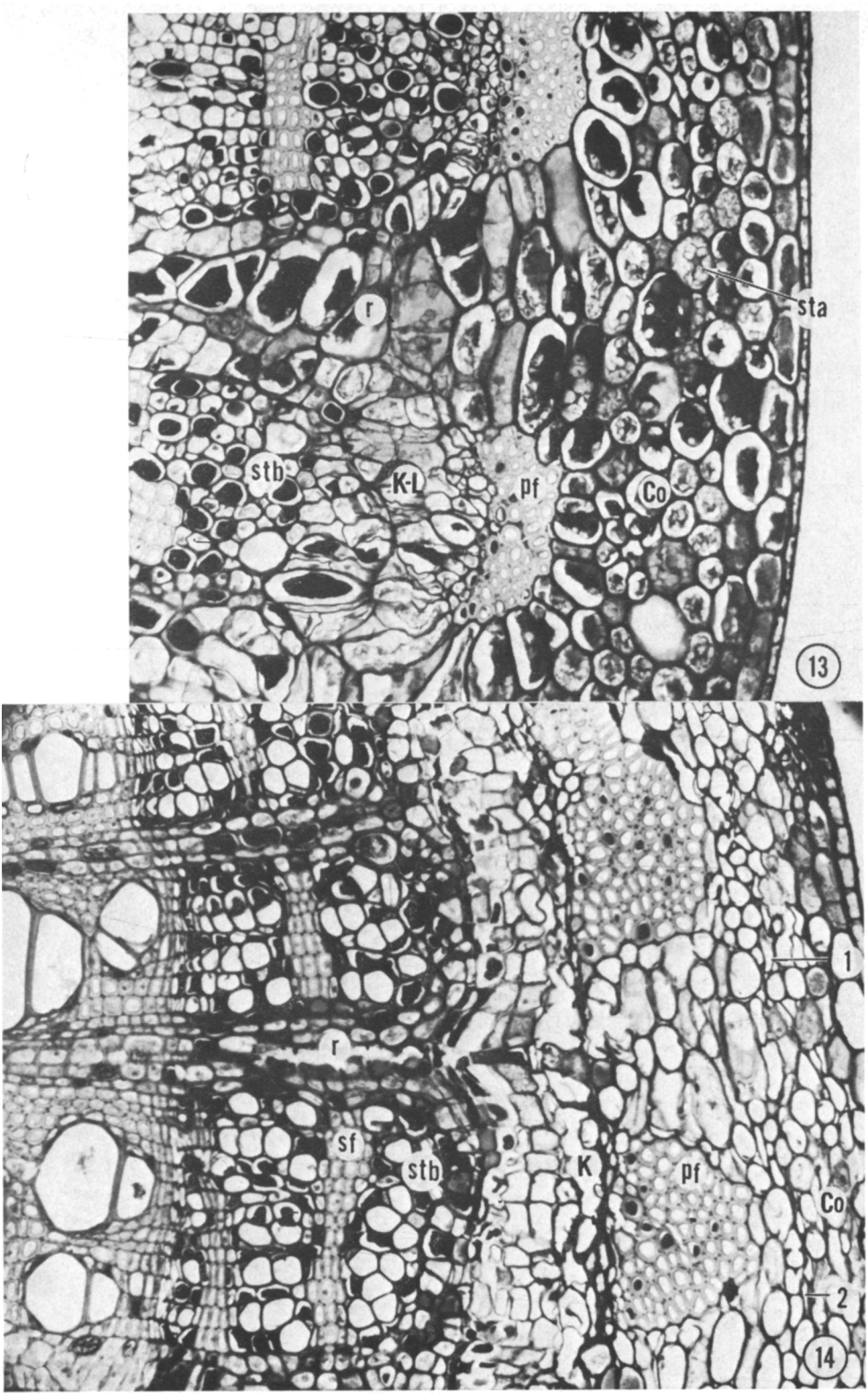

Fig. 13. Transverse section of the 11th internode of a diseased LN-33 cane collected in October 1963. The dilated ray and the presence of starch grains in the cortical cells are obvious. Between the primary phloem fibers and the sieve tube band some "cork-like" cells are in an early stage of development. No true cork cambium is present, however. × 170.

Fig. 14. Transverse section of the 18th internode of a healthy LN-33 cane collected in October 1963. Between the primary phloem fibers and a sieve tube band there is a well developed cork layer. The cells in the cortex show signs of collapse as normally would be expected at this stage of development. × 170. Details: Co, cortex; K, cork; K-L, cork-like cells; pf, primary phloem fibers; r, ray; sf, secondary phloem fibers; sta, starch, stb, sieve tube band; 1 and 2, collapsed cells of cortex.

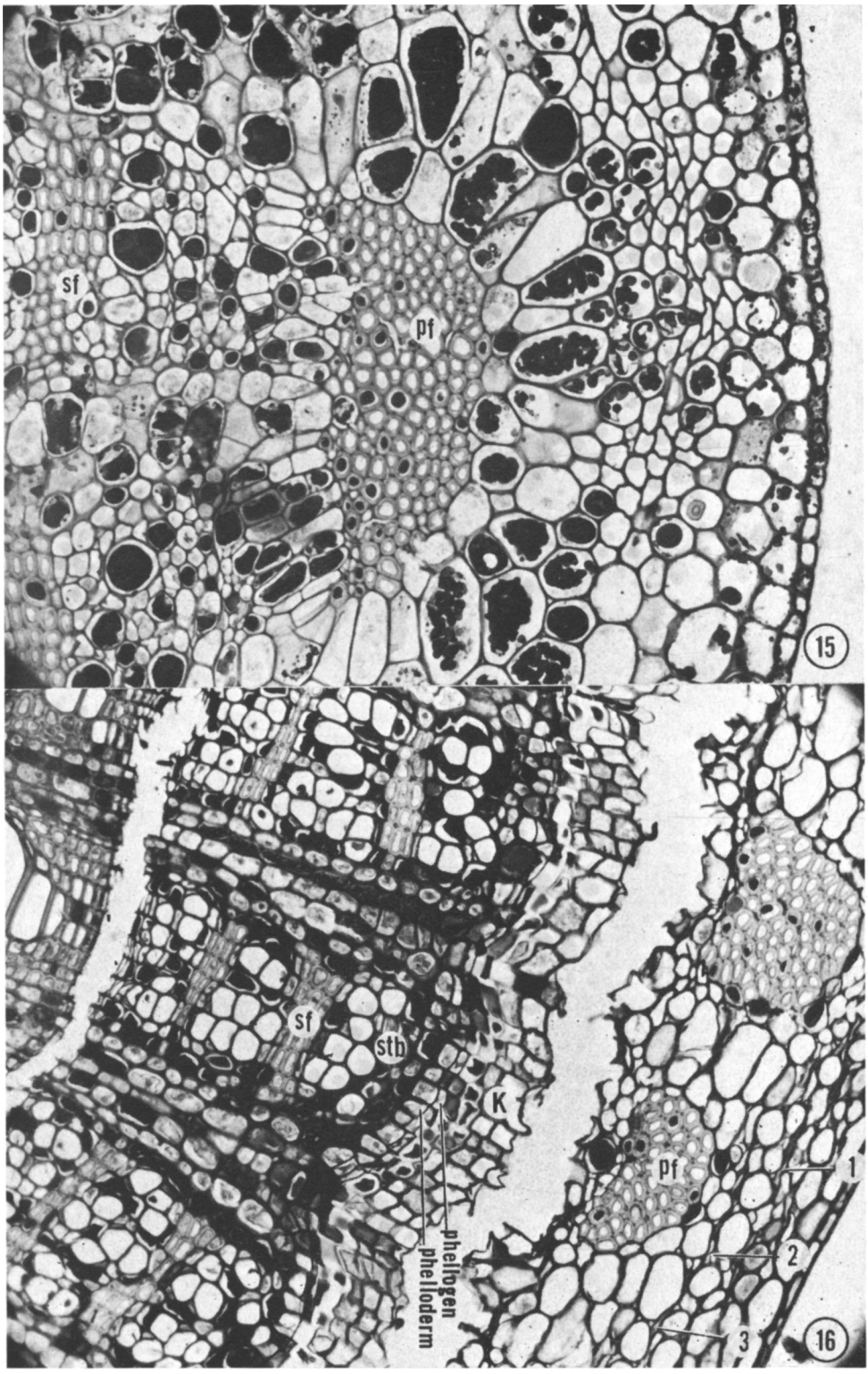

Fig. 15. Transverse section of the 9th internode of a diseased LN-33 cane collected in October 1963. A common symptom in corky bark infected canes is the inflated appearance of the cells around the primary phloem fibers. × 170.

Fig. 16. Transverse section of the 18th internode of a healthy LN-33 cane collected in October 1963. The cortex and primary phloem fibers have become separated from the rest of the stem. Some of the cells in the cortex are collapsed, × 170. Details: K, cork; pf, primary fibers; sf, secondary phloem fibers; stb, sieve tube band; 1, 2, 3, collapsed cells of cortex.

Fig. 17. Transverse section of the 19th internode of a diseased LN-33 cane collected in November 1964. In this stage all the cells of the cortex are filled with a "suberin-like" substance. × 170.

Fig. 18. Transverse section of the 10th internode of a diseased LN-33 cane collected in October 1963. In this stage the "cork-like" cells are elongated more than in fig. 13. This particular section is on the edge of a crack in the stem, and the dead and crushed cells can be seen. × 170. Details: pf, primary phloem fibers; K-L, cork-like cells; 1, edge of crack.

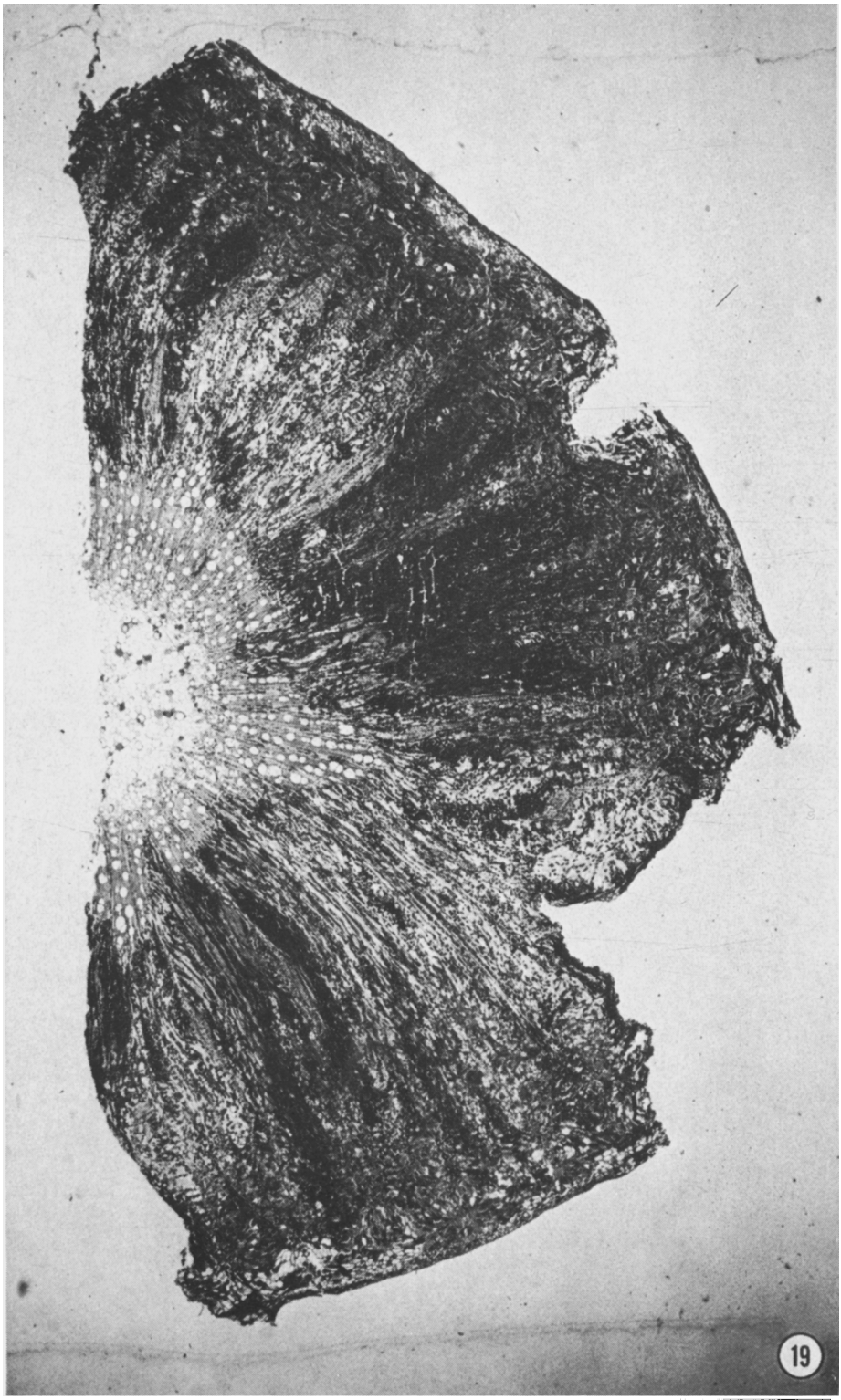

Fig. 19. Transverse section of a corky bark infected LN-33 cane collected in November 1964. Note extensive proliferation, impregnation of unknown material (perhaps suberin) and cracking of the stem. × 15.

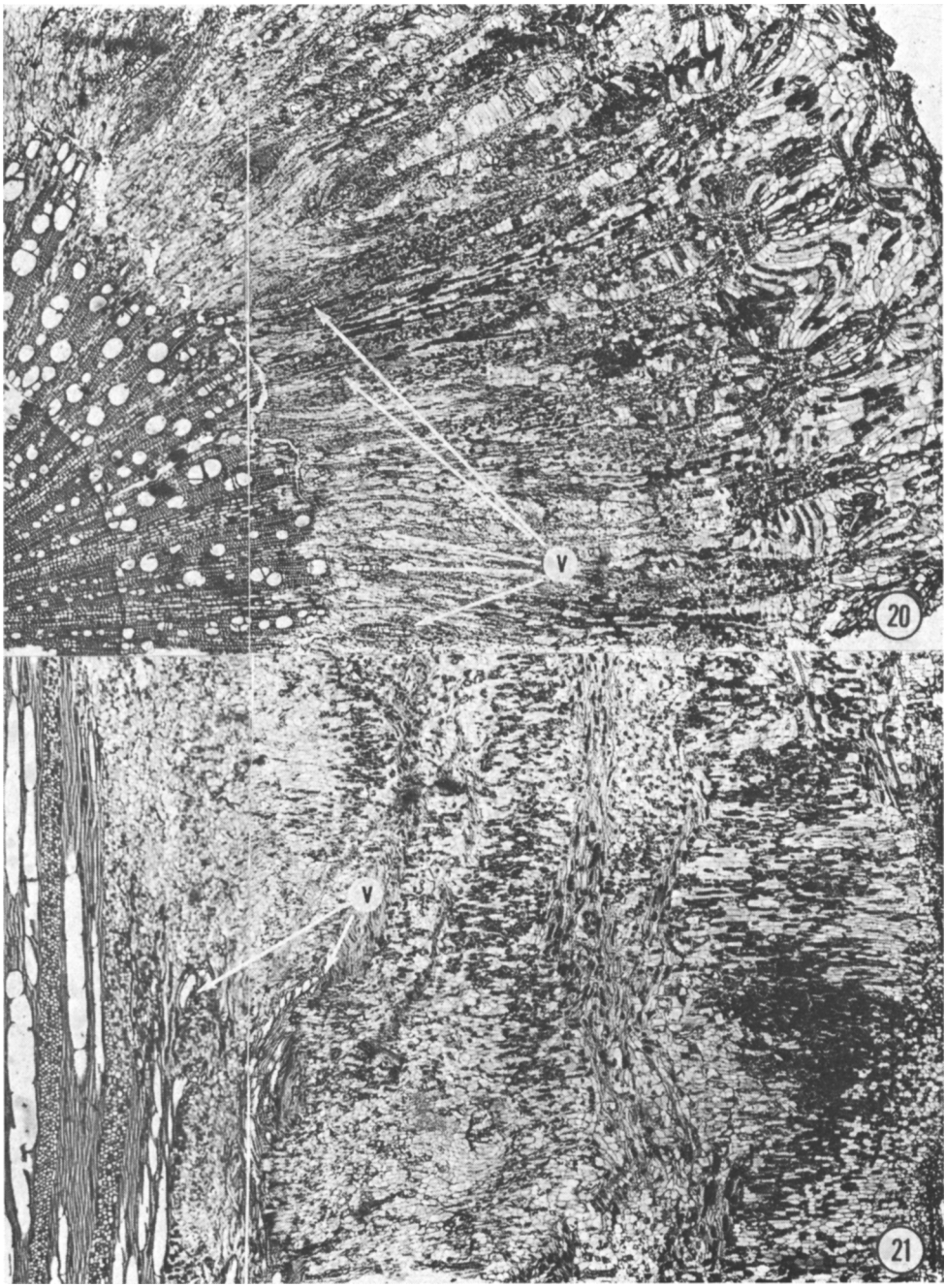

Fig. 20. Transverse section of a two-year-old cane of a diseased LN-33 vine collected in October 1963. An extensive area of proliferated tissue is present between the normal xylem and the phloem. Isolated in this proliferated tissue are groups of vessel members (indicated by arrows). × 27.

Fig. 21. Longitudinal section (radial) of a two-year-old diseased LN-33 cane collected in October 1963. Short abnormal vessel members can be seen in the proliferated tissue. × 27. Details: V. vessel member.

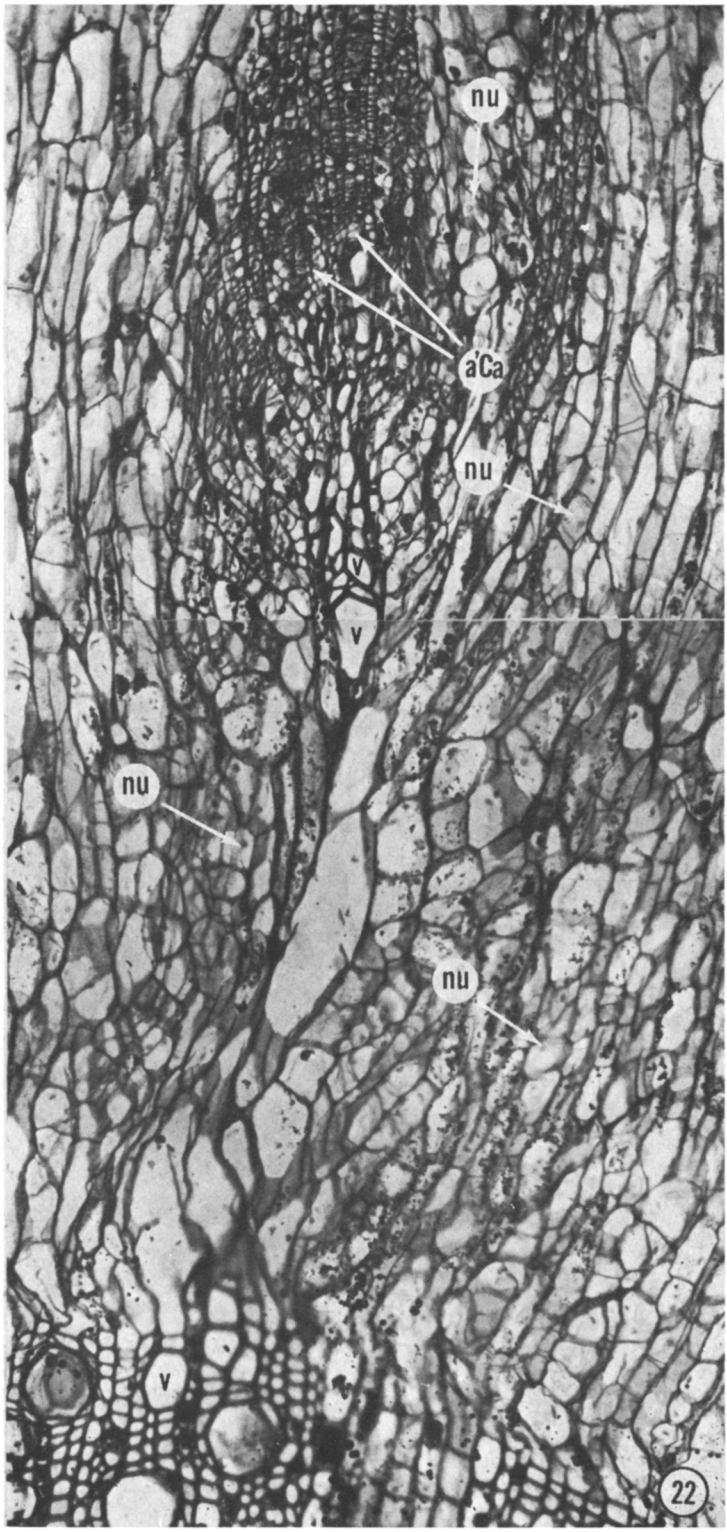

Fig. 22. Transverse section of a two-year-old cane of a diseased LN-33 vine collected in October 1963. The isolated vessel members can be seen surrounded by proliferated cells which have prominent nuclei. On one side of the group of isolated vessel members is an anomalous cambium. × 170. Details: a'Ca, anomalous cambium; nu, nucleus; V, vessel member.

Fig. 23. An LN-33 indicator vine in November 1965. This vine was rooted as a cutting in March and a Carignane bud infected with corky bark grafted on to it in April. It was planted in June and pulled out for photographing in November. Numbers 1–4 represent the levels of the transverse sections in figures 24–26. × 5/8.

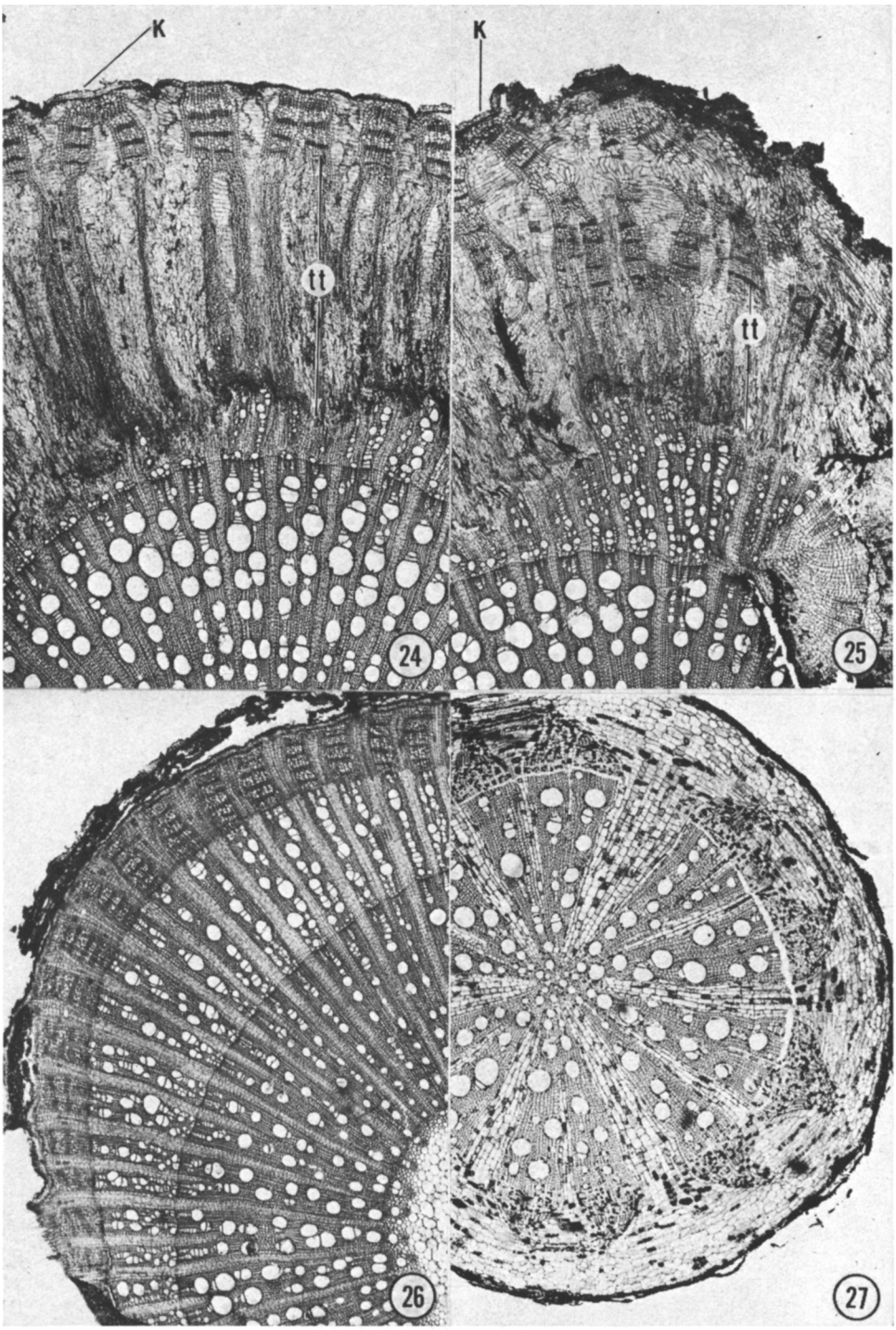

Fig. 24. Transverse section of a two-year-old cane just below the one-year-old cane (Number 1, figure 23). The first year's (1964) xylem is normal. Only a few normal cells have formed in 1965, however; the remainder is proliferated tissue. Cork formed in 1964 can be seen on the periphery of the section. × 20.

Fig. 25. Transverse section of the two-year-old cane just above the graft union (Number 2, figure 23). Somewhat more normal xylem was formed in one region at this level than in the previous one. Note the proliferated tissue on the inner and outer sides of the regions where secondary phloem fibers occur. × 20.

Fig. 26. Transverse section of the two-year-old cane below the graft union (Number 4, figure 23). The xylem of both 1964 and 1965 is perfectly normal. The phloem is normal and cork is formed as in healthy canes. × 20.

Fig. 27. Transverse section through the root of a diseased LN-33 vine collected October 1963. No visible abnormality can be detected. Rupture in the cambial zone is due to sectioning. × 23. Details: K, cork; tt, proliferated tissue.

Secondary phloem fibers are not found in the phloem of diseased canes after symptoms appear. In the more advanced stages much more phloem is present than in healthy canes (cf. figures 1, 2 and 3, 4). This excess of phloem production is in contrast to the reduced formation of xylem in diseased canes.

Sections of young canes from diseased vines (figure 6) differ little from those of healthy ones (figure 5). In both, primary phloem fibers are prominent, and inwardly the sieve tubes and companion cells can be observed. No secondary phloem fibers are yet present. The only detectable difference is the presence of more starch in the diseased cane.

In the 16th internode of the healthy cane (figure 8) two bands of secondary phloem fibers are present which alternate with regions containing sieve tubes and companion cells (sieve tube bands according to Esau, 1948a). The cell sizes of the phloem rays are normal. Cork is present; its significance with regard to corky bark infection will be discussed under a separate heading. In a section of the 9th internode of the diseased cane (figure 7) only one band of secondary phloem fibers is present. The phloem tissue between the one band of secondary phloem fibers and the primary phloem fibers appears normal when compared with the healthy phloem (figure 8). The phloem cells centripetal to the secondary phloem fibers become progressively less differentiated. Adjacent to the secondary phloem fibers, sieve tubes and companion cells are present; near the cambial zone the cells are more poorly differentiated, but remain phloem-like. The cells in the rays show an increase in size (hypertrophy) and the nuclei in these ray cells are prominent.

In a region of a cane with advanced symptoms (figure 12), the first formed phloem is normal while the region between the last formed secondary phloem fibers and the xylem is abnormal. The cambium is obscure in this section. It can be noted that the cambial zone between normal phloem and xylem is much wider than the expected region in a healthy cane (figure 8). The cells below the last band of secondary phloem fibers resemble those in the normal cane, but they soon taper off in narrow bands of sieve tube elements and companion cells that extend to the xylem. Hypertrophy as well as hyperplasia has taken place in the phloem rays. The ray cells are very much enlarged, irregularly shaped, and have large nuclei. These cells divide in several planes, forming irregular masses or panels of cells occupying the spaces between the bands of inner phloem.

The diseased regions in the phloem are, therefore, characterized by the absence of lignified cells, comparable to those of phloem fibers. The sieve tube members, formed in narrow bands between the much enlarged rays, are slightly abnormal in appearance.

Vascular cambium.—In a healthy cane (figure 8) the vascular cambium, or more correctly the cambial zone, can be seen as two or three layers of thin walled cells. In the diseased cane the cambial zone is not so easily identified (figure 7). The cambial cells do not differ much in morphology from their immediate derivatives. In the healthy cane it is easy to distinguish the thin-walled calls of the cambium from fully differentiated phloem and xylem cells. When the derivatives do not differentiate into distinct cell types it is not easy to distinguish the cambial cells. The region indicated as the cambial zone (Ca) in the diseased cane (figure 7) is a much wider zone than in the healthy cane (figure 8). The cambial zone is situated near mature xylem in the diseased cane as illustrated in figure 9. The cambium can be seen surrounding the protrusion of secondary xylem into

the proliferated tissue. This situation illustrates the fact that the last tissue formed towards the xylem side is usually normal, or, at most, only slightly abnormal.

From the above results it would appear that the vascular cambium loses its ability to form cells to the xylem side while it overproduces phloem-like cells. The vascular cambium may produce a few abnormal xylem cells centripetally, but it produces mainly abnormal phloem cells centrifugally.

Cork cambium and cortex.—As soon as secondary growth reaches a certain stage, cork cells are produced in the healthy cane (figure 2). In the diseased cane of comparable development no evidence of cork can be found (figure 1).

At a later stage of development the epidermis and the cortex are sloughed off in the healthy cane (figure 4).

In the early stages of development, the cork cambium or phellogen is formed in the outer phloem just centripetal the bundles of primary phloem fibers. The phellogen forms the phelloderm to the inside and the phellem, or cork, to the outside of the cane. About two layers of cork cells have been formed in the healthy stem illustrated in figure 14. The cork layer is continuous around the phloem and the ray areas and the cork has separated the cortex and primary phloem fibers from the phloem (figure 14). Some of the cortical cells seem near a state of collapse (at right, at numbers 1 and 2). The diseased cane at the same stage of development has no cork cambium (figure 13). The vascular bundle at the top in figure 13 shows very little activity in the region where the cork is normally formed. In the bundle at the lower edge of the figure proliferated cork-like cells are being formed in an irregular fashion. The cells are also elongated in an opposite direction from that of the normal cells; no such cells are being formed in in the ray region. The cells of the cortex are enlarged and contain a large amount of starch.

In a later stage, layers of cork (five cells thick) are present in a section of the healthy cane (figure 16). The cortex is broken in this cane and some of the cortical cells show signs of being crushed (see numbers 1, 2 and 3, figure 16). In the diseased cane of the same stage of development hypertrophy of the cortical cells can be seen (figure 15). The cells are much inflated and oriented in a spokelike manner around the primary phloem fibers. In figure 18 the cork-like cells are even more elongated. This particular section is at the edge of a crack in the cane and the cells on the edge of the fissures are collapsed and dead.

Late in the season (November) some of the cells in the cortex of diseased canes are impregnated with a substance that stains with safranin (figure 17). The extent of the tissues containing cells impregnated with the same substance can be seen in figure 19. This impregnation gives the illusion of corkiness and led to the name "corky bark" for this disease. From the anatomical standpoint, corky bark is a misnomer because no regular cork is formed in the diseased canes of LN-33.

Proliferative growth.—Under the previous headings the effects of a virus on different tissues of the cane were discussed. The responses of these various tissues of the cane were discussed in terms of increased or retarded growth. Apart from such tissue changes, some uncontrolled growth takes place that cannot be placed under any tissue classification.

In an advanced stage of proliferation, the proliferative cells tend to occur frequently in the ray tissue (figure 9). In that figure prominent nuclei can be

seen, and it can be noticed that the cells are very irregular in shape and size. In a more advanced stage the phloem rays consist mostly of proliferative tissue (figure 12). The wide rays, formed as a result of this activity, contain large irregular cells with prominent nuclei.

Early stages of anomalous growth can be seen in figure 9. There is an interruption in the radial series in the xylem, and then a continuation of a few more vessel members in the radial series.

Isolated groups of normal vessels within the proliferated tissue can be seen in the two-year-old cane (figure 20). Generally only a few tracheary occur in small groups and are surrounded by proliferative cells. These vessel members are very short when seen in a longitudinal section (figure 21).

A higher magnification of a pocket of xylem cells in transverse section (figure 22) shows that the cell walls of the xylem elements are irregular and partially crushed. On the far side of this pocket of xylem an anomalous cambium, with some phloem-like cells centrifugal to the anomalous cambium, can be observed. The entire area is surrounded by proliferative cells with prominent nuclei.

Starch accumulation.—The presence of abundant starch is observed frequently in diseased canes. Even before the presence of corky bark can be detected anatomically, abundant starch is present in the cortical cells of the diseased cane (figure 6). Much less is present in the healthy cane (figure 5). The difference in starch content between the healthy and diseased canes is also illustrated in figures 13 and 14.

Inclusion bodies.—The cells of both healthy and diseased canes contain large quantities of tannin, but no particles were observed that could be interpreted as virus-induced inclusion bodies in any of the material studied.

DISTRIBUTION OF SYMPTOMS IN THE CANE (LN-33)

A LN-33 vine which was rooted as a cutting in March 1965 and budded in April 1965 with a bud from a cane of Carignane infected with corky bark, is used to illustrate the spreading of symptoms from the graft union (figure 23). Sections of the two-year-old cane were made at the levels indicated by numbers 1 to 4 in figure 23: section number one, just below the one-year-old cane; section number 2, just above the graft union; section number 3, through the graft union; and section number 4, well below the graft union.

In section number 1 (figure 24) it is clear that the xylem formed in 1964 appears healthy. In 1965 only a small portion of fairly normal xylem was formed. The xylem is separated from the normal phloem by a wide zone of proliferated tissue. It is interesting to note that a cork cambium did develop in this instance and is evident near the edge of the section. The cork cambium apparently developed before the virus interfered with the normal growth processes.

In section number 2 (figure 25) the same phenomena are present. Proliferation can be seen to the inside and to the outside of the normal phloem.

In section 4 (figure 26), made below the inoculation bud, the growth of the xylem, phoem, and cork for 1965 is normal, and no corky bark symptoms can be detected.

It is therefore evident that the virus must have moved first toward the growing tip and that symptoms developed in an apical direction from the inoculation point. The symptoms did not spread in the direction of the roots. A section of root from a corky-bark-diseased LN-33 vine shows no visible abnormality (figure 27).

PETIOLES AND LEAVES

Sections of petioles and veins of leaves of diseased and healthy canes were studied. In some diseased leaves there is gum formation in the vessels associated with derangement and necrosis in the phloem. The effects are, however, not consistent and more samples at different stages need to be examined before the internal symptoms of diseased leaves can be described with any degree of certainty.

PATHOLOGICAL ANATOMY OF CARIGNANE

As mentioned before the variety Carignane, although a carrier of corky bark disease, shows only mild symptoms. The leaves on the tip of the canes are a light yellow color. When a bud from Carignane is budded on to LN-33, the LN-33 plant shows severe symptoms.

DISCUSSION

The virus nature of corky bark reported by Hewitt *et al.* (1962) is confirmed by this work. It was clearly illustrated that the anatomical symptoms of corky bark in a LN-33 vine were found at the level of the graft union of the LN-33 indicator and the bud from the Carignane vine, infected with the disease (see figure 23). Also, it was established that the symptoms of corky bark move in an acropetal direction. This fact is supported by visual observations in the field and by an examination of sections of the stem at various levels (figures 24 to 26). The symptoms do not move down from the point of inoculation (figure 26) and do not occur in the roots of infected LN-33 vines (figure 27).

The acropetal expression of external and internal symptoms of corky bark disease indicates that the virus itself may be moving in that direction also. This observation rules out the likelihood of the virus moving in the phloem of the plant, because photosynthates move down the cane as well as acropetally toward meristematic regions. Viruses do move upwards in the xylem of plants, but the movement is usually rapid. Although the rate of movement of the corky bark particle itself is not known, the symptoms appear relatively slowly towards the apex of the grape vine. Presumably the virus itself moves at the same rate.

From our histological study it can be concluded that corky bark virus belongs to the histologically nonlimited viruses (Esau, 1961) and that it moves from cell to cell probably by means of protoplasmic streaming. The tissue in which the virus moves is possibly the cambial region. This is supported by the fact that the initial symptoms appear at the cambial zone (figure 7). Another indication that the virus may move in the vascular cambium is the fact that symptoms of corky bark are only found after secondary growth has proceeded for some time. This suggests that an active vascular cambium must be present before the virus or the products of a deranged metabolism can move up in the plant. Finally there is no noticeable necrosis in the xylem before the symptoms occur in the cane.

Although it has not been demonstrated anatomically, there must be movement of the virus in a basipetal direction. The downward movement also could be a

cell-to-cell movement of the virus. The evidence for the downward movement is the fact that canes which emerge low on LN-33 vines the season after the canes of the vines were killed in winter, develop corky bark symptoms. Initially these canes show no corky bark symptoms (May or June) but later (July or August) symptoms appear from the base upwards.

Anatomical effects of the virus disease on the canes of LN-33.—The drooping of canes of corky-bark infected vines is caused by the lack of lignified cells that are present in a healthy plant which support the cane. No lignified xylem elements are formed after first corky bark symptoms are recognized at a specific level in the cane (see figures 1, 3, 12 and 22). To a lesser extent the absence of secondary phloem fibers in diseased canes, after the symptoms have appeared (see figures 1, 3 and 12), may also contribute to the drooping effect of the diseased canes.

The immature appearance of canes of corky-bark infected vines are due partly to the absence of secondary phloem fibers, but principally because no true cork is formed in the diseased canes. Without the formation of cork the cortex cells do remain alive and the canes retain a nonwoody appearance even late in the season. The absence of secondary phloem fibers is also reported in plants infected with curly top disease (Esau, 1961). According to Caudwell (1964) secondary phloem fibers are formed irregularly, if they are present at all, in grapevines affected by Flavescence dorée.

Concurrently with the discontinuation of lignification in the diseased cane, the amount of phloem formed is abnormally high in comparison with the normal cane (see figures 1, 3 and 12). The overproduction of sieve tubes was also observed in curly top disease (Esau, 1948b), and hyperplastic and anomalous phloem is formed in grapevines affected by "Legno ricco" (Graniti, 1964).

No severe necrosis of either the phloem or xylem was observed; thus corky bark differs from diseases such as dwarf virus of barley (Esau, 1957b), and yellow leafroll virus of peach in celery (Esau, 1958) for which there is only a necrotic effect, or the buckskin disease of peach (Schneider, 1945) and the curly top disease in sugar beet (Esau, 1957b) where necrotic obliteration follows the formation of abnormal phloem. According to Shapovalov (1939) some viruses may cause morphological changes without necrosis. This agrees with the observation made on corky bark disease.

In the corky bark disease secondary xylem is no longer produced after the first symptoms make their appearance. The xylem that was produced before this event remains intact, however, and differs from diseases such as Pierce's disease of the grapevine where a gummous degeneration takes place (Esau, 1948b) in the vessel elements, or the appearance of trabeculae in the vessel members of fanleaf diseased grapevines (Schneiders, 1937; Maier, 1939; Gifford *et al.*, 1956). The reduction in the amount of xylem produced is also reported in two other diseases of the grapevine: Flavescence dorée (Caudwell, 1964) and "Legno ricco" (Graniti, 1964).

Corky bark is definitely a disease of secondary tissue and thus differs from leafroll which is a disease of primary tissues (Hoefert and Gifford, 1965). The first symptoms of corky bark appear in the vascular cambium (see figure 7). As soon as the virus affects cells in the cambial region the derivatives of the cambium remain nonlignified in both the xylem and phloem. The position of the vascular cambium (figure 10) is an indication of a reduction in the number of

cells formed by the vascular cambium to the xylem side. The vascular cambium in advanced stages of corky bark infection is difficult to recognize (see figures 12 and 20).

The observation that no normal cork is formed in diseased LN-33 canes points to the fact that the corky bark virus influences the cambial regions of the cane. Although the normal cork is absent there is a stimulation of cork-like cells in the area of the phloem where the cork-cambium usually arises (see figures 13 and 18). In the disease Flavescence dorée, suberized cork cells are absent but a layer of cork-like cells occurs nearer to the wood than it is usually. (Caudwell, 1964).

The cortical cells are retained in corky bark diseased canes and are usually full of starch (figures 6 and 13). Starch accumulation in the pith of virus-infected plants is recorded by Samuel *et al.* (1933). This is, however, in opposition to the observations of Bawden (1964) that there is a decrease of starch in plants infected with the mosaic group of viruses.

No inclusion bodies have been noted in this work and this is in accordance with the findings of Samuel *et al.* (1933) for "big bud" disease of tomatoes.

As described above, the first major aspect of internal symptoms of corky bark is the lack of differentiation of derivatives of the vascular cambium. A second aspect of the disease is the presence of proliferation of cells in the tissue of the cane (see figures 7, 9, 11 and 12). No definite cambial zone can be detected among these thin-walled cells with prominent nuclei and they probably multiply by uncontrolled division. Proliferation of the cambium was reported in Fiji disease of sugar cane (Wood, 1937), curly top disease in sugar beet (Artschwager and Starrett, 1936), wound-tumor virus in the roots of *Hyocyamus,* and wound-tumor virus in *Lychnis* (Kelly and Black, 1949).

The presence of prominent nuclei in tumorous tissue was reported by Samuel *et al.* (1933) in "big bud" virus disease. This is an indication that corky bark might be a tumorous disease. It is true, however, that the proliferated cells do not develop into excessive masses of cells that would be expected in a true tumor. On the other hand the proliferated tissue causes a swelling of the infected canes of LN-33 vines. Finally, as a result of excessive proliferation and the lack of a sloughing-off mechanism, the canes become cracked mechanically.

The anomalous growth and the isolated vessel members seen in the two-year old wood of corky bark infected LN-33 vines (see figures 20–22) resemble the abnormal xylem illustrated by Kelly and Black (1949) in a study of the wound-tumor virus in the roots and stem of *Melilotus alba.* The xylem elements formed were short cells with reticulate thickenings. No vessel elements in the tumor tissue were found, however.

It can be concluded that corky bark is probably closely related to wound-tumor virus.

The mechanism of symptom development of the corky bark disease.—The question arises how morphological changes in diseased plants are caused by the virus. It was shown that necrosis is absent in the symptoms of corky bark. Therefore, a localized effect by the virus is absent in corky bark. Furthermore it was seen that the symptoms started from the two cambial areas and that normal differentiation was upset. In other words, the control of normal morphogenesis was upset by the virus. The fact that viruses may constitute a potent factor in morphogenesis was mentioned by Shapovalov (1939).

According to Esau (1957b) a parallel exists between the effects of viruses and growth regulating substances on plants. The first clue that growth substances may be involved in corky bark disease was evident in the observation that the leaves of diseases vines do not abscise normally in the fall, but remain on the vines until late in the winter.

CONCLUSION

Corky bark, a virus disease, moves acropetally in a vine if it is introduced into the vine by grafting. The virus may move from cell to cell in the vascular cambial region. The differentiation of cells in the vascular cambial zone is upset and tumorous growth takes place by the production of excessive amounts of abnormal phloem and proliferative tissue. There is no normal cork cambium.

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