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PHLOEM ANATOMY OF TOBACCO AFFECTED WITH CURLY TOP AND MOSAIC

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PHLOEM ANATOMY OF TOBACCO AFFECTED WITH CURLY TOP AND MOSAIC[1]

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INTRODUCTION

THE PHLOEM OF PLANTS affected by the curly-top disease undergoes profound histologic and cytologic changes. Among these, the degeneration and death of certain cells and the hyperplastic increase in the number of sieve-tube elements are the most striking symptoms (Esau, 1933, 1935*a*, 1935*b*).[3] The inception of phloem degeneration in a developing organ depends on the presence of mature sieve tubes, while the first internal symptoms are localized in areas directly connected by phloem with the inoculated leaf (Esau, 1935*a*, 1935*b*).

The interpretation of the nature of phloem degeneration in curly-top plants has been based almost entirely upon studies of a single host, the sugar beet (Esau, 1933, 1935*a*, 1935*b*; Artschwager and Starrett, 1936); a limited consideration of the anatomy of curly-top tobacco showed comparable pathologic changes in this host (Bennett and Esau, 1936).

The present paper is a result of rather comprehensive studies on the phloem of the Turkish variety of *Nicotiana Tabacum* affected with curly top. It also briefly considers phloem degeneration in tomato affected by the same disease and compares the effects of curly top and of tobacco mosaic upon the phloem of tobacco.

Nicotiana Tabacum was selected for study because this species has served repeatedly in investigations concerning translocation and tissue relations of the curly-top virus (Bennett, 1934, 1937; Bennett and Esau, 1936). At the same time, the choice of tobacco offered an opportunity to examine the course of phloem degeneration in a plant that differed considerably from the sugar beet in its development, its morphology, and its reaction to the curly-top virus. Tobacco, unlike the beet, produces a stem with secondary vascular tissues during the first year of growth, has internal phloem and no specialized underground storage organ nor anomalous secondary growth. With regard to curly-top infection, tobacco commonly recovers from the disease (Bennett, 1934; Wallace, 1939), a tendency not characteristic of the sugar beet.

Nicotiana glauca, a species also used in virus-translocation studies, was to be considered in this paper. This species, however, is a symptom-

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[3] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

less carrier (Bennett, 1934) and—judging from the curly-top material used in the present study—appears to be free of phloem abnormalities.

The development and structure of the phloem of the aerial parts of healthy tobacco plants have been previously described (Esau, 1938*a*). Reference to normal structure is, therefore, very restricted in the present paper, except regarding the root tips, which have not been considered before.

MATERIAL AND METHODS

The curly-top material was obtained from potted plants grown in greenhouses.[4] The plants were inoculated by the usual method of placing viruliferous *Eutettix tenellus* upon one or more young leaves of the experimental plants. Curly-top-diseased sugar beets served as the initial source of the virus.

The permanent slides were prepared by the ordinary paraffin method, with the normal butyl alcohol as paraffin solvent. Chrom-acetic-formalin and formalin-acetic-alcohol mixtures were employed for killing and fixing.

The iron-alum-hematoxylin staining combination was largely resorted to. Part of the slides were stained by the common procedure of overstaining with hematoxylin and destaining with an iron-alum solution until the desired differentiation was secured. This method, though satisfactory for the cytological details, did not leave sufficient stain in the walls. The other part of the material was mordanted in the usual way with iron-alum, but was stained lightly, without overstaining, in a very dilute solution of hematoxylin in water and then counterstained with safranin.[5] This staining clearly defined the cell walls. The photomicrographs were taken from slides prepared in this manner.

LOCALIZATION OF THE FIRST EXTERNAL CURLY-TOP SYMPTOMS IN TOBACCO

Although *Nicotiana Tabacum* shows only moderate susceptibility to curly top (Bennett, 1934), infection of young plants causes a severe initial shock with marked symptoms, such as translucency and swelling of veins, puckering, and rolling of the leaf blade.

The systemic symptoms of virus diseases appear only in those organs and tissues of the plant that are immature at the time of virus entry. (See review by Esau, 1938*c*.) The same relation prevails in curly-top tobacco: leaves developing systemic symptoms are younger than the inoculated

[4] The writer is grateful to Dr. C. W. Bennett of the United States Department of Agriculture, Riverside, California, for furnishing part of the material used in this study.

[5] This procedure was recommended by Dr. R. H. Wetmore of Harvard University.

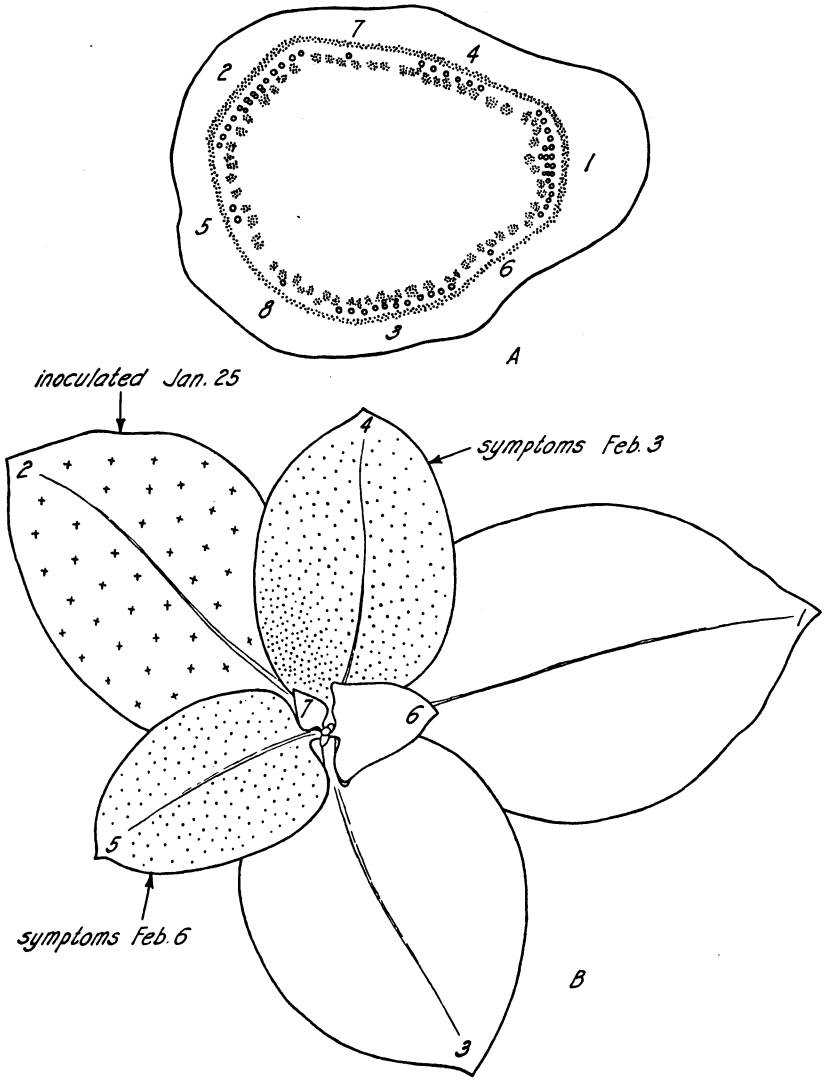

Fig. 1.—*A*, Transverse section of tobacco stem showing arrangement of leaf traces in a plant like the one in *B*. The circles indicate xylem; the stipples phloem. (× 16.) *B*, Diagram of a young tobacco plant showing the spatial relation between the inoculated leaf (marked with crosses) and the leaves that show the first systemic symptoms of curly top (marked with stipples).

one. Moreover, the young leaves with the first systemic symptoms are located on the same side as the inoculated leaf.

Six transplanted seedlings with six to eight foliage leaves were each inoculated by means of four leafhoppers caged on the fourth to sixth leaves from the oldest. Systemic symptoms appeared 9 to 13 days after inoculation. In four plants the first symptoms occurred on a leaf removed, in time of origin, two plastochrones[6] from the inoculated leaf (fig. 1, *B*). In two plants whose inoculated leaves were somewhat older than those in the first four plants, the initial symptoms appeared on a leaf three plastochrones younger than the inoculated one. Next, the symptoms developed on the leaf two plastochrones from the inoculated.

Fig. 1, *B*, illustrates the spatial relation between the inoculated leaf (marked by crosses) and the leaves showing the earliest systemic symptoms (marked by stipples). The dense stippling in leaf 4 indicates the portion of the leaf that showed the most pronounced symptoms in the plant selected for the illustration. This localization of the most severe symptoms depends partly on the proximity of the inoculated leaf, partly on the later maturation of the basal as compared with the apical portion of the leaf (Esau, 1935*b*).

As previous investigations have shown, phloem is the channel of translocation of curly-top virus in the host plant. (See reviews by Esau, 1938*c*, and Crafts, 1939.) The localization of the first symptoms in tobacco plants also is related to the phloem connection between the leaf receiving the initial supply of virus and the leaves developing the first symptoms. Figure 1, *A*, shows the arrangement of the traces of leaves depicted in figure 1, *B*. The trace of leaf 2 is flanked by those of leaves 5 and 7. If these two leaves were in a primordial state, leaf 4 would be the nearest to leaf 2 to receive the virus from it through vascular connection; at stem levels below that depicted in figure 1, *A*, the traces of leaves 2 and 4 approach each other, and their phloem strands interconnect by anastomoses. (See also Esau, 1938*a*, figs. 2, 3, and plate 5.) If leaf 5 had mature phloem at the time of inoculation, it could be the first to show systemic symptoms because it connects with leaf 2 at a higher level than leaf 4. Thus, the symptoms could appear on a leaf either two or three plastochrones removed from the leaf receiving the initial dose of virus, the location depending on the relative size of the leaves connected with the inoculated one.

These observations on the localization of the first systemic symptoms in tobacco agree with previous observations on the sugar beet (Esau, 1935*b*).

[6] *Plastochrone* as used here designates the time interval between the origin of two successive leaf primordia (Askenasy, 1880; Priestley and Scott, 1933 and 1938).

PHLOEM DEVELOPMENT IN LEAVES AND STEMS OF CURLY-TOP TOBACCO

The developing primary phloem of curly-top tobacco passes through stages of degeneration similar to those of the sugar beet affected by the same disease. The terms used in describing the pathologic changes in the beet phloem (Esau, 1935*a*, 1935*b*) are therefore applicable to the tobacco also.

Reducing to a minimum the description of phenomena that are similar in the beet and tobacco, and stressing those that are peculiar to tobacco, one may present the general course of phloem degeneration in tobacco as follows. Degeneration commences after maturation of the first sieve tube in a leaf or in its trace in the stem. Cells adjacent to this sieve tube usually increase their chromaticity, develop inclusions, and show diverse nuclear abnormalities (Esau, 1935*b*, Artschwager and Starrett, 1936). Entire cells or their nuclei enlarge (primary hypertrophy). Finally these cells die, and many of them collapse either before or after disappearance of the protoplasts (primary necrosis). If the cells collapse when degenerated protoplasm is still present, deep-staining material is evident in the area of necrosis. Some cells undergoing primary necrosis do not collapse; rather, their walls disintegrate at the same time as those of the hyperplastic tissue formed later.

In healthy tobacco several sieve tubes develop on the abaxial margin of the procambium strand before centripetal differentiation of the phloem begins (Esau, 1938*a*). The first sieve tube appears in the median abaxial position; then several more sieve tubes differentiate to the right and to the left from the first in gradual progression toward the margins of the bundle. The first degenerating cells form a developmental pattern similar to that of the sieve tubes; they appear first in the median abaxial part of the bundle (fig. 2, *A*), then to the right and left from this position, always in association with just-matured sieve tubes (fig. 2, *B*).

The peripheral adaxial sieve tubes themselves (the protophloem sieve tubes, according to Esau, 1938*a*), and also their companion cells, appear to be free of abnormalities. When, however, centripetal differentiation of the phloem begins, the meristem shows abnormally active cell division, has very dense cytoplasm, and produces phloem with excessive numbers of disorderly-arranged cells (primary hyperplasia) strikingly different from normal metaphloem. Most of the hyperplastic cells develop thick walls (fig. 2, *B* and *C*) and are, at maturity, almost devoid of cytoplasm (plates 2, *A*, and 3, *A*). This hyperplastic tissue later disintegrates (secondary necrosis), while the procambium continues to produce more similar cells.

In the sugar beet, secondary necrosis is accompanied by proliferations of adjacent parenchyma cells, which replace the dying cells (secondary hypertrophy and hyperplasia). This growth is so intense that the necrotic cells appear to be crushed in large masses. Considerable dark-

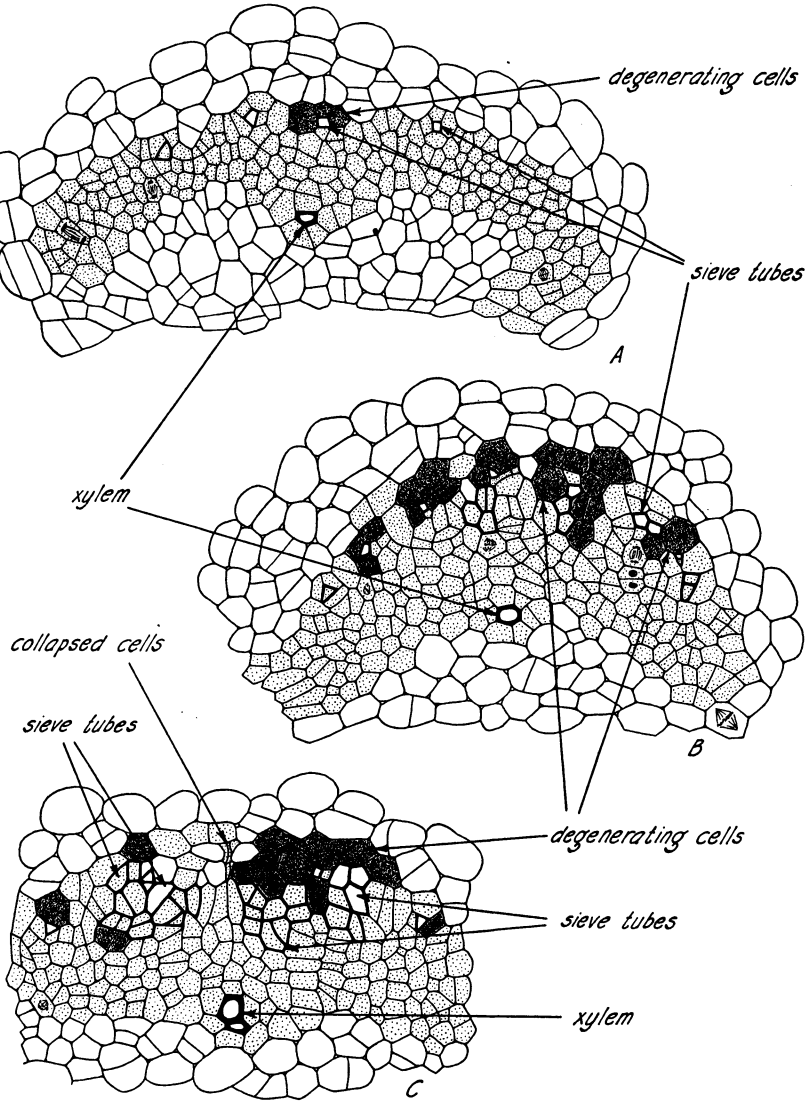

Fig. 2.—Transverse sections through median portions of curly-top tobacco petioles, showing three stages of phloem degeneration. The light stippling indicates the vascular regions; the heavy stippling the degenerating cells. All sieve tubes have rather thick walls; the immature ones are also stippled. (All × 281.)

staining material accumulates in these areas and eventually passes through intercellular spaces to the surface of the organ as droplets of exudate rich in virus (Bennett, 1934; Bennett and Esau, 1936). In tobacco the accumulation of dark-staining material is less extensive than in the beet, and no exudation seems to occur (Bennett and Esau, 1936). The secondary hypertrophy and hyperplasia are also less pronounced. They occur when phloem degeneration is not very severe. Commonly, however, large cavities develop where abnormal tissue disintegrates (plates 2, *B*, and 13, *A*). Before this disintegration takes place, the walls of the hyperplastic cells become thin and then disappear entirely. The protoplasmic content, scarce before necrosis, usually leaves no traces.

In early stages of secondary necrosis, some deep-staining material may occur on the periphery of the cavity. This material stains bright red with safranin and black with hematoxylin. It showed no lignin reaction with phloroglucinol in hydrochloric acid and with the Mäule reagent. A test with orcinol disclosed no pentosan groups.[7] Judging from these results, either the substance resulting from disintegration of cells is not wound gum, or the wound gum is masked by other substances.

The primary hyperplastic degeneration may spread also centrifugally from the first abaxial sieve tubes. In such cases, groups of hyperplastic cells may occur on the adaxial side of the cavity far removed from the vascular bundle (plate 13, *A*). If degeneration is less severe and no cavity is formed, hyperplastic cells may become separated from the vascular bundle by secondary hypertrophy and hyperplasia (plate 4, *A*).

Like the abaxial (external) phloem, the adaxial (internal) phloem first differentiates normal sieve tubes, then degenerates. Since the internal phloem differentiates later than the external (Esau, 1938*a*), it begins to degenerate in an older leaf than the external, but eventually shows the same degree of pathologic modification (plate 13, *A*).

Although the hyperplastic cells show abnormally dense cytoplasm during differentiation, they closely resemble normal sieve-tube elements in their ontogeny and mature structure. They develop slime bodies that later disintegrate, as does the nucleus. The cells have plastids with starch that stains red with iodine. The walls between the individual elements have sieve plates with connecting strands. The latter pass through pores lined with callus that gives the characteristic sky-blue color reaction with anilin blue applied after treatment of fresh sections with I_2KI. Like normal sieve tubes, the hyperplastic cells have thick walls and, when mature, show scant cytoplasm that does not stain deeply. In view of all these characteristics, these cells are interpreted, as previously in the

[7] The microchemical tests were made by Dr. W. B. Hewitt of the Division of Plant Pathology.

sugar beet (Esau, 1935*a*, 1935*b*), as sieve-tube elements. For convenience they will be called "abnormal," "hyperplastic," or "supernumerary" sieve-tube elements, sieve cells, or sieve tubes.

In contrast to the normal sieve tubes, the hyperplastic elements appear in compact masses lacking companion and phloem-parenchyma cells (plate 2, *A*). The masses are so extensive that they overlap the regions normally occupied by the primary rays. Occasional hyperplastic sieve-tube elements have companion cells, while parenchyma cells, commonly with degenerated protoplasts, appear on the periphery of the hyperplastic masses. The meristem cells from which the abnormal sieve tubes differentiate in the primary phloem show such numerous transverse and longitudinal divisions that the resulting cells are shorter than normal sieve-tube elements (plates 3, *A*, and 18, *B*). Because the abnormal sieve cells are small and appear in continuous masses, the sieve plates are strikingly abundant in the hyperplastic tissue (plate 18, *B*).

In the primary phloem the definitive callus (Esau, 1938*a*, 1939) is not common in the hyperplastic tissue, probably because the cells disintegrate before its formation.

The sieve plates of the abnormal sieve tubes are generally less distinct in the sugar beet than in tobacco. Artschwager and Starrett (1936) found no sieve plates in the hyperplastic cells of roots of curly-top sugar beets, concluded that the cells were intermediate between sieve tubes and phloem parenchyma, and named these cells "pseudosieve tubes." The same investigators found protuberances resembling callus on the walls of the abnormal cells (Artschwager and Starrett, 1936, plates 5 and 6) and called the structures "pseudocallus" because they were said to give negative tests with anilin blue.

Since the hyperplastic sieve tubes of tobacco showed only typical callus, a reëxamination of the so-called "pseudocallus" in the sugar beet seemed pertinent. Formations resembling those described by Artschwager and Starrett (1936) were abundant in prepared and fresh sections of diseased beet roots. In slides stained with hematoxylin and orange G, these structures were bright yellow; in fresh sections treated with I_2KI and anilin blue, they showed the typical sky-blue color of callus. In every instance they were associated with sieve plates, lateral or terminal. The substance was, undoubtedly, callose in definitive stage of development. Plate 18, *C* and *D*, shows definite callus in hyperplastic sieve cells of curly-top sugar-beet root in sectional views; plate 18, *E*, in face view. For comparison, plate 18, *F*, illustrates definitive callus on sieve plates from a root of a healthy beet plant. Because the callus overlaps the sieve plate (plate 18, *E*), sectional views may show only the callus and not the sieve plate to which it belongs (plate 18, *C*, below).

As in tobacco, definitive callus is not characteristic of the first-formed hyperplastic cells in the sugar beet (Esau, 1935*a*), but is common in later phloem. The callus is more abundant in diseased than in healthy roots, because of excessive numbers of sieve tubes in the latter.

The normal and hyperplastic sieve tubes of the sugar beet contain discoid plastids (plate 18, *D* and *F*). Previously, in the phloem of leaves, the plastids were found to stain brown with iodine (Esau, 1935*a*). In the present study the plastids, in healthy and diseased roots, had a reddish color upon treatment with iodine. This shows that the sieve-tube plastids of the sugar beet are similar to those of other plants (Esau, 1939).

The "pseudosieve tubes" and "pseudocallus" depicted by Artschwager and Starrett (1936) in their plates 5 and 6 might be sieve tubes and definitive callus. On the other hand, the structures appearing on one side of sieve plates in their plate 6, *A* and *B*, and described as callus of normal sieve tubes, resemble slime accumulations characteristic of killed sieve tubes. (Compare figure 12 in review by Esau, 1939.) In contrast to Artschwager and Starrett (1936, p. 641), other workers describe the normal callus not as a uniform deposit over one side of the sieve plate, but as a substance that first lines the pores of the sieve plate and then spreads over both surfaces of the plate, gradually constricting the connecting strands. (See review by Esau, 1939.)

To illustrate the progress of degeneration in successively older leaves in tobacco, one severely diseased plant was selected as an example. Fourteen leaves, the smallest 70 microns in height, appear in the transverse section at the level of the growing point. (For orientation regarding position and relative size of the first eleven leaves, figure 2 from the paper on phloem of healthy tobacco by Esau, 1938*a*, is here reproduced as figure 3.) Leaf 4 shows one sieve cell and one xylem element, both immature. One sieve tube in leaf 5 is mature and is surrounded by four parenchyma and one companion cell, two of the parenchyma cells showing the first signs of degeneration. Leaf 6 has two mature sieve tubes and two xylem elements. All cells associated with the first sieve tube, except the companion cell, show degeneration of cytoplasm and nuclei.

In leaf 7 four sieve tubes and one xylem element are mature. Near the first sieve tube, which is still intact and which has a normal companion cell, degeneration has progressed to primary hypertrophy in some cells, to primary necrosis and collapse in others. It has also commenced near two other mature sieve tubes.

In leaf 8 the first sieve tube and its companion cell have collapsed. According to previous observations (Esau, 1938*a*), in healthy plants obliteration of the first sieve tube occurs in the tenth to twelfth leaves. Leaf 8 has five intact normal sieve tubes, all associated with degenerating cells.

The procambial cells in centripetal direction from the first four sieve tubes have commenced hyperplastic divisions. The first cells resulting from these divisions have thick walls. In this leaf only one xylem element is mature.

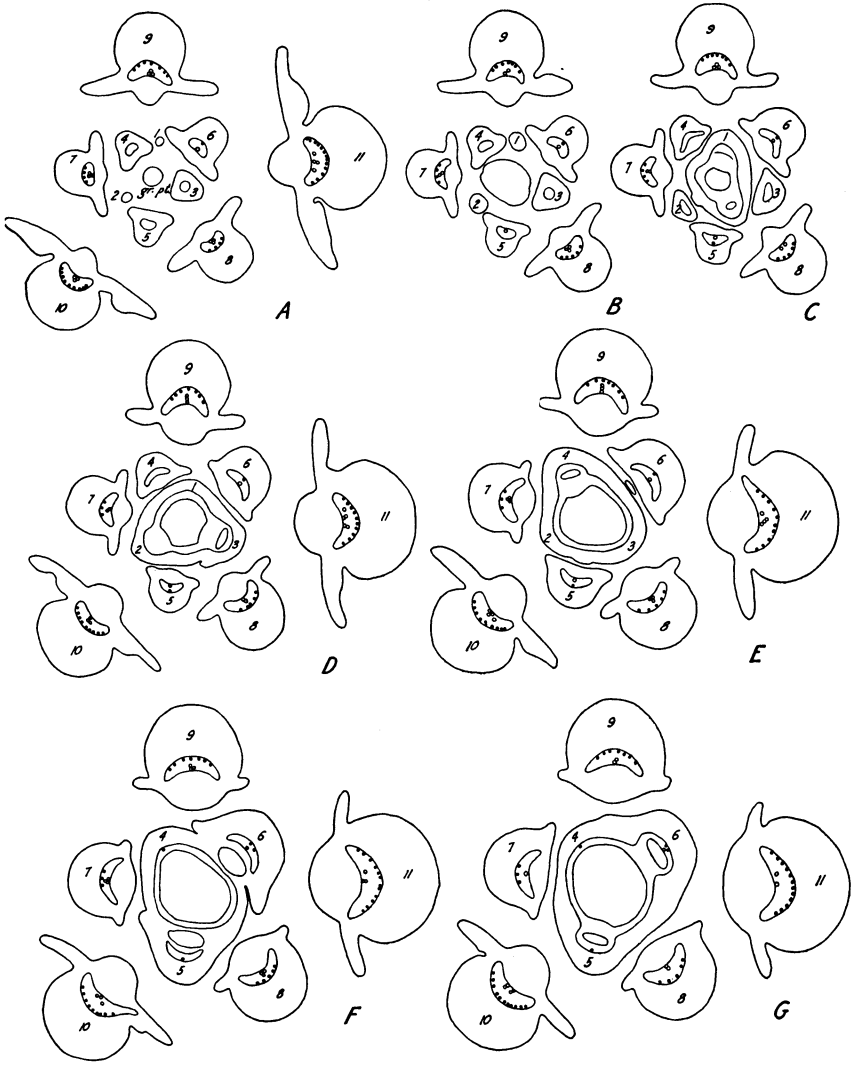

Fig. 3.—Successive transverse sections through apex of a three-months-old *Nicotiana Tabacum* plant, showing the stem and young leaves. *A*, Section through the growing point (*gr. pt.*). The succeeding sections were taken the following number of microns below the growing point: *B*, 40; *C*, 110; *D*, 190; *E*, 260; *F*, 300; G, 340. The areas outlined within the leaves and stem indicate the procambium regions. The sieve tubes are represented by dots; the xylem elements by circles. (From Hilgardia, vol. 11, no. 8.)

Leaf 9 shows remnants of the first sieve tube and six mature normal sieve tubes, all in contact with degenerating cells but with normal companion cells. In the median part of the bundle, some cells that underwent primary necrosis have lost their protoplasts, whereas others have collapsed. The hyperplastic tissue centripetally from the oldest normal sieve tubes shows thick walls and slime bodies in many cells. Some hypertrophied cells with thick walls appear among the hyperplastic cells. Two vessels are mature.

Leaf 10 contains about eight normal sieve tubes and companion cells, all on the periphery on the adaxial side of the bundle. Some hyperplastic cells deeper in the bundle have matured into sieve tubes. Healthy leaves at this stage of development have only peripheral sieve tubes. Four mature xylem elements occur in leaf 10. Divisions initiating the internal phloem have begun on the adaxial side of the procambium strand.

A healthy leaf corresponding in age to leaf 11 has about twelve sieve tubes, all on the outer periphery of the bundle (fig. 3). In the diseased plant considered here, leaf 11 shows about as many hyperplastic sieve tubes as plate 2, *A*. Occasional normal sieve tubes and companion cells are discernible on the abaxial periphery of the hyperplastic tissue. The oldest portion of the latter has begun to disintegrate (secondary necrosis). On the flanks of the bundle are three normal sieve tubes, associated with cells in early stages of degeneration. Three sieve-tube mother cells occur in the internal phloem. Twelve vessels have differentiated, two of these being already partly crushed. The meristem continues to produce hyperplastic cells toward the abaxial side.

In leaf 12 the mature hyperplastic tissue has largely disintegrated, together with the nearest normal sieve tubes. The view is comparable to that in plate 2, *B*. About ten normal sieve tubes and companion cells can be distinguished on the flanks of the bundle, most of these associated with cells in early stages of degeneration. Eight xylem elements and three sieve tubes of the internal phloem are mature. One of these sieve tubes is accompanied by cells beginning to degenerate.

In leaf 13 only two normal abaxial sieve tubes are discernible on one of the flanks of the bundle. Secondary necrosis has caused a large cavity on the abaxial side of the bundle. Right and left of the cavity, new hyperplastic cells have matured. The bundle contains eleven mature xylem elements. In the internal phloem are seven sieve tubes—six associated with cells showing early degenerative changes, one with primary necrosis.

Leaf 14 shows further progress of primary hyperplasia toward the flanks of the bundle, where four normal sieve tubes still occur. In the internal phloem, hyperplastic sieve tubes have matured. About eighteen xylem elements are present.

According to the previous studies (Esau, 1938*a*), the protophloem sieve tubes of tobacco differentiate acropetally in leaf trace and leaf; in other words, the lower portion of the sieve tube matures earlier than the upper. Probably because of this order of differentiation, the lower portion of the recently matured first sieve tube tends to be associated with cells showing more pronounced symptoms than the upper portion of the same structure.

If degeneration is studied in the leaf trace instead of in the leaf itself, the progress of development of the affected phloem may be followed after the vascular cambium begins to function.

The secondary phloem usually shows less necrosis and collapse than the primary, but develops pronounced hyperplasia. The supernumerary sieve tubes, however, appear not in continuous masses, as in the primary phloem, but interspersed with parenchyma cells. Some of the latter show typical degenerative changes; others resemble normal cells.

Although the cambium is composed, as in healthy plants, of ray and fusiform initials, the differentiating phloem does not reproduce the pattern of cell distribution of healthy phloem. Most striking is the differentiation of sieve tubes from ray as well as fusiform initials. (Compare figure 5 in the present paper with figure 12 in Esau, 1938*a*.) The resulting tissue, therefore, shows sieve tubes in every radial row of cells (fig. 4, *A*), whereas in the healthy plants certain radial rows, the rays, are free of sieve-tube elements (fig. 4, *B*). Moreover, the phloem mother cells of diseased stems undergo more numerous divisions and produce more and smaller cells than in healthy plants (fig. 4).

For convenience of description a distinction was made between normal and abnormal sieve tubes in the primary phloem, although fundamentally the two were alike. The first sieve tubes of the primary phloem, called "normal" in the preceding description, appear in the same order and position, and have the same shape as similar sieve tubes in healthy plants. The subsequent sieve tubes—the abnormal, or hyperplastic—differ strikingly from the normal elements in shape, size, distribution, and order of appearance. Whether the usual absence of companion cells in diseased phloem constitutes a fundamental difference between normal and abnormal sieve tubes is uncertain because the function of a companion cell is not understood.

A distinction between normal and abnormal sieve tubes is not practical in the secondary phloem. The secondary sieve-tube elements of curly-top plants range from those that appear normal in shape and size to those that are extremely short (fig. 5). Some have companion cells; others have none. The smallest sieve cells are those derived from ray initials (fig. 5, *a* and *b;* plate 18, *A*, cells marked *st*), although the fusi-

form mother cells may become much subdivided before they differentiate into sieve tubes. Frequently even a ray cell divides once or twice before forming sieve cells. In plate 18, *A*, for example, the three lower cells marked *st* are daughter cells of one ray initial.

The ontogeny and mature structure of ray sieve cells show that they essentially resemble sieve-tube elements derived from fusiform initials. When immature, the ray sieve cells contain slime bodies and nuclei

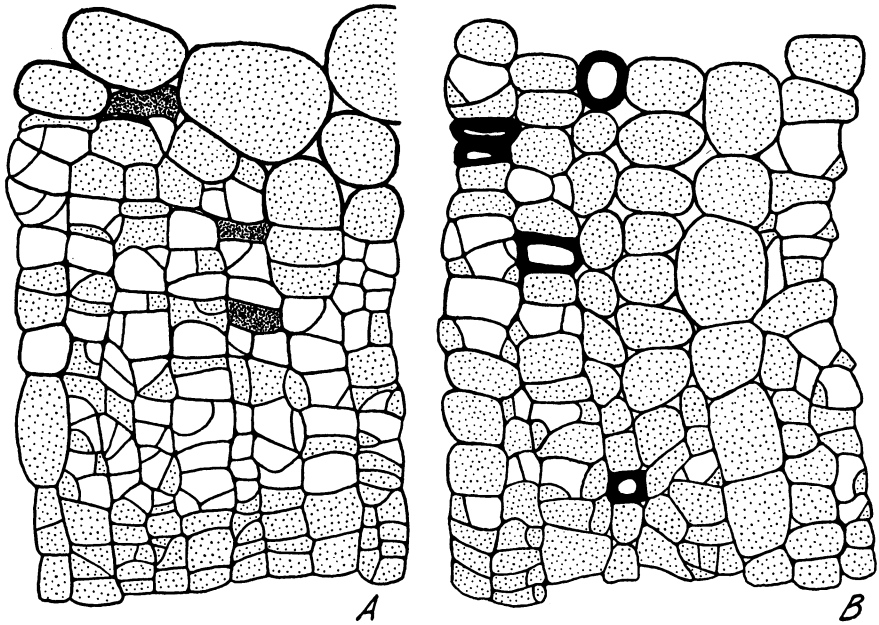

Fig. 4.—Transverse sections of secondary phloem from stems of a curly-top (*A*) and a healthy (*B*) tobacco plant. All cells except the mature sieve tubes and the fibers are stippled. The dense stippling in *A* indicates degenerating cells; the heavy walls in *B*, fibers. (Both × 325.)

(plate 18, *A*, cell marked *st*, at lower left); and they develop typical sieve plates (plate 18, *A*). If presence of companion cells should be used as one criterion of normality, some of the ray sieve cells could be called normal because they have companion cells (fig. 5, *b*).

In healthy plants, fibers appear in connection with the external and internal primary phloem on the adaxial and the abaxial sides of a vascular bundle. These fibers differentiate immediately outside and among the first sieve tubes after the latter become obliterated (Esau, 1938*a*). Since in curly-top plants, the cells associated with the first sieve tubes undergo the most severe degenerative changes, fibers are usually absent in the primary phloem of such plants. The cells that normally become fibers

either rapidly undergo necrosis or take part in hypertrophic and hyperplastic changes (plate 2, *A*, above). Sometimes fiber mother cells elongate; but instead of becoming multinucleate (Esau, 1938*b*), they form

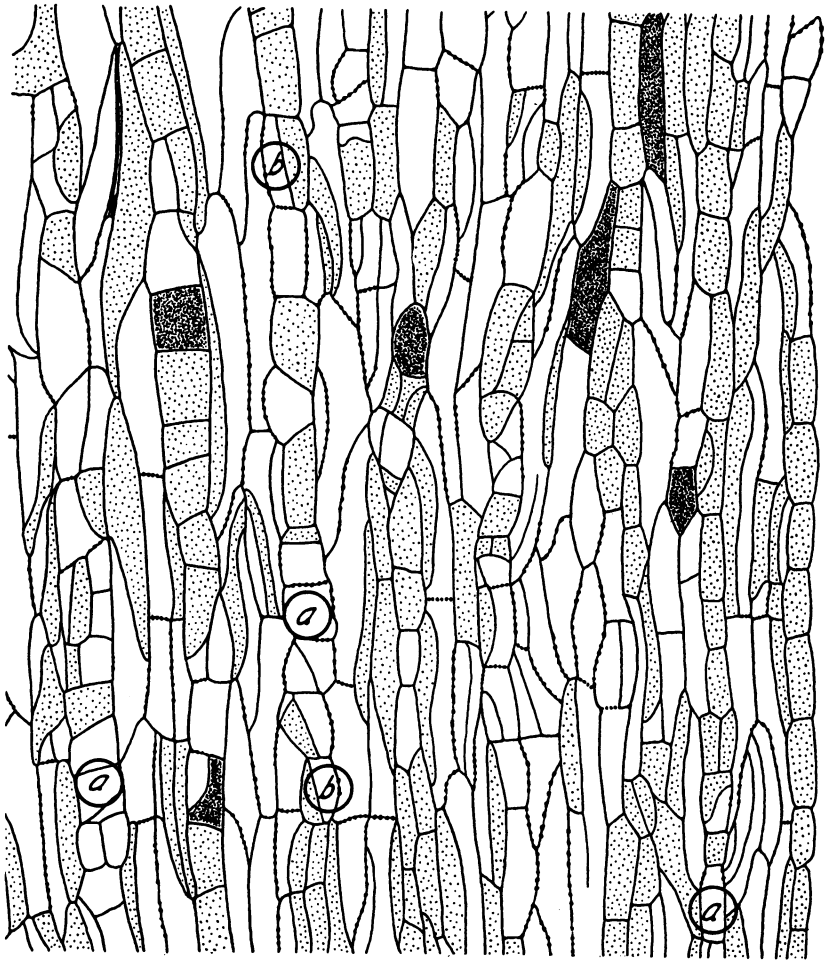

Fig. 5.—Tangential longitudinal section of secondary phloem from the stem of a curly-top tobacco plant. All cells except the mature sieve-tube elements are stippled. The heavy stippling indicates degenerating cells; the beaded lines show sieve plates. At *a* and *b* are sieve cells derived from ray cells, those at *b* having companion cells. (×200.)

cell walls following nuclear divisions and appear as longitudinal series of small cells. Fiber mother cells also may become multinucleate, with all nuclei, however, showing degenerative changes. Occasional fibers complete their development and appear, as individual cells, here and there around the periphery of the phloem.

Fibers normally formed in the secondary phloem (fig. 4, *B*, and Esau, 1938*a*) are usually absent in diseased plants (fig. 4, *A*).

Sometimes hyperplastic divisions spread in centripetal direction so rapidly that procambium cells destined to form xylem are affected and produce phloem. Consequently a phloem bridge between the internal and external phloem is formed in the internode (plate 13, *B*). Normally, the two phloem regions are connected only through the leaf gaps at the nodes (Esau, 1938*a*).

Another striking anomaly in curly-top plants is the occurrence of xylem on the adaxial side of the internal phloem (plate 13, *A*, at *x*).

EFFECT OF CURLY TOP UPON THE ROOT TIPS OF TOBACCO

Structure of Normal Root Tips.—The tips of lateral roots of two- to three-months-old tobacco plants show, at the apex, three tiers of initials: those of the stele, those of the cortex, and the common initials of the epidermis and the rootcap. Although the photographs in plate 6 were taken from a root tip of a curly-top diseased plant, they may be used to illustrate normal conditions: the cell arrangement in the region of initials is not affected by the disease. Plate 6, *B*, shows at *c* an initial cell of the cortex. Above this cell are the initials of the stele (*s*), which is bounded on the outside by the pericycle (*p*). Beneath the cortical initials, in the photograph, is the meristem giving rise to the rootcap and the epidermis. In plate 6, *A*, the same cortical initial as in plate 6, *B*, appears in the extreme right-hand corner.

By anticlinal divisions the common initials of the rootcap and the epidermis maintain their position at the apex of the meristematic cone and cut off the cells that cover the sides of the cone. By periclinal divisions at the apex these initials form successive layers of the rootcap (plate 6, *B*, below). At the margins of the common initial layer, the cells undergo final periclinal divisions (plate 6, *A*, arrow at right). The innermost daughter cell undergoes anticlinal and periclinal divisions (plate 6, *A*, arrows), the latter finally setting aside the epidermis as the cell layer next to the cortex. The outer cells are added to the rootcap.

The cortex arises from a single tier of initials that perpetuates itself at the apex of the root by anticlinal divisions. The same kind of divisions cut off, at the margins of the initial layer, cells which through subsequent periclinal and anticlinal divisions give rise to the cortex. The periclinal divisions follow one another and occur, almost invariably, in the layer next to the pericycle (plates 6 and 8, *A*). After the last periclinal division five or six layers of cells are formed, the innermost becoming the endodermis (plate 6, *A*, at *e*).

The stele also arises from one tier of initials which maintain their position at the apex through anticlinal divisions. At the margins of this layer, anticlinal divisions may add cells directly to the pericycle (plate 6, *B*, to the left from *s*) or may cut off a cell which, by dividing periclinally, contributes one cell to the pericycle, one cell to the layer subjacent to the pericycle (plate 6, *B*, to the right from *s*). In both cases the pericycle becomes a distinct layer very close to the region of initials (fig. 6 and plate 8, *A*). The inner portion of the stele is cut off from the initials by periclinal divisions.

TABLE 1

DIFFERENTIATION OF FIRST VASCULAR ELEMENTS IN ROOT TIPS OF TOBACCO

Kind of root	Distance in microns from apex		Number of protoxylem or protophloem poles
	To first sieve tube	To first xylem element	
Healthy	290	970	3
	260	580	2
	270	400	3
	470	Over 1,220	2
	460	Over 3,000	3
	410	About 2,500	2
Curly top, severe	420	1,009	4
	420	1,340	5
	360	970	4
	380	930	3
Curly top, recovery	380	About 3,000	3
Mosaic	340	1,750	2
	640	Over 6,000	2
	400	...	2

The stele may be diarch, triarch, tetrarch, or pentarch (table 1). As usual in roots (Esau, 1939, 1940), the protophloem sieve tubes are the first vascular elements to mature in the stele. They appear, however, at variable levels in different root tips (table 1).

Figure 6 illustrates the order of maturation of tissues in one of the root tips. In this example the xylem matured at more than twice the distance of the sieve tube from the initials. The endodermis, as a cell layer, becomes defined before the sieve tubes differentiate (plate 8, *B*), but develops Casparian strips slightly below the level where the first xylem elements mature. In figure 6 the endodermis is indicated only at the levels where Casparian strips are present.

Plate 7 shows at *st* a series of differentiating elements of a protophloem sieve tube, the youngest cell being located below. (This section, though

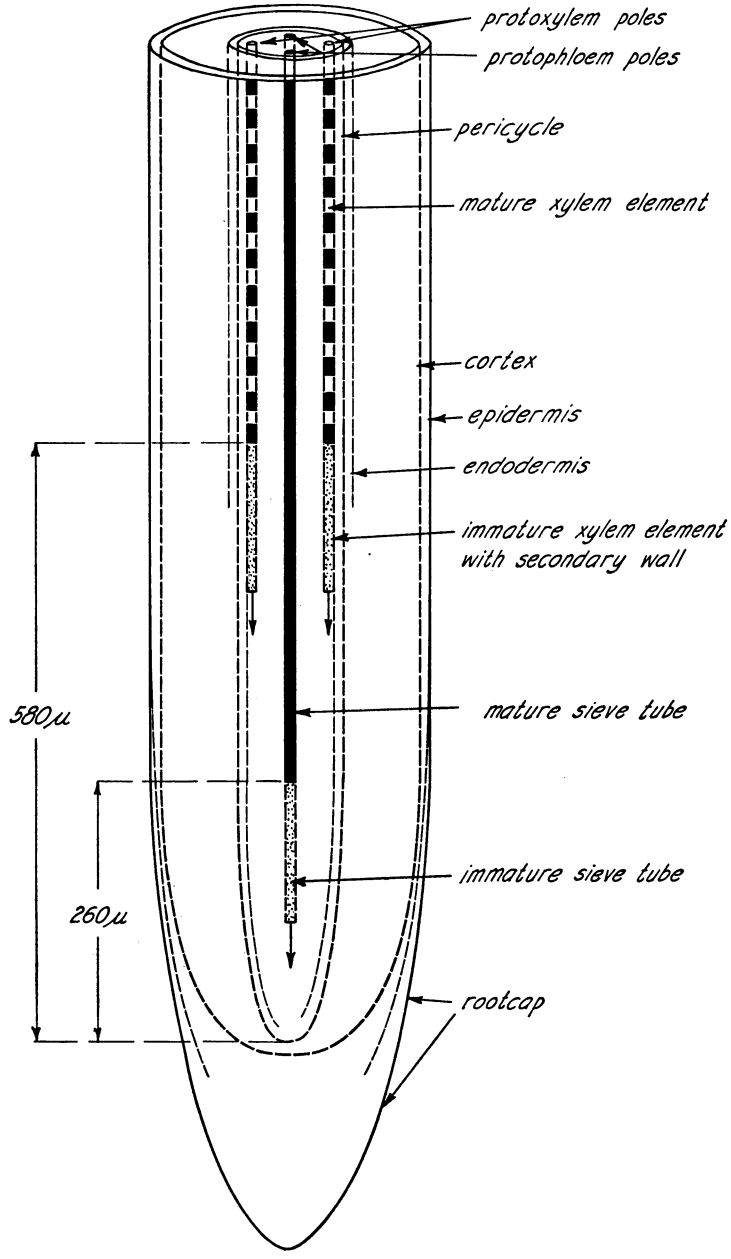

Fig. 6.—Diagram of a diarch tobacco root tip, showing spatial relations of different regions of the root and of the first vascular elements. (× 130.)

taken from a root tip of a mosaic plant, showed no abnormalities.) The youngest sieve-tube elements are very short, and their cytoplasm contains no large vacuoles. Counting from below in plate 7, elements 5 to 8 inclusive are considerably vacuolated, and their cytoplasm is somewhat contracted—an artifact that was caused, no doubt, by treatment in preparation of material. These cells also had begun to elongate. Cells 7 and 8 from below have remnants of nuclei, not easily discernible in the photograph. The nucleus is, however, clearly outlined in cell 9. It is vacuolated, and its chromatin appears in clumps—it is disintegrating in a manner characteristic of nuclei of normally differentiating sieve tubes. The cytoplasm of element 9 is less contracted than that of the younger cells.

Shortly before maturation of the sieve cells, their cytoplasm contains, in fixed material, more or less coarse strands. Plate 7 does not bring out this phenomenon.

Cell 10, the first clear cell from below, is the first mature element in the series. Devoid of nucleus, it has scant cytoplasm. The subsequent morphological changes of sieve-tube elements involve elongation, thinning down of walls, narrowing of lumen, and, finally, obliteration.

The first sieve cells elongate after maturation much more than before. The elements in plate 7, beginning with the fifth from below, measured the following number of microns in length: cells 5 to 9 (still containing cytoplasm), 16.7, 16.7, 23.3, 28.6, 23.2; cells 10 to 12 (clear cells), 23.4, 35.2, 50.0. Several additional mature elements were available for measurement above the view in the photograph and in adjacent sections. These were cells 13, 14, 15, 16, 18, and 20 of the same series as in plate 7 and measured, respectively, 40.2, 58.0, 60.0, 108.2, and over 148.0 microns in length. In another root tip, elements were measured up to 246 microns in length; and these had not yet completed their elongation. The longer the elements, the more difficult it is to see both end walls in the same section. The available measurements indicate a more-than-tenfold increase in length of the element after maturation.

Since the walls bearing sieve plates in the protophloem sieve tubes are very thin, their details cannot be well represented by photography. The sieve plates become evident shortly before the element clears and have connecting strands surrounded by translucent areas—the callus cylinders. The translucent spots in face views of the plates are the most readily discernible identifying structures. The terminal walls bearing sieve plates may be transverse or inclined.

The thickened nacré walls of sieve cells (see review by Esau, 1939) appear before these cells are mature. During further elongation the walls again become thin. In the dehydrated material, especially after fixa-

tion with the chrom-acetic-formalin solution, the nacré layer is not pronounced but stains somewhat deeper than other walls.

The appearance of the root tip used in figure 6 in successive transverse sections may serve to outline the ontogeny of the primary root. Plates 8, 9, and 11, *B*, will be employed to illustrate certain details, although the photographs were taken from two root tips other than that in figure 6. The numerical values used in this outline should be regarded as an example of spatial relations, but not as an average condition. (See table 1.)

The stele is outlined immediately above the initial region, with the pericycle as a continuous uniseriate layer around it. Some 30 microns above the initials, the central cells of the stele—the future innermost xylem elements—are larger and show more pronounced vacuolation than the surrounding stelar cells. The arrangement of the vaculoating cells indicates, at this early stage of root development, the number of protoxylem poles that the root will have later. The root in plate 8, *A*, for example, would be diarch. The central cell, prominently vacuolated, is surrounded by somewhat less vacuolated cells. Two of these, right and left of the central cell, form with the central cell a series of three disposed on the long diameter of the future diarch xylem plate.

Cell divisions are not equally numerous in different parts of the stele; they are few in the vacuolating center but abundant in the peripheral portion, particularly in the regions of the future protophloem poles. Plate 8, *A*, shows recently divided cells at *ph*. Divisions in the pericycle are only anticlinal; they occur in all planes in other parts of the stele.

The cortex in plate 8, *A*, had not yet completed the periclinal divisions in the innermost layer.

Some 160 to 200 microns from the apical initials, the phloem areas become defined through the continued vacuolation of the xylem region (plate 8, *B*). The sieve-tube mother cells, containing normal vacuolating cytoplasm and nuclei (cut through the nuclei in plate 8, *B*), are also evident. The cortex has completed its periclinal divisions and shows intercellular spaces. The future endodermis forms a continuous layer around the pericycle (plate 8, *B*) but has no Casparian strips. Some of the innermost cortical cells may lag behind the others in completing the periclinal divisions or may omit the last division entirely. In plate 9, *A*, at *e* two cortical cells had recently completed the last periclinal division, while other cells in similar position had already rounded off. In plate 9, *B*, at *e* two cortical cells have omitted one division.

At the 160-to-200-micron level, the cell layer common to the epidermis and rootcap is completing its periclinal divisions setting apart the two regions.

In the root tip selected for this outline, the sieve-tube mother cells showed nacré walls about 200 microns from the apex and matured at the 260-to-270-micron level. Usually the different protophloem sieve tubes of the same root mature at slightly different levels. Both protophloem sieve tubes of the diarch root in plate 9, *A*, are nearly mature, with somewhat shrunken protoplasts; the element to the right shows the deeply stained nacré wall. In plate 9, *B*, the two protophloem sieve tubes are mature.

The stele increases in diameter mostly before the sieve-tube mother cells are defined (plate 8). Longitudinal cell divisions and vacuolation of protoplasts account for this increase. Later, longitudinal divisions slow down, and the stele shows little increase in diameter (plates 8, *B*, to 9, *B*) until secondary growth commences.

The enlargement of the stele, together with the addition of the innermost cortical cells, causes root circumference to increase. Tangential enlargement of cells and occasional anticlinal longitudinal divisions accommodate the outer cortex to the increasing circumference. Recent anticlinal longitudinal divisions in the outer cortex are evident to the right in plate 8, *B*. Similar divisions occasionally take place in the innermost cortical layer (plate 8, *B*, at *e*; plate 9, *A*, at e^1).

About 480 microns from the apical initials, the xylem elements show secondary walls. Somewhat farther, Casparian strips develop in the endodermis. The xylem elements are mature 580 microns from the apex.

The second sieve tube and the second xylem element at each protophloem and protoxylem pole, respectively, differentiate centripetally from the first elements about three times as far from the apex as the first sieve tubes. Plate 11, *B*, shows the arrangement of the vascular elements at this stage of development in a triarch root. At points marked by *st* are the three first sieve tubes indicating the protophloem poles. Toward the center from each of these elements is another sieve tube (two more at the pole to the right), with deeply stained nacré walls and clear lumen. The new sieve tubes are smaller in diameter than the first elements. At points marked by *x* are the three pairs of xylem elements.

Between the position where the first sieve tubes mature (plate 9, *B*) and the one depicted in plate 11, *B*, the root rapidly elongates—partly by cell division, partly by cell elongation. The epidermal, the cortical, and the pericyclic cells divide transversely and elongate; the stelar cells, other than those of the pericycle, mostly elongate, and thus become longer than the cortical and pericycle cells (plate 5, *A*). They are narrow because they divide longitudinally without increasing much in diameter between divisions. The elongation of the mature sieve-tube elements, considered in connection with plate 7, occurs in this part of the root.

As was pointed out in another paper (Esau, 1940), the rapidly elongating and vacuolating portion of the root suffers considerable distortion with ordinary methods of preparation of paraffin material (plate 11, *B*). Whether defective fixation alone accounts for the comparatively small size of the stele in this region (compare plates 9, *B*, and 11, *B*) has not been determined.

Whereas the first sieve tubes elongate mostly after maturation, apparently the subsequent primary sieve tubes increase in length almost entirely during differentiation. Because of this ontogenetic difference, the first sieve tubes are here referred to as the protophloem sieve tubes, the later elements as the metaphloem sieve tubes.

The sieve plates of metaphloem sieve tubes occur on lateral and on the usually somewhat inclined end walls. Companion cells are not commonly associated with the protophloem sieve tubes, but usually occur in the metaphloem. Plastids, though apparently occurring in all sieve tubes, stain very faintly in paraffin material. Slime bodies have been observed in the metaphloem but not in the protophloem sieve tubes.

The root tips sampled for this study were about 1 centimeter long and did not carry the primary development to completion.

Development of Root Tips of Diseased Plants.—The architecture of the meristematic apices of roots from severely diseased plants showed no deviations from that of healthy roots. The arrangement of cells in cortex and stele, before sieve tubes differentiated in the latter, was likewise entirely similar to normal. The photographs in plate 6 were taken from a root tip that showed severe symptoms of curly top beginning about 350 microns from the apex.

Although cell arrangement in the meristematic apex was not disturbed by the disease, the cytoplasm tended to be somewhat denser, less vacuolated, and, therefore, more homogeneous than in the corresponding portion of a healthy root. Plates 9, *A* (healthy), and 10, *A* (diseased), were photographed under the same set of conditions, from slides prepared in similar manner; but the healthy root tip gave a "contrasty" negative, the diseased a flat one. Although soft paper was used in plate 9, *A*, and hard paper in plate 10, *A*, the latter developed into a less "contrasty" picture than the former.

As in healthy roots, sieve tubes mature first in the stele of diseased roots, at variable distances from the apex (table 1). As in the shoot, the maturation of the sieve tubes is accompanied by degeneration of the adjacent pericycle and phloem cells (plate 10, *B*). The first degenerative changes may appear in connection with sieve-tube elements that have not yet cleared but that contain highly vacuolated disorganizing nuclei and stringy cytoplasm. The differentiating sieve tube sometimes degenerates

also (plate 10, *B*, above) ; one to several elements preceding the first clear cells may show deeply stained degenerated cytoplasm. Sometimes such elements collapse. Mature sieve tubes may be clear for a distance, then filled with degenerated material for another limited distance. If the differentiating sieve tubes show no degenerated cytoplasm, they pass through developmental stages similar to those of the protophloem sieve tubes in healthy roots.

The protoplasm of cells surrounding the first sieve tubes shows the typical degenerative changes preceding primary necrosis. Hypertrophy of entire cells may be inconspicuous, but nuclei usually enlarge. The first degenerative changes are quickly followed by more or less active cell division in the procambium and pericycle (primary hyperplasia) and by a differentiation of most of the resulting cells into sieve-tube elements. Some of these supernumerary sieve tubes occur in the normal position for metaphloem sieve tubes. Others appear near the protoxylem poles, in the pericycle, and (in most severely affected roots) even in the region where xylem normally develops. Occasionally, abnormal sieve-tube elements occur in the endodermis.

The differentiation of the hyperplastic sieve tubes precedes the development of xylem and continues after the xylem elements mature. Frequently sieve tubes are in contact with xylem elements. In plate 3, *B*, a double series of sieve cells that originated in the pericycle (*p*) are located next to protoxylem elements (*x*). In the tetrarch root in plate 11, *A*, where all the clear cells without secondary walls are sieve tubes, several of these elements are in contact with xylem elements of the lower left-hand xylem pole. The upper left-hand group of xylem cells is isolated from other xylem cells by sieve tubes and is surrounded by hyperplastically dividing cells.

The endodermis has more or less dense cytoplasm (plate 5, *B* and *C*; plate 11, *A*) and, when severely affected, shows hypertrophy of entire cells, or of nuclei alone, together with inclusions. The Casparian strips appear here and there in cells that seem more nearly normal; frequently they are limited to a few cells opposite the protoxylem points.

The sieve cells in diseased root tips vary in shape. Some, long and narrow, resemble normal elements; others, the majority, are short and wide (plate 3, *B*, and 5, *B* and *C*). Companion cells are infrequent. Plate 3, *B*, shows one—the small, dense cell with nucleus next to xylem.

When certain cells at the protophloem poles undergo primary necrosis, the nearest living cells of pericycle or cortex enlarge (secondary hypertrophy) and appear to compress the dead cells. Sometimes the enlarging cells form protrusions resembling tyloses (plate 3, *C*). The enlarged cells may differentiate into sieve-tube elements.

The root tips from diseased plants were studied to completion of primary growth. Up to this stage secondary necrosis, involving disintegration of hyperplastic cells, was about as inconspicuous as in plate 11, *A*. In the latter, the large structure with the faintly outlined nucleus (*n*) at the top of the photograph resulted from fusion of several pericyclic cells and some abnormal sieve tubes. Later, presumably, the structure would become a cavity; but at this stage it still had cytoplasm and several disintegrating nuclei.

The structure of successive transverse sections of the tetrarch root used in plates 10 and 11, *A*, may serve to outline the sequence of ontogenetic changes at different levels of a diseased root. The cell arrangement and order of cell division up to the region of differentiating protophloem sieve tubes is the same as in healthy roots and need not be repeated.

The four sieve-tube mother cells are vacuolating 150 microns from the apex; they show nacré walls and stringy cytoplasm at the 250-micron level (plate 10, *A*). One of the differentiating sieve tubes is associated with degenerating cells 340 microns from the apex and matures at 380 microns. Two other sieve tubes are clear at 360 microns, and both are surrounded by degenerating cells. Beginning with the 450-micron level, the fourth sieve tube shows degenerated cytoplasm and is surrounded by degenerating cells (plate 10, *B*, above). This sieve tube is collapsed 560 microns from the apex.

In the meantime periclinal divisions have commenced near one, later at another, of the protoxylem poles. These divisions are not associated with secondary-root formation or with cambial activity, because the root is too young for such processes. These are hyperplastic divisions. Plate 10, *B*, shows periclinal walls in the pericycle near the two letters *p*.

About 560 microns from the apex, sieve tubes begin to differeniate centripetally and laterally from the three sieve tubes that had matured normally. Approximately seventeen of these supernumerary sieve tubes are mature 630 microns from the apex. At this level the fourth sieve tube is still collapsed; but 60 to 80 microns further it is open, and new sieve tubes are differentiating near it. In the meantime another of the first four sieve tubes collapses, together with the adjacent cells (primary necrosis).

The xylem elements begin to develop secondary walls 690 microns from the apex and mature at the 970-micron level, while about forty-four sieve tubes develop in the phloem. In healthy roots the number of sieve tubes and xylem elements is about equal in early stages of xylem development. The healthy triarch root shown in plate 11, *B*, has six xylem elements and seven sieve tubes. In the somewhat older tetrarch root from a diseased plant in plate 11, *A*, there are eleven xylem elements and about seventy-five sieve cells.

The elongation of cells after the first sieve tubes mature is, in the diseased roots, less pronounced than in the healthy. Hyperplastic cell divisions produce numerous small cells where few elongated cells normally occur. Not only the phloem elements are relatively short, however, but also the cells of xylem, cortex, and pericycle. Plate 5 illustrates the differences in cell size and shape between healthy and diseased root tips in the region of elongation.

The abnormal manner of growth of diseased root tips apparently affects the reaction to treatment during preparation of material for slides. Whereas the elongating part of a healthy root shows, as a rule, considerable distortion, the diseased root of similar stage of development gives a very good preparation. The cells of diseased roots have comparatively thick walls—probably because of low rate of elongation—and cytoplasm denser than normal. These characteristics perhaps account for the resistance of the diseased material to the adverse effects of treatment in slide making.

THE STRUCTURE OF PHLOEM IN TOBACCO RECOVERING FROM CURLY TOP

Tobacco plants recovering from curly top were sampled at different stages of recovery. Three of these plants (plates 14–17) were inoculated January 25, when they had four to six foliage leaves. They developed the first systemic symptoms between February 4 and 7 and, in the middle of March, were severely stunted by the disease.

Plant 1 was only 6.5 cm high on March 13, whereas the check plant measured 70 cm (plate 14). About March 15 the diseased plant showed a flower bud, which by April 11 developed into a 4-cm-long flower (plate 15, *A*). The plant itself measured 8 cm in height to the flower. The corresponding check had reached a height of 120 cm from base to the inflorescence and bore a flowering panicle 20 cm high, with numerous 5-cm-long flowers.

The flower of plant 1 (plate 15, *A*), sampled April 12, though healthy in outward appearance, showed mild internal symptoms of curly top in all parts examined—calyx, corolla, stamens, and style. The last-mentioned structure had the most obvious symptoms—primary hypertrophy and hyperplasia and some primary necrosis. The other floral parts showed no hyperplasia, only some nuclear degenerati··

On May 19 axillary shoots lacking external symptoms began to develop on plant 1. On May 29 the old shoot was 11.5 cm high up to the point where the flower was previously removed (at *a* in plate 15, *B*), whereas the new shoots were 16 cm (shoot *b* in plate 15, *B*) and 14 cm (shoot *c* in plate 15, *B*) tall. On May 29 various parts of this plant were sampled for

microscopic study. The old shoot, sectioned in the internodes below the insertion of the recovered shoots, displayed degeneration of the old phloem. There were secondary hyperplasia and necrosis; accumulations of deeply stained material, particularly in the internal phloem; connections between the external and the internal phloem; and partial suppression of fiber development on the periphery of the primary phloem. The newly developing phloem, however, seemed normal. The apical portion of the stem, beneath the pedicel, was weakly developed and appeared free of symptoms. As was mentioned previously, the flower that came off this shoot showed some phloem degeneration.

Although shoots *b* and *c* (plate 15, *B*) were free of external symptoms, they exhibited mild, but unmistakable, degenerative changes in the phloem. These shoots were sampled at their bases and apices and had internal symptoms in both regions. At the bases some hyperplasia was present, particularly in the most external phloem; but the development of the primary-phloem fibers was little affected. The internal phloem was apparently normal.

Sections through the apices that included leaves in different stages of development showed nuclear degeneration of cells associated with mature sieve tubes. Some primary necrosis and hyperplasia occurred in young leaves. In the older leaves of shoot *b*, the first few degenerated cells were obliterated, while the new phloem was seemingly normal. In shoot *c*, phloem collapse in older leaves was more marked than in shoot *b*; some cavities were also present.

The internal phloem, which developed later than the external (Esau, 1938*a*), showed only the earlier stages of degeneration in the oldest leaves of the series.

The symptoms were not equally severe at different levels of a given leaf or leaf trace. The identification of symptoms was at times very difficult, particularly in the more mature portions of vascular bundles where the first degenerated cells had, presumably, been obliterated. Sometimes the presence of disease was revealed only by the occurrence of a few supernumerary sieve tubes that disturbed the normal cell pattern. The diagnosis was more certain when the hyperplastic sieve tubes appeared on the abaxial side of a strand instead of in the normal position of the centripetal phloem. In plate 4, *A*, depicting a section from young stem region of shoot *b* (plate 15, *B*), a hyperplastic strand appears in the cortex and is separated from the rest of the phloem by cells which hypertrophied after certain other cells collapsed. The hyperplastic strand was about 200 microns long and had no connection with the vascular bundle.

Plant 2 (plate 16, *A*) was first sampled on February 21, when it showed severe external symptoms of curly top. This was one of the plants

used, in an earlier part of the paper, to describe phloem degeneration in severely affected leaves and stems. At sampling, the shoot apex was removed; but one of the axillary buds quickly produced a new shoot. On April 11 (plate 16, *A*) the younger leaves on this shoot were free of symptoms, while the older leaves showed mild symptoms. In a sample of the midvein of one of the older leaves (leaf *b* in plate 16, *A*) the degenerated portion of the phloem had collapsed, but the new phloem appeared normal. Samples of midveins of the younger leaves (*d* and *c* in plate 16, *A*) revealed no phloem abnormalities. The leaves on the old shoot—the portion that remained after the first sampling—were still severely affected. A young leaf from this shoot (leaf *a* in plate 16, *A*) manifested typical severe phloem degeneration that had reached the stage of secondary necrosis.

On April 25 the shoot that had recovered and grown to 24 cm in height was reinoculated by means of ten infective leafhoppers placed on the youngest leaves. No external symptoms developed; the plant continued to grow. On May 29 (plate 17, *B*) it was in full bloom, and the stem was 76 cm high up to the inflorescence. The old shoot, from which the apex had previously been removed, was present at the base of the flowering shoot. It showed, in sections, that primary hyperplasia had occurred in the primary and in the first secondary phloem. The hyperplastic tissue had largely collapsed (plate 1, *B*), whereas the new secondary phloem appeared normal. Primary-phloem fibers occurred only occasionally. Sections of stem at the base of the flowering shoot displayed a normal complement of fibers, had no excessive amount of collapsed tissue, and showed no abnormalities in the secondary phloem (plate 1, *A*). In the upper part of the flowering stem, the phloem was still primary, with some hypertrophied nuclei and hyperplastic areas. These degenerative changes were rather inconspicuous and scattered. The abnormality of the tissue was revealed mainly by the presence of sieve tubes in the rays and by the shortness and deformity of sieve-tube elements as seen in longitudinal sections. Figure 7 compares two phloem sections taken close to each other from the same stem, one (fig. 7, *A*) normal in appearance, the other (fig. 7, *B*) with excessive numbers of sieve tubes, some of these derived from the large primary-ray cells. The fibers are large in the section of the normal phloem, small in the degenerated phloem. The functioning phloem of the taproot of plant 2 manifested no degenerative changes. Whether the old collapsed phloem had previously degenerated was uncertain.

In plants 1 and 2, recovery was studied in axillary shoots that developed after the apices of the main shoots were removed. Plant 3, however, was left to recover undisturbed. The main shoot gradually lost symptoms

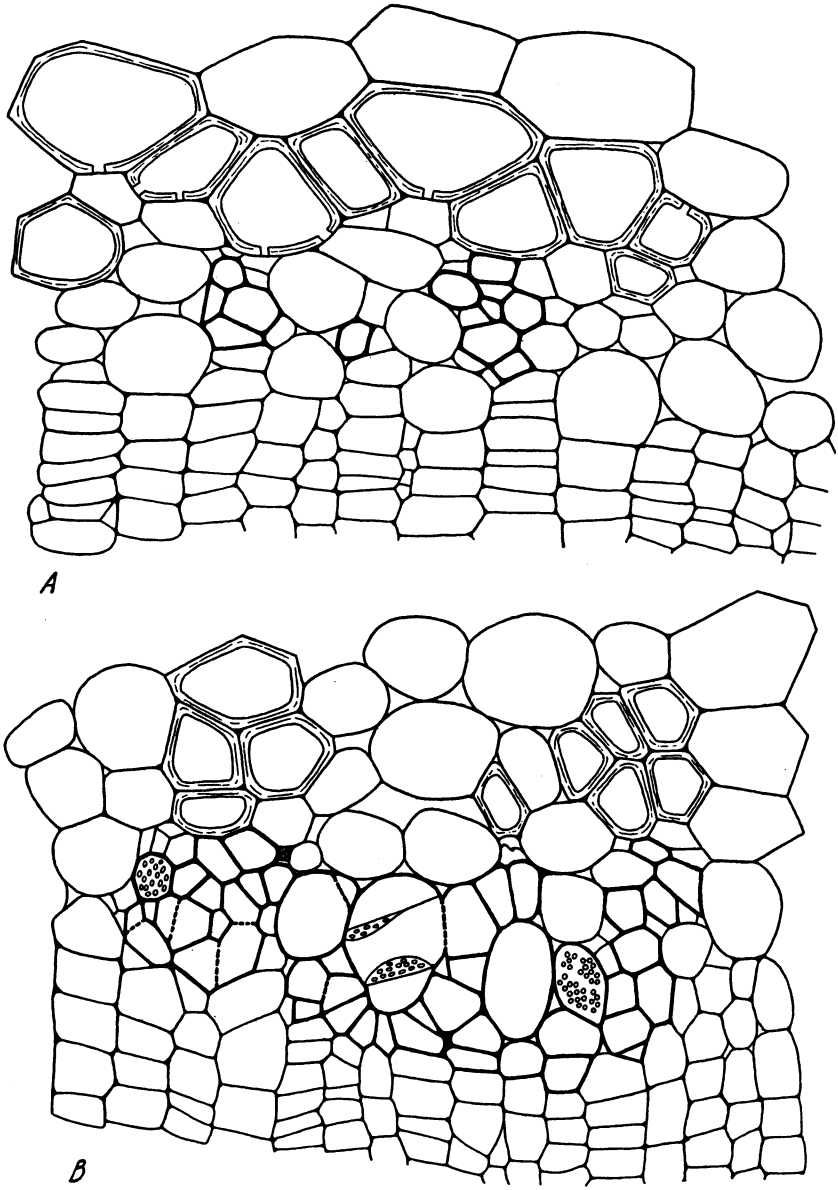

Fig. 7.—Transverse sections of phloem of stem below inflorescence of tobacco plant 2 (plate 17, *B*), which recovered from curly top; *A* is without and *B* with hyperplasia. In both sections the thick-walled fibers appear at the top, and the sieve tubes have rather thick solid-black walls. In *B*, some sieve plates are visible in face views (areas covered with circles), others in sections (broken lines). (Both × 358.)

and produced flowers. On April 11 (plate 17, *A*) the stem from base to inflorescence was 27 cm high; the lowest leaves exhibited distinct external symptoms, the median were only mildly affected, and the uppermost were free of external symptoms. Sections of the principal vein of leaf *a* in plate 17, *A*, had large cavities in the external and internal phloem; in leaf *b* the cavities were small. No abnormalities occurred in a midvein sample of leaf *c*. A flower sampled on the same day showed some primary hypertrophy and hyperplasia in the pedicel, corolla, stamens, and style.

On May 29 three axillary shoots with mild symptoms were developing on the upper part of plant 3.

Two more plants recovering from curly top were examined. These, about two months old when inoculated, showed recovery of terminal shoots on the day of sampling (one month after inoculation). Serial sections through the shoot apices and the young leaves revealed milder symptoms than in similar parts of plant 1 (plate 15, *B*). Occasionally, nuclear abnormalities alone or primary hypertrophy was recorded. Certain portions of the phloem showed abnormal distribution and excess of sieve tubes; others resembled phloem of healthy leaves. As previously noted in other plants, the hyperplastic sieve tubes occurred either within the phloem or abaxially on the periphery. In plate 4, *B*, a hyperplastic strand (*st*) appears on the outer limit of a vascular bundle. In plate 4, *C*, taken 40 microns lower than 4, *B*, the hyperplastic strand is absent; but the normal protophloem sieve tube, near which the hyperplastic strand arose, is evident at *st*. Plate 4, *C*, also shows some hyperplasia to the right in the phloem proper.

Root tips also were collected from one of the two plants described above. Some of these were apparently free of symptoms; others showed mild phloem degeneration.

When the various observations on tobacco plants recovering from curly top are combined, the following general picture results. Phloem degeneration is commonly present in plant organs that show recovery, but it is mild and of unequal severity in different parts of the same organ. The more pronounced the external symptoms, the more severe the phloem degeneration; but organs showing phloem abnormalities may be free of external symptoms.

Phloem degeneration in recovering organs follows the same course as in severely diseased plants; but it progresses more slowly, fewer cells become involved, and the amount of hyperplastic tissue is smaller. Because the number of degenerating cells is comparatively small, collapse and necrosis are not nearly so prominent as in severely affected plant parts.

As the phloem develops in a recovering organ, this tissue itself shows recovery. The first phloem may show unmistakable symptoms, yet the subsequent may become more and more like the phloem of healthy organs. Since, in a given organ, the internal phloem differentiates later than the external, it is also late in developing symptoms. It may never reach the same degree of degeneration as the external phloem before recovery sets in.

The remarkable phenomenon of increased production of sieve tubes in diseased phloem is a very characteristic symptom of curly top even when the disease is most mild.

PHLOEM DEGENERATION IN TOMATO AFFECTED WITH CURLY TOP

For the sake of completeness, the other common solanaceous host of curly top, *Lycopersicon esculentum,* was included in this study. Several plants of this species were inoculated with the curly-top virus. One of the diseased tomato plants is shown to the right in plate 16, *B,* with the healthy check plant to the left. The diseased plant was inoculated on February 14, when it had two cotyledons and two foliage leaves. On March 13 the cotyledons were still alive, and five leaves in total had developed, the fifth being 4 cm long. The entire plant was 9 cm high. The youngest leaf had faintly translucent veins; the larger ones were purple on the abaxial side and were slightly curled toward the adaxial side.

The healthy check plant was 21 cm high on March 13 and had two cotyledons and nine leaves, the youngest being 6.5 cm long.

On April 14 the diseased plant was sampled, together with its control plant. Other plants were inoculated and sampled at earlier stages of development.

Tobacco and tomato resemble each other in phloem anatomy, including the details of sieve-tube structure. Phloem degeneration in curly-top tomato follows essentially the same course as in the beet and the tobacco.

Some normal sieve tubes mature before degeneration sets in. In the taproot and hypocotyl of a plant having only two foliage leaves on the day of sampling, severe hyperplasia occurred in the stele, with abnormal sieve tubes developing in the pericycle and endodermis. Particularly striking were the longitudinal views of the masses of hyperplastic sieve cells, each with a sinuous slime body. Occasionally a slime body and an inclusion body occurred in the same cell. In the plants examined, cell divisions (secondary hyperplasia) took place after the collapse of the primary hyperplastic tissue, so that no cavities were formed.

The two sections of tomato stem in plate 12 were taken just above the cotyledons from the two plants in plate 16, *B*—plate 12, *A,* from the

healthy, plate 12, *B*, from the diseased plant. The latter shows hypoplastic xylem and small-celled hyperplastic external phloem, with the sieve-tube groups continuous across the rays. Secondary hyperplasia occurred above the collapsed part of the external phloem at *hp* in plate 12, *B*. These cells are in the position normally occupied by primary phloem fibers (*fb* in plate 12, *A*).

Because of hyperplasia the internal phloem (*iph* in plate 12) is abnormally massive in the diseased as compared with the healthy stem. The strands of internal phloem in plate 12, *B*, are surrounded by hyperplastically dividing cells that form radial rows like cambium cells. On the abaxial side of the internal phloem, some of these groups of radially arranged cells have differentiated into hyperplastic sieve-tube strands.

EFFECTS OF TOBACCO MOSAIC ON THE PHLOEM OF TOBACCO

The virus of curly top is largely localized in the phloem, its movement in the host is clearly related to food transport, and it induces highly characteristic changes in the phloem. The causal agent of tobacco mosaic is not localized in any particular tissue; can move, apparently, through the living cells of any tissue; and, as far as is known, does not affect the phloem of the host in any specific manner. The rapid transport through the plant of the nonlocalized viruses, particularly those of the tobacco mosaic, is now known, however, to be definitely related to food movement (Bennett, 1939; also reviews by Esau, 1938*c*, and Crafts, 1939). Thus the translocation of the tobacco-mosaic virus fundamentally resembles that of the localized virus of curly top. This similarity makes it particularly interesting to compare the phloem of tobacco affected by curly top and by tobacco mosaic.

Johnson's tobacco mosaic strain number 1[8] was used in this study. The plants were inoculated on July 13, developed systemic symptoms on July 16, and were sampled on July 28 when they were approximately one month old and had seven leaves with mosaic symptoms.

With Goldstein's (1926) work and review of literature as the principal basis for comparison, the mosaic material was examined for the typical histologic and cytologic symptoms of tobacco mosaic. The green leaf areas had palisade and spongy parenchyma; the yellow ones showed undifferentiated mesophyll and lacked intercellular spaces. The chloroplasts were few in the yellow mesophyll, many appearing swollen and partly disintegrated. In the most severely affected areas, nuclei were distinctly hypertrophied. The typical inclusions, the *x*-bodies (or ameboid

[8] The virus supply was received through the courtesy of Dr. W. N. Takahashi of the Division of Plant Pathology.

bodies), and the striated material were, of course, present and showed the usual uneven distribution: in some areas they were abundant; in others scarce or absent. Some mesophyll cells were hypertrophied, with their contents, including the *x*-bodies and striated material, in various stages of disintegration. Some of the hypertrophied cells were empty or contained fine, hairlike material.

Goldstein (1926) completely surveyed the occurrence of inclusions in different tissues and found them in cells of all types and ages, including sieve tubes and xylem elements. In the latter, however, she noticed only striated material.

In the present study, inclusions were found in all types of cells of the vascular elements. Vessels and tracheids contain both *x*-bodies and striated material during differentiation while the protoplasts are still intact. Mature xylem elements seem to be free of inclusions. Striated material and *x*-bodies are very common in undifferentiated phloem cells. In severely diseased areas, immature sieve-tube elements containing striated material, slime bodies, and nuclei occur frequently. The *x*-bodies seem less common in these elements. No mature sieve tubes have been found to contain inclusions. The parenchymatous members of xylem and phloem contain both types of inclusions in the mature state.

As in the mesophyll, inclusions are not generally distributed in the vascular bundles. They are most common in severely affected areas within the small bundles imbedded in the mesophyll.

The *x*-bodies differ from the slime bodies in being rounded and vacuolate; the slime bodies are usually elongated, somewhat fibrous, and frequently sinuous in shape. When the two kinds of structures are rather small and are not deeply stained, they appear less distinct from each other. The vacuolation of the *x*-body and its comparatively sharp outline distinguish it from the inclusions in cells affected by curly top.

Inclusion bodies were the only abnormality observed in the phloem of mosaic tobacco used in this study.

Two plants were inoculated with mosaic and curly top together. These showed the typical degeneration of phloem induced by curly top. Mosaic symptoms, though evident in the mesophyll, were comparatively mild. The study was not carried far enough to determine whether inclusions and degenerative changes characteristic of the two diseases may occur in the same cell.

SUMMARY

The curly-top disease induces in the phloem of *Nicotiana Tabacum* degenerative changes similar to those in sugar beet. In apices of shoots and roots, degeneration usually occurs after the first sieve tubes differen-

tiate and appears first in cells adjacent to these sieve tubes. Sometimes the differentiating sieve cells themselves degenerate in root tips.

The first degenerative changes are quickly followed by hyperplastic divisions resulting in the production of numerous short sieve-tube elements. In the primary phloem the hyperplastic tissue differs strikingly from the normal phloem in cell arrangement and in the proportion of sieve tubes. In the secondary phloem the cell pattern differs less markedly from the normal than in the primary, although many ray cells differentiate into sieve tubes and the phloem mother cells divide excessively.

The hyperplastic tissue disintegrates, particularly in the primary phloem. Commonly cavities are formed where the tissue disintegrates, although in less severe infection the hyperplastic tissue often collapses, being replaced by new cells derived from the adjacent living cells.

In plants recovering from curly top, phloem degeneration is comparatively mild. It is commonly present, however, even if the organ shows no external symptoms. The severity of degeneration varies in different parts of the same organ and is sometimes not perceptible at all. If present, it almost invariably involves the production of supernumerary sieve tubes.

The external symptoms appear on leaves that are most closely connected with the inoculated leaf by vascular tissue. This observation, together with the results of studies on phloem degeneration, confirms the concept that the curly-top virus is largely localized in the phloem.

The tomato shows the same degenerative changes as the tobacco and the sugar beet, the presence of supernumerary sieve tubes being a very conspicuous feature.

Having comparatively large phloem cells, the tobacco and the tomato are more favorable genera for the study of the hyperplastic sieve tubes than the sugar beet. These elements show the fundamental characteristics of normal sieve tubes except that they usually lack companion cells.

The phloem of tobacco affected by mosaic contains inclusions typical of this disease, but apparently has no other abnormalities.

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PLATES

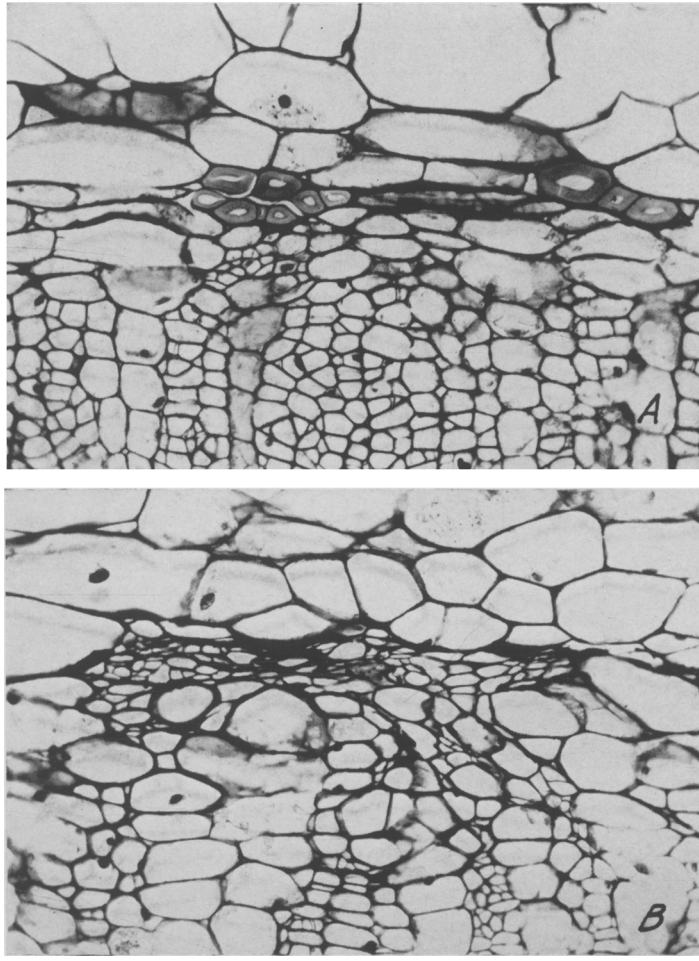

Plate 1.—Transverse sections of phloem of tobacco plant 2 (plate 17, *B*) that recovered from curly top. *A* was taken at the base of the recovered flowering shoot; *B*, at the base of the old diseased shoot. (Both × 180.)

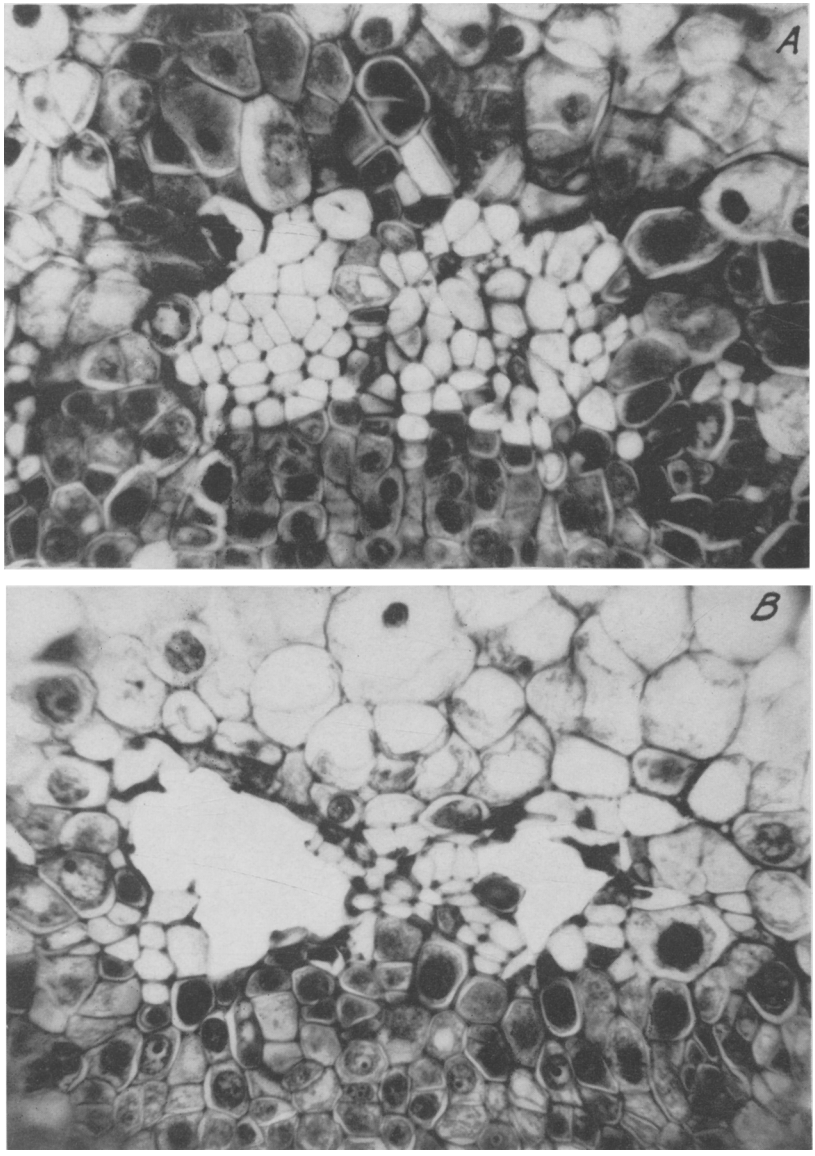

Plate 2.—Transverse sections of primary phloem of young leaves of tobacco severely affected by curly top. *A* shows in the center a large group of hyperplastic sieve tubes (primary hyperplasia); *B* shows cavities that resulted from disintegration of the hyperplastic tissue (secondary necrosis). (Both × 540.)

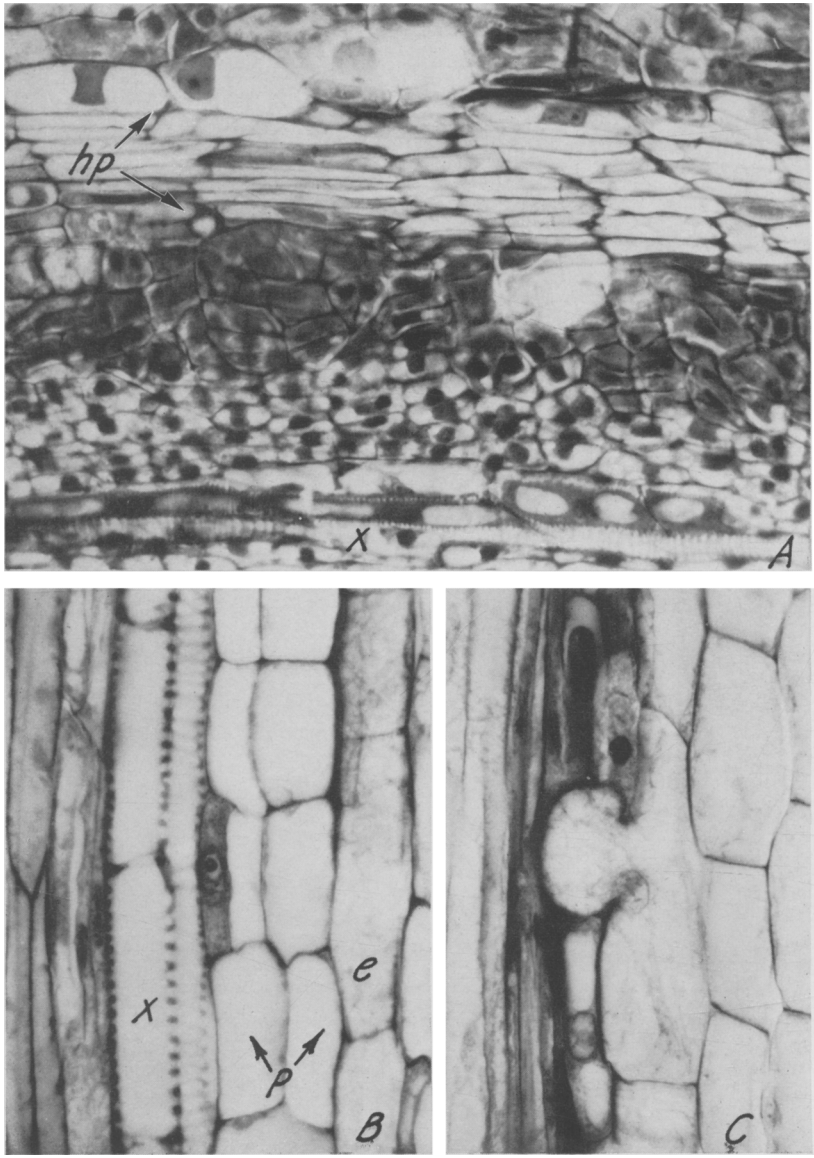

Plate 3.—*A*, Radial longitudinal section of a vascular bundle from a young leaf of tobacco with severe curly top, showing (top to bottom) hypertrophied cells, hyperplastic sieve tubes (*hp*), differentiating phloem (dense stain), procambium and xylem (*x*). (× 375.) *B*, *C*, Longitudinal sections of vascular region from a root tip of tobacco with severe curly top. In *B* pericyclic sieve tubes (*p*) appear next to the xylem (*x*). In *C* a hypertrophied cell has formed a protrusion next to the collapsed phloem. (*B* and *C*, × 540.)

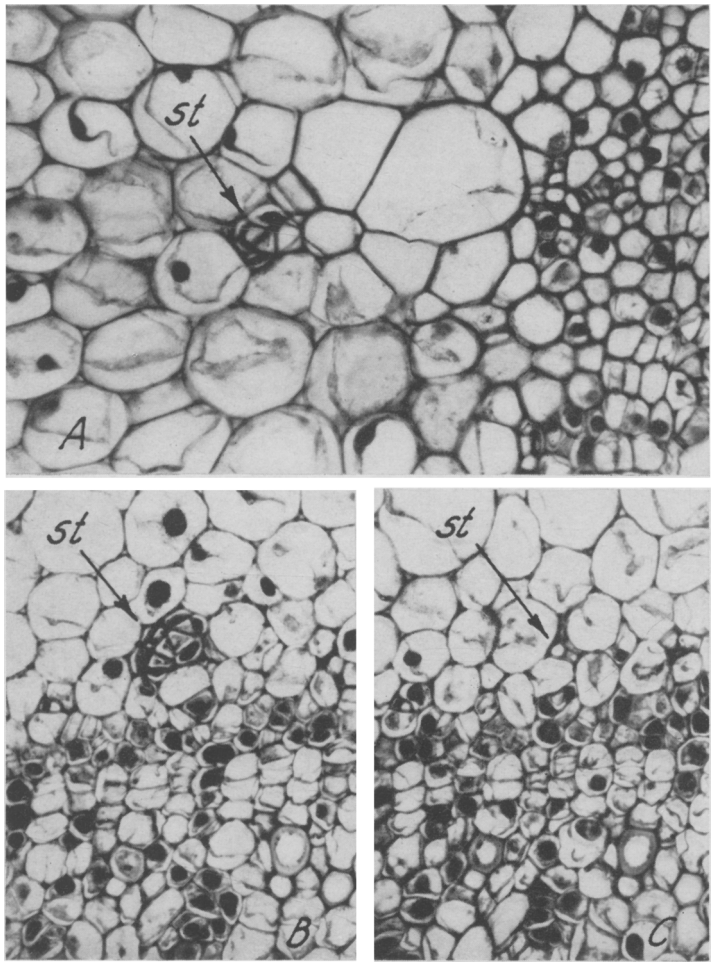

Plate 4.—*A*, Transverse section of phloem and cortex of stem near the apex of tobacco shoot *b* (plate 15, *B*), recovering from curly top. Hyperplastic sieve-tube strand at *st*; then follow (to the right) hypertrophied cells, phloem, and procambium. *B* and *C*, Two transverse sections (40 microns apart) of vascular tissues from a young leaf of a tobacco plant recovering from curly top. At *st* in *B* is a hyperplastic sieve-tube strand; at *st* in *C* a normal sieve tube. The hyperplastic strand in *B* arose near the normal sieve tube in *C*. (All × 360.)

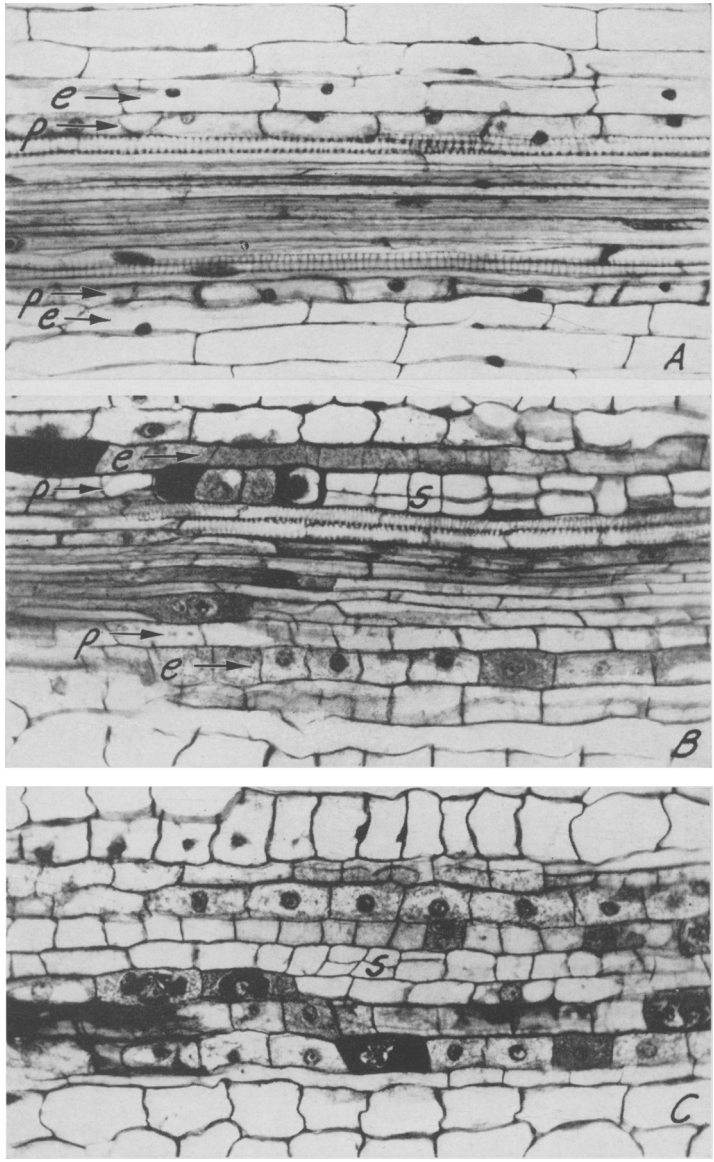

Plate 5.—Longitudinal sections through steles from root tips of healthy (*A*) and curly-top (*B* and *C*) tobacco plants. Details are: *e*, endodermis; *p*, pericycle; *s*, sieve cells. Further explanations in the text. (All × 180.)

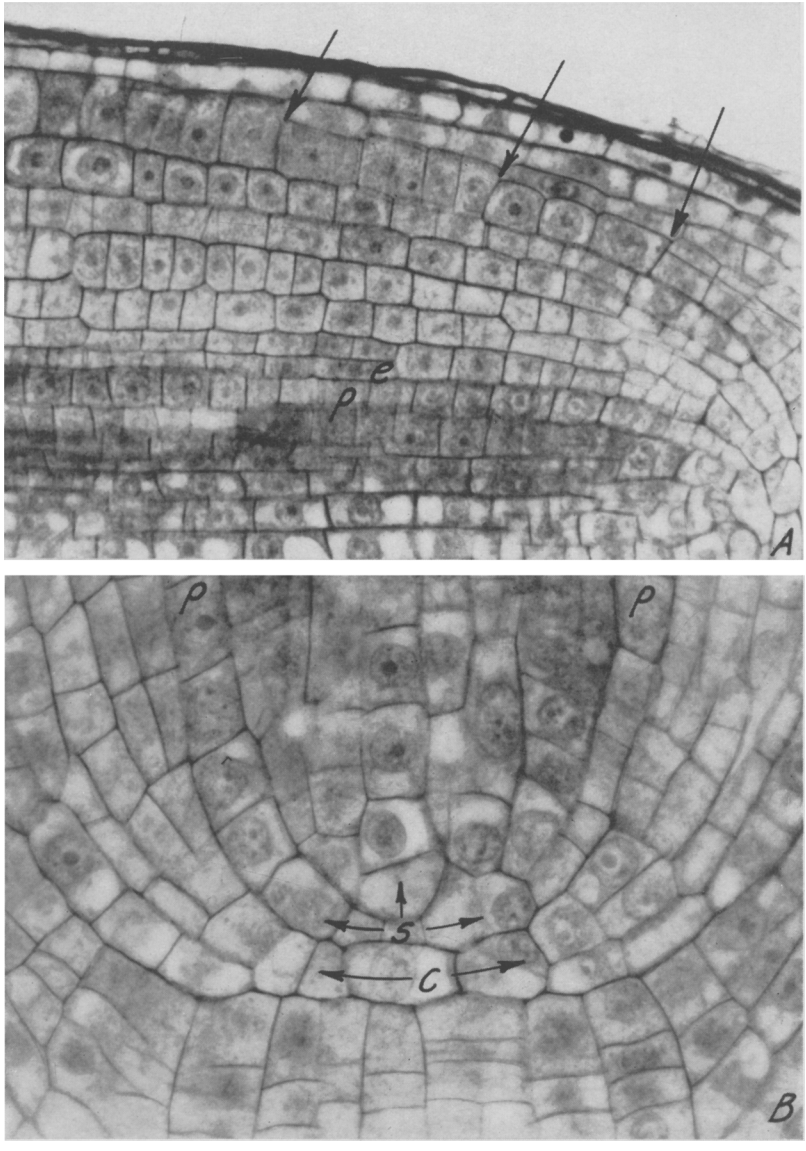

Plate 6.—Longitudinal sections of the apex of a root of curly-top tobacco. Details are: *c*, region of cortical initials; *e*, endodermis; *p*, pericycle; *s*, region of stele initials. Further explanations in the text. (*A*, × 455; *B*, × 750.)

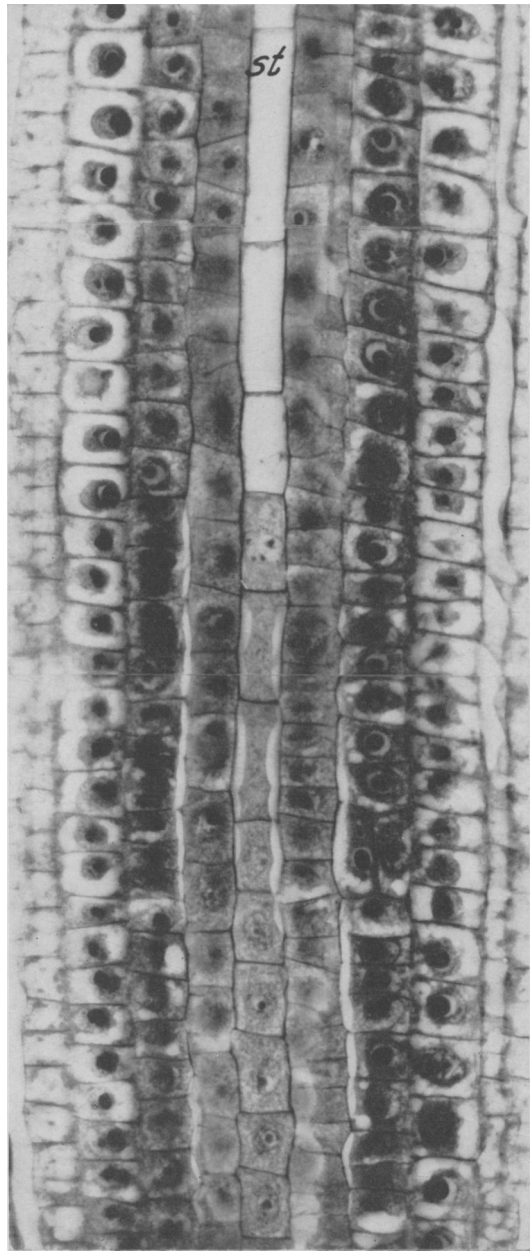

Plate 7.—Longitudinal section through stele of a tobacco root tip, showing normal differentiation of a sieve tube (*st*). Further explanations in the text. (× 570.)

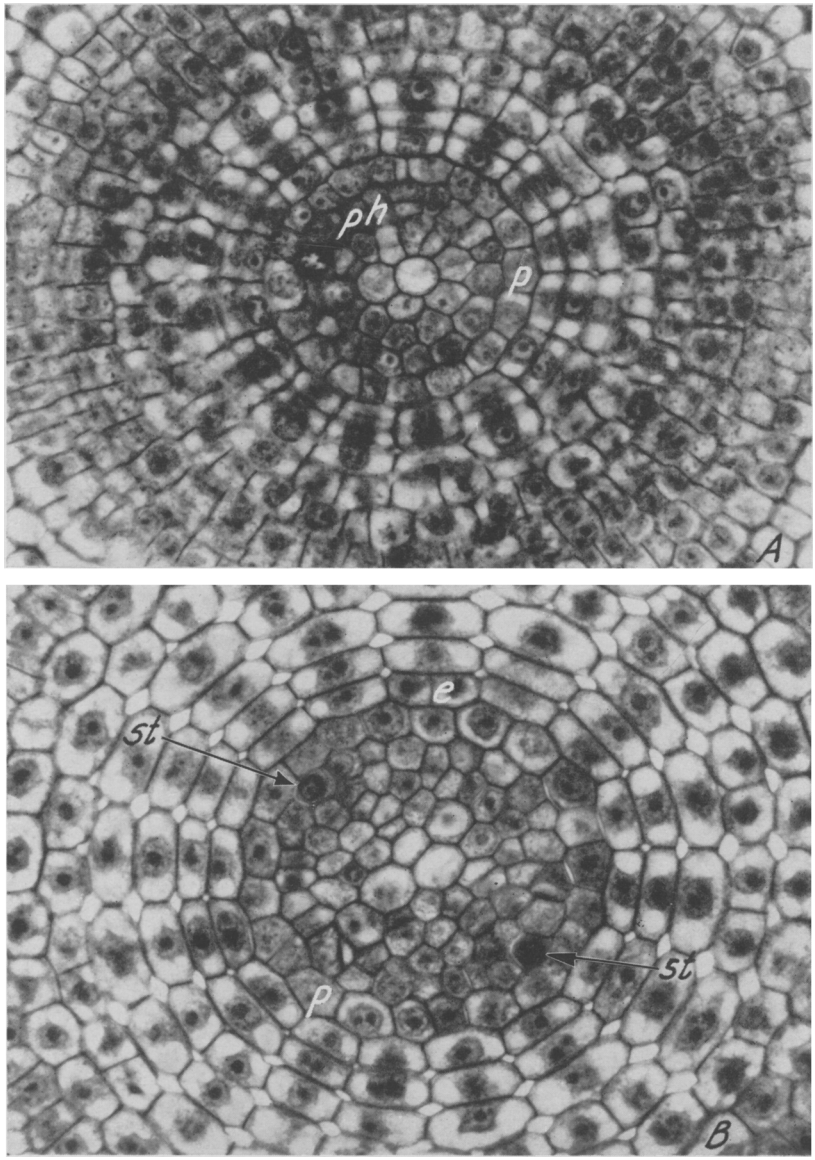

Plate 8.—Transverse sections of a young diarch root of healthy tobacco, showing stele and part of the cortex. *A* was taken 50 microns from the region of initials; *B*, 200 microns. The central xylem cells are vacuolating and the pericycle (*p*) forms a uniseriate layer around the stele. Two sieve-tube mother cells (*st*) are shown in *B*. Other details are: *e*, endodermis; *ph*, phloem. (Both × 455.)

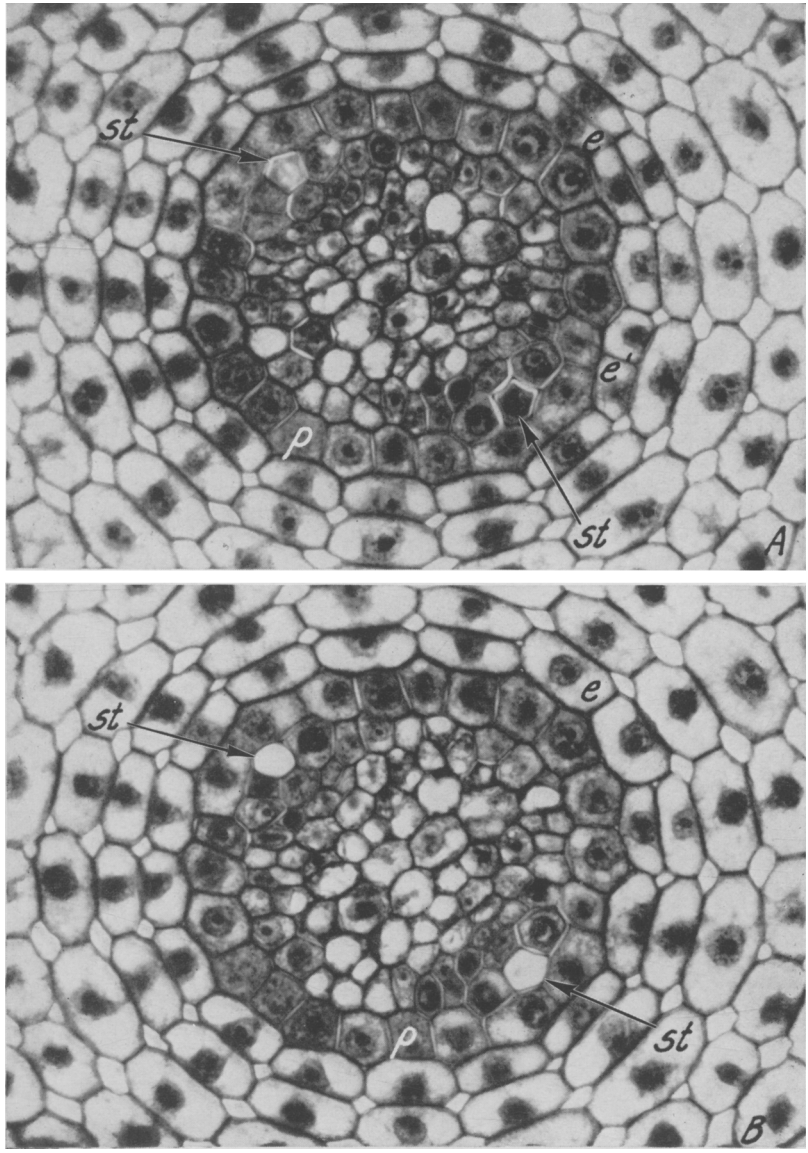

Plate 9.—Transverse sections of a young diarch root of healthy tobacco, showing stele and part of the cortex. These sections were taken from the same root as those in plate 8; *A* was taken 450 microns from the apex; *B*, 500 microns. The pericycle (*p*) is still a uniseriate layer. The two sieve tubes (*st*) are immature in *A*, mature in *B*. The endodermis is marked by *e* and e^1. (Both × 455.)

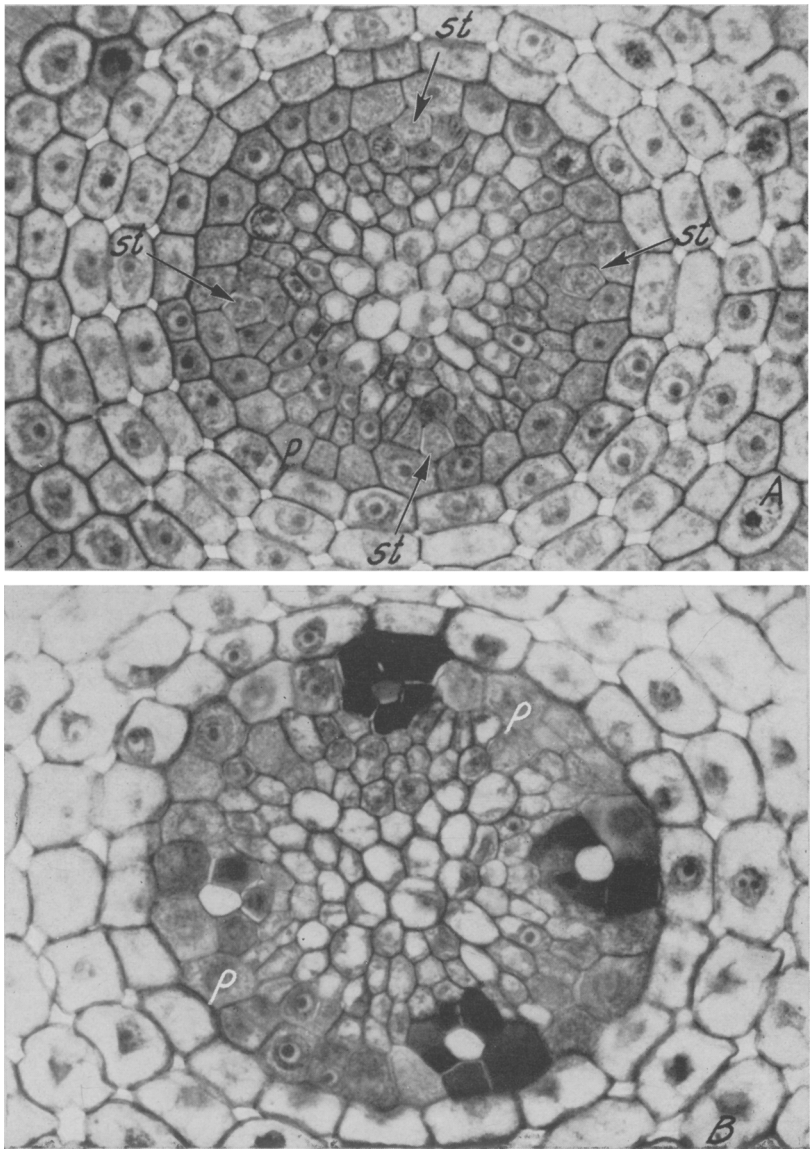

Plate 10.—Transverse sections, showing stele and part of the cortex of a young tetrarch root of tobacco with severe curly top. *A* was taken 230 microns from the apex; *B*, 500 microns. The first sieve tubes (*st*) are not yet mature in *A*. In *B* the mature sieve tubes are surrounded by degenerating cells. The pericycle (*p*) is a uniseriate layer in *A*, but shows some periclinal walls in *B*. (Both × 455.)

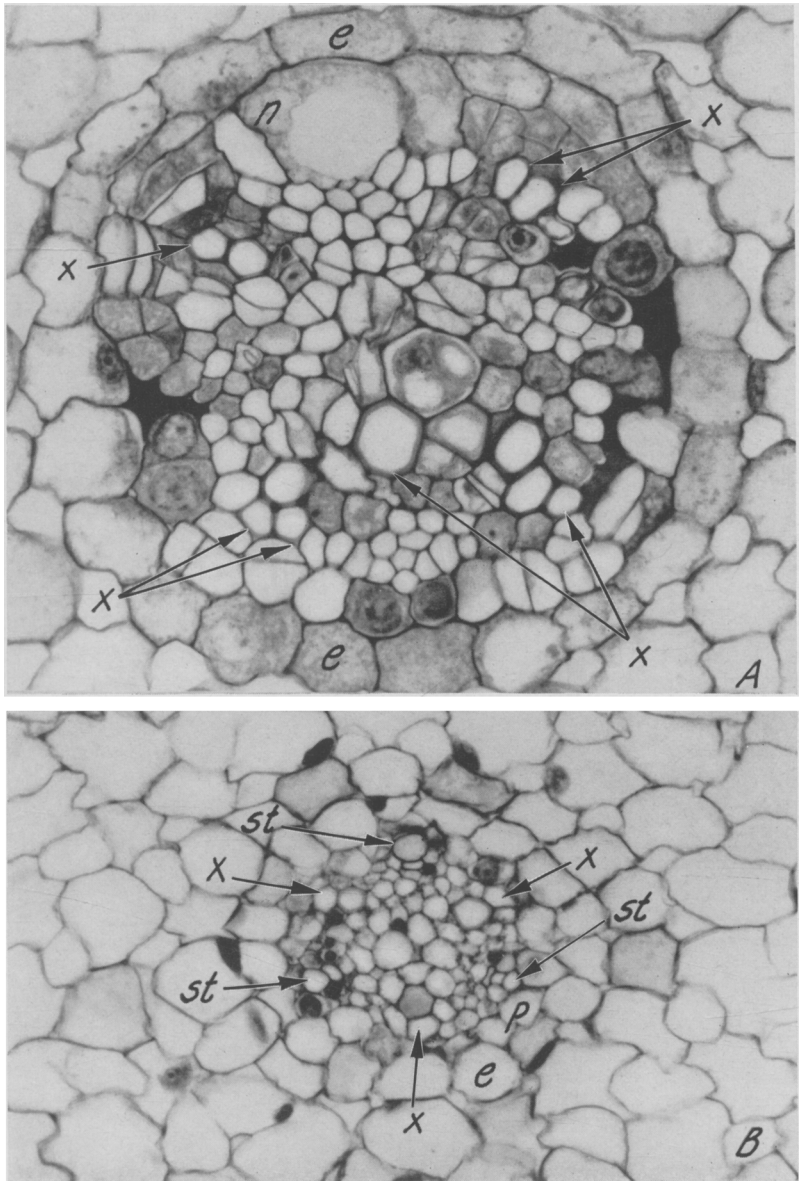

Plate 11.—*A*, Transverse section, showing stele and some cortex of a young tetrarch root of curly-top tobacco. This section was taken 2,200 microns from the apex of the same root as in plate 10. The cells in the stele showing no contents are xylem elements (with very thick walls) and sieve cells (with moderately thick walls). Primary necrosis is evident in the blackened areas to the right and left. *B*, Transverse section of a young tetrarch root of healthy tobacco. It was taken 1,120 microns from the apex and shows stele and cortex. Details are: *e*, endodermis; *n*, nucleus; *p*, pericycle; *st*, sieve tubes; *x*, xylem. (Both × 455.)

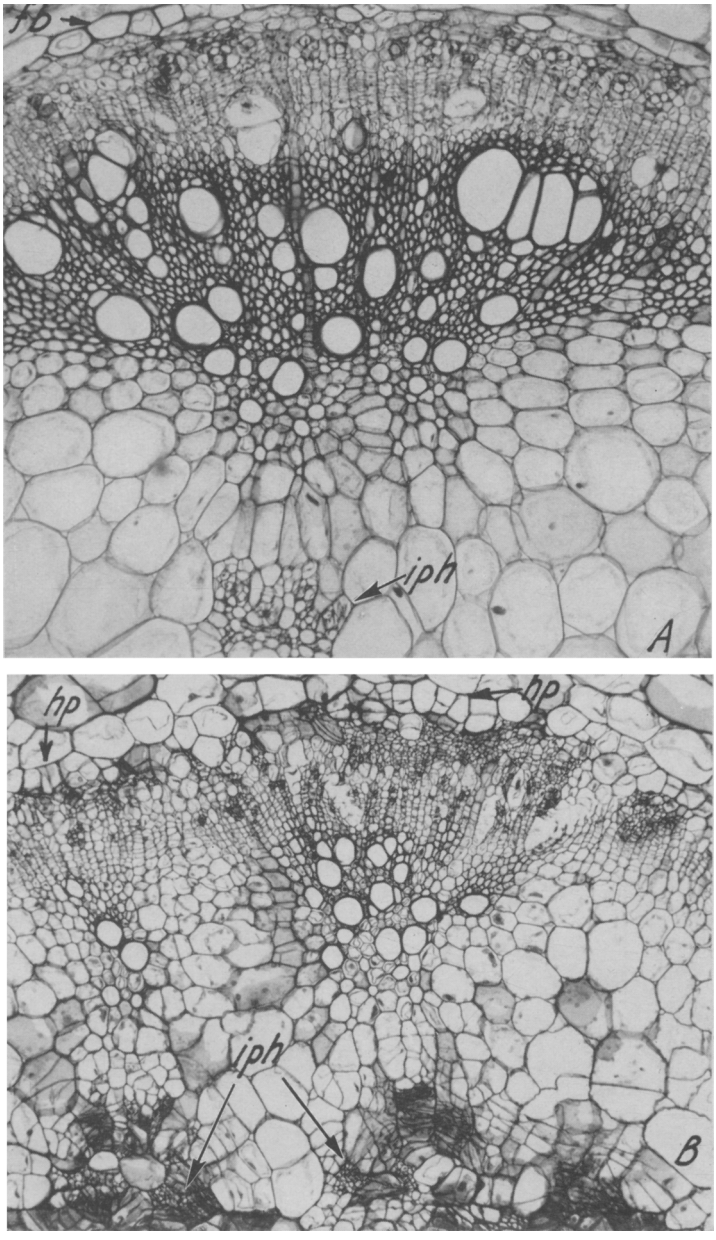

Plate 12.—Transverse sections of tomato stems showing vascular tissues. The sections were taken just above the cotyledons from the healthy (*A*) and curly-top diseased (*B*) plants in plate 16, *B*. In *A* the external phloem is fringed by fibers (*fb*); in *B*, by hyperplastic cells (*hp*). *B* shows hypoplastic xylem and abnormally massive internal phloem (*iph*). (Both × 72.)

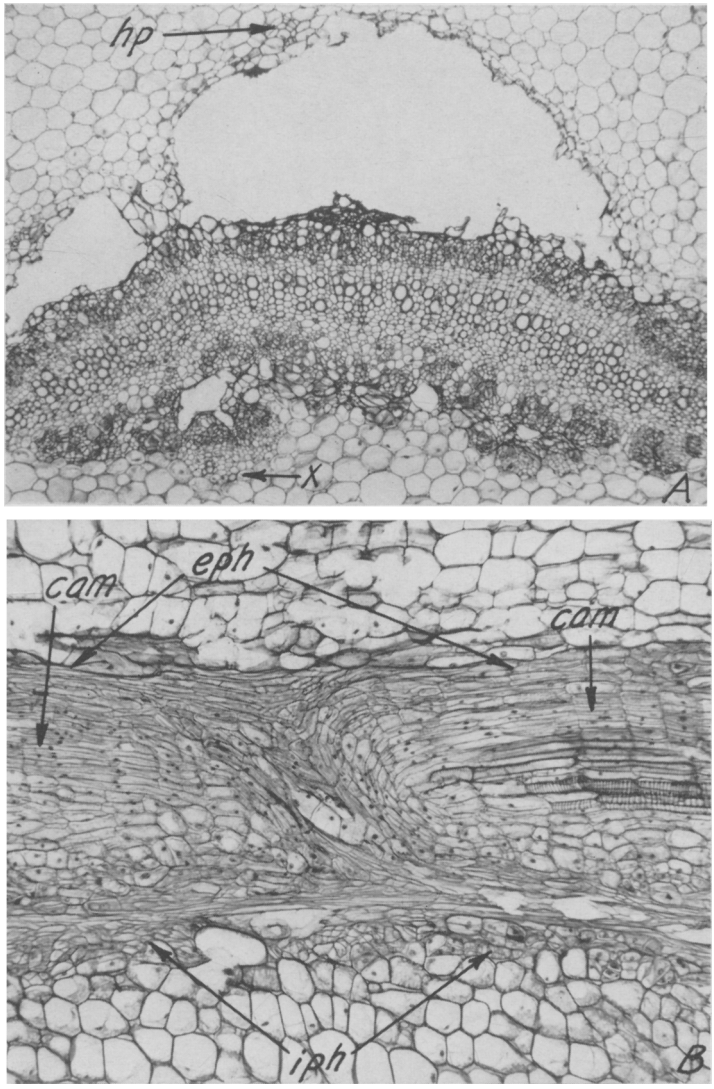

Plate 13.—*A*, Transverse section of vascular bundle of petiole of tobacco with severe curly top, showing cavities that resulted from disintegration of diseased phloem. Hyperplastic sieve tubes at *hp*. *B*, Radial longitudinal section of vascular tissues of internode of tobacco with severe curly top, showing, in the center, a phloem strand intersecting the cambium (*cam*) and xylem, and connecting the external phloem (*eph*) with the internal (*iph*). (Both × 72.)

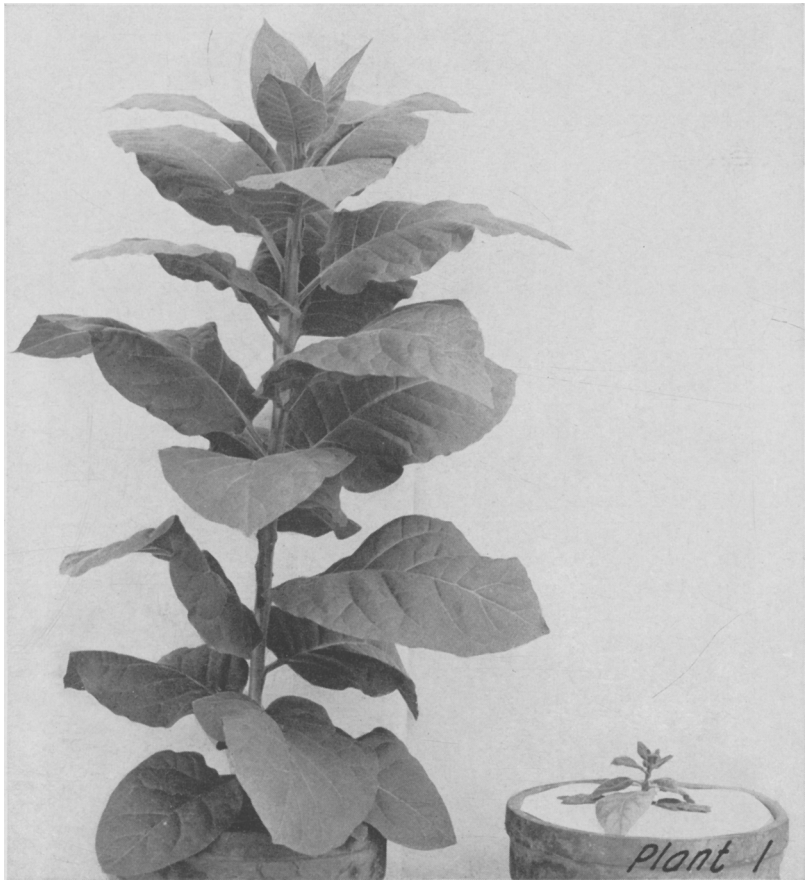

Plate 14.—A plant affected by curly top (plant 1) and a healthy check of *Nicotiana Tabacum*. The diseased specimen was inoculated January 25, 1933; and both plants were photographed March 13, 1933. (× 0.15.)

Plate 15.—Curly-top-diseased *Nicotiana Tabacum* plant 1 (also in plate 14) during two stages of recovery from curly top. *A*, Photographed April 11, 1933; *B*, May 29, 1933, when the plant had produced two axillary shoots (*b* and *c*) that lost the external symptoms. The old shoot appears at *a* in *B* and shows the scar where the flower had been previously removed. (Both × 0.36.)

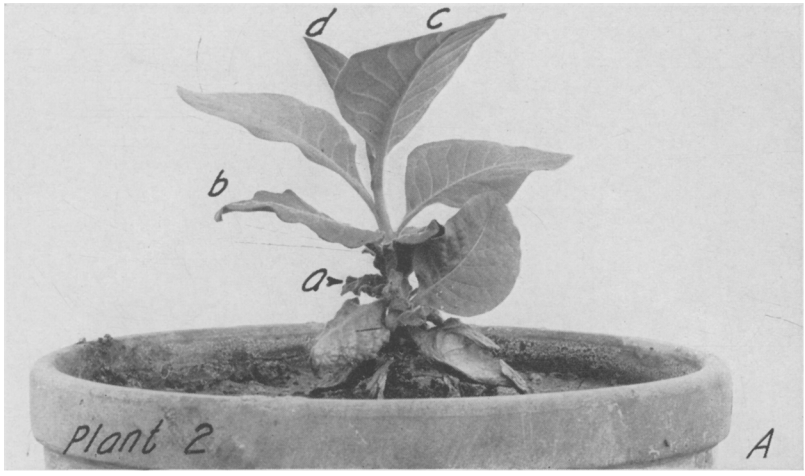

Plate 16.—*A*, Curly-top-diseased plant of *Nicotiana Tabacum* showing (below) a portion of the old shoot with symptoms and (above) an axillary shoot recovering from the disease. The plant was inoculated January 25, 1933, and photographed April 11, 1933. (× 0.36.) *B*, Tomato plant affected with curly top (right) and a healthy check (left). The diseased plant was inoculated February 17, 1933, when it had two foliage leaves, and photographed March 13, 1933. (× 0.15.)

Plate 17.—*Nicotiana Tabacum* plants showing recovery from curly top. *A*, Plant 3 inoculated January 25, 1933, photographed April 11, 1933. External symptoms are evident on the lower but not on the upper leaves. (× 0.36.) *B*, Plant 2 (plate 16, *A*) photographed May 29, 1933. A portion of the diseased shoot occurs at the base. The recovered shoot bearing flowers, arose in a leaf axil of the old shoot. (× 0.14.)

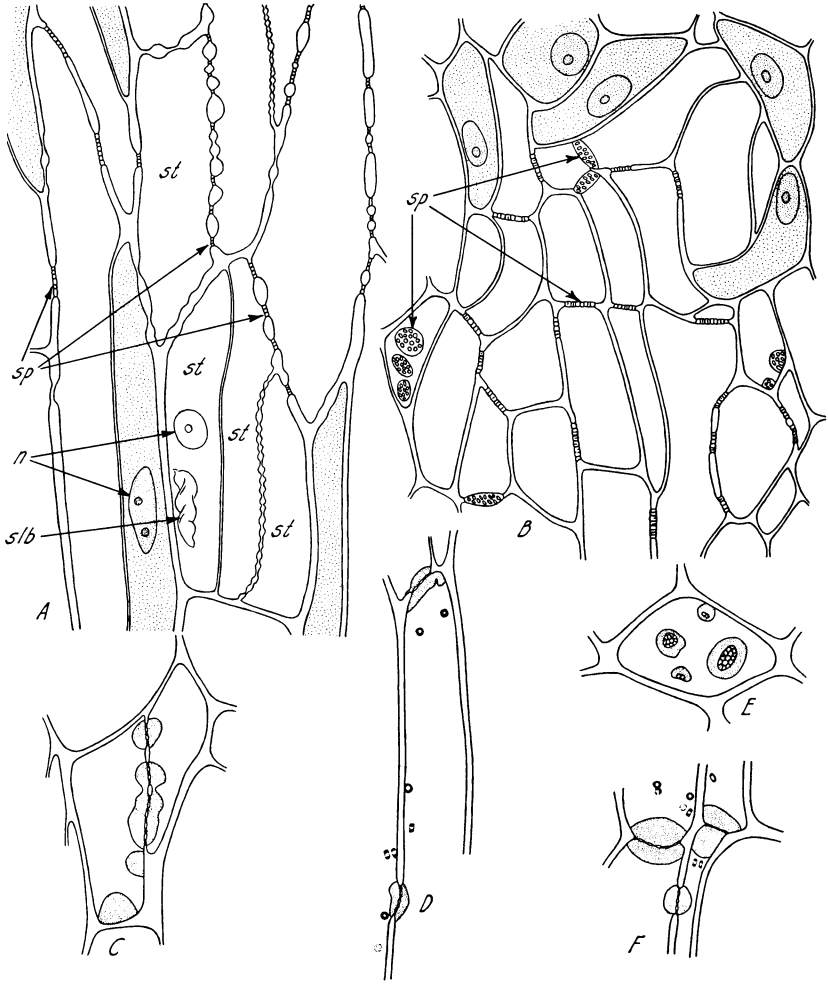

Plate 18.—Longitudinal sections of secondary (*A*) and primary (*B*) phloem from stem of curly-top tobacco, showing sieve-tube elements (without stipples) and parenchyma (stippled cells). The sieve cells marked *st* in *A* were derived from ray cells. Details are: *n*, nucleus; *slb*, slime body; *sp*, sieve plate; *st*, sieve tube. *C–F*, Sections of sieve tubes from sugar-beet roots. *C–E*, Hyperplastic elements of a curly-top plant; *F*, section of sieve cells of a healthy plant. The sieve plates covered with definitive callus (stippled structures) are seen in sectional views in *C*, *D*, and *F*, and in face views in *E*. (All × 689.)