Histopathology of Phloem Necrosis in Ulmus americana

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ABSTRACT

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Symptoms of phloem necrosis in the secondary phloem of elms (*Ulmus americana*) were studied using light- and electron microscopy. The first symptom, abnormally rapid deposition of callose within sieve tubes, was followed by collapse of sieve elements and companion cells. Hyperactivity of the cambium followed, resulting in formation of replacement phloem. Sieve elements in replacement phloem, which were smaller than normal, soon became necrotic. Starch accumulated in diseased, but not in healthy phloem. Mycoplasmalike organisms, seen only in mature sieve elements, were erratically distributed within the symptomatic phloem. Histological aberration in secondary phloem was correlated with intensity of discoloration of the tissue. Normal sieve tube necrosis, observed in tissue of healthy elms collected in November, proceeded more slowly than that seen in degenerating phloem of diseased trees. During the winter, all sieve tubes in stems of both healthy and diseased trees were either occluded with callose or crushed. In roots, samples of secondary phloem collected in January from both diseased and healthy trees showed some sieve elements that had not degenerated. It is postulated that the pathogen overwinters within these cells.

Phloem necrosis is a lethal disease of elms. Infected American elms (*Ulmus americana* L.) usually die within one year after symptoms appear. The causal agent is transmissible by leafhoppers (1) and by grafting (20). Wilson et al. (23) reported mycoplasmalike organisms (MLO) in the phloem of diseased, but not in healthy trees. Gross symptoms in several elm species have recently been described (17).

In *U. americana,* foliar symptoms (epinasty, yellowing, and premature defoliation; or wilting) are usually preceded by extensive necrosis of feeder roots. Characteristic discoloration of the most recently differentiated phloem occurs in large roots, stems, and sometimes in large branches of infected trees. This tissue, initially almost white, becomes yellow or light tan, then progressively darker. Necrotic phloem is dark brown.

In 1944, McLean (11) reported anatomical changes in phloem necrosis-diseased American elm. The objectives of the present work were to more fully characterize phloem degeneration and to reevaluate the older information about phloem necrosis in relation to recent histopathological studies of plant diseases caused by MLO.

MATERIALS AND METHODS

Sampling.—Phloem samples were taken at Ithaca, New York, between 30 July and 10 September in 1973 and 1974 from trunks and branches of six symptomatic and three healthy *U. americana* trees ranging in age and size from a one-year-old seedling to a tree of 20 cm diameter 1.4 m above the ground.

To reduce surging of sieve tube contents during sampling, phloem tissues were obtained as follows. Most of the corky tissue was first removed with a sharp razor. Two parallel longitudinal incisions about 1 cm apart and 8 cm long were then made through the phloem and into the xylem. Next, using two razors, transverse incisions were made simultaneously through both ends of the strip of bark. The strip was peeled from the tree and immediately placed into one of the fixatives used for electron microscopy (EM). The central 1-cm of each strip was removed under fixative and processed as described below for transmission EM. The two ends of each strip were transferred to formalin-acetic acid-alcohol (FAA) in preparation for light microscopy (LM).

Phloem showing various intensities of discoloration, nondiscolored phloem from branches of diseased trees, and phloem from healthy trees were examined. Symptom progression was assumed to coincide with increasing phloem discoloration. Development of symptoms within individual trees was not followed.

Samples for observation of the winter condition of phloem in healthy and diseased stems and roots were collected on 20 November 1974 and 8 January 1975. On these dates the bark could not be peeled from trees, so the phloem and some adjacent xylem were removed using a hammer and chisel. Some of the healthy phloem from the November collection was processed for EM. All other samples were prepared for LM.

Light microscopy.—Tissue was fixed in FAA, dehydrated in ethanol, and embedded in celloidin (10). Radial and transverse sections, 15- to 20-μm thick, were cut with a sliding microtome and stained with tannic acid-ferric chloride-resorcin blue. Callose (β-1,3-glucan) is stained selectively by the resorcin blue (2). Starch was identified using an iodine-potassium iodide stain (IKI) (8). Some tissue samples were fixed in 10% acrolein, embedded in glycol methacrylate and stained with toluidine blue (7).

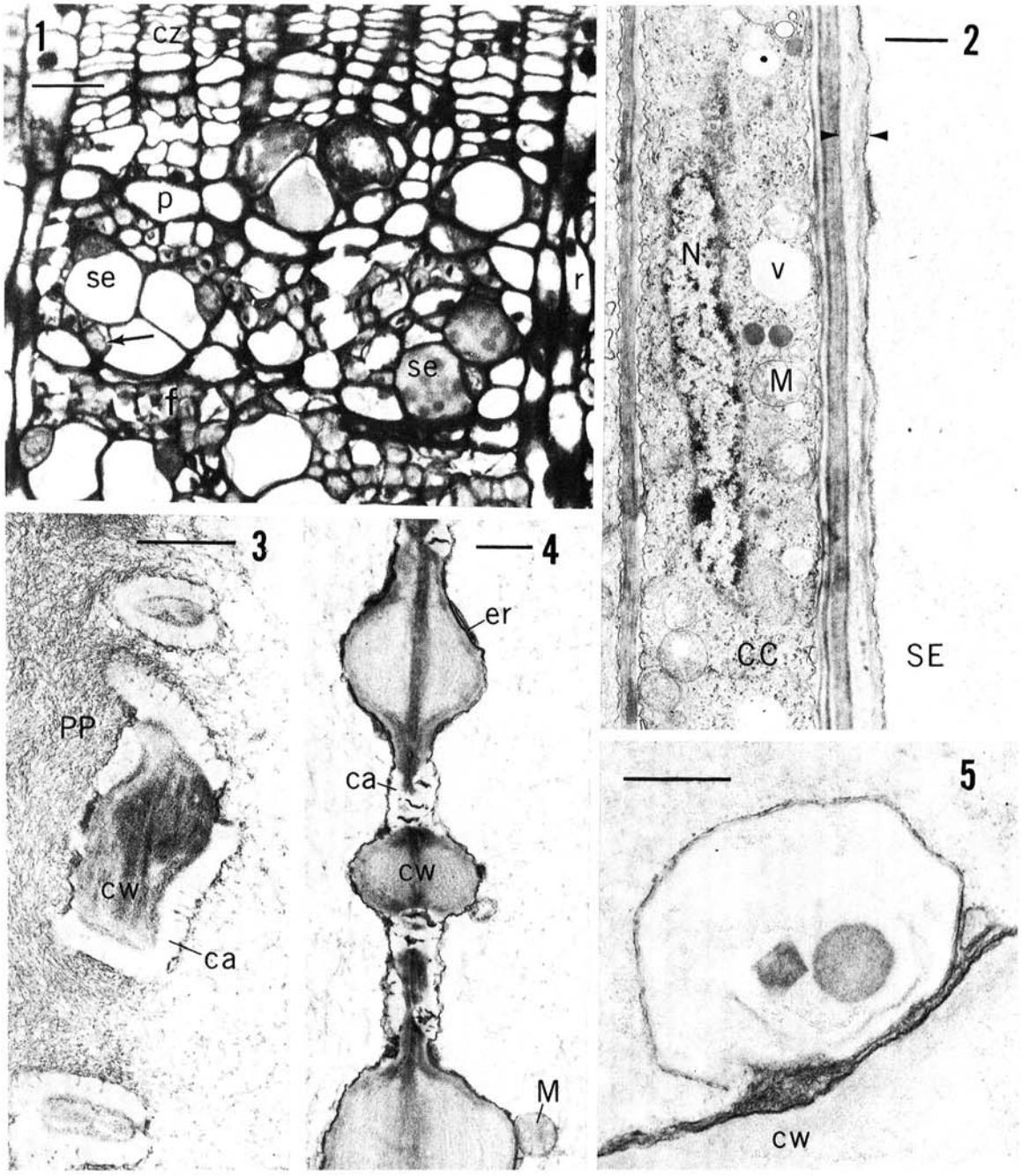


Fig. 1-5. Secondary phloem of healthy American elm. **1)** Cross section stained with toluidine blue (light micrograph); cz, cambial zone; p, phloem parenchyma; r, ray parenchyma; se, sieve elements; f, fibers; arrow indicates companion cell; scale bar = 25 μm. **2)** Longitudinal section of sieve element (SE) and companion cell (CC); sieve element wall shows distinct inner layer (darts); N, nucleus, M, mitochondrion; v, vacuole; scale bar = 1.0 μm. **3)** Longitudinal section of sieve plate showing normal callose deposits; ca, normal callose deposits; cw, cell wall; PP, phloem protein plug; scale bar = 1.0 μm. **4)** Longitudinal section of lateral sieve area with normal callose (ca) deposition in pores; M, mitochondrion; er, endoplasmic reticulum; cw, cell wall; scale bar = 1.0 μm. **5)** Transverse section of sieve element plastid containing protein crystal and round grain of sieve element starch; cw, cell wall; scale bar = 0.5 μm.

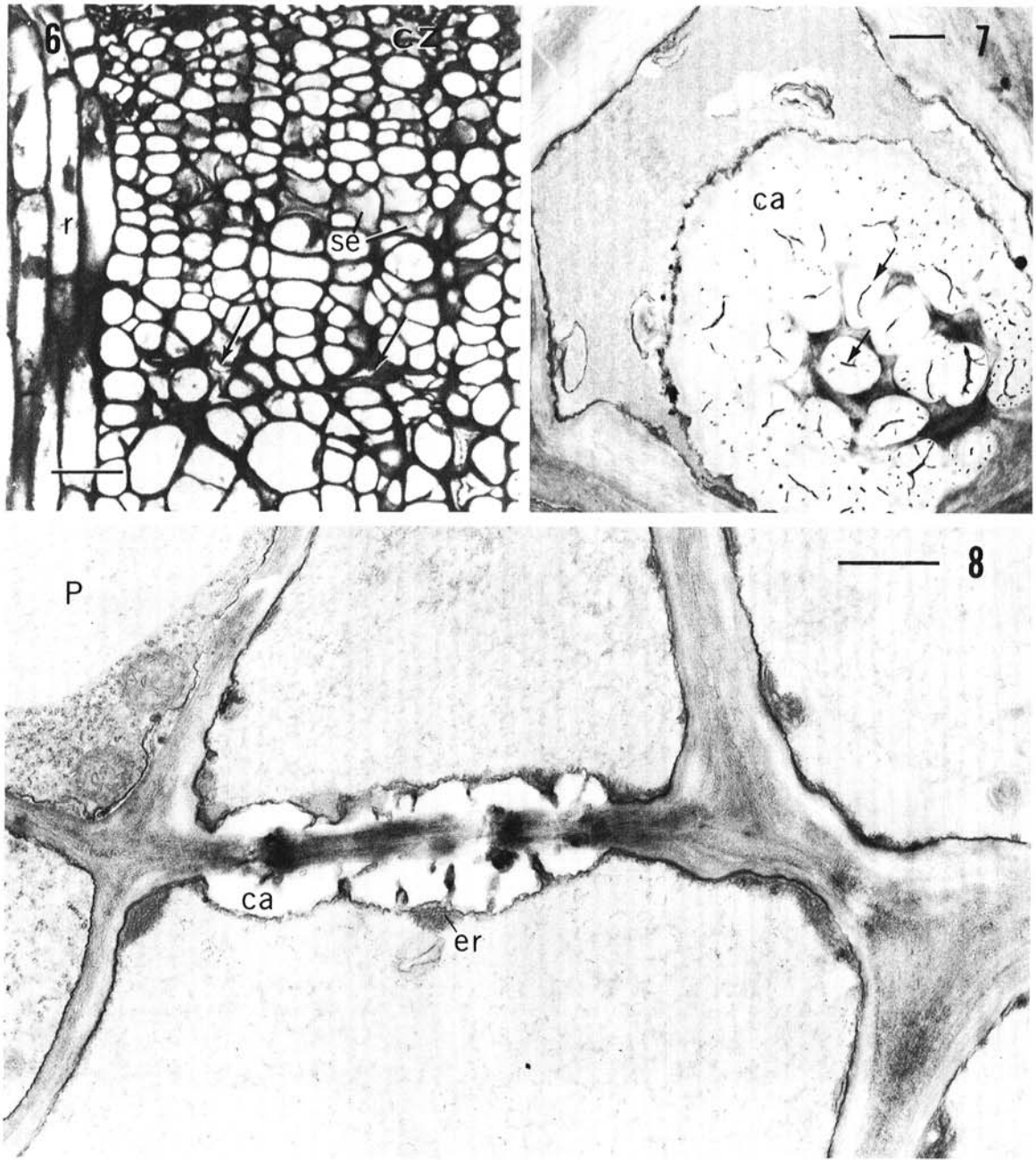


Fig. 6-8. Cross sections of secondary phloem in diseased American elm. **6)** Cross section of replacement phloem stained with toluidine blue (light micrograph); cz, cambial zone; r, ray parenchyma; se, sieve element; arrows indicate crushed sieve elements; scale bar = 25 μm. **7)** Cross section through sieve plate with callose (ca) deposit; arrows indicate occluded sieve plate pores; scale bar = 1.0 μm. **8)** Sieve elements in replacement phloem. A large callose deposit (ca) is present on the lateral sieve area; P, parenchyma cell; er, endoplasmic reticulum; scale bar = 1.0 μm.

Electron microscopy.—Tissues were cut under fixative into blocks 1-2 mm in largest dimension and fixed overnight at 5 C in either 4% glutaraldehyde or a formaldehyde-glutaraldehyde-acrolein mixture (FGA) (12). Both fixatives were prepared in 0.1 M cacodylate buffer, pH 6.8. Fixation was more consistent with FGA. After being washed in buffer, the tissue was postfixed for 3 hours at 5 C with 2% osmium tetroxide in the same buffer. Tissue blocks were then washed in distilled water at room temperature, block-stained with 0.5% aqueous uranyl acetate for 1 hour, dehydrated in an acetone series, and embedded in Spurr's epoxy resin (19). Thirteen tissue blocks from healthy, and 36 from diseased trees were sectioned using glass or diamond knives. Sections were picked up on Formvar-coated or noncoated copper grids, and stained with uranyl acetate and lead citrate.

RESULTS

Healthy tissue.—The conducting secondary phloem in the stem of American elm consists of sieve tube elements, companion cells, phloem parenchyma, fibers, and ray parenchyma (Fig. 1). The multiseriate rays are continuous across the cambium with the xylem rays. Fibers and sometimes tannin-containing parenchyma cells are distributed in tangential bands; sieve elements, companion cells, and parenchyma cells occupy the intervening spaces (Fig. 1, 13). Evert et al. (6) and Evert and Deshpande (5) have described sieve element ontogeny in *U. americana*.

In the tissue examined, the cells of greatest diameter in cross section were the sieve elements (Fig. 1). Immature sieve elements contained all the protoplasmic components usually present in parenchymatous cells. Some organelles such as nuclei and dictyosomes were lost during differentiation; mitochondria, plastids, and endoplasmic reticulum (ER) assumed a parietal position at maturity (Fig. 4, 5). After the breakdown of the tonoplast, the lumen of the mature sieve element contained a net-like reticulum of phloem protein (P-protein) (5). The P-protein originated in immature sieve elements as distinct accumulations, P-protein bodies, which became dispersed throughout the cytoplasm. Most sieve elements at a distance of 60-85 μm from the cambium were fully matured.

Accumulations of P-protein were observed at some sieve plates (Fig. 3). These plug-like accumulations were thought to be artifacts caused by the surging of sieve tube contents during sampling (4).

Contiguous sieve elements were found connected via pores in sieve plates (Fig. 3) and lateral sieve areas (Fig. 4). The plasmalemma was continuous from one element to the next through the pores, and the pores were always lined with callose. The pores of lateral sieve areas were about 0.5 to 0.75 μm in diameter, whereas most pores in sieve plates ranged from 1.0 to 5.0 μm. Most sieve tube walls had a distinct inner layer of variable thickness (Fig. 2).

Numerous, branched plasmodesmata connected companion cells to their respective sieve elements. The companion cells contained rather dense cytoplasm with abundant mitochondria, ER, ribosomes, an elongated nucleus (Fig. 2), and plastids without starch. Vacuolation was variable. Companion cells were often somewhat shorter than their associated sieve elements. In cross sections, therefore, some sieve elements appeared to lack companion cells.

Symptom development.—The extent of histological aberration was positively correlated with intensity of discoloration in the secondary phloem. Nondiscolored phloem from diseased trees appeared normal in all respects.

The first symptom observed was abnormal deposition of callose in the oldest sieve tubes. The callose accumulated on sieve plates (Fig. 7), lateral sieve areas (Fig. 8), and plasmodesmata between sieve elements and companion cells. Callose deposition became massive and widespread until only the youngest sieve tubes remained free of large deposits. At this stage, many of the older sieve elements and companion cells were seen to be in various stages of collapse.

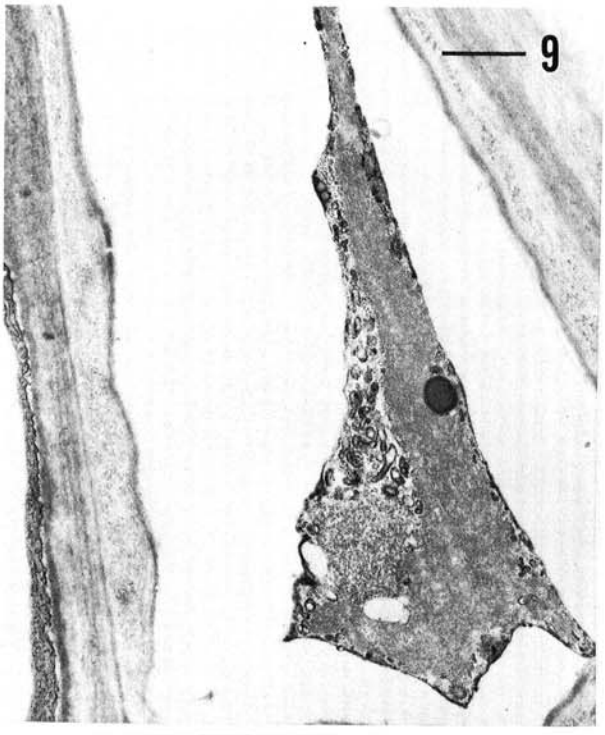
Sieve tube necrosis progressed until most of the sieve elements and companion cells interpreted to have been present before the onset of discoloration had collapsed. At about the time the sieve tubes near the vascular cambium began collapsing, the cambium became hyperactive and produced large amounts of new phloem, termed replacement phloem (16). Formation of new xylem was not detected. The replacement phloem consisted of sieve elements, companion cells, and parenchyma, but no fibers (Fig. 6).

Cambial derivatives, exclusive of ray parenchyma, were counted (using LM) in a randomly selected field on each of 16 sections, eight from healthy and eight from diseased trees. Of the cells produced by the hyperactive cambium (average of 105 per field), 42% were sieve elements and companion cells. These comprised 16% of the cells produced by healthy cambium. About 40 randomly selected sieve elements in replacement phloem and 40 in healthy phloem of mainstems were measured. Sieve elements in the replacement phloem averaged 12.1 μm in diameter compared to 28.7 μm in healthy tissue. Sieve elements ranged from 86 to 216 μm long in the healthy tissue with an average length of 146 μm. In the replacement phloem, they ranged from 60 to 175 μm with an average of 102 μm.

Cytologically, sieve elements in the replacement phloem appeared to differentiate normally; they usually matured between 25 and 40 μm from the cambium. Sieve tube walls generally were thinner than those in the healthy phloem and the inner wall layer was often absent (Fig. 8). Callose deposition occurred in the replacement phloem, and often involved sieve tubes very close to the cambial region. Most of the abnormal sieve elements and companion cells collapsed when only about 150 μm from the cambium. In the stem of a healthy elm, by contrast, all the sieve tubes produced during the growing season collapse during a period of a few weeks in the autumn.

In one of the diseased trees, most of the sieve elements in the replacement phloem contained clumped (rather than dispersed) P-protein (Fig. 12). Longitudinal sections indicated that this distribution was not due to surging of the sieve tube contents. These cells lacked callose even in the sieve plate pores. Associated companion cells appeared normal. The significance of this observation is unknown.

In diseased elms, sieve tubes of the replacement phloem and those present before the cambium became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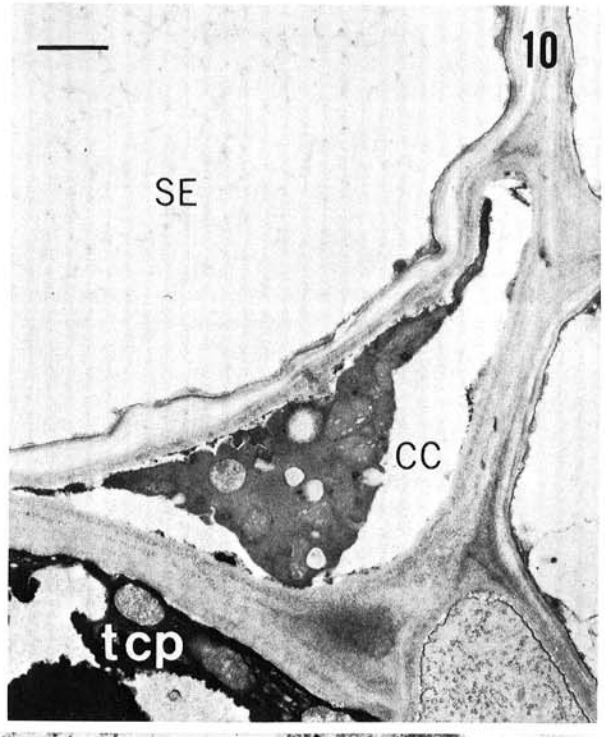
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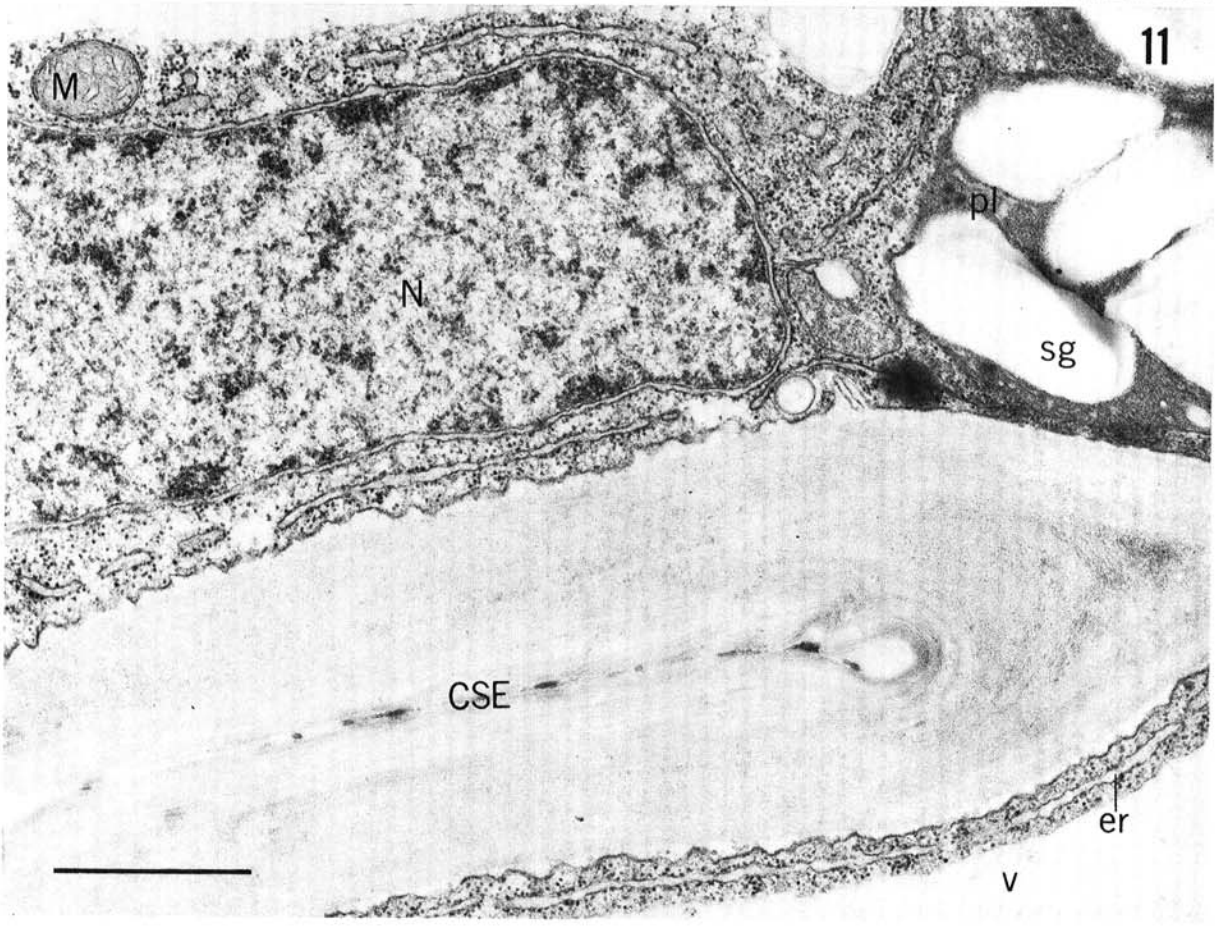
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hyperactive collapsed in the same manner. In both cases, sieve tube necrosis (generally preceded by callose deposition) seemed to have occurred rapidly. Few partially-collapsed cells were seen. In these, the plasmalemma seemed to pull away from the cell wall leaving a dense, shrunken protoplast in the cell lumen (Fig. 9, 10). No signs of impending collapse were recognized. In most cases, a sieve element and its associated companion cell were both found to have collapsed, but often a necrotic companion cell was associated with a normal-appearing sieve element (Fig. 10).

The crushing of necrotic cells was apparently the result of expansion of the surrounding parenchyma cells. Hypertrophied phloem and ray parenchyma were seen in some sections. Hyperplasia of phloem parenchyma was common. Parenchyma cells contained many large, starch-filled plastids and most samples of diseased tissue showed an abnormal accumulation of starch when compared to healthy tissue (Fig. 13, 14). Aside from the starch-filled plastids, the parenchyma cells showed no ultrastructural abnormalities, even when adjacent to collapsed sieve tubes (Fig. 11).

Mycoplasmalike organisms.—Mycoplasmalike organisms (MLO) were observed only within mature sieve elements in diseased tissue, never in the healthy tissue. Sieve elements that contained MLO appeared normal in other respects. Mycoplasmalike organisms were seen in sieve tubes produced both before and after the cambium became hyperactive, but their distribution in the secondary phloem was unpredictable. In cross section, their outlines were spherical, oval, or filamentous (Fig. 15-17). Spherical and oval forms usually ranged from 0.2 to 0.9 μm in diameter. Filamentous bodies were up to 2.2 μm long (Fig. 18). Very small spherical forms 0.08 to 0.1 μm in diameter probably represented cross sections of filamentous cells. The ultrastructure of the MLO was similar to that described for the aster yellows agent (9). Bodies were bounded by a unit membrane. In some sections, ribosomes and fibrils presumed to be DNA were seen (Fig. 15, 16). Mycoplasmalike organisms were seen in pores in sieve plates and lateral sieve areas (Fig. 18), suggesting a pathway of movement of the organism between sieve elements.

Winter condition of phloem.—Samples collected in November from stems of healthy trees exhibited massive callose deposition on sieve plates and lateral sieve areas. Protoplasts had collapsed in all but the youngest sieve elements. Older necrotic sieve tubes appeared empty, but were not crushed. Naturally-senescing sieve elements and companion cells were seen. Prior to collapse, companion cells usually contained irregular aggregations of ribosomes. Plasmolysis of companion cells and sieve elements was evident, whereas adjacent parenchyma cells

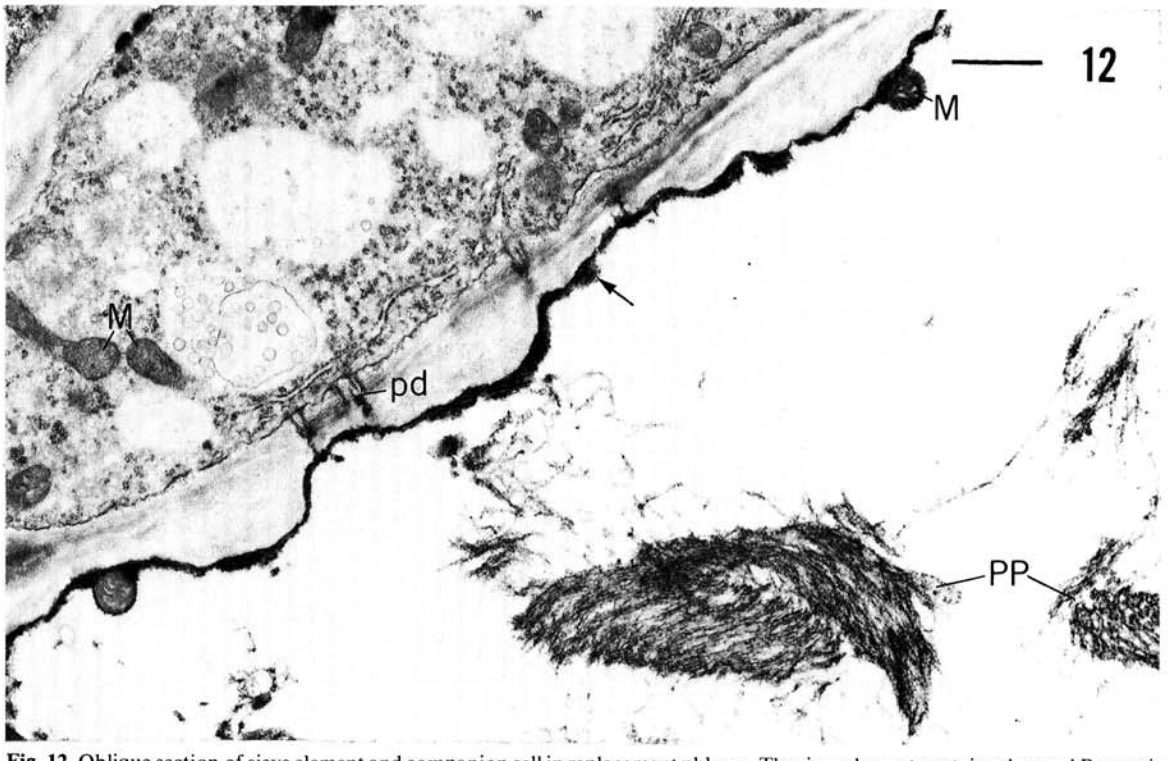


Fig. 12. Oblique section of sieve element and companion cell in replacement phloem. The sieve element contains clumped P-protein (PP); M, mitochondrion; pd, plasmodesmata; arrow indicates remnants of endoplasmic reticulum; scale bar = 1.0 μm.

Fig. 9-11. Collapsed cells in phloem of diseased elms. **9)** Sieve element with collapsed protoplast; calibration = 1.0 μm. **10)** Cross section of collapsed companion cell (CC) associated with normal appearing sieve element (SE); tcp, tannin-containing parenchyma cell; scale bar = 1.0 μm. **11)** Cross section of parenchyma cells and crushed sieve element (CSE); N, nucleus; M, mitochondrion; pl, plastid; sg, starch grain; er, endoplasmic reticulum; v, vacuole; scale bar = 1.0 μm.

appeared normal (Fig. 19).

In trunk phloem from diseased elms, virtually all sieve tubes in the replacement were crushed; those not crushed were occluded with callose.

Samples of secondary phloem from roots of three diseased trees and one healthy tree were collected in January and examined by LM. In phloem of healthy roots, all sieve elements contained callose deposits, although sieve plate pores were open in some sieve tubes (Fig. 20). In diseased roots, although there was considerable callose deposition, many sieve tubes contained only normal amounts of callose. Sieve tubes near the cambial region were not yet crushed. The sieve elements in this tissue were small and appeared similar to those in the replacement phloem of the stem.

DISCUSSION

These results corroborate the histological observations of McLean (11) and the association of MLO with PN symptoms as reported by Wilson et al. (23). In addition, the similarity of PN symptoms to those of other "yellows" diseases was confirmed; probable sequences of events in phloem degeneration were identified, and the discontinuous association of MLO with histological changes was made clear.

The histopathology of most plant diseases caused by MLO is characterized by sieve tube necrosis, which is followed by hyperactivity of the cambium (or procambium) and formation of excessive replacement phloem (16). Elm phloem necrosis fits this pattern well. The observations presented here are in basic agreement with those of McLean (11). Although McLean did not stress the sequence of degenerative changes in the secondary phloem, or test for starch or callose, he did notice sieve tube necrosis, excessive cambial activity, and hypertrophy and hyperplasia of phloem parenchyma.

Wilson et al. (23) reported MLO in root and stem phloem in diseased American elm, but did not specify the cell type within which the MLO were observed. They also reported that mitochondria in infected phloem cells (cell-type unspecified) contained dark-staining bodies that were released into the cell lumen when the mitochondria disintegrated. This condition was not seen in our material.

In the present study, MLO were sought by EM only in the secondary phloem of stems, and were seen only within mature sieve elements. Cells densely packed with MLO were not seen. Although individual sieve elements were not serially sectioned, many extensively sectioned blocks of affected tissue lacked MLO. Pores in sieve plates and lateral sieve areas were almost invariably occluded with callose prior to collapse of the sieve element protoplast. It is assumed that many collapsing sieve elements and companion cells did not contain MLO at the time of necrosis, although MLO may have been present at an earlier time. The observations gave no evidence that presence of MLO within a sieve element was a prerequisite for its eventual collapse. Within a diseased tree, no obvious differences in appearance or distribution of organelles, or in the structure of associated companion cells, were found between sieve elements that contained MLO and those that did not. Parthasarathy (13) found

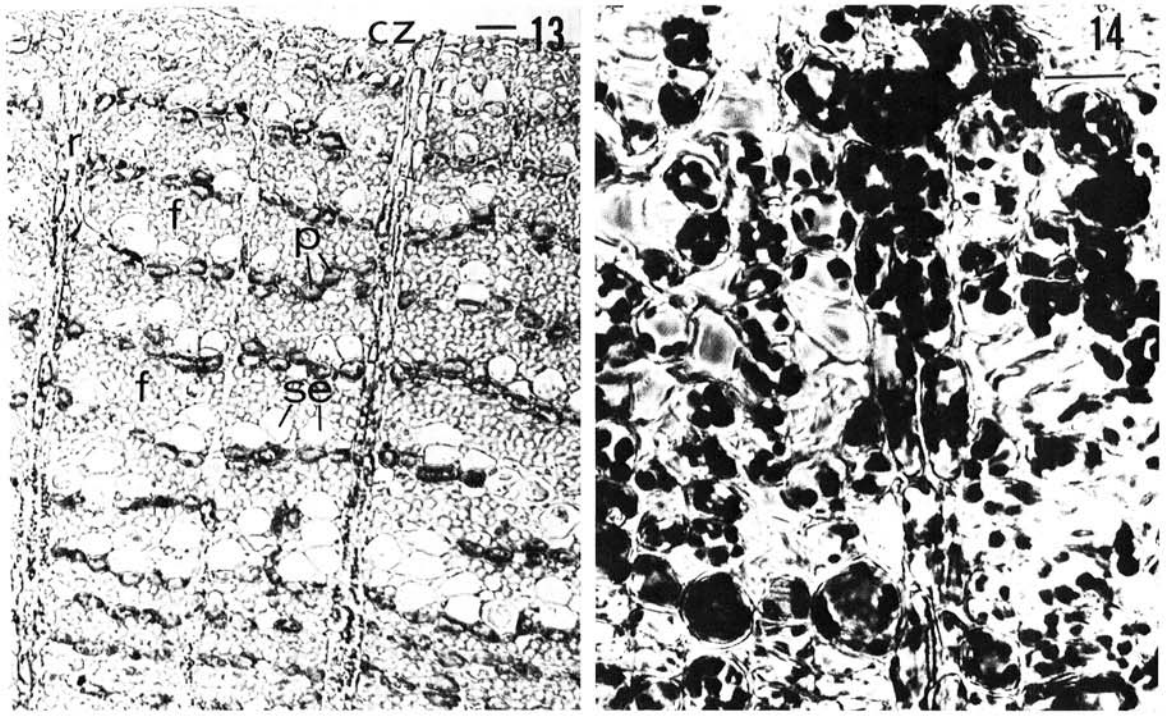
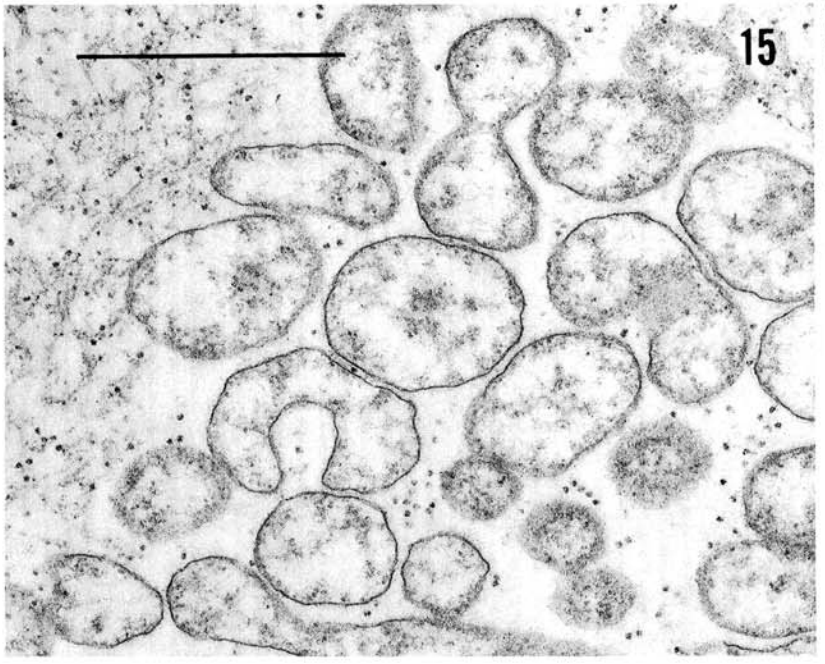


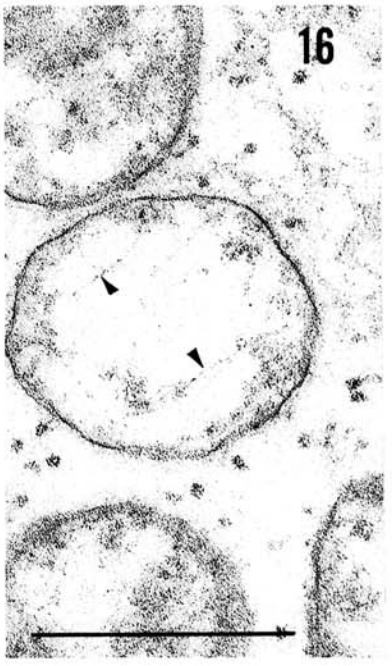
Fig. 13, 14. Light micrographs of cross sections through healthy and diseased secondary phloem respectively, stained only for starch. **13)** Healthy phloem showing tangential bands of fibers (f) and phloem parenchyma (p). Starch is present in phloem parenchyma and rays (r); se, sieve element; cz, cambial zone; scale bar = 25 μm. **14)** Increased starch deposition in replacement phloem (phase contrast); scale bar = 25 μm.

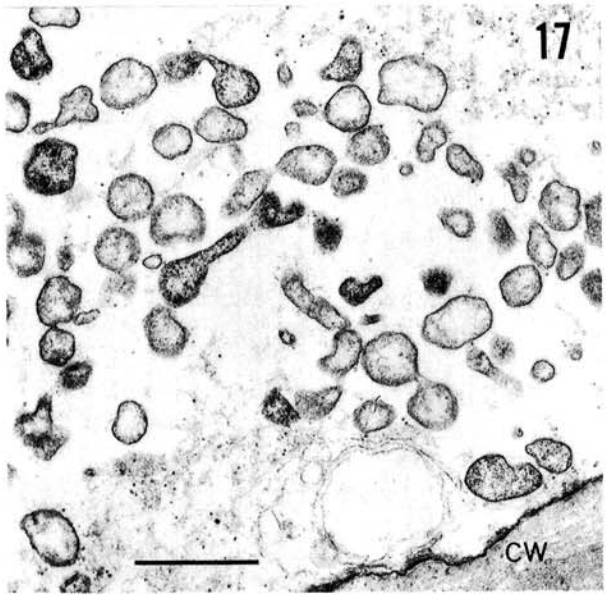
that most MLO-containing sieve elements in palms affected by lethal yellowing also appeared normal.

The sparse population of MLO observed in the aboveground portions of diseased elms, and the poor correlation between the presence of MLO and sieve tube necrosis, suggest that phloem degeneration is a secondary response to infection. McLean (11) noted that in diseased elms, feeder root necrosis was followed by death of larger roots, with necrosis of phloem eventually extending into the stem. Massive necrosis of phloem in the larger roots and root collar region could effectively girdle the tree. Many symptoms of phloem necrosis may be due, in part, to this girdling effect.

Schneider (14) found that girdling of healthy peach trees elicited anatomical responses somewhat like those seen in peach trees with X-disease. Distal to the girdle, callose deposition and necrosis of sieve elements and companion cells began with the oldest sieve tubes. Eventually the cambium became hyperactive, and produced both xylem and phloem. The new sieve elements were smaller than normal. These responses, except for the formation of new xylem, are similar to







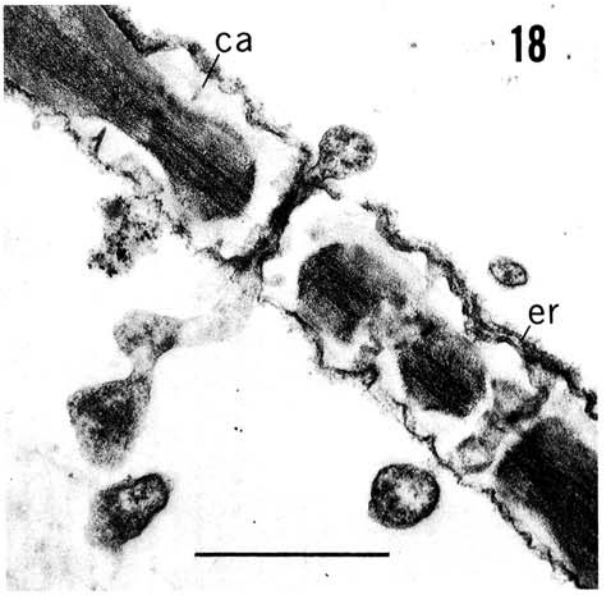


Fig. 15-18. Mycoplasmalike organisms (MLO) in diseased American elm. **15)** Group of MLO in sieve element showing various profiles; scale bar = 1.0 μm. **16)** MLO in sieve element; strands presumed to be DNA are visible (darts); scale bar = 0.5 μm. **17)** MLO, some with very dense cytoplasm, within sieve element; scale bar = 1.0 μm. **18)** MLO in pore of lateral sieve area; ca, callose; er, endoplasmic reticulum; scale bar = 1.0 μm.

those seen in elms with phloem necrosis.

Starch accumulation in the trunks of diseased elms could be a response to girdling. Starch accumulation has been noted in other plant diseases caused by MLO (16, 18), but whether this is due to impaired translocation or to a direct effect on starch metabolism is not known. Mechanical girdling of *Citrus aurantium* seedlings induced starch accumulation throughout the plant, including roots, even though carbohydrate transport from the leaves to roots was inhibited (22).

If one assumes that MLO move passively along the source-sink gradients in the phloem, as has been proposed for phloem-limited viruses (3), then observation of MLO only within mature sieve elements suggests that the phloem in diseased trees, including the replacement phloem, remains functional at least for a time.

Soma and Schneider (18) described sieve tube necrosis in leaves of pear trees affected with pear decline. They noted that cytoplasmic structures in companion cells seemed to "dissolve" before cell collapse. Whether this occurs in diseased elms was not ascertained. Not enough collapsing companion cells were seen to determine a sequence of events.

By contrast, collapsing companion cells were easily found in phloem tissue undergoing natural senescence (November collection), and some cells showed definite structural abnormalities prior to collapse. It seems likely that natural collapse occurs more slowly than that in diseased phloem.

Knowledge of the winter condition of sieve elements in *U. americana* is necessary for an understanding of the overwintering behavior of the pathogen. Sieve tubes in the phloem of trunks of both healthy and diseased plants appeared to be nonfunctional when sampled in November. They were either occluded with callose or crushed. Tucker (21) reported that some narrow, late-formed sieve elements in stems of *U. americana* remain viable through the dormant season and become functional again when spring growth resumes. We did not find such cells in stems. In secondary phloem from roots of both diseased and healthy trees sampled in January, some apparently normal sieve tubes were noted with LM. It seems possible that MLO could overwinter within these cells. Electron microscopy would be required for detection of such MLO.

Schneider (15) found that graft transmission of the pear decline agent was difficult when scions were taken from dormant branches which had undergone winter chilling. He also noted that after chilling, trees usually made a flush of normal growth which later was invaded by the pathogen. He hypothesized that the organism was inactivated by chilling or was confined to degenerated phloem, and that it remained viable only in roots or large branches. We have made similar observations, not detailed here, for elm phloem necrosis. Bark patches taken from stems of diseased trees in early spring and grafted into healthy stems were not infectious. It was not until a few weeks after spring growth began that patches

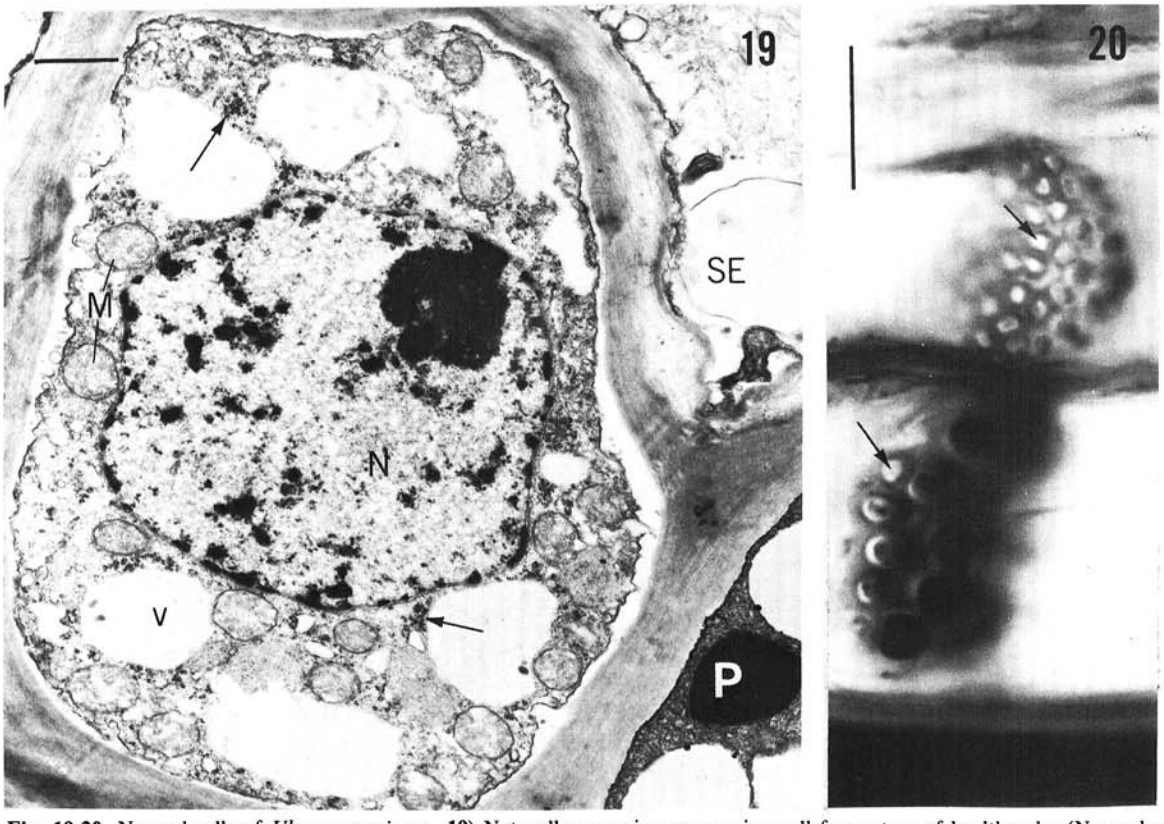


Fig. 19-20. Normal cells of *Ulmus americana*. **19)** Naturally-senescing companion cell from stem of healthy elm (November collection); arrows indicate aggregations of ribosomes; P, parenchyma cell, SE, sieve element; M, mitochondrion; N, nucleus; v, vacuoles; scale bar = 1.0 μm. **20)** Sieve plates from secondary phloem of healthy roots collected in January (light micrograph), stained with tannic acid-ferric chloride-resorcin blue; arrows indicate open sieve plate pores, scale bar = 10 μm.

from the stems of diseased trees contained effective inoculum. The transmission data and histological observations suggest that MLO overwinter in the phloem in roots of infected trees, moving into the upper parts of the plant after new functional phloem is produced in the spring.

LITERATURE CITED

1. BAKER, W. L. 1948. Transmission by leafhoppers of the virus causing phloem necrosis of American elm. Science 108:307-308.
2. CHEADLE, V. I., E. M. GIFFORD, and K. ESAU. 1953. A staining combination for phloem and contiguous tissues. Stain Technol. 28:49-53.
3. CRAFTS, A. S., and C. E. CRISP. 1971. Phloem transport in plants. W. H. Freeman, San Francisco. 481 p.
4. ESAU, K. 1969. The phloem. Part 2 of Vol. V *in* W. Zimmerman, P. Ozenda, and H. D. Wulff, eds. Handbuch der Pflanzenanatomie, 2nd ed., Gebruder Borntraeger, Berlin. 505 p.
5. EVERT, R. F., and B. DESHPANDE. 1969. Electron microscope investigation of sieve element ontogeny and structure in Ulmus americana. Protoplasma 68:403-432.
6. EVERT, R. F., C. M. TUCKER, J. D. DAVIS, and B. DESHPANDE. 1969. Light microscope investigation of sieve element ontogeny and structure in Ulmus americana. Am. J. Bot. 56:999-1017.
7. FEDER, N., and T. P. O'BRIEN. 1968. Plant microtechnique: Some principles and methods. Am. J. Bot. 55:123-142.
8. HINCHMAN, R. 1973. A permanent stain-mountant combination for starch in plant tissues. Stain Technol. 48:344-347.
9. HIRUMI, H., and K. MARAMOROSCH. 1973. Ultrastructure of the aster yellows agent: mycoplasmalike bodies in sieve tube elements of Nicotiana rustica. Ann. N. Y. Acad. Sci. 225:201-222.
10. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.
11. MC LEAN, D. M. 1944. Histo-pathologic changes in the phloem of American elm affected with the virus causing phloem necrosis. Phytopathology 34:818-826.
12. MOLLENHAUER, H. H., and C. TOTTEN. 1971. Studies on seeds. I. Fixation of seeds. J. Cell Biol. 48:387-394.
13. PARTHASARATHY, M. V. 1974. Mycoplasmalike organisms associated with lethal yellowing disease of palms. Phytopathology 64:667-674.
14. SCHNEIDER, H. 1945. Anatomy of buckskin-diseased peach and cherry. Phytopathology 35:610-635.
15. SCHNEIDER, H. 1970. Graft transmission and host range of the pear decline causal agent. Phytopathology 60:204-207.
16. SCHNEIDER, H. 1973. Cytological and histological aberrations in woody plants following infection with viruses, mycoplasmas, rickettsias, and flagellates. Annu. Rev. Phytopathol. 11:119-146.
17. SINCLAIR, W. A., and T. H. FILER, JR. 1974. Diagnostic features of elm phloem necrosis. Arborist's News 39:145-149.
18. SOMA, K., and H. SCHNEIDER. 1971. Developmental anatomy of major lateral leaf veins of healthy and pear-decline diseased pear trees. Hilgardia 40:471-504.
19. SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.
20. SWINGLE, R. U. 1938. A phloem necrosis of elm. Phytopathology 28:757-759.
21. TUCKER, C. M. 1968. Seasonal phloem development in Ulmus americana. Am. J. Bot. 55:716 (Abstr.).
22. WALLERSTEIN, I., R. GOREN, and S. P. MONSELISE. 1974. The effect of girdling on starch accumulation in sour orange seedlings. Can. J. Bot. 52:935-937.
23. WILSON, C. L., C. E. SELISKAR, and C. R. KRAUSE. 1972. Mycoplasmalike bodies associated with elm phloem necrosis. Phytopathology 62:140-143.