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Pathological Anatomy of Valsa Canker of Yellow Birch

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THE PATHOLOGICAL ANATOMY OF *VALSA* CANKER OF YELLOW BIRCH

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ABSTRACT

The purpose of this study was to determine by anatomical observation the pathogenicity of Valsa ambiens (Pers.) Fr. on yellow birch, Betula alleghaniensis Britton (B. lutea Michx. f.), seedlings. The results indicate that V. ambiens is only weakly pathogenic in active, vigorous seedlings. Some evidence of a pathogen produced toxin was present. In most instances the wounding reactions of the host were effective barriers to the spread of infection. These wounding reactions include production of tannin, gum formation, lignification of cell walls, suberization, cellular hypertrophy, callus production and periderm formation. The fungus resides intracellularly primarily in dead tissue. Longitudinally it moves easily through vessels in the xylem. Lateral movement is slow, the hyphae passing from cell to cell through the pits in the cell walls. The study includes a discussion of the healthy, wounded and infected anatomy of yellow birch.

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INTRODUCTION

Yellow birch, Betula alleghaniensis Britton (B. lutea Michx. f.), is the most valuable single tree species in the northern hardwood forest. Consequently much of the silvical research in this forest type has concentrated on problems associated with managing this species for timber production.

During the past decade research has solved, for the most part, the basic problem of prompt and adequate natural regeneration (Filip 1969, Tubbs 1969, Burton, et al. 1969). However, these as well as other studies of cultural techniques revealed several heretofore unknown pathological problems of young birch trees (Kessler 1970, Burton, et al. 1969). One of the diseases found was a canker disease caused by Valsa ambiens (Pers.) Fr. (Kessler 1970).

Reported here are the results of one of a series of studies initiated by the North Central Forest Experiment Station to determine the pathogenicity of Valsa on first year yellow birch seedlings. The study concerns the anatomical development of the fungus in the tissues of the tree after wounding. The healthy, wounded, and infected anatomy are therefore compared.

The main objectives were (a) to make observations of resistance mechanisms in the host and (b) to determine if the pathogen produces toxic substances acting on tissues in advance of hyphal invasion. Towards the end of the study additional inoculations were performed on dormant trees in a brief attempt to discover what effect the absence of wound healing had on pathogenicity of the fungus.

LITERATURE REVIEW

A. Valsa canker

The genus Valsa has long been recognized as a member of a complex causing extensive cankers and dieback in peach after pruning (Hildebrand 1947, Willison 1932). There are at least 22 species associated with 21 genera of hosts (Gilman, et al. 1957). Valsa sordida Nit. is commonly associated with cankers of poplar but is pathogenic in these species only when the host is in a weakened condition (Boyce 1948). Hubbes (1960) considers V. sordida to be a synonym for V. ambiens. Recently, some hybrid poplars have proved to be very susceptible to Valsa cankering (Bloomberg and Ferris 1963).

The causal fungus.--Valsa ambiens (Ascomycetes, Sphaeriales, Diaporthaceae, Valseae) has a wide host range including species of Pyrus, Morus, Ulmus, Celtus, and Betula. It is the perfect state of Cytospora ambiens Sacc. (Fungi Imperfecti, Sphaeropsidales). The imperfect pycnidial stage (fig. 1) of the life cycle is the one more commonly observed. Pycnidial stromata develop in the bark and break through as convex protrusions. Pycnidial chambers open into a common pore in the stroma. The hyaline, allantoid conidia (Pycnidiospores) develop in large numbers on the ends of short hyphae within the conidial chambers. The conidia are small, 1 μ x 2-11 μ (Hubbes 1960). Sticky white to yellowish conidial tendrils ooze through the pycnidial pore in moist weather (fig. 2). When moisture is excessive the spore masses may accumulate as globular masses at the pore mouths. The spores become distributed primarily by windblown rain thus traveling relatively short distances.

The perithecial perfect stage develops infrequently. An indistinct stroma of host and fungus elements forms under the bark with only the neck of the black flask-shaped perithecia projecting above the surface of the substratum. The ostioles (pores) are arranged around the edge of a grayish to black disk. Within the perithecia are narrow club-shaped asci that contain eight hyaline allantoid ascospores which are about twice the size of conidia, 3-5 x 14-24 μ , (Gilman et al. 1957). The spores that find their way into open wounds on trees may germinate and take part in stem cankering.

The disease.--A canker is defined as a localized wound or necrotic lesion which is often sunken below the surface of the stem and surrounded by healthy tissue (Husain and Kelman 1959). Valsa is a wound parasite establishing itself in the injured and dying cells on wound margins. Its invasion into surrounding tissues and subsequent canker development is dependent upon the developmental rapidity of defense mechanisms in the host. Generally, defense mechanisms are histologically manifest as a demarcation of infected lesions, callus-like swellings, cicatricial layers, abcission cells, tyloses, gum, or callosities formed on cell walls (Akai 1959).

Studies of Valsa canker in peach and poplar establish resistance mechanisms to be periderm barriers (Butin 1955), development of wound-gum (Willison 1932), tyloses (Butin 1955), tannin deposition in cell walls (Bloomberg and Ferris 1963), and callus formation (Willison 1932). Lignin deposition (callosities on walls) and tannins within a cell were found to have no effect on resistance in poplar (Bloomberg and Ferris 1963). The wound responses occur equally well with or without the presence of the fungus in poplar thus no irritating effect was

attributed to the fungus (Butin 1955). Peach exhibited more extensive wound-gum in infected material, a response thought to be indicative of a fungal toxin (Willison 1932). These gums slowed hyphal advance but did not stop it.

Conditions that reduce the normal host responses to wounding and healing favor development of the pathogen. Moisture deficits in stems were found to slow development of periderm and inhibit wound-gum and tannin formation in poplar (Bloomberg and Ferris 1963). Host dormancy and sub-optimal cool temperatures for growth favored development of V. cincta in peach (Willison 1932). V. cincta was found to be virulent in freshly made wounds during late autumn, winter and spring. It was also capable of invading callus during the dormant season thus giving rise to perennial cankers (Willison 1936). Rarely was infection observed during June, July, and August. The hyphae of V. cincta in peach (Willison 1932, 1936; Hildebrand 1947) and V. sordida (C. chrysosperma) in poplar (Bloomberg and Ferris 1963, Butin 1955) are considered to be intercellular in the tissues of the bark. Intracellular development in wood tissues has been observed for V. cincta (Willison 1936). Willison feels that cytospora survives in infected wounds from year to year in the wound gum region of the xylem being capable of bypassing gum plugs or penetrating them with slender penetration hyphae and appresoria.

B. Anatomical considerations of other cankers of hardwoods

Endothia parasitica in chestnut.--This fungus establishes itself in wounded bark of chestnut by mass action of mycelial mats. Secondary invasion occurs as individual hyphae further penetrate the tissues (Bramble 1936). Parenchyma cells are invaded and the cellular

contents change to an amorphous yellow-brown to orange substance which Bramble reports as oxidation products of tannins and generally termed wound gums. A lignin reaction was found to occur in cell walls of infected cells and in uninfected cells immediately adjacent to infection. The lignin reaction did not interfere with hyphal spread (Keefer 1914).

Vigorous seedlings inoculated with E. parasitica produced wound periderm adjacent to mycelial fans which were sometimes penetrated before the protective cork barrier had fully formed (Bramble 1936). This barrier was not found in rapidly killed seedlings. The formation of wound cork was preceded by disappearance of starch and accompanied by positive tannin reactions. The wound periderm was found to contain three or more layers of periderm as opposed to secondary periderm which contained only one. Hypertrophy of tissues occurred directly below wound periderm producing swelling on the stem. In rapidly killed seedlings, no wound periderm formed and no hypertrophy. Instead a constriction occurred due to drying out of dead tissues. In a later study Bramble (1938) reported deposition of gums and the formation of tyloses in xylem vessels, resulting in cessation of water movement.

Nectria canker on apple (Crowdy 1949).--Nectria galligena is a parasite that exploits all tissues outside the xylem but may also be found in the wood, invading xylem parenchyma, vessels and fibers. This pathogen is capable of bypassing wound periderm through direct penetration by aggregations of hyphae (i.e., mycelial mats) or by growing in the lumen of xylem and phloem fibers and emerging behind periderm barriers. The result of this invasion of barriers causes the host to produce a second barrier. The successive and repetitive invasion of the pathogen, followed by callus and periderm formation in

the host, produces a perennial canker that gives a target effect on the stem (thus the name "target canker").

The hyphae are checked by tyloses and gums in the vessels, and gums in parenchymatous tissues. In fibers there appears to be no defense mechanism thus allowing hyphae to spread beyond gum barriers.

Wiltshire (1922) reports that N. galligena is unable to directly penetrate living tissues. The effect of the mycelial mats in penetrating periderm barriers was reported as mechanical, but Crowdy (1949) speculates that an abnormal concentration of toxin from the fungal aggregate acts to kill tissues behind the periderm barriers.

C. Wound healing in woody plants

Initial phases of wound tissue development, cell division, and growth, generally are associated with decomposition processes of cells.

In the Valsa studies made on peach and poplar some note was made of the cellular responses to wounding that lead to the development of resistance mechanisms. Immediately after wounding, there is a general disappearance of starch from the cells in the wound vicinity. Willison (1936) found this to be followed by the formation of yellowish globules believed to be the precursors of gums. Butin (1955) reports a disappearance of chlorophyll and migration of cell nuclei in the direction of the cut surface.

Further information on the response of woody tissues to wounding are reported by Bloch (1941 and 1952), Kuster (1903), and Esau (1953). The following term descriptions will serve to clarify and review general tissue responses to wounding or other stimulus.

Callus is a proliferation of parenchyma tissue as a result of wounding or other stimulus. It arises from cambial zones and other

tissues that have not developed thick secondary walls. The cells show little or no differentiation, are homogeneous and have thin walls. If callus develops rapidly after wounding the cells may be loosely connected. More slowly developing callus lacks air spaces and is often arranged in regular rows with end walls aligned (Bloch 1952). Callus usually develops perpendicularly to the wounded surface. The following tissues are reported to take part in callus formation: cambium and cortical cells, phloem parenchyma, ray cells and undifferentiated xylem tissues (Esau 1953, Kuster 1903). The callus exposed to the air on external surfaces soon develops a layer of periderm or wound cork, which is one of the most striking processes of dedifferentiation in the callus.

Wound-wood refers to the layers of new xylem that develop after injury of stimulus. Anatomically the wound-wood is simpler than normal wood. According to Kuster (1903), with the resumption of tangential activity the cambial initials divide perpendicular to their long axis, resulting in short membered cells. The shortest cells occur closest to the wound. These increase in length as distance from the wound increases. Finally, a transition occurs from typical wound-wood to normal looking xylem elements and the wood again regains its normal composition. Kuster (1903) describes the early wound-wood cells as polyhedric parenchymatic cells; generally resembling those in medullary rays in normal wood; only a few developing characteristic tracheid-like wall thickenings; and with wood fibers and broad ducts lacking. A further complication of wound-wood development may be caused by the presence of parasites such as fungi (French 1969).

Hypertrophy is used to describe the enlargement without division that occurs to cells after injury. It may lead to the formation of callus (hyperplasia: Esau 1953, Kuster 1903) or to cell necrosis.

Tyloses are hypertrophies of parenchyma cells through pits into adjacent cells. They usually grow into non-conducting xylary tissues. Tyloses have a cell wall which may become lignified. The nucleus may move into the tylose. Kuster (1903) reports an increase in the number of tyloses after injury. In the young stems of birch tyloses are uncommon.

Wound gums frequently develop upon injury and/or infection and appear to be breakdown products of degenerating cells. These gums may collect in the tissues of the plant (xylem ducts, fibers, parenchyma, intercellular spaces, etc.) or if the cells are not completely broken down, form a layer on wounded surfaces or around zones of infection (fig. 32). This necrotic layer, referred to as a cicatrice, is broken down and absorbed as the wound heals (Esau 1953). Besides gums, the cicatrice contains lignin and suberin which serve as a temporary barrier to infection and further injury. They may also prevent moisture loss from underlying tissues and initiate conditions favorable for periderm formation (Kuster 1903, Esau 1953, Bloch 1941).

Tannins commonly accumulate in cells located near injuries or infections (Esau 1953). They are complex phenolic compounds with high molecular weight (between 500-3000) which are capable of forming effective cross links between proteins and other macromolecules (Swain 1965). Upon treatment with hydrolytic reagents, tannins tend to polymerise forming amorphous substances known as phlobaphenes (Swain 1965). The phlobaphenes generally become brown to yellow brown which

is very conspicuous in sectioned material (Esau 1953). They often appear as coarse or granular masses in the cell and stain deeply. In general, the term tannin is used in this paper in the wide sense to cover phlobaphenes and other tannin compounds. According to Esau (1953) the tannins may be found in vacuoles, in the cytoplasm and impregnated in the cell wall . She cites their function as substances protecting the protoplast against desiccation, decay, or injury by animals; as reserve related in some undetermined manner to starch metabolism; associated with the formation and transport of sugars; as antioxidants; and as colloids maintaining the homogeneity of the cytoplasm.

The greatest concentration of tannins appears to occur in dead or dying cells. "...their ability to inhibit enzyme systems may be important...(in these cells)..., and more especially in aiding the resistance of such cells to attack by fungi and other pathogens (Swain 1965)."

Swain further notes that tannins may not be responsible for all resistance of plants to infection, but merely assist the true factors of plant resistance by reducing the growth of pathogenic fungi.

MATERIALS AND METHODS

Sixty seedlings approximately 4 inches in height were selected from outdoor seedbeds and planted singly in 4-inch pots in the fall (October 15) before the trees had become dormant. They were transferred to the greenhouse and allowed to grow until they had reached a height of 10 to 18 inches in January. Fifteen seedlings were inoculated with distilled water as controls, and the remaining forty-five with a conidial spore suspension of Valsa ambiens prepared according to Kessler (Appendix). Inoculations were done with a thin dissecting

needle which was used to make puncture wounds through drops of the spore suspension placed on the second internode. After wounding, the plants were kept in a moisture saturated chamber for a period of 48 hours. The first seedlings were harvested 4 days after inoculation followed by harvests twice a week. Controls were harvested at every second collection of infected material. At each harvest the portion of stem around the inoculation point was fixed in F.A.A. under vacuum, dehydrated through a graded series of alcohols (70, 85, 95, and 100 percent) and imbedded in Tissuemat at 61° C. Serial transverse and longitudinal sections were made at 12 microns and affixed to slides with Haupt's affixative. A xylene spray technique published by Lin and Corlett (1969) was used to increase flattening of some paraffin sections. Several staining techniques were utilized (safranin-fast green; safranin-aniline blue; thionin-light green-orange G-erythrosin) but Johansen's (1940) quadruple stain was used most extensively. This was altered by reducing the staining with safranin from 24 to 12 hours. In some tissues even 12 hours of safranin staining produced very heavy "over" staining so that it was necessary to use a picric acid/alcohol solution to de-stain. Johansen lists the following staining effects:

Dividing chromatin-red

Resting chromatin-purplish

Nucleoli-red (occasionally violet)

Lignified cell walls-bright red

Cutinized cell walls-reddish-purple

Suberized walls-red

Cellulose cell walls-greenish-orange

Cytoplasm-bright orange

Middle lamellae-green

Starch grains-purple with green or orange halos

Plastids-purplish to greenish

Invading fungal mycelium-green

The tissues stained for this study show the following modifications and additions:

Cellulose cell walls - yellowish green to bright green

Invading hyphae - yellowish to only slightly green

Tannins - red to reddish purple

Degenerating nuclei - bright red to deep red

Cicatrice - bright red

(Other specific effects as described in the body of the paper).

All staining reactions discussed are those observed with Johansen's quadruple stain unless otherwise indicated.

Photomicrographs were made with a Zeiss microscope using Panatomic X and Ectachrome film.

The needle point method of inoculation resulted in low percentages of fungal infection. To improve the percent infection I devised the following technique: To produce the inoculum, V. ambiens was cultured on a 15 percent malt agar extract medium for 10 to 14 days. The inoculum was then applied to year old seedlings, individually potted and approximately 10 to 14 inches in height, into wounds made by scraping the bark with a sterile razor blade. The wounding was done at the base of the stem and an attempt made not to enter the wood. Small portions of the agar culture inoculum were then placed on top of the wound and lightly wrapped with dry sterile cotton, which served to hold the agar in place and reduce air-borne contamination on the agar

from outside sources. Controls were made with sterile agar. After inoculating, the trees were placed on greenhouse tables and allowed to incubate for 10 days after which the cotton wrappings were removed and the stems examined. At this time there appeared to be 100 percent infection as indicated by bark discoloration around the wound.

The scraping inoculation technique was also tried on dormant first year seedlings in an attempt to ascertain the best time for onset of infection.

RESULTS

Observations of cankers resulting from infections in which Valsa ambiens is present indicate they develop more rapidly in a vertical direction and towards the base of the tree initially forming an elliptical area of browning (fig. 3). Eventually the infected cells collapse and the bark becomes sunken in this region. The cankers produced by needle wounds and subsequent infection, develop around the wound which tends to determine the canker shape. Those cankers found in the field on larger stems are less elliptical and wound sites are not always visible. Field cankers show no particular shape except for a tendency to be somewhat elliptical. Many of these natural cankers believed to be infected with Valsa have developed around broken and injured branch stubs.

In an attempt to explain how Valsa ambiens develops in yellow birch seedlings the healthy, wounded and infected anatomy were simultaneously studied and compared. For clarity and ease of handling the results of these three areas are reported separately.

A. Anatomy of the normal first year stem[1]

The anatomy described here is that of the young differentiating stem tissues from the second and third internodes. It is at these sites that inoculations were made. These tissues are undergoing pronounced differentiation.

Epidermis.--The youngest epidermis consists of a single layer of cells and is covered by a cuticle. The surface is characterized by numerous unbranched unicellular hairs of two lengths (short hairs approximately 50 μ, long hairs approximately 1.5 mm) that stain heavily with safranin indicating lignified cell walls. In surface view the epidermal cells are more or less elongated in the direction of the stem axis with hairs appearing circular (fig. 15-f). In tangential section the cells are rectangular with an external surface convex to hemispherical. The internal surface is flat as in more mature regions. With maturation of tissues in the internode, the epidermal cells die and collapse, eventually being sloughed off as the phellogen begins to develop phelloderm. Stomata are not numerous, their long dimension directed along the stem axis (fig. 4).

Cortex.--The cortex is a simple tissue consisting of collenchymatous cells. The cells tend to be more than four sided in cross section and show intercellular spaces typical of the lacunar type as named by Mueller in Esau (1953). The cells are variable in size and may contain chloroplasts, starch, crystals (druses and prismatic crystals), oil drops and tannins. In general, cortical cells are reported to possess the ability to undergo reversible changes in wall thickness and to engage in meristematic activity (Esau 1953). Their walls consist mainly of cellulose and pectic substances and the pitting

1 Refer to figures 4 through 15, Plates I-VII

is simple. In longitudinal section the cells are rectangular and tend to be arranged in rows parallel to the stem axis. The cells are larger and more parenchymatous near the phloem. Cells containing druses are numerous and may be arranged in longitudinal rows or singly. Many cells are filled with an amorphous substance that stains purple with Johansen's quadruple stain. The substance is probably tannin.

In the subepidermal layers of the young cortex, periderm development begins from a row of cells that differentiate into phellogen by periclinal division (fig. 11). The inner cell becomes the phelloderm and undergoes no further division. The other cell becomes the phellogen which continues to develop and divide, producing the phellem whose walls eventually become suberized. Periderm is superficial and persistent for the life of the tree with cells conspicuously elongated tangentially (Eames and MacDaniels 1947). Initiation of periderm development is rapid, the appearance being simultaneous around the stem in the first year of growth. The periderm consequently is absent in very young stems and varies from one to eight layers with maturation. Lenticels begin to develop just prior to the general phellogen (fig. 5). Birch lenticels are large and elongate across the stem and continue to enlarge as the stem matures. Lenticels are always seen developing directly below stomata.

Pith.--The cells of the pith are homogeneous. In cross section they tend to be rounded leaving small intercellular spaces, while in the longitudinal plane they are rectangular and arranged in rows (figs. 8-9). The cytoplasm is filled with starch grains and occasionally oil droplets. The pitting in the walls is simple and readily visible.

Phloem.--The phloem tissue consists of sieve tubes (made up of sieve tube members), companion cells, sclerenchyma in the form of fibers and stone cells, and parenchyma (figs. 11-14). The sclerenchyma is arranged in a regular ring at the outside edge of the phloem tissue. Bundles of fibers with thick cellulose cell walls (stain light green) and very small lumens are interspersed with groups of stone cells. The fibers are very long and pitting is not readily evident. The stone cells are characterized by thick secondarily lignified walls and simple pits with long canals (fig. 15c). These cells eventually lose their protoplasts and the lumens may become filled with tannins. When longitudinal sections of stem are viewed the stone cells are arranged in rows consisting of a few to many of the generally box-like cells (they may take on irregular shapes).

Cells containing crystals (prismatic crystals and druses) are common in the phloem just as they are in the cortex. Cells containing druses are usually arranged in regular longitudinal rows (fig. 15a), and are of the parenchymatous type. The phloem parenchyma is located in the rays and also interspersed throughout the other tissue cells. Starch and lipids are often seen in this parenchyma. The cytoplasm in some cells is granular and stains lavender to a light reddish-lavender. This is indicative of tannin.

The sieve tubes have sieve areas on their side walls as well as on the terminal sieve plate (fig. 15a). Esau (1953) reports a nonstratified phloem in Betula with elongated sieve tube members bearing mostly compound sieve plates on the inclined end walls with the sieve areas of the sieve plates distinctly more differentiated than the lateral sieve areas. Older phloem cells exhibit slime plugs

(figs. 10-11). Usually two companion cells are associated with a sieve tube, the three cells being derived from the same meristematic initial (Esau 1953).

Xylem.--This tissue consists of vessel members (cells perforate on their common end walls), tracheids (imperforate cells having only pit pairs on their common walls), parenchyma (wood ray and intervascular), and fibers. The wood is diffuse porus with the vessels small in cross section and arranged in a loose oblique pattern commonly in groups of 2 to 5 cells (figs. 11-13). The tangential diameter of vessels is 50-100 with from 9 to 31/sq.mm (Metcalfe and Chalk 1950). These figures are different for younger stems with vessel member tangential diameter being approximately 30-70 with from 70 to 100/sq.mm. The perforation plates on common oblique end walls are scalariform having approximately 20 or fewer bars (figs. 7, 8, 9, & 15b). The mean member length of vessels is 0.6 to 1.2 mm (average 0.9 mm) (Metcalfe and Chalk 1950). Pit pairs are typically alternate and minute with the pits to ray and wood parenchyma similar to the intervascular pitting but only half bordered. The pits in vessels are bordered (fig. 9).

Tracheids have no perforation plates but long tapered ends. They are longer than the vessel members and have larger bordered pits in their walls (figs. 9 & 15b). The fiber types are not highly specialized and are generally fiber-tracheids. As Esau (1953) notes, extreme fiber types most highly specialized as supporting elements are probably lacking in the Betulaceae since the vessel members are tracheid-like and also serve a supporting function. Primary tracheary elements vary from annular and helical to scalariform (fig. 10). Late primary and

early secondary wood elements intergrade. Bordered pits in fibers are not numerous and the border is considerably smaller than the pit itself (fig. 9).

Parenchyma is distributed throughout the entire annual ring (a feature not usually found in the higher dicotyledons (Hoar 1916) and is apotracheal and rather sparse (Metcalfe and Chalk 1950). The medullary rays are one or two cells wide in young stems, rarely wider. The cells (figs. 15b, 15d, & 15e) are homogeneous and elongated in the radial direction. The parenchyma cells contain starches, tannins and crystals. The wall is interrupted with simple pitting.

Cambium.--The cambium is unstoried (fig. 16b) which phylogenetically groups the birches with angiosperm species of a primitive nature. The medium to very long vessel members with highly inclined tapered ends (fig. 8) and scalariform perforation plates verifies this position, as does the parenchyma distributed throughout the annual ring.

B. Effect of wounding on yellow birch tissues[2]

Wounding incites distinct anatomical changes in the plant tissues which vary according to cell type, age and position. Injured cells generally become necrotic and dry up soon after wounding and exposure to the atmosphere. The cell walls collapse and cell contents degenerate into gums forming a cicatricial layer (fig. 17). Unstained layers are yellowish brown to red-brown. Stained material is usually bright red.

Those cells immediately adjacent to the wound that are not completely destroyed begin to hypertrophy. Within 5 to 10 days after wounding callus development has begun and continues until the wound has healed over. In birch, callus develops from cambium parenchyma

2 Refer to figures 17-33, Plates VIII, IX, X, XI, and XII

of the xylem and phloem, cortex and most other undifferentiated living cells. In reference to callus in general, the cells may be much larger than those from which they were derived and contain large nuclei. This could be an indication of polyploidy which is reported to occur in callus although it can be neither proved or disproved by this study. Multinucleate cells in callus have been frequently observed in this study (fig. 20). When the wound is not severe, such as might occur from shallow lesions, or in areas adjacent to more severe breaks, the cambium forms a wedge of callus and wound-wood (figs. 18, 21, 26, 27, & 28). In large openings callus grows out from all edges (particularly the lateral sides) forming callus rolls which meet at the wound center (fig. 33). The callus cells adjacent to existing cambium accept the role of cambial initials and a layer of cambium redifferentiates through the callus. Esau (1953) likens this action to the closing of a diaphragm.

A general wounding response observed in living cells is an increased affinity for stain (figs. 18, 21, 22, & 27). This is probably due to tannins and gums. A nitroso test (Jensen 1962) performed on injured healing tissues gave a strong positive tannin reaction. This test, which is recommended for fresh tissue, was performed on tissues fixed in F.A.A. so there may be some question of the validity in the results.

The staining response continues to increase in stems harvested toward the end of the study. The substances are not necessarily associated with vauoles but homogeneously distributed in the cytoplasm tending to mask other cell contents. Developmentally the deeply staining material follows the diminuition of starch and begins as globules (fig. 49) that accumulate and fuse, eventually filling the

entire cell. This is probably the same material that leads to gums in vessels and on exposed surfaces. This reaction will be further discussed in the infected anatomy as the response was more widespread in the presence of the hyphae.

The needle wounds introduced in these small stems were not uniform resulting in variable wound healing. Long slit like wounds show less wound response than wide wounds. In the long wounds callus formation was not as abundant, gumming not as severe and less wound-wood was produced. All responses described occurred in the first month after wounding.

Cortex and periderm.--The cortical cells do not respond with profuse callus production immediately after wounding. Injured cells atrophy and become part of the cicatricial layer (fig. 17). Below this a wound periderm develops from cells dividing parallel to the wound surface. Eventually the wound periderm becomes connected with the primary periderm. The wound periderm is different from primary periderm in that the phellogen produces several layers of phelloderm, as compared to the single layer found in primary periderm (figs. 23 & 24).

The process of suberization can be observed as periderm develops in young stems and around injuries. The cells external to the phellogen begin to undergo cytoplasmic changes (fig. 24). The cytoplasm stains lavender and is finely granular. The nucleus, which is oval to orbiculate and stains differentially in recent phellogen derivatives, shrinks and stains deep red. Eventually the cytoplasmic material becomes darker and the nucleus disappears. This same process occurs under layers of cicatrice and around areas of infection isolated by fungi, although in these instances the cells seem to hypertrophy before

suberization. This often results in an irregular layer of suberized cells as compared to the very regular cells in normal cork (fig. 23).

The cells in the region of the wound show a marked decrease in starch. Sometimes callus forms before periderm is produced. Whatever callus does develop from the cortical cells soon joins that from the cambium and phloem parenchyma to form a continuous mass. Callus cells containing druses are numerous in the cortical region but show no apparent pattern of arrangement.

The periderm and epidermal cells loosen for some distance on all sides of the wound, forming cell layers that are very irregular. This appears to be the result of pressures from internal callus development (hyperplasia).

Phloem and cambium.--Wound response in the phloem tissue was the most difficult to interpret. The cells formed in the region of the phloem after wounding are mostly parenchymatic callus type (figs. 23, 26, & 28). Since the cambial initials divide transversely and become shorter cells the first sieve tube elements produced after resumption of tangential division are short (fig. 25). As in xylem, the first new rows of phloem are not as differentiated as normal tissue. The tissue passes through a transition before returning to normal (fig. 29). In the parenchymatic callus type phloem, fibers and stone cells are conspicuously absent as are the barrel shaped, regularly arranged rows of parenchyma cells in uninjured phloem.

Sclerenchyma tissue is not readily affected by wounding, especially fully developed cells. Those young cells in the processes of forming secondary wall may be stopped from developing further where wounds are incurred. It does not appear that any extensive amount of

wall readsorption occurs so that the cells may return to a meristematic condition. Stone cells are seen to contain wound gums and tannins in their lumens (fig. 21). Fibers are only affected mechanically, as when sclerenchyma is displaced from its normal position by the development of callus to its interior (figs. 18-33). It is not unusual to see stone cells surrounded by a mass of callus (fig. 28). It appears that the callus cells differentiate into sclereids.

Xylem.--Xylem ray parenchyma responds to wounding by dedifferentiation and division. This activity acts to fill in an area opened by a wound, producing a "filler" callus (figs. 17, 18, 28, & 30), or may form raylike structures several times wider than normal with larger, irregular cells (fig. 30). This tissue often produces pressures inside the stem causing external swelling and distortion in the wood structure (figs. 29 & 30). The cells have simple pitting and are lignified. Secondary wall thickenings develop as the cells mature. Usually the ray initials are stimulated to the greatest amount of activity. Depending on the severity of the wound, the rays eventually return to normal ray structure, one or two cells wide.

Wound-wood consists of short tracheid and vessel-like cell members, but with larger pit apertures and narrower borders (fig. 31). Besides being short the cells may be slanted and twisted with respect to the longitudinal axis of the stem resulting in a gnarled appearance (figs. 26, 27, & 29) (some cell pairs have perforation plates which are seen to cross at almost 90°). In cross section, large vessel members are absent for some distance beyond the site of wound response (fig. 28).

In longitudinal section there are cells with characteristic vessel patterns but they are quite short and no wider than adjacent fiber-tracheid like cells or the parenchymatic cells (fig. 29). Vessel members and fiber tracheids in the wounded region often contain gums which increase as the wound becomes older.

In a few instances tylose development is noticed in vessels above and below wounds. They are not extensive, although it is possible that they would increase as the stem continues to develop.

Pith.--Upon injury pith cells may react by cell division and form small amounts of callus (fig. 32). In some instances, pith cells just divide without producing a callus (fig. 19) and most often there is no response by division. Stored products decrease, and starch disappears more rapidly than oils if they are present. Cell contents are often altered so as to become homogeneous and deep staining (as described for parenchyma), granular (fig. 39), or globular (fig. 50).

C. Anatomy of infected stem[3]

Much of the response observed in the presence of infection was the same type of response made by the wounded stem just described with the exception that less wound healing occurs and tissue reactions to wounding are in general more intense. Living cells are easily differentiated from necrotic cells by their differential staining, lack of plasmolysis, and lack of cytoplasmic disintegration. At wound sites the pathogen occupies dead tissues and is often present in hyphal masses in and around the collapsed tissues (fig. 35). These hyphae could be termed intercellular (or extracellular) although there is no definite cellular structure. When tissue organization is present, the hyphae develop within the cells. In all the tissues

3 Refer to figures 33-50, Plates XII-XVI

examined only once was a hypha viewed to be growing in the middle lamella. This occurred in the xylem tissue between two fibers. Because of this one exception the possibility of this pathogen existing intercellularly in organized tissues cannot be excluded. Hyphal size varies from very thin elongating strands (diameter 5μ or less) to short thick, almost globose, structures (up to 15μ in diameter). Hyphae in open wounded areas are in general of the smaller type. In recently living cells much coiled, often thick, hyphae are usually observed (fig. 36). This size difference outwardly appears to be a function of available foodstuffs. The hyphae traveling in the vessels of the xylem will be large near wounded and necrotic areas but thin out in mass and size as distance from the area of maximum nutrient availability increases. In review, the following responses occur in wounded tissues: Tannin production (within cell vacuoles, cytoplasm, and impregnated in cell walls); gum formation; lignification of cell walls; suberization; cellular hypertrophy usually leading to necrosis or division, diminuition of starch; callus production and periderm formation.

Cortex and periderm (epidermis).--To introduce infection into stem tissues these layers must be damaged. Valsa is a wound parasite and does not directly penetrate healthy cortex or periderm. The parasite establishes itself in the injured cortical cells, initially moving from cell to cell through the pits. As the number of infected cells increases walls become disorganized and collapse resulting in the brown sunken area on the stem surface. Cortical and epidermal cells may contain numerous hyphae (fig. 35). Suberized cells of the primary

periderm are not attacked although they appear loosened and buckled with hyphae growing on the surface.

The cortical cells, subsequent to invasion, are devoid of starches and chloroplasts (fig. 49). Druses are unaltered in structure, even though the cells that contained them are gone. Gums are sometimes present in the intercellular spaces. Cells on the margins of infection divide parallel to this area and form a "wound" periderm (fig. 37). On open surfaces callus usually forms, the outer layers of which usually produce a periderm (fig. 33). Callus may also develop around infection as if to produce an enclosure (fig. 38). Callus without periderm, but bearing a layer of cicatrice have hyphae inhabiting the outer layers. In these instances it appears as though the cicatrice is enhanced by the presence of infection. There is no evidence from any of the slides examined that hyphae are capable of penetrating wound periderm or callus tissue once it is well established. Of course, it is possible for these barriers to be by-passed.

It appears that uninjured turgid cells are not easily infected by this pathogen.

The tissues examined seem to indicate that hyphae are capable of keeping a wound open by causing necrosis of outer layers of callus without direct cellular penetration. It does not occur in all tissues which could be an indication of plant vigor. While Valsa may not be the responsible pathogen for natural cankers, it may produce environments suitable to more pathogenic successors. Once the periderm or callus is developed no further infection occurs in cortical cells.

Phloem and cambium.--After wounding, uninjured cells of the phloem respond to produce very intense staining reactions which act to obscure cell contents making observation of hyphal development extremely difficult if not impossible. Some inference can be made of hyphal movement from the amount of necrotic tissue present beyond the wound site. Infected cambium shows the same response as phloem. The cells are necrotic. This subsequently results in cessation of normal stem development (fig. 34). The wound response of undamaged phloem and cambium tissue to produce callus is usually rapid and the hyphae are walled off. Callus and subsequent periderm formation from cortex, phloem and cambium together produce an effective barrier to invasion of "bark" by the pathogen (fig. 37).

Mature sclerenchyma in the phloem shows little direct response to hyphae other than that mentioned as a response to wounding. Fibers that have not completed secondary wall development may become infected with hyphae.

Xylem.--Hyphae are found abundantly in the vessels (figs. 36 & 41) where they travel easily through the scalariform perforation plates (fig. 40). This leads to great progress in a vertical direction. Lateral movement occurs through pit pairs and is not rapid. A hyphae becomes much constricted when passing through a pit pair only to enlarge again once the walls have been traversed (fig. 42). No direct penetration of cell wall was observed but these are young infections and the cell walls may not be sufficiently weakened.

Young medullary rays react much the same as other parenchyma cells by division (fig. 38) or by accumulation of deeply staining cytoplasm (fig. 36). These reactions are preceded by loss of starch. The globules

give a positive test for tannins in old infections. Older medullary cells plasmolyse, the cytoplasm appears viscous and does not always accept stains readily. Sometimes the cytoplasm appears granular (fig. 40). Nuclei may lyse followed by cell degeneration. Ray cells may finally succumb to hyphal penetration through the pits. Vessels often contain gums as a result of exudation from adjacent parenchyma cells (figs. 43, 44 & 45). The gums form plugs of varying staining characteristics which may be indicative of chemical activity (fig. 44). Although this might be a response to the hyphae it appears to be a lignification process. Initially the gums seem to interfere with hyphal spread but many instances of hyphal penetration are observed (fig. 45). Gumming appears to be more severe in the presence of the pathogen. Instead of gums, granules may accumulate in vessels. Sometimes they accumulate around pit apertures. Tyloses are infrequently observed and, although they seem to be more numerous than in wounded stems, probably pay no role in resistance at this early stage of infection. Wound-wood exterior and adjacent to infected xylem was never observed to be infected.

Pith.--When heavily infected this tissue is greatly disorganized (fig. 46). Cell walls are broken with particulate matter scattered in the openings. It probably consists of cell wall fragments, dead hyphae, and dry cytoplasmic contents. The breakdown products and gums observed on exposed surfaces are not observed. Small tyloses from adjacent pith cells may grow into open areas (figs. 47 & 48). In living tissues of the pith the middle lamella normally stains dark green yet in some instances it takes on a red color near infection. Cell walls of living cells show similar alteration in stain affinities. Normally

staining bright green (for cellulose) they may stain bright red. This is probably an indication of a lignification response to infection. According to Esau (1953), the lignification processes begin in the middle lamella and spread inward through the cell wall. In one stem examined the walls seemed swollen with no color change as if in the process of breakdown. All cells in an infected region are not always attacked so that nucleate cells may be in close association with the pathogen.

The cytoplasm of pith cells may show a deep staining response similar to that observed in medullary rays or be very granular (figs. 39 & 43). Starch abundantly seen in uninjured stems is lacking although oil droplets are numerous. This response is similar in injured uninfected stems. In a 30-day-old canker, a mycelial mat had formed in the region of the pith but one side of this wound had remained open.

D. Results of scrape wounds

Although time did not permit histological observation of fungal development in scrape wounds on actively growing and dormant trees, the following morphological observations were made.

On actively growing trees the wounds covered by agar containing hyphae had an area of brown sunken bark around the wound that was significantly larger than on check wounds. This observation was made 10 days after inoculation, a period during which the wound and agar were covered by cotton. Subsequent observation indicated that callus had developed completely over all wounds, checks and infected, except one infected stem.

On dormant trees the scrape inoculations were performed and the trees allowed to remain in dormancy for 5 weeks. The trees were then moved to the greenhouse and allowed to break dormancy after which time observations of inoculation sites were made. With new activity commencing in the stem, areas of sunken brown bark occurred around the wound sites that were larger than in "growing" trees. Signs of callus development were already visible in check stems but not on infected stems.

It appears that tree dormancy may allow V. ambiens to develop in the tissues of the bark. To what extent the fungus grows and the kind and extent of wound responses after the tree breaks dormancy is speculative at this point, presenting an area for further study.

DISCUSSION

This study illustrates the complexity of interaction between host and pathogen. V. ambiens appears to be a facultative parasite in yellow birch seedlings. In actively growing trees the reaction to wounding is effective as a defense mechanism to the spread of infection. Callus production, cicatrice, and periderm formation devloping over tissues external to the wood, prevent hyphae from spreading in the "bark" tissues. The cells of the xylem, being incapable of forming callus except at the rays, do contain hyphae which were introduced by means of spore suspension at the time of inoculation. The hyphae spread in the xylem in a longitudinal direction encountering only gum plugs which they are capable of penetrating. Unlike peach infected with V. cincta (Willison 1932) there were no appresoria or specialized penetration hyphae observed. From all indications in this study, the gums are degradation products of starch and protoplasm. Because hyphae are

observed to grow in necrotic parenchyma cells that contain gums, it is quite possible these gums provide a source of food material. The gum reactions following wounding are greater in infected material although gums and hyphae dwindle as the distance from wound site increases.

Hyphal development in a lateral direction is not rapid as the hyphae must pass through the pits in the cell walls. The unhindered longitudinal movement in the tissues of the xylem probably accounts for the external elliptical shape of cankers. There is no indication that, at this stage of development, wall breakdown has occurred in xylem. Neither was there evidence of mycelial mats, as are observed in Endothia and Nectria cankers (Bramble 1936, Crowdy 1949), which mechanically move through tissues.

To be pathogenic, the fungus should cause damage to cambium and phloem so as to girdle the tree. Girdling often occurs in natural cankers from which Valsa is isolated (along with other fungi). This did occur on one stem examined in which there was an absence of normal wound response. The hyphae had occupied the cortical cells, phloem and cambium within 8 days after inoculation.

The extent to which tissues are killed in advance of the fungus is not altogether clear. The damage to tissues by wounding was quite variable in these very small trees resulting in a range of healing processes. In general, the wound reactions were more intense in the presence of the hyphae. The fungus appeared to be responsible for increasing necrosis in tissues adjacent to infected regions thus acting to keep wound sites open. If this occurs in field cankers, these sites are suitable for invasion by other wound pathogens. Hindrance of wound healing may certainly indicate toxin production by the pathogen,

acting directly on cells, or causing further stimulation of hormonal action on the part of the host. The fungus was never observed directly in living cells and was usually associated with necrotic tissues. In only a few isolated instances were nucleate, differentially staining (living) cells observed in close proximity to hyphae. This would seem difficult to explain if the hyphae produce toxins capable of causing necrosis of living tissues in advance. A longer period of observation on developing stems after inoculation might possibly lead to more conclusive evidence on the presence of toxins.

Yellow birch is capable of walling off infection from V. ambiens during the growing season especially in wounds that do not enter the xylem. The evidence available from inoculations on dormant trees suggests that conditions interfering with natural wound healing favors spread of infection in host tissues. Temperatures suitable for fungal growth are likely to occur during the fall and spring months thus giving the fungus an opportunity to establish within inactive host tissues. Any further stresses that favor fungal growth and interfere with tree growth could lead to stem girdling with subsequent dieback or death in seedlings. Field cankers seem to advance most rapidly the year following infection. It is very possible that V. ambiens behaves like V. cincta (Willison 1932) invading callus during the dormant season.

In summary, Valsa ambiens seems to be only weakly pathogenic in active and vigorous seedlings. Evidence of toxin production is present but not conclusive. This study indicates that the reactions of the tree to wounding are sufficient, in most cases, to wall off the spread of infection during the growing season and makes clear the importance of investigation on stress factors and subsequent canker development.

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FIGURES

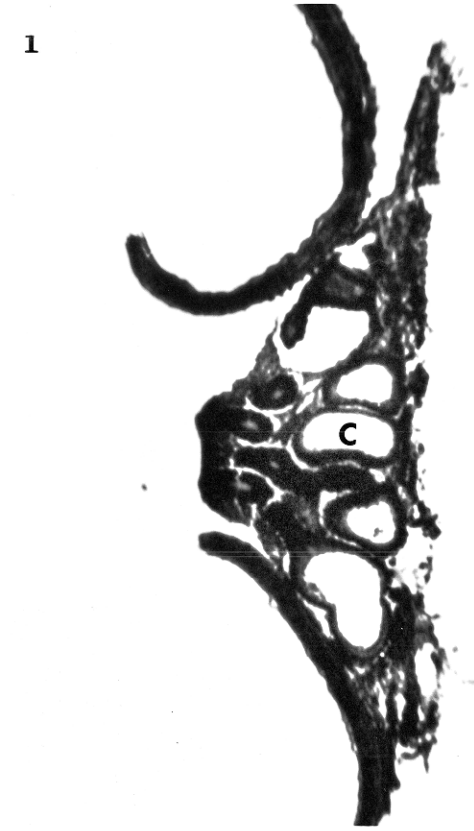
PLATE I

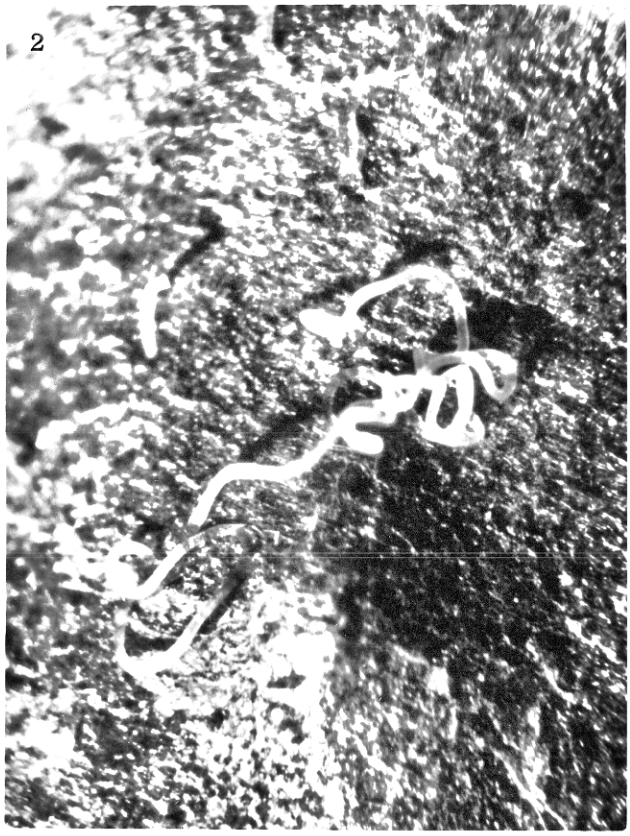
Fig. 1. Pycnidia of Valsa ambiens (Pers.) Fr. (Cytospora ambiens Sacc.) showing pycnidial chambers (C) erupting through bark (P). x40.

Fig. 2. Conidial tendril. x40.

Fig. 3. Valsa canker on year old yellow birch seedling. Note elliptical shape. x½.

Fig. 4. Stomate in surface view. Lenticels develop directly below stomata. (Fresh, unstained stem peal) x600.





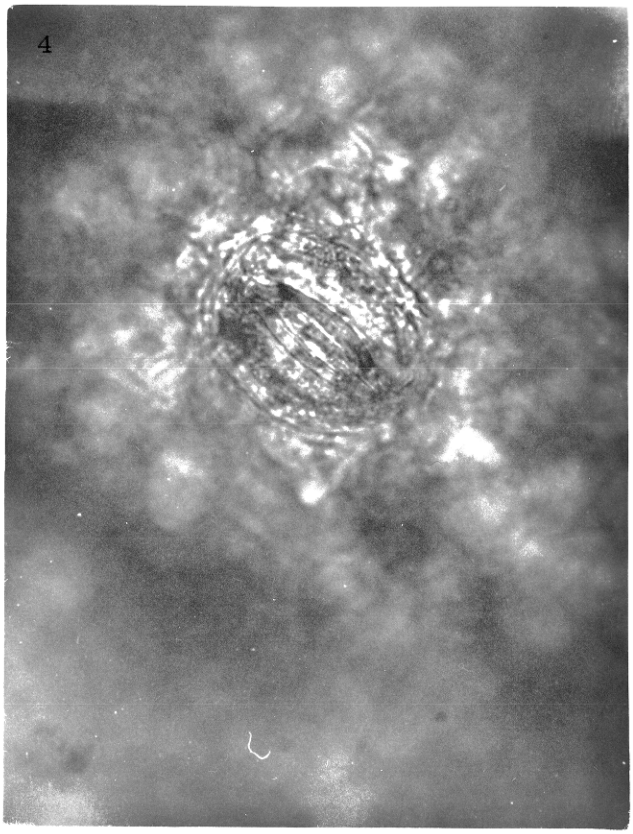


PLATE I

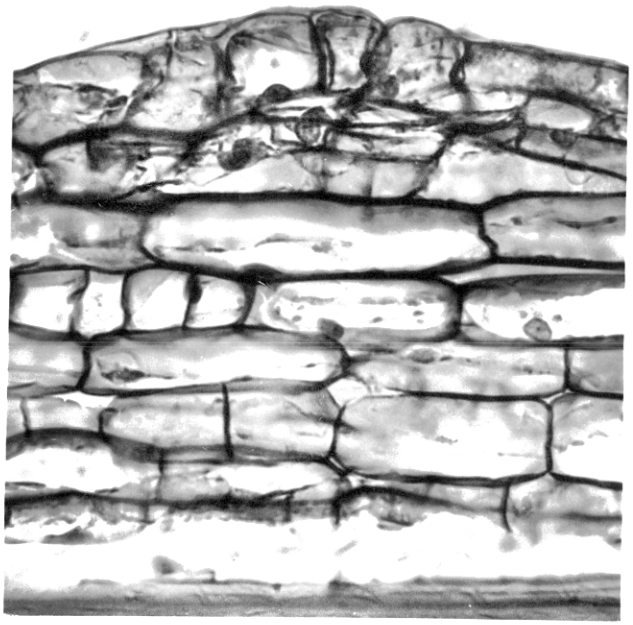
PLATE II

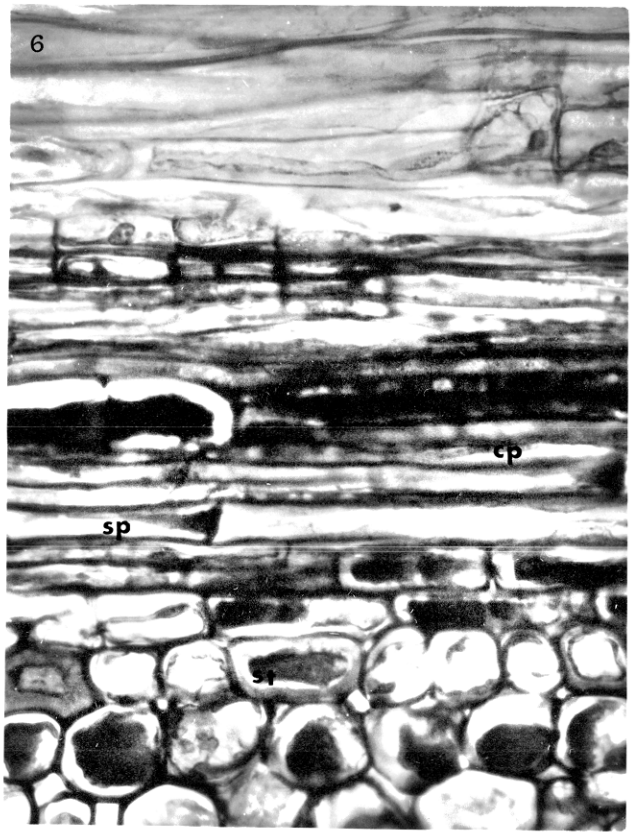
Fig. 5. Lenticel developing below stomate. Longitudinal section. x600.

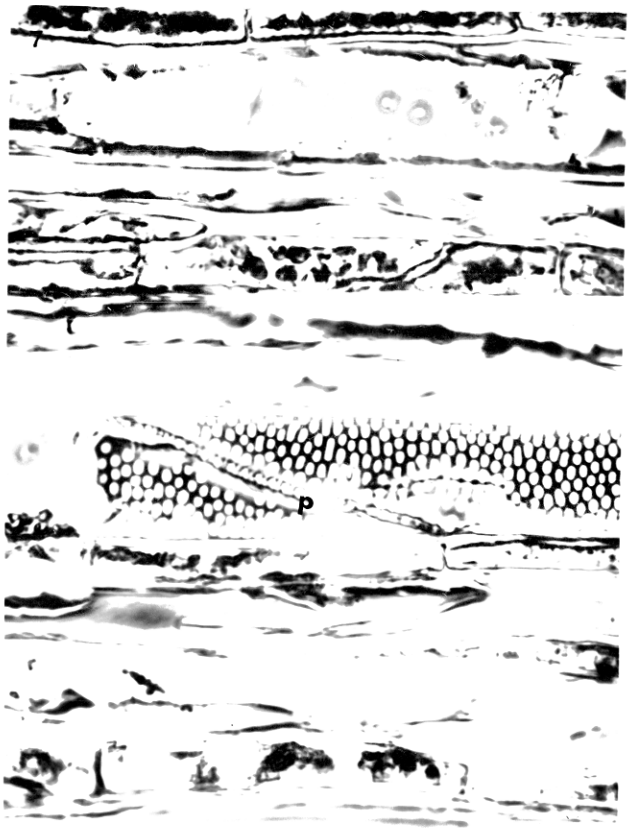
Fig. 6. Longitudinal section of phloem showing slime plug (sp); companion cell (cp); and stone cell (st). x530.

Fig. 7. Tangential section of vessel member showing perforation plate (p). High contrast copy film. x600.

Fig. 8. Radial section of vessel member. Note scalariform perforation plate and long tapered end to vessel member. x600.







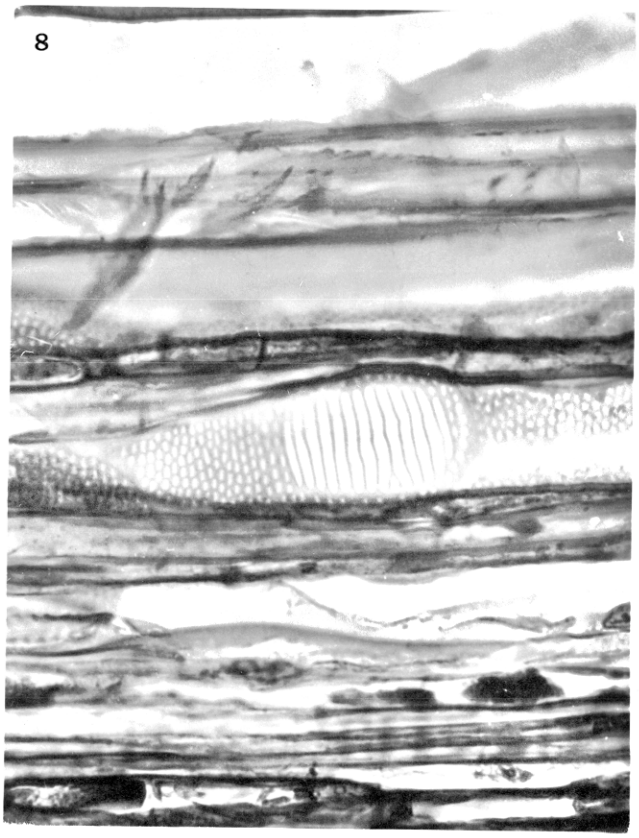


PLATE II

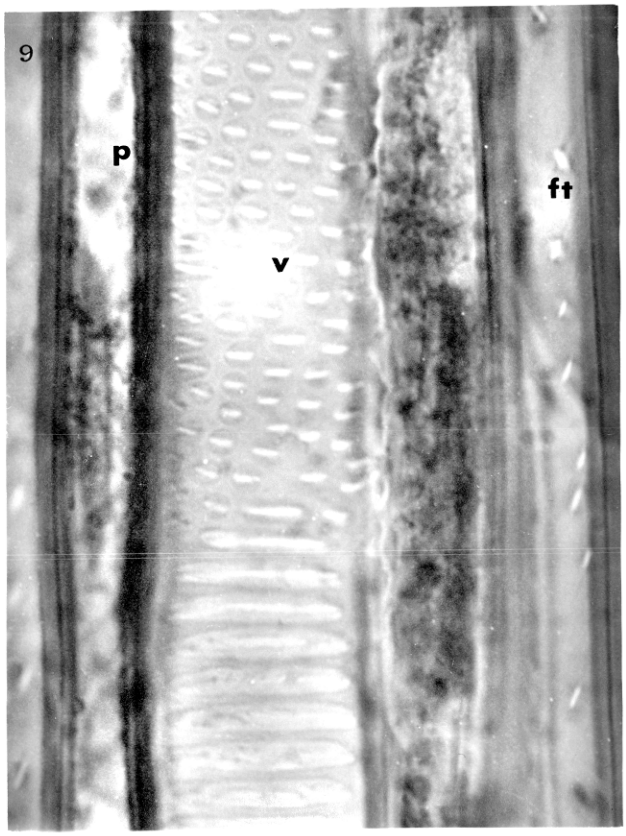
PLATE III

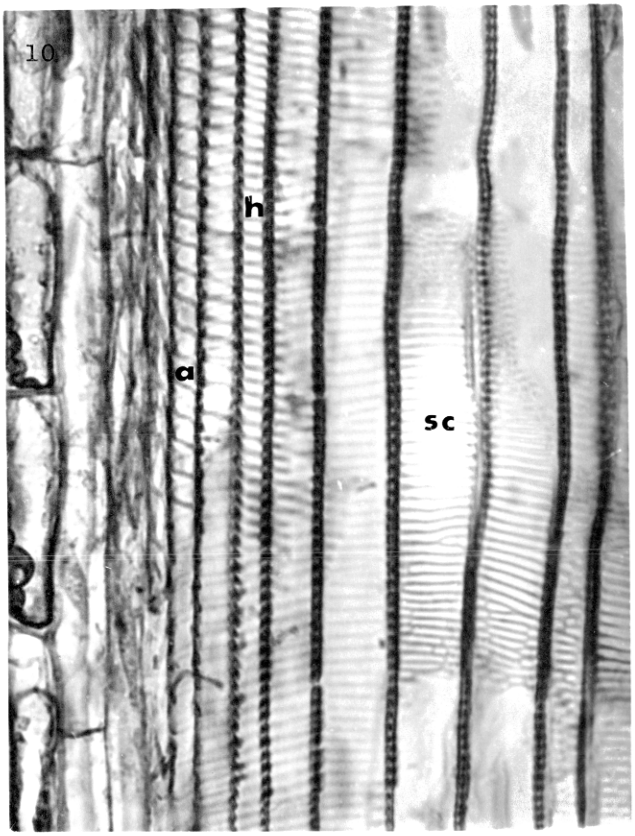
Fig. 9. Radial section of xylem showing vessel (v) member and bordered pits. Fiber tracheids (ft) exhibit pit apertures that extend beyond the pit border. Parenchyma (p). x1300.

Fig. 10. Tangential section of primary wood showing patterns in secondary walls. Annular thickenings (a), helical (h), and scalariform (sc); pith (P). x530.

Fig. 11. Transverse section of healthy yellow birch seedling. Epidermis (e), unicellular hair (h); developing periderm (pd); cortex (c); fibers (f); phloem (p); xylem (x); and pith (pi). x 130.

Fig. 12. Tangential section of stem. (Same notation as above) Medullary ray (ra). x130.





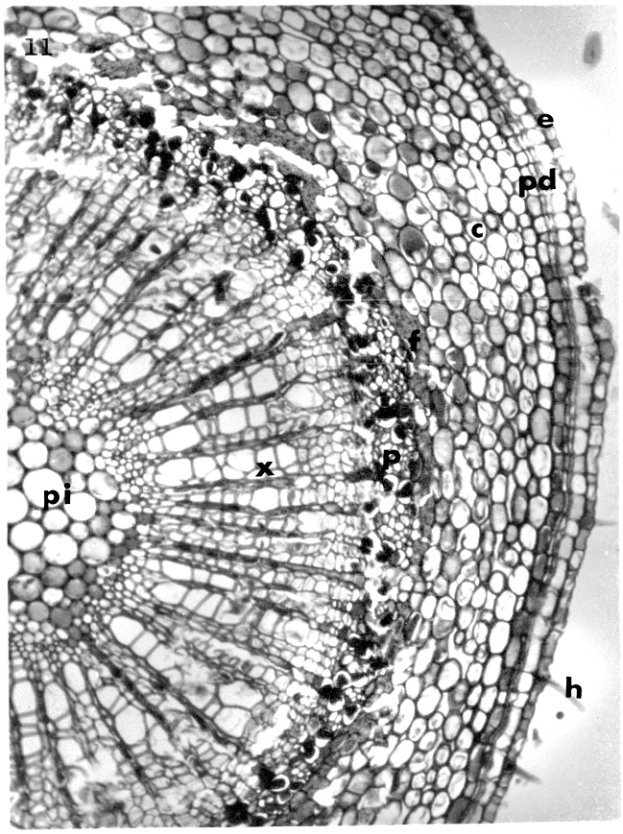


PLATE III

PLATE IV

Fig. 13. Diagram of yellow birch stem in transverse section.

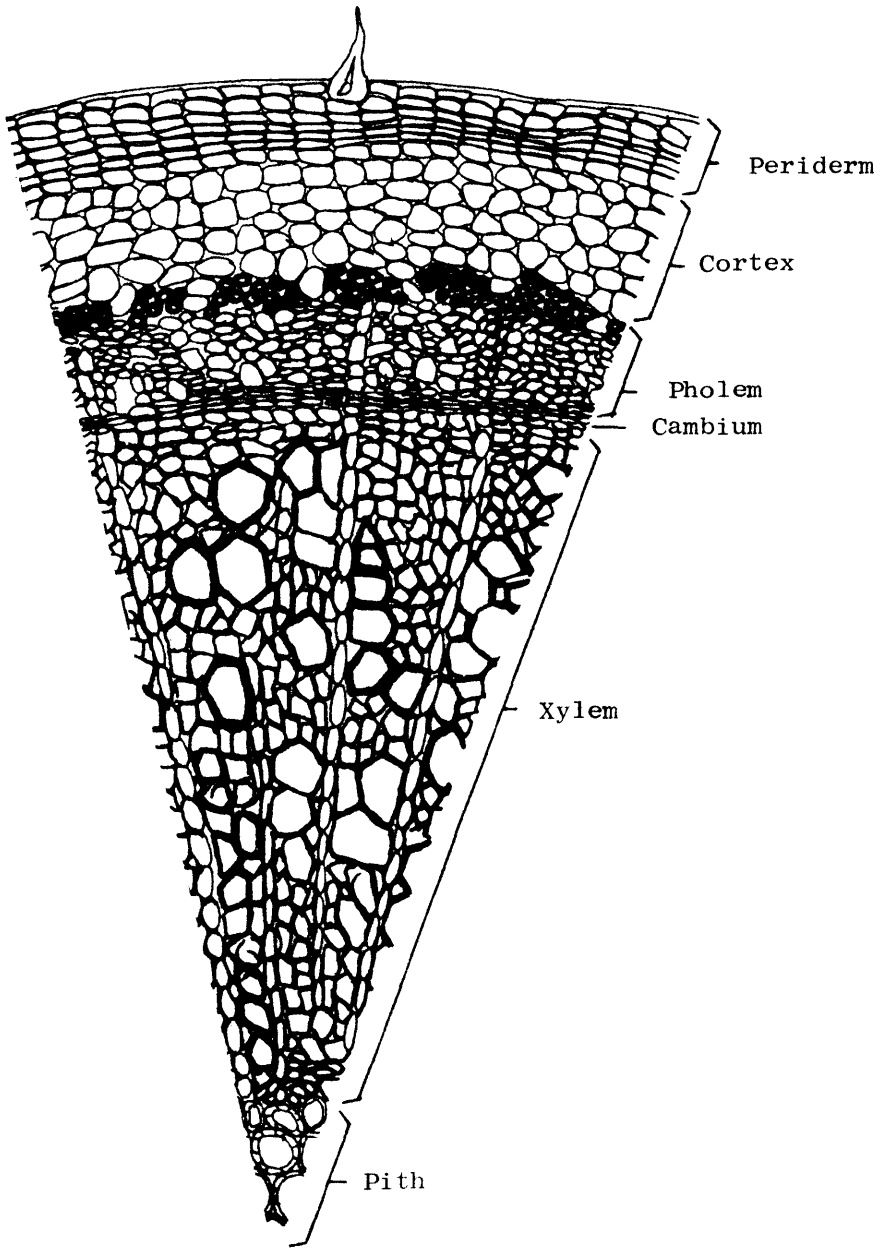


Fig. 13

PLATE IV

PLATE V

Fig. 14. Diagram of yellow birch stem in radial section. Stone cells (st), fibers (f), epidermal hair (h), medullary ray (ra), phelloderm (pd).

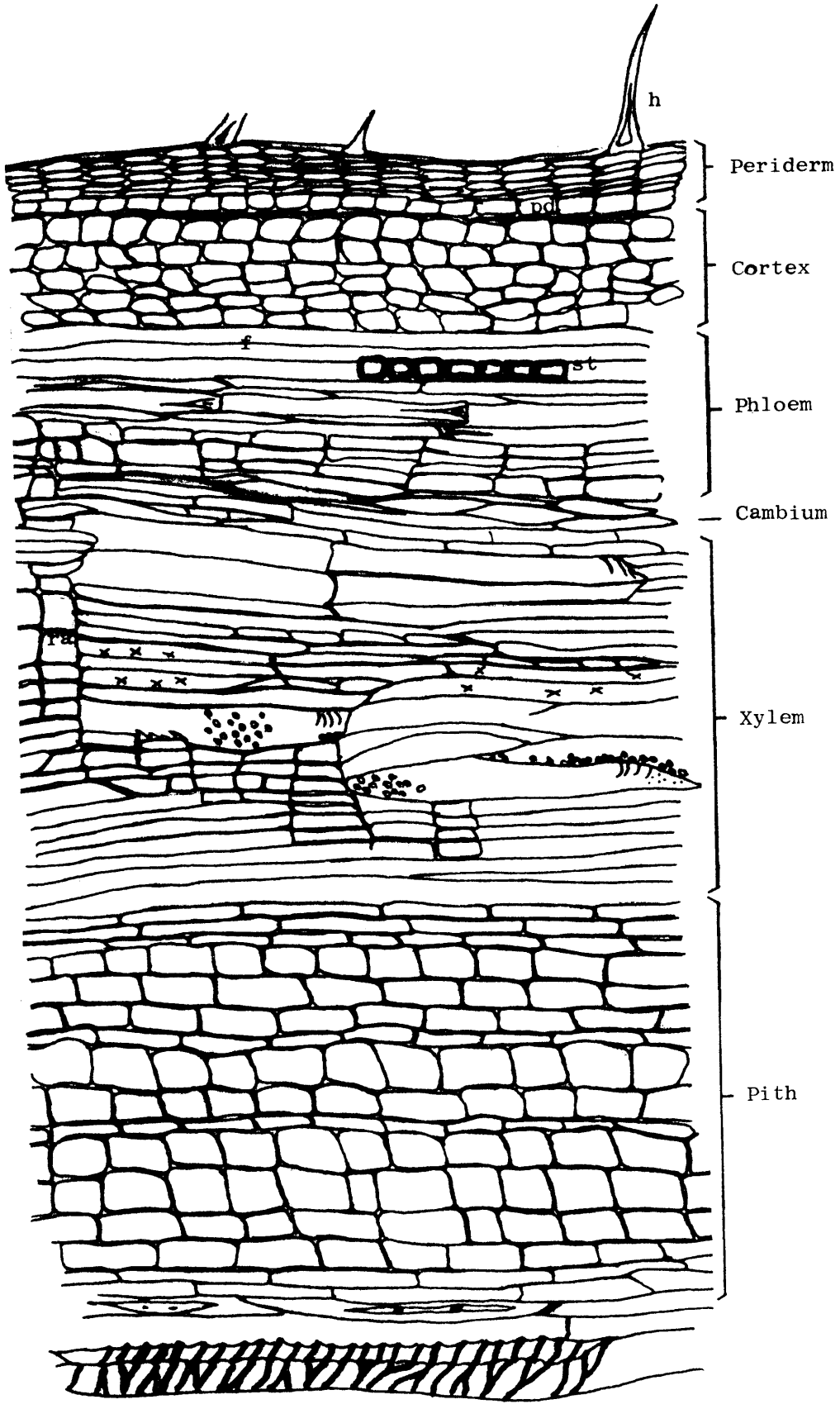


Fig. 14

PLATE V

PLATE VI

Fig. 15. A. Diagram of phloem in longitudinal section. Sieve tube (ST), parenchyma (pa), companion cell (c), druse (d), sieve plate (sp).

B. Diagram of xylem in radial section. Parenchyma (pa), fiber (f) showing pits with short borders, scalariform perforation plate (sp).

C. Stone cells. Figure 1 is completely lignified while in figure 2 the wall is still developing.

D. Ray parenchyma of xylem in radial section showing simple pitting pattern. Starch (s).

E. Ray parenchyma in tangential section.

F. Surface view of epidermis showing numerous hairs (h).

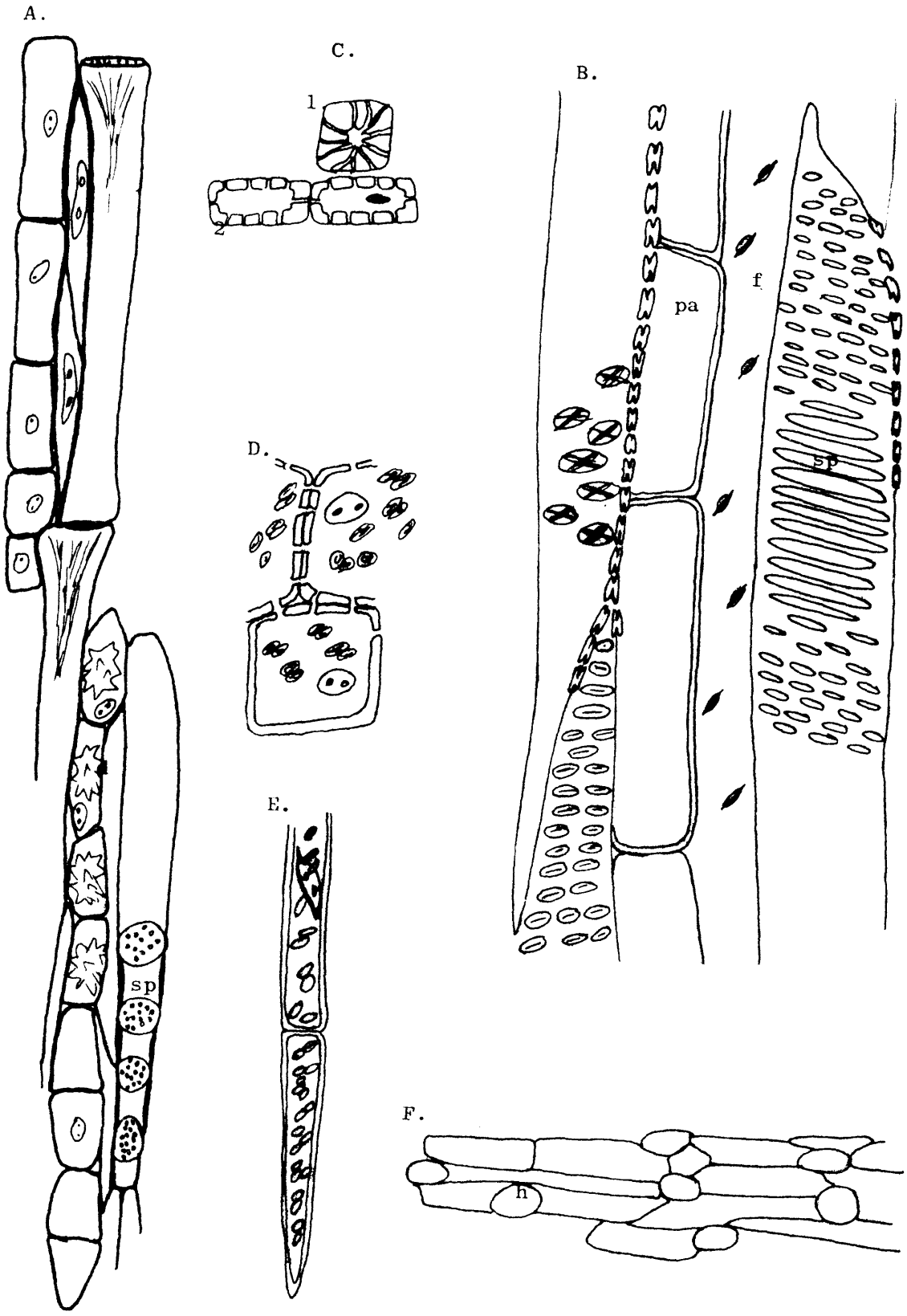


Fig. 15

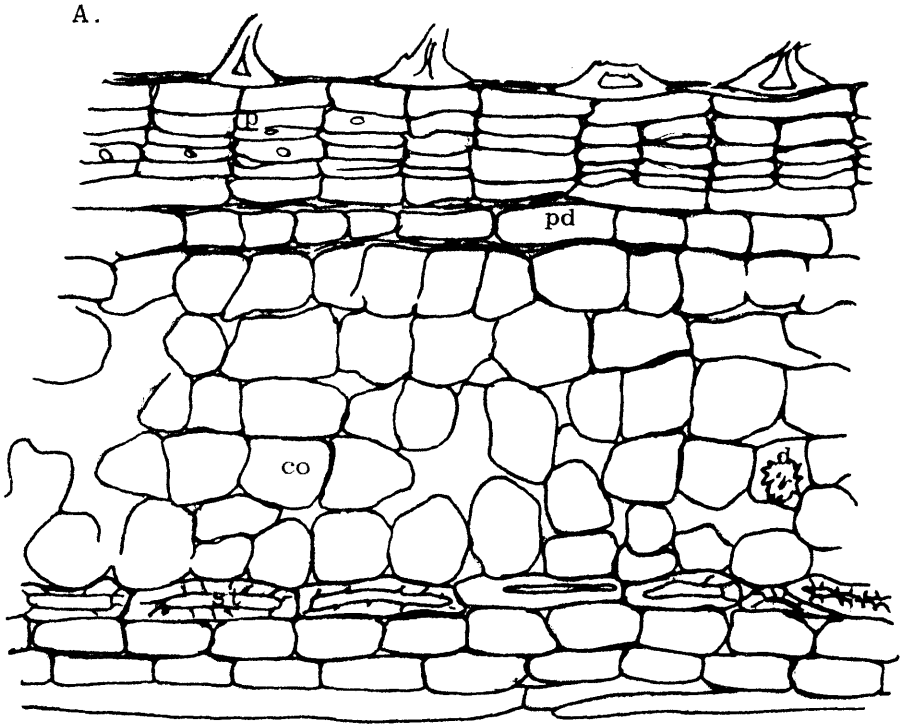
PLATE VI

PLATE VII

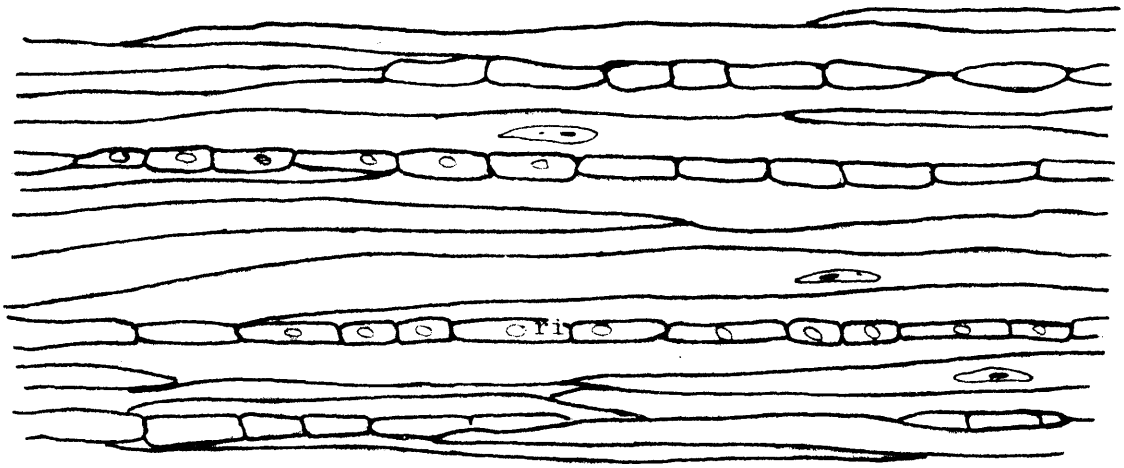
Fig. 16. A. Diagram. Cortex of yellow birch in tangential section showing regular rows of phellem (p) and single row of phelloderm (pd); collenchyma (co) and druses (d) of the cortex; and stone cells (st) from the phloem.

B. Diagram. Unstoried cambium in tangential section. Ray initials (ri).

A.



B.



Fi 16

PLAT VII

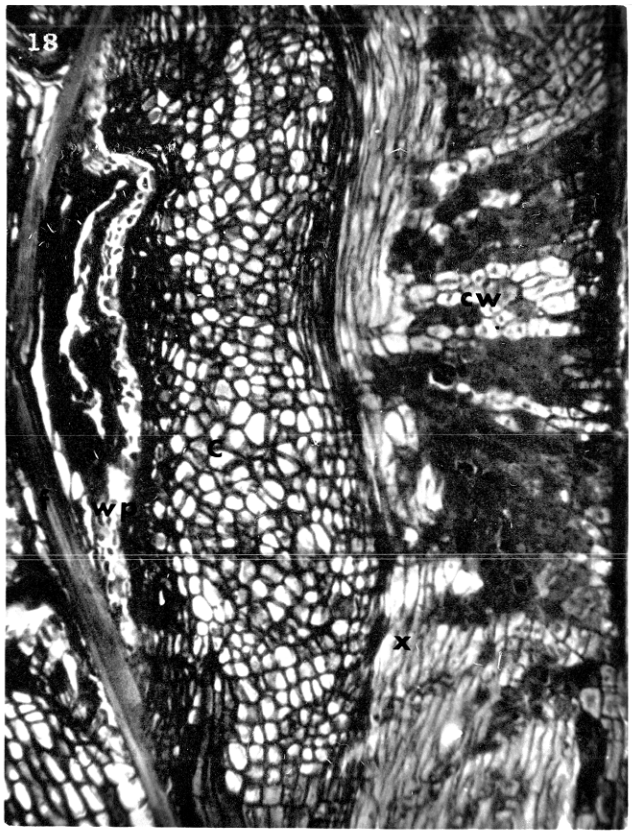
PLATE VIII

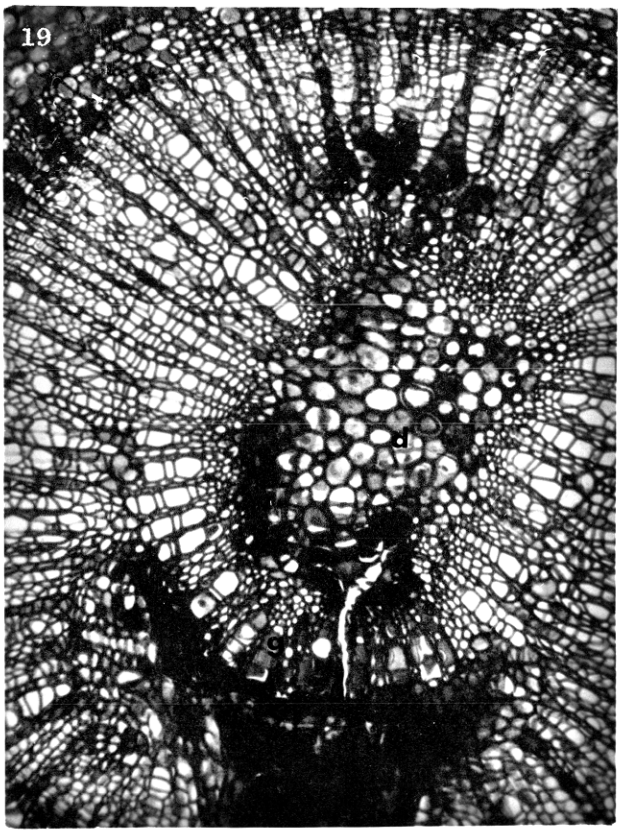
Fig. 17. Tangential view of stem showing healing processes 28 days after wounding. Note wound-wood and callus wedge (CW) in the xylem derived from ray parenchyma. Redifferentiation of xylem and phloem is also apparent. Wound periderm (wp) has developed over the wound edge of the cortex. x40.

Fig. 18. Close up of Fig. 17. Note the displacement of phloem fibers (f) caused by callus (C). A wound periderm (wp) is developing below the fibers in the outer layers of the callus below the layer of cicatrice (ci). x150.

Fig. 19. Transverse section of stem 28 days after wounding. Gums (g) are evident in vessels members below the wound. Ray proliferation produced the callus. See also some cell division occurring in the pith. This section is several mm from the wound which passes through the pith into the xylem on the opposite side thus causing the gums and ray disturbance seen in the upper right hand portion of the picture. x130.

Fig. 20. Section of callus developing in the cortex showing binucleate cells (b). x530.





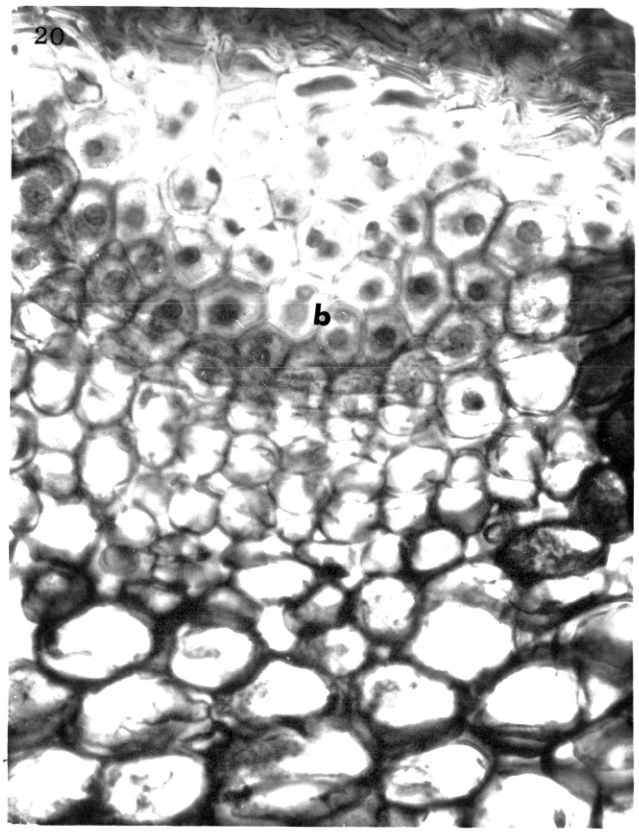


PLATE VIII

PLATE IX

Fig. 21. Transverse section showing a row of fibers (f) and stone cells (st) enclosed by a region of dead cells heavy with gums. The stone cell lumens also contain gums. Druses (d) are visible in the callus (C). x100.

Fig. 22. Twenty-eight days after wounding. Note how ray cells have produced callus, the presence of deeply staining gums, the absence of large vessels in wound-wood and the early signs of normal wood structure returning. x33.

Fig. 23. Radial section showing development of wound periderm with its several rows of phelloderm (ph). x130.

Fig. 24. Close up of wound periderm showing hypertrophy (h) of cells just before suberization, phelloderm (Ph), and dead cells of cortex (C) with druses (d). x530.

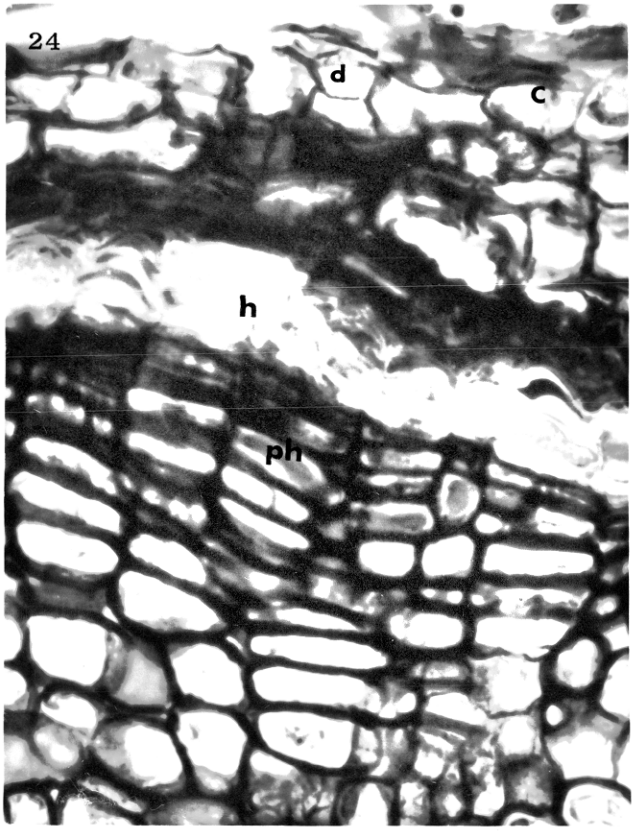


PLATE IX

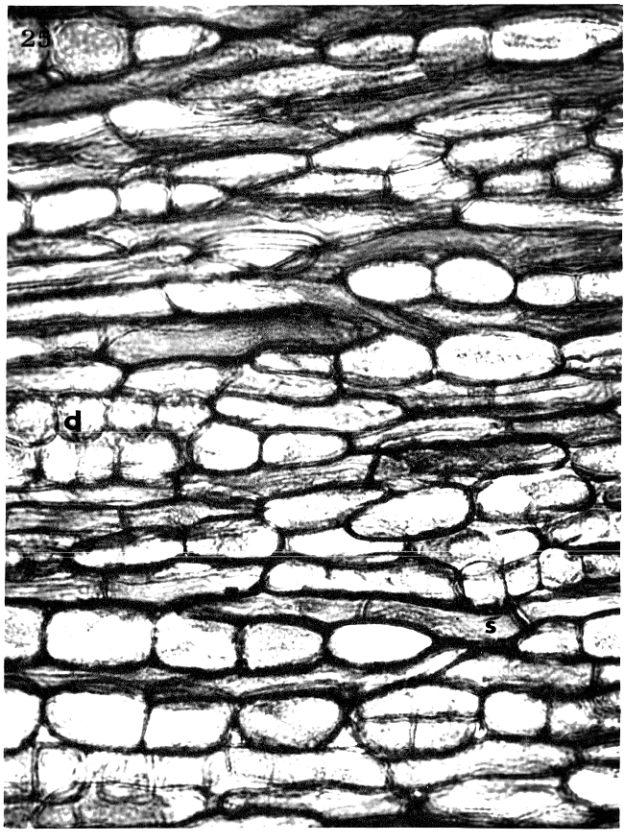
PLATE X

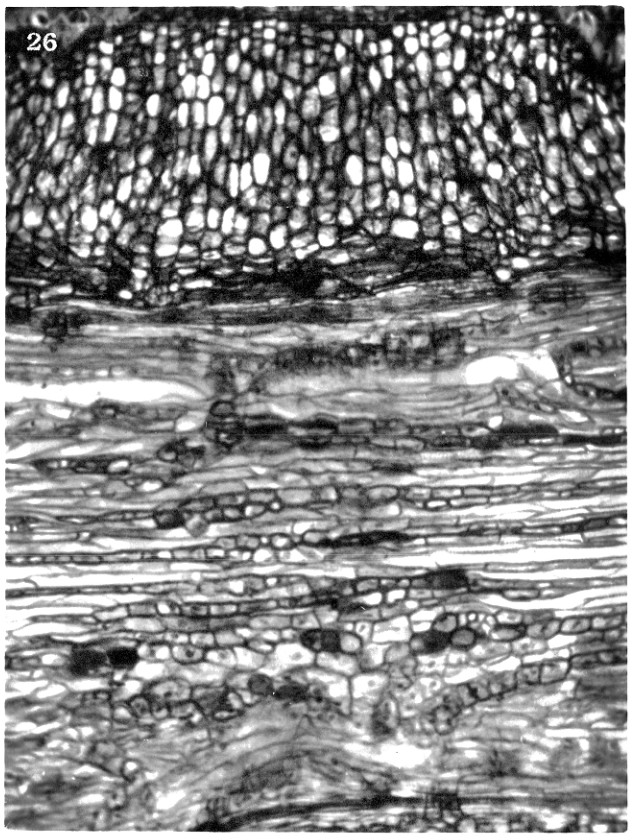
Fig. 25. Short sieve tube (s) members in redifferentiating phloem. Rows of barrel-shaped parenchyma are evident, some of them containing druses (d). x500.

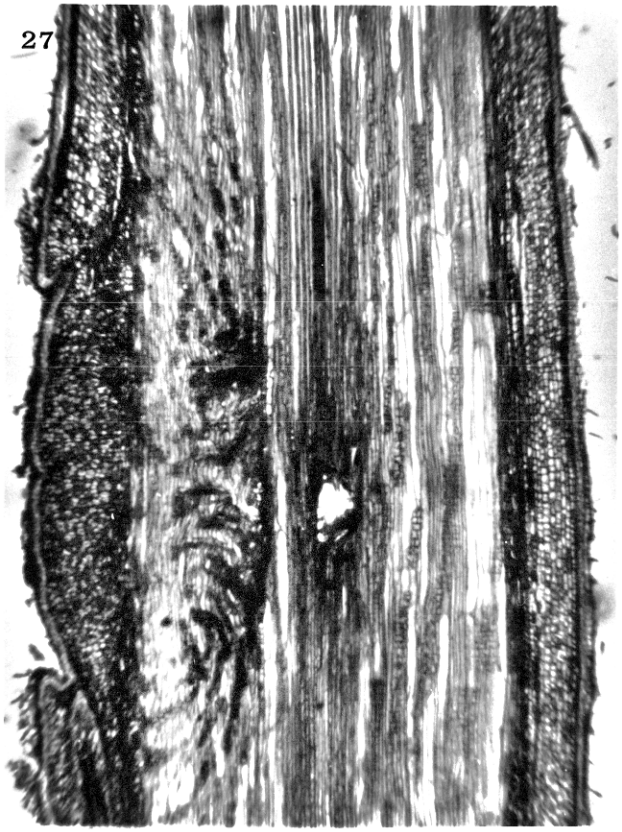
Fig. 26. Tangential section of stem showing short vessel-like cells in irregular arrangement giving a "gnarled" appearance to the wood. Note also the redifferentiation of normal appearing xylem and phloem. x130.

Fig. 27. (See also Fig. 29). View of entire section for comparison of the tissue unaffected by wounding to that affected. Note also gumming and callus development. Thirty-five days after wounding. x33.

Fig. 28. (See also Fig. 30). Transverse section showing callus wedge (cw), gums in vessels (G), stone cells (st) trapped in callus, and the redifferentiation of normal appearing xylem and phloem. Thirty-five days after wounding. x130.







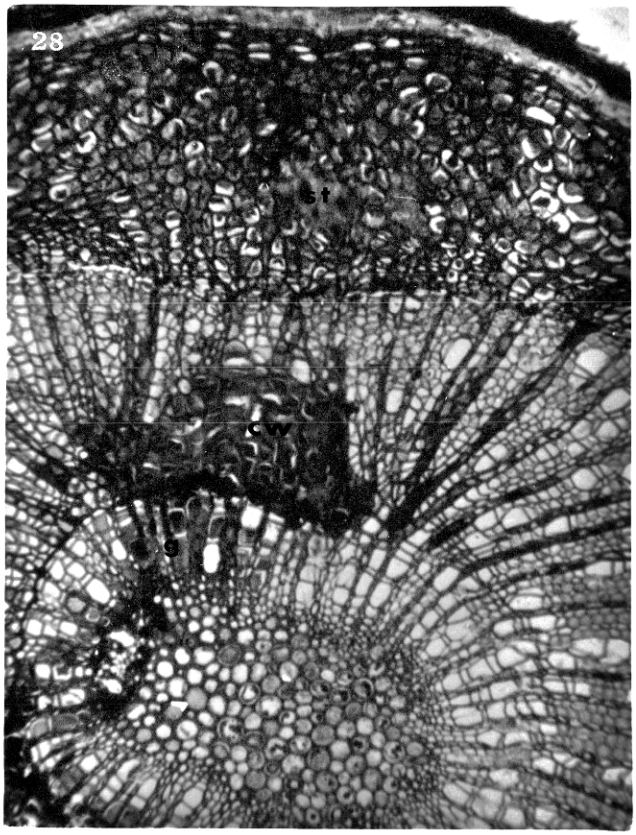
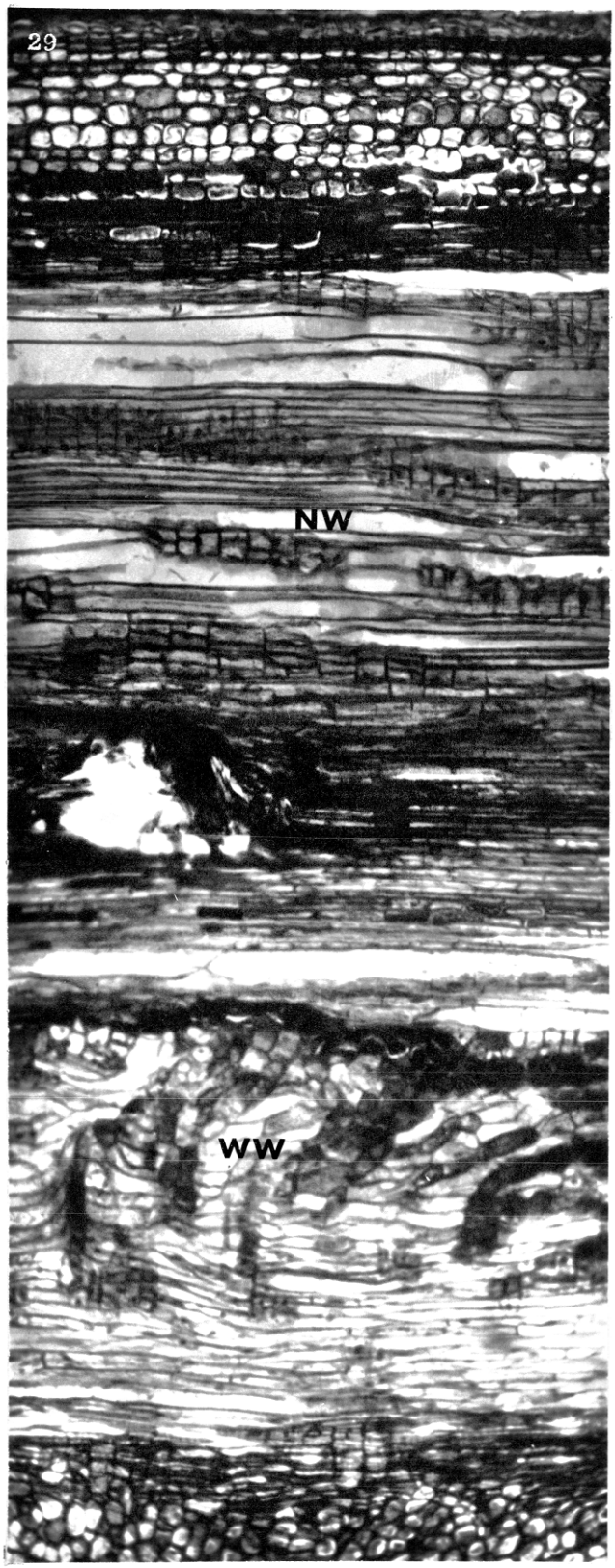


PLATE X

PLATE XI

Fig. 29. Enlargement of Fig. 27, giving a closer view of wound-wood (WW) and normal wood (NW). Note the transition to normal wood and phloem. x130.

Fig. 30. Thirty-five days after wounding. This section is from the same stem as Fig. 28, but at the wound site. The area of inoculation (I) is visible. Gums (g) are present in the dead tissues at the wound site as well as in adjacent areas. Callus (C) developed from xylary rays, phloem, cortex, and cambium. x130.



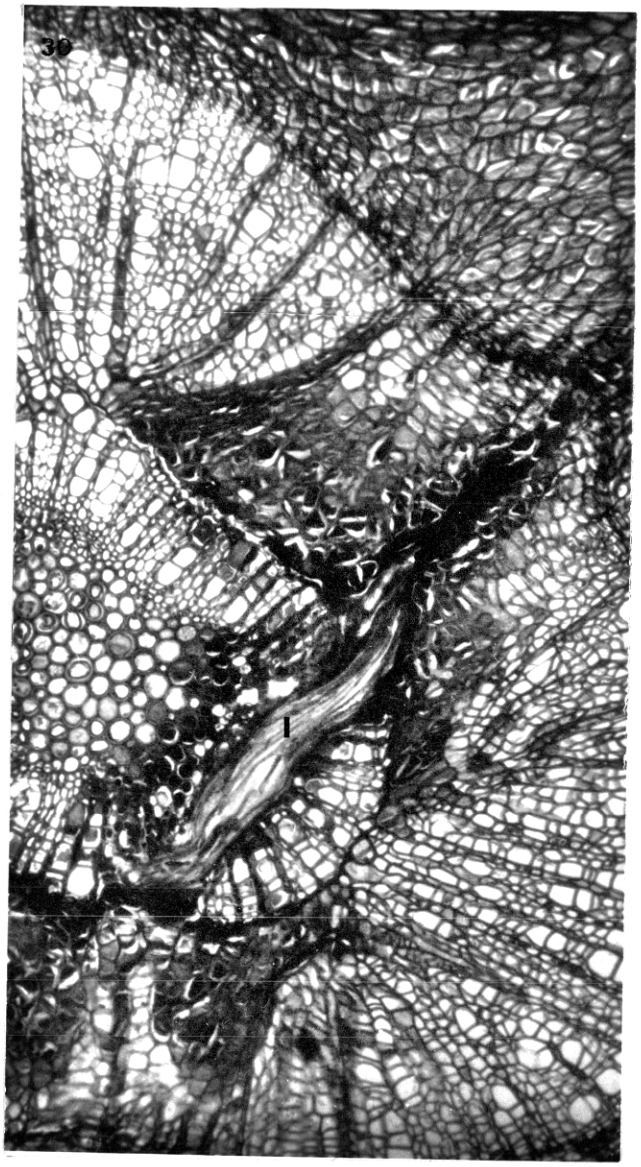


PLATE XI

PLATE XII

Fig. 31. Pits in the wall of a vessel from wound-wood. Note the large apertures and narrow borders. x1300.

Fig. 32. Radial section of stem 9 days after wounding with callus developing from the pith. Cicatrice (ci). x116.

Fig. 33. Section of a surface type wound on older stem showing typical callus rolls developing from the cortex (c), callus from phloem (C), displaced fibers (f) and cicatrice (ci). x33.

Fig. 34. Transverse section of cankered 6 year old stem believed to be infected with Valsa. A portion of the cambium is destroyed, thus new wood is not developed. Note also the disturbance in the rays and the deeply staining cells of the cortex. (Stained with safranin and fast green) x30.

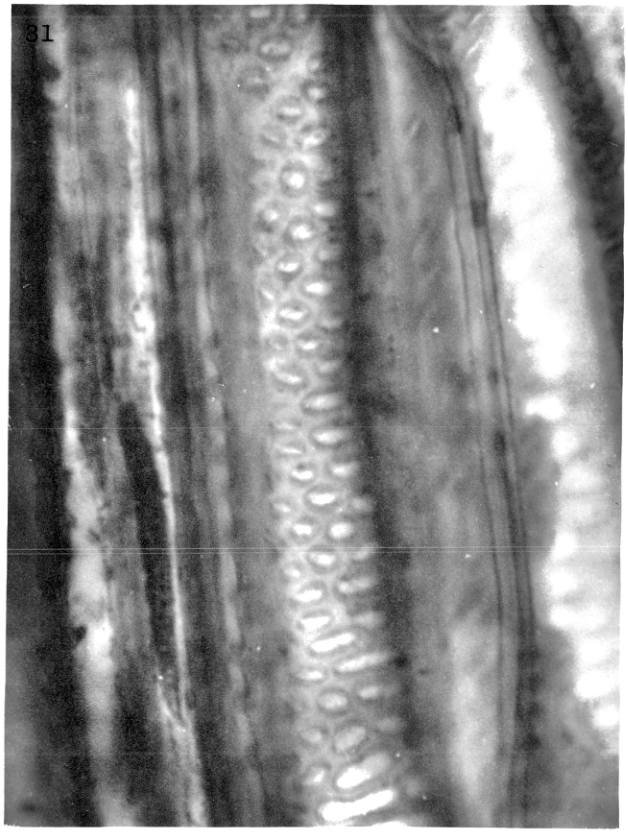


PLATE XII

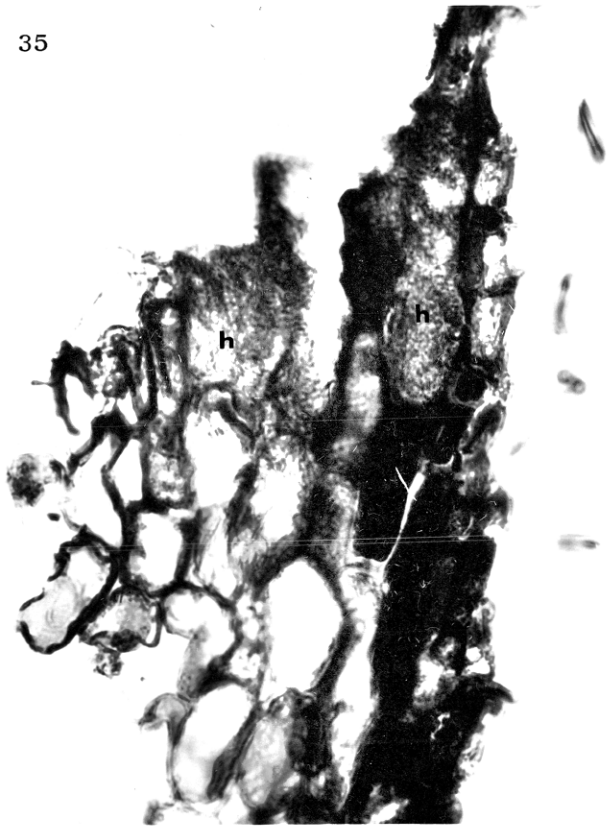
PLATE XIII

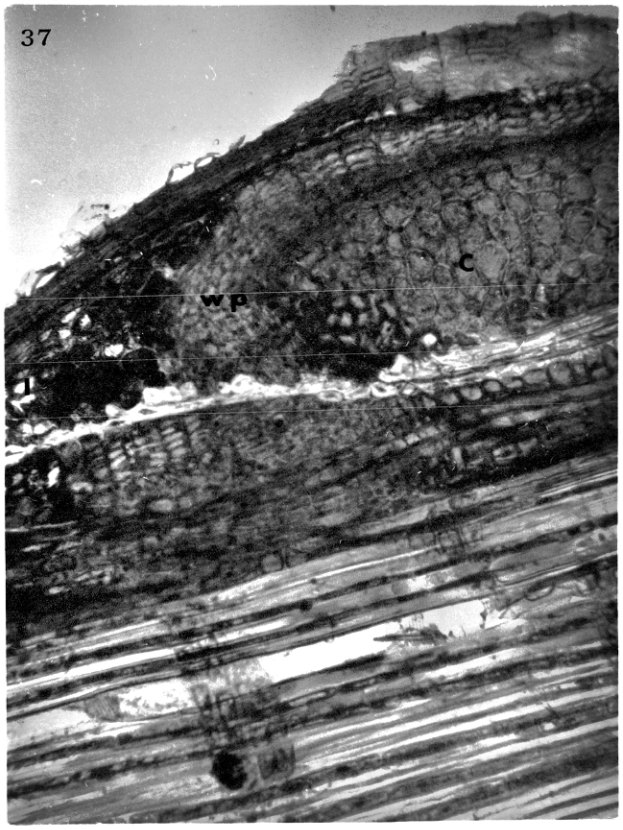
Fig. 35. Infected cortical cells at the site of wounding. The hyphae (h) completely kill some cells. Transverse section. x530.

Fig. 36. Transverse section showing infected xylem and phloem. Hyphae (h), fibers (f), xylem (X), phloem (P). x530.

Fig. 37. Radial section through the cortex of an infected stem. A wound periderm has developed between the dead infected cells (I) and healthy cortex (C). x 130.

Fig. 38. Necrotic zone (N) in the xylem in tangential section. The center of this zone contained dead cells and hyphae. Callus has developed around the area and will eventually form a layer of periderm; note also the hyperplasia (hp) of the medullary rays. x130.





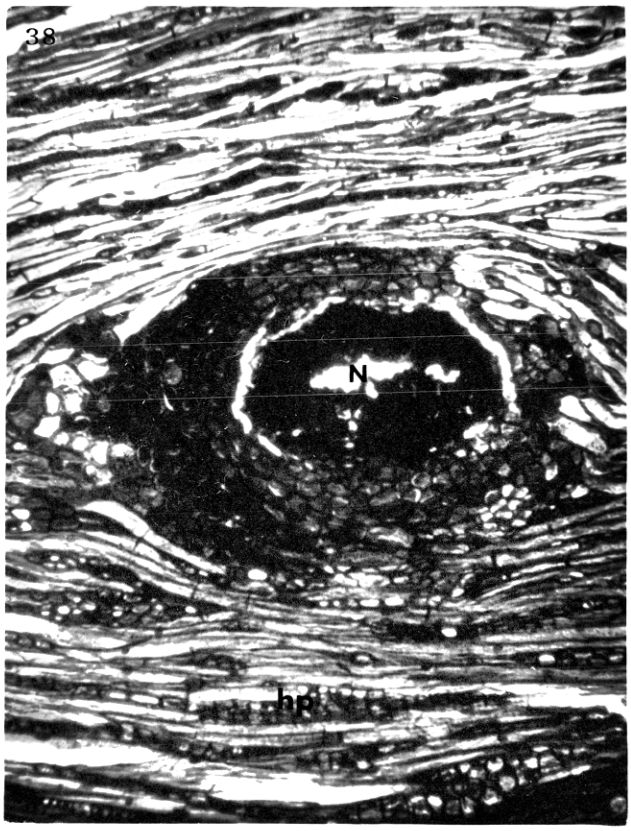


PLATE XIII

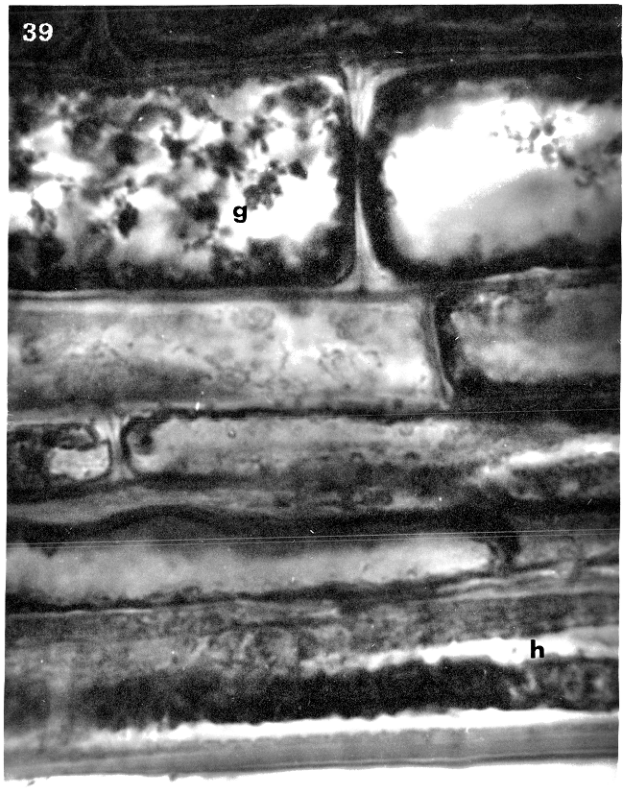
PLATE XIV

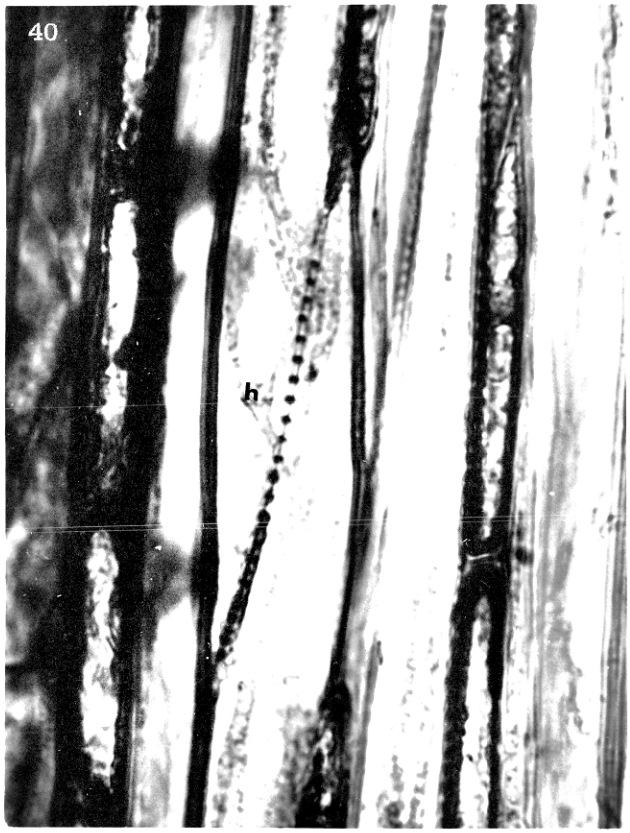
Fig. 39. Radial section through portion of pith and xylem of an infected stem. Note the granular cytoplasm (g) and the large hyphae (h). x600.

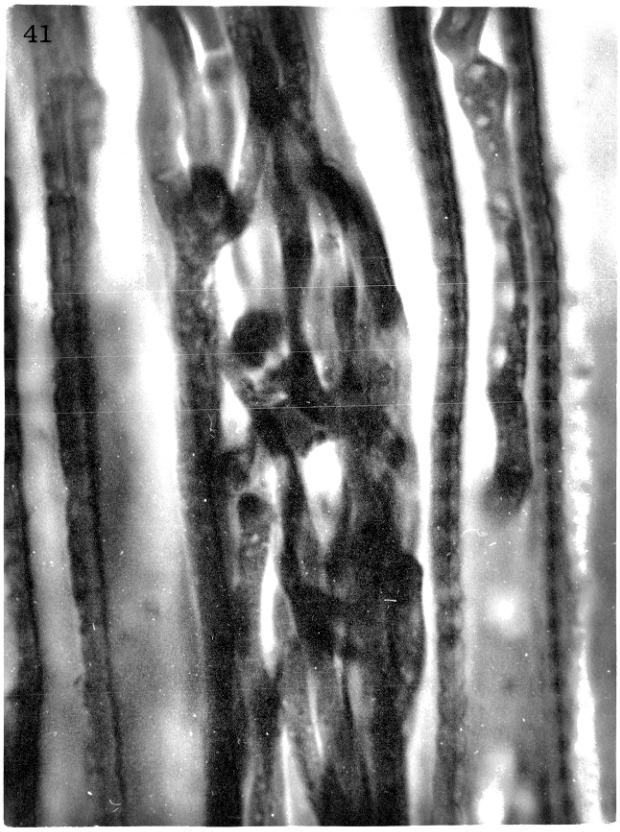
Fig. 40. Tangential section of xylem showing hyphae (h) passing through the perforation plates of a vessel. x650.

Fig. 41. A mass of large hyphae growing in a vessel. x1650.

Fig. 42. A hypha (h) passing through pit pair. Note how it becomes constricted. x1650.







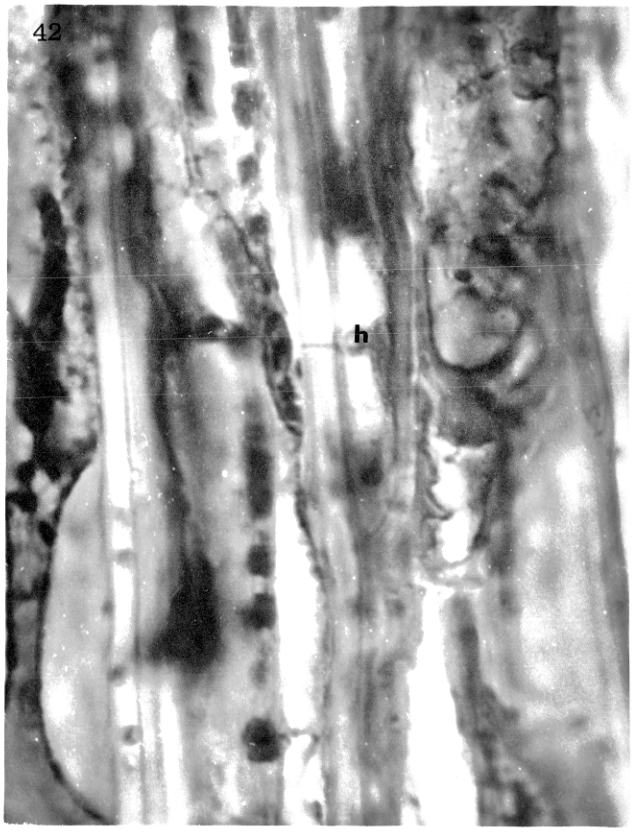


PLATE XIV

PLATE XV

Fig. 43. Tangential section of xylem and pith showing gums in the vessel lumens and granular cytoplasm in the pith cells. x200.

Fig. 44. Differentially staining gum plugs in vessel lumens. x200.

Fig. 45. Hyphae transversing a gum plug (gp). x500.

Fig. 46. Heavily infected pith cells in radial section. x500.

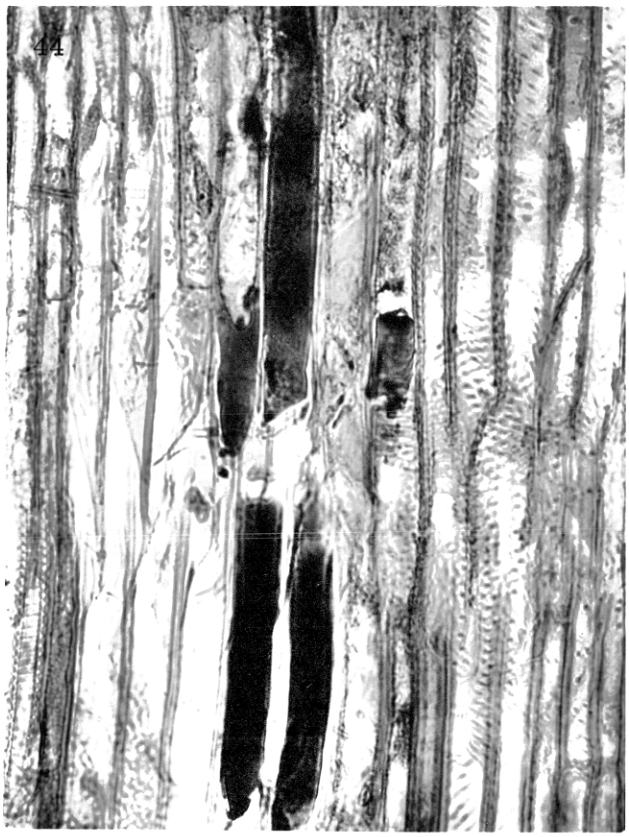


PLATE XV

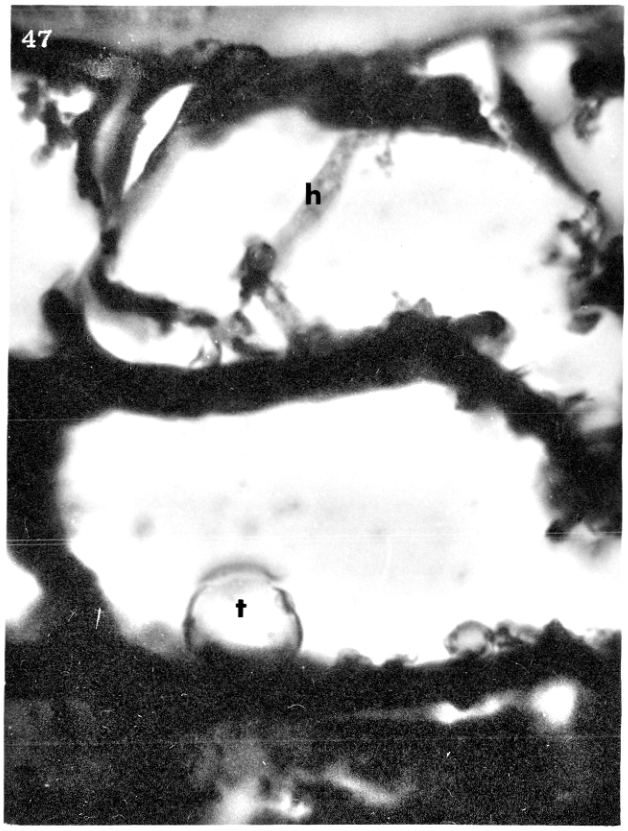
PLATE XVI

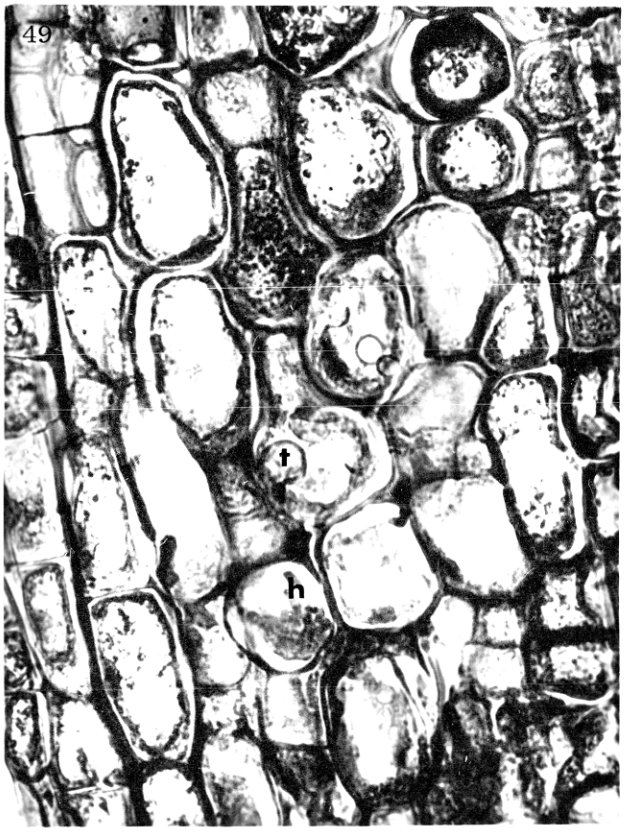
Fig. 47. Pith cells with hyphae (h) and tylose (t). x1500.

Fig. 48. Tyloses and pith cells of infected stem. Radial section. x300.

Fig. 49. Cortex in tangential section showing granular cytoplasm, hyphae (h), and a tylose (t). x250.

Fig. 50. Globules in pith cells of infected stem. Radial section. x160.





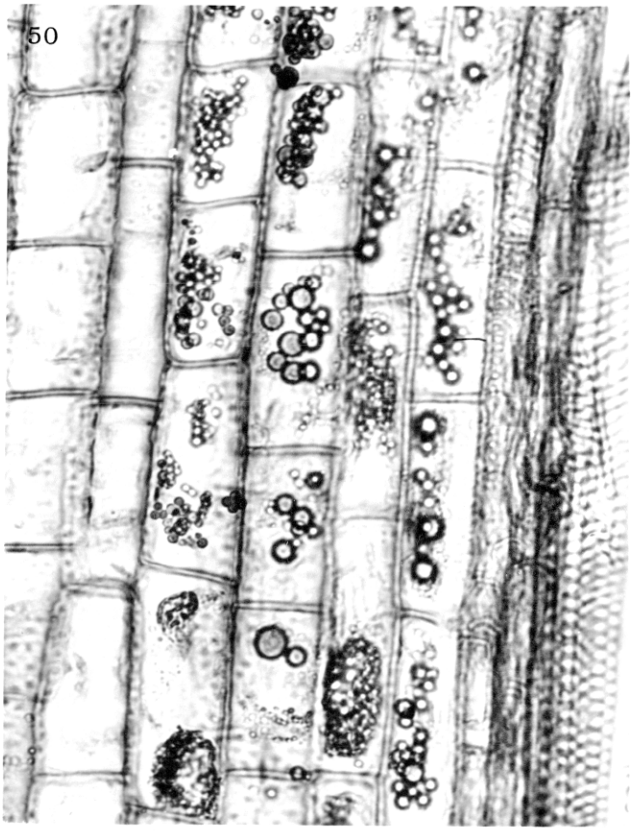


PLATE XVI

APPENDIX

YELLOW BIRCH GREENHOUSE INOCULATION METHOD*

I. Obtaining conidia. Take a sterile needle and pick up a pycnidial mass from a pycnidium produced in plate culture. Add the spores to a sterile water blank, agitate and pour aliquots of the suspension onto petri plates containing several yellow birch bark pieces. Incubate plates for one to three weeks until pycnidia with oozing masses of spores have developed on the bark pieces.

II. Preparing the inoculum. Place 100 ml of sucrose-yeast extract solution (5g:1g/liter) in a 250 ml flask, add a teflon-covered magnet bar, and autoclave. After cooling add several spore masses collected from oozing pycnidia which have developed on the sterilized yellow birch bark pieces. Place the flask on the magnetic stirrer and agitate vigorously for one hour. At the end of the hour and while the stirrer is still working, pipet two 10 ml aliquots into deep petri plates. Set these aside for 24 hours and then determine percentage germination by checking 100 spores from each plate. While the flask suspension is still being agitated take a transfer loop and make two counts of spore concentration with a hemacytometer slide (see the hemacytometer manual for multiplication factors to determine spores/mm^3). If there are from 25 to 100 spores per cubic millimeter the suspension can now be used for plant inoculations. If the concentration is less than 25/mm^3 additional spores will have to be added.

* According to Kenneth J. Kessler, Jr., Plant Pathologist, North Central Forest Experiment Station, St. Paul, Minn. unpublished.

If there are more than 100/mm^3 an appropriate dilution will have to be made.

III. Inoculating the plants. During the inoculation procedure the suspension should be kept agitated with the magnetic stirrer. Drops of spore suspension are picked up from the flask and usually placed at the first or second internode. A needle wound is then made through the drop and the plant transferred to a moist chamber for incubation. If the drop runs a second one should be added after wounding.