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Title ROOT INITIATION AND DIFFERENTIATION IN PSEUDOTSUGA MENZIESII (MIRBEL) FRANCO

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Tissue differentiation of the primary root and its associated laterals is reported. Secretory elements are the first of the primary tissues to mature. They appear to be located between the precursory phloem and pericycle in the primary root, but are more closely associated with the pericycle in long lateral roots. The stele of the primary root is generally triarch, but may also be tetrarch. Lateral roots, however, are diarch except for an occasional triarch arrangement in rapidly elongating first order laterals. Some second and third order long laterals are mycorrhizal. Short roots are either degenerate or mycorrhizal. These short roots have no secondary growth or resin ducts. The degenerate short roots fail to develop a well organized meristem and abort soon after emergence from the parent root. This occurs prior to invasion by either saprophytic or mycorrhizal fungi so that fungus activity cannot be responsible for

the development of a degenerate root. Mycorrhizal short roots are commonly racemose. The extent to which the fungus mantle covers the root tip appears to govern the development of the apical meristem and subsequent growth in length of the mycorrhizae. Root regeneration following pruning is reported in relation to the age of the seedling and the method of pruning. Adventitious laterals originate primarily from cambium at the end of a protoxylem pole. In two and three year old pruned roots, adventitious laterals originate in the cambial region of a vascular ray. Aside from their differences in origin, adventitious laterals develop in much the same manner as the laterals from which they are derived.

# ROOT INITIATION AND DIFFERENTIATION IN *PSEUDOTSUGA MENZIESII* (MIRBEL) FRANCO

by

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# ROOT INITIATION AND DIFFERENTIATION IN PSEUDOTSUGA MENZIESII (MIRBEL) FRANCO

## INTRODUCTION

The anatomy of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) has been intensively investigated in recent years. Crafts (11) described vascular differentiation in shoot apices, Sterling (31, 32, 33) discussed the structure and organization of the shoot, and Allen (4, 5, 6) described the development of the embryo and structure and development of the apical meristems. Anatomical development of the hypocotyl of Douglas-fir was reported (27) and the anatomy of heat damaged seedlings was thoroughly investigated by Smith and Silen (28). In other investigations, Grillos and Smith (13) studied the secondary phloem of Douglas-fir, and Owens (24) the development of the seed cone. Studies on the root regenerating potential of Douglas-fir seedlings (34) and the formation of mycorrhizae on seedlings (42) have been reported.

This paper is concerned with: (1) origin and differentiation of primary and secondary tissues of the primary root; (2) origin and development of lateral roots, and anatomical differences between long and short roots; and (3) the initiation and development of adventitious roots following pruning.

The term "long" root used in this paper refers to the elongated first, second and third order lateral roots. "Short" roots are

anatomically different from other lateral roots, and are not distinguished by length alone. Immature but potential long laterals, therefore, are not considered as short roots. The term is used here to include (1) the mycorrhizal short roots and (2) the degenerate short roots, commonly referred to by some investigators as "pseudomycorrhizae."

# MATERIALS AND METHODS

Seedlings of Douglas-fir ranging in age from germination to three years were used in this study. Seeds and seedlings of different ages were supplied by the Pacific Northwest Forest and Range Experiment Station from a single source within the Willamette National Forest. Seedlings of all ages were planted in containers filled with a mixture of sandy loam and peat moss, with an upper two inch layer of forest soil. The seedlings were grown in a greenhouse with temperatures ranging from 50-90°F. The average daily maximum and minimum temperatures were approximately 74° and 59°F.

Three different pruning procedures were used to determine the origin and development of regenerated roots on one, two and three year old seedlings. They consisted of: (1) pruning all first order laterals to the surface of the primary root, from the uppermost lateral downward approximately 8 cm; (2) pruning the upper first order laterals to within 2 cm of the primary root; and (3) pruning horizontally all lower laterals and the primary root at a level approximately 8 cm from the uppermost first order lateral. In the first two pruning methods the lower roots were not intentionally removed. The lower primary root and lower laterals, however, were often dead or lost in transplanting.

Development of the primary root and associated laterals was determined from materials collected from 4 to 200 days following germination, while regenerated roots were sampled from 10 to 100 days after pruning. Roots were killed and fixed in Randolph's modified Navashin fluid (16, p. 45) and dehydrated with tertiary butyl alcohol. Paraplast, a new embedding medium, replaced the more commonly used Tissuemat in the final steps of embedding. The embedded roots were softened by placing them in a mixture of glycerol and detergent (2) at 38°C. for 2 to 30 days, sectioned at 10μ and stained progressively with safranin and chlorozol black E.

# OBSERVATIONS

## Primary Root

Apical organization of the primary root of Douglas-fir has been thoroughly investigated by Allen (6). It will suffice to point out the three generally recognized mother-cell zones (Figure 1). They are: (1) the stelar mother-cell zone, which gives rise to the tissues of the embryonic stele; (2) the column mother-cell zone, where cell divisions are predominately transverse, forming the column and peripheral layers of the rootcap; and (3) the cortical mother-cell zone, from which develops the cells of the cortex and rhizoderm.

The secretory cells are the first of the primary tissues to differentiate. They arise from the outer initials of the stelar mother-cell zone and elongate to beyond 1,000μ without further divisions. Their exact length is not easily determined since secretory elements extend into the region of elongation where the primary tissues often collapse while preparing the material for sectioning. In longitudinal sections the secretory elements are identified as long, slender cells with darkly stained protoplasts or, less commonly, by their complete lack of cellular material (Figure 2). In transverse sections the secretory elements appear between the outer primary phloem and the pericycle (Figure 3). Whether the secretory elements are part

of the primary phloem or the pericycle is difficult to determine. These elements, however, do mark the outer limits of the phloic procambium.

Primary phloem is used here to include the enlarged outer elements designated as "precursory phloem" by Chaveaud (8, p. 14-21) and the smaller, inner phloem elements which differentiate prior to the development of secondary tissues. The outer precursory phloem elements are the first to differentiate (Figure 3). Further development proceeds in a centripetal direction with the inner primary phloem elements the last to differentiate. In addition to their smaller diameter, the inner primary phloem elements have sieve areas on their walls. The sieve areas found on the outer precursory phloem elements of the hypocotyl by Smith (27, p. 65) were not observed in the roots of Douglas-fir seedlings.

The cells that differentiate into protoxylem are arranged between the precursory phloem groups and are generally distinguished by their smaller size and large nuclei (Figure 3). Vacuolation of immature metoxylen elements occurs above 150μ for the apical initials but these elements mature only after differentiation of the protoxylem is complete. Protoxylem elements are clearly differentiated low in the region of maturation, some 5 mm from the apical initials (Figure 4). The stele of the primary root is either triarch or tetrarch, with the former condition most common.

The xylem elements differentiate centripetally. The first formed elements are scalariform, while the walls of the larger metaxylem elements may have simple pits at first (33, p. 277), but elements that develop later have circular bordered pits. Differentiation of the primary vascular tissues is completed in seedlings with an average root length of 8 cm (Figure 5). Primary vascular tissues mature later, however, in more rapidly growing roots.

A multiseriate pericycle forms the outer boundary of the stele. The irregular rows of pericycle cells are difficult to distinguish until the primary vascular tissues have matured (Figures 3 and 4). At this point, however, pericycle cells generally contain ergastic substances in the form of droplets (Figure 5). Frequent divisions also help to distinguish the pericycle from the rest of the stele.

The cells of the endodermis are also difficult to determine at an early age, for they resemble the cortical cells in size. Faint casparian thickenings appear on the radial walls of the endodermis in transections, shortly after the maturation of the first protoxylem elements. Suberization of the cell walls is generally completed by the time cambial activity becomes evident. As secondary growth develops, the endodermis appears to be the first of the primary tissues to collapse (Figure 5).

Allen (6, p. 207) described the ontogeny of the cortex in Pseudotsuga. The cells of the cortical mother-cell zone divide transversely at first, then cell divisions become longitudinal and oblique. Elongation of the cortical cells ultimately takes place parallel to the long axis of the root. New cell lineages develop in the inner layers of the cortex, forcing the older layers outward. This type of displacement increases the difficulty of distinguishing between cortex and rootcap cells near the apex (Figure 6). Back of the apex, the outer layers of rootcap cells become irregular in shape and more loosely arranged (Figure 7), and are sloughed. The innermost layer of rootcap cells persists as an uniseriate layer that completely covers the cortex for some distance back from the apex (Figure 8). The rootcap cells become lightly suberized and persist until they are pushed off by the formation of root-hairs (Figure 9) from the rhizodermis, or outer layer of cells of the cortex.

The first resin ducts in Pseudotsuga roots are vertical and located near the ends of the protoxylem poles (Figure 11). Resin producing parenchyma cells are formed by the segmentation of the pericycle cells. The ducts then develop by schizogenous separation of the central parenchyma cells, which then differentiate into the epithelial cells lining the duct. In Douglas-fir the epithelial cells soon become lignified and are, presumably, non-functional.

Vertical resin ducts that form later originate from fusiform initials and are scattered throughout the secondary xylem (7, p. 28). Horizontal resin ducts first appear in the vascular rays when the primary root is approximately three months old.

Tangential divisions of the procambium adjacent to the inner primary phloem strands mark the first cambial activity in the seedling root. These divisions occur prior to the time of maturation of the inner metaxylem elements (Figure 10). The vascular cambium later becomes continuous by cell divisions of the pericycle opposite the protoxylem poles and outside the vertical resin canals. In longitudinal sections, the vascular cambium appears to be made up of compact, tapered cells with prominent nuclei. The first tracheids produced by the vascular cambium generally have bordered pits and lack the tertiary spirals which are characteristic of the later secondary xylem of Douglas-fir. These spiral markings appear first in the secondary xylem laid down after the primary root is several months old. The secondary phloem of Douglas-fir has been investigated by Grillos and Smith (13), and a detailed description will be omitted here. It should be pointed out, however, that the first secondary phloem elements to appear are sieve cells and phloem parenchyma. Fusiform phloem parenchyma and vertical albuminous cells occur later, when the seedling radicle is about two months old.

The cortical cells begin to collapse sometime prior to the origin of a cork cambium (Figure 5), possibly because of suberization and early collapse of the endodermis. The cork cambium is initiated by tangential divisions of the outer pericycle cells (Figure 12) at approximately the time the first secondary xylem is produced. Radial divisions occur frequently which allows adjustment for growth in diameter of the roots. The phellogen produces phellem or cork cells to the outside, but apparently does not produce a phelloderm (Figure 13). In three year old roots, the phellem may consist of three to four layers of suberized cork cells. In cells of both the phellogen and newly initiated cork, the protoplasts contain resinous and tanniferous compounds. These ergastic substances disappear, however, as the cork cells collapse.

Lenticels are not clearly evident on one year old seedling roots. Such structures are well developed in older roots and may occur in pairs, one on either side of a lateral root, as described by Eames and MacDaniels (12, p. 263), or single as described for pine by Addoms (1, p. 211).

## Long Lateral Roots

The exact location and number of first order lateral roots appears to be determined by the growth rate of the primary root. In fast growing primary roots, the first laterals that develop are

located a greater distance from the root apex, and additional lateral roots are spaced further apart. Normally, lateral roots originate soon after the protoxylem elements mature in the primary root. Anticlinal and periclinal divisions in a group of outer pericycle cells at the end of a protoxylem pole indicate the initiation of a lateral root. The endodermis is crushed by the developing primordium and is not involved with the origin of lateral roots. The cells at the periphery of the primordium soon become heavily tanninized (Figures 14 and 15) and persist as a protective layer throughout early growth and development. Considerable primordial tissue is formed before there is any indication of distinct zonation of the apical initials (Figure 16). Then the initials appear deep within the primoridal tissues from the innermost radially elongated cells. In young lateral roots the apex is not as well organized with regard to zonation as that of the primary root (Figure 17). A more typical zonation pattern becomes evident, however, when the lateral has elongated several inches (Figure 18). A poorly developed rootcap column helps distinguish anatomically between long laterals and primary roots.

In long lateral roots the secretory cells appear to be located in the pericycle (Figure 19), rather than between the pericycle and precursory phloem as described for the primary root. In long laterals, the protoxylem elements are scalariform with some of the later

formed elements slightly reticulate (Figure 20). Metaxylem elements develop bordered pits. Most long laterals are diarch (Figure 19), but an occasional first order lateral may be triarch.

The remaining primary vascular tissues, cortex and root-hairs all develop exactly as described for the primary roots. Secondary growth is also the same as in primary roots. Vertical resin ducts appear at the ends of the protoxylem poles shortly after the first secondary xylem is formed. The periderm, on the other hand, is not fully developed until at least three months after the long root is initiated.

Second and third order long laterals may be converted to ectotrophic mycorrhizae. In long laterals of the third order the fungus hyphae form both a Hartig-net and a mantle. In long second order laterals a Hartig-net develops, but no mantle (Figure 21). Linnemann (cited by Harley 14, p. 37) has reported similar mycorrhizal development in long laterals of Douglas-fir. Morphologically, the mycorrhizal second order long laterals appear as normal roots. Their identity, therefore, can only be determined by anatomical means.

## Degenerate Short Roots

Degenerate short roots, or pseudomycorrhizae (17, p. 148), vary in length from 0.5 to 3.0 mm. Morphologically, they could be confused with mycorrhizal roots that lack a fungus mantle. The degenerate roots are found on first, second and third order laterals, are always unbranched and usually somewhat smaller in diameter than other short roots. Anatomically, degenerate roots are more similar to mycorrhizae than to normal laterals.

Degenerate roots originate in the same manner as normal lateral roots, and cannot be identified with certainty until some time after they emerge from the cortex of the parent root. In resume, a primordium of a normal lateral consists at first of a group of cells dividing in all planes, then periclinal divisions give rise to a rudimentary rootcap just prior to emergence of the root from the cortex. The highly organized zoned apex is not attained until the lateral is approximately two inches long. A primoridum that will give rise to a degenerate root follows the same pattern of development up to the time of emergence. Thus at this time the foremost cells of the apical meristem are dividing periclinally to form a rudimentary rootcap, but distinct column mother-cells and stelar mother-cells cannot be identified. Just back of this region, cells are dividing transversely

and periclinally, increasing the length and diameter of the root.

The characteristic feature of the degenerate root is reduction in activity of the apical meristem and a gradual reduction in degree of organization. In other words, instead of developing a more active and zoned meristem as does a normal lateral, the meristem of a degenerate root gradually loses both its activity and organization. This occurs prior to invasion by either saprophytic or mycorrhizal fungi so that fungus activity cannot be responsible for the development of a degenerate root. For that reason, these should be considered as actually degenerate roots and not as pseudomycorrhizae.

The time at which activity of the apical meristem becomes reduced to the stage typical of degenerate roots varies with individual roots. If the meristem aborts early, the degenerate root reaches a length of only approximately 0.5 mm. The meristem can abort any time after this but no degenerate short roots longer than 3 mm were observed. Activity in an aborted meristem consists of very infrequent periclinal divisions of the extreme apex that add cells to the stele but not to the rootcap. As a result, more and more cells are lost from the rudimentary rootcap until in later stages the apical initials are covered by only one or two layers of cells. These apical initials might be considered to correspond to the stelar mother-cells of a normal root tip. Some periclinal divisions add cells to the cortex,

but the zone of cortical mother-cells characteristic of the normal root is missing. Because of this, the cortical cells are laid down in rows that diverge toward the root tip and outward as the cells enlarge, instead of being parallel with the root axis (Figure 22). These obliquely arranged cortical cells are very characteristic of degenerate roots. If the apex aborts early, all the cortical cells are obliquely arranged. In larger degenerate roots, only the later formed cortical cells are divergent.

Degenerate short roots less than 1 mm in length never develop a rhizodermis and, therefore, have no root-hairs. On longer degenerate short roots a rhizoderm exists on the lower portion of the root, but root-hairs were not observed. The early occlusion of the cortical tissues with tannins and resins is believed responsible for the lack of root-hairs on these longer roots. Many of the cortical cells die and collapse soon after the root aborts. Saprophytic fungi then invade the deteriorating tissues. The endodermis is lightly suberized and often occluded with yellow droplets. Like normal roots, the endodermis is the first of the root cells to collapse.

The primary vascular cylinder appears as an elliptical structure in transverse sections (Figure 23). The stele is diarch, and the protoxylem groups are located near the narrow ends of the ellipse. Protoxylem elements are scalariform to scalariform-reticulate,

while metaxylem elements have bordered pits. Xylem elements are differentiated close to the root meristem after the short root aborts. In longer degenerate short roots both precursory phloem and inner primary phloem elements are evident (Figure 23). In roots less than 1 mm long, only the precursory phloem is present. Positive identification of secretory elements was never made in degenerate short roots. The outer limits of the stele is flanked by one or two rows of pericycle cells (Figure 23). Various ergastic substances in the form of granules and droplets fill the pericycle cells much the same as in long laterals and the primary roots.

Resin ducts and secondary growth never develop in degenerate short roots. A Hartig-net of mycorrhizal forming fungus may form in patches along the degenerate short root, but will persist only until the cortical cells die or collapse. It seems, therefore, that degenerate short roots may become partially mycorrhizal during their development, but the complete development of a typical ectotrophic mycorrhiza is unlikely.

## Mycorrhizal Short Roots

Mycorrhizal short roots are lateral roots infested with a mycorrhizal forming fungus at an early age. Mycorrhizae vary in length from emergence to approximately 15 mm. Like the degenerate

roots, mycorrhizal short roots are found on first, second and third order long laterals. Mycorrhizae near the tips of the parent roots eventually form a racemose pattern. The digitate types reported by Wright and Ching (43, p. 6) for Douglas-fir seedlings were not observed.

The mycorrhizae on Douglas-fir appear to be ectotrophic and show a characteristic intercellular net or Hartig-net between the cortical cells, and generally a fungus mantle over the root surface. Endotrophic mycorrhizae were not observed, but Lewis (21, p. 860) and McDougall and Jacobs (22, p. 258) reported that endotrophic mycorrhizae do occur on Douglas-fir roots.

The present study reveals two different mycorrhizal formations. Both produce a racemose pattern, but one forms a shiny, smooth fungus mantle (Figures 24 and 25) while the other develops a rough, white mantle (Figures 26 and 27). The smooth mycorrhizae generally are not heavily branched. Their color varies from white to light brown at first, but changes to dark brown or purple in older roots. The rough surfaced mycorrhizae develop a compact racemose pattern and soil particles adhere to the mantle surface giving it a speckled black and white appearance. No attempt was made to determine whether these two formations were caused by different mycorrihizal forming fungi. In the late spring and early summer months

fruiting bodies of a species of Inocybe occurred in many of the containers in which the seedlings were growing. The root systems on these seedlings were predominantly mycorrhizal, and of the smooth, shiny type described above. A possible mycorrhizal association between Douglas-fir and Inocybe has been reported by Trappe (37, p. 93), who has also reported 50 probable mycorrhizal associations of Douglas-fir in the Pacific Northwest (35, 36, 37, 38).

The time at which a lateral root becomes mycorrhizal varies with individual roots. If the parent root is mycorrhizal, and especially if there is a fungus mantle over the surface, a new lateral that develops is covered by a mantle from the time it emerges from the cortex (Figure 28). This type of mycorrhiza rarely elongates more than 3 mm and may produce even shorter branch roots. Some laterals reach several millimeters in length before being invaded by mycorrhizal forming fungi. Here the hyphae may be found at the tip (Figure 29), the base or in scattered patches along the root. Eventually a Hartig-net and fungus mantle forms, followed by frequent branching.

The difference between long mycorrhizal lateral roots and mycorrhizal short roots is primarily a matter of when fungus invasion occurs. Aside from periodic, but limited growth of the mycorrhizal short roots, few elongate beyond 5 mm. Furthermore, the majority

of the mycorrhizal short roots do not appear to live longer than a year. Long laterals that become mycorrhizal are far better developed anatomically and probably live for more than one year.

Like other laterals, the mycorrhizal short roots are originated by periclinal and anticlinal divisions of the pericycle adjacent to the protoxylem poles (Figure 30). The cells of the endodermis of the parent root may divide transversely at first, but are soon crushed by the expanding primordium and do not contribute significantly to the development of the lateral mycorrhiza. In one instance, proliferations of the inner cortical cells were observed outside the developing primordium.

A primordium that will give rise to a mycorrhizal short root follows the same pattern of development as other laterals up to the time of emergence from the parent root. From this point on there appears to be a direct correlation between the development of the fungus mantle and the apical initials. A primordium that is covered with a fungus mantle from the time of emergence develops a rounded apex with two or three layers of rootcap cells, and the meristem is slightly more organized than a degenerate short root (Figure 31). Anticlinal and periclinal divisions of the apical cells are very infrequent. For this reason the meristem does not have a well defined zonation pattern and growth in length is limited to 3 mm or less.

Since obliquely arranged cortical cells are not present in laterals covered by a fungus mantle at the time of emergence, one must assume that a relatively inactive cortical mother-cell zone exists. It appears, therefore, that periclinal divisions at the extreme apex add cells to the stele, but relatively few cells to the rootcap or cortex.

The meristem of roots that become mycorrhizal after they have elongated several millimeters is similar to that of a normal lateral of equal length (Figure 32). The root apex generally extends beyond the developing fungus mantle during periods of growth, but the mantle will eventually cover the apex as the growth rate of the root declines. As long as the root tip remains free from the fungus mantle reasonably well defined apical zones occur (compare Figures 17 and 32). On the other hand, should the fungus mantle extend over the tip of the apex, the activity of the apical meristem is considerably reduced.

The effect the fungus mantle may have on the activity of the meristem is not necessarily permanent. In some mycorrhizal short roots, the apex will rupture the mantle and elongate approximately 2 mm. As the fungus invades the new growth, the meristem becomes less active and finally the mantle completely covers the tip. Masui (cited by Kelley 18, p. 121) has reported a similar situation with

infected short roots of Abies firma, Abies Mayriana, Alnus japonica and Pinus densiflora, where vigorous growth occurs, followed by transformation into mycorrhizae once more. McMinn (23, p. 116) also reported the occurrence of elongated mycorrhizal short roots on Douglas-fir in Canada.

Ergastic materials, especially resins, appear in the outer cortical tissues and, less frequently, throughout the cortex (Figure 27). At first glance one might mistake these materials for hyphae of endotrophic fungi, but closer observation reveals the structures to be yellowish droplets like those found in the pericycle and endodermis of normal and degenerate roots. The outermost cortical cells and persistant rootcap cells are all heavily tanninized. In many cases the fungus hyphae appear to have completely surrounded the cells of the rootcap and rhizodermis. This may account for the lack of development of root-hairs on mycorrhizal short roots.

Some hypertrophy in the cortical tissues is evident. The increased diameter of mycorrhizae over other short roots, however, is also attributed to the thickness of the fungus mantle and the presence of intercellular hyphae in the cortical region. In addition, the cortical cells do not collapse early, as they do in a normal root, where the Hartig-net is well developed. The Hartig-net is located between older cortical cells and is not found in the tip region. The

fungi may extend to the endodermis, but the presence of tanniferous material in the endodermis and outer pericycle cells is believed to be responsible for stopping further penetration by the hyphae (14, p. 28). When lateral roots elongate several millimeters before becoming infested with mycorrhizal forming fungi, the mantle generally does not form over the surface until after the Hartig-net appears.

Mycorrhizal short roots have a vascular cylinder which is elliptical in transverse sections, the stele is diarch and the protoxylem poles are located at the narrow ends of the ellipse. Secretory elements occur in the mycorrhizae of Douglas-fir (Figure 32), but due to the small size of the stele, it was impossible to determine whether they are located in the phloem or pericycle.

Since cell elongation and meristematic activity is reduced in mycorrhizae, the level of tissue differentiation is altered. Vascular tissues differentiate to within a few cells from the root meristem in mycorrhizal short roots, which is much closer than in noninfected lateral roots. Protoxylem elements are scalariform to reticulate, while metaxylem elements have bordered pits. The branch laterals that develop on mycorrhizal short roots have bordered pits which appear on the xylem elements at the base of the lateral when it is often less than 500$\mu$ long (Figure 28). The development of primary phloem, pericycle and endodermis appears to be identical to

degenerate short roots. Also, like degenerate roots, the mycorrhizal short roots have no secondary growth or resin ducts.

## Root Regeneration

Considerable variation exists in the development of new laterals following pruning, and without rigid controls and detailed statistical records these differences are not easily resolved. Repeated observations, however, give reason to suspect that the age of the seedling or level or pruning might affect the length, number and location of regenerated roots. As a general rule, the older seedlings produce the fastest growing adventitious laterals. This is especially true where the upper first order laterals are pruned approximately 2 cm from the primary root, or pruned back to the surface of the primary root. New roots that develop on pruned lower laterals elongate much less than those on upper laterals.

Some differences are noticed between the development of adventitious roots on upper laterals. Where the upper first order laterals are pruned back to the surface of the primary root, adventitious laterals develop on the primary root directly above and below the base of the pruned lateral (Figure 33). Less frequently, adventitious roots will originate several millimeters from the pruned lateral. The new roots elongated to an average of five inches in 95 days after

pruning, with one regenerated lateral elongating 11 inches. Adventitious roots originate near the cut surface on upper first order laterals pruned to within 2 cm of the base of the root (Figure 34). On two and three year old seedlings, an average of six adventitious laterals developed from each pruned root. Adventitious roots that developed in this manner had an average length of 10 cm 95 days after pruning. On pruned one year old seedling roots, regenerated laterals were less numerous and slower growing.

Lower laterals pruned near the tips also regenerated new roots near the pruned surface. The new laterals usually equalled the number of protoxylem poles, and seldom elongated more than three inches in 95 days after pruning (Figure 35). A number of blackened lower laterals failed to produce adventitious roots when pruned. Others formed primordia, but the new laterals never developed beyond this early stage. A closer examination revealed that these dark roots were infected, in various stages of decay, and would soon disintegrate.

The development of adventitious laterals should have a significant value in regard to seedling transplant survival. This is especially true where the upper first order laterals are pruned back to 2 cm from the primary root (Figure 34). In this method of pruning, the upper laterals that are less than 2 cm long escape pruning.

These roots probably are not a part of the original root system, but could be adventitious laterals that developed as a result of an earlier accidental pruning. These unpruned upper laterals make up the majority of the new root system within the first 30 days (Figure 36). At the end of the 30 day period most of the adventitious laterals are still in their primordial stages, but a few may have elongated 1-2 mm. After 95 days, however, the initiation and subsequent elongation of adventitious roots on the pruned laterals far exceeds the elongation of the unpruned upper laterals (Table 1).

Wound healing in Douglas-fir is quite similar to that described for Abies procera Rehd. by Wilcox (40, p. 223-228). At the pruned surface there is a zone of dried up cells followed by a zone of disorganization and necrosis (Figure 37). Behind this necrotic region there develops a callus zone characterized by wound tracheids and anomalous parenchyma tissues (Figure 38). A transition region between normal (Figure 39) and callus tissue marks the end of the wound area, and the region where regenerated roots usually develop.

When only the tips of older laterals are removed, there is an increase in the number of new laterals that originate from pericycle in the region where normal laterals ordinarily appear. As the older part of the root is pruned, new laterals develop near the pruned surface. New roots no longer originate in the pericycle if pruning occurs

Table 1. Relationship between the development of adventitious roots on pruned upper first order laterals and of unpruned upper first order laterals. Two and three year old seedlings were grown in the greenhouse for 150 days (March-August, 1963) after pruning the upper first order laterals to within 2 cm of their base.

| Tree No. | Number of laterals | | Total length of laterals (cm)[1] | |
|---|---|---|---|---|
| | Multi-[2] laterals | Single[3] laterals | Multi - laterals | Single laterals |
| 1 | 86(11) | 17 | 841 | 143 |
| 2 | 54( 6) | 8 | 628 | 130 |
| 3 | 24( 6) | 11 | 223 | 140 |
| 4 | 58(14) | 11 | 616 | 100 |
| 5 | 31( 9) | 15 | 504 | 315 |
| 6 | 84(13) | 11 | 792 | 142 |
| 7 | 95(14) | 10 | 738 | 122 |
| Totals | 432(73) | 83 | 4342 | 1092 |
| Range | 71( 8) | 9 | 618 | 215 |
| Average per tree | 61.7(10.4) | 11.9 | 620 | 156 |
| Total dry weight (gms.) | | | 9.708 | 3.950 |
| Average per tree | | | 1.386 | 0.564 |
| Average per lateral | | | 0.0224 | 0.0475 |

[1]The measurements represent the length of adventitious mother roots or first order laterals and not their branch roots. The branch roots, however, are included in the dry weights.

[2]The first number represents the total number of adventitious laterals that develop near the pruned surface (multilaterals). The number in parenthesis is the total number of pruned upper first order laterals.

[3]The single laterals may be either short lateral roots present at the time the roots were pruned or they may be adventitious laterals formed from tissues of the primary root as the result of accidental pruning.

where the cambium has formed outside the protoxylem poles. Instead, the adventitious laterals originate from cambium along with limited proliferations of phloem parenchyma and pericycle.

On pruned one and two year old roots, adventitious laterals originate opposite a protoxylem pole (Figures 40 and 41). Regenerated laterals may originate away from the poles on pruned two and three year old roots, but are always located at the end of a vascular ray (Figure 42). In view of their location at the ends of vascular rays, the ray initials probably contribute to the development of regenerated roots. As the primordium develops, the innermost pericycle cells proliferate to form a sheath over the new growth (Figures 41 and 42). These cells soon become tanninized and persist as a protective layer until the apical initials of the new root are differentiated. In other respects, the meristem of adventitious laterals appears to be identical to that of normal long laterals (Figure 43 and 44).

The stele of adventitious roots is commonly diarch, but occassionally triarch in fast growing upper roots. No differences were noticed between the development of primary phloem, pericycle and endodermis in regenerated roots and normal long laterals. Development of secondary growth, resin ducts, rhizodermis and root-hairs also appear to be the same as in normal laterals. There are two

major differences, however, between the primary vascularization of normal and regenerated lateral roots. In adventitious roots the first tracheary elements to appear have bordered pits as opposed to the scalariform elements of normal laterals. The second difference is that the primary xylem elements make their connections with the secondary xylem of the parent root rather than with the protoxylem.

# DISCUSSION

The organization of the root apical meristem has been reported for Douglas-fir (6). Tissue differentiation of the primary root and its associated laterals has never been fully described, however. As previously stated, the secretory cells are the first of the primary tissues to mature. These cells are common to gymnosperms and have been described principally in reference to the primary phloem region (30, p. 189; 39, p. 813; 40, p. 225). Allen (5, p. 78) has described secretory elements in Pseudotsuga and suggested that they make possible rapid conduction of soluble substances through regions lacking functional xylem and phloem. He recognized the precursory phloem as being more or less associated with secretory cells, but did not consider them analogous as suggested by Smith (27, p. 67). The viewpoint advanced by Smith is that the secretory cells in the phloem region should be considered as the first elements of the primary phloem that become elongated due to their early differentiation.

The present study reveals that in the primary root the secretory cells are located between the precursory phloem and pericycle. Here, their exact origin is questionable, but in the long lateral roots secretory elements appear to be in the inner pericycle layer.

Wilcox (39, p. 813) described the secretory cells in Abies procera as being in between the precursory phloem and pericycle. For the roots of incense cedar, however, Wilcox (41, p. 225) reported that the secretory elements are in pericycle.

The above information, then, provides a basis for assuming that secretory elements in the roots of Douglas-fir might be associated more directly with the pericycle than with the phloic procambium. Also, there appears to be some difference between the location of these elements in the primary root and lateral roots.

In the ontogeny of the primary root, metaxylem elements are distinguished first by their early vacuolation, but mature after the differentiation of the protoxylem. The protoxylem matures soon after the cells complete their elongation, while the metaxylem elements differentiate concomitantly with the development of the first secondary xylem. This type of vascular development has been reported many times in the past, and more recently by Wilcox for the roots of noble-fir (39, p. 815) and incense-cedar (41, p. 229).

Allen (5, p. 76-77), after reviewing the literature on the protoderm of gymnosperms, concluded that for the roots of Pseudotsuga there is no true epidermis. The term "rhizodermis" is generally accepted which describes the dermal system in Douglas-fir. According to Plaut in 1910 and Von Guttenberg in 1941 (cited by

Allen 5, p. 76) the rhizodermis develops endogenously and is not considered homologous with the epidermis of the shoot and hypocotyl which arises from surface cells. Kroemer (cited by Allen 5, p. 76) defined the rhizodermis as being one to several layers thick. In Douglas-fir it is difficult to determine which cell layers should be included in the rhizodermis. The outermost cell layer in the region prior to the development of root-hairs is not epidermis or cortex, but a persistant, uniseriate row of rootcap cells (Figures 8 and 9). These cells should not be included as part of the rhizodermis, since they are soon pushed off by the formation of root-hairs from the outermost cortical layer. Root-hairs, then, develop from the outer cortical layer as rhizodermis, and not hypodermally as reported by Addoms (1, p. 208) for Pinus.

The heterorhizic root system of Douglas-fir has been described by Stone, Jenkinson and Krugman (34). They recognized the long first and second order laterals, as well as various combinations of shorter roots. The development of long laterals is very similar to that of the primary root except: (1) the secretory cells appear to be located in pericycle and not between the pericycle and precursory phloem; (2) the stele is usually diarch rather than triarch; (3) the protoxylem elements, aside from being scalariform, are also reticulate (Figure 20); and (4) the second and third order long laterals

may become mycorrhizal, which tends to reduce their growth rate.

Short roots, on the other hand, are more varied in their development. In the past, most of the research has focused on the mycorrhizal short root. In 1885, Frank (cited by Kelley 17, p. 147) coined the term "mycorrhiza," which is a compound of Greek terms meaning "fungus root." Although the structure was described as being analogous to the thallus of the lichen, the term today is generally restricted to such an organ formed from short roots (15, p. 86).

Three general types of short roots have been recognized by others and are discussed in detail here. They are: (1) the uninfected short root; (2) the "pseudomycorrhiza", a short root infected with non-mycorrhizal forming fungus, and (3) the ectotrophic mycorrhiza with a typical fungus mantle and Hartig-net. Endotrophic mycorrhizae were reported by Lewis (21, p. 860) and McDougall and Jacobs (22, p. 258) to occur in Douglas-fir roots, but were not observed in the present study.

Many so called short roots are simply immature laterals that will eventually elongate. It is only natural to assume that a certain number of these young roots will abort or become mycorrhizal, and thus have their growth restricted. It has been pointed out earlier in this paper that the anatomy of long lateral roots and mycorrhizae is quite different. Aldrich-Blake (3, p. 23) stated, however, that there

are no marked qualitative differences in anatomy between short and long roots in pine. Hatch and Doak (15, p. 93) believed that rapid elongation is the primary reason long roots of pine ordinarily escape attack by mycorrhizal fungi. They also postulated that, for Pinus, every root tip is theoretically capable of conversion into a mycorrhiza. Preston (25, p. 447) and Harley (14, p. 25), on the other hand, agree that short roots are restricted in their growth in length, are short lived, and often become mycorrhizal. From the above discussion, it would seem that some investigators might consider certain lateral primordia as predestined short roots. Such is not the case for Douglas-fir.

As opposed to immature, but potential long laterals, mycorrhizal short roots have their teminal growth retarded and often become heavily branched (Figures 24 and 26). According to Laing (19, p. 13), the cortex disintegrates in the unbranched mycorrhizal roots of Pseudotsuga, but such early disintegration is rare in the branched forms. The present study reveals that the unbranched, disintegrating roots are actually degenerate short roots. A Hartig-net may form in patches along these degenerate short roots and will persist only until the cortical cells collapse. In Douglas-fir the mantle which forms over the tip of the mycorrhiza probably does not decompose the rootcap cells to any great extent. The apical initials,

however, are less active where a fungus mantle forms over the tip (Figure 31). For the mycorrhizae of Fagus sylvatica, Clowes (10, p. 526-528) gave anatomical evidence for believing that rootcap cells of ectotrophic forms are decomposed within the fungus mantle.

In slow growing mycorrhizae vascular tissues differentiate close to the root meristem (Figure 31). In addition, there is a tendency for slow growing mycorrhizae to omit the scalariform xylem elements. Clowes (9, p. 12) reported a similar tendency of the mycorrhizae of Fagus sylvatica to omit the early types of xylem elements. Smith and Kersten (29, p. 223-233) studied roots of Vicia faba grown from seeds irradiated with soft x-rays and concluded that the type of wall thickening in primary xylem is determined by the extent of elongation of the surrounding tissue. Furthermore, in the slowest growing roots the first elements to differentiate near the apex had pitted secondary walls.

The meaning of the term "pseudomycorrhiza" varies considerably among different investigators. Melin (cited by Kelley 17, p. 148) first described short roots that were infected with non-mycorrhizal forming fungi and called them "pseudomycorrhiza." Implications by Hatch and Doak (15, p. 94-95) and Kelley (18, p. 118) are that the attack by the fungus results in cessation of growth and structural changes of the short root. In the present study, it appears

that such a parasitic relationship between fungus and the short root of Douglas-fir does not exist, and the term pseudomycorrhiza does not apply. Latham, Doak and Wright (20, p. 14) recognized the advantages of mycorrhizae over the pseudomycorrhizae, for the latter have lower absorption qualities. On uninfected short roots of Pinus sylvestris, Robertson (26, p. 266) noticed that the cortical cells die rapidly. He suggested that this leaves the short root more susceptible to attack by pseudomycorrhizal fungi. Here a saprophytic relationship is implied, but the term pseudomycorrhiza and its inherent meaning remain. In this study, then, what has usually been called a pseudomycorrhiza is considered to be actually a degenerate short root. The gradual loss of apical activity and organization preceeds the invasion by either saprophytic or mycorrhizal fungi. It seems doubtful that these degenerate roots would ever become truly mycorrhizal.

The hypothesis is now advanced that all short roots on Pseudotsuga menziesii (Mirb.) Franco, are potential long second and third order laterals. That these roots do not all develop into long laterals can be explained on the basis of: (1) the failure of the meristem to develop, resulting in the subsequent degeneration of the young root; or (2) the formation of mycorrhizae while the lateral root is in its early stage of development.

Stone, Jenkinson and Krugman (34) studied root regeneration of Douglas-fir seedlings on a seasonal basis. Their main objective was to measure both the lateral root elongation potential and the lateral root initiation potential, which together were referred to as the "root-regenerating potential." In their studies they recognized three areas where regenerated roots originate: (1) young unbroken laterals that begin to elongate rapidly (Figure 36); (2) adventitious lateral roots that originate near where the older roots have been removed (Figures 33 and 34); and (3) lateral roots that originate away from the wound tissue. In the present study on the seedling roots of Douglas-fir these areas are easily recognized.

Stone and his colleagues found that over a 30-day period the root-regenerating potential was primarily an expression of lateral root elongation rather than initiation of new lateral roots. Under many field conditions, a high internal moisture stress develops within the seedling in the absence of root elongation. These investigators point out, therefore, that it is important to prevent injury to the young lateral roots present at the time the seedling is transplanted.

In the coastal forests of the Pacific Northwest the problem of high internal moisture stress is less critical. Here the development of adventitious laterals should have a significant value with regard to

seedling transplant survival. When the upper laterals are pruned, and new roots are allowed to develop for 95 days, the root-regenerating potential is typically the result of initiation and subsequent elongation of adventitious roots on the pruned laterals (Figures 33 and 34).

Aside from their differences in origin, adventitious roots develop in much the same manner as the laterals from which they are derived. Adventitious roots that develop from a pruned upper lateral are generally longer and larger than new roots formed on higher order pruned laterals, or on the lower laterals. The origin of these adventitious laterals is not as easy to determine as is their later development. If a root is pruned near the tip, then new root primordia will originate from pericycle as do normal laterals. New roots no longer originate in pericycle if pruning occurs where the cambium has formed outside the protoxylem poles. In pruned one and two year old seedling roots, then, the lateral primordia originate in the cambial region opposite the protoxylem poles (Figure 40). In addition to the cambium, proliferations of the pericycle and phloem parenchyma also take part in the development of the primordium. Adventitious laterals may originate away from the poles on older pruned roots, but are always located in the cambial region of the vascular rays (Figure 42).

# SUMMARY

The anatomical development of Douglas-fir seedling roots from germination to three years, and the development of adventitious laterals following pruning is described. The lateral roots include: (1) long first, second and third order roots; (2) mycorrhizal long roots of the second and third order; (3) degenerate short roots; and (4) mycorrhizal short roots.

Secretory elements are the first of the primary tissues to differentiate in the primary root and long laterals. In the primary root the secretory elements appear to be located between the precursory phloem and pericycle, while in long laterals they are found in the inner pericycle.

Long first, second and third order laterals are nearly identical in their origin and development, and are initiated in the pericycle opposite the protoxylem poles. Vascular tissue differentiation varies somewhat in all laterals in relation to their growth rates. In mycorrhizal and degenerate short roots, however, vascular tissues differentiate much closer to the root meristem. The tendency of the mycorrhizal roots to omit the early types of xylem elements is predictable.

All short roots of Douglas-fir are potential long laterals. Young short lateral roots fail to develop a well organized meristem and either die early, or are converted into mycorrhizae. These degenerate and mycorrhizal short roots never produce secondary growth or resin ducts.

In one and two year old pruned roots, adventitious laterals originate primarily from cambium at the end of a protoxylem pole. In older pruned roots, new laterals may develop away from the poles, but originate in the cambial region of a vascular ray. In other respects, adventitious laterals develop in much the same manner as normal long laterals. Regenerated roots that develop for 95 days become an important part of the root system, and should have a significant value with regard to seedling transplant survival in the Pacific Northwest.

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## FIGURES 1-4

Figures 1-2. Median longitudinal sections of the primary root tip.

Figure 1. Diagram showing the root tip of a five day seedling. The black arc represents the root initiation zone. X50. (Allen, 1947).

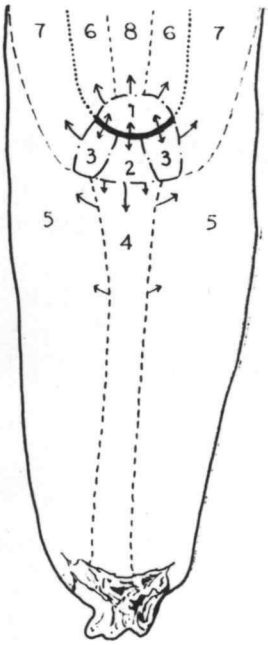
Figure 2. Ten day seedling root. Secretory cells are present. X100.

Figures 3-4. Cross sections of elongating primary roots showing stages of vascular development.

Figure 3. Early stage of vascular differentiation in the primary root. Sectioned 640μ back from the apical initials. X200.

Figure 4. Maturation region approximately 5 mm from the apical initials. X100.

1 - stelar mother-cell zone
2 - column mother-cell zone
3 - cortical mother-cell zone
4 - column
5 - peripheral tissue of rootcap
6 - procambium of embryonic stele
7 - embryonic cortex
8 - medulla of the embryonic stele
e - endodermis
p - inner primary phloem
s - secretory elements
x - protoxylem poles

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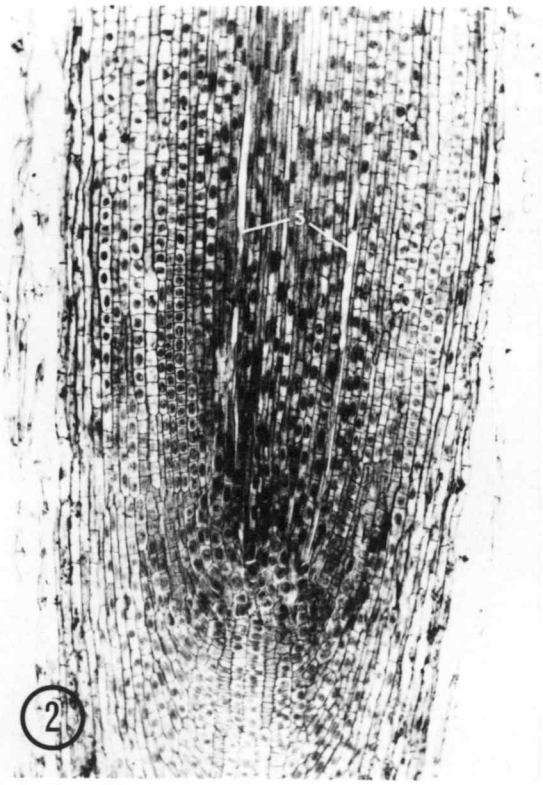


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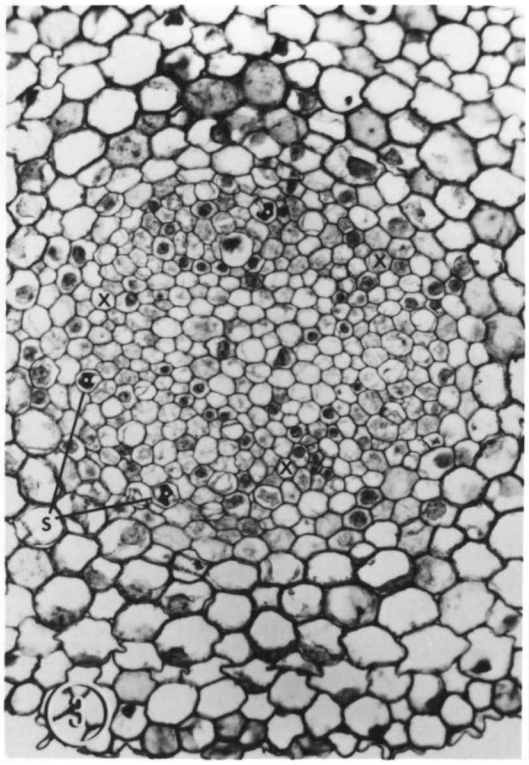


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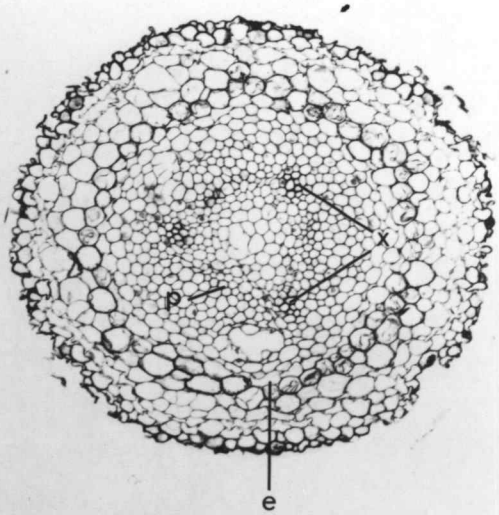


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FIGURES 5-8

Figure 5. Cross section of a 37 day old seedling root, sectioned approximately 7 cm back from the apical initials. Note the collapsed endodermis and cortex. X100.

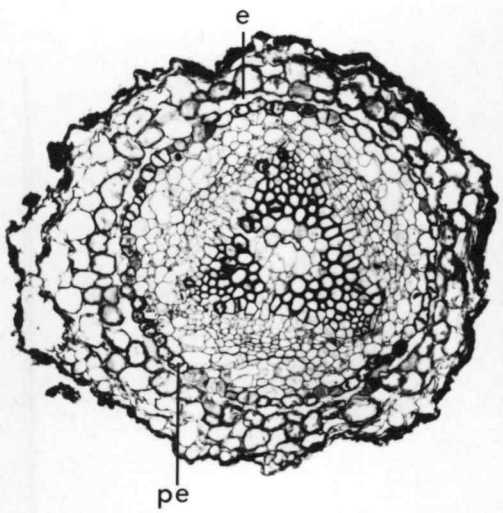
Figures 6-8. Cross sections of the primary root showing the development of the rhizodermis. X400.

Figure 6. Cortical region 290μ back from the apical initials. Note the tangential divisions of the innermost cortical cells and the poorly defined cortical-rootcap zone.

Figure 7. Primary root sectioned 800μ back from the apical initials. All but the innermost rootcap cells are sloughed.

Figure 8. Rhizodermis of root of Douglas-fir illustrating the persistant, uniseriate row of rootcap cells.

c - cortex
e - endodermis
pe - pericycle
rc - rootcap
r - rhizodermis

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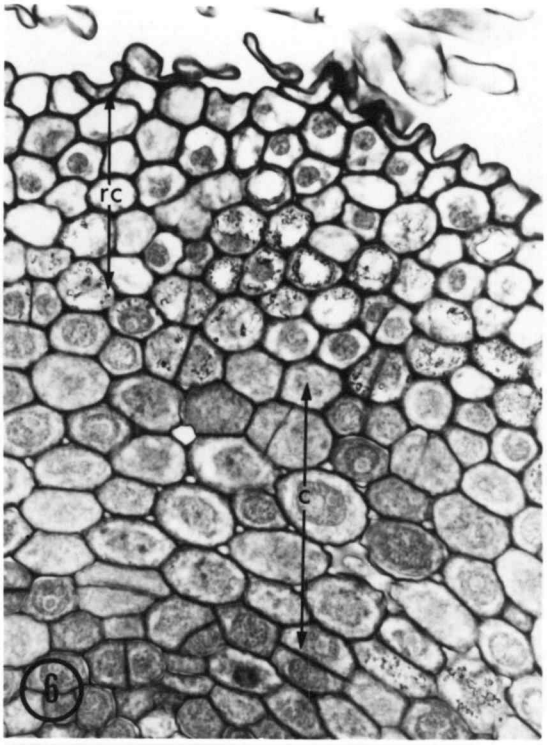


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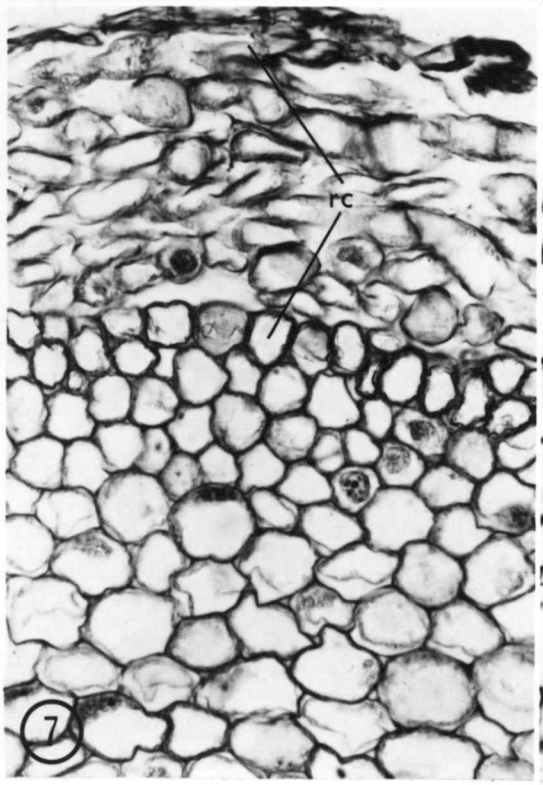


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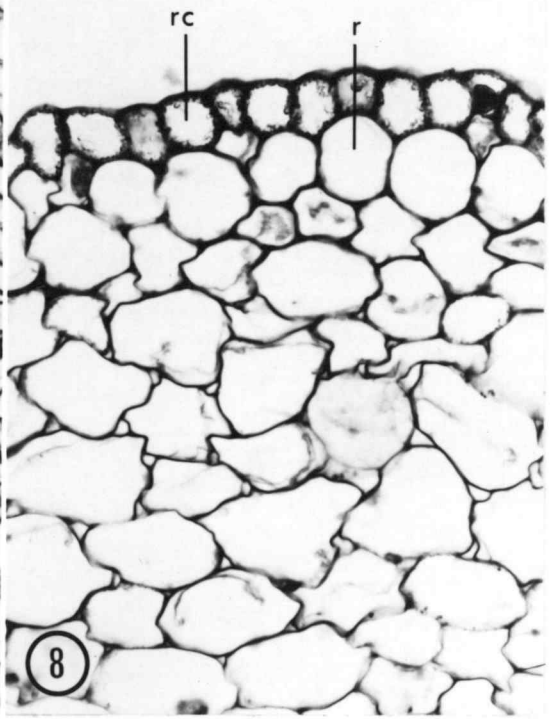


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FIGURES 9-12

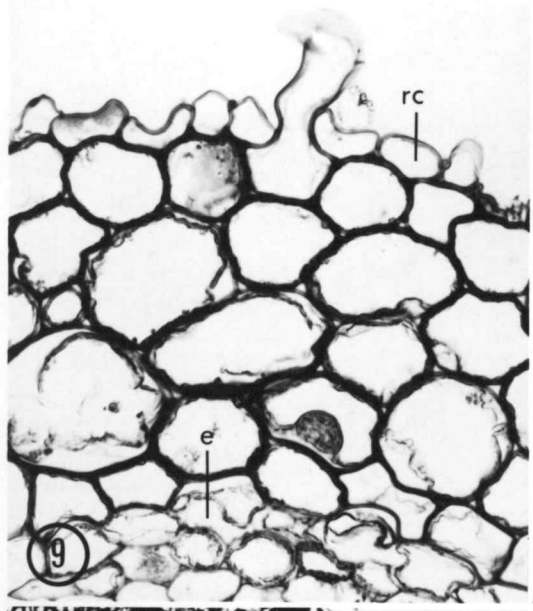
Figure 9. Cross section of the primary root showing root-hair. X400.

Figure 10. Cross section of the primary root showing first cambial activity. X100.

Figure 11. Cross section of the primary root showing the location of the first vertical resin dusts. X100.

Figure 12. Cross section of a 37 day old seedling radicle showing early periderm development. Note cell divisions. X400.

e - endodermis
ph - phellogen
rc - rootcap
rd - resin ducts
vc - vascular cambium

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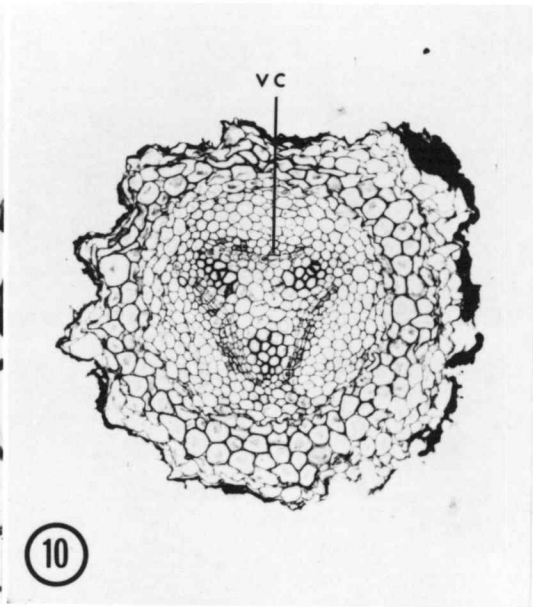


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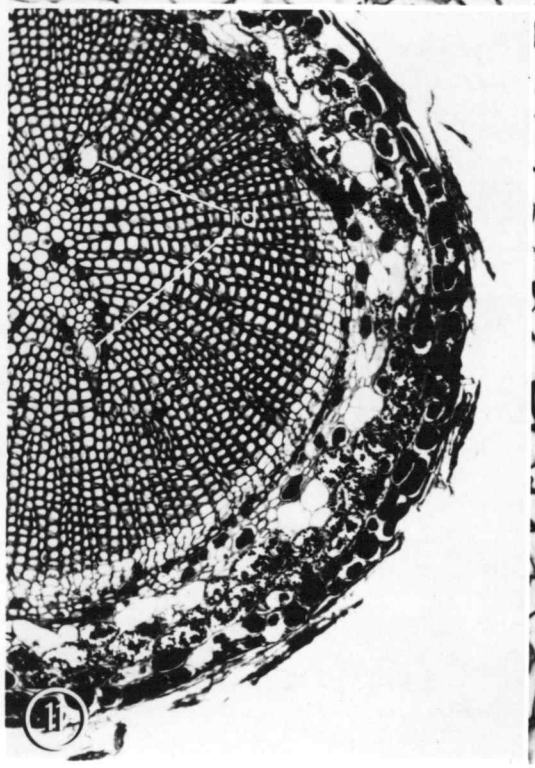

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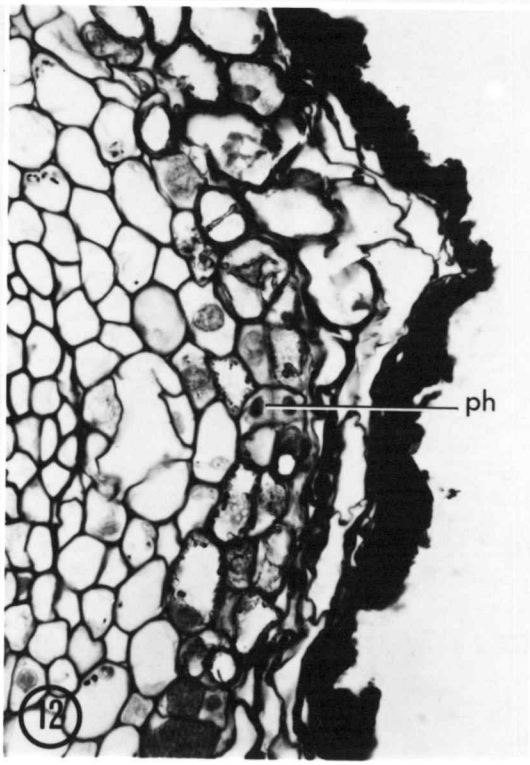


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FIGURES 13-16

Figure 13. Cross section of a three month old primary root showing development of periderm. X400.

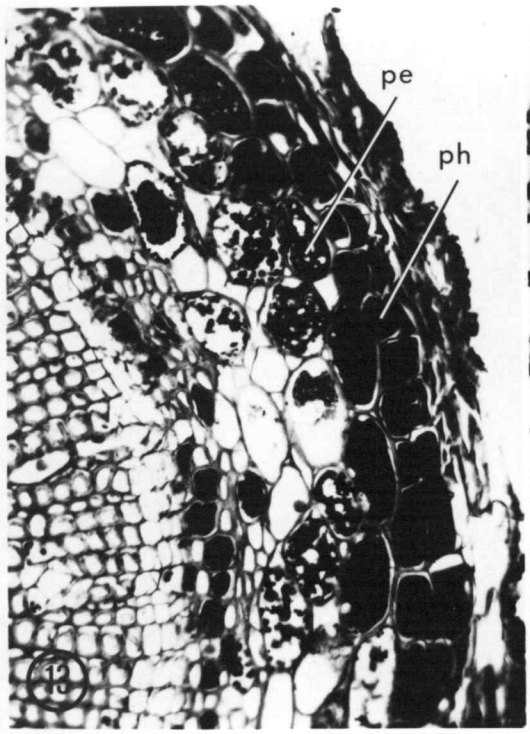
Figures 14-15. Cross sections showing the origin of lateral roots from pericycle. X200.

Figure 14. First order lateral root primordium. Note heavily tanninized cells over the primordium.

Figure 15. Second order lateral root primordium.

Figure 16. Longitudinal section of a first order lateral root. X200.

pe - pericycle
ph - phellogen


pe
ph
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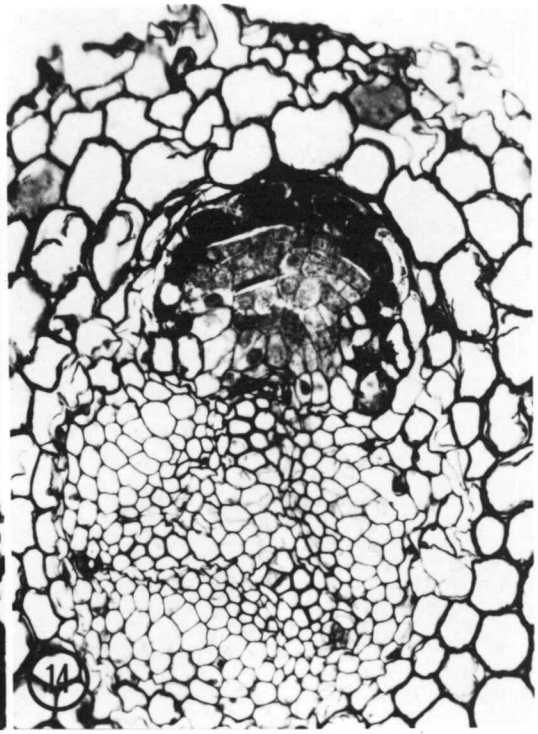
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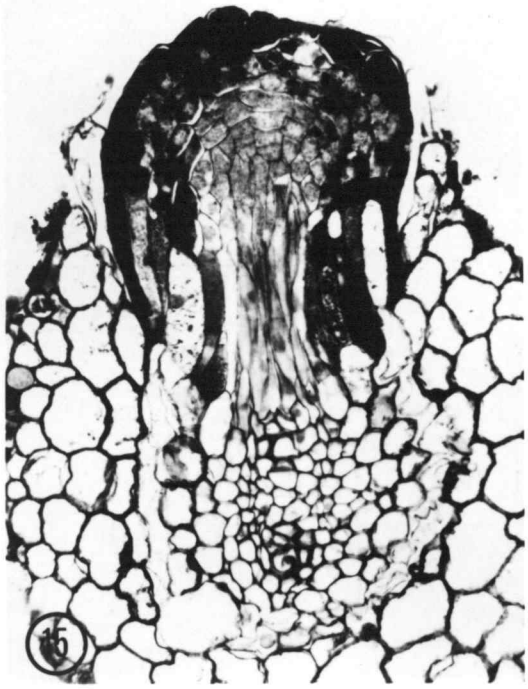
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## FIGURES 17-20

Figures 17-18. Lateral root apices in longitudinal sections.

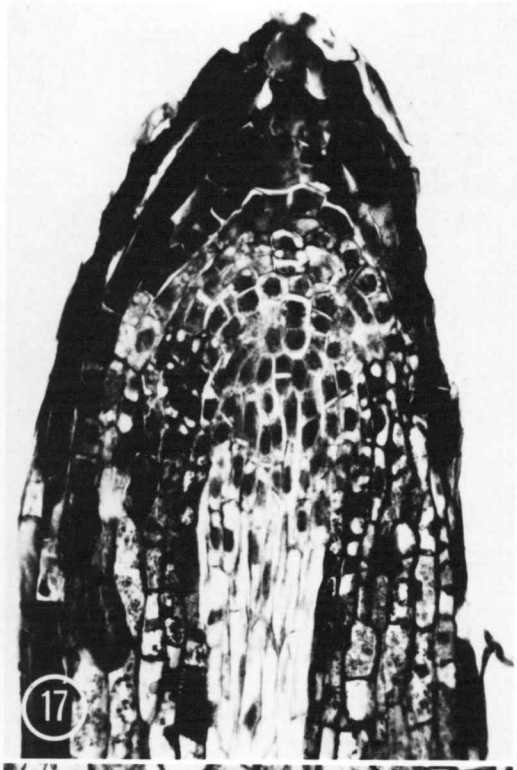
Figure 17. Young second order lateral root (2 mm long) X200.

Figure 18. Older first order lateral root (4 inches long) X100.

Figure 19. Early stage of vascular differentiation in the lateral root. Sectioned 550μ back from the apical initials. Secretory elements are marked with dots. X200.

Figure 20. Longitudinal section showing a reticulate primary tracheary element. Typical of later formed protoxylem elements in lateral roots. X400.

p - primary phloem
s - secretory elements
x - xylem poles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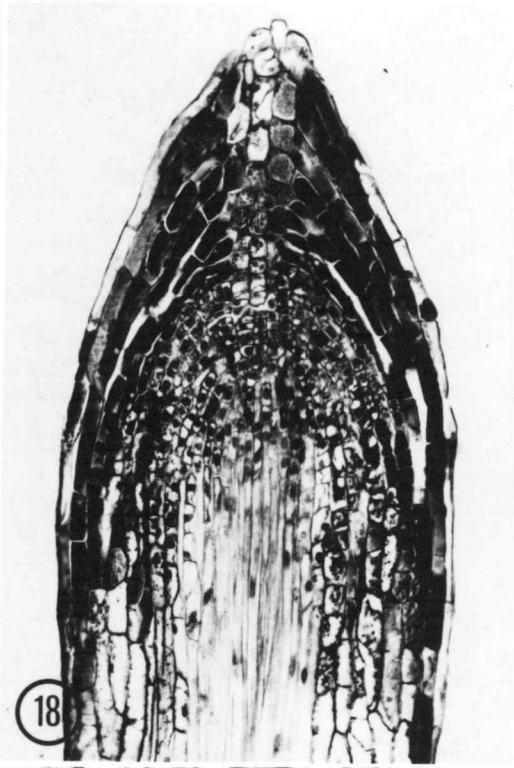
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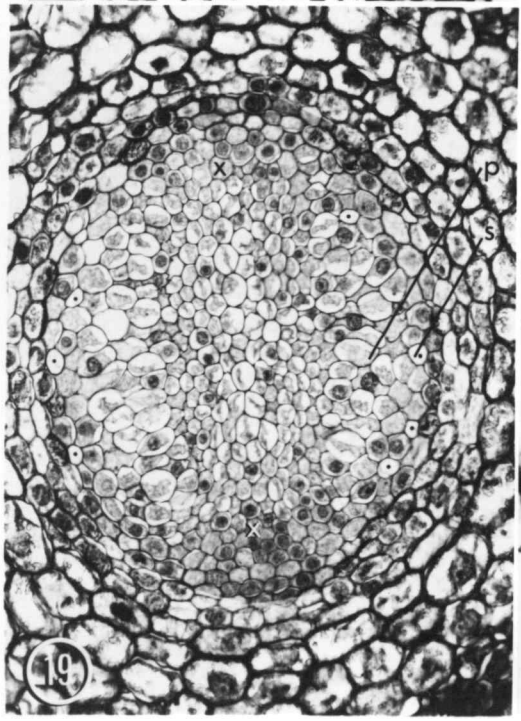

X
X
X
P
S
4
19


20

## FIGURES 21-24

Figure 21. Cross section of a mycorrhizal second order long lateral. Hartig-net present, but no mantle. X200.

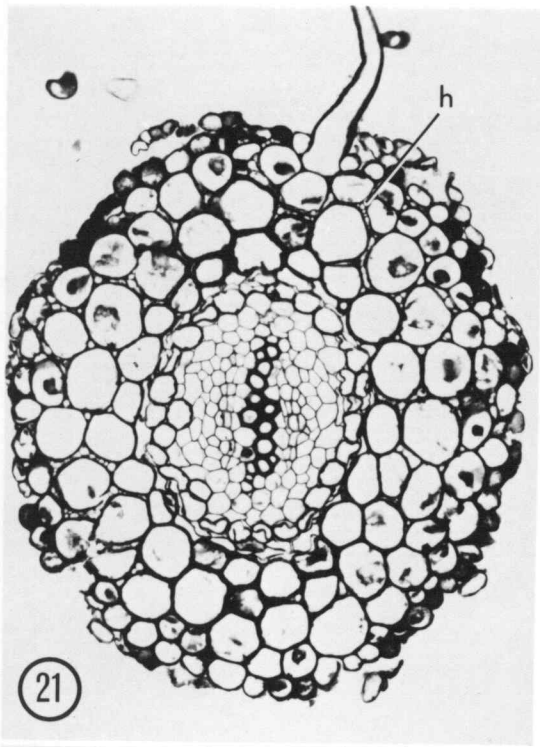
Figures 22-23. Structure of degenerate short roots.

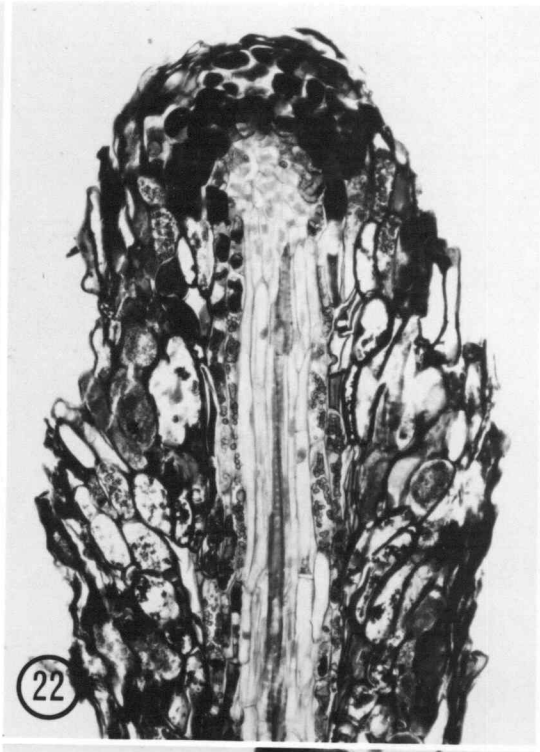
Figure 22. Longitudinal section showing the aborted meristem and obliquely arranged cortical cells. Note also that the xylem elements are differentiated close to the root meristem. X135.

Figure 23. Cross section of a degenerate short root. X200.

Figure 24. Portion of the root system of Douglas-fir showing the characteristic racemose mycorrhizae. Note the shiny, smooth fungus mantle. X5.

e - endodermis
h - Hartig-net
p - primary phloem
pe - pericycle


h
21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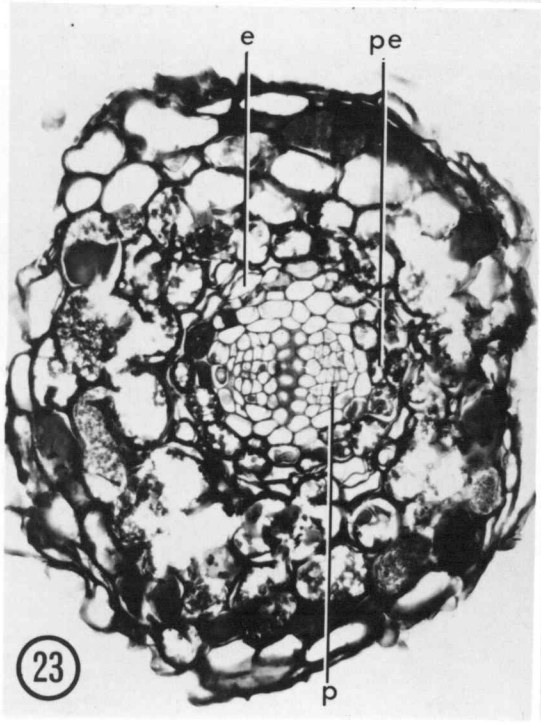
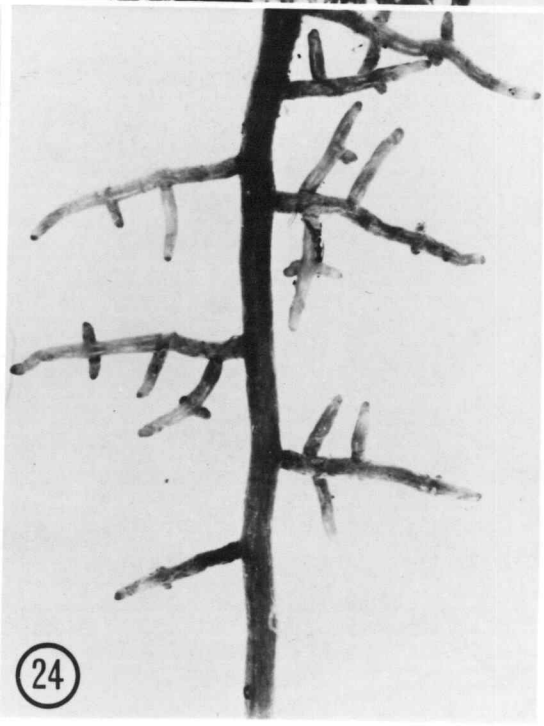

e
pe
p
23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FIGURES 25-28

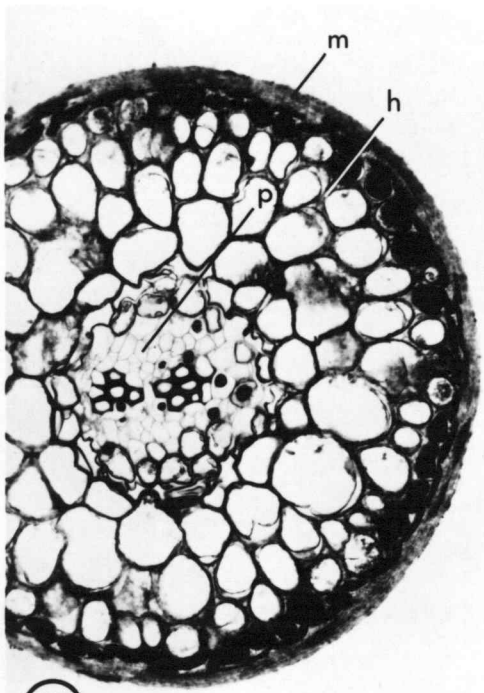
Figure 25. Cross section of a mycorrhizal short root with a smooth fungus mantle. X200.

Figure 26. Portion of the root system of Douglas-fir showing compact racemose mycorrhizae with a rough, white mantle. X6.

Figure 27. Cross section of a mycorrhizal short root with a rough fungus mantle. X200.

Figure 28. Cross section of a mycorrhizal short root showing a newly developed branch lateral. Note the fungus mantle over the root tip and the bordered pits on the primary xylem elements. X200.

e - endodermis
h - Hartig-net
m - fungus mantle
p - primary phloem
pe - pericycle
x - primary xylem

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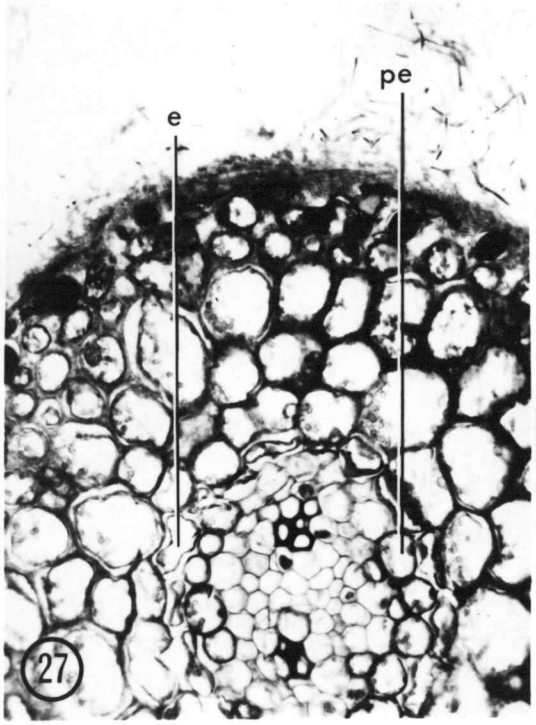


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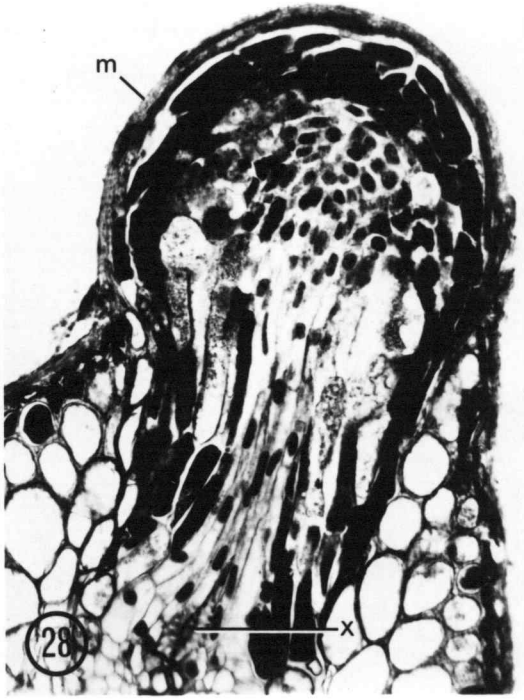


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FIGURES 29-32

Figure 29. A young lateral root which has become mycorrhizal near the tip. Note the shiny fungus mantle (arrow). X9.

Figure 30. Cross section of a mycorrhizal short root showing the origin of a branch lateral adjacent to a protoxylem pole (arrow). X200.

Figures 31-32. Longitudinal sections of mycorrhizal short roots showing the correlation between the development of the fungus mantle and the apical initials. X200.

Figure 31. Mycorrhizal short root with apex nearly covered by a fungus mantle.

Figure 32. Mycorrhiza with its apex free from the fungus mantle.

m - fungus mantle
s - secretory element

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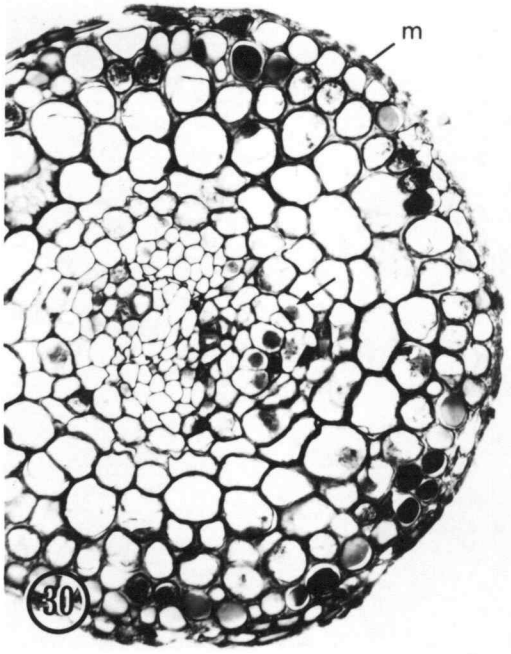


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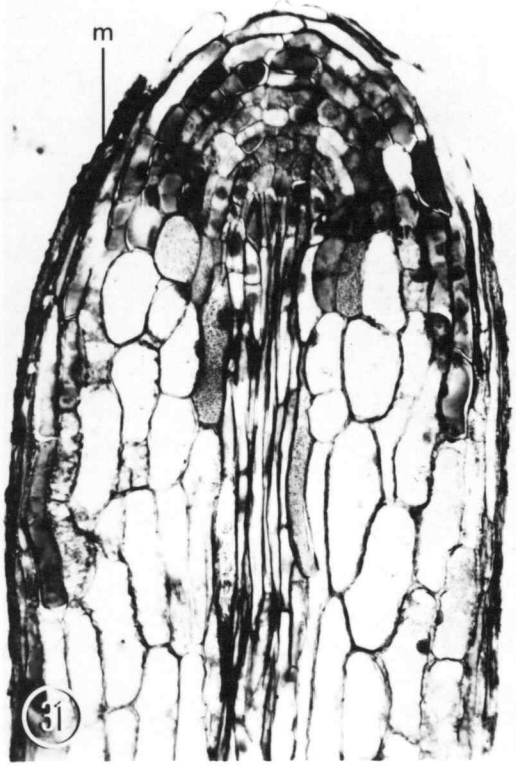


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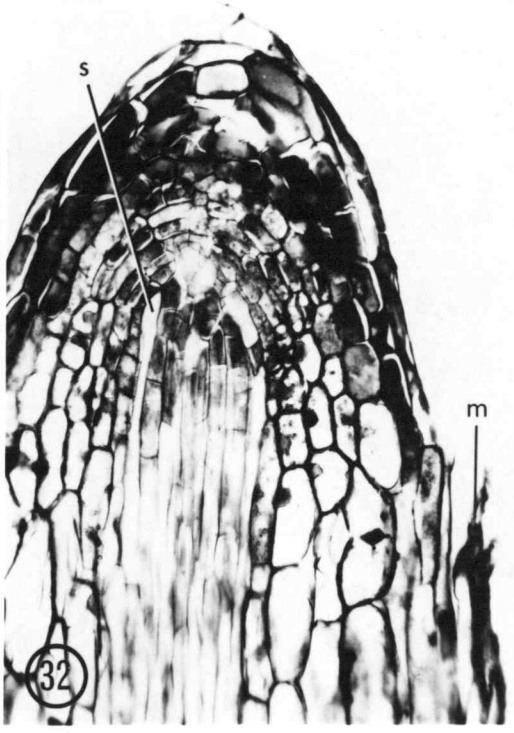


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## FIGURES 33-36

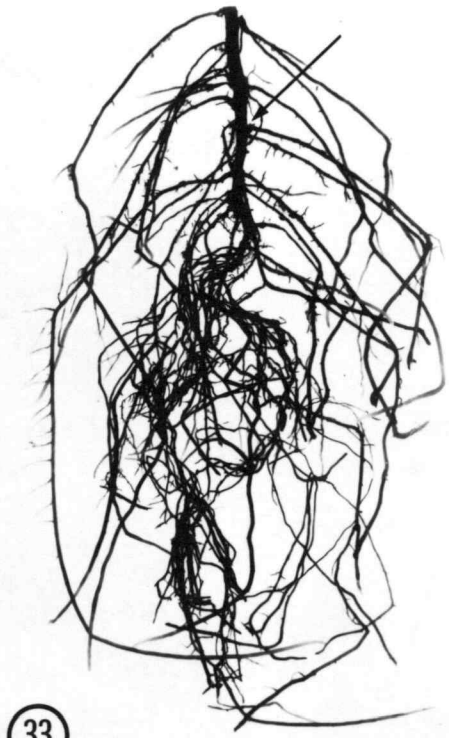
Figures 33-35. Root regeneration on two year old seedlings 95 days after pruning.

Figure 33. First order laterals pruned (at arrow) to the surface of the primary root, from the uppermost lateral downward approximately 8 cm.

Figure 34. Upper first order laterals pruned (at arrow) to within 2 cm of the primary root.

Figure 35. All lower laterals pruned horizontally at a level approximately 8 cm from the uppermost first order lateral.

Figure 36. A two year old seedling root system 30 days after pruning the upper first order laterals to within 2 cm of the primary root. The newly elongated upper laterals were less than 2 cm long at the time of pruning (arrow).



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## FIGURES 37-40

Figures 37-39. Cross sections showing wound healing on pruned lateral roots. X100.

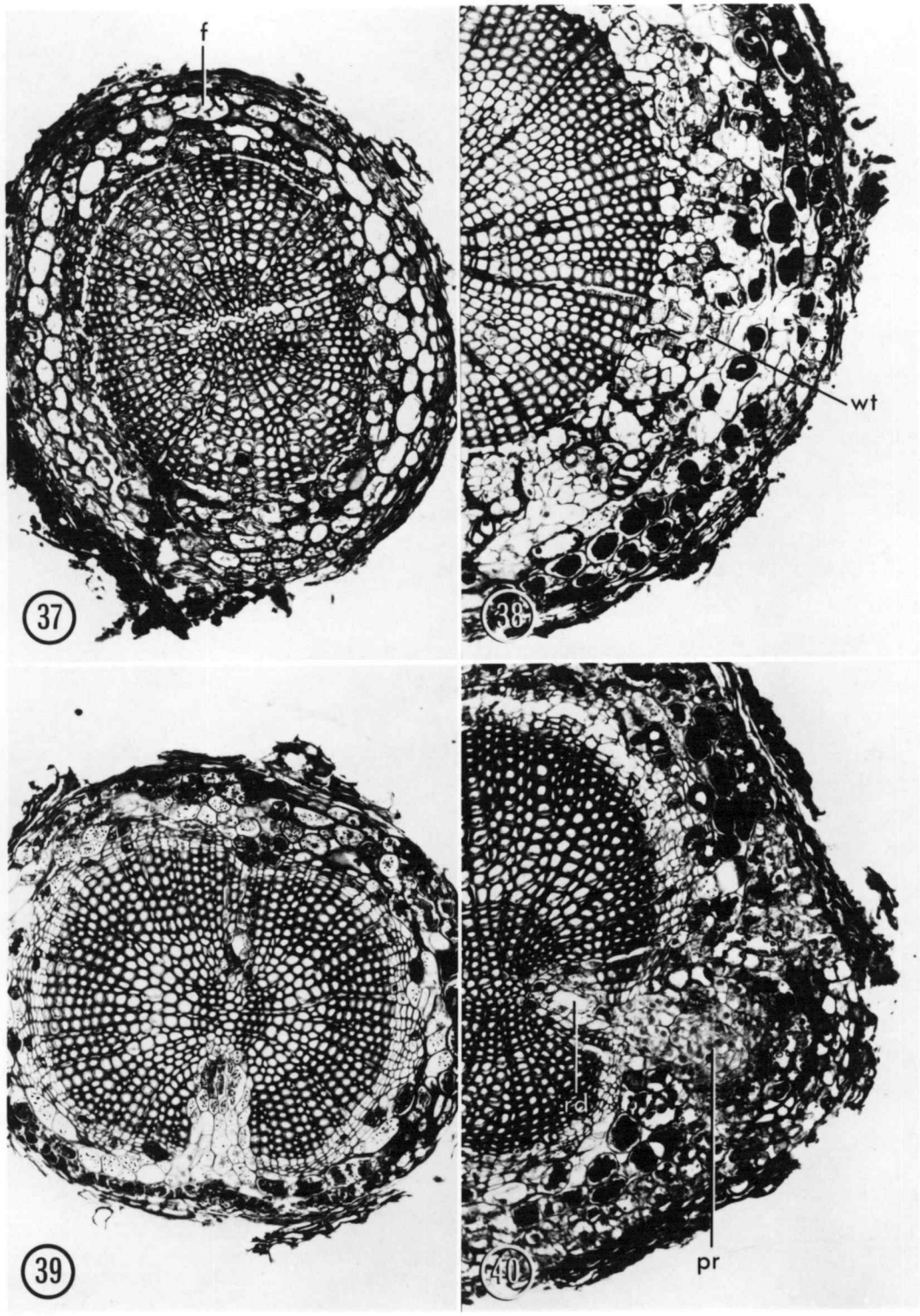
Figure 37. Zone of disorganization and necrosis nearest the pruned surface.

Figure 38. Callus zone characterized by wound tracheids and anomalous tissues.

Figure 39. Normal tissue back of callus zone.

Figure 40. Cross section of a pruned upper first order lateral showing the origin of an adventitious lateral opposite a protoxylem pole. X100.

f - saprophitic fungi
pr - primordium
rd - resin duct
wt - wound tracheids


f
wt
rd
pr
37
38
39
40

## FIGURES 41-44

Figures 41-42. Cross sections showing adventitious root primordia.

Figure 41. Root primordium located opposite a protoxylem pole on a pruned lower lateral. X100.

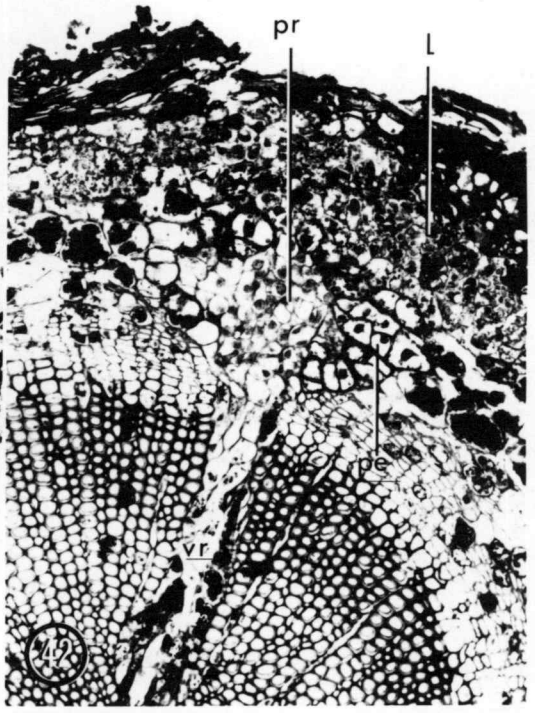
Figure 42. Cross section of the primary root showing an adventitious root primordium at the end of a vascular ray. X100.

Figure 43. Longitudinal section of an adventitious lateral showing the meristem as it emerges from the primary root near wound tissues. X200.

Figure 44. Longitudinal section of an adventitious lateral which has originated on the primary root away from wound tissues. X100.

l - lenticel
pe - pericycle
pr - primordium
rc - rootcap


pe
41


pr
l
pe
vr
42


rc
43


44