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ANATOMY OF ADVENTITIOUS ROOT FORMATION IN STEM CUTTINGS

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The art of propagating plants from cuttings is old and may have started with man's initial interest in ornamental, medicinal, orchard and field plants. But as a science, a series of facts systematically arranged to explain or predict the operation of general laws, the propagation of plants from cuttings did not begin to develop until the eighteenth century (24). Probably the first paper describing the scientific approach to plant propagation was written by the French dendrologist Duhamel du Monceau in 1758 (see fig. 1 and ref. 10). My paper this morning will be an attempt to summarize what is known concerning the anatomy of adventitious root formation in stem cuttings. And when compared with the volumes written by Duhamel, my talk will simply resemble a drop in the bucket.

The rooting of stem cuttings is one of the widely used forms of asexual propagation, a fact which you all know. It involves the placing of stem pieces with one or more buds under conditions that favor the regeneration of roots. Stem cuttings we generally classify as hardwood, softwood and herbaceous depending upon the degree of lignification of the stems, the presence or absence of leaves, and the plant species involved.

Plant propagators are aware of the fact that several easy-to-root plants bear aerial roots or roots in early stages of development within the tissues of the bark. This last group has been referred to by some authors as preformed, hidden, latent and initial roots or root germs. The term morphological roots, first used by the Dutch investigator van der Lek (37), designates all of the roots present when the stems are cut from the stock plants. Figures 2 and 3 show aerial roots that have form-

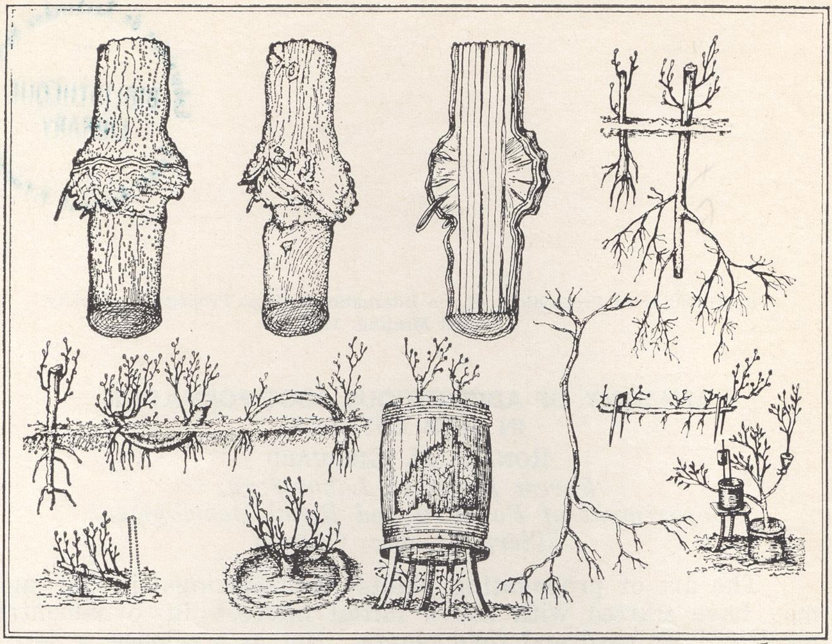


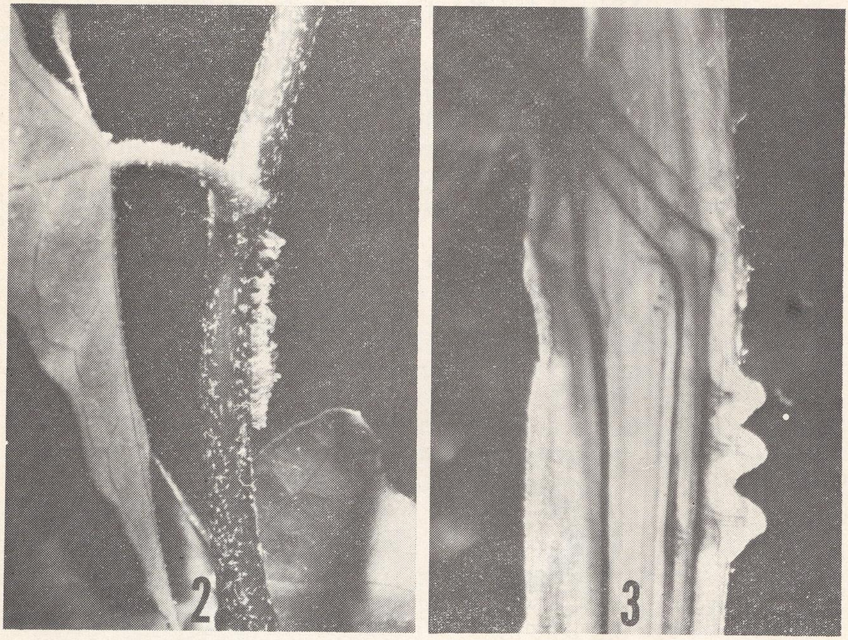
Figure 1. As early as 1758 efforts were made to study plant propagation from a scientific viewpoint. Girdling, grafting, polarity of shoots used as cuttings, and layering were a few of the subjects studied. Redrawn from H. L. Duhamel du Monceau. La physique des arbres. Guerin & Delatour, Paris. Plate XV, volume 2. 1758.

ed just below a node on stems taken from a juvenile plant of *Hedera helix*. In figure 3 the stem was stained with basic fuchsin, cleared and photographed while in methyl salicylate; the vascular bundles have retained the stain. A list of woody plants with stems bearing aerial and latent roots (morphological roots) is given in table 1.

The most common adventitious roots of stems are those that form completely after the cuttings are made; they develop near the base of stems from wounded tissues or above the cuts. It is not surprising that these structures have been named wound or wound-induced roots (18,22,37).

Since most adventitious roots arise endogenously, that is, in tissues located at some distance from the periphery of the stems, it is only appropriate that we should be familiar with some features of the internal structure of stems. First, let us consider the subdivisions of the vascular system, the vascular bundles or fascicles (traces) which function as the transport network of young shoots.

In stems of conifers and woody dicotyledons, the internodes have vascular bundles arranged in rings but separated by panels of cells high in metabolic activity. In cross-sections of stems of a number of herbaceous dicotyledons and many monocotyledons, the vascular fascicles appear scattered. Figure 4 gives us a general idea of the distribution of the bundles as found in several of the plants that we propagate. A closer look at one of the vascular bundles of a woody dicotyledon (fig. 5) reveals two smaller strands or traces: one composed of xylem



Figures 2 and 3. Aerial roots that have formed below a node on stems taken from a juvenile plant of *Hedera helix* are readily seen. In figure 3, the stem was stained with basic fuchsin, cleared and photographed while in methyl salicylate, a clearing agent. The development of vascular traces into the new roots is noted. Figure 3: from Girouard, R. M. Canad. J. Bot. *45*:1877-1881.

Table 1. Woody plants with stems bearing aerial and latent roots. *

Campsis radicans	*Populus nigra thevestina* (37)
Cotoneaster dammeri (38)	*Populus simonii* (37)
Eugeissona spp. (35)	*Populus trichocarpa* (2,3,37)
Ficus carica (11)	*Ribes alpinum* (37)
Hedera helix (36)	*Salix alba* (36)
Jasminum spp. (18)	*Salix alba chermenisa* (37)
Lonicera japonica (27)	*Salix alba sericea* (37)
Maricaria spp. (35)	*Salix amygdalina* (37)
Populus Section *Aegeiros* (4)	*Salix babylonica* (37)
Populus alba (25)	*Salix discolor* (22,37)
Populus x *canadensis* (2,3)	*Salix fragilis* (6,7)
Populus deltoides (25)	*Salix myrsinifolia* (37)
Populus x *euramericana* (25)	*Salix viminalis* (36)
Populus nigra italica (29,36,37)	*Vershaffeltia* spp. (35)

* Numbers refer to references cited.

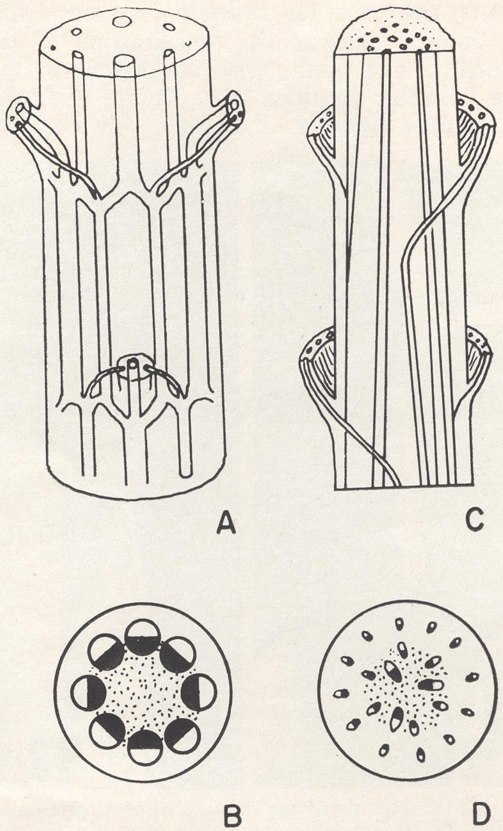


Figure 4. The vascular bundles of conifers and woody dicotyledons at the internodes are arranged in rings (A, B). In cross-sections of stems of a number of herbaceous dicotyledons and many monocotyledons the vascular fascicles are scattered (C,D). Fig. 4 A, C From: B. Huber. 1961. Grundzuge der Pflanzenanatomie. Springer-Verlag, Berlin. p. 84.

(wood) and another of phloem elements. Between the strands of xylem and phloem are cells of a lateral meristem — the fascicular cambium. At a later stage, cambial cells differentiate between the bundles to form the interfascicular cambium. It is the vascular cambium made up of the fascicular and interfascicular cambia which is responsible for the secondary growth of stems. Much of this growth is readily identified as annual growth rings in the secondary xylem. Now for a few minutes let us consider the vascular system at the nodes.

The presence of a leaf results in one or more bundles bending away from the main formation of the vascular system and in connecting the leaf to the stem (fig. 6). Bundles of this kind can be traced down through the stem to a point where they join other strands. These links between the leaf and the stem are the leaf traces. Immediately above the bending part of the traces in the stem, parenchyma cells with unspecialized properties contrast with the highly specialized cells of the vascular tissues. These groups of parenchyma cells make up the leaf gaps. Buds that form in the axils of leaves are compressed shoots or miniature branches. Nevertheless they too are linked to the main stem but by branch traces; they also have branch gaps.

I am certain that many of you like myself have wondered just where in stems the adventitious roots originate and develop, and what anatomical factors influence their development. The answer to these questions have not always been easy to obtain and they have varied considerably from one plant species to the other. I will attempt to summarize studies that have come to my attention.

It is definitely known that adventitious roots have a tendency to arise in the vicinity of differentiating tissues of the vascular bundles; the proximity of the new roots to the main axis of the stems permits a rapid vascular connection between the plant parts. In young stems of dicotyledons and gymnosperms, roots are initiated by cells located near the periphery of the vascular fascicles, often interfascicular parenchyma cells, while in relatively old stems the seats of initiation are

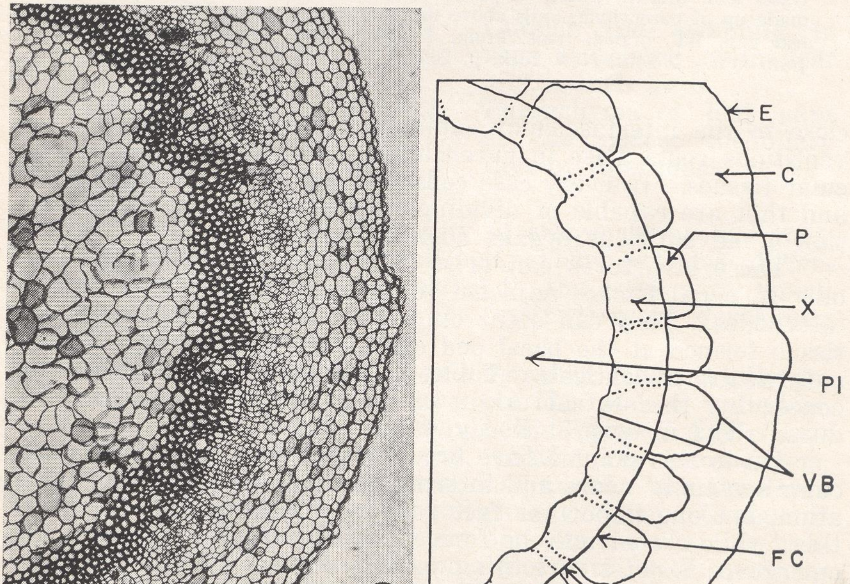


Figure 5. A close look at a cross-section of a young woody dicotyledon shows vascular bundles arranged in a ring and individual bundles composed of two strands: one of xylem (wood) and another of phloem elements. E, epidermis; C, cortex; P, phloem; X, xylem; PI, pith; VB, vascular bundle; FC, fascicular cambium; IC, interfascicular cambium. From: Girouard, R. M. 1967. Canad. J. Bot. *45*:1877-1881.

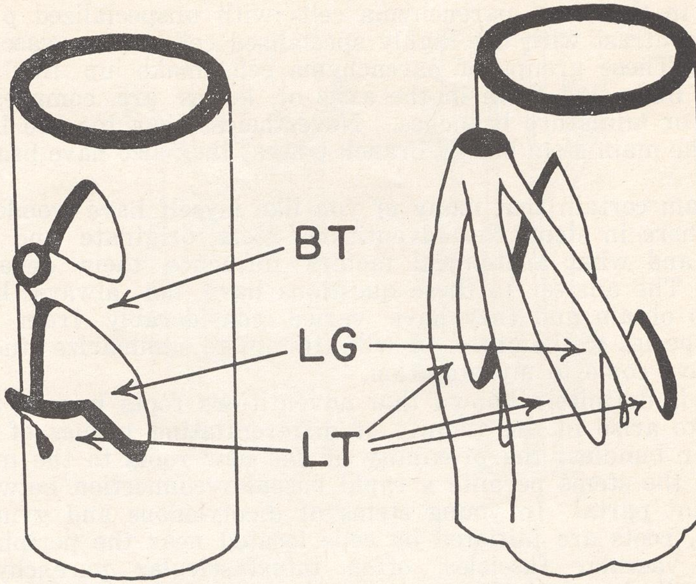


Figure 6. Leaves and buds are connected to the vascular system of stems by leaf traces and branch traces, respectively. Both leaves and buds have gaps made up of parenchyma cells above their respective traces. LT, leaf trace; LG, leaf gap; BT, branch trace. From: K. Esau. 1960. Anatomy of seed plants. John Wiley & Sons, New York, p. 209.

close to the interfascicular cambium. In monocotyledons, adventitious roots arise in parenchyma cells external to the vascular tissues. In every case cells that are alive, not just walls and that are capable of dividing are necessary for the formation of adventitious organs such as roots.

These structures can arise internally (endogenously) not only in nodal tissues adjacent to leaf and branch gaps but in internodes. They can occur externally (exogenously) in callus tissue formed at the basal end of cuttings and in hyperhydric outgrowths of lenticels. Tables 2 and 3 provide information concerning the seat of origin of morphological and wound-induced roots in several woody plants.

Up to this point I have brought to your attention the vascular system at nodes and internodes and the sites of root initiation, but one important fact remains to be mentioned. It is this: while a root may be formed within tissues of a stem, its emergence is not a sure thing. A correlation between the presence of many thick-walled cells such as fibers and sclereids (two components of sclerenchyma) and poor rooting of shoots has been reported by several investigators (1,8,9,17,23). Some workers believe that the groups of fibers act as mechanical barriers preventing the lateral emergence of roots and promoting basal emergence through callus tissue. Recently a few scientists (15,16,~~23~~) have been unable to find simple relation-
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Table 2. Origin of aerial and latent roots (morphological roots) in stems of some of the woody plants listed in table 1.

Plant	Reference	Site
Cotoneaster dammeri	35	Parenchyma cells of divided leaf and bud gaps.
Ficus carica	13	Leaf traces, leaf gaps and medullary rays.
Hedera helix	36	Between the bark and vascular bundles.
Lonicera japonica	27	From cells between the cambium and pericycle at both nodes and internodes.
Populus Section *Aegeiros*	4	External to the cambium but in front of primary rays.
Populus nigra italica, *P. n. thevestina*, *P. simonii*, *P. trichocarpa*	37	Opposite medullary rays.
Ribes alpinum	37	Opposite medullary rays.
Salix alba chermenisa, *S. a. sericea*, *S. amygdalina*, *S. discolor*	37	Parenchyma of leaf gaps at the nodes; medullary rays of internodes.
Salix frailis	6,7	Outside the cambium in rays associated with leaf and branch gaps.
Salix myrsinifolia	37	Parenchyma of leaf gaps at the nodes; medullary rays of internodes.

ships between density or continuity of sclerenchyma and rooting potential in woody stem cuttings. They consider differences in ease of rooting to be related to the ease with which root initials are formed, not to the restriction of developing root primordia by sclerenchyma.

To end what I have to say this morning, I will briefly decribe a few illustrations to give you an idea of how wound-induced roots arise in stems of *Hedera helix*. Figure 7 A, B shows cells at the outermost end of a ray, phloem ray parenchyma, with dense cytoplasm and deeply stained nuclei. The two large cells are the product of a recent anticlinal division. Groups of fibers (F) are present external and opposite the vascular bundles (VB). In figure 8 A, B a young root initial (RI) made up of a slightly organized group of cells is seen developing between two phloem traces. Fibers (F) flank the outer portion of the vascular bundles but they do not hinder the development of the young root. In a few cases (Fig. 9 A, B), vascular rays abut at their distal end on perivascular fibers. Examples of this kind are found even in easy-to-root stems of the juvenile growth phase. The rays often stain densely as shown in this illustration. As the root initials continue to develop, cells divide and differentiate and begin to form organized tissues typical of root primordia (see fig. 10 A, B and 11). Vascular strands quickly differentiate into the actively growing primordia. Phloem elements of at least two vascular bundles and three rays take part in the formation of each root primordium. Scattered groups of fibers are unable to stop the last few stages of adventitious root formation (fig. 12). Roots with rootcaps

Table 3. Origin of wound-induced roots in stems of woody plants.

Plant	Reference	Site
Abelia grandiflora	37	Callus.
Abies spp.	28	Callus.
Acanthopanax spinosa	37	Callus.
Acanthus montanus	34	Near vascular cambium.
Caragana arborescens	33	Secondary phloem; callus.
Chamaecyparis spp.	28	Vascular rays.
Clematis spp.	30	Vascular cambium.
Cryptomeria spp.	28	Vascular rays.
Cupressus spp.	28	Vascular rays.
Forsythia suspensa	33	Leaf and bud traces.
Hedera helix (juvenile)	15	Phloem ray parenchyma.
Hedera helix (mature)	16	Phloem ray parenchyma and callus.
Ilex opaca	21	Vascular cambium extended into callus; young phloem after auxin treatment.
Larix spp.	28	Bud traces.
Ligustrum vulgare	20	Outgrowths of lenticels.
Picea spp.	28	Leaf traces, callus.
Pinus spp.	28	Leaf traces, callus.
Ribes alpinum	33	Secondard xylem, cambium
Rosa 'Dorothy Perkins'	5	Secondary phloem.
Rosa dilecta 'Better Times'	31	Phloem ray parenchyma.
Rubus idaeus	32	Primary rays close to leaf traces.
Rubus occidentalis	32	From leaf and bud traces.
Sambucus nigra	20	Outgrowths of lenticels.
Sciadopitys spp.	28	Branch trees.
Tamarix aphylla	14	Outgrowths of lenticels.
Taxus cuspidata	19	Phloem ray cells and adjacent parenchyma away from basal cuts; secondary phloem near basal cuts.
Thuja spp.	28	Vascular rays.
Thujopsis spp.	28	Rays, leaf traces.
Thujopsis dolobrata	37	Callus.
Ulmus campestris	20	Outgrowths of lenticels.
Vaccinium corymbosum	23	Cambium and phloem.

present emerge above the basal cuts almost at right angle to the main axis of the stems. In cuttings made from stems of the mature growth phase roots also form from cells of the callus tissue (fig. 13 A, B).

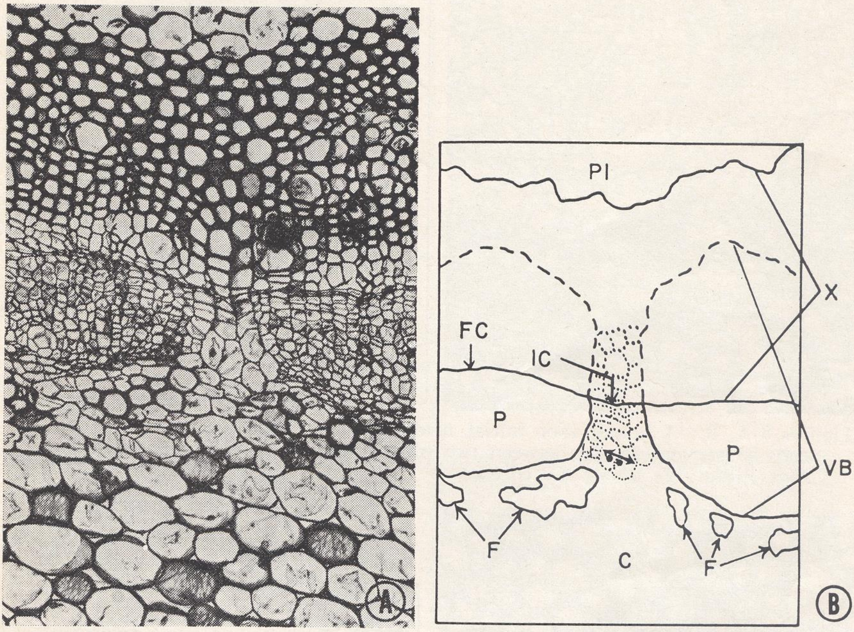
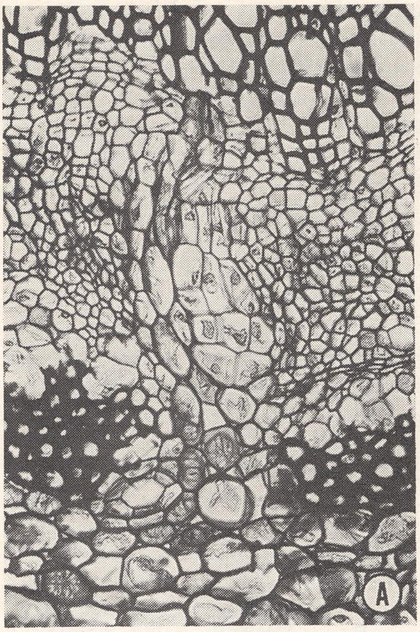
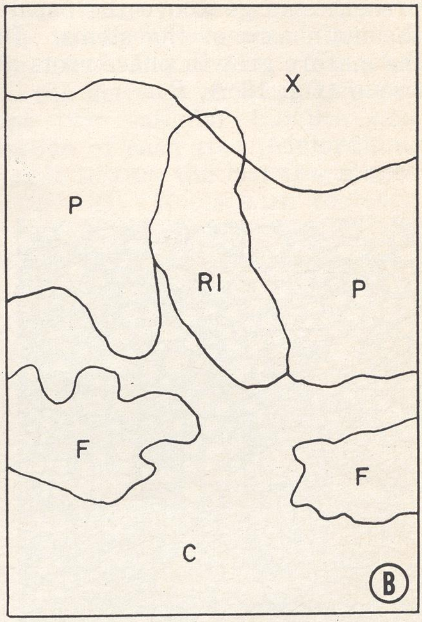
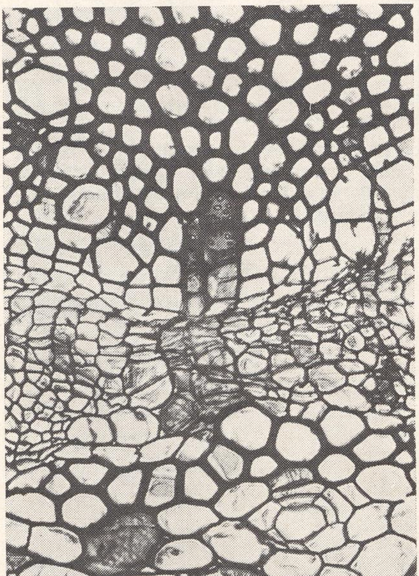


Figure 7 A, B. Two cells of phloem ray parenchyma are the product of a recent anticlinal division. These cells have dense cytoplasm and deeply stained nuclei. PI, pith; P, phloem; X, xylem; VB, vascular bundles; F, fibers; IC, interfascicular cambium; FC, fascicular cambium.





Figures 8 A, B. A young root initial made up of a slightly organized group of cells is seen developing between two phloem traces. X, xylem; P, phloem; RI, root initial; F, fibers; C, cortex.



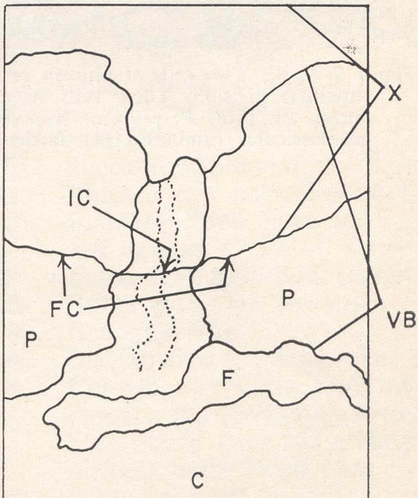


Figure 9 A, B. A densely stained vascular ray abuts at its distal end on a group of fibers. X, xylem; P, phloem; F, fibers; C, cortex; FC, fascicular cambium; IC, interfascicular cambium.

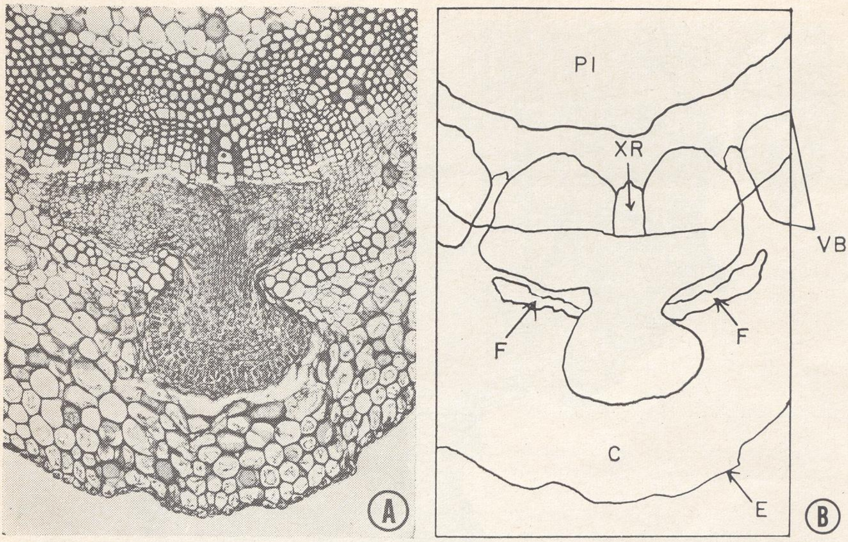
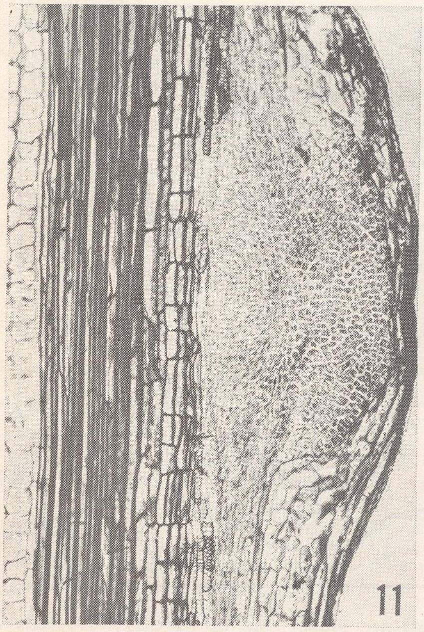


Figure 10 A, B. A young root primordium with well organized tissues. Phloem elements of at least two vascular bundles and three rays take part in the formation of a root primordium. PI, pith; XR, xylem ray; F, fibers; C, cortex. From: Girouard, R. M. 1967. Canad. J. Bot. *45*:1877-1881.



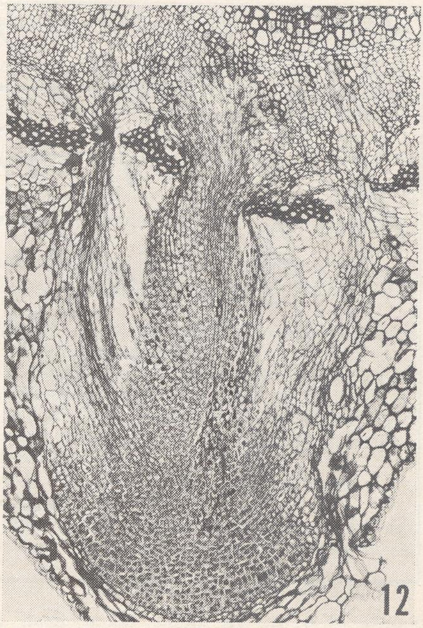


Figure 11. Longitudinal section of an adventitious root primordium at a stage similar to or slightly younger than that shown in figure 11 A.

Figure 12. Transverse section of a stem with an adventitious root developing through cortical and epidermal tissues. Groups of fibers are unable to stop the last few stages of root formation. From: Girouard, R. M. 1967. Canad. J. Bot. *45*:1883-1886.

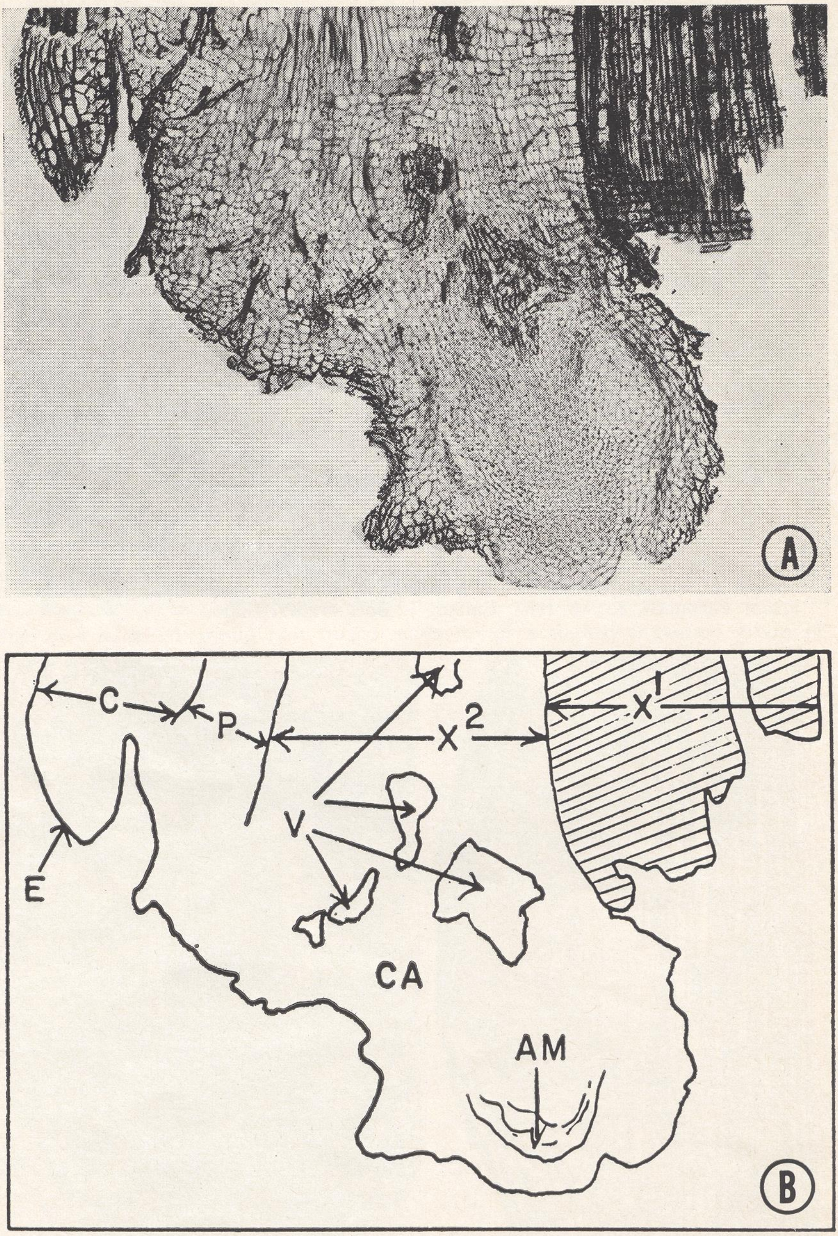


Figure 13 A, B. In stems taken from mature phase plants adventitious roots also form in callus tissue at the bascal end of cuttings. X^1, primary xylem; X^2, secondary xylem; P, phloem; C, cortex; E, epidermis; V, vessels; CA, callus; AM, apical meristem.

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