ANATOMY AND MORPHOLOGY OF *HUMULUS LUPULUS* L.

by

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A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1954

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Thesis presented May 14, 1954

Typed by Edythe Miller

ACKNOWLEDGEMENTS

The present investigation was part of a hop research program undertaken at the Oregon State College Agricultural Experiment Station, Corvallis, Oregon, under the sponsorship of the United States Brewers Foundation.

I wish to express my appreciation to all of those who rendered aid to me throughout the course of this study. I am particularly indebted to Dr. Frank H. Smith for his interest and thoughtful guidance. To Dr. S. M. Dietz, I am grateful for the opportunity to have been able to work in his department and for his many considerations offered me. I am also indebted to Dr. H. K. Phinney for the innumerable suggestions and material aid rendered.

Finally, I wish to acknowledge my humble gratitude to my wife, whose patience, understanding, and assistance made the rendering of this dissertation possible.

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INTRODUCTION

While hops have been cultivated for hundreds of years, the anatomy of the hop plant has never been thoroughly investigated. There have been only relatively few studies made on either its structure or development under field conditions. The morphology of the perennial portions of the plant is only partially understood and the information that is available has been accumulated largely through agronomic practices. Some studies on the structure of this plant have been made from time to time in the past, but primarily on its gross morphology. The existent literature concerning the vegetative parts of the plant is limited as well as widely scattered.

Only the root and stem structures of the hop plant are described and discussed in this study. Though developmental studies were also made, the work is essentially descriptive in nature. As research in various phases of hop culture progresses, a more thorough knowledge of the structure of this plant should aid materially in helping to solve certain problems as they are encountered. This investigation is by no means to be considered as an exhaustive study; however, it is hoped that it will serve as a source of reference concerning the anatomy and morphology of the hop plant, as well as perhaps be considered as a further contribution to the literature on certain structural aspects of still another dicotyledonous plant.

The common hop, Humulus lupulus L., is a perennial climbing plant arising from an underground stem or rhizome which bears large numbers of adventitious roots. The aerial stem portions or vines are herbaceous at first, later become woody, and possess long, hollow internodes that tend to be somewhat angular and "rough hairy", especially those nearer the shoot apex. Many axillary lateral branches commonly arise from the upper nodes of the young succulent vine. The vine has a twining habit which appears to be always in a clockwise or dextrorse manner about any support (Plate 29, C). The leaves of the hop plant are opposite and broad, palmately-veined and usually three- to five-lobed, with serrate margins. They are decussately arranged on the vine. At the base of each petiole are two stipules that usually become coalesced into what appears as one broad stipule. Ordinarily, under natural conditions, the lower decussate leaves remain attached to the plant. However, in commercial hop culture practices, the lower leaves are stripped off fairly early during the training of the vine in order to suppress mildew infections as well as certain insect infestations (Hoerner and Rabak, 20).

The hop plant is typically dioecious but an occasional plant may bear both staminate and pistillate inflorescences. The panicles of staminate inflorescences and drooping clusters of strobiloid pistillate inflorescences are borne in the axils of the upper leaves. The flowers of the latter comprise the so-called "hops" of commerce and are often spoken of as "burrs" or " strobiles" by the hop tradesmen. The fruit of the hop plant is a small, dry, indehiscent one-seeded achene which is almost completely invested by a persistent urceolate

perianth or perigone; the whole is more or less enclosed by the base of a subtending bracteole. In the mature hop "cone" the outer surface of the bracteoles, the perianths, and to a lesser extent, the bases of the bracts which subtend the lateral flower axes are covered with numerous, more or less bright yellow multicellular trichomes. These cupulate glandular structures become filled with a volatile resinous secretion called lupulin, and are referred to in the hop trade as "hop-meal" or just "lupulin".

The cultivated hop plant is propagated primarily from cuttings because of the lack of uniformity shown by seedlings. Then too, the seedlings require more than one growing season in which to produce satisfactory yields (Hoerner and Rabak, 20). The cuttings are obtained from the rhizomes of the hop plant and are often referred to by the hop growers as " seed pieces", " strap cuttings", " root cuttings", or just "roots" although they are not true roots but stems (Plates 49, 50).

The perennial hop plant sends out numerous subterranean rhizomes from the crown of the plant which is usually situated immediately below the surface of the soil (Plates 57, 58). In the spring when the hop plants are pruned, these rhizomes are usually removed and the better portions are often saved for the purpose of making cuttings. From the commercial standpoint an ideal cutting (Plate 49) is approximately one-half inch in diameter and some six to eight inches in length and bears at least two pairs of buds or "eyes". Occasionally, hop growers will obtain cuttings by layering portions

of the aerial vines with mounds of earth or by burying entire vines while still attached to the parent plant (Plate 53). Cuttings may also be made from various aerial regions of the vine and then planted in moist sand or culture solutions but this method is not a commercial practice (Plate 52).

The majority of the hop vines on a well-established plant originate primarily from the crown and lateral leaf buds that develop from the cuttings. Formation and development of adventitious buds do not occur. The first few short internodes of the very young growing vine appear blanched or etiolated before breaking the surface of the soil (Plate 49,B). The young vine apices bear small, brownish stipules along with undeveloped leaves and axillary buds at the closely-placed nodes. Most of the adventitious roots arise from the internodes of cuttings (Plates 50, 52). As a plant becomes established other adventitious roots may develop from the internodes of the aerial vines that may come into contact with the soil. The adventitious roots originate from divisions in the cambial region of the cutting, or the vine. On older plants, the original cutting usually becomes short and thick in appearance and the lower portion may rot away. Often, the old cuttings will become more or less deeply cleaved or split. This may be the result of tensions that build up during the formation of secondary tissues or as a result of injury. It is also possible that once a split has occurred alternating soil temperatures may further the cleavage, along with the activity of soil fungi. The older woody roots present often appear

very much like the old cutting itself, so that it becomes rather difficult at times to determine one from the other without sectioning the material (Plates 57, 58).

From an anatomical point of view, the disposition of the tissues in the rhizomes is essentially similar to that in the aerial portions of the vine. Those portions of the rhizomes that are utilized as cuttings and become the so-called "rootstocks" or woody subterranean perennial axes of the hop plants will undergo considerable thickening and cork formation.

Humulus lupulus (and other supposedly closely related species) has been placed in a number of plant families over the years. From time to time in the past the plant systematists have had occasion to consign it to the Cannabinaceae (Engler, Hutchinson, Wettstein), Moraceae (Bailey, Bessey, Coulter), and the Urticaceae (Gray, B. de Jussieu, Sachs). At present it is placed by Lawrence (33) in the Moraceae and by Metcalf and Chalk (39) in the Cannabinaceae.

REVIEW OF THE LITERATURE

As mentioned previously, the literature on the vegetative structures of the Humulus lupulus is limited. The larger part of the literature on the structure of the hop plant is concerned primarily with floral morphology and cytogenetics. However, these aspects are not included in this investigation and only brief mention will be made of the hop inflorescences.

The first work of any morphological significance is that of Wydler (60) on the inflorescences of Cannabis, Humulus, Urtica and Parietaria. Since then, a considerable number of contributions by other workers have accumulated in the literature on hop inflorescences and fruit. An anatomical study of the central axis or "strig" of the pistillate inflorescence was made by Blattny and Vukolov (4). This work, along with investigations of their own, was later reviewed in detail by Goodwin and Salmon (19) who concluded that hop varieties could be divided into three distinct categories according to their strig structure. Particularly complete and thorough descriptions of the structure and development of the pistillate inflorescences is found in the work of Irmisch (28), and Lermer and Holzner (34, 35). Further contributions with regard to the morphology of the hop inflorescences were made by Moreau and Moreau (40) and Zinger (61). Developmental studies on the inflorescences of both the Urticaceae and Moraceae were undertaken by Golenken (17). Recently the morphology of the "cone" has been reinvestigated by Georlette (15).

In his critical review of the literature on the cytology of the hop plant, Dark (5) comments upon the fact that two sex chromosome

mechanisms have been described in different dioecious hop plants by workers in Denmark and Japan. The occasional condition of bi-sexuality among hop inflorescences is briefly considered by Stockberger (55). A number of investigations have been conducted with regard to the pollination and fertilization of hops. These processes are well described by Winge (59) and most of the references to the literature on the subjects up to 1917 are included in his report. A cytologic investigation concerning the formation of pollen cells was made by Homedes (26), and the viability of the pollen of hops and related species was investigated by Holubinsky and Rybatschenko (21). The influence of pollination upon the gross development of the hop was investigated by both Howard (27) and Salmon (48). Because of the many diverse and ambiguous terms that were used by various workers in their descriptions of the inflorescences of the hop plant, a significant contribution toward a more correct nomenclature was offered by Holzner (22).

The first investigation with regard to the vegetative structures of Humulus lupulus was that of Nageli (41) on the leaves, and nothing further was accomplished until the excellent contributions by Holzner and Lermer (23-25, 36-38). Their work was concerned chiefly with the gross anatomy and development of various vegetative organs of the hop plant. Most of the literature references to date cite the work of these two investigators, and since their significant contributions, there have been but few investigations on the vegetative structures of the hop plant. These contributions vary much in nature and are

concerned chiefly with gross morphology. A study was made by Franz (14) on the development of the anvil-shaped emergences on the uppermost regions of the young hop vine. The fibers of the hop plant have been investigated by several workers (Schwede, 52; Sutcliffe, et al, 56). Studies on the formation and development of hop roots have been made by Beard (3); Golubinski (18); Lermer and Holzner (38); Runner (47).

Darwin (6) and Poli (43) made studies with regard to the circumnutation of the hop vine and found that the spiral movement of the vine occurs in a dextrorse manner.

MATERIALS AND TECHNIQUES

The material on which these studies were based was obtained mostly from plants growing under field conditions in the hop yard of the Experimental Farm at Oregon State College. Studies of the seedlings were made from material grown in the greenhouse in Vermiculite. Collections were made at varying intervals beginning in the Spring of 1951 and carried through the 1952 and 1953 growing seasons. The vegetative parts of plants of different ages, ranging from seedlings to and including some perennial portions of three year old plants, were examined. Most of the collections were made from the Fuggles and Late Clusters varieties of Humulus lupulus, although other varieties were also studied. However, the comparisons that were made indicated no essential differences in anatomical structure between varieties.

All material was killed and fixed in either formalin-acetic acid-alcohol or Craf III fixatives (Sass, 49). The best results were obtained with the latter fixative. Most of the material was subjected to aspiration while in the killing and fixing fluids. Some of the material was used in the fresh state. Entire seedlings were cleared by aspirating in undiluted Chlorox, rinsed thoroughly in tap water, and dehydrated in an ethyl alcohol series and finally stored in xylene. The cleared seedlings were especially useful in facilitating the rapid determination of the transition region prior to the sectioning of the material.

Root and shoot apices were dehydrated in a tertiary butyl alcohol series according to Johansen's (30) schedule and then gradually infiltrated with small pieces of aerated Parowax. The soft paraffin was finally completely replaced by 54° - 56° C. Fisher Tissuemat and embedded. The material was sectioned on a rotary microtome in which single-edge, safety-razor blades were used. Sections of the primary root and shoot apices were cut at 7 to 10 microns in thickness. The majority of other sections of the plant were cut 10 to 12 microns thick. To facilitate the sectioning of larger roots and stems, the material was embedded in hard paraffin and sectioned on a sliding microtome. Varrelman's (57) technique was used to prevent curling of the sections by using a small water-moistened piece of paper which was placed upon the top of the paraffin block. The paper together with the section slid onto the knife blade as the latter passed across the block. Sections by this method were cut at from 20 to 40 microns in thickness.

Since almost all vegetative structures of the hop plant contain mixtures of cells of varying degrees of refractoriness, together with softer thin-walled cells, several techniques were resorted to in order to facilitate sectioning the material without tearing (Foster and Gifford, 13; Gifford, 16: Pearlman and Cole, 42). Among the most effective was a modified dehydration series (Smith, 54) of tertiary butyl alcohol - amyl acetate. After embedding the paraffin blocks were trimmed to expose the material and then were soaked for a

period of 3 to 5 days at 37° C. in a solution containing glycerol and a detergent (Alcorn and Ark, 1). A similar solution with paraffin oil substituted for the glycerol was tried but a relatively longer exposure time was required and the results were somewhat less effective.

Most of the slides to be stained were brought down to distilled water and then immersed in ferric chloride solution, one minute for woody tissues and ten minutes for soft tissues, rinsed well, and then stained in iron hematoxylin for the same length of time they were in the mordant. The slides were then thoroughly rinsed and placed for 20 to 30 minutes in a counterstain of 1% aqueous safranin. Destaining, where necessary to obtain the desired degree of differentiation, was accomplished in a very weak acid-alcohol solution. All sections were then again thoroughly rinsed and finally cleared and mounted in H-S-R neutral synthetic resin. The use of tannic acid and iron chloride (Foster, 11) for staining meristematic tissues was not satisfactory on hop material.

A staining schedule was devised whereby resorcin blue, following iron hematoxylin and safranin, could be utilized to stain callose depositions on the sieve plates of secondary phloem. The sectioned material is brought down to water and then treated as follows:

1. Mordant in 3% iron chloride, 15-20 seconds.
2. Wash thoroughly in four changes of distilled water.
3. Ripened iron hematoxylin (20-30 drops per stender), 10-15 seconds. Destain in weakly acidulated alcohol if necessary.

4. Wash thoroughly in four changes of distilled water.
5. 1% aqueous safranin, 5-10 minutes. Destain if necessary.
6. Wash thoroughly in four changes of distilled water.
7. Resorcin blue (0.25% in 30% ethyl alcohol), 2-5 mimutes.
8. Rinse in two changes of distilled water.

Any excessive resorcin blue is removed in the lower alcohols prior to clearing and mounting. In properly accomplished preparations, parenchyma cells and contents are stained gray; xylem, pink; phloem and xylem fibers, pink; phloem - gray, with the callose on the sieve plates gray-blue to blue; resin and tannin cells appear yellow or reddish-brown. The permanency of this staining combination is not known at present. Slides that are a year old still appear to retain their original brilliancy.

The equipment used for photomicrography consisted of a Kodak Recomar 18, 6 x 9 cm. camera; a 10 x 12 cm. view camera; Eastman Panatomic-X and Commercial Process Pan Film; 10X Planoscopic, 5X and 10X Huygenian oculars; fluorite objectives; and Wratten gelatin filters. A modified Köhler illumination was used to obtain the photomicrographs.

Most of the structural detail drawings were made from prepared slides with the aid of a camera lucida.

DEVELOPMENT AND VASCULARIZATION OF THE SEEDLING

The dormancy of many seeds, including those of Humulus lupulus, is broken by treatment with low temperature. Optimal development occurred when hop seed was planted in Vermiculite and kept moist for six weeks at 35° F.

A period of approximately 10 days elapses before the seedling of the hop plant first makes its appearance above the ground from the time it is planted (Plate 1). Upon germination the primary root or radicle emerges from the stylar end of the seed. The cotyledons remain within the seed coat until they are pulled above the ground by the growth of the hypocotyl. The primary root grows rapidly and then appears to become retarded. Within a period of 3-4 days lateral roots are formed. During this time the hypocotyl elongates and becomes erect, pulling the cotyledons out of the seed coat. Once free from the seed coat the cotyledons expand rapidly and become obovate to spatulate in outline with more or less tapering bases. They tend to function both photosynthetically and as storage organs for approximately 2-3 weeks before withering and dropping off.

Shown in Plate 4 is a diagrammatic representation of the vascularization of the seedling of Humulus lupulus. The two cotyledons enclose the relatively small epicotyl between them, and below them is the hypocotyl merging with the primary root which possesses the typical diarch plate of primary xylem, which in turn is flanked by the primary phloem strands. The median vascular strands of the cotyledons are in direct line with the protoxylem

poles of the root. Lateral traces are derived from the median strand and diverge outwardly toward the edges of the cotyledon blade.

The lower hypocotylary vascular tissues are arranged similarly to those of the primary root. A few millimeters below the cotyledonary node there is a gradual reorientation of the primary vascular tissues at successively higher levels until the vascular tissues no longer resemble the condition existent in the root proper. In the root the xylem is exarch and the metaxylem occupies the center of the root axis. The protoxylem maintains its peripheral position but the metaxylem no longer differentiates toward the center of the root. Instead, each metaxylem strand separates into two which begin to diverge laterally from the protoxylem (Plate 4,B-D). This leaves the center devoid of vascular elements and a pith becomes differentiated in this part of the seedling axis.

At successively higher levels, the distance between the protoxylem poles becomes greater as the axis of the hypocotyl becomes wider in its approach to the cotyledonary node. Instead of one xylem plate, as is found in the root, there are two distinctly separate xylem traces in the upper hypocotyl as well as in the bases of the cotyledons with the protoxylem maintaining its exarch position. The protoxylem maintains its exarch position not only high in the hypocotyl, but also for a considerable distance into the median strand of the cotyledon where it gradually comes to occupy an endarch position as the strands of the metaxylem converge in an abaxial direction into one double strand (Plate 4,E-J).

The two phloem strands of the primary root branch to form four distinctly separate strands higher in the hypocotyl where they each become associated with one of the metaxylem strands as the latter undergo their lateral divergence from the protoxylem. Later, the phloem undergoes still another branching, along with the xylem, as the lateral veins are formed in the cotyledon blades as collateral bundles (Plate 4,G-J).

The two vascular traces of the first pair of leaf primordia of the epicotyl appear relatively early in the pith of the hypocotyl. In comparison with those of the root, the xylem of the first vascular traces of the epicotyl is endarch in position, and the bundles are collateral in arrangement. At successively higher levels in the hypocotyl these, and other differentiating strands of the epicotyl, become arranged to form more or less of a cylinder of discrete bundles internal to the cotyledonary traces (Plate 4,D-I).

Thus, the so-called transition region between the cotyledons and the seedling root is relatively short and occurs in Humulus lupulus over but a few millimeters in the upper portion of the hypocotyl. Transition is completed when the exarch xylem has attained the endarch condition.

The seedlings of Humulus lupulus are often abundant in tannin- and resin-containing cells. These cells are distributed among the vascular tissues of the hypocotyl, epicotyl, and cotyledons as well as occasionally in the primary root. These cells often become relatively long and arranged in a discrete series, or they may appear individually. Their end walls do not become perforated or

resorbed. These elongated cells appear to be similar to those found in the internodes of the hop vine and they occur early in the ontogeny of the seedling, making their first appearance in the phloem tissue of the primary vascular bundles. Their contents appear to be resinous in nature, with the resinous matrix containing finely - as well as coarsely - granular bodies.

ONTOGENY OF THE PRIMARY TISSUES IN THE ROOT

An examination of median longitudinal sections of the apical meristems of 19 seedling hop roots shows that there is no clear-cut differentiation of the root initials into discrete cells which would give rise to the various tissue regions of the primary root. Thus, the developing central cylinder, cortex, epidermis, and rootcap cannot be traced to separate initials, but arise rather from a common set of poorly defined initials located a short distance behind the physical apex of the primary root (Plates 2, 3).

There are several accounts of the structure and ontogeny of the root but these do not agree in all respects. Lermer and Holzner (38) reported, in their interpretation of the root initials region of Humulus lupulus, that though the periblem and plerome have common initials a special cell layer serves to form the epidermis; "--- the rootcap being formed by tangential divisions of the dermatogen". Runner (47) described the root apical meristem as comprised of three sets of initials, namely, "distal initials --- which produce the rootcap and also the dermatogen, --- the medial initials produce the periblem, --- the inner initials produce the plerome". The findings and interpretations of these workers, however, are not entirely borne out by the present investigation. In terms of Schuepp's (51) classification of root meristem organization, the root of Humulus lupulus has an apical meristem that appears to fall into his Type IIIcb, wherein the meristem is comprised of a "transverse layer forming the cortex, central cylinder and the central

part of the rootcap".

Transectional and median longisectional views indicate that cell divisions occur in a periclinal and anticlinal as well as oblique manner, so that all the tissues derived from the apical initials of the root arise from a common initial zone (Plates 2,3). Further examination of the root apical meristem indicates that the protoderm arises from the same initials as the rootcap, so that the two have a common origin. The blunt cone-shaped rootcap is produced by periclinal as well as oblique divisions, from the outermost lateral derivatives of the root initials. The protoderm becomes differentiated by periclinal divisions in the meristematic cortex beginning a short distance back of the initials region. The inner cells of these divisions differentiate the protoderm while the outer cells are added to the rootcap. In many of the roots examined, the protoderm assumes a somewhat "stepped" appearance as it undergoes its differentiation (Plate 3). This differentiation of the protoderm from cells ontogenetically related to the rootcap is not an uncommon occurrence among the roots of dicotyledonous plants (Esau, 9). The cells of the distinct rootcap are quite large, thin-walled, and contain large nuclei. They are characteristically well-filled with starch grains, especially those cells in the immediate vicinity of the initials region as well as those farther down in the rootcap (Plate 2,C). The peripheral cells that are no longer alive are entirely devoid of starch grains and nuclei.

Immediately behind the initials region, anticlinal as well as oblique-periclinal divisions give rise to the meristematic cortex, which is at first approximately three cell layers in thickness. It increases usually to five layers mainly by periclinal divisions of the innermost layer adjacent to the differentiating central cylinder. After the last division, the layer adjacent to the pericycle differentiates into a thin-walled endodermis (Plates 3, 11). There is no evidence of a so-called hypodermis being formed. The younger cortical cells near the initials region tend to appear isodiametric in form, but they assume a more or less tangential elongation further back (Plate 7,B). The older cortical cells vary in size, with the larger cells more predominant in number over the smaller ones.

The cells of the cortex become vacuolated and relatively large intercellular spaces make their appearance early and close to the root initials region, even while the cells of the central cylinder still appear dense with their cytoplasmic contents (Plate 6). As the cortex develops further, more intercellular spaces occur which soon become relatively large and in instances assume the superficial appearance of cells themselves. Because of the difficulty in obtaining good fixation of the cortical cells in the elongation region of the young hop root, distortion of the cortical cells and the endodermis often occurs. At no time do the cortical cells of the young root assume a radially seriated appearance. Sclerenchyma cells were not observed in the cortex of the young hop root.

Occasionally, the young primary roots of some plants possess cortical cells that contain a tetraploid number of somatic chromosomes rather than the usual twenty (Runner, 47; Winge, 59). While these cells do not appear to be particularly different from the cortical cells with the normal complement of chromosomes, they are nevertheless quite noticeable because of their somewhat larger nuclei, increased number of chromosomes, and occasional overall increase in cell diameter. They occur in a sporadic fashion rather than in any particular sector of the young cortex. The term "polysomaty" was applied by Langlet (32) to the condition of those cells in somatic plant tissue which contain multiples of the typical chromosome number. Whether this condition in Humulus lupulus roots is the result of a chromosome duplication in resting nuclei has not been determined in this particular study. According to Sharp (53), this aberrant condition is not uncommon in roots that are subjected to abnormal cultural conditions.

The meristematic central cylinder shows a cytologic differentiation and is distinctly set off from the immature cortex a very short distance behind the initials region (Plates 2; 5,B). At the periphery of the meristematic central cylinder, the pericycle is conspicuously the first region to become differentiated. Histogentically, it is part of the central cylinder. Transections taken at approximately 40 microns above the initials show that the pericycle undergoes a series of anticlinal as well as periclinal divisions and becomes biseriate in appearance (Plate 3). The

pericycle appears continuous as the outer boundary of the central cylinder and is not broken up by either the primary phloem or primary xylem.

In the course of the delimitation of the pericycle, the interior cells of the meristematic central cylinder continue to divide and the innermost cells enlarge and undergo considerable vacuolation. Few divisions of a longitudinal nature occur here; however, the divisions are more numerous than in the young cortex and, as a result, the cells of the cortex appear transversely larger than do those within the central cylinder (Plate 8,A). Within the central cylinder the cells of the pericycle and primary xylem become transversely larger than do the cells of the primary phloem.

Because of the periclinal divisions, the cells of the peripheral region retain their meristematic appearance for a longer time than do the cells in the center of the meristematic central cylinder. As a result of the many cell divisions, as well as the vacuolation of the protoplasts, the central cylinder increases considerably in diameter. This is particularly noticeable from the initials region upward until about the level where the protophloem mother cells become differentiated (Plate 6). As a result of the anticlinal and periclinal divisions of the innermost cells of the young cortex and the tangential enlargement of these cells and those of the central cylinder, the circumference of the young primary root is increased. From then on the young root shows little actual increase in diameter until secondary growth begins.

The distances from the initials region to the level of maturation of the various tissues were determined by measurements obtained from roots growing in greenhouse cultures as well as from those growing under field conditions. In depicting the spatial relationships in the diagram on Plate 11, the numerical values given only approximate the average condition.

Approximately 300 microns above the initials region, the first of the primary phloem becomes discernable as isolated phloem mother cells (Plate 6,A). They appear next to the pericycle in a more or less median position on opposite sides of the differentiating primary xylem plate. These solitary protophloem cells are conspicuously lighter in appearance than the surrounding cells which have denser cytoplasm. The protophloem cells are often more or less hexagonal-shaped in outline. They do not possess companion cells, or at least do not appear to be associated with any cells that could be interpreted as such. Although an attempt was made to follow the development of the protophloem mother cells from their initial appearance upward, no instances of longitudinal divisions of these cells were observed. The lack of companion cells among the first sieve-tube elements is apparently a common characteristic of the roots of dicotyledonous plants (Esau, 9).

The two primary phloem strands appear to mature at approximately the same level. At approximately 100 microns above the initial appearance of the protophloem mother cells, the protophloem sieve tubes become mature (Plates 6-8). This maturation occurs

considerably before the maturation of the first primary xylem elements. The nuclei disappear as the protophloem sieve tubes undergo elongation and the highly vacuolated cytoplasm becomes positioned parietally. The side walls undergo varying degrees of thickening. The oblique to transverse end walls develop simple sieve plates. In some roots, additional protophloem sieve-tube elements, also without discernable companion cells, mature at slightly higher levels but laterally adjacent to the first sieve tubes in each strand. These additional elements are equal in diameter to those of the first protophloem elements (Plate 8).

The metaphloem differentiates in a centripetal manner with respect to the protophloem. The first metaphloem sieve elements differentiate adjacent to the protophloem elements; the later ones differentiate in progressively deeper layers of the primary phloem strand. All metaphloem sieve tubes appear to possess closely attendant companion cells prior to the completion of primary growth in the young root. Sieve areas were not observed on the side walls of either the protophloem or metaphloem sieve-tube elements. Certain cells of the primary phloem are parenchymatous in appearance and these are interpreted as phloem parenchyma cells, while others are more elongated, relatively thick-walled and differentiate quite early as primary phloem fibers. The first fibers mature at approximately 10 millimeters above the root initials region. Occasional phloem parenchyma cells contain tanniferous compounds in their vacuoles.

Previous to the differentiation of the primary xylem, the cells of the meristematic central cylinder as a whole appear highly vacuolated, with the result that the mature primary sieve tubes become much less conspicuous and more difficult to distinguish from the adjacent companion cells and phloem parenchyma cells (Plate 9).

About 150 microns above the root initials region, a median plate of cells in the interior of the young central cylinder becomes conspicuous because of the enlargement and vacuolation of its components. This comprises the xylary procambium of the protoxylem and the metaxylem and develops at a right angle to the differentiating primary phloem (Plate 9). It does not remain as a one-celled row, however, since other laterally adjacent cells also undergo enlargement and become vacuolated, and eventually differentiate into xylem elements. The cells of the immature xylem plate become early distinguishable from those of the primary phloem by the developmental pattern of the future metaxylem cells. These cells undergo conspicuous enlargement and vacuolation before those of the protoxylem. However, the cells of the protoxylem are the first to mature and to develop secondary walls; thus maturation of the primary xylem plate occurs in a centripetal manner. Though the outermost cells of the plate are the first to mature, the cells in the center attain a larger size.

The primary root is usually diarch but triarch roots may occur. Protoxylem elements at the two poles in most instances appear to become differentiated at the same level in the primary root. Like

the protophloem, the protoxylem differentiates adjacent to the biseriate pericycle, with the subsequent primary xylem developing in a centripetal manner. The protoxylem reaches maturity at approximately 400 microns above the initials region at the ends of the radiating arms of the plate of primary xylem mother cells (Plates 9, 10). The protoxylem cells are considerably narrower in diameter than the developing metaxylem. The mature protoxylem elements develop annular and helical or spiral secondary wall thickenings. The first elements of the protoxylem appear to be tracheids since in no instances, excepting in later-formed metaxylem elements, could perforation plates be observed.

The metaxylem reaches maturity and secondary wall formation is completed before a vascular cambium becomes active (Plate 10,B). The first appearance of secondary walls in the metaxylem occurs at approximately 5 millimeters above the initials region. The secondary walls of the first maturing metaxylem elements are scalariform, the later maturing elements more or less reticulate, and still later ones pitted. These metaxylem elements, especially the later-formed ones, are vessels inasmuch as they possess perforation plates on their more or less oblique to transverse end walls. At approximately 15 millimeters above the initials region all of the metaxylem is mature (Plate 10,B).

The thin-walled, uniseriate endodermis first becomes clearly demarcated at about the time that the protophloem reaches maturation; however, Casparian strips were not observed to develop on the radial

walls until maturation of the protoxylem occurred.

Under field conditions the regions of root hair formation in Humulus lupulus occurs approximately one to two millimeters above the physical apex of the root and extends upward for approximately 6-8 millimeters. In water cultures this region may be extended somewhat higher.

As the young seedling undergoes development, the primary root as well as the hypocotyl becomes considerably thickened (Plate 23). The main increase in diameter of the primary root is due to cambial activity. The fleshy radicle and hypocotyl of the seedling may develop lateral fleshy roots along with many fibrous roots. The cells of the pericycle undergo a series of tangential and radial divisions from which a cork cambium is differentiated (Plate 27). With further expansion of the radicle and hypocotyl the epidermis and cortex become stretched and ruptured and finally slough off and continuous periderm covers the entire outer surface of the fleshy storage organ (Plate 28,A-B).

The amount of xylem tissue is comparatively small in relation to the massive storage parenchymatous tissue of the surrounding phloem (Plate 24). Relatively few sieve tubes and companion cells are present, and phloem fibers are almost completely absent. Narrow phloem rays occur up to the inner limits of a rather broad zone of considerably larger, tangentially-stretched phloem parenchyma cells located on the outer periphery of the phloem tissue. All of the thin-walled phloem parenchyma cells contain an abundance of

comparatively large starch grains. It is interesting to note that in relation to the starch grains that occur elsewhere in the hop plant, those of the fleshy storage organ of the seedling are 2-3 times as large, as well as being relatively more abundant (Plate 28,C). For example, the starch grains of the aerial vines and underground stems average around 3 microns in diameter as compared to those of the seedling storage organ, which range from 6.5 microns to 10 microns in diameter.

The cambium also produces a small amount of storage parenchyma cells in the xylem, aside from the xylem rays. Tannin cells occur throughout the phloem and xylem tissues.

Root contraction, which is supposedly a common and widely distributed phenomenon among many plants (Esau, 10), was not observed to occur in the seedling of the hop plant.

SECONDARY GROWTH IN THE ROOT

The vascular cambium originates from parenchyma cells situated between the primary xylem and primary phloem. These undergo periclinal divisions which result in a series of narrow, thin-walled, radially-aligned cells which demarcate the position of the vascular cambium for the first time. Plate 10,B depicts the structure of the vascular cylinder at the time of initiation of secondary growth. The first of the vascular cambium cells are located as isolated strips on each side of the xylem plate, outwardly flanked by the primary phloem. As secondary xylem is cut off toward the inside of the cambium and secondary phloem toward the outside, the cambium takes on the appearance of two arcs, more or less bulging toward the outside of the vascular cylinder (Plate 12). The cambium becomes a more or less cylindrical layer of tissue around the circumference of the differentiating xylem, except for the interruptions by the protoxylem. In the meantime, the pericycle immediately abutting the protoxylem undergoes periclinal divisions so that cambial cells are formed in this region also. With the formation of these cells, the gaps in the vascular cambium are filled and the cambium completely encircles the xylem (Plate 13,A).

The cambium of the root of Humulus lupulus is composed of both fusiform and ray initials. Those pericyclic cells abutting the protoxylem which become meristematic, give rise to ray initials. The developing rays eventually radiate from the protoxylem poles outwardly through the secondary vascular tissues. These are the first rays of

a multiseriate nature to occur in the secondary xylem.

With further development the cambial region or zone assumes an undulated appearance in its encirclement of the vascular cylinder. In roots in which an actively dividing cambium is prominent it is rather difficult at times to distinguish clearly between the actual cambial initials from their more immediate derivatives.

Runner (47) reported that the vascular cambium was observed to be of an "anomolous type" with the cambium "---multiseriate" between the vascular rays. Sieve tubes and companion cells are differentiated in the center of this region, while the cells to the inside and outside remain meristematic. The present study did not reveal this condition in the vascular cambium of either young or older roots.

Shortly after the vascular cambium has completely encircled the xylary tissue, the pericycle undergoes many anticlinal as well as occasional periclinal divisions as it keeps pace with the increase in diameter of the root. As mentioned earlier, a cork cambium develops in the outer layers of the pericycle just beneath the tangentially stretched endodermis (Plate 13,A). The cells of the cork cambium become aligned in more or less radial rows, with the walls of the outermost cells undergoing suberization. This periderm, comprised of thin-walled, suberized cork cells external to the phellogen or cork cambium and one or two layers of non-suberized parenchyma cells internal to the phellogen, keeps pace with the expansion of the root and appears to persist throughout its life.

With the increase in diameter of the root the epidermis, cortex, and endodermis become sloughed off. In certain portions of the cork cambium zone the cells undergo a proliferation resulting in the formation of a mass of relatively loose periderm cells that function as lenticels (Plate 56,A).

Depicted in Plate 18,B is a transection of an adventitious hop root at the end of the first year's growth. As a result of the numerous divisions of the vascular cambium and formation of cork cambium the cortex and endodermis have sloughed during the increase in the diameter of the root. The primary xylem is completely enclosed by the secondary xylem and appears as two "wings" on either side of a centrally-located vessel element. Multiseriate pericyclic rays extend from the poles of the primary xylem. These and other parenchymatous rays of the secondary xylem and secondary phloem are often filled with starch grains, crystals, or tanniferous compounds. Whereas the tissue of the primary xylem is devoid of fibers, the secondary xylem abounds in relatively long and slender, small-lumened lignified fibers with thick walls which appear to be hygroscopic in nature. When examined in transection, the secondary wall appears to separate away from the primary wall. These fibers occur as individuals or in various-sized groups among the other cells of the xylem (Plate 19) and, along with the distinct and remarkably wide-diametered vessels, lend considerable toughness to the root. It is the relative abundance of the xylem and phloem fibers that makes for the extreme refractoriness of the root in

sectioning.

The relatively wide-pored vessels appear singly as well as in radial multiples and clusters. These vessels possess simple perforations of their more or less transverse end walls, and possess bordered pits on their side walls where they adjoin other vessels or parenchyma. In many vessels of the secondary xylem, abundant tyloses may occur through the pit-pairs connecting adjoining parenchyma cells (Plate 21). There are relatively few thin-walled, xylem parenchyma cells in comparison with the rather abundant thick-walled, simple-pitted parenchyma cells which surround the vessels and enclose them in one or two layers. Some of the thin-walled parenchyma cells, which occasionally occur also in the xylem and phloem of the aerial and underground stems, appear in a close longitudinal series and contain fairly large prismatic or rhomboid crystals of calcium oxalate (Plate 47).

The greater portion of the root consists of secondary phloem tissue (Plate 18,B). The primary phloem elements, principally the sieve tubes and companion cells, become crushed and obliterated with the increase in the amount of secondary phloem during the enlargement and expansion of the vascular cylinder. The parenchyma cells at the outer periphery of the secondary phloem become conspicuously enlarged and many of them contain tanniferous compounds and resins. Occasionally large druse crystals also occur in these cells. Many of the phloem parenchyma cells contain an abundance of starch grains. Starch was not observed to occur in the companion cells.

The sieve tubes are relatively large-diametered with 1-4 companion cells per element. Numerous fibers are distributed throughout the phloem in irregular groups or strands. In comparison with the fibers of the secondary xylem, those of the secondary phloem are somewhat fewer in number and appear to be mucilaginous in nature (Record, 46). Numerous multiseriate parenchymatous phloem rays are present which are continuous with the rays of the xylem and which become more or less widely extended laterally, or "dilated" in appearance, in the outer portions of the phloem.

According to Lermer and Holzner (38), latex ducts are found in the roots of Humulus lupulus. In this investigation, neither lactiferous canals nor resin ducts were found. However, as mentioned earlier, many parenchymatous cells scattered throughout both the primary and secondary phloem as well as the secondary xylem, contain resins and tannins (Plates 20,A; 22,B). Tannins or their derivatives also often appear among the outer cells of the rootcap.

Approximately 5 to 8 millimeters above the root initials region, cells of the pericycle in the region opposite the protoxylem poles of the root undergo a series of anticlinal and periclinal divisions which result in the initiation of the branch or lateral roots (Plates 14, 15, 16,A). The endodermis divides anticlinally and keeps pace with the growth of the lateral root primordium. This endodermis persists until the lateral root emerges from the primary root tissue and is ruptured as the root penetrates the soil substrate.

Besides the pericycle, the endodermis appears to be the only other part of the root that takes part in the initial growth of the lateral root. The cells between the lateral root and the main root are derivatives of the pericycle. They differentiate into tracheary and sieve elements in continuity with similar elements of the main root. The overall architecture of the mature secondary roots is similar to that of the primary root.

If the hop plant is left undisturbed over a period of years, the roots become quite thickened as a result of the activity of the vascular cambium, and the cork cambium produces a more or less spongy periderm a few millimeters in thickness.

According to Beard's studies (3), the roots of Humulus lupulus are of two types, namely, (1) "horizontal, tough and wiry, with considerable small root and fibre", and (2) "vertical, fleshy and brittle, with little branching and no fibre". Most of the work in the present investigation was done upon the fleshy roots and in contrast to the findings of Beard, these roots contained an abundance of fibers. Lermer and Holzner (38) have reported the occasional appearance of large, irregularly-shaped and very much thickened fleshy roots. These roots were found to contain exceptionally large amounts of phloem tissue in relation to xylem tissue. The phloem fibers were reported as occurring in very small amounts.

The fibrous roots of the hop plant are considerably smaller, relatively fragile, and much shorter-lived than those of the larger fleshy storage roots. Although they occur as laterals of the larger roots and may be found at various depths, these small, slender

fibrous absorbing roots tend to be more prominent in the upper layers of the soil. The initiation of the fibrous laterals occurs similarly to that described for the initiation of lateral or secondary roots from the primary root. A group of pericycle cells undergoes periclinal and anticlinal divisions resulting in the formation of the lateral root primordium. Prior to the emergence of the primordium, the primary tissue regions of the young root axis as well as the rootcap become delimited by oriented cell divisions. By continued growth the primordium gradually penetrates the cortex of the parent root. Eventually the secondary tissues of the parent root and the primary tissues of the lateral root differentiate in continuity with each other, and the secondary xylem of the base of the lateral root becomes embedded in the xylem of the parent root.

A transverse section of a small mature fibrous root is shown in Plate 18,A. The fragility of the fibrous root makes it difficult to section without a certain amount of compression. The architecture of the mature fibrous root appears somewhat similar in tissue arrangement as that of the primary root at the time of completion of primary growth in the latter. The small central cylinder is comprised principally of primary xylem tissue flanked by a relatively small amount of primary phloem. A vascular cambium is not differentiated. Neither the pericycle nor the endodermis are distinctive in appearance. Fibers are completely absent. The central cylinder is enclosed by relatively large

cortical parenchyma cells with few intercellular spaces. Occasional tannin cells occur in the cortex as well as in the phloem. A cork cambium does not develop in the fibrous absorbing root.

THE SHOOT APICAL MERISTEM

The vegetative shoot apices of the vines of Humulus lupulus appear rather narrow and conical in form (Plates 30; 31,A). The diameter of these apices varies somewhat during their period of development. It was found that the average growing vine, measuring around 12 feet in length during the height of its growing season, has a shoot apex diameter of approximately 175 microns. An average was obtained from measurements taken between the youngest decussate leaf primordia of 23 apices.

The apical meristem of the vegetative shoot has the type of organization in which two definite tissue zones are readily distinguishable, namely, the tunica and the corpus (Schmidt, 50; Type VII of Popham, 45). This holds true not only for the apices of the main vines and their many lateral branches, but also for the apical organization of the adventitious buds arising from cuttings as well as for those buds arising from the rhizomes attached to the parent plant. Shoot apices were examined from many locations on the same plant, as well as from many separate plants (including two varieties), and also from plants in various stages of development, and they were all found to possess a similar type of organization. No study was made, however, of the epicotylary apex of the hop embryo.

The shoot apex, which varies in its convexity but appears to be always elevated above the leaf primordia, is differentiated into two rather discrete growth regions or zones. The regions are

distinguished by contrasting planes of cell division. An outermost uniseriate layer, the tunica, consists of uniform-sized cells from which the protoderm originates. Occasionally, what appears to be a biseriate tunica arrangement will occur; however, this was found not to be a constant feature.

The cells of the tunica undergo only anticlinal divisions. Immediately below the tunica is the corpus, a region comprised of a mass of more or less irregularly arranged cells which are slightly larger than those of the tunica, and which gives rise to all the underlying tissues. In comparison to the tunica, the cells of the corpus divide both periclinally and anticlinally as well as obliquely (Plates 30; 31,B). Approximately the first 100 microns of the shoot apex contain cells that are fairly dense and comparatively uniform in size. Within this mass of cells the corpus becomes delimited as a more or less large-celled central zone which is flanked by smaller and more densely cytoplasmic cells directly derived from the corpus, comprising the peripheral zone (Kaplan, 31). The overall effect of the smaller cells of the tunica is to give the appearance of a sort of mantle surrounding and enclosing the corpus.

Variously oriented divisions in the peripheral region of the corpus initiate the leaf primordia and the young cortical and procambial tissues. Immediately below the corpus and derived directly from the central cells of the latter, is the rib meristem comprised of more or less vertical files of cells somewhat more vacuolated than the cells of the central zone. Small intercellular

spaces are present in the rib meristem. This tissue eventually differentiates further below into the parenchymatous pith cells of the young hop vine. The vacuolation of the pith cells becomes evident considerably higher than that of the cortical cells. Enlargement and differentiation of the pith cells occurs rather rapidly. In the more mature regions of the vine, the more centralized cells of the pith become stretched and are finally torn by expansion of the stem during its growth. The level at which this tends to occur varies among different vines (Plates 37, 41).

Periclinal and anticlinal divisions in the peripheral layer or layers of the corpus result in the formation of slightly elongated cells. In median longisections of the shoot apex these cells may be followed downward into the axis of the shoot apex to a level where they definitely assume the characteristic appearance of the elongated narrow shape of the procambial cells (Plate 31,A; 51,A). Thus these cells are the uppermost components of an acropetally differentiating procambium.

A sharp demarcation between the procambium and the adjacent cells is lacking in the early developmental stages of the shoot apex. In transverse sections the procambium strands increase in diameter for a time. This occurs not only as a result of cell division within the strands themselves but also by the addition of cells on the periphery of the strands by cell divisions in the surrounding tissue. Plate 31,C shows the appearance of a procambial strand in its earliest

development, and in Plate 34,A can be seen the procambial strands at the beginning of protophloem differentiation.

In transverse sections the first protophloem elements are readily distinguishable by their somewhat thick primary walls. The mature elements are enucleate and their cytoplasm becomes thin and attenuated so that the cells appear to be devoid of contents. The immature protophloem elements have dense protoplasts. Wall thickening takes place before the nucleus disintegrates. Median longisections of the young vine apex reveal the presence of sieve plates, therefore these phloem elements are sieve-tube elements. Companion cells are absent in the protophloem, but are present among the sieve-tube elements of the metaphloem.

Plate 32,B shows a procambium strand during the late developmental stages of the first sieve-tube element. During the maturation of this element, the procambial cells continue to undergo periclinal divisions. As a result of these divisions, cells of the procambial strand orient themselves in radial rows. Before many of these periclinally-formed cells are produced, the primary wall of a cell on the innermost periphery of the procambial strand takes a deeper stain and soon shows a more or less conspicuous thickening similar to that of the differentiating sieve tubes. These cells are the first xylem elements (Plate 32,A).

The first protoxylem elements are conspicuously narrower than those that develop later and tend to overlap on their oblique end walls which do not appear to be perforated. These are therefore

interpreted as tracheids. In their subsequent stages of development the secondary walls of the protoxylem elements develop annular or helical thickenings. In the fully mature state these elements lack protoplasts. The elements of the metaxylem are considerably larger in diameter than those of the protoxylem. The secondary walls of the first mature metaxylem elements possess simple pits with the walls of the later-forming ones developing bordered pits.

As primary vascular bundle development progresses further, there is a tendency for the procambial cells to become tangentially oriented between the first phloem and xylem. They tend to become increasingly vacuolated and eventually are distinctly set off from the densely cytoplasmic phloem and xylem regions as radially seriated, vacuolated cells, assuming the appearance of a somewhat indistinct fascicular cambium. The cells of the interfascicular areas keep pace with the radial increase in the size of the differentiating vascular bundles by occasional periclinal divisions.

As the procambial tissue undergoes further development the protoxylem and protophloem become differentiated into discrete collateral strands arranged in a cylinder around the outer periphery of the developing pith (Plate 35). As the young vine expands in its development, these strands increase radially in size as a result of lateral growth and additional procambial strands develop between them. An interfascicular cambium develops between the bundles and eventually becomes continuous with the fascicular cambium.

Numerous secretory cells containing resinous or tanniferous substances make their appearance early in the ontogeny of the young vine (Plates 32,B; 51,A). Individual cells often become quite elongated and extend without ramifications throughout the cortical and phloem tissue. They first make their appearance as typical small meristematic cells among the other promeristem cells. By rapid elongation they keep up with the growth of the surrounding meristem and penetrate between the new cells.

In many instances these secretory cells make their earliest appearance at the base of the first internode of the young vine, distributed among the cells of the procambial tissue. They appear to be confined to the primary phloem tissue in the young leaves also. The cotyledons and young leaves of the seedlings may contain similar secretory cells. Other less elongated cells which also contain resins and tannins are of frequent occurrence among the cells of the epidermis and cortex of the young leaves of the hop vine as well as the young leaves of the seedling. Large intercellular spaces that form secretory ducts also appear among the primary tissues of the young vine. This is further considered in the description of the tissues comprising the young vine.

It is interesting to note that the "crown buds" of stem cuttings of the previous year's growth contain large quantities of starch grains throughout the subepidermal, cortical, and pith tissues. Of further interest is the occurrence of larger starch grains in the lower regions of the bud and bud scales with the

starch grains becoming successively smaller in size in approaching the apical meristem. Very small starch grains were observed to occur immediately below the region of the corpus where the rib meristem begins to differentiate.

STRUCTURE OF THE YOUNG VINE

Transverse sections of the very young portions of the developing hop vine, near the shoot apex, at first appear more or less round or ovoid in shape (Plates 33,B; 34). The first two or three internodes are very short. Several internodes below, the vine assumes a somewhat angled appearance (Plate 35,A) and still further down it becomes distinctly hexagonal in shape, with prominent ridges which give the young vine at this stage of development an appearance of a fluted or stellate structure in transectional view (Plate 35,B). In still lower portions of the vine, the hexagonal shape becomes less and less pronounced so that the mature regions of the vine finally appear more or less round with only a faint suggestion of its previous angularity (Plate 44,B).

The young hop vine possesses a uniseriate cuticularized epidermis bearing diversified types of appendages. These epidermal trichomes (Eames and McDaniels, 8) develop early on the young vine, appearing among the first few elongated and following internodes (Plate 35,A). They consist of single unicellular hairs; pointed, non-glandular hooked hairs; knob-like multicellular glands; and peltate, multicellular discoid glands. Emergences (Foster, 12) in the form of non-glandular, anvil-shaped prickles ("spindle hairs" of De Bary, 7; "climbing hairs" of Lermer and Holzner, 36) are formed on the older elongating portions of the young vine. They are particularly prevalent on the ridges of the upper regions of the young vine (Plates 36,A; 37). These emergences are multicellular,

but the "anvil" part is unicellular and is lignified at maturity. The emergences are formed by the proliferation of both epidermal and subepidermal cells. The cells tend to break down and eventually disintegrate so that in older non-elongating, woody regions of the vine only the bases of these emergences may be evident (Plate 43,B). No attempt was made to determine the developmental aspects of the various trichomes in this study.

Stomata (Plate 43,A) occur in relatively few numbers in the internodes of the young vine. For the most part, the epidermal cells of the younger internodes, excluding their appendages, are approximately equal in their tangential and radial diameters. In older regions of the vine, the tangential diameter tends to become two or three times that of the radial diameter. In those areas of the vine in which the collenchyma cells occur almost immediately below the epidermis, the epidermal cells appear to remain relatively small and equal in size. In older regions of the vine, the epidermis becomes more heavily cuticularized.

Immediately adjacent to the epidermis is a distinct layer of subepidermal cells which extends completely around the periphery of the vine. These subepidermal cells are distinctly larger than those of the epidermis and become thicker-walled as they mature. They very frequently contain considerable amounts of tannins and also what often appears to be resinous substances (Plate 45,A). Occasionally, cells containing chloroplasts may also be found in this

cell layer.

Beneath the zone of subepidermal cells, longitudinal strands of thick-walled, compact collenchymatous cells form a part of the ridges of the young vine (Plate 38,A). These prismatic cells are relatively short with oblique or transverse end walls and tend to appear like the adjacent thin-walled cortical cells, excepting for their somewhat polygonal appearance in transverse sections. It is interesting to note that the anvil-shaped emergences are situated primarily on the ridges of collenchyma tissue. In more mature regions of the vine, the collenchyma cells may become twice as long as the adjacent subepidermal and cortical cells. As the vine matures, the collenchyma tissue tends to become compressed radially.

The remainder of the cortex of the young hop vine is composed of thin-walled parenchymatous cells, 4-5 layers in thickness. The outermost layers adjacent to the subepidermal cells are chlorenchymatous. The innermost layer of the cortex is composed of relatively larger cells and contains an abundance of fairly large starch grains. This starch sheath appears as a regular continuous layer around the inner periphery of the cortex, and does not develop Casparian strips on any of its cell walls (Plate 38). In more mature sections of the vine it loses its distinctive appearance and resembles the cortical parenchyma. As the vine becomes older, resins and tannins are deposited in cells widely distributed throughout the subepidermal, collenchyma, and cortical tissues.

There is no evidence of an endodermis developing in the hop

vine. No distinct layer that could be interpreted as a pericycle separates the cortex of the aerial vine from the vascular tissue. The cells immediately abutting the starch sheath are protophloic in nature. Thus, the external limit of the vascular cylinder is found to lie immediately adjacent to the starch sheath. The tissue comprising the primary phloem forms a rather wide band separated from the relatively narrow strands of primary xylem by a fairly distinct cambium region. During the elongation of the young internodes, certain of the primary phloem cells located on the periphery of the vascular cylinder adjacent to the cortical starch sheath develop thick secondary walls and differentiate as narrow bands of primary phloem fibers. Excepting for short breaks in their continuity, these fibers almost completely encircle the vascular cylinder (Plate 45,A).

The metaphloem consists of sieve tubes with oblique sieve plates, and with 1-3 companion cells per sieve-tube element; single or grouped relatively thick-walled phloem fibers; thin-walled phloem parenchyma; and elongated secretory cells. Many large and distinctive secretory ducts surrounded by what appear to be epithelial cells also develop and occur at random throughout the primary phloem tissue (Plate 38,B). These ducts are considered by Holzner and Lermer (36) to be distinct latex tubes. No latex was found to occur in any cell, tissue, or organ of the Humulus lupulus plant. The ducts are not tubes with discrete cell walls but are rather schizogenous intercellular spaces in the form of comparatively long intercellular canals lined by

thin-walled parenchymatous secretory cells (Plate 39). The ducts contain various reddish and yellowish resinous substances in granular or liquid form; often large, spindle-like structures occur within the resinous matrix. Occasional tanniferous cells also occur in the young phloem tissue.

As mentioned earlier, a fairly distinct cambium separates the primary xylem tissue from the primary phloem. The protoxylem consists of tracheary elements whose secondary wall thickenings may be annular or helical in nature. Metaxylem elements possess bordered pits on their secondary walls. The elements comprising the primary xylem are differentiated in more or less radial rows, with each row consisting of a single series of progressively larger protoxylem and metaxylem elements that are separated from those of adjacent rows by parenchymatous xylem rays usually one to two cells wide. These radial rows appear in groups which are separated by parenchymatous pith rays which may be several to many cells in width (Plate 38,A). Thus, the primary xylem has an appearance at this stage of development of an indistinct series of bundles of radially aligned cells which are separated from each other by bands of pith rays. Small thin-walled parenchymatous pith cells, some of which are to undergo secondary wall thickening, lie adjacent to the protoxylem. Aside from the vessels no other sclerenchymatous cells are apparent in the primary xylem tissue of the young internodes.

Prior to the completion of primary growth in the vines of the hop plant, the cells of the fascicular and interfascicular cambia

undergo a series of periclinal divisions which results in the formation of a wide band of prosenchymatous cells. These cells are differentiated on the outer periphery of the primary xylem elements immediately adjacent to the last-formed wide-lumened metaxylem cells from which they are distinctly set off. This band may be 4-6 or more cells in width and forms a distinctive region which completely encircles the primary xylem (Plate 45,B). These fiber-tracheid cells are relatively thick-walled and elongated with tapering ends and possess small pits with reduced or vestigial borders. The inner apertures of the pit-pairs are often crossed with each other.

The pith in the interior of the young vascular cylinder is composed of thin-walled, simple-pitted, living parenchyma cells among which are numerous small intercellular spaces. The cells of the pith often contain small crystals and are occasionally filled with resinous and tanniferous substances. Sclereids were not observed to occur among any of the pith cells of the hop vine.

STRUCTURE OF THE MATURE VINE

With the further development and maturation of the hop vine, the overall external appearance becomes altered from the more or less succulent, hexagonally-ridged condition of the younger stem with the so-called "climbing" prickles, to that of a rigid, almost round, woody stem one centimeter or more in diameter and 15 to 20 feet or more in length and devoid of prickles. Plate 44,B shows a transverse section of a mature hop vine at the time of harvesting. Several varieties of Humulus lupulus were examined and there appeared to be no significant differences in their overall morphology at this stage.

The epidermal cells become more distinctly flattened tangentially in the regions between the more or less compressed ridges than do the much smaller epidermal cells immediately external to the flattened collenchyma zones. All of the walls of the epidermal cells undergo considerable thickening and in addition the external walls become impregnated with a relatively heavy coat of cutinous material. Functional stomata are absent.

A greater number of considerably larger subepidermal cells contain resinous and tanniferous substances than occurs in the younger regions of the vine. Occasionally, some of the epidermal cells themselves will contain these substances. All subepidermal cells tend to become somewhat thicker-walled also. Where the cells of the subepidermis lie opposite the compressed collenchyma, they appear relatively larger than the other subepidermal cells. The strands

of collenchyma tissue become compressed radially as the vine matures (Plate 54,A). As the diameter of the vine increases with the formation of considerable amounts of xylem and phloem tissue, the ridges of the vine become flattened out. Thus, the mature portions of the vine lose their former angular appearance and become more or less rounded (Plate 43,B).

Immediately below the thick-walled subepidermal cells, one to two layers of cortical chlorenchymatous cells are still present. They do not extend completely around the vine, but are interrupted by the compressed collenchyma strands.

Whereas the cells in the median region of the cortex in the young hop vine are devoid of starch grains, these cells in the mature vine do contain starch grains. Many of the cells in this region are filled with resinous and tanniferous compounds. The starch sheath loses its distinctive appearance as its cells become stretched tangentially. However, it still retains some starch grains.

Large cortex-like phloem parenchyma cells constitute a rather broad area immediately below the region occupied by the primary phloem fibers. These large cells contain an abundance of starch grains and many of them are also filled with resinous and tanniferous substances (Plate 45,A). Crushed sieve tubes also occur throughout the outer regions of the phloem tissue below the primary phloem fibers. Occasional cells containing rhomboid crystals are also present. This region has been referred to by Lermer and Holzner (37) as an inner starch sheath, although it is several cell layers in width. Occasional

rather thick-walled tanniferous cells appear in this region also. Some of these particularly large cells are often encircled by epithelial-like parenchyma cells. Latex tubes or secretory ducts do not occur in the phloem tissue of the mature vine. In fact no such structures develop in any of the mature tissues of the hop vine. However, numerous large resin and tannin cells are distributed throughout the vascular tissues and particularly in the phloem (Plate 45, A).

Along with numerous sieve tubes and companion cells occur phloem parenchyma, as well as parenchymatous phloem rays and secondary phloem fibers. The sieve tubes have clearly-defined, simple sieve plates which are primarily oblique with occasional ones appearing transverse. Numerous distinct lattices (Eames and McDaniels, 8) are present on the side walls. The average length of the mature sieve-tube element is approximately 230 microns, with a diameter of about 30 microns. Companion cells are intimately connected with the sieve-tube elements and tend to vary in number from 1-4 per element. They are variable in size and form a longitudinal series adjacent to each element. No starch was observed to occur in the companion cells.

The rays of the phloem appear as radially-disposed strands, two to several cell layers in width, and are in direct continuity with those of the xylem. Tannin cells are of common occurrence within the phloem rays.

Lermer and Holzner (37) reported the mature phloem fibers as

averaging 10 millimeters or more in length, "---rarely shorter, often longer". Measurements made in the present investigation indicated that these fibers averaged slightly more than 14.6 millimeters in length, some shorter and others longer. They occur individually or in numerous strands dispersed among the sieve tubes and companion cells. At maturity these fibers do not appear to possess living protoplasts nor do they tend to become lignified. They are to be considered as gelatinous or mucilaginous fibers (Record, 46) inasmuch as their secondary walls appear to be hygroscopic in nature. Transverse sections of these fibers show that the secondary walls are not cemented to the primary wall and often appear not only detached from the primary wall, but are also more or less separated into two or more layers. The secondary walls are often marked by numerous threadlike horizontal grooves or stria. Whether these result from the nature of the walls or are mere artifacts was not determined in this study. The stria do not appear to be part of the adjacent cell wall boundaries nor do they appear to connect with adjacent cells. The numerous fibers present in the mature hop stem have been found to be economically unsuitable because of the low pulp yields and high chemical requirements for processing (Sutcliff, et al, 56).

The vascular cambium appears as an undulating zone of cells, forming a complete cylinder (Plate 45,A). Because the immediate derivatives of the vascular cambium cannot be readily distinguished from those of the cambial initials themselves, the cells of the

cambium region assume the appearance of a comparatively wide radial series. As mentioned earlier, the cambial initials are of two primary types, namely fusiform and ray initials (Plate 44,A). The fusiform initials are of the storied or stratified type with overlapping ends and give rise to the vertical systems of phloem and xylem tissue. The vascular rays arise from the ray initials and occur as the horizontal systems of the phloem and xylem.

The xylem of the mature hop vine is conspicuously distinguished by its numerous wide-lumened lignified vessels which occur singly or in radial bands. This feature of the xylem becomes evident comparatively early in the maturation of the vine. As seen in transverse sections, these large vessels appear circular or oval in outline (Plate 45,B). The elements of the larger vessels may have a diameter of approximately 270 microns and be approximately 360 microns in length. Vessel members for the most part possess transverse end walls with simple perforation plates. End walls that are somewhat oblique are also not uncommon (Plate 46). The secondary walls of the vessel elements contain numerous bordered pits which are closely arranged in an alternate manner. All of the xylem tissue, with the exception of the rays, undergo lignification.

As the various vessel members expand during their development, the adjacent cells tend to become crowded out of their original positions, with the result that the original seriation which was present when they were in the cambial zone no longer exists (Plate 43, A). The xylem rays also become deflected somewhat from their

original position as a direct result of this expansion.

The larger vessels are often more or less enclosed by one or more layers of paratracheal-like, reticulate or simple-pitted parenchyma cells that may contain starch grains (Plate 45,B). Frequently, adjacent to these cells are other relatively thinner-walled cells which are arranged in a longitudinal series with the end cells in the series tending to taper. As many as 23 of these cells have been found in a series. Although their appearance at first suggests a septate fiber, they are interpreted as xylem parenchyma cells. Each cell contains a living protoplast with a single calcium oxalate crystal that is prismatic or rhomboid in form (Plate 47). Lermer and Holzner (37) described these cells as being crystal fibers. Many comparatively longer thick-walled libriform fibers, possessing simple pits, occur randomly as individuals or in strands throughout the vessel regions of the secondary xylem.

Immediately inside the region with the large secondary xylem vessels lies a broad band of lignified, thick-walled prosenchymatous cells. Reference to this region was made earlier in the discussion of primary xylem development. In the mature hop stem this region sharply demarcates the primary xylem from the secondary xylem (Plate 45,B).

Pith or medullary rays set off the radially seriated primary xylem into distinct bundle-like groups. The cells of this region in the younger portions of the vine are entirely devoid of starch, whereas the cells may contain an abundance of starch grains, as well

as occasional resins, tannins, or crystals, in the mature vine. The majority of the pith cells in the mature hop vine are thin-walled and more or less isodiametric in shape, with relatively small intercellular spaces among them (Plate 45,C). Those cells of the pith that form the medullary rays usually possess secondary wall thickening with simple pits and may become somewhat lignified. The innermost cells of the pith come to be stretched radially and consequently become torn so that the pith of the hop plant is partially destroyed during the growth and maturation of the vine, resulting in the formation of a hollow zone in the center of the vine (Plate 44,B).

Although the aerial internodes of the hop vine become hollow, the nodes themselves retain their pith so that an internodal diaphragm remains (Plate 48). It is interesting to note that the rhizomes from which hop cuttings are made usually do not possess a hollow pith in their internodes. No attempt was made in this investigation to study the nodal anatomy of the hop plant. Nothing occurs in the literature on the hop plant concerning a study of this nature.

As stated earlier, the cultivated hop plant is propagated primarily by cuttings or rootstocks taken from the perennial subterranean vines of the plant. The anatomy of the cutting is essentially similar to that of the aerial vines of the plant. The principle difference is the development of periderm in the cutting. No such periderm was observed to develop in the aerial vines of the

plant. However, the internodes immediately above the soil surface, as well as those immediately below the surface, develop a periderm in the subepidermal tissues. The periderm is first initiated by periclinal divisions in the outer portions of the cortical tissue, immediately below the subepidermal cells, as a relatively distinct cork cambium or phellogen (Plate 54,A). The cork cells or phellem that are produced to the outside of the phellogen appear in a short radial series extending completely around the circumference of the underground portions of the vine. Those of the outer layers undergo enlargement and become suberized. The epidermis and adjacent cells become sloughed off.

Further development results in the formation of a deeper situated cork cambium within the innermost layers of the cortex (Plate 55,A), with the earlier formed periderm and outer cortical cells eventually becoming sloughed off. A cork cambium is finally differentiated from among the outermost parenchyma cells of the secondary phloem. The cutting or rootstock may possess a periderm a millimeter or more in thickness. A periderm of several millimeters or more in thickness is not uncommon among cuttings that have remained in the ground over a period of several growing seasons, especially among those that have become deeply furrowed as a result of longitudinal splitting.

It is interesting to note that when earth layerings of the aerial vines are made a cork cambium very shortly develops immediately below the subepidermal cells (Plate 54,A). Apparently the absence

of adequate amounts of aeration along with the presence of moisture creates internal conditions which are favorable for meristematic activity with the resultant formation of a periderm. It is of further interest that the underground portion of the vines arising from cuttings develop a cork cambium in the innermost layers of the cortex adjacent to the primary phloem fibers, whereas the aerial portions of the same vines are devoid of a cork cambium.

Longitudinal lenticels are fairly numerous on the external surface of the subterranean portions of the vines as well as on the cuttings. According to a count made by Holzner and Lermer (24), they occur about 40 per square centimeter. The lenticels originate from the subepidermal cells. These cells undergo a series of divisions which finally result in a more or less extensive mass of parenchymatous, complementary cells which are produced toward the outer periphery of the underground vine portions. After the epidermis and cortex become sloughed, the lenticels arise from the periderm where certain of the continuous cork cambium cells stop producing cork temporarily and instead form complementary tissue. The cells of the complementary tissue formed by the cork cambium tend to not only protrude above the surface of the suberized cork that has previously been formed in that region, but to develop inwardly also by proliferation (Plate 56,B). The complementary cells remain thin-walled and do not undergo suberization. Lenticels of the hop plant never appear to be in any direct continuity with the tissue of the vascular rays.

With the sloughing of some of the outer portion of the phloem tissue, the remainder of the phloem consists of very large amounts of irregular strands of thick-walled fibers with small lumina; smaller amounts of relatively large phloem parenchyma cells; sieve tubes with companion cells; and numerous phloem rays 2-3 or more layers wide, that tend to appear somewhat dilated near the periphery of the phloem. These rays separate the phloem into rather discrete radial strands of tissue. Collapsed sieve tubes occur throughout the outer regions of the phloem tissue. The phloem parenchyma cells are to a large extent filled with resinous and tanniferous substances. Some starch grains as well as large druse crystals may occur in the parenchyma cells situated on the outer periphery of the phloem immediately below the periderm. The rays of the phloem, as well as those of the xylem, contain an abundance of very small starch grains.

The tissue of the xylem is also well-filled with numerous strands of non-septate fibers whose lumina are nearly occluded by their thick secondary walls. Considerably fewer thin-walled xylem parenchyma cells, containing prismatic or rhomboid crystals, also occur intermingled with the ordinary fibers as well as among the thicker-walled parenchyma cells. The vessels occur mostly in radial series or multiple groups enclosed by the thick-walled parenchyma cells. Groups of thinner-walled parenchyma cells also occur and are well-filled with starch grains. Many of these cells become filled with resinous and tanniferous compounds, especially those

close to the pith region. The xylem rays are thin-walled and may be from 2-4 or more cells wide, appearing in direct continuity with those of the phloem.

Toward the close of the growing season, tylose development is of rather common occurrence in the last-formed vessels of the xylem. They may be few in number or quite numerous within the vessels and usually contain a living protoplast along with its distinct nucleus. Their appearance is similar to those occurring in the mature vessels of the fleshy storage roots (Plate 21).

As mentioned earlier, the pith of the cuttings taken from rhizomes is not hollow. It contains many small, thin-walled parenchymatous cells with intercellular spaces among them. The cells contain large quantities of minute starch grains as well as occasional druse crystals. Distributed throughout the pith and within the pith rays also are many resin and tannin cells that may be somewhat larger than the other cells of the pith.

If the cuttings are allowed to remain undisturbed in the soil for several growing seasons, new wood in the form of annual growth rings is added each season by the vascular cambium so that the persistent cuttings become quite wide in diameter. They develop a periderm that may be a millimeter or more in thickness (Plate 59).

SUMMARY

The common hop, Humulus lupulus L., is a dioecious, climbing plant with herbaceous vines arising from a perennial underground stem. The cultivated hop plant is propagated primarily from rhizome cuttings. These cuttings become the so-called rootstocks of the cultivated hop plant.

In the apical meristem of the hop root there is no clear-cut differentiation of the root initials into discrete regions. Instead, the central cylinder, cortex, epidermis, and rootcap arise from a common set of rather poorly defined initials. Vascular differentiation begins with the delimitation of the xylem plate through the expansion and vacuolation of the future tracheary elements. Despite the early vacuolation of the xylary procambium, the protophloem elements are the first to mature and are followed by the centripetally-maturing protoxylem and metaxylem. In the primary state the hop root shows a diarch xylem plate with two groups of primary phloem flanking the primary xylem.

A relatively short transition region occurs in the upper part of the hypocotyl between the vascular tissues of the primary root and the cotyledons. The transition takes place from a radially alternate arrangement of the vascular tissues in the root to a collateral arrangement in the cotyledons.

The seedling develops a fleshy storage organ through secondary growth of the taproot and hypocotyl. The hypocotyl resembles the fleshy taproot throughout most of its extent. The vascular cambium

forms highly parenchymatous secondary vascular tissues. Relatively little secondary xylem is formed. The bulk of the secondary tissue is composed of phloem parenchyma and phloem rays which become well-packed with large starch grains. A phellogen is differentiated from the pericycle and develops a persistant periderm on the outer surface of the fleshy storage organ after the cortex is sloughed.

Lateral roots of the fleshy storage organ are initiated in the pericycle opposite the protoxylem poles. The architecture of the secondary roots appears similar to that of the primary root. The adventitious root system of the cuttings and rhizomes consists of numerous short, fibrous absorbing roots as well as many larger fleshy storage roots.

The vascular cambium differentiates large amounts of secondary xylem and phloem in the fleshy storage roots. Numerous thick-walled, lignified fibers develop throughout both the xylem and phloem. Resin and tannin cells occur in great numbers. The secondary phloem contains a high percentage of phloem parenchyma and ray cells and constitutes the larger portion of the fleshy root. The outer surface of the fleshy root consists of a persistent periderm.

The vegetative apical meristem of the hop shoot is differentiated into a single-layered tunica and a corpus. The tunica forms a mantle of cells around the corpus which consists of a mass of irregularly-shaped, highly parenchymatous cells. The first elements to become differentiated in the provascular strand of the young vine are sieve tubes, with protoxylem elements maturing somewhat lower. As the procambial tissue undergoes further development, the

protophloem and protoxylem become differentiated into discrete collateral strands arranged in a cylinder around the outer periphery of the differentiating pith. At the end of primary growth of the young stem axis a fascicular cambium becomes organized within the vascular bundles, and an interfascicular cambium develops between the bundles.

The young, more or less succulent, hop vine is deeply furrowed and prominently ridged, and bears numerous trichomes and emergences. The ridges of the young vine are supported internally by relatively large strands of collenchyma tissue. A prominent single-layered starch sheath encloses the vascular cylinder which contains strands of thick-walled mucilaginous primary phloem fibers on its outer circumference.

The primary phloem consists of sieve tubes with oblique simple sieve plates. Companion cells are absent from the protophloem but are present in the metaphloem. Phloem parenchyma cells occur in abundance along with numerous tannin cells. Many large resin-containing secretory ducts surrounded by epithelial cells are present in the young phloem. Relatively thick-walled fibers are also present in abundance.

A rather indistinct vascular cambium separates the primary xylem from the primary phloem. The primary xylem consists of tracheary elements whose secondary walls may contain annular or helical thickenings (protoxylem) or bordered pits (metaxylem). Fibers are not present in the primary xylem.

Parenchymatous pith rays separate the primary xylem tissue into

radial strands. Prior to the completion of primary growth, a wide band of relatively thick-walled prosenchymatous cells is differentiated by the fascicular and interfascicular cambia on the outer periphery of the primary xylem. This band, 4-6 cells or more in width, forms a distinctive zone which completely encircles the primary xylem and sharply demarcates it from the remainder of the vascular tissues.

The pith is composed of thin-walled parenchymatous cells among which are numerous small intercellular spaces. Resins and tannins as well as small crystals are often present in the cells.

As the hop vine matures, its external architecture is angular at first and then becomes more or less round in appearance and loses the epidermal appendages. The strands of collenchyma become radially compressed. Many cortical cells become filled with resins and tanniferous substances. The starch sheath loses its distinctive appearance and becomes compressed tangentially. The phloem of the mature hop vine contains numerous sieve tubes which possess slightly oblique simple sieve plates. Numerous mucilaginous phloem fibers are present. Secretory ducts do not occur in the mature secondary phloem tissue but numerous resins and tannin cells are prevalent. Latex ducts are not present among any of the older tissues of the mature hop vine.

The secondary xylem tissue of the hop vine, and root, is conspicuous because of the many wide-lumened, thick-walled lignified vessels that occur in clusters or radial series. Xylem parenchyma

cells containing large crystals are of frequent occurrence adjacent to the numerous libriform fibers. Toward the end of the growing season numerous tyloses may develop in the vessels of the secondary xylem of the cuttings, rhizomes, and roots.

A parenchymatous pith is entire in the young vine, but becomes ruptured in the older portions of the vine so that in the internodal regions of the mature aerial stem the central portion is hollow. The pith of the internodal regions of the rhizomes does not become hollow.

Anatomically, the architecture of the aerial vine and that of the rhizome is essentially similar. No periderm is formed in the aerial portions of the vine, but a cork cambium is differentiated in the rhizome. The cork cambium is first initiated immediately below the subepidermal cells and develops in successively deeper layers of the cortex as the first periderm and cortical cells are being sloughed. Rootstocks that have remained in the ground over a period of several growing seasons may develop a cork cambium from the outermost parenchyma cells of the secondary phloem. Longitudinal lenticels are of common occurrence on the external surface of the underground portions of the hop plant.

The cuttings or rootstocks of the cultivated hop plant usually persist for several years providing they do not rot away. If allowed to remain undisturbed, new wood in the form of annual growth rings is added each year by the vascular cambium so that the cuttings may become quite thick in appearance. The outer surface

may be covered with a more or less persistent periderm several millimeters in thickness.

Plate 1. Seedlings of Humulus lupulus in successive stages of development. These plants were collected the following number of days after the seed had been sown: (1) 3; (2) 5; (3) 6; (4) 7; (5) 9; (6) 13. The primary root and hypocotyl have not as yet become fleshy. (All X2)

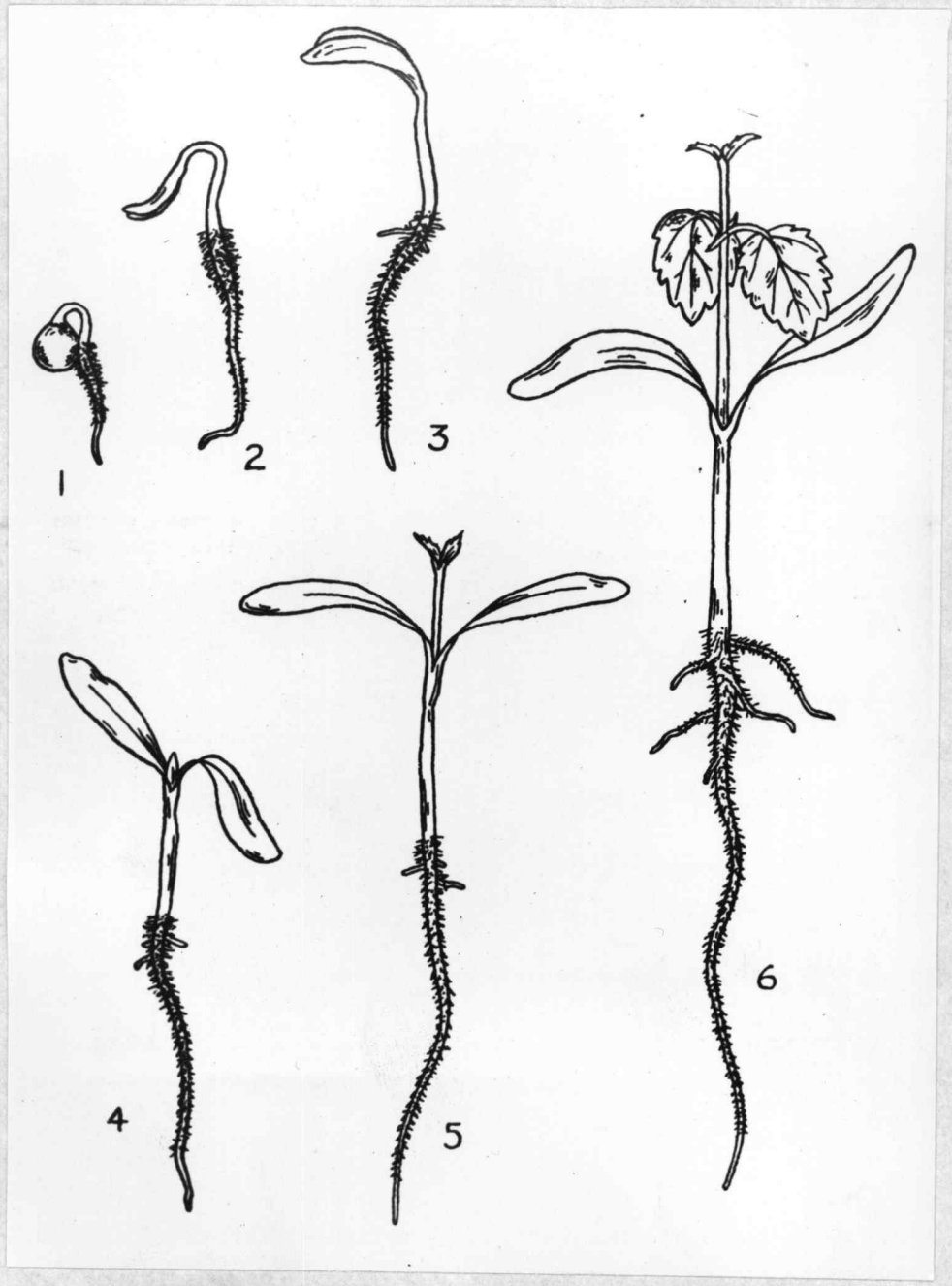
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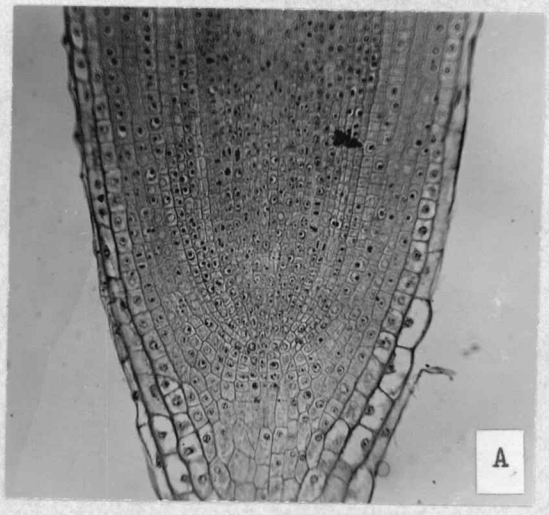
Plate 2. Longitudinal sections of the primary root apex of Humulus lupulus showing the organization of the apical meristem. B and C are enlargements of A. Notice the poorly-defined initials region, a. See Plate 3. (A, X150; B, X300; C, X600)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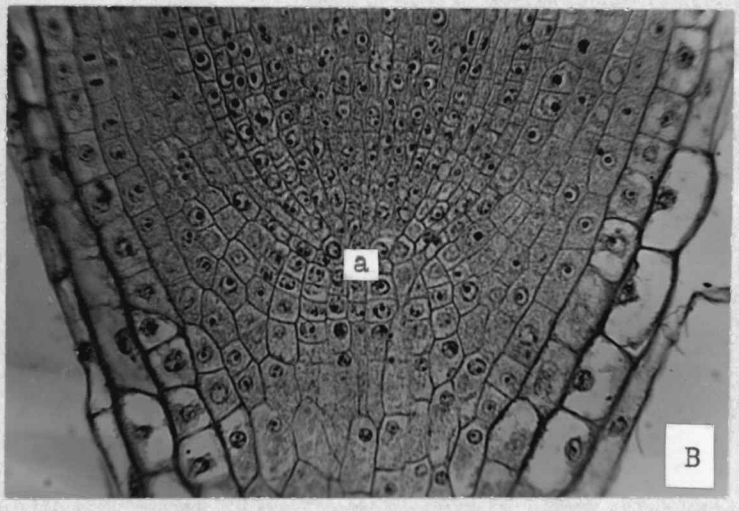
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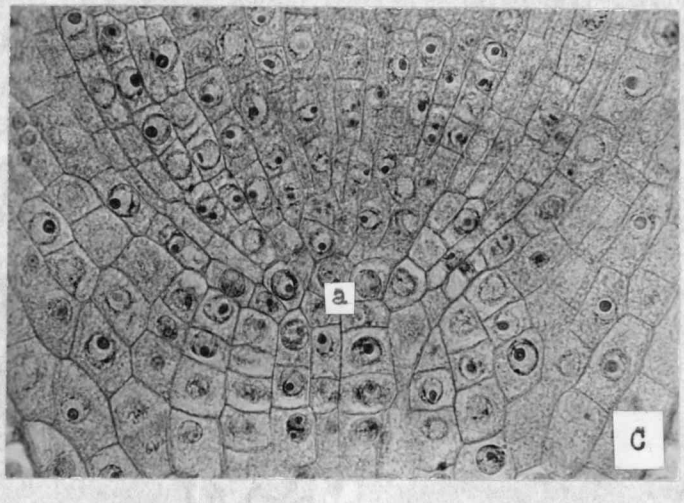
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Plate 3. Root tip of Humulus lupulus in longitudinal section, drawn from the same root shown in Plate 2. The heavy lines were made to demarcate the various tissue regions. a, root initials region; b, central cylinder or stele; c, cortex; d, epidermis; e, rootcap. (Camera lucida drawing, X300)

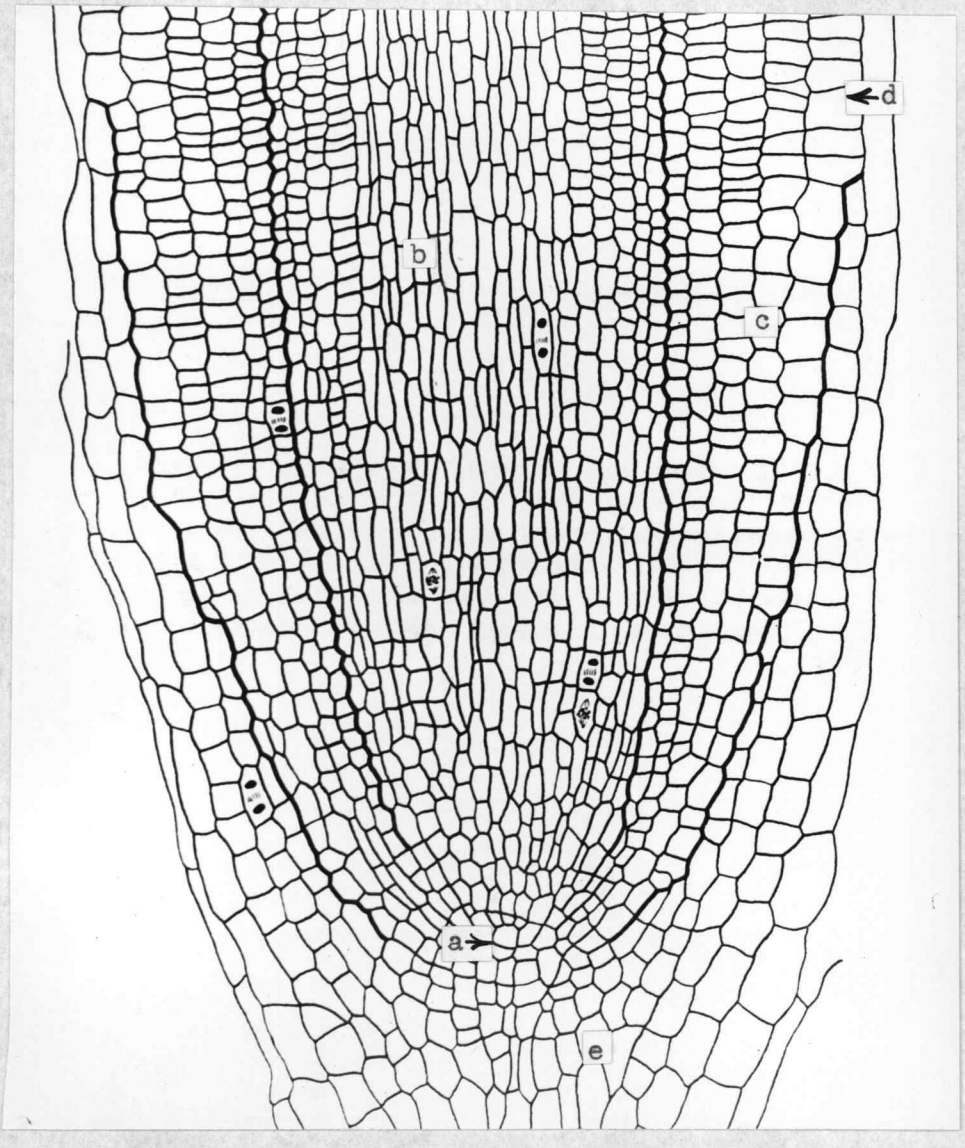
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Plate 4. Vascularization of the hop seedling. These diagrams illustrate the connection between the root and the cotyledons. The vascular system of the primary root, A, diverges upwardly into the two cotyledons.

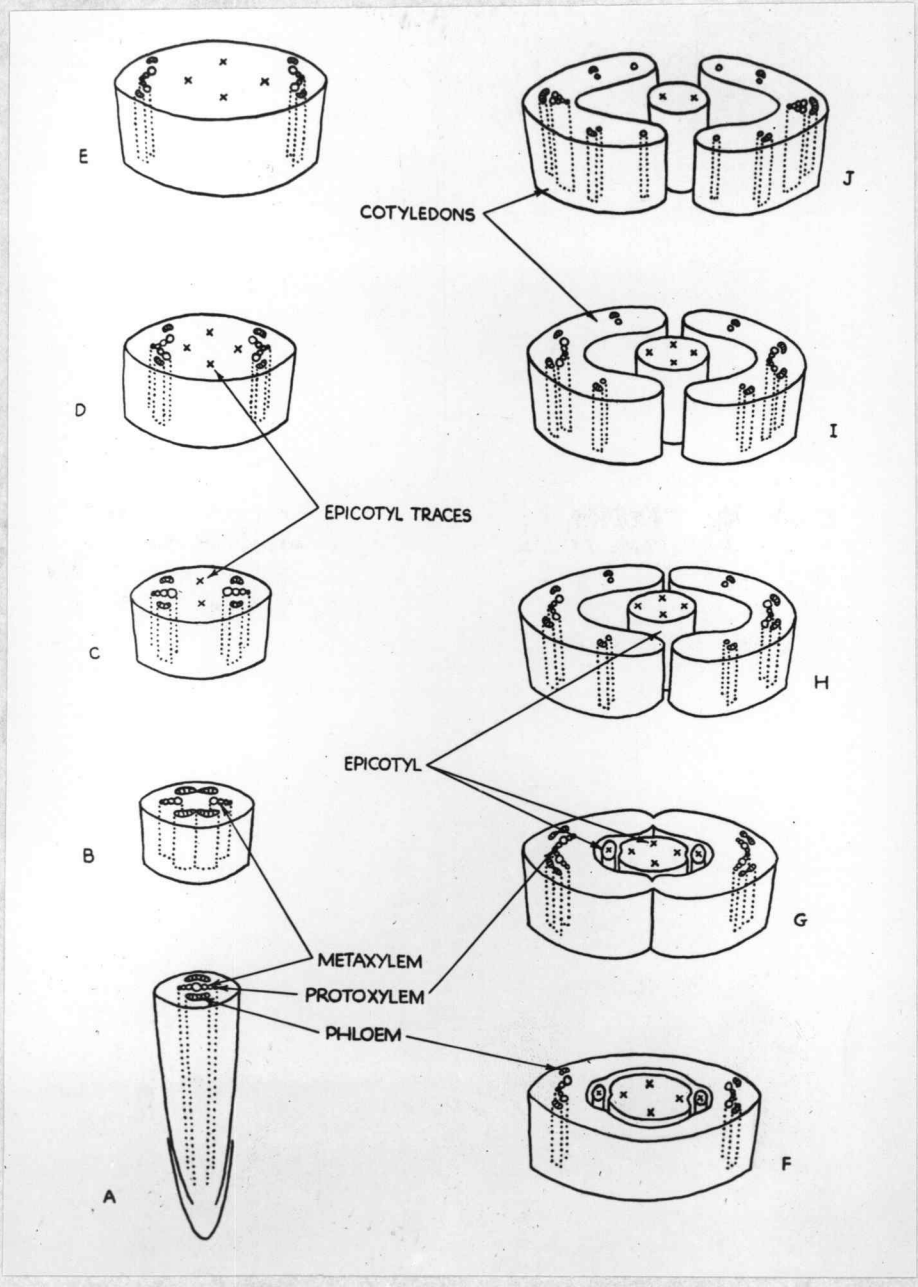
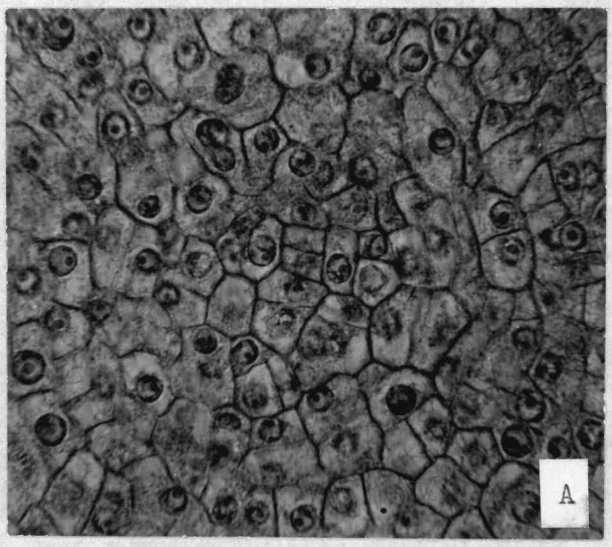

E
J
COTYLEDONS
D
I
EPICOTYL TRACES
C
H
EPICOTYL
B
G
METAXYLEM
PROTOXYLEM
PHLOEM
F
A

Plate 5. Transverse section through the root apical meristem. In A, the root initials are shown as they appear immediately above the cells of the differentiating rootcap. B, shows the region slightly above that of A and depicts the differentiation of the pericycle, a. (Both X600)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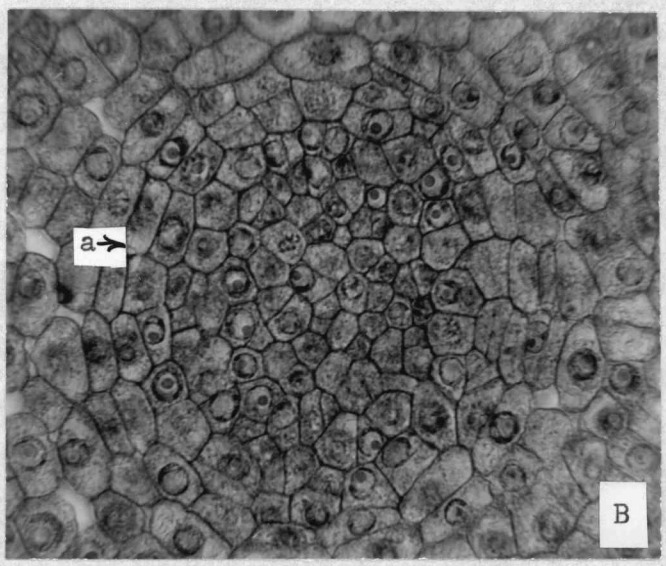
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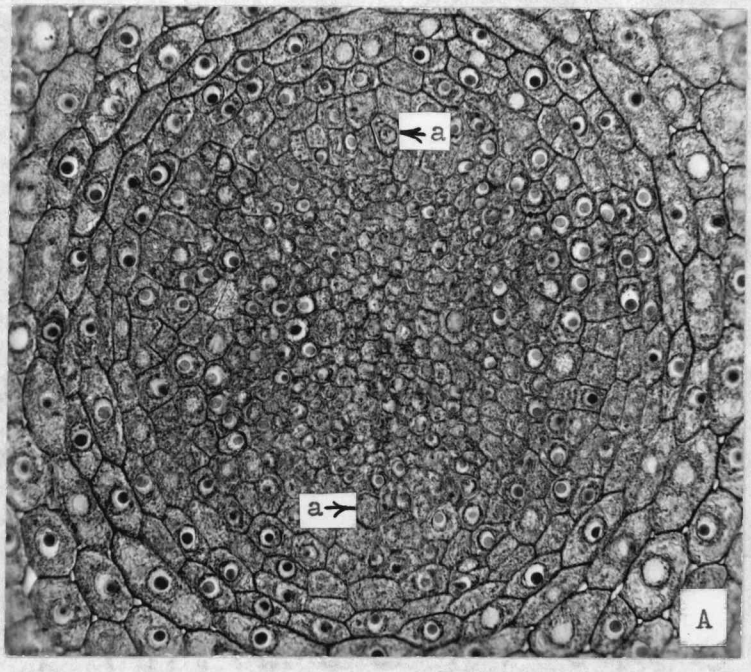
Plate 6. Differentiation of the protophloem. In A, the first of the protophloem is evident at opposite poles, a; note that the xylary procambial cells are not as yet distinct. B, the first sieve-tube elements are mature and appear as light, hexagonal-shaped structures, a. (A, X400; B, X300)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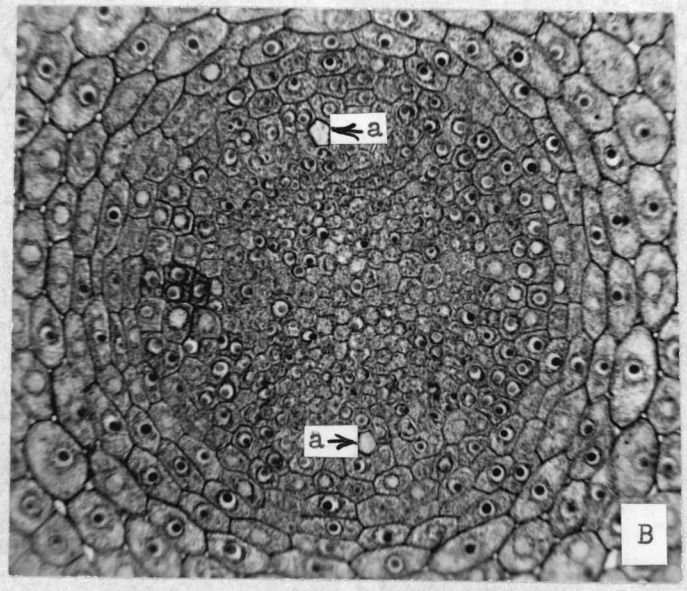
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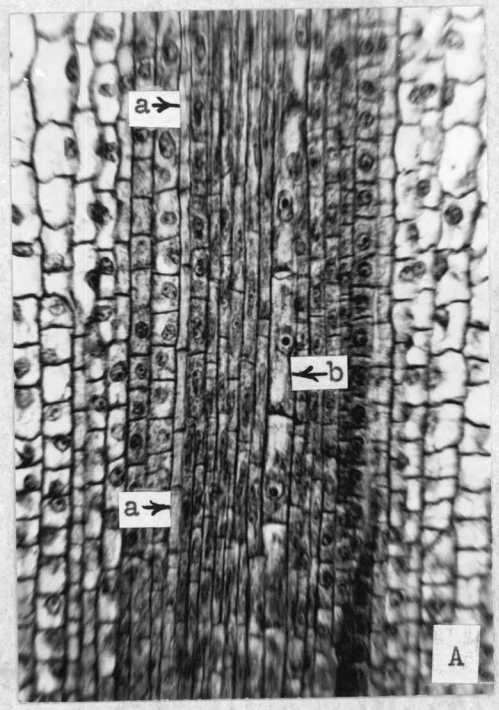
Plate 7. A, longitudinal section of the primary root slightly above the apex. Note the differentiation of the protophloem sieve-tube elements, a, and the appearance of the immature metaxylem vessels, b. B, transverse section of the entire primary root as it appears at the level of protophloem maturation and beginning of protoxylem differentiation. (A, X300; B, X200)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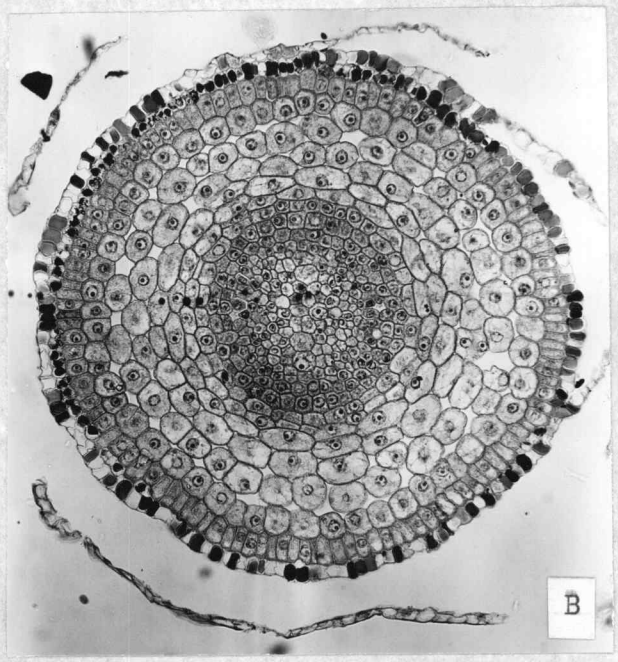
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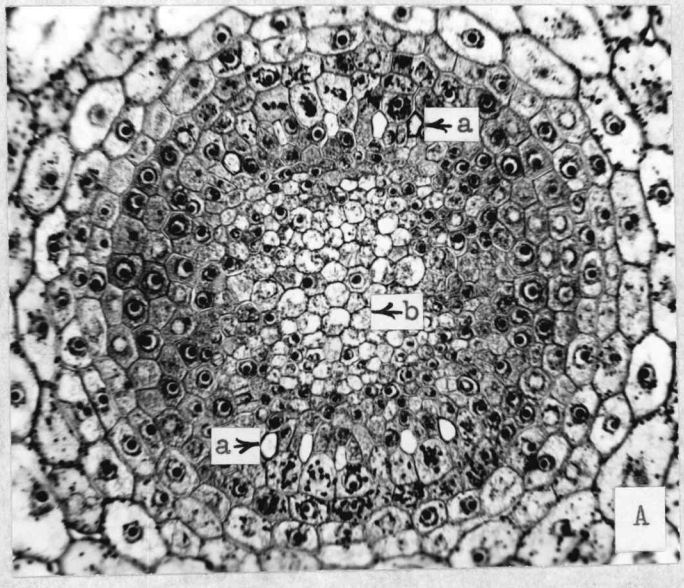
Plate 8. A, transverse section of a primary hop root depicting the vascular cylinder with several differentiated sieve-tube elements, a, and the vacuolation of the xylary procambium, b. B, an enlargement of the upper portion of the vascular cylinder in A; note the position of the pericycle, c. (A, X300; B, X970)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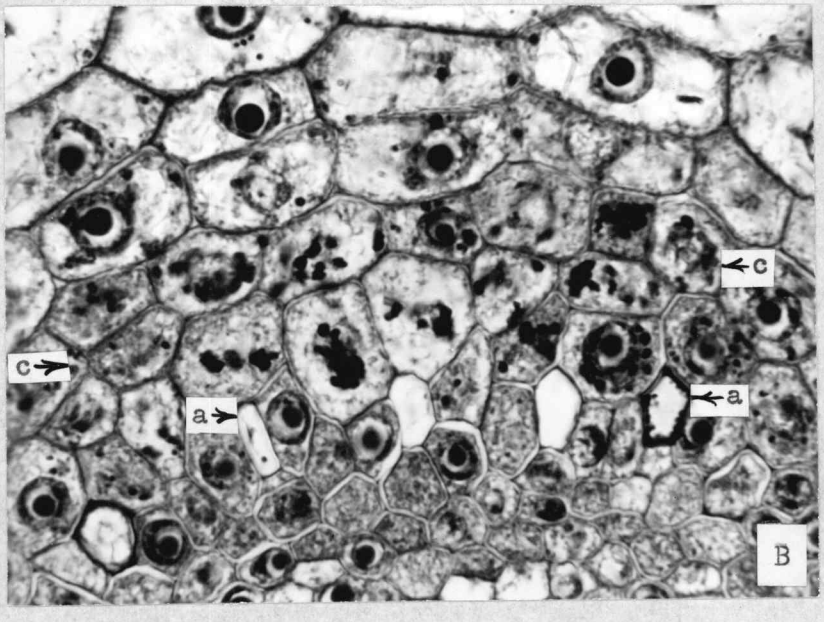
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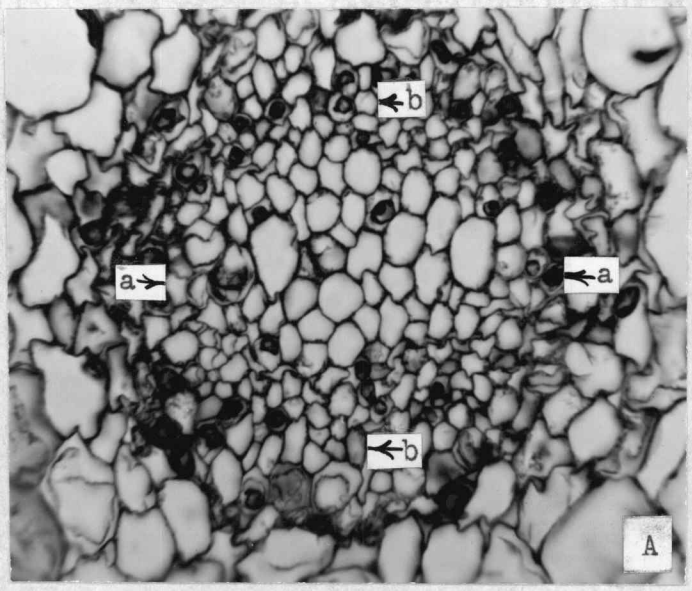
Plate 9. A and B are transverse sections of the primary hop root in the region of the first differentiating protoxylem, a; b, differentiated sieve-tube element. (Both X600)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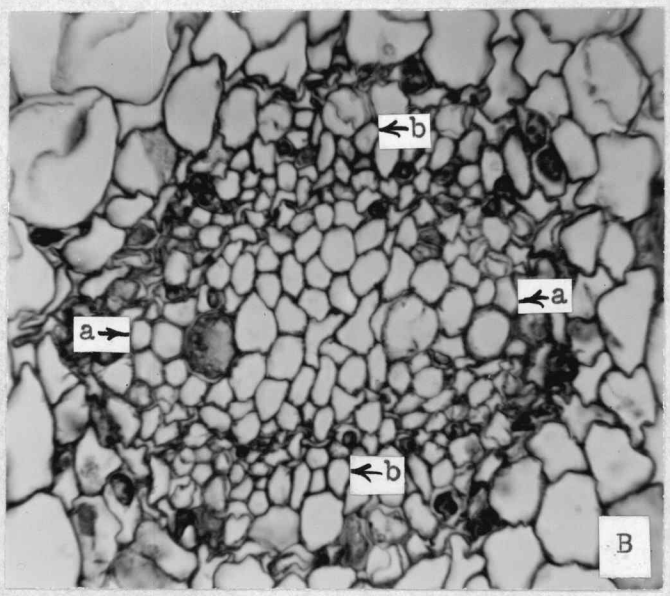
b
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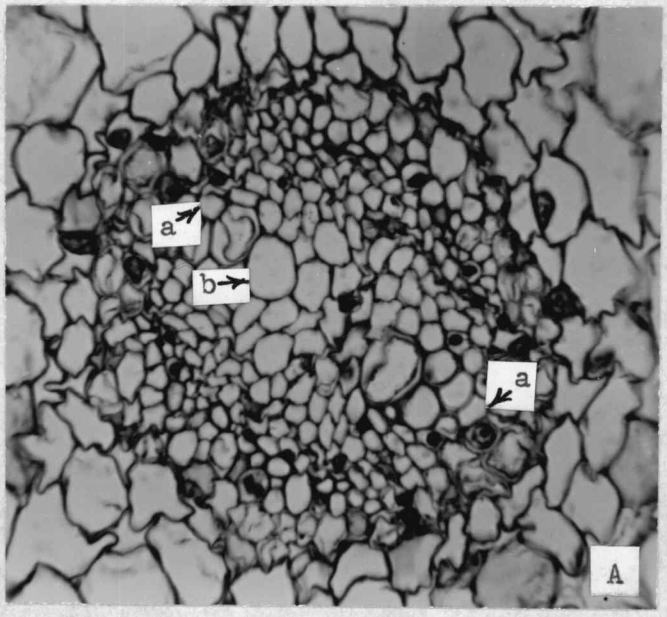
B

Plate 10. Further development of the primary xylem. In A, the protoxylem is differentiated at both poles, a, with the maturation of the metaxylem occurring in a centripetal manner, b. B, shows the completion of maturation of the primary xylem in the young hop root. The cambial tissue which has formed, c, has been somewhat crushed in sectioning of the material. (Both X600)


a
b
a
A

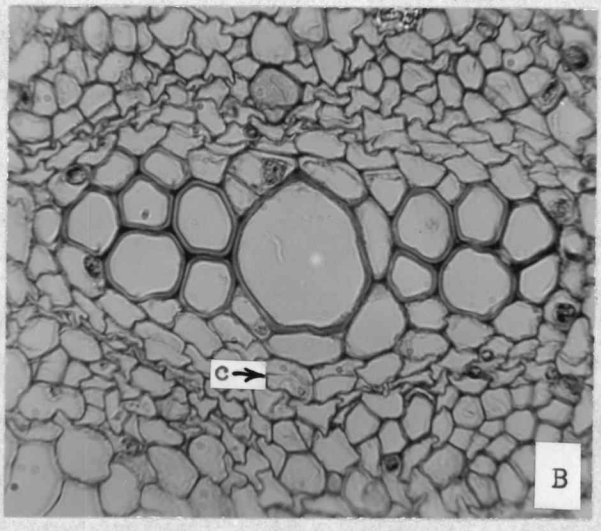

c
B

Plate 11. Diagrammatic longitudinal section of a root tip of Humulus lupulus to illustrate certain features in the differentiation of its various tissues. All of the tissues have a common origin. See Plates 2, 3, and 4.

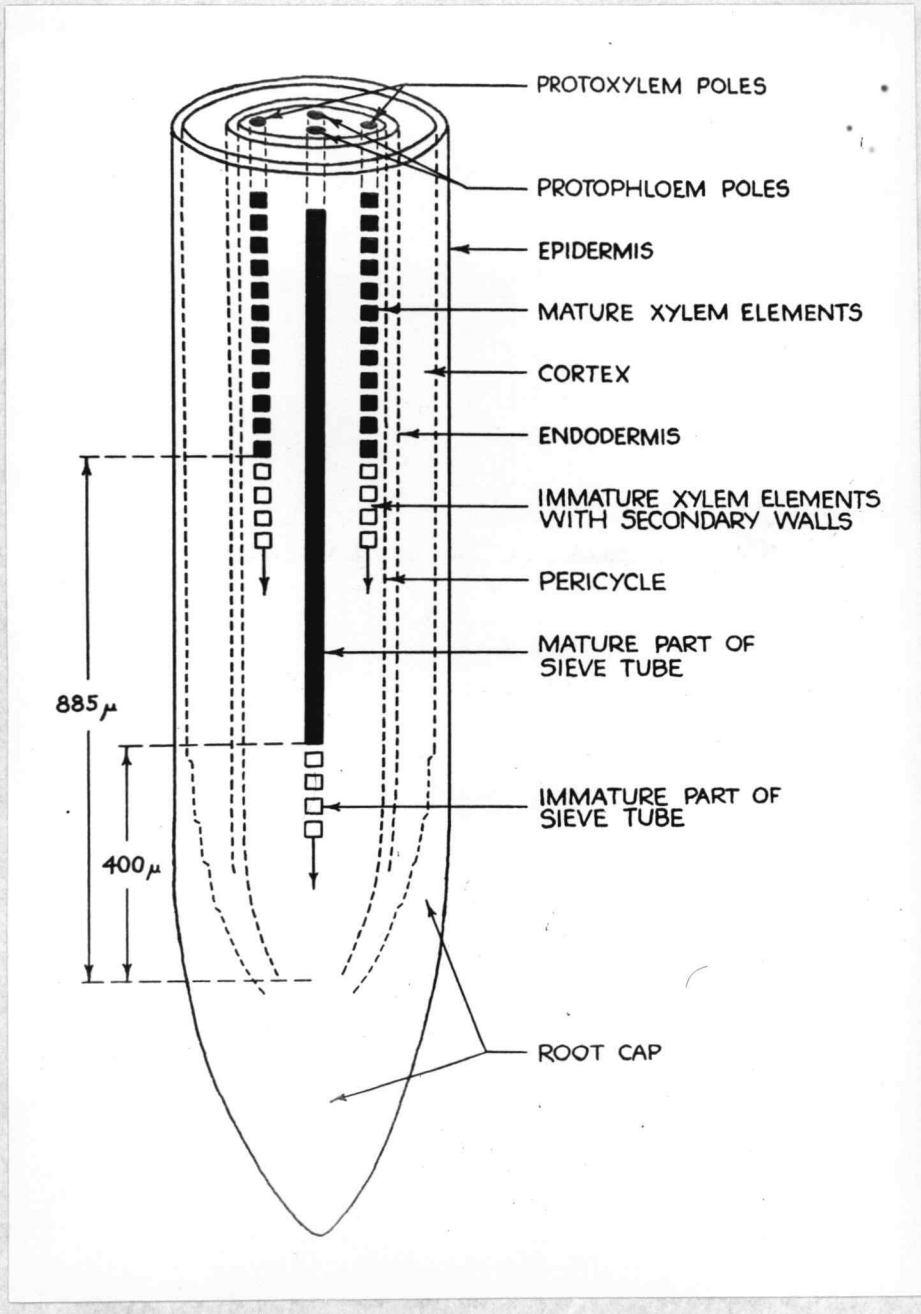
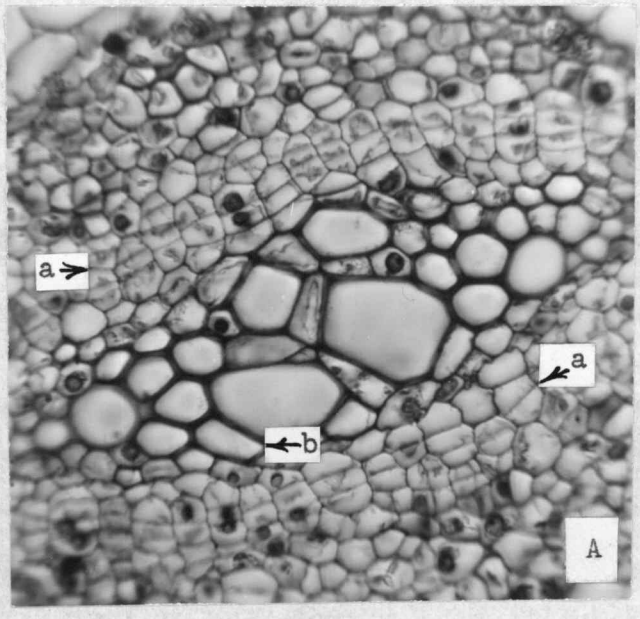

PROTOXYLEM POLES
PROTOPHLOEM POLES
EPIDERMIS
MATURE XYLEM ELEMENTS
CORTEX
ENDODERMIS
IMMATURE XYLEM ELEMENTS WITH SECONDARY WALLS
PERICYCLE
MATURE PART OF SIEVE TUBE
885 μ
IMMATURE PART OF SIEVE TUBE
400 μ
ROOT CAP

Plate 12. A, transverse section of the central portion of the vascular cylinder of the primary hop root showing the development of the cambium, a, and the primary xylem, b. In B, the transverse section shown is one similar to that of A but enlarged to show more detail: a, cambium; b, primary and developing secondary phloem; c, sieve-tube element; d, companion cell; e, part of the primary and developing secondary xylem. (A, X300; B, X970)


a
a
b
A

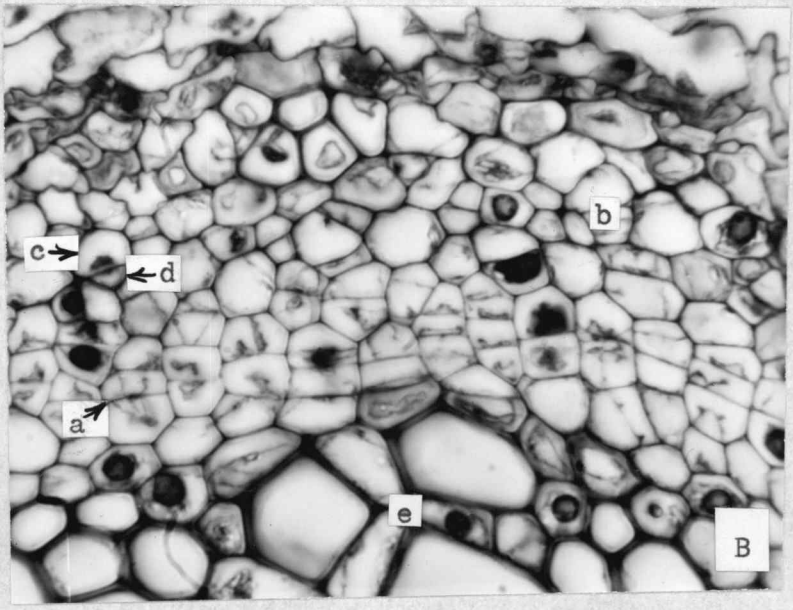
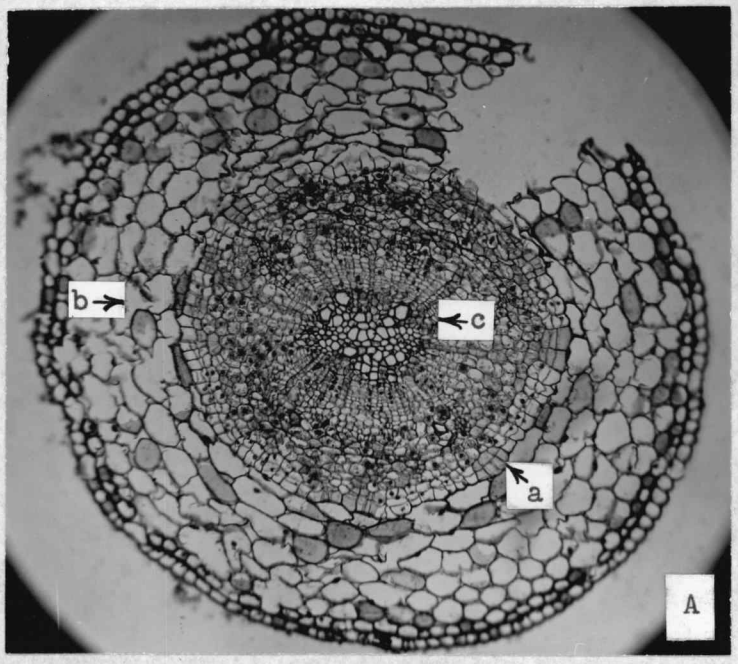

b
c
d
a
e
B

Plate 13. A, transverse section of a mature primary root in which the pericycle, a, has undergone periclinal division in its initiation of a cork cambium, and the cortex, b, has become ruptured in the meantime. In B, the transverse section depicts the appearance of the vascular tissues in the lower part of the hypocotyl. Note that the cambium, a, has not as yet completely enclosed the xylary tissue; b, pith. (A, X150; B, X300)


b
c
a
A

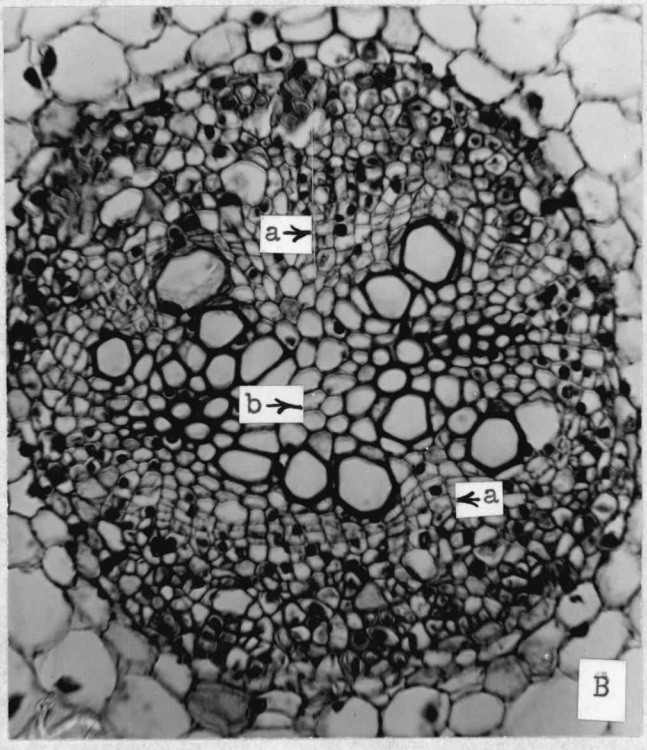
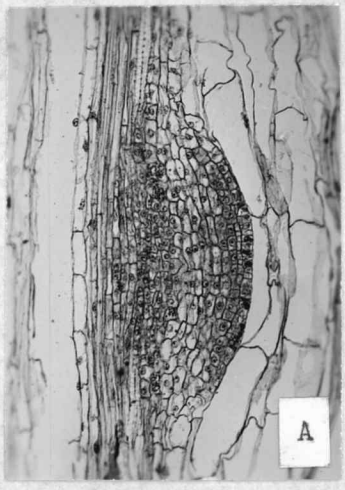

a
b
a
B

Plate 14. Longitudinal sections showing the development of lateral roots in the hop plant. A, the lateral root primordium is compressing the cortical cells in its outward growth. B, the root primordium has undergone further development and is beginning to protrude externally to the ruptured cortex. In the lower left is a part of another root primordium. (Both X120)


A

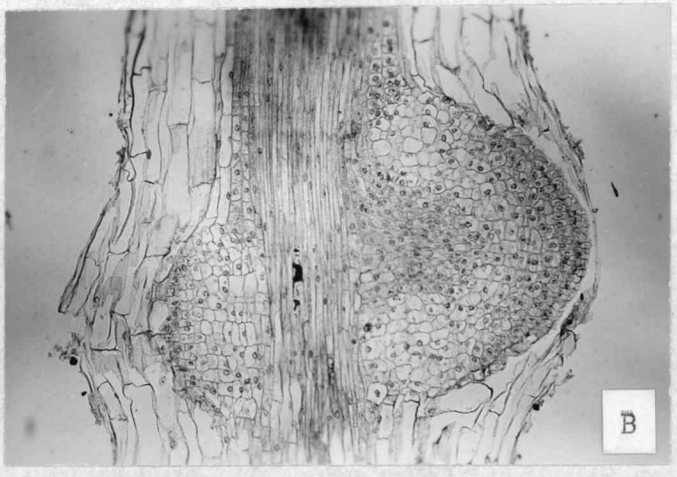
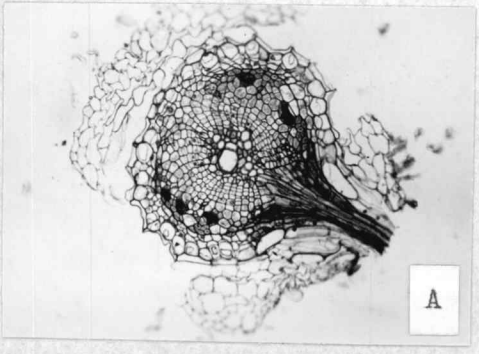

B

Plate 15. Transverse sections of a primary root showing the further development of a lateral root. B is an enlargement of A. a, primary phloem fibers; b, cambium; c, cortex. (A, X120; B, X450)


A

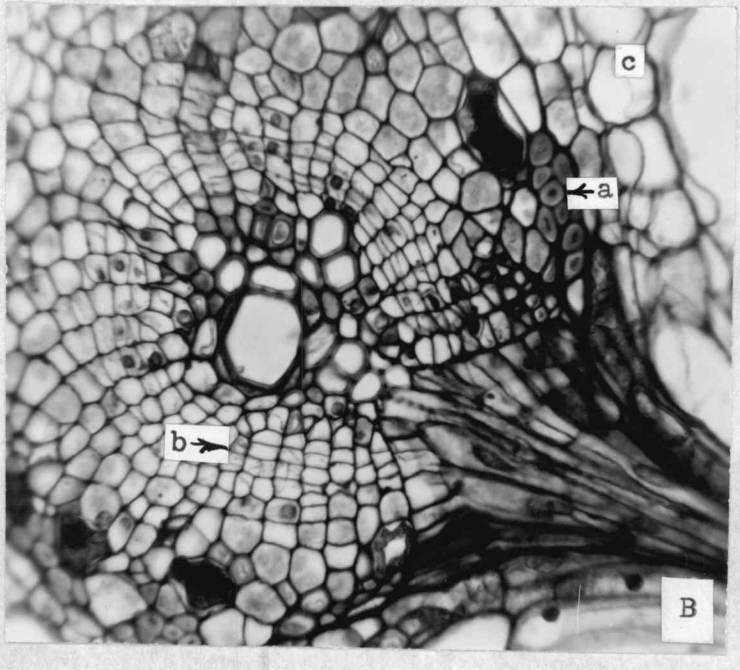
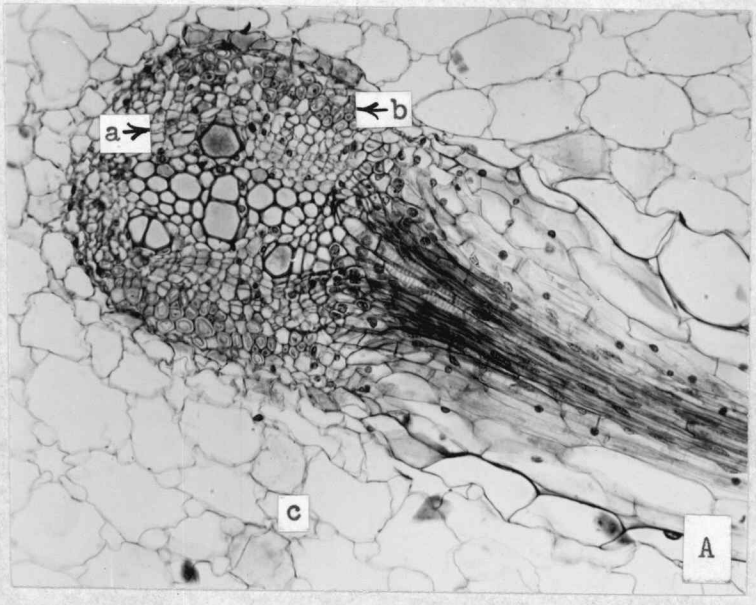

c
a
b
B

Plate 16. A, transverse section of a primary root older than the one shown in Plate 15 and showing the appearance of the tissue arrangement between the main root and the lateral. The vascular tissue of the lateral root can be seen to connect directly with that of the main root. a, cambium; b, primary phloem fibers; c, cortex. B, is a transverse section of a part of a well-developed first year hop root. a, secondary phloem; b, secondary xylem; c, vascular cambium; d, cork cambium. The outlined area represents the part that is enlarged in Plate 17. (A, X200; B, X120)


a
b
c
A

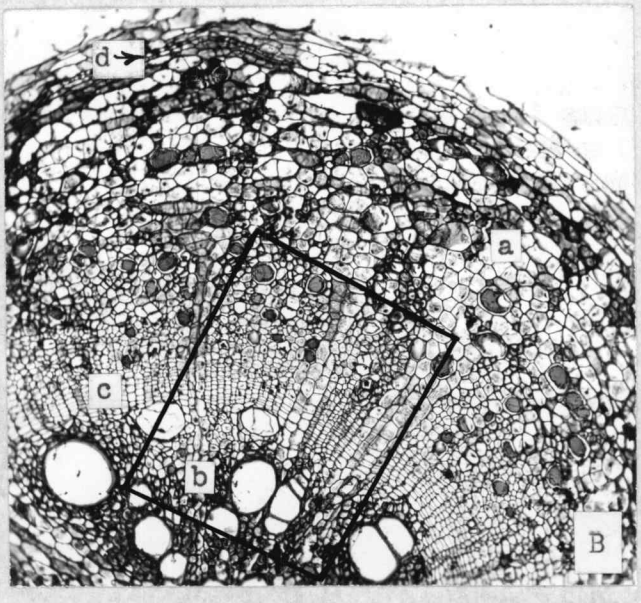
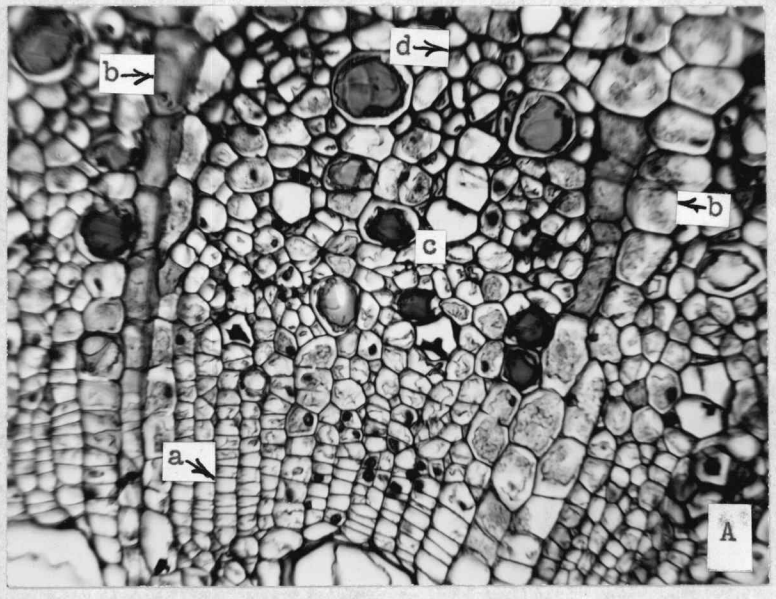

d
a
c
b
B

Plate 17. A and B are enlarged transverse sections of the area outlined in Plate 16, B. A, is a portion of the secondary phloem while B is a portion of the secondary xylem. a, cambium region; b, phloem rays; c, secondary phloem; d, secondary phloem fibers; e, secondary xylem; f, xylem rays. (X300)


b
d
b
c
a
A

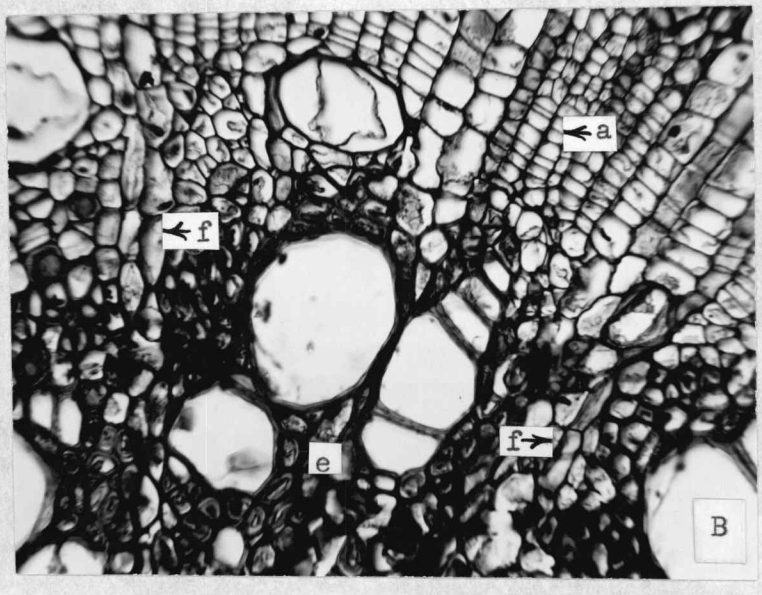
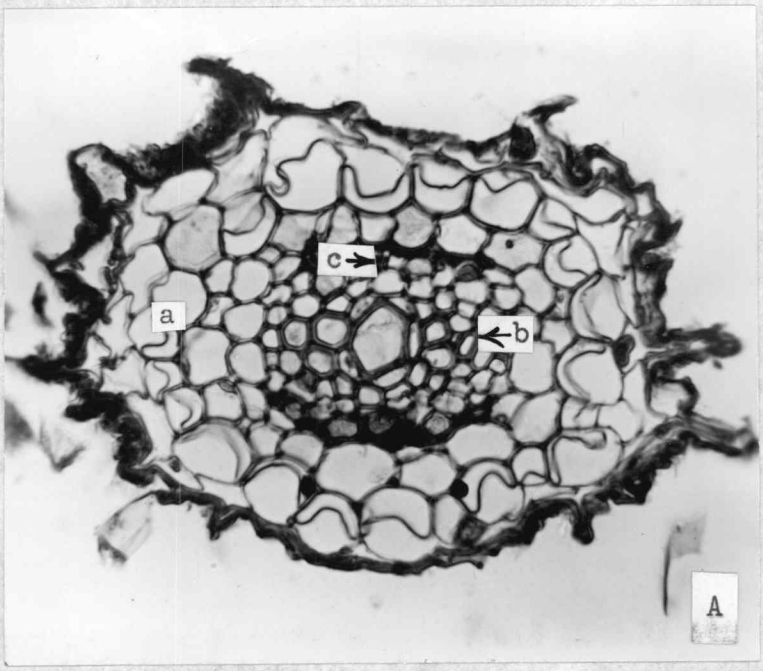

a
f
f
e
B

Plate 18. A, transverse section of a mature fibrous absorbing root. Note the absence of secondary tissues. a, cortex; b, primary xylem; c, primary phloem. In B, the transverse section shows a small well-developed primary storage root at the end of the first year's growth. a, remnants of cork tissue; b, secondary phloem, note the numerous resin and tannin cells which appear as black spots. In the process of sectioning much of the older cork tissue becomes lost. (A, X450; B, X13.5)


c
a
b
A

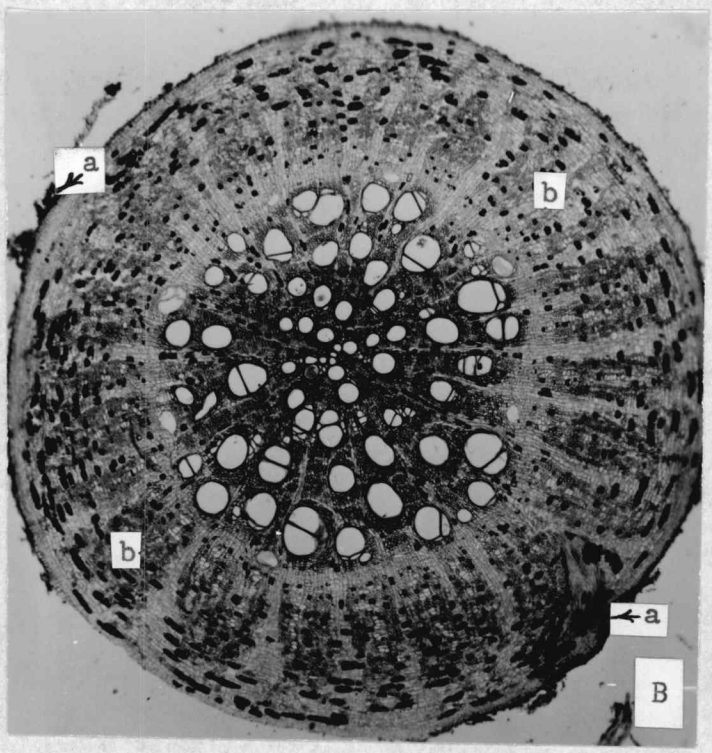
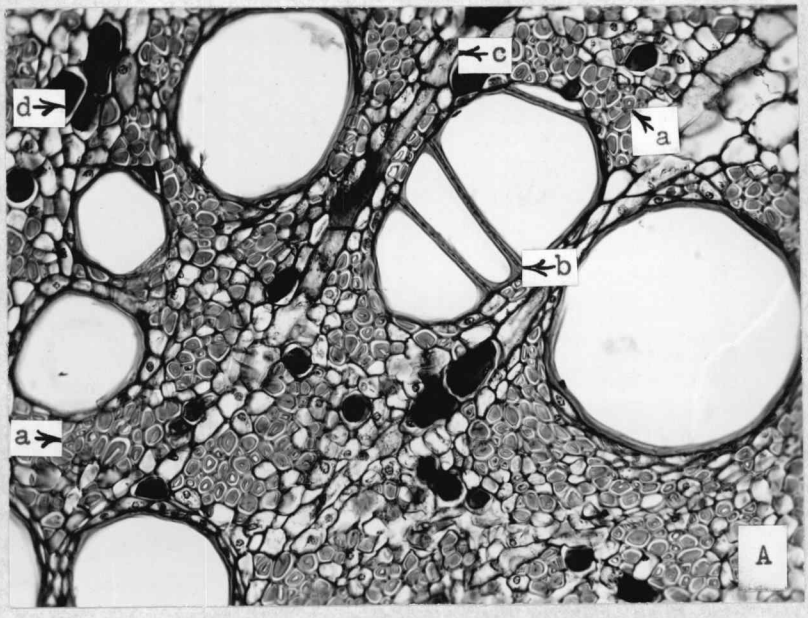

a
b
b
a
B

Plate 19. A, transverse section of a portion of a fleshy storage hop root. Note the numerous secondary xylem fibers, a; b, vessels; c, xylem ray; d, tannin cell. B, the central region of a fleshy storage root showing the relationship of the secondary xylem to the primary xylem. Note how small the primary xylem appears in comparison to the enclosing secondary xylem. a, secondary xylem fibers; b, vessel; c, secondary xylem ray; d, tannin cell. (A, X200; B, X100)


c
d
a
b
a
A

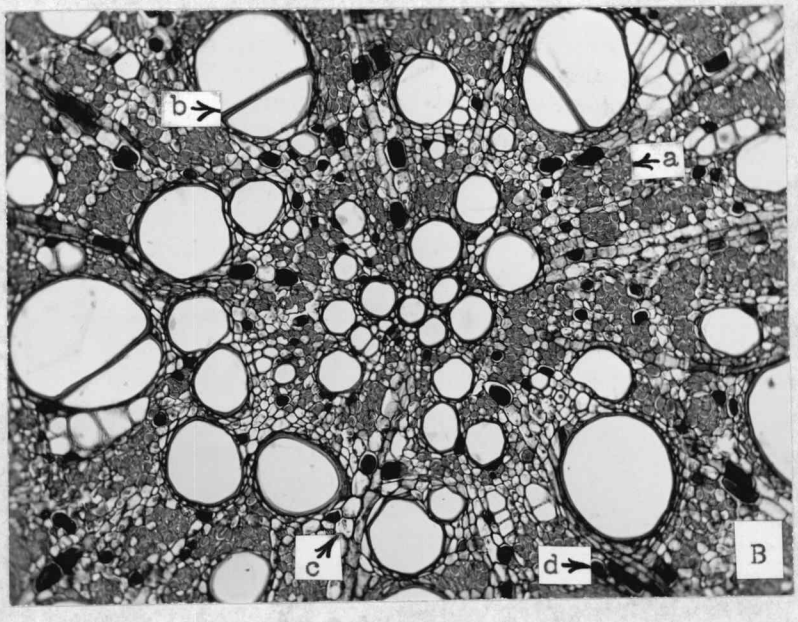
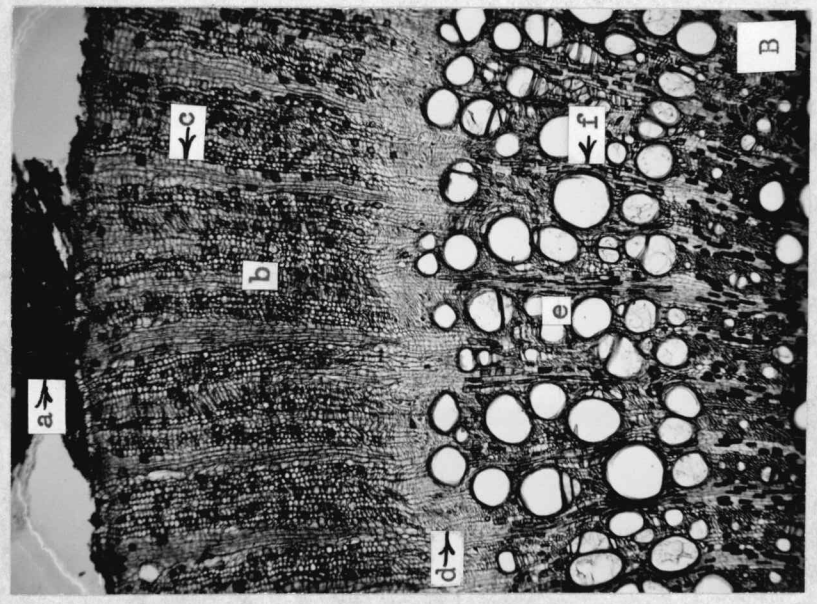

b
a
c
d
B

Plate 20. Transverse sections of storage roots to show the appearance of a younger root A, and an older root B. Note the difference in the respective phloem areas in particular. In A, the cork cambium has become differentiated from the proliferated pericycle, a, and in B, a, represents sloughing phloem tissue as well as older cork cells. b, secondary phloem; c, expanded phloem rays; d, cambial region; e, secondary xylem; f, xylem rays; g, tannin cells. (A, X100; B, X150)


a
b
c
d
e
f
B

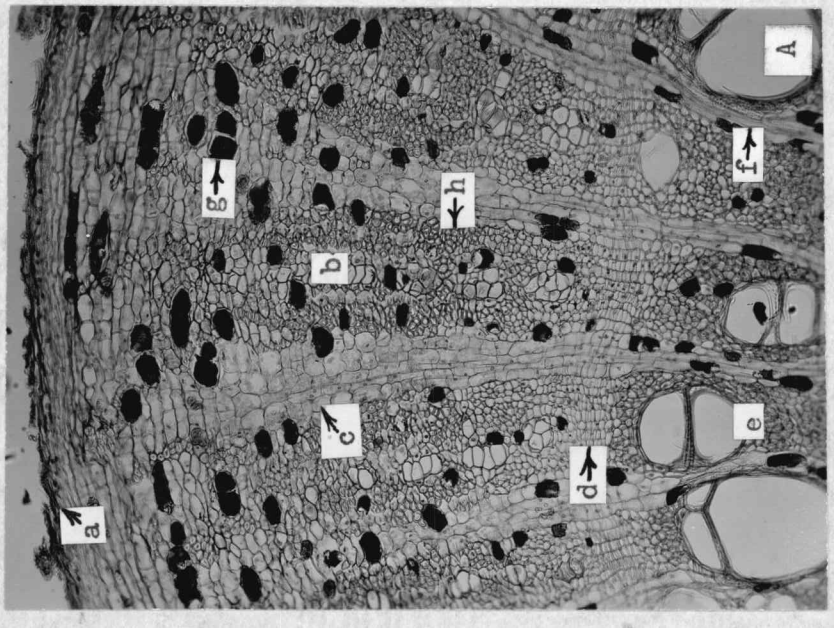
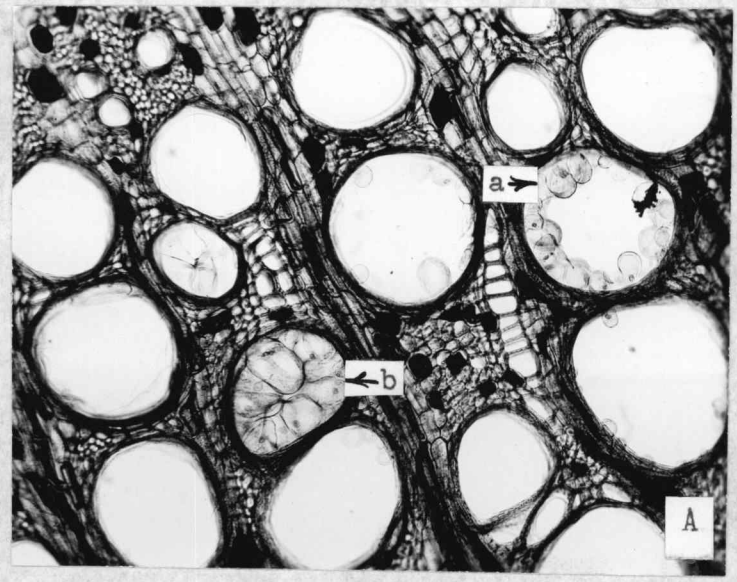

a
b
c
d
e
f
g
h
A

Plate 21. Various stages of tylose development in mature vessels of the root. In A, the tyloses are protruding into the vessel, a. Note the presence of the distinctive nuclei in the tyloses. The tyloses have almost completely occluded the vessel in b. B, is an enlargement of the central part of A. (A, X100; B, X200)


a
b
A

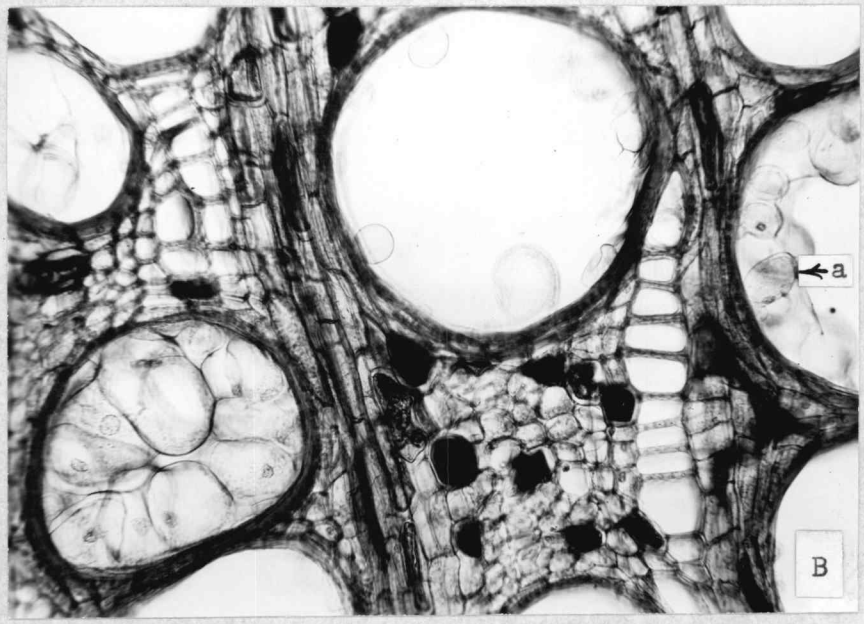
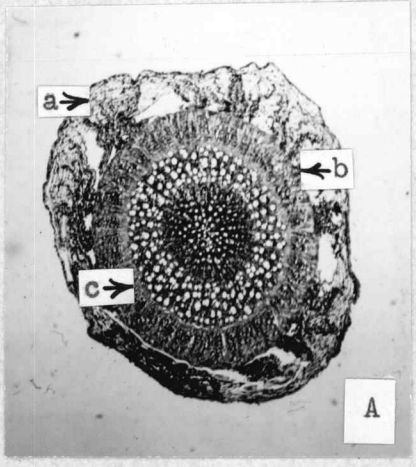

a
B

Plate 22. A, transverse section of a second year storage root. Note the appearance of the old secondary phloem tissue, a, which looke like a massive cork formation. A new cork cambium occurs immediately below the inner edge of the cavities of the old phloem, b; c, vascular cambium. B shows the relatively abundant tannin cells that occur in the secondary xylem of the mature hop root. The numerous black areas represent these cells. (A, X4; B, X150)


a
b
c
A

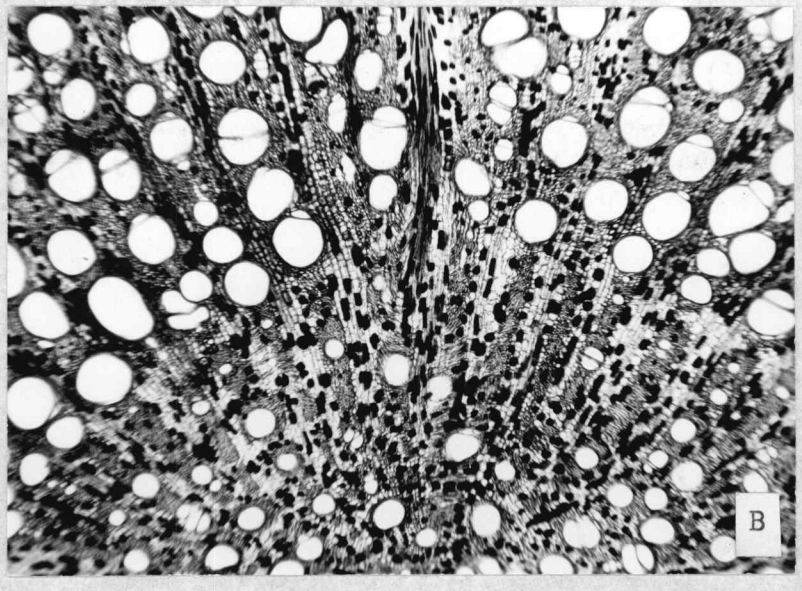

B

Plate 23. The fleshy primary root and hypocotyl of hop seedlings, with the leaves removed. The seedlings were obtained the following number of weeks after the seeds had been sown in the greenhouse: a, 3; b, 4; c, 5; d, 6. The arrows represent the approximate length of the hypocotyl. See Plate 1. (All X1)

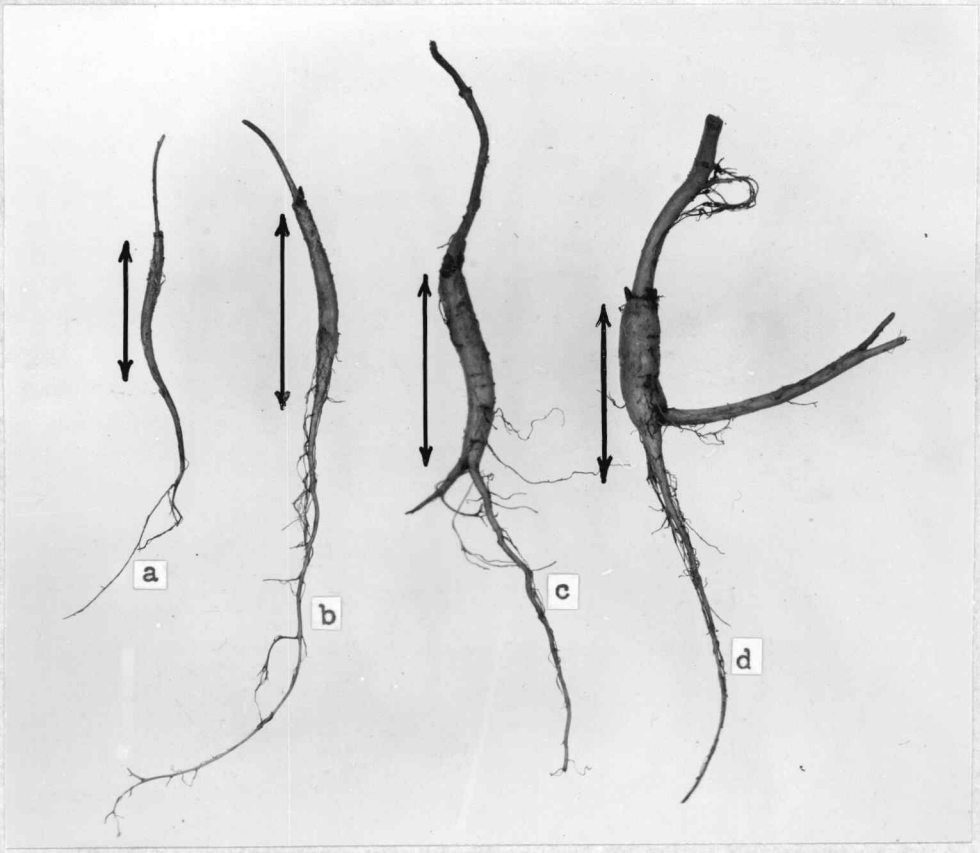
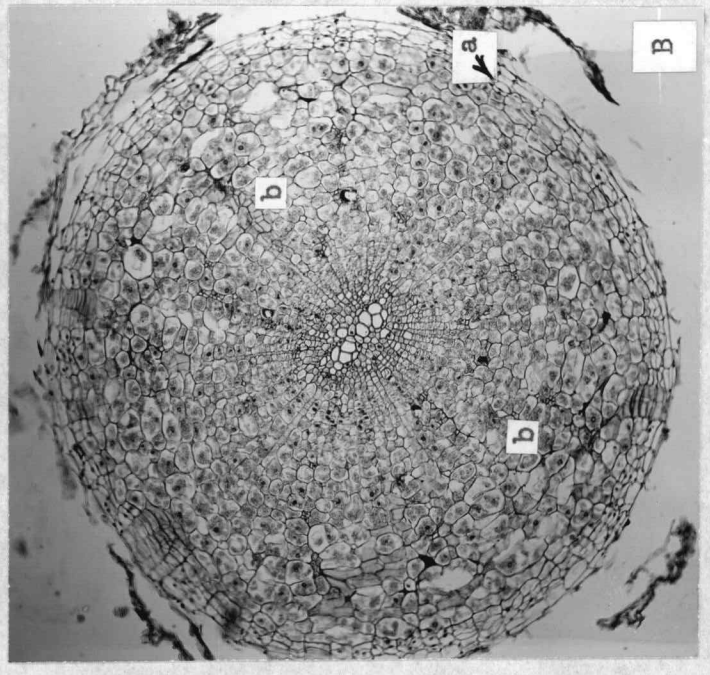

a
b
c
d

Plate 24. Showing the overall appearance of a hop seedling, approximately three months old, grown in the greenhouse. Note the fleshy lateral root and smaller fibrous roots as well as the main fleshy primary root and hypocotyl. (X 1)

Plate 25. A four month old hop seedling with several fleshy lateral roots and an abundance of smaller fibrous roots. The large buds at the cotyledonary node may develop into lateral shoots. See Plate 26. (X1)

Plate 26. A hop seedling approximately five months old. Note the numerous fibrous and fleshy lateral roots arising from the massive fleshy storage organ. The etiolated protruberance growing out from the latter, in the upper left region, is a young shoot developing from a lateral bud similar to those shown in Plate 25. (X1)

Plate 27. Transverse sections of the fleshy storage organ of the hop seedling. A, is a section taken from the radicle. The cortex has practically completely sloughed, c; cork cambium, a; the secondary phloem, b, phloem parenchyma cells well-packed with starch grains. B, represents a section taken from the lower part of the hypocotyl. Cork cells, a; secondary phloem, b. (Both X100)


a
B
b
b

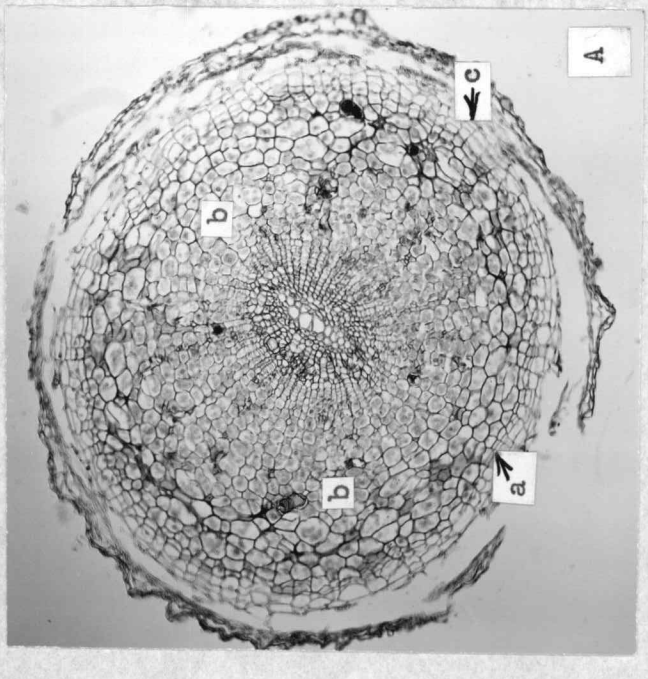
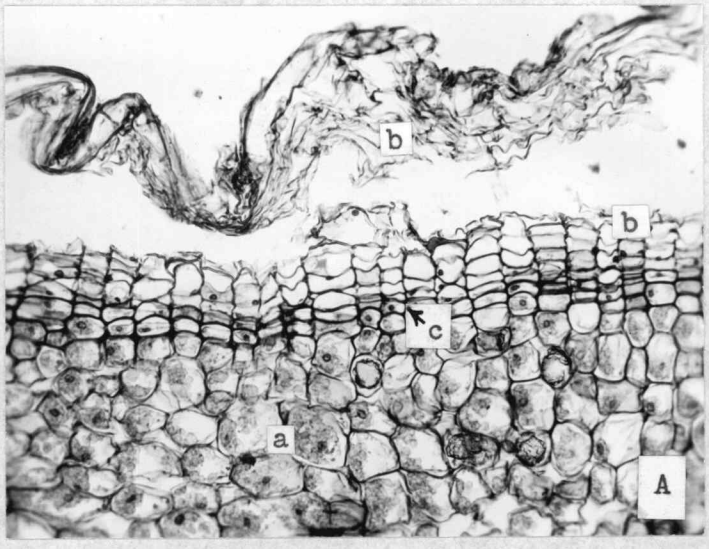
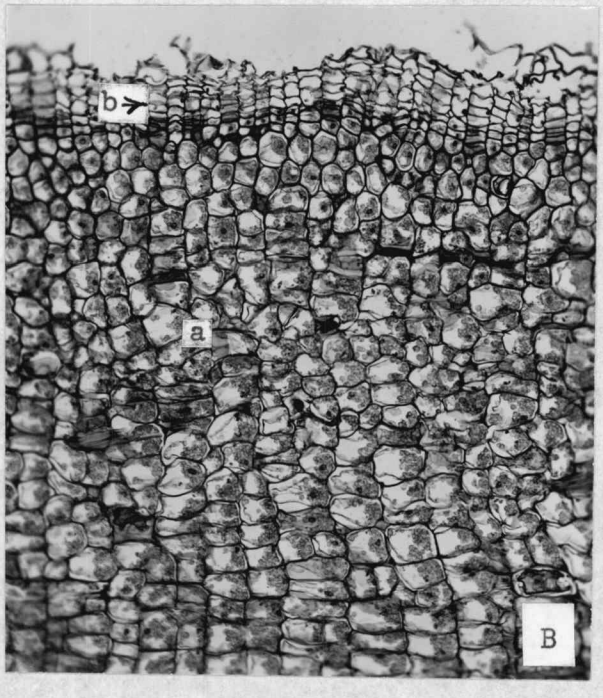

A
c
b
b
a

Plate 28. A, portion of a longitudinal section of the upper region of the fleshy primary root showing the proliferation of the pericycle and the formation of a cork cambium. a, primary phloem parenchyma; b, sloughing cortex; c, pericycle with cork differentiating externally. In B, the longitudinal section of the hypocotyl shows the cork layers on the external surface, b, and the abundant secondary phloem parenchyma cells containing numerous starch grains, a. C, is an enlargement of a few cells of the secondary phloem parenchyma as depicted in B showing the starch grains as they appear with polarized light. (A, X160; B, X100; C, X540)


b
b
c
a
A


b
a
B

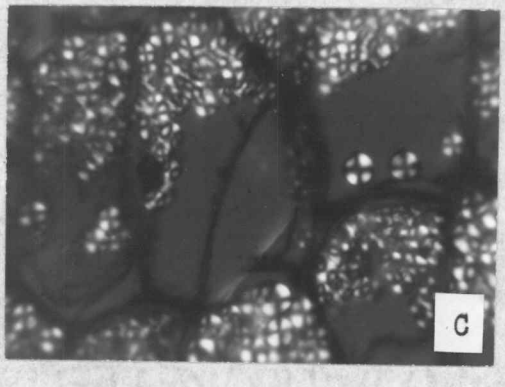

C

Plate 29. Hop plants in various stages of growth under field conditions. A, the young shoots as they are emerging from the soil; B, the young vines prior to their being trained; C, trained vines with the lateral shoots removed; D, the underground stems and root system of a four-year old hop plant.


A


B


C


D

Plate 30. Drawing of a vegetative shoot apex of Humulus lupulus in longitudinal view. Plate 31, B, shows in part the area from which this camera lucida drawing was made. (X300)

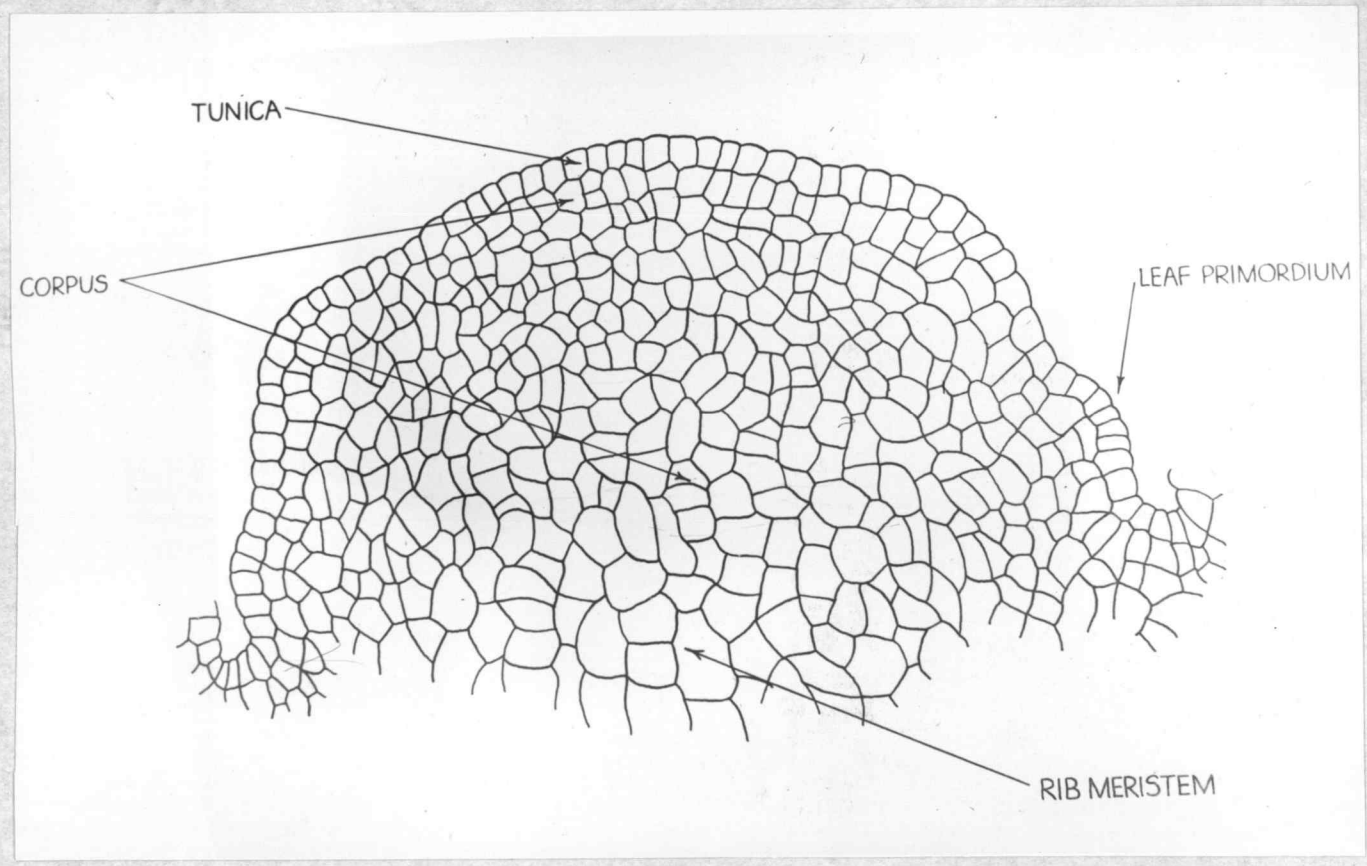
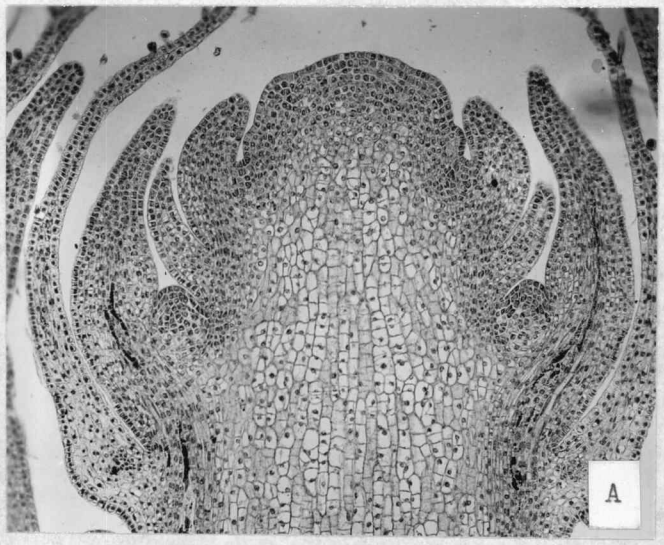
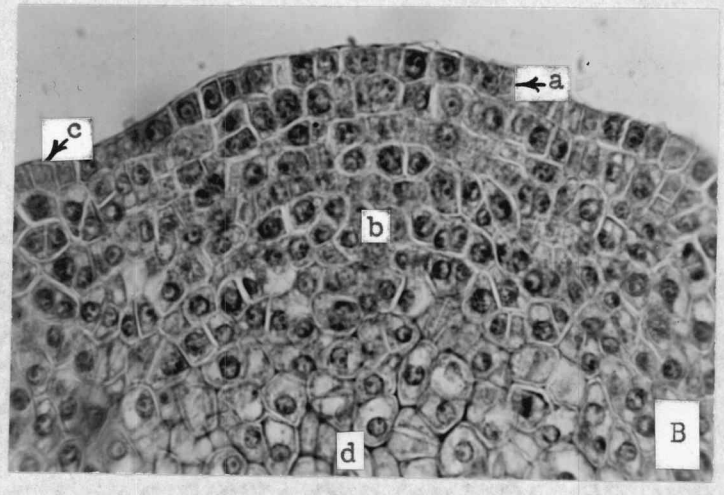

TUNICA
CORPUS
LEAF PRIMORDIUM
RIB MERISTEM

Plate 31. **A and B are median longitudinal sections** of the vegetative shoot apex of the hop plant. Note the appearance of the large bud primordia in the axils of the young leaves. In B, the single-layered tunica, a, encloses the massive corpus, b; c, leaf primordium; d, the upper portion of the rib meristem. C, transverse section of a portion of the young vegetative hop stem approximately 119 microns below the shoot apex, showing the appearance of the first provascular strand, a; b, rib meristem. (A, X100; B, X450; C, X300)


A


a
c
b
d
B

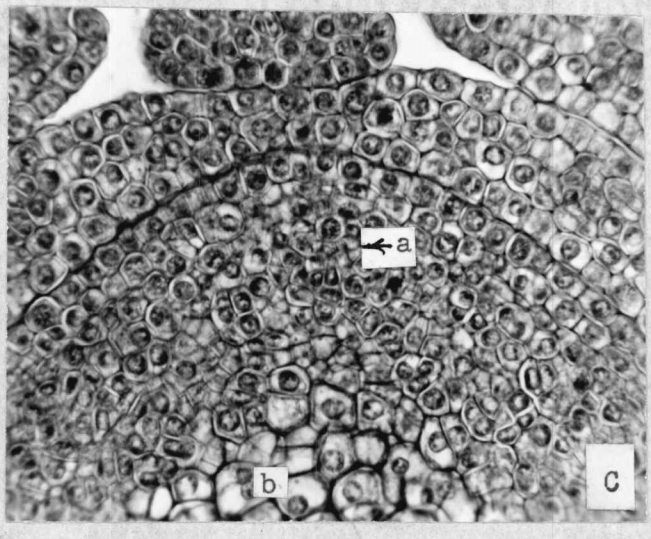
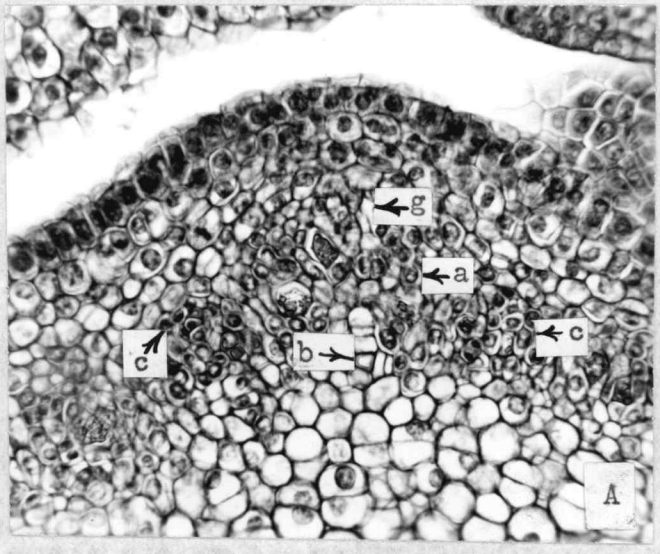

a
b
C

Plate 32. Transverse sections of the young vegetative hop shoot showing further differentiation of the provascular strands. In A, which is approximately 378 microns below the shoot apex, provascular strands are differentiating at a and c; the first protoxylem element is evident at b; protophloem sieve tube, g. B is approximately 567 microns below the shoot apex, and the vascular bundles are more fully differentiated; d, tannin cell; e, epidermis; f, cortex; g, sieve tube; h, pith; i, secretory duct. (Both X300)


g
a
c
c
b
A

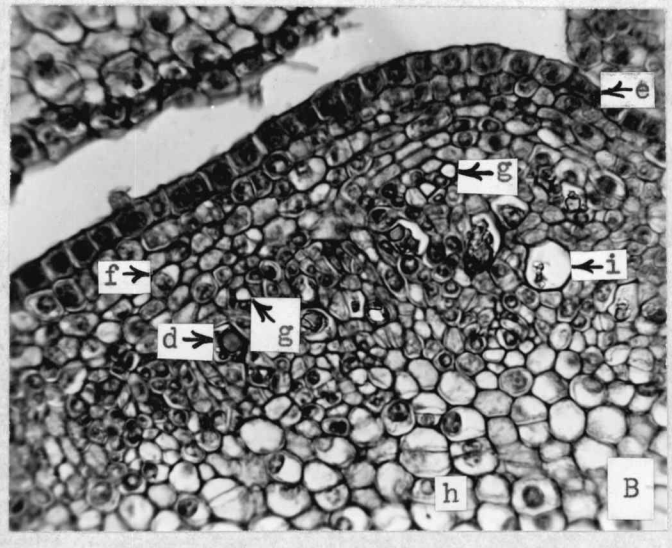
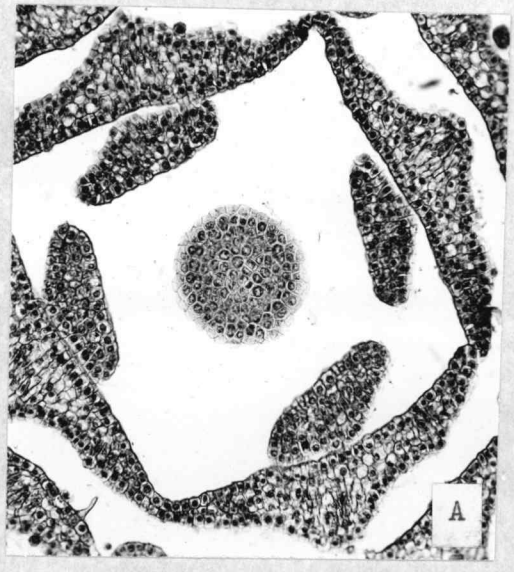

e
g
f
i
d
g
h
B

Plate 33. Transverse sections immediately below the shoot apex showing the appearance of the very young stem before the initial stages of vascularization takes place. A, 14 microns, and B, 56 microns, respectively, below the shoot apex. (Both X200)


A

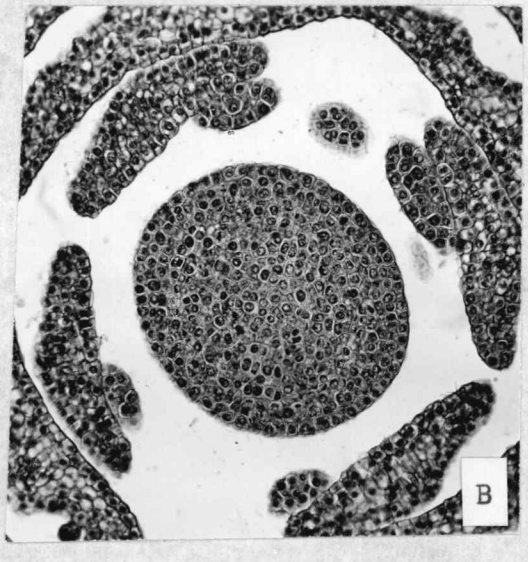
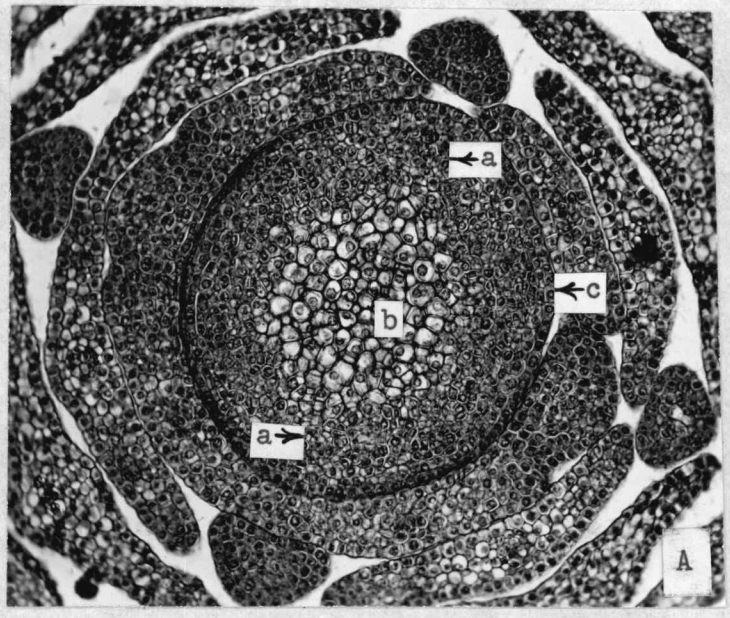

B

Plate 34. A, transverse section approximately 133 microns below the shoot apex. The provascular strands are differentiating at a; note the appearance of the highly vacuolated cells of the rib meristem, b; c, epidermis. In B, approximately 200 microns below the shoot apex, other provascular strands have made their appearance, a; the young pith is evident at b; the cortex has become distinctly differentiated c; d, epidermis. (Both X200)


a
b
c
a
A

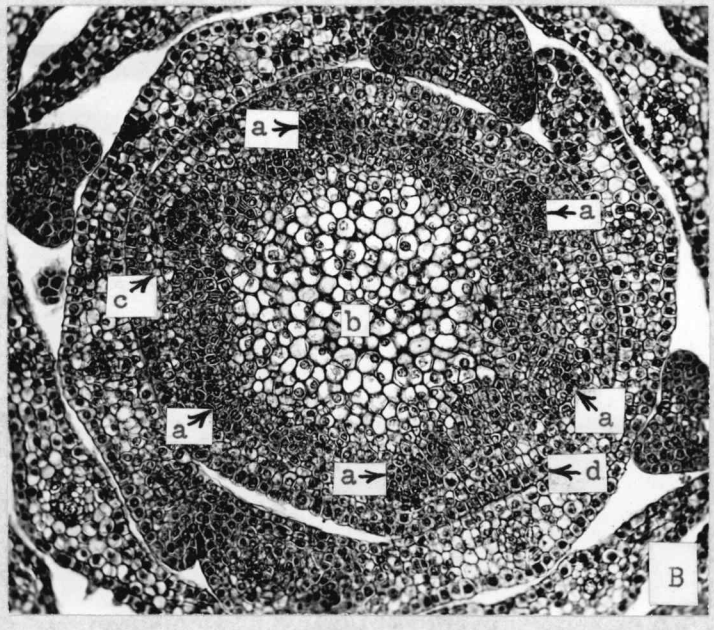
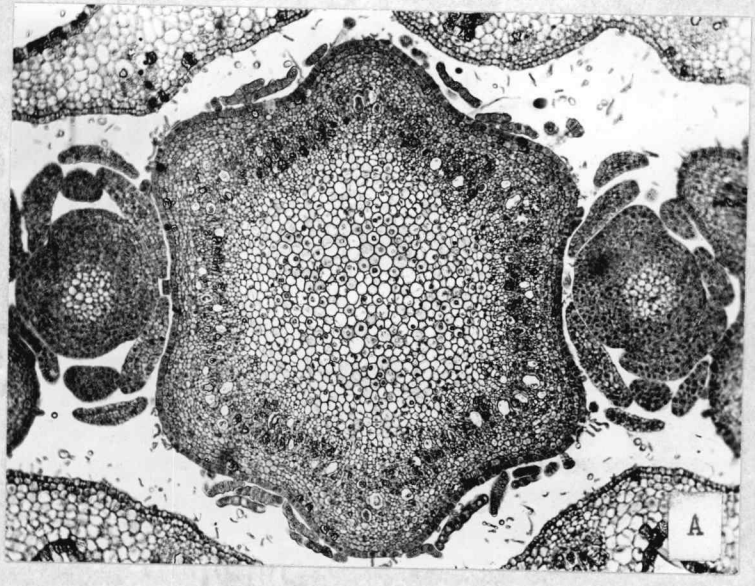

a
a
c
b
a
a
a
d
B

Plate 35. A, transverse section approximately 1071 microns below the shoot apex of the hop plant. The vascular tissue still appears in more or less discrete bundles which now possess fascicular cambia. Note the appearance of the knob-like and peltate trichomes on the periphery of the young stem. In B, the transverse section shows an older portion of the young hop stem. Note the pronounced ridged appearance of the stem at this stage of its development. The starch sheath, a, appears as a white line following roughly the contour of the stem. (A, X100; B, X72)


A

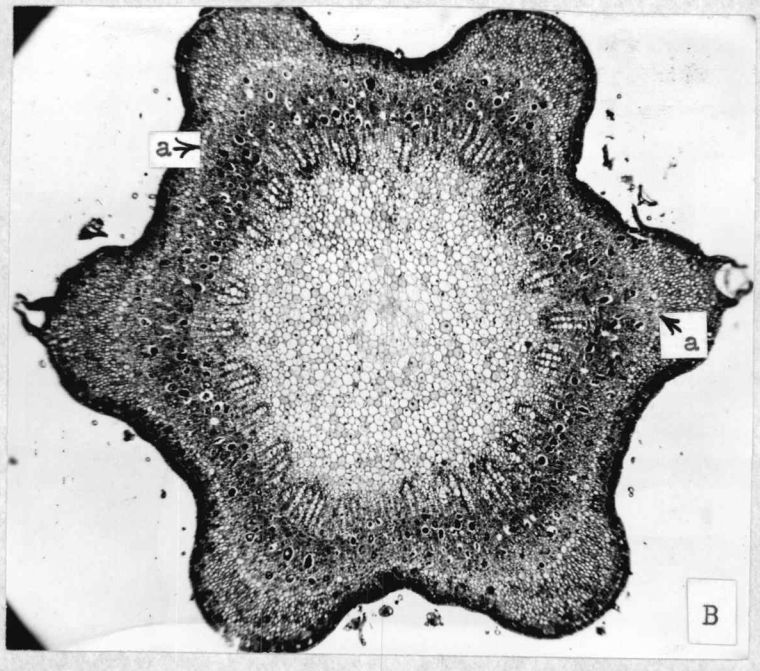
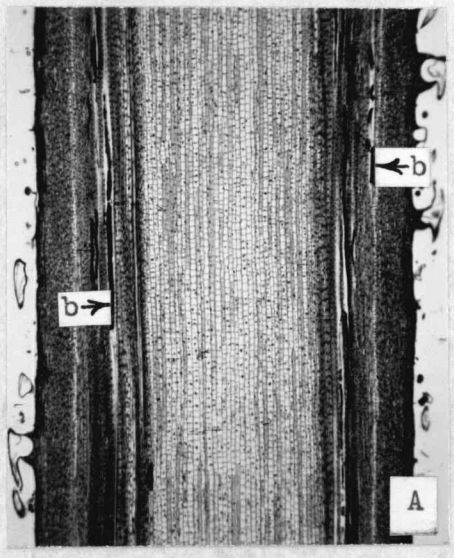

a
a
B

Plate 36. Longitudinal sections of young hop stems. A is a section comparable to that of Plate 35, B. The pith has not as yet become torn and appears as longitudinal files of thin-walled parenchyma cells. Secretory ducts containing resinous and tanniferous substances are present in the primary and secondary phloem tissue, b. Note the appearance of the anvil-shaped emergences on the external surface of the stem. B is a somewhat older portion of a young stem and shows the occurrence of tannin cells in the pith, a; b, secretory ducts in the phloem tissue. Note the absence of epidermal appendages. (Both X30.5)


b
b
A

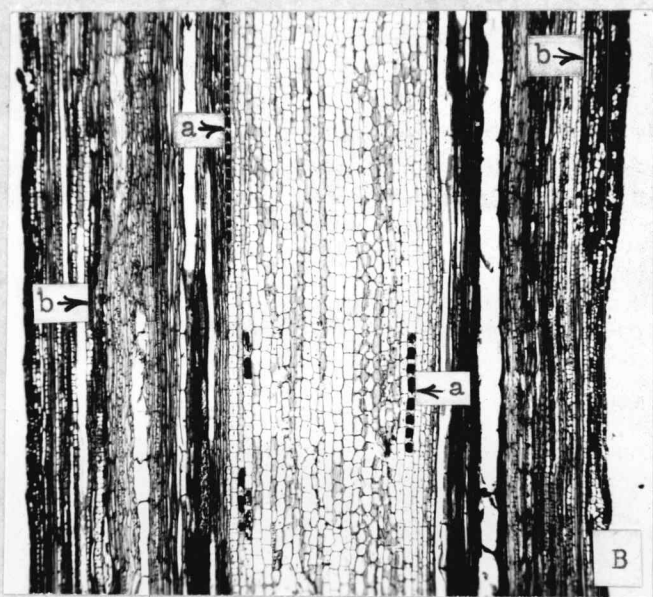
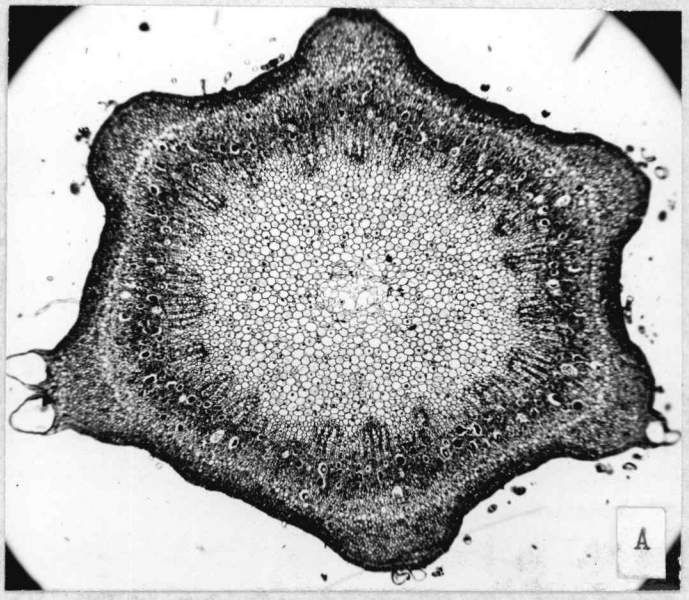
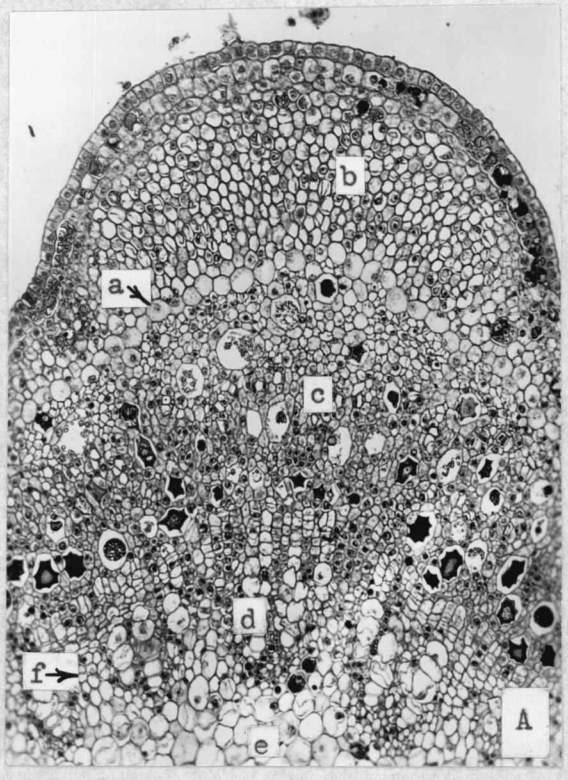

a
b
b
a
B

Plate 37. A, transverse section of a still older region of the young hop stem than that shown in Plate 35,B. The vascular cambium has now become more or less distinct. Note the beginning of pith disintegration in the central part of the vascular cylinder. The large hollow-appearing structures on the lower left of the stem are remnants of the anvil-shaped emergences; see B. B, a radial longitudinal section of the young stem slightly lower than that of A. Note that the pith has now broken down more or less completely to form a hollow center. (A, X52.5; B, X30.5)


A


B

Plate 38. Transverse sections of a young hop stem showing one of the ridges in detail. In A, the starch sheath, a, is the innermost boundary of the cortex; b, collenchyma strand; c, primary phloem; d, primary xylem; e, pith; f, pith ray. B is an enlargement of a portion of A. Note the large secretory duct surrounded by epithelial cells in the phloem, c; starch sheath, a; collenchyma, b. (A, X100; B, X450)


b
a
c
d
f
e
A

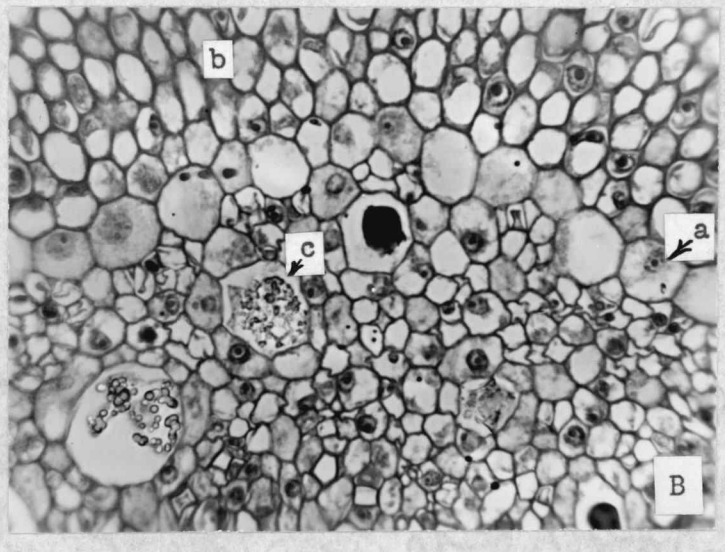
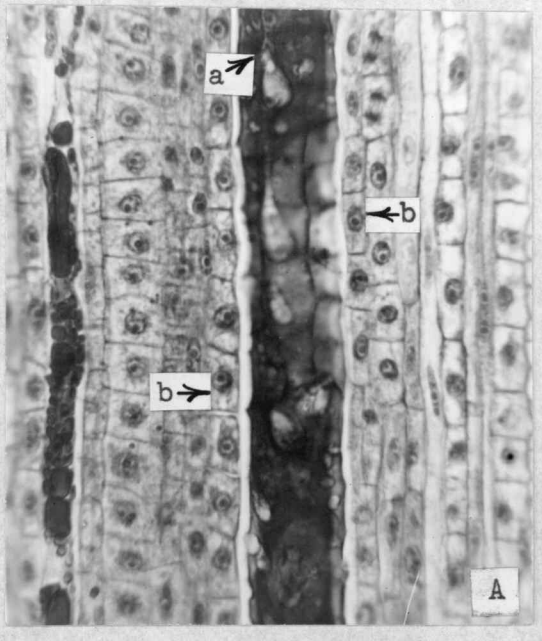

b
c
a
B

Plate 39. A, longitudinal section through a portion of a young hop stem showing the appearance of the large secretory duct, a, containing resinous substances, surrounded by the parenchymatous epithelial cells, b. B is a transverse section of a portion of the young hop stem similar to that shown in A. a, secretory ducts; b, epithelial cells. (Both X450)


a
b
b
A

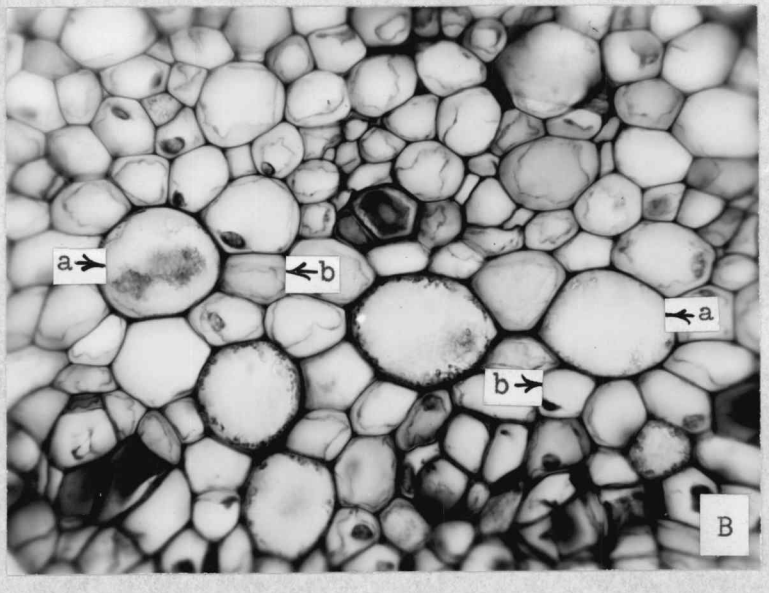
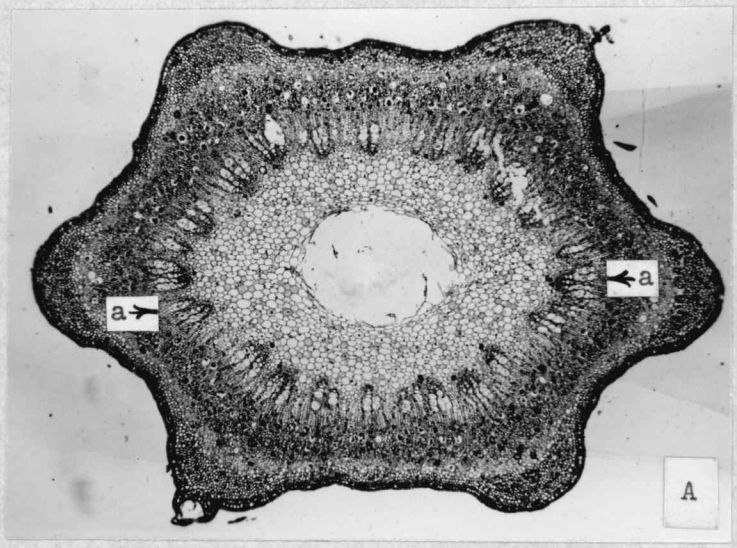

a
b
a
b
B

Plate 40. A, transverse section of an older portion of the young stem showing its appearance at the beginning of secondary vascular tissue development. Note the fluted appearance and that the stem has become more hollow with the further disintegration of pith cells. Thick-walled prosenchyma cells are being differentiated external to the primary xylem, a. B, tangential longi-section through a part of the secondary xylem of a slightly older portion of stem than A. a, vessels; b, xylem parenchyma; c, xylem fibers. (A, X30.5; B, X100)


a
a
A

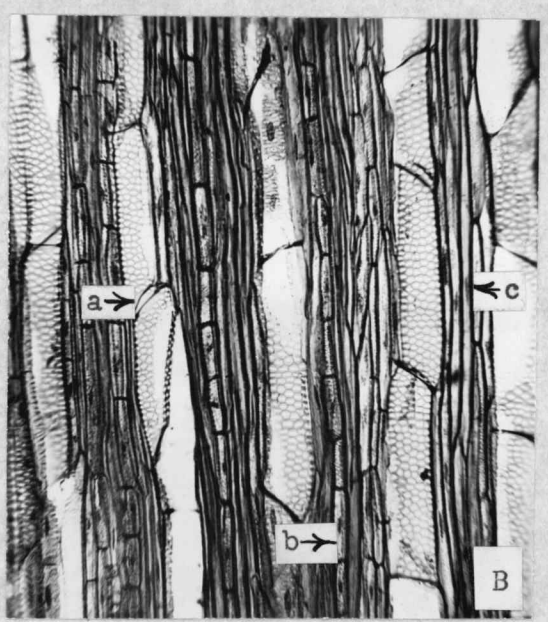
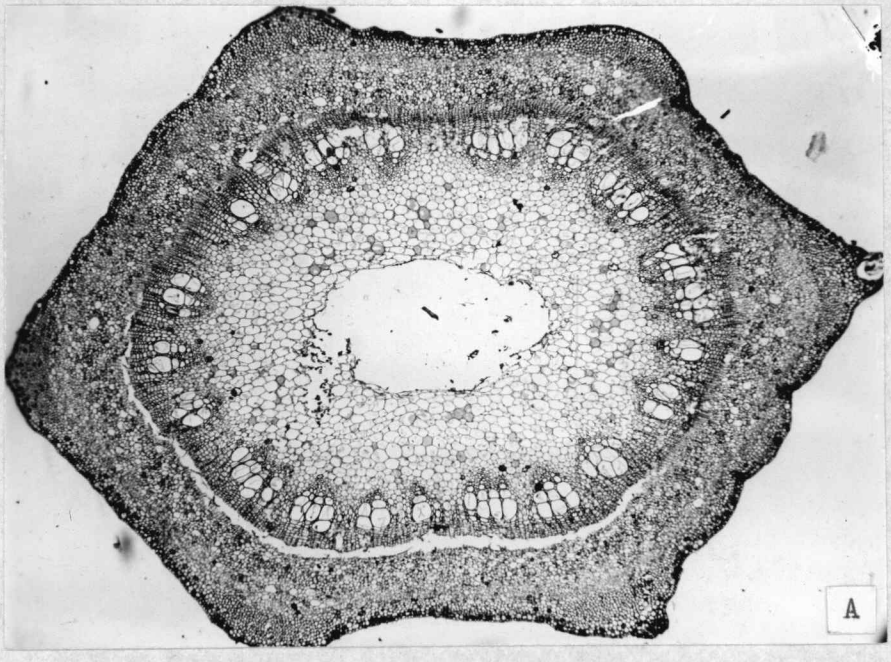

a
c
b
B

Plate 41. Transverse sections of older portions of the hop vine. Note how the fluted architecture has given way to the angular appearance in A, and even less angularity in B. In B, the collenchyma tissue has become flattened and is no longer prominent. Remnants of "climbing hairs" are present on some of the remaining ridges in both A and B. (Both X30.5)


A

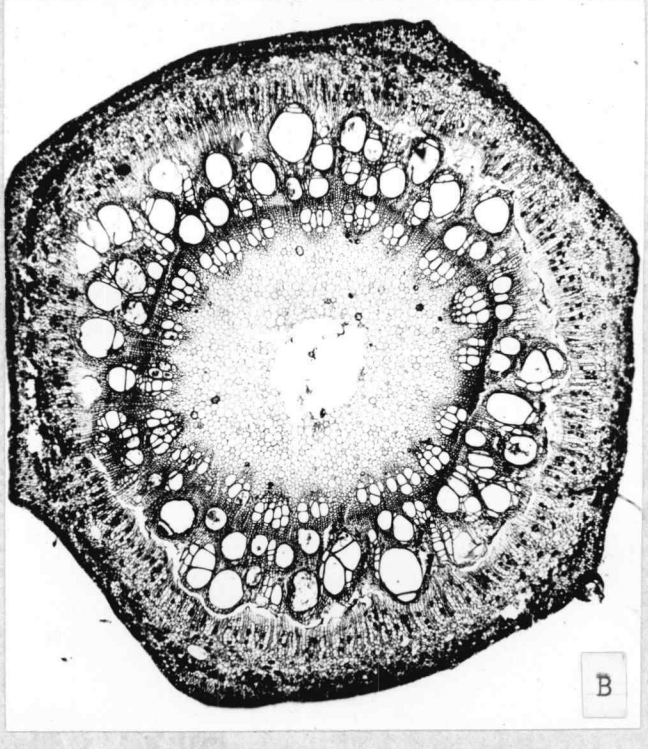
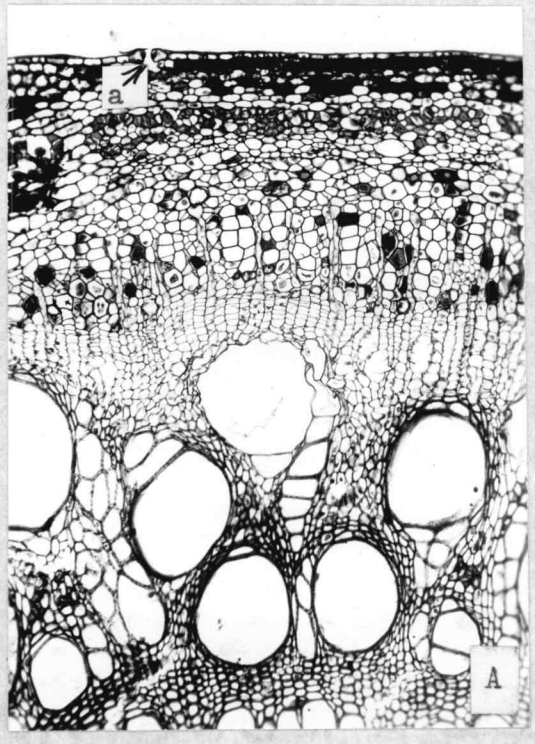

B

Plate 42. Radial longitudinal sections of a nearly mature hop stem. A, subepidermal cells, a; cortex, b; primary phloem fibers, c; secondary phloem, d, note the slime plugs on the sieve plates; vascular cambium, e. B, secondary xylem, f; xylem prosenchyma, g; primary xylem, h; pith, i. (Both X100)

Plate 43. A, transverse section of a nearly mature aerial stem in the sixteenth internodal region below the shoot apex. Note the stoma with two guard cells at the left of the epidermal surface, a. B, a nearly mature hop stem in transverse section. Note the absence of epidermal appendages and the large hollow pith region. (A, X100; B, X10)


a
A

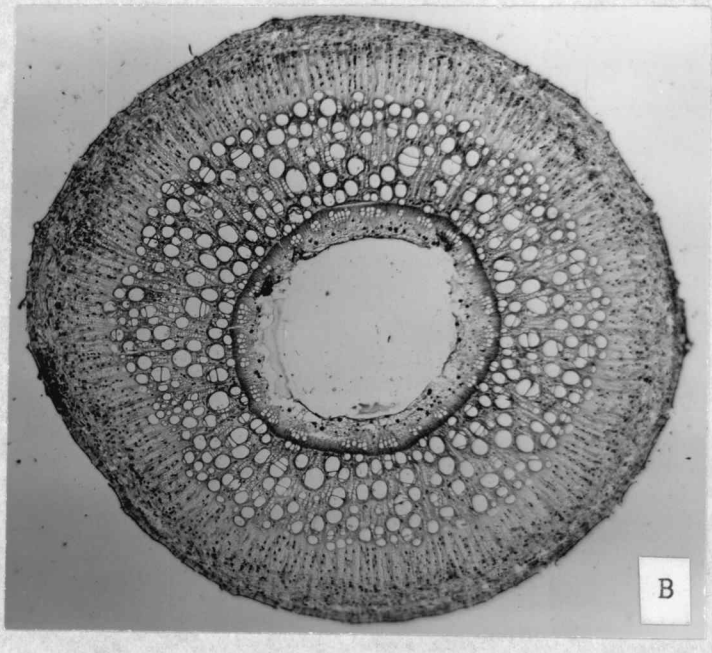
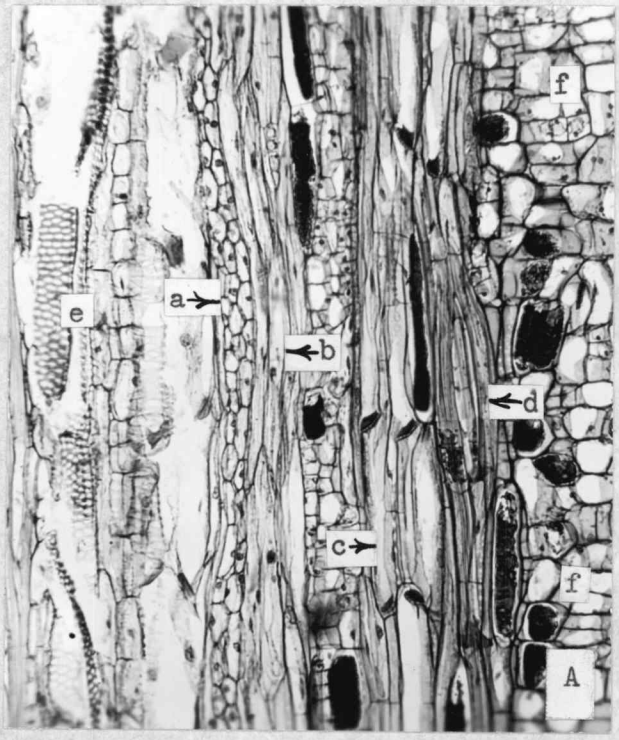

B

Plate 44. A, tangential longitudinal section of a portion of a mature stem. a, ray initials; b, fusiform initials; c, sieve-tube element of the secondary phloem; d, secondary phloem fibers; e, secondary xylem; f, phloem parenchyma. B, transverse section of a mature hop stem similar to that shown in A. Note that the secondary xylem comprises the larger part of the tissues of the stem at this stage of its development. Note also the large hollow pith region. The area outlined indicates the portion of the stem that is enlarged in Plate 45. (A, X160; B, X10)


f
e
a
b
d
c
f
A

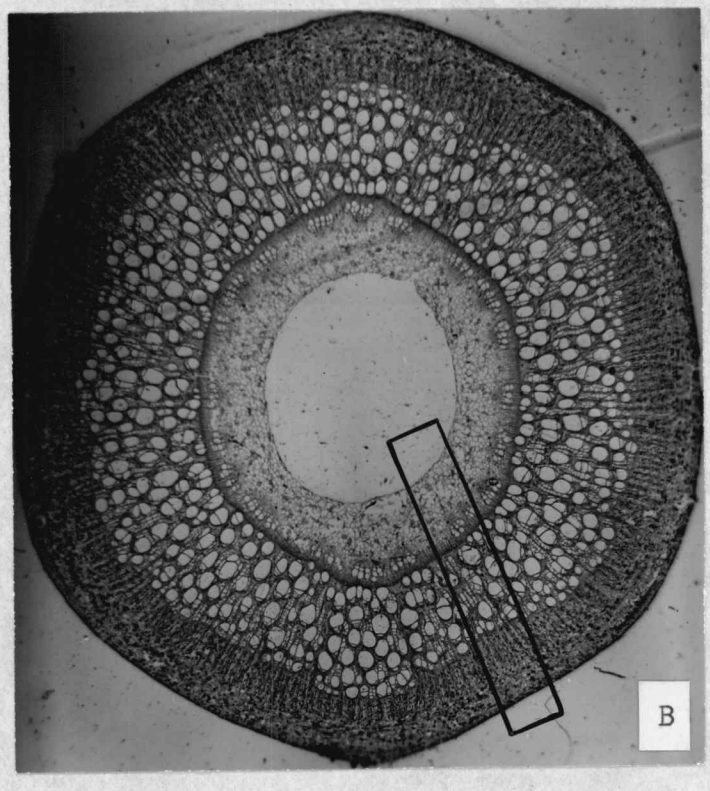

B

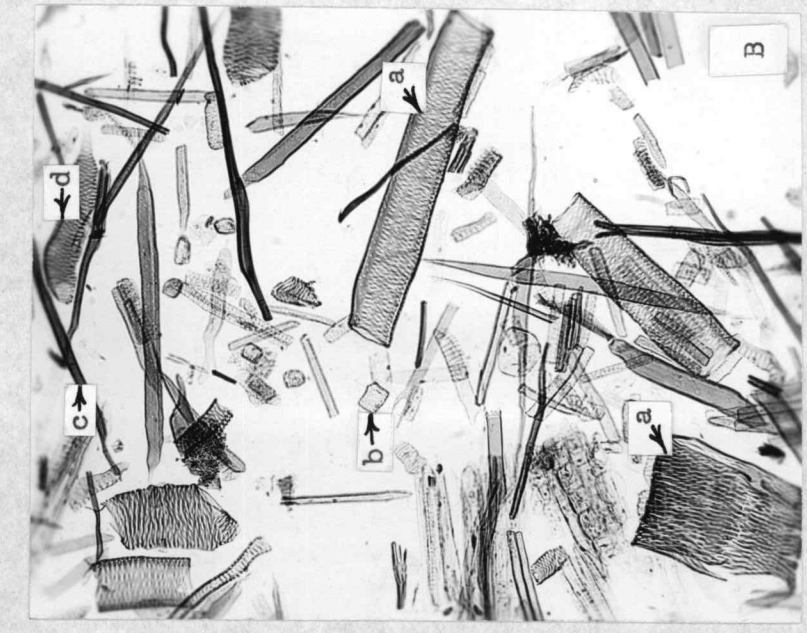
Plate 45. Transverse sections of a mature hop stem showing the various tissues in greater detail. These are enlarged sections taken from an area as outlined in Plate 44,B. In A, a, epidermis; b, subepidermis; c, cortex; d, primary phloem fibers; e, primary and secondary phloem parenchyma; f, tannin cell; g, secondary phloem fibers; h, phloem ray; i, sieve tubes of the secondary phloem; j, cambium region; k, xylem ray. In B, l, secondary xylem vessels; m, thick-walled xylem parenchyma; n, secondary xylem fibers; o, prosenchyma. In C, p, metaxylem vessels; q, protoxylem; r, pith ray; s, pith. (X75)

a
b
c
d
e
f
g
h
i
j
k
A

l
m
n
o
B

p
q
r
s
C

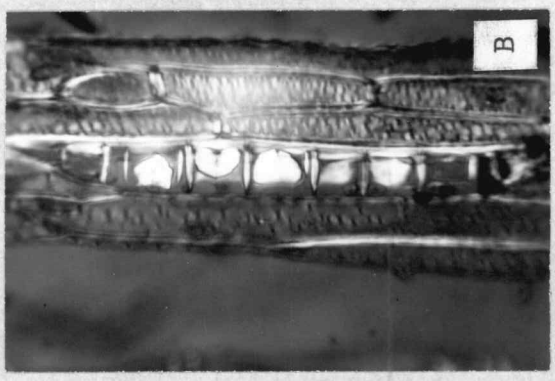
Plate 46. A and B contain disassociated cells from the aerial region of a mature hop vine showing various elements. a, vessels of the secondary xylem; b, thick-walled xylem parenchyma cells; c, secondary xylem fibers; d, tracheids. See Plate 45. (X100)


B
a
d
c
b
a


A
d
b
c
a
b
a

Plate 47. Tangential sections of secondary xylem parenchyma cells containing large prismatic crystals. Note the size of the individual crystal in comparison to the cell nucleus which appears as a small dark structure. In A and B thick-walled xylem parenchyma with pits appear to the right of the crystal-containing cells. A and B are the same cells, but the latter was photographed with polarized light. (All X540)


C


B

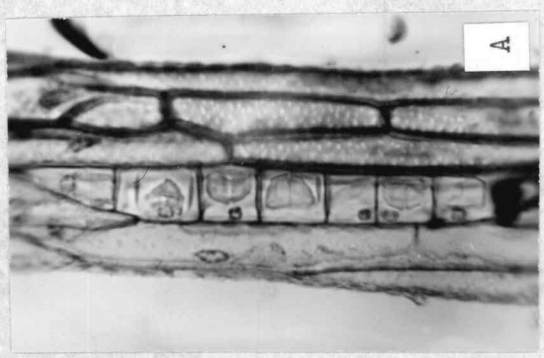
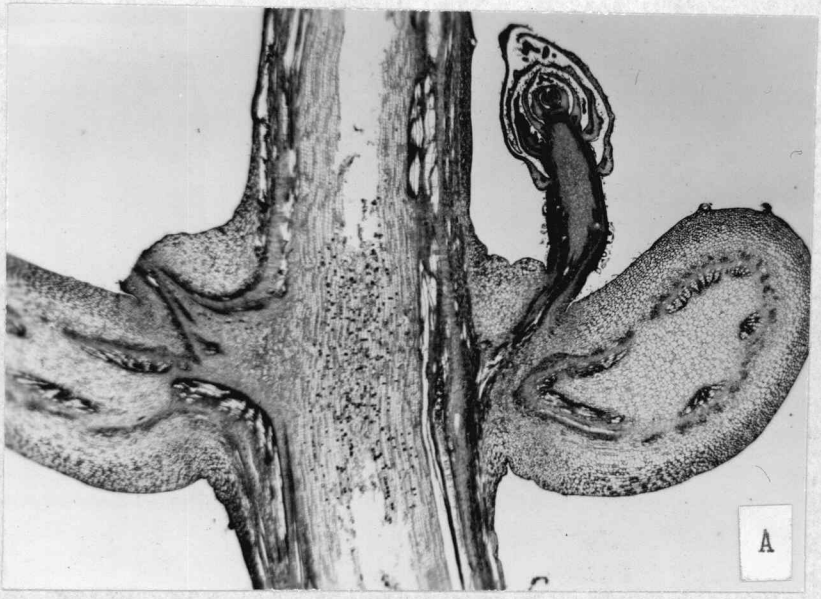
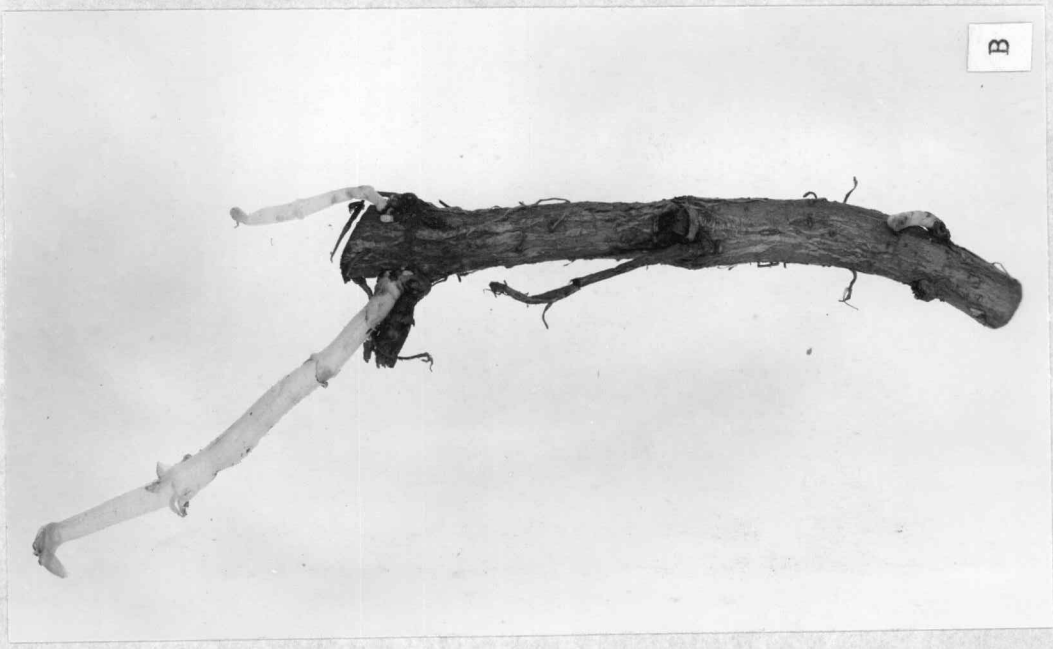

A

Plate 48. A, longitudinal section and B, transverse section through a nodal region of a young hop stem. Note the retention of the pith cells in their entirety at the node. The axillary structure in A is a developing bud. Lateral branch traces are shown in B, a. (A, X10; B, X20)


A


a
a
B

Plate 49. "Seed" pieces or cuttings taken from underground portions of the hop stem for transplantation. Note the lenticular structures on their external surfaces. Note also the hooked-shaped etiolated shoots in B which are derived from lateral buds similar to the ones shown in A. In A the cutting appears as planted. B shows the appearance of a cutting approximately 3 weeks after planting. (Both X1)


B

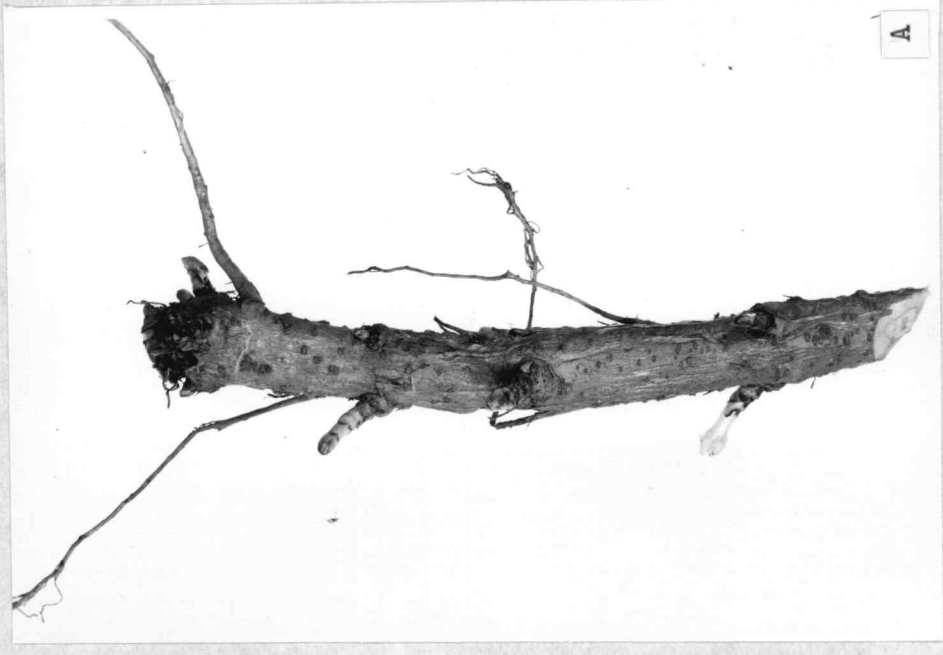

A

Plate 50. Hop cuttings showing advanced stages of growth during the early part of the growing season. These are one-year old cuttings of the variety Early Clusters on the right and Late Clusters on the left. (X1)

Plate 51. A, longitudinal section of a lateral bud taken from a hop cutting similar to the one shown in Plate 49, A. The vegetative apical meristem is similar to that shown in Plate 31, A. a, provascular tissue; b, pith; c, secretory cells. B, transverse section of a bud similar to A, taken approximately 950 microns below the apex and shows the overall architecture of the bud at this level of development. a, pith; b, primary xylem; c, primary phloem; d, axillary bud. Note the abundant resin and tannin cells in both A and B. (Both X20)

c
a
c
b
c
A

b
d
a
c
B

Plate 52. Cuttings made from young greenhouse-cultured hop plants. The cuttings were grown in tap water in an attempt to develop roots. This is not ordinarily done by commercial hop growers; instead, hop cuttings are usually obtained from the rhizomes of the parent plant. Successive stages in adventitious root formation are shown from right to left. (X3/4)

Plate 53. Adventitious roots arising from the internodal regions of the mature hop vines (Late Clusters variety). The vines had been layered with soil in the hop yard while still attached to the parent in the attempt to propagate vegetatively aside from the usual cutting methods. The leaves on this particular plant had been completely stripped. When the leaves were allowed to remain on the layered vines very few adventitious roots formed. A period of six weeks elapsed prior to final examination with the shown results. (X3/4)

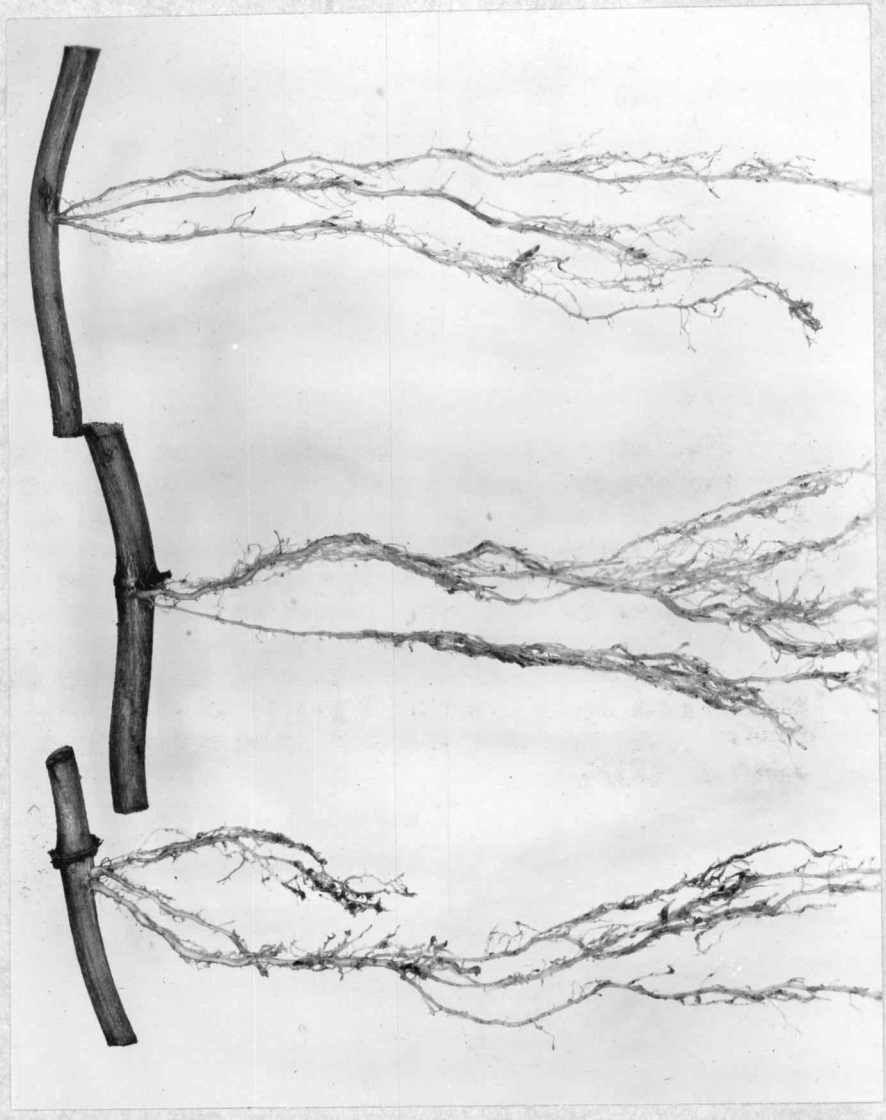
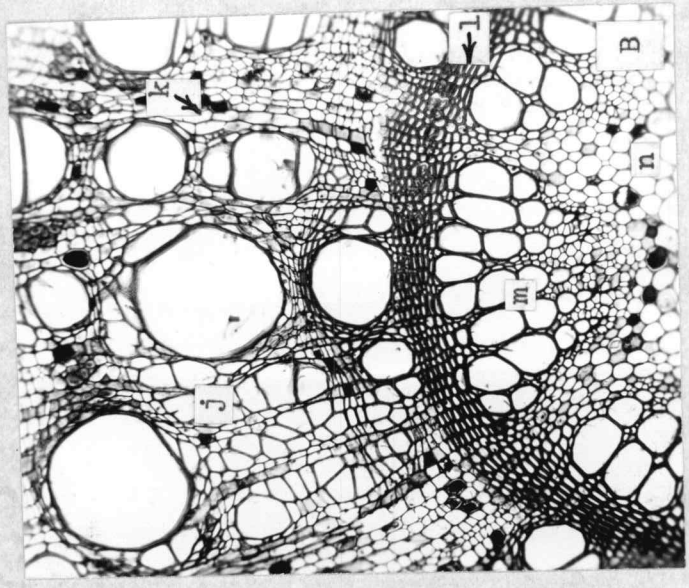


Plate 54. A and B are transverse sections taken from a layered vine as shown in Plate 53. Note the development of a cork cambium from the subepidermal cells, a; cortical parenchyma, b; flattened collenchyma, c; primary phloem fibers, d; phloem parenchyma; e, tannin cells, f; sieve tubes and companion cells, g; phloem ray, h; cambial region, i; secondary xylem, j; xylem ray, k; prosenchyma, l; primary xylem, m; pith, n. (X100)


B
l
i
n
m
j

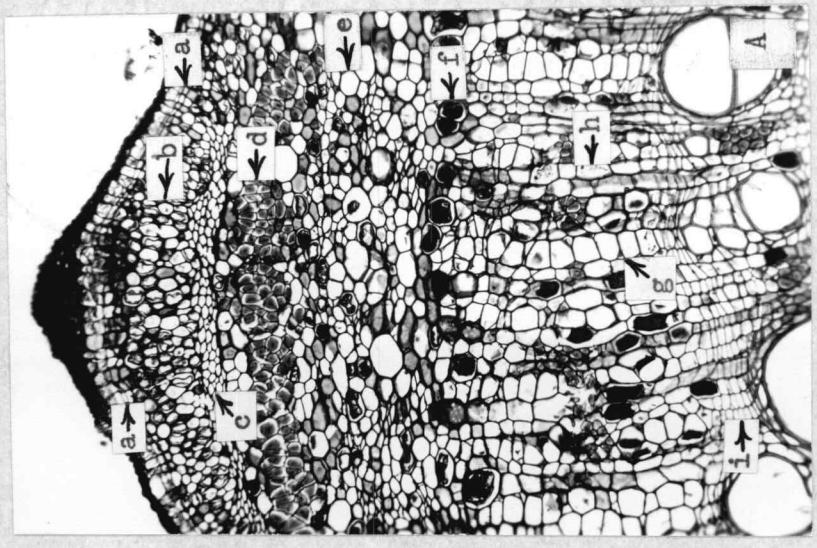
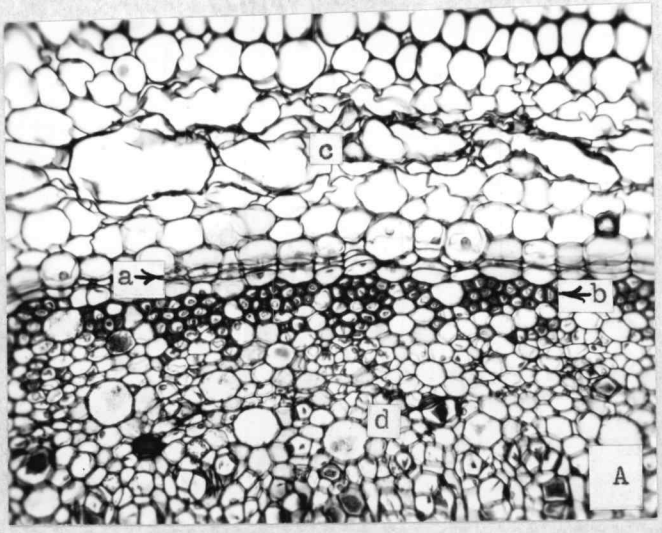

A
a
b
d
e
f
h
c
a
i

Plate 55. A, transverse section of a portion of a young hop vine growing below the surface of the soil while still attached to the parent plant. A cork cambium, a, is developed from the innermost parenchyma cells of the cortex, c. The tissues external to this cambium are rupturing and will eventually be sloughed. b, primary phloem fibers; d, primary and secondary phloem. B, transverse section of a small fleshy storage root showing the sloughing of the primary phloem tissue. a, new cork cambium; b, primary phloem tissue; c, old cork cambium; d, secondary phloem. (Both X160)


c
a
b
d
A

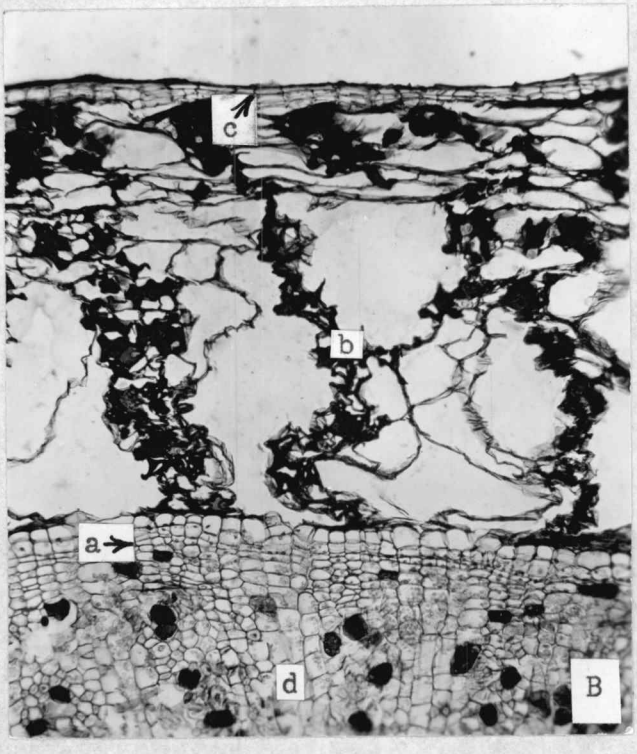
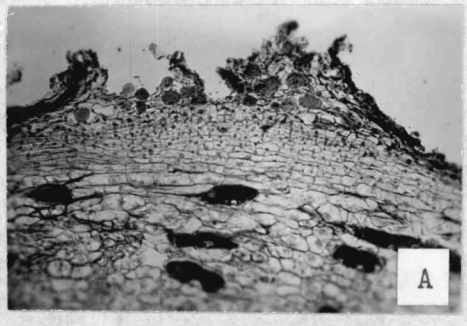

c
b
a
d
B

Plate 56. A, transverse section of a portion of a fleshy storage root showing the appearance of a lenticel on its external surface. B, transverse section of a portion of a hop cutting showing the structure of a lenticel in detail. a, complementary cells; b, cork cambium; c, cortex. (Both X100)


A

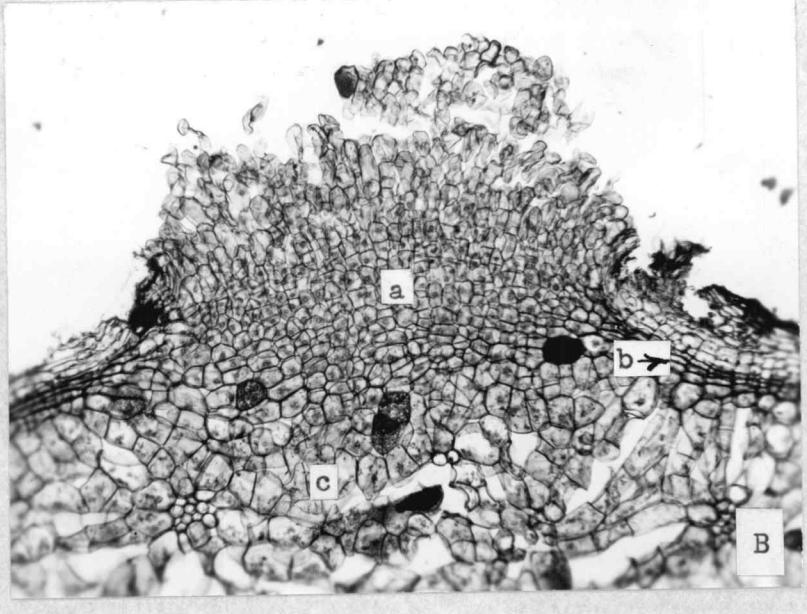

a
b
c
B

Plate 57. A Late Clusters variety of a two-year old hop plant at the beginning of the third growing season. Note the massive corky adventitious roots in the lower foreground. The upper region or crown of the cutting has become somewhat furrowed and enclosed with a large amount of cork. (X3/4)

Plate 58. A Fuggles variety of a two-year old hop plant at the beginning of its third growing season. Note the extensive adventitious root system of massive fleshy storage roots and smaller fibrous absorbing roots. (X1/2)

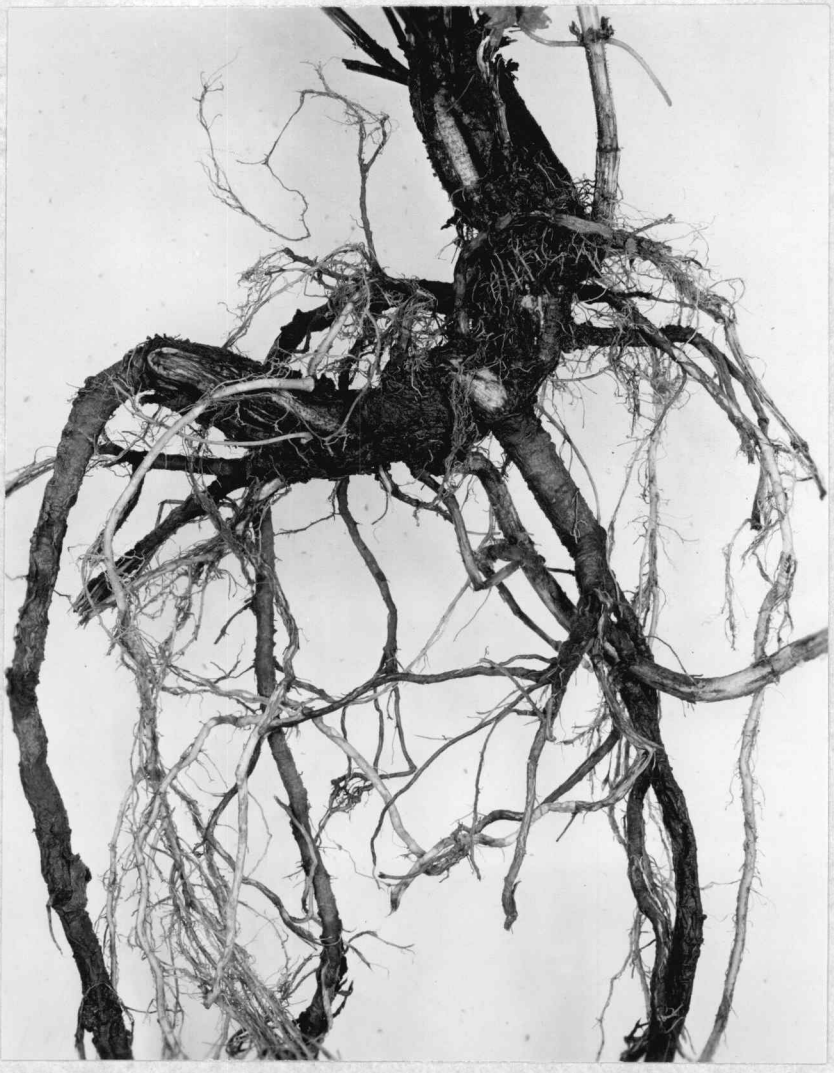
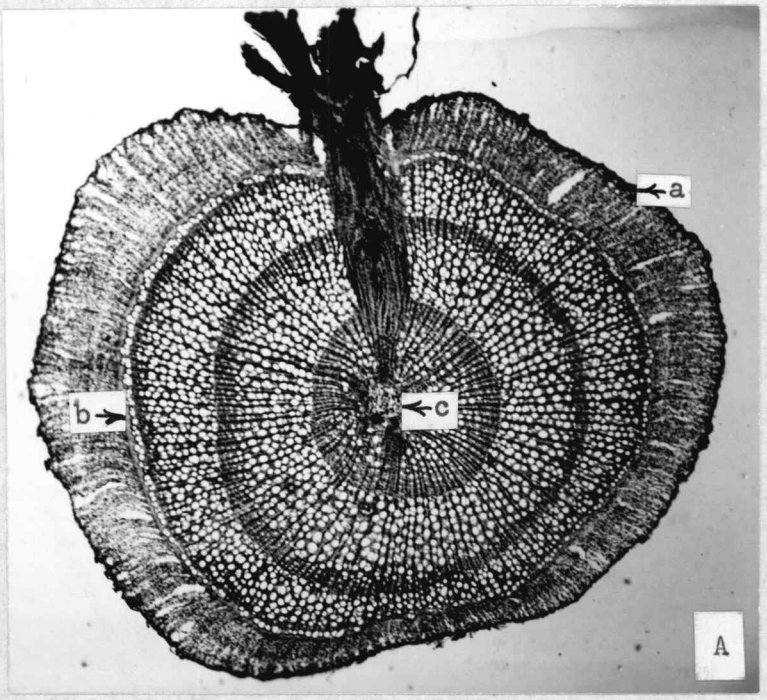
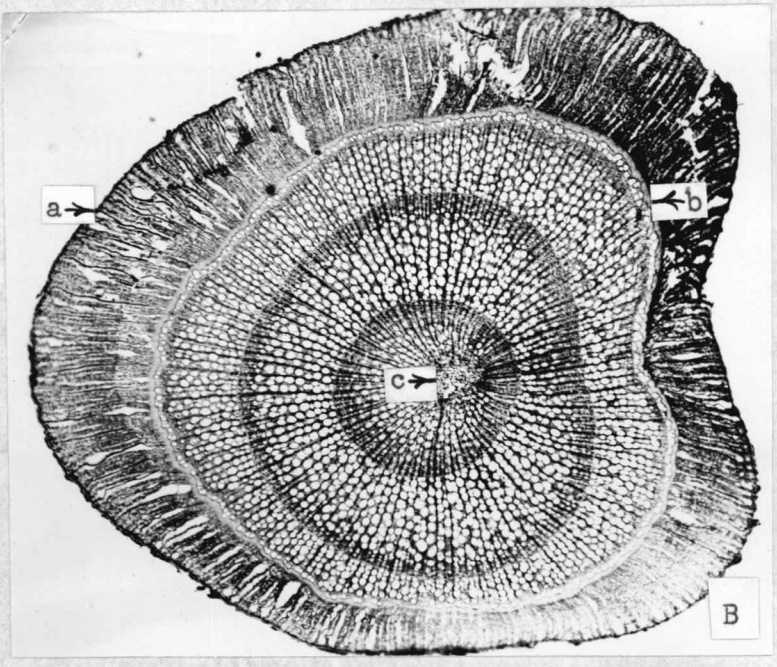


Plate 59. Transverse sections of three-year old hop cuttings at the beginning of their fourth growing season. Note the annual growth rings. The tissue enclosing the massive xylem is secondary phloem. Dead cork is seen on the periphery of the sections, a; cambial zone, b; pith, c. A remnant of an adventitious root is seen in A. (Both X4)


a
b
c
A


a
b
c
B

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