SECONDARY THICKENING IN THE STEM AND ROOT OF *STELLERA CHAMAEJASMAE* LINN.

BY A. C. JOSHI.
(From the Department of Botany, Benares Hindu University.)

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Introduction.

DUE to the investigations of a number of workers, but chiefly Van Tieghem[8] and Supprian[7], the anatomy of the vegetative parts of the family Thymelæaceæ, to which group *Stellera Chamæjasmæ* L. belongs, at least from the systematic standpoint, is much better known than that of many other families of flowering plants. These two authors between themselves have covered the anatomy of almost all the genera of the family and Van Tieghem has even prepared a key to distinguish the various genera (about 45) on the basis of anatomical characters alone. Further a number of anomalies have been described in the axis of the various members of the family, including intra-xylary phlœm in the stem of all the genera except *Drapetes*[6], inter-xylary phlœm in the genera *Linostoma*, *Lophostoma*, *Synaptolepis*, *Aquilaria*, *Gyrinops* and *Gyrinopsis*[6], and liane-structure in the stem of *Daphne Julia*[4]. In spite of such knowledge, a rough study of the anatomy of *Stellera Chamæjasmæ* L. revealed a type of secondary growth in the stem and the root as yet unrecorded in the family and also not exactly comparable with that described in any other vascular plant so far. A detailed investigation, therefore, of all the available material was undertaken.

The investigation is based partly on material preserved in formalin-acetic-alcohol and partly on dry herbarium material. All this material was collected from Dochen (Central Tibet ; height 14,700 ft.) by the late Professor S. R. Kashyap of the Punjab University during the month of August 1930. The formalin-acetic-alcohol material comprised a piece of main root about 1 inch long and 1¼ inch in diameter, another piece of root about ¾ inch in diameter and 1½ inch long, three pieces of secondary roots about ¼ inch in diameter and each about 2 inches long and a piece of leafy annual ærial shoot bearing a head of about 15 flowers. There were a few small rootlets coming out from the secondary roots and five were collected for study. There was no material of older underground stem in this collection, so that the secondary thickening in the stem was studied only from the dry material.

The habit of the plant has been figured and described by Kashyap[8] as follows : " The individual plants form tufts scattered at a distance from each other. Each has a thick woody underground stem which may be 2½ inches or even more in circumference and remains alive year after year. The stem gives rise to a number of thick woody roots below which go deep into the soil and similarly forms a few short thick branches above still under the soil, each of which produces a large cluster of erect green leafy aerial shoots, 8–10 inches high, during spring. These shoots die away at the end of summer, leaving their bases behind." The writer finds this description correct, except what has been described as the main thick underground stem, is seen from its anatomy to be the main primary root, and the lower roots are to be regarded as secondary roots.

Structure of the Stem.

(*a*) *Annual green stem.*—The annual green aerial stems show the primary structure of the organ and the early phases of secondary growth. The epidermis consists of somewhat elongated and nearly rectangular cells. These have well-developed cuticle and gelatinised internal walls. The long axis of the stomata is nearly parallel to the long axis of the stem. Their guard cells may be superficial, but usually these are more or less sunken below the general level of the epidermis to a varying extent, and sometimes there is even the differentiation of cells looking like subsidiary guard cells. The cortex is differentiated into an outer chlorenchymatous and an inner merely parenchymatous regions. There is no distinct endodermal layer, but the cortex is delimited from the stele by a broken or unbroken chain of pericyclic fibres. The vascular elements form a closed ring, and not separate bundles, though the protoxylem elements are in a number of separate groups. In addition to the external phlœm, there is a ring of internal phlœm patches around the pith. There are no hard bast fibres either in the internal phlœm or in the primary external phlœm. The pith is homogeneous and parenchymatous. There are no stone cells such as have been described in some other species of the genus.

(*b*) *Old underground stem.*—The old stems are characterised by an anomalous type of secondary growth in the central cylinder which results in the development of several zones of wood and bast separated by tangential parenchyma (Plate XXXVI, Fig. 1). The separation between the different vascular zones is very clear in the inner part of the stem, as there are several layers of parenchyma separating two adjacent zones ; but in the outer part of the stem, parenchyma is present only to the outside of the phlœm groups and at some places two adjacent zones of xylem are continuous (Plate XXXVI, Fig. 3, right-hand side), so that a transition is made to interxylary phlœm, which, as mentioned previously, has already been recorded in several other Thymelæaceæ. To the outside of all the vascular zones just beneath the periderm is found a large amount of phlœm, though mostly in a disorganised state. The exact method of development of this type of structure is not clear, as material was not available for such a study. It may be taking place through a succession of secondary cambiums, or may be the result of a periodic change in the activity of the same cambial ring. On account of the presence of a large amount of phlœm to the outside of all the vascular zones, the latter interpretation seems to be more likely. An unusual feature of secondary growth in this plant is that each of the different vascular rings begins on the inside with the larger xylem elements (especially vessels), and ends towards the outside with smaller xylem elements (Plate XXXVI, Fig. 3). The difference between the two is such as is found between spring and summer wood in plants growing in regions with well-marked winters. This makes it probable that each of the successive rings of xylem and phlœm is a result of one year's growth. This can be due to either each active cambial ring dying in the beginning of every winter period of rest and a new cambium developing in the next spring to the outside of the previous vascular zone, or due to the periodic change in the activity of the single cambial ring taking place about the end of each growing season just before winter.

The secondary growth is equal all round only in the central stems. In the peripheral ones, as is well exemplified by Fig. 1 (Plate XXXVI), it is stronger towards one (the outer) side. This is due to the shoots in *Stellera Chamæjasmæ* being very closely packed and lack of space for equal development on all sides.

Another fact of ecological interest shown by Figs. 1 and 2 (Plate XXXVI) is about the development of buds. One of these (marked *b* in Fig. 1) is seen to arise on an underground stem inside the cork of the main stem, and is itself seen to be surrounded by another layer of cork. The origin of the phellogen of this second cork layer is not clear, but it may have arisen by a proliferation around the bud of the phellogen of the main stem. The material from which Figs. 1 and 2 (Plate XXXVI) have been prepared was collected in the month of August. It was late in the growing season, and the aerial parts of the plants were expected to die shortly after this. This bud is consequently for the next year. Similar buds have been seen on the sectioned material of many other underground stems, and it may be concluded that in *Stellera Chamæjasmæ* probably buds for one year develop adventitiously at the end of the growing season of the previous year on the underground stems, and are well protected, owing firstly to their situation inside the cork of the mother stem, and secondly due to the possession of a separate cork sheath of their own.

Structure of the Root.

(*a*) *Primary structure and the primary cambium.*—The primary structure of the root was studied from the small rootlets coming out from some of the secondary roots, though these were already fairly advanced and some amount of secondary growth had taken place in them. A photograph of the central part of a transverse section of the youngest of these is reproduced in Fig. 4 (Plate XXXVI). The cambium and the phlœm are found to be crushed in all these rootlets, probably due to the underground parts of the plant having been pulled out of the hard soil while collecting the material. There is a strong development of the periderm on the outside. From the distribution of the xylem, the roots appear to be diarch. Although there is no large pith, the proto-xylem elements do not form a continuous plate. There are a few cells of parenchyma separating the two groups of them.

The first or the primary cambium both in its origin and behaviour appears to be perfectly normal. To the outside it forms phlœm all round; to the inside secondary xylem opposite to the primary metaxylem elements, and broad rays of parenchyma to the outside of the protoxylem elements. The structure of the primary metaxylem and the secondary xylem is quite similar, so that the two merge into each other. Both consist of vessels and only slightly thickened prosenchymatous elements.

In all the roots at this stage secondary increase in xylem is very unequal on the two sides. Further this feature is seen not only in the small rootlets, but also in the main roots. It is not possible to guess the exact cause of this development. It may be that the configuration of the soil has got to do something with it, but its occurrence in every root examined appears to be against such a supposition.

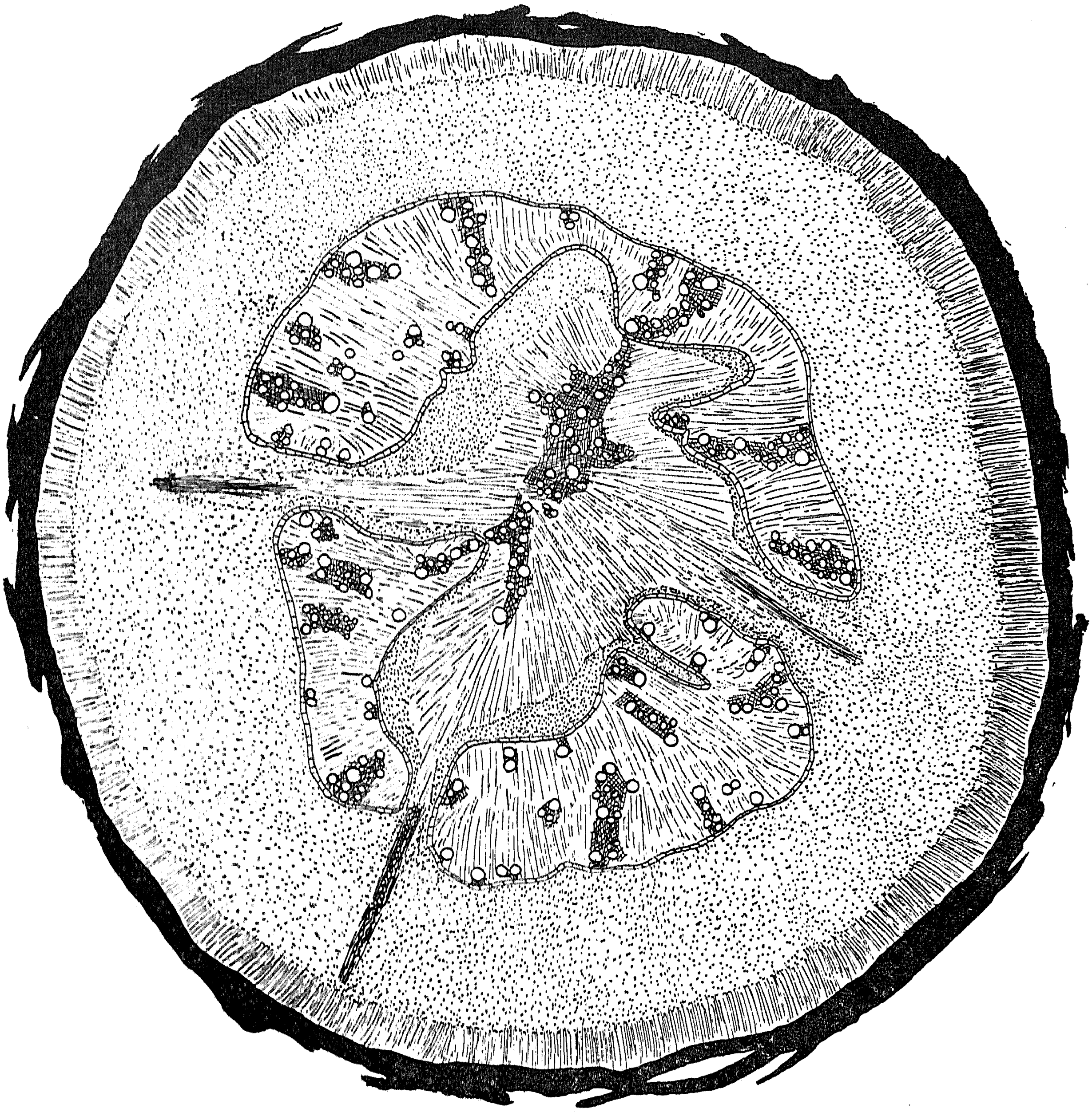
The next stage in the development of the root is shown by Fig. 5 (Plate XXXVII). This is a photograph of a transverse section made from a dry root. There is much greater difference here between the growth of the two sides than is shown even by Fig. 4 (Plate XXXVI). This figure further shows that the primary cambium after forming xylem on each side for some time in one patch begins to form it in the form of a number of plates (3–5) on either side and in the intervening areas gives rise only to parenchyma. Secondary rays thus develop, and they are almost as broad as the primary rays. The entire parenchyma of the rays is full of starch grains.

(*b*) *Secondary cambium and later growth.*—The next stage in the development of the root is the establishment of a second cambium to the inside of the primary cambium. Its origin is shown in Fig. 5 (Plate XXXVII) and it is seen in a fully established condition in Text-Fig. 1 and in Figs. 6 and 7 (Plate XXXVII). In Fig. 5 (Plate XXXVII), the places where the secondary cambium has been formed are marked *s.c.* In the rays it arises to the inside of the primary cambium from some of the parenchyma cells, and in the xylem bands by the transformation of some of the living cells of the xylem. At some places in the broad rays it is continuous with the primary cambium, and may be described to have arisen by an inward extension of the latter.

When fully formed the second cambium extends all round the circumference of the root. It forms another ring inside the ring of the primary cambium, though it is not so regular as the outer cambium. Its course is very tortuous and it shows a large amount of looping to the inside and outside. During its activity, it gives rise to xylem and storage parenchyma towards the outside and phlœm towards the inside (Text-Fig. 1, and Fig. 6, Plate XXXVII). Thus it is inversely orientated as compared with the normal primary cambium.

The stele of the root of *Stellera*, therefore, at this stage has the following structure. In the centre there is in the midst of storage parenchyma a small island of vascular tissue consisting of primary xylem and a part of secondary xylem elements formed from the primary cambium. On the outside it is surrounded by the two cambial rings, one outer primary cambium, normally orientated, and forming xylem and storage parenchyma to the inside and phlœm to the outside, and an internal secondary cambium of an irregular form and inversely orientated. The xylem elements of the two cambiums, where the internal cambium has been formed in the middle of a xylem band, are continuous; while at other places, where the internal cambium has taken its origin from storage parenchyma cells, the xylem formed from the two cambiums and the two cambiums themselves are separated by a broad band of parenchyma.

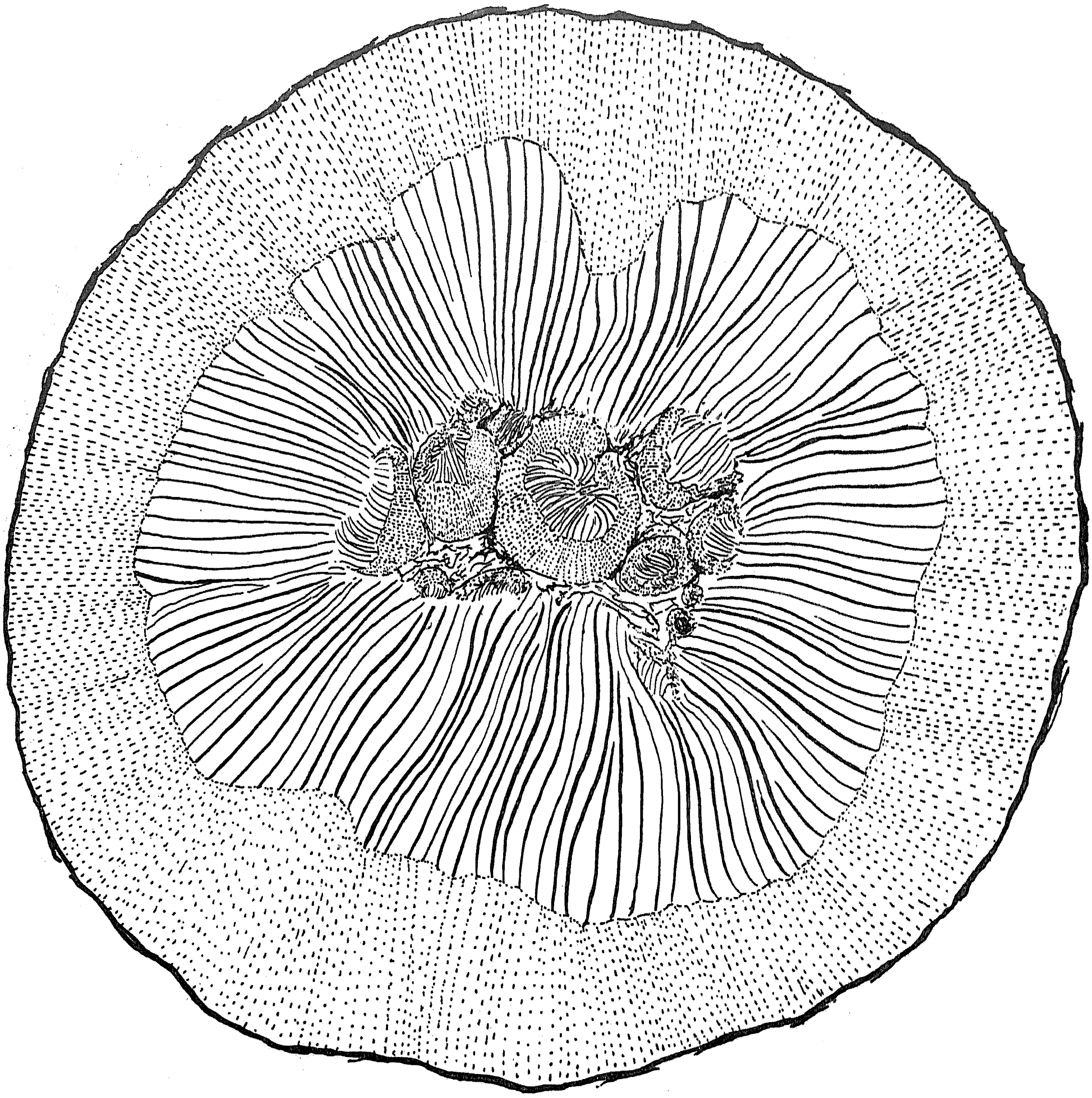
The internal secondary cambium may remain perfectly free from the outer primary cambial ring and may never show any connection with the latter, as is the case in Text-Fig. 2, which represents the cross-section of a root about $\frac{3}{4}$ inch in diameter. In other parts of the root the internal and the external cambiums are seen to be continuous with each other in the broad rays of parenchyma. This is the case in Text-Figs. 1 and 3, and is also shown by Fig. 7 (Plate XXXVII), which is a photograph of a part of Text-Fig. 1, showing a region where the two cambiums are continuous with each other. In a number of instances these spots have been seen to be related to the rootlet traces (Text-Fig. 1), but it is not possible to say if this is so in every case. When the internal and external cambiums are continuous at a number of



TEXT-FIG. 1. *Stellera Chamæjasmæ*. T.S. of a root about 6 mm. in diameter showing a fully established secondary cambium forming xylem and phlœm in an inverse manner, and its union with the primary cambium at three places where the rootlet traces are passing out. The cambium is shown as a line of cells, phlœm by dots, xylem vessels as circles, xylem prosenchyma by cross lines, and parenchyma by simple lines. × 22.

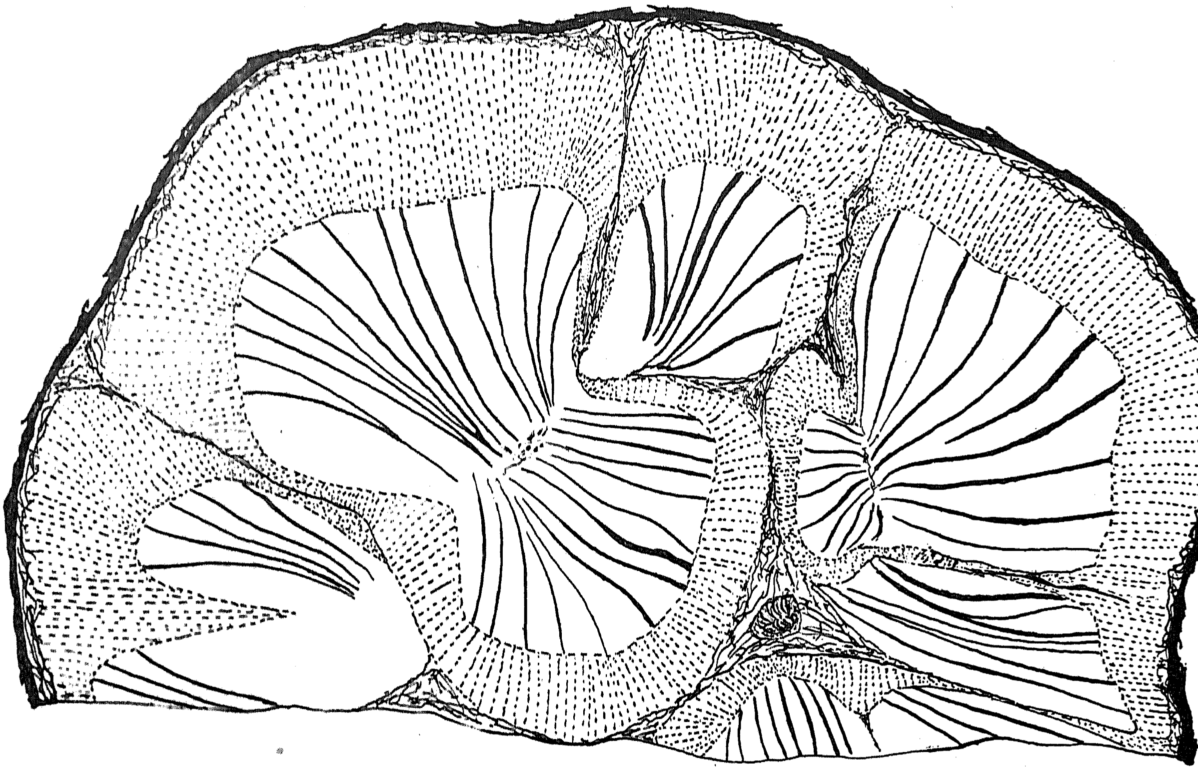
places, there may be described to be a number of separate circles of cambium at the periphery of the central cylinder, each forming xylem and storage parenchyma to the inside and phlœm to the outside.

Still older stages in the development of the stele of the root are shown in the Text-Figs. 2 and 3. When the external and the internal cambiums



TEXT–FIG. 2. *Stellera Chamæjasmæ.* T.S. of an older root than the one shown in Text-Fig. 1, about 18 mm. in diameter. The primary and the secondary cambiums in this case have been perfectly free from each other. The primary one has formed a large cylinder of wood and bast in the normal manner to the outside, while the secondary cambium at some places has formed xylem to the outside and phlœm to the inside, and at other places has formed by a process of looping a number of separate cylinders of wood and bast. Phlœm is represented on the figure by dots, and wood by lines. × 7½.

remain perfectly separate from each other, the former forms a large cylinder of wood and bast towards the outside, while the latter at some places forms simply xylem to the outside and phlœm to the inside, and at other places by looping and proliferation gives rise to a number of small separate steles



TEXT-FIG. 3. *Stellera Chamæjasmæ*. Part of a T.S. of a still older root than the one shown in Text-Fig. 2 about 29 mm. in thickness. The external and the internal cambiums in this case have been continuous at a number of places forming a number of cambial rings. Each of these has developed into a separate cylinder of wood and bast. Representation of the parts is in the same manner as in Text-Fig. 2. × 4½.

in the central part of the root (Text-Fig. 2). In parts of the root, where the internal and the external cambiums are continuous with each other at a number of places, each of the circles of cambium grows into a separate stele (Text-Fig. 3). Further by the looping of the internal cambium and the meeting of the two sides of a loop a number of small steles may be formed to the inside of the circle of large steles. The root may thus come to have a highly complicated type of apparently polystelic structure with a ring of large steles to the outside and a number of small steles in the central region. The growth of the outer steles is much greater towards the outside than on the inner side due apparently to the availability of space, but in some it may be almost equal all round. The phlœm is formed much more towards the outside and the xylem is several times greater on the outer side than on the inside. Even growth in each stele towards the outside is not equal, and it adds another complication to the configuration of the central cylinder as a whole. The xylem of these steles consists of radial bands of vessels and prosenchyma, alternating with large rays of storage parenchyma (Fig. 8,

Plate XXXVII). The phlœm is also similarly broken up by rays into a number of radial patches.

When the radial bands of xylem are studied more closely, it is found that they are not composed of xylem elements alone. At intervals there are patches of phlœm in all the bands at nearly the same distance from the centre of the stele (Fig. 8, Plate XXXVII). Thus inter-xylary phlœm is also present in the roots of *Stellera Chamæjasmæ*, but owing to the lack of necessary material it is not possible to say anything about its development, nor can one say definitely if its formation is related to the winter period of rest just as appears to be the case with the development of internal phlœm in the stem. The occurrence of phlœm in all the radial bands of xylem at the same level, however, is a point in favour of such an interpretation.

Discussion.

The main characteristic of the secondary thickening in the vascular cylinder of the stem is the development of a series of zones of wood and bast separated by tangential bands of parenchyma. Towards the periphery it leads to the formation of inter-xylary phlœm. The important features of root anatomy are the formation of a secondary cambium to the inside of the primary one by transformation of some of the living cells of the rays and the wood, the functioning of this cambium in a manner inverse to that of the outer primary cambium, the union of the two cambiums at a number of places in some parts of the root to form a number of cylinders of wood and bast, the formation in the central region of the root of a number of small cylinders of xylem and phlœm by the looping of the internal cambium, and the development of inter-xylary phlœm. The method of secondary thickening in the stem of *Stellera Chamæjasmæ* is also seen in the axis of many other families of angiosperms. Similarly, the method of secondary growth in the root in its broad outlines, particularly in the formation of an internal inversely orientated secondary cambium, is seen at least in the roots of one plant, but in no case both the features have been seen in the same species. The structure of *Stellera Chamæjasmæ* is thus noteworthy in that the process of secondary thickening in the stem and the root, while of an anomalous type in both, is so very different in the two organs.

An important feature of secondary thickening in the stem of *Stellera Chamæjasmæ* is the possible relation of the different vascular rings to the annual growth in the plant, though it needs evidence of seasonal connection to be definite about this point. Each xylem ring begins with larger elements and ends with smaller elements, from which it appears that it is related to one growing season. In other plants in which this mode of anomalous growth has been observed the different vascular rings have in no case been seen to bear any relation to the annual growth of the plant. In *Iresine paniculata* and *Alternanthera polygonioides*, for instance, according to Schmid[5], four rings of wood and bast are formed in a year and somewhat similar is the case in most other plants showing such anomalous construction.

The plant, to the root of which, the roots of *Stellera Chamæjasmæ* in their structure show the nearest approach is *Myrrhis odorata*, a member of the family Umbelliferæ. Long ago, it was shown in this plant by Trècul that after normal thickening in the roots had continued for some time, " a radially-seriate secondary meristem appears in the internal parenchyma of the wood at some distance from the middle. It begins at one point and extends through an annular zone which surrounds the root. This zone assumes the properties of an independent normal cambium which starting from its outerside bordering on the peripheral wood and proceeding inwards, that is in centrifugal succession forms strands of bast of normal structure, alternating with medullary rays, and subsequently on the side remote from the bast layer, that is facing outwards, it forms strands of wood which insert themselves exactly on the surrounding portions of the strands of wood, and increase in a centripetal, that is, a reversed direction."* The resemblance with the roots of *Stellera*, however, is only so far. The later history of the roots of *Myrrhis* is quite different. In them a tertiary cambium may appear in the same way as the secondary one between the primary and the secondary cambial rings, and may in its turn be succeeded by another cambium. A feature common to the roots of both the plants is that they store a large amount of starch, and as Haberlandt[2] has stated, it is not improbable that this type of anomaly may be related to this feature, as it in the first place tends to ensure the development of a suitable parenchymatous storage tissue, and secondly leads to the multiplication and appropriate arrangement of the conducting strands required for the deposition and removal of the reserve materials.

In the family Thymelæaceæ, the secondary growth in the roots of *Stellera Chamæjasmæ* may be compared to that seen in *Daphne Julia* by Lashevsky[4]. He has described in the underground stem of this plant a continued growth of the parenchyma of the pith, wood, and wood rays, which leads to the breaking up of the vascular cylinder into fan-shaped strands arranged in rosettes. He, however, does not mention the organisation of a definite inversely orientated internal cambium.

The list of plants belonging to the family Thymelæaceæ, in which inter-xylary phlœm has been found, has already been given in the introductory

* From a quotation in De Bary.[1]

portion of this paper. These all belong to the sub-family Aquilarioideæ. In the sub-family Thymelæoideæ, the wood is known to be without inter-xylary phlœm, and Supprian[7] has already proposed this as a distinguishing anatomical feature between the two groups. The present investigation by showing the presence of this character both in the wood of the root and the stem of *Stellera Chamæjasmæ* destroys this criterion of distinguishing the Thymelæoideæ from the Aquilarioideæ.

The formation of buds for the next year's growth at the end of the previous growing season inside the cork on the underground stems, and their protection besides this by a separate cork sheath of their own, are features of ecological interest and may be read as adaptations to the habitat in which the species grows.

Summary.

The paper describes the anatomy of the stem and root of *Stellera Chamæjasmæ*, a member of the family Thymelæaceæ.

The annual aerial green stems have a structure normal for the family, but the old stems show anomalous type of secondary growth which leads to the development of a series of zones of wood and bast separated by parenchyma. At some places, especially in the outer vascular rings, this leads to the formation of inter-xylary phlœm as well. A peculiar feature of anomalous secondary growth in the stem of *Stellera Chamæjasmæ* is that the wood of each of the vascular rings begins with larger vessels and ends with smaller vessels, showing the probability of each ring being formed in one growing season (one year). The annual green aerial shoots of one season are probably laid down as buds on the perennial underground stems at the end of the previous growing season. Besides being situated inside the cork of the mother stem, these are further protected by a separate cork sheath of their own.

The root is characterised by a different type of secondary growth as compared with the stem. In this case a secondary cambium is formed to the inside of the primary cambium from the living cells of the rays and the xylem. This functions in an inverse manner. It may remain perfectly free from the primary cambium, or in other parts of the root it may be connected with the primary cambium at a number of places, resulting in the formation of a number of cambial rings, and later on of separate vascular cylinders in the peripheral part of the root. The secondary cambium is very irregular in outline and by a process of looping gives rise to a number of small vascular cylinders in the centre of the root. Besides these anomalies, the root also shows the presence of inter-xylary phlœm.

This type of secondary growth in the stem and root together is not found in any other member of the family Thymelæaceæ, nor in any other vascular plant studied so far, though the type found in the stem itself is found in many other families of angiosperms, and the secondary growth in the root shows in its early stages points of similarity with the secondary growth in the roots of *Myrrhis odorata* in the differentiation of a secondary inversely orientated cambium.

The presence of inter-xylary phlœm in the root and to some extent in the stem of *Stellera Chamæjasmæ* breaks down a distinguishing character between the two tribes of the family Thymelæaceæ, Aquilarioideæ and Thymelæoideæ.

The writer takes this opportunity of expressing his cordial thanks to Professor J. H. Priestley of Leeds University for his valuable criticism made during a revision of the manuscript. To his late teacher, the late Professor S. R. Kashyap of the Punjab University, he is indebted for the material.

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EXPLANATION OF PLATES.

(All the photographs are from *Stellera Chamæjasmæ* Linn.)

PLATE XXXVI.

FIG. 1.—Transverse section of an old underground stem showing a number of vascular rings, one-sided growth, and a bud on one side marked *b*. × 20.

FIG. 2.—A part of Fig. 1 showing the bud at a higher magnification. × 60.

FIG. 3.—A part of Fig. 1 showing a portion of the two outermost rings at a higher magnification. Phloem is marked *ph*. × 200.

FIG. 4.—Central part of a transverse section of a rootlet showing the primary structure and the beginning of secondary growth. × 250.

PLATE XXXVII.

FIG. 5.—Transverse section of an older root showing a fully developed primary cambium (*p.c.*), one-sided growth of the stele, and the origin of the secondary cambium (*s.c.*). × 60.

FIG. 6.—Part of text-figure 1 showing a region where the two cambial rings are quite free from each other. The outer (primary) cambium is forming wood to the inside and phloem to the outside. The inner (secondary) cambium is forming wood to the outside and phloem to the inside in an inverse manner. × 140.

FIG. 7.—Another part of text-figure 1 showing a region where the external and the internal cambiums are continuous with each other. × 150.

FIG. 8.—A portion of the root wood showing radial bands of vessels and prosenchyma alternating with broad rays of storage parenchyma, and also showing the presence of inter-xylary phloem (*ph.*). × 75.