THE ORIGIN AND DEVELOPMENT OF THE DIAPHRAGM
AT THE BASE OF THE INTERNODE OF EPHEDRA CORYI

THESIS

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I. THE PROBLEM OF INTERCALARY GROWTH IN PLANTS

A. Types of growth in length

The phenomenon of growth in length of stems has been known for many years. It is recognized that increase in length may occur by apical growth or by intercalary growth. Apical growth has been investigated and reported for numerous species of plants. Most elementary college botany texts discuss apical meristems rather completely, but tend to pass by the matter of intercalary growth with only a few brief statements.

B. Literature of intercalary growth

Eames and MacDaniels (1947, pp. 60-61 and 66-67) stated that in plants with little or no tissue specialization, cell division may occur throughout the plant body or in any part of it, thereby increasing the size of the plant by the increase in the number of cells. In plants with tissue differentiation, new cells are formed chiefly in organized regions known as meristems. The term "meristematic" indicates a resemblance to a meristem, but not necessarily consisting

of or constituting a meristem. Since nodal regions are mature, the intercalary meristems are internodal. In early stages, the internode is more or less uniformly meristematic, whereas later some parts mature more rapidly than the rest, so that a definite sequence in development within the internode is set up and maintained. It is generally believed that the intercalary meristems are separated from the mother meristem at an early stage, and that they retain a layer of promeristem derived from the apical meristem. Although the region containing the intercalary meristem consists partly of promeristem cells, vascular strands with protophloem and protoxylem cells in various stages of maturation pass through it.

Miss Buchholz (1921) said that Meyer (1808) was the first to describe intercalary growth and that this description was for the grasses.

The articles by Grisebach (1843) are considered the basic works for all more recent studies of intercalary growth. His work at first was not characterized by definite measure-

ments, but only by rather crude observations. He thought that all growth in length is due to the formation of new cells, but failed to refer to them as being meristematic. Later, by measurements, he found that an area becomes "inserted" a short distance above the node, which area, if it increases in length to twice its original, he called an intercalary region. By comparing longitudinal sections from various levels of the internode, he reached the conclusion that for true intercalary growth there must be an increase in the number of cells. If intercalary growth were due only to the elongation of cells already present, then there would have to be present in the growing zone a layer of short cells. By his microscopic measurements in the grasses, Grisebach was forced to assume that the formation of new cells was not the only cause of intercalary growth in plants. He stated that in Dianthus plumarius in the older, upper part of the internode, vessels are numerous; while in the lowermost (intercalary) zone they are few in number and of the spiral-vessel type.

Unger (1846, p. 125) noted that a stem could grow in length by the formation of a number of new internodes at the apex, and also that in different species these internodes later become elongated.

Harting (1847) made actual measurements of the cells in different parts of the internode, primarily in the pith, the bark, and the epidermis; these, Schmalfluss (1929) said, represent the first instance of such measurements. Harting recognized the fact that growth of the internode is dependent upon (1) the formation of new cells, (2) the increase in size of cells, and (3) the thickening of the cell walls. In his report, growth was said to cease first at the base and lastly at the upper end of the internode, this placing the intercalary zone at the opposite end of the internode from that reported by Grisebach. It is to be noted, however, that Grisebach worked with plant material having an apical intercalary zone.

The intercalary growing zone in the leaves of Equisetum hyemale was described by Duval-Jouve (1860), as reported in his later paper of

1864, page 58. The increase in length of the internode was found to take place exclusively by the multiplication of the cells at the base of the leaf.

Hofmeister (1868, pp. 405-665) described intercalary growth in both the apical and basal position, these being found in different species. He said that the longest internodes of the plant kingdom attain their length by intercalary growth.

Sachs (1873, p. 142) mentioned intercalary growth in several plants, such as *Equisetum*, grasses, *Liliaceae*, and other monocotyledons. He expressed the idea that intercalary growth is responsible for the development of the spur of such flowers as *Aquilegia* and *Diclytra*.

Van Tieghem (1884, pp. 261-265), in discussing the morphology of the internal growth of the stem said that the node itself does not grow in length (leaving one to draw the conclusion that the internode does all of the growing in length).

Tschirch (1889, p. 238) lists intercalary growth as occurring in grasses, most young leaves, and the flower, even stating that cambial divisions can, in a sense, be considered a type of intercalary growth.

Warming-Johansen-Meinecke (1907 and 1909, pp. 30 and 190) give a general statement concerning intercalary growth, containing the major ideas expressed above.

Buchholz (1921) gave a rather extensive review of the earlier literature on intercalary growth. In describing the older internode, she said that mature vessels are present, whereas in the intercalary zone the vessels are few and of the "stretchable" type (meaning either annular or spiral, or both). This idea had been earlier stated by Grisebach. The typical mature vascular bundle is not present throughout the internode until internodal growth has been completed.

Blagovescensky et al (1927) proved that elongation of the internodes of barley occur in the "hidden growth" covered by the sheath or the base of the leaf, and that elongation occurs first in the second and third internodes, followed by the first internode. This is a relatively new idea of growth in length. Apparently the Ephedras do not follow such a pattern as herein described.

Schmalfluss (1929) stated that intercalary growth occurs mainly in plants having unbranched shoots, such as certain grasses, and long flower stalks, which are leafless and nodeless. The zone is protected by leaves or scales; this protection makes the arrangement an ideal one for growth in length.

In bamboo, Porterfield (1932) found that elongation passes over into cell differentiation and specialization increasingly from the growing point until the region of growth is restricted to the base of an internode. The cortical intercalary meristematic cells were found to elongate 12.5 times their original length. The basal intercalary zone of the leaf is mentioned but is not discussed anatomically.

Goebel (1932, pp. 1779-1780) discussed growth in length of the stem from two different angles. One is the condition in which the leaf and its axillary bud are displaced by the growth of the stem at the base of the leaf, thereby pushing the leaf and bud away from the main axis. The other is the situation in which the stem grows

in length between the leaf attachment and the axillary bud, thereby moving the bud up the stem and away from the leaf. The author considers these types of growth to be intercalary.

Foster (1942, p. 18), in his discussion of intercalary meristems, locates them between areas of permanent or mature tissues. He also made the statement that a more critical study of so-called intercalary meristems is urgently needed.

Jacobs (1947), working with the gynophore of the peanut, found that the intercalary growth was due to a resumption of meristematic activity by cells which were apparently stimulated by some phenomena associated with pollination. He said that according to recent workers on grasses, the intercalary meristem formed at the base of the internode is merely the last portion of the internode to differentiate. This zone retains the ability to divide which was previously common to the entire internode.

In 1949, Williams attributed the presence of a large differentiated lamina in the alga,

Laminaria agardhii, to a well developed intercalary meristematic region.

In summarizing the literature, mostly taken from Buchholz (1921), intercalary growth has been reported in a number of species and a number of plant organs. The following are representative for stems: Zea mays (Moldenhawer, 1812), Narcissus (E. Meyer, 1832), Equisetum (Hofmeister, 1851), Nitella flexilis (Askenasy, 1881), some mints (Eames and MacDaniels, 1947, p. 66). For flower stalks we have Tradescantia and Commelina (Burkom, 1913) and Taraxacum and Plantago (Eames and MacDaniels, 1947, p. 66). Intercalary growth is reported to occur in the gynophore of the peanut (Jacobs, 1947). For leaves we have Crocus and Hyacinthus (Munter, 1841), Umbelliferae and grasses (Grisebach, 1844), Equisetum (Duval-Jouve, 1860), Welwitschia mirabilis (Hooker, 1864), Allium cepa (Stebler, 1878), Isoetes (Goebel, 1883), Liliaceae (Nathanson, 1898), Cannaceae (Pfeffer, 1904) and Pinus leaves (Eames and MacDaniels, 1947, p. 67). For the algae we have some of the kelps (Jacobs, 1947) and Laminaria agardhii (Williams, 1949).

II. THE PROBLEM OF INTERNODAL DIAPHRAGMS

A. Types of diaphragms

The preliminary discussion in the foregoing paragraphs on intercalary growth was necessary for an understanding of the internodal diaphragm. As was earlier stated, an intercalary zone is bounded on either side by permanent tissues (mature tissues). In the genus *Ephedra*, an intercalary meristematic zone is found in the mature internode of the stem a short distance above the node; this internodal diaphragm is the major subject of this paper.

Such internodal diaphragms have apparently been found only in the genus *Ephedra*. Attention is here called to the fact that such a diaphragm differs radically from the well-known diaphragms found in certain *Juglandaceae*, as walnut and butternut, where they divide the pith into a vertical row of chambers (Trelease, 1931, p. 17). They also differ from the nodal diaphragm described by Brown (1939) for *Equisetum kansanum*. Again, they have no similarity to the diaphragms found commonly in the underwater petioles, stems and

roots of many aquatic plants (Snow, 1914). We have, therefore, at least four distinct meanings for the term, diaphragm.

B. Literature on Ephedra in general

Much of the literature dealing with Ephedra discusses it as problems in pharmacy and chemical analysis of the various vegetative structures. Some of the early investigations of various Ephedras were anatomical, but with no mention in the text of a diaphragm, even though such was shown in some of their drawings. There is very little on the origin and development of the layer in question.

Strasburger (1872) has a diagram of a longitudinal section through the node of an Ephedra. The description of the figure (his plate 22, figure 11) emphasizes buds, but does not mention the nodal or internodal structures present. The figure shows crudely what later authors call the diaphragm.

Evans (1888) in several Ephedras found a basal diaphragm of three or four cells in thickness completely cutting through the pith and often into the xylem, apparently extending into

the phloem. He said that the tracheids which penetrate this layer are long and heavy-walled. He is not prepared to make a definite statement concerning the layer, but surmises that it may be composed of active meristematic cells and that growth of the internode may take place here. This region furnishes easy separation of the stem, as it always seems to break at this point. His diagram (his figure 11) shows a diaphragm above the node almost approaching the margins of the internode. His figure 12 is an enlarged section of the diaphragm from the pith to the region of the phloem, apparently not penetrating the cortex of the stem. This follows his explanations as to the lateral limits of the diaphragm.

Stapf (1889) described the origin of the diaphragm of Ephedra altissima as a meristem dividing indefinitely until ultimately there is a differentiation into permanent tissues, with the exception of this narrow band. In this species he says the zone traverses the entire internode, and remains in a resting condition until it becomes differentiated or forms

an abscission layer. The abscission layer is recognizable externally by a constriction and a lighter color of the internode. The cells of this layer are not lignified and are rich in protoplasm. His figure 14 shows a straight diaphragm and a constriction in the outer part of the stem. The path of this layer is more or less indefinite after passing out of the vascular bundle. His figure 15, a transverse section, suggests a difference in the types of cells present, those of the cortical region being small, parenchyma-like, with rich cytoplasm.

Graham (1908) has approximately the same point of view as Stapf. He does not, however, show a constriction in the external walls of the internode, even though he mentions it in the body of the article. This layer cuts partially through the vascular tissues, the xylem connections being kept up by short-reticulate tracheids.

He adds:--

In the first internode after the apical the stelar elements have begun to differentiate, while in the second internode they are well developed. In this internode elongation of the stem has set in, but is more pronounced in the

succeeding two or three internodes. At first the whole internode is meristematic (Plate III, figure 17), but later elongation comes from a meristem situated at the base of the internode.

His figure 17 shows a full length young internode which is partially differentiated at the apex while the basal portion remains meristematic. There is no indication of the presence of a diaphragm.

Thompson (1911, p. 1078) said that the origin of the diaphragm seems to be conceived as comparable to that of a cork cambium. Nodes are recognized by the constriction of the pith, and the layers of thick-walled cells. The diaphragm is sharply differentiated from the rest of the pith by the arrangement, size, and contents of its cells. He made no reference to Strasburger (1872), Evans (1888), Stapf (1889) or Graham (1908).

Coulter and Chamberlain (1917, p. 366) used the works of others in their book and commented on the diaphragm of the plate-like cells which occurs at the base of each internode as a peculiar anatomical feature of the genus *Ephedra*.

Greguss (1920) has the same point of view as to the description of the diaphragm as earlier

authors; however he said that the primary xylem does not extend through the zone, but ends at the diaphragm. The "bridging over" is accomplished by very short spiral tracheids which are grouped closely together. The cambium also penetrates the diaphragm. In maturation, the walls of the diaphragm cells thicken, lose cytoplasm but do not become lignified, even though the surrounding cells are lignified. Walls of this layer remain cellulose. His figure 1 shows a well-developed diaphragm through the wood to the cambium; it shows less clearly through the phloem and cortex to the external "notch". Cells elongate transversely to the stem. Vessels do not penetrate this zone. His figure 2 shows an older stem with branches, the diaphragm crossing only pith and xylem, dotted lines showing the path of future abscission. His figure 5 apparently shows the origin of the diaphragm in the pith. This information was taken from the three page German summary to the original article.

Markgraf (1926), as did earlier writers, calls attention to the basal meristematic zone in each internode. In his figure 216 (c) he shows the internode, but fails to show a diaphragm.

Pearson (1929, p. 22-23) failed to cite many of the earlier writers of Ephedra and intercalary growth. He mentioned the absciss layer above the node, stating that the origin and its behavior had not been investigated up to that time.

Cutler (1939), while discussing the continuity of the vascular system, said that the fiber system of the stem is interrupted at the node, and that a peridermal diaphragm is produced at the base of each internode. This interruption of the vascular tissue allows easy fragmentation of the stem at this point.

One of the first references definitely giving the time of beginning of the diaphragm is Johansen (1940, p. 445). He said that longitudinal sections of the first two to four nodes show a meristematic region at the base of each internode; also that there is developed an abscission layer.

C. Ephedra coryi

An examination of the literature cited for this investigation failed to find mention of a diaphragm as being present in Ephedra coryi, even though it is now accepted that internodal diaphragms are characteristic of the genus Ephedra. It has been reported that the diaphragm is absent in Ephedra monostrachya (Evans, 1888).

Ephedra coryi belongs to the order of plants known as the Gnetales. This group of plants is considered to belong to the gymnosperms, even though it approaches the angiosperms in both anatomical and reproductive features. The common names sometimes applied to the genus Ephedra are joint-fir, ma huang, Mormon's tea, and many others. Perhaps the most important related species economically is Ephedra sinica from which ephedrin, a drug commonly administered as an astringent and as a mild substitute for adrenalin is obtained.

The particular species used in this investigation, namely Ephedra coryi, was discovered and described by Dr. E. L. Reed (1936), who was then a member of the Biology Department of this insti-

tution. It is considered to be a native plant of the plains of West Texas, with a restricted distribution in western Texas and southeastern New Mexico. Other species of *Ephedra* are found in most arid regions of the world. The genus is typically a xerophyte.

Ephedra coryi is found growing in sandy soil among native grasses, scrub oak and mesquite thickets. It is of importance to this region as a "soil binder" and furnishes some early grazing for livestock.

During the growing season the above ground stems are green and elongating. Upon superficial examination of the growing stem, it will be noticed that the part of the internode toward the apex is always darker in color and usually extremely tough, while the basal portion of the internode is a pale green to yellow in color with soft tissues. The first visible internode is usually one of the shortest, being approximately equal to the basal internode of the same branch. The intermediate internodes are much longer. Growth in length seems to resemble that of the grasses and of the Equisetineae.

III. MATERIALS AND METHODS

The plants of Ephedra coryi used in this investigation were collected from Studhalter's site 26 (1.5 miles north of Tahoka public schools) and site 184 and 188 (south of Tahoka on U. S. Highway 87) by Dr. R. A. Studhalter. The plants, partly staminate and partly carpellate, were placed in moist paper towelling to prevent undue drying until examinations and selections were made for killing and fixing in 70 percent formalin-aceto-alcohol.

The embedding procedure used was the tertiary-butyl alcohol dehydration method of Johansen (1940, p. 130). The selected materials were later embedded in paraffin. Due to extremely hot 1949 summer temperatures, some of the material was embedded in a paraffin-bayberry wax mixture in order to make the paraffin more firm. Johansen's safranin and fast green schedule was used for staining.

Approximately 500 slides showing transverse or longitudinal sections were prepared for this investigation. Most of the slides are serial sections, with a few showing only selected

sections. Some slides were made of free-hand sections from old wood to ascertain the lateral extremities of the diaphragm. A series of slides was made of consecutive nodes from the apex to the ninth definite node.

Difficulty in sectioning this material was encountered, perhaps due to an impregnation of the cells and their walls with silica or calcium oxalate. Only a few sections could be made at one time without resharpening of the microtome knife. It was discovered that by soaking the paraffin block for 24-72 hours in water sectioning was made more satisfactory.

Drawings were made by the use of a RayOScope; however, most of the illustrations are photomicrographs.

IV. THE ORIGIN AND DEVELOPMENT OF THE DIAPHRAGM IN EPHEDRA CORYI

A. The apical meristem

In Ephedra coryi, as in nearly all other species of the genus, the internodal diaphragm has its origin in the meristem found at the growing apex of each young aerial stem. The cells of this apical meristem have the characteristics of any meristematic region (Figs. 1, 2). They have extremely dense cytoplasm, large prominent nuclei (in proportion to the size of the cell), and relatively thin walls. Their general shape is isodiametric; only at the top and lateral edges of the cone-shaped region is cell shape changed appreciably so as to give it a smooth, rounded surface (Fig. 2). Vacuoles, if present, are very small; most of the cells do not possess easily visible vacuoles. It is in this region that the cells are actively dividing, and from this region all of the cells of the older portions of the stem are formed and differentiated.

In the formative region of the stem tip the nodes are not prominent. There are several sets of leaf primordia which are not present in most

sections since the leaves are oppositely arranged on the stem. The first node as referred to in this paper is the one shown just above the middle of Fig. 1; other nodes are numbered in sequence from here downwards.

Most of the work reported herein is taken from young growing stems which are composed of from six to nine prominent nodes. The mature stem at the end of the growing season was found to have from ten to fifteen prominent nodes.

The stages of development gone through by any single internode may be successfully traced by studying a number of successive internodes in a single stem from the stem tip downward.

B. From the apical meristem to the diaphragm

The recognition of the node in the early stages of development is rather difficult. Immediately above the node, there is a constriction to which the leaf primordia are attached (Fig. 1). The cells in the nodal region very early in their development show a slight elongation vertically (Fig. 2). The differentiation of cells in the region of the fourth node is principally one of increase in trans-

verse thickness (Figs. 3, 4). Here the beginning of a dome-shaped structure at the apex of the internode is noticed (Figs. 9, 10). In the fifth node these cells are considerably elongated (Figs. 5, 6). In the sixth node there is the formation of several groups of spiral vessels (Figs. 11 to 13), which at this time are non-lignified. In the seventh node each of these groups is marked by several strands of vessels (Figs. 14, 15), most of which are tracheid-like and partially lignified. The vessels more or less "fan out" at the node as they are more numerous here than just above the node (Fig. 20). The parenchyma cells which remain have begun to have deposits of materials in the corners of the cell walls so that they are now collenchyma tissue. The walls of the cells in the nodal region possess bordered pits. Ultimately more and more vessels are produced until the major part of the node is made up of lignified vessels (Fig. 38). The mature condition is found approximately at the eighth or ninth node, where it has maintained its dome-shaped character.

The major changes which are linked with the formation of the diaphragm occur in the pith.

In the early developmental stages the cells of the entire internode tend to be more or less meristematic, as stated above. The first change noticed in differentiation is the slight amount of elongation and vacuolation of the pith cells at the upper end of the internode.

In the region of the first node there is no histological evidence of a prospective diaphragm. Very little differentiation has occurred in the entire second internode (Fig. 1). In the third internode (Fig. 7), however, the cells previously reported have increased in size in all dimensions. At the upper end of the internode they are approximately twice their original length; as one goes down the length of the internode, the cells become gradually shorter until a horizontal zone is reached at a short distance above the lower node, in which zone the cells have retained more or less their original size and shape. In other words, differentiation begins at the top of the internode and progresses in wave-like fashion downwards.

At the same time, the cells immediately above the lower node also show a differentiation which at first consists primarily of a transverse broadening accompanied by an increase in length and in the thickness of the cell walls. From this lower zone, as one goes upwards in the internode, the cells show progressively less differentiation until one arrives at the zone mentioned above in which the cells retain their original size and shape. Hence, at the lower end of the internode there is an upward progression of differentiation comparable to the downward progression in the upper part of the internode.

The undifferentiated zone in the third internode, therefore, is not located in the middle of the long axis of the internode, but at a relatively short distance above the lower node, approximately eight or nine cells above the node, or about one-eighth of the entire internode. This zone of meristematic tissue may be looked upon as a residual meristematic region, since the original undifferentiated cells of the internode are all showing more or less differentiation with the single exception of this layer. It may be considered as the early stage

of an intercalary growing zone, also of the diaphragm (Fig. 8). In longitudinal section, this residual zone in the third internode varies in vertical thickness. At the center it is found to be three to five cells thick; it becomes progressively thicker toward the periphery, where there may be from five to eight cells in vertical thickness.

The differentiation noted above, both in the upper and in the lower ends of the internode, begins at the center of the pith; there is again a wave of differentiation outwards toward the periphery in both zones.

It should be emphasized that the entire discussion of the third internode thus far has been confined to the pith region.

The differentiation described above for the pith in the upper end of the internode involves not only a change in cell size and shape, but also some prominent cytological alterations. The cell wall becomes thick and, with the staining technique used, a vivid red in color. The protoplasm dies and the interior of the cell becomes impregnated with a red sub-

stance of unknown chemical composition. In the lower end of the internode, the cytological changes are much less pronounced in the third internode, involving mainly the thickening of the cell wall, which remains cellulosic in composition.

In the fourth internode the cells of the pith are further elongated (Figs. 9, 11). Microscopically these in the upper end are approximately three to five times as long as they are broad. In most of the pith cells, cytoplasm is almost extinct (Figs. 12, 13). There is a tendency for the walls of the larger cells to rupture (Figs. 14, 15). There appears to be some form of granulation and gelatinization of both walls and contents of the cells. The diaphragm is well formed by this time, being composed of from two to five cells in thickness, mostly limited to the pith at the lower end of the internode. The cells of the internode on both sides of the diaphragm are approximately two times their width in the longitudinal section (Figs. 16 to 19).

In the fifth internode there is a further rupturing of cell walls accompanied by the

disappearance of cell contents. All of the pith cells, except those of the diaphragm, now show differentiation in that they have elongated and have become vacuolated to some degree (Figs. 21 to 26). Nuclei are fewer in number. The uppermost pith cells of the internode apparently are no longer functional living cells. Their cell walls are thicker. The diaphragm cells are extremely short vertically as compared to the cells on each side; the latter are still elongating.

As differentiation continues below the fifth node virtually all of the cells of the pith ultimately reach their mature length, which in some cases is in excess of twenty-five times the length of the cells of the diaphragm. Complete differentiation includes the heavy lignification of the cell wall.

In or below the fifth internode, the pith cells at the extreme upper part are seen to be completely differentiated at an earlier stage than is true of the lowermost cells of the same internode; however, differentiation is completed in all the cells between the lower node and the

diaphragm some time before this is true of the cells immediately above the diaphragm. When all the cells below have completed their differentiation, those immediately above the diaphragm are still in an elongating stage, being only one-half to three-fourths their ultimate length. The latter reach their complete differentiation in about the ninth or tenth prominent internode. This situation is probably due to the fact that the upward wave of differentiation has a much shorter distance to travel, therefore reaching the diaphragm long before the downward wave. The various stages of cell differentiation from the diaphragm both upwards and downwards are shown in Fig. 46.

In the third internode cells belonging to the provascular strands make their first appearance. In the fourth internode are found the first vessels of the vascular bundle. These vessels are primary xylem, as yet non-lignified; lignification is first seen in the fifth internode. Thus far, the vessels are all of the spiral type. The possession of vessels of any type gives Ephedra coryi a characteristic of

the angiosperms. The vessels are more numerous at the node than in the internode. In general the primary xylem of the bundles does not pass through the diaphragm (Figs. 27, 28). The vessels of the secondary xylem however, are found present in the diaphragm, where they effect the necessary vascular continuity up and down the stem. These vessels are much shorter than those above and below in the same vascular strand (Figs. 37 to 45); they are tracheid-like in character with spiral thickenings; and their walls are not lignified. Their walls contain bordered pits.

The vessels in those portions of the vascular bundle outside of the diaphragm are longer than those enclosed by it; their walls are also prominently lignified. They apparently retain the thickenings found in the younger elongating vessels contrary to the statement made by Graham (1908).

At the time of the first appearance of the primary xylem in the fourth internode, the primary phloem can also be distinguished. Secondary phloem cells are added soon afterwards. These cells soon elongate and are rather dense

with cytoplasm in the earlier stages of development. The cambium is not easily distinguishable in longisections. Just outside of the phloem region there are scattered bundles of fibers (Figs. 29, 30, 31, 33, 35, 36), which are here interpreted as belonging to the pericycle. There is no distinct boundry between the pericycle and the cortex.

The cortical cells are apparently the last cells of the internode to differentiate. In the first several internodes these cells are found to elongate as do other cells of the internode. The cells adjacent to the epidermis apparently divide to produce additional cells compactly arranged, which soon become broader radially than they are long, somewhat resembling the palisade layer of a leaf blade. Still later, by further cell divisions, the "palisade" layer becomes a double one (Fig. 29 to 36). The other cells of the cortex, which at first are angular, round out at the corners, later to become rather large and irregular in outline. With the irregularity large intercellular spaces are developed, so that this region bears a close resemblance to the spongy mesophyll of a leaf.

Chloroplasts are found in abundance in both layers. Hence, the cells of the cortex at this stage somewhat resemble the mesophyll of a leaf blade both in structure and function. It should be remembered that in the genus *Ephedra* photosynthesis is carried on primarily by the stem, the leaves being scale-like. At about the sixth prominent internode, numerous small bundles of cortical "fibers" become apparent (Figs. 29, 31, 33, 35); their walls apparently remain unlig-nified, at least for some time.

The last region of the internode to be considered is the epidermis. Its cells are isodiametric in their early development. In the first prominent internode they have elongated somewhat, with no cutinization of the outer wall, whereas in the second prominent internode cutinization is first apparent. No guard cells are present in this internode. In the third prominent internode there is a heavy deposit of cutin on the epidermis; also, stomates and guard cells are present. At maturity the ordinary epidermal cells are approximately three times as long as they are broad radially; however in the

tangential view they are only one and a half times as broad as they are thick. All of their walls are thickened, the outer one being the heaviest.

C. The mature diaphragm

The mature diaphragm retains its position a relatively short distance above the lower node. Since, however, the cells below it have elongated considerably in the course of the development, the diaphragm at maturity is found at a somewhat greater distance from the node than had been the case in its earlier stages of development. Its distance from the upper node has been increased many fold since there are so many more cells above the diaphragm to elongate than below it. Relatively, therefore, the diaphragm is close to the lower node (Fig. 37). Since the node has the normal shape of a dome and the diaphragm that of an inverted dome, the distance between node and diaphragm increases materially from the center of the stem outwards. The mature diaphragm retains the concave or inverted dome shape which it had earlier (Fig. 37). In radial extent the diaphragm of Ephedra coryi appears to be rather

limited. In its more typical form, it cuts through the entire pith and outward between the vascular bundles at least as far as the margins of the fascicular cambium (Fig. 38 to 45). Beyond the cambium the diaphragm region is only indistinctly present as a rather thick group of pointed, thick-walled, non-lignified cells. In this respect Ephedra coryi appears to vary radically from other species as briefly described and pictured by Stapf (1889) and Greguss (1920), where it is shown to extend laterally to and even through the epidermis as typical short, thin-walled cells.

The mature diaphragm in Ephedra coryi is from two to four cells in vertical thickness. They remain more or less meristematic in appearance (Figs. 39 to 44), although there is no indication that they divide after the diaphragm has reached its maturity. They are short vertically and elongated both radially and tangentially. As has already been shown, the spiral vessels which occur within the boundary of the diaphragm are short and unlignified. The location of the mature diaphragm may be approxi-

mated on the outer surface of the stem by a local yellow color between it and the node. It has already been stated that no constriction is present in this species as is described for Ephedra altissima and others (Stapf, 1889).

V. SUMMARY AND DISCUSSION

In Ephedra coryi the very young internode is meristematic throughout its length. The first cells to differentiate and mature into permanent tissues are those at the upper end of the internode, leaving the balance in a meristematic condition. A wave of differentiation and maturation progresses down the internode, thus gradually shortening the zone of meristematic cells and restricting it to a shorter and shorter area. At the same time, another wave of differentiation and maturation begins at the base of the internode and moves upwards. While the lower zone matures earlier than the upper one, it nevertheless occupies only a fraction of the length of the upper zone. As these two waves of differentiation approach one another, they all but meet at a horizontal level a short distance above the lower node, at which time further differentiation ceases and there is left a horizontal cup-shaped area of meristematic cells two to four cells in thickness in the longitudinal

axis of the stem. This intercalary area is the diaphragm which represents the residual portion of the meristematic cells which at first occupies the entire length of the internode.

The diaphragm is at first seen in the central region of the pith from which location it extends outwards on all radii to the edges of the fascicular cambium. Secondary spiral vessels of modified form are found in the diaphragm, these cells being short and remaining unlig-nified.

The future history of the diaphragm presents some interesting problems. In the field, a "plant" of Ephedra coryi is found to arise from a rhizome, to branch freely at and above the ground and to consist of branches of varying age, up to four or five years. A mature diaphragm is found in every mature branch; in other words, the diaphragm is semi-permanent and does not differentiate completely into permanent tissues, at least for a period of five years. It is suggested by Greguss (1920) that the diaphragm of *Ephedra* may remain in the resting condition for twenty-five or thirty years before

complete differentiation occurs.

The abscission of branches rather than of leaves may be considered a prominent characteristic of the genus *Ephedra*. The time of abscission as hinted above varies considerably, many internodes abscissing in their second year, whereas others remain attached indefinitely. There seems little doubt that an absciss layer is formed within or at the diaphragm; branch abscission may therefore be considered to be the main function of the diaphragm. From the above it is further evident that many diaphragms form absciss layers in the second year of their existence, while at the other extreme many retain their meristematic appearance for many years. The factors responsible for such differences are unknown. Greguss (1920) adds to the thought mentioned above that in other internodes the diaphragm may remain in the resting condition for twenty-five to thirty years before finally forming an absciss layer. Since aerial stems of *Ephedra coryi* do not reach such an advanced age the longevity of its diaphragm is much shorter.

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Figure 1. Longitudinal section through the stem tip of Ephedra coryi showing the first two visible nodes. X 65. Note lack of differentiation in the apex as compared with internode below the first pair of leaves. In the second internode pith is being differentiated.

Figure 2. Stem tip of the same section showing embryonic nature of cells and lack of differentiation. Cells toward the center are elongated vertically. X 300.

Figure 3. Diaphragm region just above second node of Figure 1. Some cells are flattened longitudinally while others show growth in both transverse and longitudinal dimensions. X 300.

Figure 4. An approximately nodal view at second node of Figure 1, showing differentiation above the node toward Figure 3. X 300.

Figure 5. Continuation downwards from Figure 1, showing the third node. Note progressive amount of differentiation in the pith from the diaphragm region both upwards and downwards. Beginnings of vascular tissue. Pith cells are prematurely dead and filled with dark contents. X 65.

Figure 6. Diaphragm region of above figure. Note increase in size of cells adjacent to the diaphragm on both sides. X 300.

Figure 7. Region above the fourth node in the same stem as Figure 1. Note length of cells in upper portion as compared with those in diaphragm region (near the bottom of the figure). X 65.

Figure 8. Diaphragm region of Figure 7 enlarged. Note longitudinal flattening of the diaphragm cells, while other cells are elongated. X 300.

Figure 9. Longitudinal section of a stem slightly older than that in Figure 7, showing diaphragm region and nodal region below it. X 65.

Figure 10. Enlarged view of nodal region of Figure 9, showing the type of differentiation in this region. Note irregular arrangement of these cells and the beginning of the dome-shaped pith region just below the node. X 300.

Figure 11. Longitudinal section of nodal region of a still slightly older stem, showing early stage of diaphragm and lateral branches in which diaphragms can also be distinguished. X 65.

Figure 12. Diaphragm region of Figure 11. Note elongation of cells above and below the diaphragm. Vessels are present below the diaphragm but not above it. The spatial relation of Figure 13 to Figure 12 is shown below. X 300.

Figure 13. Diaphragm region in
Figure 11; continuation of Figure 12.
X 300.

Figure 14. Longitudinal view of still older developing diaphragm. Note spiral vessels in the vascular region and rapid differentiation within the pith region. X 65.

Figure 15. Pith region of Figure 14, at the level of the diaphragm, showing greater differentiation of cells adjacent to either side of the diaphragm. Vessels of the primary xylem on either edge of the figure. X 300.

Figure 16. Longitudinal view of well developed diaphragm; the lower node at bottom of the figure. Note vascular tissue along left side of diaphragm. Pith is hollow at the bottom of photomicrograph, in the next older internode. X 65.

Figure 17. Diaphragm region of cortex from Figure 16, showing a region of meristematic cells lying between partially differentiated cells. X 300. The spatial relation of Figures 17, 18, and 19 is shown below.

Figure 16. Central portion of diaphragm from Figure 16, showing greater elongation of pith cells and flattening of diaphragm cells. Note rupturing of part of cell walls of pith. X 300.

Figure 19. Part of diaphragm from Figure 16, showing its marginal limit confined to central part of the stem. Differentiation occurring in pericycle, cortex and epidermis. X 300.

Figure 20. Nodal region from Figure 16, showing spiral vessels of the primary xylem concentrated at the lower node and becoming fewer in number toward top of photomicrograph. X 300.

Figure 21. Longitudinal section of a nearly mature diaphragm slightly off median, showing further differentiation of pith and xylem cells. Note increased number of spiral vessels and lignification of pith cells above and below the diaphragm. The cells below the diaphragm are more nearly mature than those above the region. Lower node below. X 65.

Figure 22. Section including the upper left margin of diaphragm from Figure 21. Note elongation of cells in cortex and pericycle region; some cells are not differentiating as rapidly as others. The spatial relation of Figures 22 to 26 is shown below. X 300.

Figure 23. Diaphragm region from far left side of Figure 21. Note shortened vessels, increased size and lignification of pith cells, and dense cytoplasm of the diaphragm cells. X 300.

Figure 24. Pith region from Figure 21, showing well defined diaphragm. Note greater lignification of cells below diaphragm than those above. Here the diaphragm is 2 to 4 cells in thickness. X 300.

Figure 25. Diaphragm region from right side of Figure 21. Outer margin ends at the spiral vessels of the primary xylem. X 300.

Figure 26. Continuation beyond the diaphragm region of Figure 25. Note especially the spiral vessels with bordered pits. X 300.

Figure 27. Longitudinal section near the median of a nearly mature internode showing continuity of vascular tissues. X 65.

Figure 28. Diaphragm region of Figure 27, showing that the diaphragm does not penetrate vessels of the primary xylem at this stage. X 300.

Figure 29. Transverse section of stem of Ephedra coryi 1 cm above the bottom of the mature diaphragm. The pith cells are dead and filled with dark, granular contents; their cell walls are thick and in the process of rupturing; the pith condition is comparable to that shown in the upper part of Figure 5. Note mesophyll-like cortex and pericycle with fibers. X 65.

Figure 30. Enlarged view of the same. Note rupture and lignification of the walls and dark deposits within certain pith cells; also the absence of nuclei. X 300.

Figure 31. Transverse section of stem in region of internodal diaphragm just below that shown in Figure 29. Note arrangement of vascular tissue, lack of lignification of pith cells, and the presence of nuclei. X 65.

Figure 32. Diaphragm region of a section adjacent that in Figure 31. Note cytoplasm and nuclei. The cell walls are thick but not lignified. X 300.

Figure 33. Transverse section of the same stem a short distance below Figure 31, between the diaphragm and node (0.4 mm below the diaphragm). Some apparent lignification of cell walls with deposits in certain cells. On either side of stem are embryonic leaves of two lateral branches, which together with the stem is enclosed by a leaf sheath.

Figure 34. A portion of region between diaphragm and node in a section adjacent to the one shown in Figure 33, showing lignification and rupturing of cell walls, dark deposits in the cells, and a portion of the vascular bundle. Nearly all pith cells are dead. X 300.

Figure 35. Region just above the lower node of the same internode as in figures 29 to 34 in transverse section. Bounded on opposite sides by leaf bases. X 65.

Figure 36. Details from a serial section, three removed from Figure 35. Cells similar to those in Figure 34. X 300.

Figure 37. A mature diaphragm,
well defined in the pith region,
much less so at its periphery. The
diaphragm is typically cup-shaped.
X 65.

Figure 38. Free hand longitudinal section of a mature stem showing diaphragm slightly in oblique section. Note differentiation of cells above and below the diaphragm as compared with earlier figures. There is an indication that cells in xylem region do not completely differentiate within the boundaries of the diaphragm. X 65.

Figure 39. Upper left border of diaphragm region of Figure 38. Note the shortened cells in this region and the bordered pits. The spatial relation of Figures 39 to 44 is shown below. X 300.

Figure 40. A view from Figure 38 overlapping with Figure 39. The vessels are spirally thickened and lack lignification. X 300.

Figure 41. The next adjacent and overlapping zone to Figure 40. The diaphragm cells, here at the pith, are typical in size, shape, density of cytoplasm, and presence of nuclei. X 300.

Figure 42. Central pith region of Figure 38. Note increased length of adjacent pith cells, both above and below, and the absence of nuclei in the pith cells. Diaphragm cells are typical. X 300.

Figure 43. Diaphragm region to the right of Figure 42 and overlapping with it. Note spiral thickenings and bordered pits in the primary xylem. X 300.

Figure 44. Area to the far upper right of diaphragm zone of Figure 38. Cellular detail not very clear, but shortened cells are noticeable in the xylem. X 300.

Figure 45. Free hand longitudinal section of lower part of an internode. Cellular detail not clear, but there is a suggestion that there may be a continuation ultimately of the diaphragm completely across the internode. X 65.

Figure 46. Differentiation and changes in cell size in the pith during the formation and development of the diaphragm. The cells on the dotted line represent, from left to right, different stages in the development of the diaphragm from an essentially undifferentiated to a mature internode. The vertical columns show various stages of differentiation at various levels of the internode, both above and below the diaphragm. The last vertical column shows that all cells of the internodal pith have become differentiated except the two to four cells which constitute the diaphragm.