On the Seedling Structure of *Acer Pseudoplatanus*.

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With seventy-four Figures in the Text.

INTRODUCTION.

THE morphology of the later stages in the development of the embryo of the sycamore and the germination of the seed were worked out by Lubbock (11) and described in his classic memoir 'On Seedlings' in 1892, and since then it has frequently figured in elementary text-books as an example of epigeal germination. A further description, again morphological, of the polycotylous seedlings so frequently occurring in this plant is given by Thiselton-Dyer (15), whilst Tansley and Thomas (14) make a brief reference to its anatomy in a short abstract dealing with their work on the vascular structure of seedlings. A more detailed knowledge of the early anatomy of the normal seedling was a necessary preliminary to projected work on polycotylous and syncotylous specimens, and the present paper is primarily the outcome of that need.

THE FOOD RESERVES IN THE EMBRYO AND YOUNG SEEDLING.

The embryonic food reserves comprise at least four substances. The first of these, *starch*, is distributed through the whole of the general parenchyma of the embryo and rapidly disappears as growth proceeds, until, apart from that located in the starch sheath of the hypocotyl, it is absent by the time that the cotyledons are expanded. It has been recently shown by Briggs (2) that the sycamore seedling belongs to a type in which assimilates are produced immediately on exposure to light, so that these must be

utilized as rapidly as they are produced in addition to the stored starch of the embryo during the early phases of rapid growth. Following on the expansion of the cotyledons, which apparently coincides with the advent of full assimilatory activity, the formation of storage starch commences. This appears to be closely associated with the developing vascular structures, the major portion being situated in the outer part of the pith and the inner part of the cortex. As the secondary xylem of the hypocotyl develops, abundant starch also occurs in the xylem parenchyma and in the medullary rays.

In addition to the starch reserves there appear to be at least two *proteins*. During the early stages of the investigation free-hand sections were cut from embryos which had been preserved in methylated spirit, and it was noticed

FIG. 1. Cell from the cotyledonary parenchyma mounted 'dry'.

FIG. 2. The same cell irrigated with tap-water, showing the emulloid phase of precipitation.

FIGS. 3-5. Cells showing variations in the final phase of precipitation. The starch grains are indicated as black granules throughout.

that the cell-contents were obscured by numbers of globules which stained brown with iodine (Fig. 3). Subsequent investigation showed that the globules were also present in sections of material fixed in formalin or chromacetic fixatives, and of *fresh* material mounted in tap-water. It was not until the precaution was taken of mounting sections of fresh material in tap-water and examining at once that the clue to the formation of these globules was obtained. It was then discovered that they were the result of precipitation, presumably from colloidal solution. The first stage in precipitation is the development of a fine haziness in the cell-contents, succeeded by the formation of minute granules showing active Brownian movement (Fig. 2). The coalescence of these granules produces the globules (Figs. 3-5). Sections of fresh material cut and mounted in distilled water developed a haziness of the cell-contents similar to that of the preliminary stages of precipitation produced by tap-water, but no further precipitation was observed.

Sections were also mounted in dilute glycerol, and here precipitation in the form of globules was immediate, there being no emulloid phase. After some time the cell-contents became clear owing to the solution of the globules, and this suggested the probability that, with higher concentrations of glycerol, no precipitation would occur. Owing to the difficulty of obtaining anhydrous glycerol the 'pure glycerine' of the British Pharma-

copoeia was taken as a standard and the effects of varying dilutions of this on the precipitation phenomena were studied, with the results indicated in the following table :

<i>Parts 'Pure Glycerine', B.P.</i>	<i>Parts Aq. Dest.</i>	<i>Result.</i>
100	—	No globules formed.
75	25	No globules formed as a general rule : a few transient globules occurred in one section.
50	50	Immediate precipitation of globules, followed by rapid disappearance within twenty minutes.
25	75	Precipitation of globules almost immediate. Solution of globules complete in about two hours.
10	90	Precipitation of globules within a few minutes, followed by slow solution. Several hours elapse before solution is complete.

It would seem probable that the proteins are immediately soluble in glycerol which contains relatively small amounts of water, and that the explanation of the slow disappearance of the globules in the higher dilutions lies in the gradual concentration of the glycerol owing to the evaporation of the water. Although the usual chemical tests for proteins did not give clearly defined results owing to the obscuring effects of the general protoplasm, a number of digestion experiments were tried with sterile pepsin and trypsin extracts. Whilst the majority of the globules were dissolved by the pepsin some were resistant : all, however, were digested by the trypsin. This difference in behaviour indicates the presence of at least two proteins, and is borne out by the fact that whilst, when mounted in 10 per cent. KOH, all the globules disappear within 24 hours, the rate of disappearance is differential, some going relatively quickly, others very slowly, and also by the staining reactions, some staining much more deeply than others with iodine solution, whilst some take aqueous gentian violet well and others remain clear and refringent. Like the starch grains, the reserve proteins are generally distributed through the parenchyma of the embryo and young seedling : they are absent from older seedlings.

The third type of reserve substance occurring in the sycamore is of a *fatty nature*,¹ and was chiefly studied in sections which had been treated with trypsin solution to remove the proteins, and in some cases with diastase solution also to remove the starch. The fatty substance, which, like the proteins, occurs as globules, is also generally distributed, but appears to be specially abundant in and near the outer cell layers of the cotyledons. After preliminary treatment to remove the proteins, the action of various solvents was tested in two ways, namely, by immersing thin sections in each of the solvents for 24 hours and then staining with a specific stain, and also by staining with a specific stain and mounting in each of the various solvents

¹ The authors are indebted to Professor J. H. Priestley, of Leeds, for several helpful suggestions in this section.

under direct microscopic observation. Judged by these tests this substance is insoluble in alcohol, xylol, or benzene, but readily soluble in acetone, ether, and chloroform. It stains well with both Scharlach R and Sudan III, and gives an intense black with 1 per cent. osmic acid solution.

Although its general staining and solubility characters are sufficiently indicative of its fatty nature it does not agree entirely with either the true fats or the lipoids described by Cramer (7), and it may be that more detailed investigations will show that it is a mixture of substances.

THE VASCULAR SYSTEM.

(i) *The Embryo and Young Seedling.*

The earliest stage studied in connexion with the development of the

FIG. 6. Transverse section of a portion of the upper end of the hypocotyl of a very young seedling before the radicle has penetrated the testa. Note the isolated protoxylem (*px.*) and the first-formed metaxylem elements (*mx.*). $\times 750$.

vascular system was that of the embryo at the stage when the fruit is shed from the tree in autumn. There is no differentiation of the xylem at this stage, but the position of the vascular strands is clearly indicated owing to the presence of large secretory sacs in the phloem areas. Embryos examined in the spring show the earliest stage of xylem differentiation, the protoxylem being well marked and the first metaxylem, though very immature, also being present (Fig. 6). Each seed-leaf is traversed by five main vascular strands, namely, the midrib, two laterals, and two marginals (Fig. 7). Towards the base of the leaf the midrib divides into three parts, these consisting of a central isolated protoxylem with a group of metaxylem and phloem forming a collateral bundle on either side (Fig. 8). These collateral bundles *move outwards* and unite with the lateral and marginal bundle so that at the cotyledonary node there are two isolated median protoxylem strands with four collateral bundles in the diagonal planes (Fig. 9). At some dis-

tance below the cotyledonary node the xylem of each of the four diagonally placed bundles divides into two, so that at this level there are eight groups of metaxylem associated in pairs, each pair lying within one large group of phloem (Fig. 10). This arrangement of the vascular tissues is maintained

FIGS. 7-13. Series of semi-diagrammatic figures, showing the behaviour of the vascular strands during the transition from the cotyledons to the younger part of the root. Figs. 11 and 12 are from sections below the collet, and illustrate the leisurely manner in which root structure is attained.

FIGS. 14-21. Fig. 14. Young seedling from which the sections illustrated in Figs. 15-21 were drawn. Fig. 15. Cotyledonary midrib. Fig. 16. Bifurcation of midrib. Fig. 17. Isolated protoxylem and one diagonal bundle at the top of the hypocotyl. Fig. 18. Portion of section from the middle of the hypocotyl: the xylem of the diagonal bundle has divided into two. Figs. 19-21. Successive stages in the formation of the cotyledonary root pole. Fig. 21 is near the lower limit of differentiation. (Figs. 15-21 $\times 600$.)

in the very young seedling to the lower limits of differentiation, thus showing that the bulk of the initial growth is intercalary in character. The epicotyledonary leaves, two in number, are very minute at this stage and show very little differentiation of tissues. At the cotyledonary node six desmogen strands are present in the intercotyledonary plane, these being in two groups

of three, one associated with each of the two leaves. In older seedlings (root $\frac{1}{2}$ "– $\frac{3}{4}$ ") (Fig. 14) the structure of the hypocotyl is essentially the same, but it is noteworthy that secondary thickening is very evident in the cotyledonary midrib (Fig. 15) and is beginning at the upper end of the hypocotyl (Fig. 17) whilst the protoxylem is already disorganizing. The transition to root structure takes place *below the collet* and proceeds very slowly with the ultimate establishment of tetrarchy (Figs. 11–13). During the assumption of root structure the metaxylem groups flanking the cotyledonary plane gradually approach the protoxylems in that plane and fuse with them, thus constituting two root poles (Figs. 19–21). This method is constant in all seedlings. The behaviour of the strands concerned in the formation of the root poles in the intercotyledonary plane is, however, somewhat variable. In some seedlings a central protoxylem group appears in the intercotyledonary plane, the adjacent metaxylems fusing with this to form a root pole. In other cases there is no such differentiation of a central isolated protoxylem, but the two metaxylems simply approach one another and after fusion become organized into a normal root pole. The approximation of the metaxylem groups may be accomplished by the tangential development of elements in continuity with the main body of elements, or in many cases elements may appear irregularly on the inner side of each metaxylem group, first in isolation, then linked up with the main group.

The tetrarch root in all cases possesses a large pith throughout its whole length.

It is interesting to note that from the time that the cotyledons are unrolled the bulk of the cotyledonary xylem (Fig. 22) and that associated with it in the hypocotyl are secondary and the primary elements occupy a subordinate position in the conducting system. The six plumular strands connected with the two epicotyledonary leaves show little differentiation at this stage. The median strand consists of a fairly well developed phloem group, whilst the development of the xylem is just beginning, a single element appearing within the phloem. The two lateral strands, which are small and undifferentiated, move outwards to join the adjacent diagonal phloem groups. The behaviour of the median strand is very variable. In some cases it bifurcates, each half moving across as a small collateral strand to join the adjacent lateral strand, whilst in others the phloem alone divides and the xylem remains central to the lowest level of differentiation. In other cases, again, the bundle does not divide at all and moves as a unit to the right or left. Even in the same seedling the behaviour is inconstant, one midrib behaving in one way and one in another. The plumular xylem does not appear to have any influence whatever on the formation of the intercotyledonary root poles, since many seedlings occur in which the plumular midrib forks at the cotyledonary node or moves laterally, whilst a new intercotyledonary root protoxylem is organized below the collet.

(ii) *The Anatomy of the Older Seedling.*

The period following the full expansion of the cotyledons is one of rapid anatomical change which is to be correlated with the growth and expansion of the plumular structures. The change, as far as it affects the hypocotyl, consists essentially of the development of a purely cauline system of secondary xylem, the upper portion of which connects directly with the epicotyledonary leaf-traces. The cotyledonary vascular supply and its connexions have attained practically their full dimensions at the beginning of this period and only add slightly to their bulk subsequently. The further vascular history of the seedling is one of steady change in the relative importance of cotyledonary and epicotyledonary constituents, the former

FIG. 22. Transverse section of the midrib of the cotyledon when completely unrolled. Note the disorganized and partly resorbed protoxylem and the fact that the bulk of the functional xylem is secondary. The large cells are secretory sacs. $\times 750$.

assuming a subsidiary position as the latter increase in size. Although it is impossible to correlate precisely the various stages in vascular development with the morphological changes, since these vary slightly from seedling to seedling, the broad features are as follows: At the time that the cotyledons are first expanded the isolated cotyledonary protoxylems are disorganized and in some cases partly resorbed (cf. Fig. 15), but are still recognizable. This disorganization and resorption is shared to some extent by the older elements of the primary metaxylem which lie on the inner faces of the four large diagonally situated bundles. The epicotyl, which has not yet begun to elongate, contributes six well-differentiated strands to the hypocotyledonary vascular system, namely, the midrib and laterals of the first pair of epicotyledonary leaves. Near the apex of the hypocotyl the lateral bundles are reinforced by desmogen strands representing the vascular supply of the axillary buds of the cotyledons. The three epicotyledonary bundles of each side are united by a continuous band of phloem which, at a slightly lower level, connects with that of the cotyledonary traces (Fig. 23). The xylem of the lateral bundles also soon unites with that of these traces, but,

as was noted in the case of younger seedlings, the behaviour of the midrib is somewhat variable. In the majority of cases it bifurcates and the half-bundles move apart and fuse with the cotyledonary strands; but occasionally the phloem alone bifurcates, the xylem moving as a single unit to one side or the other. In none of the seedlings of this age examined does the midrib retain its original median position in the intercotyledonary plane. Below the level of fusion of the epicotyledonary bundles with those from the cotyledons the general vascular structure of the hypocotyl shows no significant change from that of the earlier stages and its elements are wholly associated with the cotyledonary traces.

In seedlings in which the first pair of leaves projects about a quarter of

FIGS. 23-6. Camera lucida outlines of hypocotyls from progressively older seedlings ($\times 20$). Fig. 23 is from a seedling in which the cotyledons are just fully expanded; Fig. 24, from one in which epicotyledonary growth has commenced; Fig. 25, from one in which the first epicotyledonary leaves are expanded; Fig. 26, from one in which the secondary pair of epicotyledonary leaves are expanded. The cotyledonary xylem is outlined; the epicotyledonary xylem and its connexions are solid black; the phloem is indicated by fine dots, and the pericyclic fibres by coarse dots.

an inch above the median fissure between the cotyledons considerable changes have taken place. The upper part of the elongating epicotyl shows six well-differentiated strands derived from the first pair of leaves and, alternating with these, six desmogen strands representing the vascular supply of the second pair of leaves. At a lower level fascicular cambium is present in the larger strands, whilst the smaller strands each possess a group of three or four xylem elements. The bundle fusions characterizing the epicotyledonary nodes, which will be described in detail below, occur in this instance at or near the apex of the hypocotyl, so that there are six large strands in two equal groups at this level. These are united at a slightly lower level by a band of secondary xylem, and as the diagonally placed cotyledonary bundles move inwards they come to lie at the extremities of the arcs of xylem so produced (Fig. 24). At or near this level the site of the now completely disintegrated protoxylem is frequently occupied by a small group of cambiform cells, whilst still farther down the hypocotyl the eight strands which result from the bifurcation of each of the cotyledonary bundles are linked by a thin but continuous zone of secondary xylem often only one cell in width. In some seedlings of this age the continuity of the secondary xylem is broken in the lower half of the hypocotyl, but in others it remains constant down to root level.

In older seedlings a steady increase in the thickness of the secondary xylem occurs, the cambium adding elements external to the cotyledonary system and linking up with a small collateral strand which fills the position formerly occupied by the isolated cotyledonary protoxylem in each half of the hypocotyl (Fig. 25). By the time the second pair of epicotyledonary leaves have expanded there is a continuous ring of secondary xylem five or six elements in width throughout the hypocotyl and the lower half of the first epicotyledonary internode (Fig. 26).

Accompanying these changes there occurs a development of pericyclic fibres. These first appear as clearly differentiated arcs in the hypocotyl lying outside the four diagonal cotyledonary bundles about the time the first epicotyledonary leaves are fully expanded. They are succeeded in older seedlings by others which develop both in the epicotyl and hypocotyl opposite the strands derived from the midribs of the first pair of leaves (Figs. 25, 26). It is interesting to note that traumatic stimulus may induce their formation in younger seedlings even though the wound is relatively slight and local in character. Somewhat deeper but still superficial wounds frequently result in an excessive development of secondary xylem adjacent to the injured area. The cork cambium is sub-epidermal in origin (Fig. 27). The period of its inception is variable, since many seedlings gathered in 1921, with the first pair of epicotyledonary leaves alone expanded, had a well-developed cork cambium, whilst in the wetter season of 1922 it was only just commencing to show in seedlings in which the second pair of epicotyledonary leaves were expanded. It first appears at the top of the hypocotyl and often involves the bases

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FIG. 27. Sub-epidermal formation of cork in the hypocotyl: the granular contents of three outer cells only shown.

FIG. 28. Pericyclic cork formation in the region of the collet involving more than one layer of cells. Immediately outside these is a layer of crushed and disorganized cells (indicated in solid black), and beyond these the dead outer cortical tissues.

FIG. 29. Pericyclic cork formation in the root. The layer of solid black indicates the same region as in Fig. 28.

of the cotyledons, the cells of which divide to a depth of several layers. From this region it spreads downwards and in some cases is remarkably irregular, frequently leaving one or two vertical strips of green tissue bounded by the epidermis when all the rest of the hypocotyl shows the characteristic grey-brown resulting from cork formation. The cork cambium in the root is pericyclic in origin, as is normally the case (Fig. 29), but it is interesting to note that in the collet, where the pericycle is becoming several

cells deep, more than one layer of cells is involved in the subdivision to form cambiform elements (Fig. 28). We were unable to detect any indication of junction between the pericyclic cork cambium of the root and that arising sub-epidermally in the stem.

(iii) *The Vascular System of the Leaf and Petiole.*

The midrib, at the tip of the leaf, is represented by a small collateral strand which, as it is added to by lateral veins, gradually forms a shallow

FIGS. 30-5. Diagrammatic figures illustrating the building up of the petiolar vascular system. Figs. 35 A, B, C show the common variants derived from Fig. 34. The bundles *a* and *b* are lateral bundles which may undergo displacement near the base of the lamina, and *d* and *e* are portions of the adaxial bar which may undergo similar alterations in position: the bundle *x* is one which is occasionally displaced at a higher level.

gutter-shaped system of collateral bundles the concavity of which is adaxial (Figs. 30, 31). At a lower level, as further contributions from the lateral veins occur, it becomes 'C'-shaped, as seen in transverse section (Fig. 32). The incurving of the free margins appears to be due to the displacement towards the adaxial side of some of the components from a higher level and, nearer the basal portion of the lamina where the largest lateral veins join the midrib, results in the formation of a closed ring of bundles (Figs. 33, 35 B). The adaxial portion of this closed ring is flattened and undergoes considerable secondary thickening, so that it is sometimes difficult to determine the limits of the original components. Whilst the formation of the closed ring is characteristic of all the leaves examined, a number show from one to three small internal strands in addition. These may occasionally consist only of phloem (Fig. 51 *a*), but are usually collateral and show an inverted orienta-

tion compared with the adaxial portion of the outer ring. Their formation is due to further bundle displacements, and is apparently the result of the compression of the adaxial portion of the ring or the lateral bundles on either side of it (Figs. 35 A, 35 C). This compression causes an infolding or displacement of vascular tissue from the part affected to the inside of the ring, the xylem and phloem rotating during their passage inward, so that they come to have an inverse orientation to the system from which they are derived. More complex internal bundle systems of the same type often occur in the vascular systems of the leaves of mature plants, and earlier observers appear to regard the more elaborate types as an expression of greater size and vigour. Thus Col (4) says, 'Le pétiole des plus larges feuilles montre dans sa moelle un arc, surmonté d'un plus petit, ayant tous deux leur bois tourné en avant. Dans d'autres pétioles, il n'y a qu'un arc interne. D'autres encore ont montré un très petit nombre de fascicules médullaires. Ainsi, sur quatre feuilles d'une pousse d'automne, toutes offrent au milieu du pétiole, un ou trois faisceaux libéroligneux médullaires très petits.' The size of the leaf, in the early stages at any rate, does not seem to us to have any direct bearing on the presence or absence of internal bundles, since we have noted their absence in the larger of one of the first pair of leaves and their occurrence in the smaller, and also their occurrence in seedling leaves distinctly below the average size.

The vascular grouping prevailing at the base of the lamina is maintained throughout the greater part of the petiole, but the adaxial portion often forms a continuous bar, or two contiguous bars, as a result of secondary thickening. The first indication of rearrangement is shown by the internal bundles where these are present. Although too small a number of petioles showing this feature were examined to enable us to make a general statement, it appears to be the rule that, as the base of the petiole is approached, the internal bundles undergo a reversal of the series of changes which resulted in their formation. Thus those derived from the central portion of the adaxial system rotate and reunite with this (Fig. 54), a gap being developed to admit of their insertion, whilst those derived from the lateral portions of the ring behave similarly.

As the base of the petiole is approached there is a considerable increase in its transverse diameter, and this is accompanied by a series of bundle fusions which result in the passage into the stem of three bundles from each leaf (Fig. 40). These bundle fusions are somewhat complex, but they always involve two processes. These are (i) the concentration and fusion of the lateral bundles, and (ii) the moving outwards of the bundles constituting the adaxial system and their union with one or another of the abaxial series. The regroupings may be arranged in a series showing progressively greater complexity, although there is no evidence that this constitutes an evolutionary sequence.

The simplest type, (a), which is relatively rare, is that shown in Figs. 36-9, in which the adaxial bar divides into two bundles, each of which rotates and unites directly with the midrib. An advance, (b), on this type

FIGS. 36-42. Camera lucida outlines of successively lower sections through the young plumular bud, showing the simplest type of bundle fusions occurring at the base of the petiole (Figs. 36-40) and the passage into the epicotyl of the three bundles from each leaf. $\times 20$.

FIGS. 43-9. Transverse sections through a petiole in which a slightly more complex type of fusion of the adaxial bundles with the abaxial constituents occurs. Figs. 44-9 are from the petiole which is fellow to that shown in Fig. 43, the adaxial bundle on the right behaving similarly, whilst that on the left shows a still further complexity. (Camera lucida outlines $\times 20$.)

is shown by those petioles in which each of the two bundles derived from the adaxial bar rotates and unites with a small lateral strand adjacent to the midrib, the compound strand thus formed then uniting with the midrib (Fig. 43). A still further advance, (c), is illustrated by the division of each of the two original bundles derived from the adaxial bar (Figs. 54, 55). Of

the four bundles so produced the two more centrally placed ones rotate and unite with small lateral strands which in turn join the midrib as in the previous type. The two distally situated bundles also rotate, but unite with strands farther from the midrib which contribute to the formation of the large laterals passing into the stem. An additional complication is introduced by the fact that irregularities occur, not only in any two petioles

FIGS. 50-5. Transverse sections of a petiole in the upper part of which three feebly developed medullary bundles occurred locally, but died out at a lower level without fusion. The one persisting longest (Fig. 51a) was represented by phloem only for the greater part of its course. Figs. 54 and 55 show the bundle fusions near the base of the petiole. Fig. 51a $\times 750$, the remainder $\times 20$.

constituting a pair, but even on opposite sides of the same petiole. Such a case is shown in Figs. 44-9, in which the adaxial bundle on the right behaves in the manner described under (b) above, whilst that on the left bifurcates and its halves behave in the manner described under (c), but with the further peculiarity that the two bundles to which they unite are in their turn produced by the bifurcation of a parent bundle (Figs. 46-9).

A further irregularity is occasionally produced by the union of the whole of one of the adaxial bundles with a strand which forms part of the main basal lateral instead of its uniting with one supplementing the midrib (Fig. 58). Whatever the type of bundle fusion, the ultimate result is always

the production of three bundles at the base of the petiole, so that six bundles enter the epicotyl at each node (Fig. 40), these alternating with the six bundles entering the stem from the node above (Fig. 42).

FIGS. 56-9. Transverse sections of a petiole, showing a single asymmetrically situated medullary bundle (Fig. 56) which at a lower level unites with half the adaxial bar (Fig. 57). Figs. 58 and 59 show differing methods of bundle fusion on opposite sides of the same petiole. (Camera lucida outlines $\times 20$.)

FIG. 60. Diagrammatic representation of the vascular system of the young seedling shown as if cut through in the intercotyledonary plane and spread out flat. M., midribs; L., laterals.

The bundles derived from each pair of leaves, with the exception of those at the first epicotyledonary node, travel down through two internodes. At the base of the second internode each bundle bifurcates to admit of the

insertion of the six bundles from the leaves arising at that node (Fig. 41). The half-bundles so produced move apart and unite with the bundles entering the stem at the previous node. The bifurcation of the midrib bundles is always somewhat precocious compared with that of the laterals (Figs. 41, 60), this admitting of the insertion, on the adjacent faces of the two half-bundles, of the strands representing the vascular supply of the axillary buds. The six bundles entering the epicotyl at the first epicotyledonary node only traverse a single internode before entering the hypocotyl. The laterals unite with the diagonally placed cotyledonary metaxylems *without bifurcation*, whilst the midrib, as has been stated previously, generally bifurcates, its halves behaving similarly. The six strands from the second epicotyledonary node are also involved in the bundle fusions occurring at the apex of the hypocotyl. Of these the midribs bifurcate in the usual way, but become joined to the laterals from the node below a little earlier than is generally the case. No division of the laterals has been detected, but they appear to move outwards with the half-bundles of the midribs of the first epicotyledonary node and so link with the cotyledonary strands. It is, however, somewhat difficult to determine their exact fate, since by the time that they are sufficiently differentiated in this region the limits of the individual bundles are obliterated by secondary thickening.

ABNORMALITIES.

Of the fifty-three seedlings examined, nine show some degree of departure from the normal in the behaviour of their vascular strands during transition and are best dealt with individually. With two exceptions they may be arranged in two groups. In the first of these the abnormality is generally the result of the persistence of plumular contributions, chiefly phloem, beyond the upper part of the hypocotyl and in some cases down into the root. It is not always possible, however, to refer the abnormality to plumular strands (e. g. in seedlings *E* and *H* below).

In the second group the abnormality takes the form of the development of accessory groups of metaxylem in the root, which may or may not give rise to additional root poles.

Group 1.

Seedling A. This is a very young seedling in which the plumular elements are not well differentiated. A single phloem group appears in the intercotyledonary plane of one side and after persisting for some time dies out.

Seedling B. This is also very young and is characterized by two groups of phloem in the intercotyledonary plane on opposite sides of the stele, one of these developing at a lower level than the other. Both persist to the

limit of differentiation, but this seedling is too young to determine the effect of the persistence of the phloem groups on the root structure.

Seedling C. This is an older seedling of the same type as seedling *A*, the phloem of one of the plumular midribs persisting throughout the hypocotyl and below the collet, though subsequently dying out. Preparation for the formation of the intercotyledonary root poles is evident below the collet, small xylem elements appearing associated with the adjacent margins of the lateral metaxylem groups. These are separated at first by the intrusive plumular phloem, but approach each other after this has disappeared.

Seedling D. This is similar in type to seedling *C*, except that the phloems of both plumular midribs are persistent to the limits of differentiation.

Seedling E also resembles seedling *C*, but in this case the persistent plumular strand consists of both xylem and phloem, a small collateral vascular strand appearing therefore in the intercotyledonary plane throughout the hypocotyl. As in the seedlings previously described, small xylem elements appear on either side of the persistent bundle in preparation for the formation of the intercotyledonary root pole, the xylem of the bundle bifurcating, and the halves moving towards these small elements. Meanwhile, a group of phloem has appeared on the other side of the axis and small xylem elements are differentiated on either side of this second phloem also. At the limit of differentiation these arrangements still prevail, but the phloem group which was last to appear is becoming much reduced in size and seems about to disappear.

Group 2.

Seedling F. In this seedling normal structure obtains throughout the hypocotyl and the upper part of the root. When the organization of typical root structure is almost completed, however, a group of metaxylem appears lying inside the phloem between a cotyledonary and an intercotyledonary pole. This does not cause any disturbance of root symmetry, and in the lowest sections is approaching the adjacent cotyledonary pole.

Seedling G is obviously of the same type as seedling *F*, and like it shows normal structure in the hypocotyl and upper part of the root. At this stage, however, a fifth xylem group appears within one of the four phloem groups. The phloem bifurcates and a fifth root pole is established, this being followed at a slightly lower level by a sixth pole developed as the result of the intrusion of a new xylem group directly opposite the fifth. Hexarchy prevails throughout the remaining part of the root.

Seedling H, though showing distinct affinities with the two seedlings of Group 2, has additional peculiarities of considerable interest. The behaviour of the epicotyledonary strands is normal, as is that of the cotyledonary strands, except that the central isolated protoxylems die out. One appears again sporadically as one or two small elements throughout the hypocotyl

and only becomes well defined in the region of the collet. The site of the other is occupied, a little way below the apex of the hypocotyl, by a small but well-defined collateral bundle which persists for a little over a centimetre but gradually dies out. As it disappears a similar bundle appears in the intercotyledonary plane at one side and persists for about a centimetre and a half before it too dies out. Meanwhile, the four diagonally situated xylem groups, with one exception, have each divided into two in the normal way. The one showing delay is in close proximity to a wound involving the greater part of the cortex in that particular part of the hypocotyl, and

FIGS. 61-7. Series of semi-diagrammatic outlines, showing the transition features of seedling *J*. The small crosses in Figs. 63 and 64 mark the positions of the single protoxylem-like elements which appear sporadically in the hypocotyl; the arrow in Fig. 64 marks the position which would be occupied by *Cpx. 2* if it had persisted.

the traumatic stimulus has led to a precocious development of secondary xylem which obscures the behaviour of the affected strand. Transition to root structure, however, apart from this disturbing factor, is typical. Subsequently there appear, at slightly different levels, three xylem groups lying inside three of the four root phloems and, apart from the repetition, resembling the condition found in seedling *F*. Of the three groups of xylem one persists for about half a centimetre and then dies out, whilst the other two are still well developed in the lowest sections obtained.

Seedling J is one which stands quite apart from the remainder and is peculiarly complex. Only one epicotyledonary leaf is developed, the fusion of the cotyledons being slightly earlier on the side opposite to this. In one cotyledon the vascular strands behave in the normal manner, so that a protoxylem strand (*Cpx. 1*) and two widely separated collateral groups (*A* and *B*) enter the hypocotyl from it. In the second cotyledon the vascular strands of one side fuse in the usual manner to form one large bundle (*C*), whilst on the other side the half-midrib bundle fuses with the

lateral bundle only, producing a compound strand (*D*) the marginal (*E*) remaining independent. From this cotyledon, therefore, a median protoxylem strand (*Cpx. 2*) enters the hypocotyl with a single collateral (*C*) on one side and two such strands (*D* and *E*) on the other. A few small xylem elements become detached from the inner side of the marginal strand (*E*) and move away from the parent group until they occupy a position midway between strands *D* and *E*. Although at first they lie within the phloem of the main bundle they ultimately resemble a protoxylem group closely (Fig. 63, *Px. 3*) and during the development of root structure behave as such. This condition is maintained for some distance down the hypocotyl, the only change being the division of the metaxylem groups *B*, *C*, and *E* into *B*¹, *B*², *C*¹, *C*², *E*¹, *E*²; the metaxylem group *A* does not divide until considerably later and then becomes resolved into two very unequal portions. During the passage of these half-bundles down the hypocotyl a solitary, small protoxylem-like element appears and disappears between *A* and *E* and a similar one, at a slightly lower level, between *B* and *C*. Towards the base of the hypocotyl the protoxylem from the cotyledon showing abnormal transition (*Cpx. 2*) dies out and the metaxylem group *C*¹ divides into two.

From this complex five root poles are organized as follows:

1. A normal cotyledonary pole is formed from *B*¹ and the bulk of *A*.
2. The remnant of *A* forms a second pole with *E*².
3. Strands *E*¹ and *D* concentrate on *Px. 3* to form a third pole.
4. Strand *C*² divides into two, half forming a fourth pole with *B*² and half forming a fifth pole with *C*¹: the remaining part of *C*¹, though persistent, takes no part in root-pole formation.

It is worthy of note that the poles formed by the remnant of *A* with *E*², and that of *B*² with *C*², occupy the positions of the isolated protoxylem-like elements which appeared and disappeared higher up in the hypocotyl.

DISCUSSION.

In considering the possible theoretical significance of the seedling anatomy of the sycamore it will perhaps be well to outline the ontogenetic succession which Chauveaud (3) has shown to be characteristic of a large number of angiosperm seedlings. This investigator has established the fact that the vascular elements pass through a series of phases which are remarkably uniform and which he regards as constituting a phylogenetic sequence. He considers that each xylem group has its phylogenetic origin in a radial file of vessels developing centripetally, the phloem at this stage being represented by a series of tangentially distributed elements occupying alternate radii to the xylem. This condition is termed the 'disposition alterne'. The xylem and phloem elements developed subsequently to those exhibiting

the 'alterne' grouping show a characteristic alteration in relative position. In this phase, showing what Chauveaud terms the 'disposition intermédiaire', the xylem vessels arise in a tangentially extended series to the right and left respectively of the plane occupied by those of the 'alterne' phase, whilst the new phloem elements develop in a plane roughly parallel with the earlier ones, but are situated nearer the xylem. In continuity with the latest formed xylem elements of the second phase ('disposition intermédiaire') arise other elements developed in a centrifugal series, whilst the new phloem elements lie still nearer the xylem and thus lie on the same radii as the centrifugal vessels. As a result of this, two collateral bundles are formed, one on either side of the original plane of xylem differentiation. This third phase is termed the 'disposition superposée'.

The xylem of all three phases may be represented at one time, or the development of each of the later phases may be accompanied by the disappearance of the preceding one.

In the absence of the first phase the elements of the second appear as two separate groups of vessels inclined to one another like the arms of the letter V, whilst the third phase, in the absence of the two earlier ones, takes the form of two quite separate collateral bundles. Chauveaud has shown that in the ontogeny of the seedling vascular system not only the root, but also the hypocotyl and even the basal portion of the cotyledon, may exhibit the 'alterne' grouping of the xylem and phloem. In the root this vascular grouping is persistent, and though further conducting elements possessing the 'intermédiaire' arrangement may augment the original units (e.g. *Phaseolus vulgaris*), they do not obscure its character. In the hypocotyl and cotyledon, however, it is far otherwise, since the development of each new phase is followed by the partial, or total, resorption of the vascular elements of the phase preceding it. The result is that in these organs the early stages in vascular development are only to be observed during the initial stages of germination and those which immediately follow it. Chauveaud considers that seedlings exhibit in their vascular development an acceleration from below upwards, so that whilst the primitive exarchy is retained in the root it is transient in the aerial parts of the seedling, this transient character being shared to a less degree by the intermediate elements. Where this acceleration is accentuated it leads to the total elimination of the earlier phases from the ontogeny in the upper portion of the hypocotyl and in the whole of the cotyledon and to their early resorption at lower levels. It will thus be seen that, according to Chauveaud, the differences in orientation exhibited during the transition from the radial distribution of the xylem and phloem in the root to their collateral distribution in the stem are simply the result of the persistence of only the phyletically more recent stages at successively higher levels of the hypocotyl and cotyledons.

Whilst Chauveaud's theory supplies a consistent explanation of the

march of events in the vascular development of many seedlings, there are a number which present difficulties requiring further elucidation and explanation. The chief of these are two in number, and reference has already been made to them by previous investigators. The first is the difficulty presented by the behaviour both of the protoxylem and metaxylem which constitute the intercotyledonary poles in tetrarch roots, and here, as Dr. E. N. Thomas (16) points out, Chauveaud's theory breaks down to some extent, the metaxylem of these poles being 'continuous with the "elements superposés" of the lateral strands of the cotyledon'. Although the collateral segments may be almost wholly concerned in augmenting the cotyledonary vascular supply, they frequently combine this function, as Compton (6) has shown, with that of ensuring a closer contact with the epicotyledonary strands. Occasionally, as in *Juglans nigra* (8) and *Caesalpinia sepiaria* (6) for example, this function may be the only one, and the intercotyledonary root poles are then directly continuous with the epicotyledonary traces and contribute no elements to the cotyledons. This extreme condition is one which is frequently associated with hypogeal germination, and the fact of its occurrence raises the question as to what extent the intercotyledonary root protoxylems generally, in tetrarch types, are referable to epicotyledonary sources. Miss Davey, in a paper dealing with the seedling anatomy of the *Amentiferae* (8), suggests that this may be the case in certain species (e.g. *Myrica Gale*, *Alnus*, *Carpinus*) even though direct connexion between the epicotyledonary protoxylem on the one hand, and that of the root on the other, cannot be established. This lack of continuity is held to be due to the delay in plumular development. As a result of this delay 'differentiation is not complete at the upper end of the seedling, so that the protoxylem centre "dies out" in ascending the hypocotyl, and actual connexion cannot be established'. The same author (8, p. 598) cites Compton as describing a similar distribution of the intercotyledonary vascular units between cotyledons and plumular leaf in the Leguminosae 'as a phenomenon of replacement in which the cotyledons are being supplanted by plumular leaves'. It would appear, however, that Miss Davey had misinterpreted Compton's views, since this investigator (6, pp. 102-4), after stating the following alternatives: '(i) The plumular traces may tend to replace the intercotyledonary root poles, or vice versa their relation to one another being *complementary* and one of supplantation. (ii) The intercotyledonary xylem may combine with the leaf-trace to form a joint conducting channel, their relation to one another being *supplementary*,' and after discussing the evidence for each, pronounces in favour of the latter.

The evidence in the case of the sycamore seems definitely against the view that the intercotyledonary protoxylems are plumular in origin. In the majority of cases the midrib bundles fork at a relatively high level in the

hypocotyl, though they may move laterally as a whole to join the adjacent cotyledonary strands, and subsequent to such displacement it is difficult to see how the intercotyledonary root protoxylems which appear below the external collet can be referred to them. Further, when, as in some of the abnormal types described in Group 1, the epicotyledonary midribs persist below their normal level, they appear to delay the formation of the intercotyledonary poles rather than assist in their development. It should also be noted in this connexion, although we do not wish to stress the point, that in the abnormal seedling *J*, although only *one* epicotyledonary leaf is present, *two* root poles are formed in the positions which would normally be occupied by the intercotyledonary poles.

The second criticism of Chauveaud's theory was originally put forward by Compton (5), who pointed out that the sequence of alternate, intermediate, and superposed phases is not realized in types such as *Lupinus hirsutus*, in which, at the base of the cotyledon, an isolated median protoxylem is present, flanked on either side by a widely separated collateral bundle, from which it may be separated by as many as fifteen parenchymatous cells. It is equally evident that this sequence is not realized in the sycamore. If the upper part of the hypocotyl is examined in the very young seedling (Fig. 6) it is found that the formation of the central protoxylem is followed by the development of metaxylem elements in the diagonal plane, and that a series, often exceeding twenty, of parenchymatous cells intervenes between the protoxylem and the metaxylem. It requires a good deal of faith to believe that this parenchymatous gap represents a region occupied ancestrally by intermediate xylem elements, and the facts seem to be met better by a different explanation.

We may say, generally speaking, that in each seed-leaf of a dicotyledonous seedling the vascular components consist of a midrib and a lateral system (Fig. 68). These may react on one another in various ways. Thus there may be a *median concentration* of vascular components leading to their aggregation in the cotyledonary plane so that *two compound strands* enter the hypocotyl (Fig. 69 *a, b*); alternatively the midrib and laterals may remain independent so that *six simple strands* enter the hypocotyl, two in the cotyledonary plane and four in the diagonal planes (Fig. 70 *a, b*). Finally, there may be a *lateral concentration* of the vascular components so that the metaxylem and phloem of the midrib are detached from the central protoxylem, each half moving outwards and frequently uniting with its adjacent lateral. In this event *four compound strands* will enter the hypocotyl in the diagonal planes, whilst the cotyledonary plane will show two isolated protoxylems (Fig. 71 *a, b*). Any of these three types of grouping may produce either a diarch or a tetrarch condition in the root, so that there does not appear to be any close connexion between the number of root poles and the type of vascular arrangement found in the hypocotyl.

Of the three types we believe the last to be the most recently evolved, and to be the one which gives the clue to the origin of the majority of those forms showing diagonal tetrarchy in the root (e. g. *Calycanthus*).

We consider that the sycamore exhibits this lateral concentration and that there has been *a definite moving outwards* of the major portion of the midrib constituents. It represents a relatively early stage, however, of this line of evolution, and the vascular components forming the compound diagonal bundle are soon dissociated, probably in part at least owing to the importance of the laterals which form the intercotyledonary poles. We may trace the later stages in a number of seedlings. In *Lupinus hirsutus*,¹ *L. albus*, and *L. mutabilis*, for example, the diagonal arrangement extends throughout the hypocotyl, and a still later stage is exhibited by the seedlings of *Abronia* spp. (9). Here the diagonal grouping is not only continued through the whole of the hypocotyl, but involves the upper part of the root. The result is that well-developed root poles are developed in the diagonal position, whilst only a rudimentary pole, represented by the isolated protoxylem, is present in the cotyledonary plane. This condition may be persistent, but more usually there is a gradual return to the older symmetry and the diagonally situated poles dwindle whilst the cotyledonary poles become progressively more important, so that the tap-root is ultimately diarch. The situation is complicated somewhat in *Abronia* owing to the fact that the lateral concentration only affects one cotyledon, the other, which is much reduced in size, showing median concentration (Fig. 73 a). The final term in this series is shown by forms like *Calycanthus*. Here the diagonal position is permanent throughout the seedling, so that four diagonal root poles occur without normally any reversion to an ancestral diarchy. It is interesting to note, however, that Thomas (16) records a reversion to the diarch state as an occasional abnormality in *Calycanthus*. A further point of similarity in the series is also worthy of mention. There exist in *Calycanthus* a number of small secondary collateral bundles at the base of the cotyledons between the widely separated diagonal strands, these appearing quite soon after germination has commenced. A single strand of a similar character is frequent in *Lupinus mutabilis*, though here it appears later, and it is also present as a rare abnormality in the sycamore. There is thus good reason for regarding the series *Acer-Lupinus-Abronia-Calycanthus* as illustrating a well-marked tendency in seedling anatomy.

It is not proposed to discuss in this paper the possible significance of the vascular structure of the leaves and epicotyl, as it is felt that more work of a comparative character is necessary before its real bearing can be adequately determined.

¹ Thomas (16) records a number of species showing a condition similar to that found in *Lupinus hirsutus*, e. g. *Decaisnea Fargesii*, *Laurus Sassafras*, *Liquidambar styraciflua*.

FIGS. 68-71. Diagrams illustrating the three types of transition from the cotyledons to the hypocotyl described in the text.

FIGS. 72-4. Comparative diagrams illustrating the transition features in *Acer*, *Abronia* and *Calycanthus*.

SUMMARY.

1. The development of the seedling vascular system of the sycamore, *Acer Pseudoplatanus*, is described from an early stage in its differentiation up to a period in which the epicotyl is beginning to assume a dominant rôle.
2. It is held to illustrate an early stage in the evolution of diagonal root symmetry of which the stable tetrarch stage is shown by *Calycanthus*.
3. An account is also given of the food reserves found in the embryo and young seedling. These include starch, a fatty substance, and at least two proteins, all of which are digested and utilized in the early stages of seedling growth.

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