AGRICULTURAL UNIVERSITY WAGENINGEN PAPERS
88-4 (1988)

Morphogenesis of primary vascular tissue and regeneration

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Received 27-09-1988
Date of publication 22-12-1988

Cip-data Koninklijke Bibliotheek, Den Haag

ISBN 90-6754-139-7

Printed in the Netherlands by Drukkerij Veenman b.v., Wageningen

Contents

Abstract

The morphogenesis of primary vascular tissue was studied of the vein endings in the leaves of *Hedera*. The observed differentiation has been related to the development of stomata and the transpiration stream. The sequence of characteristics of the developing of vascular tissues between adventitious buds and developing vascular tissues in the petioles of leaf cuttings of *Lunaria* has been studied accurately. Finally the morphogenesis of vascular tissue between the scion and stock and other regeneration phenomena in graftings of *Piper* species have been studied in detail. The place of first initiation of the promeristem of the adventitious bud and the course of differentiation of the vascular tissues in *Lunaria* and *Piper* regeneration processes are discussed in relation to the morphogenesis of procambium and apoplastic transpiration transport in the vein endings of the *Hedera* leaf. On the basis of the followed route of the differentiation process the theory is defended that the rapid apoplastic transpiration transport must play a major role in morphogenesis of primary vascular tissue.

1. Introduction

The transport in plant tissues is apoplastic as well as symplastic (e.g. Läuchli, 1976; Esau and Thorsch, 1985; Canny, 1986). The primary cell walls form an apoplastic space that is pervious to the transpiration stream (Tanton and Crowdy, 1972; Burbano et al., 1976; Pizzolato et al., 1976). The rate of apoplastic transport has been estimated to be about 50 times faster than the rate of symplastic transport; the apoplast forms the major pathway for water and the symplastic pathway is a very slow 'by-pass' (Weatherly, 1963, 1970; Boyer, 1985). In both the symplast and the apoplast stimuli and nutrients occur, the incidence of which is much lower in the apoplast (Madore and Webb, 1981; Minchin and Thorpe, 1984; Minchin et al., 1984). A continuous exchange of substances takes place between apoplast and symplast. Transport of the substances in the apoplast together with the transpiration stream seems unavoidable and the near vicinity of the cells probably can change quickly. Both pathways may become entirely specialized and during these differentiation processes the transport capacity is enlarged greatly. Magendans (1985) studied the differentiation processes of both pathways in the vein endings of young *Hedera* leaves. Leist (1976) mentioned the procambium as the supply route of substances to the cells of the plate meristem in a young leaf. The apoplast of the procambium will differentiate into tracheary elements and in this way into the largest volume of the apoplast in the xylem. The symplast in the procambium will differentiate into sieve elements and with that into an important part of the symplast in the phloem. In this article the results of three groups of experiments will be given and considered in the light of the importance of the transpiration stream for morphogenesis of vascular tissue.

2. Materials and methods

2.1 Plant material and culture conditions

The first group of observations was made on the veins of white leaves in totally white shoots of *Hedera canariensis* Willd.cv. Gloire de Marengo. The plant grew in a potometer in a conditioned growth cabinet (Weiss, W. Germany). The examined leaves grew in an atmosphere of 22.5 ± 1% r.h. and a light intensity of about 48 Watt/m² at plant level. The dark period was from 20.30 p.m. unto 08.00 a.m. with 39 ± 1% r.h. The temperature was controlled at 31 ± 1 °C during the light period and 21 ± 0.5 °C in the dark period.

The second group of observations was on glasshouse-grown leaf cuttings of *Lunaria annua* L. The cuttings were taken from two groups of seedlings: six weeks and twelve weeks after sowing. In each group of cuttings half of the number received a cold treatment of 5 °C during 12 weeks immediately after striking the cuttings in order to stimulate the regeneration process. In the first group of plants the first pair of leaves was used and of the other plants the second, third or fourth pair of leaves was chosen in order to get leaves of about the same size. The petiole was about 8 cm long and cut perpendicularly to the long axis with a sharp razor-blade and disinfected in a solution with tetramethylthiuramdisulfide (TMTD) before putting into the medium. The cuttings were raised in a mixture of peat and sand in one to two ratio and were sampled after three months and later.

The third group of observations was on graftings of *Piper nigrum* L. cv.'s Kaluvally, Kuching and Cheriakaniakadan, and *Piper hirsutum* Swartz as scion material on rootstocks of *Piper colubrinum* Link. These grafts were made and cultivated in a greenhouse of the Research Station for Nursery Stock and Urban Greenery in Boskoop, The Netherlands. Temperature could be adjusted to a value of 20 °C and the light period was 12 hours; relative humidity was kept above 65%. The veneer side graft method (Garner, 1979) was applied (Fig. 10). After making the grafts, at first the plants were placed under a plastic tent for about four weeks and after that the rootstocks were cut back and the grafts were hardened off to standard greenhouse conditions.

2.2 Microtechnique

For the first group of observations square leaf tissue pieces (25 mm²) were punched out of *Hedera* leaves near the base of leaves of various ages and immediately fixed in F.A.A. The air in the tissue was extracted, the tissue was dehydrated with the TBA method and embedded in paraplast (Lancer, Sherwood)

paraffin wax. Paradermal and transverse sections of 7 μm were made at various leaf ages. The sections were made with a Leitz rotary microtome and stained with safranin and fast green. All observations were made with a Wild microscope using oil immersion and 1,500 × magnifying optics.

For the second group of observations fixations with Randolph's modified Navashin fluid (Johansen, 1940) were made of the underside of the petiole of the *Lunaria* leaf cuttings at points of time dependent on the development of the regeneration processes. Microtoming was done with a sliding microtome without embedding. The plant material was put in a small block of wood with a hole in it of about the same shape and size as the piece of material. After that the plant material was attached to the block by melted paraffin, filling up the small spaces between material and wood. After placing in position the plant material and the paraffin around the tissue, the petiole had to be kept wet with ethanol. Serial transverse and longitudinal sections were made in this way of the underside of the petioles of the cuttings and of the thickened roots. Clearing of sections was done with Eau de Javelle and chloral hydrate. Suberized and cutinized cell walls were stained with Sudan III (Gerlach, 1969).

The third group of observations was done on F.A.A. fixed *Piper* grafts. After embedding in paraplast (Lancer, Sherwood) sectioning was carried out with a sliding microtome. Serial sections were stained with safranin and fast green. Observations were made with a Wild microscope, sometimes using oil immersion and 1,500 × magnifying optics.

3. Results

3.1 Initiation of vein endings in the leaf of *Hedera canariensis* Willd. cv. Gloire de Marengo

In the plate meristem of a young *Hedera* leaf (Fig. 1) the minor veins normally differentiate in the 5th cell layer. Differentiation of the vein endings proceeds over a period of leaf growth between 15% and 40% of the final length of the lamina (% F.L.L.) (Magendans, 1985). The transpiration stream in the young leaf is possible due to the development of the large intercellular spaces in the layers 6-11 of the plate meristem being coordinated with the initiation of stomata in the abaxial epidermis. At 15% F.L.L. functioning i.e. open stomata are already present. The vein endings (mostly 6th or 7th vein order, Fig.2) differentiate acropetally and show intrusive growth in the areoles (Magendans, 1985), surrounded by small veins that are of the 4th order mostly. Though the distal ends of the procambium strands of the free ends of the venation terminate somewhere above the (developing) stomata (Fig. 3), this relation is not very strict in the Hedera leaf. In the six cell layers between the differentiating vein endings and the stomata many large intercellular spaces develop. At the same time the transpiration transport takes place along them from the procambium of the vein endings at ± 30% F.L.L. (Magendans, 1985).

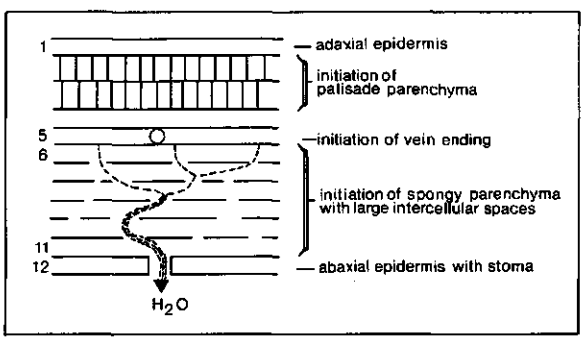

Fig. 1. Schematic drawing of transverse section of young *Hedera* leaf at about 35% of the final length of the lamina. The vein endings mostly differentiate in the 5th layer of the plate meristem; in this layer no intercellular spaces have been formed yet, except at the end of the vein ending. 1-12, layers of plate meristem. ----, apoplastic streaming pattern.

Fig. 2. Light micrograph of paradermal section of young *Hedera* leaf at 38% of the final length of the lamina. The free end of the venation consists entirely of procambium and is composed of three segments (1, 2, 3).

3.2 Initiation of adventitious buds in petiole and root of *Lunaria annua*

Adventitious apical meristems originate in a layer of periderm (Figs 4, 6c, d, 7a). This layer differentiates in the cooled cuttings almost entirely after cessation of the cold treatment; therefore no direct influence of the cold treatment on the regeneration process of adventitious buds could have taken place. Under

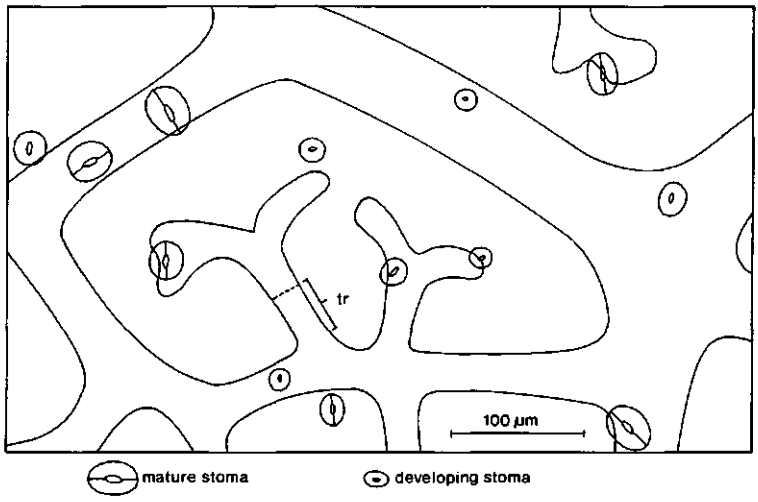

Fig. 3. Reconstruction by means of 34 paradermal sections of an areole in a young *Hedera* leaf at 29% final lamina length as seen from the abaxial side. The free ends of the venation at this age of the leaf consist entirely of procambium; only in the left ramified complex of free ends in the areole 1 tracheid (tr) is formed in the most basal part. Stomata are already developed; also younger and smaller stomata are present.

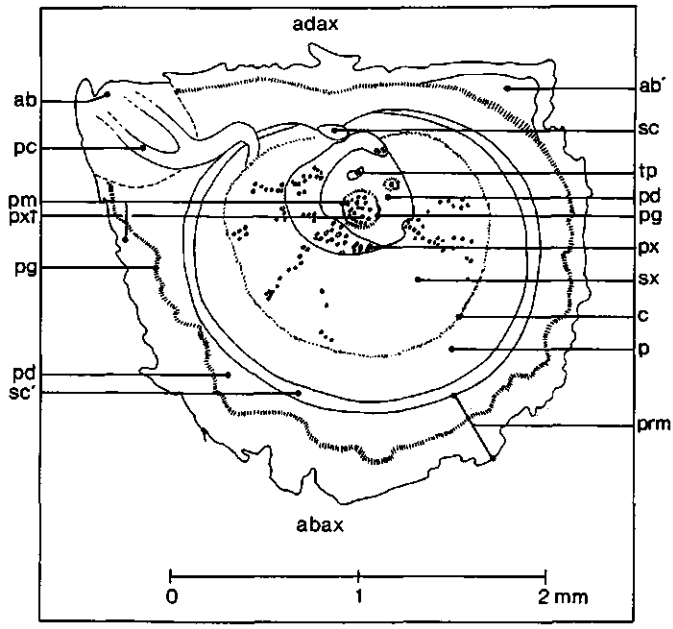

Fig. 4. *Lunaria annua*, transverse section of isolated median vascular bundle in the basal part of the petiole of a leaf cutting (cp. Fig. 8), four and a half month after striking the cutting (with cold treatment). A periderm layer originates from a starch sheath around the bundle while the other ground tissues of the petiole and the epidermis degenerated. Within the bundle the primary xylem and the adaxial group of sclerenchyma are still visible; in the primary xylem a periderm originated with some tertiary phloem in the phelloderm. Much secondary xylem and phloem were produced by the vascular cambium. Mostly at the places left and right of the adaxial side of the bundle adventitious buds were initiated in the phellogen. The connection between basipetally differentiating procambium of the bud and the vascular cambium of the petiole is easiest at places where the layer of unlignified sclerenchyma in pericycle and obliterated phloem is thin or absent locally. ab, adventitious bud; ab[1], ditto, grazed only; abax, abaxial; adax, adaxial; c, vascular cambium; p, primary and secondary phloem; pc, procambium; pd, phelloderm; pg, phellogen; pm, phellem; prm, periderm; px, primary xylem; px†, dead, isolated primary xylem; sc, sclerenchyma, often with some lignin; sc', sclerenchyma without lignin; sx, secondary xylem with few tracheary elements; tp, tertiary phloem.

the surface of the phellem at first an extra meristematic activity of the phellogen cells is found (Figs 5, 6a, b). The cells of the phellogen are periclinally smaller within the initiation and they contain much more cytoplasm. From these meristematic cells a young apical meristem with leaf primordia and procambium strands belonging to them arises (Figs 6c, d). The procambium joins under the apical meristem to one strand (Fig. 7b) that differentiates progressively to join the vascular cambium (Figs 4, 6d). This differentiation is hampered by a layer of thick walled elements in the pericycle and obliterated primary phloem (Figs 4, 7b; sc'). In a more adaxial direction this layer becomes thinner and after dividing of some cells with thin walls in this layer further differentiating of the procambium through pericycle and phloem in the direction of the vascular cam-

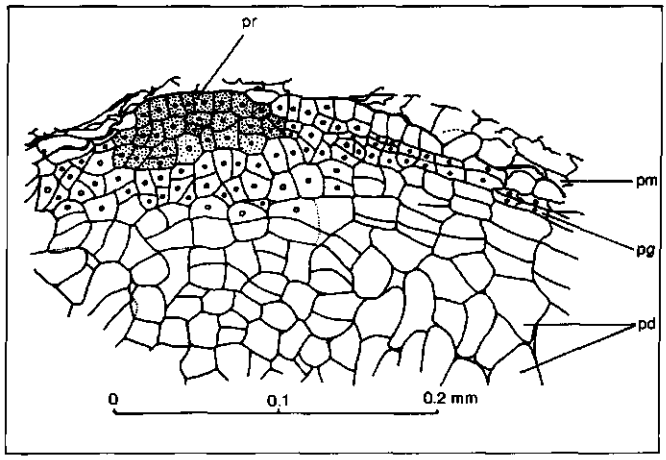

Fig. 5. *Lunaria annua*, initiation of adventitious bud in the phellogen. pd, phelloderm; pg, phellogen; pm, phellem; pr, protoderm of young apical meristem. The small rings indicate the cells of protomeristem (pr and below) and of phellogen.

bium has become possible. In Fig. 8 a transverse section of the basal part of the whole petiole is shown. Adjacent to one of the five regular vascular bundles an older adventitious bud has originated in the periderm layer around the vascular bundle. After examination of other transections of this young bud, the procambial development in the phelloderm became evident. At several places the differentiation of procambium in the direction of the vascular cambium has taken place, at first at the most adaxial side of the vascular bundle. This longest connection has already been accomplished and along this route the layer of cells with thick walls is absent. Other initiations of procambium differentiation to reach the vascular cambium along a shorter connective route were not yet successful (Fig. 8).

In Figs 7c, d, 9 a developing adventitious bud is shown. This bud originated in the phellogen of a rather thick periderm layer initiated in the pericycle of the thickened root. This root contains much parenchyma tissue filled with starch in phelloderm, phloem and secondary xylem. There is no layer with thick walled elements. The progressively differentiating procambium strand from the bud will reach the vascular cambium directly along a straight path, without meeting any obstruction (Fig. 7d). Also the transport of morphogens and nutrients into the direction of the developing primordium will have been along the same, shortest route and will keep this route afterwards.

3.3 Processes of graft union in *Piper* species

In Fig. 11 a small part is shown of a transverse section through a 44 days old grafting of two varieties of pepper as indicated in Fig. 10. Above the wound

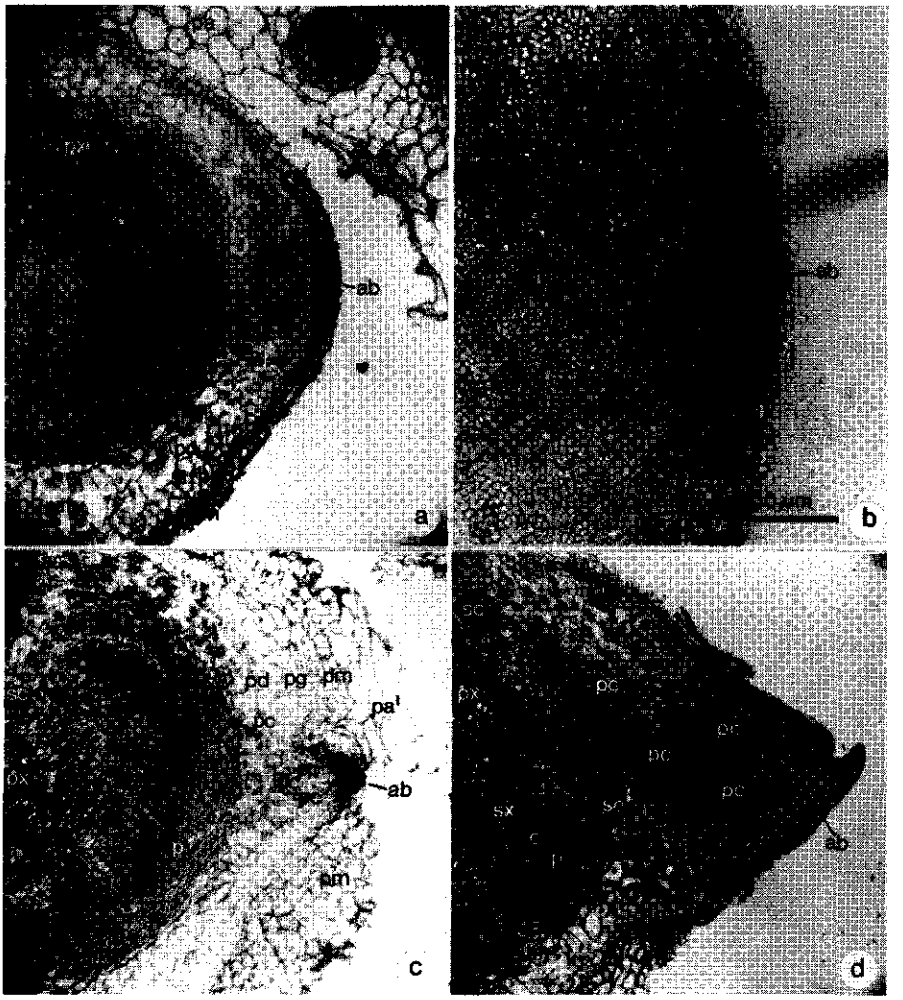

Fig. 6. Light micrographs of transverse sections of the basal part of the petiole of leaf cuttings of *Lunaria annua*. Adaxial side is above. a,b, three months old leaf cutting with initiation of adventitious bud in the phellogen at a thin spot or rupture in the phellem; b, detail, the protoderm of the young apical meristem lies at the surface while the phellem is interrupted; c, same section as a, b, other initiation of young adventitious bud below rupture of phellem against median vascular bundle of petiole; d, isolated median vascular bundle with adventitious bud initiated in the phellogen. For abbreviations see legend to Fig.4 and sc, group of sclerenchyma cells at the adaxial side of the vascular bundle; pa, parenchyma of ground tissue of petiole; pa', ditto, degenerating.

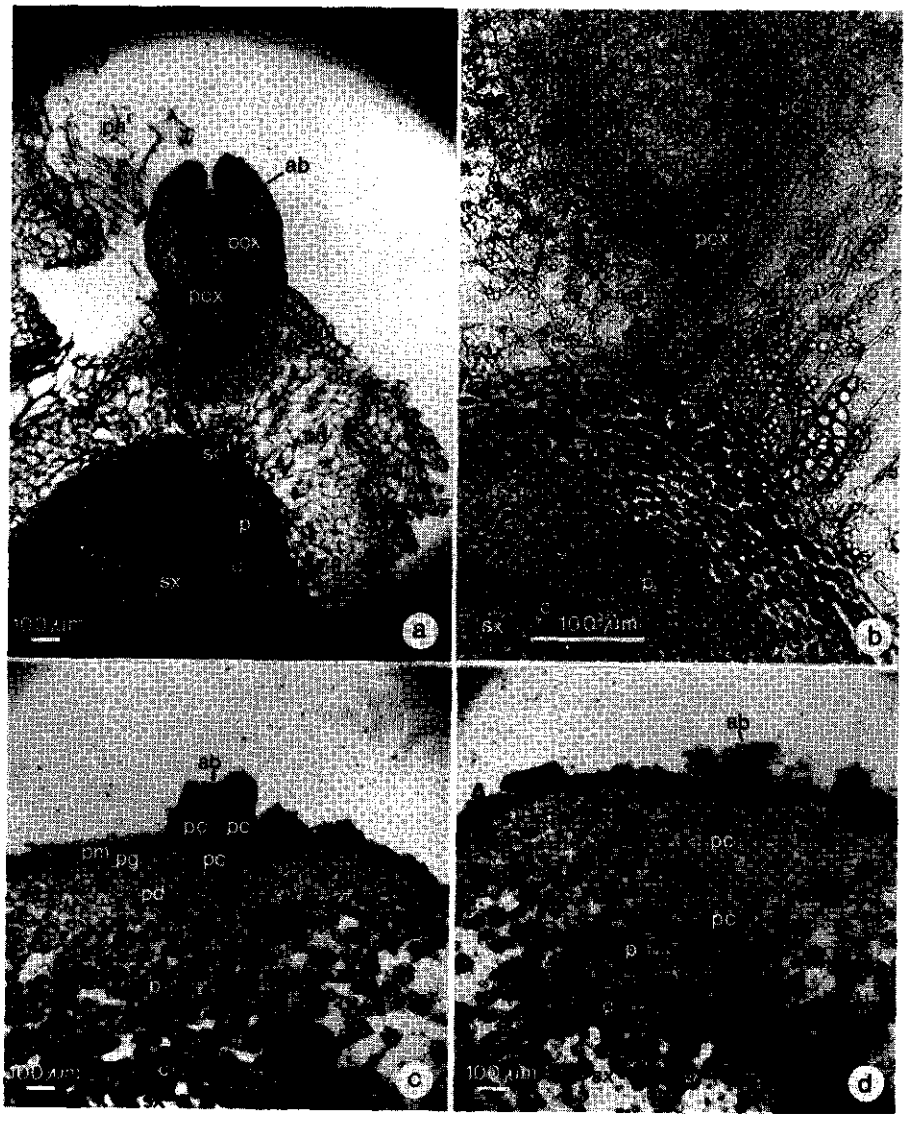

Fig. 7. Light micrographs of transverse sections of the basal part of the petiole with adaxial side to the left (a, b) and of an adventitious root (c, d) of three months old leaf cuttings of *Lunaria annua*. a, adventitious bud initiated in the phellogen around one of the bundles next to the median vascular bundle; b, detail, basipetal differentiation of procambium stops against the layer of sclerenchyma of pericycle and primary phloem and after that the differentiation is directed to the adaxial side; c, d, young adventitious bud initiated in the phellogen of a thickened root; c, section through the bud; d, section through the procambial strand differentiating basipetally to the cambium. For abbreviations see legend to Fig. 4 and: pcx, procambium in which tracheary elements differentiated.

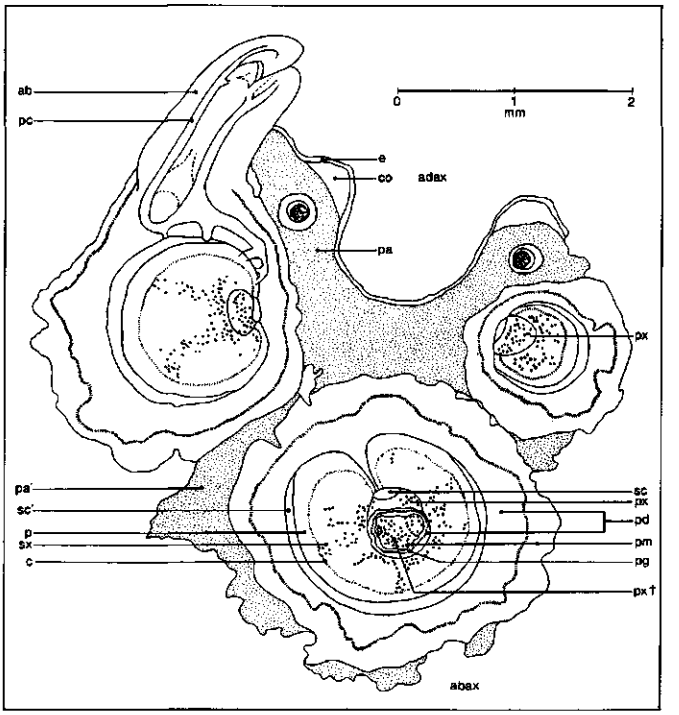

Fig. 8. *Lunaria annua*, transverse section of the basal part of the petiole with five vascular bundles of a leaf cutting, three months after striking the cutting (without cold treatment). The connection between basipetally differentiating procambium of the adventitious bud and the vascular cambium of the petiole succeeded only at the most adaxial position in which the pericycle layer of sclerenchyma without lignin is thinnest or absent. For abbreviations see legend to Fig. 4 and: co, collenchyma; e, epidermis; pa, parenchyma of ground tissue of petiole; pa', ditto, degenerating.

surfaces a part of the small slice of the rootstock is visible and under the wound surfaces a small part of the cut scion. In the zone 1-2 of Fig. 11 the wound surface of the cut cortex of the scion shows many dividing cells and a wound periderm has been formed. There was no possibility to realize a graft unification in this zone 1-2 because no tissue of the graft partner is found opposite this living parenchyma tissue.

In Fig. 13 a survey is given of scores of the reaction (division of cells) of all types of tissue that can be found in the wound surfaces of rootstock and scion owing to the grafting of an other type of tissue at the opposite place in the wound surface.

The reactions of the wound surfaces in scion and rootstock and their interactions can be summarized as follows. The living cells in epidermis, cortex and central cylinder (vascular bundles and surrounding parenchymatic pith) always show dividing of cells near the wound surface. The cells of sclerenchyma and xylem cylinder do not divide; these cells are dead or lignified. The extent of

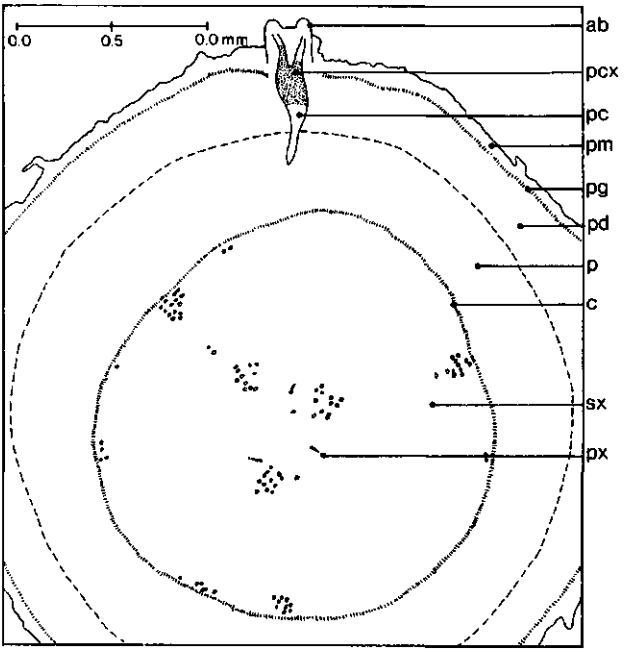

Fig. 9. *Lunaria annua.* Transverse section of thickened triarch root of leaf cutting, three months after striking the cutting (without cold treatment). An adventitious bud originated in the phellogen. While differentiating to the vascular cambium of the root the procambium of the bud will not be hampered by a layer of sclerenchyma and progresses straight onto the vascular cambium. For abbreviations see legend to Fig. 4 and: pcx, procambium in which tracheary elements differentiated already.

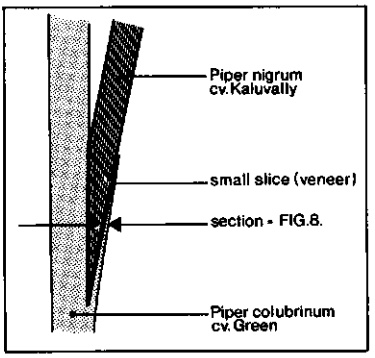

Fig. 10. Veneer side graft (Garner, 1979) of *Piper nigrum* cv. Kaluvally (scion) onto *Piper colubrinum* cv. Green (rootstock).

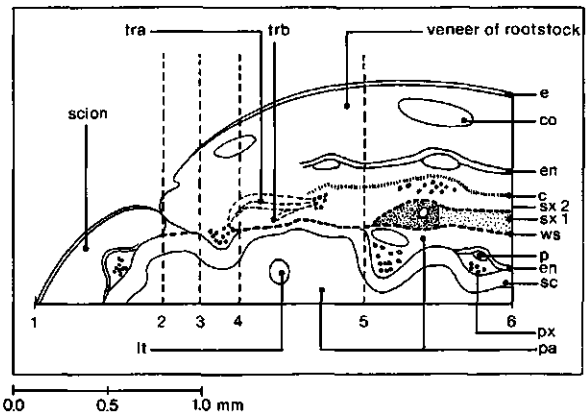

Fig. 11. Camera lucida drawing of a small part of the transverse section of a graft union, 44 days after grafting and as indicated in Fig. 10. Many possibilities of contact between the different tissues in the rootstock and the scion are shown (summarized in Fig. 13). The zones of the wound surfaces at which different tissues of rootstock and scion are grafted opposite to each other have been indicated with 1-6. c, cambium (in rootstock only); co, collenchyma in cortex; e, epidermis; en, endodermis; lt, latex tube; p, phloem of vascular bundle; pa, parenchyma of ground tissue; px, xylem of vascular bundle; sc, sclerenchyma layer; sx1, secondary xylem initiated before grafting: lignified ray parenchyma, and lignified libriform to the left (darker shaded); sx2, secondary xylem formed after the grafting; tra, differentiating tracheary elements to vascular bundle of scion; trb, idem, to parenchyma cells of scion in interruption of sclerenchyma layer; ws, wound surface(s).

dividing activity of the living cells of the scion is strongly dependent on the type of tissue of the rootstock that happened opposite this tissue after grafting. Opposite living cells of the rootstock the reaction is small, but the dividing activity of the cells in the wound surface of the rootstock is much more intense (Fig. 13: $\pm$, resp. $++$).

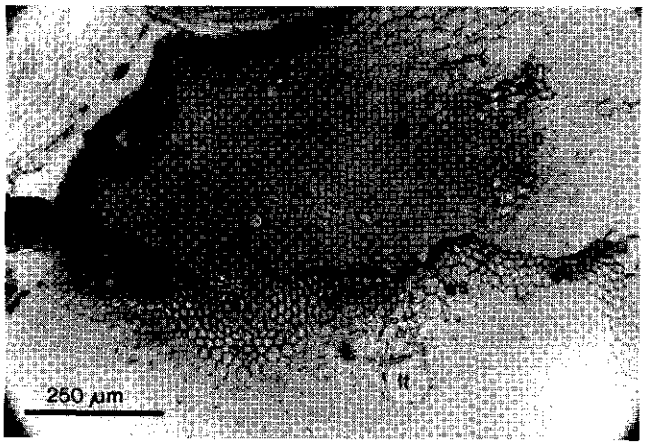

Fig. 12. Light micrograph of transverse section of graft union 44 days after grafting and as indicated in Fig. 10 and 11. The possibilities of contact between the different tissues in the rootstock and the scion are shown in Fig. 11. For abbreviations see legend to Fig. 11.

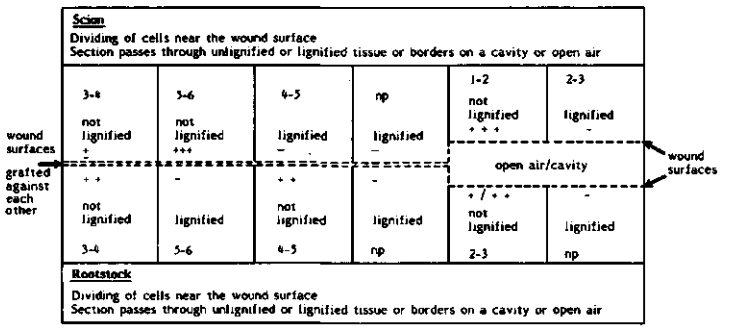

Fig. 13. Summary of the reaction of different tissues (cell divisions) of scion and rootstock in the near vicinity of the wound surfaces after grafting. Twenty four different graftings have been examined (three types of graftings and four different combinations of graft partners, varieties of *Piper nigrum*, *P. hirsutum* and *P. colubrinum*). The reaction of the tissues in the wound surfaces is dependent of the type of tissue (lignified or not) that can be found opposite this tissue after graftings. −, no cell divisions; ±, cell divisions only locally; +, cell divisions; + +, rather many cell divisions; + + +, many cell divisions; np, not present in Fig. 11. The numbers 1-2, 2-3, etc. refer to the numbered zones in Fig. 11.

The living cells of the scion near the wound surface react with a strong dividing activity (Fig. 13: + + +) when graft union fails to occur because the grafting took place in such a way that the tissue of the scion came to rest opposite lignified tissue of the rootstock. Also when the grafting resulted in the position of living cells of the scion opposite open air or a cavity, a strong reaction takes place (Fig. 13: + + +), followed by the initiation of periderm. The reaction of the living non-lignified tissues near the wound surface of the rootstock was always rather strong (Fig. 13: + +); only the reaction of living cells opposite open air or a cavity was sometimes weaker.

The differentiation of vascular tissue in the graft union, shown in Figs 10 and 11, occurred about six weeks after the date of grafting. From the cut vascular bundle of the stock (Figs 11 and 12, tr a, zone 4-5) differentiation of tracheary elements in the dividing parenchyma cells near the wound surface of the stock took place in the direction of the nearby vascular bundle of the scion (Fig. 11: zone 3-4). Also in other graft unions examined the initiation of new tracheary elements occurred from vascular tissue of the stock to vascular tissue of the scion. In Fig. 11 a special case of differentiation of vascular tissue is shown also (tr b). Near an interruption of the periderm in the wound surface and the absence of the sclerenchyma, differentiating tracheids branching off in the direction of the living parenchyma cells of the scion can be observed in some sections lower in the graft union.

4. Discussion

4.1 Introduction

In the symplast as well as in the apoplast stimuli (morphogens) do occur of which the concentration in the apoplast is much lower, but this solution is probably in a continuous situation of exchange with the solution in the symplast (e.g. Fritz et al., 1983; Gifford and Thorne, 1986; Minchin et al., 1984). Thus via the apoplast the direct surroundings of a cell can change quickly. Both transport routes may undergo a strong specialization, during which process the transport capacity of both does increase very much. The apoplast of the procambium (tr o) specializes via stages of differentiation of tracheary elements (e.g. tr 1, tr 2, tr 3, Magendans, 1985) into the largest volume of the apoplast of the xylem, and the symplast in the procambium (se o) via stages of differentiation of the sieve elements (e.g. se 1, se 2, se 3) into the most important part of the symplast in the phloem concerning transport.

The mutual relation between the specialization of apoplast and symplast varies to the organ, to the plant species, and is also dependent on the climate (Esau, 1975; Esau and Kosakai, 1975; Larson, 1984; Magendans, 1983; Sivaramakrishna and Vijayaraghavan, 1983). For example this relation proves to be dependent on the prevailing water potential and with that probably on the supplied stimuli.

4.2 The vein endings

Owing to the relative low water potential, caused by transpiration via the intercellular spaces and the stomata, a transpiration stream arises through the small veins surrounding the areoles and through the procambium of young vein endings (Magendans, 1985 and Figs 2, 3). In the areole-bounding small veins a relative high water potential prevails and in many places a streaming originates out of the areole-bounding veins to points in the areole with lower water potentials. The direction of the cell walls in the two cell layers between the differentiating palisade chlorenchyma and the spongy chlorenchyma is probably of importance for the preferred direction of the transpiration stream following the onset of transpiration (e.g. the origin of a main stream in a meristem). The main stream originates along the route of the largest drop of water potential and this route is also dependent on the direction of the cell walls. The starting-point of an ultimate main stream probably arises at that point where the direction of the cell walls is parallel with the line of largest drop of water potential (Fig. 14). The transpiration sink can be a stoma for instance, as in a young leaf. Along the cell walls indicated with arrows the drop of water potential will be largest,

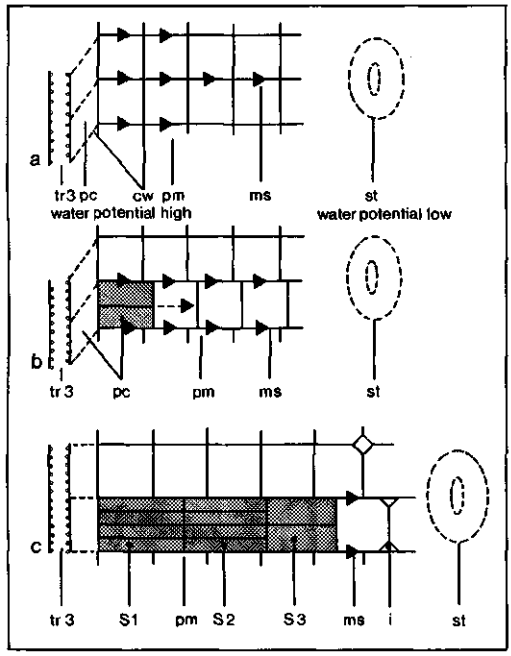

Fig. 14. Paradermal sections of young leaves of *Hedera canariensis* through 5th or 6th cell layer of plate meristem (cp. Fig.1, 2). Acropetal differentiation of vein endings. a, b: 15% final lamina length; c: 38% final lamina length.

a. Main stream of transpiration (ms) develops between tracheary elements (tr3) differentiated already in veins of lower order via the walls (cw) of procambial cells (pc) and the cells of the plate meristem (pm) to the stomata in the lower epidermis (st).

b. Main stream of transpiration will be directed along the newly developed procambial cells in the plate meristem. At the distal end of the new procambial cells a higher concentration of stimuli per unit of time will be found (→) that causes the adjoining plate meristem cell to differentiate into procambial cells; i.e. acropetal differentiation of vein ending.

c. Vein ending originated in three cells of the plate meristem and therefore consists of three segments (s1, s2, s3). At the distal end of the lengthwise fully differentiated vein ending intercellular spaces (i) developed. The main stream of transpiration will be directed along the vein ending to the stomata via cell walls and intercellular spaces. At this moment differentiated tracheids will be present near the proximal end of the young vein ending; in a and b they are still at a larger distance from the proximal end.

depending also on the positions of intercellular spaces in the cell layers 6-11 of the plate meristem (Figs 1, 14a). Through these cell walls, generally forming the shortest connection between the nearest tracheary element and the intercellular spaces near the stoma, the main stream of transpiration will take place (Magendans, 1985 and Fig. 3).

Together with the generated main stream a larger number of stimuli (as IAA, cp. Bruck and Paolillo, 1984) arrives, probably also coming from the distal front of the acropetally differentiating phloem at a position in the young venation farther away yet (Magendans, 1985). At first these stimuli reach the cells at the

starting-point of the main stream nearest the procambial cells and tracheary elements of the young vein surrounding the areole. Then cell division, cell extension and a strong intrusive growth of the new procambial cells do occur, at first at the proximal end of the row of cells along the main stream (Magendans, 1985 and Fig. 14b). Meanwhile the procambial cells become fusiform. The new walls in the first segment of the new vein ending (Fig. 14c) are anticlinal walls and are always parallel with the main stream, thus increasing the apoplastic space and decreasing the resistance of streaming between tr 3 and the stoma. Now the transpiration stream will be attracted by the route of least resistance, i.e. by the new procambial cells ('autocatalytic effect'). Then a bigger number of stimuli will be supplied at the distal end of the young procambial cells (Fig. 14b) and the adjoining mesophyll cells along the route of the largest drop of water potential will be incited to divide and extend. Thus continuation of the differentiation process of procambium takes place and in the first instance the acropetal differentiation of a procambium strand will be the result (Fig. 14c) constructed by forming several segments of successive longitudinal groups of procambial cells. Soon more cell divisions occur in the proximal segment (S1) and the new walls are always formed parallel with the longitudinal axis of the vein ending at this stage of development (Magendans, 1985).

At the same time the course of the transpiration stream has been investigated (Magendans, 1985). This course has been shown in the young leaf tissue ($\pm$ 30% F.L.L.) by means of colouring with a solution of eosin. In this young tissue the eosin was found in high concentration in the free ends of the venation (i.e. procambium) already after about 15 min. in a totally white shoot. Many stomata appeared to be open after microscopical examination.

An abrupt end to the distal differentiation of procambium takes place at about 38% F.L.L. and coincides with the development of large intercellular spaces in this layer of the plate meristem, at first exactly at the distal end of the procambial strand (Magendans, 1985); the transpiration stream will bend away to the stomata from now on.

Meanwhile more functional stomata are initiated and more, larger intercellular spaces are formed in the differentiating spongy parenchyma. Therefore the transpiration becomes stronger and more stimuli will be supplied. In the vein endings of the *Hedera* leaf tracheary elements differentiate first in the procambial strand. These tracheary elements differentiate directly from procambial cells in the adaxial part of the strand (specialization of the apoplast). At a later stage, when the lamina of the leaf almost has reached its ultimate length, the first sieve elements differentiate in the vein endings. These sieve elements differentiate in the abaxial part of the procambial strand not before many cell divisions in the procambial cells have taken place (specialization of the symplast, Magendans, 1985). Finally, the minor veins normally differentiate in the 5th cell layer, this is indicative of a preferential apoplastic transport of the transpiration stream in this layer.

4.3 The adventitious buds

Adventitious buds develop from small shoot apical meristems that are seemingly initiated at casual places in the plant. The origin of those meristems is exogenous in *Lunaria* as opposed to the endogenous origin of the apical meristems of the adventitious roots. The investigated shoot apical meristems (e.g. Figs 4, 6, 15) arise in the phellogen close to a thin spot or rupture in the phellem (a place where apoplastic water may evaporate and the water potential is lower, compare the extra activity of the phellogen cells underneath a stoma as introduction to the initiation of the first lenticels; Esau, 1965) (Figs 5, 6a, b). Besides these initiations arise at places where the distance of apoplastic transport from the vascular cambium to the initiation of the bud is shortest (Figs. 4, 15). The layer of unlignified sclerenchyma in pericycle and obliterated phloem seems to be an obstacle for this transport (Fig. 8). The interpretation of this regeneration phenomenon might be as follows and is summarized in Fig. 15.

The osmotic potential of the meristematic cells of the young shoot apex may be 1.5 to 4 bar below that of the vascular system (Boyer, 1985), and moreover

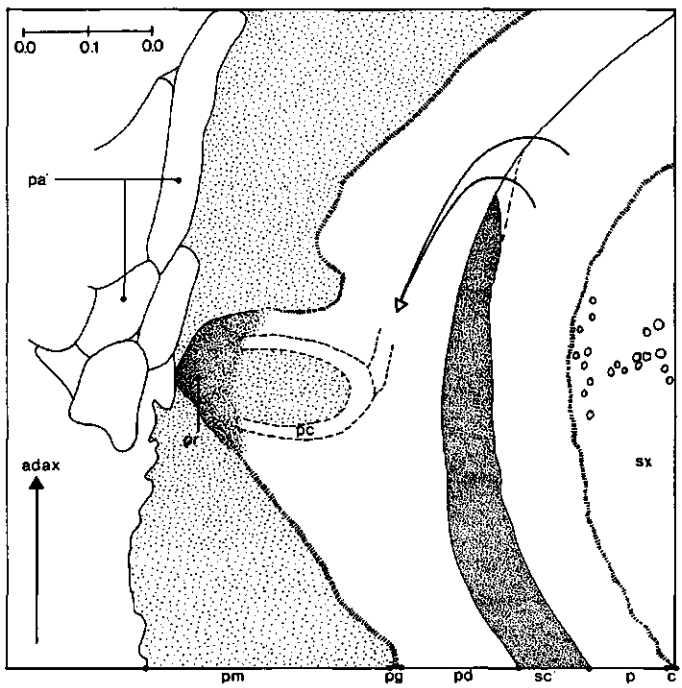

Fig. 15. *Lunaria annua*, transverse section of the basal part of the petiole of a leaf cutting, about 4 mm from the plane of section of the cutting and three months after striking the cutting. A very young adventitious bud originated in the phellogen of the median vascular bundle, cp. Fig. 8. Under the protomeristem the young procambium is bowl-shaped. At the bottom of this procambium one strand differentiates along the route that the transport stream to the bud primordium might be expected to follow (arrow). For abbreviations see legend to Figs 4, 5 and 8.

transpiration will be possible through the thin spot of the phellem (see also Fig. 5). A transport stream comes into being from the vascular cambium (compare procambium in a young *Hedera* leaf, Magendans, 1985) to the protomeristem along the line of greatest decline of water potential (cp. Fig. 8: along the route of first connection of the procambium to the vascular cambium in earlier stages of development of the primordium). In this stream morphogens will be transported and the 'cells respond to the signal flux by gradually differentiating to become the preferred or facilitated channels for this flux' (Sachs, 1981, 1984). The route of vascular differentiation indicates that the resistance to streaming through the cell walls is not equal in every part of tissue between the primordium and the vascular cambium (cp. the direct way of differentiation of the procambium from the adventitious bud to the vascular cambium in the root, Figs. 7d, 9). The layer of unlignified sclerenchyma and obliterated phloem (Figs 7b, 15: sc') is avoided; the main stream bends around the adaxial extremity of this layer and approaches the young shoot apex from a certain angle. The differentiation of procambium takes place along the same 'line of least resistance' according to the concept of flux-dependent differentiation (see Warren Wilson and Warren Wilson, 1984; Sachs, 1984). After a first initiation of procambium (see Fig. 15, at the bottom of the bowl-shaped procambium in the primordium) these first procambial cells of the strand, connecting the vascular cambium and the primordium, attract the main stream to themselves because of a local decrease of resistance to streaming. Also morphogens will reach this place of initiation of procambium in higher concentrations. So the process of differentiation into procambium is probably the result of an autocatalytic effect; this differentiation continues along the route of the main stream and the differentiation takes place in opposite direction, contrary to the situation just below the protomeristem (Fig. 15, pr) and in the young vein ending (Fig. 14).

4.4 Vascular differentiation in graft unions

The differentiation of procambium and vascular tissue in graft unions takes place along the apoplastic route along which the first transpiration transport will occur; it is the only possible route for transpiration transport structurally and the scion does not wilt. This way of morphogenesis of the vascular connection between stock and scion, viz. differentiation from the vessels of the stock to the vessels of the scion indicates that this process is connected with the apoplastic transpiration transport. The origin of an apoplastic transport route of the transpiration stream probably leads to the differentiation of new tracheary elements under the influence of the resultant of dissolved differentiation stimuli in the acropetal transpiration stream and of (basipetal) symplastic transport of stimuli.

In summary it can be concluded that the direction of differentiation of the vascular tissue in the three treated examples is in agreement with the following

interpretation. This differentiation is brought about by stimuli transported through the apoplast and stimuli produced by the symplast. The stimuli produced by the symplast are in a certain equilibrium with the apoplastic solution (Madore and Webb, 1981; Fritz et al., 1983). The direction of transport in a plate meristem of the apoplastic solution is dependent on the transpiration stream whereas the direction of transport of the symplastic stimuli has not yet been determined because polarity in the young meristem is not established yet (Jacobs, 1979). In case of sufficient supply of apoplastic stimuli, the direction of differentiation of vascular tissue will correspond with the direction of the transpiration stream in the meristem.

The direction of transport in older tissue in which polarity has been established is also dependent on the stimuli in the transpiration stream, but the direction of transport of the stimuli in the symplast is basipetal (to the roots). The direction of differentiation of vascular tissue will be dependent in this case of the stimulus that is limiting. The differentiation of the procambium underneath the primordium of an adventitious bud in *Lunaria* leaf cuttings (Fig. 15) is an example of this situation. The initially slow, symplastic transport of stimuli (IAA) in a new polar direction determines the differentiation of procambium in a direction opposed to the transport of the apoplastic stimuli, because the quantity of symplastic stimuli is limiting in this case. The main transpiration stream is drawn to the walls of the new elongating procambium cells and the autocatalytic process can go on. Finally the differentiation of vascular tissue in a graft union will be realized in a similar manner. In this case, however, the reverse condition seems to hold true as in the graft union; the vascular differentiation proceeds from stock to scion. The slow developing transpiration stream in the graft union or the quantity of apoplastic stimuli seems to be limiting now.

The practical significance of the given explanation of the observations concerning the initiation of vascular tissue during the ontogeny of leaf tissue, as in regeneration and graft unions is the importance of inducing the development of a transpiration sink. Transpiration out of a certain place of the phellogen will lead to extra activity in that part of the phellogen and the initiation of an adventitious bud. Concerning the graft union the maintaining of some transpiration in the scion will be a prerequisite for the differentiation of vascular tissue from the stock to the vascular system of that scion, in accordance with the practical experience that a graft union which is kept in humid conditions will not survive.

In this article it has been tried to link several phenomena in differentiation of vascular plant tissue in three varied examples. This study of the structural aspects of the development, normal ontogeny and regeneration is partly a reflection of the process and with that it provides the initial impetus to the physiological approach to the problem of vascular development.

5. Acknowledgements

Thanks are due to Prof.dr. M.T.M. Willemse for discussions and reading and correcting of the manuscript. I am grateful to Mr. A.B. Haasdijk for the drawings and to Mrs G.G. van de Hoef-van Espelo for typing the manuscript.

6. References

Boyer, J.S., 1985. Water transport. Ann. Rev. Plant Physiol. 36, 473-516.

Bruck, D.K. and Paolillo, D.J., 1984. Replacement of leaf primordia with IAA in the induction of vascular differentiation in the stem of *Coleus*. New Phytol. 96, 353-370.

Burbano, J.L., Pizzolato, T.D., Morey, P.R. and Berlin, J.D., 1976. An application of the Prussian blue technique to a light microscope study of water movement in transpiring leaves of cotton (*Gossypium hirsutum* L.). J. Exp. Bot. 27, 134-144.

Canny, M.J., 1986. Water pathways in wheat leaves. III. The passage of the mestome sheath and the function of the suberised lamellae. Physiol. Plant. 66, 637-647.

Esau, K., 1965. Plant Anatomy, 767 pp. 2nd edn, John Wiley & Sons, Inc., New York.

Esau, K., 1975. The phloem of *Nelumbo nucifera* Gaertn. Ann. Bot. 39, 901-913.

Esau, K. and Kosakai, H., 1975. Laticifers in *Nelumbo nucifera* Gaertn.: Distribution and structure. Ann. Bot. 39, 713-719.

Esau, K. and Thorsch, J., 1985. Sieve plate pores and plasmodesmata, the communication channels of the symplast: ultrastructural aspects and developmental relations. Amer. J. Bot. 72, 1641-1653.

Fritz, E., Evert, R.F. and Heyser, W., 1983. Microautoradiographic studies of phloem loading and transport in the leaf of *Zea mays* L. Planta 159, 193-206.

Garner, R.J., 1979. The grafter's handbook, 319 pp. 4th edn, Faber and Faber, London.

Gerlach, D., 1969. Botanische Mikrotechnik, 298 pp. Georg Thieme Verlag, Stuttgart.

Gifford, R.M. and Thorne, J.H., 1986. Phloem unloading in soybean seed coats: Dynamics and stability of efflux into attached 'Empty ovules'. Plant Physiol. 80, 464-469.

Jacobs, W.P., 1979. Plant hormones and plant development, 339 pp. Cambridge University Press, Cambridge.

Johansen, D.A., 1940. Plant microtechnique, 523 pp. McGraw-Hill Book Company, New York.

Larson, P.R., 1984. Vascularization of developing leaves of *Gleditsia triacanthos* L. II. Leaflet initiation and early vascularization. Amer. J. Bot. 71, 1211-1220.

Läuchli, A., 1976. Apoplasmic transport in tissues, pp. 16-29. In Encyclopedia of Plant Physiology, eds A. Pirson and M.H. Zimmermann, N.S., vol. 2B, Springer, Berlin.

Leist, N., 1976. Uber die Blattnervatur bei Farnen. Ber. Deutsch. Bot. Ges. 89, 225-242.

Madore, M. and Webb, J.A., 1981. Leaf free space analysis and vein loading in *Cucurbita pepo*. Can. J. Bot. 59, 2550-2557.

Magendans, J.F.C., 1983. Anatomy of vein endings in *Hedera* leaves; influence of dry and wet conditions. Meded. Landbouwhogeschool Wageningen, 83-6, 1-34.

Magendans, J.F.C., 1985. Anatomy of vein endings in *Hedera* leaves; aspects of ontogeny. Agric, Univ. Wageningen Papers 85-5, 1-74.

Minchin, P.E.H. and Thorpe, M.R., 1984. Apoplastic phloem unloading in the stem of bean. J. Exper. Bot. 35, 538-550.

Minchin, P.E.H., Ryan, K.G. and Thorpe, M.R., 1984. Further evidence of apoplastic unloading into the stem of bean: Identification of the phloem buffering pool. J. Exp. Bot. 35, 1744-1753.

Pizzolato, T.D., Burbano, J.L., Berlin, J.D., Morey, P.R. and Pease, R.W., 1976. An electron microscope study of the path of water movement in transpiring leaves of cotton (*Gossypium hirsutum* L.). J. Exp. Bot. 27, 145-161.

Sachs, T., 1981. The control of the patterned differentiation of vascular tissues, pp. 151-262. In: Advances in Botanical Research, ed. H.W. Woolhouse, vol. 9, 279 pp., Academic Press, London.

Sachs, T., 1984. Axiality and polarity in vascular plants, pp. 193-224. In Positional controls in plant development, eds P.W. Barlow and D.J. Carr, 502 pp., Cambridge University Press, Cambridge.

Sivaramakrishna, D. and Vijayaraghavan, P., 1983. Levels of maturation of protoxylem and protophloem elements in aerial roots of *Cissus* and *Ficus*. Can. J. Bot. 61, 1140-1149.

Tanton, T.W. and Crowdy, S.H., 1972. Water pathways in higher plants. III. The transpiration stream within leaves. J. Exp. Bot. 23, 619-625.
Warren Wilson, J. and Warren Wilson, P.M., 1984. Control of tissue patterns in normal development and in regeneration, pp. 225-280. In Positional controls in plant development, eds P.W. Barlow and D.J. Carr, 502 pp., Cambridge University Press, Cambridge.
Weatherly, P.E., 1963. The pathway of water movement across the root cortex and leaf mesophyll of transpiring plants, pp. 85-100. In The water relations of plants, eds A.J. Rutter and F.H. Whitehead, 394 pp., Blackwell Scientific Publications, London.
Weatherly, P.E., 1970. Some aspects of water relations, pp. 171-206. In Advances in Botanical Research, ed. R.D. Preston, vol. 3, 309 pp., Academic Press, London.

ISBN 90-6754-139-7