

**ANATOMY OF VEIN ENDINGS IN HEDERA LEAVES
ASPECTS OF ONTOGENY AND INFLUENCE OF DRY
AND WET CONDITIONS**

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ANATOMY OF VEIN ENDINGS IN HEDERA LEAVES

Aspects of ontogeny and influence of dry and wet conditions

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C. C. Oosterlee,
in het openbaar te verdedigen
op woensdag 13 november 1985
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

WAGENINGEN

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STELLINGEN

1.

Tussendringende groei en de spoelvorm zijn kenmerken die voor procambiumcellen en de lange cambiumcellen beide gelden.

Dit proefschrift.

2.

De mate van predifferentiatie van xyleem met betrekking tot de floeemdifferentiatie in het procambium van de nerfuiteinden van het blad, is afhankelijk van de vochtpotentiaal in en rondom het blad.

Dit proefschrift.

3.

De differentiatie van primair xyleem moet functioneel als een vergroting van de apoplastruimte in de procambiumstreng worden beschouwd.

Dit proefschrift.

4.

Voor de morfogenese van de nerfuiteinden is de apoplastische, acropetale transpiratiestroom met de daarin voorkomende stimuli, het belangrijkste.

Dit proefschrift.

*
5.

Het maken van een theoretisch model voor de morfogenese van een biologische structuur is slechts zinvol als dit gepaard gaat met nauwkeurige waarnemingen van de ontogenie.

MEINHARDT, H., 1978. Rev. Physiol., Biochem. Pharmacol. **80**: 47-104

1979. Biologie in unserer Zeit **9**: 33-39

1984. In: Barlow, P. W. and D. J. Carr (eds.), Positional controls in plant development. Cambridge Univ. Press. Cambridge, London, New York, New Rochelle, Melbourne, Sydney

MITCHISON, G. J., 1980. Proc. R. Soc. Lond. B **207**: 79-109

1981. Phil. Trans. R. Soc. Lond. B **295**: 461-471.

6.

De presentatie van anatomische gegevens van een 'typical' kleine nerf, berustend op enige, tamelijk willekeurig gekozen dwarse doorsneden van kleine nerven uit het blad van *Amaranthus*, is onbetrouwbaar.

FISHER, D. G. and R. F. EVERT, 1982. Amer. J. Bot. **69**: 1375-1388.

7.

De formulering: 'the phloem accompanies the xylem' met betrekking tot de nerfuiteinden van het blad, is morfogenetisch onjuist.

ESAU, K., 1969. Handbuch der Pflanzenanatomie, 2 Aufl. Bd 5 Teil 2, Zimmermann, W., P. Ozenda und H. D. Wulf. Berlin-Stuttgart

1977. Anatomy of seed plants. 2nd ed. John Wiley and Sons. New York-Santa Barbara-London-Sydney-Toronto.

8.

De opvatting dat de plasmodesmata 'die Bahnen liefern für den Stofftransport zur Ernährung der Zellen, denn die Organisation der Pflanzen mit ihren rigiden Zellwänden hat die Differenzierung von interzellulären Räumen zu Transportbahnen nicht erlaubt' is onjuist.

KLEINIG, H. and P. SITTE, 1984. Zellbiologie. Ein Lehrbuch. Fisher. Stuttgart-New York.

*

9.

Een homogeen, doordacht, didactisch gefundeerd onderwijsprogramma, leidend tot een optimaal verlopend leerproces, kan niet worden verwacht op basis van democratische besluitvorming, politieke uitgangspunten of vele wensen van alle meer of minder betrokkenen.

10.

Een vergaande verstrengeling van de morfologie, anatomie en cytologie in één onderwijsselement is strijdig met de beoogde duidelijkheid van opzet van het onderwijs in ieder onderdeel, en draagt niets bij tot het inzicht in de morfologie in brede zin.

11.

In het universitair onderwijs vormt het bezit van vak-didactische ervaring in steeds hogere mate een handicap voor de docent.

12.

De lange praktijktijd van studenten als deel van het onderwijs aan de Landbouwhogeschool is meer in het belang van de onderwijsinstelling dan van de student.

*

13.

Planning van fundamenteel onderzoek leidt tot onderzoek dat niet fundamenteel is.

14.

Het pleidooi voor het stimuleren van de geboorten door het CDA (Weijers, NRC, 26-06-1985) kan rationeel beschouwd slechts gebaseerd zijn op religieuze opvattingen betreffende de voortplanting.

15.

Dat het Nederlandse publiek genoeg neemt met de hyperbetutteling in het omroepbestel door de overheid, moet zijn oorsprong hebben in een zeldzame tolerantie jegens elkaanders roeping tot het bedrijven van zendingsactiviteit.

16.

Middels het op veel plaatsen overmatige aantal lantaarnpalen maakt het gemeentebestuur het publiek er op heldere wijze op attent dat de kas nog overvloedig gevuld is.

*

J. F. C. MAGENDANS
Anatomy of vein endings in Hedera leaves
Wageningen, 13 november 1985

In herinnering aan mijn oud-docenten
in de Algemene Plantkunde:
Prof. Dr. E. Reinders
Prof. Dr. C. A. Reinders-Gouwentak
Dr. J. de Zeeuw

WOORD VOORAF

De inhoud van dit proefschrift vormt het resultaat van vijf jaar onderzoek met nadruk op de laatste jaren. Mijn promotor, professor dr. M. T. M. Willemse, ben ik zeer erkentelijk voor het vertrouwen dat hij stelde bij de aanvang van deze studie en voor de zorgvuldige aandacht bij de voorbereiding van de manuscripten. Evenzo dank ik dr. ir. G. A. Pieters voor gesprekken over relevante onderwerpen uit de plantenfysiologie.

Van de overige medewerkers van de vakgroep Plantencytologie en -morfologie van wie ik hulp ontving, wil ik noemen de heer dr. ir. R. W. den Outer voor zijn waardevolle opmerkingen na lezing van de manuscripten en Mw. M. G. Boersma voor assistentie bij de submicroscopische microtechniek. De heer A. B. Haasdijk, wiens incasseringsvermogen voor het verwerken van telkens weer nieuwe tekenopdrachten en wijzigingen groot is, ben ik zeer erkentelijk. Ook de heer P. A. van Snippenburg voor het tekenen van de grafieken en Mw. G. G. van de Hoef-van Espelo voor het zorgvuldig typen van de manuscripten ben ik dankbaar.

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INTRODUCTION

Transport, rigidity and leaf shape are aspects belonging to the functions of the venation of a leaf. The function of transport of the free vein endings, last developed within an areole, is suitable for studying the ontogeny of the vein endings, also in case of changes of type of climate. Leaf shape and rigidity which vary little along the *Hedera* stem, permit such a study.

Examinations of the influence of the climatic conditions on the anatomy of the vascular tissue mostly concern the secondary vascular tissue, especially the wood (BAAS, 1973; CARLQUIST, 1975, 1977). It is important, however, to study the influence of the climate on primary vascular tissue, especially in leaves, because changes of climate likely affect more directly the origin and the development of this primary vascular tissue. For example a change of the rate of transpiration through the stomata does affect the ontogeny of the vascular tissue in the leaf. In submerged plant parts sometimes no xylem occurs in the smaller vascular bundles; if present, the xylem is relatively poor developed (ESAU and KOSAKAI, 1975; ESAU, 1975). These observations suggest a reduction of the differentiation of xylem under very wet circumstances.

Since the variability of the structure of vein endings in a leaf is enormous (cp. STRAIN, 1933), at first the need of finding some ordering principles in this great variability arised. PRAY (1955) remarked 'that some new ontogenetic factor, which previously had not been operative during the differentiation of the earlier developing portion of the venation, has become effective'. These free vein endings originate more independently of, and later than the remaining venation in the leaf development. Vein endings develop progressively from strands delimiting the ultimate areoles (PRAY, 1955). An influence of the climatic conditions, notably the relative humidity, will probably be expressed at first in the development of the vein endings. Moreover from the end of the sixties interest arised in the anatomy of the vein endings in connection with the study of the sugar accumulation into the phloem of these veinlets in green leaf tissue (e.g. GEIGER and CATALDO, 1969; GEIGER, MALONE and CATALDO, 1971; FELLOWS and GEIGER, 1974; TURGEON, WEBB and EVERT, 1975; TURGEON and WEBB, 1976; FISHER and EVERT, 1982).

When analyzing the structure of the vein endings the basic question arises why during the initiation of the vascular tissue in bundles the differentiation of the xylem turns out to be so strictly connected with the differentiation of the phloem. Would the study of the ontogeny of the vein endings provide more clarity in this matter? Perhaps this provides a contribution to the resolution of the problem: what is a vascular bundle. To take up the problem three questions can be formulated: does the structure of the vein endings in green leaf tissue differ from that in white leaf tissue without chloroplasts, or to what extent a structural response can be perceived to different physiological conditions around the developing vein endings; does the structure of the vein endings, differentiated in leaf tissue in very dry climate differ from the anatomy of those veinlets differentiated in very wet climate, or can the water potential affect the anatomy

of the vein endings; and finally the question: can a change in the anatomy of the vein endings in response to different climatic conditions be explained on the basis of the ontogeny, or at which moment in the developmental process the change does appear. This way of approaching to the problem provides insight into the morphogenesis of the vein endings and not only this, but also as to how far this structure is a response to wet and dry conditions.

As experimental plant *Hedera canariensis* Willd. var. 'Gloire de Marengo' is very suited for trying to answer the questions above-mentioned. This chimeral plant has variegated leaves. The type of variegation is characterized by several shades of green centrally of the leaf and irregular areas of white marginally. Besides totally white shoots did arise of which the white leaves have some small chloroplasts in the epidermis only, especially in the guard cells. This plant is able to grow and develop well in a conditioned growth cabinet in a cooled potometer with an aerated nutrient solution. In the cabinet the long and flexible stems grow unlimited and regularly and can be led easily. These stems form many nearly identical leaves. The leaves have many free vein endings in the mesophyll and these vein endings show a great number of tracheids at their distal ends. The volume of these tracheids does vary experimentally. The leaves of *Hedera* show a broad structural adaptability under several climatic conditions (WATSON, 1942; WYLIE, 1943). This change of leaf anatomy under the influence of change of climate can be studied by means of these long stems with many leaves, also during the ontogeny. Finally it turns out to be possible to elaborate the vein endings in the *Hedera* leaf well microtechnically, and the growth of these vein endings can be expressed well by means of mathematical equations.

The regular occurrence of a distinct maximum of the number of tracheids near the distal extremity of the phloem in the vein endings, that soon became apparent, made one think also of the xylem differentiation being influenced by the differentiation of the phloem, at least under sink conditions. For this reason totally white leaf tissue and later totally white shoots have been used in these examinations.

After gaining an insight into the structure of the vein endings of the *Hedera* leaf and the specific influence of the relative humidity on this structure, the question of the ontogeny arised, also with a view to a possible more accurately directed, experimental approach to the problem.

Then the question still exists how the venation comes into being and what the cause might be that there is a response to particular conditions. Models are known concerning the venation, and these models can be compared with the results of our examinations into the venation of the *Hedera* leaf. Physiological stimuli influence the realization of the structure, but these stimuli are tied down to this structure also.

Finally the morphogenesis of the vein endings and the role of possible differentiation stimuli is discussed. In this discussion the different theories of the development of vein endings (as MEINHARDT, 1979; MITCHISON, 1980, 1981) are involved. In their studies the anatomical structure of the developing tissue is not involved in the theoretical considerations.

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ANATOMY OF VEIN ENDINGS IN
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CONDITIONS

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ABSTRACT

In variegated and totally white leaves of *Hedera canariensis* Willd. var. 'Gloire de Marengo', the anatomical structure of the vein endings has been studied by means of serial cross-sections through each analysed vein ending. Two groups of observations were made: a comparison in structure between vein endings in green and in white leaf tissue and a comparison in structure between vein endings in white leaf tissue in a very dry ($7.0 \pm 2\%$ r.h.) and in a very wet ($97.0 \pm 2\%$ r.h.) atmosphere. The elements of the phloem in the vein endings are sieve elements (se), intermediary cells (ic; companion cells included) and vascular parenchyma cells (vp). In the xylem tracheids with spiral thickenings (tr), and vascular parenchyma cells (vpx) have been found. In general one may find four different zones along a vein ending: l_{se} (part of the vein ending with sieve elements), l_{ic} (distad of l_{se} , containing intermediary cells), l_{vp} (distad of l_{ic} , containing vascular parenchyma cells in a direct line with the distal end of the zone l_{ic}) and l_{tr} (at the ultimate extremity of the vein ending, containing only tracheids and sometimes vascular parenchyma cells belonging to the xylem). The percentage of living elements decreases fairly regularly in the direction of the distal extremity of the vein ending, whereas the percentage of tracheids increases. The average length of the extremities of the vein endings without sieve elements ($l_v - l_{se}$) is independent of the total length of the vein ending (l_v). It has been possible to construct a model of a vein ending with a rather constant type of curve when relating the xylem part of the vein ending with the phloem part. This curve shows a distinct maximum, situated near the distal end of the zone l_{se} . It was found that under influence of the presence of the zones l_{ic} and l_{vp} this tracheid maximum shifts to a more distal position. Any part of a vein ending consisting of e.g. the zones $l_{ic} + l_{vp} + l_{tr}$, $l_{vp} + l_{tr}$ or l_{tr} may also be found as lateral branches of the vein ending. The point of branching may occur on any spot along the vein ending; however, the zones l_{ic} and l_{vp} are continuous each and are connected with a zone l_{se} . The rather constant length of the extremities ($l_v - l_{se}$) of the vein endings in a given climatic condition is significantly longer, however, in a very dry atmosphere than in a very wet atmosphere. Models have been constructed of vein endings in very dry and in very wet atmosphere. The tracheary volume of the ultimate endings formed in a dry climate, turned out to be 1.9 times as great as those produced under wet conditions. The possibility of the distal extremity of the phloem being a sink of differentiating factors for the xylem is discussed.

INTRODUCTION

In the leaves of *Hedera canariensis* Willd. var. 'Gloire de Marengo' (a clone, Meded. Landbouwhogeschool Wageningen 83-6 (1983)

propagated vegetatively; CHRISTENSEN, 1976) 'freely ending veinlets' (vein endings or veinlets for short) occur in the areoles. Near the distal extremity of these veinlets one tracheary element may be found in transections of the shortest veinlets and up to 26 tracheary elements occur in transections of the longer ones. Leaves of *Hedera* show a broad structural adaptability under several climatic conditions (WATSON, 1942; WYLIE, 1943a). The leaves do not have bundle sheath extensions (WYLIE, 1943b; SHERIFF and MEIDNER, 1974), a xeromorphic characteristic. The number of tracheids forms an important water reservoir at the distal ends of the veinlets, completely isolated from the transpiring epidermis for some time (WYLIE, 1943b). Especially under dry circumstances these 'storage tracheids' (PRAY, 1954) play an important role in loading into the terminal sieve elements and during basipetal transport of assimilates within sieve elements (see PATE, LAZELL and ATKINS, 1980).

This *HEDERA* is a chimeral plant with plastid variegations. The type of variegation is characterized by several shades of green centrally of the leaf and irregular areas of white marginally. There is also a difference in pattern between the two surfaces of leaves; this is an important characteristic of true variegations that are of genetic origin (DERMEN, 1960). STEWART (1966) concluded from observations of plastid variegations in English Ivy, that up to five independent histogenic layers could exist in the leaves. Totally white shoots are of GWWW composition which means that its white leaves have some chloroplasts in the epidermis only.

The anatomical structure of the minor veins and the vein endings has been investigated several times (e.g. FISCHER, 1885; PRAY, 1954, 1955b; MORRETES, 1962; ESAU, 1967, 1972; ESAU and HOEFERT, 1971; and TURGEON, WEBB and EVERT, 1975). ESAU (1967) and ESAU and HOEFERT (1971) found that the conducting cells in the phloem of minor veins are typical angiosperm sieve elements. Special interest for the types of vascular parenchyma cells in minor veins has been given by ESAU (1973) in *Mimosa pudica* L. She clearly distinguished companion cells and parenchyma cells, the companion cells having denser protoplasts. But in the beet (ESAU, 1967) the companion cells in the minor veins of the leaves cannot be singled out specifically because other cells in the vicinity of the sieve elements may have the same appearance. The parenchyma cells usually resemble the companion cells in density of cytoplasm. Many names for these parenchymatous elements in vein endings have been used. They constitute a group of cells that intergrade in function and structure (ESAU, 1969). In this article the parenchymatous elements will be indicated as intermediary cells (see ESAU, 1969; TURGEON, WEBB and EVERT, 1975) when these cells are relatively richer in cytoplasmic contents than are the other vascular parenchyma cells in the phloem and when they are in contact with a sieve element. The other parenchyma cells in the phloem will be called vascular parenchyma cells.

The intermediary cells of the phloem are functionally 'transfer cells' in the sense of GUNNING, PATE and BRIARTY, 1968 (ESAU, 1972). In the white leaves of *Hedera* these intermediary cells could function as permanent sinks for the translocated carbohydrates. The contents of the intermediary cells indicate metabolically active protoplasts.

It is well established that all important phytohormones can move within the sieve tubes (cf. ZIEGLER, 1975). The differentiation of sieve tubes precedes that of tracheary elements as in major and minor venation (PRAY, 1955a, b, c); as in the stem referred to by ESAU (1965); as in callus (ALONI, 1980). ALONI (1980) proposed that phloem is formed in response to auxin, while xylem is formed in response to auxin together with some added factor which reaches it from the phloem. Sucrose may reach the differentiating tracheary elements from the free space (ESCHRICH, 1980).

In submerged plant parts sometimes no xylem occurs in the smaller vascular bundles; if present it is relatively poorly developed (ESAU and KOSAKAI, 1975; ESAU, 1975). These observations suggest a reduction of the differentiation of xylem under very wet circumstances.

This anatomical study analyses the differences in vein endings under wet and dry conditions.

MATERIALS AND METHODS

Plant material and culture conditions

The first group of observations was made on the veins of one variegated leaf of *Hedera canariensis* Willd. var. 'Gloire de Marengo' (Fig. 1), grown in a green house under conditions normally prevailing in summertime with a daily temperature range of $25 \pm 12^\circ\text{C}$ and with a maximum light intensity of about 15,000 lux. The pieces of leaf tissue fixed for examination were chosen out of green and white parts in such a way that more or less corresponding spots in the leaf were used in respect of the larger veins of the leaf. All figures and tables concerning the results of this first group of observations have been given the indication '(green house conditions)' in the text belonging to them. The second group of observations was made on leaves grown in a conditioned growth cabinet (Weiss, W. Germany). The first part of this second group of experiments was made with one variegated leaf on a plant grown in an atmosphere of $7.0 \pm 2\%$ relative humidity (r.h.) (dry climate) and a light intensity of about 16,000 lux at plant level (Fig. 16). From this variegated leaf only white parts were fixed. The second part was done with one entirely white leaf in an atmosphere of $97.0 \pm 2\%$ r.h. (wet climate) and a light intensity of about 14,000 lux. The dark period of both parts of the second group was from 20.30 p.m. unto 08.00 a.m. and the temperature was controlled at $31^\circ\text{C} \pm 1^\circ\text{C}$ during the light period and $21^\circ\text{C} \pm 0.5^\circ\text{C}$ in the dark period. The lamps used were Philips HPI/T 375 W mercury halide and the air velocity in the cabinet 0.4–0.5 m/sec. In the culture chamber the relative humidity was measured by means of the dry and wet bulb method making use of a calculation ruler based on the Mollier diagram relating air temperature, dew-point and water content of the air. Light intensity was measured with a Metrawatt lux meter (MetruX K, cos. corrected). The *Hedera* plant of the

second group of observations grew in a potometer consisting of a 'perspex' acrylate vessel, darkened by a plastic foil. This vessel had double walls and was cooled by means of a refrigerating system. A Hoagland nutrient solution modified by STEINER (1968) was aerated and renewed after three days. Around the vessel opening the stem was secured by means of a split rubber stop with lanoline paste.

Measurements of transpiration have been done with the potometer method determining the water absorption of the whole plant every 24 hours. Growth curves have been made of the leaves. The examined leaves grown in a dry or wet climate were fixed 6 respectively 12 days after reaching their final laminar length.

Microtechnique

For the first group of observations round (Fig. 1), for the second square leaf tissue pieces (25 mm^2) were punched out and immediately fixed in FAA. The air in the tissue was extracted, the tissue was dehydrated with the TBA method and embedded in paraplast (Lancer, Sherwood) paraffin wax. Transections of $7 \mu\text{m}$ were made with a Leitz rotary microtome and stained with safranin and fast green. The flattening out of the ribbons has been done carefully. Samples which had been cut cross-wise entirely or nearly so along the whole length of the vein endings were selected. As the vein endings may point at any direction, this selection is indifferent. The vein length was estimated by multiplying the number of sections by $7 \mu\text{m}$, and corrected by means of estimating microscopically the angle of obliqueness and calculating the real distance. In the second group of observations the straight sides of the sections were used; calculating of vein length was therefore possible by means of the number of sections and the possible change of distance to the straight edge of the sections. All analysed veins are in sequence of finding them in the slides in that way. All observations were made with a Wild microscope using oil immersion and $1,500\times$ magnifying optics. Determining of the surface areas of cross sections of tracheids has been done with camera lucida drawings of the transections and after that using an image analyser (MOP-30 of the firm Kontron, W. Germany).

Statistical methods

Statistical analysis and tests of significance were by Wilcoxon's test. Significances were determined between green and white leaf tissue concerning vein lengths in groups of veins with and without phloem, phloem lengths, numbers of tracheids in the tracheid maxima and the total amount of transections of tracheids in relation to the total amount of transections of sieve elements. Also the Student's t-test was applied to determine possible significant differences between vein lengths, vein lengths minus phloem lengths and numbers of tracheids in the tracheid maxima between dry and wet conditions.

RESULTS

The composition of vein endings

In the used *Hedera* the irregular white leaf margins (Fig. 1) and the totally white shoots contained small chloroplasts only in the epidermis, especially in the guard cells. The central green parts of the leaf mostly contain 4–6 layers of cells in the middle of the mesophyll with large chloroplasts.

The free vein endings can be divided into six types (Fig. 2). In the vein endings with phloem (i.e. vascular tissue with one or more sieve elements) the most distal end of the xylem (i.e. vascular tissue with one or more tracheary elements) always differentiates beyond the last sieve elements of the vein endings. The elements of the vein endings are mentioned in Table 1.

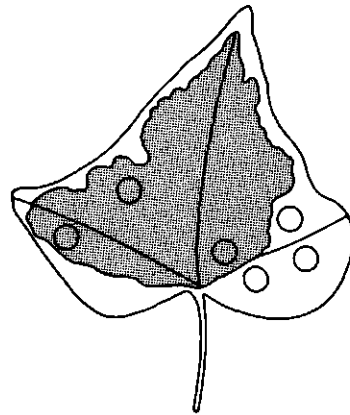


FIG. 1. *Hedera canariensis*: variegated leaf used for analysis of veinlets in three circular pieces of green tissue (dotted) and white tissue (green house conditions).

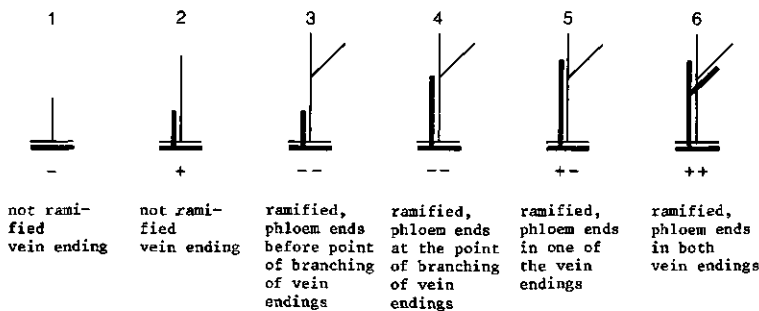


FIG. 2. Six types of vein endings. — = phloem, — = xylem,¹ + = phloem in vein ending, — = no phloem in vein ending.

¹Symbols:

- = phloem, i.e. vascular tissue with sieve elements (se)
- = phloem with intermediary cells (ic) only
- = phloem with vascular parenchyma cells (vp) only
- = xylem, i.e. vascular tissue with tracheary elements (tr)

TABLE 1.

Phloem	Abbrev.	Xylem	Abbrev.
sieve elements	se	tracheids with spiral thickenings	tr
intermediary cells (companion cells included)	ic	vascular parenchyma cells	vpx
vascular parenchyma cells	vp		

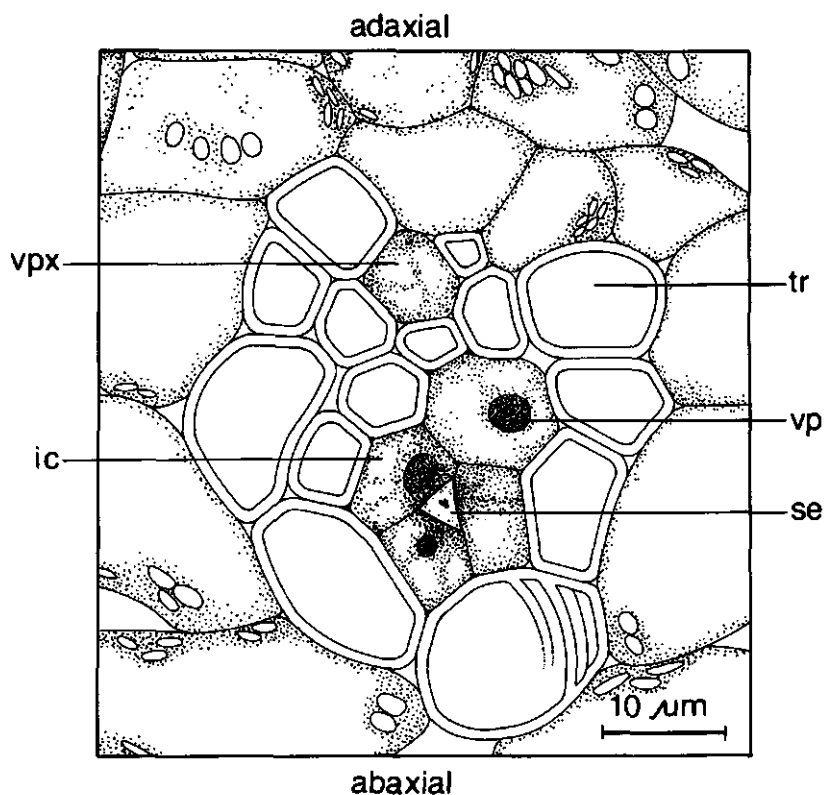


FIG. 3. Transection of vein ending not far from the distal end of the vascular bundle. It shows an amphivasal structure. ic = intermediary cell, se = sieve element, tr = tracheid with spiral thickenings, vp = vascular parenchyma cell, vpx = vascular parenchyma cell in the xylem.

Anatomy of the vein ending

The sieve elements of the veinlets are very small in cross sections (Fig. 3). Their walls are mostly straight, somewhat rounded in the corners and slightly thickened showing a bright colour after staining with fast green. These extremely narrow elements mostly show nearly any cytoplasm. The sieve elements can nearly always be found in the middle of a small group of much larger parenchyma cells (Fig. 3). The sieve elements never become obliterated. The intermediary cells are much larger than the adjoining sieve elements. These cells have dense cytoplasm and a large distinct nucleus, mostly situated near the wall bordering upon the sieve element. The one or two intermediary cells that sometimes protrude beyond the last sieve element of the veinlet, are in terminal contact with that sieve element.

The vascular parenchyma cells do not have markedly dense cytoplasm and they have a large vacuole (Table 2). In between and next to the tracheary elements vascular parenchyma cells can be present also. The limitation between vascular parenchyma cells in the xylem and those in the phloem is not sharp. Parenchyma cells isolated from the cells around the complex of sieve elements and intermediary cells by tracheary elements will be regarded as belonging to the xylem in this article. The limitation to the cells of the bundle sheath is unsharp, especially in the distal part of the vein ending. The vascular parenchyma cells in the xylem are usually as small as medium-sized tracheids and smaller

TABLE 2. Detail of 14 sections (16, distad – 29, proximad) of a complex of 4 parenchyma cells being in a direct line with the distal end of the phloem complex of the veinlet. The configuration of the parenchyma cells is a square of 4 cells. The parenchyma cell with most cytoplasm (ic) adjoins the distal sieve element in section 28.

For abbreviations see Table 1 and: central vac. = visible central vacuole (+); n = nucleus (cut in the section); pl = section of the cell almost entirely filled up with cytoplasm; vp 1–3 = vascular parenchyma cells 1–3 (white leaf tissue, dry climate).

Section no.	ic		vp 1		vp 2		vp 3	
	Cyto-plasm	Central vac.	Cyto-plasm	Central vac.	Cyto-plasm	Central vac.	Cyto-plasm	Central vac.
16	pl							
17	pl							+
18	pl			+	pl			+
19	pl		<div style="border: 1px solid black; padding: 2px;">n</div>			+		+
20	pl		<div style="border: 1px solid black; padding: 2px;">n</div>			+		+
21		+		+		+		+
22	pl		pl			+		+
23	<div style="border: 1px solid black; padding: 2px;">n</div>			+		+		+
24	<div style="border: 1px solid black; padding: 2px;">n</div>			+	<div style="border: 1px solid black; padding: 2px;">n</div>		<div style="border: 1px solid black; padding: 2px;">n</div>	
25	<div style="border: 1px solid black; padding: 2px;">n</div>		<div style="border: 1px solid black; padding: 2px;">n</div>		<div style="border: 1px solid black; padding: 2px;">n</div>		<div style="border: 1px solid black; padding: 2px;">n</div>	
26	<div style="border: 1px solid black; padding: 2px;">n</div>		<div style="border: 1px solid black; padding: 2px;">n</div>			+		+
27		+		+		+		+
28	pl			+		+		+
29	pl		pl			+		+

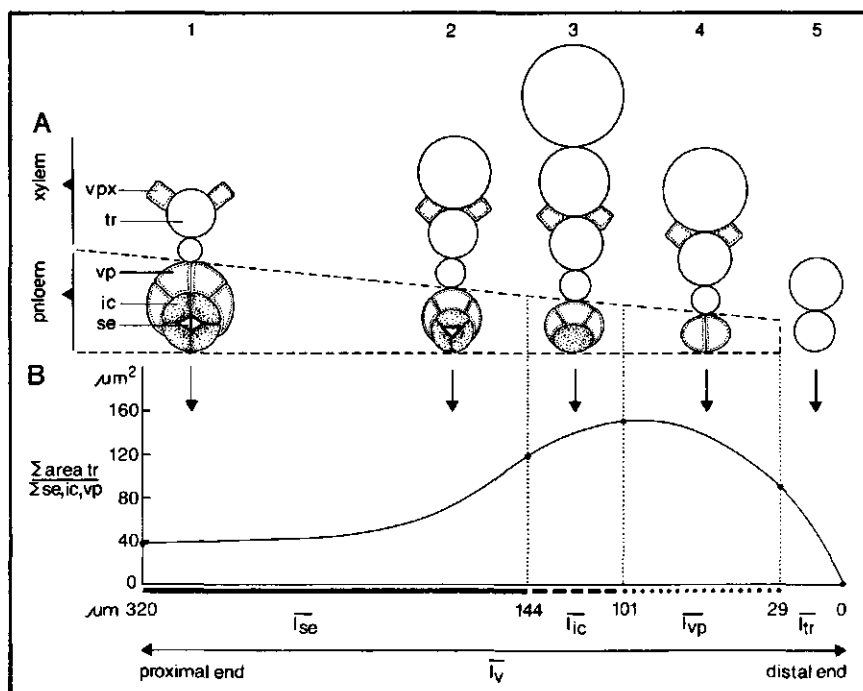


FIG. 4. Analysis of vein endings with phloem in white leaf tissue (growth cabinet).

A. Schematic drawings of sections of the vein endings at the place of the proximal ends (1), of the distal extremities of the phloem parts with sieve elements (2), of the phloem parts with intermediary cells and vascular parenchyma cells only (3), of the phloem parts with vascular parenchyma cells only (4) and of the parts with tracheids only (5). Cells of the bundle sheath are not given.

B. Diagram composed of values calculated as averages of 10 vein endings in total, 3 in dry climate and 7 in wet climate.

Abscissa: average length in μm , reckoned from the tip, of: lengths until the distal extremity of vascular parenchyma cells, lengths until the distal extremity of intermediary cells and lengths until the distal extremity of the sieve elements. The vein endings were 320 μm long on an average. Ordinate: averages of the (Σ transections area tr/number of se + ic + vp) values² in the sections of 10 vein endings at the place of the distal extremity of the sieve elements (117) of the intermediary cells (148) and of the vascular parenchyma (93). Near the proximal end this value amounts to 37.

For abbreviations see Table 1 and: l_{se} = length with sieve elements, etc., l_{tr} = length with tracheids only or with vascular parenchyma cells in addition (vp), \bar{l}_v = average length of the 10 vein endings.

² Calculation for each veinlet: Σ transections area tr means the sum of the section areas of the total of tracheary elements per transection; number of se + ic + vp (denominator) means the sum of the number of sieve elements, intermediary cells and vascular parenchyma cells in the same transection. Along the zone l_{se} the denominator is variable; distad of the zone l_{se} it is kept constant with a value as in the distal extremity of the zone l_{se} , cp. Fig. 13.

(Σ transections area tr/number of se + ic + vp) is abbreviated as ($\Sigma \text{ area tr} / \Sigma \text{ se, ic, vp}$).

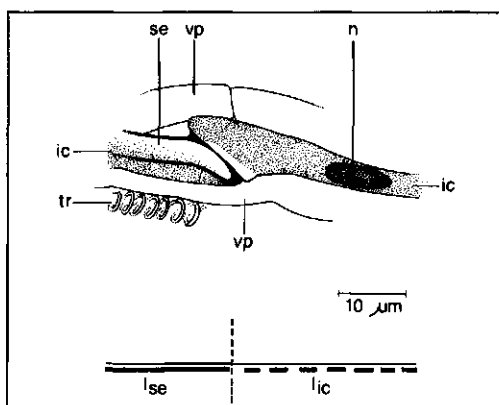


FIG. 5. Detail of a small connecting vein of white leaf tissue (dry climate; see also Fig. 15B) at the distal end of the zone l_{se} and the proximal extremity of the zone l_{ic} .

In the intermediary cell on the right hand side (ic) some plasmolysis has taken place at the proximal extremity. The vascular parenchyma cells (vp) only have a thin layer of cytoplasm against the walls (not drawn).

l_{ic} = zone with intermediary cell, l_{se} = zone with sieve element, n = large nucleus in ic, se = terminal sieve element, from which the contents are not drawn; the walls are thickened especially in the corners (these terminal sieve elements are sometimes wider and they often have more cytoplasm than the other sieve elements), tr = tracheary element.

than the neighbouring cells of the bundle sheath. These cells possess large vacuoles and less dense cytoplasm.

The tracheary elements are tracheids with spiral thickenings of the walls. The tracheids are very small and obliterated sometimes (protoxylem) or they are larger up to very large and intact (metaxylem).

Close to the distal end of the veinlets the vascular bundle often becomes amphivasal, i.e. the tracheary elements completely surround the phloem (Fig. 3). Adjacent to and in a direct line with the distal end of the phloem with sieve elements, usually a narrowing complex of parenchyma cells occurs towards the ultimate top of the veinlet (Fig. 4A). In this complex of parenchymatous elements often one or two cells are found in direct contact with the distal extremity of the distal sieve element (Fig. 5). These cells have dense cytoplasm and large nuclei and will be called intermediary cells also (Table 2, Fig. 4A). In the direction of the ultimate top of the veinlet in a direct line with the distal end of these intermediary cells, frequently some parenchymatous elements will follow. In the majority of veinlets these vascular parenchyma cells have distinctly less dense cytoplasm. Still nearer to the ultimate top of the veinlet only tracheids and sometimes also some vascular parenchyma cells constitute the top of the veinlet.

Analysis of vein endings

The vein ending can be divided in four zones (Fig. 4B).

- Zone 1: l_{se} = length of the vein ending along which sieve elements can be found;

- Zone 2: l_{ic} = length of the vein ending with intermediary cells (no sieve elements);
- Zone 3: l_{vp} = length of the vein ending with vascular parenchyma cells (no sieve elements and no intermediary cells);
- Zone 4: l_{tr} = the most distal part of the vein ending in which only tracheids and sometimes also a few vascular parenchyma cells among them (vpx).

The vascular parenchyma cells of the xylem (vpx in zone l_{tr}) abut on the tracheids and the cells of the bundle sheath. In general these cells are not in direct contact with the cells of the phloem.

This division in zones is not always complete: the zones l_{se} , l_{ic} or l_{vp} may be lacking. Finally the limits are not always sharp. The limit between the zones l_{se} and l_{ic} is nearly always distinct. In one case this line could be drawn sharply only after some difficulty because of the occurrence of an intermediary cell in a direct line with a terminal sieve element and the fact that the general shape and the thickness of the wall of the intermediary cell was similar to that of the adjacent sieve element. Because this intermediary cell had among other things much cytoplasm and a large nucleus with distinct nucleoli, the identification could take place without doubt. The limit between the zones l_{ic} and l_{vp} was determined by comparing the density of cytoplasm of an intermediary cell with the density of the more distal oriented vascular parenchyma cells.

In one variegated leaf (Fig. 1) 33 vein endings were analysed of which 16 vein endings differentiated in the green leaf tissue and 17 vein endings differentiated in the white tissue. In both parts of the leaf 10 vein endings have been analysed in which a zone l_{se} was present, i.e. the types 2, 5 and 6 according to Fig. 2.

Classes of vein length in the vein endings

In Table 3 some results of vein length are given; the analysed vein endings are arranged into groups of 100 μm difference in length each. In every group the average values were determined of the total length of the vein endings ($\overline{l_v}$),

TABLE 3. (Green house conditions)

Total number	Length of vein endings, μm	$\overline{l_v}$	$\overline{l_{se}}$	$(\overline{l_v} - \overline{l_{se}})$	% of $\overline{l_v}$
<i>green</i>					
1	0 - 100	80	14	66	82,5
1	100 - 200	110	40	70	63,6
8	200 - 300	259	129	130	50,0
0	300 - 400	-	-	-	-
<i>white</i>					
0	0 - 100	-	-	-	-
5	100 - 200	168	65	103	61,2
4	200 - 300	260	157	102	38,6
1	300 - 400	377	265	112	29,7

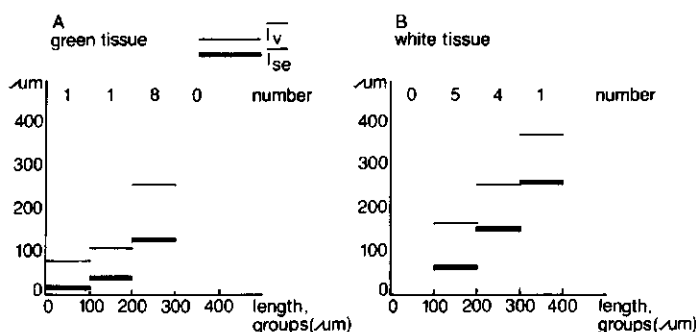


FIG. 6. Diagram of the mean values of the total length (l_v) and of the length of zone l_{se} (l_{se}) per group of vein endings. Each following group of vein endings is 100 μm longer. The number of vein endings in each group is noted above (variegated leaf, green-house conditions).

of the lengths of l_{se} (l_{se}), of the lengths of $(l_v - l_{se})$ and of $(l_v - l_{se})$ as a percentage of l_v .

From Table 3 it becomes clear that the length of the extremities of the vein endings ($l_v - l_{se}$) becomes proportionally smaller (in % of l_v) as the veinlet length (l_v) becomes longer.

In the diagram of Fig. 6B the mean values of the total length and the length of zone l_{se} are given. There is a tendency towards a rather constant value of the length of the xylem tips ($l_v - l_{se}$) of the vein endings independent of the length of the veinlets in white leaf tissue. Fig. 7 is a detailed representation of one vein ending in white tissue. A tendency exists to a maximum of the calculated sum of areas of transections of tracheids ($\Sigma \text{area tr}$). This maximum coincides approximately with the maximum number of tracheids in one transection. The position of this tracheid maximum also coincides approximately with the position of the extremity of the zone l_{se} .

It may be noticed that the terminal sieve element appears wider; this phenomenon can be found frequently, but not in every veinlet. In this terminal sieve element more cytoplasm is often present too. It is also evident that the tracheids of the distal end of the veinlet are wider (metaxylem) than those of the proximal end (more protoxylem).

The position of the tracheid maximum

The position of the tracheid maximum has been located for 33 analysed vein endings of one variegated leaf (Fig. 8). The numbers of tracheids at the proximal end of most analysed vein endings are approximately equal in both green and white leaf tissue; the short vein endings in D possess a somewhat greater number of tracheids. The maximum number of tracheids in the vein endings with a zone l_{se} ($l_{se} \geq 15 \mu\text{m}$) is about 20.

In white tissue this tracheid maximum is always situated in the vicinity of the extremity of the zone l_{se} (in 10 out of 10 analysed vein endings) and in green tissue there seems to be more diversity: the tracheid maximum has been found

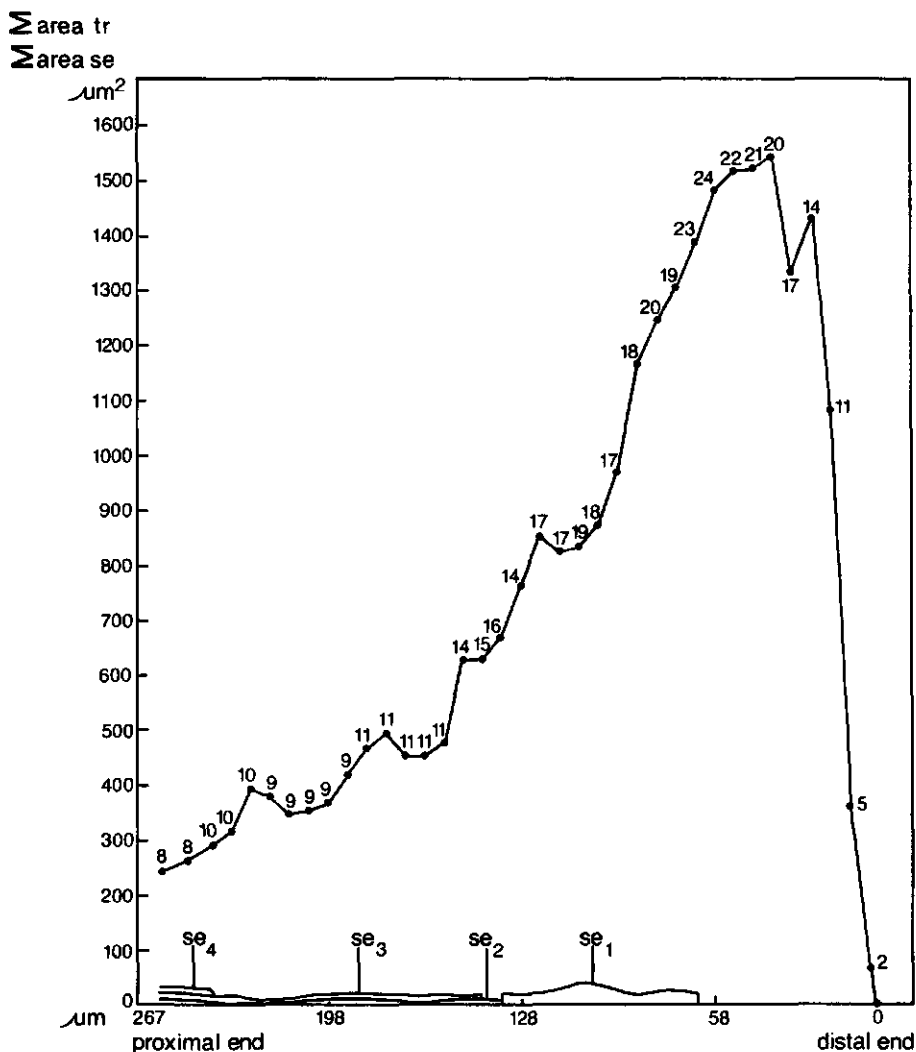


FIG. 7. One vein ending in white leaf tissue of type 2 (see Fig. 2). Variegated leaf, green house conditions. Abscissa: length of the vein ending l_v and of the phloem with sieve elements (l_{se} , with sieve elements se_i , etc.). Ordinate: calculated sum of areas of transverse sections of tracheids (Σ area tr) in each transverse section of the veinlet and for the transverse sections of sieve elements (Σ area se) in μm^2 . The number of tracheids is noted belonging to each transection.

to occur at the proximal side of the extremity of l_{se} ($1 \times$) and also at the distal side of the extremity of l_{se} ($4 \times$). In the vein endings without a zone l_{se} (types 1, 3 and 4) the tracheid maximum is always close to the proximal end of the veinlet in white leaf tissue. In green tissue this tracheid maximum may be found also further removed from the proximal end. The results in Fig. 8B and 8D

TABLE 4. The average length of the vein endings (\overline{l}_v), the average length of the phloem with sieve elements (\overline{l}_{se}), the average number of tracheids in the tracheid maxima and the average number of sieve elements close to the proximal end of the vein endings per group of vein endings in green and in white leaf tissue, with and without a zone l_{se} (variegated leaf, green house conditions).

Leaf tissue	Number of veinlets	Types of veinlets	\overline{l}_v , μm	\overline{l}_{se} , μm	Max. number of tracheids in maxima (average)	Number of sieve elements near proximal end (average)
green	10	2, 5, 6	226,1	108,5	17,5	1,6
green	6	1, 3, 4	137,8	—	11,8	—
white	10	2, 5, 6	225,7	123,5	19,7	1,9
white	7	1, 3, 4	89,0	—	14,0	—

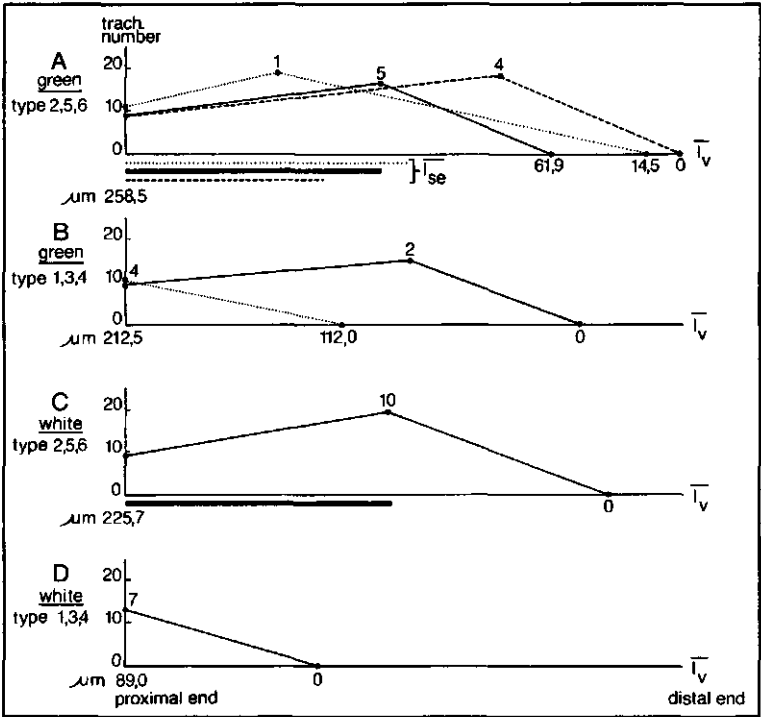


FIG. 8. Position of the tracheid maximum in vein endings in green and in white leaf tissue (variegated leaf, green house conditions). Types of veinlets (see Fig. 2): 2, 5 and 6 (10 in A and 10 in C) and types 1, 3 and 4 (6 in B and 7 in D). In each group of vein endings the average numbers have been determined of (a) the total length (abscissa) of the vein endings (\overline{l}_v), of (b) the number of tracheids (ordinate) close to the proximal extremity, of (c) the maximum number of tracheids, of (d) the position of the tracheid maximum along the veinlet, and of (e) the average sizes of the length of the phloem with sieve elements (\overline{l}_{se}) belonging to the group of vein endings. The number at the top of the curves indicates the number of vein endings in that group. The length of \overline{l}_{se} is expressed with special corresponding types of lines.

correspond with those in Fig. 8A and 8C in this respect; and this creates the impression that the vein endings without a zone l_{se} are to be considered as the extremities of the vein endings with a zone l_{se} in the corresponding green and white leaf tissue.

Table 4 shows that the average lengths of the vein endings with a zone l_{se} are equal in green (226.1 μm) and in white (225.7 μm) tissue. The vein endings without a zone l_{se} are significantly shorter, however, than the veinlets with a zone l_{se} , Wilcoxon test, $P = 0.025$ in green and $P = 0.001$ in white tissue. The number of tracheids in the tracheid maxima is significantly lower in vein endings without a zone l_{se} , Wilcoxon test, $P = 0.05$ in green and $P = 0.025$ in white tissue. The critical values are given for one-tailed probability.

From the above mentioned data one may conclude that a further analysis is desirable for a determination of the position of the tracheid maximum. For an experimental approach of the nature of these structures in the leaf of *Hedera*, the white leaf tissue seems to be most suited because its tracheid maximum

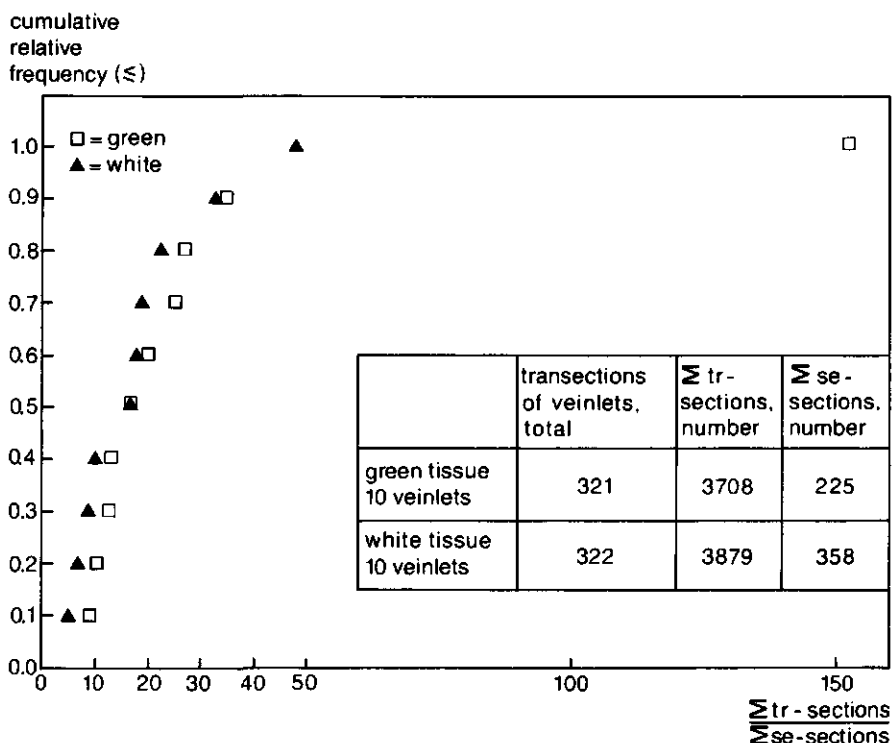


FIG. 9. Comparison of the total amount of the xylem with the total amount of the phloem by means of the quotient Σ tr-sections/ Σ se-sections per vein ending. 10 vein endings with a zone l_{se} in green and in white leaf tissue were used for calculation. Ordinate: fraction smaller than or equal to the value of the quotient.

Insert: total of transverse sections of the veinlets, total of cross-sections of the tracheids and the total of cross-sections of sieve elements (variegated leaf, green house conditions).

turned out to be more constantly in the vicinity of the extremity of the zone l_{sc} .

The amounts of phloem and xylem in vein endings

The total amount of phloem and xylem in the vein endings can be specified by the length and the diameter from each of these tissues. An estimation of these values may be achieved by scoring the total quantity of transections of tracheids (Σ tr-sections) and of transections of the sieve elements (Σ se-sections) in every transverse section of the vein ending (Fig. 9). The quotients Σ tr-sections/ Σ se-sections are not significantly higher in the green leaf tissue than in the white tissue; this means that the difference between the given number of tracheids in relation to the phloem part with sieve elements belonging to it in the green and in the white leaf tissue, is not significant.

Distribution of phloem and xylem elements in a long not ramified vein ending

The percentage of tracheids in every transverse section of a vein ending, calculated for the total number of elements in every section, usually regularly increases towards the distal extremity of the veinlet (Table 5, from 28 to 100%). The percentage of living elements in every transection decreases regularly (Table 5, from 72 to 0%). Of 12 vein endings these percentages have been calculated and they are shown in Table 6.

The vein endings nos 5, 6, 7, 9, 11 and 12 (Table 6), which showed a distinct tracheid maximum in the veinlet, a very gradual decline of the percentage of tracheids was found in all the six veinlets from the distal end to the proximal end (as in Table 5).

The percentage of living elements per transection increases fairly regularly in the same direction from 0 to 66.7 on an average in these six vein endings. The tracheid maximum in these vein endings appears independent of the total number of elements in the transections of the veinlet. Another structural cause for the origin of this tracheid maximum (such as a greatly overlapping of the extremities of the tracheids at the end of two vein segments) has not been found.

In order to investigate the cause of appearance of the tracheid maximum, a long and not ramified vein ending (type 2, Fig. 2) was reexamined (Table 5). In this vein ending three tracheid maxima do appear properly, viz. in transections 10, 23 and 33. These maxima do not clearly coincide in each case with a maximum of the total number of elements in the sections.

Position of the tracheid maximum in detail

It has been shown (Figs 4, 7 and 8) that the position of the tracheid maximum is associated with the distal end of the zone l_{sc} in the vein ending, especially in white leaf tissue. It is of importance to compare the positions of the appearing tracheid maxima in the vein ending given in Table 5 with corresponding changes in character and dimension of the phloem. This comparison can be made by means of the graphs in the Figs 10, 11, 12, 13 and 14.

In Fig. 10 the three tracheid maxima are visible in the distal half of the vein

TABLE 5. Survey of the numbers of all elements found in the transections no. 1 (distal end) to no. 76 (proximal end) of a not ramified vein ending, type 2 (Fig. 2), with a zone l_{se} and 588 μ m in length. Those vascular parenchyma cells (vp) situated in the distal extremity of the veinlet abaxial of the xylem and in a direct line with the distal end of the zones l_{se} or l_{ic} , have been put under the heading phloem. This vein ending is grown in a wet climate.

Section number	Phloem			Xylem		% living elements of total	% tr of total	Total number of elements
	se	ic	vp	vpx	tr			
1					3	0	100	3
2					3	0	100	3
3			1		3	25	75	4
4			1		4	20	80	5
5			3		10	23	77	13
6			5	1	16	27	73	22
7			5	1	17	26	74	23
8			5	1	16	27	73	22
9		1	6	1	16	33	67	24
10		1	6	1	18	31	69	26
11		1	6	1	17	32	68	25
12		1	7	2	16	38	62	26
13		1	7	2	14	42	58	24
14		1	7	1	15	38	62	24
15		1	7	1	13	41	59	22
16	1	3	7	1	13	48	52	25
17	1	3	4	2	13	43	57	23
18	1	3	3	3	13	43	57	23
19	1	3	3	4	15	42	58	26
20	1	3	6	1	17	39	61	28
21	1	5	5	2	17	43	57	30
22	2	6	7	1	15	52	48	31
23	2	6	5		18	42	58	31
24	2	6	6		17	45	55	31
25	2	6	7		15	50	50	30
26	3	6	6	1	13	55	45	29
27	3	6	6	1	12	57	43	28
28	3	6	9	1	13	59	41	32
29	3	6	8	1	12	60	40	30
30	3	6	8	1	12	60	40	30
31	3	6	7	1	14	55	45	31
32	3	6	8	1	16	53	47	34
33	3	6	9	2	21	49	51	41
34	4	7	11	3	20	56	44	45
35	4	9	9	3	17	60	40	42
36	5	10	3	3	18	54	46	39
37	4	11	4	3	18	55	45	40
38	4	11	11	3	14	67	33	43
39	4	11	13	3	13	70	30	44
40	4	12	13	3	12	73	27	44
41	4	12	10	3	12	71	29	41
42	4	12	8	3	13	68	32	40
43	4	12	6	3	13	66	34	38
44	3	11	8	3	13	66	34	38
45	3	11	10	3	12	69	31	39

Section number	Phloem			Xylem		% living elements of total	% tr of total	Total number of elements
	se	ic	vp	vpx	tr			
46	3	10	12	3	12	70	30	40
47	3	10	12	3	12	70	30	40
48	3	10	10	3	12	68	32	38
49	4	11	8	3	13	67	33	39
50	4	11	8	2	13	66	34	38
51	4	9	9	3	14	64	36	39
52	6	9	10	2	12	69	31	39
53	5	10	10	3	12	70	30	40
54	5	9	11	3	12	70	30	40
55	5	9	9	3	12	68	32	38
56	4	10	7	2	11	68	32	34
57	4	10	10	1	10	71	29	35
58	4	10	9	1	10	71	29	34
59	4	10	10	1	11	69	31	36
60	4	10	10	1	11	69	31	36
61	4	10	9	1	11	69	31	35
62	4	10	9	1	10	71	29	34
63	4	10	8	1	10	70	30	33
64	4	10	7	1	10	69	31	32
65	4	9	8	1	10	69	31	32
66	4	9	8	1	10	69	31	32
67	4	9	6	1	10	67	33	30
68	5	9	6	1	9	70	30	30
69	5	9	7	1	9	71	29	31
70	4	8	9	1	8	73	27	30
71	4	6	10	1	8	72	28	29
72	4	6	10	1	8	72	28	29
73	4	6	10	1	8	72	28	29
74	4	6	8	1	8	70	30	27
75	4	5	9	1	9	68	32	28
76	5	6	11	1	9	72	28	32

ending. In this part of the veinlet the number of sieve elements decreases to zero and still more in distal direction a zone with one intermediary cell (l_{ic}) follows. Closer to the extremity of the veinlet the zone with vascular parenchyma cells (l_{vp}) follows, being in a direct line with the distal end of the intermediary cells. Finally a short zone with tracheids only (l_{tr}) forms the ultimate end of the veinlet.

In Fig. 11 the added section areas of tracheids (a better estimation of the total quantity of the xylem) and sieve elements per transection, all along the vein ending, have been indicated. The three tracheid maxima are still clearly visible in the graph; however, the maximum appearing at the distal end of the zone l_{ic} is most striking and reaches a much higher value than the other two.

In Fig. 12 the number of tracheids in each transection has been plotted in relation to the quantity of the phloem, expressed in the quotient of the total number of tracheids in a transection divided by the total number of sieve elements, intermediary cells and vascular parenchyma cells in the same transection.

TABLE 6. Percentages of tracheary elements with regard to the total number of elements at the proximal end and at the distal extremity in 12 veinlets of types 2,5 and 6 (with a zone l_{se} , Fig. 2). The type of vein ending is indicated in more detail according to the classification of Table 7. In two veinlets the vascular parenchyma cells in a direct line with the distal end of the intermediary cells or the sieve elements reach to the distal extremity (numbers 1 and 10). l_v = length of the veinlets in μm .

Veinlet number	Types of veinlets (Table 7)	l_v , μm	Proximal end		Distal end		Climate type
			Total number of elements	% trach. elements of total	Total number of elements	% trach. elements of total	
1	s	143	20	45	4	25	dry
2	t	240	28	56	1	100	wet
3	n	285	33	42	1	100	wet
4	m	286	31	35	4	100	wet
5	n	295	30	43	2	100	dry
6	q	304	38	35	1	100	dry
7	p	325	31	23	1	100	wet
8	t	332	29	38	1	100	wet
9	t	345	29	34	1	100	wet
10	t	363	26	46	2	50	dry
11	s	403	28	36	1	100	dry
12	t	588	30	30	3	100	wet
average		325.8	29.4	38.6	1.8	89.6	

These quotients could be calculated in the zones l_{se} and l_{ic} . The three tracheid maxima above mentioned are found again in the same positions, while the third maximum again is the highest. In the zone l_{vp} the quotient of the sum of the tracheids and the total number of vascular parenchyma cells has been determined in each transection. These points in the graph show an indistinct path, and no quotients can be calculated in the zone l_{tr} . If one wishes to express a relation between the numbers of tracheids and the quantity of the phloem in these zones l_{vp} and l_{tr} , a comparison of the number of tracheids in these zones with the quantity of the phloem somewhere at the distal extremity of the phloem is obvious. If these quotients are calculated by dividing the sum total of tracheids per transection by the value of $\Sigma se, ic, vp$ at the distal extremity of the zone l_{ic} ($se = 0, ic \geq 1$), a rather regular decrease of the curve from the third maximum to zero at the extremity of the veinlet is obtained.

In Fig. 13 the added section areas of tracheids have been plotted in relation to the quantity of the phloem, as quotients again in the zones l_{se} and l_{ic} . In the zone l_{vp} the quotients have been determined of the sum of the section areas of tracheids and the sum total of vascular parenchyma cells per transection. The points in the graph determined in this way do not show a distinct continuation of the curve in the zones l_{se} and l_{ic} . However, when calculating these quotients in the zones l_{vp} and l_{tr} using the constant value of $\Sigma se, ic, vp$ at the distal end of the zone l_{ic} ($= 7; se = 0; ic = 1; vp = 6$) as the denominator, one

FIGS. 10–14. Abscissa: length of the non ramified vein ending of Table 5 in μm ; left: proximal and right: distal end. The zones l_{se} , l_{ic} , l_{vp} and l_{tr} are indicated as in Fig. 4.

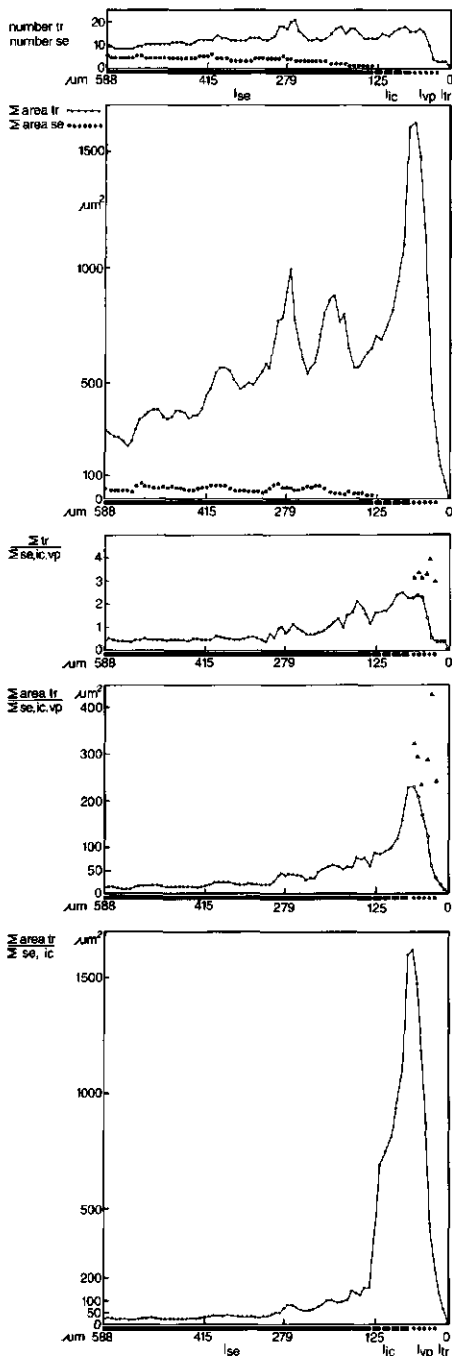
FIG. 10. The number of tracheids (—●—●—) and sieve elements (●●●●●) in each of the 76 transections of the vein ending of Table 5.

FIG. 11. The sum of the section areas of the total of tracheary elements ($\Sigma \text{ area tr}$, —●—●—) and the sum of the section areas of the total of sieve elements ($\Sigma \text{ area se}$, ●●●●●) in μm^2 per transection of the vein ending all along the veinlet.

FIG. 12. The quotients of the sum of the number of tracheids and the sum of the number of sieve elements, intermediary cells and vascular parenchyma cells ($\Sigma \text{ tr}/\Sigma \text{ se, ic, vp}$) per transection all along the zones l_{se} and l_{ic} (—●—●—). In the zone l_{vp} the quotients of the sum of the tracheary elements and the sum of the number of vascular parenchyma cells ($\Sigma \text{ tr}/\Sigma \text{ vp}$) have been determined per transection (▲▲▲). Moreover in the zones l_{vp} and l_{tr} the quotients of $\Sigma \text{ tr}$ on the one side and $\Sigma \text{ se, ic, vp}$ at the place of the distal extremity of the zone l_{ic} on the other side, have been determined per transection (—○—○—○—).

FIG. 13. The quotients ($\Sigma \text{ area tr}/\Sigma \text{ se, ic, vp}$) per transection all along the zones l_{se} and l_{ic} (—●—●—). In the zone l_{vp} the quotients ($\Sigma \text{ area tr}/\Sigma \text{ vp}$) have been determined per transection (▲▲▲). Moreover in the zones l_{vp} and l_{tr} the quotients of $\Sigma \text{ area tr}$ on the one side and the constant value of $\Sigma \text{ se, ic, vp}$ at the place of the distal extremity of the zone l_{ic} on the other side, have been determined per transection (—○—○—○—).

FIG. 14. The quotients of $\Sigma \text{ area tr}$ and the sum of the number of sieve elements and intermediary cells ($\Sigma \text{ area tr}/\Sigma \text{ se, ic}$) (—●—●—) per transection all along the zones l_{se} and l_{ic} . However, in the zones l_{vp} and l_{tr} the quotients of $\Sigma \text{ area tr}$ per transection and the constant value of $\Sigma \text{ se, ic}$ at the place of the distal extremity of the zone l_{ic} , have been determined.



can see the very regular continuation of the curve from the third maximum to zero at the extreme point of the veinlet, just like the regular steep descent of the curve in Fig. 11. Moreover it is striking that only the third tracheid maximum still appears as a pronounced maximum and it arises exactly at the extremity of the zone l_{ic} of this veinlet.

Finally in Fig. 14 the added section areas of tracheids have been plotted in relation to the sum of sieve elements and intermediary cells per transection. The quantity of the phloem has now been characterized only by the total of $\Sigma se, ic$. Also in this graph the zones l_{vp} and l_{tr} have been calculated using as denominator the constant value of $\Sigma se, ic$ at the distal end of the zone l_{ic} ($= 1$) in the quotients. In this curve the most pronounced tracheid maximum also appears at the distal end of the zone l_{ic} .

In summary: the position of the already described tracheid maximum at the distal end of the zone l_{ic} can be shown in the most expressive way when the added section areas of the tracheids (and therefore also the total volume of the tracheary elements), are given in relation to the quantity of the phloem in every transection of the veinlet. The quantity of the phloem can be described best by $\Sigma se, ic, vp$, because of the fact that the difference between the intermediary cells and the vascular parenchyma cells may be difficult to determine in some parts of the veinlet. The procedure as represented in Fig. 13 will be followed therefore in the next part of this article in case of doubt of the exact position of the tracheid maximum in the veinlet.

The localization of the tracheid maxima in different types of vein endings

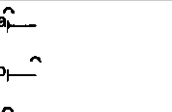
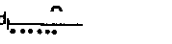

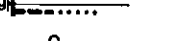
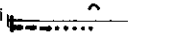

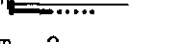
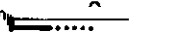


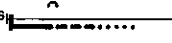





After calculation on 20 vein endings it turned out to be possible to represent the quantity of xylem in the vein endings in relation to the quantity of the phloem in the shape of a more or less constant type of curve as in the Figs 4 and 13. Now the question arises whether the position of the tracheid maximum can be located more exactly in relation to the zones l_{se} , l_{ic} , l_{vp} and l_{tr} .

The possible types of vein endings (21 types, a-u) have been brought together in Table 7. The determining of the position of the tracheid maximum runs up against difficulties when one or more zones are relatively short, or when a broad maximum appears within which more than one zone terminates. In these cases the position of the maximum has been located by estimating the shortest distance between the position of the maximum and the two adjacent limits of the zone. The limit between the zones l_{ic} and l_{vp} was determined by paying attention to cytoplasmic density. Finally the distal limit of the zone with vascular parenchyma cells is not sharp sometimes caused by the fact that the distinction between vascular parenchyma cells and cells of the bundle sheath is difficult to determine.

Based on these theoretical types it can be concluded that:

- veinlet type b has not been found. This means that one does never find the tracheid maximum along that part of the vein ending only consisting of xylem (tracheids and possibly vascular parenchyma cells, vpx);
- veinlet types j, l, o and r have not been found. This indicates that the tracheid maximum along that part of the vein ending in which sieve elements do occur

TABLE 7. Theoretically possible types in dry and wet climate of free vein endings (a-u) in relation to the position of the tracheid maximum (\cap). The position of the maximum is only determined by the observed number of tracheids, or after calculation of the quotient Σ area tr/Σ se, ic, vp. The zones l_{se} , l_{ic} and l_{vp} are always $\geq 21 \mu m$ in length, with the exception of the zone l_{se} in one of the vein endings of type k and one of the vein endings of type m (column no. 7), which are only about $15 \mu m$ in length both.

tr. max (∩) in vein endings, zones ≥ 21 μm	dry				wet				dry total	wet total	total
	not ram.		ramified		not ram.		ramified				
	position of tracheid maximum determined by:										
	number of trach	calcula- tion of quotient	number of trach	calcula- tion of quotient	number of trach	calcula- tion of quotient	number of trach	calcula- tion of quotient			
	1		4	1	1		3		6	4	10
											
	1		2				1	1	3	2	5
											
											
			1				1	1	1	2	3
								1		1	1
				1				1	1	1	2
								1		1	1
											
								2		2	2
											
	4		2		1		1	1	6	3	9
		1		1				1	2	1	3
											
							1			1	1
	2								2		2
			1	1					2		2
			3	1	1	1	1	3	4	6	10

not can be expected;

- veinlet type a has been found frequently (10 ×). In these cases at the origin of this vein ending
 1. sieve elements do occur at the proximal extremity, for example in the vein of lower order (5 ×);
 2. intermediary cells do occur at the proximal extremity (2 ×); and
 3. vascular parenchyma cells do occur at the proximal extremity (3 ×).

One may conclude that the veinlet type a can be considered entirely to be a zone l_{tr} .

- the tracheid maximum does always occur near the distal extremity (= near the limit of the zone in the direction of the extremity of the vein ending) of the zones l_{se} , l_{ic} or l_{vp} (also when respectively se , ic or vp are present at the point of branching, viz. in types a, c and g). This becomes visible

1. in the end of zone l_{se} , as in type a (5 ×), c (4 ×), g (1 ×), k (2 ×), m (9 ×), p (1 ×), and s (2 ×), altogether 24 ×;
2. or in the end of zone l_{ic} , as in type a (2 ×), f (3 ×), h (2 ×), q (2 ×) and t (10 ×), altogether 19 ×;
3. finally in the end of zone l_{vp} , as becomes clear in type a (3 ×), i (1 ×) and n (3 ×), altogether 7 ×.

It appears that the tracheid maximum has been found most often close to the distal extremity of the zone l_{se} , but the maximum also does occur near the distal end of the zone l_{ic} frequently if this zone is present. In the presence of a zone l_{vp} the tracheid maximum does appear near the end of this zone sometimes.

The conclusion is that a strong tendency exists of the tracheid maximum to shift to the right (Fig. 15A) under the influence of an extension of the zone l_{se} by a zone l_{ic} or the zones $l_{ic} + l_{vp}$ or sometimes by a zone l_{vp} only.

Any part of a vein ending consisting of the zones ($l_{ic} + l_{vp} + l_{tr}$), ($l_{vp} + l_{tr}$) or l_{tr} may be found also as lateral vein branches. Thus the small branches often represent the extremities of the longer free vein endings (cp. Fig. 8 and the text belonging to it). The point of branching may occur at any place along the vein ending; however, the zones l_{ic} and l_{vp} are continuous each and communicate with a zone l_{se} .

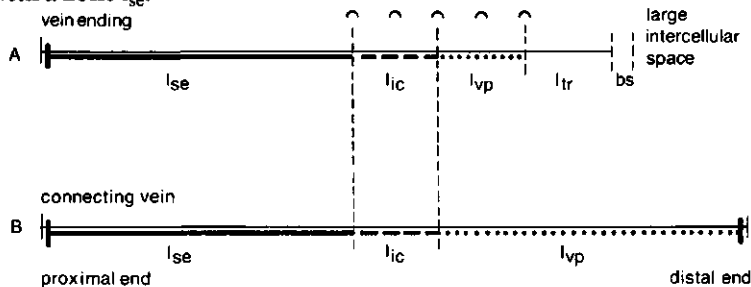


FIG. 15A. Schematic drawing of a free vein ending with zones l_{se} , l_{ic} , l_{vp} and l_{tr} ; \cap : possible positions of the tracheid maxima, bs = bundle sheath.

B. Schematic drawing of a small vein connecting two veins of lower order. In the connecting vein the same zones appear but a tracheid maximum is absent (see also Fig. 5).

TABLE 8. Average values of the number of tracheary elements in the tracheid maximum, expressed in the average number of observed tracheids in a transection of a vein ending on the spot of the tracheid maximum in three different groups of vein endings. \bar{l}_v = average length of the vein endings in a group.

Group number	Types of veinlets	Dry climate			Wet climate		
		Number of veinlets	\bar{l}_v , μm	Max. number of tracheids (average)	Number of veinlets	\bar{l}_v , μm	Max. number of tracheids (average)
1	only with zones l_{vp} and l_{tr}	9	101.8	13.7	6	92.3	10.7
2	only with zones l_{ic} , l_{vp} and l_{tr}	2	151.0	12.0	5	145.4	16.4
3	with zones l_{se} , l_{ic} , l_{vp} and l_{tr}	16	347.7	19.3*	13	258.0	17.7*
	total	27	251.1	16.9	24	193.1	15.7

* cp. Table 4: the number of tracheids in the maxima of 10 veinlets in white leaf tissue averages 19.7.

TABLE 9. Vein endings with a zone l_{se} (type 2, 5, 6) arranged in order of increasing length (l_v) in dry and in wet climate.

Veinlet number	Dry climate			Wet climate		
	l_v , μm	l_{se} , μm	$l_v - l_{se}$, μm	l_v , μm	l_{se} , μm	$l_v - l_{se}$, μm
1	131	42	89	62	15	48
2	143	55	88	63	15	49
3	195	71	124	68	26	42
4	231	42	189	189	40	149
5	275	80	195	207	68	139
6	295	128	167	240	104	136
7	304	156	148	285	171	114
8	339	143	196	286	195	91
9	342	236	106	325	224	101
10	363	98	265	332	165	167
11	378	218	160	345	171	174
12	379	235	144	364	203	161
13	403	245	158	588	463	125
14	513	383	130			
15	615	470	145			
16	657	433	224			

In Fig. 15B a small vein has been drawn schematically being the connection between two veins of lower order. Fig. 5 depicts a detail of this connecting vein, viz. the marginal area near the distal extremity of zone l_{se} and the proximal end of zone l_{ic} . There is no tracheid maximum in this vein, the number of tracheids is 6 proximad (left) and 7 distad. The intervening part contains 5–7 tracheids on an average. The zone l_{vp} abuts on very large intermediary cells next to a big sieve element on one of the sides of the phloem of a large vein.

External influences on vein ending composition

The influence of the moist percentage of the air in the growth cabinet might cause a change of types of veins and/or a change of the number of tracheary elements in the vein endings in respect of the phloem tissue. The transpiration of the test object has been measured. When the relative humidity was low ($7.0 \pm 2\%$ r.h.) the water absorption by the whole plant (32 leaves larger than 1 cm) appeared to be about 115 ml and in a wet atmosphere ($97.0 \pm 2\%$ r.h.) the absorption by the plant (40 leaves larger than 1 cm) amounted to about 55 ml during 24 hours. The average length of the 25 mature leaves in the dry atmosphere was about 7.5 cm and of the 30 mature leaves in the wet atmosphere about 6.5–7 cm.

It appears from Table 7 that there is not a clear difference in the distribution of types of vein endings in a dry and a wet climate. All types found frequently do occur in both climates and the types found less frequently do appear regularly spread over both climates. The influence of the moisture content of the air on the number of tracheary elements may be considered with respect to the height of the tracheid maximum (Table 8) and also regarding the length of the terminal part of the veinlet without sieve elements (i.e. the length of the zones $l_{ic} + l_{vp} + l_{tr}$; Table 9 and Fig. 16) in proportion to the total length of the vein endings.

After some statistical calculation (t-test) on the data of which Table 8 is a summary, it appeared that the total length of the vein endings as well as the height of the tracheid maximum do not differ significantly in dry and in wet climate ($P = 0.05$). The calculated dispersion showed rather homogeneous groups of data. After statistical calculation (t-test) on the values ($l_v - l_{se}$) from Table 9 in dry and in wet climate, the sum of the terminal zones ($l_{ic} + l_{vp} + l_{tr}$) of the vein endings appeared to be shorter in wet climate ($P = 0.05$). This means a decrease of differentiation of xylem in respect to the phloem part of the vein ending within an areole. (The critical values of P are given for one-tailed probability).

In Fig. 16 the average values of l_v , l_{se} and l_{ic} , calculated for groups of veinlets of 100 μm difference in length each, have been put in a diagram. This diagram confirms the diagram of Fig. 6; the lengths of the extremities ($l_v - l_{se}$) of the vein endings are rather constant and highly independent of the length of the vein ending l_v . The shortest veinlets may be looked upon as exceptions (like the short ones in Fig. 6) in which these extremities are shorter. In wet climate these extremities ($l_v - l_{se}$) thus appear to be shorter than in dry climate.

As in Fig. 9 the total amounts of the xylem and the phloem in the vein endings

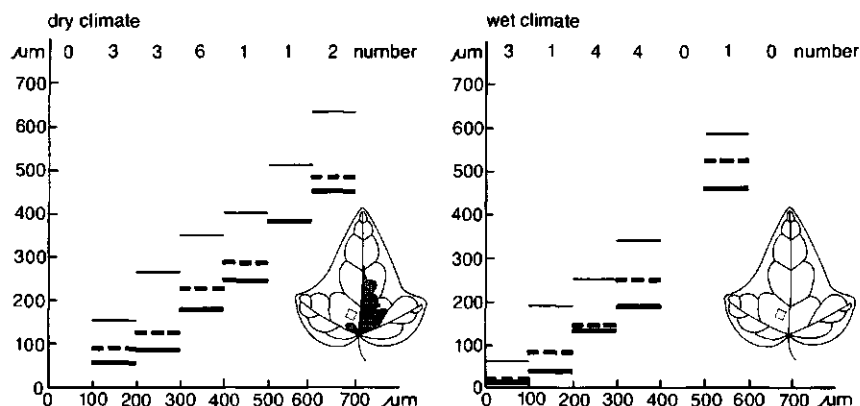


FIG. 16. Diagram with the data from Table 9. The vein endings have been divided into groups of 100 μm difference in length each (abscissa) and in every group of veinlets the average values of l_v , l_{se} and l_{ic} have been determined (ordinate).

— = l_v , --- = l_{ic} , — = l_{se} .

The two drawn leaves, with a schematic major venation characteristic for *Hedera* leaves, have been used for this experiment; on the left a variegated leaf with little chlorenchyma (dotted), on the right a white leaf without chlorenchyma. The small squares indicate the pieces of tissue that have been used in analysing.

TABLE 10. Comparison of the total amounts of the xylem and the phloem in the vein ending by means of the quotient $Q = \Sigma \text{tr-sections} / \Sigma \text{se-sections}$ per vein ending in dry and wet climate. Vein endings with a zone l_{se} , arranged in order of increasing length (l_v).

Veinlet number	Dry climate				Wet climate			
	l_v , μm	Σ tr-sections, number	Σ se-sections, number	Q	l_v , μm	Σ tr-sections, number	Σ se-sections, number	Q
1	131	246	6	41.00	62	37	2	18.50
2	143	119	7	17.00	63	57	2	28.50
3	195	338	10	33.80	68	67	4	16.75
4	231	388	6	64.67	189	393	11	35.73
5	275	482	17	28.35	207	431	14	30.79
6	295	590	23	25.65	240	467	24	19.46
7	304	468	51	9.18	285	765	44	17.39
8	339	630	41	15.37	286	575	75	7.67
9	363	569	16	35.56	325	500	72	6.94
10	379	602	79	7.62	332	641	48	13.35
11	403	793	85	9.33	345	642	58	11.07
12	615	1061	157	6.76	364	662	48	13.79
13	657	1030	128	8.05	588	1007	220	4.58
total	4330	7316	626	11.69	3354	6244	622	10.04

may again be approximated by determining the quotient $Q = \Sigma \text{ tr-sections} / \Sigma \text{ se-sections per vein ending}$ (Table 10). It appears from this table that Q is dependent on the length of the veinlets; the longer the veinlet the lower Q will be. This is in conformity with data in Fig. 4. It may be imagined that a veinlet lengthens (the narrow part of the veinlet on the left lengthens out proximally), then the length and usually width of the phloem increase (Fig. 7), while at the mean time the size of the xylem diminishes relatively. In spite of the larger total length of the vein endings in dry climate, the average value of Q seems to be higher in dry climate (11.69). Because the extremities ($l_v - l_{se}$) of the veinlets in dry climate are longer than in wet climate (Table 9), it could be expected that Q was higher in dry climate than in wet climate. The difference is small because of an appearing difference in vein length between both groups of veinlets.

The comparison of the distal extremities of the vein endings in dry and wet climate.

The relation of the value of the quotient Q to the vein length is given in Fig. 17. In short vein endings Q is high, and Q is maximal in veinlets 100–200 μm in length (wet climate) and in veinlets 200–300 μm in length (dry climate). In very short vein endings the average value of Q drops rapidly, because the height

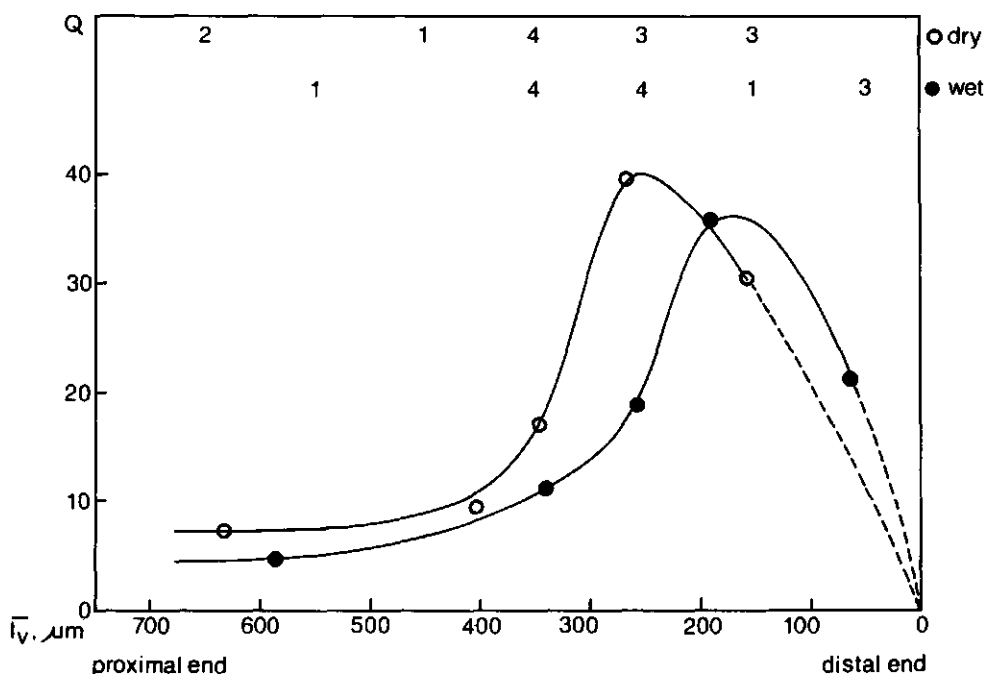


FIG. 17. The value of Q in relation to the vein length in dry and in wet climate. The value of Q ($= \Sigma \text{ tr-sections} / \Sigma \text{ se-sections per veinlet}$) has been noted as an average per group of vein endings; these groups are 0–100 μm , 100–200 μm , etc. in length (l_v). The numbers above the curves indicate the numbers of veinlets in every group.

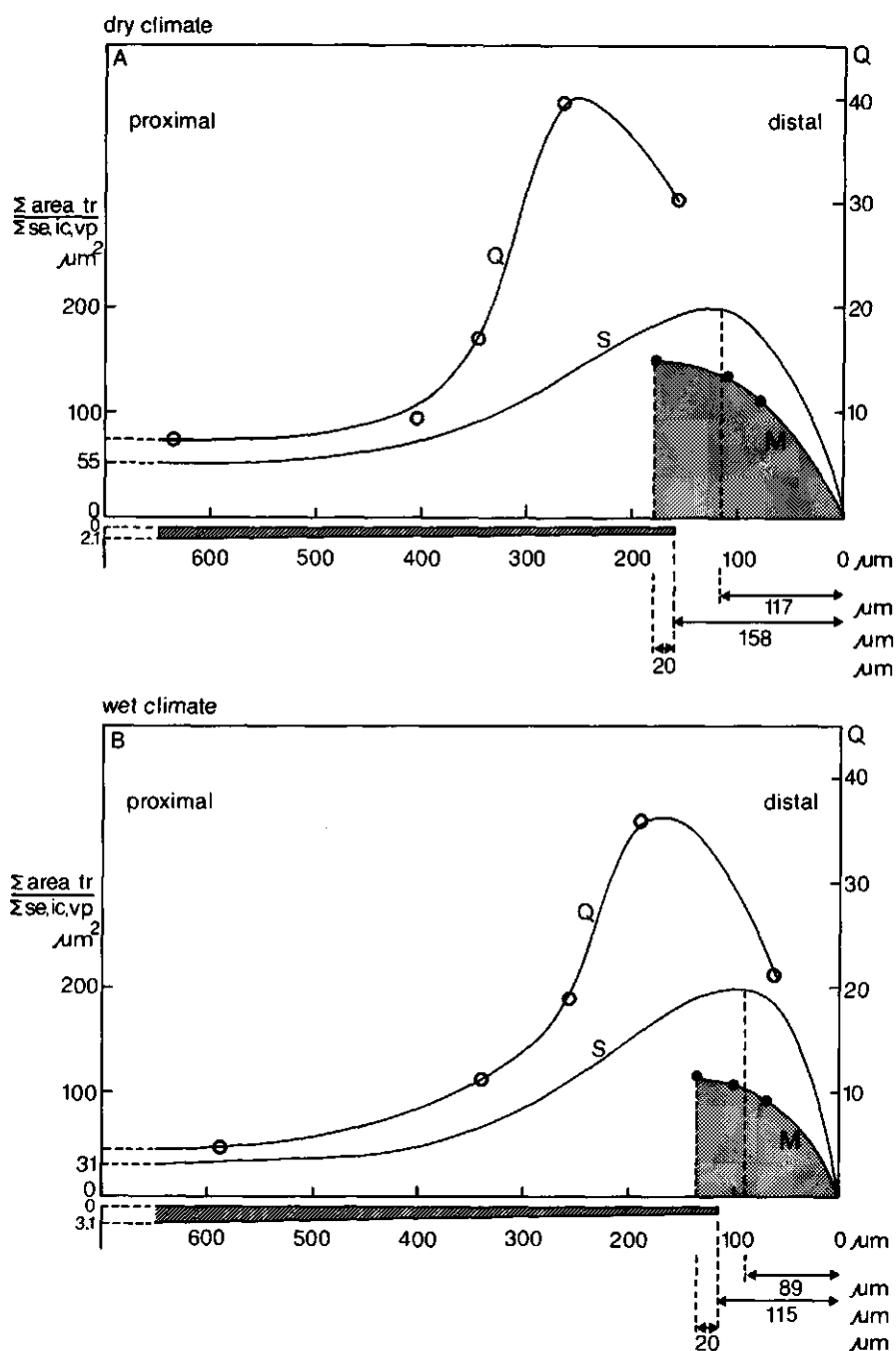


FIG. 18. Vein endings with a zone l_{sc} : schematic drawings of calculated specimen in a dry (A) and a wet climate (B) based on averages (curves S). The $\Sigma \text{ area tr} / \Sigma \text{ se, ic, vp}$ values are indicated on the left (left ordinate). The curves M have been drawn between three calculated points and zero. The calculated points are averages of the height of tracheid maxima in three groups of short veins, shorter than or equal to a certain length (Table 12B).

The curves Q are the same as in Fig. 17 (right ordinate). Below the abscissa the length (μm) and the width (number of sieve elements) of the phloem are indicated at the proximal and at the distal end. Further explanation in the text.

of the tracheid maximum (number of tracheids) is much reduced. At the same time it is obvious that in vein endings longer than about 200 μm the total amount of the xylem in respect of the total amount of the phloem is higher in dry climate than in wet climate. This is in conformity with the result: the extremities ($l_v - l_{se}$) are longer in dry climate than in wet climate.

In Fig. 18 the three curves Q, S and M have been drawn for dry and for wet climate. The curves Q are the same as in Fig. 17; the cause of shift of the maximum of the curve Q to the right in a wet climate will be evident when considering the curves Q in connection with the curves S. The curves S deal with all vein endings with a zone l_{se} and are constructed after the example of Fig. 4, for data given in Table 11.

The average vein length, \bar{l}_v , did not appear to be significantly different in dry and in wet climate and this is not essential either for the purpose for which Fig. 18 as a summary of results has been made. It concerns mainly the differences at the ultimate ends of the veinlets in a dry and a wet climate. The value of Σ area tr/Σ se, ic, vp at the proximal end has been calculated only for three vein endings in dry climate. The result is in agreement with the found values of Q when comparing both results with those at the proximal end in wet climate.

The values of Σ area tr/Σ se, ic, vp in the tracheid maximum do not differ significantly in both climates. Consequently the value of 200 μm^2 has been taken for both S curves as an approximation of the average value.

The average length of the pieces of vein ending from the tracheid maximum to the ultimate end of the veinlet in a dry climate and a wet climate has been used for the position finding of the tracheid maximum in the figure. The lengths of the extremities ($l_v - l_{se}$) appeared significantly different in a dry and in wet

TABLE 11. Data required for the schematic drawings of vein endings with a zone l_{se} in dry and in wet climate (Fig. 18, the curves S). The numbers in brackets indicate the numbers of veinlets that were available for calculating the averages.

	Dry climate		Wet climate	
Γ_v , μm (Table 8)	347.7 (16)		258.0 (13)	
<i>xylem</i>				
Σ area tr/Σ se, ic, vp, proximal, μm^2	55	(3)	31	(7)
Σ area tr/Σ se, ic, vp, maximum, μm^2	184	(4)	204	(7)
Σ area tr/Σ se, ic, vp, distal, μm^2	0		0	
average length between the spot of the tracheid maximum and the distal extremity of the veinlet, μm	116.94 (16)		88.70 (10)	
<i>phloem</i>				
$(l_v - l_{se})$, μm (from Table 9)	158.00 (16)		115.08 (13)	
number of se, proximal	2.14 (14)		3.10 (10)	
number of se, distal,	1.00 (16)		1.00 (13)	
along the length, μm	> 58		34	

TABLE 12A. Survey of all short vein endings. In the dry as well as wet climate a length of phloem does occur in the longest specimen of at least about 20 μm on an average and a size of the phloem of only 1 sieve element (see Fig. 18).

Veinlet number	Dry climate			Wet climate		
	$l_v, \mu\text{m}$	$l_{se}, \mu\text{m}$	tracheid maximum (number)	$l_v, \mu\text{m}$	$l_{se}, \mu\text{m}$	tracheid maximum (number)
1	38	—	9	55	—	10
2	80	—	12	56	—	5
3	84	—	12	62	15	7
4	98	—	14	63	15	10
5	108	—	11	68	26	11
6	110	—	17	77	—	12
7	120	—	21	82	—	17
8	122	—	11	89	—	7
9	131	42	22	106	—	13
10	135	—	20	110	—	15
11	143	—	9			
12	143	55	9			
13	180	—	13			

climate and the averages have been used to find the position of the distal end of the zone l_{se} in Fig. 18.

The dimension of the phloem has only been indicated as the average number of sieve elements at the distal and at the proximal end of the phloem. It is apparent from Fig. 18 that the volume of the xylem, being in front of the distal end of the phloem, must be smaller in wet climate than in dry climate, for the area below the curve S to the abscissa is an approximation of the total volume of this part of the xylem. Besides the short vein endings may be considered the extremities of the long ones. Comparing the curves S and Q it is evident that in a wet climate the maximum of curve Q, reckoned from the distal end, is reached sooner than in a dry climate. In both climates the value of Σ se-sections

TABLE 12B. The average number of tracheids in the tracheid maxima of all the veinlets shorter than 657 μm (Table 8), 180 μm , 110 μm etc. in dry and in wet climate.

dry climate	vein endings $\leq 657 \mu\text{m}$: average tracheid maximum = 16.9
	vein endings $\leq 180 \mu\text{m}$: average tracheid maximum = 13.9
	vein endings $\leq 110 \mu\text{m}$: average tracheid maximum = 12.5
	vein endings $\leq 80 \mu\text{m}$: average tracheid maximum = 10.5
wet climate	vein endings $\leq 588 \mu\text{m}$: average tracheid maximum = 15.7
	vein endings $\leq 135 \mu\text{m}$: average tracheid maximum = 10.7
	vein endings $\leq 100 \mu\text{m}$: average tracheid maximum = 9.9
	vein endings $\leq 70 \mu\text{m}$: average tracheid maximum = 8.6

is small yet in the class of veinlets 0–100 μm in length, and in dry climate this value is also small in the class of veinlets 100–200 μm in length (see also Table 11). In the same classes of veinlets the value of Σ tr-sections rises rapidly in proximal direction especially in wet climate. Consequently the quotient Q increases in proximal direction much more in wet climate. Not until the number of tracheid sections decreases and the number of sieve element sections rises, the quotient Q will decline. This bend downwards in the curve in proximal direction takes place earlier in a wet climate.

Curve M is given to make a more exact comparison possible between the total tracheary volume, differentiated against and also protruding in front of the 20 μm long extremity of the phloem (with one sieve element), in a dry as well as in a wet climate. Data of Table 12 were used to construct curve M. In Table 12A one finds a survey of all the short vein endings and in Table 12B the average numbers of tracheids in the tracheid maxima belonging to groups of veinlets shorter than a certain length. Curves M were drawn between zero and 3 other points. The average values have been used of the tracheid maxima belonging to all the veinlets shorter than or equal to a certain length between 38 and 180 μm in dry climate and between 55 and 135 μm in wet climate (Table 12B). Plotting the three calculated points, a scale has been chosen (as ordinate) with a maximum of 18.5, corresponding with the average number of tracheids of the tracheid maximum in all the vein endings with a zone l_{se} in dry and in wet climate (Table 8: 19.3 and 17.7 respectively, and not significantly different). This value 18.5 of the tracheid maxima has been chosen at the same height as the maxima of the curves S. The calculated averages of the tracheid maxima of the 3 groups of short veinlets in Table 12B may all be thought at the proximal end with a small error. This means that the abscissa values of the three points of the curves M correspond with the lengths of veinlets chosen to divide the short vein endings into three groups. When connecting the three points with zero the curve M arises that forms the upper limitation of an area that is an approximation of the average tracheary volume at the extremity of the veinlet. In Fig. 19 one finds the relation between the number of tracheids in the tracheid maxima (abscissa) and the total area of the transections of the tracheids in the maximum. The denominator in the quotients $\Sigma \text{ area tr} / \Sigma \text{ se, ic, vp}$ varies in the extremities of the vein endings. The calculated averages at the distal extremity of the zones l_{se} and l_{ic} , and also in the zones l_{vp} and l_{tr} are for dry climate 6.75 (4 veinlets) and for wet climate 6.11 (9 veinlets). After correcting the left scale with these averages, it is possible to estimate the tracheary volume in the extremities of the vein endings by calculating the shaded area.

Calculation with the image analyser gives a value for dry climate and one for wet climate; the ratio of both values is 1.91. Calculation from the figure, in which the abscissa values are found by determining of, for instance, 7 μm -parts of length multiplied by the ordinate values belonging to them, gives the values of the tracheary volumes in μm^3 : in dry climate 126,700 μm^3 and in wet climate 66,600 μm^3 . The ratio between these two values is 1.90.

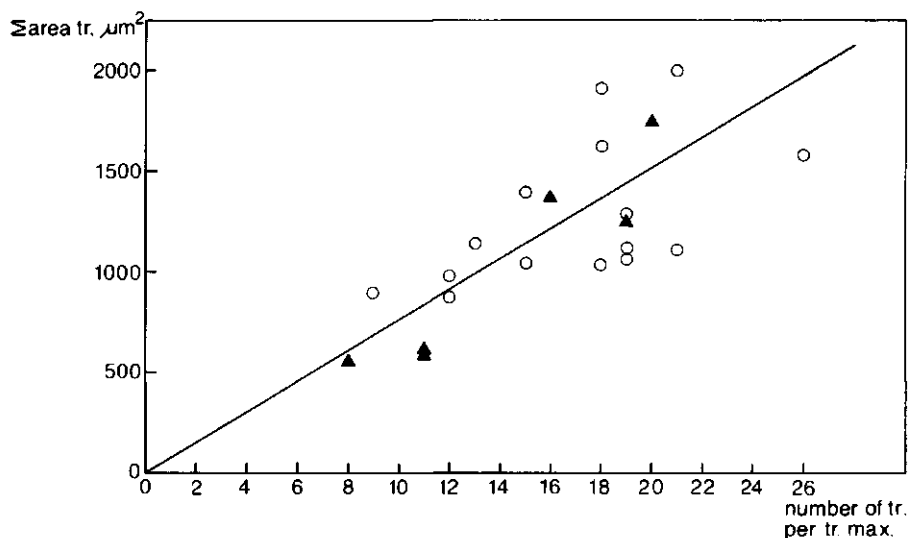


FIG. 19. Relation between the number of tracheids in the tracheid maximum, and the total area of transections of the tracheids in the maximum. 6 vein endings in dry climate (▲) and 15 vein endings in wet climate (○). Straight line not calculated.

DISCUSSION AND CONCLUSION

The term intermediary cell (Übergangszelle, FISCHER, 1885) has been used in the sense of TURGEON, WEBB and EVERT (1975). Since the difference between companion cells (direct ontogenetic relation with the sieve element) and other parenchyma cells rich in cytoplasm next to the sieve elements is difficult to determine, and also because the function of both types of elements is probably the same (a presumptive role in the exchange of photosynthates between the mesophyll and the sieve elements), it did make sense to use the name 'intermediary cell' for both elements only.

The most general partition in zones of veinlets which end blindly within the arcole of *Hedera* leaves, reckoned from proximal end to distal extremity, is: l_{se} (part with sieve elements), l_{ic} (part with intermediary cells), l_{vp} (part with vascular parenchyma cells) and l_{tr} (part with tracheids and sometimes also vascular parenchyma cells belonging to the xylem). The sometimes quoted example in *Hosta* (PRAY, 1955b) of the phloem extending beyond the limits of the xylem (e.g. ESAU, 1969), may be interpreted as the extending of the zone l_{ic} or l_{vp} beyond the limits of the xylem.

The vascular parenchyma cells and the intermediary cells intergrade in structure (ESAU, 1969); there is also evidence that the intermediary cells and the terminal sieve elements intergrade in structure. In the light microscope examples of enucleate terminal sieve elements have been found in true transections which

were wider and contained distinctly more cytoplasm than the more proximal sieve elements. Thus the intergrading from parenchyma cells to sieve elements may be more completed in some vein endings. It may be that those intermediate cellular forms show aspects of evolutionary trends in differentiation of a parenchyma cell into a sieve element.

The relation of the quantity of xylem to the quantity of the phloem has been expressed by means of the quotient $\Sigma \text{ area tr} / \Sigma \text{ se, ic, vp}$. In this way it has been possible to represent the quantity of xylem along the vein endings by means of a more or less constant type of curve with a distinct maximum (Fig. 4) corresponding with the actual volume of tracheary elements per unit of phloem along the veinlet. It is consistent to relate the xylem in the extremities of the veinlets with the quantity of phloem in the distal end of the phloem tissue. The result of this way of calculating was a descending curve like the diminishing of the number of tracheids in the distal end of the veinlet. Theoretically it is important that the denominator of the quotient mentioned above, will remain larger than one, which means that also in zones l_{ic} , l_{vp} and l_{tr} a relation exists between the volume of tracheary elements and the terminal phloem. The influence of the characteristics of the phloem on the volume of the xylem is also evident from the shifting of the tracheid maximum in the direction of the distal extremity under influence of the presence of the zones l_{ic} and l_{vp} , suggesting an extension of the phloem by the zones l_{ic} and l_{vp} . The tracheid maximum can always be found at the distal extremities of the zones l_{se} , l_{ic} or l_{vp} (Table 7, Fig. 15) and this corresponds with the absence of veinlet types b, j, l, o and r in Table 7. The percentage of phloem elements in the transections decreases rather regularly in distal direction along the veinlets; the percentage of tracheary elements increases (Table 5, the number of vascular parenchyma cells in the xylem, vpx , is small). This means that the influence of the phloem on the volume of the xylem increases in distal direction up to the distal ends of the zones l_{se} , l_{ic} or l_{vp} when present. The value $\overline{l_v - l_{se}}$ appeared to be constant in a certain climate. It seems that an ontogenetic influence of the phloem on the differentiation of the xylem at the extremities of the vein endings is a constant one, independent of the lengths of the veinlets.

The system of zones l_{se} , l_{ic} and l_{vp} is uninterrupted; that means that a probable ontogenetic influence is able to reach every small veinlet, i.e. the zone complexes ($l_{ic} + l_{vp} + l_{tr}$), ($l_{vp} + l_{tr}$) and l_{tr} may be found as separate vein endings.

ALONI (1980) concluded from the spatial relation of phloem and xylem while differentiating in callus tissues, that the xylem possibly is formed in response to auxin together with some added factor which reaches it from the phloem. This work on *Hedera* veinlets confirms ALONI's proposition; the differentiation of xylem in vivo is not only exactly dependent on the dimension of the phloem tissue, but also on the character of the phloem composition as sieve elements, intermediary cells and vascular parenchyma cells. Especially the distal ends of the phloem appear also as sinks of differentiating factors, from which a distribution takes place of growth regulators, important for the differentiation of xylem. This leaching from the 'phloem' (i.e. sieve elements) into the free space (ESCH-

RICH, 1980) can probably take place via the intermediary cells (zone l_{ic}) and perhaps the vascular parenchyma cells (zone l_{vp}). The quantity of xylem and therefore probably also the amount of nutrients and growth regulators released by the phloem turned out to be dependent on climatic circumstances. At least a great increase of the relative humidity of the air and correlated with that a sharp lowering of transpiration of the plant, are able to bring about a lowering of the quantity of xylem per unit of phloem in the vein endings. The availability of the nutrient solution to the roots was unlimited, so the limitation of the transpiration was because of the high relative humidity of the air only. The temperature of the roots was kept constant at about 12.5°C. The distal extremities of the veinlets ($l_v - l_{se}$) are significantly shorter in wet climate. It is shown (Fig. 18) that in veinlet tips (along 20 μ m of the zone l_{se} and more distad) the value of the tracheary volume in wet climate was about 0.53 times smaller than in dry climate. This difference was smaller when longer vein endings were considered, but the quotient Q (Fig. 17) remained always higher in a dry climate. The tracheid maximum (Table 8) is less high in wet climate, but anyhow this difference, compared with a dry climate, is not significant on the basis of the available data.

Finally it may be asserted that hardening off the *Hedera* plant means, among other things, the differentiation of more xylem in the leaves. Therefore more carbohydrates, i.e. more assimilatory energy is used for the anatomical structure of the leave in dry climate as compared with wet climate.

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ANATOMY OF VEIN ENDINGS
IN HEDERA LEAVES;
ASPECTS OF ONTOGENY

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ABSTRACT

In totally white leaves of *Hedera canariensis* Willd. var. 'Gloire de Marengo', the anatomical structure of the vein endings in developing leaves in moderate climate has been studied by means of paradermal and serial transverse sections. Near the leaf base a nearly equal growth rate of the leaf tissue in all directions is found and, based on the identical growth curves of the tissue near the leaf base and the whole leaf, the percentage final lamina length (F.L.L.) is used as parameter for the ontogeny of the vein endings. The procambium cells of the vein endings originate from young mesophyll cells in an acropetal sequence and the young procambium cells show a manifest intrusive growth after obtaining polarity. At about 40% F.L.L. the procambium of the vein ending has been differentiated; measurements of the lengths of the young vein endings showed an average increase which can be written as a linear equation. Geometrically it follows that the length of every young veinlet can be converted to its length at 100% F.L.L., and vice versa. The differentiation into the tracheids follows that of the procambium from about 43% F.L.L.; at first rapidly and along a narrow adaxial route of the procambium strand and after that slower and broadwise. The differentiation into the sieve elements occurred not until about 87% F.L.L., and took place by means of a new continuous and acropetal division activity of the cells in the abaxial part of the procambium strand. The interval of leaf age in which the tracheid maximum near the distal extremity of the phloem length differentiates from the remaining procambium cells in that position, ranges from about 85% to 100% F.L.L.

The morphogenesis of the vein endings is discussed in relation to the transpiration stream and the differentiation stimuli transported by it, possibly coming from the differentiating phloem.

INTRODUCTION

In the leaf of *Hedera canariensis* Willd. var. 'Gloire de Marengo' freely ending veinlets (vein endings or veinlets for short) occur in the areoles (MAGENDANS, 1983). Commonly one may find four different zones along a vein ending: l_{se} (part of the vein ending with sieve elements), l_{ic} (distad of l_{se} , containing intermediary cells), l_{vp} (distad of l_{ic} , containing vascular parenchyma cells in a direct line with the distal end of the zone l_{ic}) and l_{tr} (at the ultimate extremity of the vein ending, containing only tracheids and sometimes vascular parenchyma cells belonging to the xylem). It has been possible to construct a model of a vein ending, represented as a rather constant type of curve when relating the xylem part of the vein ending with the phloem part. This curve showed a distinct tracheid maximum; it was found that the exact position of this tracheid maximum was depen-

dent on the position of the distal end of the zones l_{sc} , l_{ic} or l_{vp} .

In the literature, it is generally agreed upon the first phloem differentiating before the first xylem in the procambium strands (ESAU, 1965; HERBST, 1972; JAIN, 1984; KUEHNERT and LARSON, 1983; LERSTEN, 1965; OLSON et al., 1969; PRAY, 1955a, 1962; SHININGER, 1979; SUNDBERG, 1983) until recently LARSON (1984) and SIVARAMAKRISHNA and VIJAYARAGHAVAN (1983) found the reverse situation in resp. very young leaves and in aerial roots.

Many authors consider auxin (I.A.A.) as the differentiation stimulus of xylem (e.g. ALONI and ZIMMERMANN, 1984; BRUCK and PAOLILLO, 1984b and MINOCHA, 1984), and also the apoplast is mentioned as transport route of I.A.A. (JACOBS and GILBERT, 1983; MORRIS and THOMAS, 1978; PERBAL et al., 1982).

ALONI (1980) concluded from the spatial relation of phloem and xylem while differentiating in callus tissues, that the xylem possibly is formed in response to auxin together with some added factor which reaches it from the phloem. The close relation between the tracheid maximum and particular positions in the phloem also point out an ontogenetic relation between the phloem and the xylem. In order to get more information about a possible ontogenetic relation between the differentiation of the phloem and the xylem in the *Hedera* leaf, a close examination has been carried out of the differentiation of the phloem and the xylem in the vein endings in the young leaves. Besides an exact time scale is made of the various processes of differentiation in relation to the growth rate of the leaf. Finally the morphogenesis of the vein endings and the role of possible differentiation stimuli will be discussed. In this discussion the different theories of the development of vein endings (as MEINHARDT, 1979; MITCHISON, 1980, 1981) are involved; the real structure of the developing tissue was not involved in these theoretical considerations.

MATERIALS AND METHODS

Plant material and culture conditions

The observations were made on the veins of white leaves in totally white shoots of *Hedera canariensis* Willd. var. 'Gloire de Marengo'. This plant with variegated leaves produced two or three totally white shoots that contained some small chloroplasts in the epidermis only, especially in the guard cells. The plant grew in a potometer (as in the preceding examinations, MAGENDANS, 1983) in a conditioned growth cabinet (Weiss, W. Germany). The examined leaves grew in an atmosphere of $22.5 \pm 1\%$ relative humidity (r.h.) and a light intensity of about 17,000 lux at plant level. The dark period was from 20.30 p.m. unto 08.00 a.m. with $39 \pm 1\%$ r.h. The temperature was controlled at $31^\circ\text{C} \pm 1^\circ\text{C}$ during the light period and $21^\circ\text{C} \pm 0.5^\circ\text{C}$ in the dark period. The lamps used were Philips HPI/T 375W mercury halide and the air velocity in the cabinet 0.4–0.5 m/sec. Measurements of light intensity and relative humidity were taken corresponding

MAGENDANS (1983). Growth curves of the leaves were made continually. Leaf age was calculated and is indicated in this article as a percentage of the final length of the lamina of the leaf (% F.L.L.). The lamina length has been measured from the point of junction of the major veins in the leaf base till the tip of the lamina (fig. 3: L). Measurements of the part of the lamina near the leaf base (figs 2, 3) was difficult in very young leaves. It proved to be possible to measure particular distances between observed points of intersection of larger veins by means of a pocket-lens and the transmitted light of the lamps of the growth cabinet.

Microtechnique

The preliminary observations were made on square leaf tissue pieces (25 mm²) punched out of leaves near the leaf base (fig. 2B) at various ages and fixed in F.A.A. Next the pieces of tissue were cleared in 5% NaOH during 3 days and an aqueous solution of chloral hydrate during 3 days and after that stained in a solution of safranin in 50% alcohol (BERLIN, G. P. and J. P. MIKSCH, 1976: 126, 127). After evaluating the results of observations on leaf tissue between about 47% and 97% F.L.L. more exactly chosen fixations have been made, a survey of these is given in table 19. The procedure of fixing, embedding in paraffin wax, sectioning and staining is the same as in MAGENDANS (1983). The measure of staining with safranin has been regarded as a measure of the lignification of the spiral thickenings in the tracheids. The length of the vein endings was estimated also by making use of the straight sides of the sections. All analysed veinlets are in sequence of finding them in the slides in this way; in the group of 20 veinlets at 96% F.L.L. a closer search has taken place because of shortage of material. All observations were made with a Wild microscope using oil immersion and 1,500x magnifying optics. Camera lucida drawings have been made by means of a Wild drawing tube. The electron micrographs of plate 1 were prepared after fixation of small sections of mature leaves in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 3 hrs and subsequent postfixation in 1% osmium tetroxide in the same buffer for 5 hrs at room temperature. The material was dehydrated in a graded ethanol series and embedded in Epon 812 through propylene oxide. Ultrathin sections were stained with uranyl acetate and lead citrate.

Statistical methods

Statistical analysis and many tests of significance were by Wilcoxon's test. Also the t-test was applied to determine possible significant differences between the length of zones ($l_v - l_{se}$) in moderate and in dry and wet climate.

RESULTS

Measurements of the growth of leaves in length and width

Studying the ontogeny of vein endings one must base oneself on measurements of the growth of leaves. The vein endings in the leaf of *Hedera canariensis* Willd. var. 'Gloire de Marengo' develop in de areoles in all directions (MAGENDANS, 1983). This means that in studying the ontogeny one must be acquainted also with the growth of leaves in length as well as in width, if one wants to avoid that the study will be confined to vein endings which develop in one certain direction in the leaf only.

The course of length and width of a lamina is shown in fig. 1.

In the given growth period (abscissa) the length and width have increased with the factors 3.15 and 3.11 resp.; the difference is 1.3%. In an other leaf a larger difference was noted (9.8%). In case of these growth curves the final length of the leaf is known and consequently the percentage F.L.L. of the stages of growth in which the measurements were carried out.

The research into the developing vein endings was confined, however, to a small area near the leaf base (fig. 2B). The increase of length and width of the leaf tissue in this small area is given in fig. 2.

The dimensions of l_1 , l_2 , b_1 and b_2 have been measured every two days. The

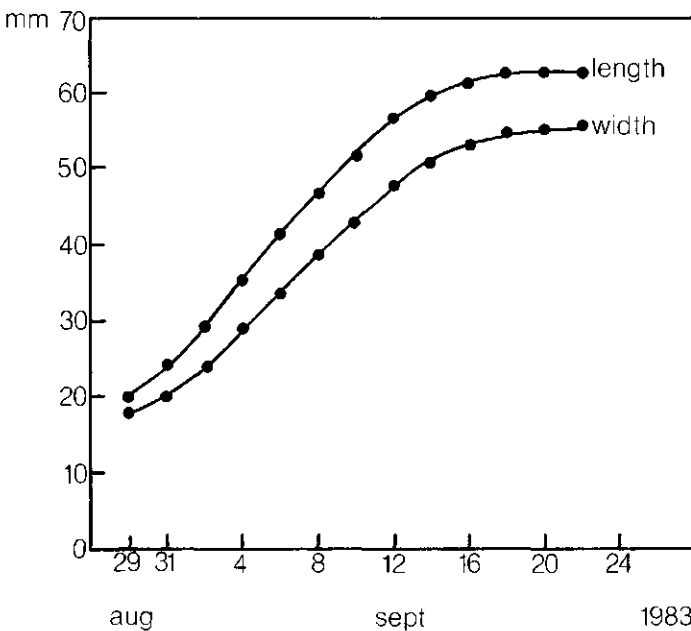


FIG. 1. Increase of length and width of one lamina in mm during 24 days. Start and finish of the measurements resp. at $\pm 31\%$ and 100% F.L.L.

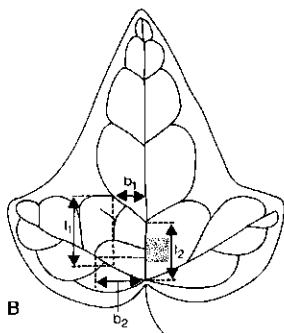
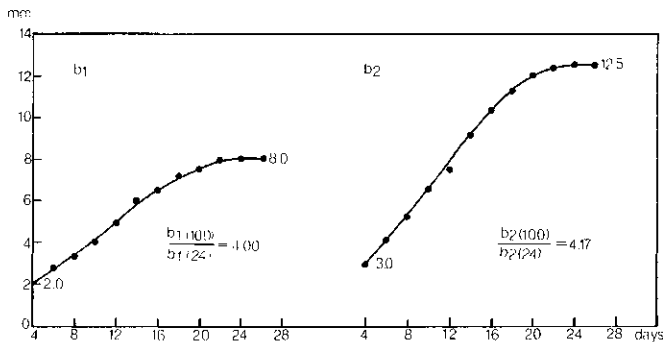
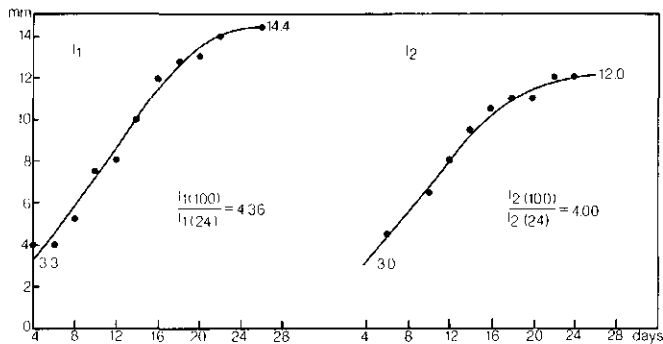


FIG. 2. A. Increase of length of l_1 , l_2 , b_1 and b_2 in the leaf, indicating the growth of the leaf tissue near the leaf base. Start and finish of the measurements resp. at $\pm 24\%$ F.L.L. (4 days) and 100% F.L.L. (26 days).

B. Leaf with a schematic major venation characteristic for *Hedera* leaves with indications of the measured lengths l_1 , l_2 , b_1 and b_2 near the leaf base. The total lamina length was 18 mm at 24% F.L.L. and 74 mm at 100% F.L.L. The small square (dotted) indicates approximately the piece of tissue that has been used in analysing.

measuring-points could be located by means of special, identifiable, fixed points in the venation of second and third order. The dimensions of l_1 at 100% F.L.L.

TABLE 1. Ratios of the l_1 , l_2 , b_1 and b_2 (fig. 2B) values, measured at 100% F.L.L. and at resp. 24%, 41% F.L.L., etc.

%F.L.L.	24	41	51	72	89
l_1	4.36	2.50	2.00	1.44	1.15
l_2	4.00	2.18	1.88	1.33	1.11
b_1	4.00	2.35	1.95	1.38	1.11
b_2	4.17	2.36	1.89	1.36	1.12

($l_{1(100)}$) and at 24% F.L.L. ($l_{1(24)}$) are in the ratio of 4.36 to 1.00. This ratio (4.36) indicates the growth of the leaf from 24% to 100% F.L.L. near the leaf base in the direction of l_1 and is, combined with the data of other directions of growth for various percentages F.L.L., shown in table 1.

The data of table 1 are obtained by means of the graphs in fig. 2A for the purpose of eliminating small errors, appearing especially when measuring within small leaves. The l_2 , b_1 and b_2 values only show very small differences at the various percentages F.L.L. The l_1 values are somewhat larger, 6.1% on an average over the various percentages F.L.L. (cp. ERICKSON, 1966 and PIETERS, 1974). The vein endings differentiate in all directions, independent of the direction of the midvein. Measuring the length of the vein endings, a small error will be made when one assumes an equal growth rate in all directions near the leaf base. It must be considered, however, that the fixed piece of 5×5 mm leaf tissue (fig. 2B, dotted square) is always entirely or for the most part included in the measured part of the leaf base, that the studied vein endings never occur near the margins of the fixed piece of leaf tissue, and that only those analysed vein endings differentiating parallel to the midvein (and to l_1) and also differentiating at the same distance from the midvein as l_1 , will show a maximum deviation of growth in length of 6.1%.

It may be concluded that the average deviation of the increase in length of the analysed vein endings, based on the percentage of growth of the leaf tissue near the leaf base, will only amount to a few per cents. The measuring of lengths of vein endings in the following dissertation is based on an equal growth rate in all directions near the leaf base.

Comparison of growth in length of the total lamina with the growth of leaf tissue near the leaf base

When comparing the growth curve of a leaf (fig. 3A) with the growth curves of the dimensions l_1 , l_2 , b_1 and b_2 near the leaf base (fig. 3B), it becomes apparent that the growth of the entire lamina and the part near the leaf base elapse synchronous.

The tissue near the leaf base attains maximum length (l_1 , l_2) at about the same moment as the total length (L , fig. 3B) of the lamina. This also applies to the dimensions b_1 and b_2 . Any possible, measurable difference is small; perhaps the tissue near the leaf base completes its extension growth 1–2 days earlier.

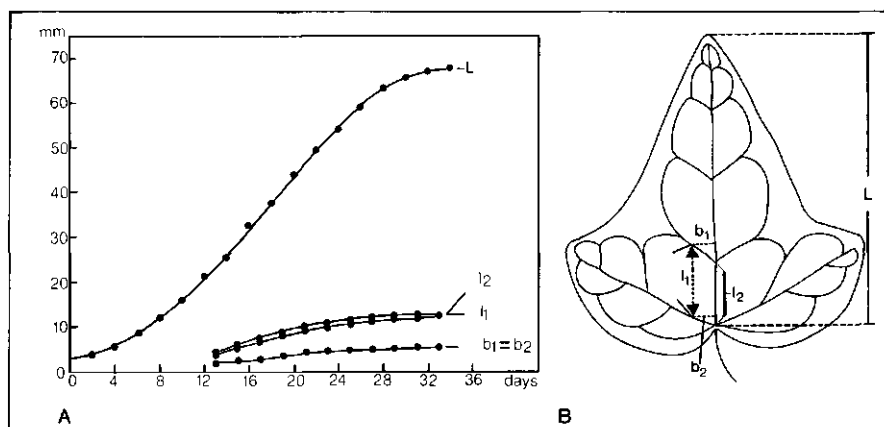


FIG. 3. A. Growth curves of a leaf with lamina length L ($L_{(100)} = 68$ mm) and of a part of the lamina near the leaf base (l_1 , l_2 , b_1 and b_2).

B. *Hedera* leaf (cp. text fig. 2B) with the measured part of the lamina near the base as indicated.

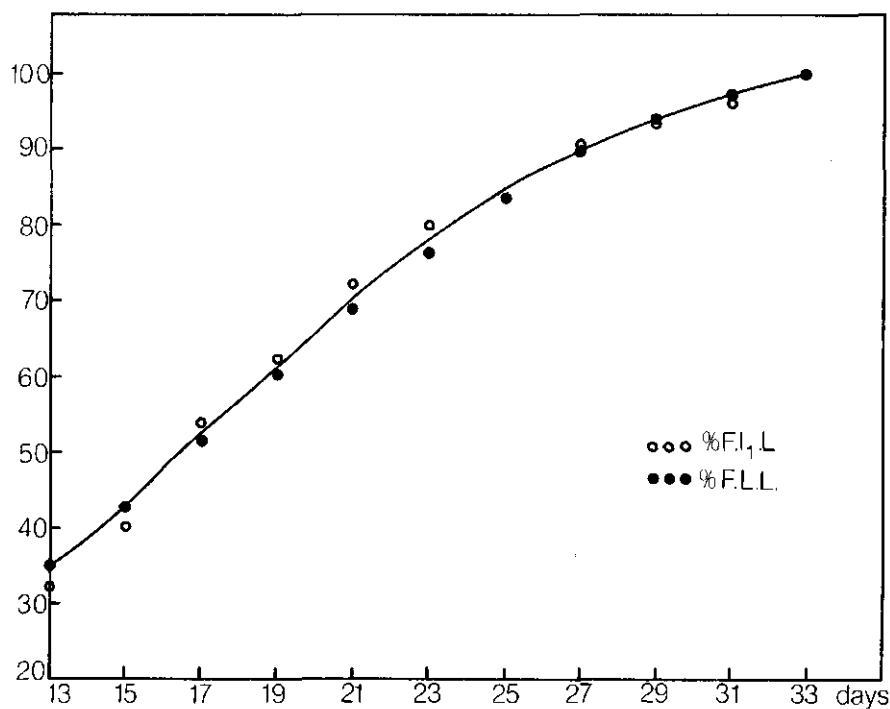


FIG. 4. Relation between growth of the lamina expressed in % F.L.L. and expressed in % F.l.L. (l_1 in fig. 3B) and time (days) between $\pm 35\%$ and 100% F.L.L.

The same comparison can be made with the percentage F.L.L. as parameter (fig. 4).

From this figure one may draw the conclusion that the growth of the entire lamina, expressed in time as a percentage of the final length, almost entirely corresponds with the growth of the tissue near the leaf base with the percentage of the final length of that part of the leaf tissue used as parameter. The measured length l_1 is the same as in fig. 3B and this part of the leaf tissue is the same as the analysed tissue for the study of the anatomy of the vein endings.

One must conclude that the application of the relative time-scale for analysis of the ontogeny of the vein endings in this part of leaf tissue, expressed in the percentage F.L.L., corresponds with an equal relative time-scale expressed in the percentage of growth of a part of tissue near the leaf base. In studying the ontogeny of the vein endings the percentage F.L.L. will be applied as parameter of the growth of leaf tissue in which the studied vein endings have been found.

Comparison of growth in length of the leaves along the shoot

In fig. 5 a number of growth curves of successive leaves along the shoot are shown; the leaf length in mm has been measured every two days. The curves are not identical: the final lamina length varies from 58 to 68 mm in this part of the shoot. The laminae do approach to their final length gradually and to make possible a uniform way of determining the final lamina length, this value will be assessed by means of the angle of inclination ($\text{tg}\alpha$) of the growth curve. The final lamina length is defined as the length at which $\text{tg}\alpha$ becomes ≤ 0.1 ; i.e. the moment at which the growth curve does not rise more than 1 mm in a four days' period.

Assessment of the final length of a leaf and determination of the percentage of final lamina length (F.L.L.) of a younger leaf

In fig. 6 the relation is shown between the final lengths of the laminae of

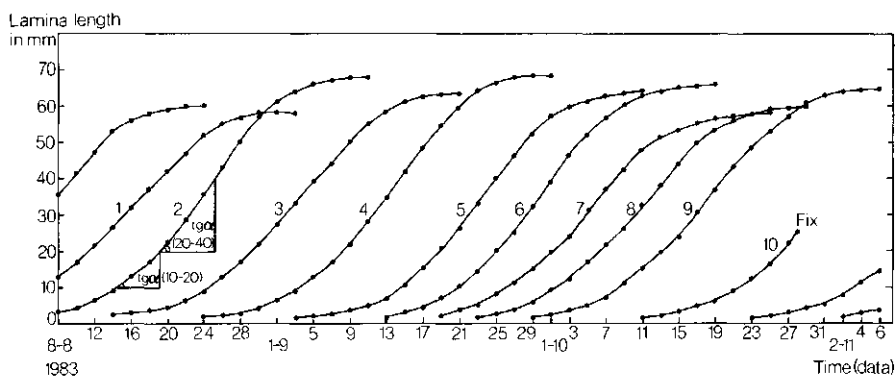


FIG. 5. Growth curves of 9 successive leaves on a totally white stem. Lamina length is maximal (F.L.L. = 100%) at $\text{tg}\alpha \leq 0.1$. Determination of $\text{tg}\alpha$ at intervals of 10–20, 20–40 mm etc. lamina length. Leaf 10 has been used for analysis of vein endings (Fix; at 37% F.L.L.).

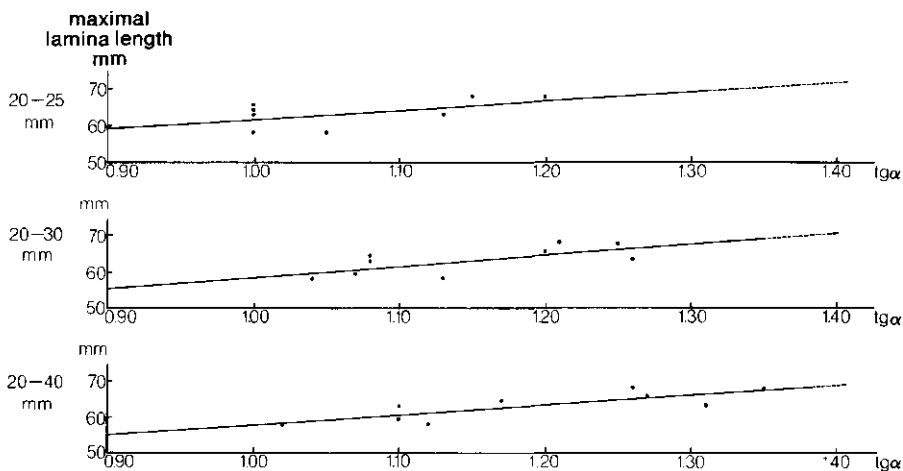


FIG. 6. Relation between the maximal lamina length (leaves 1-9, fig. 5) and the angle of inclination of the growth curves ($\text{tg } \alpha$) in intervals 20-25, 20-30 and 20-40 mm. The straight lines have been calculated.

the leaves 1-9 (fig. 5) and the angles of inclination of the growth curves in intervals 20-25, 20-30 and 20-40 mm. The straight lines have been calculated with the least squares method. By means of these lines it is possible to determine approximately the final length of a still growing lamina. For instance leaf 10 in fig. 5: $\text{tg } \alpha_{(20-25)} = 1.25$; the final length will be 68 mm in this case and the percentage leaf length 37% F.L.L.

In fig. 7 the progress of the magnitude of the correlation coefficient is shown between the values of $\text{tg } \alpha$ at lamina lengths 10-20, 10-25, 20-30, 20-40 and 20-50 mm (ordinate) and the final lengths of the laminae of leaves 1-9 (fig.

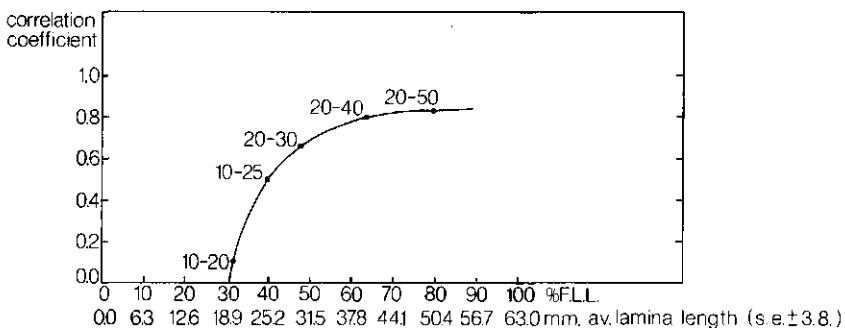


FIG. 7. Correlation coefficients between the values of $\text{tg } \alpha$ at lamina lengths 10-20, 10-25, 20-30, 20-40 and 20-50 mm and the final length of the lamina belonging to each value of $\text{tg } \alpha$. The highest values of lamina length in the interval have been noted in the graph. As parameter has been used % F.L.L., based on the average final length of the laminae. Data of the shoot with 9 leaves of fig. 5.

5) belonging to each value of $\text{tg}\alpha$ (abscissa). The leaf age at which the angle of inclination has been determined is indicated on the abscissa as percentage F.L.L. of the average lamina length at 100% F.L.L. (63.0 ± 3.8 mm). The average lamina length has been noted in mm below the abscissa as well. It appears from the graph that determining of the angle of inclination of the growth curve as a means of approximating the final length of a young lamina, is significant above 37% F.L.L. The correlation coefficient with the angle of inclination of the growth curve is higher than 0.4 in that case. In the following study the probable final lengths of young laminae has been determined in this way above 37% F.L.L.

In still younger leaves it is better to base the calculation of the final lengths of the laminae on the average lengths of the mature leaves in the last period of growth. This last period of growth may be chosen as the period in which 10 leaves matured in the shoot, i.e. a period of 2–3 months in which changes in growth of the plant as a result of changes in growth of the roots for instance, will be small. Probably the mentioned values of the correlation coefficient between $\text{tg}\alpha$ and the final lengths of the laminae (fig. 7) only apply to a growing shoot of *Hedera canariensis* with undisturbed development. After removing a leaf the correlation coefficients appeared to be lower than in fig. 7. The fixation of leaf tissue has been carried out from shoots from which no leaves were taken away before.

The length of vein endings in leaves at different ages

Square pieces of leaf tissue near the leaf base (fig. 2B) of different ages have been examined by means of paradermal sections. Not ramified vein endings (fig. 12B) have been selected and the lengths have been measured (l_v , tables 2–7). The average length of the vein endings at each leaf age was noted in the graph of fig. 8. The 6 located points are on a straight line approximately which was to be expected in case of extension growth of this leaf tissue in all directions to the same extent (fig. 2, table 1). All anatomical structures in this piece of leaf tissue, consequently the vein endings of all dimensions and directions as well, will be made longer with about the same factor.

The calculation of line 1 through the 6 points in fig. 8 occurred by means of the least squares method. The equation of this line is:

$$\bar{l}_{v(\% \text{ F.L.L.})} = 3.244 \times (\% \text{ F.L.L.}) - 45.472 \mu\text{m} \quad (1)$$

($\bar{l}_{v(\% \text{ F.L.L.})}$ = average length of the vein endings in leaf tissue of a particular age (% F.L.L.) in μm).

Suppose a piece of the midvein of the leaf that is as long as the average length of the vein endings in the adjoining, investigated leaf tissue. Then this piece of midvein is, as procambium, already present when the leaf primordium is not yet 1 μm long (cp. ESAU, 1965); that means at $\pm 0\%$ F.L.L. Suppose this piece of midvein attains the length of 279 μm finally, corresponding with the average

TABLE 2-7. Results of observations on 20 or 21 (57% F.L.L.) not ramified vein endings in paradermal sections of 6 leaves of different ages. l_v = length of the vein ending; l_{tr} = length in which (differentiating) tracheids are present. Further explanation in the text.

TABLE 2. Leaf at 38% F.L.L.

Veinlet number	l_v , μm	l_{tr} , μm	Number of tr		Number of segments
			proximal	distal	
1	37	0	0	0	1
2	49	0	0	0	1
3	63	0	0	0	2
4	66	0	0	0	2
5	66	0	0	0	2
6	77	0	0	0	2
7	79	0	0	0	3
8	82	0	0	0	3
9	82	0	0	0	3
10	87	0	0	0	3
11	87	0	0	0	3
12	87	0	0	0	3
13	89	0	0	0	3
14	91	0	0	0	3
15	94	0	0	0	3
16	105	0	0	0	3
17	117	0	0	0	3-4
18	126	0	0	0	4
19	135	0	0	0	3
20	157	28	1	0	3-4
average	88.8	1.4	0.05	0	2.7

TABLE 3. Leaf at 48% F.L.L.

Veinlet number	l_v , μm	l_{tr} , μm	Number of tr distal	Over μm	Number of segments
1	47	0	0	0	2
2	53	0	0	0	2
3	72	0	0	0	2
4	75	0	0	0	3
5	79	0	0	0	3
6	84	0	0	0	3
7	89	0	0	0	3
8	91	0	0	0	3
9	100	0	0	0	3
10	100	0	0	0	3
11	101	0	0	0	3
12	101	40	1	40	3
13	119	17	1	17	3
14	124	26	1	26	4
15	131	49	1	49	4

TABLE 3. (continued)

Veinlet number	$l_v, \mu\text{m}$	$l_{tr}, \mu\text{m}$	Number of tr distal	Over μm	Number of segments
16	131	74	1	67	3
17	135	0	0	0	4
18	135	26	2	26	3
19	145	0	0	0	4
20	150	0	0	0	4
average	103.1	11.6	0.35		3.1

TABLE 4. Leaf at 57% F.L.L.

Veinlet number	$l_v, \mu\text{m}$	$l_{tr}, \mu\text{m}$	Number of tr distal	Over μm
1	56	53	1	24
2	68	51	1	51
3	84	59	1	59
4	84	63	2	63
5	107	54	1	54
6	122	122	1	122
7	128	84	2	59
8	129	105	1	56
9	133	114	1	114
10	138	98	2	14
11	145	140	1	9
12	150	108	1	37
13	150	126	1	37
14	154	133	1	10
15	175	161	1	87
16	177	117	2	51
17	177	147	2	98
18	180	168	1	23
19	182	173	1	170
20	212	206	1	37
21	213	171	1	52
average	141.1	116.8	1.24	

TABLE 5. Leaf at 66% F.L.L.

Veinlet number	$l_v, \mu\text{m}$	$l_{tr}, \mu\text{m}$	Number of tr distal	Over μm
1	54	0	0	0
2	65	54	1	54
3	87	80	1	80
4	87	0	0	0
5	89	45	1	10
6	94	86	2	13

TABLE 5. (continued)

Veinlet number	l_v , μm	l_{tr} , μm	Number of tr distal	Over μm
7	105	75	1	28
8	117	110	2	31
9	133	123	1	30
10	140	140	1	31
11	145	140	1	45
12	145	105	1	14
13	156	124	1	53
14	164	142	2	56
15	178	154	3	42
16	192	140	1	35
17	206	196	3	35
18	213	175	1	17
19	252	213	1	10
20	466	456	1	35
average	154.4	127.9	1.25	

TABLE 6. Leaf at 82% F.L.L.

Veinlet number	l_v , μm	l_{tr} , μm	Number of tr distal	Number of segments
1	105	105	± 4	1
2	131	131	2	2
3	133	133	2	2
4	142	135	± 3	2
5	147	147	1	2
6	152	152	5	1
7	157	124	3-4	2
8	157	157	2	2
9	175	166	2-3	2
10	189	189	± 3	3
11	199	199	± 4	2
12	224	224	± 4	2-3
13	234	234	1	4
14	236	236	3-4	2
15	241	241	± 3	3-4
16	339	339	1	4-5
17	353	353	3-4	5
18	353	353	> 5	4
19	406	399	± 5	4
20	437	437	3	5
average	225.5	222.7	± 3.05	2.8

TABLE 7. Leaf at 97% F.L.L.

Veinlet number	$l_v, \mu\text{m}$	$l_{tr}, \mu\text{m}$	Number of differentiating tr ¹⁾	
			subdistal ²⁾	distal ³⁾
1	66	66	0	0
2	107	107	1	0
3	119	119	0	0
4	178	178	1	0
5	182	182	0	1
6	196	196	0	0
7	210	210	1	1
8	231	231	0	1
9	238	238	1	1
10	245	245	1	0
11	252	252	1	1
12	257	257	4	1
13	313	313	1	1
14	322	322	3	4
15	329	329	2	0
16	336	336	2	1
17	443	443	1	1
18	451	451	0	1
19	455	455	2	0
20	528	528	3	2
average	272.9	272.9	1.2	0.8

¹⁾ i.e. tr with a nucleus and cytoplasm;

²⁾ i.e. not in distal segment, but in the other segments;

³⁾ i.e. in distal segment.

length of the vein endings at 100% F.L.L. ($l_{v(100)}$, based on 121 observations). Line 2 (fig. 8) represents the growth (lengthening) of this supposed piece of midvein near the leaf base. This piece of midvein is 139.5 μm long at 50% F.L.L.; however, $\bar{l}_{v(50)}$ is only 117 μm long (formula 1). This means that the length of the vein endings does increase more rapidly than the supposed piece of midvein and the surrounding mesophyll: intrusive growth (fig. 9 and 10).

Intrusive growth of procambium cells

In transverse sections of the leaf tissue one can find longitudinally sectioned vein endings (fig. 9). This vein ending, originated from the 5th cell layer of the plate meristem (cp. fig. 20D), intrudes at the distal extremity between the 4th and 5th cell layer. In fig. 10 a median longitudinal section of a vein ending is shown that originated partly from the abaxial half of cells belonging to the 5th cell layer. The procambium cells of the distal part (segment) originally had the same length as the adjacent cell (marked with *) in the 5th cell layer. It is evident that the procambium cells showed a strong extension growth in longitudinal direction and meanwhile the cells have become fusiform. The proximal extremi-

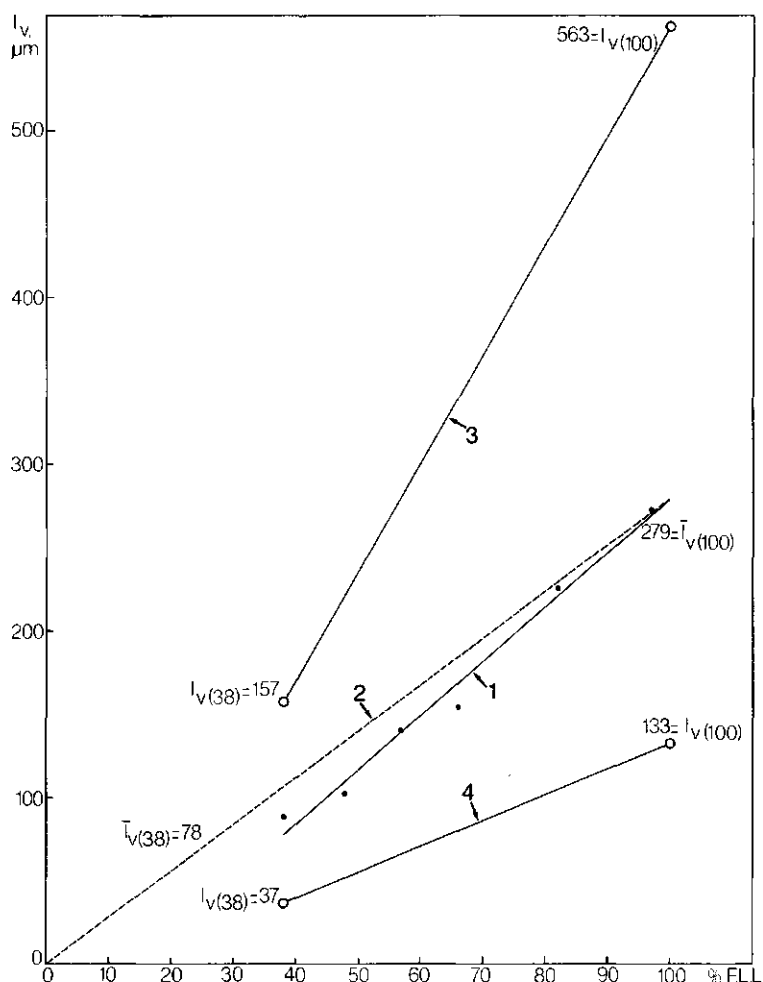


FIG. 8. Relation between \bar{l}_v and age of the leaf tissue (% F.L.L.); data (6 average values) from tables 2-7. Straight line (1) calculated. Line 2: lengthening of a piece of midvein with a length at 100% F.L.L. equal to $\bar{l}_{v(100)}$. Line 3: growth of the longest veinlet from table 2 during 38-100% F.L.L. period (formula 3). Line 4: growth of the smallest veinlet from table 2 during 38-100% F.L.L. period (formula 3).

ties of the procambium cells of this distal segment intruded between the adjacent fusiform procambium cells of the proximal segment and the distal extremities intruded between the cells in the 5th and 6th cell layer of the plate meristem. Consequent on this intrusive growth, the cells in the 5th and 6th cell layer in front of the distal extremity of the veinlet have been somewhat pushed up, so that wall foldings originated (big arrow). This vein ending ought to be $\pm 64 \mu\text{m}$ long considering the outlines of the cell (*) in the 5th cell layer. However, the measured length after intrusive growth is $\pm 78 \mu\text{m}$; the extension factor

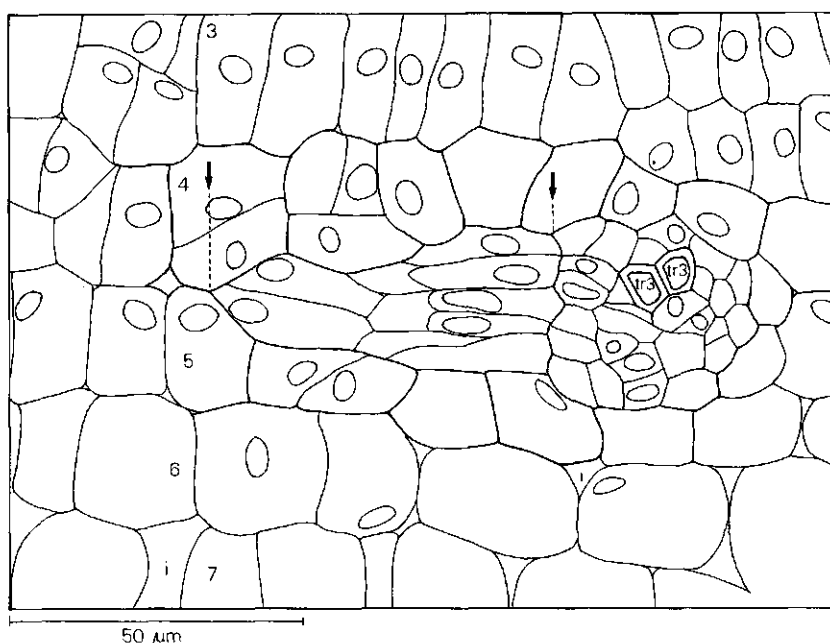


FIG. 9. Oblique longitudinal section of vein ending (between arrows) in leaf tissue of 38% F.L.L. At the proximal end of the vein ending the rather tortuous boundary-line is located by the position of the procambium cells of the vein of lower order; these procambium cells are often almost perpendicular to the cells of the vein ending. This veinlet arised entirely from the 5th layer of the plate meristem and becomes wider by intrusive growth between the adjoining cells of the 4th and 5th layer. Other sections clearly show two segments in this veinlet. 3-7 = layers of plate meristem; tr3 = differentiated tracheids in small vein of lower order; i = intercellular space.

is 1.22 in this case. It appears, both on the ground of calculations based on observations in paradermal sections and from observations of longitudinally sectioned vein endings in transverse sections, that the vein endings individually extend more than the other tissues in the mesophyll by means of intrusive growth. The extension factor of the vein endings can be calculated by means of the straight line through the 6 points in fig. 8, and the extension factor of the surrounding leaf tissue can be calculated by means of line 2 (table 8).

At the end of the growth period between 38% and 100% F.L.L. the extension of the leaf tissue is 2.63x and the lengthening of the vein endings 3.58x; the ratio between these numbers is 1.36, i.e. 36% of the lengthening consists of intrusive growth. At the end of the growth period between 38% and 57% F.L.L. this ratio amounts to 1.19; this is only 19% intrusive growth (cp. the ratio 1.22 in fig. 10). Therefore at 57% F.L.L. intrusive growth in the young leaf tissue still occurs.

TABLE 8. Comparison of growth of the leaf tissue near the leaf base (cp. fig. 2) and the intrusive growth of the vein endings. As in fig. 8 $l_{v(100)} = 279 \mu\text{m}$, the same length as an imaginary piece of midvein or adjoining piece of leaf tissue at 100% F.L.L. (cp. fig. 8, line 2). The lengths of both types of tissue at 38% F.L.L. and 57% F.L.L. have been calculated (formula 1 and fig. 8). Then the relation between the extension factors can be calculated.

	38% F.L.L.	57% F.L.L.	100% F.L.L.	$\frac{57\% \text{ F.L.L.}}{38\% \text{ F.L.L.}}$	$\frac{100\% \text{ F.L.L.}}{38\% \text{ F.L.L.}}$
leaf tissue (small square, fig. 2B), μm	106	159	279	1.50	2.63
$l_v, \mu\text{m}$	78	139	279	1.78	3.58

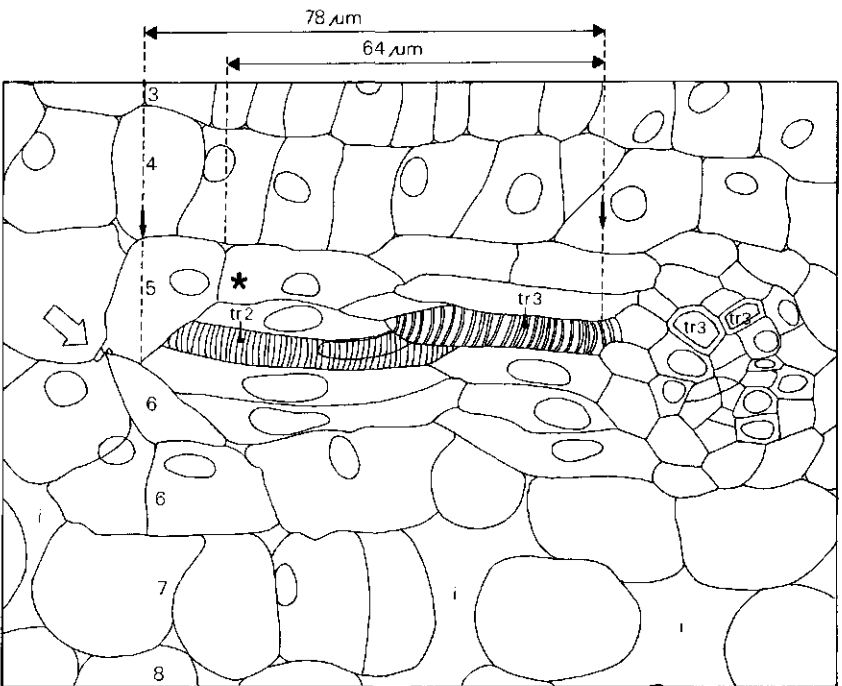


Fig. 10. Median longitudinal section of vein ending (between arrows) in leaf tissue of 57% F.L.L. This veinlet consists of 2 segments. The proximal segment contains one differentiated tracheid (tr3) like the two tracheids (tr3) in transverse section in the small vein of lower order. The distal segment contains one differentiating tracheid (tr2, with nucleus). The distal segment is much longer than the abaxial part of the cell (*) in the 5th layer of the plate meristem from which this segment partly arised. 3-8 = layers of plate meristem; double arrow = folding of wall parts in front of the intrusive apical elongation of fusiform procambium cells; i = intercellular space.

Conversion of the length of vein endings in leaves at different ages

The straight line no. 1 in fig. 8, based on the calculation of 121 examined vein endings, has been drawn again in fig. 11. The calculated point of intersection of the abscissa is 14.0% F.L.L. The calculated value of $\bar{l}_{v(38)}$, based on this straight line, is 78 μm ; $\bar{l}_{v(100)} = 279 \mu\text{m}$. From the similarity of the two drawn right-angled triangles, it follows that the proportion mentioned in fig. 11 does apply to this case.

Hence we may write:

$$\bar{l}_{v(\% \text{ F.L.L.})} = [(\% \text{ F.L.L.}) - 14]/86 \times \bar{l}_{v(100)} \quad (2)$$

$$\text{or: } \bar{l}_{v(100)} = [86/(\% \text{ F.L.L.}) - 14] \times \bar{l}_{v(\% \text{ F.L.L.})} \quad (3)$$

and: $38\% \leq \% \text{ F.L.L.} \leq 100\%$ (cp. fig. 16)

By means of these equations between % F.L.L. and the average length of the vein endings it is also possible to approximate the length of a vein ending at 100% F.L.L. when for instance the value of $\bar{l}_{v(38)}$ of this vein ending is known. In table 2 the longest vein ending is 157 μm ($\bar{l}_{v(38)} = 157 \mu\text{m}$); then a good approximation of $\bar{l}_{v(100)}$ is 563 μm (fig. 8). This vein ending really belongs to the longest specimen (MAGENDANS, 1983: table 9). The shortest vein ending is $\bar{l}_{v(38)} = 37 \mu\text{m}$; then an approximation of $\bar{l}_{v(100)}$ is 133 μm . And this latter vein ending does

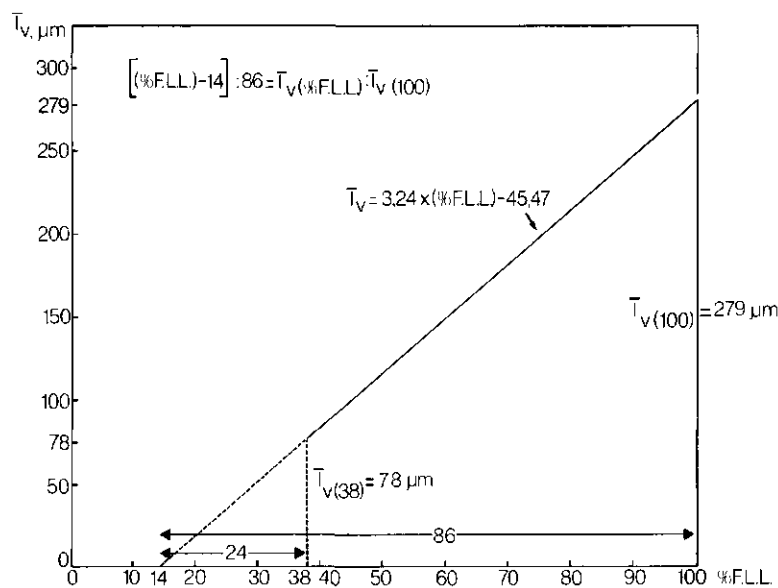


FIG. 11. Calculating of $\bar{l}_{v(\% \text{ F.L.L.})}$ and $\bar{l}_{v(\% \text{ F.L.L.})}$ on the base of similar right-angled triangles that arise after drawing of respectively line \bar{l}_v (= line 1, fig. 8, equation 1) completely, and other lines through the point 14% F.L.L. (abscissa) and any value of $\bar{l}_{v(\% \text{ F.L.L.})}$ as in fig. 8.

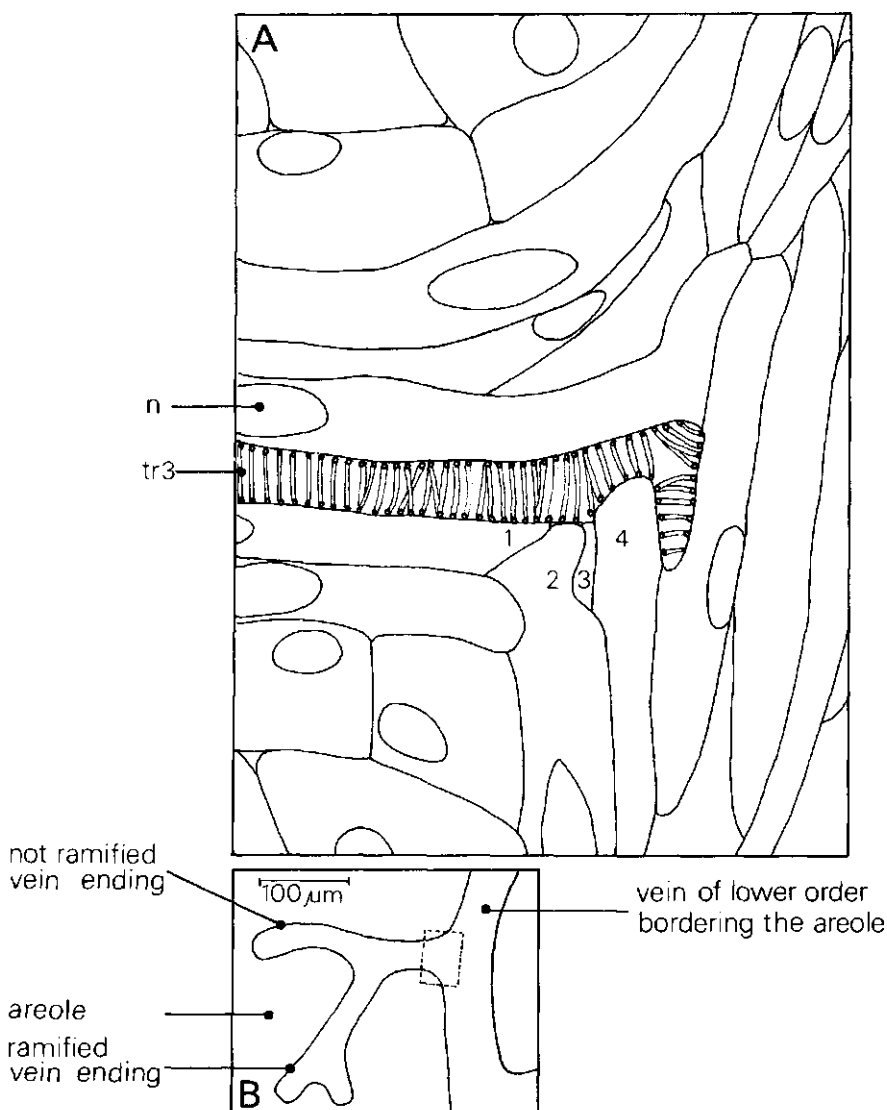


FIG. 12. A. Paradermal section of leaf tissue (82% F.L.L.). Strong intrusive apical elongation of fusiform procambium cells. 1, 2, 3, 4 = growing extremities of procambium cells; 2, 3, 4 push against the tracheid. In the adjoining adaxial section the right curved extremity of the tracheid is in contact with tracheids in the vein of lower order. n = nucleus, tr3 = differentiated tracheid.

B. Survey of part of an areole with three vein endings. The uppermost vein ending is considered as not ramified because of the connection with a vein that is ramified. The dotted rectangle indicates the portion of which fig. 12A is a detail.

not belong to the shortest specimen in mature leaf tissue (MAGENDANS, 1983: table 12A). The cause lies in the fact that in leaf tissue at 38% F.L.L. very short vein endings still differentiate that consist of only one procambium cell's length. The equations above-mentioned are useful only when calculating the lengths of vein endings and parts of them at various % F.L.L. of the *Hedera* leaf. All other (pieces of) veins of lower order lengthen to the same extent as the other leaf tissues, corresponding with the extension growth. However, intrusive growth of the procambium cells does also occur in these veins of lower order (fig. 12A).

In conclusion we may state that it is possible by means of the equations 2 and 3 to convert the length of vein endings at different ages, necessary to analyse the differentiation process in the veinlets.

Origin of the segmentation of the vein ending

In very young leaves (15% F.L.L. and 22% F.L.L., fig. 13) free ends of the venation do occur in the mesophyll. These free ends are not identical to the vein endings in the mesophyll of mature leaves. In young leaves these free ends still differentiate acropetally; the examined specimen were at such a distance from other veins that a distal contact with another vein was not probable to occur. Some details of the process of differentiation are illustrated by means of fig. 13. In the youngest leaf tissue (15% F.L.L.) mesophyll cells are found around free ends of the venation, represented as squares. The dimensions of these squares are in a distinct proportion to the average largest diameter of the mesophyll cells, that surround a free end. Now let us consider the distal procam-

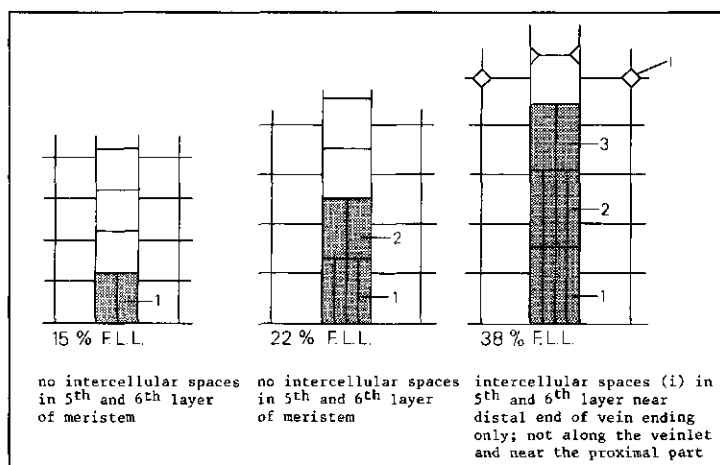


FIG. 13. Schematic drawings of free endings of veins in young leaves (15%, 22% and 38% F.L.L.) as seen from the adaxial side. The procambium cells are dotted. Originating of the segments 1, 2 and 3 from the mesophyll cells. Open stomata do occur in the abaxial epidermis of leaves of the three ages. Further explanation in the text.

bium cells of the free ends in this young leaf tissue, or those free ends that are only slightly longer than an adjacent mesophyll cell (av. 17.82 μm , table 9). In this latter case one of these mesophyll cells divided in such a manner that the new partition becomes an anticlinal cell wall (cp. fig. 20C-1 and D section 1) and is about at right angles to the vein of lower order. The daughter cells, that at first were no longer than the surrounding mesophyll cells, have already become longer at the moment of observation by intrusive growth in longitudinal direction (table 9: av. 21.47 μm , and statistically significant, $P < 0.005$, Wilcoxon's test; cp. also fig. 10).

In somewhat older leaf tissue (22% F.L.L.) slightly larger mesophyll cells are found (cp. PIETERS, 1974) than in leaf tissue at 15% F.L.L. (average of 20 longest diameters is 21.31 μm and is statistically longer than at 15% F.L.L.; $P < 0.001$, Wilcoxon's test). These larger mesophyll cells have been represented by means of squares also, and to the same scale as in the leaf tissue at 15% F.L.L. In this somewhat older leaf tissue free ends of the venation can be found also. Now we consider the two most distal segments of these free ends. When we assume that the procambium cells of segment 1 after dividing longitudinally several times, have originated from the procambium cells of segment 1 in leaf tissue at 15% F.L.L. (segment 1₍₁₅₎), then it will be understandable that these procambium cells have grown longer in this somewhat older leaf tissue: both intrusive growth and cell extension played a part ($\bar{l}_{\text{pc}(22)} > \bar{l}_{\text{pc}(15)}$, table 9).

To segment 2₍₂₂₎ the same does apply as to segment 1₍₁₅₎. Segment 2₍₂₂₎ originated from a somewhat larger mesophyll cell and consequently it was as long as that

TABLE 9. Data required for the schematic drawings of vein endings in young leaves (15%, 22% and 38% F.L.L.) in fig. 13. The numbers in brackets (2nd column) indicate the numbers of measurements that were available for calculating the averages

$\bar{\text{cell}}_{(15)}$, μm (average of largest diameter of mesophyll cells around distal end of free endings at 15% F.L.L.; 5th and 6th layer of plate meristem)	17.82	(20)
$\bar{l}_{\text{pc}(15)}$, μm (average length of procambium cells of segment 1; distal cells at 15% F.L.L.)	21.47	(9)
$\bar{\text{cell}}_{(22)}$, μm	21.31	(20)
$\bar{l}_{\text{pc}(22)}$, μm (segment 1 at 22% F.L.L.)	27.81	(10)
$\bar{l}_{\text{pc}2(22)}$, μm (segment 2 at 22% F.L.L.)	25.90	(10)
$\bar{\text{cell}}_{(38)}$, μm	20.64	(23)
$\bar{l}_{\text{pc}1(38)}$, μm (segment 1 at 38% F.L.L.)	32.24	(10)
$\bar{l}_{\text{pc}2(38)}$, μm (segment 2 at 38% F.L.L.)	33.04	(10)
$\bar{l}_{\text{pc}3(38)}$, μm (segment 3 at 38% F.L.L.)	28.35	(10)

larger cell initially. However this segment $2_{(22)}$ was already longer than the surrounding mesophyll cells at the moment of observation by intrusive growth that immediately occurred (table 9: $\bar{l}_{pc2(22)} > \overline{cell}_{(22)}$), the difference is statistically significant, $P < 0.005$, Wilcoxon's test).

In still older leaf tissue (38% F.L.L.) mesophyll cells do occur of which the average dimensions are the same as in younger leaf tissue at 22% F.L.L. (table 9: 20.64 μm on an average). In this leaf tissue free ends of the venation can be found also and now we consider the three most distal segments of these free ends. When we assume again that the procambium cells of segment $1_{(38)}$, after dividing longitudinally several times, have originated from the procambium cells of segment $1_{(15)}$, then it will be understandable that these procambium cells have become much longer in this tissue at 38% F.L.L. This lengthening was brought about by intrusive growth as well as cell extension. To segment $2_{(38)}$ the same does apply with respect to segment $2_{(22)}$. When we assume that the procambium cells of segment $2_{(38)}$, after dividing longitudinally, have originated from those of segment $2_{(22)}$, then it will be understandable that these procambium cells have grown longer also consequent on intrusive growth and cell extension. Meanwhile it becomes more difficult, however, to determine the right border-line between segment $1_{(38)}$ and segment $2_{(38)}$; this is brought about by further dividing of procambium cells also, especially at the abaxial side of the procambium strand. The limit of these segments has been determined by locating the middle of the bounding line that has become wider and less distinct. More causes of the limits of the segments becoming blurred are: more intrusive growth of the procambium cells, local division of these cells transversely, an oblique end wall coupled with a longitudinal division, and some remodelling of the procambium cell with an (oblique) division afterwards. The cause of the fact that $\bar{l}_{pc2(38)}$ is about as long as $\bar{l}_{pc1(38)}$ may be found in the difficulty to determine exactly the limits of these two segments.

To segment $3_{(38)}$ the same does apply again as to segment $2_{(22)}$ with regard to the origin from a mesophyll cell at the distal end of the developing vein ending. This segment arised from a mesophyll cell which was larger than the one from which segment $1_{(15)}$ originated, but was as large as the mesophyll cell from which segment $2_{(22)}$ originated. Therefore the procambium cells of segment $3_{(38)}$ will be as long as, or slightly longer than those of segment $2_{(22)}$ (table 9, the difference is not significant statistically).

The size of the mesophyll cells does not increase any more from 22% to 38% F.L.L. This is possible only because cell division still occurs very regularly and this can be observed also in the leaf tissue at 38% F.L.L.

The smallest veins mostly originate in the 5th and sometimes (also) in the 6th layer of cells of the plate meristem (fig. 10 and 20D). In these layers no intercellular spaces can be found yet at 15% and 22% F.L.L. At 38% F.L.L. intercellular spaces do occur in the 5th and 6th layer of cells, exclusively in the vicinity of the distal ends of the vein endings (fig. 14, and represented schematically, but about the correct average sizes in fig. 13). These intercellular spaces are in contact with the already larger intercellular spaces in cell layers 7-12 and

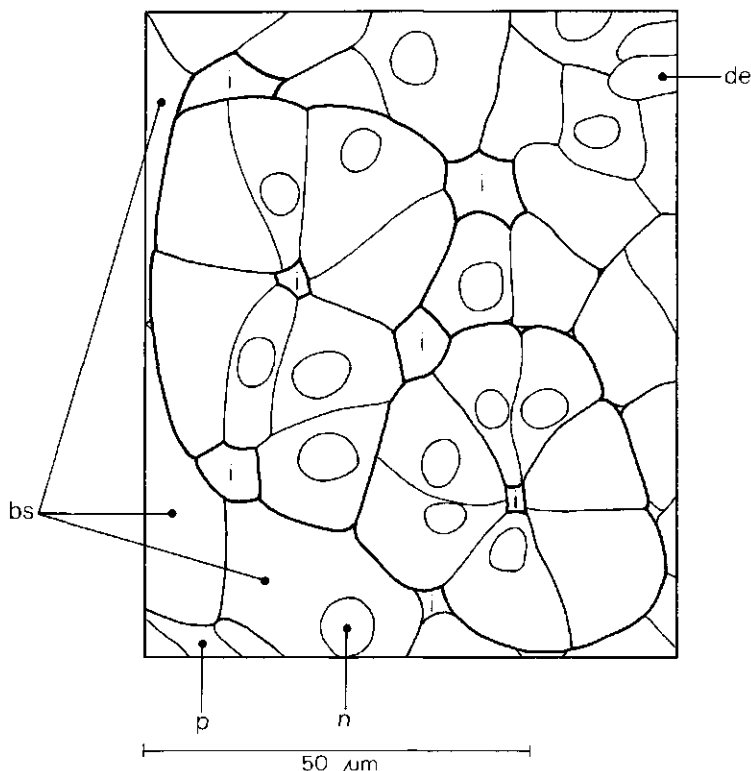


FIG. 14. Paradermal section of mesophyll cells in the vicinity of the distal extremity of a young vein ending, 5th or 6th layer of plate meristem. Development of intercellular spaces (i). New cell walls are formed by cell wall formation radiating from the intercellular spaces. 38% F.L.L. bs = bundle sheath, de = distal end of veinlet (procambium), n = nucleus, p = procambium of larger vein.

with the latter via the stomata with the surrounding air. Transpiration by way of the apoplast of the procambium cells in the young vein ending and the future cells of the bundle sheath, and afterwards via the intercellular spaces and stomata, is possible at 38% F.L.L. anyhow. At 97% F.L.L. the distribution of the intercellular spaces in an areole has been changed completely (fig. 15). Then the vein endings are entirely surrounded by large intercellular spaces.

Finally we may come to the conclusion that the procambium cells of the vein endings originate from young mesophyll cells in an acropetal sequence and that the young procambium cells, after obtaining polarity, lengthen extra with regard to the surrounding cells in the direction of the vein ending. The very specific way of originating of the first intercellular spaces in the 5th or 6th layer of the plate meristem makes one think of the possibility that the morphogenesis of the vein endings is related to the first route along which the transpiration transport takes place.

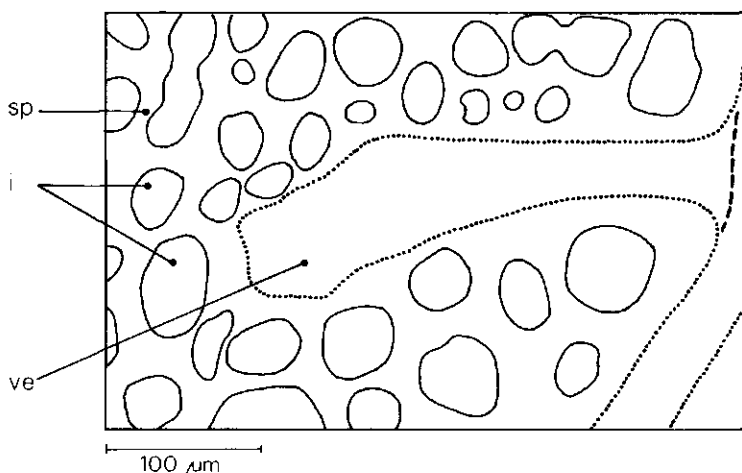


FIG. 15. Position of intercellular spaces around and in the cell layers in which the vein ending is situated in leaf tissue at 97% F.L.L. The intercellular spaces near the distal end of the veinlet develop earlier ($\pm 35\%$ F.L.L.) than the others and are important for determining the position of the distal limit of the young vein ending. The position of intercellular spaces within the vein ending is indicated in fig. 3, MAGENDANS, 1983. i = intercellular space, sp = spongy parenchyma, ve = vein ending.

Number of segments of the vein ending

Is the number of 3 segments as represented in fig. 13, at 38% F.L.L., a real image of the average number of segments of which completely differentiated vein endings at 100% F.L.L. are composed? The appearance of the distinct intercellular spaces at the distal end of a free end of the venation (fig. 13) is an indication that the free end has reached its total length of differentiation. Soon the limits of the segments can not be located exactly any longer in leaf tissue older than 38% F.L.L. However, the number of segments of which a vein ending is composed, mostly can be determined well yet. In tables 2, 3 and 6, at resp. 38%, 48% and 82% F.L.L. the number of segments has been given of the examined vein endings in paradermal sections. The average number of segments is 2.7 (38%), 3.1 (48%) and 2.8 (82% F.L.L.). No statistically significant difference exists between these numbers (Wilcoxon's test), this means that the number of segments in the vein endings remains unaltered in leaf tissue at the age of 38% F.L.L. and older. So it appears that the vein endings in leaf tissue at 38% F.L.L. do not increase in length any more owing to differentiation of mesophyll cells into new procambium cells at the distal end. From the age of 38% F.L.L. the vein endings have been differentiated completely in this respect. In fig. 16 the straight line \bar{l}_v is the same as in fig. 8. In addition the average length of the segments is represented by the straight line \bar{l}_{pc} (the value of \bar{l}_v divided by the average number of segments, 2.87, at each age of the leaf). The average segment's length at 40% F.L.L. amounts to about 30 μm.

In table 10 data are shown concerning the free ends of the venation as are

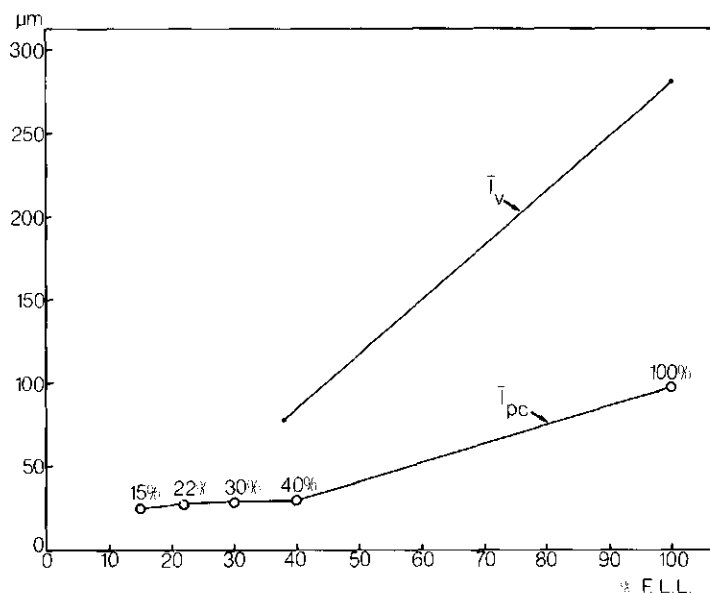


FIG. 16. \bar{l}_v at different % F.L.L. (as in fig. 8, 11) and $\bar{l}_{pc} (= \bar{l}_v/2.87)$. The number 2.87 is the average number of segments per veinlet, see tables 2, 3 and 6: $(2.7 + 3.1 + 2.8) \times \frac{1}{3}$. The part of \bar{l}_{pc} below 40% F.L.L. gives the average length of all segments present in the free ends at 15%, 22% and 30% F.L.L. (table 10) and in the vein endings at 40% F.L.L. ($\bar{l}_{v(40)}/2.87$), resp. 25.0, 27.1, 28.5 and 29.4 μm .

found in leaf tissue at 15%, 22% and 30% F.L.L. In this very young leaf tissue free ends of the venation do occur in the mesophyll (fig. 13); of these free ends one cannot be sure new differentiating segments will appear at the distal end or even they will ramify also. These free ends we will call free ends of the young venation. In order to assess now the approximate leaf's age at which the vein endings have been differentiated, i.e. the number of segments has been fixed, one can measure the average length of the segments of the free ends of the venation in very young leaf tissues. In leaf tissue in which this average segment's length of the free ends equals that of the vein endings, and the average number of segments corresponds also with that of the differentiated vein endings, the prolongation of the free ends of the young venation has come to an end. Or one can say that the average segment's length in very young leaf tissue (\bar{l}_{pc}) at a particular age of the leaf approaches to the value at which $\bar{l}_v/\bar{l}_{pc} = 2.87$ (fig. 16, at about 40% F.L.L.). The value 2.87 (average of 2.7, 3.1 and 2.8, see tables 2, 3 and 6) remains constant when the leaf tissue grows older. The segment's length of $\pm 30 \mu\text{m}$ is too long below 40% F.L.L. for the leaf to be able to compose vein endings with 2.87 segments on an average; unless the vein endings in younger leaf tissue would have about the same length as in older leaf tissue, and that is impossible because of the growth of the leaf. In table 10 the average segment's lengths are given of the free ends with 1–3 segments at 15%, 22%

TABLE 10. Average length of the 1-3 distal segments (\bar{l}_{pc1-3}) of free endings of the young venation at 15%, 22% and 30% (resp. $\bar{l}_{pc1-3(15)}$, etc.). F.L.L. For each percentage F.L.L. also the number of segments is noted. In the last column the average length of the segments of a representative number of 10 vein endings at 38% F.L.L. (from table 2) is given and also the number of segments for each vein ending.

$\bar{l}_{pc1-3(15)}$ μm	Number of segments	$\bar{l}_{pc1-3(22)}$ μm	Number of segments	$\bar{l}_{pc1-3(30)}$ μm	Number of segments	$\bar{l}_{v1-4(38)}$ no of segm. μm	Number of segments
31.0	1	22.5	2	31.0	1	37.0	1
21.0	2	24.5	2	24.0	2	31.5	2
24.5	2	24.5	2	26.0	2	33.0	2
25.5	2	26.0	2	27.0	2	26.3	3
29.5	2	26.0	2	28.0	2	27.3	3
19.0	3	28.0	2	31.0	2	29.0	3
24.0	3	29.5	2	38.0	2	30.3	3
24.0	3	31.0	2	24.0	3	35.0	3
25.0	3	31.0	2	25.0	3	33.4	3.5
26.0	3	28.0	3	31.0	3	45.0	3
av. 24.4 ± 3.1	2.4	27.1 ± 2.7	2.1	28.1 ± 4.2	2.2	32.5 ± 5.5	2.65

and 30% F.L.L. At 38% F.L.L. the average segment's length has been determined in vein endings with 1-4 segments (table 2). The number of segments at 38% F.L.L., determined in this way, does not differ significantly from the number of segments at 15% F.L.L. (Wilcoxon's test). When comparing, however, the length of the segments at 15% F.L.L. with the segment's length at 38% F.L.L., the difference is significant: $\bar{l}_{pc(38)} > \bar{l}_{pc(15)}$ (Wilcoxon's test, $P < 0.001$). The cause of this difference is based on the fact that the distal segments originated in larger mesophyll cells (fig. 13), and also because the older segments 1 and 2 grew more in length already. The values of the average length of the segments 1-3 in the free ends at 22% and 30% F.L.L. ($\bar{l}_{pc1-3(22)}$ and $\bar{l}_{pc1-3(30)}$) are intermediate (table 10).

The average segment's length of the vein endings at 38% F.L.L. is $32.5 \mu\text{m}$ (table 10). The calculated value is lower: $\bar{l}_{v(38)}/2.87 = 77.8/2.87 = 27.1 \mu\text{m}$. This difference is caused by the higher value noted for $\bar{l}_{v(38)}$ ($88.8 \mu\text{m}$ in table 2), than could be expected, basing oneself on the linear equation between \bar{l}_v and % F.L.L. ($77.8 \mu\text{m}$, fig. 8). It is possible that the bending-point in the line \bar{l}_{pc} at 40% F.L.L. should be corrected into a smoothly continuous curve, in consequence of which the calculated point (29.4) at 40% F.L.L. must be higher. Still the point of junction at about 40% F.L.L. between the first, almost flat part of the line and the ascending part of the line \bar{l}_{pc} is clearly present. This means that the definitive length of differentiation of the vein endings is established at about 40% F.L.L., corresponding with the first appearance of rather large intercellular spaces in the 5th and 6th layers of cells of the plate meristem near the distal extremities of the vein endings (fig. 13, 14).

In conclusion we may state, based on the average number of segments (2.87)

and the value of the average segment's length in the free ends of the venation (table 10), that the differentiation of the free ends has been completed at about 40% F.L.L. and that the free ends changed into vein endings at that moment.

First differentiation of tracheary elements in vein endings.

In tables 2-7 the length of the vein endings along which the differentiation of tracheids becomes visible, is indicated by l_{tr} , expressed in μm . The vein endings

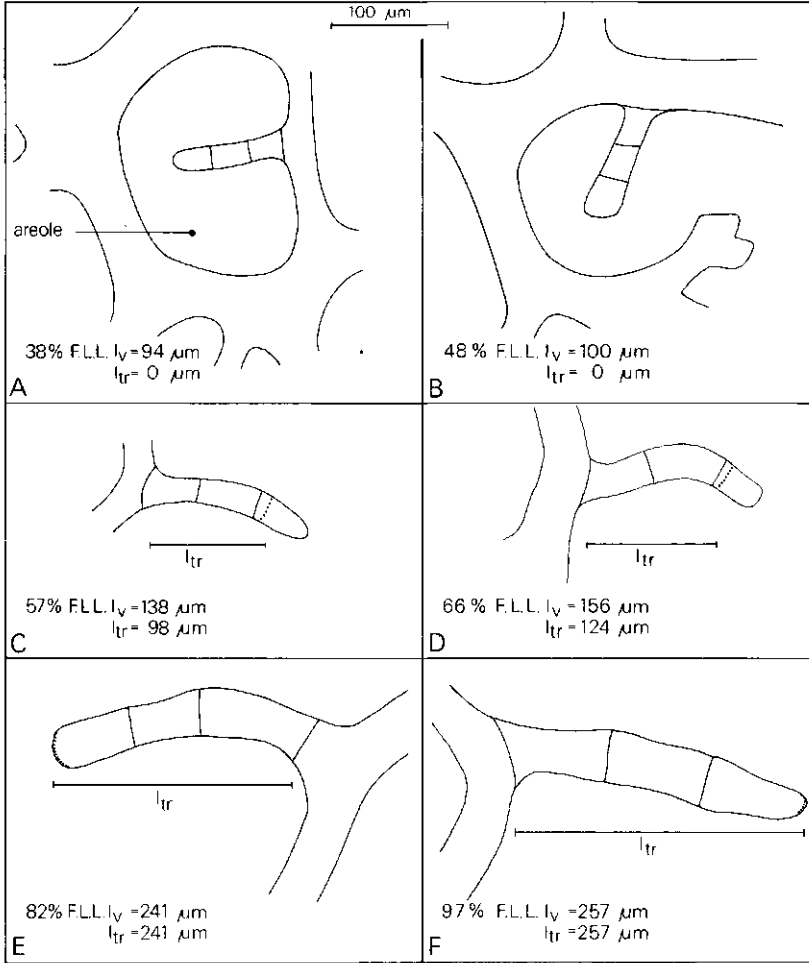


FIG. 17. Not ramified vein endings (A-F) in paradermal sections. The veinlets are representative of the length of the vein endings for each % F.L.L., and of the number of segments (3). The limits of the segments are indicated with transverse lines. The differentiation of tracheids has taken place up to the dotted lines. This differentiation of tracheids extends beyond the limits of the second segments because of intrusive growth of the procambium cells. l_{tr} = length of veinlet along which differentiation of tracheids has taken place.

in the young leaf at 38% F.L.L. (table 2) almost consist of procambium cells only along the whole length (fig. 17A). Only in the longest specimen (157 μm) the differentiation of one tracheid was beginning along a distance of 28 μm .

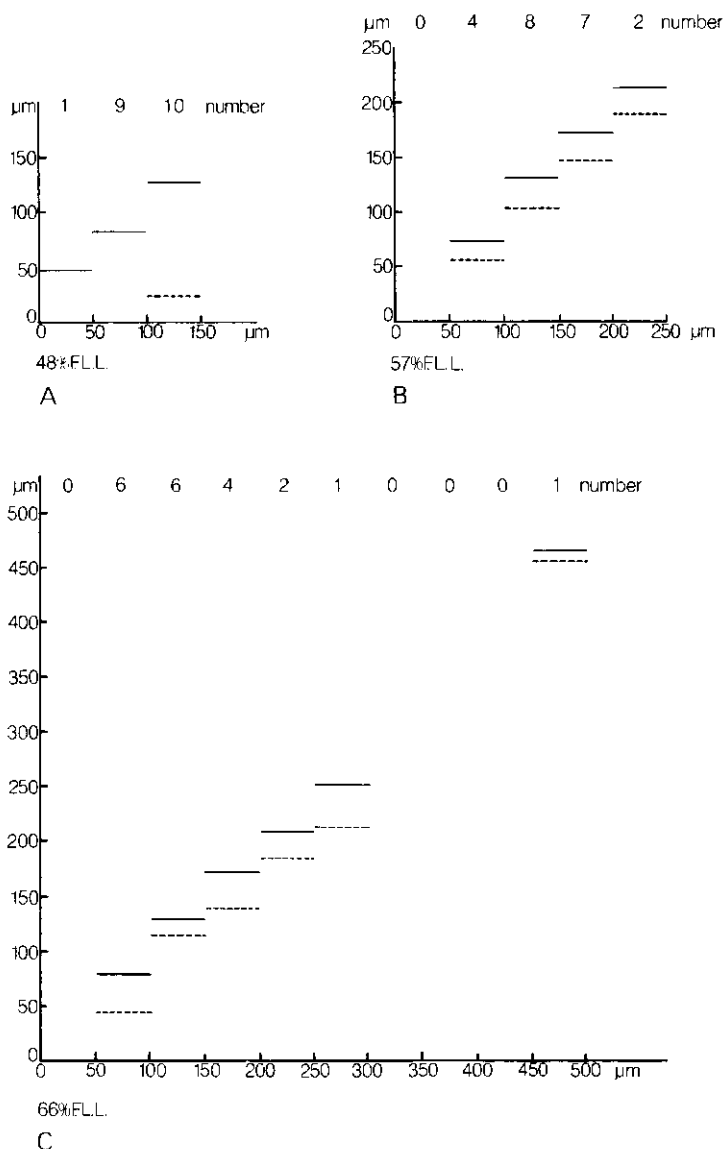


FIG. 18. Diagram with data from tables 3, 4 and 5. The vein endings in young leaves at 48% (A), 57% (B) and 66% F.L.L. (C) have been divided into groups of 50 μm difference in length each (abscissa) and in every group of veinlets the average values of l_v (—) and l_{tr} (-----) have been determined (ordinate).

This differentiation took place at the proximal end, linking up with the differentiation of xylem in the vein of lower order. In 6 vein endings in the leaf tissue at 48% F.L.L. (table 3) differentiation of tracheids took place in the proximal part. In 5 specimen this differentiation concerned only one tracheid along 40 μm on an average and in one specimen 2 tracheids were differentiating along a distance of 26 μm . In most vein endings no differentiation of tracheids took place at all yet along the vein ending (fig. 17B). In fig. 18A it is shown that differentiation of tracheids has taken place only in the group of 10 vein endings to which applies $100 \mu\text{m} \leq l_{v(48)} \leq 150 \mu\text{m}$ ($\bar{l}_{v(48)} = 127.2 \mu\text{m}$ in this group); this differentiation took place the length of 23.2 μm on an average.

Off the growth rate diagram of vein endings in fig. 19, based on the formulae 2 and 3 and on an average growth curve (fig. 28), one can read that this situation has been reached about 15 days after the beginning of the growth of the leaf. Well over 3 days later (66% F.L.L.) the diagram reads that this same group of 10 vein endings has reached the length $153.0 \mu\text{m} \leq l_{v(66)} \leq 229.4 \mu\text{m}$ and $\bar{l}_{v(66)} = 155 \mu\text{m}$ in this group (table 5, fig. 18C).

In all vein endings in leaf tissue at 57% F.L.L. (table 4, fig. 17C) differentiation into tracheids occurred. The total length along which differentiation into tracheids is found, now amounts to 83% of $\bar{l}_{v(57)}$ on an average. This differentiation does occur continuously and the progress is acropetal without exception. The maximum number of tracheids is 3 broadwise. This means that the first stage of xylem differentiation consists of a rather rapid proceeding of a differentiation process that is transmitted continuously via a small number of cells at the adaxial side of the procambium strand (fig. 21 and 22). In the specimen $l_{v(57)} = 182 \mu\text{m}$ (table 4) one can see that the width of xylem differentiation along a distance of 170 μm is only one tracheid. At the distal end of the zone in which tracheids differentiate, only 1–2 tracheids can be found always. In fig. 18B the relation is given between \bar{l}_v and \bar{l}_{tr} at 57% F.L.L. in the various groups of vein endings that differ 50 μm in length. Now it appears from this figure that the differentiation of tracheids in every group of vein endings has arrived up to about 24 μm from the distal end of the procambium strand. Supposing that the distal segment in the procambium strand differentiates at about 35% F.L.L. (fig. 13, 16), then one may come to the conclusion that the first tracheid differentiates in this distal segment about 4 days after the differentiation of procambium (fig. 19).

In the group of vein endings $l_{v(48)} = 100\text{--}150 \mu\text{m}$ (fig. 18A) we find that $\bar{l}_{tr(48)} = 23.2 \mu\text{m}$, i.e. 18.2% of \bar{l}_v in this group of veinlets. In the group of vein endings $l_{v(57)} = 126.5\text{--}189.7 \mu\text{m}$ (fig. 19) we find that $\bar{l}_{v(57)} = 155 \mu\text{m}$ and $\bar{l}_{tr(57)} = 129 \mu\text{m}$, i.e. 83% of $\bar{l}_{v(57)}$ (table 4) and that occurs after about 1.5 day. In the group $l_{v(66)} = 153.0\text{--}229.4 \mu\text{m}$ (fig. 19) we can find that $\bar{l}_{v(66)} = 185 \mu\text{m}$ and $\bar{l}_{tr(66)} = 155 \mu\text{m}$, i.e. 84% (table 5), and that is almost 2 days later again (fig. 19). Then in the interval between 48 and 57% F.L.L. (± 38 hrs) a rapid first differentiation into tracheids takes place, especially in longitudinal direction. Broadwise the number of tracheids amounts to 3 maximally at 57% F.L.L. In the interval between 57% and 66% F.L.L. (± 45 hrs) a much slower differentiation of tracheids proceeds in longitudinal direction (fig. 17C, D). However, more differentiation

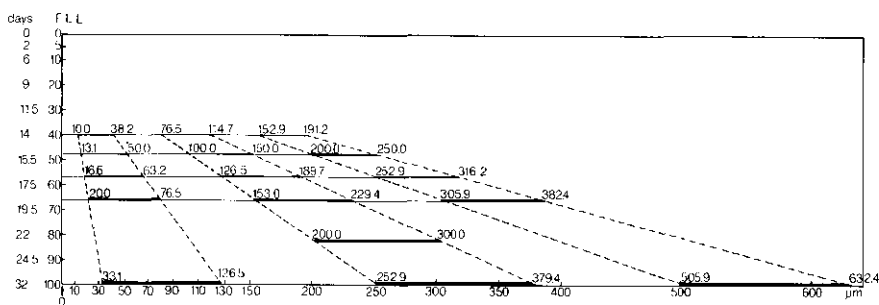


FIG. 19. Growth rate diagram of vein endings in *Hedera* leaves. The diagram is based on the formulae 2 and 3 of growth of vein endings and on the average growth curve in fig. 28. This diagram has been constructed to make possible comparisons between the diagrams A, B and C in fig. 18. The number of segments of the vein endings is complete at 40% F.L.L. (fig. 16), from that time on the formulae are valid. The strips between the broken lines with alternating thick and thin parts in the horizontal lines indicate the growth of the groups of veinlets (13.1–50.0; 50.0–100.0; 100.0–150.0 μm , etc. at 48% F.L.L., fig. 18A) from 40% to 100% F.L.L.

takes place broadwise, for the number of tracheids is already 6 at 77% F.L.L. on the spot of the future tracheid maximum (MAGENDANS, 1983), see fig. 30A. In the group of vein endings $l_{v(82)} = 200\text{--}300\text{ }\mu\text{m}$ (table 6, fig. 19) $l_{tr(82)}$ has reached 100% of $l_{v(82)}$ in each of the four veinlets (fig. 17E). This percentage amounts to 99% on an average over all 20 veinlets and at the distal extremity already 5 tracheids can be found. At 88% F.L.L. already 8 tracheids on an average can be found in one transverse section near the place of the future tracheid maximum (fig. 30B).

In the first stage of differentiation into tracheids between 43% (fig. 26) and 57% F.L.L. (fig. 18) 1–3 tracheids differentiate rapidly along a narrow adaxial strip of the procambium (fig. 21, 22) until not far from the distal extremity of the procambium strand. In the second stage the differentiation slowly proceeds acropetally but the number of tracheids especially increases broadwise until about 70–80% F.L.L. After that the last stage of tracheid differentiation in the vein endings begins, in which especially the upbuilding of the tracheid maximum takes place. In table 7 it is shown that $l_{v(97)} = l_{tr(97)}$ applies to all vein endings (fig. 17F). Besides in this table the number of developing tracheids is given, as visible in paradermal section. Those tracheids in which the spiral wall strengthening structures are clearly perceptible already, but in which a nucleus and cytoplasm are still be found, will be considered as being in differentiation. It is shown that the number of differentiating tracheids seems to be distributed regularly along the vein ending; no clear maximum of differentiation activity is perceptible (fig. 30C).

Interpretation of some transverse sections of a vein ending in young leaf tissue

In fig. 20A a small part of an areole is represented schematically with one entirely straight and not ramified vein ending, occurring as a young procambium

strand in leaf tissue at 38% F.L.L. This strand is represented in fig. 20B as a series of procambium cells about 30 μm wide on an average. This strand is 114 μm long, which means that $l_{v(100)} = 408 \mu\text{m}$ (formula 2). The value of $(\overline{l_v - l_{se}})_{(100)}$ in this climate is $170.8 \pm 30.1 \mu\text{m}$ (table 19).

The 95% prediction lower bound of $l_{se(100)}$ will be:

$$408 - [(\overline{l_v - l_{se}})_{(100)}] - t_{0.95}(12) \sqrt{1/13 + 1} \times \text{SE} =$$

$$408 - 170.8 - 1.9191 \times 30.1 = 179.4 \mu\text{m}.$$

Then $l_{se(38)}$ will be 50.1 μm (formula 2); this means that in the transverse sections 9 and 10 (fig. 20B, D) phloem most probably will differentiate.

The tissues of the mature *Hedera* leaf originate from a plate meristem consisting of 10–13 cell layers; the adaxial epidermis originates from the first cell layer. The two layers of palisade cells originate from the 2nd and 3rd cell layers and the cells of the bundle sheath originate from the 4th layer at the adaxial side of a vein ending. Sometimes the cells of the bundle sheath originate from the adaxial part of cells in the 5th layer (fig. 20D: sections 1–4).

The procambium strand in fig. 20 originated from the 5th cell layer (fig. 20D: sections 2, 3), as is the case normally; by way of exception the 6th cell layer

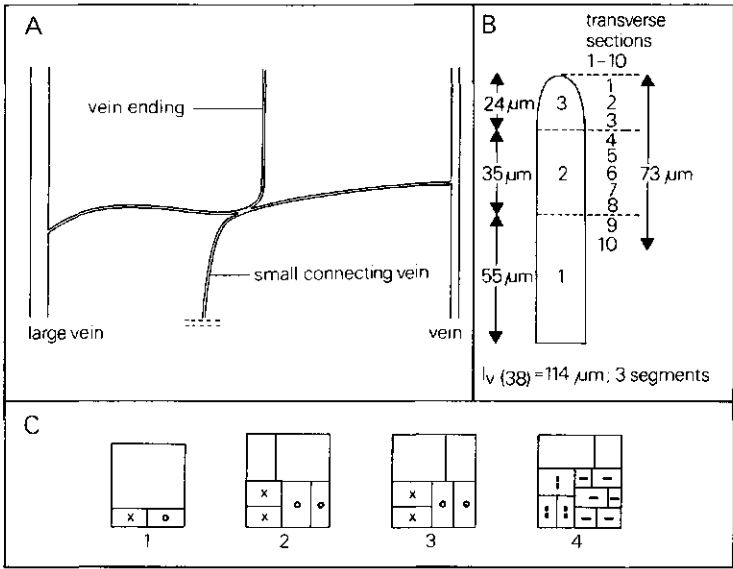


FIG. 20A. Part of an areole with one, not ramified vein ending (38% F.L.L.).
 B. The straight schematic vein ending in A has been sectioned transversely (sections 1–10). Three sections hit the distal segment 3, five sections went across segment 2 and two sections across segment 1. $l_{v(38)} = 114 \mu\text{m}$; $l_{v(100)} = 408 \mu\text{m}$; expectation value for $l_{se(100)} = 237 \mu\text{m}$ and $l_{se(38)} = 66 \mu\text{m}$; i.e. in that part of the veinlet, hit by the sections 7–10, differentiation of sieve elements may be expected.
 C. Sections 1–4 drawn schematically. Sections 1–3 in segment 3 and section 4 in segment 2. The same symbols are used for the same procambium cells. The cells without symbols are cells of the future bundle sheath.

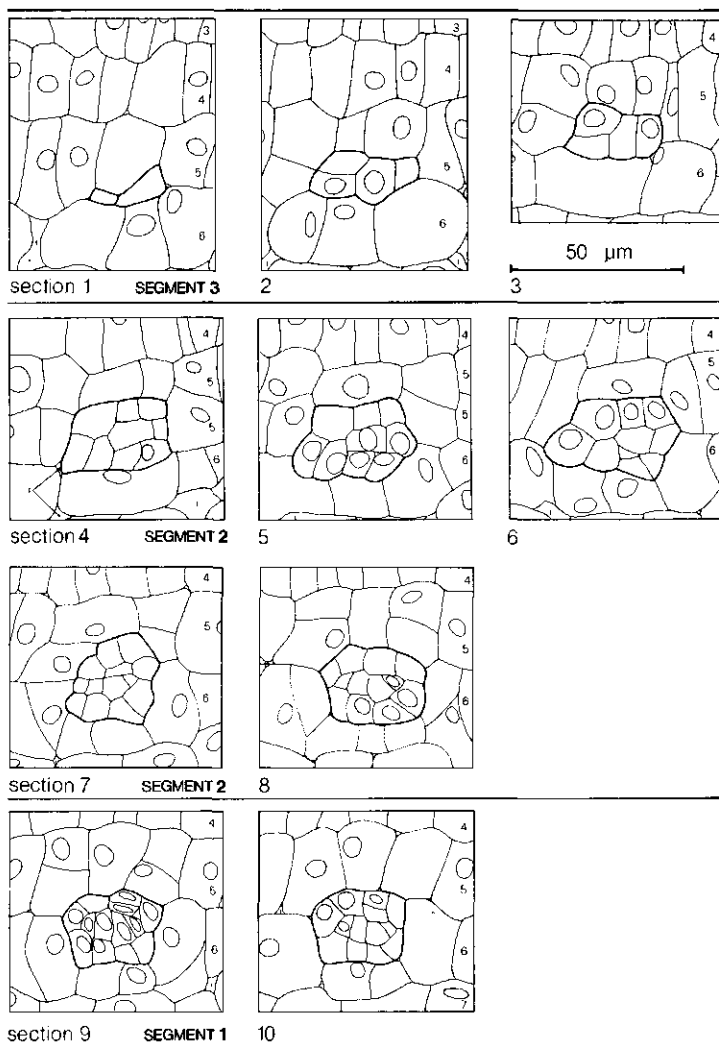


FIG. 20. D. Sections 1–10 in detail, adaxial side is at the top. Drawn layers of the plate meristem are indicated 3–6. Layer 3 will differentiate into palisade parenchyma, cells of the 4th layer and sometimes an adaxial part of the 5th layer differentiate into part of the bundle sheath adaxial of the veinlet. The oval to round structures in the cells are nuclei. i = intercellular space.

may also contribute to the differentiation into a vein ending. Mostly the procambium strand does not originate from the complete anticlinal measure of the 5th cell layer but from the abaxial half of the cells only (fig. 20D: sections 2, 3). The remaining cell layers 7–13 form the spongy parenchyma and the abaxial epidermis. The vein ending in fig. 20 is composed of 3 segments. The positions of the transverse sections 1, 2 and 3 (fig. 20B) are in the distal segment 3, the

sections 4–8 are located in the middle segment 2 and the sections 9 and 10 concern the distal part of segment 1. The bounding line between the segments 2 and 3 is clearly perceptible by comparing the sections 3 and 4, see the positions of longitudinal walls (fig. 20C–3, 4, at the right, below) and nuclei (fig. 20D, sections 2, 3, 4 and 5). However, the bounding line between the segments 1 and 2 has become indistinct in the transverse sections by vigorous intrusive growth of the individual procambium cells and because dividing of procambium cells took place, especially in the abaxial part of the procambium strand. The length of segment 2 can be deduced well from the three relative long and wide procambium cells at the adaxial side of the procambium strand, of which the nuclei are visible in section 6. The intrusive growth of the procambium cells is easily perceptible in section 1 also (cp. fig. 10, the distal extremity).

The initiation of the first tracheids takes place by direct differentiation of adaxial cells of the procambium strand (fig. 21); the initiation of the first sieve elements takes place after repeated cell division in the abaxial part of the procambium strand (fig. 23) and is to be expected in sections 9 and 10 (fig. 20D).

Analysis of differentiation of tracheids in vein endings in transverse sections

The differentiation of tracheids in procambium strands in young leaf tissue can be followed more closely when the differentiation process into a completely differentiated tracheid can be divided into a number of anatomically defined stages which are distinct from each other in a considerable measure. In regard to this differentiation process in the vein endings of the *Hedera* leaf the classification in table 11 appeared to be useful. This classification is based on differences in the wall strengthening structure and cell contents. It is possible that the limit between the stages tr1 and tr2 as perceived in longitudinal section, does not exactly coincide with that in transverse section; but this difference must be very small. The tr3 stage is clearly different from tr2, both in longitudinal and in transverse section, provided that all sections through the element can be examined. In figs 21, 22 and 23 the various stages have been represented.

In table 12 the results are given of observations on 5 vein endings in transverse sections of these veinlets in young leaf tissue (38% F.L.L.). In the shorter veinlets (1–4) no differentiation of tracheids has taken place at all. Also the first stage of differentiation (tr1) has not been found yet in these veinlets, not even in the proximal part. Only in the longest veinlet (5) a single tracheid in the last stage of differentiation (tr3) is found in the proximal part the length of 14 μm . For the most part, however, this tracheid belongs to the vein of lower order in which the differentiation into tracheids did make more progress. One of the extremities of this tracheid bended into the vein ending (intrusive growth?). The value of $\bar{l}_{v(38)}$, that is to be expected, amounts to 77.8 μm (formula 1). This value of the 5 examined vein endings in table 12 is considerably higher (100.8 μm). When correcting $\bar{l}_{tr3(38)} = 2.8 \mu\text{m}$ of the 5 vein endings, corresponding with a lower calculated value of $\bar{l}_{v(38)}$, then $\bar{l}_{tr3(38)}$ will have a lower value also and approaches near to the real value of zero (fig. 26).

TABLE 11. Three stages (tr1, tr2 and tr3) of the differentiation process of a tracheid in the veinlet procambium, as perceptible with the light microscope (1500×). The three stages are characterized by differences in wall strengthening structure and cell contents. The measure of staining with safranin has been regarded as a measure of the lignification of the spiral thickenings in the tracheids.

Stages of differentiation of tracheids	Wall strengthening structure		Cell contents	Figure
	longitudinal section	transverse section		
tr1	spiral in shape; the windings just becoming visible; colour green	wall a bit and evenly thickened; no spiral windings visible yet	nucleus and cytoplasm	21-3a 23-A
tr2	spiral in shape and clearly perceptible; partly or entirely lignified; colour faintly green and (locally) becoming faintly red	wall partly or entirely lignified; if not lignified, yet clearly thickened spiral windings present	mostly with nucleus and cytoplasm, or remnants of these present	21-3b, 4 22-C 23-B, C
tr3	spiral windings thickened and lignified; colour red	thickened spiral windings entirely lignified	no nucleus, no remnants of cytoplasm	22-A, B, C 23-A, B, C

TABLE 12. Leaf tissue at 38% F.L.L. Analysis of 5 not ramified vein endings in transections. Only in the longest veinlet differentiation of 1 tracheid occurred over a short distance: a tr3 in the vein of lower order bended partly into the vein ending. l_{tr1} , l_{se1} , etc. = length of veinlet tr1, se1, etc. elements differentiated (Table 11 and 18).

$l_{v(100)} = 3.58 \times l_{v(38)}$ (formula 3) and $l_{v(38)} = 77.8 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(38)}$ μm	l_{tr1} μm	l_{tr2} μm	l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	$l_{v(100)}$ μm	$l_{se(100)}$ μm
1	80	0	0	0	0	0	0	287	—
2	89	0	0	0	0	0	0	319	—
3	98	0	0	0	0	0	0	351	—
4	114	0	0	0	0	0	0	408	—
5	123	0	0	14	0	0	0	441	—
av.	100.8	0	0	2.8	0	0	0	361.2	—

In table 13 the results are given of observations on 5 vein endings in less young leaf tissue (46% F.L.L.). In the shortest vein ending the length along which differentiation of tracheids took place, is still zero; the differentiation has not arrived yet in the proximal part of this vein ending that originated perhaps later. In the four longer vein endings, which are older usually, differentiation of tracheids has taken place already from the proximal end. The differentiation of tracheids does occur irregular in the different vein endings, but in the individual veinlets this differentiation, once caused to begin, proceeds rapidly; more rapid than at which the differentiation of procambium took place (fig. 26). The value of

TABLE 13. As table 12. Leaf tissue at 46% F.L.L. The differentiation of the first tracheids is acropetal and continuous into the provascular tissue of the veinlet. This differentiation proceeds more rapid than did the differentiation of the procambium (cp. table 12 and fig. 26).

$l_{v(100)} = 2.69 \times l_{v(46)}$ (formula 3) and $\bar{l}_{v(46)} = 103.8 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(46)}$ μm	l_{tr1} μm	l_{tr2} μm	l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	$l_{v(100)}$ μm	$l_{se(100)}$ μm
1	73	0	0	0	0	0	0	196	—
2	122	42	42	0	0	0	0	328	—
3	132	114	0	0	0	0	0	355	—
4	150	0	95	0	0	0	0	403	—
5	153	107	107	31	0	0	0	411	—
av.	126.0	52.6	48.8	6.2	0	0	0	338.6	—

$\bar{l}_{v(46)}$, that is to be expected, amounts to $103.8 \mu\text{m}$ (formula 1). This value of the 5 examined vein endings in table 13 is considerably higher ($126.0 \mu\text{m}$). The found values of $\bar{l}_{tr1(46)} = 52.6 \mu\text{m}$ and $\bar{l}_{tr3(46)} = 6.2 \mu\text{m}$ can be corrected by lowering these values, basing oneself on a fairly constant value of \bar{l}_{v-tr1} and \bar{l}_{v-tr3} in leaf tissue of a particular age (fig. 18, table 19).

In table 14 the results are given of observations on 5 vein endings in leaf tissue at 57% F.L.L. In all the veinlets differentiation of tracheids has taken place; in the veinlets with the numbers 2 and 5 tracheids are found already, which differentiated completely in the proximal part. The value of $\bar{l}_{v(57)}$ that is to be expected, amounts to $139.4 \mu\text{m}$ (formula 1). This value of the 5 examined vein endings in table 14 is much lower ($85.4 \mu\text{m}$). Therefore the values of $\bar{l}_{tr1(57)} = 52.2 \mu\text{m}$ and $\bar{l}_{tr3(57)} = 22.8 \mu\text{m}$, given in the table, should be raised to obtain a better general view by means of which comparison with other data becomes possible (fig. 26). When correcting these data one can base oneself again on the fairly constant value of \bar{l}_{v-tr1} in leaf tissue of a particular age (fig. 18, table 19).

In table 15 the results are given of observations on 5 vein endings in leaf

TABLE 14. As table 12. Leaf tissue at 57% F.L.L. The first, acropetal differentiation of tracheids ($tr1$) approaches the distal extremity of the procambium strand in some of the young veinlets.

$l_{v(100)} = 2.00 \times l_{v(57)}$ (formula 3) and $\bar{l}_{v(57)} = 139.4 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(57)}$ μm	l_{tr1} μm	l_{tr2} μm	l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	$l_{v(100)}$ μm	$l_{se(100)}$ μm
1	55	0	29	0	0	0	0	110	—
2	59	0	0	36	0	0	0	118	—
3	62	59	21	0	0	0	0	124	—
4	125	86	86	0	0	0	0	250	—
5	126	116	94	78	0	0	0	252	—
av.	85.4	52.2	46.0	22.8	0	0	0	170.8	—

tissue at 77% F.L.L. Now the acropetal differentiation of tracheids approaches the distal extremity in all vein endings. Also the completely differentiated tracheids (tr3) are found close to the distal ends of the veinlets already. The value of $\bar{l}_{v(77)}$, that is to be expected, amounts to 204.3 μm (formula 1) and this value does not differ much from the calculated average of the 5 vein endings (213.4 μm). Therefore the values of $\bar{l}_{tr1(77)} = 205.6 \mu\text{m}$ and $\bar{l}_{tr3(77)} = 195.4 \mu\text{m}$, given in the table, will be representative of this group of vein endings in leaf tissue of this age and this is apparent from fig. 26 also.

In table 16 the results are shown of observations on 5 vein endings in leaf tissue at 88% F.L.L. In this somewhat older leaf tissue the total number of vein endings in the transections, suitable for analysis, is smaller already; in the first instance only 4 useful (= sectioned transversely at full length nearly), not ramified specimen were available. The acropetal differentiation of tracheids has arrived in the distal extremity of the vein endings with all 3 stages of development almost entirely. The value of $\bar{l}_{v(88)}$, that is to be expected, amounts to 240.0 μm

TABLE 15. As table 12. Leaf tissue at 77% F.L.L. The acropetal differentiation of tracheids approaches the distal extremity of the procambium strands with all stages of development (tr1, tr2 and tr3, cp. fig. 26). No differentiation of sieve elements is found yet. $l_{v(100)} = 1.37 \times l_{v(77)}$ (formula 3) and $\bar{l}_{v(77)} = 204.3 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(77)}$ μm	l_{tr1} μm	l_{tr2} μm	l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	$l_{v(100)}$ μm	$l_{se(100)}$ μm
1	137	130	130	123	0	0	0	187	—
2	160	153	153	146	0	0	0	218	—
3	173	173	173	166	0	0	0	236	—
4	204	193	193	179	0	0	0	278	—
5	393	379	379	363	0	0	0	536	—
av.	213.4	205.6	205.6	195.4	0	0	0	291.0	—

TABLE 16. As table 12. Leaf tissue at 88% F.L.L. Analysis of 4 not ramified and 1 ramified vein ending in transections. The acropetal differentiation of tracheids has arrived in the distal extremity of all young veinlets with all stages of development (tr1, tr2 and tr3, cp. fig. 26). In the longest veinlet two young sieve elements (se1) are found along 59 μm in the proximal part, cp. fig. 26. $l_{v(100)} = 1.16 \times l_{v(88)}$ (formula 3) and $\bar{l}_{v(88)} = 240.0 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(88)}$ μm	l_{tr1} μm	l_{tr2} μm	l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	$l_{v(100)}$ μm	$l_{se(100)}$ μm
1	199	199	199	199	0	0	0	231	—
2	204	204	204	204	0	0	0	237	—
3	220	220	220	213	0	0	0	256	—
4	295	295	295	290	0	0	0	343	—
5	334	334	334	334	59	0	0	388	—
av.	250.4	250.4	250.4	248.0	11.8	0	0	291.0	—

(formula 1) and this value does not differ much from the calculated average of the 5 vein endings (250.4 μm).

In table 17 the results are shown of observations on 20 vein endings in leaf tissue at 96% F.L.L. The acropetal differentiation of tracheids has arrived in the distal extremity of the vein endings with all 3 stages of development. The average length of these veinlets ($\bar{l}_{v(96)} = 206.9 \mu\text{m}$) is considerably lower than the value that was to be expected (266.0 μm , formula 1). Probably this larger difference has been caused by a deficiency of suitable not ramified vein endings in the available leaf material.

In conclusion we may say that it appears from the examination of the transverse sections of the vein endings that the differentiation of xylem in all three stages of differentiation (tr1, tr2 and tr3) proceeds entirely continuously and acropetally. From about 40% F.L.L. this differentiation begins in the proximal part of the procambium strand with stage 1 (tr1) and this differentiation terminates lengthwise at about 90% F.L.L. when all 3 stages have arrived in the distal extremity of the procambium strand. This type of differentiation proceeds rapidly along the procambium strand, more rapid than the differentiation process of the procambium (fig. 26).

Aspects of division activity of cells in a procambium strand and stages of differentiation of sieve elements

In a very young procambium strand, especially at the distal extremity, all cells divide by means of new longitudinal partitions (fig. 20C, 21-1, resp. 38% and 46% F.L.L.). Soon the division activity shifts to the abaxial side of the young procambium strand (fig. 21-2). In the vein ending of fig. 21-3 a tracheid in the first stage of differentiation (tr1) is shown in the distal part on the adaxial side (fig. 3A), and dividing cells on the abaxial side. In the proximal part (fig. 21-3B) one tracheid is shown in the second stage of differentiation (tr2) and dividing of cells on the abaxial side of the tracheid. In the vein ending of fig. 21-4 differentiating tracheids are shown in the proximal part on the adaxial side, and dividing of cells on the abaxial side. Consequently in the adaxial part of a procambium strand only differentiation of tracheids can be found yet after a very short period of cell division took place; in the abaxial part of the procambium strand the cell division activity continues meanwhile.

In leaf tissue at 57% F.L.L. no cell division can be found any more in the adaxial part of the young vein ending, however, in the abaxial part cell division continues. In fig. 22A it is shown that the procambium strand widens on the abaxial side of the tracheid (tr3) about half the length of the vein ending. This cell division activity seems to continue without interruption till the moment of arrival of the phloem differentiation front, i.e. the last divisions in the abaxial part of the procambium are the preparatory divisions before the differentiation into sieve elements and associated cells. Generally only very few cell divisions are found in the mesophyll of this still young leaf tissue; however, cell divisions are found rather frequently in the abaxial part of the procambium (and future position of the phloem). In figs 22B, C sections are shown through the common

TABLE 17. As table 12. Leaf tissue at 96% F.L.L. Analysis of 10 not ramified and 10 ramified vein endings in transections. It appeared to be necessary to examine 10 ramified veinlets owing to deficiency of not ramified ones in the available leaf material. All stages of differentiation of tracheids (tr1, tr2 and tr3) are present in the distal extremity of the young veinlet. The first sieve elements differentiate acropetally and continuously. In the long vein endings all three stages of development (se1, se2 and se3) are present. The indication of the type of analysed vein ending is as follows. tr = with zone l_{tr} ; se = with zone l_{se} ; v = vein ending; v.tr and v.tr.se = not ramified vein endings (other, composed indications mean ramified types); tr/se = basal part of ramified veinlet, at the distal extremity of which two vein endings are attached; all along this basal part phloem is present (MAGENDANS, 1983; fig. 2, 4-6). Underlined vein endings (13, with zone l_{se}) have been used for calculations (fig. 26) and for determination of the value $(\frac{l_{v(100)} - l_{se(100)}}{l_{v(100)}})$ in this type of climate (table 20).

$l_{v(100)} = 1.05 \times l_{v(96)}$; $l_{se(100)} = 1.05 \times l_{se(96)}$ (formula 3) and $l_{v(96)} = 266.0 \mu\text{m}$ (formula 1).

Veinlet number	$l_{v(96)}$ μm	l_{tr1} , l_{tr2} , l_{tr3} μm	l_{se1} μm	l_{se2} μm	l_{se3} μm	(n) r = (not) ramified	Type of vein ending	$l_{v(100)}$ μm	$l_{se(100)}$ μm	$(\frac{l_{v(100)} - l_{se(100)}}{l_{v(100)}})$ μm
1	83	83	0	0	0	nr	v.tr	87	0	-
2	101	101	0	0	0	r	tr/se; v.tr.se, v.tr	106	0	-
3	106	106	0	0	0	r	tr/se; v.tr.se, v.tr	111	0	-
4	112	112	0	0	0	nr	v.tr	117	0	-
5	129	129	0	0	0	r	tr/se; v.tr, v.tr	135	0	-
6	158	158	24	0	0	nr	v.tr.se	166	25	141
7	181	181	21	21	0	nr	<u>v.tr.se</u>	190	22	168
8	182	182	0	0	0	nr	v.tr	191	0	-
9	184	184	15	0	0	nr	<u>v.tr.se</u>	193	16	177
10	189	189	59	59	0	r	tr/se; v.tr.se, v.tr	198	62	136
11	199	199	14	14	0	r	tr/se; v.tr.se, v.tr	209	15	194
12	204	204	77	77	7	nr	<u>v.tr.se</u>	214	81	133
13	212	212	0	0	0	r	tr/se; v.tr, v.tr	222	0	-
14	233	233	45	45	0	nr	<u>v.tr.se</u>	244	47	197
15	251	251	82	82	0	r	tr/se; v.tr.se, v.tr.se	263	86	177
16	269	269	45	45	0	r	tr/se; v.tr.se, v.tr.se	282	47	235
17	292	292	137	137	0	nr	<u>v.tr.se</u>	306	144	162
18	316	316	169	169	70	r	tr/se; v.tr.se, v.tr.se	331	177	154
19	362	362	224	150	61	nr	<u>v.tr.se</u>	380	235	145
20	374	374	182	182	70	r	tr/se; v.tr.se, v.tr.se	392	191	201
av. (20 veinlets)	206.9	206.9	54.7	49.1	10.4			216.9	57.4	
av. (13 veinlets, with zone l_{se})	247.1	247.1	84.2	75.5	16.0			259.1	88.3	170.8 \pm 30.1

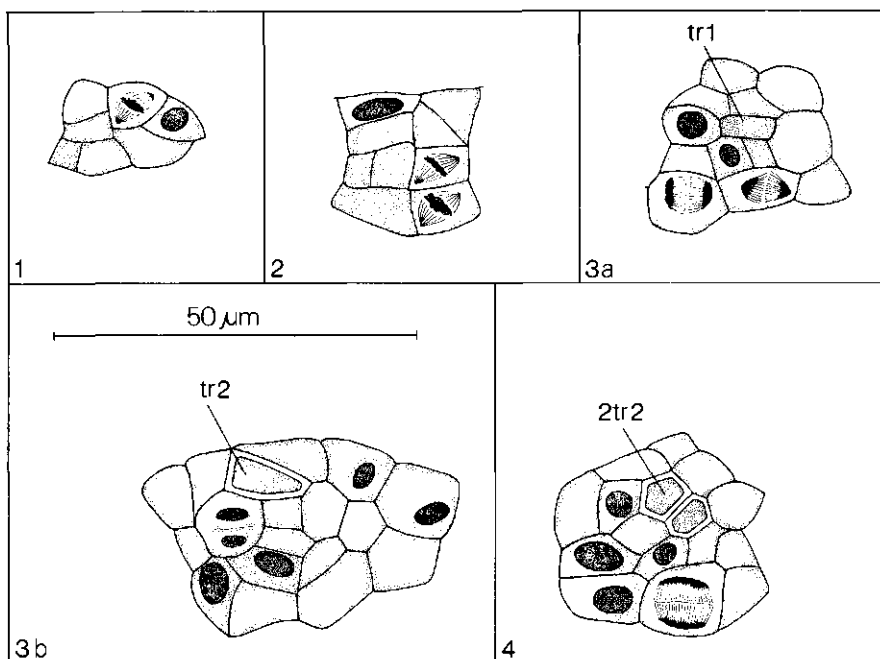


FIG. 21. Transections of 4 vein endings in young leaf tissue (46% F.L.L.). Bundle sheath not drawn. Adaxial side is at the top of the figures. tr1,2: see table 11.

1. Vein ending, $\pm 10 \mu\text{m}$ from distal extremity. Dividing of adaxial cells still happens in the distal segment in this young leaf tissue.

2. Vein ending, $\pm 35 \mu\text{m}$ from distal extremity. Cell division in the abaxial part of the procambium strand.

3a, b. Transections of the same vein ending ($l_{v(46)} = 160 \mu\text{m}$).

a. $\pm 50 \mu\text{m}$ from distal extremity; adaxial side with 1 tr1, cell division in abaxial part.

b. $\pm 125 \mu\text{m}$ from distal extremity; adaxial side with 1 tr2, cell division in abaxial part.

4. Vein ending, $\pm 70 \mu\text{m}$ from distal extremity ($l_{v(46)} = \pm 110 \mu\text{m}$). On the adaxial side 2 tr2; in the abaxial part cell division (in this part sieve elements will differentiate probably).

foot of resp. 3 and 2 vein endings. In these parts of the venation differentiation of sieve elements did not occur yet. However, cell division does take place regularly in the abaxial parts of the procambium which will certainly differentiate into phloem comprising sieve elements.

In fig. 23 sections are shown of a smaller (A) and of larger (B, C) connecting veins in leaf tissue at 77% F.L.L. In fig. 23A cell division and differentiation into a sieve element are visible in the abaxial part of the procambium strand. The sieve element is in the first stage of differentiation and can be identified by means of a not yet clearly perceptible thickening of the wall, that begins in the corners of the element. Besides the identification is aided by the general shape and orientation of the element with regard to the adjacent cells. Moreover this very young sieve element (se1, table 18) is at its proximal end in contact

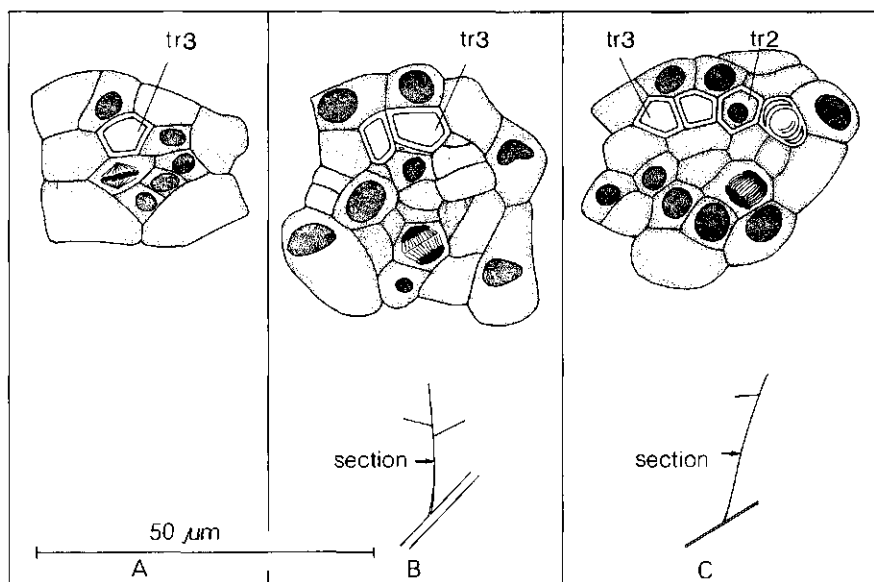


FIG. 22. Transections of a vein ending (A) and small veins of lower order (B, C, see schematic drawings underneath) in young leaf tissue (57% F.L.L.). Bundle sheath not drawn. Adaxial side is at the top of the figures. tr2, 3: see table 11.

A. Vein ending ($l_{v(57)} = 133 \mu\text{m}$), $\pm 70 \mu\text{m}$ from distal extremity and probably distad of zone l_{se} . On the abaxial side the procambium strand enlarges continuously by cell division.

B. Small vein, transection as indicated below. Cell division in abaxial part of procambium strand, i.e. in the part where phloem will differentiate.

C. Small vein, transection as indicated below. Cell division in abaxial part in which phloem will differentiate. Many cells have dense cytoplasm in the abaxial part.

with the distal extremity of a sieve element in the second stage of differentiation (se2). The sieve element (se1) still contains a nucleus, as the contiguous older sieve element (se2).

In fig. 23B a larger connecting vein is shown with cell division and cells with dense cytoplasm in the abaxial part. In adjacent sections the neighbouring cells in the same abaxial part divided. This section is $\pm 15 \mu\text{m}$ distad from the first differentiating sieve element (se1), the position of which is in a direct line with the represented dividing cell, and the distal extremity of this se1 is in contact with the dividing cell.

In fig. 23C a still larger connecting vein is shown in which cell division takes place and in which cells with dense cytoplasm and also a completely differentiated sieve element (se3) are present in the abaxial part. In this larger vein the phloem differentiation front has passed by already and still dividing cells are found in the abaxial part yet. No cell division takes place in the xylem any more.

Just as the differentiation of tracheids the differentiation process into a sieve element can be divided in a number of anatomically defined stages which are

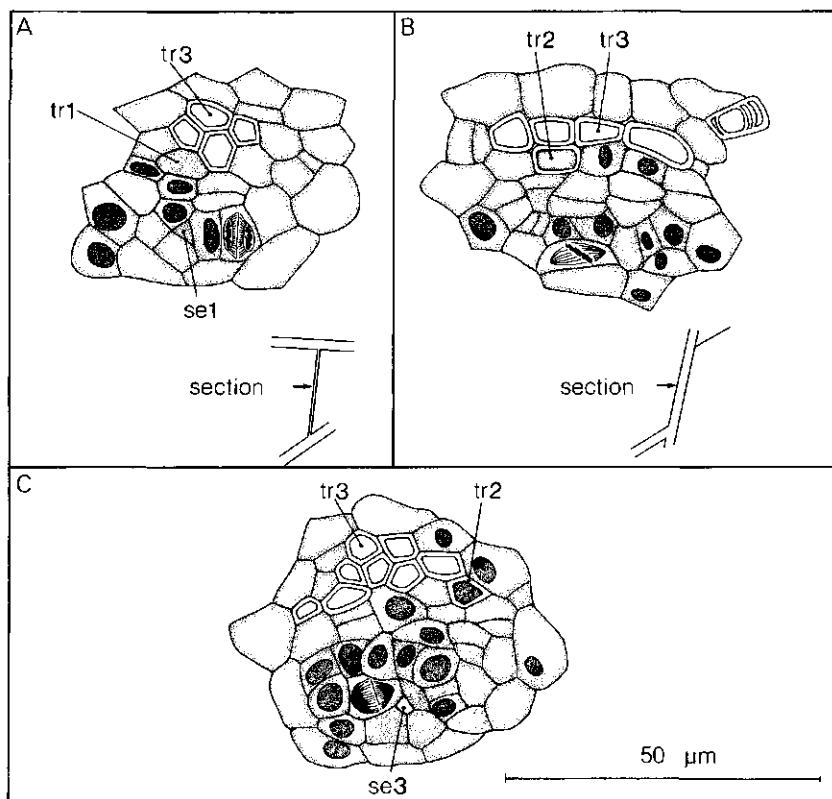


FIG. 23. Transections of slightly larger connecting veins in young leaf tissue (77% F.L.L.). Bundle sheath not drawn. Adaxial side is at the top of the figures. tr1, 2, 3 and se1, 3: see resp. table 11 and 18.

A. Small connecting vein, transection as indicated below. Cell division in abaxial part and a sieve element in the first stage of differentiation (se1).

B. Larger connecting vein, transection as indicated below. Cell division in the abaxial part and cells with dense cytoplasm. In adjacent sections the neighbouring cells in the same abaxial part divided. This section is $\pm 15 \mu\text{m}$ distad from the first identifiable se1. This se1 is in terminal contact with the drawn dividing cell.

C. Larger connecting vein. In the abaxial part cell division takes place and cells with dense cytoplasm are present and also a completely differentiated sieve element (se3).

distinct from each other in a considerable measure. The differentiation of the procambium into phloem in a young *Hedera* leaf can be followed more closely when using the classification in table 18. The thickening of the wall is a first distinguishing mark of differentiation into a sieve element (ESAU, 1965; SINGH, 1980; ELEFTHERIOU and TSEKOS, 1982; THORSCH and ESAU, 1981). This wall thickening of the sieve elements in *Hedera* leaves is well perceptible by means of the light microscope. Moreover as good marks of recognition in a very young stage of development may also be considered the orientation of the small sieve

TABLE 18. Three stages (se1, se2 and se3) of the differentiation process of a sieve element in the procambium of the vein ending and other veins, as perceptible with the light microscope (1500 \times). The three stages are characterized by differences in wall thickness and the condition of the protoplasm.

Stages of differentiation of sieve elements	Wall thickness, viewed in transection	Contents of sieve element	Figure
se1	thickening of wall becomes visible, first in the corners	much cytoplasm, with nucleus	23-1
se2	thickened wall clearly visible	cytoplasm entirely or for the most part present, protoplast often somewhat constricted to a central strand, sometimes with nucleus	
se3	thickened wall clearly visible	almost no myctoplasm visible along considerable part of element, sometimes myctoplasm only as constricted strand at the end of the element	23-3 plate 1

element in respect of the larger surrounding cells, the mostly straight walls and the proximal extremity of the se1 primordium being in contact with the distal end of an older sieve element in adjacent sections. The second stage of differentiation can be correctly identified by means of the straight, somewhat thickened wall and moreover by means of the different appearance of the protoplast. The cytoplasm of the surrounding cells becomes denser of appearance; the quantity of the myctoplasm of the sieve element diminishes and appears as a constricted strand sometimes. The third stage of differentiation is distinct because of the very small quantity of myctoplasm, especially in relation to the surrounding cells with dense cytoplasm.

Analysis of differentiation of sieve elements by means of transections of the vein endings

No differentiation of sieve elements takes place in vein endings in young leaf tissue till the age of about 80% F.L.L. (tables 12, 13, 14 and 15). Only in the longest veinlet of table 16 ($l_{v(88)} = 334 \mu\text{m}$) two sieve elements differentiated in the most proximal part the length of 59 μm . These sieve elements are in the first stage of differentiation (table 18). The value of $\bar{l}_{se(88)} = 12 \mu\text{m}$ seems to be a tolerable estimate, because the value of $\bar{l}_{v(88)} = 250.4 \mu\text{m}$ of these 5 veinlets nearly corresponds with the value that is to be expected (240 μm). In fig. 24 six transverse sections (A-F) are shown of the 4th vein ending in table 16 ($l_{v(88)} = 295 \mu\text{m}$ and $l_{v(100)} = 343 \mu\text{m}$). Diagrams of this veinlet are given below at 88% F.L.L. and 100% F.L.L. The positions of the 6 transections are indicated in both diagrams, in the lower diagram after conversion to 100% F.L.L. Besides

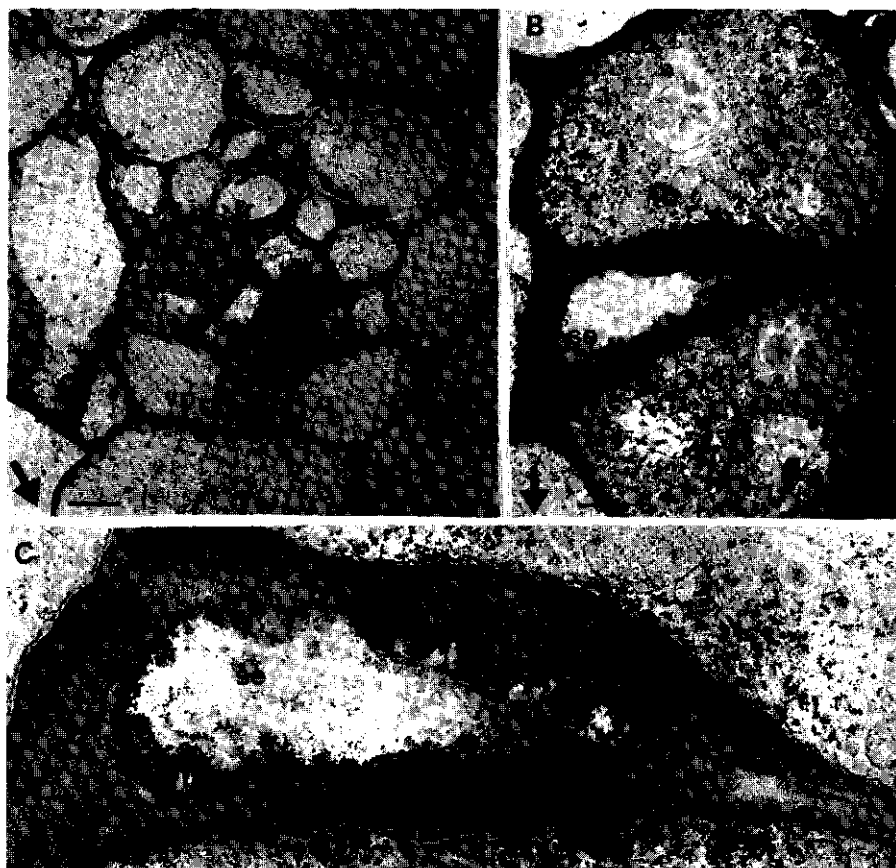


PLATE I A-C. Electro micrographs of one straight vein ending in a white *Hedera* leaf, 100% F.L.L. Transverse sections at different positions not far from the distal extremity of zone I_{se} . The arrows indicate abaxial side of the leaf. A. One sieve element (se3), three intermediary cells, six vascular parenchyma cells and seven tracheids in one transection of the vein ending. Identification by means of many other transections also (scale bar $5.15\ \mu\text{m}$). B. One sieve element connected by so called branched plasmodesmata with intermediary cell (scale bar $1.09\ \mu\text{m}$). C. One sieve element connected by branched plasmodesmata with both intermediary cells (scale bar $0.39\ \mu\text{m}$).
ic = intermediary cell; se = sieve element; tr = tracheid; vp = vascular parenchyma cell.

the calculated value of $I_{se(100)}$ and its standard deviation are given in the lower diagram ($(\overline{I_v - I_{se}})_{(100)} = 170.8 \pm 30.1\ \mu\text{m}$ in this climate, table 20). In transections A and B, 7 tracheids are shown in the aggregate and moreover a number of procambium cells that did not differentiate and does not contain much cytoplasm. Part of these procambium cells will differentiate into tracheids yet. In transection C, 10 tracheids are shown in the aggregate with a number of procambium cells that did not differentiate and does not contain much cytoplasm. This transection corresponds approximately with the future position of the tracheid

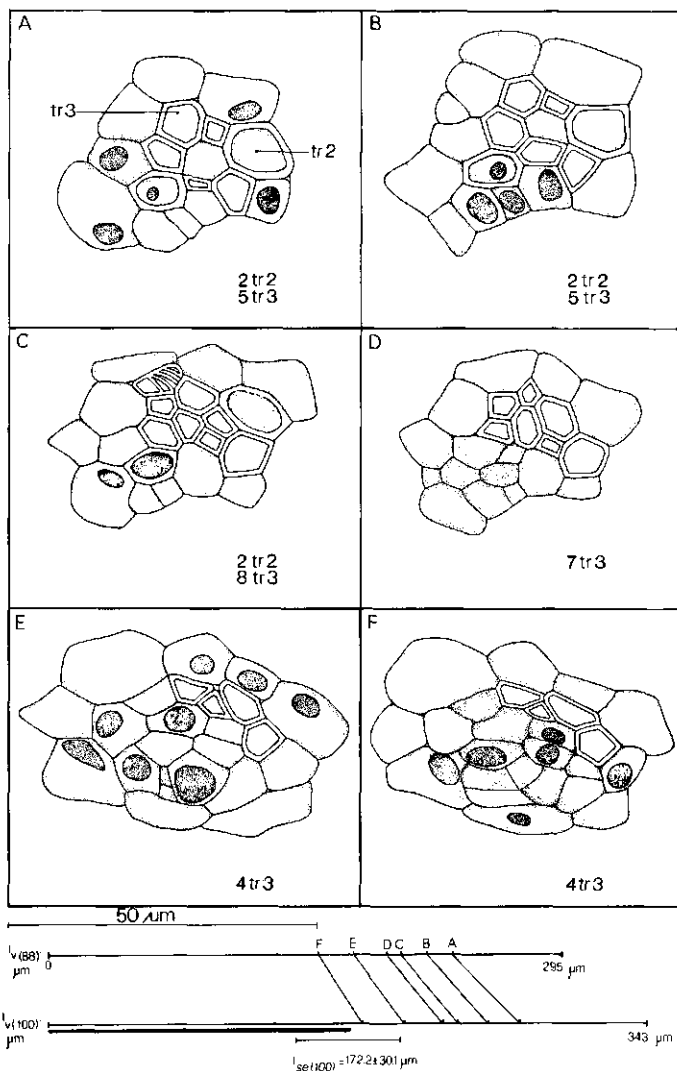


FIG. 24. Transections (A-F) of part of the 4th vein ending in table 16 (88% F.L.L.). Veinlet not ramified, bundle sheath not drawn. Adaxial side is above in the figures. Diagrams of this veinlet are given below at 88% F.L.L. and 100% F.L.L. The positions of the 6 transections (A-F) are indicated also, in the lower diagram after conversion to 100% F.L.L. Besides the calculated value of $l_{se(100)}$ and its standard deviation is given for this climate. See text. — = $l_{se(100)}$.

maximum (MAGENDANS, 1983). In transection D the number of tracheids (7) diminishes again, while in the abaxial part smaller cells are found which especially contain more cytoplasm (future distal extremity of the zone l_{vp} or l_{ic} probably). In transection E the number of tracheids (4) went on decreasing, while in the

abaxial part small cells originated which are acute-angled and contain dense cytoplasm. These latter small cells are surrounded by larger ones which also contain much cytoplasm relatively. One or perhaps both of these small angular cells will differentiate into a sieve element. Therefore the position of transection E will be at the place already where the zone l_{se} differentiates. In the diagram of fig. 24 (below) is shown that transection E has been made at a place that is about as far as the distal extremity of zone l_{se} can reach. The cells on both sides of the small angular cells will differentiate into intermediary cells probably. In section F the number of tracheids is only 4 also, whereas many cells with much cytoplasm originated in the abaxial part after cell division (thin wall between pairs of cells). In this abaxial part with dividing cells the phloem will differentiate. The distal limit of the zone l_{se} can be located approximately in vein endings in leaf tissue at 88% F.L.L. by paying attention to the distal limit of the abaxial zone containing cells with much cytoplasm and cell division activity, to the occurrence of small angular cells among larger ones with much cytoplasm, and considering the length of the zone $l_{se(88)}$ that is to be expected, resulting from the value of $(l_v - l_{se})_{(100)}$ belonging to the prevailing type of climate. In transections proximad from F qualitative changes of significance do not occur any more, corresponding with the data in table 16 (veinlet 4).

In table 17, the results are given of observations on 20 vein endings in nearly mature leaf tissue (96% F.L.L.). In the shorter veinlets (nos 1-5, 8 and also 13) no identifiable sieve elements do occur yet in this leaf tissue. In the longer vein endings the first sieve elements that can be identified while moving our observations from distal to proximal positions, are sieve elements in the first stage of differentiation (se1), more proximad somewhat older sieve elements (se2) can be perceived and finally close to the proximal extremity of the longest vein endings sieve elements in the third stage of differentiation (se3) are found. Therefore the differentiation of the phloem does occur acropetally and continuously in these vein endings and long after the first tracheary elements differentiated acropetally and continuously along the procambium strand (fig. 26). The distal extremity of the zone l_{se} can be located well, paying attention to the anatomical and cytological features in the abaxial part of the vein endings as mentioned above. These features have become much more distinct yet in this older leaf tissue.

In fig. 25 the average values of $l_{v(96)}$, $l_{se1(96)}$, $l_{se2(96)}$ and $l_{se3(96)}$ are given belonging to the 20 veinlets in table 17. It appears from the figure that the differentiation of the phloem takes place in distal direction. This differentiation activity did not arrive yet in the shorter veinlets, or has just arrived at the proximal extremity of these veinlets. In the longer veinlets this differentiation proceeded in the direction of the distal extremity of zone l_{se} already. At the same time an obvious tendency towards an arrangement in particular zones is shown. It appears that a proxima with young sieve elements (se1) can be found most distad, behind (= proximad) a zone with older sieve elements (se2) does appear and the zone with mature sieve elements (se3) is the last to enter into the vein ending. In some vein endings the difference between se1 and se2 is not distinct, or the zone se1

is as long as the zone se2. It can be seen also that the distal zone $(l_v - l_{se1})_{(96)}$ has a rather constant value on an average; this value does not change any more, except for a small fraction in connection with the growth (extension) of the leaf tissue.

In the next columns of table 17 the type of each vein ending is given, ramified or not ramified (fig. 12B). The type of branching can be indicated in more detail: the classification of table 17 corresponds with the classification mentioned earlier (MAGENDANS, 1983, fig. 2). The influence of whether the veinlets are ramified or not, on the differentiation of the phloem in these vein endings, has been tested. The values (7) of $(l_v - l_{se1})_{(100)}$ in not ramified vein endings are not statistically different from the values (6) of $(l_v - l_{se1})_{(100)}$ in ramified veinlets (Wilcoxon's test).

It may be concluded that the distal limit of the zone l_{se} in leaf tissue from the age of 88% F.L.L. may be identified using cytological features and considering the length of the zone l_{se} that is to be expected resulting from the value of $(l_v - l_{se})_{(100)}$ belonging to the prevailing type of climate. The differentiation of the phloem does occur acropetally and continuously in the vein endings and long after the first tracheary elements differentiated along the procambium strand (fig. 26). As with the differentiation of xylem an obvious tendency towards an arrangement in three zones (se1, se2 and se3, fig. 25) can be distinguished with the differentiating of the phloem into young veinlets.

Summary and comparison of observations in paradermal and transverse sections of vein endings

In table 19 the average values of observations in paradermal sections (tables 2-7) are compared with those of the transverse sections (tables 12-17). In the first column the various percentages of leaf length at which the observations have been taken, are shown and in the second column the average lengths of the vein endings that are to be expected at the various % F.L.L. (formula 1) are given. The values of $\bar{l}_{v(\% \text{ F.L.L.})}$ as found in paradermal sections (column 4), vary little from the calculated values (formula 1) and this must be expected because formula 1 is based on the 121 measurements of l_v in paradermal sections. The values of $\bar{l}_{tr(\% \text{ F.L.L.})}$ in tables 2-7 have been noted down as values of $\bar{l}_{tr1(\% \text{ F.L.L.})}$ (column 5). This is allowed approximately; in paradermal section the difference between the stages of differentiation tr1 and tr2 is not exactly the same, however, as in transverse section. Probably the value of $\bar{l}_{tr(\% \text{ F.L.L.})}$ in paradermal sections corresponds for the most part with $\bar{l}_{tr1(\% \text{ F.L.L.})}$, but for a small part with $\bar{l}_{tr2(\% \text{ F.L.L.})}$ and consequently the value of \bar{l}_{tr} will be too low. This is visible in fig. 26 because the position of the curve through the 6 points (\bar{l}_{tr1} , paradermal) is found at the right side of the curve through the 3 points with symbols Δ (\bar{l}_{tr1} , transverse) that has not been drawn. The values of $\bar{l}_{v(\% \text{ F.L.L.})}$ as found in transverse sections (column 7), differ in greater degree from those calculated by means of formula 1. Especially the value 85.4 (57% F.L.L.) is much too low; it is shown in table 14 that all the not ramified vein endings (5), that could be examined in transverse sections, were rather short (compare $l_{v(46)}$ and $l_{v(77)}$ in tables 13 and 15). This means that the values of $\bar{l}_{tr1(57)} (= 52.2 \mu\text{m})$ and $\bar{l}_{tr3(57)} (= 22.8 \mu\text{m})$ will be too

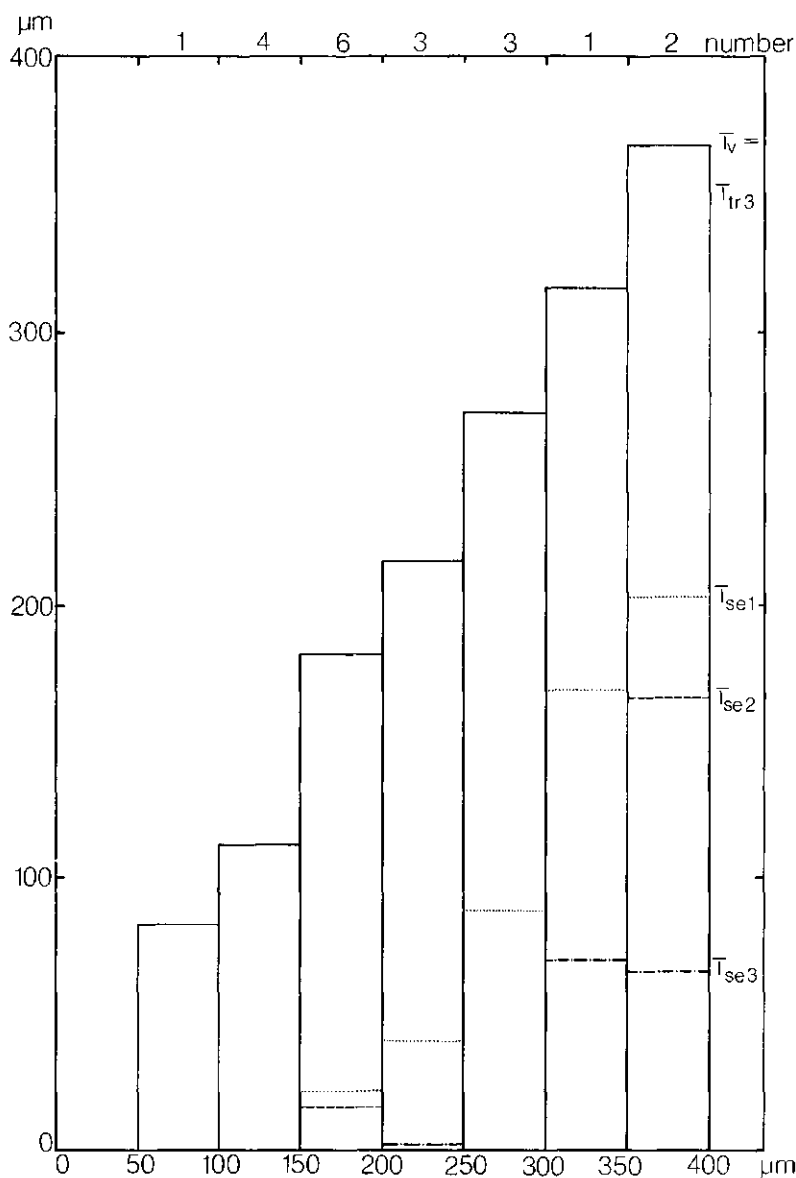


FIG. 25. State of phloem differentiation at 96% F.L.L., diagram with the data from table 17. The vein endings have been divided into groups of 50 μm difference in length each (abscissa) and in every group of veinlets the average values of $l_v(96)$, $l_{se1}(96)$, $l_{se2}(96)$ and $l_{se3}(96)$ have been determined (ordinate).

— = $\bar{l}_v(96)$ (= $\bar{l}_{tr3}(96)$), = $\bar{l}_{se1}(96)$ (and $\bar{l}_{se2}(96)$ if equal to $\bar{l}_{se1}(96)$), ---- = $\bar{l}_{se2}(96)$,
 -.-.- = $\bar{l}_{se3}(96)$.

TABLE 19. Comparison of results of observation in paradermal and transverse sections at various % F.L.L. At each % F.L.L. $\bar{l}_{w\%F.L.L.}$ has been calculated (formula 1, column 2). The values $\bar{l}_{w\%F.L.L.}$ and $\bar{l}_{tr(\%F.L.L.)}$ found in paradermal sections (tables 2-7) are given in columns 4 and 5 (\bar{l}_{tr} has been considered equal to \bar{l}_{tr}). The values $\bar{l}_{w\%F.L.L.}$, $\bar{l}_{tr(\%F.L.L.)}$ and $\bar{l}_{tr3(\%F.L.L.)}$ found in transverse sections (tables 12-17), are noted resp. in columns 7, 8 and 10. The values $\bar{l}_{tr(\%F.L.L.)}$ and $\bar{l}_{tr3(\%F.L.L.)}$ (columns 9, 11) are found by correcting the values in resp. columns 8 and 10 by means of \bar{l}_v (formula 1) and the rather constant values of $(\bar{l}_v - \bar{l}_{tr})/\%F.L.L.$ and $(\bar{l}_v - \bar{l}_{tr3})/\%F.L.L.$ (see text). The corrected values (columns 9, 11) are indicated in fig. 26 by resp. the symbols Δ and \circ .

% F.L.L.	$\bar{l}_{w\%F.L.L.}$ μm (formula 1)	Paradermal sections			Transverse sections					
		Number of veinlets	$\bar{l}_{w\%F.L.L.}$ μm	$\bar{l}_{tr(\%F.L.L.)}$ μm	Number of veinlets	$\bar{l}_{w\%F.L.L.}$ μm	$\bar{l}_{tr(\%F.L.L.)}$ μm	$\bar{l}_{tr(\%F.L.L.)}$ $\mu\text{m, corr.}$	$\bar{l}_{tr3(\%F.L.L.)}$ μm	$\bar{l}_{tr3(\%F.L.L.)}$ $\mu\text{m, corr.}$
1	2	3	4	5	6	7	8	9	10	11
38	78	20	88.8	1.4	5	100.8	0	0	2.8	0
46	104	—	—	—	5	126.0	52.6	30	6.2	0
48	110	20	103.1	11.6	—	—	—	—	—	—
57	139	21	141.1	116.8	5	85.4	52.2	115	22.8	53
66	169	20	154.4	127.9	—	—	—	—	—	—
77	204	—	—	—	5	213.4	205.6	198	195.4	186
82	221	20	225.5	222.7	—	—	—	—	—	—
88	240	—	—	—	5	250.4	250.4	240	248.0	238
96	266	—	—	—	20	206.9	206.9	266	206.9	266
97	269	20	272.9	272.9	—	—	—	—	—	—

low also. This divergence, consequence of the small number of 5 examined vein endings, has been corrected as follows. The divergence at 57% F.L.L. above-mentioned will be discussed as an example. It appears from fig. 18B that the value $(\bar{l}_v - \bar{l}_{tr})$ is fairly constant in vein endings of different length. From the data, on which fig. 18B is based, it can be calculated that $(\bar{l}_v - \bar{l}_{tr})_{(57)} = 23.3 \mu\text{m}$ (21 veinlets). Looking at the two long vein endings 4 and 5 in table 14 it appears that $(\bar{l}_v - \bar{l}_{tr})_{(57)} = 125.5 - 101 \mu\text{m} = 24.5 \mu\text{m}$. This latter value does not differ much from the value first mentioned, found in paradermal sections ($23.3 \mu\text{m}$). Hence a good estimate of the value $\bar{l}_{tr(57)}$ is: $\bar{l}_{v(57)}$ (formula 1) - $(\bar{l}_v - \bar{l}_{tr})_{(57)} = 139 - 24 \mu\text{m} = 115 \mu\text{m}$, noted down in column 9. The values of other % F.L.L. have been corrected in the same way.

The low value of $\bar{l}_{v(96)} = 206.9 \mu\text{m}$ cannot be explained by pointing out to the small number of examined vein endings. In this case the 20 vein endings (table 17) could not be chosen at random as in the mature leaf (MAGENDANS, 1983). The cause of the low value of $\bar{l}_{v(96)}$ will probably be the limited quantity of fixed leaf tissue at 96% F.L.L. in which fewer long (and straight) vein endings

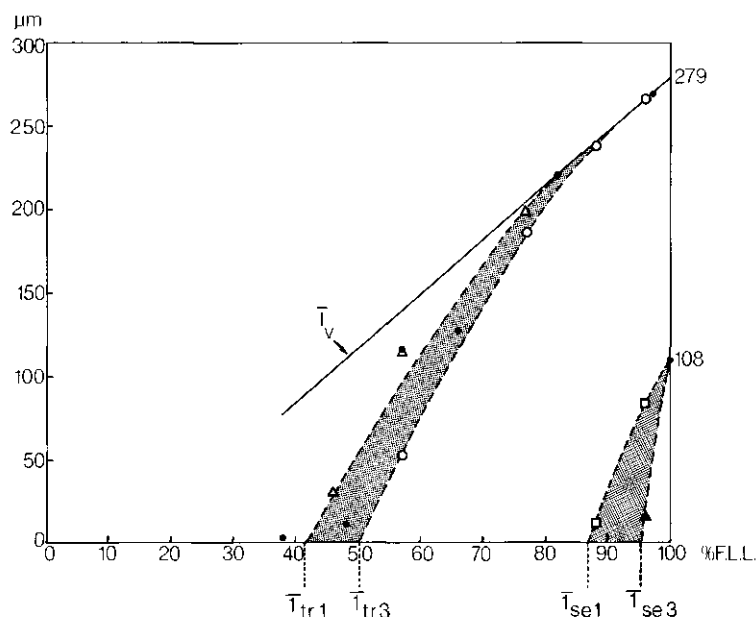


FIG. 26. Comparison of the differentiation process of tracheids and sieve elements along the procambium strand of the vein ending. The straight line (\bar{l}_v) is the same as in fig. 11 and 16. Between the lines \bar{l}_{tr1} and \bar{l}_{tr3} one finds the interval of leaf age within which the first differentiation of tracheids along the procambium strand comes about. Between the lines \bar{l}_{se1} and \bar{l}_{se3} one finds the interval of leaf age within which the first differentiation of sieve elements along the procambium strand takes place. Both processes of differentiation are transmitted continuously and acropetally along the procambium strands with a time lapse of about 45% F.L.L. See the text.

... = \bar{l}_{tr1} , from tables 2-7; $\triangle\triangle\triangle$ = \bar{l}_{tr1} , from tables 13-17 (corrected); $\circ\circ\circ$ = \bar{l}_{tr3} , from tables 12-17 (corrected). $\square\square$ = \bar{l}_{se1} , from tables 16, 17; $\blacktriangle\blacktriangle\blacktriangle$ = \bar{l}_{se3} , from table 17.

can be found within the same area. In younger leaves the number of suitable vein endings was much greater within 25 square millimetres.

The values of \bar{l}_{tr3} (column 10) have been determined only by means of the transverse sections (tables 12–17). The corrections of these values have been carried out in the same way as with \bar{l}_{tr1} (as above, column 11). Only in the longest vein ending at 46% F.L.L. differentiation of tracheids till the 3rd stage has taken place in the proximal part (table 13). After correction, however, i.e. bringing into accordance with the value of $\bar{l}_{v(46)}$, $\bar{l}_{tr3(46)}$ is only 0 μm yet (column 11). In fig. 27 diagrams of differentiation of tracheids (tr3) at 57% and 77% F.L.L. are shown. The proceeding of the differentiation till the tr3 stage along the vein ending can be clearly seen by means of the diagrams. This first differentiation of tracheids always takes place continuously and acropetally.

In fig. 26 the curve \bar{l}_{tr3} forms the connection between the 4 observed points based on the values of $\bar{l}_{tr3(46\% \text{ F.L.L.})}$ after correction (table 19, column 11). The shaded zone between the curves \bar{l}_{tr1} and \bar{l}_{tr3} now indicates the interval of leaf age within which the first differentiation of tracheids takes place acropetally along the young vein endings. It is clearly visible that this differentiation proceeds much more rapid along the procambium strand than the increase in length of the procambium strand, resulting for the most part from the extension growth of the leaf tissue in this interval of leaf age. Besides the progress of the differentiation till the tr3 stage seems to overtake the progress of the first stage of differen-

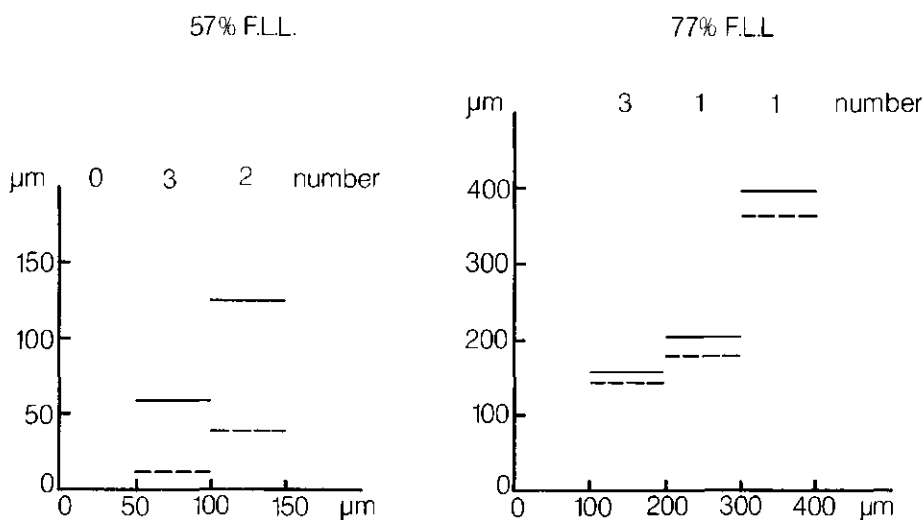


FIG. 27. Diagrams of the proceeding of differentiation of tracheids (tr3) (data from tables 14 and 15). The 5 vein endings in the diagram (57% F.L.L.) have been divided into groups of 50 μm difference in length each and the 5 vein endings in the other diagram (77% F.L.L.) have been divided into groups of 100 μm difference in length (abscissa). In every group of veinlets the average values of \bar{l}_v and \bar{l}_{tr3} have been determined (ordinate).

— = \bar{l}_v , ---- = \bar{l}_{tr3} .

tiation (tr1). Whereas this first stage of acropetal differentiation of tracheids along the procambium strands of the vein endings is limited to the shaded zone between the curves \bar{l}_{tr1} and \bar{l}_{tr3} , further differentiation of tracheids in breadth of the procambium strand proceeds till beyond the leaf age of 96% F.L.L. In vein endings in leaf tissue at 96% F.L.L. many tracheids in the tr1 and tr2 stage of differentiation will be found as well yet.

In table 16 the value of $\bar{l}_{se1(88)}$ ($= 11.8 \mu\text{m}$) is shown and in table 17 the values of $\bar{l}_{se1(96)}$ ($= 84.2 \mu\text{m}$) and $\bar{l}_{se3(96)}$ ($= 16.0 \mu\text{m}$). The latter values derived from table 17 concern the averages of the 13 vein endings with phloem (moreover this group of vein endings has an average length ($\bar{l}_{v(100)} = 259.1 \mu\text{m}$) that does not differ much from the value of $\bar{l}_{v(100)}$, resulting from formula 1: $278.9 \mu\text{m}$). The value of $\bar{l}_{se(100)}$ is: $278.9 - 170.8 = 108.1 \mu\text{m}$ (formula 1 and table 17). The curve \bar{l}_{se1} in fig. 26 originated from the connection of the two calculated points above-mentioned of \bar{l}_{se1} at 88% and 96% F.L.L. and the calculated point of the distal extremity of the zone l_{se} at 100% F.L.L. ($108.1 \mu\text{m}$). The curve \bar{l}_{se3} originates from the connection of the calculated point of \bar{l}_{se3} at 96% F.L.L. and the calculated point of the distal extremity of the zone l_{se} at 100% F.L.L. The shaded zone between the curves \bar{l}_{se1} and \bar{l}_{se3} now indicates the interval of leaf age within which the first differentiation of sieve elements takes place continuously and acropetally along the vein endings. It is clearly visible that this differentiation proceeds much more rapid also along the procambium strand than the increase in length of the procambium strand. The progress of differentiation till the se3 stage seems to overtake the progress of the first stage of differentiation (se1). Whereas this first stage of acropetal differentiation of sieve elements along the young vein endings is limited to the shaded zone between the curves \bar{l}_{se1} and \bar{l}_{se3} , further differentiation of sieve elements in breadth of the procambium strand proceeds till beyond the leaf age of 96% F.L.L..

In vein endings in leaf tissue at 96% F.L.L. sieve elements in the se1 and se2 stage of differentiation can be found as well yet. The rapidity at which the differentiation process moves acropetally along the vein ending is about equal to the rapidity at which the differentiation of the tracheids proceeds along the vein endings. The difference in time between the moments at which the two processes of differentiation enter the proximal end of the vein endings, is large, however, in proportion to the total duration of leaf growth. The difference in time amounts to about 45% F.L.L., i.e. about 10 days, viz. from about the 15th day till the 25th day of the average growth curve. In fig. 28 the moment is shown at which the tr3 stage of differentiation reaches the distal extremity of the vein ending and also the moment at which the differentiation of se3 reaches the distal extremity of the zone l_{se} .

Finally we may come to the conclusion that the comparison of observations in paradermal and transverse sections of vein endings is summarized best in fig. 26. In this figure the growth of the procambium strand of the young vein ending, the first differentiation of tracheids and about 10 days later the first differentiation of sieve elements along the procambium strand are visualized. The last two processes of differentiation are transmitted continuously and acro-

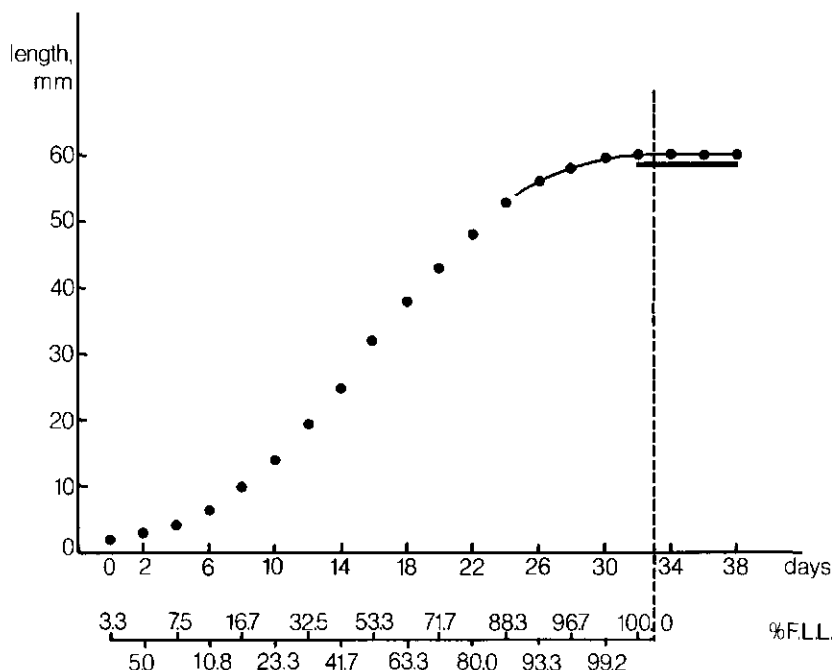


FIG. 28. Growth curve of a leaf of average leaf length (60 mm). The curve shows a normal course (cp. fig. 5). The leaf lengths reached at the noted points of time (abscissa) have been turned into % F.L.L. (noted below abscissa). On the 32nd day maximal leaf length was reached and on the 33rd day $\tan \alpha = 0.1$. From about 90% F.L.L. the differentiation of tr3 reaches the distal extremity of the vein endings (——) and from about 100% F.L.L. the differentiation of se3 reaches the distal extremity of the zone l_{se} (——, below curve).

petally along the procambium strands with a time lapse of about 45% F.L.L.

Type of climate and the length of the distal extremities of the veinlets with tracheary elements only

The prevailing type of climate in the growth cabinet was about intermediate with regard to the very dry and the very wet climate, adjusted during the previous series of observations (MAGENDANS, 1983). In the diagram of fig. 29 some data of table 17 (moderate climate) are shown, calculated for 100% F.L.L. The value of $(\overline{l_v} - \overline{l_{se}})_{(100)}$ is $170.8 \pm 30.1 \mu\text{m}$ in this moderate climate (table 17, 13 vein endings); in very dry climate this value is $158.0 \pm 47.9 \mu\text{m}$ and in very wet climate $115.1 \pm 46.1 \mu\text{m}$ (MAGENDANS, 1983, table 9: resp. 16 and 13 vein endings). When comparing these results statistically the contrast appears as shown in table 20 (t-test). The comparison of climate in table 20 shows that the adjusted intermediate type of climate as appears from this analysis of ontogeny of the vein endings, has been experienced by the *Hedera* plant as a very dry climate. The value of $(\overline{l_v} - \overline{l_{se}})_{(100)}$ does not differ significantly from the one that originates in

TABLE 20. Statistical comparison between the values $(\bar{l}_v - \bar{l}_{se})_{(100)}$ in μm of three groups of vein endings: dry and wet climate (data of MAGENDANS, 1983) and in moderate climate (data of table 17).

	158.0 dry	170.8	moderate	115.1	wet
158.0 dry n = 16	0	$P > 0.10$	-12.8 (SE = 15.9) $t = -0.8$ not sign.	$P < 0.01$ (one sided)	+42.9 (SE = 15.9) $t = 2.7$ sign.
170.8 moderate n = 13	+12.8		0		+55.7 (SE = 16.7) $t = 3.3$ sign.
115.1 wet n = 13	-42.9		-55.7		0

a very dry climate. However, the value of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ in wet climate does show a significant difference.

In fig. 29 the diagram is shown of the values of $\bar{l}_{v(100)}$ and $\bar{l}_{se(100)}$ of the 13 vein endings at 96% F.L.L. (table 17), divided into three groups of 100 μm difference in length each (abscissa). This diagram has been represented exactly in the same way as fig. 16, MAGENDANS, 1983; the possible comparison through this also shows that the values of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ are fairly constant in this moderate climate and comparable in length with those in dry climate. The values of $(\bar{l}_v - \bar{l}_{se})_{(100)}$

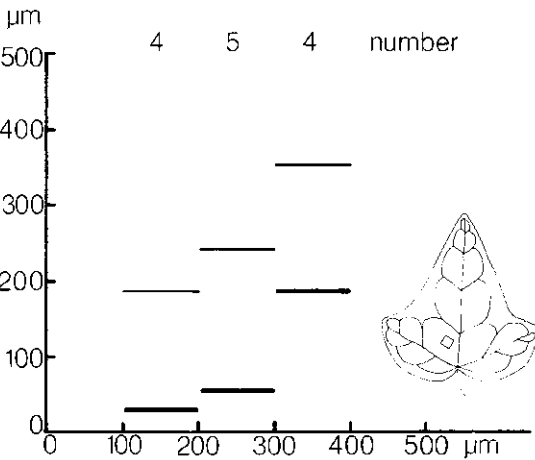


FIG. 29. Diagram with data from table 17. The vein endings have been divided into groups of 100 μm difference in length each (abscissa) and in every group of veinlets the average values of $\bar{l}_{v(100)}$ and $\bar{l}_{se(100)}$ have been determined (ordinate).

— = $\bar{l}_{v(100)}$, — = $\bar{l}_{se(100)}$.

The drawn leaf, with a schematic major venation characteristic for *Hedera* leaves, has been used for this experiment; the small square indicates the piece of tissue that has been used in analysing. This figure is entirely comparable with fig. 16 in MAGENDANS, 1983, concerned in two other climatic circumstances.

in the groups of short veinlets till 200 μm , are often lower in each climate (cp. also fig. 6, MAGENDANS, 1983).

Determination of the interval of leaf age in which the tracheid maximum arises

The tracheid maximum in vein endings of *Hedera* originates in 43 of 50 vein endings near the distal extremities of zones l_{se} or l_{ic} ; in the remaining 7 veinlets near the end of zone l_{vp} (MAGENDANS, 1983, p. 22). In order to determine the way of originating of this tracheid maximum and to assess the leaf age at which this maximum originates, calculations have been made by means of the data of three groups of vein endings in leaf tissue at 77%, 88% and 96% F.L.L. (table 21). In each of these three groups only long vein endings have been used, in which a zone l_{se} is always present ($l_{v(100)} \geq 200 \mu\text{m}$, $l_{se(100)} \geq 42 \mu\text{m}$, MAGENDANS, 1983 and table 17, i.e. concerning 21 vein endings in the aggregate). The values of \bar{l}_{se} at these three leaf ages have been calculated by making use of the fairly constant value of $(\bar{l}_v - \bar{l}_{se})$ (MAGENDANS, 1983 and fig. 29). This value amounts to $170.8 \pm 30.1 \mu\text{m}$ at 100% F.L.L. (table 17). Converting by means of formula 2 gives the value of $(\bar{l}_v - \bar{l}_{se})_{(\% \text{ F.L.L.})}$. Then the value of l_{se} that is to be expected, is: $l_{v(\% \text{ F.L.L.})} - (\bar{l}_v - \bar{l}_{se})_{(\% \text{ F.L.L.})}$.

TABLE 21. Calculation of the values to be expected of l_{se} at 77%, 88% and 96% F.L.L. (see tables 15, 16 and 17). The calculation is based on the fairly constant value of $(l_v - l_{se})$ in vein endings to which applies $l_{v(100)} \geq 200 \mu\text{m}$ (cp. fig. 29). The value $(\bar{l}_v - \bar{l}_{se})_{(\% \text{ F.L.L.})}$ can be calculated with formula 2, making use of $(\bar{l}_v - \bar{l}_{se})_{(100)} = 170.8 \pm 30.1 \mu\text{m}$ (table 17).

% F.L.L.	Veinlet number ($l_{v(100)} \geq 200 \mu\text{m}$; from tables 15, 16 and 17)	$l_{v(\% \text{ F.L.L.})}$ μm	$l_{v(100)}$ μm	$(\bar{l}_v - \bar{l}_{se})_{(\% \text{ F.L.L.})}$ μm	$l_{se(\% \text{ F.L.L.})}$ μm
77	2	160	218	125 ± 22	35
77	3	173	236	125 ± 22	48
77	4	204	278	125 ± 22	79
77	5	393	536	125 ± 22	268
88	1	199	231	147 ± 26	52
88	2	204	237	147 ± 26	57
88	3	220	256	147 ± 26	73
88	4	295	343	147 ± 26	148
88	5	334	388	147 ± 26	187
96	12	204	214	163 ± 29	41
96	14	233	244	163 ± 29	70
96	15	251	263	163 ± 29	88
96	16	269	282	163 ± 29	106
96	17	292	306	163 ± 29	129
96	18	316	331	163 ± 29	153
96	19	362	380	163 ± 29	199
96	20	374	392	163 ± 29	211

In order to examine the formation of the tracheid maximum, we now consider only the distal parts of the vein endings with a length, chosen in such a way that in this distal part of the veinlet the length of the distal part of the zone l_{se} will be 50 μm at 100% F.L.L. Then the total length of the chosen distal parts will be $(50 + 171 \mu\text{m}) = 221 \mu\text{m}$ at 100% F.L.L. This length almost corresponds with the two shortest vein endings in table 21 (77% F.L.L., no. 2: $l_{v(100)} = 218 \mu\text{m}$ and 96% F.L.L., no. 12: $l_{v(100)} = 214 \mu\text{m}$). Now the lengths can be determined of the distal part of zone l_{se} at the other leaf ages with formula 2: at 77% F.L.L. = 37 μm , at 88% F.L.L. = 43 μm and at 96% F.L.L. = 48 μm . Now for each of these three leaf ages a model of the distal part of the vein endings can be made (fig. 30). The abscissa has been divided in two parts: $\bar{l}_{se}(\% \text{ F.L.L.})$ at the left of point zero, and $(\bar{l}_v - \bar{l}_{se})(\% \text{ F.L.L.})$ at the right of point zero. In the latter part

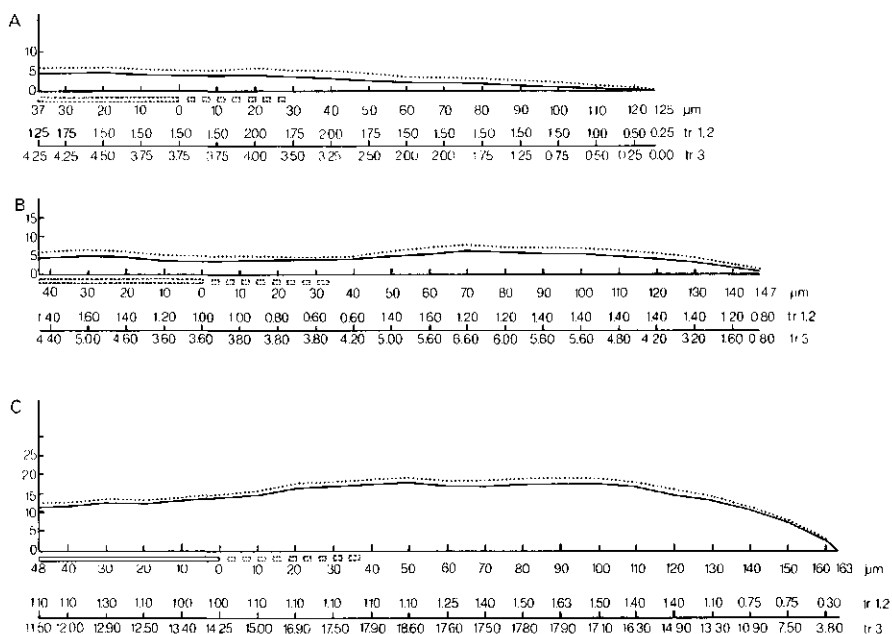


FIG. 30. Average numbers of differentiated tracheids (tr3, —) and differentiated tracheids plus differentiating tracheids (tr3 and tr1, 2, ...) (ordinate and below abscissa), at indicated distances (μm) from the calculated distal extremity of the zone l_{se} (point zero). The noted averages apply to 4 (A), 5 (B) and 8 (C) vein endings at resp. 77% 88% and 96% F.L.L., and $l_{v(100)} \geq 200 \mu\text{m}$ (table 21).

A. $(\bar{l}_v - \bar{l}_{se})_{(77)} = 125 \mu\text{m}$ and the chosen value of $l_{se(100)} = 50 \mu\text{m}$ as proximal part of the model means that $\bar{l}_{se(77)} = 37 \mu\text{m}$. $\bar{l}_{ic(77)} = 28 \pm 27 \mu\text{m}$.

B. $(\bar{l}_v - \bar{l}_{se})_{(88)} = 147 \mu\text{m}$ and the chosen value of $l_{se(100)} = 50 \mu\text{m}$ means that $\bar{l}_{se(88)} = 43 \mu\text{m}$. $\bar{l}_{ic(88)} = 33 \pm 32 \mu\text{m}$.

C. $(\bar{l}_v - \bar{l}_{se})_{(96)} = 163 \mu\text{m}$ and the chosen value of $l_{se(100)} = 50 \mu\text{m}$ means that $\bar{l}_{se(96)} = 48 \mu\text{m}$. $\bar{l}_{ic(96)} = 37 \pm 36 \mu\text{m}$.

— = future position of zone l_{se} , - - - = future position of zone l_{ic} (A, B) and position of not differentiated zone l_{ic} (C), — = position of differentiating zone l_{se} .

the value of $\bar{l}_{ic(\%F.L.L.)}$ has been indicated also yet. The value of $\bar{l}_{ic(100)}$ (MAGENDANS, 1983) amounts to $38.6 \pm 37.2 \mu m$ in dry climate (16 vein endings), and we will assume that this value in the moderate climate in which the data of table 21 and fig. 30 have been determined, does differ little from the value of $\bar{l}_{ic(100)}$ in dry climate, as does the value of $(l_v - l_{sc})$ (table 20). By means of formula 2 the value of $\bar{l}_{ic(\%F.L.L.)}$ can be determined again.

Now we consider the observations on the individual vein endings, to begin with the group at 77% F.L.L. (fig. 30A). All observations in transverse sections are now transferred to the accurate positions of the abscissa in the figure after a precise correction of length in the veinlet also and with the calculated position of the distal extremity of zone l_{sc} as fixed point (point zero). In this group of vein endings this point zero cannot be perceived microscopically yet; this point is determined by locating all transections within the exactly corrected length of $125 \mu m$ from the distal extremity, in the zone $(\bar{l}_v - \bar{l}_{sc})_{(77)}$. The number of observed, entirely differentiated tracheids (tr3) and differentiating tracheids (tr1,2) in all transections is now converted for positions at every $10 \mu m$ and noted at the correct position along this zone for each vein ending. Then these results are summed up and averaged for all 4 vein endings at the corresponding positions with respect to point zero. The calculated averages are given in the figure below the abscissa. It appears from the graph that there is no question of the developing of a tracheid maximum yet. The activity of differentiation into tracheids in the procambium is about the same along the entire, represented distal part of the vein endings, but at this moment (77% F.L.L.) this activity is at its maximum near the distal extremity of the zone $\bar{l}_{ic(77)}$ (2.00 tr1,2 on an average per transection). The number of tracheids that differentiated already (tr3) is maximal in the proximal part of fig. 30A, in the zone l_{sc} , and decreases uniformly distad. The uniform increase of the number of tracheids per transection along the vein ending, an increase that moves forward in distal direction along the vein ending, may be considered as a second stage of the differentiation of xylem in the veinlet (in succession of the first and linear stage). This second stage continues a long time and is combined later with a third stage in which an extra activity of differentiation appears near the distal extremity of the procambium strand as a first impulse to the formation of the tracheid maximum. In the leaf tissue at 88% F.L.L. (fig. 30B) it seems as though the foremost limit of the progressing wave of activity of tracheid differentiation in distal direction, arrived at the distal extremity of the procambium strand, is stopped for lack of procambium cells in distal direction. From this moment the only way out for the progressing wave of activity of tracheid differentiation is formed by the remaining procambium cells broadwise of the strand. Then this is the cause of the originating of a greater number of tracheids per transection in the distal part of the procambium strand. This greater number of tracheids, as a first perceptible indication of extra activity of tracheid differentiation (stage 3 of the tracheid differentiation in these vein endings), does not originate exactly in the position of the future tracheid maximum, but more distad.

At 88% F.L.L. the acropetal wave of activity of phloem differentiation has

not arrived yet in the proximal part of fig. 30B.

At 96% F.L.L. (fig. 30C) the activity of tracheid differentiation along the vein endings does still differ little from the activity at 77% F.L.L. (compare the number of differentiating tracheids (tr1,2) per transection along the vein endings in the three leaf tissues). The maximum activity can also be found at about half-way the zone (l_v-l_{se}), as at 88% F.L.L. This greater activity brought about a larger number of tracheids (tr3) in this position. Along the zones l_{se} and l_c the activity of differentiation seems to decrease again (cp. fig. 30A, B).

At 96% F.L.L. the acropetal wave of activity of phloem differentiation did arrive already at the distal extremity of zone l_{se} (point zero).

Meanwhile the number of available procambium cells at the distal extremity of the procambium strand has been used up almost entirely; in many vein endings this number has been reduced to zero, in others it is still varying from 1 to 5 in the distal 50 μ m of the vein ending. Therefore the number of differentiating tracheids (tr1,2) must decrease sharply in the distal extremity generally.

In the proximal part of the zone (l_v-l_{se}) more procambium cells are available and in the zone l_{se} the number of procambium cells still goes on increasing, in connection with the acropetal progressing of abaxial cell division activity in the procambium (fig. 22, 23). At 88% F.L.L. cell division activity does not take place any more in the zone (l_v-l_{se}) (fig. 24); the differentiation of tracheids cannot go beyond the number of available procambium cells, which number decreases in distal direction. This does also apply to leaf tissue at 96% F.L.L., in which still less procambium cells have been left over in the distal part. The distal limit of zone l_{se} is already characterized at 88% F.L.L. by a continual cell division activity in the abaxial part of the veinlet, proximad of the distal limit of zone l_{se} and a short distance distad from this limit. Consequently in this part more potentialities are created for tracheid differentiation and in older leaf tissue the developing tracheid maximum will perhaps shift a bit to a more proximal position. At 96% F.L.L. the tracheid maximum has been formed partly already; the exact position of this maximum is not clear from fig. 30C, however.

Finally we may come to the conclusion that the whole process of the forming of a tracheid maximum in the vein endings of *Hedera* has some resemblance to a flat wave of differentiation activity, rather rapidly proceeding along the procambium and probably coming from the foremost part of the acropetally differentiating phloem. This wave swells up far in front of the differentiating phloem; in this stage 1 of tracheid differentiation $\pm 1-2$ tracheids are formed along the procambium strand (tables 3 and 4). This wave reaches its maximum activity before or at about 77% F.L.L. in the proximal half of fig. 30A; after this moment the activity decreases again. At the distal extremity the moment of maximal activity is somewhat later (about 88% F.L.L., fig. 30B). Meanwhile the differentiation of tracheids can only take place broadwise of the vein ending (stage 2). At 96% F.L.L. the activity of tracheid differentiation almost stopped at the distal end of the veinlet for lack of procambium cells and from this moment the rest of the slowly decreasing wave of differentiation activity of tracheids can find a way out in proximal direction only, broadwise of the vein ending

(stage 3, fig. 30C) because of remaining procambium cells in that region yet. At 96% F.L.L. most tracheid differentiation takes place distad of the distal extremities of zones l_{se} and l_{ic} yet, probably because of acropetally transported factors from the differentiating phloem and the availability of procambium cells in this part of the vein ending.

The position of the tracheid maximum in leaf tissue at 96% final lamina length

In order to obtain an impression of the position of the tracheid maximum in leaf tissue at 96% F.L.L. data have been gathered of all three longest, analysed vein endings (nos 18, 19 and 20 in table 17). In table 22 the estimated numbers of procambium cells in the xylem part of the vein endings that did not differentiate into tracheids yet ($pc_{(xyl)}$), the numbers of differentiating tracheids ($tr1, 2$), the numbers of differentiated tracheids ($tr3$) and the numbers of sieve elements, differentiating and differentiated ($se1, 2, 3$) of these vein endings can be found. This table is divided into a part with observed numbers in the zones l_{se} (on the left of point 0 μm) and a part with data from the zones ($l_v - l_{se}$) (on the right of point 0 μm). Therefore the observed numbers at the distal extremity of zone l_{se} can be found in a vertical row below the point 0 μm . The observations were taken by means of transverse sections of the vein endings and after that converting for positions at every 10 μm took place. Besides a precise correction of length occurred because some sections were not exactly transverse. The shown parts of the veinlets on the left of point 0 μm have been shortened till 160 μm for each vein ending. When comparing these 3 vein endings it is conspicuous that only few procambium cells are found yet in the xylem of the veinlets 18 and 20, whereas the number of differentiating tracheids in the zones ($l_v - l_{se}$) is also small and differentiating tracheids are entirely lacking in transections at about 50 μm from point 0 μm for instance. On the other hand many still not differentiated procambium cells can be found in the xylem of vein ending 19, and the number of differentiating tracheids in this veinlet is greater also. Apparently vein ending 19 has developed less far yet than the other two. Obviously the possibility of further differentiation of this vein ending is amply present: many procambium cells have not been differentiated yet and the differentiation into tracheids is not yet over. But the number of sieve elements in zone l_{se} is also greater than in the other two vein endings; veinlet 19 is initiated as a wider structure and therefore it has probably been differentiated less far than the other two.

In order to form an idea of position and development of the tracheid maximum, the data of all three veinlets can be summed up and after that the quotients $\Sigma tr1,2 / \Sigma se1,2,3$ and $\Sigma tr3 / \Sigma se1,2,3$ can be determined, in accordance with MAGENDANS, 1983: fig. 12. In this case it must be allowed that the value of $\Sigma se1,2,3$ is substituted for the value of $\Sigma (se, ic, vp)$. Now it appears that in a vein ending, analysed before already (MAGENDANS, 1983: table 5), $\Sigma se / \Sigma (se, ic, vp) = 0.173 \pm 0.028$ on an average ($l_{se} = 456 \mu m$, 60 transections) and that this value does vary little along the entire zone l_{se} . In groups of 10 transections, proximad from the distal extremity of zone l_{se} , these 6 values are: 0.125 ± 0.024 ; 0.181 ± 0.012 ;

TABLE 22. Three vein endings at 96% F.L.L. (18, 19 and 20, table 17) and their numbers of procambium cells in the xylem part of the veinlet ($pc_{(xyl)}$), differentiating tracheids ($tr1,2$), differentiated tracheids ($tr3$) and sieve elements in three stages of development ($se1,2,3$). The numbers are noted with calculated intervals of 10 μm per section and corrected for exactly transverse sections. The distal limits of the zones l_{se} (point zero) have been put in a vertical row. Of zones l_{se} only 160 μm has been noted ($l_{se} \geq 160 \mu m$ in all three vein endings). The total numbers of $tr1,2$, $tr3$ and $se1,2,3$ in the three vein endings per transverse section, and also the quotients of these totals ($tr1,2$ and $tr3$) and the total of $se1,2,3$ per transverse section, have been noted below.

Veinlet number	Noted elements	$l_{se}, \mu m$																
		160	150	140	130	120	110	100	90	80	70	60	50	40	30	20	10	
18	$pc_{(xyl)}$	2	2	2	2	2	2		1	1	1	1	1	1	3	3	4	
	$tr1,2$	1	1	1	2	2	2	2	2	1	1	1	1	1	1	1	1	
	$tr3$	11	11	11	11	11	15	17	17	16	13	13	13	13	11	11	18	
	$se1,2,3$	3	3	3	3	3	3	2	1	1	1	1	1	1	1	1	1	
19	$pc_{(xyl)}$	6	6	5	5	5	5	5	6	6	7	7	8	8	8	12	12	
	$tr1,2$			1	1	1	1	1				2	2	2	2	2	2	
	$tr3$	14	15	12	12	12	12	13	16	16	18	16	14	14	14	14	17	
	$se1,2,3$	5	5	5	5	5	5	5	3	2	1	1	1	1	1	1	1	
20	$pc_{(xyl)}$	2	3	3	3	3	3	3	4	4	4	4	3	3	3	3	2	
	$tr1,2$	2	1	1	1	1	1	1	1	2	2	2	2	1	2	2	1	
	$tr3$	8	7	7	7	8	8	10	14	15	14	13	11	9	12	11	17	
	$se1,2,3$	3	3	3	3	3	2	2	2	1	1	1	1	1	1	1	1	
$\Sigma tr1,2$		3	2	3	4	4	4	4	3	3	3	5	5	4	5	5	4	
$\Sigma tr3$		33	33	30	30	31	35	40	47	47	45	42	38	36	37	36	52	
$\Sigma se1,2,3$		11	11	11	11	11	10	9	6	4	3	3	3	3	3	3	3	
$\Sigma tr1,2/\Sigma se1,2,3$		0.27	0.18	0.27	0.36	0.36	0.40	0.44	0.50	0.75	1.00	1.67	1.67	1.33	1.67	1.67	1.33	
$\Sigma tr3/\Sigma se1,2,3$		3.0	3.0	2.7	2.7	2.8	3.5	4.4	7.8	11.8	15.0	14.0	12.7	12.0	12.3	12.0	17.3	

TABLE 22 (continued)

	$(l_v - l_{gc}), \mu m$																			
	0	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190
4	4	4	2	2	2	2	2	3	4	3	3	4	4	3	2	2				
1	1	1	1	1				1	1	1	2	2	2	2	1	1	1			
21	27	26	24	24	22	21	21	24	23	16	16	16	14	11	4	1				
1																				
12	13	11	11	10	8	8	8	6	3	4	4	2	2	1						
2	2	2	2	2	2	2	2	2	2	2	2	2	2							
17	17	18	19	21	22	22	22	23	17	17	16	13	6	4	1					
1																				
2	2	2	1	1	1	1	1				1	1	1	1	1					
1	1	1	1					1	1	1	1	1	1	1						
18	16	16	17	18	17	22	22	22	22	19	17	16	15	16	11	8	7	7	5	2
1																				
4	4	4	3	2	2	2	2	4	4	4	5	5	3	3	1	1	1			
56	60	60	60	63	61	65	69	62	52	49	45	35	31	16	9	7	7	5	2	
3																				
1.33	1.33	1.33	1.00	0.67	0.67	0.67	1.33	1.33	1.33	1.33	1.67	1.67	1.00	1.00	0.33	0.33	0.33			
18.7	20.0	20.0	20.0	21.0	20.3	21.7	23.0	20.7	17.3	16.3	15.0	11.7	10.3	5.4	3.0	2.3	2.3	1.7	0.7	

0.169 ± 0.046; 0.175 ± 0.041; 0.178 ± 0.010; 0.212 ± 0.020. Calculation of merely the position of the tracheid maximum by means of the quotient $\Sigma tr / \Sigma se_{1,2,3}$ (table 22) must therefore be comparable with the calculation by means of the quotient $\Sigma tr / \Sigma (se, ic, vp)$. From fig. 31 this position, determined in this way, becomes clear. In leaf tissue at 96% F.L.L. a definite tracheid maximum (tr3) is developing already. This maximum originates in all three vein endings at a short distance distad from the distal extremity of the zones l_{se} . The tracheids that are differentiating yet, can be found also in this part of the vein endings mainly. It seems, however, that the differentiation on the spot of the tracheid maximum stops owing to shortage of available procambium cells (veinlets 18 and 20, between 30 and 70 μm in the zone ($l_v - l_{se}$)). On either side of this arising maximum differentiation of tracheids is still possible; distad as long as the stock of procambium cells lasts and proximad also dependent on the still occurring

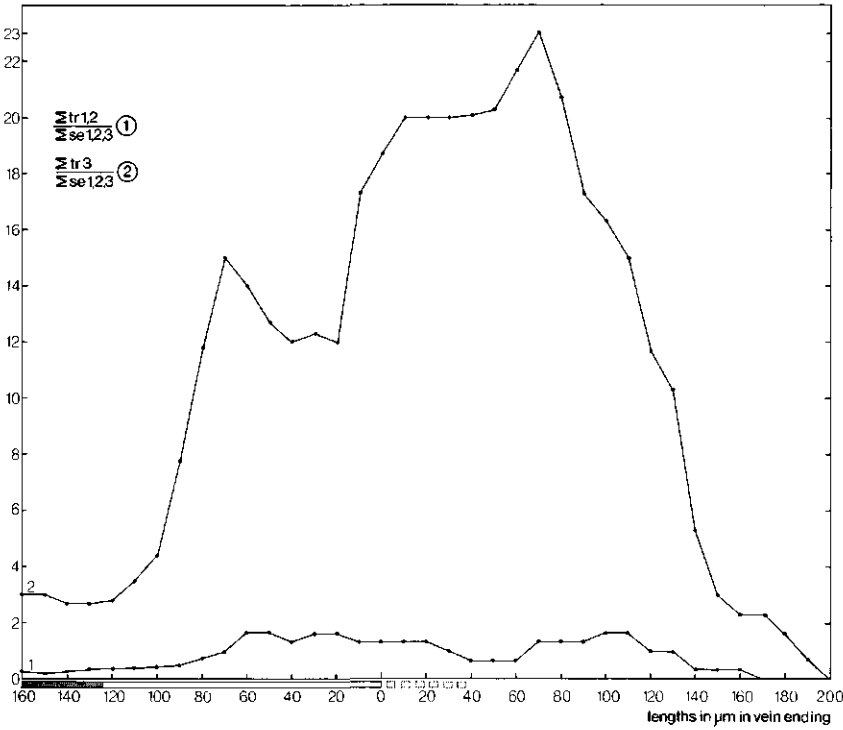


FIG. 31. The quotients of the sum of the number of differentiating tracheids and differentiated tracheids, and the sum of the total number of sieve elements in all 3 stages of development ($\Sigma tr_{1,2} / \Sigma se_{1,2,3}$ (1) and $\Sigma tr_3 / \Sigma se_{1,2,3}$ (2)) per transverse section, calculated for 3 vein endings (table 22, 96% F.L.L.). Of zones l_{se} only 160 μm has been noted ($l_{se(96)} \geq 160 \mu m$ in the three veinlets). The transections have been calculated 10 μm apart and corrected for true transverse sections.

$\text{hatched box} = l_{se3}; l_{se3} = 37 \mu m$
 $\text{solid box} = l_{se1,2}; l_{se1,2} = 123 \mu m$
 $\text{dotted box} = l_{ic}; l_{ic(96)} = 37 \mu m$ (average of 16 veinlets, dry climate, MAGENDANS, 1983).

division activity in the procambium strand. Then the decline of the curve 2 (tr3) between 10 and 70 μm in zone I_{sc} will be smoothed out for the greater part yet.

Therefore we may conclude that the tracheid maximum is formed at a short distance distad from the position, and at about the same time, of the last division activity in the phloem part of the procambium strand, as an introduction to the most distal phloem differentiation (cp. fig. 24).

The quantitative relation between the differentiating xylem and the differentiating phloem along the vein ending

It appeared that the tracheid differentiation after the distal extremity of the procambium strand was reached (completion of stage 1), continued broadwise along the procambium strand (stage 2, fig. 30A). In the distal part of the procambium strand an extra differentiation of tracheids set in at 88% F.L.L. (fig. 30B) between the probable position of the distal end of zone I_{sc} and the distal extremity of the vein ending. The thought of a wave of activity of tracheid differentiation arises, that could not proceed on its way along the vein ending for lack of procambium cells, but could find a way out broadwise of the veinlet because of still available procambium cells in that direction, especially more proximad. The transport of the wave of activity of tracheid differentiation probably continues to be distal, but can find possibilities of realization only broadwise of the vein ending near the end of the veinlet. Now it is possible to examine in what position along the vein ending this realization takes place, and one may think of the possibility that places of phloem differentiation activity in the procambium strand are the source of the wave of activity of tracheid differentiation. The sum total of transections of differentiating sieve elements ($\Sigma\text{se1,2}$) can serve as a 'measure' for activity of phloem differentiation, both in the terminal part of the acropetally differentiating phloem and in the other parts of the vein ending.

It appears from the data of table 23 that $\Sigma\text{se1,2}$ per veinlet increases sharply when the length of the vein endings increases. However, $\Sigma\text{tr1,2}$ attains its maximum value in the group of vein endings with the length of 250–300 μm and this value of $\Sigma\text{tr1,2}$ remains about constant in longer vein endings. The ratio $\Sigma\text{tr1,2}/\Sigma\text{se1,2}$ sections per veinlet shows a distinct maximum in the groups of vein endings 200–300 μm long; to these groups belong the vein endings in which the tracheid maximum is comparatively long (i.e. with regard to I_v). Consequently therefore no constant relation between $\Sigma\text{tr1,2}$ sections and $\Sigma\text{se1,2}$ sections is found along the vein endings. The maximum value of $\Sigma\text{tr1,2}/\Sigma\text{se1,2}$ is found at the place of the future tracheid maximum, and the moment at which this maximum value manifests itself about coincides with the arrival of the distal front of the acropetally differentiating phloem in this position.

Probably the origin of the tracheid maximum is the result of extra differentiation of tracheids in that position by slowing down of the distal transport of the differentiation factors and/or an increase of the concentration of these factors from the approaching, differentiating phloem (cp. fig. 24). This results in the differentiating into tracheids of every or almost every available procambium cell near the terminal differentiating phloem (fig. 31), after which some tracheid

TABLE 23. Survey of data of the 20 analysed vein endings in leaf tissue of 96% F.L.L. The vein endings have been divided into groups of 50 μm difference in length each. $\Sigma\text{se1,2}$ means the sum of the number of transections of differentiating sieve elements, etc.

$l_v, \mu\text{m}$	Number of veinlets	$\bar{l}_{v(96)} \mu\text{m}$	$\bar{l}_{\text{se1,2}(96)} \mu\text{m}$	$\bar{l}_{\text{se3}(96)} \mu\text{m}$	$\Sigma\text{se1,2}$ sections per veinlet ¹⁾	tr1,2 number per veinlet	$\Sigma\text{tr1,2}$ sections per veinlet ¹⁾	$\Sigma\text{tr1,2}$ sections/veinlet $\Sigma\text{se1,2}$ sections/veinlet
50-100	1	83	0.0	0.0	0.0	0.0	0.0	0.0
100-150	4	112	0.0	0.0	0.0	1.0	6.5	1.5 ²⁾
150-200	6	182	22.2	0.0	4.2	1.2	8.5	2.0
200-250	3	216	40.7	2.3	5.7	2.7	22.3	3.9
250-300	3	271	88.0	0.0	14.7	4.7	49.7	3.4
300-350	1	316	169.0	70.0	34.0	5.0	52.0	1.5
350-400	2	368	203.0	65.5	66.0	5.5	51.0	0.8

¹⁾ = calculated at regular intervals of 7 μm

²⁾ = 6.5/4.2, cp. MAGENDANS, 1983: 18.

differentiation finally occurs yet proximad and distad of the distal extremity of the phloem. Thus an extra big mass of tracheids is formed around the distal extremity of zone l_{se} , partly dependent on the prevailing humidity (MAGENDANS, 1983).

DISCUSSION AND CONCLUSION

The most important features of the differentiation processes in a vein ending with average length in the Hedera leaf

A survey is given in table 24 of the characteristics and the duration of stages of the differentiation processes in the developing vein endings. The data have been derived from the diagrams and tables in the previous parts. It is not possible to indicate the exact duration of the differentiation of one segment in the procambium strand, nor the exact duration of the differentiation into the zones l_{se} , l_{ic} , l_{vp} and l_{tr} can be determined. The mentioned % F.L.L. have been given in classes of leaf lengths.

As is shown in previous chapters the biometrical results indicate a highly ordered process. During the developmental stages, expressed in % F.L.L., it is possible to predict the mean length of the vein ending by the formula 1: $\bar{l}_{v(\% \text{ F.L.L.})} = 3.24 \times (\% \text{ F.L.L.}) - 45.47 \mu\text{m}$. From this biometrical analysis, suggesting a relative constancy in growth, and the ontogenetic development with the ordered sequential differentiation into the procambium strand, the composition and growth of the vein ending seems to be a highly determined and programmed process. Vein endings seem to show a greater independence in their development than the venation of lower order.

TABLE 24. Scheme of the most important features of the differentiation processes in a vein ending with average length in the *Hedera* leaf.
 l_v = average length of the vein endings belonging to the noted time after leaf initiation.
 l_{pc} = average length of segments 1, 2 and 3 in the procambium strand.
The mentioned times have been estimated in days.

l_{pc} μm	l_v μm	Differentiation into	Way of differentiation	Part of the vein ending	Synchronous processes	Time after leaf initiation % F.L.L. days
21		procambium	directed cell division in mesophyll cells	differentiation of proximal segment 1		
28			ditto	differentiation of middle segment 2	extension and longitudinal division in segment 1	till 38
32	78		ditto	differentiation of distal segment 3	extension, and longitudinal and local transverse divisions in segments 1 and 2. Shifting of division activity into abaxial part	till 13.5
94-214		tracheids	continuously and acropetally in the procambium strand	mainly the linear stage (stage 1), adaxially only; when approaching the distal extremity stage 2 takes place in the proximal part	differ. into tr1, tr2 and tr3 differ. broadwise takes place from about 70% F.L.L.	40-80 14.5-22
199-279			continuously and acropetally, broadwise in the procambium strand	adaxially in zone 1 _{se} ; in zone ($l_v - l_{se}$) both adaxially and abaxially (stage 2)	differ. into tr1, tr2 and tr3 differ. into se1, se2 and se3 from about 87% F.L.L.	70-100 21-32
230-279			broadwise along the procambium strand, but especially in the subdistal part	especially near distal extremity of zone 1 _{se} (stage 3) mainly adaxially, sometimes abaxially also \rightarrow amphivasal structure (MAGENDANS, 1983, fig. 3).	differ. into tr1, tr2 and tr3 differ. into se1, se2 and se3 from about 87% F.L.L.	85-100 23-32
237-279		sieve elements	continuously and acropetally, after repeated cell divisions	in zone 1 _{se} , abaxially only	differ. into tr1, tr2 and tr3 differ. into se1, se2 and se3	87-100 24-32

The differentiation processes into a vein ending in the *Hedera* leaf show a very close connection between the differentiation of the xylem and the phloem, both in place and time, leading to the conclusion that the differentiation of the xylem is highly dependent on the differentiation of the phloem.

Vein endings are suitable structures when studying the differentiation into procambium and primary vascular tissue

Vein endings show, also in the *Hedera* leaf, a greater independence in their development than the venation of lower order. PRAY (1955a) remarked already 'that some new ontogenetic factor, which previously had not been operative during the differentiation of the earlier developing portion of the venation, has become effective'. The differentiation into procambium and vascular elements can be studied simpler in free vein endings in areoles of a young leaf than in the stem and in the root. The advantages can be summarized by pointing out the two dimensions of the plate meristem in which these differentiation processes take place, and besides the fact that these processes are confined to 1-2 cell layers in the plate meristem only (cp. FOSTER, 1952; PRAY, 1955a, b). The cells in the plate meristem are about equal in shape and size in these layers before the differentiation into procambium. The measurements of the developing vein endings are always limited and occur amply within the boundaries of an areole. It is only a question of difference in time between the various processes of differentiation in the procambium that lead to the forming of tracheids and sieve elements.

The results summarized in table 20 have been based on different leaves. In theory it is possible that the observed differences are not founded on the influence of the climate, but on differences in the various leaves only. This last possibility seems improbable. The examined leaves always came from the same plant and were identical morphologically. The environment around the roots has always been kept exactly constant, with regard to composition, nutrient solution, temperature, aeration and also regarding the room for growth of the young roots. The results of the third group of vein endings in moderate climate exclude a possible influence of a small part of leaf tissue with chloroplasts (MAGENDANS, 1983: fig. 16, dry climate), because no statistical difference has been found between both groups of veinlets. The results of examination of the vein endings of the heterophyllous plant *Oenanthe fistulosa* L. (MAGENDANS, data not published) also show a (greater) difference of the value $(\bar{l}_v - \bar{l}_{se})$ of the vein endings in leaf tissue above the water-level and in leaf tissue that has grown below water-level. The value of $(\bar{l}_v - \bar{l}_{se})$ was much smaller in vein endings developed below water-level. The value of $(\bar{l}_v - \bar{l}_{se})$ of the vein endings in the *Hedera* leaf in wet climate is statistically significantly lower than in dry and moderate (dry) climate and it seems evident to hold responsible the difference in relative humidity for this.

This examination of the ontogeny of the vein endings in *Hedera* leaves only concerns the longer veinlets: $120 \mu\text{m} < l_{v(100)} < 650 \mu\text{m}$ approximately. The ontogeny of these longer veinlets will have taken place more independently, less

influenced by the vein of lower order with which the proximal extremity of the vein ending is in contact. When considering the length of $(l_v - l_{se})_{(100)}$ in this type of climate, only those 13 vein endings have been investigated that were in possession of a zone l_{se} (table 17). The reason of this restriction is found in the error that can arise in case of the length of zone l_{se} in the vein endings becoming negative. This negative value cannot be determined and therefore the value of $(l_v - l_{se})$ cannot be determined also.

The observed intrusive growth of the young vein endings (figs 9, 10), that continues a long time (table 8), has become possible without much further pushing up of the walls of neighbouring mesophyll cells because of the vigorous two-dimensional enlargement of the plate meristem and the developing intercellular spaces in this meristem. The otherwise not scarce microscopical images of this phenomenon of pushing up of cell walls still remain limited in extent for that reason.

Ontogenetic models of leaf growth, some theories

MEINHARDT (1979) starts from the principle of a model with morphogenetic gradients in a system of signals. A theory has been developed by means of which spatial inhomogeneous distributions can be imagined to come into being by biochemical interactions. In his model differentiated cells are added sequentially at the tips of a branching structure. A competitive interaction among cells near to a tip decides which will be added. This choice is influenced by the distribution of a substance 'S', whose presence favours the competitive balance. 'S' is assumed to be produced by all cells and removed by the vascular system. MEINHARDT (1978) suggests that auxin may play the role of substance 'S'. MITCHISON (1980) proposes against that, that it is not clear how a tip that acts as a source for 'S' could generate a well defined strand, for the local gradients are presumably the reverse of those near a tip that is part of a draining vascular system. MITCHISON (1980, 1981) then presents a model of 'pure diffusion for meristematic tissues of the leaf' and polar transport for stem segments. This model also starts from the principle of the production of a signal substance 'S' in every meristematic cell in a constant quantity and the model joins in the observations of SACHS (1969) who proposes a polar signal flow, improving the capacity of the cells for polar transport of the stimuli which control vascular differentiation, and leading to further differentiation, restricted to a defined strand. In 1975 SACHS suggests, however, that the trait that is first determined is the axis of the transport of the differentiation-inducing stimuli, rather than its polar direction. This would account for the differentiation of networks of veins. The model of MITCHISON, for a growing leaf, based on diffusion, starts from the principle of polarity acquired by cells 'de novo' at some stage in the maturation of the leaf. Meristematic cells and perhaps early strands also, would have an axiality only, defined by the direction of diffusion facilitation.

These theories cannot be stated on the structural results of the present work on *Hedera*. The results point to an other direction, namely a pure acropetal transport of a signal flow (auxin) in the apoplast at first (cp. PERBAL, LEROUX

and DRISS-ECOLE, 1982); only in a later developmental stage the principles of the theories of SACHS may become valid, also in *Hedera*, when perhaps the polar signal flow becomes more dominant.

Humidity and the length of the distal part of the vein endings without sieve elements
 $(\bar{l}_v - \bar{l}_{se})$

LARSON (1984) observed a shifting of the moment of first differentiation of xylem with regard to the moment of first differentiation into the phloem in the procambium of the midvein of a young leaflet of *Gleditsia triacanthos* L. At $\pm 3\%$ F.L.L. the differentiation into phloem occurred first and at $\pm 15\%$ the differentiation of the xylem was first; we will speak of a shift from a negative predifferentiation to a positive predifferentiation of the xylem in the procambium of the leaf. In the vein endings of the *Hedera* leaf a positive predifferentiation of xylem takes place in a great measure. Perhaps the shift of predifferentiation in the leaflets of *Gleditsia* is connected with the transpiration out of the young leaf tissue; this transpiration becoming greater as the leaves grow older. In very small leaves of *Hedera* open stomata can be found already and a greater transpiration stream out of somewhat larger leaves is coupled with an increase of length of the predifferentiation of procambium and xylem and this seems to happen in the leaves of *Gleditsia*.

MAGENDANS (1983) observed a larger value of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ in a very dry climate in a growth cabinet in vein endings of the *Hedera* leaf; in a very wet climate this value was significantly lower. This difference resulted in an almost two times greater tracheary volume at the distal ends of the veinlets in dry climate. In the moderate climate in which the examined vein endings of this article developed, the value $(\bar{l}_v - \bar{l}_{se})_{(100)}$ appeared about equal to the value in dry climate.

Of vein endings in leaves of *Oenanthe fistulosa* L., a heterophyllous, partly submerge water plant (MAGENDANS, data not published) the value of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ of vein endings in leaves grown above water-level was different from the value of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ of vein endings in submerge leaves. The value of $(\bar{l}_v - \bar{l}_{se})_{(100)}$ in submerge leaves often appeared to be zero or negative (when substituting the total length of the xylem for \bar{l}_v) and above water-level this value is always positive. For example in the vein endings in the lodicules of *Zea mays* (PIZZOLATO, 1980) with only a few stomata, the value of $(\bar{l}_v - \bar{l}_{se})$ also appears to be negative sometimes. An influence of the acropetal transpiration stream along the first procambium cell, containing differentiation stimuli for the further development of the procambium strand and differentiation into tracheids, on the length of predifferentiation of xylem and the final value of $(\bar{l}_v - \bar{l}_{se})$ in the vein endings seems highly probable.

During the examinations of the vein endings in the *Hedera* leaf the course of the transpiration stream has been investigated also. This course has been investigated in a totally white shoot with young leaves by means of colouring with a solution of eosin. In a young leaf ($\pm 30\%$ F.L.L.) the solution of eosin was found in the free ends of the venation (= procambium) already after about 15 min. Many stomata appeared to be open after microscopical examination.

Starting from the assumption that the transpiration stream can influence the ontogeny of the vein endings, a theory can be devised concerning the ontogeny of the vein ending based on a transport stream.

Ontogeny of the procambium

The direction of the development of the procambium of the vein endings is possibly determined by a 'transpiration sink' in the areole (figs 13, 14). LEIST (1976) mentions already three possible types of attraction centres, the marginal meristem, a rest meristem in the areole and the initiation of a sorus, in the direction of which the development of procambium takes place, and he speaks of a possible function of the procambium as a feeder-line of nutrients. SACHS (1975) considers 'the early stage of vascular differentiation as an improvement in the capacity of the cells for polar transport of the stimuli which control vascular differentiation'. The early stage of differentiation is considered as procambium. The results of structural examinations in the *Hedera* leaf point out the existence of such an apoplastic transport route for the transpiration stream in the young leaf. This route includes the procambium strand with intercellular spaces at the distal extremity (from 38% F.L.L.) and intercellular spaces in the cell layers 7-13 of the plate meristem to the stomata that are present already and are functioning. Perhaps the development of the sequential, directed, series of procambium cells comes into being because the procambium differentiation stimuli arrive in higher concentration via the transpiration stream along a preferential route. At the, apoplastic, extremity of a young mesophyll cell, bordering on a future vein of lower order that bounds the areole, the supplied stimuli induce the development of a series of procambium cells, in accordance with the direction of the transpiration stream. The young mesophyll cell extends and by means of the transpiration stream the polarization of the young mesophyll cell comes about in the direction of the stream and this cell is the preferential route for the differentiation stimuli after that. Apoplastic transport of these stimuli (cp. PERBAL, LEROUX and DRISS-ECOLE, 1982), leading to higher concentrations at the extremities of directed cells, and perhaps also consequent on the evaporation of water at these extremities, later leads to the development of procambium and with that to a faster, shorter and more directed transport route for the stimuli. The process of differentiation into procambium is probably the result of an autocatalytic effect (MITCHISON, 1981), to be considered as a directed self-steering, dependent on the apoplastic transport of water with, among other things, dissolved differentiation stimuli in the direction of the transpiration sink.

The first differentiation into tracheids in the vein endings

In contrast with many publications in which mention is made of a preceding differentiation of phloem, i.e. negative predifferentiation of xylem in the procambium strand, a positive predifferentiation of xylem is found recently by LARSON (1984) in the leaflets of *Gleditsia* and by SIVARAMAKRISHNA and VIJAYARAGHAVAN (1983) in aerial roots of *Ficus*. Also the present results of the examination of the vein endings in the *Hedera* leaf show a positive predifferentiation

of xylem in the procambium. Primary cell walls form an apoplastic space that is pervious to the transpiration stream (TANTON and CROWDY, 1972; BURBANO et al., 1976; LÄUCHLI, 1976; PIZZOLATO et al., 1976). An exception may be the sieve element wall of minor veins which is probably a primary wall, but very different from that of the mesophyll tissue (LUCAS and FRANCESCHI, 1982). Comparing this with the results of PERBAL, LEROUX and DRISS - ECOLE (1982) also, who showed relative impervious walls in the phloem, we may conclude that the transpiration stream can pass through the primary walls of the procambium cells of the developing vein endings, as appeared from the experiments with an eosin solution in young *Hedera* leaves. The great length of predifferentiation of tracheids in the procambium of the vein endings in the *Hedera* leaf can then be considered as a great enlargement of the apoplast in the procambium (= tr0). The very first thickening of the longitudinal walls of differentiating tracheids (tr1) can, together with the orientation of the longitudinal walls, probably be considered as a first enlargement and 'stream-lining' of the apoplast and offers with that an enlarged possibility for acceleration of the transpiration stream in the direction of the procambium. The differentiation of the adaxial procambium cells into tracheids takes place almost directly; the differentiation into sieve elements on the contrary does occur much later and after many cell divisions in the abaxial part of the procambium have appeared.

The positive predifferentiation of xylem is preceded again by the positive predifferentiation of procambium consequent on the same transport stream. The quantity of positively predifferentiated procambium is determinative of the quantity of xylem that ultimately is formed distad of the distal extremity of the zone I_{sc} in the vein endings. These quantities of xylem in the *Hedera* leaf are roughly constant in a particular type of climate. BRUCK and PAOLILLO (1984a) suppose that auxin (I.A.A.) is the differentiation stimulus of procambium; DRISS-ECOLE et al. (1984) and PERBAL et al. (1982) suppose a rapid transport of auxin through the procambium, and the transport of this stimulus through the apoplast is possible (JACOBS and GILBERT, 1983; MORRIS and THOMAS, 1978; PERBAL et al., 1982). As differentiation stimulus of tracheids I.A.A. is mentioned as well (ALONI and ZIMMERMANN, 1984; BRUCK and PAOLILLO, 1984b; and MINOCHA, 1984). The concentration of I.A.A. in the apoplast increases probably in the young vein endings because part of the water evaporates continuously via transpiration. The many substances present in the apoplast in the transpiration stream (GIAQUINTA, 1980; MADORE and WEBB, 1981; WOLSWINKEL and AMMERLAAN, 1983) are at least partly exchanged for the same substances from the sieve elements (FRITZ et al., 1983). SUNDBERG (1983) thinks that the procambium forms the first transport route in the embryo, but that a separate, different way of regulation of the differentiation into phloem and xylem occurs; SIVARAMAKRISHNA and VIJAYARAGHAVAN (1983) also conclude from their observations that the predifferentiation of xylem in one aerial root can be both positive and negative, the length of predifferentiation being dependent on the type of plant, that there must be a different way of regulation of the differentiation of the phloem and the xylem. According to these authors

these processes are independent of each other. ALONI (1980) concluded from the spatial relation of phloem and xylem while differentiating in callus tissues, that the xylem possibly is formed in response to auxin together with some added factor which reaches it from the phloem. From the examinations into the vein endings of the *Hedera* leaf (MAGENDANS, 1983) it appears that at least anatomically a very close tuning exists of the differentiation of phloem and xylem to each other. That this tuning in roots and in embryo's seems to be less close, does not necessarily mean that the differentiation stimuli in those meristems are quite different and that the processes of differentiation are independent of each other.

A young leaf in which no chloroplasts are formed, remains a sink for assimilates (cp. BLECHSCHMIDT-SCHNEIDER, 1984) in which an acropetal transport takes place in the phloem, and in which sink unloading via the symplast probably occurs permanently (cp. GEIGER and FONDY, 1980; GIAQUINTA, 1983). This quality of the plant tissue also gives an explanation for the rather constant value of $(l_v - l_{se})$ in vein endings with a zone l_{se} in *Hedera* leaves.

The first differentiation into sieve elements in the vein endings

The differentiation into sieve elements occurs latest in the vein endings after many cell divisions in the abaxial part, and remains always in contact with the phloem of veins of lower order. The first distinguishing mark of a differentiation into a sieve element is a thickening of the walls (DUTE, 1983; NEUBERGER and EVERT, 1976; SINGH, 1980; THORSCH and ESAU, 1981), at first in the corners (ELEFThERIOU and TSEKOS, 1982). This differentiation of sieve elements may be understood as further specialization of the procambium cells, particularly of the symplast (se0). This specialization of the symplast takes place abaxially and perhaps the preceding cell divisions in the procambium of the differentiating phloem are coupled with an important reduction of apoplast capacity as in the mature phloem probably (cp. LUCAS and FRANCESCHI, 1982; PERBAL et al., 1982). The transpiration stream is forced to take refuge in the adaxial part of the procambium strand which does intensify the process of xylem differentiation adaxially. In a growing young leaf sink unloading probably takes place via the symplast of resp. se3, se2 and se1 (GEIGER and FONDY, 1980), but perhaps also via the apoplast in a less degree (HENDRIX, 1983). Whereas all nutrients and hormones that are indispensable for the growing plant occur in the phloem sap (GIAQUINTA, 1980; ZIEGLER, 1975), very many of these substances can be found in the apoplast also, but in lower concentration (MADORE and WEBB, 1981, and cp. WOLSWINKEL and AMMERLAAN, 1983). A transportation of these substances together with the transpiration stream in acropetal direction via the procambium seems unavoidable. Into plant parts with little transpiration this probably leads to a smaller supply of these substances in acropetal direction and with that to a shorter predifferentiation of procambium and xylem. This appears to occur also in submerge leaves of *Oenanthe* (MAGENDANS, data unpublished) and in very wet climate in *Hedera* leaves (MAGENDANS, 1983). The length of differentiation of the phloem turned out to be independent as yet of an influence of the

humidity. Probably this differentiation of the phloem is dependent on the morphogenesis of an organ, considered in its totality, in a much more direct sense.

In morphogenesis the cell walls and the directions of the cell walls are in the first instance of importance because of the apoplastic transport that takes place through it (LEIST, 1976). After that the histogenesis, the further differentiation of the tissues in an organ, can follow in the developing plant part (HAGEMANN, 1965). The procambium differentiation of the vein endings in the *Hedera* leaf will have little or no influence on the morphogenesis of the leaf as a whole or vice versa. But this differentiation does have influence on the histogenesis of the leaf, on the ontogeny of the vascular tissues and on the future functioning as a source leaf when developing chloroplasts.

About the forming of the tracheid maximum in the vein endings of Hedera

Starting from the phenomenon of a great length of positive predifferentiation of tracheids in the vein endings, the moment will come at which, in case of a limited length of the procambium strand and a continuous, acropetal differentiation of the phloem, the differentiation into the xylem reaches the distal extremity of the procambium strand. After that the procambium length that can differentiate into tracheids, becomes smaller and only differentiation of tracheids broadwise of the procambium strand is possible yet in case of an incessant supply of differentiation stimuli in distal direction with the transpiration stream, and the approaching of the phloem differentiation front. All procambium cells are used up during this differentiation and the last differentiating will occur at places where procambium cells were available still, or possibly have been added by cell division at or in the vicinity of the distal extremity of zone I_{sc} . The tracheid maximum is formed at a short distance distad from the position, and at about the same time, of the last division activity in the phloem part of the procambium strand (fig. 31 and table 23). This indicates that the last differentiation activity especially takes place near the position of the last differentiation into phloem in the distal extremity of zone I_{sc} . Possibly the differentiation stimuli come from the dividing phloem mother cells in the phloem differentiation front. Still the differentiation into tracheids mainly takes place distad of the distal extremity of the zone I_{sc} because of the acropetal transport in the procambium.

The influence of the climate in relation to the ontogeny

It appears from the foregoing that there is question of a causal series of processes in the upbuilding of the vein endings in the *Hedera* leaf. This is possibly an expression of a for the most part constantly elapsing, steered process by means of external stimuli in and along transport routes. Also in very dry circumstances the leaf reacts within granted possibilities. It appears from examinations made before (MAGENDANS, 1983), that in dry climate more xylem differentiates in the vein endings. This points out an ontogeny influenced by an aiming at greater water capacity of the apoplast in dry climate. In this case, the differentiation of xylem also began earlier perhaps, in connection with a longer procambium strand in the zone I_{tr} .

As conclusion about the developmental and differentiation processes, it may be stated that the development of the vein endings in the *Hedera* leaf as a partly independent development, offers a suitable opportunity for experimental research into this development. Especially the important moments of initiation of various differentiation processes and the type of stimuli leading to these differentiations might be investigated well on this vascular tissue.

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SUMMARY

This dissertation consists of two articles (MAGENDANS, 1983 and 1985; abbr. as M. 1983 and M. 1985) in which the anatomy and ontogeny of free vein endings in the mesophyll of the leaf of *Hedera canariensis* Willd. var. 'Gloire de Marengo' have been studied. The examinations have been carried out by means of serial cross-sections and paradermal sections through individual vein endings grown under different conditions and in developing leaves studying the ontogeny. All observations were made with a Wild microscope using oil immersion after fixing the leaf tissue in F.A.A., embedding in paraffin wax, cutting with a rotary microtome and staining the sections with safranin and fast green. Measurements were made by means of the camera lucida. The *Hedera* leaf appeared to be a good test object because of the high number (up to 26) tracheids occurring distally in the vein endings in one transection.

The free vein endings can be divided into six types. The not ramified and ramified types, contain phloem or not and the phloem may reach till the point of ramification or further. In the ramified types the phloem can also be present in one or in both free branchings (M. 1983: fig. 2). The elements of the phloem in the vein endings are sieve elements (se), intermediary cells (ic; companion cells included) and vascular parenchyma cells (vp). In the xylem tracheids (tr) with spiral thickenings and vascular parenchyma cells (vpx) have been found (M. 1983: fig. 3). In general four different zones can be distinguished along a vein ending: l_{se} (part of the vein ending with sieve elements, l_{ic} (distad of l_{se} , containing intermediary cells), l_{vp} (distad of l_{ic} , containing vascular parenchyma cells in a direct line with the distal end of the zone l_{ic}) and l_{tr} (at the ultimate extremity of the vein ending, containing only tracheids and sometimes vascular parenchyma cells belonging to the xylem) (M. 1983: fig. 4). The percentage of living elements (se, ic, vp and vpx) decreases fairly regularly in the direction of the distal extremity of the mature vein ending, whereas the percentage of tracheids increases. The average length of the extremities of the vein endings without sieve elements, $(l_v - l_{se})$, turned out to be independent of the total length of the vein ending (l_v) and is about constant in a particular climate.

It is possible to construct a model of a vein ending, represented as a rather constant type of curve when relating the xylem part of the vein ending with the phloem part (M. 1983: fig. 13). This curve shows a distinct maximum, situated near the distal end of the zone l_{se} . It was found that under influence of the presence of the zone with intermediary cells (l_{ic}) and its distal prolongation (l_{vp}), this tracheid maximum shifts to a more distal position (M. 1983: table 7). This points to the influence of the diversity of characteristics of the phloem on the volume of the xylem, suggesting an extension of the phloem with the mentioned zones l_{ic} and l_{vp} . Any part of a vein ending consisting of e.g. the zones $l_{ic} + l_{vp} + l_{tr}$, $l_{vp} + l_{tr}$ or l_{tr} may also be found as a lateral branch of the vein ending. In other words: the short vein endings are as the tips of the longer ones. The point of branching may occur on any spot along the vein ending; however, the

zones l_{ic} and l_{vp} are both continuous and are always connected with a zone l_{se} .

A comparison in structure was made between vein endings in mature green and white leaf tissue, and between vein endings in white leaf tissue in a very dry ($7.0 \pm 2\%$ r.h.) and in a very wet ($97.0 \pm 2\%$ r.h.) atmosphere. It appeared, however, that the difference between the number of tracheids in relation to the phloem part with sieve elements belonging to it, in the green and in the white leaf tissue is not significant. But the rather constant length of the extremities of the vein endings without sieve elements in mature leaves, $(l_v - l_{se})_{(100)}$, is significantly greater in a very dry atmosphere than in a very wet atmosphere. Models have been calculated of vein endings in very dry and in very wet atmosphere (M. 1983: fig. 18) and the tracheary volume of the ultimate endings formed in a dry climate, turned out to be 1.9 times as great as the tracheary volume produced under wet conditions. The quantity of xylem and probably in connection with that the amount of nutrients and growth regulators released by the phloem also, turned out to be dependent on climatic conditions. At least a great increase of the relative humidity of the air and correlated with that a sharp decrease of transpiration of the plant, are able to bring about a decrease of the quantity of xylem per unit of phloem in the vein endings.

A biometrical foundation for studying the ontogeny of the vein endings in the *Hedera* leaf has been found in the nearly equal growth rate of the tissue in all directions near the leaf base (M. 1985: table 1). Based on the nearly identical growth curves of a small piece of tissue near the leaf base and the whole leaf (M. 1985: fig. 3), the percentage final lamina length (F.L.L.) is used as parameter for the study of the ontogeny of the vein endings. The angles of inclination of the growth curves have been used as a means of approximating the final length of the young laminae, and the calculation of the percentage F.L.L. of a young leaf is based on the estimate of the final length (M. 1985: figs 5,6).

Comparison of the length of vein endings in leaves at different ages showed a regular increase in length of these vein endings. The average length of the vein endings (\bar{l}_v) does increase according to the linear equation:

$$\bar{l}_v (\% \text{ F.L.L.}) = 3.24 \times (\% \text{ F.L.L.}) - 45.47 \mu\text{m} \quad (\text{M. 1985: fig. 8})$$

From this equation (line 1 in fig. 8) and from longitudinal sections of vein endings it appeared that the young procambium strands show a strong intrusive growth (M. 1985: figs 9, 10). From this linear equation of the growth of \bar{l}_v , it follows geometrically that the length of every young veinlet can be converted to its length at 100% F.L.L., and vice versa (M. 1985: fig. 11).

In very young leaves (till about 35% F.L.L.) free ends of the venation do occur in the mesophyll. The first, proximal procambium cells of a vein ending originate from a young mesophyll cell (M. 1985: fig. 13, represented as squares) adjacent to a veinlet of lower order after obtaining polarity due to its position. This young mesophyll cell divides about perpendicularly to the direction of the vein of lower order and the daughter cells grow strongly in this same acropetal direction. The young procambium cells show a manifest intrusive growth. The

differentiation process is repeated at the distal end of the young procambium strand in an adjacent meristematic mesophyll cell. Thus a procambium strand is built up in segments. At about 40% F.L.L. the procambium strand of the vein ending has entirely been differentiated; a conclusion based on the average number of segments (2.87) and the value of the average segment's length in the free ends of the venation. At the same time intercellular spaces are formed exclusively in the vicinity of the distal ends of the vein endings (M. 1985: fig. 14).

The acropetal differentiation into tracheids follows that of the procambium from about 43% F.L.L. (M. 1985: fig. 26). This differentiation may be divided into three stages. The first stage consists of a rather rapid proceeding of a differentiation process (M. 1985: table 11) that is transmitted continuously via a small number of cells at the adaxial side of the procambium strand (M. 1985: figs 21, 22). In the second stage the differentiation more slowly proceeds acropetally but the number of tracheids especially increases broadwise until about 80% F.L.L. After that the last stage of tracheid differentiation in the vein endings begins, in which especially the upbuilding of the tracheid maximum takes place. The interval of leaf age in which this tracheid maximum near the distal extremity of the phloem differentiates from the remaining procambium cells, ranges from about 85% to 100% F.L.L. (M. 1985: fig. 30).

The differentiation into sieve elements in the young vein endings has been studied by means of transections of the procambium strands exclusively. As with the differentiation into tracheids, three stages of the differentiation process into a sieve element (se1, se2 and se3; M. 1985: table 18) have been distinguished. By means of this approach the process of phloem differentiation in the vein endings could be followed more closely. The differentiation into sieve elements did not occur until about 87% F.L.L., and took place in continuity with the phloem in the lower order veinlets (M. 1985: fig. 26). The phloem differentiation does occur acropetally and continuously in the vein endings and long after the first tracheary elements differentiated along the procambium strand. This differentiation into sieve elements takes place by means of new cell divisions in the abaxial part of the procambium strand. As with the differentiation of xylem an obvious tendency towards an arrangement in three zones (se1, se2 and se3; M. 1985: fig. 25) can be distinguished in the differentiating of the phloem along the young veinlets.

In the moderate climate in which the ontogeny of the vein endings has been studied, the average length of the extremities of the vein endings at 100% F.L.L., $(\bar{l}_v - \bar{l}_{se})_{(100)}$, does not differ significantly from $(\bar{l}_v - \bar{l}_{se})_{(100)}$ in dry climate (M. 1985: table 20). In very wet climate, however, this value is significantly smaller (M. 1983: table 11). This means that the differentiation of procambium and tracheids with regard to the phloem are influenced by differences in humidity of the climate.

In the discussion (M. 1985: p. 68) the influence of the direction of the transpiration stream and transported stimuli is emphasized. The differentiation into procambium and vascular elements takes place in the 5th and sometimes in the 6th cell layer of the plate meristem, probably because the transpiration streams

in the young leaf prefer these middle layers. This preference in position may come about because of cell wall directions that are more suitable for apoplastic transport in these layers, between developing palisade tissue and spongy parenchyma, to the differentiating stomata ('transpiration sinks'). The differentiation into the first proximal procambium cells of a vein ending may also originate because of a preferential transpiration stream in those walls of a particular meristematic cell, adjacent to the vein of lower order, that have the correct direction as a result of which an intrusive growth of these cells takes place in the same direction. After this an adjacent meristematic cell, between the first proximal procambium cell and a place of lower water potential, for example in the direction of a partly/totally functional stoma, will divide and show intrusive growth and then this meristematic cell has become the second segment of the vein ending. The acropetal transpiration stream, containing differentiation stimuli (cp. PERBAL, LEROUX and DRISS-ECOLE, 1982, in the coleoptile of *Triticum*) passes along the first procambium cells of the proximal segment and it seems highly probable that this directed transpiration stream now affects the further development of the procambium strand. Also the differentiation into tracheids, and the length of the predifferentiation of xylem (i.e. tracheids differentiating from the procambium cells in front of the differentiating sieve elements) and the ultimate value of $(\overline{l_v - l_{se}})$ in the vein endings will be influenced by the acropetal transpiration stream and the differentiation stimuli transported in it.

The great length of predifferentiation of tracheids in the procambium of the vein endings in the *Hedera* leaf can be considered as a great enlargement of the apoplast in the adaxial part of the procambium, while the continuing cell division in the abaxial part of the procambium strand and the later differentiation of the phloem in this part of the procambium, may strongly reduce the apoplast and therefore the apoplastic transport in the abaxial part (cp. LUCAS and FRANCESCHI, 1982, and PERBAL, LEROUX and DRISS-ECOLE, 1982). The transpiration stream is thus forced to retreat into the adaxial part of the procambium strand, leading to the differentiation of more tracheids in this part.

Also the upbuilding of the tracheid maximum points to an influence of stimuli transported in acropetal direction. The maximum value of differentiating tracheids in relation to the differentiating sieve elements is found at the place of the future tracheid maximum (M. 1985: table 23). Also the moment at which this maximum value manifests itself, about coincides with the arrival of the distal front of the acropetally differentiating phloem at that place. Probably the origin of the tracheid maximum is the result of extra differentiation of tracheids at that place by slowing down of the acropetal transport of the differentiation stimuli and/or an increase of the concentration of these stimuli from the approaching, differentiating phloem.

Finally it may be stated that the biometrical results indicate a highly ordered process. A relative constancy in growth is suggested, and the ontogenetic development with the ordered sequential differentiation into the procambium strand, the composition and growth of the vein ending seems to be a highly determined and programmed process. The results mentioned in this dissertation indicate

an influence of the relative humidity of the air on the differentiation length of the procambium and the tracheid differentiation via the velocity of the transpiration stream.

The vein endings in the *Hedera* leaf seem to show a greater independence in their development than the venation of lower order. Therefore the morphogenesis of these vein endings offers a suitable opportunity for more experimental research into their development. The vein endings also offer an opportunity for the study of the reaction to environmental influences. A reaction that is restricted to limits posed by the procambium cells.

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SAMENVATTING

Deze verhandeling omvat twee artikelen (MAGENDANS, 1983 en 1985; aangeduid met M. 1983 en M. 1985) waarin de anatomie en ontogenie van vrije nerfuiteinden in het mesofyl van het blad van *Hedera canariensis* Willd. var. 'Gloire de Marengo' zijn bestudeerd. Het onderzoek is uitgevoerd met behulp van dwarse en tangential doorsneden in serie door individuele nerfuiteinden, gegroeid onder verschillende omstandigheden, en in jonge bladen voor een gedetailleerde studie van de ontstaanswijze. Alle waarnemingen werden verricht met een Wild microscoop met gebruikmaking van olie immersie na fixatie van het bladweefsel in F.A.A., inbedding in paraplast, snijden met een rotatiemicrotoom en kleuring van de coupes met safranine en fast green. Metingen werden verricht met behulp van objectmicrometer en tekenprisma. Het *Hedera* blad bleek een goed proefobject te zijn vanwege het grote aantal (tot 26) tracheiden dat in de nerfuiteinden distaal kan voorkomen in één doorsnede.

De vrije nerfuiteinden kunnen worden verdeeld in zes typen. De onvertakte en vertakte typen bevatten al of niet floem, en het floem kan reiken tot het punt van vertakken of nog verder. In de vertakte typen kan het floem ook voorkomen in een of in beide vrije vertakkingen (M. 1983: fig. 2). De cellen van het floem in de nerfuiteinden zijn zeefelementen (se), intermediaire cellen (ic; met inbegrip van de begeleidende cellen) en vasculaire parenchymcellen (vp). In het xyleem zijn tracheiden (tr) met spiraalvormige wandverstevigingsstructu-

ren, en vasculaire parenchymcellen (vpx) aangetroffen (M. 1983: fig. 3). In het algemeen kan men vier verschillende zones onderscheiden langs een nerfuiteinde: l_{se} (deel van het nerfuiteinde met zeefelementen), l_{ic} (distaal van l_{se} , met intermediaire cellen), l_{vp} (distaal van l_{ic} , met vasculaire parenchymcellen en in het verlengde van het distale uiteinde van de zone l_{ic}) en l_{tr} (aan de distale top van het nerfuiteinde, met alleen tracheiden en soms vasculaire parenchymcellen die tot het xyleem behoren) (M. 1983: fig. 4). Het percentage levende elementen (se, ic, vp en vpx) neemt vrij regelmatig af in de richting van de distale top van het volgroeide nerfuiteinde, terwijl het percentage tracheiden toeneemt. De gemiddelde lengte van de toppen van de nerfuiteinden zonder zeefelementen, $(\overline{l_v} - \overline{l_{se}})$, bleek onafhankelijk te zijn van de totale lengte van het nerfuiteinde (l_v), en is ongeveer constant in een bepaald klimaat.

Het is mogelijk om een model te maken van een nerfuiteinde, voorgesteld als een tamelijk constant type curve, wanneer men het xyleemgedeelte van het nerfuiteinde in verband brengt met het floeemgedeelte (M. 1983: fig. 13). Deze curve toont een duidelijk maximum, gelegen nabij het distale uiteinde van de zone l_{se} . Er is gebleken dat onder invloed van de aanwezigheid van de zone met intermediaire cellen (l_{ic}) en de zone met vasculaire parenchymcellen (l_{vp}) in het verlengde daarvan, dit tracheide maximum verschuift naar een meer distale positie (M. 1983: tabel 7). Dit wijst op de invloed van de verscheidenheid van kenmerken van het floeem op het volume van het xyleem, en suggereert een verlenging van het floeem met de genoemde zones l_{ic} en l_{vp} . Elk gedeelte van een nerfuiteinde dat bijvoorbeeld bestaat uit de zones $l_{ic} + l_{vp} + l_{tr}$, $l_{vp} + l_{tr}$, of l_{tr} kan ook aangetroffen worden als een zijvertakking van het nerfuiteinde. Met andere woorden: de korte nerfuiteinden zijn vergelijkbaar met de toppen van de langere. Het vertakkingspunt kan op elke plaats voorkomen langs het nerfuiteinde; de zones l_{ic} en l_{vp} zijn echter beide ononderbroken en staan altijd in verbinding met een zone l_{se} .

Een structuurvergelijking werd verricht tussen nerfuiteinden in volgroeid, groen en wit bladweefsel, en tussen nerfuiteinden gegroeid in een zeer droog ($7.0 \pm 2\%$ r.h.) en in een zeer nat ($97.0 \pm 2\%$ r.h.) klimaat in wit bladweefsel. Er bleek echter dat het verschil tussen het aantal tracheiden met betrekking tot het floeemgedeelte met zeefelementen er in, in het groene en in het witte bladweefsel niet significant is. Maar de vrij constante lengte van de toppen van de nerfuiteinden zonder zeefelementen in volgroeide bladen, $(l_v - l_{se})_{(100)}$, is statistisch significant groter in een zeer droog klimaat dan in een zeer nat klimaat. Modellen van nerfuiteinden in zeer droog en in zeer nat klimaat zijn berekend (M. 1983: fig. 18) en het tracheaal volume van de toppen van de nerfuiteinden gevormd in droog klimaat, bleek $1,9 \times$ zo groot te zijn als van de nerfuiteinden gegroeid in nat klimaat. De hoeveelheid xyleem en waarschijnlijk in verband daarmee ook de hoeveelheid voedingsstoffen en groeiregulatoren afkomstig uit het floeem, bleken afhankelijk te zijn van de klimaatsomstandigheden. Een grote toename van de relatieve luchtvochtigheid en daarmee gecorreleerd een sterke afname van de transpiratie van de plant, zijn althans in staat om een vermindering van de hoeveelheid xyleem per eenheid van floeem in de nerfuiteinden te

weeg te brengen.

Een biometrische grondslag voor de studie van de ontogenie van de nerfuiteinden in het *Hedera* blad is gevonden in de vrijwel gelijke groeisnelheid van het weefsel in alle richtingen nabij de bladvoet (M. 1985: tabel 1). Gebaseerd op de bijna identieke groeicurves van een klein stukje weefsel nabij de bladvoet en het gehele blad (M. 1985: fig. 3), wordt het percentage van de uiteindelijke bladschijflengte (F.L.L.) gebruikt als parameter voor de studie van de ontogenie van de nerfuiteinden. De hellingshoeken van de groeicurves zijn gebruikt als middel om de eindlengte van de jonge bladschijven bij benadering vast te stellen, en de berekening van het percentage F.L.L. van een jong blad wordt gebaseerd op de schatting van de eindlengte (M. 1985: fig. 5 en 6).

Vergelijking van de lengte van nerfuiteinden in bladen van verschillende leeftijd toonde een regelmatige lengtetoeename van deze nerfuiteinden. De gemiddelde lengte van de nerfuiteinden (\bar{l}_v) neemt toe volgens de lineaire vergelijking:

$$\bar{l}_{v(\% \text{ F.L.L.})} = 3,24 \times (\% \text{ F.L.L.}) - 45,47 \mu\text{m} \text{ (M. 1985: fig. 8)}$$

Uit deze vergelijking (lijn 1 in fig. 8) en uit longitudinale doorsneden van nerfuiteinden bleek dat de jonge procambiumstrengen een sterke tussendringende groei vertonen (M. 1985: fig. 9 en 10). Uit deze lineaire vergelijking van de groei van \bar{l}_v , volgt geometrisch dat de lengte van ieder jong nerfuiteinde kan worden omgerekend naar zijn lengte bij 100% F.L.L. en omgekeerd (M. 1985: fig. 11).

In zeer jonge bladen (tot ongeveer 35% F.L.L.) komen vrije uiteinden van de nervatuur voor in het mesofyl. De eerste, proximale procambiumcellen van een nerfuiteinde ontstaan uit een jonge mesofylcel (M. 1985: fig. 13, afgebeeld als vierkantjes), grenzend aan een kleine nerv van lagere orde, na het verkrijgen van polariteit in verband met de positie van de cel. Deze jonge mesofylcel deelt ongeveer loodrecht op de richting van de kleine nerv van lagere orde en de dochtercellen groeien sterk uit in deze zelfde, acropetale, richting. De jonge procambiumcellen vertonen daarbij een sterke tussendringende groei. Dit differentiatieproces wordt herhaald aan het distale uiteinde van de jonge procambiumstreng in een aangrenzende meristematische mesofylcel. Aldus wordt een procambiumstreng opgebouwd uit segmenten. Bij ongeveer 40% F.L.L. is de procambiumstreng van het nerfuiteinde geheel gedifferentieerd; een conclusie gebaseerd op het gemiddeld aantal segmenten (2,87) en de waarde van de gemiddelde segmentlengte in de vrije uiteinden van de nervatuur (M. 1985: fig. 16). Tezelfdertijd worden intercellulair gevormd uitsluitend in de nabijheid van de distale uiteinden van de nerfuiteinden (M. 1985: fig. 14).

De acropetale differentiatie tot tracheiden volgt die van het procambium vanaf ongeveer 43% F.L.L. (M. 1985: fig. 26). Deze differentiatie kan in drie stadia worden verdeeld. Het eerste stadium bestaat uit een tamelijk snelle voortgang van een differentiatieproces (M. 1985: tabel 11) dat zich continu voortplant via een klein aantal cellen aan de adaxiale zijde van de procambiumstreng (M. 1985: fig. 21 en 22). In het tweede stadium zet het differentiatieproces zich langzamer voort naar het distale uiteinde, maar het aantal tracheiden neemt vooral

in de breedte toe tot ongeveer 80% F.L.L. (M. 1985: fig. 30A). Daarna begint het laatste stadium van tracheide differentiatie in de nerfuiteinden, waarin vooral de opbouw van het tracheide maximum plaats vindt. Het bladleeftijdsinterval, waarbinnen dit tracheide maximum nabij het distale uiteinde van het floeem differentieert uit de resterende procambiumcellen, bevindt zich ongeveer tussen 85% en 100% F.L.L. (M. 1985: fig. 30B, C).

De differentiatie tot zeefelementen in de jonge nerfuiteinden is uitsluitend bestudeerd met behulp van dwarse doorsneden door de procambiumstrengen. Evenals bij de differentiatie tot tracheiden, zijn er drie stadia van het differentiatieproces tot een zeefelement (se1, se2 en se3, M. 1985: tabel 18) onderscheiden. Door deze benadering kon het proces van de floeemdifferentiatie in de nerfuiteinden nauwkeuriger worden gevolgd. De differentiatie tot zeefelementen trad niet op tot ongeveer 87% F.L.L. en vond plaats in continuïteit met het floeem in de nerven van lagere orde (M. 1985: fig. 26). De floeemdifferentiatie geschiedt acropetaal en continu in de nerfuiteinden en lang nadat de eerste tracheale elementen langs de procambiumstreng differentieerden. Deze differentiatie tot zeefelementen vindt plaats door middel van nieuwe celdelingen in het abaxiale gedeelte van de procambiumstreng. Evenals bij de differentiatie tot het xyleem kan er een duidelijke neiging tot een rangschikking in drie zones (se1, se2 en se3, M. 1985: fig. 25) worden onderscheiden bij het differentieren van het floeem langs de jonge nerfuiteinden.

In het gematigde klimaat, waarin de ontogenie van de nerfuiteinden is bestudeerd, verschilt de gemiddelde lengte van de toppen van de nerfuiteinden bij 100% F.L.L., $(l_v - l_{se})_{(100)}$, niet significant van $(l_v - l_{se})_{(100)}$ in droog klimaat (M. 1985: tabel 20). In zeer nat klimaat is deze waarde echter statistisch significant lager (M. 1983: tabel 11). Dit betekent dat de differentiatie van procambium en tracheiden met betrekking tot het floeem beïnvloed worden door verschillen in vochtigheid van het klimaat.

In de discussie (M. 1985: p. 68) wordt de nadruk gelegd op de invloed van de richting van de transpiratiestroom en meegevoerde stimuli. De differentiatie tot procambium en vasculaire elementen vindt plaats in de vijfde en soms in de zesde cellaag van het plaatmeristeem, waarschijnlijk omdat de transpiratiestromen in het jonge blad de voorkeur geven aan deze middenlagen. De voorkeur voor deze cellagen ontstaat wellicht vanwege gerichtheid van celwanden. Een bepaalde wandgerichtheid is meer geschikt voor het apoplastisch transport in deze cellagen, tussen het differentierend palissadeweefsel en sponsparenchym, naar de differentierende stomata ('transpiratie sinks'). De differentiatie tot de eerste, proximale procambiumcellen van een nerfuiteinde kan wellicht ook ontstaan vanwege een voorkeurstranspiratiestroom in die wanden van een bepaalde meristematische cel, grenzend aan de nerf van lagere orde, die de juiste richting hebben met als resultaat dat er een tussendringende groei van deze cellen in dezelfde richting plaats vindt. Hierna zal een aangrenzende meristematische cel, gelegen tussen de eerste, proximale procambiumcel en een plaats waar een lagere waterpotentiaal heerst, bijvoorbeeld in de richting van een gedeeltelijk/totaal functionerend huidmondje, delen en tussendringende groei vertonen en deze me-

ristematische cel is dan het tweede segment van het nerfuiteinde geworden. De acropetale transpiratiestroom, die differentiatie stimuli bevat (vergelijk PERBAL, LEROUX en DRISS-ECOLE, 1982, in het coleoptiel van *Triticum*), stroomt langs de eerste procambiumcellen van het proximale segment en het schijnt zeer waarschijnlijk dat deze gerichte transpiratiestroom nu de verdere ontwikkeling van de procambiumstreng beïnvloedt. Ook de differentiatie tot tracheiden, en de lengte van de vóórdifferentiatie van xyleem (d.i. tracheiden differentierend uit procambiumcellen die meer distaal gelegen zijn dan het differentierend floeem) en de uiteindelijke waarde van $(l_v - l_{se})$ in de nerfuiteinden, zullen beïnvloed worden door de acropetale transpiratiestroom en de differentiatie stimuli die met de transpiratiestroom worden meegevoerd.

De grote mate van vóórdifferentiatie van tracheiden in het procambium van de nerfuiteinden in het blad van *Hedera* kan beschouwd worden als een sterke verruiming van de apoplast in het adaxiale deel van het procambium, terwijl de voortgaande celdeling in het abaxiale gedeelte van de procambiumstreng en de latere differentiatie van het floeem in dit procambiumgedeelte, de apoplasruimte wellicht sterk kunnen verkleinen. En daarmee wordt ook het apoplas-tisch transport gereduceerd in dit abaxiaal gedeelte (vergelijk LUCAS en FRANCE-SCHI, 1982 en PERBAL, LEROUX en DRISS-ECOLE, 1982). De transpiratiestroom wordt aldus gedwongen om zich terug te trekken in het adaxiale deel van de procambiumstreng, leidend tot de differentiatie van meer tracheiden in dit gedeelte.

Ook de opbouw van het tracheide maximum wijst op een invloed van stimuli die in acropetale richting getransporteerd worden. De maximum waarde van differentierende tracheiden met betrekking tot de differentierende zeefelementen wordt gevonden op de plaats van het toekomstig tracheide maximum (M. 1985: tabel 23). Ook het moment waarop deze maximum waarde zich manifesteert, valt ongeveer samen met de aankomst op die plaats van het distale front van het acropetaal differentierend floeem. Waarschijnlijk is het ontstaan van het tracheide maximum het resultaat van extra differentiatie van tracheiden op die plaats door vertraging van het acropetale transport van de differentiatie stimuli en/of een toename van de concentratie van deze stimuli uit het naderende, differentierende floeem.

Tenslotte kan gesteld worden dat de biometrische resultaten op een sterk geordend proces wijzen. Een relatieve constantheid van de groei wordt gesuggereerd, en de ontogenetische ontwikkeling met het geordende proces van opeenvolgende differentiaties in de procambiumstreng, de samenstelling en groei van het nerfuiteinde, schijnt een sterk gedetermineerd en geprogrammeerd proces te zijn. De resultaten vermeld in deze dissertatie wijzen op een invloed van de relatieve vochtigheid van de lucht op de differentiatielengte van het procambium en de tracheide differentiatie via de snelheid van de transpiratiestroom.

De nerfuiteinden in het blad van *Hedera* schijnen een grotere onafhankelijkheid te tonen in hun ontwikkeling dan de nervatuur van lagere orde. Daarom biedt de morfogenese van deze nerfuiteinden een geschikte gelegenheid voor meer experimenteel onderzoek naar hun ontwikkeling. De nerfuiteinden bieden

ook een gelegenheid voor het bestuderen van de reactie op milieu invloeden. Een reactie die beperkt is binnen grenzen die gesteld worden door de procambiumcellen.

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CURRICULUM VITAE

Johannes, Frederik, Christiaan Magendans werd op 11 maart 1933 geboren te Amersfoort. Hij doorliep de H.B.S.-B-afdeling van het Wageningsch Lyceum te Wageningen. Na het volbrengen van de militaire dienstplicht studeerde hij aan de Landbouwhogeschool in de richting Tuinbouwplantenteelt. Tijdens deze studie was hij 6 jaren verbonden aan het laboratorium voor Plantkunde als assistent in tijdelijke dienst. Na het behalen van het ingenieursdiploma in 1966 met als specialisaties de plantkunde (verzwaard) en de phytopathologie, was hij 2 jaren als docent verbonden aan de Rijks Middelbare Tuinbouwschool te Utrecht.

Sinds 1968 is hij eerst als wetenschappelijk medewerker en nu als universitair docent verbonden aan de afdeling Plantkunde, later de vakgroep Plantencytologie en -morfologie, van de Landbouwhogeschool waarbij vele jaren werden besteed aan het zeer veel aandacht vragende onderwijs.