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

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CONTENTS

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 $(\overline{l_v - l_{se}})$ 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, 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, Layzell 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 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 poor 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 dayly temperature range of 25 ± 12°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 ± 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 ± 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°C ± 1°C during the light period and 21°C ± 0.5°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 μm 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 μm, 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 × 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 devided 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.

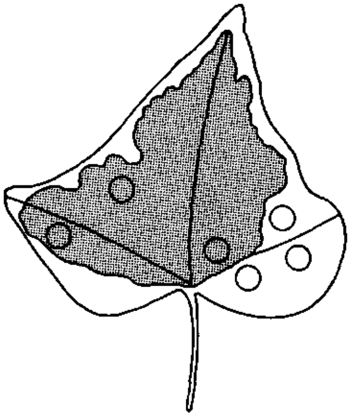

FIG. 1. *Hedera canariensis:* variegated leaf used for analysis of veinlets in three circular pieces of green tissue (dotted) and white tissue (green house conditions).

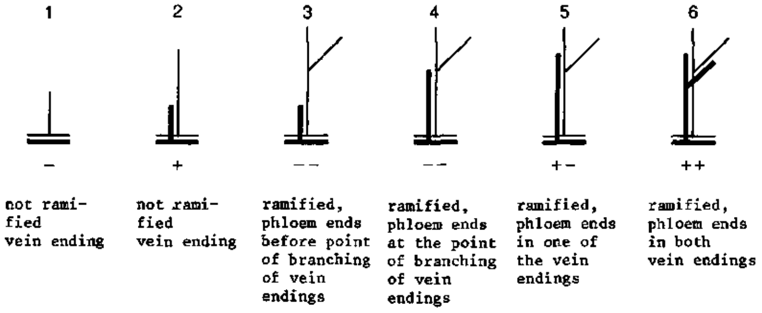

FIG. 2. Six types of vein endings. ▬▬▬ = phloem, ——— = xylem,[1] + = phloem in vein ending, – = no phloem in vein ending.

[1] Symbols:
——— = 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		

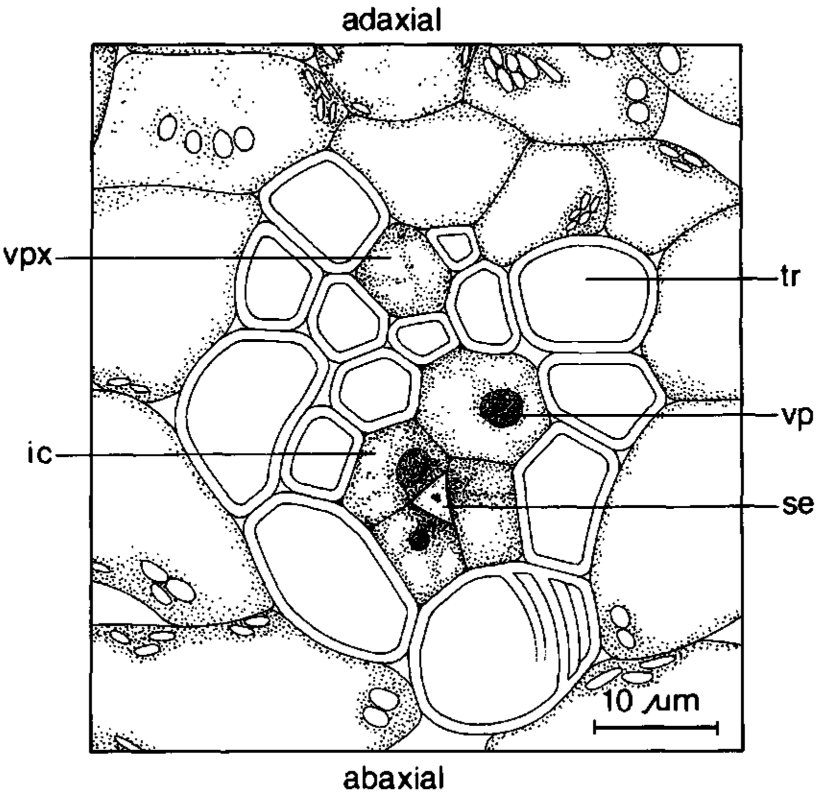

FIG. 3. Transection of vein ending not far from the distal end of the vascular bundle. It shows an amphivasal structure. ic = intermediary cell, se = sieve element, tr = tracheid with spiral thickenings, vp = vascular parenchyma cell, vpx = vascular parenchyma cell in the xylem.

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).

	ic		vp 1		vp 2		vp 3	
Section no.	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		n			+		+
20	pl		n			+		+
21		+		+		+		+
22	pl		pl			+		+
23	n			+		+		+
24	n			+	n		n	
25	n		n		n		n	
26	n		n			+		+
27		+		+		+		+
28	pl			+		+		+
29	pl		pl			+		+

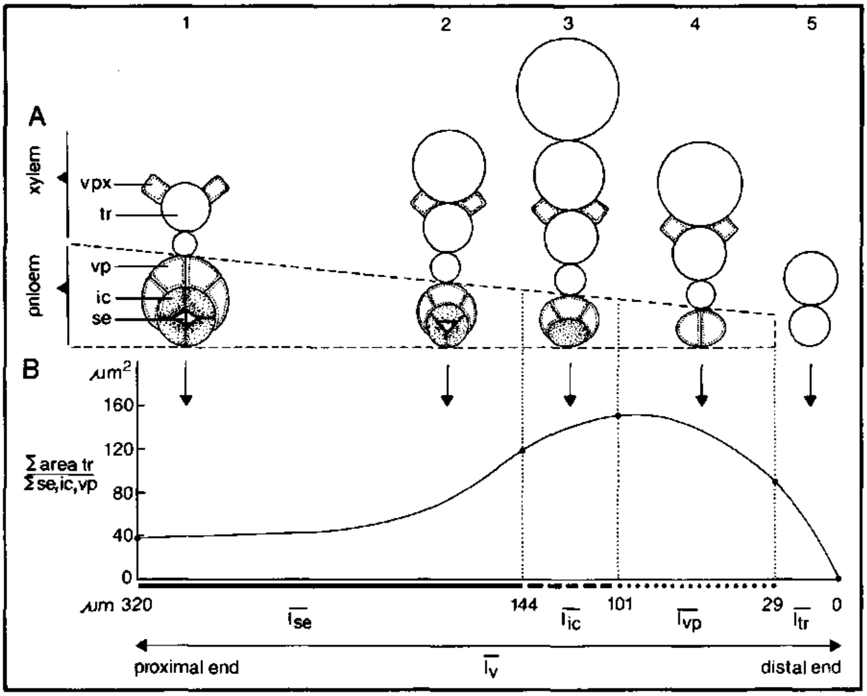

FIG. 4. Analysis of vein endings with phloem in white leaf tissue (growth cabinet).
A. Schematic drawings of sections of the vein endings at the place of the proximal ends (1), of the distal extremities of the phloem parts with sieve elements (2), of the phloem parts with intermediary cells and vascular 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) values[2] 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 (vpx), $\bar{l}_v$ = average length of the 10 vein endings.

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[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 (Σ area tr/Σ se, ic, vp).

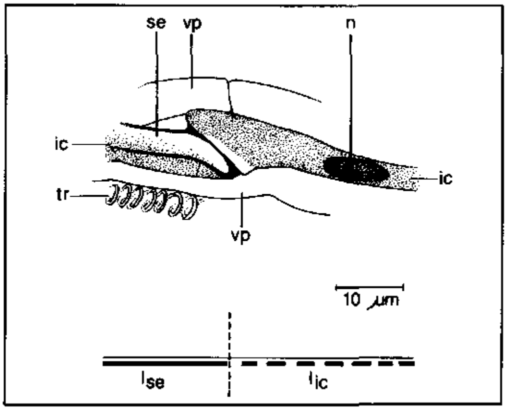


FIG. 5. Detail of a small connecting vein of white leaf tissue (dry climate; see also Fig. 15B) at the distal end of the zone l_{se} and the proximal extremity of the zone l_{ic}.
In the intermediary cell on the right hand side (ic) some plasmolysis has taken place at the proximal extremity. The vascular 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	$\overline{l_v}$	$\overline{l_{se}}$	$(\overline{l_v - l_{se}})$	% of $\overline{l_v}$
green					
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	–	–	–	–
white					
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

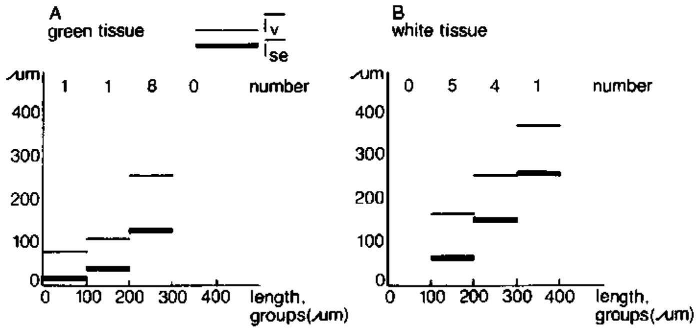


FIG. 6. Diagram of the mean values of the total length ($\bar{l}_v$) and of the length of zone l_{se} ($\bar{l}_{se}$) per group of vein endings. Each following group of vein endings is 100 µm longer. The number of vein endings in each group is noted above (variegated leaf, green-house conditions).

of the lengths of l_{se} ($\bar{l}_{se}$), of the lengths of ($\overline{l_v - l_{se}}$) and of ($\overline{l_v - l_{se}}$) as a percentage of $\bar{l}_v$.

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 $\bar{l}_v$) as the veinlet length ($\bar{l}_v$) 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 (Σ 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} ($l_{se} \geqslant 15$µm) 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

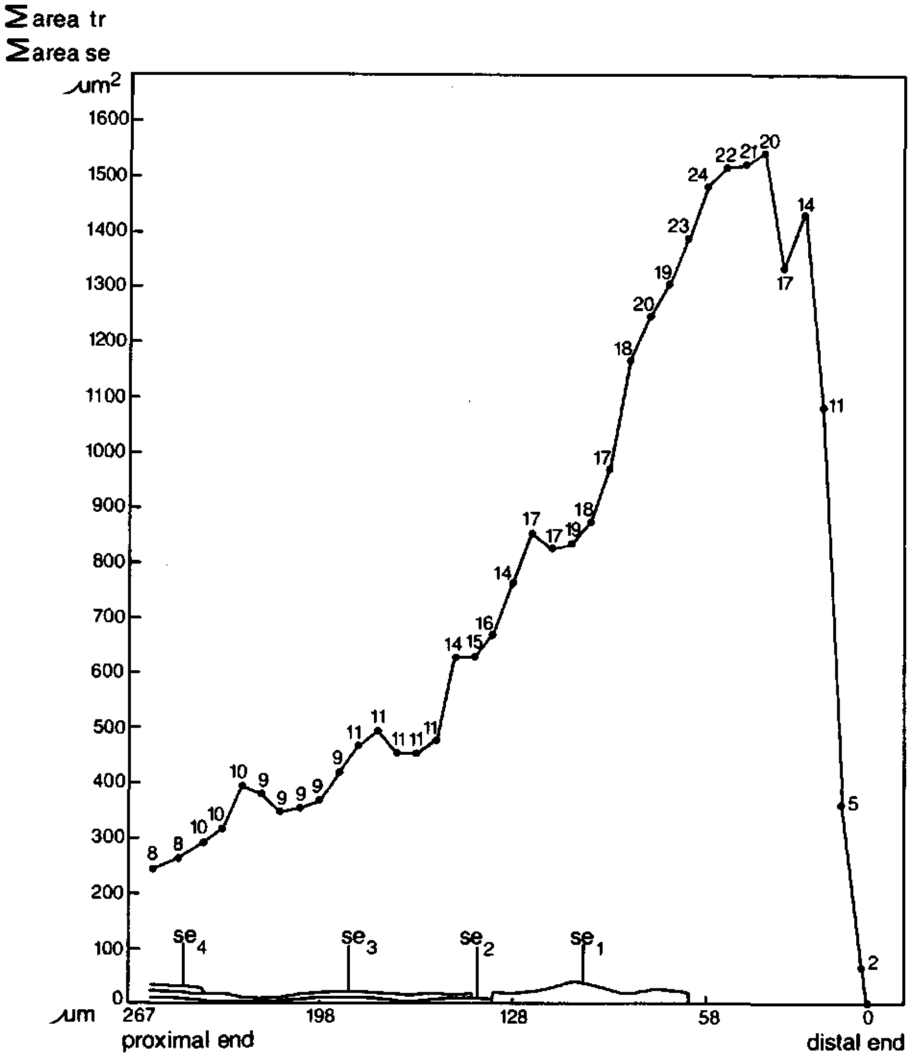


FIG. 7. One vein ending in white leaf tissue of type 2 (see Fig. 2). Variegated leaf, green house conditions. Abscissa: length of the vein ending l_v and of the phloem with sieve elements (l_{se}, with sieve elements se_1, etc.). Ordinate: calculated sum of areas of transections of tracheids (Σ area tr) in each transverse section of the veinlet and for the transections 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 ×) and also at the distal side of the extremity of l_{se} (4 ×). 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 ($\bar{l}_v$), the average length of the phloem with sieve elements ($\bar{l}_{se}$), 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	$\bar{l}_v$, µm	$\bar{l}_{se}$, 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	–

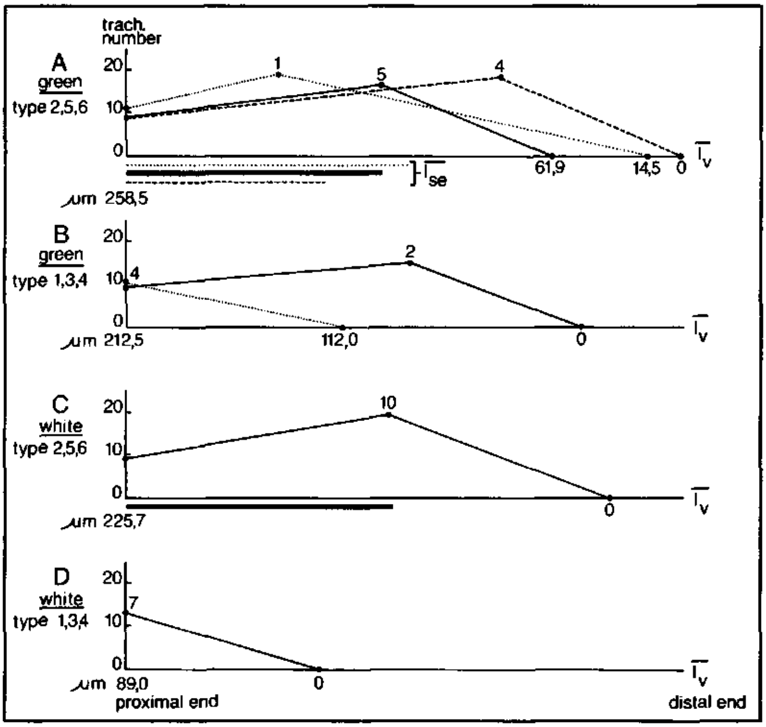


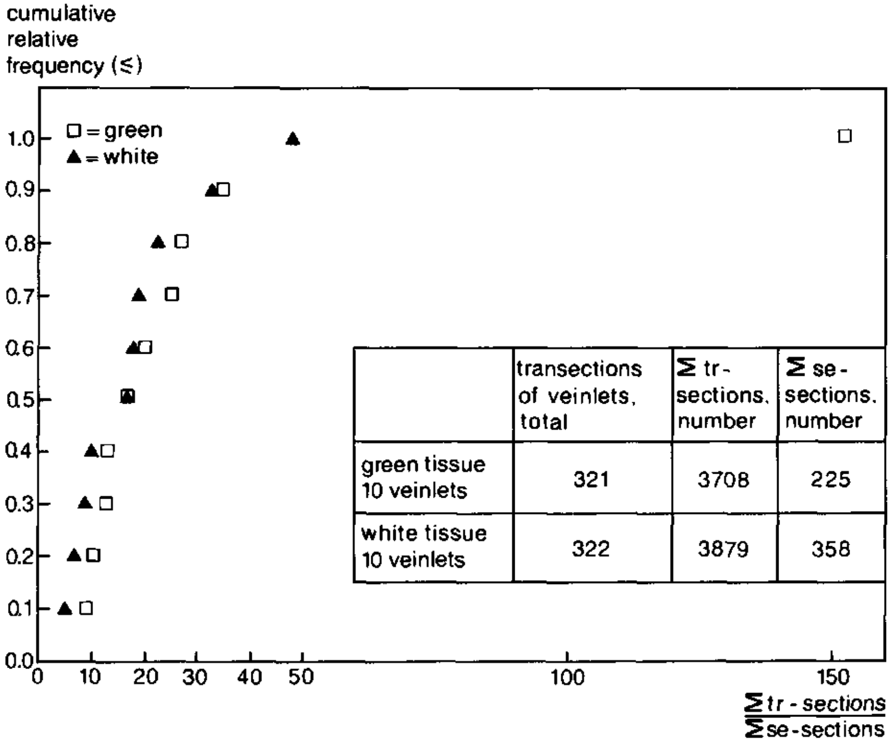
FIG. 8. Position of the tracheid maximum in vein endings in green and in white leaf tissue (variegated leaf, green house conditions).

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 ($\bar{l}_v$), 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 ($\bar{l}_{se}$) 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 $\bar{l}_{se}$ 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



	transections of veinlets, total	Σ tr-sections, number	Σ se-sections, number
green tissue 10 veinlets	321	3708	225
white tissue 10 veinlets	322	3879	358

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_{se}.

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_{se} 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 µm 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 Σ se, ic, vp at the distal extremity of the zone l_{ic} (se = o, ic $\geqslant$ 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 Σ 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.

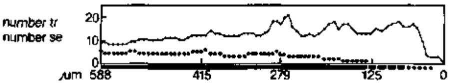


FIG. 10. The number of tracheids (-•-•-•-) and sieve elements (●●●●●) in each of the 76 transections of the vein ending of Table 5.

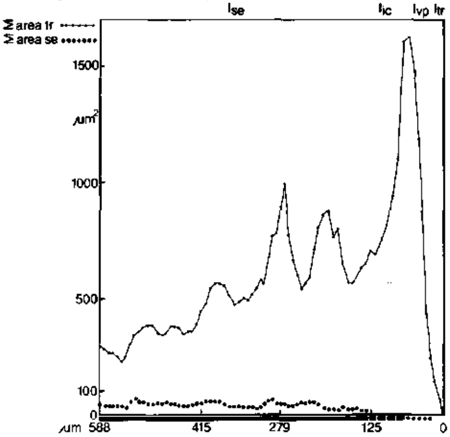


FIG. 11. The sum of the section areas of the total of tracheary elements (Σ area tr, -•-•-•-) and the sum of the section areas of the total of sieve elements (Σ area se, ●●●●) in $μm^2$ per transection of the vein ending all along the veinlet.

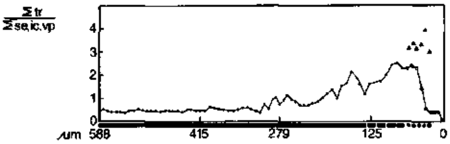


FIG. 12. The quotients of the sum of the number of tracheids and the sum of the number of sieve elements, intermediary cells and vascular parenchyma cells (Σ tr/Σ se, ic, vp) per transection all along the zones l_{se} and l_{ic} (-•-•-•-). In the zone l_{vp} the quotients of the sum of the tracheary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

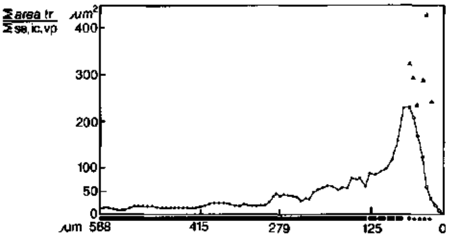


FIG. 13. The quotients (Σ area tr/Σ se, ic, vp) per transection all along the zones l_{se} and l_{ic} (-•-•-•-). In the zone l_{vp} the quotients (Σ area tr/Σ vp) have been determined per transection (▲▲▲). Moreover in the zones l_{vp} and l_{tr} the quotients of Σ area tr on the one side and the constant value of Σ se, ic, vp at the place of the distal extremity of the zone l_{ic} on the other side, have been determined per transection (-o-o-o-o-).

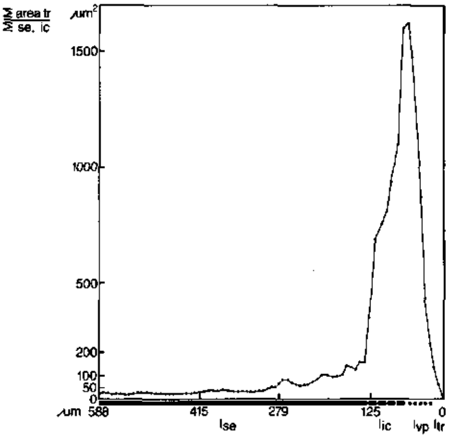


FIG. 14. The quotients of Σ area tr and the sum of the number of sieve elements and intermediary cells (Σ area tr/Σ se, ic) (-•-•-•-) per transection all along the zones l_{se} and l_{ic}. However, in the zones l_{vp} and l_{tr} the quotients of Σ area tr per transection and the constant value of Σ se, 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 Σ se, ic. Also in this graph the zones l_{vp} and l_{tr} have been calculated using as denominator the constant value of Σ 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 Σ 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

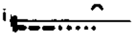
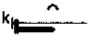
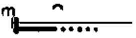
After calculation on 20 vein endings it turned out to be possible to represent the quantity of xylem in the vein endings in relation to the quantity of the phloem in the shape of a more or less constant type of curve as in the Figs 4 and 13. Now the question arises whether the position of the tracheid 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 (⌒). 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 $\geqslant$ 21 μm 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 μm 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:										
 l_{se} l_{ic} l_{vp} l_{tr}	number of trach.	calculation of quotient	number of trach.	calculation of quotient	number of trach.	calculation of quotient	number of trach.	calculation of quotient			
a 	1		4	1	1		3		6	4	10
b 											
c 	1		2				1	1	3	2	5
d 											
e 											
f 			1			1		1	1	2	3
g 								1		1	1
h 				1				1	1	1	2
i 								1		1	1
j 											
k 								2		2	2
l 											
m 	4		2		1		1	1	6	3	9
n 		1	1					1	2	1	3
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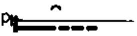
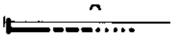
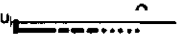
p 						1				1	1
q 	2								2		2
r 											
s 			1	1					2		2
t 			3	1	1	1	1	3	4	6	10
u 											

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}.

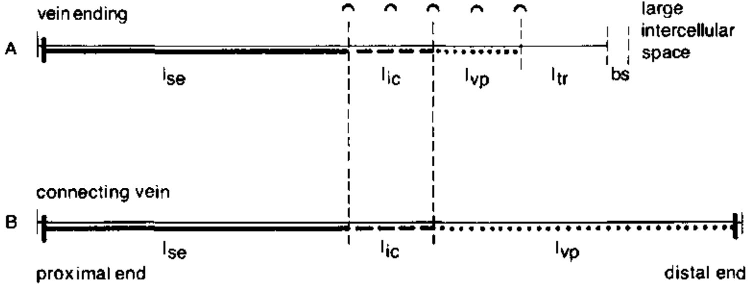


FIG. 15A. Schematic drawing of a free vein ending with zones l_{se}, l_{ic}, l_{vp} and l_{tr}; ∩: possible positions of the tracheid maxima, bs = bundle sheath.

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 ± 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 ± 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

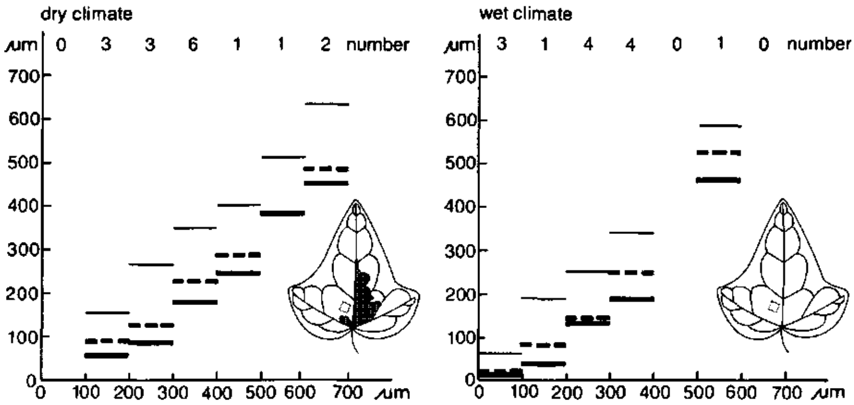


FIG. 16. Diagram with the data from Table 9. The vein endings have been divided into groups of 100 µm difference in length each (abscissa) and in every group of veinlets the average values of l_v, l_{se} and l_{ic} have been determined (ordinate).

——— = $\overline{l_v}$, – – – = $\overline{l_{ic}}$, ▬▬▬ = $\overline{l_{se}}$.

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 $Q = \Sigma$ tr-sections/Σ 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 = Σ tr-sections/Σ 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

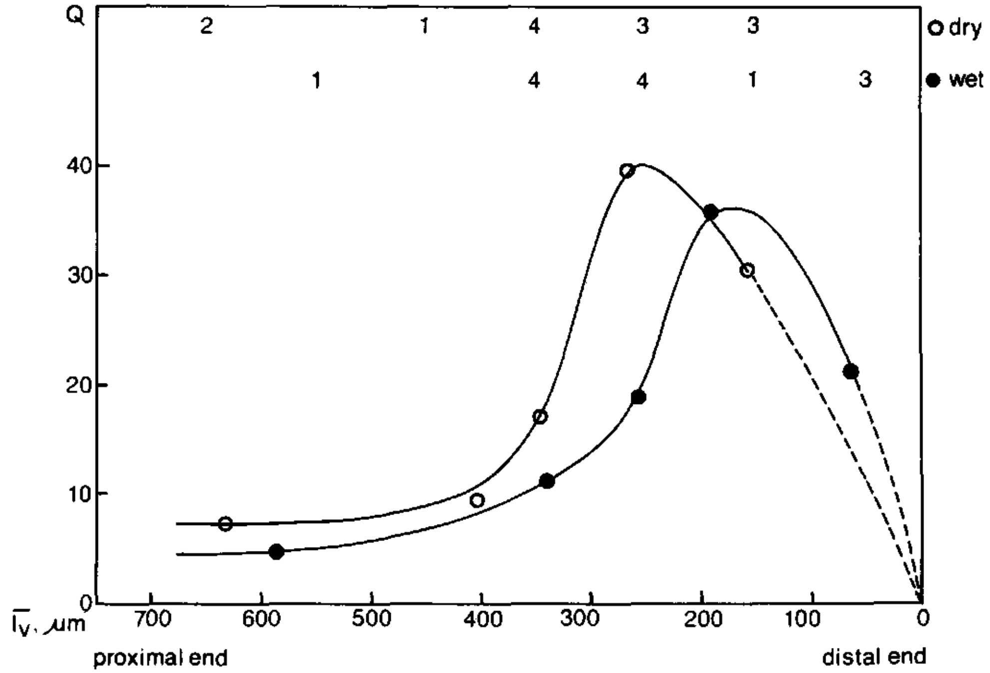


FIG. 17. The value of Q in relation to the vein length in dry and in wet climate. The value of Q (= Σ tr-sections/Σ se-sections per veinlet) has been noted as an average per group of vein endings; these groups are 0–100 µm, 100–200 µm, etc. in length ($\bar{l}_v$). The numbers above the curves indicate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

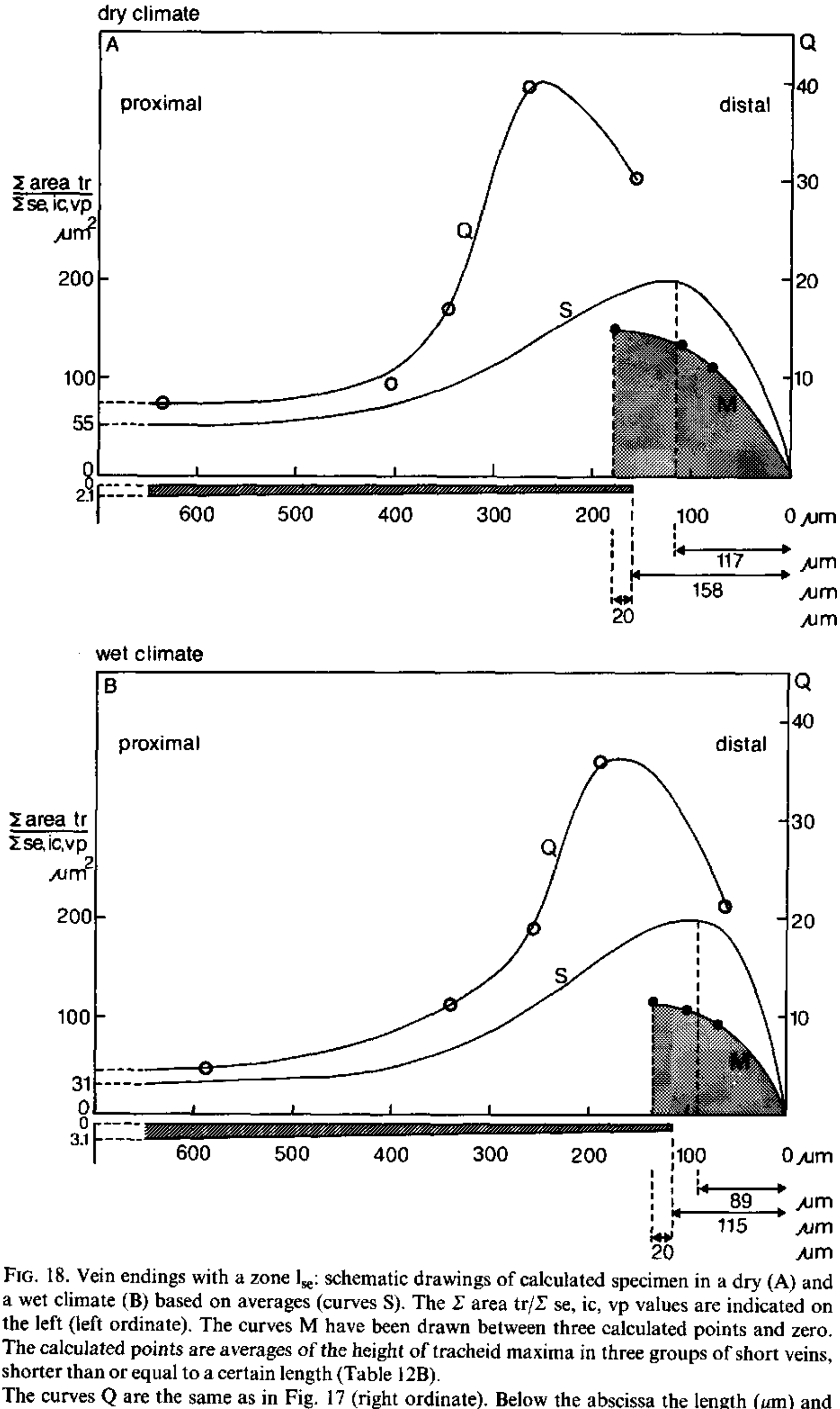


FIG. 18. Vein endings with a zone l_{se}: schematic drawings of calculated specimen in a dry (A) and a wet climate (B) based on averages (curves S). The Σ area tr/Σ se, ic, vp values are indicated on the left (left ordinate). The curves M have been drawn between three calculated points and zero. The 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, $\overline{l}_v$, 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
$\overline{l}_v$, μm (Table 8)	347.7 (16)	258.0 (13)
xylem		
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)
phloem		
$(\overline{l_v - l_{se}})$, 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, μm	l_{se}, μm	tracheid maximum (number)	l_v, μm	l_{se}, μm	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 $\leqslant$ 657 μm: average tracheid maximum = 16.9
	vein endings $\leqslant$ 180 μm: average tracheid maximum = 13.9
	vein endings $\leqslant$ 110 μm: average tracheid maximum = 12.5
	vein endings $\leqslant$ 80 μm: average tracheid maximum = 10.5
wet climate	vein endings $\leqslant$ 588 μm: average tracheid maximum = 15.7
	vein endings $\leqslant$ 135 μm: average tracheid maximum = 10.7
	vein endings $\leqslant$ 100 μm: average tracheid maximum = 9.9
	vein endings $\leqslant$ 70 μm: 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 Σ area tr/Σ 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.

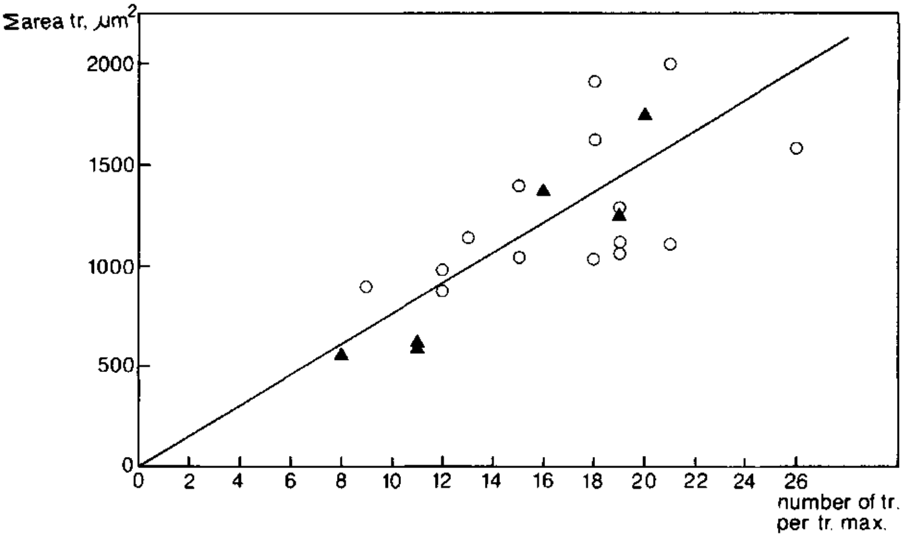


Fig. 19. Relation between the number of tracheids in the tracheid maximum, and the total area of transections of the tracheids in the maximum. 6 vein endings in dry climate (▲) and 15 vein endings in wet climate (○). Straight line not calculated.

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 areoles 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 Σ area tr/Σ 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 μm 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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