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REVIEW

Different phloem-loading machineries correlated with the climate

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INTRODUCTION

A few years ago, phloem loading in source leaves was assumed to be a universal process (reviews Giaquinta 1983; Delrot 1987). In the transfer of photo-assimilates from mesophyll to sieve element an apoplastic step was invoked to explain the accumulation of sugars in the sieve tubes. This step involves leakage from the mesophyll and subsequent

Abbreviations: PCMBS: parachloromercuribenzenesulfonic acid, SE/CC-complex: sieve element/companion cell complex, SE/IC-complex: sieve element/intermediary cell complex.

accumulation by the SE/CC-complexes of the minor vein. Sugar transport, driven by a large proton-motive force ($\Delta\mu H^+$) across the membrane of the SE/CC-complex, results in a high sugar concentration in the sieve tubes. The thermodynamic logic of this model impeded for some time the development of other views on the phloem-loading mechanism. Recent evidence, however, is beginning to turn this rigid conception into a more flexible one. There is a tendency to see phloem loading as a process which is different in various plant families. The phloem-loading mechanism may even shift, within certain limitations, in individual plants in response to changing environmental conditions.

This paper presents the ultrastructural and physiological arguments leading to a more flexible model of phloem loading: the highly variable plasmodesmatal connectivity between the mesophyll and the phloem elements (minor-vein configuration), the physiological evidence for different modes of loading, the coincidence between the minor-vein configuration and the mode of phloem loading, the correlation between the minor-vein configuration and plant taxonomy and the global distribution of plant families. The emerging picture of a multiprogrammed phloem-loading within an ecophysiological context is discussed.

ULTRASTRUCTURE OF THE PHLOEM-LOADING ZONE

Plants often possess specific metabolic properties which correlate with changes in (micro)morphology (for instance in C_3, C_4, CAM plants). Likewise, the ultrastructural variety of the minor veins amongst plant families may point to different modes of phloem loading. Here, a set of correlated anatomical, metabolic and physiological properties with regard to phloem loading is referred to as a phloem-loading machinery.

Phloem loading takes place mainly in the minor veins of the source leaves. Accordingly, the pathway from the mesophyll into the minor vein is presumed to be the highway for photosynthate transport into the phloem system. The particular degree of intercellular connectivity in the mesophyll–sieve element path is thought to be critical for determining the nature and rate of the photosynthate transport. The assumption is that the greater the number of plasmodesmata at a given interface, the greater is the potential symplastic transport through that interface. Therefore, the symplastic continuity between SE/CC-complexes and the mesophyll has played a pivotal role in the re-evaluation of the phloem-loading mechanism (Gamalei 1985, 1989, 1991; Van Bel *et al.* 1988; Van Bel & Gamalei 1991). The plasmodesmatal frequencies between SE/CC-complexes and adjacent cells can differ by more than a factor 10^3 between various families. These observations indicated differences in the phloem-loading mechanism.

Plasmodesmatal configuration of the minor vein

In a series of papers (1985, 1989, 1991), Gamalei drew attention to the diversity in phloem anatomy of the minor vein in various (140) plant families. He distinguished several types of plasmodesmatal connectivity between SE/CC-complex and adjacent cells: type 1, 1–2a, 2a, and 2b with, respectively, abundant, moderate, sporadic, and virtually no plasmodesmatal contacts (Table 1).

At first sight, classification on the basis of plasmodesmatal density alone seems to be rather arbitrary (Table 1). The quantitative diversity of symplastic contacts, however, correlates with differences in spatial and cellular organization of the minor vein (Fig. 1). In species with abundant connectivity (type 1) sieve elements are bordered by special companion cells (intermediary cells). The intermediary cells contain long cylindrical

Table 1. Plasmodesmatal frequencies between mesophyll cells and companion cells in some dicotyledonous species (Gamalei 1991). The number of plasmodesmata (n) is given per μm^2 of interface

Species	n	Species	n
Type 1: $n > 10$		Type 1–2a; $10 > n > 1$	
Fraxinus ornus	60·8	*Rhododendron caucasicum*	8·1
Ligustrum japonicum	59·1	*Phlox paniculata*	6·8
Tectona grandis	56·9	*Gossypium hirsutum*	6·2
Rosmarinus officinalis	56·4	*Ipomoea tricolor*	6·1
Teucrium orientale	55·2	*Lonicera fragrantissima*	5·4
Syringa vulgaris	54·5	*Morus alba*	4·9
Catalpa bignonioides	49·6	*Sambucus nigra*	4·3
Cucurbita pepo	48·2	*Ricinus communis*	4·1
Clerodendron trichomotum	47·2	*Castilleja pallida*	3·9
Combretum extensum	46·5		
Coleus blumei	44·8	Type 2a; $1 > n > 0{\cdot}1$	
Terminalia tomentosa	42·5	*Polemonium sibirica*	0·36
Hydrangea hortensis	41·2	*Campanula patula*	0·23
Fuchsia neglecta	40·7	*Scopolia stramonifolia*	0·16
Buddleja albiflora	40·3	*Solanum tuberosum*	0·12
Campsis fragrans	38·1	*Veronica officinalis*	0·12
Verbascum lychnitis	37·9	*Primula obconica*	0·11
Paulownia tomentosa	35·1	*Nicotiana tabacum*	0·08
Euonymus europaeus	34·1	*Beta vulgaris*	0·06
Carya ovata	33·9		
Aucuba japonica	32·8	Type 2b; $n < 0{\cdot}1$	
Vitis vinifera	30·9	*Lathyrus pratensis*	0·09
Liriodendron tulipifera	23·6	*Atropa bella-donna*	0·08
Cinchona pubescens	23·1	*Pisum sativum*	0·08
Platanus orientalis	20·9	*Vicia faba*	0·07
Nerium oleander	16·0	*Xanthium strumarium*	0·06
Tilia cordata	14·1	*Plantago major*	0·06
Salix babylonica	13·8	*Helianthus annuus*	0·06
		Antirrhinum majus	0·05
		Limonium meyeri	0·04
		Galium aparine	0·04
		Lactuca sativa	0·03
		Senecio vernalis	0·03

ER-strands oriented from the mestome sheath to the sieve element. These are inter-woven with tubular mitochondrial skeletons embedded in a matrix of dense cytoplasm. Chloroplasts are absent and other plastids are small and scarce (Gamalei & Pakhomova 1981; Gamalei 1989). In species with sporadic, or no plasmodesmatal contacts (types 2a, 2b), the companion cells are smaller and envelope the sieve elements. These companion elements have a mitochondrial network with many cristae, generally several very small spherical vacuoles and several chloroplasts within an exceptionally dense cytoplasmic matrix (Gamalei & Pakhomova 1981; Gamalei 1989) In type-2b minor veins, the walls of the companion cells are strongly invaginated (transfer cells; Pate & Gunning 1972). The degree of symplastic connectivity of the SE/CC-complex together with the corresponding ultrastructural features of the companion cell is termed the minor-vein configuration (type 1, 1–2a, 2a, 2b).

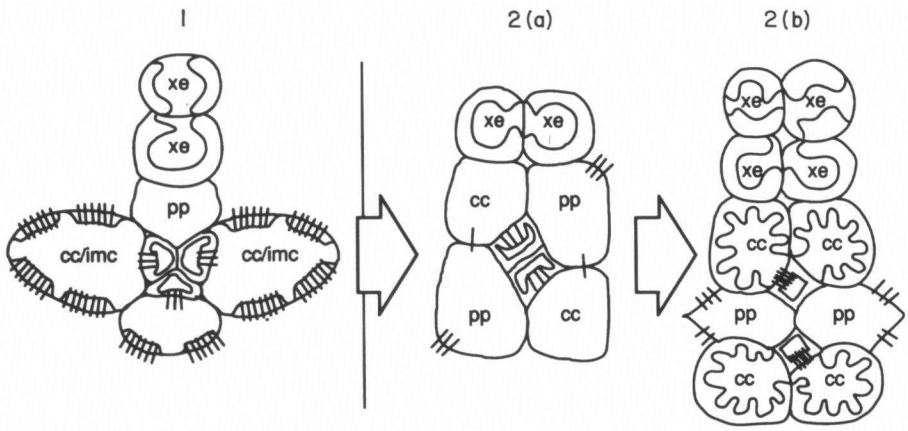

Fig. 1. Evolutionary development of the plasmodesmatal connections between the SE/CC-complex (the small central thick-walled elements are the sieve elements) and the neighbouring cells (Van Bel & Gamalei 1992; modified after Gamalei 1991). The cross-hatching in the walls indicates the degree of plasmodesmatal connectivity. The minor veins are placed in the evolutionary sequence. Type 1 with numerous plasmodesmata between SE/CC-complex and neighbouring cells, type 2 with few (type 2a) or virtually no (type 2b) plasmodesmata (see also Table 1). Intermediary cells often replace companion cells in type 1 veins. cc: companion cell, imc: intermediary cell, pp: phloem parenchyma, xe: xylem element.

The minor-vein configuration is mostly a constant family feature (Gamalei 1985, 1989). A minority (12) of the dicotyledonous families investigated so far (Van Bel & Gamalei 1991) possesses several minor-vein configurations. These families are denoted as polytypical (Van Bel & Gamalei 1991).

Occasionally, combined configurations in one minor vein have been encountered, i.e. if individual sieve elements in one minor vein are connected with the neighbouring cells in a dissimilar way. Combinations of type 1 + type 2a (Fisher 1986; Van Bel *et al.* 1988; Gamalei 1989) and, recently, of type 1 + type 2b (Van Bel *et al.* 1992) have been reported. It is likely that many more combined configurations will be discovered following further ultrastructural studies.

Plasmodesmograms of the phloem-loading zone

The route from adjacent cells to the SE/CC-complex as quantified by the minor-vein configuration, is only a part of the phloem-loading pathway. Symplastic connections between other veinal and extra-veinal cell elements are equally important for the transfer of photosynthate. A discontinuity in the extra-veinal symplastic network, as found in *Vicia faba* (Bourquin *et al.* 1990), may function as an apoplastic step in the phloem-loading pathway. Other extra-veinal symplastic constrictions have not yet been reported. Vast fields of wide plasmodesmata exist between bundle sheath and mesophyll in C_4-plants (Type 2c – Gamalei 1989, 1990; Valle *et al.* 1989; Robinson-Beers & Evert 1991b).

It is obvious that the picture of the symplastic connectivity should be extended to the whole loading-pathway. To this end, the relative plasmodesmatal frequencies in the mesophyll-to-sieve element pathway have been depicted in plasmodesmograms (Van Bel *et al.* 1988; Van Bel & Gamalei 1991). These diagrams enable an instant comparison of symplastic connectivity to be made between plant species. The plasmodesmograms, which are transformations of tabulated plasmodesmatal frequencies (Gunning *et al.* 1974; Turgeon *et al.* 1975; Evert *et al.* 1978; Kaneko *et al.* 1980; Fisher & Evert 1982; Chonan

et al. 1985; Russin & Evert 1985; Evert & Mierzwa 1986; Fisher 1986, 1990; Botha & Evert 1988; Van Bel *et al.* 1988; McCauley & Evert 1989; Bourquin *et al.* 1990; Warmbrodt & VanDerWoude 1990), disclose a wide variety in plasmodesmatal coupling between the various cell elements. The assortment of plasmodesmatal contacts suggests a great diversity of mechanisms for phloem loading.

Diagnostic value of plasmodesmograms

The diagnostic value of plasmodesmograms for the assessment of the phloem-loading pathway has been disputed (Fisher 1990) or relativized (Robards & Lucas 1990). Fisher (1990) put forward that plasmodesmograms of the phloem-loading zone were inadequate to predict the intercellular assimilate fluxes. He stated that the plasmodesmograms, based on the percentage distribution of plasmodesmata per μm^2 of interfaces between various cell elements, greatly underestimated the absolute number of plasmodesmata at the mesophyll–bundle-sheath border in relation to those at other interfaces. Robards & Lucas (1990) raised the possibility that plasmodesmograms were 'snapshots of cytoplasmic continuity'. This contention assumes a high and unevenly distributed turnover of plasmodesmata within the cells. To my knowledge, however, such a phenomenon has not been documented for mature cells within any tissue.

Obviously, the plasmodesmograms cannot be uncritically employed (Van Bel *et al.* 1988). The limitations of the plasmodesmograms are the following:

1. The plasmodesmatal frequency per interface is expressed as the percentage of the total number of plasmodesmata. The calculated frequencies of plasmodesmata in the phloem-loading zone are dependent on the numbers of plasmodesmata between the other cell types included in the calculation. Furthermore, the collection of the data (counting all plasmodesmatal connections or only those in the radial mesophyll-to-sieve tube pathway) also influences the resulting plasmodesmogram.
2. The functional diameter of the plasmodesmata may differ largely. Plasmodesmata of the bundle-sheath cells of C_4-plants (Burnell 1988) seem to be somewhat broader than those of other cells (Goodwin 1983; Erwee & Goodwin 1985; Terry & Robards 1987). The branched plasmodesmata, always encountered between sieve elements and companion cells, and the plasmodesmata between phloem parenchyma cells, appear to have quite different flux diameters (Van Bel & Kempers 1991).
3. The diverse structure of the plasmodesmata (Robards & Lucas 1990; Lucas & Wolf 1991; Robinson-Beers & Evert 1991a) suggests diverse functioning.
4. The permeability of the plasmodesmata may be radically altered by endogenous changes or changes in the cellular environment (reviewed by Robards & Lucas 1990).
5. The plasmodesmograms only provide information on the highest-order veins. Lower-order veins with a disparate plasmodesmatal connectivity (McCauley & Evert 1989) may also contribute to phloem loading (Van der Schoot 1989; Van Bel & Gamalei 1991).

Provided that the differences in symplastic continuity are large, plasmodesmograms will prove to be useful despite the impressive list of drawbacks. The relative plasmodesmatal frequencies can be used to identify potential bottlenecks in the symplastic pathway from the mesophyll to the seive element. Indeed, mapping the plasmodesmata in various ways demonstrated that plasmodesmograms facilitate the assessment of the pathway and consequently, the mode of phloem-loading employed (Botha & Van Bel, 1992).

Ultrastructural reactions to the blockage of photosynthate export

When photosynthate export was blocked by petiole chilling, the intermediary cells (in type-1 veins) and companion cells (in type-2 veins) responded differently (Gamalei 1990). Intermediary cells responded to petiole chilling by a condensation of the mitochondrial matrix and a complete collapse of the ER-strands within 4 hours. In companion cells, the mitochondria remained unchanged, but the cytoplasm became very condensed around the periphery of the cell. After some time, the vacuoles enlarged and fused, while the starch grains in the chloroplasts increased in both number and size. The differential response indicates a sharp distinction between the metabolism of intermediary cells and companion cells.

This ultrastructural reaction is part of a conglomerate of events following photosynthate accumulation in the source tissue. When photo-assimilate export was blocked by incision, heat girdling or cold jackets (Faucher *et al.* 1982; Mayoral *et al.* 1985; Ntsika & Delrot 1986), the tissues engaged in production and export of photosynthate reacted in various ways. The accumulation of photosynthate provoked an inhibition of phloem loading, an increase in apoplastic sucrose, an accumulation of starch and a decrease in photosynthesis (Mayoral *et al.* 1985; Ntsika & Delrot 1986). Similarly, starch accumulated and photosynthesis declined following sink removal (Mayoral *et al.* 1985).

CORRELATION BETWEEN MINOR-VEIN CONFIGURATION AND MODE OF PHLOEM LOADING

The challenge is now to link vein anatomy on the one hand with the physiology of phloem loading on the other. Phloem loading is very versatile both in terms of the routes employed and the substances translocated. This flexibility makes it a difficult process to analyse comprehensively. A particular problem is posed by the microscopic scale of the structures involved. It is practically impossible to directly analyse the contents or functions of the cells and tissues, and indirect approaches are, by implication, prone to error.

Multiprogrammed phloem-loading

A paucity of plasmodesmata in a phloem-loading pathway supposedly reflects a symplastic constriction which provokes apoplastic transfer. An abundance of plasmodesmata indicates a continuous symplastic pathway from the mesophyll to the sieve elements (Fig. 2) and therefore requires only one symplast domain (terminology coined by Erwee & Goodwin 1985). Apoplastic phloem-loading demands at least two symplast domains (Fig. 2): the mesophyll symplast (from which photosynthate is released) and the SE/CC symplast (by which the photosynthate is accumulated). The mode of phloem loading is defined as apoplastic if the photo-assimilates cross an apoplastic intermission anywhere in the pathway from mesophyll to sieve element (cf. Oparka 1990). On the basis of these definitions, the pictograms of the SE/CC-complex (Gamalei 1985, 1989) and the plasmodesmograms of the phloem-loading zone (Van Bel *et al.* 1988; Van Bel & Gamalei 1991) led to the concept of multiprogrammed phloem-loading (Van Bel & Gamalei 1991).

As vein configuration is a family feature (Gamalei 1989), the mode of phloem loading may vary with the plant family. This correlation permits the prediction of the following distribution of the modes of phloem loading in dicotyledons:

1. Symplastic phloem-loading in families with a type-1 minor-vein configuration (Fig. 3a). These families include many tree and shrub species (Gamalei 1989).

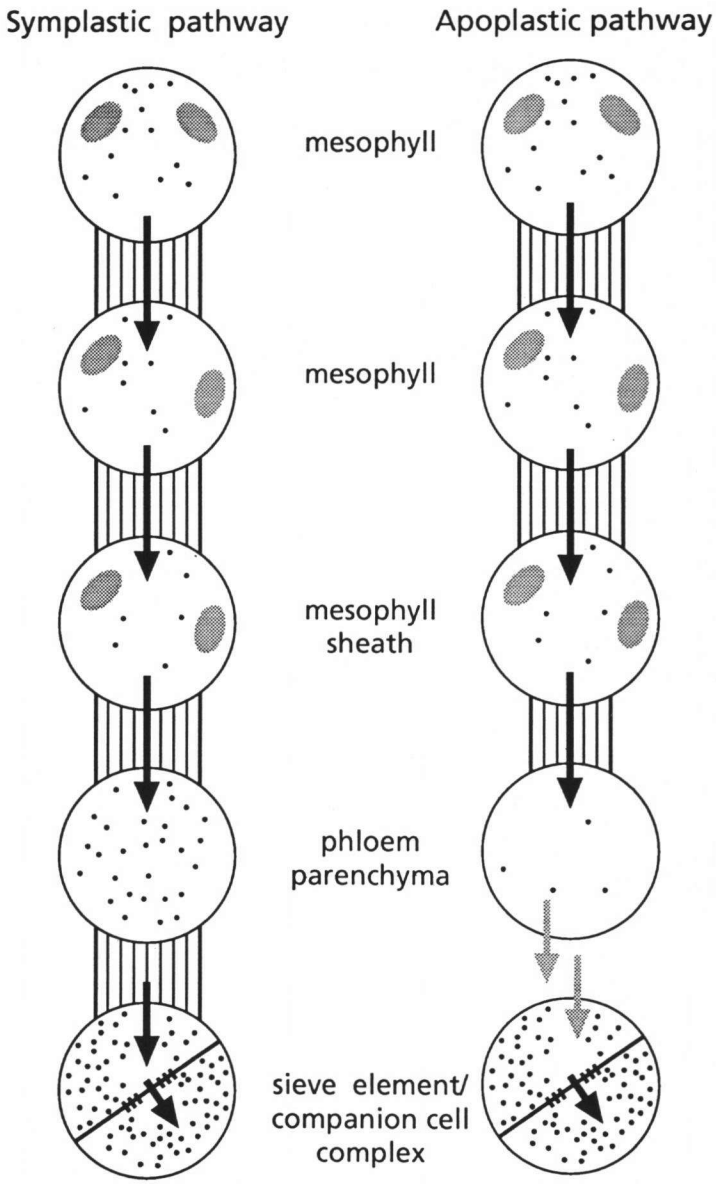

Fig. 2. Generalizations of the two potential pathways of phloem loading. In both cases, the sugar concentration in the SE/CC-complexes is presumed to be higher than in the mesophyll. The corresponding phloem loading mechanisms must be quite different to achieve sugar accumulation in the SE/CC-complex. The cross-hatching between the cells represents symplastic continuity, the short stripes between SE and CC indicate the specialized branched plasmodesmata. Dark arrows indicate the symplastic transfer of photosynthate. Light arrows indicate apoplastic transfer of photosynthate which comprises consecutive release from the mesophyll symplast, diffusion via the apoplast and retrieval by the SE/CC-complex symplast. The likely photoassimilate concentration is indicated by the density of the dotting. The term 'companion cell' also includes here the intermediary cell.

2. Apoplastic phloem-loading in families with the type-2a or type-2b minor-vein configuration (Fig. 3b). In these families, herbaceous plants predominate. This

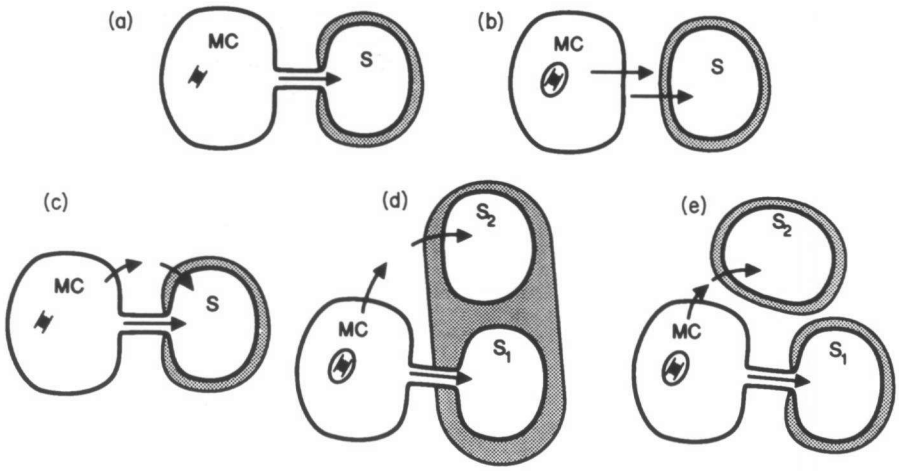

Fig. 3. The potential assortment of phloem-loading pathways in higher plants devised using the plasmodesmatal frequencies. The dotted zone represents the minor vein, the cells within the dotted zone are the SE/CC-complexes. The arrows indicate the direction of the photosynthate transport via the symplast or the apoplast. The corridors between the cells are the symplastic pathway(s). (a) Strictly symplastic loading, (b) strictly apoplastic loading, (c) mixed loading of one single sieve element which is loaded via symplast and apoplast, (d) mixed loading of two sieve elements in one vein. One sieve element is loaded via the symplast, the other via the apoplast, (e) mixed loading of two sieve elements in different veins. The sieve element in the highest-order vein is loaded via the symplast, the sieve element in the lower-order vein via the apoplast. MC mesophyll cell, S, S_1, S_2 SE/CC-complexes.

group includes important crop families of the temperate zone such as Asteraceae, Brassicaceae, Chenopodiaceae, Fabaceae, and Solanaceae.

The plasmodesmogram of *Vicia faba* (from Bourquin *et al.* 1990) deserves special attention: it shows symplastic isolation of the mestome sheath cells at the paradermal sides. This specific configuration raises the possibility of extraveinal, apoplastic intermissions in the phloem-loading pathway and a three-step mode of apoplastic loading.

3. Combined symplastic and apoplastic loading in families with the type-1-2a minor vein configuration (Fig. 3c). The limited capacity of the symplastic loading appears to be complemented by apoplastic loading. The leaves may be able to switch their mode of phloem loading in response to temporary or permanent changes in the environment.

According to recent reports on plasmodesmatal connectivity (see also Van Bel *et al.* 1988; Van Bel 1989; Van Bel & Gamalei 1991), this provisional classification seems to be an over-simplification. Sometimes, two sieve tubes in one single, minor vein (Fig. 3d) are differently connected with the mesophyll symplast (e.g. *Coleus*, Fisher 1986; *Cucumis*, Schmitz *et al.* 1987; *Commelina*, Van Bel *et al.* 1988; *Acanthus*, Van Bel *et al.* 1992). It remains to be determined whether these configurations occur in the very minor veins. For instance, the ultrastructure of rice cross veins (Chonan *et al.* 1985) is highly variable and, as a consequence, the classification of the minor veins is uncertain. It is unknown whether minor veins in dicotyledons can also exhibit such a degree of diversity.

This turns our attention to the involvement of the lower-order veins in phloem loading. As all sieve elements are equipped with retrieval systems, direct absorption of apoplastic photo-assimilates by lower-order veins cannot be ruled out. The behaviour of fluorescent dyes injected intracellularly into the mesophyll of tomato leaves, suggests a potential

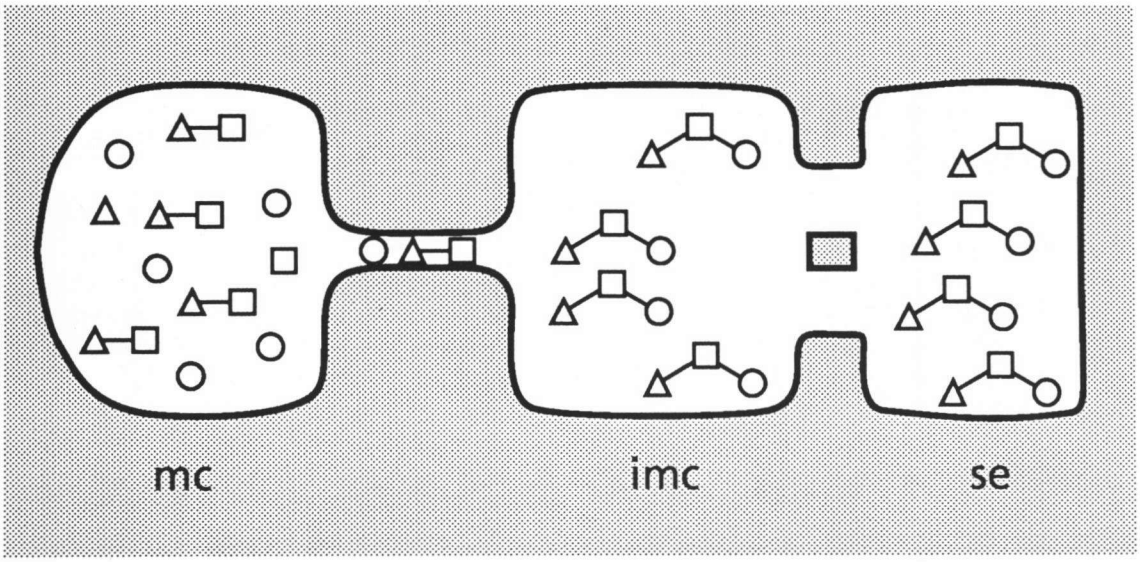

Fig. 4. Model of the polymerization trap mechanism (constructed after Turgeon 1991) to explain symplastic phloem loading against the photo-assimilate gradient. Galactinol (○) and sucrose (glucose △ and fructose □) diffuse from mesophyll to the intermediary cells where they are synthesized to raffinose. The molecular size of the raffinose prevents symplastic back-flow to the mesophyll, but allows transfer to the sieve element via broader plasmodesmata. mc: mesophyll cell, imc: intermediary cell, se: sieve element.

symplastic route for phloem loading in the fifth-order veins but an apoplastic one in the fourth-order veins (Van der Schoot 1989; Van Bel & Gamalei 1991). The reverse has been claimed for phloem loading in rice: apoplastic loading in the highest-order veins (Chonan *et al.* 1985), symplastic loading in the lower-order ones (Kaneko *et al.* 1980). Leaves with differential loading within and between veins are expected to show physiological responses similar to those of combined loading in type-1-2a veins (Fig. 3c).

Phloem loading of various photo-assimilates

The sugar composition of phloem exudate, collected from petioles or stems (Zimmermann & Ziegler 1975; Gamalei 1985), is a highly diverse mixture of mono- (glucose, fructose), di-(sucrose), and oligosaccharides (e.g. raffinose, stachyose, verbascose), sugar alcohols (sorbitol, mannitol) and other components. The minor-vein configuration appeared to correlate with the sugar components of the exudate (Gamalei 1985, 1989). Families with type 1 minor veins translocate 20–80% of the sugars in the form of raffinose-related compounds, whereas type-2 families translocate almost exclusively sucrose (Gamalei 1985).

An attractive option is that oligosaccharides are loaded via the symplastic pathway, and sucrose and the monosaccharides via the apoplastic pathway. From inspection of their plasmodesmograms, many plants would be able to combine both modes of loading. Sucrose is mostly predominant in the phloem sap, even in those families which are presumed to load primarily via the symplast (Zimmermann & Ziegler 1975). This seems to be incompatible with the fact that synthesis of oligosaccharides is an absolute prerequisite in the proposed model of symplastic phloem-loading (Fig. 4). There are three feasible explanations for this apparent inconsistency: (a) highest-order veins with a symplastic-

loading configuration execute a concealed parallel apoplastic phloem-loading, (b) leaves with a symplastic minor-vein configuration (also) load sucrose apoplastically via the lower-order veins, (c) it is possible that sugars are originally loaded as oligosaccharides, but that some of them are converted into sucrose along the transport path. It should be borne in mind that the phloem exudates analysed by Zimmermann & Ziegler (1975) were collected some distance away from the site of loading.

Limitations of experimental approaches to identify loading mechanisms

Some reviews (Van Bel 1987; Turgeon & Beebe 1991) have disputed the absolute value of virtually all phloem-loading experiments. Ironically, one of them was written to challenge the universal validity of the apoplastic phloem-loading concept (Van Bel 1987), whereas the other demonstrated the weakness of the arguments in favour of symplastic phloem-loading (Turgeon & Beebe 1991). Both papers, however, pointed out the equivocal nature of the arguments in favour of either mode of phloem loading and advocated a more flexible phloem-loading concept. A short summary of the objections may suffice to cast a critical light on the present physiological evidence for the mechanisms of phloem loading.

1. To begin with, the mode of phloem loading (apoplastic, symplastic) is difficult to assess because the minor veins are capable of both collection and retrieval (Maynard & Lucas 1982). Even SE/CC-complexes of minor veins in leaves loading via the symplast will therefore accumulate solutes from a medium and show features similar to those of 'apoplastic loaders'.
2. Conclusions based on the use of isolated mesophyll cells also present problems. The plasmodesmatal connectivity of 'apoplastic loaders' indicates that assimilates are probably released into the apoplastic space close to the SE/CC-complex. Consequently, leakage from mesophyll cells or protoplasts (e.g. Scorer 1984) is not directly pertinent to the mechanism of phloem loading unless extraveinal apoplastic transport is involved (e.g. Bourquin *et al.* 1990). Release (and resorption) in the mesophyll is therefore only pertinent to the retention of photo-assimilates in the symplastic section of the pathway to the phloem (cf. Delrot 1989; Fig. 2). It appears that experiments with isolated mesophyll cells provide no argument whatsoever for the mode of phloem loading. In addition, the techniques used to isolate the mesophyll cells or protoplasts inevitably alter them in unknown ways (see Delrot 1989; Turgeon & Beebe 1991).
3. Experiments with (stripped) discs or segments provide a more true-to-life situation for phloem loading, at least for 'apoplastic loaders'. But disc systems still fail to determine the exact site and mechanism of release from the mesophyll symplast and the subsequent uptake by the SE/CC-complex.

   Leakage from discs probably includes several simultaneous processes: loss from the mesophyll, leakage from the cells engaged in photo-assimilate release from the mesophyll symplast and, perhaps, delivery from the cut veins (Anderson 1983, 1986; Kaiser & Martinoia 1985). Furthermore, the leakage solute is contaminated to some extent with the compounds in the apoplast at the start of the washing. The involvement of several leakage processes may explain the inconsistent effect of PCMBS on photosynthate release from discs. Photosynthate release was inhibited by PCMBS in Fabaceae (Anderson 1983, 1986; Secor 1987; M'Batchi & Delrot 1988), but was promoted in *Nicotiana* (Turgeon 1984) and *Commelina* (Van Bel *et al.* 1986b).

Uptake of ^{14}C-labelled solutes by discs suffers from an unknown contribution of mesophyll, mesophyll sheath and vein cells to the bulk uptake. Micro-autoradiograms of leaf discs probably provide optically misleading information regarding the location of uptake (see for details Van Bel 1987; Madore & Lucas, 1989). The apparently dominant accumulation of organic substrates by the veins is possibly the sum of direct uptake by the veins and rapid 'by-pass' transfer via the mesophyll (Wilson *et al.* 1985; Van Bel *et al.* 1986a; Madore & Lucas 1989; Schobert & Komor 1989). The differential contribution of various leaf cells to the uptake from a medium is not well-defined. Comparative experiments with isolated veins and mesophyll cells support the early observation (Fondy & Geiger 1977) that uptake by veins and mesophyll hardly differs in absolute terms (Cataldo 1974; Wilson *et al.* 1985; Van Bel & Koops 1985). Mesophyll cells appear to possess more high-affinity low-capacity systems, while vein tissues possess more low-affinity high-capacity systems (Van Bel 1986; Van Bel *et al.* 1986a). But once again, the uptake parameters of the tissues may have been affected by the isolation procedure (see Delrot 1989).

The notion that cells other than minor-vein phloem can largely contribute to the uptake by discs from a medium, has important consequences for interpretation of the results. Making final statements about the mode of phloem loading and the functioning of sieve elements then becomes a risky undertaking. Though unlikely, massive and selective uptake of sucrose, specific conversion of sugars, and sucrose/proton co-transport may be due to the action of mesophyll cells rather than to that of sieve tubes.

4. Intracellular injection of membrane-impermeant fluorescent dyes was employed to provide support for the idea of symplastic phloem-loading. It was aimed to show a symplastic continuity between the mesophyll cells and the sieve elements. In some instances, dye was transferred to the vein, but the arrival of dye in the sieve elements could not be distinguished (Erwee *et al.* 1985; Madore *et al.* 1986). On the basis of microscopic sections of minor veins showing Lucifer Yellow distribution, Fisher (1988) claimed that the dye never reached the sieve element as it halted in the mestome sheath. Dye moved to the minor-vein phloem in leaves of *Commelina* (Van Kesteren *et al.* 1988) and *Cucurbita* (Turgeon & Hepler 1989), but the uninterrupted movement from mesophyll to sieve element was uncertain. Recent work (Farrar *et al.* 1992) demonstrates that the Lucifer Yellow injected into the mesophyll in barley leaves arrives in the sieve tubes identifiable by the luminescence of the sieve plates. One has to realize, however, that dye-coupling only shows potential cell–cell transfer and is not fully conclusive for symplastic loading of photo-assimilates.

Evidence for different modes of loading

In spite of the problems associated with the interpretation of the experiments, the body of evidence obtained for apoplastic loading remains convincing (for arguments see Giaquinta 1983). The attraction of the apoplastic step is that it is so consistent with the observed accumulatory capacity and the selectivity of sugar uptake by the SE/CC-complex (Giaquinta 1983; Turgeon & Beebe 1991).

Symplastic loading lacks such a thermodynamic elegance according to the present standards. Ever since the pioneering work of Madore & Webb (1981), physiological evidence of symplastic loading has been scarce. Good evidence for symplastic phloem-loading was obtained with micro-autoradiography of minor veins of *Cucumis* leaves (Schmitz *et al.* 1987). After feeding intact leaves with $^{14}CO_2$, labelled photosynthate

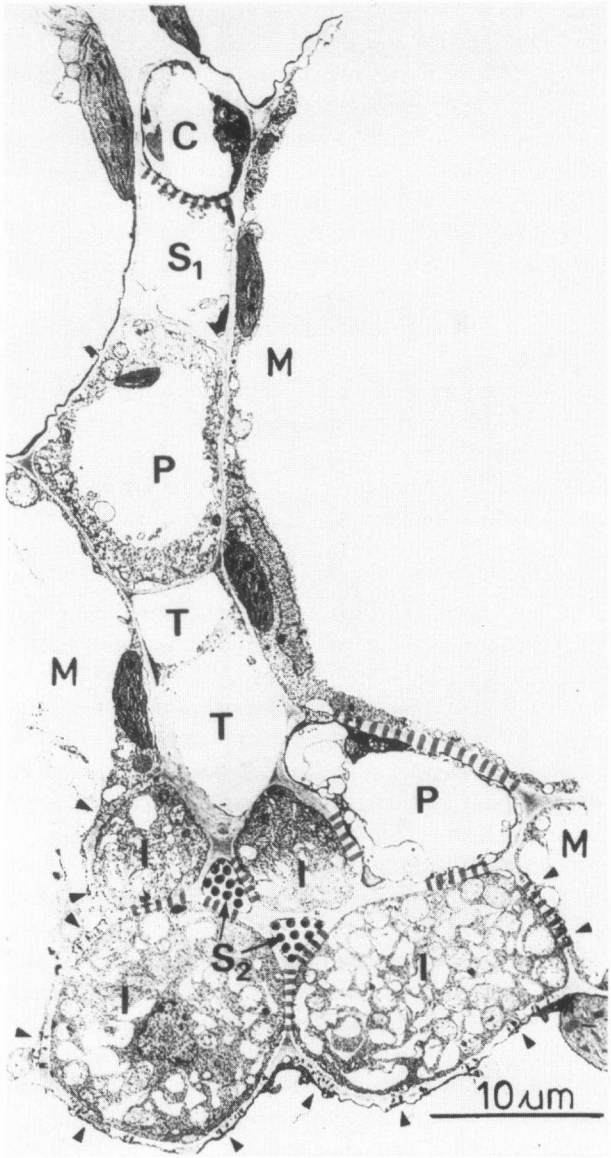

Fig. 5. The ultrastructure, the plasmodesmatal connectivity and the localization of photo-assimilates in the minor vein of *Cucumis* (reconstructed after Schmitz *et al.* 1987). The cross-hatching between the cells indicates the symplastic continuity of the two sieve elements. The stippling represents the location of labelled photosynthate following the feeding of the leaf with $^{14}CO_2$. C: companion cell, S_1: adaxial sieve element, I: intermediary cell, S_2: abaxial sieve element, P: vascular parenchyma, T: tracheid, M: mesophyll cell.

showed up exclusively in those sieve elements symplastically connected with the mesophyll symplast (Fig. 5). Furthermore, PCMBS was unable to block phloem loading (Fig. 6) in *Ipomoea* leaf discs (Madore & Lucas 1987) and in leaf discs or intact leaves of *Coleus* (Turgeon & Wimmers 1988; Turgeon & Gowan 1990). The results imply an absence of apoplastic loading in plants with a symplastic minor-vein configuration.

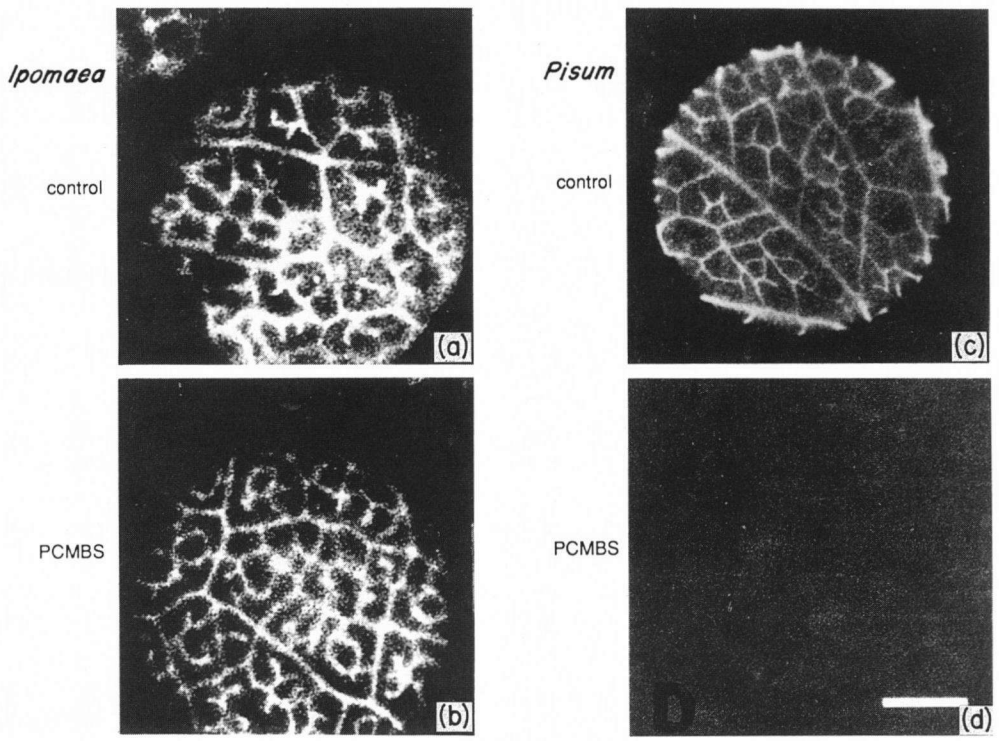

Fig. 6. The effect of PCMBS on phloem loading in the type 1–2a species *Ipomoea tricolor* (Madore & Lucas 1987) and the type 2b species *Pisum sativum* (Turgeon & Wimmers, 1988). Autoradiograms are prints of leaf discs which were fed with $^{14}CO_2$ (*Ipomoea*) or ^{14}C-sucrose (*Pisum*) with or without PCMBS.

The most convincing evidence for different modes of phloem loading comes from the contrasting responses of phloem loading to PCMBS in leaves with different minor-vein configurations (Van Bel *et al.* 1992). Species with strippable leaves were selected from families with a symplastic (type 1) and an outspoken apoplastic (type 2b) minor-vein configuration. PCMBS was unable to block phloem loading in leaf discs of all type 1 species and inhibited phloem loading in all type 2b species (Van Bel *et al.* 1992). To answer the criticism that the disc autoradiograms merely showed uptake by minor veins and not by the sieve elements, photosynthate export from intact leaves has been inhibited with PCMBS in a range of leaves with symplastic and apoplastic minor-vein configuration. The same correlation between the vein configuration and the mode of phloem loading also existed there (A. J. E. van Bel, A. Ammerlaan and A. van Dijk, unpublished results).

Hypothetical mechanisms of the different modes of loading

Although the mechanism of apoplastic phloem-loading seems to be firmly established, many aspects of the process are still unresolved. The (few) plasmodesmograms predict that the site of photo-assimilate release from the mesophyll symplast may differ greatly from family to family (Van Bel *et al.* 1988; Van Bel & Gamalei 1991). Moreover, the nature of the release is still a matter of debate. Some experiments with discs indicate active release (Anderson 1983, 1986; Secor 1987; M'Batchi & Delrot 1988), others passive leakage as part of a pump–leak system (Turgeon 1984; Van Bel *et al.* 1986b). A general belief is that

the sugars are accumulated in the sieve tubes by sugar/proton co-transport (for a review see Giaquinta 1983). The metabolic events in the apoplast of the transition zone may not be universal. Initially, it was thought that sucrose was hydrolysed by an extracellular acid invertase prior to uptake by the SE/CC-complex (Brovchenko 1970). Later, others (e.g. Giaquinta 1977) claimed that the presence of invertase was an artefact due to cutting and aging of the leaf tissue. Autoradiograms of sugar-beet leaf tissues, fed with ^{14}C-labelled sucrose and glucose, showed strong accumulation of sucrose by the veins and no accumulation of glucose at all (Fondy & Geiger 1977). That sucrose is accumulated intact by the phloem was also evidenced by experiments with asymmetrically labelled sucrose (Giaquinta 1977; Daie 1985). Moreover, yeast-derived cell wall invertase most likely prevented phloem loading in transgenic tobacco plants (Von Schaewen *et al.* 1990).

The nature of symplastic phloem-loading remains, of course, a source of controversy. First of all, it is unclear whether the transport occurs up or down the photosynthate gradient. Phloem loading is undoubtedly against the osmotic gradient in most cases (for review see Van Bel 1987). The question is whether the osmotic gradient is synonymous with the photosynthate gradient. Neither the osmotic substances nor the compartmentation of the osmotic substances have been specified. Other evidence for the direction of the photosynthate gradient in symplastic loaders is not fully equivocal. The observed absence of an osmotic gradient between mesophyll and sieve element was presumed to indicate loading down the gradient in *Cucurbita* (Richardson *et al.* 1984). By contrast, plasmolysis experiments showed that the osmotic values in the SE/IC-complex of *Cucurbita* (Turgeon & Hepler 1989) and *Coleus* (Fisher 1986) were much higher than those of the mesophyll cells. Histochemical studies also evidenced a steep, upward sugar-gradient between mesophyll and SE/IC-complex in *Cucurbita* (Pristupa 1983).

The thermodynamic implausibility of symplastic transfer against the concentration gradient has discouraged physiologists from thinking seriously about a possible mechanism. On the other hand, it has inspired others to creative solutions. It has been proposed that plasmodesmata act as valves through which solutes are displaced irreversibly (Giaquinta 1983). Precompartmentation of photosynthate in the mesophyll (Altus & Canny 1985; Wang & Canny 1985) was another attempt to explain symplastic transfer against the gradient. After quasi-loading of a small subcompartment in the mesophyll, photo-assimilates would diffuse via the plasmodesmata to the sieve element. The most elegant proposal is the polymerization trap mechanism (Turgeon 1991). It proposes that sucrose and galactinol produced by the mesophyll diffuse along their concentration gradient to the intermediary cells (Fig. 4). There, they are converted into raffinose and, eventually, stachyose or verbascose. The diameter of raffinose which exceeds the molecular exclusion limit of the plasmodesmata between mesophyll and intermediary cells, prevents any back-flow to the mesophyll. The special construction of these plasmodesmata (Fisher 1986) may hint to a specific task in phloem loading. The hypothesis of Turgeon is corroborated by immunolabelling techniques identifying sucrosylgalactosidases (stachyose synthase) mainly in the intermediary cells (Holthaus & Schmitz 1991).

THE ECOPHYSIOLOGICAL CONCEPT OF PHLOEM LOADING

Phloem loading takes place in those plant organs which are most exposed to the environmental vicissitudes. The buffering capacity of the thin leaf tissues towards these changes is clearly less than that of the thicker and compact stem and the well-protected root. The leaf shape, the shape, size, and positioning of the stomata, the temperature tolerance, the

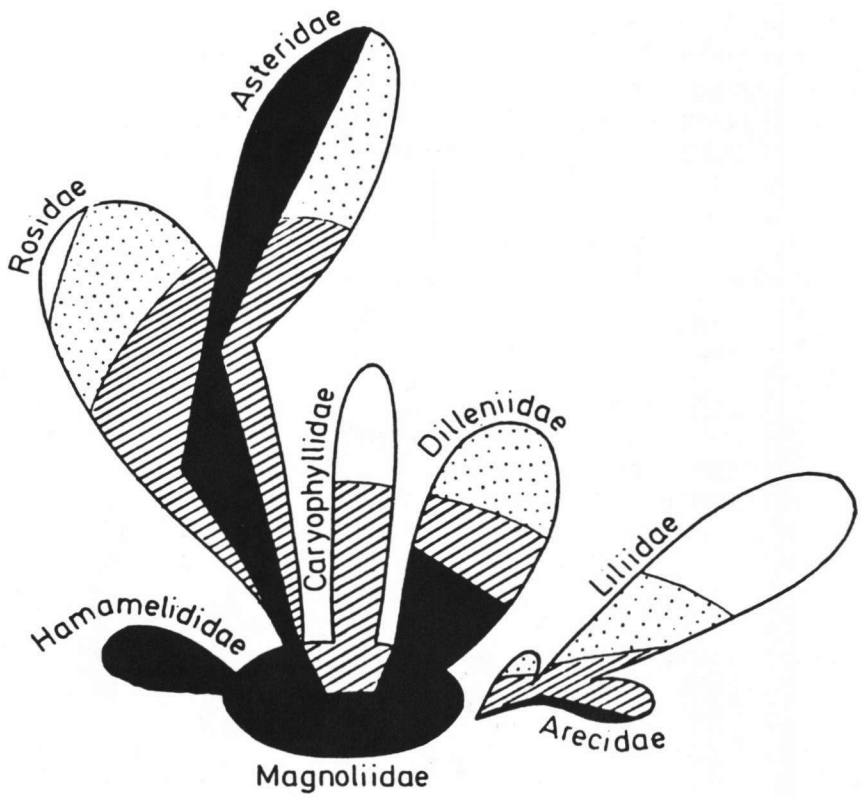

Fig. 7. Scheme of the taxonomic distribution of minor-vein configurations based on the Takhtajan system of flowering plants (Gamalei 1989). Type 1 (black), type 2a (hatched), type 2b (stippled), 2c (white).

mobility of the chloroplasts, the different types of photosynthesis, all can be considered as rigorous adaptations to the climatological regime. The morphology of the minor vein anatomy of the dicotyledons also shows some possibly adaptive specializations (Gamalei 1990). The vein endings of hygrophytes, for instance, do not contain xylem tissue. Xylem tissue is present in the free vein endings of skoto-, meso-, and xerophytes, and in the latter the xylem is topped of sclereids (Gamalei 1990). Thinking along this line, a close relationship between plasmodesmatal minor-vein configuration — and implicitly phloem loading — and habitat would not be illogical. The strong correlation between the minor-vein type and the taxonomic family has made it relatively easy to demonstrate the association between different types of phloem loading and particular growth forms, geographical distribution, and evolutionary history.

Taxonomy and evolution of the minor-vein configuration

The familial minor-vein configuration was projected onto the Takhtajan system of higher-plant evolution (Gamalei 1989). The minor-vein configuration turned out to be layered in evolutionary zones throughout the system (Fig. 7). All ancient families possessed the type 1 configuration and with progressive evolution types 1–2a, 2a and 2b emerged in this order (Fig. 7). Judging from the evolution diagram, type 2c (this is a modified type 2a vein in many C_4 and CAM-plants) is the crown on the vein evolution (Gamalei 1989). The evolutionary order of appearance leads to the speculation that symplastic phloem-loading

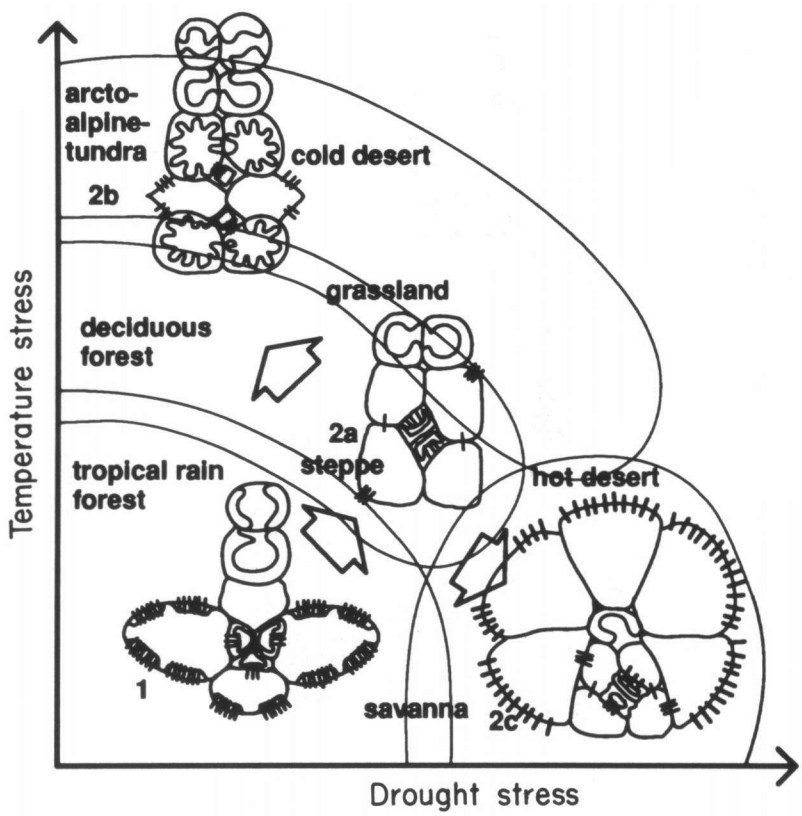

Fig. 8. The distribution of the minor-vein types over various terrestrial ecosystems (Van Bel & Gamalei 1992; modified after Gamalei 1991). With the exception of type 2c, the vein typology is explained in Figure 1. Type 2c is similar to type 2a with regard to the symplastic connections between the SE/CC-complex and the neighbouring cells, but has many conspicuous plasmodesmata between the mesophyll and the bundle sheath (C_4 plants). Typical dicotyledonous 2c-families are the Portulacaceae, Molluginaceae and Amaranthaceae. Many 2c-representatives are found amongst Chenopodiaceae, Polygonaceae, Euphorbiaceae, Poaceae, and Cyperaceae (Gamalei 1989).

is the ancient, strictly apoplastic phloem-loading the most advanced mode of phloem loading (Fig. 7).

The vein configuration is correlated with the growth form (Gamalei 1989). If the comparisons are restricted to the two extremes of the vein typology spectrum (type 1 and 2b), shrubs and trees are abundantly present in 70–80% of the type 1 families, herbs only in 10% of them. In type 2b families, the situation is just the reverse: herbs occur in all families, trees only in 10% of them (Van Bel & Gamalei 1991, 1992).

Correlation between vein configuration and climate

Confronting the global distribution of plant families (Heywood 1978) with the vein typology (Gamalei, 1989) produced an uneven geographical distribution of the minor-vein types (Gamalei 1991; Van Bel & Gamalei 1991, 1992). Type 1 families predominantly reside in the tropics; type 2b families tend to occur in the temperate and boreal zones (Van Bel & Gamalei 1991, 1992). Further specification yields a diagram (Fig. 8) illustrating the distribution of the vein types between various terrestrial ecosystems (Gamalei 1991).

The coincidence between minor-vein configuration, mode of phloem loading (Van Bel *et al.* 1992), and terrestrial ecosystem preference (Gamalei 1991) predicts that specific modes of phloem loading are correlated with specific climate types. So, typically tropical families probably load mainly raffinose-related sugars via the symplastic pathway and typically temperate families load sucrose via the apoplastic route.

Selective pressures for the evolution of minor-vein configuration

Several speculations have been formulated about the drives for evolution of the phloem-loading machinery (Van Bel & Gamalei 1992). Considering the present distribution of the vein types (Gamalei 1991), water stress and low temperature must have imposed a selective pressure on the loading system.

Temperatures below 10°C induce the apparent accumulation of photosynthesis products in the mesophyll cells of species with a symplastic configuration (Gamalei 1990). Such a reaction is absent in species with an apoplastic configuration. The sensitivity of symplastic phloem-loading to temperature may be due to plasmodesmatal dysfunctioning at low temperatures (Minorsky 1985; Van Bel & Gamalei 1992). Increased levels of cytosolic calcium (Minorsky 1985) probably induce the formation of the callose-synthetizing enzyme 1, 3-β-D-glucan synthase (Waldmann *et al.* 1988). Deposition of callose on the orifices of the plasmodesmata would be consistent with the observed blockage of intercellular contact by an elevated level of intracellular calcium (Erwee & Goodwin, 1983).

The temperature-dependence is critical, the more so as symplastic phloem-loading seems to have a limited capacity as indicated by the ultrastructure of the intermediary cells. The number of vesicles in the intermediary cells increases with the quantum flux density of light till a certain threshold, above which starch builds up in the mesophyll (Gamalei 1990). The functioning of the enzymes engaged in the formation of sucrosyl-oligosaccharides or the flux diameter of the plasmodesmata may impose limitations on the symplastic loading-process. Deficient transport in the sieve tubes is another possible reason for the limited export capacity. The mass transfer rate of C-skeletons in the sieve tubes is related to the sugar concentration and its viscosity. Maximal mass flow of organic C is attained at 750 mol m^{-3} sucrose, whereas a raffinose solution becomes saturated (at about 250 mol m^{-3}) before such an optimum is reached (Lang 1978).

Explanations of the drought-sensitivity of symplastic loading are even more hypothetical (Van Bel & Gamalei 1992) than those of the temperature-sensitivity and therefore are not treated here in detail.

Conclusion: association between multiprogrammed phloem-loading and climate

The above considerations have led to the idea that the various programs of phloem loading are correlated with the climate type. This ecophysiological multiprogrammed concept of phloem loading proposes a great diversity in (ultra)structure and metabolism of the phloem-loading zone in concert with the specific requirements of the environment. It also assumes a functional versatility in many species to enable these to deal with climatic variations during the day or the growing season. The ecophysiological concept of phloem loading is admittedly mainly a network of correlations with some scattered physiological evidence. It certainly does not yet explain every possible loading situation in any plant. It may act, however, as a working hypothesis for further experimental studies on the phloem-loading mechanisms and their association with environmental conditions.

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