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REVIEW PAPER

Photosynthetic acclimation in the context of structural constraints to carbon export from leaves

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Abstract The potential role of foliar carbon export features in the acclimation of photosynthetic capacity to differences and changes in light environment was evaluated. These features included apoplastic vs. symplastic phloem loading, density of loading veins, plasmodesmatal frequency in intermediary cells, and the ratio of loading cells to sieve elements. In initial studies, three apoplastic loaders (spinach, pea, *Arabidopsis thaliana*) exhibited a completely flexible photosynthetic response to changing light conditions, while two symplastic loaders (pumpkin, *Verbascum phoeniceum*), although able to adjust to different long-term growth conditions, were more limited in their response when transferred from low (LL) to high (HL) light. This suggested that constraints imposed by the completely physical pathway of sugar export might act as a bottleneck in the export of carbon from LL-acclimated leaves of symplastic loaders. While both symplastic loaders exhibited variable loading vein densities (low in LL and high in HL), none of the three apoplastic loaders initially characterized exhibited such differences. However, an additional apoplastic species (tomato) exhibited similar differences in vein density during continuous growth in different light environments. Furthermore, in contrast to the other apoplastic loaders, photosynthetic acclimation in tomato was not complete following a transfer from LL to HL. This suggests that loading vein density and loading cells per sieve element, and thus apparent loading surface capacity, play a major role in the potential for photosynthetic acclimation to changes in light environment. Photosynthetic acclimation and vein density acclimation were also characterized in the slow-growing, sclerophytic evergreen *Monstera deliciosa*. This evergreen possessed a lower vein density during growth in LL compared to HL and exhibited a more severely limited potential for photosynthetic acclimation to increases in light environment than the rapidly-growing, mesophytic annuals.

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Abbreviations

A	Antheraxanthin
CC	Companion cell
HL	High light
LL	Low light
PC	Phloem parenchyma cell
PFD	Photon flux density
SE	Sieve element

TC Transfer cell
V Violaxanthin
Z Zeaxanthin

Introduction

The level of sink activity in a plant contributes to modulation of photosynthesis rates: when there is a high level of carbohydrate utilization during rapid growth, photosynthesis rates are high, whereas photosynthesis is often downregulated when sink activity is lowered, e.g., if active sinks such as developing fruit are removed (Layne and Flore 1995) or as growth decreases in response to decreased nutrient availability (Paul and Driscoll 1997). Similarly, photosynthesis is downregulated in response to inhibition of sucrose export by cold-girdling of the petioles of leaves (Krapp et al. 1993; Krapp and Stitt 1995), inhibition of sucrose export resulting from overexpression of an apoplastic invertase (Stitt et al. 1991; Krapp et al. 1993), elevated CO_2 levels (Van Oosten and Besford 1996; Makino and Mae 1999), and sugar feeding of plants or leaves (Jones et al. 1996; Smeekens 2000; Rolland et al. 2002). This regulation of photosynthesis by demand occurs via modulation of photosynthetic genes (Krapp and Stitt 1995; Koch 1996; Paul and Driscoll 1997; Paul and Foyer 2001). Repression of the small subunit of Rubisco, ATPsynthase, and Chl *a*/*b*-binding genes (Krapp et al. 1993; Krapp and Stitt 1995) as well as plastocyanin (Dijkwel et al. 1996) and D1 (Kilb et al. 1996) has been reported in response to sugar repression or sink-limiting conditions where export of carbohydrates from leaves was inhibited.

Furthermore, the maximal photosynthetic capacity a plant can exhibit under conditions optimal for growth varies among different species and is higher in annuals compared with evergreen species (see e.g., Demmig-Adams et al. 2006a). Part of this variation is presumably due to differences in inherent, genetically set maximal levels of sink activity (limited by progression through the cell cycle, growth, and developmental program). In addition, the results presented here suggest that some of this limitation (possibly a co-limitation that has evolved along with a suite of other characters) may also be found at the level of foliar carbon export.

The majority of plant species that have been characterized with regard to foliar carbon export have been classified as active phloem loaders. Such species use one of two active physiological mechanisms to accumulate sugars in the sieve elements of the phloem against a concentration gradient, resulting in an increased turgor pressure where sugars accumulate. This increased turgor pressure where sugars are loaded, coupled with a decreased turgor pressure at the sink end where sugars are unloaded and the pressure potential is reduced, results in the mass flow of the contents of the sieve tubes.

In apoplastic loaders, sugars (sucrose or sugar alcohols) diffuse along a concentration gradient through the mesophyll cells to the vein where they enter the apoplast, and are then actively transported into a companion cell and/or sieve element via H^+/sucrose symporters across the cell membrane (Kühn 2003; Lalonde et al. 2003). This is facilitated by the active pumping of protons into the cell wall spaces by ATPases in the plasma membrane of the companion cells (Sondergaard et al. 2004). In species that load exclusively through the apoplast, plasmodesmata (cell wall pores that permit direct movement of substances between adjacent cells) connecting the minor vein phloem to surrounding cells are few in number (Gamalei 1989, 1991)

In model symplastic loaders, on the other hand, plasmodesmatal connections between mesophyll and phloem tissues are frequent, and sugar diffuses all the way from the mesophyll to the sieve elements via plasmodesmata (Gamalei 1989, 1991; van Bel 1993). In these species, the minor vein companion cells are very distinctive, with extremely abundant, highly branched and narrowed plasmodesmata joining them to the bundle sheath. Such anatomically specialized companion cells are known as intermediary cells, and these cells carry out the key step in concentrating sugars in the phloem of symplastic loaders. Sucrose diffuses into the intermediary cells, where units of the monosaccharide galactose are added to the sucrose, creating sugars (primarily the trisaccharide raffinose and the tetrasaccharide stachyose) that are too large to diffuse back through the plasmodesmata to the mesophyll (Beebe and Turgeon 1992; Buchi et al. 1998; Holthaus and Schmitz 1991). This polymer trapping (Turgeon and Gowan 1990) facilitates the accumulation of high concentrations of sugars in the phloem, resulting in the elevated turgor pressure to drive translocation (Haritatos et al. 1996). We hypothesized that loading of sugars through plasmodesmatal pores in symplastic loaders has the potential to impose a physical bottleneck to carbon export in the form of a limited total loading area.

Materials and methods

Plant material

All annual species [*Arabidopsis thaliana* (L.) Heyn. (ecotype Columbia), pea (*Pisum sativum* L. cv. Alaska), pumpkin (*Cucurbita pepo* L. cv. Autumn Gold), *Senecio vulgaris* L., spinach (*Spinacia oleracea* L. cv. Giant Nobel), and tomato (*Solanum lycopersicum* L.)] were grown in growth chambers under different photon flux densities (PFDs), taking care to maintain leaf temperature at a constant moderate temperature (approx. 25°C). Plants were grown in either low light (LL; 100 or 150 μmol photons m^{-2} s^{-1}), high light (HL; 1000 μmol photon m^{-2} s^{-1}), or transferred from LL to HL for 1 week, as previously described (Amiard et al. 2005). In all cases, only fully expanded, mature leaves were characterized. Special care was taken to be sure that only leaves that were fully expanded prior to the transfer from LL to HL were characterized after the week's exposure to HL.

Monstera deliciosa Liebm. was grown in LL (10 μmol photons m^{-2} s^{-1}) or HL (naturally lit greenhouse with a peak PFD of 1500 μmol photons m^{-2} s^{-1}). In addition, LL-grown plants were transferred from LL to HL (700 μmol photons m^{-2} s^{-1}, 12 h light/12 h dark photoperiod; in a temperature- and humidity-controlled growth chamber) for 1 week. Leaf temperatures in both LL and HL in the growth chamber were kept at 25°C during the photoperiod and at 22.5°C during the night.

Photosynthesis and photosystem II efficiency

The capacity of photosynthetic electron transport was ascertained as the CO_2- and light-saturated rate of oxygen evolution from leaves using a leaf disc oxygen electrode with saturating light and CO_2 (5%) (Delieu and Walker 1981; Adams et al. 2002). The efficiency of solar energy conversion in PSII at open centers was determined from the ratio of variable over maximal chlorophyll fluorescence (F_v/F_m; Kitajima and Butler 1975; Adams et al. 1990; Demmig-Adams et al. 1996; Adams and Demmig-Adams 2004). Chlorophyll and carotenoid composition and quantification were determined by high-pressure liquid chromatography using the method of Gilmore and Yamamoto (1991) as modified by Adams and Demmig-Adams (1992) with samples of leaf tissue rapidly frozen in liquid nitrogen.

Ultrastructural and anatomical characterization

Leaf tissue was fixed in glutaraldehyde, embedded in Spurr resin, and examined by electron microscopy using standard procedures (Goggin et al. 2001; Amiard et al. 2005). High-resolution electron microscopy was used to examine and quantify the ultrastructure of the phloem (Amiard et al. 2005). Morphometric analyses of plasmodesmatal densities were conducted as described previously (Volk et al. 1996; Amiard et al. 2005). Transfer wall ingrowths were characterized from electron micrographs, and the degree of wall invagination quantified using our custom-designed software (Wimmers and Turgeon 1991; Amiard et al. 2005). Vein densities were determined using cleared leaf tissue and our custom-designed software (Amiard et al. 2005).

Carbohydrate analyses

The starch content of chloroplasts in palisade cells of the annual species was quantified from electron micrographic images as percent chloroplast area occupied by starch grains (see also Amiard et al. 2005). For *Monstera deliciosa*, leaf tissue (4 cm^2) excluding all major veins was frozen in liquid nitrogen, lyophilized and ground to a fine powder in a mechanical amalgamator, and quantified as described in Logan et al. (1999). For the complete profile generated from a plant growing in high light in a glasshouse, carbohydrates were extracted and measured by high-performance anion-exchange chromatography using pulsed amperometric detection as described by Moore et al. (1997).

Statistical analyses

To test for significant differences among means, analysis of variance was applied followed by a Tukey-Kramer comparison for honestly significant differences (JMP Statistical Software; SAS Institute Inc., Cary, North Carolina). For comparisons between two means, a Student's *t*-test was performed.

Results and discussion

Survey of photosynthetic acclimation and loading vein density in two symplastic and three apoplastic loaders

In two previous studies (Adams et al. 2005; Amiard et al. 2005), the role that physical and physiological features of carbon export play in the acclimation of photosynthesis was examined. Species that load sugars into the phloem via plasmodesmata (symplastic loaders; see Haritatos and Turgeon 1995) were compared with those that export sucrose into the apoplastic space around the phloem and actively load the phloem via

H^+/sucrose symporters (and ATPases that pump protons across the cell membrane) (apoplastic loaders; see Kühn 2003; Lalonde et al. 2003). It was hypothesized that symplastic loaders, relying on a physical feature that is likely to become fixed during development, would exhibit less flexibility in their response to changes in environmental conditions than apoplastic loaders that might be able to simply adjust the numbers of membrane-bound proteins involved in facilitating carbon export. It was predicted that, under certain conditions, the limited number of plasmodesmatal connections in symplastic loaders would represent a bottleneck in the export of carbon, and that this in turn would be reflected in a lesser potential for upregulation of photosynthetic capacity.

This hypothesis was evaluated in five well-characterized species, two of which had been unequivocally shown to be symplastic loaders (pumpkin and *Verbascum phoeniceum*; Turgeon et al. 1993; Volk et al. 1996) and three species that have been shown to load sugars apoplastically (spinach, pea, and *Arabidopsis thaliana*; Wimmers and Turgeon 1991; Lohaus et al. 1995; Haritatos et al. 2000). These five species were characterized after developing under either low light (LL; 100 or 150 µmol photon m^{-2} s^{-1}, 10 h photoperiod) or high light (1000 µmol photons m^{-2} s^{-1}, 14 h photoperiod), or 1 week following transfer from LL to HL. The results from these previous studies (Adams et al. 2005; Amiard et al. 2005) are briefly reviewed here using primarily the results for spinach and pumpkin. Ultrastructural analysis of the intermediary cells (specialized companion cells with many plasmodesmata) of both pumpkin and *Verbascum* revealed no difference in the frequency of plasmodesmata on a per cell basis among the three treatments (Amiard et al. 2005). However, whereas the foliar density of minor loading veins did not vary among treatments in *Arabidopsis* (Adams et al. 2005), pea (Amiard et al. 2005), or spinach (Figs. 1a, 2), in both symplastic loaders it was significantly lower in LL or LL → HL-transferred plants than in plants grown in HL (pumpkin in Figs. 1a, 2). Thus, through the increased density of loading veins, and consequently, increased number of intermediary cells and their associated plasmodesmata on a per unit leaf area basis, HL-grown plants of pumpkin and *Verbascum* had a significantly greater cross-sectional area available for the physical export of carbon via symplastic loading compared with either LL-grown or LL → HL-transferred plants. The venation pattern in all of these species was open reticulate (net-like), as is commonly seen in dicots (Essau 1977).

Both spinach (Fig. 1b) and pea (not shown), as apoplastic loaders presumably capable of rapidly

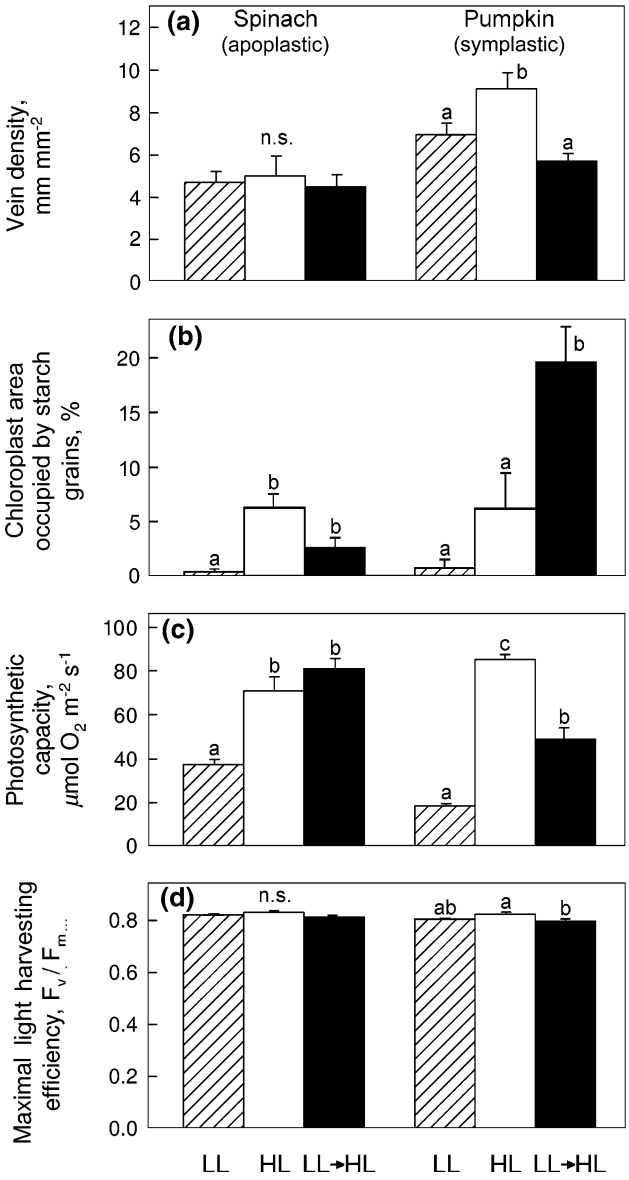

Fig. 1 Vein density ((**a**) minor loading veins only, determined from 9 mm^2 sections of leaf tissue), palisade cell starch content ((**b**) percentage of chloroplast area occupied by starch grains), photosynthetic capacity ((**c**) light- and CO_2-saturated rate of O_2 evolution at 25°C), and maximal light harvesting efficiency ((**d**) PSII efficiency, F_v/F_m after 12 h dark) in fully-expanded, mature leaves of the apoplastic loader spinach and the symplastic loader pumpkin grown in LL, HL, or in LL, and then transferred to HL for 1 week. Means ± standard deviation; significant differences among means within each panel are indicated by lower case letters at $P < 0.05$; n.s. = not significantly different. Vein density, starch data (expressed on a different basis), and photosynthetic capacity from Amiard et al. (2005)

adjusting the level of sucrose transport proteins, exhibited no accumulation of starch (beyond that observed in HL-acclimated leaves), and all three apoplastic loaders fully upregulated photosynthetic capacity (to the level seen in HL-acclimated leaves;

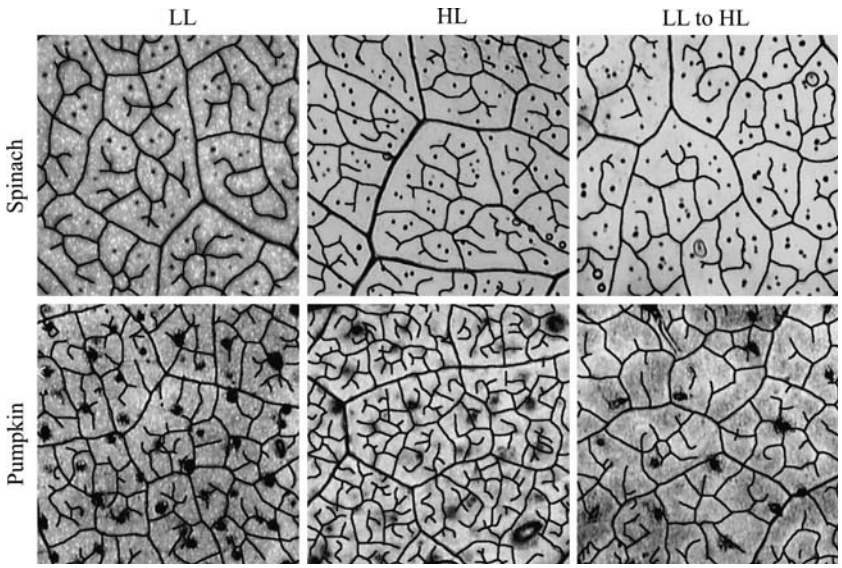

Fig. 2 Images showing the pattern and density of leaf venation in 9 mm^2 sections from mature leaves of the apoplastic loader spinach and the symplastic loader pumpkin grown in low light (LL), high light (HL), or transferred from LL to HL. Veins were highlighted in black. From Amiard et al. (2005)

Fig. 1c for spinach) within 1 week after transfer of fully expanded leaves from LL to HL (Adams et al. 2005; Amiard et al. 2005). In contrast, following an identical transfer, fully-expanded leaves of the symplastic loaders *Verbascum* (Amiard et al. 2005) and pumpkin (Fig. 1b) exhibited increased levels of starch and a photosynthetic capacity (Fig. 1c) that was elevated above LL-acclimated plants, but remained significantly below that in HL-acclimated plants. Thus, the prediction of an incomplete upregulation of photosynthetic capacity in the symplastic loaders, but not for apoplastic loaders, was supported by the results from these few species. While maximal efficiency of light harvesting was high in both species under all conditions (Fig. 1d), this efficiency was slightly lower after transfer compared to growth under high light in pumpkin, but not in spinach.

The results from this study, however, raised some additional questions. In pea, the level of transfer cell (specialized companion cells with wall invaginations; see Offler et al. 2003) wall ingrowths, and thus cell membrane surface area available for transport of sucrose, was at 33 and 178% in LL- and HL-acclimated leaves, respectively (Amiard et al. 2005). A companion cell with no ingrowths was arbitrarily set to a level of wall ingrowths equal to 0%, and thus even LL-acclimated leaves exhibited a small amount of wall invaginations. Fully expanded LL leaves transferred to HL for 1 week increased the transfer cell wall ingrowths to the same level as that in HL-acclimated leaves (Amiard et al. 2005), thus providing an increased surface area for transport and for additional proteins involved in sucrose transport and phloem loading. However, spinach possesses no such specialized companion cells, and yet fully expanded LL-acclimated leaves transferred to HL exhibited full upregulation of photosynthetic capacity to the level present in HL-acclimated leaves (Fig. 1c). Furthermore, the photosynthetic capacity of HL-acclimated and LL to HL transferred leaves of spinach (71 and 81 μmol O_2 m^{-2} s^{-1}) was almost twice that of similarly treated pea leaves (44 and 43 μmol O_2 m^{-2} s^{-1}). Although initial studies suggested that the upper limit to photosynthetic capacity might be strongly influenced by foliar loading vein density (based upon a linear correlation among pumpkin, pea, and *Arabidopsis thaliana*; Adams et al. 2005), spinach has a vein density that is similar to pea (Amiard et al. 2005) and significantly less than that of HL-acclimated pumpkin (Fig. 1a), and yet has a maximal photosynthetic capacity similar to pumpkin (Fig. 1c). Other possible explanations for the high maximal photosynthetic capacity of spinach leaves acclimated to HL were thus explored.

A comparison of the phloem anatomy of pea and spinach (Fig. 3), which have virtually identical foliar vein densities as stated above, revealed that spinach possessed almost twice as many companion cells per sieve element compared to pea (Table 1) irrespective of growth light environment. It was thus hypothesized that spinach is able to achieve its high maximal photosynthetic capacities relative to pea by virtue of a larger cross-sectional area of companion cells in the minor loading veins and a greater number of cells participating in the loading of each sieve element in the phloem, all leading to a greater sugar export capacity in spinach (Amiard et al. 2005). In fact, as additional

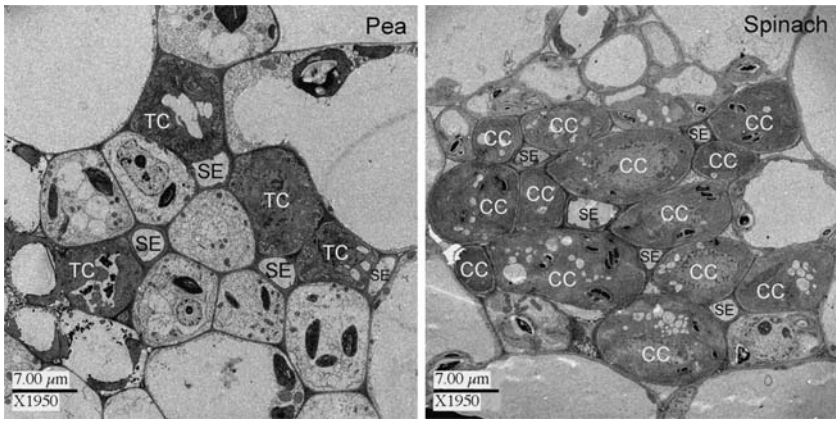

Fig. 3 Transverse images of the phloem (minor loading vein) of pea or spinach leaves. Images illustrate the arrangement of transfer cells (TC) in pea and companion cells (CC) in spinach that are likely to be involved in the export of sucrose from the leaf mesophyll cells to the phloem by means of cell membrane-localized H^+/sucrose symporters for the loading of sieve elements (SE). From Amiard et al. (2005)

species have been examined, a more general model of the factors that contribute to carbon export capacity that may influence both maximal photosynthetic capacity and the flexibility for adjustments in this capacity has begun to coalesce. Thus far, the phloem features identified as being associated with the maximal photosynthetic capacity (i.e., under otherwise favorable conditions that result in maximal rates of growth and/or maximal sink activity) that an apoplastically loading species might achieve include the total companion cell surface area available for sucrose transport (simple companion cells vs. transfer cells with wall ingrowths and thus magnified cell membrane surface area for transport) and the number of loading cells per sieve element (Fig. 3). In the present paper, additional species have been examined to determine if their features are consistent with this emerging model of anatomical and ultrastructural factors that contribute to setting the upper limit to photosynthetic capacity (see below).

Table 1 shows the loading cells per sieve element, vein density, and photosynthetic capacity, as well as an indication of where transfer cells with wall ingrowths are present for five species that load sugars apoplastically. *Arabidopsis thaliana*, with an intermediate number of normal companion cells per sieve element

Table 1 Loading cells per sieve element (SE), minor loading vein density (as mm vein length mm^{-2} leaf area), and light- and CO_2-saturated rates of oxygen evolution (photosynthetic capacity) in leaves of *Arabidopsis thaliana*, tomato, pea, spinach, and *Senecio vulgaris* grown under HL

Species loading cell type	Number of loading cells per SE	Loading vein density (mm mm^{-2})	Photosynthetic capacity (μmol O_2 m^{-2} s^{-1})
Arabidopsis thaliana			
Regular CCs	2.3 ± 0.7^{b} ($n = 42$)	3.1 ± 0.4^{a}	33 ± 3^{a}
Solanum lycopersicum			
Regular CCs	$1.8 \pm 1.0^{a,b}$ ($n = 80$)	$4.6 \pm 0.4^{a,b}$	$39 \pm 7^{a,b}$
Pisum sativum			
Transfer cells (CCs)	1.7 ± 0.5^{a} ($n = 38$)	5.3 ± 0.3^{b}	44 ± 4^{b}
Spinacia oleracea			
Regular CCs	2.9 ± 0.8^{b} ($n = 52$)	5.0 ± 1.0^{b}	71 ± 6^{c}
Senecio vulgaris			
Transfer cells (CCs + PCs)	3.8 ± 0.8^{c} ($n = 80$)	3.9 ± 0.3^{a}	74 ± 5^{c}

Means ± standard deviation are shown; number of leaves examined were three for vein density and photosynthesis, and minimally six leaves characterized for the number of loading cells per SE, with n = number of examined SEs given in brackets following each value. Significant differences among the means are indicated by the superscripted letters at $P < 0.05$. Transfer cells are specialized companion cells with cell wall ingrowths that greatly increase the total cell membrane surface area for sucrose transport and phloem loading. Vein density and photosynthetic capacity of *Arabidopsis* from Adams et al. (2005), data on pea and spinach from Amiard et al. (2005), and data on *Senecio vulgaris* and loading cells per SE in *Arabidopsis* from Amiard et al. (2007). CCs = companion cells; PCs = phloem parenchyma cells

and the lowest vein density, had the lowest photosynthetic capacity. Pea, with the lowest number of companion cells (that do, however, have an increased cell membrane area) and the highest vein density, had the next highest photosynthetic capacity. *Senecio vulgaris* and spinach may have achieved the highest photosynthetic capacities through a combination of higher numbers of loading cells per sieve element (both species), cell wall invaginations and increased cell membrane surface area (*S. vulgaris*), and a high vein density (spinach). From this, it appears that several parameters may be interacting to determine the potential maximal rate of photosynthesis (and assimilate export). Future research should address this phenomenon in an even more comprehensive manner by considering all factors that may contribute to total phloem loading surface, including those considered here, i.e., (i) the number of loading cells per sieve element and (ii) the foliar loading vein density, as well as the additional parameters (iii) companion cell wall perimeter as an estimate of cell membrane surface area and (iv) the number of sieve elements per loading vein.

Photosynthetic acclimation in an apoplastic loader that adjusts loading vein density

The results presented above showed that the apoplastic loaders tested [pea, spinach (Fig. 1), and *Arabidopsis thaliana*] possessed foliar vein densities that were constant regardless of growth light conditions, whereas the leaves of the symplastic loaders tested [pumpkin (Fig. 1) and *Verbascum phoeniceum*] developed with low vein densities in low light and high vein densities in high light (Adams et al. 2005; Amiard et al. 2005). While intriguing, these data do not allow one to broadly categorize all symplastic loaders as exhibiting less photosynthetic flexibility than all apoplastic loaders. For instance, the apoplastic loader tomato (Weise et al. 2000) behaves like the two symplastic loaders mentioned above in this respect (Figs. 4, 5). Tomato exhibits an open reticulate venation pattern like the other dicots described above, and loading vein density differs in LL vs. HL and no increase occurs during transfer from LL to HL (Figs. 4a, 5). Furthermore, tomato shows incomplete upregulation of photosynthetic acclimation during transfer from LL to HL (Fig. 4b). While maximal light harvesting efficiency remains quite high under all growth conditions, tomato shows a minor decrease upon transfer from LL to HL (Fig. 4c) as did the symplastic loader pumpkin (Fig. 1d). It thus appears that a relatively low loading vein density may limit the ability of tomato to fully upregulate photosynthetic capacity upon transfer of

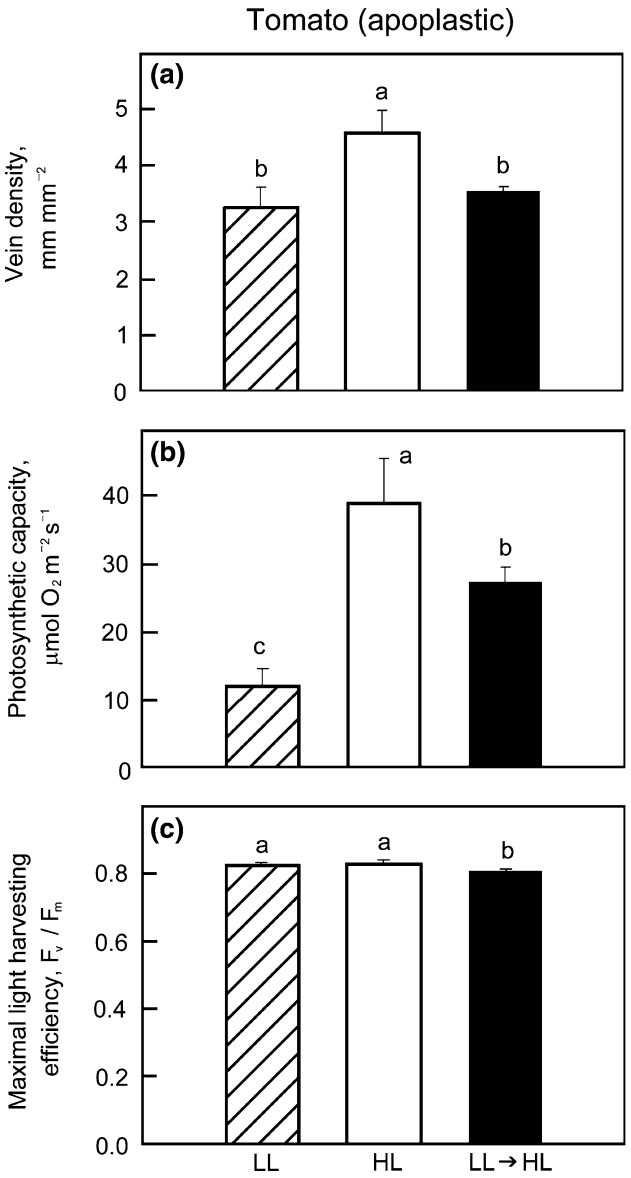

Fig. 4 Vein density ((**a**) minor loading veins only, determined from 9 mm^2 sections of leaf tissue), photosynthetic capacity ((**b**) light- and CO_2-saturated rate of O_2 evolution at 25°C), and maximal light harvesting efficiency ((**c**) PSII efficiency, F_v/F_m after 12 h dark) in fully-expanded, mature leaves of the apoplastic loader tomato grown in LL, HL, or in LL and then transferred to HL for 1 week. Means ± standard deviation; significant differences among means within each panel are indicated by lower case letters at $P < 0.05$

mature leaves from LL to HL, as was the case for the symplastic loaders (Amiard et al. 2005). Even in an apoplastic loader, there can thus apparently be a physical limitation by total loading surface. The presence or absence of vein density adjustments between long-term growth in LL vs. HL thus appears to be a better predictor for the flexibility of photosynthetic

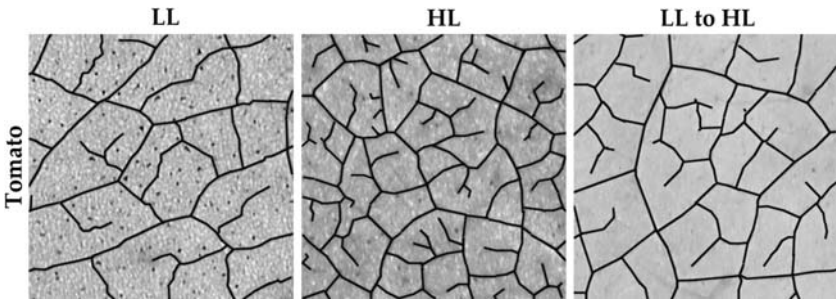

Fig. 5 Images showing the pattern and density of leaf venation in 9 mm^2 sections from the apoplastic loader tomato in mature leaves grown in LL, HL, or transferred from LL to HL. Veins were highlighted in black

acclimation than the phloem-loading mode per se or alone.

Downregulation of photosynthetic efficiency in an evergreen upon transfer from low to high light

As described above, transfer from LL to HL resulted in upregulation of photosynthesis (with variation in the degree of the response among the different species), with no strong decreases in light harvesting efficiency. However, all of those species were short-lived, rapidly growing, mesophytic plants, well characterized as either symplastic or apoplastic phloem loaders. An additional species the evergreen sclerophyte *Monstera deliciosa* that exhibits pronounced decreases in maximal light harvesting efficiency when transferred from shade to high light and no upregulation of photosynthesis (Demmig-Adams et al. 1989; Ebbert et al. 2001), was also examined. As a monocot, *M. deliciosa* does not show the parallel venation pattern typical for many monocots (Essau 1977), but instead exhibits a reticulate venation pattern (Fig. 6) more similar to that of the dicots described above (Figs. 2, 5). However, in contrast to the open reticulate pattern in these dicots (where many veinlets terminate within the mesophyll tissue), *M. deliciosa*'s venation pattern is largely closed, with the veinlets connected to each other (Fig. 6). *Monstera deliciosa* shows adjustment of loading vein density during continuous growth in different light environments (low in shade leaves; high in sun leaves; no increase in shade leaves transferred to high light; Figs. 6 and 7a; see also Adams et al. 2006), as documented in the two symplastic loaders above as well as in tomato (Figs. 1a, 2, 4a). Since the phloem-loading mode of *Monstera deliciosa* was unknown, we characterized this species and have obtained features consistent with apoplastic loading (Amiard V, Turgeon R, Adams W, & Demmig-Adams B, unpublished data). Sucrose is the primary sugar transported in the phloem (data not shown).

Similar to the other species characterized above, which adjust vein density during continuous growth in different light environments, *M. deliciosa* also failed to fully upregulate photosynthetic capacity during transfer from LL to HL (Fig. 7b). In contrast to the annual species, however, *M. deliciosa* showed no upregulation over the course of a week (Fig. 7b). In addition, *M. deliciosa* exhibited strong and sustained depressions in maximal light harvesting efficiency (Fig. 7c), while the annual species employing vein density adjustments only showed very minor decreases. Despite the absence of photosynthetic upregulation in

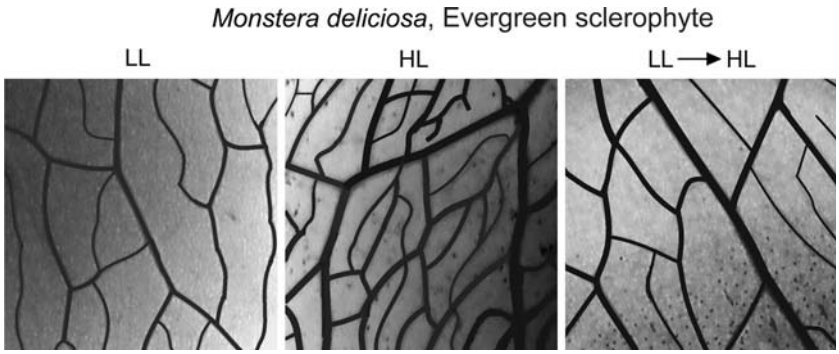

Fig. 6 Images showing the pattern and density of leaf venation in 9 mm^2 sections from mature leaves of *Monstera deliciosa* growing in low light = LL (10 μmol photons m^{-2} s^{-1}), high light = HL (peak of 1500 μmol photons m^{-2} s^{-1} in a glasshouse), and following 1 week of exposure to 700 μmol photons m^{-2} s^{-1} after transfer from 10 μmol photons m^{-2} s^{-1} (LL → HL). Veins were highlighted in black

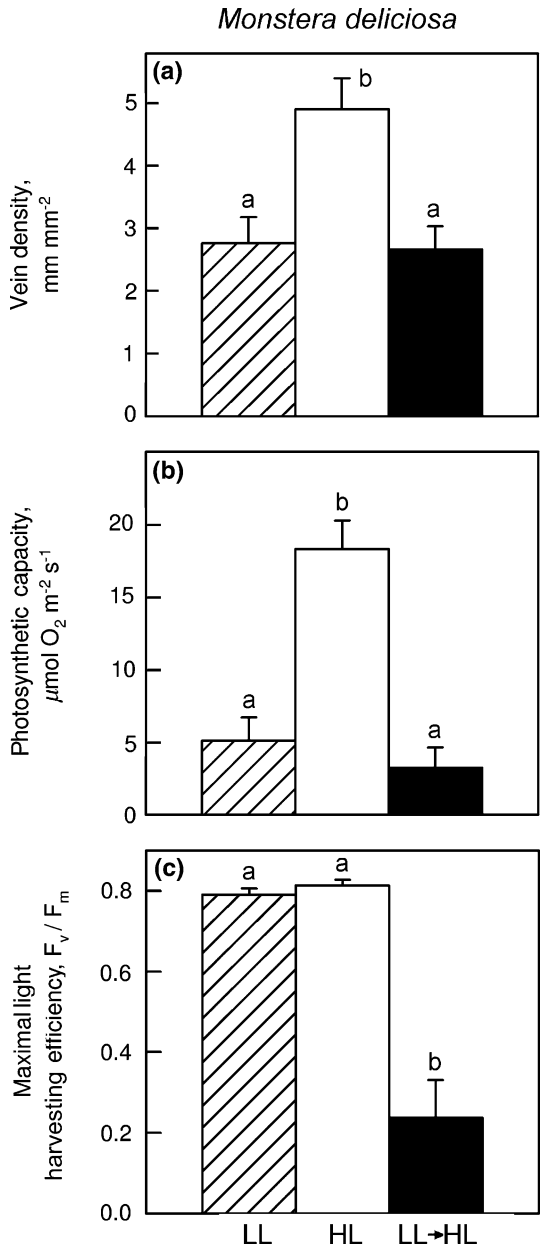

Fig. 7 Vein density ((**a**), minor loading veins only, determined from 9 mm^2 sections of leaf tissue), photosynthetic capacity ((**b**), light- and CO_2-saturated rate of O_2 evolution at 25°C), and maximal light harvesting efficiency ((**c**), PSII efficiency, F_v/F_m after 12 h dark) in fully-expanded, mature leaves of *Monstera deliciosa* grown under a PFD of 10 μmol photons m^{-2} s^{-1} (LL), in a glasshouse with a peak PFD of 1500 μmol photons m^{-2} s^{-1} (HL), and following 1 week of exposure to 700 μmol photons m^{-2} s^{-1} after transfer from 10 μmol photons m^{-2} s^{-1} (LL → HL). Means ± standard deviation; significant differences among means within each panel are indicated by the lower case letters at $P < 0.05$

HL, there were no signs of damage to the leaves. The deep green, shade-grown *M. deliciosa* leaves maintained their high chlorophyll content over the course of the week at the increased growth PFD, showing that there was no major chlorophyll bleaching. Chlorophyll content per leaf area was 707 ± 63 and 666 ± 76 μmol Chl m^{-2} before transfer and after 1 week at the increased PFD, respectively (no significant differences). This was presumably due to photoprotection by the xanthophyll cycle whose pool increased from 43 ± 1 to 93 ± 8 mmol (V+A+Z) mol^{-1} Chl ($P < 0.001$) from before transfer to 1 week following the increased PFD, respectively.

This increase in xanthophyll cycle pool was associated with an increase in Z+A levels both at the end of the day and the end of the night over the course of the week in HL as was shown previously (Demmig-Adams et al. 1989; see also Demmig-Adams et al. 1998). The sustained decreases in maximal light harvesting efficiency, coupled with nocturnal retention of large amounts of zeaxanthin and antheraxanthin (not shown), indicate employment of high levels of photoprotective energy dissipation (Adams and Demmig-Adams 2004; Adams et al. 2006; Demmig-Adams and Adams 2006; Demmig-Adams et al. 2006b). This is exactly what is expected when photosynthetic rate remains low and the level of excess light is high (Adams et al. 2006; Demmig-Adams et al. 2006b).

Moreover, changes in carbohydrate levels were also followed over an entire week upon transfer to HL (Fig. 8). Total nonstructural carbohydrates and starch

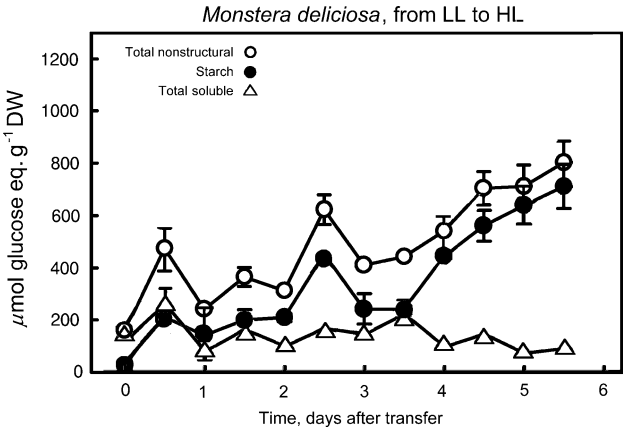

Fig. 8 Changes in total nonstructural carbohydrates (starch + sucrose + glucose + fructose), starch, and total soluble carbohydrates (sucrose + glucose + fructose), from leaves of *Monstera deliciosa* plants transferred from 10 to 700 μmol photons m^{-2} s^{-1} during the photoperiod (12 h day, 12 h night). During the first three days, end of light samples (0.5, 1.5, and 2.5 days) exhibited carbohydrate levels that were greater than the end of dark samples (0, 1, 2, and 3 days), whereas such diel variation disappeared after day 3. Where it applies, triplicate samples for carbohydrates were taken from three different plants (one leaf per plant; means ± standard deviation depicted). Statistical analysis of data from 0 vs. 168 h showed significant differences for total non-structural carbohydrates ($P < 0.0001$) and starch ($P < 0.001$), but not for total soluble carbohydrates

levels increased significantly over the course of the week. In contrast, HL-grown plants of *M. deliciosa*, that have higher photosynthetic capacities than leaves grown in LL and do not show sustained decreases in maximal light harvesting efficiency (Fig. 7b, c), did not contain high levels of accumulated carbohydrates and exhibited particularly low levels of starch. Samples collected at midday from plants growing on the south side of a sun-lit greenhouse contained only 5.2, 5.1, 46.2, and 2.4 μmol glucose equivalents per g dry weight leaf tissue of glucose, fructose, sucrose, and starch, respectively.

Carbohydrate, and particularly starch, production is thus not limiting in LL-grown *M. deliciosa* plants transferred to HL, but instead carbon utilization and export is apparently limited. Under conditions repressing photosynthesis, various aspects of carbon storage (including starch synthesis) have been shown to be stimulated, whereas processes associated with carbon export (such as sucrose synthesis) are repressed (Koch 1996).

Additional experiments were done to examine whether a single exposed leaf might show less of a decrease in maximal efficiency of light harvesting than a leaf on a plant where all leaves absorb strongly excessive light. Deep shade-grown *M. deliciosa* plants were transferred to HL either with all their leaves exposed or with only a single leaf per plant exposed and the rest of the plant kept at limiting PFD under a shade cloth (Fig. 9). This experiment was designed to test the possibility that the decrease in maximal light harvesting efficiency might respond to an imbalance in whole plant source-sink balance. However, there was no indication that the single leaf experienced less sustained depressions, as changes in maximal light harvesting efficiency developed similarly in both plants. This finding is consistent with the conclusion, drawn in the present paper, that a bottleneck instead exists at the level of carbon export from individual leaves.

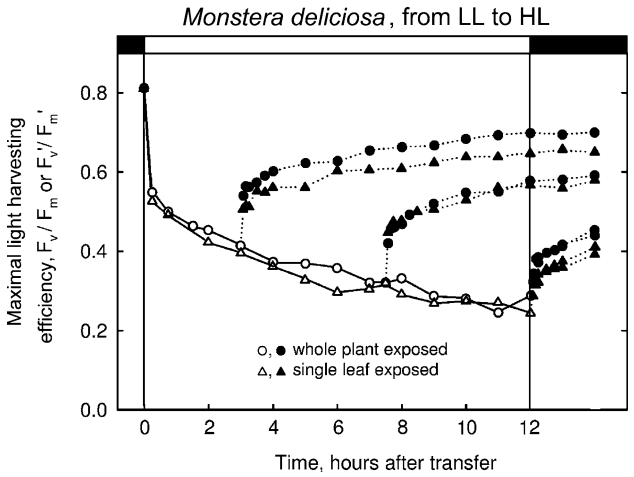

Fig. 9 Changes in maximal light harvesting efficiency (PSII efficiency; filled symbols = F_v/F_m in darkness; open symbols = F_v'/F_m' during actinic light exposure) upon transfer of *Monstera deliciosa* plants from 10 to 700 μmol photons m^{-2} s^{-1} for 1 day. Open symbols indicate measurements taken from the same leaf in light, while filled symbols are leaf discs darkened after varying lengths of high light exposure. Measurements taken from leaves of whole plants exposed to the high light are represented by triangles, while measurements taken from a single exposed leaf on a covered plant are represented by circles

The results available to date do not offer an explanation for the much more severe limitation to photosynthetic acclimation in the evergreen, sclerophyllous monocot *Monstera deliciosa* compared with the annual, mesophytic dicots. However, it may be rewarding to address, e.g., the possibility that palisade cell elongation during transfer from LL to HL may be more severely restricted in the sclerophyte than in annuals. Among the annuals characterized, most species showed an elongation of palisade cells during transfer from LL to HL, with stronger responses in those species that showed complete photosynthetic acclimation (Amiard et al. 2005). In addition, one may also address the possibility that the closed reticulate venation patterns seen in *M. deliciosa* may be more limiting to foliar sugar export than the open venation pattern seen in all of the annual mesophytes.

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