Anatomical and photosynthetic acclimation to the light environment in species with differing mechanisms of phloem loading

Véronique Amiard*, Kristine E. Mueh*, Barbara Demmig-Adams*, Volker Ebbert*, Robert Turgeon†, and William W. Adams III*‡

*Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309-0334; and †Department of Plant Biology, Cornell University, 256 Plant Science Building, Ithaca, NY 14853

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Plants load sugars from photosynthesizing leaves into the phloem of exporting veins either "apoplastically" (by using H^+/sucrose symporters) or "symplastically" (through plasmodesmata). The ability to regulate photosynthesis in response to the light environment was compared among apoplastic loaders (pea and spinach) and symplastic loaders (pumpkin and *Verbascum phoeniceum*). Plants were grown under low light (LL) or high light (HL) or transferred from LL to HL. Upon transfer, pea and spinach up-regulated photosynthesis to the level found in HL-acclimated plants, whereas up-regulation in pumpkin and *V. phoeniceum* was limited. The vein density of pea and spinach was the same in HL and LL. Although spinach did not exhibit anatomical or ultrastructural acclimation to the light environment, in pea, wall invaginations in minor vein companion (transfer) cells were more extensive in HL. Furthermore, upon transfer from LL to HL, these invaginations increased in mature pea leaves. Foliar starch levels in mature leaves of plants transferred from LL to HL were not greater than in HL-acclimated leaves of either apoplastically loading species. In the symplastic loaders, plasmodesmatal frequency per loading cell did not vary with treatment, but vein density and thus total plasmodesmatal frequency were higher in HL. Upon transfer of symplastic loaders, however, vein density remained low, and starch levels were higher than in HL; the incomplete acclimation of photosynthesis upon transfer is thus consistent with a carbon export capacity physically limited by an inability to increase vein and plasmodesmatal density in a mature leaf.

apoplastic loading | leaf vein density | photosynthesis | symplastic loading | transfer cells

Plants adjust their capacity to carry out photosynthesis in response to the demand for photosynthetic products. This balance between source (mature, photosynthesizing leaves) and sinks (growing, metabolizing, and storing tissues) is continuously adjusted in response to environmental and developmental cues (1, 2). When sink activity is high during rapid growth, photosynthesis rates are high, whereas photosynthesis is typically down-regulated when sink activity is lowered [for example, after fruit removal (3) or in response to decreased soil nitrogen availability (4)]. Similarly, photosynthesis is down-regulated in response to external sugar feeding of leaves or plants (5–7), inhibition of sucrose export resulting from overexpression of an apoplastic invertase (8–10), elevated CO_2 levels (11, 12), and inhibition of export by cold-girdling petioles (10, 13). Despite the preponderance of evidence from such manipulative experiments in support of source–sink feedback regulation of photosynthetic capacity, there has been no examination of the potential role of variation in foliar carbon export features that might influence acclimation of photosynthesis to varying environmental conditions.

One of the most profound differences among plant species in terms of carbon export from source tissues is the mode of phloem loading, which is divided into two types (14–16). In some species, sucrose from the mesophyll enters the cell wall space (part of the apoplast) and is actively taken up by the companion cells (CCs) and/or sieve elements (SEs) of the minor veins by means of H^+/sucrose symporters. Species with this mode of phloem loading are referred to as apoplastic loaders. The other type of phloem loading is found in species in which sucrose gains access to the phloem through plasmodesmata. These symplastic loaders maintain a diffusion gradient by converting the sucrose to larger sugars (raffinose and stachyose) in the minor vein CCs (17–20). They have distinctive minor vein CCs called intermediary cells. Intermediary cells are characterized by numerous plasmodesmata that permit sucrose diffusion from the adjoining bundle sheath (BS) cells but presumably prevent the larger sugars, raffinose and stachyose, from diffusing back into the BS and mesophyll cells ["polymer trapping" (21)]. A third mechanism of moving sugars within plants involves the diffusion of sugars (sucrose) via a pathway similar to that used by symplastic loaders (via plasmodesmata) but does not involve a means of concentrating the sugars in the phloem (22), and plants using this mechanism are known as nonloading species.

The present study was undertaken to examine whether photosynthetic acclimation to different light environments is different between species that load sugars into the phloem apoplastically vs. symplastically. Four species were chosen in which the mode of phloem loading has been determined: the apoplastic loaders pea (23) and spinach (24) and the symplastic loaders pumpkin (25) and *Verbascum phoeniceum* (26). These four species also provide comparable pairs in terms of growth habit; pea and pumpkin are vines, whereas spinach and *V. phoeniceum* are rosettes. Both pumpkin and members of the genus *Verbascum* have distinctive intermediary cells that are characteristic of symplastically loading species (25, 26). Pea possesses specialized minor vein CCs [termed transfer cells (TCs)] with cell wall invaginations that result in an increased plasma membrane surface area (23, 27), possibly to accommodate increased levels of H^+/sucrose symporters and ATPases. The minor vein CCs of spinach have neither abundant plasmodesmata nor wall invaginations.

Parameters characterized in the present study include photosynthetic capacity, chlorophyll and starch levels, cell wall morphology, number of plasmodesmata, vein density, and leaf thickness. We hypothesized that apoplastic loading offers greater flexibility for photosynthetic acclimation upon transfer from one light environment to another, whereas symplastic loading is less flexible due to physical constraints imposed by the

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Abbreviations: BS, bundle sheath; CC, companion cell; HL, high light; LL, low light; SE, sieve element; TC, transfer cell.

‡To whom correspondence should be addressed. E-mail: william.adams@colorado.edu.

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plasmodesmata that are formed during leaf development and serve as potential bottlenecks in carbon export.

Materials and Methods

Plant Material. *Pisum sativum* L. cv. Alaska (pea), *Spinacia oleracea* L. cv. Giant Nobel (spinach), *Cucurbita pepo* L. cv. Autumn Gold (pumpkin), and *V. phoeniceum* L. were grown from seed in Fafard Canadian Growing Mix 2 (70% Canadian sphagnum peat, perlite, and vermiculite) and received nutrients every other day. Plants were grown under low light (LL) (100 μmol of photons $m^{-2}{\cdot}s^{-1}$ for pea, pumpkin, and *V. phoeniceum* and 150 μmol of photons $m^{-2}{\cdot}s^{-1}$ for spinach with fluorescent light bulbs and a 10-h photoperiod per day) or high light (HL) (1,000 μmol of photons $m^{-2}{\cdot}s^{-1}$ with 1,000-W metal halide lamps and 14-h photoperiod per day) or transferred from LL to HL for 1 week, all at 25°C during the day and 20°C at night. During growth in HL, plants received water every second day when they did not receive nutrients. In addition, pea plants grown in HL were also transferred to LL, and the level of cell wall invagination was assessed. All plants were young and completely vegetative, and only fully expanded, mature leaves were characterized (the second true leaf for pumpkin and pea; in spinach and *V. phoeniceum*, the first true leaf was characterized).

Vein Density. Leaf pieces were cut to avoid midribs and secondary veins, immersed in a 3:1 (vol/vol) mixture of 95% ethanol and glacial acetic acid, washed in water, and cleared in glacial acetic acid. Leaves were scanned (transmission mode) at a resolution of 3,200 dots per inch, and total minor loading vein length was measured in 9-mm^2 areas by using custom-designed EEB VIEWER software.

Starch Content. Starch content of leaves that were harvested and fixed early in the day was quantified in electron microscopic images of chloroplasts as the area occupied by starch grains relative to the total length of palisade cells. The latter was chosen as a basis of expression rather than the ratio of starch area to chloroplast area because the size of the chloroplasts varied among treatments. The length of the palisade cells was chosen rather than the area of the palisade cells because the area is largely composed of vacuole that also varied among species, and the chloroplasts are aligned within the thin cytosol between the vacuole and plasma membrane on either side of the elongated palisade cells. All of the chloroplasts in cells from the uppermost palisade layer from three leaves for each species/treatment were assessed in this manner.

Photosynthetic Capacity. The capacity of photosynthetic oxygen evolution at 25°C was determined in a leaf disk oxygen electrode (28) under saturating CO_2 (5%) and light (1,475 μmol of photons $m^{-2}{\cdot}s^{-1}$ for LL leaves and 2,425 μmol of photons $m^{-2}{\cdot}s^{-1}$ for HL and LL→HL transferred leaves).

CC Ultrastructural Characterization. Leaf samples were fixed in 2% (wt/vol) glutaraldehyde/2% (wt/vol) paraformaldehyde/0.1% (wt/vol) tannic acid in 0.07 M sodium-cacodylate buffer (pH 6.9) for 1 h at 25°C. Tissue was washed three times in sodium-cacodylate buffer, postfixed in 1% OsO_4 in the same buffer for 1 h at 25°C, washed again, stained in 2% uranyl acetate for 1 h, dehydrated in acetone, and embedded in Spurr resin. Ultrathin sections (40 nm) were cut with a diamond knife and stained with 2% (wt/vol) uranyl acetate (in 30% methanol) and 0.2% (wt/vol) lead citrate. Digital pictures captured from a CM10 transmission electron microscope (Philips Electronic Instruments, Mahwah, NJ) were used for analysis. Plasmodesmatal density was determined as the number of channels on the intermediary cell side per length of contiguous wall between intermediary and BS cells in pumpkin and *V. phoeniceum*. The percentage increase of plasma membrane length due to wall ingrowths in the TCs of pea (23) was characterized by using EEB VIEWER.

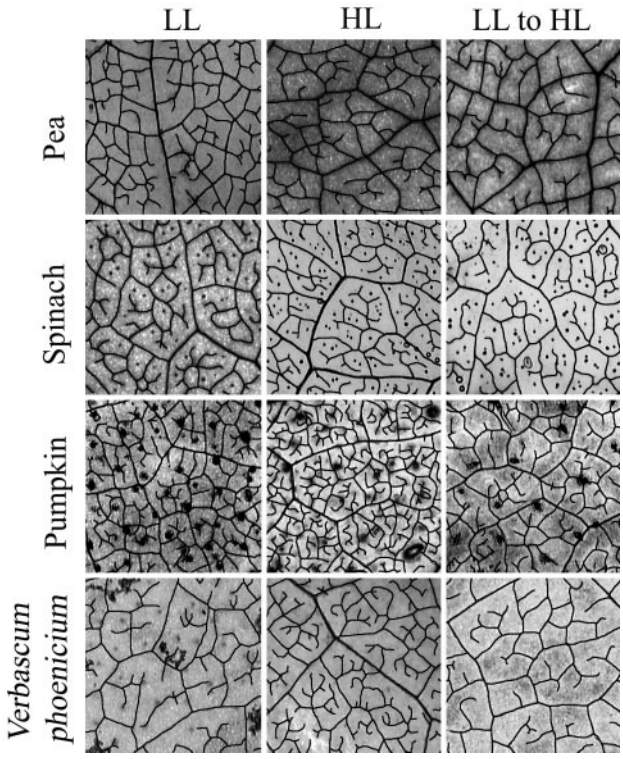

Fig. 1. Images show the pattern and density of leaf venation in the apoplastic loaders pea and spinach and the symplastic loaders pumpkin and *V. phoeniceum*. Plants were grown in LL, HL, or LL and then transferred to HL for 1 week. Each image is 3 × 3 mm (i.e., 9 mm^2). For clarity, the veins have been highlighted in black.

Leaf Thickness. Samples were fixed and embedded in resin as described for electron microscopy. Sections (500 nm) were cut, affixed to glass slides, and stained with 0.1% toluidine blue/1% sodium borate in distilled H_2O. Total leaf, palisade mesophyll, and spongy mesophyll thicknesses were determined by means of light microscopy (Zeiss Axiostar) by using a 5-mm-scale stage micrometer (Pyser-SGI, Kent, U.K.).

Foliar Chlorophyll Content. Chlorophyll content was determined from leaf samples that were collected, extracted, and quantified as described by Adams and Demmig-Adams (29) by using the high-pressure liquid chromatography procedure of Gilmore and Yamamoto (30).

Statistical Analyses. Analysis of variance was performed, followed by a Tukey–Kramer comparison for honestly significant differences among several means for all parameters, or Student's *t* test was used for comparisons of two means (JMP statistical software, SAS Institute, Cary, NC).

Results

Vein Density. There was no statistically significant difference in minor vein density between LL and HL (Figs. 1 and 2 *A* and *B*) in either pea or spinach. Upon transfer from LL to HL, vein density declined slightly in pea (Fig. 2*A*). In both symplastic loaders (pumpkin and *V. phoeniceum*), leaf vein density was significantly greater in plants grown in HL compared with those

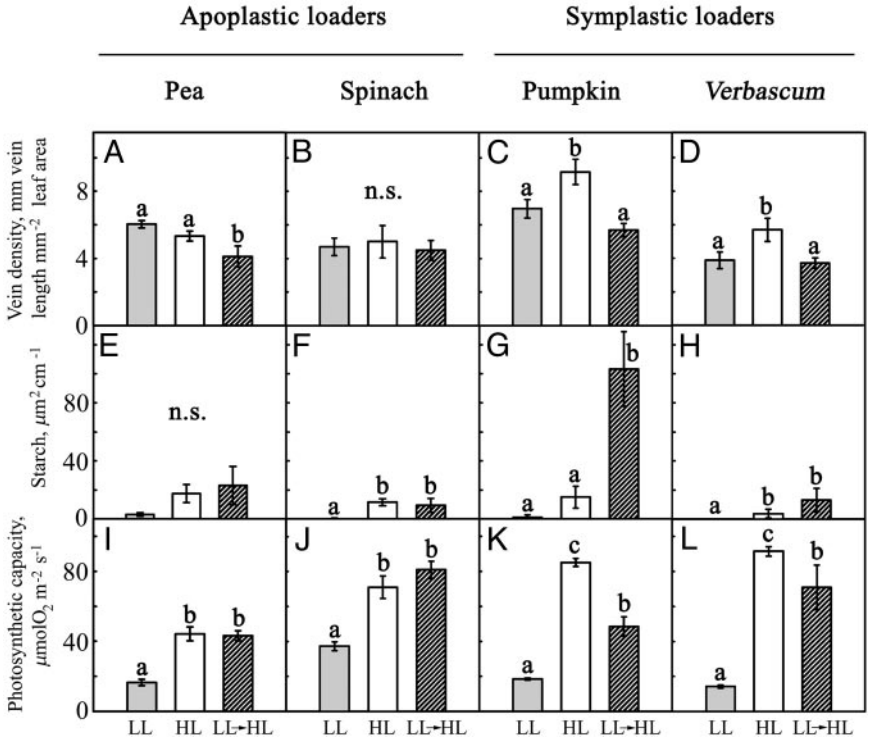

Fig. 2. Vein density, palisade cell starch content, and photosynthetic capacity in the apoplastic loaders pea and spinach and the symplastic loaders pumpkin and *V. phoeniceum*. (*A–L*) Vein density (*A–D*), estimated levels of starch as assessed through the area occupied by starch grains per unit cell length in the upper palisade layer (*E–H*), and photosynthetic capacity (light- and CO_2-saturated rates of oxygen evolution determined at 25°C) (*I–L*) in mature leaves of the apoplastic loaders pea and spinach and the symplastic loaders pumpkin and *V. phoeniceum* from plants grown in LL, HL, or LL and then transferred to HL for 1 week. Means ± SDs are shown, and significant differences among the means within each panel are indicated by the lowercase letters at $P < 0.05$. n.s., not significantly different. $n = 3$ leaves for all means, except $n = 6$ cells (two cells from each of three leaves) in *E* and *H*. For LL, HL, and LL→HL, the number of chloroplasts per palisade cell for each species and respective treatment were as follows (significant differences at $P < 0.05$ are indicated by the superscript letters): in pea, 11.3 ± 1.5^a, 19.3 ± 5.0^{ab}, and 29.0 ± 5.6^b; in spinach, 19.7 ± 4.0^a, 29.0 ± 6.1^{ab}, and 31.7 ± 1.5^b; in pumpkin, 9.3 ± 0.6^a, 18.7 ± 2.1^b, and 13.0 ± 1.7^a; in *V. phoeniceum*, 10.0 ± 1.0^a, 19.0 ± 7.5^a, and 50.3 ± 1.5^b.

grown in LL or transferred from LL to HL (Figs. 1 and 2 *C* and *D*).

Starch Content. Growth in HL resulted in greater accumulation of starch in cells of the upper palisade layer of both spinach and *V. phoeniceum* (Fig. 2 *F* and *H*) but not in pea or pumpkin (Fig. 2 *E* and *G*). Transfer from LL to HL did not lead to greater starch accumulation compared with HL in the apoplastic loaders pea and spinach (Fig. 2 *E* and *F*). In contrast, after transfer of pumpkin from LL to HL, starch accumulated to levels well above those in HL plants (Fig. 2*G*), indicating a bottleneck in carbon export. A similar trend was seen in *V. phoeniceum* (Fig. 2*H*). Similar results after transfer were obtained when the data were expressed as total starch grain area as a fraction of the total chloroplast area for all chloroplasts in a palisade cell (not shown).

Photosynthetic Acclimation. For all species, photosynthetic capacities were significantly greater in mature leaves of HL plants compared with LL plants (Fig. 2 *I–L*). For mature leaves from both of the apoplastic loaders (pea and spinach), these rates increased to a level that was not significantly different from those in HL 1 week after transfer from LL to HL (Fig. 2 *I* and *J*). In contrast, transfer of the symplastic loaders pumpkin and *V. phoeniceum* led to photosynthetic capacities in mature leaves that were significantly greater than those observed in LL-acclimated leaves but that were still significantly lower than those in HL-acclimated leaves (Fig. 2 *K* and *L*).

Plasmodesmatal Density in Intermediary Cells of the Symplastic Loaders. Fields of abundant plasmodesmata link intermediary cells and BS cells in pumpkin and *V. phoeniceum*. Plasmodesmatal frequencies at these interfaces did not change in either species when grown in LL or HL or transferred from LL to HL (Table 1, which is published as supporting information on the PNAS web site).

Ultrastructural Characterization of TC Wall Invagination in Pea. For each TC, the length of the internal cell wall (plasma membrane) was measured and expressed as a percentage increase relative to the hypothetical length of the wall (plasma membrane) in the absence of cell wall ingrowths (i.e., 0% increase in cell wall ingrowths for a cell with no ingrowths). Ingrowths were most extensive in pea leaves grown in HL (178%) and less extensive in those grown in LL (33%) (Figs. 3 *A* and *B* and 4). One week after transfer from LL to HL, wall ingrowths in leaves that had been fully expanded before transfer were just as extensive as those in mature leaves of HL plants (Figs. 3 *B* and *C* and 4). In the reverse experiment, transfer of plants from HL to LL for 2 weeks (Fig. 3*D*) led to a statistically significant decrease in wall ingrowths, although they were still more extensive than in LL plants (Fig. 4). These findings indicate that cell wall ingrowths (and thus total plasma membrane surface area) are plastic in mature leaves and subject to considerable regulation by the light environment, with flexibility to both increase and decrease.

The Anatomy of Spinach Minor Veins. The anatomy of spinach minor veins was examined to identify (*i*) factors that might be

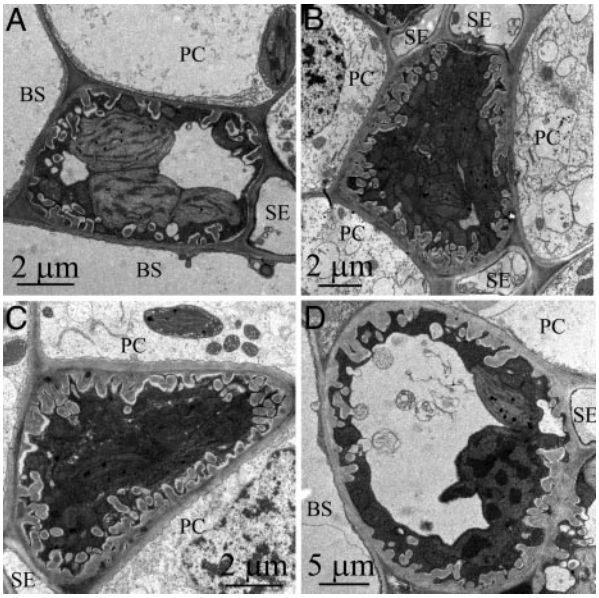

Fig. 3. Transmission electron microscopic images of pea TCs from mature leaves. Plants were grown in LL (*A*), HL (*B*), LL and then transferred to HL for 1 week (*C*), or HL and then transferred to LL for 2 weeks (*D*). BS, BS cell; PC, parenchyma cell. (Scale bars: *A–C*, 2 μm; *D*, 5 μm.)

associated with its higher photosynthetic capacity relative to pea in HL plants (Fig. 2) and (*ii*) what features might permit the full acclimation of photosynthesis in mature spinach leaves upon transfer from LL to HL in the absence of the increased TC plasma membrane surface area that occurs in mature pea leaves. The total cross-sectional area of minor vein CCs was greater in spinach relative to pea (Fig. 5). In spinach, each SE was surrounded by ≈3 (2.9 ± 0.8, $n = 52$ SEs) darkly staining cells that appear to be CCs, although we have not established their ontogenetic relationships to the SEs. The comparable number of CCs (TCs) in pea was 1.7 ± 0.5 ($n = 38$ SEs, significantly different from spinach at $P < 0.0001$). These features did not differ between leaves grown in LL vs. those grown in HL (not shown).

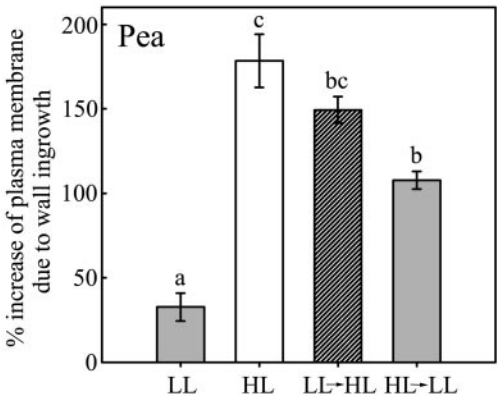

Fig. 4. Percentage increase in cell wall invaginations in TCs of mature leaves of pea from plants grown in LL, HL, LL and then transferred to HL for 1 week, or HL and then transferred to LL for 2 weeks. Means ± standard errors are shown, and significant differences among the means are indicated by the lowercase letters ($P < 0.05$). Thirty-two cells from six leaves were characterized for LL, 28 cells from six leaves were characterized for HL, 26 cells from three leaves were characterized for the LL→HL transfer, and 30 cells from three leaves were characterized for the HL→LL transfer.

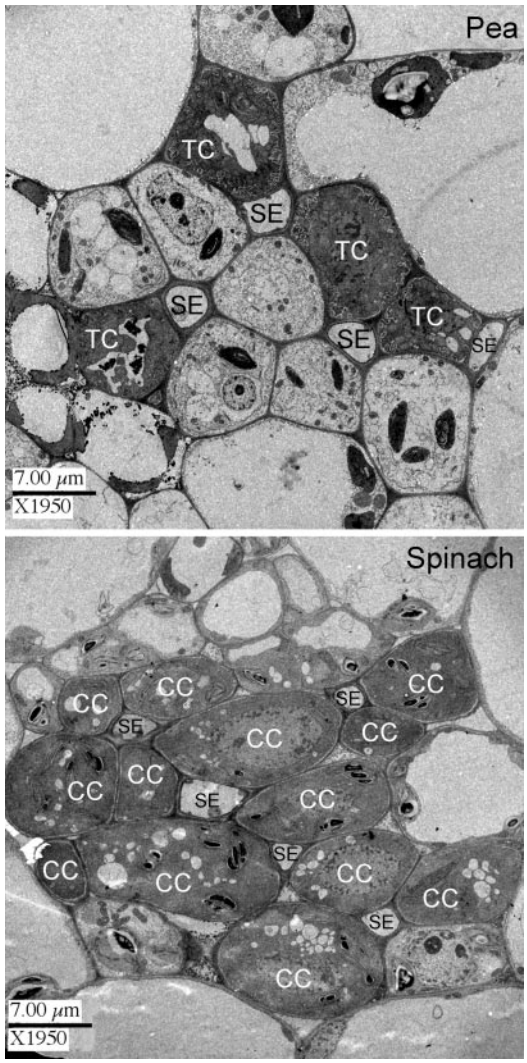

Fig. 5. Transverse images of the phloem in pea and spinach. Images illustrate the arrangement of TCs in pea and CCs in spinach that are likely to be involved in the export of sucrose by means of plasma membrane-localized H^+/sucrose symporters for the loading of SEs.

Leaf Thickness. The two vines, pea and pumpkin, had thinner leaves than the two rosette-forming species when grown in either LL or HL (Table 2, which is published as supporting information on the PNAS web site). Spinach had the thickest leaves under all growth conditions ($P < 0.05$). Within each species, total leaf thickness was significantly greater in leaves of HL plants compared with those grown in LL (Table 2). This increase in leaf thickness was due to significantly thicker palisade tissue and, in the case of the two rosette-forming species (spinach and *V. phoeniceum*), thicker spongy mesophyll tissue as well (Table 2). In pea, the increased palisade thickness was due solely to elongation of the single layer of palisade cells. In pumpkin, the palisade layer consisted of a single layer of cells in LL and two layers of cells in HL. Among the rosette-forming species, the palisade layer consisted of one to two cells (*V. phoeniceum*) or two to three cells (spinach) in LL plants, whereas in HL plants, it consisted of two to three (*V. phoeniceum*) or three (spinach) cells.

One week after transfer of pea and *V. phoeniceum* from LL to HL, total leaf and palisade mesophyll thickness was significantly greater than in LL but still significantly less than in HL (Table 2). In spinach, total leaf thickness 1 week after transfer from LL

to HL was the same as in LL, despite the fact that palisade mesophyll tissue was significantly thicker (Table 2). Mature leaves of pumpkin plants transferred from LL to HL for 1 week exhibited no adjustment in leaf thickness compared with LL leaves (Table 2).

Chlorophyll Content. Foliar chlorophyll content was constant in spinach under all growth light conditions (Table 3, which is published as supporting information on the PNAS web site). Mature leaves of pea and *V. phoeniceum* possessed higher chlorophyll contents in HL relative to LL, whereas there was no difference in chlorophyll content between pumpkin leaves from LL and HL plants (Table 3). The effect of transferring plants from LL to HL differed in the different species. In pea and spinach, there was no significant change in chlorophyll content. Significantly, transfer of pumpkin from LL to HL caused a reduction in chlorophyll content. Chlorophyll content in mature leaves of *V. phoeniceum* increased to a level that was similar to that in plants grown in HL (Table 3).

Discussion

Photosynthetic Acclimation in the Apoplastic Loaders Pea and Spinach. Pea and spinach, both of which load sugar apoplastically (23, 24), exhibited complete acclimation (up-regulation) of photosynthetic capacity in fully expanded, mature leaves of plants transferred from LL to HL. This up-regulation of photosynthetic capacity did not involve any adjustments in chlorophyll content or density of venation but was associated with increased leaf (pea) and palisade mesophyll (pea and spinach) thickness. The capacity for carbon export by means of H^+/sucrose symporters may be either nonlimiting or modulated by adjustments in the activity, type, and/or abundance of symporters. The level of sucrose transport activity is proportional to symporter abundance in sugar beet (31). It is possible that an increase in the number of H^+/sucrose symporters is facilitated by the increased level of cell wall ingrowths in HL (and after transfer from LL to HL) in the specialized phloem TCs that pea possesses (see also refs. 23 and 27). However, enhanced cell wall ingrowth is clearly not a general prerequisite for full acclimation of photosynthesis in transferred leaves, as is illustrated in spinach (without wall ingrowths). Instead, spinach was found to have a larger cross-sectional area of CCs in the minor veins and a greater number of cells likely to be participating in the loading of each SE compared with pea. This potentially greater capacity for the export of sugars from spinach leaves may also be associated with the photosynthetic capacities that were twice those found in pea leaves when acclimated to HL.

H^+/sucrose symporters are turned over rapidly (31, 32), and the level of symporter mRNA is responsive to sucrose (31). The latter experiments involved sucrose feeding to detached leaves through the xylem, leading to decreases in the level of symporter mRNA, the symporter itself, and the transport of sucrose (31, 33). These effects may have been due to elevated levels of sucrose outside of the CCs. In contrast, increased light availability to an intact plant leads to increased growth and development (including sexual reproduction) and an increased demand for photosynthate in the rest of the plant and may be associated with an up-regulation of CC H^+/sucrose symporters. Interestingly, elevated sucrose levels also induced up-regulation of another member of the sucrose transporter family that localized to the transport pathway in the petioles of tomato and is thought to play a role in sensing sugar levels and regulating the activity of symporters involved in loading sucrose into SEs (34).

Photosynthetic Acclimation in the Symplastic Loaders Pumpkin and *V. phoeniceum*. Although photosynthetic capacity in leaves of pumpkin and *V. phoeniceum* plants transferred from LL to HL increased significantly above the level observed in LL-acclimated leaves, it did not reach the level observed in HL-acclimated leaves. The observed increases in leaf and palisade tissue thickness and chlorophyll content might contribute to this partial up-regulation in *V. phoeniceum*. However, the most striking anatomical difference between these two symplastic loaders and the apoplastic loaders pea and spinach is their greater vein density in HL leaves. The increased capacity to export carbon that results from the greater total vein number and thus total number of plasmodesmata per unit of leaf area is likely to be a key feature facilitating the high capacity of photosynthesis in HL-acclimated pumpkin and *V. phoeniceum* and may also be one anatomical characteristic setting the upper maximal limit for photosynthetic capacity among species (35). The greater accumulation of starch in leaves transferred from LL to HL relative to that found in HL-acclimated leaves suggests a more limiting carbon export capacity in pumpkin (and perhaps *V. phoeniceum*), whereas excess starch accumulation was not observed in the apoplastic loaders pea and spinach. Down-regulation of photosynthesis is a common response when the synthesis of sugars far exceeds their export and utilization (1–13). Such repression of photosynthesis would be expected to impact photosynthetic capacity, as was consistently observed for both symplastic loaders compared with the apoplastic loaders in the present study. The incomplete acclimation of photosynthesis upon transfer from LL to HL in mature, fully expanded leaves of these symplastic loaders is thus consistent with their inability to increase vein/plasmodesmatal density (and presumably carbon export capacity).

Comparative Considerations. The experiments reported here suggest that phloem loading is a limiting step in export, at least in some species, because physical adjustments are apparently made to increase loading capacity (defined as the amount of solute transferred to the phloem per unit of leaf area) under high-light conditions. In principal, adjustments to loading capacity could be made by altering flux per unit of wall area or wall area itself. Flux, under limiting conditions, depends on the number of available symporters (and their capacity, their affinity for sucrose, and/or their activity) or plasmodesmata, depending on the strategy of phloem loading. Cell wall area contributing to foliar carbon export could be altered by invagination, adjustments in the size of CCs, alterations in the numbers of CCs, or changes in vein density.

Comparisons of the four species studied here indicate that different plants adjust loading capacity in different ways, subject to developmental limitations. In pea, an increase in plasma membrane surface area in TCs presumably increases the available number of sucrose symporters per unit of leaf area. In the other apoplastic loader, spinach, no adjustment in membrane surface area occurs, perhaps because of the already maximal ratio of CCs to SE. Vein density does not increase in response to HL in either spinach or pea. However, further work is needed to determine whether this strategy for up-regulating loading capacity may be available to other apoplastic loaders. In the few studies that have characterized vein density in sun vs. shade leaves (36–38), the mode of phloem loading was not firmly established for most of the species investigated, although those species capable of adjusting vein density appear to have frequent plasmodesmatal connections into the minor vein phloem (39–41).

Unlike cell wall morphology, plasmodesmatal frequency was an inflexible structural feature in the present study. Although secondary plasmodesmata formation has been observed in tissues that are undergoing developmental changes [e.g., during sink-to-source transition in minor leaf veins (25) or flower induction in the apical meristems (42)], the evidence presented here indicates that plasmodesmatal frequency in intermediary

cells is not responsive to light conditions during development. Instead, the symplastic loaders in this study responded to growth in HL with an increase in vein density. Not surprisingly, this parameter seems to be set once the leaf is mature and does not change upon transfer to HL. As a result, mature leaves in the two symplastic loaders studied here, and perhaps symplastic loaders in general, are inherently less adaptable to alterations in light regime compared with species that load by means of the apoplast. It will be interesting to determine whether the mode of phloem loading acts as a determinant of habitat exploitation in nature.

What other implications may these different approaches to facilitating higher rates of carbon export have for the two groups of species? Of course, the vascular system of plants is used not only for the transport of photosynthetic products but also, most notably, for the transport of water through the xylem to leaf mesophyll and epidermal tissues. In fact, the capacity for transporting water (hydraulic conductance) has been related to photosynthetic capacity among different species (e.g., ref. 43). It is interesting that both pea and spinach, which do not show any increase in foliar vein density with growth at HL, are rather restricted in their tolerance for growing during the summer months in sites that experience high evaporative demand (i.e., they are known primarily as cool-weather crops). Both species are typically grown in the spring and harvested before the heat of summer, or they are grown as fall crops. In contrast, pumpkin is planted in the spring and thrives throughout the summer before harvest in the fall. Members of the genus *Verbascum* also grow during the summer, setting seed in the late summer/early autumn. The ability to increase the level of foliar venation may be crucial to provide a sufficient supply of water through the xylem to support leaf hydration during summer growth.

Although the differences in the anatomical and photosynthetic responses of these apoplastic vs. symplastic loading species are clear and striking, they are thus far limited to the four species characterized in this study. Future studies should be undertaken with other apoplastic and symplastic loaders to determine whether these responses are of a more universal nature. In addition, it should be instructive to conduct comparative studies of this type in response to different environmental stresses.

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