Photosynthetic pathway alters xylem structure and hydraulic function in herbaceous plants

F. KOCACINAR & R. F. SAGE

Department of Botany, University of Toronto, 25 Willcocks St., Toronto, ON M5S3B2 Canada

ABSTRACT

Plants using the C₄ photosynthetic pathway have greater water use efficiency (WUE) than C₃ plants of similar ecological function. Consequently, for equivalent rates of photosynthesis in identical climates, C₄ plants do not need to acquire and transport as much water as C₃ species. Because the structure of xylem tissue reflects hydraulic demand by the leaf canopy, a reduction in water transport requirements due to C₄ photosynthesis should affect the evolution of xylem characteristics in C₄ plants. In a comparison of stem hydraulic conductivity and vascular anatomy between eight C₃ and eight C₄ herbaceous species, C₄ plants had lower hydraulic conductivity per unit leaf area ($K_L$) than C₃ species of similar life form. When averages from all the species were pooled together, the mean $K_L$ for the C₄ species was $1.60 \times 10^{-4}$ kg m⁻¹ s⁻¹ MPa⁻¹, which was only one-third of the mean $K_L$ of $4.65 \times 10^{-4}$ kg m⁻¹ s⁻¹ MPa⁻¹ determined for the C₃ species. The differences in $K_L$ between C₃ and C₄ species corresponded to the two- to three-fold differences in WUE observed between C₃ and C₄ plants. In the C₃ species from arid regions, the difference in $K_L$ was associated with a lower hydraulic conductivity per xylem area, smaller and shorter vessels, and less vulnerable xylem to cavitation, indicating the C₃ species had evolved safer xylem than the C₄ species. In the plants from resource-rich areas, such as the C₄ weed Amaranthus retroflexus, hydraulic conductivity per xylem area and xylem anatomy were similar to that of the C₄ species, but the C₄ plants had greater leaf area per xylem area. The results indicate the WUE advantage of C₄ photosynthesis allows for greater flexibility in hydraulic design and potential fitness. In resource-rich environments in which competition is high, an existing hydraulic design can support greater leaf area, allowing for higher carbon gain, growth and competitive potential. In arid regions, C₃ plants evolved safer xylem, which can increase survival and performance during drought events.

Key-words: C₄ photosynthesis; hydraulic architecture; water use efficiency; water transport; xylem anatomy.

INTRODUCTION

Two opposing evolutionary selection pressures act upon xylem structure and function (Zimmermann 1983; Tyree, Davis & Cochard 1994). The benefit derived from enhanced photosynthesis selects for efficient xylem consisting of relatively long and wide vessels that rapidly supply water to transpiring leaves. Wide vessels enhance conducting efficiency because flow capacity increases with the fourth-power of the conduit radius; longer vessels reduce hydraulic resistance within the xylem by reducing the number of inter-vessel pits that water must cross while flowing from roots to leaves (Sperry et al. 2002; Tyree & Zimmermann 2002). The cost of efficient xylem is a greater risk of catastrophic xylem failure, caused when high tension in xylem conduits cavitates the water column. To minimize the probability of xylem failure, an opposing selection pressure favours safer xylem characterized by reduced flow capacity and shorter, narrower and mechanically stronger vessels (Tyree et al. 1994; Wagner, Ewers & Davis 1998; Hacke & Sperry 2001). In addition, efficient xylem is more vulnerable to catastrophic failure because there is less redundancy in the conducting tissue than in safe xylem (Tyree & Sperry 1989). Cavitation of large vessels increases the probability of catastrophic xylem failure relative to cavitation in small vessels, because the loss of function in a large vessel represents a much greater loss of total transport capacity (Comstock & Sperry 2000; Hacke & Sperry 2001). In contrast to efficient xylem, evolution of safer xylem with less flow capacity requires a reduction in water use by the leaf canopy because the capacity of the stem to re-supply water lost in transpiration is reduced (Hubbard, Bond & Ryan 1999; Brodribb & Feild 2000; Hubbard et al. 2001; Sperry et al. 2002). Reduction in water use usually occurs via stomatal closure or a decline in leaf area, both of which reduce whole-plant photosynthetic capacity (Brodribb & Feild 2000; Salleo et al. 2000; Davis et al. 2002; Sperry et al. 2002).

As a consequence of the evolutionary pressures selecting for efficiency or safety, xylem structure should reflect the balance between water supply and potential canopy evaporation in environments where freezing is not an issue (Tyree et al. 1994). The optimal solution is predicted to occur when the xylem structure in a plant provides just enough flow capacity to meet the highest transpiration rate a leaf canopy normally exhibits during a growing season (Tyree & Sperry 1989; Tyree 2003). In arid environments,
the balance between safety and efficiency is weighted towards safety features, reflecting adaptations to low soil water supply and high xylem tension. In mesic environments in which competition for light is critical, the balance would shift towards hydraulic efficiency in order to support a larger leaf canopy. The evolutionary balance would also reflect unique aspects of the environment or plants, such as average humidity and temperature, soil water and nutrient status and allocation between roots and shoots. Differences in water use efficiency (WUE) could also affect xylem structure and function because WUE affects the balance between safety and efficiency (Sperry et al. 2002). In this regard, innovation of novel metabolic pathways that increase WUE, such as C₄ photosynthesis, should also influence xylem characteristics of plants.

C₄ plants have two- to four-fold greater WUE than C₃ plants and therefore have substantially lower transpiration rates, assuming equivalent growth form and environmental conditions (Osmond, Björkman & Anderson 1980; Pearcy & Ehleringer 1984; Long 1999; Sage & Pearcy 2000). For example, the C₄ Atriplex species, A. rosea, A. expansa and A. serenana, have two to three times the WUE of the C₃ Atriplex species, A. triangularis, A. hortensis and A. heterosperma (Osmond et al. 1980). By evolving C₄ photosynthesis, plants might shift the optimal balance between xylem efficiency and safety, such that the lower water requirements of the C₄ leaf canopy might allow for safer xylem than present in their C₃ ancestors. Alternatively, for the same amount of xylem, C₄ plants could exploit the benefits of higher WUE not by increasing safety, but instead by supporting a greater leaf area per unit of xylem tissue and thereby improving overall carbon gain. In either case, C₄ species should have a lower leaf specific conductivity (Kₛ; stem hydraulic conductivity relative to the leaf area supported by the stem) compared with similar C₃ plants.

Many studies have compared hydraulic properties of xylem from a wide range of species (reviewed in Hacke & Sperry 2001). Few, however, have examined hydraulic properties in C₄ plants, and none to our knowledge has specifically compared xylem hydraulics of C₃ and C₄ species of similar life forms, ecological requirements or phylogenetic affinity. The leafy CAM plant Clusia uvitana has been observed to have Kₛ values that are 1/3 to 1/30 of a range of tropical C₃ species, indicating that the photosynthetic pathway can alter xylem function (Zotz, Tyree & Cochard 1994). In woody C₄ species differing in habitat, differences in WUE have been inversely correlated with hydraulic conductivity and xylem efficiency (Pockman & Sperry 2000; Sobrado 2000; Sperry et al. 2002), which is consistent with the hypothesis that the higher C₄ WUE could promote a drop in Kₛ. For example, in a comparison of three Venezuelan mangrove species, WUE was lowest in Rhizophora mangle, intermediate in Laguncularia racemosa and highest in Avicennia germinans; Kₛ was highest in R. mangle and lowest in A. germinans (Sobrado 2000). In the Great Basin desert in Utah, stems of the C₃ shrubs Atriplex canescens and Atriplex confertifolia were more cavitation-resistant than the stems of the co-occurring C₃ shrubs Chrysanthemus nauseosus and Chrysothamnus viscidiflorus (Hacke, Sperry & Pittermann 2000; Sperry & Hacke 2002). Although this work was not designed to compare photosynthetic pathway effects on xylem properties, the data from Atriplex and Chrysothamnus spp. is consistent with the possibility that C₄ photosynthesis promotes evolutionary changes in the hydraulic pathway.

In the study described here, we evaluated whether differences in photosynthetic pathway affect xylem structure and function using 16 species of herbaceous plants having similar taxonomic and/or ecological distribution. Hydraulic properties and anatomical characteristics of the stem xylem were measured in eight C₃ and eight C₄ species segregated into four functional groups (Table 1). Group 1 consisted of four co-occurring annual species common in disturbed habitats such as old fields, cultivated lands and severely degraded habitats (wastelands). All were from the Chenopodiaceae/Amaranthaceae taxonomic complex. Group 2 consisted of five annual Atriplex species from the family Chenopodiaceae. Many of these species are from coastal habitats where wave action and sand movement create new habitat. The third group included two phylogenetically related annuals from the Sesuvioideae subfamily of the family Aizoaceae (Bittrich & Hartmann 1988; Kubitzki, Rohwer & Bittrich 1993). Group 4 consisted of trailing herbs from tropical coastal stands and roadsides of northern Australia. Because environment and phylogeny can influence xylem properties (Wagner, Ewers & Davis 1998; Sperry & Hacke 2002), we have compared these four groups to provide enough information to allow for broad inferences regarding the effects of photosynthetic pathway. If photosynthetic pathway or WUE alters xylem properties, we hypothesized that leaf specific conductivity will be lower in the C₄ species relative to the C₃ species within a comparison group.

MATERIALS AND METHODS

Plant material

The functional group, photosynthetic type, family, habitat and collection site or seed source of the plants are summarized in Table 1. Seeds of the first group, Kochia scoparia (C₃), Amaranthus retroflexus (C₄), Chenopodium album (C₃) and Chenopodium botrys (C₄) were collected from disturbed habitats in Toronto, Ontario. In the second group, Atriplex texana (C₄) seeds were collected from the desert of the American south-west, near Marathon, Texas. Atriplex rosea (C₄) seeds were courtesy of Dr Paul Hattersley from the Australian National University. Seeds of Atriplex hortensis (C₃) were obtained from the Botanical Garden of the Free University, Amsterdam, The Netherlands. Atriplex triangularis (C₃) seeds were collected on a farm field at Pigeon Point, California. Atriplex littoralis (C₃) seeds were obtained from the Antwerp Botanical Garden, Belgium. Seeds of the third group, Trianthema portulacastrum (C₃) were collected on an abandoned lot in St. George, Utah and Sesuvium verrucosum (C₃) was collected on the dry lake-
Table 1. Functional group, species names, photosynthetic type, family, habitat, and collection site or seed source of the species used for this study. Species with geographic coordinates given were collected by F. Kocacinar or R. Sage

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Species</th>
<th>Ph. type</th>
<th>Family</th>
<th>Habitat</th>
<th>Collection site/seed source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1: weedy annuals</strong></td>
<td><em>Kochia scoparia</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, disturbed land</td>
<td>Toronto, ON (43°65′ N 79°38′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Amaranthus retroflexus</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, cultivated land</td>
<td>Toronto, ON (43°65′ N 79°38′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Chenopodium album</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, cultivated land</td>
<td>Toronto, ON (43°65′ N 79°38′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Chenopodium botrys</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, cultivated land</td>
<td>Toronto, ON (43°65′ N 79°38′ W)</td>
</tr>
<tr>
<td><strong>Group 2: annual Atriplex</strong></td>
<td><em>Atriplex texana</em> S. Wats.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Arid land, roadsides</td>
<td>Marathon, TX (30°10′ N 103°15′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Atriplex rosea</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, disturbed land</td>
<td>Australian National University</td>
</tr>
<tr>
<td></td>
<td><em>Atriplex hortensis</em> L.</td>
<td>C₄</td>
<td>Chenopodiaceae</td>
<td>Waste, disturbed land</td>
<td>Free University Botanical Garden, The Netherlands</td>
</tr>
<tr>
<td></td>
<td><em>Atriplex triangularis</em> Willd.</td>
<td>C₃</td>
<td>Chenopodiaceae</td>
<td>Saline wetlands</td>
<td>Pigeon Point, CA (37°9′ N 122°25′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Atriplex littoralis</em> L.</td>
<td>C₃</td>
<td>Chenopodiaceae</td>
<td>European coastal strands</td>
<td>Antwerp Botanical Garden, Belgium</td>
</tr>
<tr>
<td><strong>Group 3: Sesuvioideae annuals</strong></td>
<td><em>Trianthema portulacastrum</em> L.</td>
<td>C₄</td>
<td>Aizoaceae</td>
<td>Arid land ephemeral</td>
<td>St. George, UT (37°12′ N 113°35′ W)</td>
</tr>
<tr>
<td></td>
<td><em>Sesuvium verrucosum</em> L.</td>
<td>C₃</td>
<td>Aizoaceae</td>
<td>Ephemeral saline wetlands</td>
<td>L. Lahonton, NV (39°19′ N 119°9′ W)</td>
</tr>
<tr>
<td><strong>Group 4: coastal-strand herbs</strong></td>
<td><em>Boerhavia dominii</em> Meikle &amp; Hewson</td>
<td>C₄</td>
<td>Nyctaginaceae</td>
<td>Waste land, saline marshes</td>
<td>Darwin, Australia (12°15′ S 131°10′ E)</td>
</tr>
<tr>
<td></td>
<td><em>Boerhavia coccinea</em> Miller</td>
<td>C₄</td>
<td>Nyctaginaceae</td>
<td>Waste land, coastal strands</td>
<td>Darwin, Australia (12°15′ S 131°10′ E)</td>
</tr>
<tr>
<td></td>
<td><em>Canavalia rosea</em> (Sw.) DC.</td>
<td>C₃</td>
<td>Fabaceae</td>
<td>Coastal strands, beaches</td>
<td>Darwin, Australia (12°15′ S 131°10′ E)</td>
</tr>
<tr>
<td></td>
<td><em>Ipomoea pes-caprae</em> (L) R. Br.</td>
<td>C₃</td>
<td>Convolvulaceae</td>
<td>Coastal strands, beaches</td>
<td>Darwin, Australia (12°15′ S 131°10′ E)</td>
</tr>
</tbody>
</table>

bed of Lake Lahonton, Nevada. Seeds of the plants in the fourth group were collected on the coastal strands along the Timor Sea about 80 km north of Darwin, Northern Territories, Australia.

All plants were grown from seeds for three months in 6 L pots on an outdoor roof at the University of Toronto, Ontario. The potting medium was a mix of 25% Promix (Sun Gro Horticulture Canada Ltd, Seba Beach, AB, Canada), 25% sand and 50% topsoil. The plants were watered regularly to avoid drought and were fertilized once a week with a full-strength Hoagland’s solution. Plants were measured approximately 3 months after germination.

**Stem hydraulic conductivity**

Stem hydraulic conductivity (Kₒ) was measured on 5–10 main stems of each species using segments 0.3–1 cm in diameter and 5–10 cm in length. Only the lower portion of the main stem was measured in order to maximize xylem content and minimize pith tissue. Stem segments of 5–10 cm were used for all measurements after preliminary assessments showed no change in Kₒ on stem segments ranging in length from 5 to 15 cm. Segments were first cut under water, and then flushed with a perfusion solution at elevated pressure (150–175 kPa) to remove embolisms (Sperry, Donnelly & Tyree 1988). The perfusion solution was deionized, degassed and filtered (0.2 μm) water. Agents such as KCl or oxalic acid were not used because the measurements lasted less than 30 min and new solution was used every 3 d; hence microbial growth was negligible. After perfusion, the volumetric flow rate through the segments was measured at 5–20 kPa. A pressure–flow plot was constructed for each segment and Kₒ was calculated from the slope of the plot multiplied by stem length (Dryden & Van Alfen 1983). Linearity was high in each case as indicated by r² values between 0.98 and 0.9999 for the linear regression equations. Xylem specific conductivity (Kₛ) was calculated by dividing Kₒ by xylem cross-sectional area determined with a light microscope (Olympus AX70, Olympus America Inc., Melville, New York, USA). Lumen specific conductivity (Kₛ) was obtained by dividing Kₒ by total vessel lumen area as determined by measuring the cross-sectional area of all the vessels in representative sectors of the xylem and extrapolating it.
to the whole xylem in a stem. $K_h$ is more informative than $K$ alone because $K_h$ takes xylem porosity and vessel frequency into account. Leaf specific conductivity ($K_i$) was measured as $K_i = k_h / A$ where $K_h$ is the leaf area distal to the segment. Leaf area was measured using a Li-Cor 3100 leaf area meter (Li-Cor, Lincoln, NB, USA).

Xylem vulnerability curves

Vulnerability of the xylem to cavitation was evaluated for C. album (C3) and K. scoparia (C4) from group 1 and for Ipomoea pes-caprae (C3) and Boerhavia coccinea (C4) from group 4. Xylem embolism was induced by drying cut shoots on laboratory benches or by withholding water from potted plants. Xylem pressure potential was measured on three different leaves or branches for each species using a pressure chamber (PMS Instruments, Corvallis, OR, USA). After a desired xylem pressure potential was reached, initial hydraulic conductivity was measured at 4–8 kPa pressure. Stem segments were then perfused under high pressure (up to 175 kPa) for half an hour until maximum conductivity was reached (Sperry et al. 1988). Two to three initial and maximum conductivity values were measured at different pressures and averaged for each plant. The percentage loss of conductivity was determined as:

$\text{Percentage loss in } K_h = \left( 1 - \frac{K_{h_{\text{initial}}}}{K_{h_{\text{maximum}}}} \right) \times 100.$

Between 20 and 25 plants were used to generate a vulnerability curve for each species (Pockman & Sperry 2000).

Stem anatomy measurements

Vessel lengths were measured using the paint-infusion method (Zimmermann & Jeje 1981). Maximum vessel length (MVL) and the percentage of vessels in 2-cm length classes were studied on three to five samples per species. The perfusion solution was a red commercial latex paint (Pratt & Lambert Co. Orange, CA, USA) diluted 1000 times with distilled water and filtered through 2.5 µm filters. The paint suspension was gravity-fed for 2 d into stems with leaves and branches still attached to facilitate transpiration. After paint infusion, the stems were cut into 2-cm segments and all paint-filled vessels were counted and used to calculate the distribution of the conduit lengths (Zimmermann 1983).

Xylem area and conduit diameters were measured on stem cross-sections obtained from the same samples as used for hydraulic conductivity measurements. For each species, six of the stem segments that had previously been used for $K_h$ determination were fixed with formalin, acetic acid and ethyl alcohol in water (FAA), paraffin-embedded and sectioned using a sliding microtome. Sections were examined under a light microscope and images analysed using imaging software (IMAGE PRO PLUS; Carsen Group Inc., Markham, Ontario, Canada). The lengths of major and minor axes across a conduit were averaged to obtain vessel diameters. Maximum vessel diameter (MVD) was measured from each cross-section on the largest vessel. The vessels mean diameter (VMD), hydraulically weighted mean diameter (HMD), mean diameter of conduits that facilitate 95% of the flow (D95) and vessel frequency (VF, the number of vessels per xylem area) were determined for each segment by measuring all xylem conduits in three randomly chosen sectors from the cambium to pith that together represented at least 20% of the stem xylem area. The hydraulically weighted mean diameter (HMD) was calculated as $D = 2(\Sigma r^2)/\Sigma r^3,$ where $r$ is the radius of a conduit (Sperry et al. 1994). Conduit efficiency value was determined by dividing the sum of the fourth power of all conduits extrapolated to whole xylem area by the leaf area supplied by these conduits. Conduit efficiency is important because it indexes the contribution of xylem anatomy to the hydraulic supply of the canopy. Porosity of the xylem was also indexed to the leaf area to produce leaf specific porosity (LSP), which is calculated as the total vessel lumen area divided by the leaf area.

Statistical analysis

Mean values of all parameters between C3 and C4 plants within each group were compared using one-way analysis of variance (ANOVA) followed by Fisher LSD multiple comparison test ($P < 0.05$). As the vessel diameters are not normally distributed, the data was log-transformed when it failed a normality test.

RESULTS

Hydraulic properties

Xylem specific conductivity, $K_h$, differed little between the species in the first three comparison groups (Fig. 1a). In the fourth group, the coastal strand C4 species – Boerhavia dominii, B. coccinea and Tribulus eichlerianus – had significantly lower $K_h$ than the co-occurring C3 species Canavalia rosea and I. pes-caprae (Fig. 1a). Lumen specific conductivity, $K_m$, is a preferred index for comparing xylem hydraulics between species because it compensates for differences in vessel size and number, and it removes the contribution of non-conducting cells in the xylem tissue. There was no obvious trend in $K_m$ between C3 and C4 species except in the Australian plants where all C3 species showed significantly lower values than C4 species (Fig. 1b). In this group, $K_m$ was between three and 12 times lower in C3 than C4 species.

When leaf area was accounted for, all C3 species within a group showed significantly lower leaf specific conductivity, $K_{l3}$, than C4 species within the same functional group (Fig. 2). $K_{l3}$ was 54 to 85% lower in the C3 than C4 plants in the first group, 27 to 80% less in the C3 than C4 species in the second group consisting of annual Atriplex spp., and 50% lower in the C3 T. portulacastrum than the C4 S. verrucosum of the third group. In the Australian coastal species, $K_{l3}$ was 50 to 92% less in the C3 than C4 members of the group. When averages from all the species were pooled together, the mean $K_{l3}$ for the C3 species was $1.60 \times 10^{-4}$.
Effect of photosynthetic pathway alters xylem structure and hydraulic function


(kg m⁻¹ s⁻¹ MPa⁻¹), which was one-third of the mean $K_t$ of $4.65 \times 10^{-4}$ (kg m⁻¹ s⁻¹ MPa⁻¹) determined for the C₃ species ($P = 0.004$).

Vulnerability of the xylem to cavitation was less in the two C₄ members of the representative pairs of C₃ and C₄ species chosen from groups 1 and group 4 (Fig. 3a & b). In the C₃ plant Chenopodium album, the xylem tension that caused 50% loss of $K_h$ was about −1.0 MPa; 2.8 MPa greater than the −3.8 MPa observed in the C₄ species K. scoparia. The point where $K_h$ declined by 75% was nearly 3 MPa greater in C. album than K. scoparia (~2.2 versus ~5.1 MPa). Similarly, the xylem of the C₃ B. coccinea was more cavitation resistant than the xylem of co-occurring C₃ I. pes-caprae (Fig. 3b). Ipomoea pes-caprae had lost 75% of $K_h$ by −2.5 MPa whereas in B. coccinea this occurred at −5.2 MPa xylem pressure potential.

Anatomical properties

Vessel size in the C₃ plants was similar to or smaller than C₃ species within a comparison group (Table 2). In most cases, one of the C₃ species exhibited the widest vessels in

**Figure 1.** Xylem and lumen area specific conductivities, $K_s$ and $K_{ls}$, of C₄ and C₃ herbaceous species. Group 1 consists of coexisting old-field annual plants. Group 2 includes annual Atriplex species. The third group includes two closely related species in the Sesuvioideae tribe of the Aizoaceae and group 4 contains species from coastal regions of northern Australia. Bars represent means (± SE) of 5–10 plants. Different letters within each group represent significant difference at $P < 0.05$. ‘ns’, not significant.
a comparison group whereas C₃ species had the smallest; these differences were readily visible in a comparison of xylem cross sections (Fig. 4). In the first group, *A. retroflexus* (C₄) had similar xylem anatomy as the C₃ species *C. botrys*: MVL, MVD, VMD, HMD and D₉₅ were all similar between the two species (Table 2). However, *C. botrys* had twice as many vessels per xylem area as *A. retroflexus*. Both C₄ *K. scoparia* and *A. retroflexus* had smaller vessels than *C. album* (Fig. 4a & b, Table 2). In group 2, *A. rosea* (C₃) exhibited similar anatomical parameters to the C₃ species *A. hortensis*, *A. triangularis* and *A. littoralis*. The other C₄ species in this group, *A. texana*, had smaller vessels on average than the other species (Fig. 4c & d, Table 2). Xylem of the species of the third group was alike except that *S. verrucosum* (C₃) had double the number of vessels per xylem area compared with the C₄ herb *T. portulacastrum* (Fig. 4e & f, Table 2). In group 4, the C₄ species within each group tended to have a greater proportion of their vessels in the shorter size classes than C₃ species in the corresponding group (Fig. 5). Notably, the distribution of vessels extended to longer size classes in the C₃ members of groups 1 and 4.

**DISCUSSION**

C₄ plants are widely noted for having superior WUE than C₃ plants and this is often presumed to increase carbon gain and drought tolerance. As a secondary consequence of the superior WUE, we hypothesized that C₄ plants have modified xylem structure and function to improve hydraulic safety and/or enhance photosynthetic potential by allowing a larger leaf area per unit of xylem. In either case, the key index for changes in functional xylem traits is leaf specific conductivity, *Kₑ*. In all comparison groups, the C₄ species consistently exhibited lower *Kₑ* than the corresponding C₃ species, demonstrating shifts in the relationship between hydraulic transport capacity and leaf water use. These differences were apparent in the two groups in which species had close taxonomic affinity (groups 2 and 3), and in the two groups in which species were less related but shared identical ecological habitats (groups 1 and 4). Differences
Effect of photosynthetic pathway alters xylem structure and hydraulic function

in $K_L$ between the species reflected differences in inherent WUE typically observed between the $C_3$ and $C_4$ pathways. $C_4$ plants are commonly noted to have a WUE that is two to four times greater than ecologically similar $C_3$ plants (Osmond et al., 1980; Larcher 1995), which corresponds to the three-fold difference in mean $K_L$ observed between the $C_3$ and $C_4$ species examined in this study. In the specific case of $C. album$ and $A. retroflexus$, WUE at 10 mbar vapour pressure difference between leaf and air was previously measured to be 7–10 mmol CO$_2$ mol$^{-1}$ H$_2$O in the $C_4$ species grown at high soil nitrogen; WUE in $C. album$ was 3–5 mmol CO$_2$ mol$^{-1}$ H$_2$O under identical conditions (Sage & Pearcy 1987b). These differences correspond to the two fold difference in $K_L$ measured here between $C. album$ and $A. retroflexus$.

Differences in $K_L$ can reflect either changes in xylem structure or the amount of leaf area produced relative to xylem tissue. Both were apparent in our samples. The $C_4$ plants *Amaranthus retroflexus*, *Atriplex rosea* and *T. portulacastrum* exhibited similar $K_s$, $K_{ls}$ and xylem anatomy to the $C_3$ species in their respective study groups. In contrast to their $C_3$ counterparts, these $C_4$ species supported substantially higher leaf areas per unit xylem, thus causing the lower values of $K_L$ and conduit efficiency. *Amaranthus retroflexus* supported two and three times higher leaf area per unit xylem than the $C_3$ *C. album* and *C. botrys*, respectively. *Atriplex rosea* had a mean leaf area per xylem area of 62.5 cm$^2$ mm$^{-2}$, which was twice that of the $C_3$ *A. hortensis* (33.2 cm$^2$ mm$^{-2}$) and *A. triangularis* (30.5 cm$^2$ mm$^{-2}$), and three-fold more than the $C_3$ *A. littoralis* (21.0 cm$^2$ mm$^{-2}$). Similarly, for the same xylem area, *T. portulacastrum* supported twice the leaf area as the $C_3$ *S. verrucosum* (72 versus 34 cm$^2$ leaf area mm$^{-2}$ xylem area, respectively; despite twice the number of vessels per area in *S. verrucosum*). The other $C_4$ species appear to have exploited the greater WUE primarily by enhancing xylem safety rather than leaf area, as indicated by relatively low $K_s$, $K_{ls}$ and anatomical values. *Kochia scoparia* and *A. tex-


![Figure 3. Xylem vulnerability curves expressed as the percentage loss of $K_L$ versus xylem tension for $C_3$ and $C_4$ species. (a) $C_3$ *Chenopodium album* and $C_4$ *Kochia scoparia* and (b) $C_3$ *Ipomoea pes-caprae* and $C_4$ *Boerhavia coccinea*. The best fit equations (shown as solid curves) are an exponential rise [$y = 91.04(1 - 0.45x); r^2 = 0.87$] for *C. album*; exponential growth [$y = -61 + 64.4(1.2x); r^2 = 0.78$] for *K. scoparia*; sigmoidal [$y = 233.2/1 + (x/4.76)^{-1.22}; r^2 = 0.66$] for *I. pes-caprae*; and exponential rise [$y = 171.2(1 - 0.9x); r^2 = 0.85$] for *B. coccinea*. Each symbol represents one stem measurement.](image-url)
Table 2. Anatomical parameters of C₃ and C₄ species

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Species</th>
<th>MVL (cm)</th>
<th>MVD (μm)</th>
<th>VMD (μm)</th>
<th>HMD (μm)</th>
<th>Dₑ (μm)</th>
<th>VF (mm²)</th>
<th>Conduit EV (m²/10⁻¹⁴)</th>
<th>LSP × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. scoparia</td>
<td>C₄</td>
<td>13.2 ± 0.9²</td>
<td>57.2 ± 1.1²</td>
<td>30.4 ± 1.4²</td>
<td>38.3 ± 1.4²</td>
<td>34.6 ± 1.4²</td>
<td>177 ± 13 ²</td>
<td>1.5 ± 0.3 ²</td>
<td>0.9 ± 0.1 ²</td>
</tr>
<tr>
<td>A. retroflexus</td>
<td>C₃</td>
<td>19.5 ± 1.9²</td>
<td>70.1 ± 1.4²</td>
<td>38.8 ± 0.8²</td>
<td>51.3 ± 3.9²</td>
<td>45.2 ± 0.9²</td>
<td>87 ± 6 ²</td>
<td>2.6 ± 0.5 ²</td>
<td>0.9 ± 0.1 ²</td>
</tr>
<tr>
<td>C. album</td>
<td>C₃</td>
<td>28.8 ± 1.3²</td>
<td>93.1 ± 3.3²</td>
<td>49.0 ± 1.8²</td>
<td>65.1 ± 2.4²</td>
<td>57.2 ± 2.2²</td>
<td>43 ± 3 ²</td>
<td>6.8 ± 6.0 ²</td>
<td>1.5 ± 0.1 ²</td>
</tr>
<tr>
<td>C. boys</td>
<td>C₃</td>
<td>20.3 ± 2.0²</td>
<td>75.8 ± 3.2²</td>
<td>38.5 ± 1.2²</td>
<td>52.8 ± 2.4²</td>
<td>46.6 ± 2.3²</td>
<td>166 ± 11²</td>
<td>15.3 ± 1.7²</td>
<td>4.5 ± 0.2²</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. texana</td>
<td>C₃</td>
<td>6.6 ± 0.4²</td>
<td>39.8 ± 2.3²</td>
<td>17.4 ± 1.2²</td>
<td>27.0 ± 1.2²</td>
<td>23.1 ± 1.5²</td>
<td>206 ± 24²</td>
<td>1.0 ± 0.2²</td>
<td>1.2 ± 0.1²</td>
</tr>
<tr>
<td>A. rosea</td>
<td>C₄</td>
<td>9.0 ± 0.9²</td>
<td>52.7 ± 0.9²</td>
<td>21.8 ± 1.4²</td>
<td>35.9 ± 1.9²</td>
<td>31.8 ± 1.9²</td>
<td>169 ± 18²</td>
<td>1.5 ± 0.3²</td>
<td>1.3 ± 0.1²</td>
</tr>
<tr>
<td>A. hortensis</td>
<td>C₃</td>
<td>8.2 ± 0.3²</td>
<td>56.4 ± 2.5²</td>
<td>19.6 ± 0.3²</td>
<td>35.9 ± 1.0²</td>
<td>27.6 ± 0.7²</td>
<td>165 ± 11²</td>
<td>2.2 ± 0.3²</td>
<td>1.8 ± 0.2²</td>
</tr>
<tr>
<td>A. triangularis</td>
<td>C₄</td>
<td>n.d.</td>
<td>51.6 ± 1.6²</td>
<td>20.0 ± 0.9²</td>
<td>33.7 ± 1.5²</td>
<td>27.9 ± 1.8²</td>
<td>225 ± 16²</td>
<td>3.1 ± 0.4²</td>
<td>2.8 ± 0.1²</td>
</tr>
<tr>
<td>A. littoralis</td>
<td>C₃</td>
<td>9.8 ± 1.4²</td>
<td>58.8 ± 1.4²</td>
<td>26.0 ± 1.2²</td>
<td>39.0 ± 1.6²</td>
<td>33.2 ± 1.6²</td>
<td>113 ± 15²</td>
<td>4.1 ± 0.2²</td>
<td>3.0 ± 0.3²</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. portulacastrum</td>
<td>C₄</td>
<td>n.d.</td>
<td>75.7 ± 3.3²</td>
<td>23.2 ± 1.1²</td>
<td>37.4 ± 3.1²</td>
<td>30.8 ± 2.3²</td>
<td>268 ± 25²</td>
<td>3.3 ± 0.8²</td>
<td>2.0 ± 0.2²</td>
</tr>
<tr>
<td>S. verrucosum</td>
<td>C₃</td>
<td>n.d.</td>
<td>55.7 ± 2.2²</td>
<td>19.8 ± 1.2²</td>
<td>34.1 ± 2.5²</td>
<td>29.6 ± 1.5²</td>
<td>581 ± 91²</td>
<td>6.9 ± 1.2²</td>
<td>6.2 ± 0.9²</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. dominii</td>
<td>C₃</td>
<td>n.d.</td>
<td>52.6 ± 5.1²</td>
<td>20.6 ± 1.0²</td>
<td>39.0 ± 3.9²</td>
<td>29.9 ± 1.8²</td>
<td>231 ± 28²</td>
<td>1.3 ± 0.2²</td>
<td>1.0 ± 0.2²</td>
</tr>
<tr>
<td>B. coccinea</td>
<td>C₄</td>
<td>14.6 ± 0.8²</td>
<td>56.9 ± 2.6²</td>
<td>25.5 ± 1.1²</td>
<td>38.9 ± 2.4²</td>
<td>32.5 ± 2.0²</td>
<td>131 ± 6²</td>
<td>2.2 ± 0.3²</td>
<td>1.4 ± 0.1²</td>
</tr>
<tr>
<td>T. eicherianus</td>
<td>C₄</td>
<td>14.5 ± 1.7²</td>
<td>67.4 ± 3.7²</td>
<td>24.4 ± 2.1²</td>
<td>42.2 ± 1.9²</td>
<td>33.5 ± 2.4²</td>
<td>245 ± 26²</td>
<td>3.4 ± 0.6²</td>
<td>1.8 ± 0.1²</td>
</tr>
<tr>
<td>C. rosea</td>
<td>C₄</td>
<td>25.6 ± 4.9²</td>
<td>143 ± 6.8²</td>
<td>45.6 ± 2.8²</td>
<td>98.2 ± 6.2²</td>
<td>75.2 ± 6.4²</td>
<td>84 ± 4²</td>
<td>10.4 ± 1.4²</td>
<td>1.3 ± 0.2²</td>
</tr>
<tr>
<td>I. pes-caprae</td>
<td>C₃</td>
<td>33.4 ± 3.9²</td>
<td>157.7 ± 7.1²</td>
<td>37.8 ± 1.7²</td>
<td>105.7 ± 6.8²</td>
<td>61.4 ± 7.1²</td>
<td>156 ± 14²</td>
<td>9.8 ± 1.4²</td>
<td>1.5 ± 0.1²</td>
</tr>
</tbody>
</table>

MVL, maximum vessel length; MVD, maximum vessel diameter; VMD, vessel mean diameter; HMD, hydraulic mean diameter; Dₑ, mean diameter of conduits that account for 95% of the flow; VF, vessel frequency, number of vessels per unit xylem area; Conduit EV, conduit efficiency value (sum of the fourth power of the radius of all conduits divided by the leaf area supplied by these conduits); Leaf specific porosity (LSP), total vessel lumen area divided by leaf area. Different letters within each functional group represent significant differences within the group (P < 0.05). Values are means (± SE) of 5–10 samples for each species. Non-determined is indicated by n.d.

ana in the first two groups, and the three C₄ species from the fourth group (B. dominii, B. coccinea and T. eicherianus) produced shorter and narrower vessels compared to their respective C₃ counterparts. Consistently, K. scoparia and B. coccinea had less xylem holes than C. album and I. pes-caprae, their respective C₃ counterparts in the comparison.

In habitats with abundant water and nutrients, high shoot growth is promoted and light availability becomes the main limiting resource that determines competitive outcomes (Bloom et al. 1985; Bazzaz 1996; Hutchings 1997). In these environments, strong competitive interactions above ground provide the evolutionary selection pressure that could favour location allocation to greater leaf area, but only as long as the hydraulic pathway is able to support the leaf canopy. The greater WUE of C₃ photosynthesis should relax the hydraulic demands of the canopy and allow for greater leaf area. This hypothesis is supported by the greater leaf area observed in *Amaranthus retroflexus*, *Atriplex rosea* and *T. portulacastrum*. *Amaranthus retroflexus* is one of the world’s worst weeds, growing in highly productive agricultural fields and other resource-rich sites such as old fields and livestock pens (Paul & Elmore 1984; Holm et al. 1997). It often grows amongst *Chenopodium* spp. in weedy situations and the two are considered be competitors in these sites (Pearcy, Tumosa & Williams 1981). *Atriplex rosea* similarly grows in old field and abandoned lots, where it often competes with C₃ *Atriplex* and *Chenopodium* species (Holm et al. 1997). Neither of these species are very drought tolerant, although the *Atriplex* spp. are tolerant of moderate salinity (Osmond et al. 1980). *Trianthema portulacastrum* and *S. verrucosum* are also fast-growing annuals, but the main characteristic of their habitat is the ephemeral presence of water. They grow on river floodplains, recently exposed mudflats and where water puddles following heavy rain, typically in arid regions and on soils with some levels of salinity (Wayne 1993; Sage, personal observation). High rates of growth appear valuable in order to set seed before soil water is depleted and salinity stress becomes extreme. In this regard, the greater leaf canopy supported by *T. portulacastrum* should lead to greater carbon gain, seed yield and potential fitness.

On drought-prone soils, relaxation of hydraulic requirements due to greater WUE could be exploited by increasing xylem safety. Improved safety would allow plants to maintain photosynthesis at lower leaf water potentials without compromising the hydraulic pathway. This could allow for longer growing seasons, as leaves could remain active later into a dry season (Osmond et al. 1980), or alternatively, leaves could reduce the level of stomatal closure during low humidity periods, such as occurs during midday (Schulze & Hall 1982; Sperry, Alder & Eastlack 1993; Sperry 1995). Comparisons of C₃ and C₄ responses in arid communities support these hypotheses. In Death Valley in California, C₄ *Atriplex hymenelytra* maintained daily leaf conductance and CO₂ uptake two to three times higher than co-

occurring C3 Larrea divaricata during the dry season (Osmond et al. 1980). In the Great Basin desert of North America, the C4 shrub Atriplex confertifolia maintains its leaf canopy in late summer conditions that cause die-back in sympatric Chrysothamnus and Ceratoideae species (Osmond et al. 1980; Sperry & Hacke 2002). In the Negev desert, C4 Hammada (= Haloxylon) scoparia maintains gas exchange under more extreme conditions of atmospheric and soil drought than the sympatric C3 plants Zygophyllum dumosum and Artemisia herba-alba (Schulze et al. 1980). This was also the case in C3 and C4 annual Atriplex species grown in a common garden under non-irrigated conditions in coastal California. Atriplex rosea (C4) maintained activity toward the end of the dry season whereas A. triangularis (C3) died early in the dry season (Nobs et al. 1972).

Most of the comparisons of C3 and C4 plants have focused on the direct advantages of the C4 pathway such as the suppression of photorespiration, enhanced photosynthetic potential in warm climates, and greater water, nitrogen and radiation use efficiency. Differences in resource use efficiency facilitate secondary evolution that relieves constraints that may occur elsewhere in the system such as in the hydraulic pathway (Bloom et al. 1985). The secondary evolutionary response to the WUE advantage in C4 plants is pronounced and complements the direct benefits of the C4 pathway by allowing C4 plants to develop adaptive traits to a greater degree than may be possible in C3 plants. For example, in resource-rich environments, the ability to carry more leaf area per stem allows for greater light capture, productive potential and competitive ability (Potter &
This explains in part the ability of C₄ species to exhibit greater yields than C₃ species, and to become aggressive weeds (Brown 1999). By contrast, in arid environments, the secondary advantages of WUE could allow C₄ species to occur in drier soils and maintain function at drier periods of the year.

The high productivity of C₄ plants has led to attempts to engineer C₄ photosynthesis into crops such as rice (Häusler et al. 2002). Our results indicate the benefits of engineering C₄ plants do not end with the insertion of C₄ photosynthesis into leaves. To fully exploit the benefits of C₄ photosynthesis, bioengineers should eventually consider modifying...
Effect of photosynthetic pathway alters xylem structure and hydraulic function

xylem properties to optimize $K_L$. As the crop yield is directly related to canopy area (Gifford & Evans 1981) shifts in $K_L$ will also be needed to maximize the productive potential of the C$_4$ pathway.

In conclusion, the results here demonstrate the WUE advantage of C$_4$ plants lead to more than just greater rates of carbon gain or water savings. As in many economic enterprises, efficiencies realized in one part of the system can allow resources to be re-allocated to functions that address the next most critical environmental challenge (Bloom et al. 1985). In arid environments, this may be greater safety. In resource-rich environments, this could be competitive potential, as indexed by leaf area production or increased fecundity. In either case, it is apparent that evolution within the hydraulic pathway in response to the advent of C$_4$ photosynthesis enhances the ability of these plants to address environmental challenges.

ACKNOWLEDGMENTS

We thank Tammy Sage, Thomas Fung, Kathy Sault and Mathew Lam for their assistance and Nancy Dengler, Spencer Barrett and Taylor Feild for helpful comments. We also thank Digdem Demir and Emin Kocacinar for their assistance during the study. This research was supported by a scholarship from the Ministry of Education of Turkey to F.K. and by NSERC grant #OGP0134273 to R.F.S.

REFERENCES


Received 22 May 2003; received in revised form 20 August 2003; accepted for publication 21 August 2003