Photosynthetic pathway alters xylem structure and hydraulic function in herbaceous plants

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ABSTRACT

Plants using the C_4 photosynthetic pathway have greater water use efficiency (WUE) than C_3 plants of similar ecological function. Consequently, for equivalent rates of photosynthesis in identical climates, C_4 plants do not need to acquire and transport as much water as C_3 species. Because the structure of xylem tissue reflects hydraulic demand by the leaf canopy, a reduction in water transport requirements due to C_4 photosynthesis should affect the evolution of xylem characteristics in C_4 plants. In a comparison of stem hydraulic conductivity and vascular anatomy between eight C_3 and eight C_4 herbaceous species, C_4 plants had lower hydraulic conductivity per unit leaf area (K_L) than C_3 species of similar life form. When averages from all the species were pooled together, the mean K_L for the C_4 species was 1.60×10^{-4} kg m^{-1} s^{-1} MPa^{-1}, which was only one-third of the mean K_L of 4.65×10^{-4} kg m^{-1} s^{-1} MPa^{-1} determined for the C_3 species. The differences in K_L between C_3 and C_4 species corresponded to the two- to three-fold differences in WUE observed between C_3 and C_4 plants. In the C_4 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 C_4 species had evolved safer xylem than the C_3 species. In the plants from resource-rich areas, such as the C_4 weed *Amaranthus retroflexus*, hydraulic conductivity per xylem area and xylem anatomy were similar to that of the C_3 species, but the C_4 plants had greater leaf area per xylem area. The results indicate the WUE advantage of C_4 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, C_4 plants evolved safer xylem, which can increase survival and performance during drought events.

Key-words: C_4 photosynthesis; hydraulic architecture; water use efficiency; water transport; xylem anatomy.

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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 C_4 photosynthesis, should also influence xylem characteristics of plants.

C_4 plants have two- to four-fold greater WUE than C_3 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 C_4 *Atriplex* species, *A. rosea*, *A. expansa* and *A. serenana,* have two to three times the WUE of the C_3 *Atriplex* species, *A. triangularis*, *A. hortensis* and *A. heterosperma* (Osmond *et al.* 1980). By evolving C_4 photosynthesis, plants might shift the optimal balance between xylem efficiency and safety, such that the lower water requirements of the C_4 leaf canopy might allow for safer xylem than present in their C_3 ancestors. Alternatively, for the same amount of xylem, C_4 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, C_4 species should have a lower leaf specific conductivity (K_L; stem hydraulic conductivity relative to the leaf area supported by the stem) compared with similar C_3 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 C_4 plants, and none to our knowledge has specifically compared xylem hydraulics of C_3 and C_4 species of similar life forms, ecological requirements or phylogenetic affinity. The leafy CAM plant *Clusia uvitana* has been observed to have K_L values that are 1/3 to 1/30 of a range of tropical C_3 species, indicating that the photosynthetic pathway can alter xylem function (Zotz, Tyree & Cochard 1994). In woody C_3 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 C_4 WUE could promote a drop in K_L. 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*; K_L was highest in *R. mangle* and lowest in *A. germinans* (Sobrado 2000). In the Great Basin desert in Utah, stems of the C_4 shrubs *Atriplex canescens* and *Atriplex confertifolia* were more cavitation-resistant than the stems of the co-occurring C_3 shrubs *Chrysotham-*

nus 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 C_4 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 C_3 and eight C_4 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 strands 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 C_4 species relative to the C_3 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* (C_4), *Amaranthus retroflexus* (C_4), *Chenopodium album* (C_3) and *Chenopodium botrys* (C_3) were collected from disturbed habitats in Toronto, Ontario. In the second group, *Atriplex texana* (C_4) seeds were collected from the desert of the American south-west, near Marathon, Texas. *Atriplex rosea* (C_4) seeds were courtesy of Dr Paul Hattersley from the Australian National University. Seeds of *Atriplex hortensis* (C_3) were obtained from the Botanical Garden of the Free University, Amsterdam, The Netherlands. *Atriplex triangularis* (C_3) seeds were collected on a farm field at Pigeon Point, California. *Atriplex littoralis* (C_3) seeds were obtained from the Antwerp Botanical Garden, Belgium. Seeds of the third group, *Trianthema portulacastrum* (C_4) were collected on an abandoned lot in St. George, Utah and *Sesuvium verrucosum* (C_3) 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

Functional group Species	Ph. type	Family	Habitat	Collection site/seed source
Group 1: weedy annuals				
Kochia scoparia L.	C_4	Chenopodiaceae	Waste, disturbed land	Toronto, ON (43°65′ N 79°38′ W)
Amaranthus retroflexus L.	C_4	Amaranthaceae	Waste, cultivated land	Toronto, ON (43°65′ N 79°38′ W)
Chenopodium album L.	C_3	Chenopodiaceae	Waste, cultivated land	Toronto, ON (43°65′ N 79°38′ W)
Chenopodium botrys L.	C_3	Chenopodiaceae	Waste, cultivated land	Toronto, ON (43°65′ N 79°38′ W)
Group 2: annual *Atriplex*				
Atriplex texana S. Wats.	C_4	Chenopodiaceae	Arid land, roadsides	Marathon, TX (30°10′ N 103°15′ W)
Atriplex rosea L.	C_4	Chenopodiaceae	Waste, disturbed land	Australian National University
Atriplex hortensis L.	C_3	Chenopodiaceae	Waste, disturbed land	Free University Botanical Garden, The Netherlands
Atriplex triangularis Willd.	C_3	Chenopodiaceae	Saline wetlands	Pigeon Point, CA (37°9′ N 122°25′ W)
Atriplex littoralis L.	C_3	Chenopodiaceae	European coastal strands	Antwerp Botanical Garden, Belgium
Group 3: *Sesuvioideae* annuals				
Trianthema portulacastrum L.	C_4	Aizoaceae	Arid land ephemeral	St. George, UT (37°12′ N 113°35′ W)
Sesuvium verrucosum L.	C_3	Aizoaceae	Ephemeral saline wetlands	L. Lahonton, NV (39°19′ N 119°9′ W)
Group 4: coastal-strand herbs				
Boerhavia dominii Meikle & Hewson	C_4	Nyctaginaceae	Waste land, saline marshes	Darwin, Australia (12°15′ S 131°10′ E)
Boerhavia coccinea Miller	C_4	Nyctaginaceae	Waste land, coastal strands	Darwin, Australia (12°15′ S 131°10′ E)
Tribulus eichlerianus (K. L. Wilson)	C_4	Zygophyllaceae	Waste land, coastal strands	Darwin, Australia (12°15′ S 131°10′ E)
Canavalia rosea (Sw.) DC.	C_3	Fabaceae	Coastal strands, beaches	Darwin, Australia (12°15′ S 131°10′ E)
Ipomoea pes-caprae (L) R. Br.	C_3	Convolvulaceae	Coastal strands, beaches	Darwin, Australia (12°15′ S 131°10′ E)

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 (K_h) 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 K_h 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 K_h 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 r^2 values between 0.98 and 0.9999 for the linear regression equations. Xylem specific conductivity (K_s) was calculated by dividing K_h by xylem cross-sectional area determined with a light microscope (Olympus AX70, Olympus America Inc., Melville, New York, USA). Lumen specific conductivity (K_{ls}) was obtained by dividing K_h 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_{ls} is more informative than K_s alone because K_{ls} takes xylem porosity and vessel frequency into account. Leaf specific conductivity (K_L) was measured as K_h divided by 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* (C_3) and *K. scoparia* (C_4) from group 1 and for *Ipomoea pes-caprae* (C_3) and *Boerhavia coccinea* (C_4) 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:

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

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 alchohol in water (FAA), paraffin-embedded and sectioned using a sliding microtome. Sections were examined under a light microscope and images analysed with 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 (D_{95}) 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 $D = 2(\Sigma r^5 / \Sigma r^4)$, 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 C_3 and C_4 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_s, differed little between the species in the first three comparison groups (Fig. 1a). In the fourth group, the coastal strand C_4 species – *Boerhavia dominii*, *B. coccinea* and *Tribulus eichlerianus* – had significantly lower K_s than the co-occurring C_3 species *Canavalia rosea* and *I. pes-caprae* (Fig. 1a). Lumen specific conductivity, K_{ls}, 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_{ls} between C_3 and C_4 species except in the Australian plants where all C_4 species showed significantly lower values than C_3 species (Fig. 1b). In this group, K_{ls} was between three and 12 times lower in C_4 than C_3 species.

When leaf area was accounted for, all C_4 species within a group showed significantly lower leaf specific conductivity, K_L, than C_3 species within the same functional group (Fig. 2). K_L was 54 to 85% lower in the C_4 than C_3 plants in the first group, 27 to 80% less in the C_4 than C_3 species in the second group consisting of annual *Atriplex* spp., and 50% lower in the C_4 *T. portulacastrum* than the C_3 *S. verrucosum* of the third group. In the Australian coastal species, K_L was 50 to 92% less in the C_4 than C_3 members of the group. When averages from all the species were pooled together, the mean K_L for the C_4 species was 1.60×10^{-4}
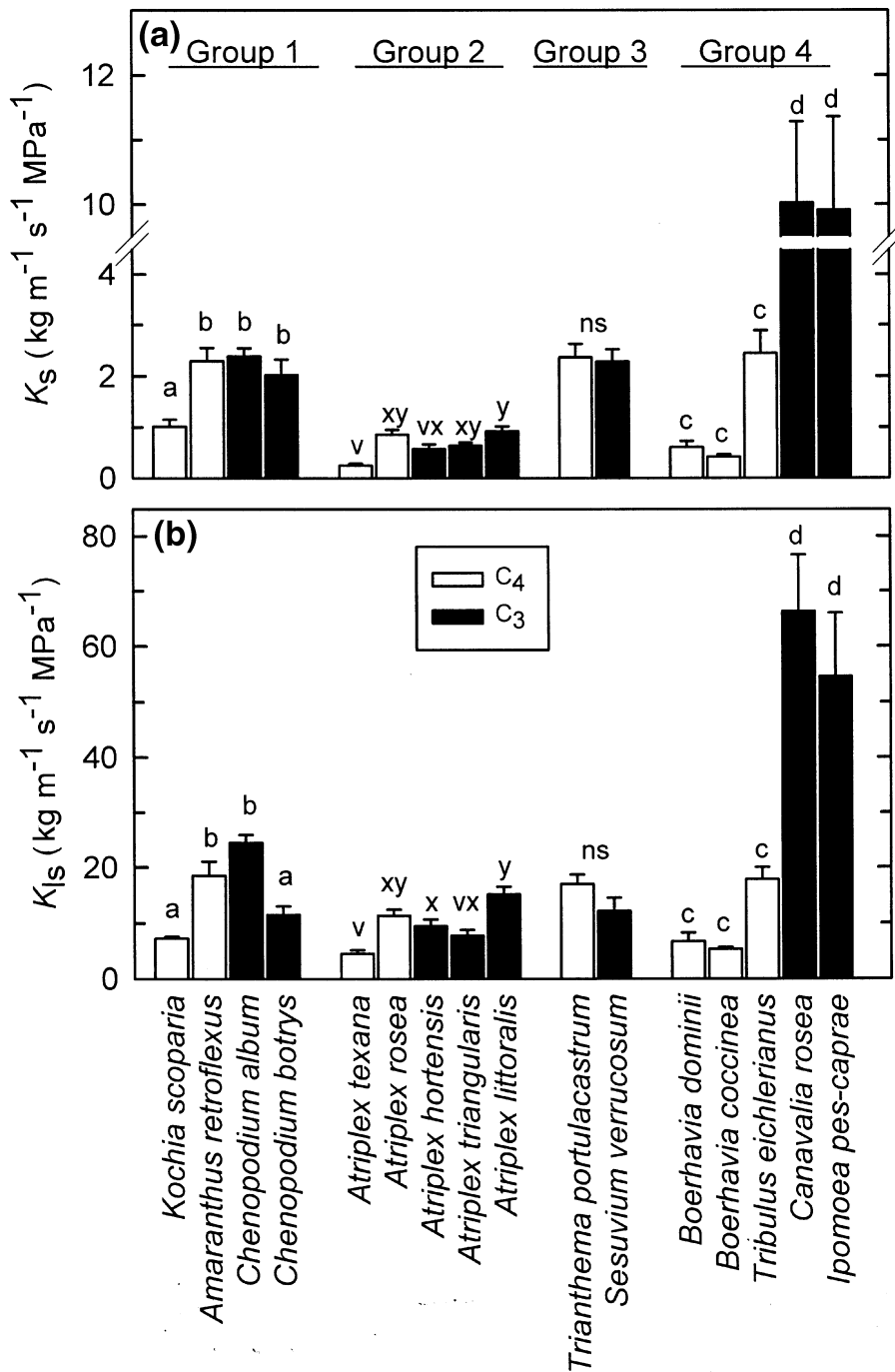
Figure 1. Xylem and lumen area specific conductivities, K_s and K_{ls}, of C_4 and C_3 herbaceous species. Group 1 consists of coexisting old-field annual plants. Group 2 includes annual *Atriplex* species. The third group includes two closely related species in the Sesuvioideae tribe of the Aizoaceae and group 4 contains species from coastal regions of northern Australia. Bars represent means ($\pm$ SE) of 5–10 plants. Different letters within each group represent significant difference at $P < 0.05$. 'ns', not significant.

$(kg\ m^{-1}\ s^{-1}\ MPa^{-1})$, which was one-third of the mean K_L of 4.65×10^{-4} $(kg\ m^{-1}\ s^{-1}\ MPa^{-1})$ determined for the C_3 species ($P = 0.004$).

Vulnerability of the xylem to cavitation was less in the two C_4 members of the representative pairs of C_3 and C_4 species chosen from groups 1 and group 4 (Fig. 3a & b). In the C_3 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 C_4 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 C_4 *B. coccinea* was more cavitation resistant than the xylem of co-occurring C_3 *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 C_4 plants was similar to or smaller than C_3 species within a comparison group (Table 2). In most cases, one of the C_3 species exhibited the widest vessels in
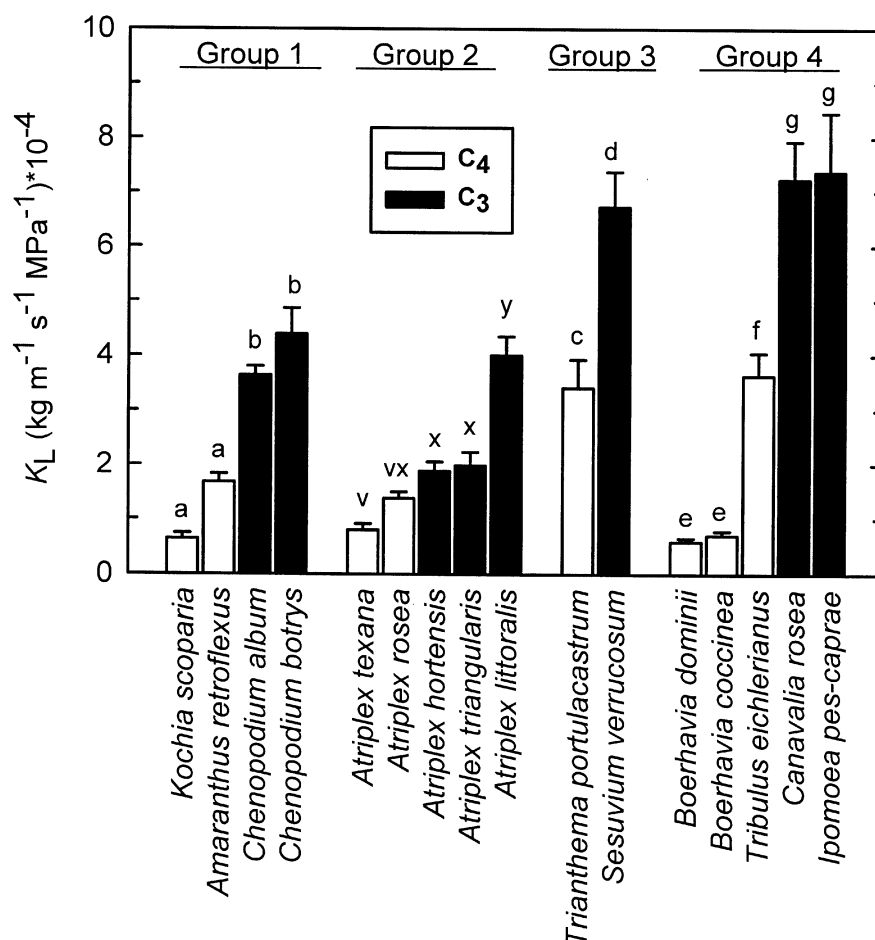
Figure 2. Leaf specific conductivity, K_L, of the four groups of C_4 and C_3 plants. Groups are as described in Fig. 1. Bars are means ($\pm$ SE) of 5–10 plants. Different letters within each group represent significant difference at $P < 0.05$.

a comparison group whereas C_4 species had the smallest; these differences were readily visible in a comparison of xylem cross sections (Fig. 4). In the first group, *A. retroflexus* (C_4) had similar xylem anatomy as the C_3 species *C. botrys*: MVL, MVD, VMD, HMD and D_{95} were all similar between the two species (Table 2). However, *C. botrys* had twice as many vessels per xylem area as *A. retroflexus*. Both C_4 *K. scoparia* and *A. retroflexus* had smaller vessels than *C. album* (Fig. 4a & b, Table 2). In group 2, *A. rosea* (C_4) exhibited similar anatomical parameters to the C_3 species *A. hortensis*, *A. triangularis* and *A. littoralis*. The other C_4 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* (C_3) had double the number of vessels per xylem area compared with the C_4 herb *T. portulacastrum* (Fig. 4e & f, Table 2). In group 4, the C_4 species (*B. dominii*, *B. coccinea* and *T. eichlerianus*) had shorter and narrower vessels than the C_3 species *C. rosea* and *I. pes-caprae* (Fig. 4g & h, Table 2). When anatomy was referenced to leaf area, C_4 species generally had lower leaf area-based ratios as indicated by significantly lower conduit efficiency values and leaf specific porosity values (Table 2). Leaf specific porosity overlapped between C_3 and C_4 species only in

group 4. The C_4 species within each group tended to have a greater proportion of their vessels in the shorter size classes than C_3 species in the corresponding group (Fig. 5). Notably, the distribution of vessels extended to longer size classes in the C_3 members of groups 1 and 4.

DISCUSSION

C_4 plants are widely noted for having superior WUE than C_3 plants, and this is often presumed to increase carbon gain and drought tolerance. As a secondary consequence of the superior WUE, we hypothesized that C_4 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, K_L. In all comparison groups, the C_4 species consistently exhibited lower K_L than the corresponding C_3 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
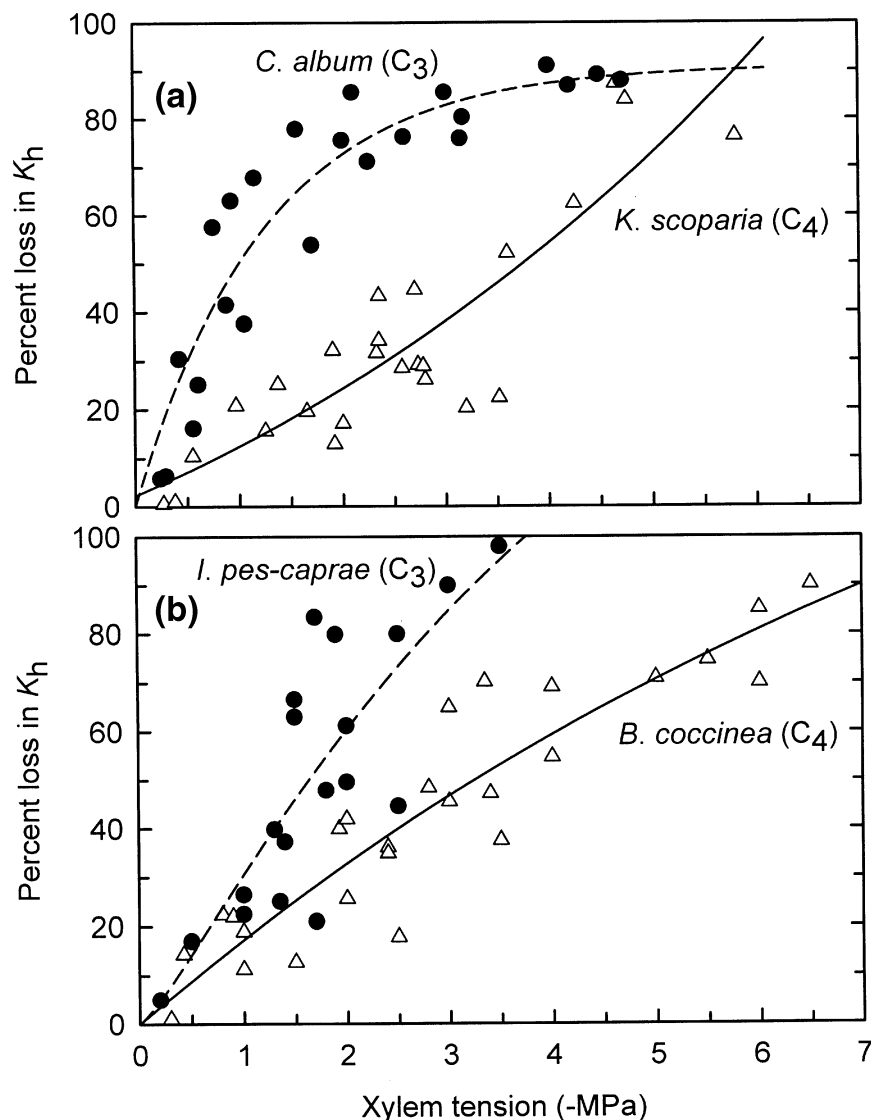
Figure 3. Xylem vulnerability curves expressed as the percentage loss of K_h versus xylem tension for C_3 and C_4 species. (a) C_3 *Chenopodium album* and C_4 *Kochia scoparia* and (b) C_3 *Ipomoea pes-caprae* and C_4 *Boerhavia coccinea*. The best fit equations (shown as solid curves) are an exponential rise [$y = 91.04(1 - 0.45^x)$; $r^2 = 0.87$] for *C. album;* exponential growth [$y = -61 + 63.4(1.2^x)$; $r^2 = 0.78$] for *K. scoparia*; sigmoidal [$y = 233.2/1 + (x/4.76)^{-1.22}$; $r^2 = 0.66$] for *I. pes-caprae*; and exponential rise [$y = 171.2(1 - 0.9^x)$; $r^2 = 0.85$] for *B. coccinea*. Each symbol represents one stem measurement.

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_2O in the C_4 species grown at high soil nitrogen; WUE in *C. album* was 3–5 mmol CO_2 mol^{-1} H_2O 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. portu-*

lacastrum 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 plants *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-*

Table 2. Anatomical parameters of C_4 and C_3 species

Functional group Species		MVL (cm)	MVD (μm)	VMD (μm)	HMD (μm)	D_{95} (μm)	VF (n mm^{-2})	Conduit EV (m^2) $\times 10^{-14}$	LSP $\times 10^{-5}$
Group 1									
K. scoparia	C_4	13.2 ± 0.9^a	57.2 ± 1.1^a	30.4 ± 1.4^a	38.3 ± 1.4^a	34.6 ± 1.4^a	177 ± 13^c	1.5 ± 0.3^a	0.9 ± 0.1^a
A. retroflexus	C_4	19.5 ± 1.9^b	70.1 ± 1.4^b	38.8 ± 0.8^b	51.3 ± 1.8^b	45.2 ± 0.9^b	87 ± 6.0^b	2.6 ± 0.5^a	0.9 ± 0.1^a
C. album	C_3	26.8 ± 1.4^c	93.1 ± 3.3^c	49.0 ± 1.5^c	65.1 ± 2.4^c	57.2 ± 2.2^c	43 ± 3.0^a	6.8 ± 0.6^b	1.5 ± 0.1^b
C. botrys	C_3	20.3 ± 2.0^{bc}	75.8 ± 4.3^b	38.5 ± 1.2^b	52.8 ± 2.4^b	46.6 ± 2.3^b	166 ± 11^c	15.3 ± 1.7^c	4.5 ± 0.2^c
Group 2									
A. texana	C_4	6.6 ± 0.4^a	39.8 ± 2.3^a	17.4 ± 1.2^a	27.0 ± 1.2^a	23.1 ± 1.5^a	206 ± 24^b	1.0 ± 0.2^a	1.2 ± 0.1^a
A. rosea	C_4	9.0 ± 0.9^a	52.7 ± 0.9^a	21.8 ± 1.4^{ab}	35.9 ± 1.9^b	31.8 ± 1.9^b	169 ± 18^{ab}	1.5 ± 0.3^{ab}	1.3 ± 0.1^a
A. hortensis	C_3	8.2 ± 0.3^a	56.4 ± 2.5^b	19.6 ± 0.3^a	35.9 ± 1.0^b	27.6 ± 0.7^{ab}	165 ± 11^{ab}	2.2 ± 0.3^{bc}	1.8 ± 0.2^a
A. triangularis	C_3	n.d	51.6 ± 1.6^b	20.0 ± 0.9^{ab}	33.7 ± 1.5^b	27.9 ± 1.8^{ab}	225 ± 16^b	3.1 ± 0.4^c	2.8 ± 0.1^b
A. littoralis	C_3	9.8 ± 1.4^a	58.8 ± 1.4^b	24.6 ± 1.2^b	39.0 ± 1.6^b	33.2 ± 1.6^b	113 ± 15^a	4.1 ± 0.2^c	3.0 ± 0.3^b
Group 3									
T. portulacastrum	C_4	n.d.	57.5 ± 5.3^a	22.3 ± 1.1^a	37.4 ± 3.1^a	30.8 ± 2.3^a	268 ± 25^a	3.3 ± 0.8^a	2.0 ± 0.2^a
S. verrucosum	C_3	n.d.	55.7 ± 2.2^a	19.8 ± 1.2^a	34.1 ± 2.5^a	29.6 ± 1.5^a	581 ± 91^b	6.9 ± 1.2^b	6.2 ± 0.9^b
Group 4									
B. dominii	C_4	n.d	52.6 ± 5.1^a	20.6 ± 1.0^a	39.0 ± 3.9^a	29.9 ± 1.8^a	231 ± 28^c	1.3 ± 0.2^a	1.0 ± 0.2^a
B. coccinea	C_4	14.6 ± 0.8^a	56.9 ± 2.6^a	25.5 ± 1.1^a	38.9 ± 2.4^a	32.5 ± 2.0^a	131 ± 6^{ab}	2.2 ± 0.3^a	1.4 ± 0.1^{abc}
T. eichlerianus	C_4	14.5 ± 1.7^a	67.4 ± 3.7^a	24.4 ± 2.1^a	42.2 ± 1.9^a	33.5 ± 2.4^a	245 ± 26^c	3.4 ± 0.6^a	1.8 ± 0.1^c
C. rosea	C_3	25.6 ± 4.9^b	143.1 ± 6.8^b	45.6 ± 2.8^c	98.2 ± 6.2^b	75.2 ± 6.4^b	84 ± 4^a	10.4 ± 1.4^b	1.3 ± 0.2^{ab}
I. pes-caprae	C_3	33.4 ± 3.9^b	157.7 ± 7.1^b	37.8 ± 1.7^b	105.7 ± 6.8^b	61.4 ± 7.1^b	156 ± 14^b	9.8 ± 1.4^b	1.5 ± 0.1^{bc}

MVL, maximum vessel length; MVD, maximum vessel diameter; VMD, vessel mean diameter; HMD, hydraulic mean diameter; D_{95}, 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 ($\pm$ SE) of 5–10 samples for each species. Non-determined is indicated by n.d.

ana in the first two groups, and the three C_4 species from the fourth group (*B. dominii*, *B. coccinea* and *T. eichlerianus*) produced shorter and narrower vessels compared to their respective C_3 counterparts. Consistently, *K. scoparia* and *B. coccinea* had less vulnerable xylem than *C. album* and *I. pes-caprae*, their respective C_3 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 allocation to greater leaf area, but only as long as the hydraulic pathway is able to support the leaf canopy. The greater WUE of C_4 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 C_3 *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 flood-plains, 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 C_3 and C_4 responses in arid communities support these hypotheses. In Death Valley in California, C_4 *Atriplex hymenelytra* maintained daily leaf conductance and CO_2 uptake two to three times higher than co-
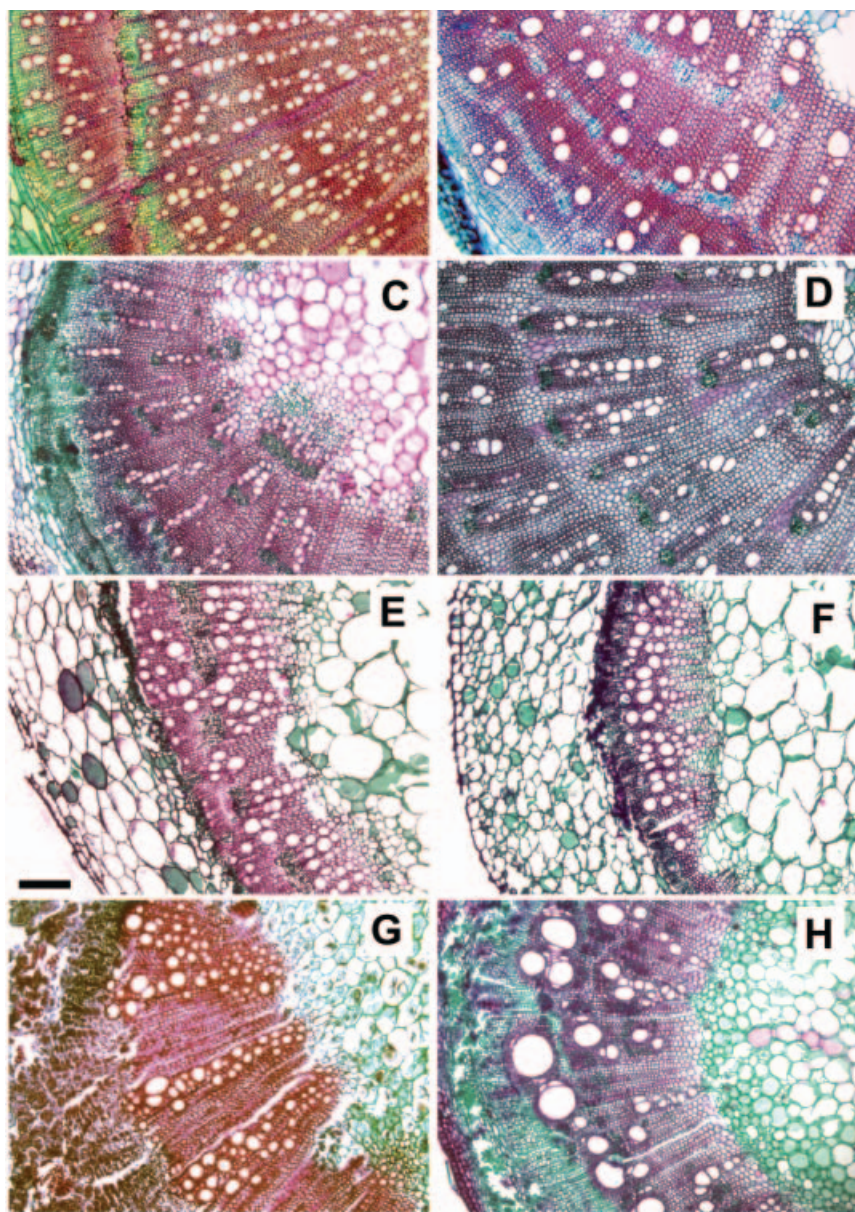
Figure 4. Cross sections of stem xylem of representative pairs of C$_4$ and C$_3$ species from each functional group. Left panels are C$_4$ and right panels are C$_3$. (a), *Kochia scoparia*; (b), *Chenopodium album*; (c), *Atriplex texana*; (d), *Atriplex littoralis*; (e), *Trianthema portulacastrum*; (f), *Sesuvium verrucosum*; (g), *Tribulus eichlerianus*, and (h) *Canavalia rosea*. The scale bar in (e) is 200 μm and applies to all panels.

occurring C$_3$ *Larrea divaricata* during the dry season (Osmond *et al*. 1980). In the Great Basin desert of North America, the C$_4$ shrub *Atriplex confertifolia* maintains its leaf canopy in late summer conditions that cause die-back in sympatric *Chrysothamnus* and *Ceratoides* species (Osmond *et al*. 1980; Sperry & Hacke 2002). In the Negev desert, C$_4$ *Hammada* (= *Haloxylon*) *scoparia* maintains gas exchange under more extreme conditions of atmospheric and soil drought than the sympatric C$_3$ plants *Zygophyllum dumosum* and *Artemisia herba-alba* (Schulze *et al*. 1980). This was also the case in C$_3$ and C$_4$ annual *Atriplex* species grown in a common garden under non-irrigated conditions in coastal California. *Atriplex rosea* (C$_4$) maintained activity toward the end of the dry season whereas *A. triangularis* (C$_3$) died early in the dry season (Nobs *et al*. 1972).

Most of the comparisons of C$_3$ and C$_4$ plants have focused on the direct advantages of the C$_4$ 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 C$_4$ plants is pronounced and complements the direct benefits of the C$_4$ pathway by allowing C$_4$ plants to develop adaptive traits to a greater degree than may be possible in C$_3$ 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 &
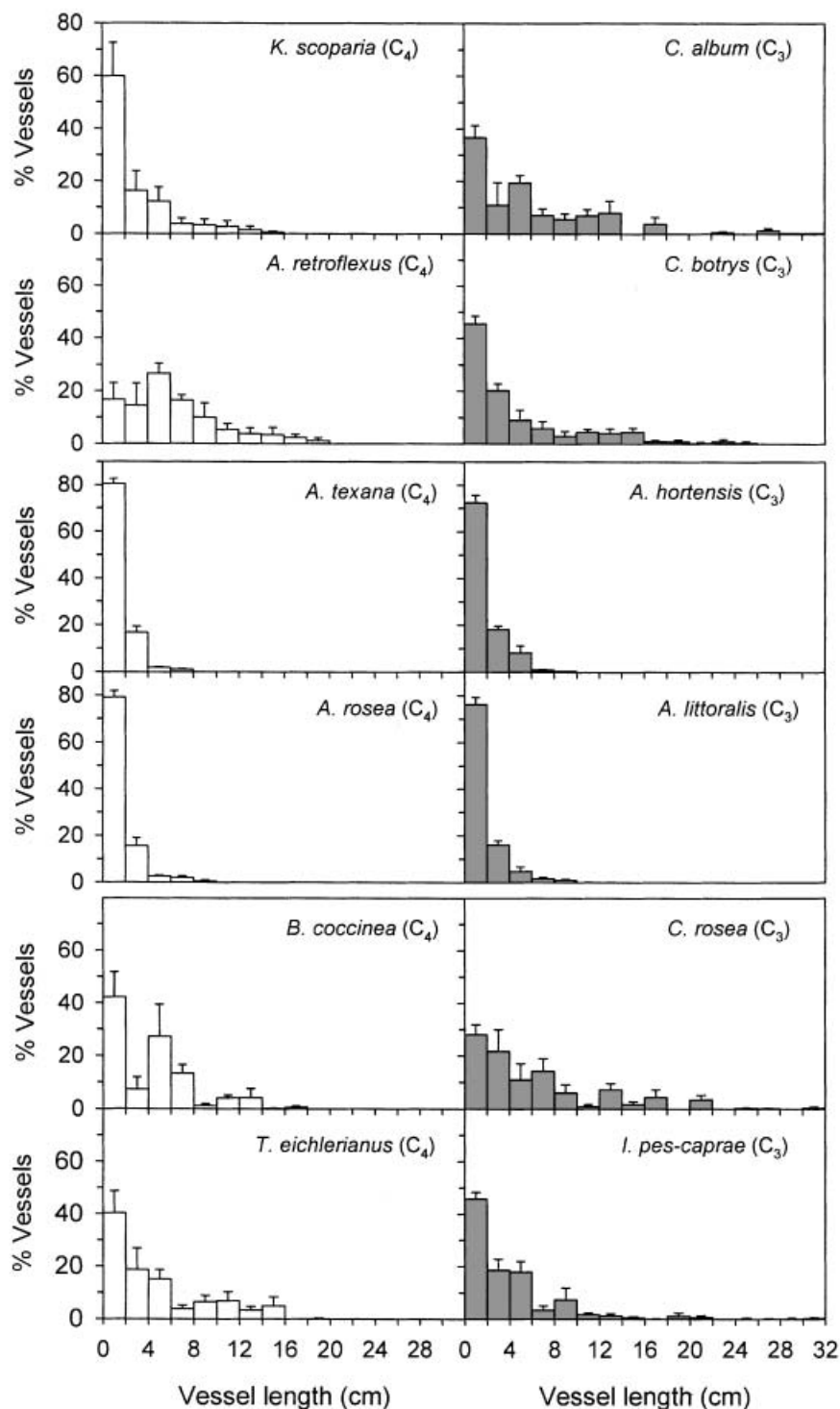
Figure 5. Vessel length distributions of C_4 and C_3 species from group 1, 2, and 4 in 2-cm length classes. Bars are means ($\pm$ SE) of three to six samples.

Jones 1977; Sage & Pearcy 1987a). This explains in part the ability of C_4 species to exhibit greater yields than C_3 species, and to become aggressive weeds (Brown 1999). By contrast, in arid environments, the secondary advantages of WUE could allow C_4 species to occur in drier soils and maintain function at drier periods of the year.

The high productivity of C_4 plants has led to attempts to engineer C_4 photosynthesis into crops such as rice (Häusler *et al.* 2002). Our results indicate the benefits of engineering C_4 plants do not end with the insertion of C_4 photosynthesis into leaves. To fully exploit the benefits of C_4 photosynthesis, bioengineers should eventually consider modifying

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.

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