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Stem anatomy and relative growth rate in seedlings of a wide range of woody plant species and types

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Abstract Stem traits were analysed in laboratory-grown seedlings of 80 European woody and semiwoody species of known potential relative growth rate (RGR) and of similar ontogenetic phase. The objectives were, firstly, to assess the relation between stem structure and plant growth potential and, secondly, to explore how stem structure varies among species differing in life form and leaf habit. Hydraulic conductance was represented by the mean diameter of the widest xylem conduits (*D*max), and structural strength by the percentage of xylem tissue occupied by cell wall material (CWx) or stem tissue density (SD). Across all species RGR showed a weak positive correlation with *D*max and weak negative ones with CWx and SD, with slow-growers showing great dispersion of stem trait values. In the RGR-*D*max relationship this dispersion disappeared when trees were removed from the analysis. None of the relationships were significant among tree species alone. It was suggested that fast-growers require a xylem with wide conduits (high *D*max) to achieve high hydraulic conductivity, and "cheaply" constructed stems (low CWx and SD) to maximise allocation to leaves. However, the possession of such traits does not guarantee fast growth, as other factors may constrain RGR elsewhere in the plant. Deciduous seedlings showed higher *D*max and lower CWx than evergreens. Higher *D*max could reflect an innate higher tolerance of conductivity loss by freeze-induced embolism in deciduous plants, which are not burdened by the maintenance of foliage in winter. In contrast, life forms were differentiated most clearly by SD. For instance, shrub seedlings had less dense stem tissues than tree seedlings, possibly because they need less investment in long-term strength and stature.

Key words Leaf habit · Life forms · RGR · Stem anatomy · Woody plants

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Introduction

Plants differ widely in the net rate of production per unit of dry weight (mean relative growth rate, RGR). These differences can be attributed partly to environmental conditions, partly to innate species traits (Grime and Hunt 1975; Poorter 1990). Many works have aimed to identify the plant attributes explaining RGR variation, such as physiology (Konings 1990; Walters et al. 1993), leaf such as morphology (Konings 1990; Poorter 1990; Cornelissen et al. 1996), leaf anatomy (Van Arendonk and Poorter 1994), nutrient partitioning (Ingestad and Lund 1986; Huante et al. 1995; Cornelissen et al. 1997), chemical composition (Niemann et al. 1995; Van Arendonk and Poorter 1994) and allocation-related factors (Poorter and Remkes 1990; Cornelissen et al. 1996). All these studies ignored stem xylem traits, in spite of their importance in the water supply of plants.

Plant stems fulfil three major roles: water transport, support and storage. Among woody species, which can attain large sizes, there must be a conflict between the need for support, which is an important sink for biomass, and the growth capacity (Givnish 1995). Additionally, as the water pathway can be very long in woody plants, xylem hydraulics might be expected to constrain

the growth capacity. Few works, however, have related stem traits to plant growth. Mencuccini and Grace (1996) suggested that hydraulic resistance can be an important factor limiting the growth of a tree as it ages. Xylem embolism has been demonstrated to prevent apical growth in grape vine under natural conditions (Schultz and Matthews 1988). Dense wood can provide mechanical support at the expense of growth rate (Lawton 1984). However, the relationship between innate wood traits and plant growth capacity has not been tested empirically for sufficiently large sets of species to justify general conclusions.

This paper investigates whether the RGR of a wide range of woody species is connected with stem traits that are indicators of the hydraulic capacity (conduit diameter), or mechanical strength (proportion of cell wall in xylem tissue and stem tissue density). The study was performed on the seedlings of 80 woody species from (Mediterranean) northeast Spain and Great Britain, grown under standard conditions, of similar ontogenetic phase, whose RGRs were known (Cornelissen et al. 1996). The comparison of species from different climatic regions ensures a wide range of RGR and stem characteristics, as plants from water-limited environments (such as the Mediterranean) tend to show lower innate RGR (Cornelissen et al. 1998) and higher frequency of xeromorphic xylem anatomical specialisations (Rury and Dickison 1984; Carlquist and Hoekman 1985; Baas and Schweingruber 1987) than those from mesic sites.

The second aim of this work is to explore how innate stem structure varies between functional groups of woody species. Constraints in the xylem of adult plants have been demonstrated to differ between leaf habits (Sobrado 1993) and life forms (Baas and Schweingruber 1987; Ewers et al. 1990; Gartner et al. 1990). Therefore, stem attributes will be compared between deciduous and evergreen seedlings and between different life forms in order to test whether such variation already exists at an early developmental stage.

Material and methods

Plant material and RGR measurements

The seedling populations and growth protocol were the same as those used by Cornelissen et al. (1996) to assess RGR, so only a brief summary is given here. Seedlings were grown from seeds from provenances in temperate and Mediterranean Europe, mostly Great Britain and northeast Spain, respectively. The set of 80 species included the most representative (semi-)woody species of both regions. A few species from other provenances were added to balance the spectra of life forms and leaf habits. After germination and at least three days before the start of the growth analysis, seedlings were transplanted into the experimental 300- or 400-l pots and filled with quarried, prewashed silica sand. The pots were put in a growth chamber, with standard environmental conditions at the Unit of Comparative Plant Ecology, Sheffield University (details in Hendry and Grime 1993). The light regime was 135 ± 10 μmol m^{-2} s^{-1} of photosynthetic photon flux density (red/far red ratio 1.4) during 14 h day^{-1}, at 20–22°C, the night temperature being 15–17°C. This "partial shade" light regime may have led some Mediterranean species to underperform in terms of growth rate. However, studies dealing with different species sets have shown that the RGR ranking did not change when species were grown under different light intensities (Williams and Buxton 1989; Kitajima 1994; Reich et al. 1998).

Population RGR is the mean net increment in dry biomass per unit of plant dry biomass over a time interval. The population of each species was divided into two halves. The first one was harvested once the modal plant of the population had opened (but not fully expanded) the first true leaf (pair). At that time, plants were considered to be in a comparable ontogenetic phase and starting to gain carbon by true leaves. Total plant dry weight was determined. The second half was grown for 21 days, supplied every 2 days with 0.25 ml of full-strength Rorison nutrient solution (N, P and K at 56, 31 and 78 mg l^{-1}, respectively, plus Ca, Mg, Fe and trace elements) per ml of sand volume (Hendry and Grime 1993). Sufficient deionised water was added to the drainless trays in which the pots stood, to provide continuous access to bottom water. These surplus conditions in the substratum and the moderate air temperature combined with a ventilation system were intended to minimise resistance to water flow from soil to the plant and from leaves to the atmosphere. Once the second half was harvested, seedlings were oven dried (48 h at 80°C), then weighed. RGR across the time interval t_2-t_1 was derived as:

$$\mathrm{RGR} = (\ln W_2 - \ln W_1)/(t_2 - t_1),$$

W being the plant dry weight, excluding any attached thick cotyledon (even if green as in some *Prunus* species).

Preparation of material for anatomical analysis

At the time of the second harvest, three to four seedlings per species were randomly selected and pickled (details in Hendry and Grime 1993). The whole stem or a portion of it was embedded in agar 5% and progressively dehydrated in 50, 70 and 95% ethanol (2 h per solution). The small blocks of agar were then infiltrated for 15 days with resin JB 4 Polysciences (Polysciences Inc., 400 Valley Road, Warington, Pa., USA). After polymerisation of the resin, 2 μm thick cross-sections were obtained with a glass ultra microtome, then sections were stained with 5% toluidine blue and permanently mounted on slides with DPX. The section point was approximately midway between the cotyledons and the first true leaves in most species, but in some seedlings there was not enough true stem and the section was made below the cotyledons.

Data collection

The stem cross-sections were studied under a microscope Zeiss Axioskop equipped with a camera/scopeman MORITEX MS-500 m-SCOPEMAN which transfers the microscopic images to a computer. Anatomical measurements were taken on the computer screen using image analysis software (*Aequitas* IA version 1.25).

The average diameter of the ten widest conduits of the section (*D*max) was measured as a correlate of the theoretical hydraulic conductivity (t-K_h). This parameter is calculated from the Hagen-Poiseuille law (Zimmermann 1983) and has been found to be strongly correlated with the measured hydraulic conductivity (Chiu and Ewers 1992; Hargrave et al. 1994). It has been shown for several woody species that the largest vessels account for a very high proportion of t-K_h (Chiu and Ewers 1992; Villar-Salvador et al. 1997). Ten conduits were found to be sufficient to provide an accurate average *D*max because many seedlings had only a few vessels.

The proportion of xylem area occupied by cell walls in transverse section (CWx) was considered to be directly related to stem strength, as the amount of other thick-walled tissues in the stem was negligible. CWx was measured in three to four microscopic fields per slide using *Aequitas* tools. Data were collected only in a subsample of 42 species (see Table 1), as not all the slides showed enough contrast between xylem cell walls and lumen to allow accurate measurements.

Stem density (SD) was also assumed to indicate tissue strength (Ryser 1996). The transverse section area of each stem was multi-

plied by the segment length of a 3- to 10-mm-long stem segment adjacent to the cut in order to obtain segment volume. Those species where this value was below 10 mm^3 were excluded from the analysis, as the error became too large. SD was calculated as stem segment dry weight over its volume.

Most samples showed a clearly developed metaxylem (cell walls were intensely stained). However, in a few of them xylem was not clearly differentiated from other stem tissues, these cases being excluded from analysis.

Statistical analysis

Relations between the stem parameters and RGR were quantified using Spearman's rank correlation, as the data were not normally distributed. Differences in traits among leaf habits or life forms were tested using two and one-way ANOVAs and Tukey's tests. Leaf habits were evergreen, deciduous and semi-deciduous and species life forms were classified into subshrubs, procumbent subshrubs, shrubs, climbers, scramblers and trees.

Analyses were first performed using species. In order to test whether the results were confounded with taxonomy above the species level, a taxonomic relatedness analysis was performed (Felsenstein 1985; Kelly and Beerling 1995). For each set of two or more subtaxa belonging to the same taxon, it was recorded whether the relationship between RGR and stem parameters showed a positive (+) or negative (−) trend. The average value of the lower taxa could subsequently be employed for a similar comparison of the next level up, up to the class level. We also tested whether, within such contrasts, the average trait values of deciduous versus evergreen taxa showed the same ranking than at species level (+) or the reverse (−). For evergreen versus deciduous contrasts (discrete variables), average values where calculated using only the taxa which had not been used in lower level contrasts. Combining the results at all taxonomic levels, χ^2-tests were carried out to investigate whether there were more pluses than minuses than expected if there were no underlying relationship.

Results

The studied traits varied widely among the species. Mean RGR ranged from 0.014 in *Ilex aquifolium* to 0.227 day^{-1} in *Solanum dulcamara* (Cornelissen et al. 1996), *D*max from 6.51 in *Erica cinerea* to 42.08 μm in *S. dulcamara*, SD from 0.04 in *S. dulcamara* to 0.57 mg mm^{-3} in *Fagus sylvatica* and CWx from 28 in *Rubus fruticosus* to 62% in *Cistus clusii* (Table 1).

In the entire set of species *D*max mean RGR showed a weak positive correlation with *D*max and weak negative ones with SD and CWx (Fig. 1A, C, E). When the tree species were removed from the analysis, the correlation improved drastically for RGR versus *D*max (Fig. 1B), but not for RGR versus CWx (Fig. 1D) nor SD (Fig. 1F). None of these relationships was significant when only tree species were considered (data not shown).

The taxonomic relatedness analysis only showed a significant (negative) trend for the relationship between RGR and SD, most consistently at the higher levels. Although not significant, the RGR versus *D*max relationship appeared more often positive than negative (Table 2).

Both leaf habit and life form influenced the distribution of stem trait values. Semi-deciduous species were added to the evergreen group for analysis because their leaves can be retained through the winter, a critical season for xylem embolism. Subshrubs and procumbent subshrubs were considered together (named SS), and so were climbers and scramblers (named C + Sc), which share their dependence on external physical support. However the distribution of species among life forms and leaf habits was non-orthogonal, with only one deciduous SS and two evergreen C + Sc. Therefore, the two-way ANOVA testing the effect of both factors on stem traits only included shrubs (S) and trees (T). *D*max was higher in deciduous than in evergreens, while the reverse was true for CWx, independently of the life form (Tables 3 and 4). Both results appeared more often than the reverse across all taxonomic levels, although the latter was not significant, maybe due to the low number of cases (Table 5). Within the deciduous species, trees showed significantly higher values of SD than other life forms, and within evergreens, *D*max was lower in SS than in shrubs or trees (Table 6). Owing to the low number of cases in some of the combinations between life forms and leaf habits, a taxonomic relatedness analysis was not possible in this case. Instead, we repeated the analysis using genus averages and obtained very similar results (data not shown).

Discussion

Stem parameters and RGR

Among the seedlings of 80 European (semi-)woody species variation in wood structure corresponded with that in plant growth capacity. The positive link between RGR and *D*max may be a consequence of the close relationship between RGR and leaf area ratio (leaf area per unit of plant biomass, LAR) found previously in these seedlings ($r = 0.88$ $P < 0.001$; Cornelissen et al. 1996). Fast growers have a higher proportion of transpiration surface and therefore, a higher potential for loosing water per unit of plant biomass. As they allocate proportionally little matter to transport organs (i.e. stems and roots), their xylem should be more efficient in water transport to meet the foliage demands for transpiration. High hydraulic conductance can be achieved by developing wide diameters among a fraction of the xylem conduits and/or by increasing the relative amount of xylem and thus the total number of conduits (Shumway et al. 1993). However, the latter strategy could reduce LAR by reducing the proportion of biomass available to the foliage (leaf weight fraction) which is a correlate of LAR (Cornelissen et al. 1996). It thus appears that the most reliable strategy to simultaneously maximise the hydraulic conductance, LAR, and therefore also RGR, is the development of xylems with high *D*max values. This trend was strong among seedlings of small woody species but was not observed among trees. Tree seedlings had lower average LAR and RGR (Cornelissen et al. 1996), but not lower *D*max, than smaller life forms. This might be expected for tall adult

Table 1 Plant and stem features of the studied species (Seed provenance: *B* British Isles, *S* Spain, *E* Elsewhere in western Europe, * from specimen in park or garden; life form: *PS* procumbent subshrub, *S* shrub, *Sc* scrambler, *C* climber, *SS* subshrub, *T* tree; leaf habit: *D* deciduous, *E* evergreen, *D*/*E* semi-evergreen; seedling stem attributes: *Dmax* average diameter of the 10 widest conduits, *SD* stem density, *CWx* xylem cell wall proportion). Nomenclature follows Stace (1991)

Species	Seed prov.	Life form	Leaf habit	*D*max (μm)	SD (mg/mm^3)	CWx (%)
Acer platanoides	B*	T	D	15.42	0.27	53%
Acer pseudoplatanus	B	T	D	21.45	0.20	37%
Aesculus hippocastanum	B	T	D	26.67	0.29	34%
Alnus glutinosa	B	T	D	19.23		31%
Arbutus unedo	S	T/(S)	E	12.06	0.23	47%
Atriplex halimus	S	S	E	16.19		41%
Berberis vulgaris	B	S	D	12.20	0.24	59%
Betula pendula	B	T	D	13.42		30%
Buddleja davidii	B	S	D	24.84	0.14	31%
Buxus sempervirens	S	S	E	6.64		56%
Calluna vulgaris	B	SS	E	8.31		38%
Castanea sativa	B	T	D	35.76	0.27	46%
Celtis australis	S	T	D	33.17	0.29	34%
Cistus albidus	S	S	E	10.45	0.31	53%
Cistus clusii	S	SS	E	8.40	0.18	62%
Cistus laurifolius	S	S	E	16.16	0.11	33%
Clematis vitalba	S	C	D	21.11	0.15	37%
Cornus sanguinea	B	S	D	25.41	0.27	38%
Crataegus monogyna	B	S	D	15.38	0.29	49%
Cytisus scoparius	B	S	E	14.43	0.21	47%
Daphne gnidium	S	S	E	12.29	0.09	44%
Dryas octopetala	B	PS	E	14.06		40%
Empetrum nigrum	B	PS	E	10.13		42%
Erica cinerea	B	SS	E	6.51		42%
Fagus sylvatica	E	T	D	20.79	0.57	45%
Frangula alnus	E	S	D	17.85	0.29	49%
Fraxinus excelsior	B	T	D	21.45	0.20	45%
Hebe x franciscana	B*	S	E	16.30		41%
Hedera helix	B	C	E	13.76	0.29	37%
Helianthemum nummularium	B	PS	E	10.80		61%
Helianthemum squamatum	S	SS	E	9.39	0.43	55%
Hippophae rhamnoides	B*	S	D	14.83	0.20	39%
Ilex aquifolium	B	T/(S)	E	13.72	0.24	
Juglans regia	E	T	D	39.28	0.38	
Laburnum anagyroides	B*	T	D	21.64	0.22	
Larix decidua	E	T	D	10.73		
Ligustrum vulgare	B	S	D/E	14.33	0.07	
Linum suffruticosum	S	SS	E	8.78	0.13	
Lonicera implexa	S	C	E	17.62	0.08	42%
Lonicera periclymenum	B	C	D	16.53	0.09	37%
Malus sylvestris	B	T	D	20.20	0.26	
Picea sitchensis	B	T	E	10.88		45%
Pinus halepensis	S	T	E	13.57	0.19	
Pinus sylvestris	B	T	E	10.77	0.21	
Prunus laurocerasus	B	S	E	19.50	0.22	
Prunus lusitanica	B*	T/(S)	E	25.38	0.21	
Prunus spinosa	B	S	D	23.99		38%
Quercus cerris	B*	T	D	23.24	0.28	
Quercus coccifera	S	S	E	13.37	0.44	58%
Quercus faginea	S	T	D	23.45	0.36	49%
Quercus ilex subsp. *ballota*	S	T	E	16.72	0.49	58%
Quercus ilex subsp. *ilex*	S	T	E	19.68	0.38	
Quercus petraea	B	T	D	28.15	0.38	
Quercus pubescens	S	T	D	25.48	0.46	
Quercus robur	B	T	D	28.49	0.46	
Quercus suber	S	T	E	25.92	0.48	56%
Rhamnus alaternus	S	S	E	10.75	0.20	56%
Rhamnus cathartica	B	S	D	12.73		
Rhamnus lycioides	S	S	E	11.63	0.28	60%
Rhododendron ponticum	B	S	E	10.70		
Ribes nigrum	B*	S	D	18.57	0.13	
Ribes uva-crispa	B*	S	D	21.02	0.25	
Rosa arvensis	B	Sc	D	19.50	0.14	39%

Table 1 (continued)

Species	Seed prov.	Life form	Leaf habit	*D*max (μm)	SD (mg/mm^3)	CWx (%)
Rosa canina	B	Sc	D	19.43	0.20	37%
Rosmarinus officinalis	S	S	E	12.51	0.10	
Rubus fruticosus	B	Sc	D/E	25.07	0.09	28%
Salix caprea	B	T	D	21.13	0.14	
Sambucus nigra	B	S	D	25.28	0.05	
Solanum dulcamara	B	Sc	D	42.08	0.04	33%
Sorbus aria	B	T	D	21.47	0.28	
Sorbus aucuparia	B	T	D	13.37	0.39	
Taxus baccata	B	T	E	13.58	0.12	
Thymus polytrichus	B	PS	E	13.24	0.12	
Tilia cordata	E	T	D	22.10		
Ulex europaeus	B	S	E	17.43	0.15	
Ulex gallii	B	S	E	16.90	0.17	
Ulmus glabra	B	T	D	18.15	0.32	
Vaccinium myrtillus	B	SS	D	7.26		
Vaccinium vitis-idaea	B	SS	E	7.83	0.22	
Viburnum opulus	B	S	D	16.04		46%

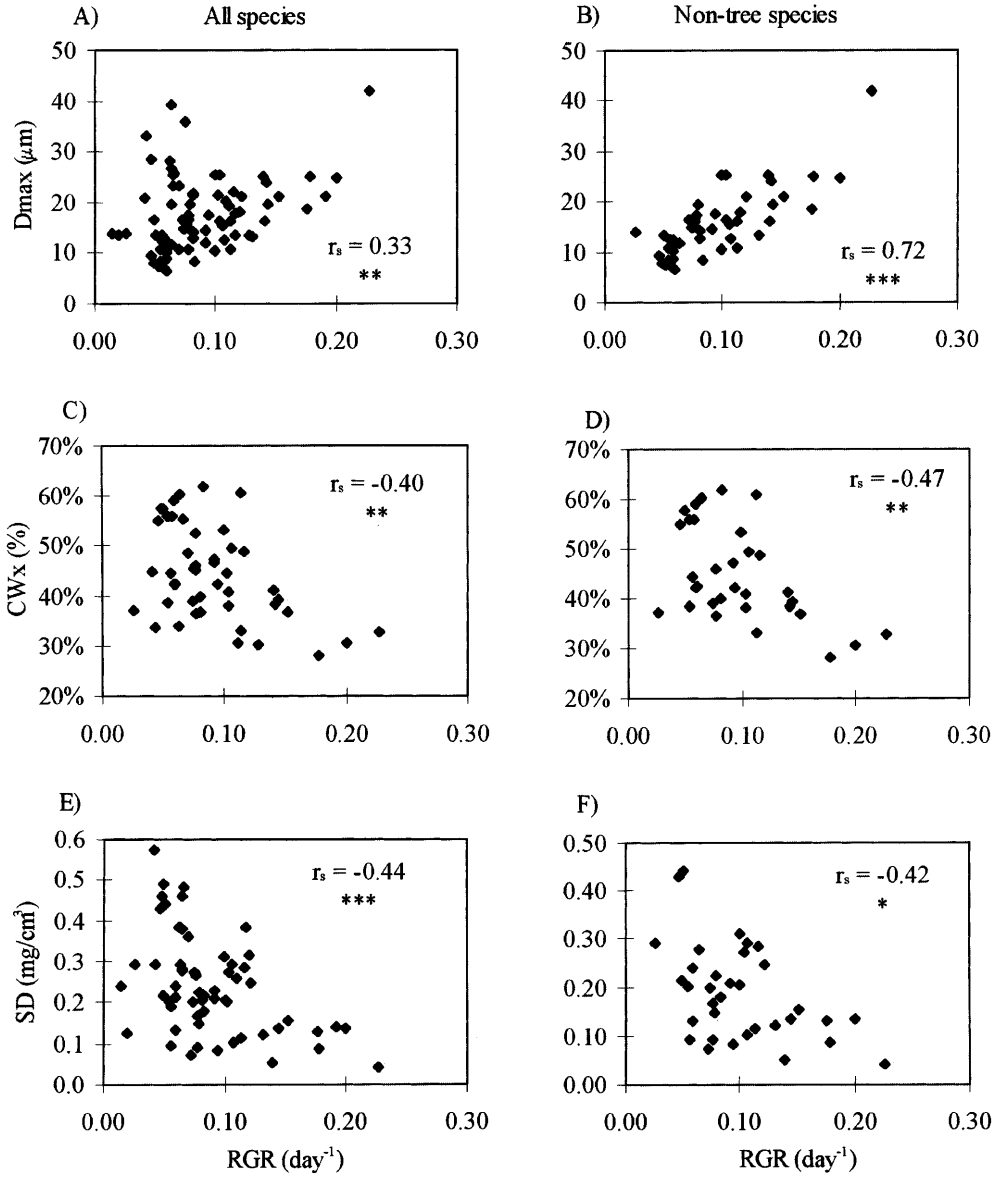

Fig. 1 Spearman's rank correlations between RGR and stem traits (*Dmax* mean maximum diameter of xylem conduits, *CWx* percentage of cell wall in the xylem, *SD* stem density) among the 80 studied species

Table 2 Taxonomic relatedness analysis of the relationship between mean RGR and stem anatomical traits. The null hypothesis states that these relationships show either positive (+, RGR *vs.* *D*max) or negative trends (−, RGR *vs.* CWx or SD) no more often than if by chance between taxa of the same taxonomic level (one-tailed χ^2 tests). The number of taxa used in each contrast is in parentheses; in the second and third columns it only appears where it differs from the number in the first column

	RGR *vs.* *D*max	RGR *vs.* CWx	RGR *vs.* SD
Species within genera			
Acer (2)	+	−	−
Cistus (3)	+	−	−
Helianthemum (2)	+	+	
Lonicera (2)	+	+	−
Pinus (2)	−		+
Prunus (3)	+		− (2)
Quercus (9)	+	− (4)	−
Rhamnus (3)	+	+ (2)	+ (2)
Ribes (2)			−
Ulex (2)	+		−
Vaccinium (2)	−		
Genera within families			
Betulaceae (2)	−	−	
Caprifoliaceae (3)	+	− (2)	− (2)
Cistaceae (2)	+	−	−
Ericaceae (5)	+	+ (3)	+ (2)
Fabaceae (3)	−		+
Fagaceae (3)	+	+	−
Lamiaceae (2)	+		+
Oleaceae (2)	+		+
Pinaceae (3)	−		
Rhamnaceae (2)	+	−	+
Rosaceae (7)	+	− (5)	− (6)
Ulmaceae (2)	−		+
Families within orders			
Ericales (2)	−	+	
Fagales (2)	−	−	
Ranunculales (2)	+	−	−
Rosales (3)	+	− (2)	−
Sapindales (2)	−	+	−
Scrophulariales (3)	+	−	
Orders within subclasses			
Asteridae (4)	+	− (3)	−
Dilleniidae (4)	+	+ (2)	− (3)
Hamamelidae (3)	−	+ (2)	−
Rosidae (10)	+		
Subclasses within classes			
Magnoliopsida (6)	−	−	− (5)
All contrasts	χ^2 ; 2.162 ns	χ^2 ; 1.080 ns	χ^2 ; 3.846 ns

* $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$; *ns* not significant

Table 3 Summarised results of two-way ANOVAs testing the effect of leaf habit (*D* deciduous *vs.* *E* evergreen) and life form (*S* shrub *vs.* *T* tree) on the three stem parameters. Subshrubs and climbers + scramblers were not considered due to the low number of cases

	Leaf habit (D/E)	Life form (S/T)	Interaction
*D*max	***	*ns*	*ns*
CWx	*	*ns*	*ns*
SD	*ns*	**	*ns*

* $0.01 \leqslant P \leqslant 0.05$; ** $0.001 \leqslant P \leqslant 0.01$; *** $P < 0.001$; *ns* not significant

Table 4 Mean stem trait values compared between deciduous and evergreen species in Student's *t*-test

	Deciduous	Evergreen	*t*-test
*D*max	21.51	13.44	***
CWx	0.40	0.48	**
SD	0.25	0.22	ns

* 0.01 *ns* $P < 0.05$; ** 0.001 *ns* $P < 0.01$; *** $P < 0.001$; ns not significant

trees because, independently of their hydraulic necessities, they require a higher proportion of stem volume and biomass in order to be mechanically stable (Givnish 1995). The small stature of tree seedlings does not justify the existence of such mechanical constraints. However, they may be programmed genetically to produce stems with higher biomechanical safety per unit of hydraulic conductance, to cope with the hazards accompanying tall stature at maturity. This is evidenced by the generally denser stem tissues of tree seedlings as compared to seedlings of shrubs, climbers and scramblers. In addition, the use of stems and roots for resource storage would reduce LAR without altering the balance between

Table 5 Taxonomic relatedness analysis comparising stem anatomy traits of deciduous (*D*) and evergreen (*E*) taxa. The ranking of the groups obtained from the whole set of species (Student's *t*-test) is indicated in parentheses in the column headings. "+" means that the result is the same as the above, while "−" means that the result is reversed. The null hypothesis states that there are no more "+" than if by chance across all taxonomic levels (one-tailed χ^2-test). Figures in parentheses indicate the number of taxa used in each higher taxon

	*D*max[a] (D > E)	CW_x[b] (E > D)		*D*max (D > E)	CW_x (E > D)
Species within genera			Families within orders		
Lonicera (2)	−		Scrophulariales (2)	+	+
Prunus (3)	+				
Quercus (9)	+	+ (4)	Orders within subclasses		
Vaccinium (2)	−		Asteridae (3)	+	
			Dilleniidae (4)	+	
Genera within families			Rosidae (10)	+	+ (7)
Fabaceae (3)	+				
Oleaceae (2)	+		Subclasses within classes		
Pinaceae (3)	−		Magnoliopsida (3)	+	−
Rosaceae (6)	+	+ (4)	All contrasts χ^2	4.57*	1.18 *ns*

* $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$; *ns* not significant

Table 6 Mean values ± 1SE of the stem variables for each group of leaf habit and life form (number of species sampled per group in parenthesis). A one-way ANOVA assessed the effect of the life form in each leaf habit group. Differences between individual life forms were tested a posteriori (Tukey's test). The same letter in different rows means that differences are not significant

	*D*max (μm)	CWx (%)	SD (mg/cm^3)
Deciduous species			
C + Sc	23.95 ± 3.8 (6)	0.35 ± 0.02 (6)	0.12 ± 0.02a (6)
S	19.01 ± 1.4 (12)	0.44 ± 0.03 (8)	0.21 ± 0.03a (9)
T	22.79 ± 1.4 (23)	0.40 ± 0.03 (10)	0.32 ± 0.03b (19)
ANOVA	*ns*	*ns*	***
Evergreen species			
SS	9.74 ± 0.75a (10)	0.49 ± 0.04 (7)	0.22 ± 0.05 (5)
S	14.41 ± 1.03b (17)	0.49 ± 0.03 (10)	0.20 ± 0.03 (13)
T	15.21 ± 1.64b (9)	0.51 ± 0.03 (4)	0.29 ± 0.05 (8)
ANOVA	**	*ns*	*ns*

* $0.01 \leq P < 0.05$; ** $0.001 \leq P < 0.01$; *** $P < 0.001$; *ns* not significant

hydraulic conductance and plant leaf area. We observed that seedlings of large-seeded trees (deciduous *Quercus* spp., *Castanea sativa*, *Juglans regia*, *Aesculus hippocastanum*) had big proportions of storage pith both in the stem and main root. Indeed, these species combined low LAR with rather high *D*max.

A word of caution seems appropriate for those trees (e.g. deciduous *Quercus* species) that expand their leaves in flushes. Ren and Sucoff (1995) found that the hydraulic conductance per unit of leaf area is not constant through the development of two leaf-flushing *Quercus* species. Therefore, the *D*max to LAR ratio may be very different in the same plant according to whether the harvest took place just before or just after a leaf flush.

Xylem cell walls constitute the main component of wood, the higher CWx the more biomass is needed to construct a unit of wood volume and the more compact it is. SD was considered as an easy-to-assess correlate of CWx, but it depends not only on the "quality" of the xylem (i.e. CWx), but also on its "quantity" in the stem. Wood density has been associated with slow plant growth in adult woody plants (Lawton 1984; Sobrado 1993). However, stem density assessment at maturity does not consider the tissues outside the cambium, so it is only partially comparable to our SD. The negative correlation of SD and CWx with RGR can be explained in terms of allocation pattern. Low tissue density, in general, has been suggested to enable a fast resource acquisition as the plant can rapidly expand its organs with low investment of dry matter (Ryser 1996). The price for low stem density and cell wall-poor xylem may be a low resistance both to mechanical stress and to fungal and/or insect attack. This would result in smaller stature or in shorter plant longevity (Loehle 1988; Brzeziecki and Kienast 1994; Givnish 1995).

There was a wide scatter of CWx and SD values among the slow-growing species, but, unlike for *D*max, this could not be attributed to the trees in the species set. Whilst the possession of dense stems and cell wall-rich xylems could constrain the growth capacity by reducing the potential biomass to be allocated to photosynthetic tissues, the reverse stem traits do not guarantee fast growth. Innate fast seedling growth may be constrained by factors other than stem quality, such as leaf morphology or physiology, or the whole-plant allocation pattern (Lambers and Poorter 1992).

Within the 80 woody species, the association between high RGRs and high *D*max seemed the result of relatively recent divergences in the phylogenetic history, although overall the positive trend seemed confounded with taxonomic relatedness. The negative relationship between RGR and SD was confirmed by the taxonomic relatedness analysis, which also suggested that this trend is mainly the result of ancient divergences at the superfamilial level. Indeed, "woodiness" has been considered a primitive plant trait (Sporne 1980).

Variation in stem traits among plant groups

Differences in stem parameters among the studied species corresponded with differentiation of leaf habit and life form. Higher *D*max values in deciduous than evergreen species, as demonstrated for 80 woody species, have also been reported for adult plants, both within (Rury and Dickison 1984; Villar-Salvador et al. 1997) and between taxa of the same life form (Gartner et al. 1990). Conduit width results from an evolutionary trade-off between xylem safety and hydraulic efficiency (Zimmermann 1983), the balance between both trends depending on macroclimatic factors (Zhang et al. 1992). However, leaf habit can modify the interactions between xylem structure and climate. The need for transport efficiency would override the need for xylem safety in those species that avoid the hazards of freeze-induced embolism in winter by shedding their leaves. Deciduous species need to "pump up" large amounts of water in spring in order to supply the flush of expanding buds. On the other side of the trade-off, the protection against embolism through narrower vessels would be a primary selective force in evergreens which keep their foliage during the cold season (Wang et al. 1992; Sperry 1995). Thus, the early appearance of these *D*max differences among seedlings could reflect a genetically fixed trade-off between leaf habit and xylem traits. The recurrence of the same trend at different taxonomical levels suggests that this trade-off is fundamental, although most consistent in the most ancient contrasts.

Evergreen seedlings had higher CWx than deciduous ones and, although not yet confirmed by the low number of contrasts, the trend was also apparent when taxonomic relatedness was taken into account. Although ecological information on xylem cell walls is scarce (Baas et al. 1983), it has been postulated that tracheary element wall thickness is increased with aridity (Rury and Dickison 1984). The fact that most evergreen species came from Mediterranean (semi-arid) or oligotrophic environments suggests that their generally higher CWx is associated with stressful conditions.

The similarity in SD between evergreen and deciduous seedlings contrasts with results reported for adult woody plants, where evergreens had denser stems (Sobrado 1993). An adult stem consists mainly of xylem while a seedling stem possesses important proportions of other tissues. Therefore, differences in xylem quality would be the main factor determining SD of adult plants, while the contributions of other tissues could be more important in seedlings.

The influence of life form on seedling stem traits is less pronounced than expected. In adult plants, mean and maximum vessel diameters are generally wider in climbers than in other life forms (Carlquist 1991; Ewers et al. 1991; Gartner et al. 1990). By using external support, climbers and scramblers face fewer constraints on traits that enhance hydraulic conductivity. The lower SD found in seedlings of this life form reflects the lower investment for stem strength. However, *D*max was not significantly higher than those of tree or shrub seedlings.

Adult trees have been reported to show wider average vessel diameters than smaller life forms, both inside the same phyletic group (Carlquist 1966; Noshiro et al. 1995; Zhang et al. 1992) and among more remotely related taxa (Baas and Schweingruber 1987). Tall plants require higher transport capacity per unit of leaf area than smaller plants to maintain the same transpiration rate (Jarvis 1975), so wider vessels in trees may have been selected for to compensate for the higher resistance of their longer pathways. Aloni (1991) suggested that plant height can partly influence conduit size through auxin gradients along the plant axes. Wide conduits would result from a slow differentiation, caused by a low auxin level, which decreases basipetally as the distance to the buds increases. If the wider vessels in trees as compared to shrubs are indeed a function of plant height, this would explain the similarity in *D*max between the similarly small seedlings of these life forms. Finally, the relatively narrow conduits of subshrubs could be positively selected in stressed environments, as most species of this life form are associated with semi-arid or oligotrophic sites (Baas and Schweingruber 1987).

In conclusion, variation in stem structure corresponded with that in RGR in seedlings of a large set of woody species, fast-growers presenting wide xylem conduits, low proportions of cell wall in xylem tissue and low stem tissue density. However, at the slow-growth end of the spectrum, other factors seem to distort the relationship between stem structure and RGR. When *D*max and SD are not limiting, RGR may be constrained by other factors. Stem trait differences between evergreen and deciduous seedlings corresponded with those described for adult plants, so they can be considered as ontogenetically fixed. In contrast differences between life forms were smaller in seedlings than in adult plants.

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