ANATOMY AND MECHANICAL AND HYDRAULIC NEEDS OF WOODY CLIMBERS CONTRASTED WITH SUBSHRUBS ON THE ISLAND OF CYPRUS

Alan Crivellaro[1,*], **Katherine McCulloh**[2], **F. Andrew Jones**[3] and
Barbara Lachenbruch[2]

SUMMARY

Climbers and self-supporting woody plants have different constraints on their stems. Although plants of both growth forms need wood that functions mechanically and hydraulically, climbers have a lower need for mechanical self-support and an elevated need for hydraulic conductance to enable longer-distance water transport. We sampled all the woody climbers (10 species) and most of the woody subshrubs (25 species) of the island of Cyprus in the eastern part of the Mediterranean, to characterize their vessel and fiber anatomies relative to hydraulic and mechanical function. Consistent with their lower need for self-support, on average the climbers had lower wood density than did the subshrubs ($0.44 \, \text{g/cm}^3 \pm 0.15$ vs. $0.59 \, \text{g/cm}^3 \pm 0.20$, means ± s.d.) and had a lower proportion of their cross section devoted to fibers ($29\% \pm 11$ vs. $49\% \pm 15$). Consistent with climbers' need for higher hydraulic conductance and total plant height, climbers had vessel sizes and frequencies closer to the theoretical packing limit than did subshrubs. Lastly, we grouped species within a growth form by site water availability (dry vs. wet site), site temperature (cold vs. hot site), site water equability (low vs. high), and xylem ring porosity (ring porous, semi-ring porous or diffuse porous). Climbers had different vessel lumen diameters or vessel frequencies for six of these eight groupings, whereas subshrubs showed no such differences, illustrating the wide variation in climber wood structure compared to the relatively conservative anatomy of the subshrubs.

Key words: Growth form, vine, shrub, biomechanics, ecological wood anatomy, Mediterranean vegetation.

INTRODUCTION

The structure of secondary xylem in a given species is a reflection of both its evolved, adaptive features, and the constraints on that evolution that have canalized which fea-

1) Department of Land, Environment, Agriculture and Forestry, University of Padova, Viale dell' Università 16, I-35020 Legnaro (PD), Italy.
2) Department of Forest Ecosystems and Society, Oregon State University, Corvallis, OR 97330, U.S.A.
3) Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330, U.S.A.
*) Corresponding author [E-mail: alancrivellaro@gmail.com].

tures were most likely to evolve. Moreover, those evolved adaptive features may be intrinsically controlled (developmentally fixed) or they may be extrinsically controlled (plastic) (Day *et al*. 2002). By comparing the wood structure of taxa of differing growth forms and from different habitats, we can infer the environmental factors that were important in shaping the evolution of the growth forms and taxa. For example, if we observe a consistent difference in wood structure between taxa growing in dry vs. wet sites, we could infer that moisture is of strong importance in the evolution of these taxa's wood structures.

This study looks at the mechanical, hydraulic, and ecological wood anatomy of 10 species of woody climber and 25 species of woody subshrubs (defined as woody chamaephytes or dwarf shrubs with less than 80 cm height) growing on the island of Cyprus in the eastern Mediterranean. In relation to its size, Cyprus has one of the richest floras in the Mediterranean region. This is due to a number of factors, including its geological structure, climatic conditions, geographic location (situated at the boundary of three continents), its insular character, and the topographical configuration (Tsintides *et al*. 2002). Subshrub species are a conspicuous part of the natural vegetation on Cyprus, with some noteworthy endemic species. The goals of this study are to better understand the suites of anatomical characteristics in each of the growth forms, to learn which growth forms exhibit more variability with changing site characteristics, and to infer the environmental factors responsible, in an evolutionary sense, for these relationships.

Climbing species have less need to provide their own support than do self-supporting species (*e.g.*, Darwin 1865; Isnard & Silk 2009), and a number of studies have compared the wood anatomy of climbers to self-supporters. In this study, climbers were compared to subshrubs because in a larger study on the ecological wood anatomy of over 250 woody taxa of Cyprus (Crivellaro 2012), subshrubs as a group showed less variability (in plant height and stem diameter) than did the other self-supporting taxa, and so appeared to be a more cohesive growth form for this comparison. Climbers typically have relatively large-diameter vessels and a high proportion of the cross section devoted to vessel lumen and parenchyma compared to self-supporters (Carlquist 1985). Among the self-supported plants, subshrubs in particular often have diffuse porous wood, small diameter vessels, and high variability in axial parenchyma arrangements (Schweingruber & Poschlod 2005; Schweingruber *et al*. 2011). Because of the partial release from the need to provide mechanical support, climbers can have wood with lower strength and stiffness (*e.g.*, Gartner 1991b). Wood density is a good index of mechanical support: the higher the density, the more mass a given unit of wood can support (Kollmann & Côté 1968; Niklas 1992). Fibers provide much of the mechanical support in woody stems. Therefore, we expect that subshrubs will have higher wood density and a higher proportion of fibers than climbers.

A second expectation is that climbers will have an anatomy in which their vessel diameters and frequencies are closer to the 'packing limit' than will subshrubs. The packing limit is the maximum number of vessels that can fit in an area for a given mean vessel cross-sectional area: it is often represented by a line on a graph of area weighted vessel diameter vs. vessel frequency (in number per mm^2) (McCulloh *et al*. 2010).

The negative correlation between these two parameters means that species with wide vessels have very few of them in a given area relative to species with narrower vessels. Because fewer and wider vessels are theoretically more efficient at water conduction than more numerous, narrower ones (Gartner 1991a; Tyree & Zimmermann 2002; Zanne *et al*. 2010), more efficiently conductive species will approach the theoretical maximum packing limit (McCulloh *et al*. 2010). Again, because climbers have less need to provide their own mechanical support, one could expect their wood to be more optimized for water transport. The higher wood-area-specific hydraulic conductivity of climbers than self-supporters has been well documented in the wet and dry tropics (Bamber 1984; Ewers & Fisher 1989; Ewers *et al*. 1990; Gartner *et al*. 1990; Gallen-müllen *et al*. 2001, 2004), and the temperate zone (Baas & Schweingruber 1987; Gartner 1991a; Chiu & Ewers 1992; Tibbetts & Ewers 2000). To our knowledge, the packing function of climbers vs. self-supporting species has not been reported.

A third expectation is that the wood anatomy of a species will be adaptive for the habitat in which it is growing, and this expectation forms the basis of extensive reviews (*e.g*., Baas 1982, 1986; Baas *et al*. 1983; Baas & Miller 1985; Carlquist 1988; Tyree & Zimmermann 2002; Rowe & Speck 2005; Wheeler *et al*. 2007). Given that climbers and self-supporters have different relative hydraulic and mechanical constraints, one would not expect the same habitat/anatomy relationship across the growth forms. Ring porosity, for example, is more common in climbers than in self-supporting taxa (Baas & Schweingruber 1987; Carlquist 1985, 1991).

The three hypotheses tested in this paper on Cyprus climbers and subshrubs are the following:

1) Climbers have less dense wood and a lower proportion of their cross section devoted to fibers than subshrubs.
2) Vessel size and frequencies are closer to the packing limit in climbers than in subshrubs.
3) Wood anatomy (porosity type, vessel diameter, vessel frequency) is related to site characteristics (temperature and water availability), and these relationships are different in climbers and subshrubs.

These hypotheses are tested by comparing values from a census of species anatomies using data from one individual of each of the climbing species and most of the subshrub species on the island of Cyprus. The basic xylem traits compared here, such as wood porosity, raylessness, and possession of successive cambia (successive layers of xylem and phloem, common in the subshrubs) are species traits and as such this census method is appropriate and allows a large sample size.

MATERIALS AND METHODS

The field sampling was carried out in the island of Cyprus (Fig. 1), which lies at the easternmost end of the Mediterranean basin (33° 2" E, 35° 12" N; 9251 km^2; maximum elevation 1952 m). The overall climate is Mediterranean, but it is strongly influenced locally by geographical position relative to relief and the Mediterranean

Table 1. Descriptive information on climbers and subshrubs studied.

1) Scientific name and family; asterisks indicate species endemic to Cyprus; 2) Total plant height (cm); 3) Sampling height from soil level (cm); 4) Wood diameter where sampled (mm); 5) Wood basic density (g/cm³); 6) Presence of successive cambia (N, not present; Y, present); 7) Presence of rays (N, not present; Y, present); 8) Wood porosity (3: ring porous; 4: semi-ring porous; 5: diffuse porous); 9) Mean annual temperature (°C); 10) Mean annual precipitation (mm); 11) Site temperature (H, hot; C, cold); 12) Water equability (L, low; H, high); 13) Water availability (L, low; H, high); 14) Elevation of sampling (m); 15) Leaf persistence during the adverse season (E, evergreen; D, Deciduous; ?, unknown but presumed to be evergreen); 16) Habitat from which the plant was sampled.

1) Species / Family	2) Plant height	3) Sampling height	4) Stem diameter	5) Wood basic density	6) Successive cambia	7) Rays	8) Wood porosity	9) Mean annual temperature	10) Mean annual precipitation	11) Site temperature	12) Water equability	13) Water availability	14) Elevation	15) Leaf persistence	16) Habitat
Climbers															
Aristolochia sempervirens L. - Aristolochiaceae	150	15	3.0	0.29	N	Y	5	19.2	474	H	H	D	358	E	Maquis and garigue
Clematis cirrhosa L. - Ranunculaceae	650	550	12.5	0.38	N	Y	3	13.0	919	C	H	W	1200	E	Maquis and garigue
Clematis vitalba L. - Ranunculaceae	500	100	8.2	0.42	N	Y	3	13.0	919	C	H	W	1200	D	Maquis and garigue
*Cyprinia gracilis (Boiss.) Browicz - Asclepiadaceae	400	4	7.7	0.36	N	Y	3	13.0	919	C	L	W	920	D	Pine forest
Ephedra fragilis Desf. subsp. campylopoda (J.C.Mayer) Asch. & Graebn. - Ephedrac.	100	20	2.5	0.83	N	Y	4	18.7	430	H	H	D	54	E	Rocky slope in maquis
Hedera helix L. subsp. poetarum (Bertol) Nyman - Araliaceae	1200	100	13.0	0.36	N	Y	3	13.0	919	C	L	W	1271	E	Moist place in pine forest
Lonicera etrusca Santi - Caprifoliaceae	400	300	8.2	0.49	N	Y	5	13.0	919	C	H	W	924	D	Rocky place in pine forest

(Climbers continued)

Species / Family															Habitat / Notes
Lonicera japonica Thumb. - Caprifoliaceae	450	150	5.1	0.56	N	Y	3	19.7	308	H	L	D	170	E	Escaped from cultivation in hedges
Rubia tenuifolia d'Urv. - Rubiaceae	300	30	5.2	0.35	N	Y	3	17.2	714	C	H	D	61	E	Rocky slopes in maquis
Vitis vinifera L. - Vitaceae	1200	100	16.0	0.40	N	Y	3	17.2	714	C	L	W	650	D	Wasteland

Subshrubs

Species / Family															Habitat / Notes
*Alyssum akamasicum B.L.Burtt - Brassicaceae	12	2	3.4	0.43	Y	Y	5	19.2	374	H	St	D	358	E	Rocky site
*Alyssum troodi Boiss. - Brassicaceae	12	2	4.5	0.56	Y	N	5	10.8	1109	C	St	W	1825	?	Rocky place
*Arabis purpurea Sibth. & Sm. - Brassicaceae	10	0	3.2	0.33	Y	Y	5	19.7	308	H	St	D	175	E	Fissures in rocks
Arthrocnemum macrostachyum (Moric.) Mois & Delponte - Chenopodiaceae	20	2	4.0	0.67	Y	Y	5	19.6	334	H	L	D	0	E	Edge of salt lake
*Brassica hilarionis Post - Brassicaceae	15	2	4.3	0.63	N	Y	5	17.2	714	C	St	D	620	E	Rocky site
Cichorium spinosum L. - Asteraceae	30	2	3.4	0.33	N	Y	5	18.7	430	C	St	D	0	E	Rock crevices on sea side
*Erysimum kykkoticum Hadjikyriakou & Alziar - Brassicaceae	15	5	3.7	0.44	Y	N	5	17.2	714	C	St	D	534	E	Rocky site
Frankenia hirsuta L. - Frankeniaceae	4	2	1.4	0.50	N	Y	5	19.6	344	H	L	D	2	E	Sandy shore
Fumana arabica (L.) Spach. - Cistaceae	35	10	2.1	0.80	N	Y	5	17.2	714	C	St	D	465	?	Rocky slope in maquis and garigue.
Halocnemum strobilaceum (Pall.) Bieb. - Chenopodiaceae	40	2	5.0	1.00	Y	N	5	19.6	344	H	L	D	0	E	Edge of salt lake
*Helianthemum obtusifolium Dunal - Cistaceae	20	8	3.7	0.71	N	Y	5	19.6	344	H	St	D	2	E	Rocky slope in maquis and garigue
Micromeria myrtifolia Boiss. & Hohen - Labiatae	20	2	2.4	1.00	N	Y	4	17.2	714	C	St	D	585	?	Forests and phrygana
Micromeria nervosa (Desf.) Benth. - Labiatae	20	10	3.7	0.75	N	Y	5	19.7	308	H	St	D	377	?	Garigue

(Table 1 continued)

(Subshrubs continued)

| 1) Species / Family	2) Plant height	3) Sampling height	4) Stem diameter	5) Wood basic density	6) Successive cambia	7) Rays	8) Wood porosity	9) Mean annual temperature	10) Mean annual precipitation	11) Site temperature	12) Water equability	13) Water availability	14) Elevation	15) Leaf persistence	16) Habitat
Nepeta troodi Holmboe - Labiatae	20	2	4.4	0.39	N	Y	5	10.8	1109	C	St	W	1800	?	Rocky place
*Odontites cypria Boiss. - Scrophulariaceae	15	0	3.1	0.50	N	Y	4	13.0	919	C	St	W	1360	?	Garigue and maquis forests
Onosma fruticosa Sm. - Boraginaceae	40	2	8.6	0.61	N	N	4	19.7	308	H	St	D	338	E	Garigue
Onosma mitis Boiss. & Heldr. - Boraginaceae	30	0	2.9	0.47	N	N	5	17.2	714	C	St	D	650	E	Pine forest
*Origanum cordifolium (Aucher & Montbret ex Benth.) Vogel - Labiatae	30	2	3.6	0.50	N	Y	5	17.2	714	C	L	D	540	E	Moist places along stream
Phagnalon rupestre DC. - Asteraceae	30	3	3.2	1.00	N	Y	5	19.7	308	H	St	D	344	?	Rocky ground
*Salvia willeana (Holmboe) Hedge - Labiatae	20	10	8.0	0.44	N	Y	4	10.8	1109	C	St	W	1830	?	Rocky place in pine forest
*Sideritis cypria Post - Labiatae	25	8	5.0	0.37	N	Y	4	17.2	714	C	St	D	715	?	South facing cliffs
*Teucrium cyprium subsp. cyprium Boiss. - Labiatae	20	10	2.8	0.67	N	Y	5	17.2	714	C	St	D	721	?	Rocky place
*Teucrium cyprium Boiss. subsp. kyreniae Boiss. P.H. Davis - Labiatae	10	2	3.0	0.45	N	Y	4	17.2	714	C	St	D	600	?	Rocky places in pine forest
*Teucrium divaricatum Heldr. subsp. canescens (Celak.) Holmboe - Labiatae	38	12	11.7	0.60	N	Y	4	17.2	714	C	St	D	654	?	Rocky place in maquis and garigue
*Thymus integer Griseb. - Labiatae	15	8	1.2	0.60	N	Y	5	19.7	308	H	St	D	277	?	Ophiolite formation in mountain range
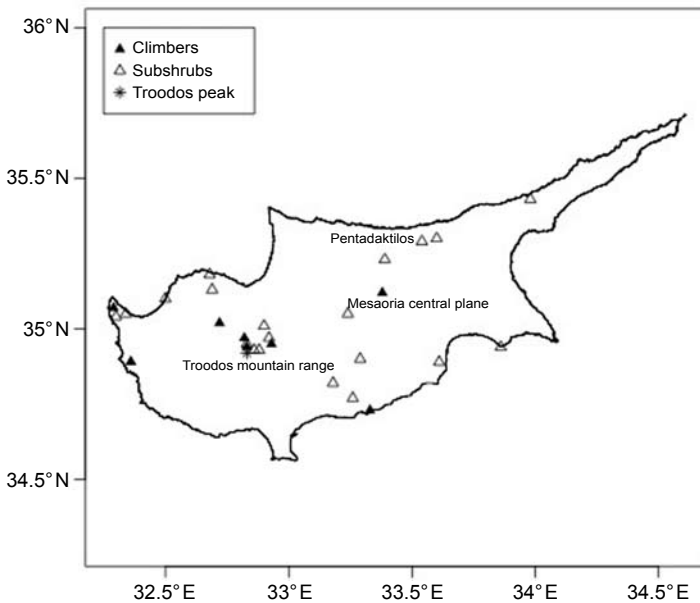
Figure 1. Location of sampling sites for climbers and subshrubs on the island of Cyprus.

Sea. Annual rainfall averages about 480 mm, with 60 % falling between the months of December and February. It ranges from about 300 mm in the central plain to about 1100 mm in the central upper Troodos Mountain Range. The mean daily temperature in July and August ranges between 29 °C on the central plain and 22 °C at the higher altitudes in the Troodos, whereas the respective mean daily temperatures in January are 10 °C and 3 °C (Cyprus Meteorological Service 2011).

In March and September 2009 we sampled all of the endemic and indigenous climbers (10 species) and most of the subshrubs (25 of approximately 40 species) belonging to the flora of Cyprus. We used the range descriptions in the standard references of Cyprus' woody flora (Meikle 1977, 1985; Tsintides *et al*. 2002) to choose a sampling area that was representative of the geographic distribution and habitat range for each species, and then at that site, sampled one individual. The individual chosen appeared normal and healthy, and was one of the tallest individuals of that species at the site. A portion of one stem was cut near the plant's basis (with the height dependent on the plant's stature, Table 1), and from this location we took a segment 5–8 cm tall including bark. The segment was stored in a sealed plastic bag to which we added several drops of 40 % ethanol and kept it at 3–4 °C until it was sectioned.

During harvest, we recorded plant height, height of the midpoint from which the segment was taken, and diameter at that point (under bark) for each sample. Elevation and coordinates of the site (from a GPS unit) and site name (from the nearest geographic feature) were recorded. We also described the site location relative to natural features such as bodies of water, position along the slope, and physical features of the surrounding landscape.

Stem samples were sawn to expose the wood at the segment's mid-point for sectioning. We made transverse and longitudinal sections (15–25 μm) using a disposable blade and a sliding Reichert microtome. The sections were stained with Astra blue and safranin, dehydrated with alcohol and xylene, and mounted in Canada balsam (Schweingruber *et al.* 2008). The cell walls richer in cellulose stained blue, and cell walls richer in lignin stained red. With this double staining, parenchyma cells were usually blue and the remainders of the cells were usually red, facilitating the study of the wood patterns in the cross sections. General anatomy was described (Crivellaro 2012) including the IAWA features (Wheeler *et al.* 1989). For this paper, we report presence or absence of successive cambia, presence or absence of rays, and the type of porosity (ring porous, semi-ring porous, or diffuse porous).

Sections were imaged using a digital camera mounted on a Nikon Eclipse E 400 compound microscope, and analyzed with the image-analysis program ImageJ v. 1.45d (National Institutes of Health, Bethesda, MD, USA; http://rsb.info.nih.gov/ij/). Under the microscope, we characterized a zone of the transverse section that included the outer complete growth ring between two rays and that was large enough to contain at least 80 vessels. For this zone, we estimated lumen area of all vessels, and then converted these areas to lumen diameters, assuming vessels were circular in cross section. Next, we estimated the total area of the zone (excluding one of the two side rays). Then, knowing the number of vessels it contained, we calculated vessel frequency as number of vessels per mm^2. Determining vessel frequency in ring porous woods is not usually done, but in this study it was examined to determine the packing function. We accounted for the influence of varying growth ring width and latewood proportion in ring porous species by counting the number of both earlywood and latewood vessels in an area constrained tangentially by rays and radially by ring boundaries. The mean vessel hydraulic diameter was calculated as $d_h = (\Sigma d^4/n)^{1/4}$, where d is the diameter of each vessel and n is the number of conduits measured. The area-weighted mean vessel diameter (d_A) was calculated as $d_A = (\Sigma d^2/n)^{0.5}$ (McCulloh *et al.* 2010) to allow us to examine the packing function, d_A vs. vessel frequency.

We estimated the proportion of this same zone that was occupied by vessel, fiber, and parenchyma (axial plus ray) (termed vessel area, fiber area, and parenchyma area) by using a line tool. We manually drew multiple polygons that enclosed each tissue type, and determined the area of that tissue using ImageJ. Knowing the total area we could then calculate proportion of transverse area occupied by each tissue. Lastly, we measured the tangential outer wall diameters of 15 earlywood fibers from this same zone.

Wood density was measured on stem sample portions after the bark and the pith were removed. Fresh wood volume was measured using Archimedes' principle by immersing the segment in a beaker containing distilled water on a balance, recording the grams of water displaced, and converting that value to volume using the relationship of 1 g water at room temperature is equal to 1 cm^3 volume. After fresh wood volume was measured, the sample was dried at 103 ± 2 °C for 48 hours, and then weighed. Wood density was calculated on a dry mass/fresh volume basis (g/cm^3).

From the GPS information, we found the closest meteorological data (Cyprus Meteorological Service 2011) for each of the 35 species sampling sites and calculated mean annual precipitation (MAP) and mean annual temperature (MAT). We developed three site characteristics for each species. Site water availability was listed as dry if the species came from a site with MAP less than or equal to the median MAP for the 35 species pooled (714 mm); otherwise, it was listed as wet. Site temperature was listed as cold if the species came from a site with MAT less than or equal to the median MAT for the 35 species pooled (17.2 °C); otherwise, it was listed as hot. Site water equability (the degree to which water would be available during the growing season) was determined by observing the very local plant growing conditions at the sampling site. Water equability was listed as low when it appeared water would be removed slowly enough to keep the soil moist for a significant part of growing season, and high when the soil would be moist only for short periods following precipitation. For example, a sample growing on the edge of a pond or year-round creek would be listed as having high water equability but a sample growing on a slope, which would be presumed to have wet soil in the rainy season and dry soil in the dry season, would be listed as having low water equability.

Lastly, we recorded leaf persistence during the adverse season (evergreen, deciduous) from the standard references of the woody flora of Cyprus (Meikle 1977, 1985; Tsintides *et al.* 2002).

We plotted the relationships between pairs of anatomical and geometric variables for climbers and subshrubs separately, and then compared the curves between these growth forms statistically. These paired variables included outer fiber diameter vs. stem diameter, distance from the apex, and vessel lumen diameter; vessel lumen diameter vs. distance from apex; and vessel lumen diameter vs. both vessel frequency and the value -2, which is the slope of the theoretical packing limit. We also analyzed vessel area vs. fiber area. These variables could have different degrees of correlation in the two growth forms, even though there are constraints on the correlation because the sum of vessel area, fiber area and parenchyma area is 100 %.

We used a Standardized Major Axis (SMA) line-fitting method to estimate the relationship between the two variables. We used the software SMATR (Warton *et al.* 2006, http://www.bio.mq.edu.au/ecology/SMATR/) to test for differences in slope between climbers and subshrubs.

Next, we compared the relationships of two anatomical factors (vessel lumen diameter, fiber diameter) with two stem characteristics (stem diameter and distance of sample from the stem apex) for climbers and subshrubs. Slopes were compared as above. Means were compared using Student's t-tests with the software R (R Development Core Team 2009).

To control for lack of independence in species comparisons, a phylogeny including the species in the study was generated using the program PHYLOMATIC (Webb & Donoghue 2005). We performed phylogenetic independent contrasts (PICs) for each variable using the method of Felsenstein (1985) and implemented in the R package "ape" (Paradis *et al.* 2004).

Lastly, we categorized each taxon by four criteria: the three environmental factors (site water availability, site temperature, site water equability), and ring porosity. For each of the categories within each criterion, we plotted vessel diameter, vessel frequency, and fiber diameter of climbers and subshrubs. Means were compared as above using Student's t-tests. We also inspected plots qualitatively to learn the extent to which climbers and subshrubs had the same pattern of wood variation with these environmental factors.

<div align="center">RESULTS</div>

Climbers were much taller than the subshrubs, and generally were sampled at much higher locations above ground (Table 1). Sample diameters were similar in the two growth forms (Table 1 & 2). None of the climbers had successive cambia or lacked rays but six of the subshrubs had successive cambia, and five of the subshrubs were rayless. Most of the climber species were ring porous and most of the subshrub species were diffuse porous (Table 1). The 10 climber species represented 8 families, whereas the 25 subshrub species represented only 8 families.

Climbers had significantly less dense wood compared to subshrubs (0.44 g/cm^3 ± 0.15 vs. 0.59 g/cm^3 ± 0.20, means $\pm$ s.d.) (Table 2). The climber wood had lower fiber area but greater fiber diameters, greater vessel area, greater vessel diameters, and lower vessel frequency (Table 2).

Table 2. Sample characteristics of climbers (n = 10 species) vs. subshrubs (n = 25 species); probabilities are from paired T-tests.

	Climbers			Subshrubs			P
	mean ± s.d.	min	max	mean ± s.d.	min	max	
Stem diameter (mm)	8.4 ± 4.9	2.5	18.0	4.1 ± 2.3	1.2	11.7	0.02
Wood density (g/cm^3)	0.44 ± 0.15	0.29	0.83	0.59 ± 0.20	0.33	1.00	0.03
Fiber area (%)	29.4 ± 11.3	16.0	51.0	49.3 ± 15.0	17.0	82.0	< 0.01
Vessel area (%)	44.5 ± 12.9	26.0	64.0	22.9 ± 9.3	8.0	47.0	< 0.01
Parenchyma area (%)	25.9 ± 14.7	6.0	56.0	27.7 ± 12.3	6.0	51.0	0.72
Fiber diameter (μm)	14.4 ± 2.60	11.86	19.9	9.69 ± 1.6	6.9	14.0	< 0.01
Vessel diameter, d_h (μm)	63.3 ± 46.3	23.3	165.9	19.0 ± 6.7	8.0	34.0	0.01
Vessel frequency (no./mm^2)	368 ± 367	28	1199	631 ± 364	153	1520.	0.07

In climbers, vessel anatomy did not appear to be related to fiber anatomy: vessel lumen diameter had no relationship with fiber diameter (Fig. 2a, $r^2 = 0.04$, $P = 0.57$), and vessel area (the percentage of the cross section comprised of vessels) had no relationship with fiber area (the percentage of the cross section comprised of fibers, Fig. 2b, $r^2 = 0.08$, $P = 0.44$). The three climbers with the largest diameter vessels, all of which were ring porous, drove the relationship of vessel lumen diameter vs. fiber diameter, but not the relationship of vessel area vs. fiber area (Fig. 2a, b).

In subshrubs, in contrast, vessel anatomy was strongly related to fiber anatomy within a sample, showing that the subshrub cambium tends to make wider fibers in species
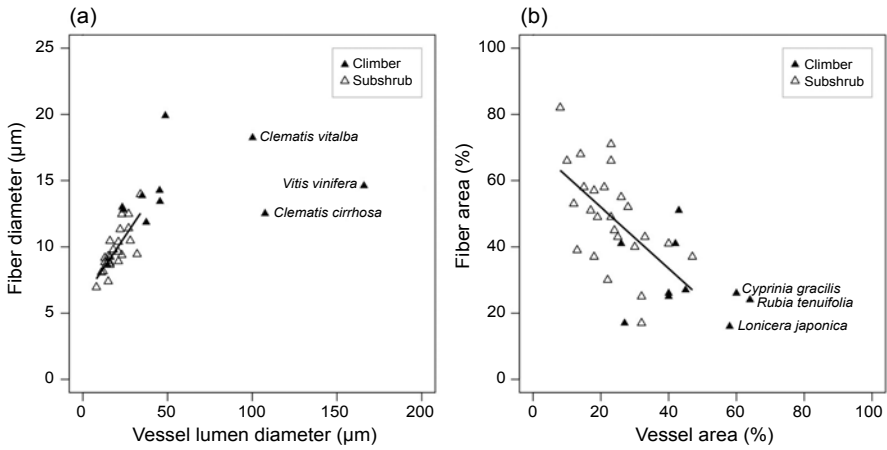
Figure 2. Fiber diameter vs. vessel lumen diameter (a), and the percent of the wood area comprised of fiber area vs. vessel area (b), in climbers vs. subshrubs from the island of Cyprus. Each data point represents a species. The symbols for three species are identified to emphasize their relative influence on the overall pattern of each graph. See text for details.

that have wider vessels. In subshrubs, vessel lumen diameter was positively correlated with fiber diameter (Fig. 2a; $r^2 = 0.60$, P < 0.01), and vessel area (%) was negatively correlated with mean fiber area (%) (Fig. 2b; $r^2 = 0.33$, P < 0.01).

Vessel diameter and stem diameter were correlated and the relationship was significant for climbers ($r^2 = 0.70$, P < 0.01) but not for subshrubs ($r^2 = 0.19$, P = 0.03) (Fig. 3a). There was no significant relationship for either growth form between fiber diameter and stem diameter (Fig. 3b). Climbers and subshrubs showed a common slope for vessel diameter vs. distance from the apex (P = 0.30, Fig. 3c), but had different y-intercepts (P < 0.01). Fiber diameter vs. distance to the stem apex also showed a common slope in climbers and subshrubs (P = 0.36, Fig. 3d), and had different y-intercepts (P = 0.02).

Vessel frequency was plotted against vessel lumen diameter (d_A) to examine the packing functions of the climbing vs. the subshrub species (Fig. 4). The slope of the

Table 3. Pairwise Pearson correlation coefficients for phylogenetically independent contrasts (lower diagonal) and original data (upper diagonal). Numbers in bold have P > 0.05.

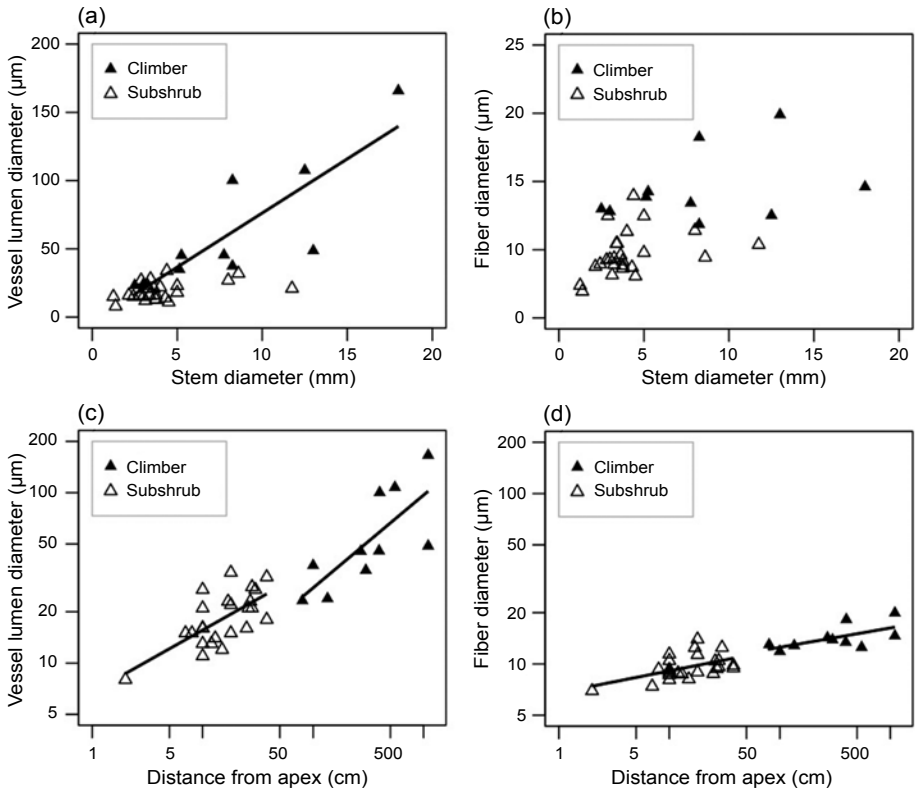|	Distance from apex	Fiber diameter	Fiber area	Ring porosity	Stem diameter	Vessel area	Vessel diameter
Distance from apex	–	0.735	-0.493	-0.713	0.799	0.470	**0.080**
Fiber diameter	0.764	–	-0.583	-0.678	0.568	0.676	0.586
Fiber area	-0.493	-0.565	–	0.527	**-0.292**	-0.666	-0.455
Ring porosity	-0.772	-0.726	0.507	–	-0.655	-0.677	-0.649
Stem diameter	0.783	0.607	**-0.289**	-0.666	–	**0.289**	-0.798
Vessel area	0.500	0.664	-0.677	-0.642	**0.281**	–	0.349
Vessel diameter	0.797	0.676	-0.513	-0.688	0.781	**0.371**	–

Figure 3. (a) Vessel lumen diameter vs. stem diameter, (b) fiber diameter vs. stem diameter, (c) vessel lumen diameter vs. distance from the stem apex, and (d) fiber diameter vs. distance from the stem apex in climbers vs. subshrubs from the island of Cyprus. Each data point represents one species. The axes in c and d are shown on log-log plots.
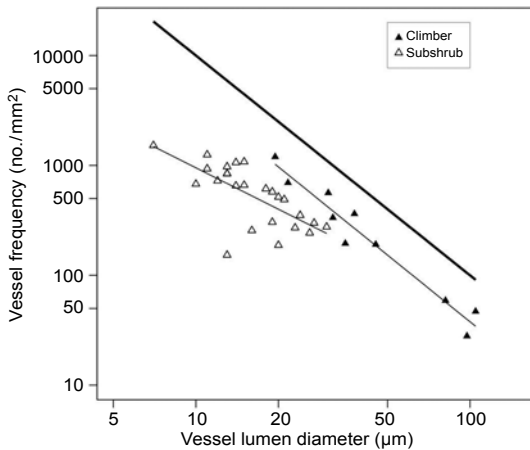

Figure 4. Vessel frequency vs. vessel diameter (d_A) in climbers vs. subshrubs from the island of Cyprus. Each data point represents one species. The heavy solid line shows the packing function limit. The axes are shown on log-log plots.

relationship did not differ significantly between the two growth forms nor did either slope differ from the slope of the packing limit, which is -2 (P = 0.46). Climbers had a stronger correlation between vessel diameter and frequency than did subshrubs ($r^2 = 0.95$, $P < 0.01$, and 0.47, $P < 0.01$, respectively), suggesting more constraints on packing limit in climbers than subshrubs.

Results of PICs analysis show that correcting for lack of independence due to species relatedness in our dataset does not qualitatively change our results. Similar correlations are found when comparing Pearson pairwise comparisons among PICs and among non-transformed data (Table 3).
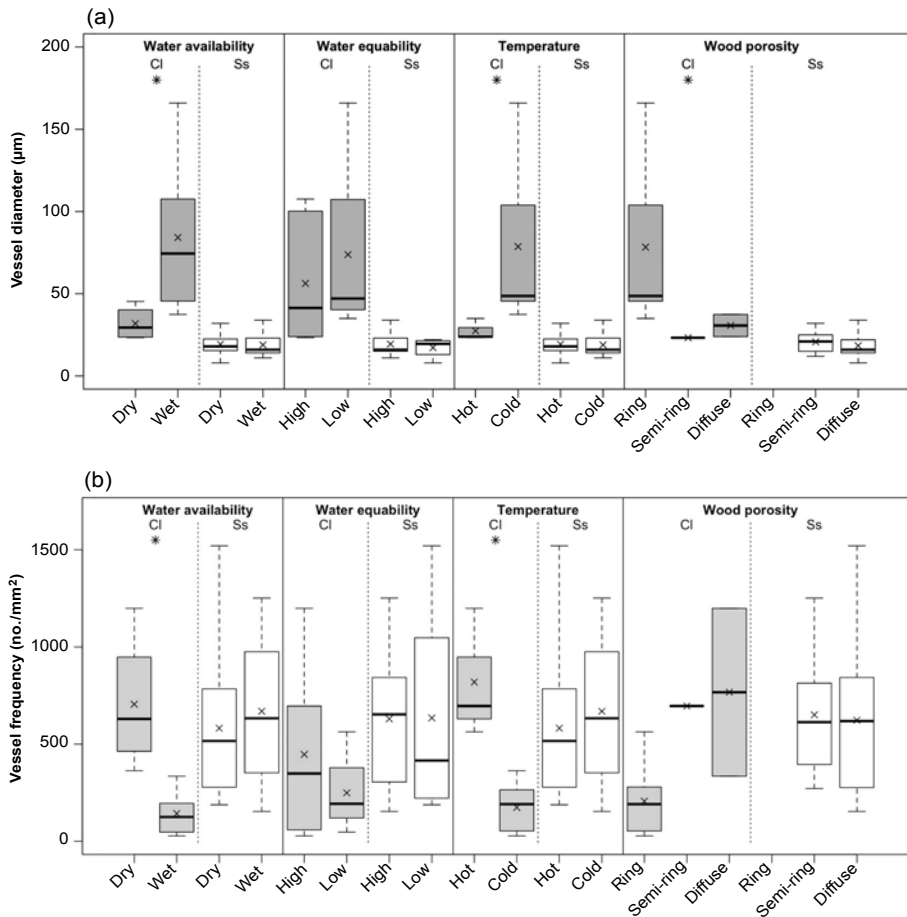
Figure 5. (a) Vessel lumen diameters and (b) vessel frequency vs. site water availability, site water equability, site temperature, and wood porosity in climbers and subshrubs. Box and whisker diagrams, where the heavy bar shows the median, the × shows the mean, the extent of the box show 50 % of the variance, and the light bars show the maximum and minimum values. Asterisks indicate significant differences within the growth form (T-test, P<0.05).

Climbers differed markedly in vessel diameter or vessel frequency across environmental and anatomical categories (Fig. 5), showing the wide variation in climber anatomical structure. In climbers fiber diameter was not significantly different across these categories. In contrast, subshrubs had remarkably constant values for vessel diameter, vessel frequency and fiber diameter across the categories (Fig. 5).

Climbers were significantly taller in wet relative to dry sites (mean plant height of 725 vs. 250 cm, respectively, t = 2.73, df = 7.14, P = 0.03) and in cold relative to hot sites (664 vs. 233 cm, respectively, t = 2.38, df = 7.47, P = 0.04). The two climatic variables, MAP and MAT, were significantly and inversely related to each other (r^2 = 0.89, P < 0.001). No statistically significant plant height differences were observed on the basis of water equability in climbers vs. subshrubs.

DISCUSSION

The analysis of all the woody climbers and many of the woody subshrubs of the island of Cyprus supported all the hypotheses in this study and showed that climbers have less dense wood and a lower proportion of their cross section devoted to fibers than subshrubs, that vessel size and frequencies are closer to the packing limit in climbers than in subshrubs, and that the ecological wood anatomy of climbers differs from that of subshrubs.

Biomechanically, we expected that climbers would have a wood structure that was less specialized for self-support than the subshrubs (Hypothesis 1). This expectation was supported by the climbers having lower wood density and lower fiber area than subshrubs (Table 2), and by climber anatomy exhibiting greater diversity, suggesting it has been less constrained evolutionarily in the structures and their combinations that were able to evolve. For example, climbers showed no correlation between the vessel and fiber area in a stem, because a wide variety of combinations of cell types were exhibited (Fig. 2b). In contrast, subshrubs had a strong negative correlation between these variables. Another example is the degree to which fiber and vessel diameter were correlated (Fig. 2a). In climbers, the two variables were not correlated but in subshrubs there was a strong positive correlation.

The wide range of patterns in which climbers' anatomical characteristics were combined was consistent with our understanding of their growth form. We think of climbers as having more slender stems, and thus more flexibility than self-supporting plants of the same height. Flexibility in climbers is often interpreted as a mechanical advantage for a growth form that may hang, swing, or be coiled while slipping or falling from host vegetation (Haberlandt 1914; Putz & Holbrook 1991; Isnard & Silk 2009). The stem stiffness is given by the products of material stiffness and a geometric factor (second moment area). Because the geometric factor is proportional to the fourth power of stem radius, narrower stems are much more flexible than wider stems of the same material (Niklas 1992). Climbing plants commonly have a much smaller rate of radial growth than do self-supporting species (Putz 1990; Ewers *et al*. 1991), and they are quite small in stem diameter relative to the amount of foliage they supply (Putz 1983). The climbers we studied had thin stem diameters (maximum 18 mm, Table 2),

which is consistent with the common habit of Mediterranean and dry region climbers. Apparently climbers compensate for their narrow stem diameters by producing wider vessels, and a greater number of vessels per area than closely related self-supporting species (Ewers 1985), in relation to climate and local site growing conditions.

However, what is the adaptive significance of the observed anatomical constraints on the subshrubs? One possibility is related to the path-length for water transport in different growth forms, with the assumption that subshrubs may have had little selective pressure to have wood with wide diameter vessels. Many woody plants produce wood that is more conductive near the base than the top of the plant, presumably mitigating the increased resistance on the water column due to path-length and height (Ryan & Yoder 1997; Enquist *et al.* 2000; McDowell *et al.* 2002; Barnard & Ryan 2003; McCulloh *et al.* 2010; Savage *et al.* 2010; Lachenbruch *et al.* 2011). This interpretation is consistent with Figure 3c, showing that vessel lumen diameter was positively correlated with the distance from the apex of the plant: wood near the base of the taller plants had much wider vessels than did wood at the base of short plants. If there was little selective pressure in subshrubs for wood with wide vessels and high selective pressure for wood that could support self-weight, then one may expect that small-diameter vessels and large fiber areas may have evolved. An interesting observation is that in the subshrubs, with their apparent need for a large fiber area and their consequent constraint on vessel area, there could have been selective pressure to evolve larger-diameter vessels to maintain high water flux: this pattern, however, was not observed.

Fiber diameter is related to distance from the apex in both climbers and subshrubs (Fig. 3d). This relationship may explain the higher wood density in subshrubs. In fact, previous findings show that wood density in shrubs can be driven by a reduction in fiber cell sizes (Jacobsen *et al.* 2005, 2007; Martínez-Cabrera *et al.* 2009).

Fiber diameters tended to be larger in ring porous climbers than in the other types of climbers and all of the subshrub types (Table 1). This result reflects the many roles that fibers can have. It is possible that the narrow-diameter fibers, typically found in the subshrubs (Fig. 3b) and diffuse porous climbers function primarily in mechanical support but that the larger-diameter fibers in the ring porous climbers function in water storage and release (Carlquist 1958, 1966, 2001). This idea is supported by observed negative correlations between wood density and fiber diameter (Jacobsen *et al.* 2005; McCulloh *et al.* 2012), and the positive correlations found between wood density and water storage capacity and daily use of stored water (Meinzer *et al.* 2008; McCulloh *et al.* 2012).

As predicted by Hypothesis 2, climbers had vessel diameters and frequencies that were closer to the packing limit than did subshrubs. Climbers had a higher vessel frequency for a given vessel lumen diameter than did subshrubs (Fig. 4), reflected in climbers' higher vessel area and lower fiber area (Table 2). As discussed above, this functional allocation of stem area is consistent with our understanding that climber xylem has less of a role in self-support than subshrub xylem. The fact that climbers tended to be shifted to the right on the packing-limit graph (Fig. 4) is consistent with the water transport efficiency that climbers often exhibit by having fewer wide vessels compared to subshrubs.

As predicted by Hypothesis 3, there were differences between growth forms in the basic wood anatomy and in the distribution of anatomies with respect to climate and site factors. Regarding anatomy, only climbers had species that were ring porous (Table 1 and Fig. 5), and only subshrubs had species with successive cambia and/or that were rayless (Table 1). One possible mechanism for the ring porosity in climbers as well as the axial gradient in vessel diameter for all species pooled is the auxin hypothesis that predicts an increase in vessel diameter with distance from apical meristem (Aloni & Zimmermann 1983). The alteration of vascular increments with parenchyma provided by successive cambia may offer an ideal stem organization for storage and retrieval of photosynthate and water (Carlquist 2007). Raylessness may cause the loss of radial conduction of assimilates and possibly water and hormones, and occurs only in stems that have relatively small diameters. If rayless stems do experience the addition of secondary xylem to an appreciable extent, they tend to develop rays. Raylessness can also be regarded as a means of achieving a rapid substitution of mechanical tissue for ray tissue (Carlquist 2001). The rays may aid in giving climber stems flexibility, as was proposed for the role of axial parenchyma (summarized in Haberlandt 1914).

Regarding site factors, the climbing species showed differences in their mean vessel diameters and vessel frequencies by site water availability and site temperature. For the subshrub species, in contrast, we detected no site-related variation in vessel anatomy (Fig. 5), and for fiber characteristics, neither growth form exhibited consistent site-related variation. These results underscore the wider range of diversity of wood anatomies found within climbers than within subshrubs. The lack of fiber diameter differences by site characteristic for either growth form may have resulted from 'fiber' actually representing more than one functional role, as discussed above.

CONCLUSIONS

This study showed that within the woody flora of the island of Cyprus, the structure of the secondary xylem of climbers appeared to be less specialized for mechanical support and more specialized for long-distance water transport than did the secondary xylem of subshrubs. The support for this statement included the greater vessel areas, vessel lumen diameters and higher incidence of ring porosity in climbers than in subshrubs, and the packing-limit functions that were closer to the maximum possible in climbers than in subshrubs. Additionally, the anatomy of climbers varied systematically with site water availability and temperature, whereas the subshrub anatomy did not.

Our understanding of the plastic vs. genetic nature of these adaptations could be furthered by studies of the ecological wood anatomy of selected species over a range of environments. It would also be interesting to compare the habitats, ranges, plant architectures, and physiologies of ring porous or diffuse porous species of a variety of growth forms to learn if there are specific associations that would suggest constraints of the wood anatomy on the ecology of species.

REFERENCES

Aloni, R. & M.H. Zimmermann. 1983. The control of vessels size and density along the plant axis: a new hypothesis. Differentiation 24: 203–208.

Baas, P. 1982. Systematic, phylogenetic and ecological wood anatomy. History and perspectives. In: P. Baas (ed.), New perspectives in wood anatomy: 23–58. Nijhoff/Junk, The Hague.

Baas, P. 1986. Ecological patterns in xylem anatomy. In: J. Givnish (ed.), On the economy of plant form and functions: 327–352. Cambridge Univ. Press, Cambridge.

Baas, P. & R.B. Miller. 1985. Functional and ecological wood anatomy – Some introductory comments. IAWA Bull. n.s. 6: 281–282.

Baas, P. & F.H. Schweingruber. 1987. Ecological trends in the wood anatomy of trees, shrubs and vines. IAWA Bull. n.s. 8: 245–274.

Baas, P., E. Werker & A. Fahn. 1983. Some ecological trends in vessel characters. IAWA Bull. n.s. 4: 141–159.

Bamber, I.W. 1984. Wood anatomy of some Australian rainforest vines. In: S. Sudo (ed.), Proceedings of the Pacific Regional Wood Anatomy Conference: 58–60. Wood Technology Division of the Forestry and Forest Products Research Institute, Ibaraki, Japan.

Barnard, H.R. & M.G. Ryan. 2003. A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*. Plant, Cell & Environment 26: 1235–1245.

Carlquist, S. 1958. Wood anatomy of Heliantheae (Compositae). Trop. Woods 108: 1–30.

Carlquist, S. 1966. Wood anatomy of Compositae: a summary, with comments on factors controlling wood evolution. Aliso 6: 25–44.

Carlquist, S. 1985. Observation on functional wood histology of vines and lianas: vessels dimorphism, tracheids, vasicentric tracheids, narrow vessels, and parenchyma. Aliso 11: 139–157.

Carlquist, S. 1988. Comparative wood anatomy. Systematic, ecological, and evolutionary aspects of dicotyledon wood. Springer Verlag, Berlin.

Carlquist, S. 1991. Anatomy of vine and liana stems: a review and synthesis. In: F.E. Putz & H.A. Mooney (eds.), The biology of vines: 53–71. Cambridge University Press, Cambridge.

Carlquist, S. 2001. Comparative wood anatomy. Systematic, ecological, and evolutionary aspects of dicotyledon wood. Springer Verlag, Berlin.

Carlquist, S. 2007. Successive cambia in Aizoaceae: products and process. Bot. J. Linn. Soc. 153: 141–155.

Chiu, S.T. & F.W. Ewers. 1992. Xylem structure and water transport in a twiner, a scrambler, and a shrub of *Lonicera* (Caprifoliaceae). Trees. 6: 216–224.

Crivellaro, A. 2012. Wood, bark and pith structure in trees and shrubs of Cyprus: anatomical descriptions and ecological interpretation. PhD thesis.

Cyprus Meteorological Service. 2011. Meteorological reports. Accessed March 2011. http://www.moa.gov.cy/moa/ms/ms.nsf/DMLindex_en/DMLindex_en?opendocument.

Darwin, C. 1865. On the movements and habits of climbing plants. Bot. J. Linn. Soc. 9: 1–118.

Day, M.E., M.S. Greenwood & C. Diaz-Sala. 2002. Age- and size-related trends in woody plant shoot development: regulatory pathways and evidence for genetic control. Tree Physiol. 22: 507–513.

Enquist, B.J., G.G. West & J.H. Brown. 2000. Quarter-power allometric scaling in vascular plants: functional basis and ecological consequences. In: J.H. Brown & G.B. West (eds.), Scaling in biology: 167–198. Oxford University Press, Oxford.

Ewers, F.W. 1985. Xylem structure and water conduction in conifer trees, dicot trees, and lianas. IAWA Bull. n.s. 6: 309–317.

Ewers, F.W. & J.B. Fisher. 1989. Variation in vessel length and diameter in stems of six tropical and subtropical lianas. Amer. J. Bot. 76: 1452–1459.

Ewers, F.W., J.B. Fisher & S.T. Chiu. 1990. A survey of vessel dimensions in stems of tropical lianas and other growth forms. Oecologia 84: 544–552.

Ewers, F.W., J.B. Fisher & K. Fichtner. 1991. Water flux and xylem structures in wine. In: F.E. Putz & H.A. Mooney (eds.), The biology of vines: 127–160. Cambridge University Press, Cambridge, UK.

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist 125: 1–15.

Gallenmüllen, F., U. Müller, N. Rowe & T. Speck. 2001. The growth form of *Croton pullei* (Euphorbiaceae) – Functional morphology and biomechanics of a neotropical liana. Plant Biol. 3: 50–61.

Gallenmüllen, F., N. Rowe & T. Speck. 2004. Development and growth form of the neotropical liana *Croton nuntians*: the effect of light and mode of attachement on the biomechanics of the stem. J. Plant Growth Regulation 23: 83–97.

Gartner, B.L. 1991a. Stem hydraulic properties of vines vs. shrubs of western poison oak, *Toxicodendron diversilobum*. Oecologia 87: 180–189.

Gartner, B.L. 1991b. Structural stability and architecture of vines vs. shrubs of poison oak, *Toxicodendron diversilobum*. Ecology 72: 2005–2015.

Gartner, B.L., S.H. Bullock, H.A. Mooney, V.B. Brown & J.L. Whitbeck. 1990. Water transport properties of vine and tree stems in a tropical deciduous forest. Amer. J. Bot. 77: 742–749.

Haberlandt, G. 1914. Physiological plant anatomy. Translated from the 4th German edition by M. Drummond. Macmillan & Co. Ltd, London.

Isnard, S. & W.K. Silk. 2009. Moving climbing plants from Charles Darwin's time into the 21st century. Amer. J. Bot. 96: 1205–1221.

Jacobsen, A.L., F.W. Ewers, R.B. Pratt, W.A. Paddock III & S.D. Davis. 2005. Do fibers affect vessel cavitation resistance? Plant Physiol. 139: 546–556.

Jacobsen, A.L., R.B. Pratt, F.W. Ewers & S.D. Davis. 2007. Cavitation resistance among 26 chaparral species of southern California. Ecol. Monographs 77: 99–115.

Kollmann, F.F. & W.A. Côté. 1968. Principles of wood science and technology. Springer-Verlag, Berlin.

Lachenbruch, B., F.C. Meinzer & T.E. Dawson (eds.). 2011. Size- and age-related changes in tree structure and function. Springler Verlag, Berlin.

McCulloh, K., J.S. Sperry, B. Lachenbruch, F.C. Meinzer, P.B. Reich & S. Voelker. 2010. Moving water well: comparing hydraulic efficiency in twigs and trunks of coniferous, ring-porous, and diffuse-porous saplings from temperate and tropical forests. New Phytol. 186: 439–450.

McCulloh, K.A., D.M. Johnson, F.C. Meinzer, S.L. Voelker, B. Lachenbruch & J.C. Domec. 2012. Hydraulic architecture of two species differing in wood density: opposing strategies in co-occurring tropical pioneer species. Plant, Cell & Environment 35: 116–125.

McDowell, N.G., N. Phillips, C. Lunch, B.J. Bond & M.G. Ryan. 2002. An investigation of hydraulic limitation and compensation in large, old Douglas-fir trees. Tree Physiol. 22: 763–774.

Martínez-Cabrera, H.I., C.S. Jones, S. Espino & H.J. Schenk. 2009. Wood anatomy and wood density in shrubs: Responses to varying aridity along transcontinental transects. Amer. J. Bot. 96: 1388–1398.

Meikle, R.D. 1977 & 1985. Flora of Cyprus. 2 vols. Royal Botanic Gardens, London.

Meinzer, F.C., P.I. Campanello, J.C. Domec, M.G. Gatti, G. Goldstein, R. Villalobos-Vega & D.R. Woodruff. 2008. Constraints on physiological function associated with branch architecture and wood density in tropical forest trees. Tree Physiol. 28: 1609–1617.

Niklas, K.J. 1992. Plant biomechanics. An engineering approach to plant form functions. The University of Chicago Press.

Paradis, E., J. Claude & K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.

Putz, F.E. 1983. Liana biomass and leaf area of a "tierra firme" forest in the Rio Negro Basin, Venezuela. Biotropica 15: 185–189.

Putz, F.E. 1990. Liana stem diameter growth and mortality rates on Barro Colorado Island, Panama. Biotropica 22: 103–105.

Putz, F.E. & N.M. Holbrook. 1991. Biomechanical studies of vines. In: F.E. Putz & H.A. Mooney (eds), The biology of vines: 73–97. Cambridge University Press.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org.

Rowe, N. & T. Speck. 2005. Plant tree growth forms: an ecological and evolutionary perspective. New Phytol. 166: 61–72.

Ryan, M.G. & B.J. Yoder. 1997. Hydraulic limits to tree height and tree growth. Bioscience 47: 235–242.

Savage, V.M., L.P. Bentley, B.J. Enquist, J.S. Sperry, D.D. Smith, P.B. Reich & E.I. von Allmen. 2010. Hydraulic trade-off and space filling enable better predictions of vascular structure and functions in plants. Proceedings of the National Academy of Science 107(52): 22722–22727.

Schweingruber, F.H., A. Börner & E.D. Schulze. 2008. Atlas of woody plant stems. Evolution, structure and environmental modifications. Springer-Verlag, Berlin.

Schweingruber, F.H., A. Börner & E.D. Schulze. 2011. Atlas of stem anatomy in herbs, shrubs and trees. Springer-Verlag, Berlin.

Schweingruber, F.H. & P. Poschlod. 2005. Growth rings in herbs and shrubs: life span, age determination and stem anatomy. Forest, Snow and Landscape Research. 79: 195–415.

Tibbetts, T.J. & F.W. Ewers. 2000. Root pressure and specific conductivity in temperate lianas: exotic *Celastrus orbiculatus* (Celestracea) vs. native *Vitis riparia* (Vitaceae). Amer. J. Bot. 87: 1272–1278.

Tsintides, T.C., G.N. Hadjikyriakou & C.S. Christodoulou. 2002. Trees and shrubs in Cyprus. The Anasasios G. Leventis Foundation, Nicosia.

Tyree, M.T. & M.H. Zimmermann. 2002. Xylem structure and the ascent of sap. Springer, Berlin.

Warton, D.I., J.I. Wright, D.S. Falster & M. Westoby. 2006. Bivariate line-fitting methods for allometry. Biological Reviews 81: 259–291.

Webb, C.O. & M.J. Donoghue. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes 5: 181–183.

Wheeler, E.A., P. Baas & P.E. Gasson (eds.). 1989. IAWA List of microscopic features for hardwood identification. IAWA Bull. n.s. 10: 219–332.

Wheeler, E.A., P. Baas & S. Rodgers. 2007. Variations in dicot wood anatomy: a global analysis based on the InsideWood database. IAWA J. 28: 229–258.

Zanne A.E., M. Westoby, D.S. Falster, D.D. Ackerly, S.R. Loarie, S.E.J. Arnold & D.A. Coomes. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. Amer. J. Bot. 97: 207–215.