

RESEARCH PAPER

Contrasting seasonal overlaps between primary and secondary growth are linked to wood anatomy in Mediterranean sub-shrubs

J. J. Camarero¹, S. Palacio² & G. Montserrat-Martí³

¹ ARAID – Pyrenean Institute of Ecology (IPE-CSIC), Zaragoza, Spain

² Pyrenean Institute of Ecology (IPE-CSIC), Jaca (Huesca), Spain

³ Pyrenean Institute of Ecology (IPE-CSIC), Zaragoza, Spain

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Correspondence

J. J. Camarero, Instituto Pirenaico de Ecología, IPE-CSIC, Avenida Montañana 1005, Zaragoza E-50192, Spain.

E-mail: jjcamarero@ipe.csic.es

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ABSTRACT

Whole-plant approaches allow quantification of the temporal overlap between primary and secondary growth. If the amount of time available to grow is short, there may be a high temporal overlap between shoot growth and wood formation. We hypothesise that such overlap depends on the duration of the growing season and relates to wood anatomy. We evaluated wood anatomy, shoot longitudinal and radial growth rates, fine root production and the concentrations of non-structural carbohydrates (NSC) in the wood of six sub-shrub species growing in sites with contrasting climatic conditions (*Lepidium subulatum*, *Linum suffruticosum*, *Salvia lavandulifolia*, *Satureja montana*, *Ononis fruticosa*, *Echinopartum horridum*). Sub-shrub species living in sites with a short growing season displayed a high overlap between above-ground primary and secondary growth and formed wide vessels, whereas species from the warmest and driest sites presented the reverse characteristics. The highest overlap was linked to a rapid shoot extension and thickening through the enhanced hydraulic conductivity provided by wide vessels. The reductions in NSC concentrations when growth peaked were low or moderate, indicating that sub-shrubs accumulate NSC in excess, as do trees. The temporal overlap among primary and secondary growth in woody plants may be connected to the duration and rates of shoot and wood growth, which in turn depend on the vessel lumen area.

INTRODUCTION

Integrated approaches are required to understand growth processes in woody plants. However, research on both primary and secondary growth in woody plants is extremely scarce (but see Yañez-Espinosa *et al.* 2006). Comparative approaches considering above- and belowground growth processes in different organs and using complementary data sets may allow determination of how wood anatomy and formation is related to other growth processes within the plant, such as shoot extension and root production. Similarly, although several studies have analysed the impact of growth processes on the storage and use of non-structural carbohydrates (NSC) by woody plants (Newell *et al.* 2002; Palacio *et al.* 2007a; Gruber *et al.* 2012), integrative information at the whole-plant level is missing. Consequently, it is not known how aboveground primary and secondary growth and root production affect NSC dynamics.

Trees pose practical difficulties to address questions at the whole-plant level in mature individuals, which are often impossible to overcome because of their large size and usually long lifespan. However, chamaephytes – hereafter termed sub-shrubs – (plants that develop buds at a maximum height of 80 cm in the case of Mediterranean species; Orshan 1982) provide an opportunity to study how secondary growth interacts simultaneously with primary aboveground (*e.g.* shoot growth)

and belowground (*e.g.* fine root dynamics) growth processes because they are smaller and usually have shallower root systems than trees (Palacio & Montserrat-Martí 2007). Hence, sub-shrubs constitute a valuable material to test how primary and secondary growth interact, if both tend to avoid overlap with other growth components (*e.g.* root development) and how growth components of woody plants interact with NSC use and storage. In spite of the aforementioned advantages and despite the fact that they constitute the dominant plant growth form in many regions of the world with harsh climate conditions, such as Mediterranean areas under continental conditions (Di Castri 1981), sub-shrubs have rarely been studied.

The amount of time available in the year for sub-shrubs to complete their growth cycle under continental Mediterranean climate conditions may be very limited, either because of a marked summer drought or due to cold conditions in winter (Montserrat-Martí *et al.* 2011). In trees from continental Mediterranean areas the season of wood formation is mainly shortened by cold and also by dry conditions (Cherubini *et al.* 2003; Camarero *et al.* 2010). However, sub-shrubs may have a shorter growing season than trees to avoid both of these climate stressors (Schenk *et al.* 2008). To date, it is not known how sub-shrubs arrange growth processes, including wood formation (xylogenesis), to withstand the restrictive environmental conditions of the inland Mediterranean sites where they dominate the

woody flora. Pioneering studies evidenced that Mediterranean sub-shrub species form less wood when water availability decreases (Aljaro *et al.* 1972; Avila *et al.* 1975; Liphschitz & LevYadun 1986). Nevertheless, data on wood anatomical features (ring distinctiveness, vessel area and theoretical hydraulic conductance) and seasonal wood formation of Mediterranean sub-shrub species related to above- and belowground phenology are lacking. However, wood anatomical data such as vessel lumen area, a proxy of hydraulic conductance and shoot growth, may link primary and secondary growth dynamics.

In this study, we analyse how wood anatomy and formation, shoot extension, fine root production and NSC concentrations interact in six sub-shrub species growing in sites with contrasting climate conditions and hence different durations of the vegetative period. For instance, in the warmest sites the studied species achieve maximum photosynthetic biomass values in early spring, whereas in the coldest sites these are attained in summer (Palacio *et al.* 2006). We expect that species from cold and relatively moist sites, such as *Echinopartum horridum* and *Ononis fruticosa*, will have the shortest vegetative periods, whereas species from warmer and drier sites (*Satureja montana*, *Lepidium subulatum*, *Linum suffruticosum*, *Salvia lavandulifolia*) will have longer vegetative periods. We aimed to evaluate: (i) how secondary growth (wood anatomy and formation, shoot thickening) interacts with primary aboveground (shoot extension) and belowground (fine root dynamics) growth processes, *i.e.* to quantify the temporal overlap that pri-

mary and secondary growth types display; and (ii) how the growth components of sub-shrubs, particularly secondary growth, interact with NSC use and storage. In relation to the first objective, we hypothesised that aboveground secondary (wood formation) and primary growth (shoot extension) will show the highest overlap in the coldest sites because low temperatures will constrain both growth types in autumn and winter. Therefore, such strong overlap should be related to rapid shoot extension and thickening, which may be enhanced by the production of wide vessels providing high hydraulic conductivity. In addition, wood and root formation will show a low temporal association since fine root production in Mediterranean sub-shrubs peaks in autumn (Palacio & Montserrat-Martí 2007). As to the second objective, we expected that the high overlap among aboveground secondary and primary growth types in species from the coldest sites will also involve a strong but negative association between secondary growth and NSC concentrations in wood.

MATERIAL AND METHODS

Studied species and sites

The studied species were distributed along a climate gradient in northeast Spain (41°43'–42°31' N, 0°26'–0°44' W) with a continental Mediterranean climate, ranging from cold–wet to warm–dry conditions (Fig. 1, Table 1). Sampling was

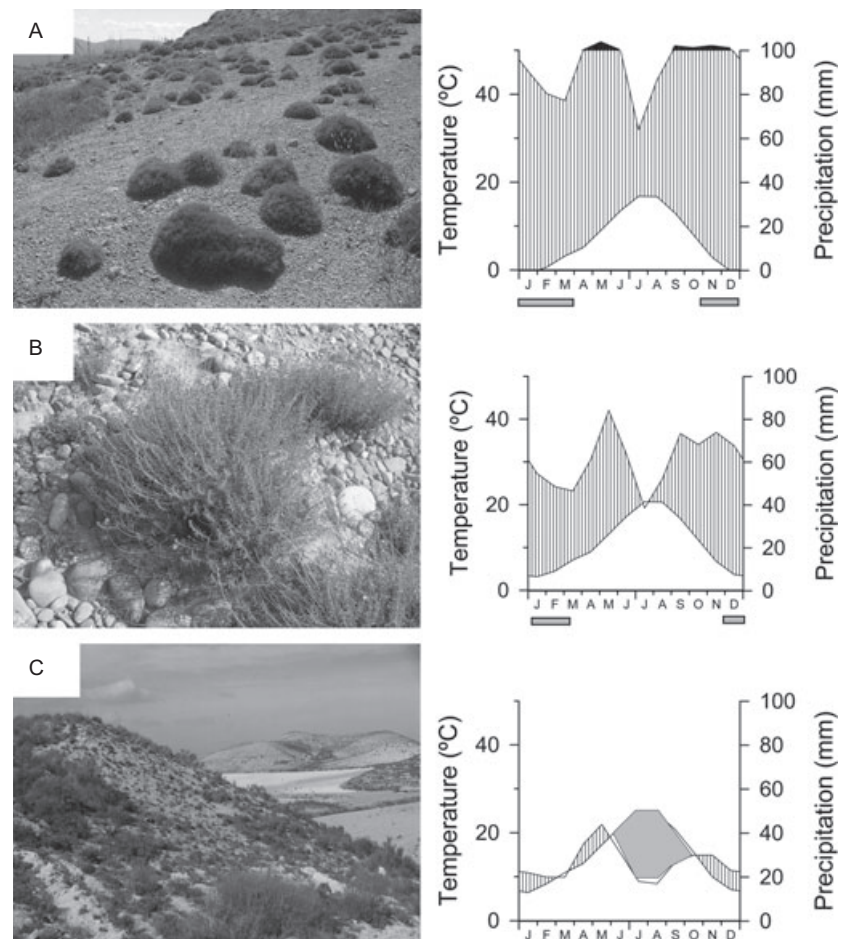


Fig. 1. Views of three study sites dominated by (A) cushion *Echinopartum horridum* individuals, (B) *Satureja montana* plant, and (C) *Linum suffruticosum* and *Lepidium subulatum* populations. The climate diagrams were built using data from nearby meteorological stations (see Table 1) and correspond to cold (upper graph), mild (middle graph) and warm (lower graph) climate conditions.

Table 1. Characteristics of the studied sub-shrub species and sites. Height values are means \pm SE.

species (family)	study site	sampling period (sampling frequency in days)	elevation (m)	mean annual temperature ^a (°C)	total annual precipitation (mm)	soil	life form	height (cm)
<i>Linum suffruticosum</i> L. (Linaceae)	Villamayor	Oct 2002 – Jan 2004 (29)	320	14.6	335	Gypsum	Seasonally dimorphic	40 \pm 90
<i>Lepidium subulatum</i> L. (Brassicaceae)	Villamayor	Oct 2002 – Jan 2004 (29)	320	14.6	335	Gypsum	Summer deciduous	30 \pm 70
<i>Salvia lavandulifolia</i> L. (Lamiaceae)	Villamayor	Sep 2002 – Nov 2003 (30)	420	14.0	453	Limestone, gypsum	Seasonally dimorphic	30 \pm 50
<i>Satureja montana</i> L. (Lamiaceae)	Lasieso	Jan 2004 – Jan 2005 (31)	670	12.1	654	Calcareous alluvium	Seasonally dimorphic	20 \pm 40
<i>Ononis fruticosa</i> L. (Fabaceae)	Bernués	Dec 2003 – Dec 2004 (30)	1,030	10.9	735	Marl	Winter deciduous	60 \pm 100
<i>Echinospartum horridum</i> (Vahl.) Rothm. (Fabaceae)	S. Juan de la peña	Oct 2002 – Nov 2003 (32)	1,380	8.0	1,247	Limestone	Evergreen cushion	50 \pm 80

^aClimate data were obtained from the following meteorological stations (site): Zaragoza-Aeropuerto (41°40' N, 1°00' W, 247 m) and Perdiguera (41°45' N, 0°38' W, 452 m) (Villamayor); Apies (42°14' N, 0°24' W, 680 m) (Lasieso), Arguis (42°19' N, 0°26' W, 1,039 m) (Bernués) and Santa Cruz de la Serós (42°31' N, 0°41' W, 820 m) (San Juan de la Peña).

conducted each month in 2003 and 2004 for a minimum of 13 months per species. Weather conditions were similar between the 2 years of study. Three of the selected species are seasonally dimorphic (*S. montana*, *L. suffruticosum*, *S. lavandulifolia*), i.e. they normally bear two different types of branch (short and long branches) with different leaf types displayed during unfavourable (summer to winter) and favourable (spring) periods, respectively (Orshan 1989). Since wood anatomy may change as a function of branch type in Mediterranean seasonal dimorphic species, we restricted our analyses to long branches or dolichoblasts (De Micco & Aronne 2009). The remaining species are the summer deciduous *L. subulatum*, the winter deciduous *O. fruticosa* and the evergreen thorny cushion plant *E. horridum*. For further details on the studied species and sites see Palacio & Montserrat-Martí (2005, 2006).

Wood anatomy and growth

On each sampling date, 15 3-year-old branches were sampled from 15 individuals randomly selected within one population per species. To check for differences in radial growth among different cohorts of shoots, the diameter of the three cohorts of shoots, including the bark, in each 3-year-old branch was measured for each species and sampling date (see Fig. S1).

Cross-sections were cut (10–20- μ m thick) with a sliding microtome (AS 200; Anglia Scientific Instruments, Cambridge UK) from the middle position of each branch following Schweingruber & Poschold (2005). They were stained with 1% aqueous solutions of safranin, methylene blue or astra blue and embedded permanently in synthetic resin (Eukitt; Merck, Darmstadt, Germany). Mounted cross-sections were photographed with a digital camera (Leica DFC 290) under a light microscope (Olympus BH2) at 100–400 \times magnification. Photographs of each ring were processed and converted into black-and-white images and processed with Adobe Photoshop CS2 (Adobe Systems, San Jose, CA, USA) after excluding the pith and bark.

Vessel area and number were analysed for each annual ring using the ImageJ software (Rasband 2011; Fig. S2). We measured all vessels within the 0.0001–0.01 mm² range. The vessel area (%) was calculated as the ratio between the sum of the areas of all individual vessels and the measured xylem area. The predicted hydraulic conductance (*Kh*) was calculated, according to the Hagen–Poiseuille law, as the sum of the fourth power diameters of all the vessels from each species assuming vessels had a circular shape. The relative contribution of each vessel class, according to area, to the total *Kh*, was also obtained and expressed as a percentage for 1-, 2- and 3-year-old branches. Radial growth was calculated by measuring the ring width in two opposite radii in the latest formed ring of photographed cross-sections from 3-year-old branches.

Primary shoot and root growth

Data of primary shoot and root growth were obtained from published studies on the same species and sites (Palacio & Montserrat-Martí 2005, 2006, 2007). Data presented are mean values for each sampling date and for the same studied populations and years. Briefly, shoot growth was measured each month as the mean increase in shoot length of 15 marked individuals between two consecutive samplings. Fine root growth was assessed by carefully digging three to five individuals per species to a depth of 30–40 cm. Plants with their surrounding soil were taken to the laboratory, where roots were carefully washed. A representative subsample of fine roots was collected at three different depths within the root system of each plant (upper, mid and lower third). The number and diameter of growing fine roots within each subsample were measured under a stereomicroscope. Fine roots were considered growing when their apex was hyaline and their cortex remained white (Kummerow *et al.* 1978). Subsampled fine roots were oven-dried at 60°C to a constant weight and dry weights were measured to the closest 0.01 mg. Numbers of fine, growing roots of the three subsamples were pooled and root growth data

expressed as the percentage of mean monthly maximum amount of growing fine roots per biomass of sampled roots recorded during the sampling period for each species.

Chemical analyses

Data on non-structural carbohydrate (NSC) concentrations in stems and roots (SS, soluble sugars; starch and total NSC) were obtained from studies on the same sites considering five individuals per species (Palacio *et al.* 2007a,b). Carbohydrate data were expressed as means for each sampling date and for the same studied populations and years.

Statistical analyses

To compare the distribution of vessels according to their transverse lumen area among species, we used *G* tests. To quantify growth patterns, cumulative shoot length and radial increment were converted into growth rates by subtracting consecutive growth data and dividing them by the elapsed time between sampling dates (mean = 30 days). We evaluated the associations among growth variables (longitudinal and radial growth rates, fine root production) and NSC (SS, starch and NSC separately) concentrations in stems and roots using the non-parametric Spearman correlation coefficient (r_s). Finally, to estimate the temporal overlap between primary and secondary growth, we calculated the area below the curves corresponding to monthly longitudinal and radial growth rates. The rates were converted to relative values (0–1) by dividing by the maximum rate of each growth type. Then, we graphically estimated the temporal overlap as the percentage of non-overlapping and overlapping areas between the areas underneath longitudinal and radial growth rates curves.

RESULTS

Wood anatomical features

All studied sub-shrub species formed distinct rings and had semi-ring-porous wood (Fig. 2). *L. subulatum* presented highly packed vessels in radial multiples, and ring shake (rings break along the ring boundary) was common in old branches (Fig. 2). In all species, most vessels had areas smaller than 0.0005 mm^2 . The vessels with the widest lumens (up to 0.004 mm^2 in area) were observed in *E. horridum*, followed by *O. fruticosa*, and the highest frequency of smaller vessels occurred in *S. lavandulifolia* and *L. suffruticosum* (Table 2, Fig. 3). The highest vessel area (3.4%) was recorded in *E. horridum* and *O. fruticosa*, whereas the minimum (1.1%) value corresponded to *L. subulatum*. The vessel distributions according to their lumen area differed significantly among species ($P < 0.001$ in all cases) for all ages (1-year-old vessels, $G = 116$; 2-year-old vessels, $G = 527$; 3-year-old vessels, $G = 608$).

Considering the predicted hydraulic conductance (K_h), vessels whose lumen area was smaller than 0.001 mm^2 accounted for all of the K_h in *L. suffruticosum*, while wide vessels with areas from 0.001 up to 0.005 mm^2 accounted for most (89% in 3-year-old shoots) of the K_h in *E. horridum* (Fig. 3). In *S. montana* and *O. fruticosa* vessels with lumens of intermedi-

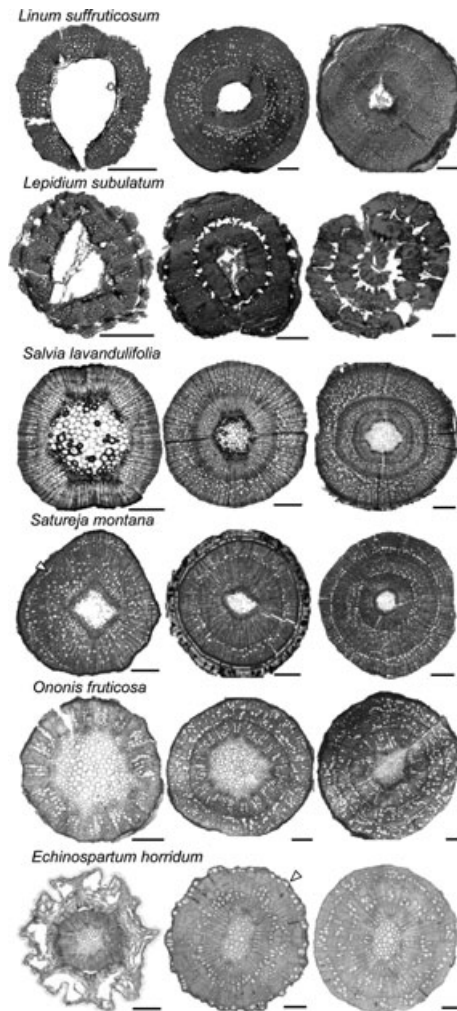


Fig. 2. Cross-sections of 1-, 2- and 3-year-old branches of the six studied species (shown from left to right). Triangles indicate spring growth in the case of the selected 1- and 2-year-old *Satureja montana* and *Echinopartum horridum* branches, respectively. Scale bars correspond to $250 \mu\text{m}$.

ate area ($0.001\text{--}0.002 \text{ mm}^2$) also accounted for ca. 40% of the K_h .

Wood formation and radial growth

The studied species started radial growth between March and April (Fig. 4). The maximum increase in vessel area was observed in the early phases of cambial growth in spring (April–May), *i.e.* when the widest vessels were formed, and at that time vessel area varied between 2% (*S. lavandulifolia*, *S. montana*, *L. suffruticosum*, *L. subulatum*) and 5% (*O. fruticosa*, *E. horridum*). All species had maximum radial growth rates from May (*L. subulatum*, *L. suffruticosum*, *S. lavandulifolia*, *E. horridum*) to June (*S. montana*, *O. fruticosa*), while most of the ring (at least 80%) was formed before the summer drought started in July (Fig. 4). Mean radial growth rates ranged from $0.003\text{--}0.004 \text{ mm day}^{-1}$ (*L. subulatum*, *L. suffruticosum*) up to ca. $0.005 \text{ mm day}^{-1}$ (*S. montana*, *S. lavandulifolia*, *O. fruticosa*, *E. horridum*). In some of the studied species there were differences in the timing of maximum radial growth rates when

Table 2. Morphological and wood anatomical characteristics of the sub-shrubs studied. Mean (\pm SD) values ($n = 15$).

Species	shoot length (cm)			shoot diameter (mm)			vessel area (%)			vessel area ($\text{mm}^2 \cdot 10^{-3}$)		
	age (years)	1	2	3	age (years)	1	2	3	age (years)	1	2	3
<i>Linum suffruticosum</i>	5.2 \pm 2.4	4.8 \pm 1.8	4.3 \pm 1.7	1.5 \pm 0.5	2.2 \pm 0.5	1.9 \pm 1.2	2.0 \pm 0.8	2.1 \pm 1.0	0.19 \pm 0.09	0.20 \pm 0.11	0.21 \pm 0.11	
<i>Lepidium subulatum</i>	5.3 \pm 2.3	4.5 \pm 1.8	4.8 \pm 2.0	1.1 \pm 0.3	1.5 \pm 0.4	1.1 \pm 0.6	1.2 \pm 0.7	1.2 \pm 0.2	0.21 \pm 0.16	0.24 \pm 0.18	0.25 \pm 0.32	
<i>Salvia lavandulifolia</i>	3.3 \pm 1.0	2.6 \pm 1.1	2.7 \pm 1.1	1.5 \pm 0.3	1.8 \pm 0.4	1.5 \pm 1.3	1.6 \pm 0.8	1.8 \pm 1.0	0.16 \pm 0.06	0.17 \pm 0.07	0.19 \pm 0.09	
<i>Satureja montana</i>	6.0 \pm 2.6	4.1 \pm 1.4	3.0 \pm 1.1	1.0 \pm 0.2	1.5 \pm 0.4	1.1 \pm 0.4	1.5 \pm 0.7	1.8 \pm 1.2	0.18 \pm 0.08	0.26 \pm 0.16	0.27 \pm 0.17	
<i>Ononis fruticosa</i>	5.0 \pm 1.3	5.1 \pm 1.4	4.4 \pm 1.5	1.3 \pm 0.2	1.6 \pm 0.3	3.1 \pm 0.9	3.5 \pm 1.1	3.7 \pm 1.3	0.25 \pm 0.12	0.26 \pm 0.17	0.30 \pm 0.20	
<i>Echinopartum horridum</i>	3.7 \pm 1.1	2.4 \pm 0.9	3.0 \pm 1.2	1.3 \pm 0.2	1.4 \pm 0.2	2.6 \pm 1.0	2.9 \pm 1.0	3.0 \pm 0.6	0.33 \pm 0.23	0.50 \pm 0.24	0.53 \pm 0.30	

comparing shoots of different ages. For instance, in the case of *S. lavandulifolia*, *O. fruticosa* and *E. horridum* the youngest shoots had the highest radial increment one month later than the oldest ones (Fig. S1).

Relationships between growth type and NSC concentration

The period comprising the most active shoot extension occurred from April to May in *L. subulatum* and *L. suffruticosum* and from May to June and July in *E. horridum* and *O. fruticosa* (Fig. 4). This period of active primary growth extended up to August in *S. montana* and *S. lavandulifolia*. In autumn and winter, fine roots grew actively in all species, while spring was also an important period of belowground growth in *L. suffruticosum*, *S. montana* and *S. lavandulifolia*. Only *S. montana* presented root growth in summer, probably because this population was located near a riverbed.

Secondary and primary aboveground growth clearly overlapped in all studied species (Fig. 4). The rates of both growth types were positively associated in all species except *L. suffruticosum*, which had rapid shoot extension (Table 3). The temporal overlap between primary and secondary growth was maximum (98.5%) in *E. horridum* (the species growing in the coldest site) and minimum (38.5%) in *L. suffruticosum* (growing in the warmest site; Fig. 5). The other species from cold sites (*O. fruticosa*) also presented a very high overlap between the two growth types (92.9%), being followed by *S. lavandulifolia* (71.5%), whereas the remaining species showed intermediate overlap values (*L. subulatum*, 61.2%; *S. montana*, 50.2%). In *L. suffruticosum* and *L. subulatum* secondary growth rates and root production were negatively related (Table 3).

Stem NSC reached maximum concentrations in late summer and autumn (e.g. *L. suffruticosum*, *S. montana*, *O. fruticosa*), winter (*S. lavandulifolia*, *E. horridum*) or late spring (*L. subulatum*) (Fig. 6). The SS and NSC concentration in the stems, and also the SS concentration in roots, were inversely related to radial growth rates in all species except *L. suffruticosum* and *L. subulatum* (Table 3). Primary shoot and root growth were not significantly related to SS, starch and NSC concentration in any of the six studied species ($P > 0.10$ in all cases, results not presented), except *S. montana*, which showed a negative correlation between shoot longitudinal growth rates and SS concentrations in the stems ($r_s = -0.57$, $P = 0.05$).

DISCUSSION

We found that Mediterranean sub-shrubs display different temporal overlaps between primary and secondary growth. Such diverse overlap patterns seem to be linked to the different durations of the growing season in the diverse environmental conditions where these sub-shrubs grow, which has implications for their contrasting wood anatomical properties and growth rates. The highest overlap between growth types occurred in species growing in sites characterised by a short vegetation period.

Wood anatomy and secondary growth

All studied Mediterranean sub-shrub species had semi-ring-porous wood and distinct annual rings, but formed vessels with

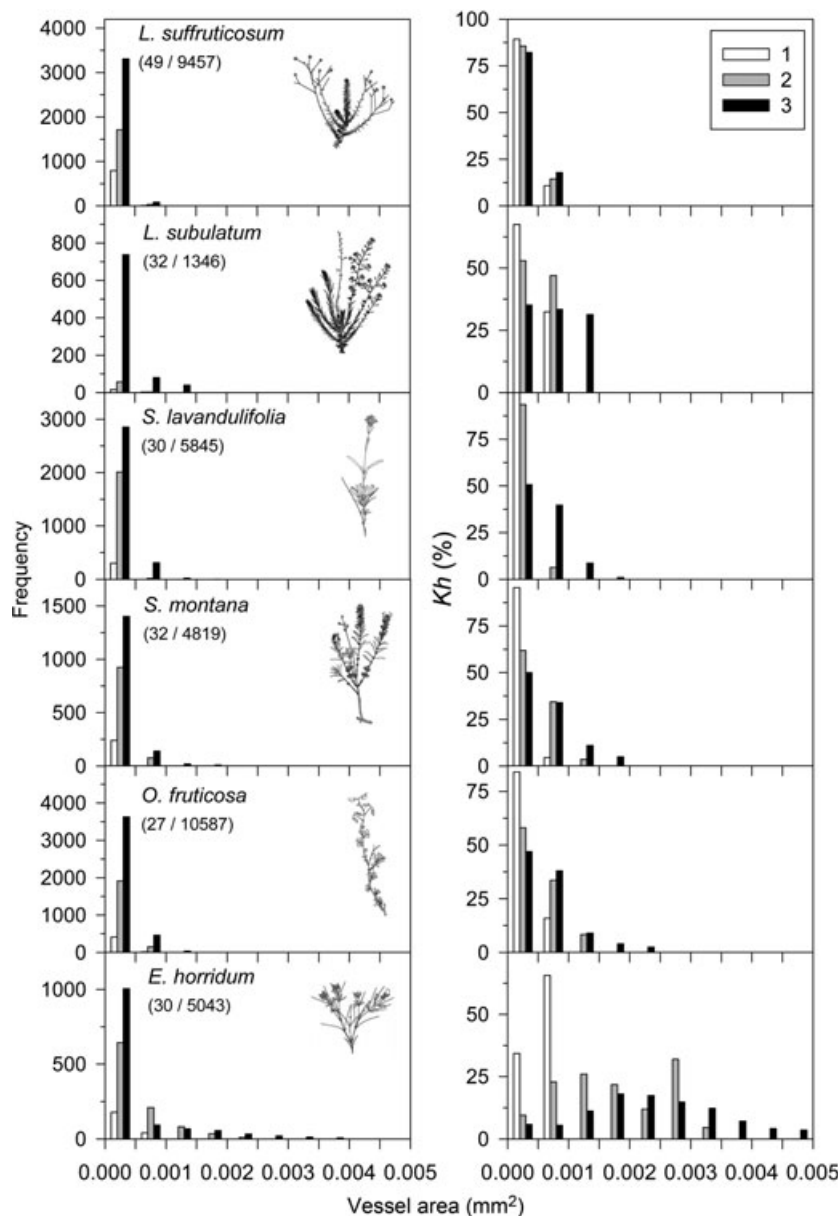


Fig. 3. Distribution of vessels in 1-, 2- and 3-year-old branches (bars of different colours) according to their area and their relative contribution to the total predicted hydraulic conductance (K_h) in the six sub-shrub species studied. Numbers located below species names indicate total number of samples and vessels measured. A scheme showing the general morphology of the branches of each species is also displayed.

variable lumen size and showed contrasting seasonal patterns of radial growth. Species from the warmest and driest sites (e.g. *L. suffruticosum*, *L. subulatum*) formed narrow vessels along an extended xylogenesis period, while species from the coldest and moistest sites (e.g. *O. fruticosa*, *E. horridum*) formed wide vessels over a short period of secondary growth. The wide vessels of the species from high-elevation sites may also be a response to increasing water availability (Table 1), which may enhance the formation of wide early-wood vessels in Mediterranean sub-shrubs (De Micco *et al.* 2008). Although drought stress is absent in these cold sites, and the highest vessel area values were observed in either deciduous (*O. fruticosa*) or evergreen (*E. horridum*) species, our findings indicate that xylem properties seem to be tuned to the length of the growing season. This suggests that the length of the growing season for the studied species is more constrained by low temperatures than by drought periods that usually occur in the summer.

Most secondary growth occurred from early to late spring, *i.e.* a pattern that matches that described for seasonally dimorphic species (Liphschitz & LevYadun 1986). All species formed most of their wood almost one month before summer drought started; however, some local deviations from this pattern were also observed. For instance, primary and secondary growth were recorded in autumn in *S. montana*, which was sampled near a riverbed where soil water availability is high and thus may allow growth even during the dry summer (Palacio *et al.* 2006). In this species and also in *S. lavandulifolia* and *E. horridum*, radial growth peaked earlier in oldest than in youngest shoots, which may be related to hydraulic or growth constraints. For example, the rapid extension of current-year shoots of *E. horridum* may require an increased amount of water and thus enhanced hydraulic conductivity supplied by the first-formed wide vessels (Palacio & Montserrat-Martí 2006; Palacio *et al.* 2008). Indeed, young shoots in species with rapid shoot

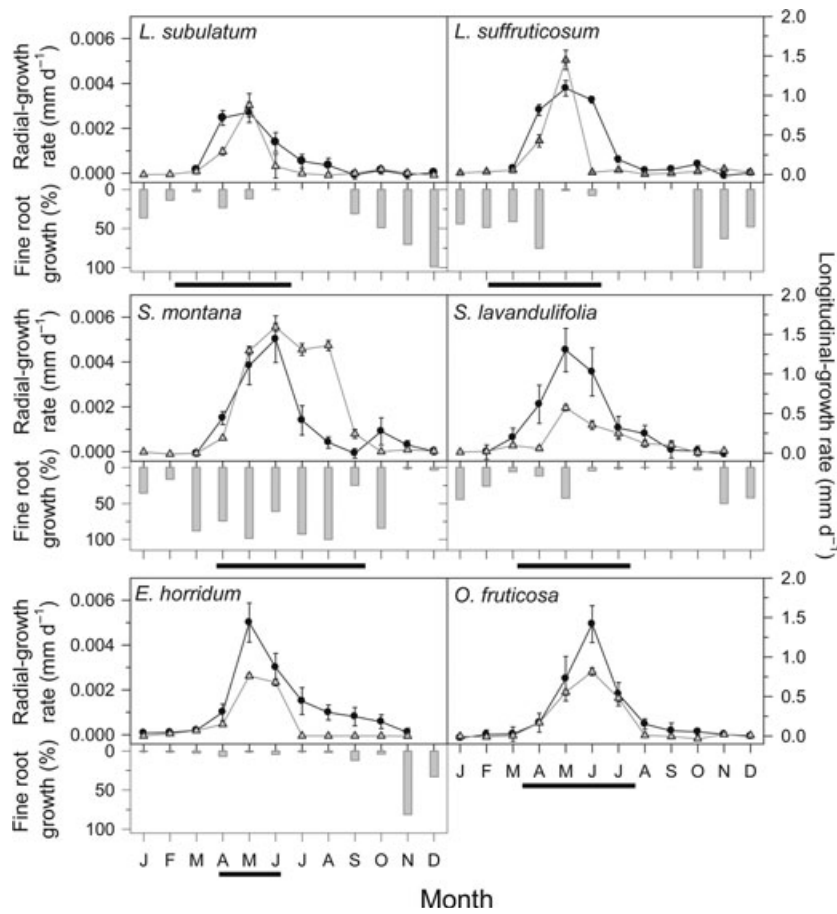


Fig. 4. Monthly radial (circles) and longitudinal (triangles) shoot growth rates as compared to fine root growth (bars, percentage scales) of the six studied sub-shrubs. The lower black lines indicate the periods of active primary shoot growth. Data are means ± SE (n = 15).

Table 3. Main relationships (Spearman correlation coefficient) between radial growth rates of studied species and the primary growth (shoot longitudinal growth rate), fine root growth and non-structural carbohydrate (SS, soluble sugars; starch and total NSC) concentrations of their main stems and roots (n = 13). Only correlations with P ≤ 0.05 are shown.

species	shoot growth	fine root growth	SS		starch		NSC	
			root	stem	root	stem	root	stem
<i>Linum suffruticosum</i>		−0.50 (0.05)						
<i>Linum subulatum</i>	0.56 (0.03)	−0.52 (0.04)	−0.49 (0.05)		0.55 (0.03)			
<i>Salvia lavandulifolia</i>	0.78 (0.004)		−0.48 (0.05)	−0.49 (0.05)				−0.51 (0.05)
<i>Satureja montana</i>	0.72 (0.005)		−0.68 (0.03)	−0.65 (0.04)	−0.58 (0.05)		−0.72 (0.006)	−0.57 (0.05)
<i>Ononis fruticosa</i>	0.64 (0.03)	no data	−0.79 (0.001)	−0.72 (0.005)				−0.65 (0.03)
<i>Echinopartum horridum</i>	0.60 (0.05)		−0.63 (0.04)	−0.79 (0.003)				−0.64 (0.04)

extension are fully elongated before the start of lignification, whereas in species with a more extended period of shoot growth, lignification and extension partly overlap. Hence, there seems to be a more pronounced delay in the secondary growth of young shoots as compared with old ones in species with high overlap between primary and secondary growth, such as *S. lavandulifolia*, *O. fruticosa* and particularly *E. horridum*.

Phylogenetic constraints on xylem characteristics and phenology must also be taken into account, since the two species with the largest vessel lumens and highest relative vessel areas belong to the Fabaceae, whereas the two Lamiaceae species formed the smallest vessels. It is also remarkable that Brassicaceae woody species growing under cold conditions and sup-

porting a short growing season usually form distinct rings, as we observed for *L. subulatum*. Interestingly, Schweingruber (2006) also noted a trend towards ring porosity through formation of wide vessels and enhanced hydraulic transport under arid conditions in this last family.

Temporal overlap among growth types and changes in NSC concentration

We found that secondary growth overlapped with primary aboveground growth in all studied sub-shrub species. As expected, the major periods of shoot extension and thickening overlapped more in the species from the coldest site (e.g.

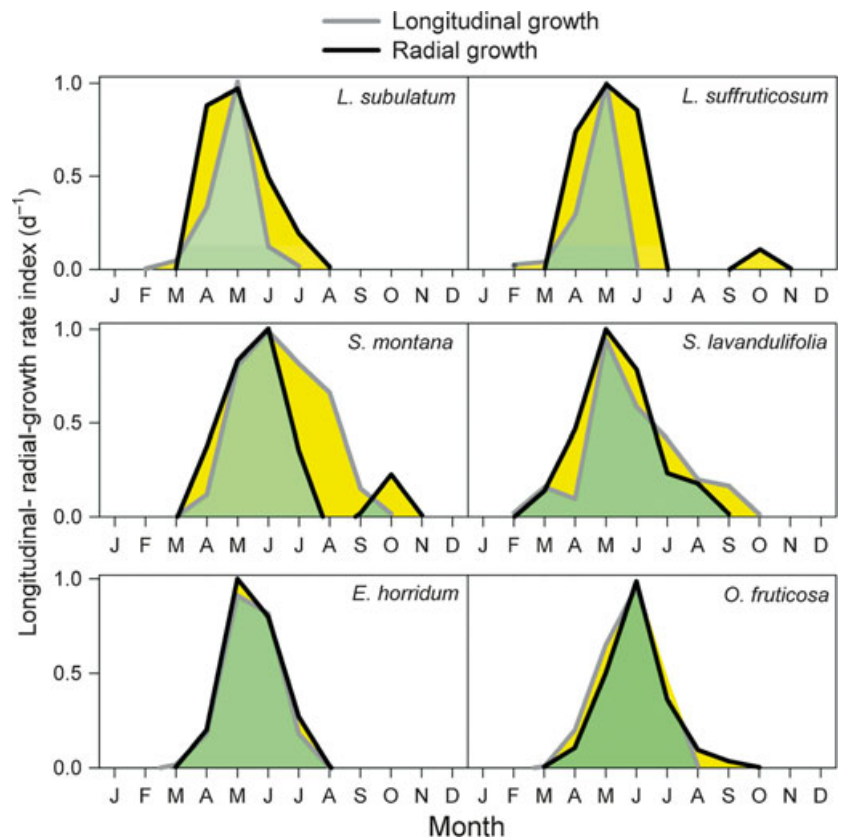


Fig. 5. Periods of overlapping (green area) and non-overlapping (yellow areas) primary and secondary growth types. The periods were calculated by graphically estimating the areas below the curves corresponding to the indexed longitudinal and radial growth rates (range 0–1) of the sub-shrub species.

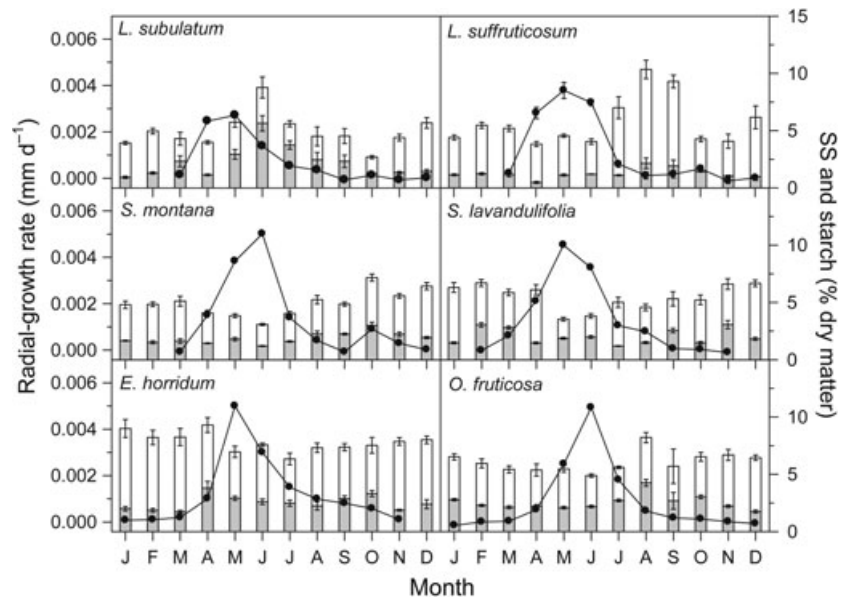


Fig. 6. Radial growth rates (circles) and seasonal dynamics of non-structural carbohydrate concentrations (soluble sugars – white, starch – grey bars) in the main stems of the six studied sub-shrubs. Data are means ± SE (n = 15).

E. horridum) than in those from warm sites (e.g. *L. suffruticosum*). *E. horridum* had a short period of wood growth that allowed it to develop shoots quickly (Palacio & Montserrat-Martí 2007). This species and *O. fruticosa* have the widest vessels and are characterised by rapid shoot extension during the short growing seasons in the cold sites. Our findings indicate that the wide vessels of these species allow them to increase hydraulic conductivity, with efficient rapid growth and a high

overlap between primary and secondary growth. According to our results, such overlap seems to be linked to the rapid consumption of available carbohydrates for growth, leading to a sharp reduction in NSC concentrations during shoot growth (Table 3).

At the opposite extreme, primary and secondary growth in warm but dry sites showed a relatively lower temporal overlap, either because of the protracted period of shoot thickening as

compared to shoot extension (*L. suffruticosum*) or because the primary growth extended from spring to autumn under humid conditions (*S. montana*). For instance, *L. suffruticosum* forms vessels with narrow lumens, has small leaves and shows low overlap among growth types, which may explain why radial growth rates were not related to changes in NSC concentration in this species.

In the warmest and driest sites, primary and secondary growth overlapped less in the summer deciduous *L. subulatum* than in *L. suffruticosum*. This may be related to the relatively longer shoot extension period of *L. subulatum*, which has to rebuild its whole canopy after summer drought (Palacio *et al.* 2006). Nevertheless, in these species, wood formation and root growth clearly occurred in the two favourable seasons of the year, namely spring and autumn. This suggests that *L. suffruticosum* and *L. subulatum* finish most of their xylem development before summer drought, arrest most growth processes during summer (except bud formation, see Palacio & Montserrat-Martí 2005) and resume growth in autumn, probably by diverting carbohydrates for active root growth to uptake water and nutrients available in the soil (Palacio & Montserrat-Martí 2007).

Although we observed a negative relationship between stem NSC concentration and secondary growth in plants with increasingly shorter vegetative periods and the highest overlaps between primary shoot growth, the observed seasonal change in NSC concentrations was only moderate. This may indicate that sub-shrubs accumulate NSC in excess, similar to trees (Millard *et al.* 2007). Possible explanations for immobilising NSC in the stems may be to use them as a safeguard against potential disturbances or to maintain hydraulic integrity (Sala *et al.* 2012). The lack of a strong association between primary shoot growth rates and SS, starch and NSC concentrations may be because recently formed shoots and leaves are carbon sinks only during their initial development, becoming net C sources thereafter (Keel & Schädel 2010).

To conclude, Mediterranean sub-shrubs displayed different degrees of temporal overlap between primary and secondary growth processes, which seem to be linked to their wood ana-

tomical properties and growth rates. The highest overlap between growth types occurred in species from cold and relatively moist sites characterised by a short vegetation period (*E. horridum* and *O. fruticosa*). These plants showed rapid shoot extension and radial growth rates and formed vessels with a relatively wide lumen. In fact, the temporal overlap among primary and secondary growth seems to be positively associated with mean vessel diameter in the youngest stems ($r = 0.75$, $P = 0.09$). This association was marginally significant and was based on only six species, but it opens up interesting questions as to how xylem anatomy is linked to the temporal arrangement of the different growth processes of woody species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Monthly cumulative radial growth (diameter) of shoots of different ages (1-, 2- and 3-year-old shoots) of the six sub-shrub species. Data are means \pm SE ($n = 15$).

Figure S2. Example of the processing protocol of an image based on a section of a 2-year old branch of *Ononis fruticosa* sampled in August 2004 (A). The original grey image was processed and converted into a black and white image (B). Then, the pith area was removed and the oldest (C) and youngest (D) rings defined to quantify the total and individual areas of vessels located within each annual ring.

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