Bud Morphology and Shoot Growth Dynamics in Two Species of Mediterranean Sub-shrubs Co-existing in Gypsum Outcrops

S A R A   P A L A C I O * and G A B R I E L   M O N T S E R R A T - M A R T Í

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• *Background and Aims* Understanding the effects of the environment on the morphology and shoot growth activities of plants is crucial to identifying plant ecological strategies. This study analysed the bud morphology, bud activity, shoot growth dynamics and shoot water content at full hydration (WC_h) of two species of Mediterranean sub-shrubs, *Lepidium subulatum* and *Linum suffruticosum*, co-existing in gypsum outcrops in north-east Spain.

• *Methods* Sampling was conducted monthly over 2 years in one population per species. Buds were dissected under a stereo-microscope. Shoot growth was measured as the mean increase in shoot length of 15 marked individuals between two consecutive samplings. Bud activity was studied following the variations in the number of leaf primordia shorter than 1 mm and longer than 0·025 mm in the buds.

• *Key Results* Both species bore naked buds and displayed discontinuous seasonal patterns of shoot growth, leaf primordia formation and WC_h. The number of leaf primordia in the bud peaked before the beginning of shoot expansion. In both species, organogenesis and expansion were uncoupled throughout the year. The time lapse between these two processes varied throughout the year, and was greatest for those elements differentiated in autumn. WC_h was more closely related to shoot expansion than to organogenesis.

• *Conclusions* Both species displayed similar bud morphology and similar seasonal patterns of bud and shoot growth, and WC_h as a result of the strong seasonality of the Mediterranean climate in gypsum outcrops. The beginning of the spring period of expansion of long branches coincided with maximum values of WC_h, while the rest period of summer matched minimum values. These results support the hypothesis that the growth of long branches is strongly related to WC_h.

Key words: Shoot growth, bud morphology, Mediterranean sub-shrubs, gypsum, *Linum suffruticosum*, *Lepidium subulatum*.

INTRODUCTION

Plants growing in highly seasonal environments must adjust their morphology and the activity of their renewal structures and shoots to maximize survival and productivity (Meloche and Diggle, 2001). An understanding of these features is crucial to identifying the ecological strategies of plants (Nitta and Ohsawa, 1999). Mediterranean trees and shrubs normally bear cataphyllary or hipsophyllary buds in which meristems are protected during unfavourable seasons by specialized protective organs (Hoffmann, 1972; Ginocchio and Montenegro, 1996; but see Hoffmann and Hoffmann, 1976). Conversely, the buds of some species of Mediterranean sub-shrubs lack these protective organs (Montserrat-Martí *et al.*, 2004; Palacio-Blasco *et al.*, 2004). Most Mediterranean sub-shrubs bear their naked buds in the apices of two distinct types of branches: long and short branches. The latter usually develop in the axils of the leaves of long branches (Orshan, 1989). Few studies have addressed the morphology and growth dynamics of buds and shoots of Mediterranean sub-shrubs (but see Orshan, 1972; Gray and Schlesinger, 1981; Montserrat-Martí *et al.*, 2004; Palacio-Blasco *et al.*, 2004). In particular, very little is known about bud and shoot growth dynamics during the unfavourable seasons of winter and summer, or about the relationship between the growth of these organs. The growth of axillary short branches is maintained throughout the year, at least in some species growing in semi-arid environments (Orshan, 1972).

However, it is unlikely that a continuous pattern of shoot growth and morphogenesis occurs under the seasonal conditions of a Mediterranean climate. Furthermore, shoot expansion may be rapid in Mediterranean sub-shrubs, thereby taking advantage of the short favourable periods of spring and autumn. Therefore, the shoots of these sub-shrubs may be at least partly preformed, with buds containing many leaf primordia prior to shoot expansion (Kozlowski and Clausen, 1966).

For shoot growth to occur, plants require increased bud and shoot hydration (Bradford and Hsiao, 1982; De Faÿ *et al.*, 2000). The potential to hydrate might therefore vary through the year, and might be related to the growth activity of these two organs (De Faÿ *et al.*, 2000). Several authors have studied the relationship between growth processes and the water content of buds and stems of woody plants (Jones and Laude, 1960; Cottignies, 1983, 1990; Essiamah and Eschrich, 1986; Tousignant *et al.*, 2003). However, water content is highly dependent on the weather conditions at the sampling time (Tousignant *et al.*, 2003) and is therefore not a good predictor of plant hydration capacity. In contrast, the water content at full hydration (WC_h, %) is a measurement of the maximum amount of water a given organ can hold, expressed as the percentage of the fresh weight of the fully hydrated organ. In a previous study, both water content and water content at full hydration of the shoots of four species of Mediterranean shrubs were analysed monthly over one year (G. Montserrat-Martí, unpubl. data). According to these results, water content
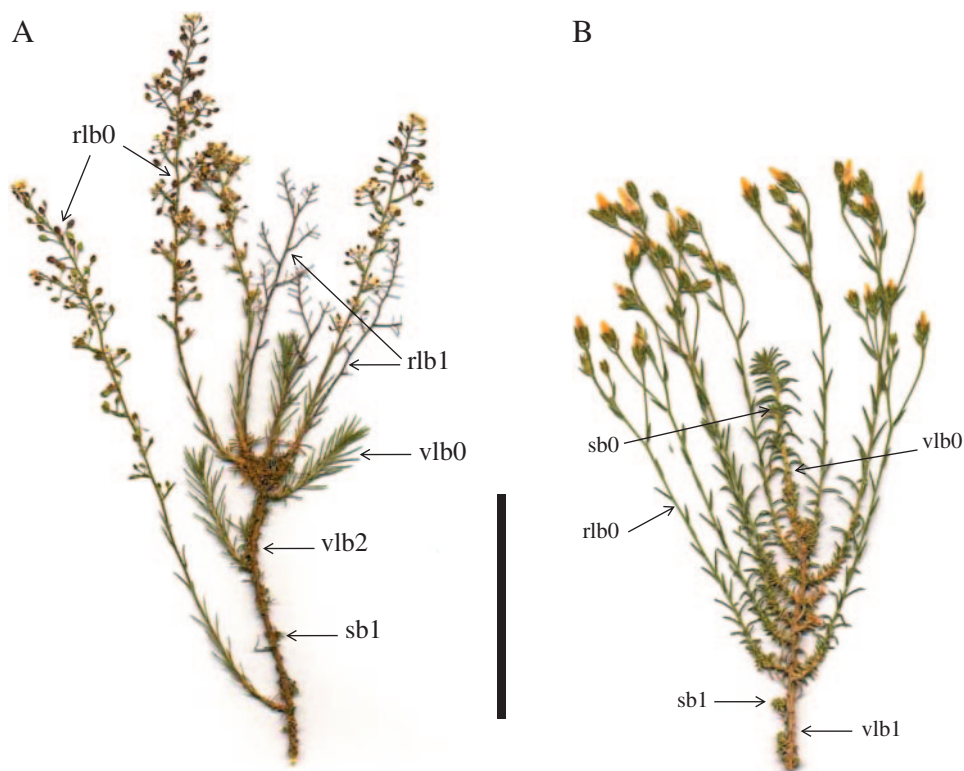
FIG. 1. General morphology of a 3-year-old branch of (A) *Lepidium subulatum* and a 2-year-old branch of (B) *Linum suffruticosum* on 12 and 5 May 2003, respectively. vlb0, vlb1 and vlb2: vegetative long branches from 2003, 2002 and 2001, respectively; rlb0, rlb1: reproductive long branches from 2003 and 2002, respectively; sb0, sb1: short branches from 2003 and 2002, respectively. Scale bar = 5 cm.

varied following rainfall, while the seasonality of water content at full hydration appeared to be related with shoot growth. Few studies have addressed WC_h in relation to shoot growth. Davis and Mooney (1986) reported a sharp increase in WC_h of the shoots of two co-occurring chaparral species during the growing season and concluded that this increase was related to organogenetical processes. More recently, the growth peaks in *Cistus laurifolius* shoots and leaves have been reported to coincide with maximum values of WC_h (Montserrat-Martí *et al*., 2004). No studies relating WC_h to shoot growth processes have been conducted in Mediterranean sub-shrubs.

Mediterranean gypsum outcrops such as the ones considered here are suitable to study the relationship between climatic seasonality and shoot growth processes as they are subjected to sharp environmental variations during the year (Rivas-Martínez and Costa, 1970; Nelson and Harper, 1991). Pure gypsum soils have a very low water retention capacity, and therefore do not buffer the effects of drought on plant performance (Guerrero Campo *et al*., 1999*b*). These stress factors are more intense in the ridges of pure gypsum hills, such as those considered in this study (Guerrero Campo *et al*., 1999*a*).

Here we describe the bud morphology and shoot growth dynamics of two species of Mediterranean sub-shrubs co-existing in gypsum outcrops. The relationship between shoot growth processes and the potential of shoots to become fully hydrated is also analysed. Between September 2002 and August 2004, a study was made of the phenology, bud morphology and activity, shoot growth dynamics and WC_h of *Lepidium subulatum* and *Linum suffruticosum* plants co-existing in pure gypsum outcrops in north-east Spain.

MATERIALS AND METHODS

Species and study site

Linum suffruticosum L. (Linaceae) is an evergreen sub-shrub with a maximum height of 70 cm that inhabits low scrublands on gypsum soils and limestones in south-west Europe (Ockendon and Walters, 1968). *Lepidium subulatum* L. (Brassicaceae) is an evergreen sub-shrub up to 60 cm in height that is restricted to open scrublands on gypsum soils in eastern Spain and north-west Africa (De Carvalho e Vasconcellos, 1964). For ease of presentation, from here on *L. suffruticosum* and *L. subulatum* will be referred to as *Linum* and *Lepidium*, respectively.

Both species were selected for a comparative analysis of shoot growth processes due to their similar crown morphology and architecture. Their leaves are linear, rigid and helicoidally arranged. Both species bear two distinct types of branches: long and short branches (Fig. 1). In the present study, short branches are those shorter than 2 cm in length, while long branches are those longer than 2 cm in length. Long branches either bear terminal inflorescences or remain vegetative, while short branches develop mainly in the axils

FIG. 2. General view of the study area on 3 February 2003. Gypsum outcrops of Villamayor, near Zaragoza, in north-east Spain (UTM: 30TXM8820; 320 m a.s.l).

of the leaves of vegetative long branches, but also grow at the base of some reproductive long branches, or on older stems (epicormic short branches), especially in *Lepidium*.

The study site was located on the slope of a nearly pure gypsum hill in the gypsum outcrops of Villamayor, near Zaragoza, in north-east Spain (UTM: 30TXM8820; 320 m a.s.l; Fig. 2). The dominant substratum in this area is almost pure gypsum, with a few thin inserted outcrops of marls and clays (Quirantes, 1977). The climate at this site is semi-arid and highly seasonal, with a mean annual temperature of 14·6 °C, and an average annual rainfall of 334·5 mm, which falls mainly during spring and autumn (Rivas-Martínez, 1987). Summers are hot and dry and the mean maximum temperature of the warmest month (July) is 31·4 °C. Winters are cool and dry and the mean minimum temperature of the coldest month (January) is 1·6 °C (De León *et al.*, 1987). At this site, these two species co-exist and display a similar degree of abundance. The area is covered by open low scrubland (termed tomillar) dominated by *Ononis tridentata*, *Helianthemum syriacum*, *Helianthemum squamatum*, *Thymus zygis*, *Herniaria fruticosa* and *Gypsophila struthium* subsp. *hispanica*, and the two sub-shrubs studied here (Braun-Blanquet and Bolòs, 1957). The area was partly ploughed for *Pinus halepensis* reforestation 15 years ago, although only some isolated pines survive on the upper part of the hill. Since then, the site has remained abandoned.

Phenology

Above-ground phenology was studied monthly from September 2002 to August 2004 on 15 marked plants of each species. Phenological processes such as flowering, fruiting, shoot expansion and leaf shedding were assessed by visual inspection. Estimations of the approximate amount of green biomass were also recorded for each individual. To do this, one standard 3-year-old branch was examined in each of the 15 marked individuals, and the percentage of its green biomass was visually estimated. At each sampling date, representative plant material was collected, pressed and stored in a herbarium for future verification.

Bud morphology and composition

Sampling was conducted monthly during an 18-month period, from September 2002 to February 2004. Ten 2-year-old branches were collected randomly from ten non-marked individuals within each population at each sampling date. Samples were kept at 4 °C until bud dissection, which was performed within the following 48 h. To avoid within-branch variability, only those buds located in standard short branches were dissected, thereby excluding extremely large or small short branches or those that were apparently malformed or damaged. Buds were examined under a stereo-microscope fitted with an ocular micrometer (MS5 Leica Microsystems, Heerbrugg, Switzerland) at ×10 or ×40. The properties (colour, vigour and developmental stage) of most leaf primordia were recorded. Because of the large number of leaf primordia in the buds of these two species ($n > 20$ in some months), only those shorter than 1 mm and longer than 0·025 mm were counted (hereafter N_p) and measured. The resolution limit of the stereo-microscope was approximately 0·025 mm. Given that the maximum length of leaf primordia varied through the year (see below), we set an arbitrary upper limit of 1 mm on the basis of the lowest value of the maximum lengths of leaf primordia recorded. Adult leaves were distinguished from leaf primordia by the increased toughness of their epidermis, which indicated the cessation of their expansive growth. The length of leaf primordia was measured from their insertion point on the immature stem to their distal end.

Shoot growth

In September 2002, we selected and marked 15 well-developed adult individuals of each species. Sampling was conducted monthly over two years, from September 2002 to August 2004. At each sampling date, three 2-year-old branches were collected from distinct positions within the canopy of each plant. Repeated cuttings from the same branch were avoided so that the effect of the sampling method on plant growth was minimized. To check for possible interactions between sampling method and plant performance, 15 plants of each species, similar to those used for shoot growth analyses, were marked and left uncut. No differences were found in the survival and shoot vigour of sampled and control plants at the end of the sampling period. Therefore, the effect of sampling on plant performance was negligible. Samples were pressed at constant pressure and stored in a herbarium until measurements of shoot length were conducted under the stereo-microscope. For each branch, the lengths of the longest shoot and its three closest shoots were measured. Shoot length was measured from the insertion point on the stem to the tangent line between the apices of the most apical green leaves. Leaves were considered green when more than 50 % of their lamina was green. Destructive analyses were required because of the small size of undeveloped short branches. The relative growth rate (*RGR*) of the shoots of marked plants at each sampling date was calculated using the following formula:

$$RGR = (L_n - L_{n-1})/T$$

where L_n (mm) is the mean shoot length of month n, L_{n-1} (mm) is the mean shoot length of month $(n - 1)$, and T (d) is the period between $(n - 1)$ and n.

Water content at full hydration

Sampling was conducted monthly between October 2002 and December 2003. Ten branches over 3 years old were randomly collected from ten non-marked individuals at each sampling date. Branches were placed in individual plastic bags and taken to the laboratory in a cooler. Once in the laboratory, branches were set at full hydration. To do this, the three most proximal centimeters of the stem of each branch were cut under water, and the remaining material was kept at 4 °C, with the first 3–4 cm of the stem immersed in distilled water, and covered by a wet plastic bag for 24 h. Full hydration weights of samples of whole short branches (including leaves and stems) and stems of the long branches were obtained for each hydrated branch. Subsequently, samples were oven-dried at 60 °C to a constant weight and dry weights were obtained. All weighing was conducted using a precision scale (MC1, Sartorius AG, Goettingen, Germany). Water content at full hydration (WC_h, %) was calculated using the following formula:

$$WC_h = (W_h - W_d) \times 100/W_h$$

where W_h(mg) is the weight of a given sample at full hydration and W_d (mg) is the dry weight of the same sample.

Statistical analysis

All data were checked for normality, homocedasticity and outlier detection. RGR for each sampling date was analysed using a Student t-test in which the mean RGR was compared with a theoretical mean value equal to zero. In cases in which normality and homocedasticity were not attained, data were analysed using the non-parametrical Wilcoxon t-test for paired samples. Differences in N_p and WC_h between distinct sampling dates were analysed using one-way ANOVA. To study the relationship between WC_h and growth, WC_h data from short and long branches were sorted in two groups: 'growth' and 'no growth'. The former included those WC_h values of dates with mean RGR significantly greater than zero ($\alpha = 0.05$); while the latter comprised the remaining WC_h values. All data except those from the short branches of *Linum* lacked normality and/or homocedasticity. Therefore, to detect differences in WC_h between 'growth' and 'no growth' groups, a Student t-test was run in the short branches of *Linum*, while the remaining data were analysed using the non-parametrical Mann–Whitney U-test for unpaired samples. All statistical analyses were conducted using SPSS 11.0 (SPSS Inc., Chicago, USA).

RESULTS

Phenology

Lepidium and *Linum* flowered in spring. The former began flowering before mid April and continued up to May/June,
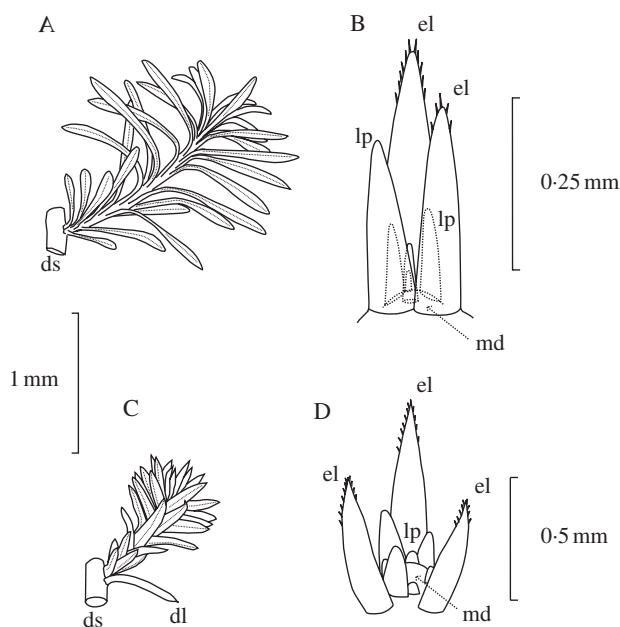

FIG. 3. Morphology of the short branches and apical meristems of *Lepidium subulatum*, (A) and (B) respectively, and *Linum suffruticosum*, (C) and (D) respectively, in December 2003. For clarity, only the inner structures of the buds are represented. ls, long branch stem; ll, long branch leaf; lp, leaf primordia; md, meristematic dome.

while the latter began flowering slightly later, in late April, and concluded by early June. Fruiting began by late April in *Lepidium* and finished by late June. *Linum* began fruiting by late May, ending by early July. *Lepidium* dispersed its seeds during late spring and summer, beginning in late May/June. In contrast, *Linum* began seed dispersal by late September. Both species showed protracted seed dispersal over several months. For both species, dry leaves tended to remain attached to the stems for several months and were gradually shed throughout the year. *Lepidium* plants were apparently dry in summer, with few green leaves in many cases. In contrast, *Linum* kept many green leaves through the summer. In early autumn, short branches restarted growth, slowly increasing the amount of green biomass of both species, which reached maximum values in spring.

Morphology of the renewal structures

The meristematic tissues that gave rise to the long branches of *Linum* and *Lepidium* were the apical buds of axillary short branches and, less frequently, those of vegetative long branches (Fig. 3A, C). In *Linum*, buds consisted of a dome-shaped meristem surrounded by six to >30 helicoidally arranged leaf primordia in different stages of development (Fig. 3D). In *Lepidium*, buds comprised a dome-shaped meristem surrounded by two to >28 helicoidally arranged leaf primordia at different stages of development (Fig. 3B). No specialized protective organs, such as cataphylls or scales, were found in either species, indicating that bud formation is attained simply by the arrest of apical growth of short branches. Therefore, in *Linum* and *Lepidium*, leaf primordia are protected only by the

expanded leaves of short branches, and hence these buds should be considered as being naked (Nitta and Ohsawa, 1998).

Shoot growth

Linum and *Lepidium* showed very similar shoot growth dynamics (Fig. 4). Two periods of shoot extension were recorded that lasted for most of the year. The first occurred in spring and comprised long branch expansion from the apical buds of axillary short branches formed the previous year, and the concurrent growth of a new cohort of axillary short branches. The second period occurred in late summer and autumn and involved a slight expansion of short branches. During mid-summer and winter, the two species showed arrested shoot growth. In both species, long-branch expansion began by mid-March and finished around June (July in the case of *Linum* in 2004) with the development of terminal inflorescences in reproductive long branches and the formation of apical buds in vegetative ones. Not all short branches elongated to long branches in spring. Some remained unexpanded, increasing just some millimeters in length and drying out in early summer. The *RGR* of long branches peaked in May for the two species in both years of study, reaching 0·88 mm d^{-1} and 0·63 mm d^{-1} in 2003 and 2004, respectively, for *Lepidium*, and 1·45 mm d^{-1} and 1·05 mm d^{-1} for *Linum*. The mean *RGR* for the period of long-branch expansion was 0·40 mm d^{-1} and 0·25 mm d^{-1} in 2003 and 2004, respectively, for *Lepidium*, and 0·64 mm d^{-1} and 0·45 mm d^{-1} for *Linum*.

The expansion of the new cohort of axillary short branches overlapped that of long branches in both species. This expansion was first detected by mid-March and was arrested by the end of June in *Lepidium* and by late July in *Linum*. Subsequently, the *RGR* of short branches reached close to zero in *Linum* during August and September, while in *Lepidium* it attained significant negative values during July ($P < 0·01$) and August ($P < 0·05$) because of the drying of most leaves caused by summer drought. The expansive growth of short branches was resumed in September in *Lepidium* and in October in *Linum*, thereby coinciding with late-summer and autumn rainfall, and was arrested again in December. During winter (from December to the end of February), both species maintained short branch *RGR* values close to zero, with the exception of *Linum*, which had an isolated pulse of short-branch growth in February ($P < 0·01$). The expansion of short branches that gave rise to the next cohort of long branches was resumed by mid-March.

Leaf primordia formation

The number of leaf primordia shorter than 1 mm and longer than 0·025 mm (N_p) in buds varied significantly between dates ($P < 0·001$), and showed similar patterns in the two species (Fig. 5). The maximum length of leaf primordia also varied through the year, peaking in spring and reaching minimum values in summer (Fig. 6). During autumn and winter, the apical meristem produced leaf primordia almost continuously. The older ones grew into

adult leaves. However, most of these went through a slow period of development that resulted in an accumulation of leaf primordia at the end of winter (early March), when maximum N_p values were reached in both species (Fig. 5). During early spring, the apical meristem continued to produce new primordia. The development of these into adult leaves was then very quick, and the sizes of leaf primordia and adult leaves were at their greatest (Fig. 6). The number of leaf primordia present in buds diminished steadily. At the end of spring, the formation of new structures by the meristem slowed down. Leaf primordia matured quickly, giving rise to shorter adult leaves. With the onset of summer drought, the formation of leaf primordia was apparently arrested. The tissues of most of the remaining leaf primordia within the bud hardened quickly, giving rise to tiny adult leaves. By this time, N_p and the maximum length of leaf primordia reached minimum values in both species (Figs 5, 6).

In spite of the similarities in their general patterns, *Linum* and *Lepidium* differed in the dynamics of N_p during the first six months of short-branch expansion. In the former, the N_p of the newly formed short branches increased during early summer, reaching a peak in July, while in the latter it decreased from the initial value after short-branch formation, reaching a minimum also in July (Fig. 5). From then on, N_p values increased steadily in *Lepidium* until late winter, while in *Linum* they declined again to reach a new minimum in September and then rose again progressively until late winter. In both species, N_p values peaked before the onset of the rapid expansion of long branches, while they were low before the slow autumn growth of short branches.

Water content at full hydration

The variations in WC_h were greater in *Lepidium* than in *Linum* (Fig. 7). However, both species displayed similar patterns of variation in this parameter during the course of the year, with significant differences between dates ($P < 0·001$; Fig. 7). Minimum values of WC_h were reached in August and September for *Lepidium* and *Linum*, respectively. The extremely low WC_h values of the short branches of *Lepidium* during the summer might be due to the presence of dry leaves and the difficulty of excluding them prior to weighing due to their small size. Dry leaves are not re-hydrated when the branches are set at full hydration, but they are included in the dry weight of short branches, resulting in abnormally low WC_h values. Maximum values of WC_h were attained in early May and November in *Lepidium*, while in *Linum* they were reached in April and October. During the autumn–winter period between these two maxima, WC_h displayed nearly constant values in *Lepidium*, while in *Linum* WC_h diminished to reach a second minimum. Therefore, the WC_h values closely followed those of shoot *RGR* in both species; attaining maximum values in spring and minimum values in summer (Figs 3, 6). In *Linum*, minimum autumn and winter WC_h values matched the reduced autumn and winter growth of short branches. In the short and long branches of *Linum*, WC_h was significantly higher in months with significant positive
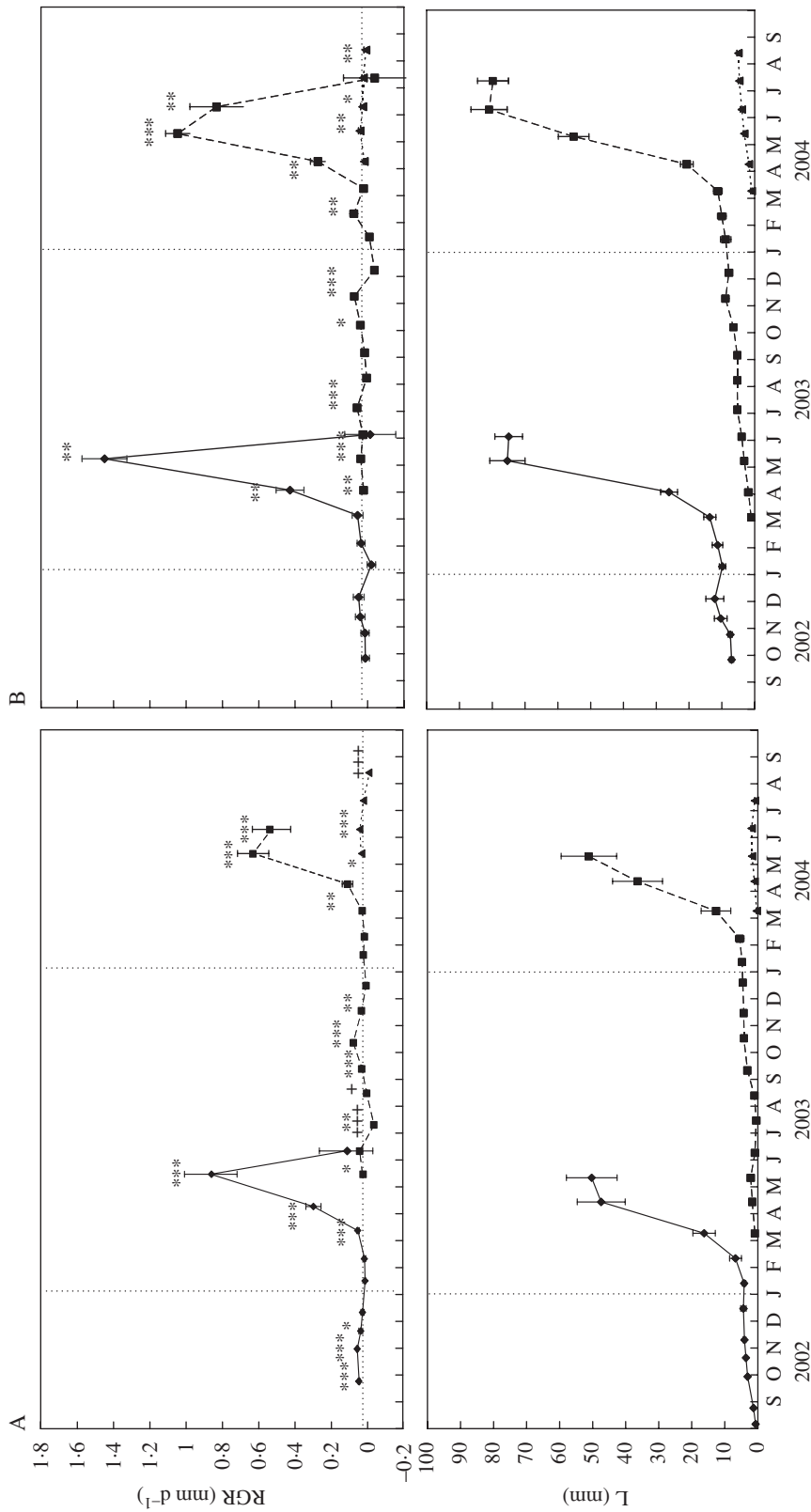
FIG. 4. Relative growth rate dynamics (top) and mean length of the shoots (bottom) of (A) *Lepidium subulatum* and (B) *Linum suffruticosum*. Values are means of 15 shoots ± s.e. Solid lines represent shoots recorded in 2002, dashed lines are those from 2003, and dotted lines are those from 2004. Asterisks indicate RGR values significantly higher than zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and crosses indicate RGR values significantly lower than zero (+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$), after a Student's *t*-test, in case of normal data, or by Wilcoxon *t*-test otherwise.
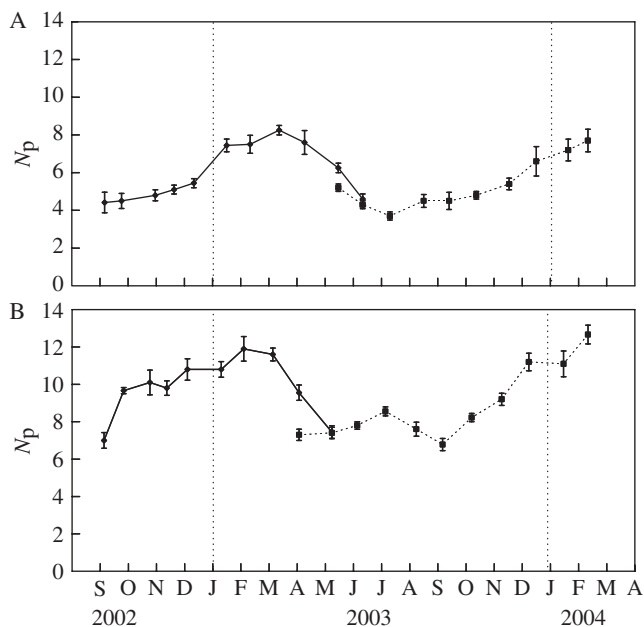
FIG. 5. Number of leaf primordia smaller than 1 mm and longer than 0·025 mm in the renewal buds (N_p) of (A) *Lepidium subulatum* and (B) *Linum suffruticosum*. Solid lines represent buds from shoots recorded in 2002 and dotted lines are those from 2003. Values are means of ten buds ± s.e.

FIG. 7. Water content at full hydration (WC_h) of the short and long branches of (A) *Lepidium subulatum* and (B) *Linum suffruticosum*. Dotted lines represent short branches while solid lines show the stems of long branches. Open circles are shoots recorded in 2002 while closed circles are those from 2003. Values are means from ten shoots ± s.e.
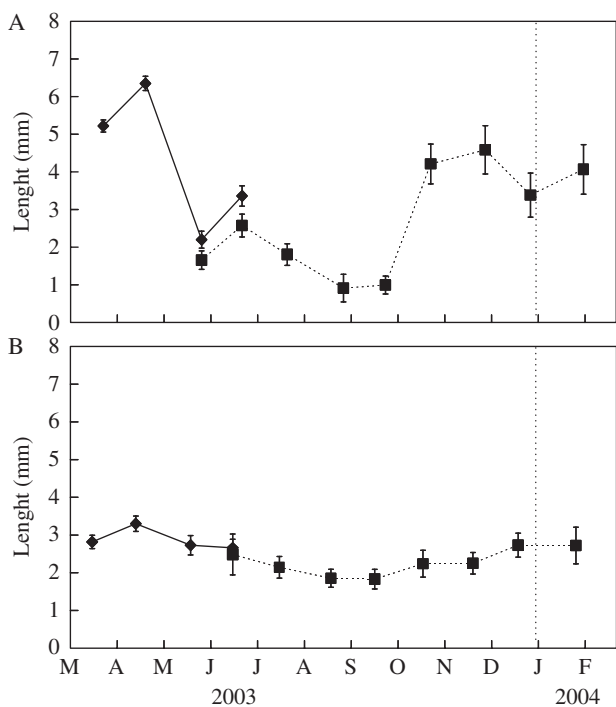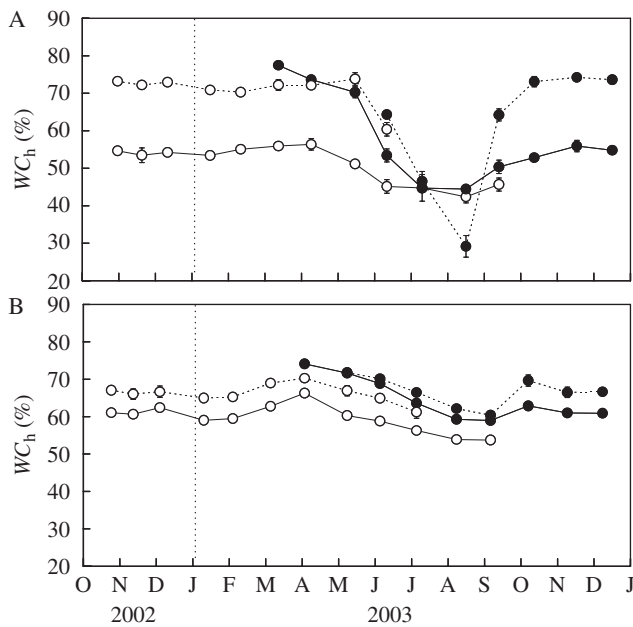

FIG. 6. Maximum length of the leaf primordia of (A) *Lepidium subulatum* and (B) *Linum suffruticosum*. Solid lines represent shoots recorded in 2002 and dotted lines are those from 2003. Values are means of ten shoots ± s.e.

TABLE 1. *Relationship between shoot growth and water content at full hydration (WC_h, %) after comparison of the WC_h values of the 'growth' and 'no growth' groups in the long and short branches of* Lepidium subulatum *and* Linum suffruticosum *by Mann–Whitney U-test or Student's t-test*

Species and Branches	WC_h (%), 'growth'	WC_h (%), 'no growth'	U (or t)	P
Lepidium subulatum				
Long branches	54·0 ± 0·7	50·3 ± 2·0	21·0	0·126
Short branches	73·0 ± 0·3	60·6 ± 6·4	8·0	0·038
Linum suffruticosum				
Long branches	65·7 ± 2·0	60·5 ± 0·4	10·0	0·008
Short branches	68·6 ± 0·9	65·3 ± 0·8	(−2·5)	0·022

Values of WC_h are means ± s.e. U, Mann–Whitney U-statistic; t, Student's t-statistic (*Linum* short branches only).

DISCUSSION

Bud morphology

Linum and *Lepidium* bore naked buds with no specialized protective organs. In this type of bud, protection is given by the surrounding leaves of the shoot, which are arranged compactly around the meristem (Nitta and Ohsawa, 1998). Naked buds have been reported in other species of Mediterranean sub-shrubs, and also in several tree species from other climates (Hallé *et al.*, 1978; Nitta and Ohsawa, 1998, 1999). However, within woody Mediterranean species, trees and shrubs normally bear cataphyllary or hipsophyllary buds (Hoffmann, 1972; Ginocchio and Montenegro, 1996), while naked buds are more frequent

growth compared with those months with negative or no growth (Table 1). This positive relationship between shoot growth and WC_h was also significant in the short branches of *Lepidium*, but not in its long branches (Table 1).

in sub-shrubs. This observation opens up questions as to the adaptive significance of naked buds and their phylogenetic origin. Some authors have interpreted these buds as less specialized structures than scaled ones (Puntieri *et al.*, 2002*a*). Cataphylls and scales might have evolved from foliar structures (Goffinet and Larson, 1981) to increase protection under harsh environmental conditions (Nitta and Ohsawa, 1998; Puntieri *et al.*, 2002*a*). However, this hypothesis does not explain the presence of naked buds in Mediterranean sub-shrubs, which are frequently exposed to harder environmental conditions than Mediterranean trees or shrubs (Shmida and Burgess, 1988; Orshan, 1989).

Shoot growth and organogenesis

The growth activity of *Linum* and *Lepidium* shoots varied widely throughout the year, following the strong seasonality imposed by the Mediterranean climate on gypsum substrates. Most vegetative and reproductive activities of these two species occurred in the favourable periods of spring and autumn, hence avoiding summer drought and winter cold. These results contrast with the observations of maintenance of short-branch growth for other sub-shrubs of semi-arid environments (Orshan, 1972). These discrepancies could be attributed to the different methodologies applied to assess shoot growth in the two studies. The visual estimations of shoot growth used by Orshan might be inappropriate to distinguish low autumn growth rates from the inactivity of winter and summer.

Branch growth is the result of two processes: the differentiation of organ primordia from meristems, i.e. organogenesis, and the extension of these primordia into fully developed organs (Champagnat *et al.*, 1986; Puntieri *et al.*, 2002*b*). In *Lepidium* and *Linum*, the timing of these two processes was uncoupled. Hence, most leaf primordia that were differentiated in autumn and winter expanded next spring, giving rise to the leaves of long branches. This phenomenon of leaf primordia accumulation is similar to the preformation of leaves reported in the winter buds of temperate woody species (Kozlowski and Clausen, 1966; Marks, 1975; Inouye, 1986). In these plants, organogenesis and expansion of leaf primordia are separated by a period that normally coincides with winter dormancy (Puntieri *et al.*, 2002*b*). During dormancy, the activity of the meristem is normally arrested (Owens and Molder, 1973; Gregory, 1980; Jordy, 2004). However, in *Lepidium* and *Linum* organogenesis was protracted throughout the year, including autumn and winter, although the rate of primordia differentiation might vary throughout the year. Therefore, the period of time between organogenesis and extension of leaf primordia varied widely through the year. The leaves differentiated in autumn remained in an immature state for longer than those formed in early spring or summer.

Water content at full hydration and its relation to shoot growth processes

The capacity of *Lepidium* and *Linum* shoots to become fully hydrated was closely related to shoot expansion. This relationship could be attributed to the necessity of expanding cells to maintain an adequate turgor pressure during the growth process (Bradford and Hsiao, 1982). The mechanisms used by cells to maintain this pressure, such as increasing osmotic potential, will also lead to an increased capacity of the organ to reach high WC_h values. In their study on the WC_h of two co-occurring chaparral species, Davis and Mooney (1986) ascribed the high spring WC_h values to increased organogenetical activity of shoots. However, in our study maximum values of N_p preceded maximum values of both RGR and WC_h, and hence the values of WC_h paralleled better the RGR values than those of N_p. Therefore, in the shoots of *Lepidium* and *Linum*, WC_h may be more closely related to expansion growth than to organogenetical processes.

Shoot growth differences between Lepidium *and* Linum

In spite of the general similarities of *Lepidium* and *Linum*, these two species presented differences in the performance of their phenological and growth activities, which might explain their distinct ecological strategies. *Lepidium* finished flowering, fruiting and shoot expansion earlier than *Linum*, hence avoiding summer drought more efficiently. Furthermore, *Lepidium* dried most of its transpiring body during summer, while *Linum* maintained many of its green leaves alive. The capacity of Mediterranean sub-shrubs to dry out part of their transpiring biomass with the onset of summer has been interpreted as a strategy to reduce water loss during summer drought (Orshan and Zand, 1962; Orshan, 1963, 1972). However, this strategy might entail several drawbacks, like reduced nutrient retention efficiency (Reich and Borchet, 1982), or reduced net growth at the end of the growing season. Therefore, with the onset of autumn, *Lepidium* must rebuild more green biomass than *Linum*. This is accomplished by increasing relative growth rate or by extending shoot growth. Given the lower RGR of *Lepidium* shoots, this shrub can attain a similar shoot development to *Linum* by the end of autumn only by extending the period of autumn shoot growth. This might explain why *Lepidium* resumes autumn growth of its short branches in September, while this growth is resumed in October in *Linum*. In addition, *Lepidium* seems also to avoid potential freezing damage to newly initiated tissues more efficiently than *Linum*. While the short-branch growth of the former is arrested during winter (from January to March), the latter is prone to isolated pulses, as recorded in February 2004. Therefore, *Lepidium* seems to follow a stress-avoiding strategy, while *Linum* is more stress-tolerant (*sensu* Grime, 2001). This stress-tolerant strategy might explain the presence of *Linum* in the stressful gypsum outcrops.

Concluding remarks

The seasonality of the Mediterranean climate in gypsum outcrops leads to a similar phenology, bud morphology and similar patterns of WC_h and bud and shoot growth in *Lepidium subulatum* and *Linum suffruticosum*. These two species bear naked buds and undergo shoot expansion during the favourable periods of spring and autumn. In both species, the reduced duration of the suitable period

for long-branch expansion leads to an accumulation of leaf primordia in late winter. Organogenesis and expansion are uncoupled in both species, and the lapse of time between these two processes varies throughout the year, being greatest for leaf primordia initiated in autumn. Finally, long-branch expansion coincides with maximum WC_h values in both species. This observation supports the hypothesis of a strong relationship between high WC_h and shoot expansion.

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