

Relative Growth Rate and Biomass Allocation in Ten Woody Species with Different Leaf Longevity Using Phylogenetic Independent Contrasts (PICs)

J. Ruiz-Robledo^{1,2} and R. Villar¹

¹ Area de Ecología, Campus de Rabanales, Universidad de Córdoba, 14071, Spain

² Facultad de Ciencias Ambientales y Agrícolas, Universidad Rafael Landívar, Campus Central, Vista Hermosa III, zona 16, Guatemala City, Guatemala

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Abstract: In this study, we compare the relative growth rate (RGR) and biomass allocation of 10 woody species (5 deciduous and 5 evergreen) from the Mediterranean region using phylogenetic independent contrasts (PICs) to test if these two functional groups differ in these traits. In general, the results were similar when using PICs or without taking into account phylogenetic relations. Deciduous species had a higher RGR than evergreen species, due to the higher net assimilation rate (NAR). Deciduous species had a higher specific leaf area (SLA) but a lower leaf mass ratio (LMR), resulting in a similar LAR for deciduous and evergreen species ($LAR = SLA \times LMR$). In some cases, the use of PICs revealed patterns that would not have appeared if phylogeny had been overlooked. For example, there was no significant correlation between RGR and final dry mass (after 4 months of growth) but PICs revealed that there was a positive relation between these two variables in all deciduous-evergreen pairs. In general, RGR decreased with time and this temporal variation was due primarily to NAR variations ($r = 0.79$, $p < 0.01$), and also to variations in LAR ($r = 0.69$, $p < 0.05$). Considering the phylogeny, the only variable constantly different for all deciduous-evergreen pairs was SLA. This result, and the fact that SLA was the best correlated variable with RGR ($r = 0.81$, $p < 0.01$), reinforce the value of SLA as a variable closely associated to growth and to the functional groups (deciduous vs. evergreen).

Key words: Leaf area ratio, net assimilation rate, specific leaf area, deciduous, evergreen.

Introduction

Woody species are normally classified as deciduous or evergreen as a function of their leaf longevity. These two functional groups have been studied extensively to understand different characteristics that might explain their distribution in nature. Various studies have concluded that, generally, deciduous species are characterized by higher photosynthetic rates, specific leaf area (SLA), and relative growth rate (RGR) than evergreen species (Chabot, 1982; Sobrado, 1991; Reich et al., 1992; Villar

et al., 1995; Cornelissen et al., 1996; Reich et al., 1997; Cornelissen et al., 1998; Reich, 1998; Eamus et al., 1999; Antúnez et al., 2001; Villar et al., 2004; Wright et al., 2004). The higher RGR of deciduous species could be advantageous in habitats with abundant resources and this could partly explain the differences in distribution of these two functional groups (Aerts, 1995; Cornelissen et al., 1996; Nielsen et al., 1996; Lusk et al., 1997; Montoya and López-Arias, 1997; Cornelissen et al., 1998; Antúnez et al., 2001).

In general, the majority of comparative studies regarding RGR have tried to elucidate the causes of differences in RGR between species. A classic technique has been the factorization of RGR into two components: LAR (leaf area ratio) and NAR (net assimilation ratio) ($RGR = NAR \times LAR$). At the same time, LAR is partitioned in SLA (specific leaf area) and LMR (leaf mass ratio), where $LAR = SLA \times LMR$. Most studies have concluded that the factor that best explains the differences in RGR is LAR, with NAR not being as important (Garnier, 1992; Lambers and Poorter, 1992; Reich et al., 1992; Huante et al., 1995; Cornelissen et al., 1996, 1998; Poorter and Van der Werf, 1998; Eamus et al., 1999; Antúnez et al., 2001; Hoffmann and Franco, 2003). Besides, these studies have shown that the most important factor within LAR is SLA, thus a morphological variable of the leaf (SLA) is strong and positively correlated with RGR.

However, Shipley (2002) in a recent experiment with herbaceous and woody plants in high radiation conditions, found that RGR was positively correlated with NAR and not with LAR. Ryser and Wahl (2001) also found, in a study with 24 herbaceous species grown in full daylight, that NAR contributed more than LAR or SLA to interspecific variation in RGR. Shipley (2002) proposed that the importance of SLA and NAR on RGR depends of the levels of radiation, so that with high levels of radiation the importance of NAR on RGR will be higher. In fact, Villar et al. (2005) found, in a field study with high levels of radiation, that for short growth periods, the variation in RGR in 22 congeneric herbaceous species was due to NAR and that the importance of NAR increased with increasing the radiation.

The importance of having a higher RGR is that it can determine a higher biomass (Van Andel and Biere, 1989) and this can make the plant more competitive and provide advantages in environments rich in resources. However, the plant mass is determined not only by RGR, but also by the initial mass. If one

considers the first stages of plant development, the initial mass is usually correlated positively with seed mass (Choe et al., 1988; Villar et al., 1998). Therefore, at the initial stages, the plant mass could be determined by RGR as well as the seed mass or both (Villar et al., 1998). Different studies have found that these two variables (RGR and seed mass) are not independent and that, in general, they are negatively correlated. Thus, in general, there is a negative correlation between seed mass and RGR (Marañón and Grubb, 1993; Huante et al., 1995; Swanborough and Westoby, 1996), although some studies have shown that this relationship is not clear (Shipley and Peters, 1990; Paz and Martínez-Ramos, 2003).

The majority of studies have determined RGR in seedlings over a relatively short period of time (two to three weeks), with the objective being to obtain the maximum RGR. Few studies have considered RGR over longer periods of time (several months) or what the variation in RGR over time can be. In this study we have estimated the RGR of 10 woody species over a growth period of four months.

Another unique aspect of this study is that we have compared deciduous and evergreen species using phylogenetic independent contrasts (PICs) (Harvey and Pagel, 1991; Saverimuttu and Westoby, 1996). Every PIC is a different branch of the phylogenetic tree and so the evolutionary divergence in one PIC is independent of another PIC. Including the phylogeny has the advantage that we can interpret the results from an evolutionary point of view and also correct possible biases when phylogeny is not taken into account (Harvey and Pagel, 1991). For that, in this study, we considered five pairs of species that belong to the same genus or family and that at the same time differ in leaf longevity (deciduous vs. evergreen). Therefore, every pair (deciduous-evergreen) is considered as a phylogenetic independent contrast (PIC) and we can use them to compare different characters: RGR, SLA, biomass allocation, and dry matter proportion (DM) between the deciduous and evergreen species. These contrasts will tell us which characteristics are closely related to the deciduous or evergreen character and will allow us to discuss the possible causes of their different distribution in nature.

The objectives of this study are: 1) to recognize differences in RGR between deciduous and evergreen species and to determine the possible causes of such differences, 2) to determine the importance of including phylogenetic relations in comparative studies between species, 3) to determine the relationship of seed mass to different growth variables, 4) to identify the causes of temporal variations in RGR, and 5) to elucidate any differences between deciduous-evergreen species that will help us to understand their implications for plant growth, resistance to stress, and the ecology of the two functional groups.

Materials and Methods

Growth conditions and harvesting

Ten woody species were selected from the Mediterranean region to form 5 pairs of deciduous-evergreen species within the same genus or family (Table 1). These ten species were all different from those used in a preliminary paper (Antúnez et al., 2001). The nomenclature of the species and the ecological

characteristics (preferred soil type, cold and drought resistance, and community type) were taken from Galán-Cela et al. (2000). The growth forms of the ten species were either shrubs or trees, except for *Lonicera implexa* which is a climber (Table 1). The species soil preference was from acid to basic, but six species do not show any soil preference (Table 1). Most of the deciduous species studied grow in habitats with high water availability (high rainfall or riparian habitats) and are generally tolerant to cold winters. In contrast, most of the evergreens are xerophytes, being able to tolerate summer drought, but sensitive to frost (Table 1).

For each species, the fresh mass of a group of 30 seeds was taken. Prior to sowing, the seeds were treated to enhance germination following Catalán-Bachiller (1991). Seeds from *Lonicera* spp., *F. ornus*, *F. alnus*, *P. angustifolia*, and *R. alaternus* were soaked in cold water for 3 days, changing the water every day. They were dried with filter paper and then planted. The seeds of *Q. faginea*, *Q. ilex* ssp. *ilex*, *P. dulcis*, and *P. lusitanica* ssp. *lusitanica* were washed with water and planted.

Seeds were planted in January–February 1998, in plastic trays with a sand–peat mix 3 : 1, except for *Q. faginea* and *Q. ilex* ssp. *ilex* where acorns were planted in the same medium but into forestry trays. All trays were placed in a greenhouse and watered once a week. Between March and April 1998, the germinated seedlings were transplanted into individual black polyethylene bags (12.7 × 35 cm diameter and height; 3.5 l capacity) with the same substrate. Seedlings of homogeneous size were selected to obtain a better estimate of RGR (Poorter and Garnier, 1996). Seedlings of different species were randomly placed in the greenhouse to avoid that any possible environmental heterogeneity might have affected the results.

Each plant received a 13.6 g dosage of a slow release fertilizer (Basacote 6 M, BASF with 14% N; 10% P₂O₅; 13% K₂O; 2% MgO; 7.5% SO₃, and other micronutrients) every three months. Daily automatic irrigation was used. The daily mean radiation throughout the experiment was 31.6 ± 11.7 mol m⁻² day⁻¹, but there was a decrease from the first (0–69 days, see below) to the second growth period (69–124 days) (40.0 ± 8.96 mol m⁻² day⁻¹ and 21.4 ± 7.2 mol m⁻² day⁻¹, respectively).

For each species three harvests were done (harvest 1: day 0; harvest 2: day 69 ± 3; harvest 3: day 124 ± 7). The first harvest (day 0) was done 3 months after transplanting the plants to individual bags. At each harvest, 10 ± 3 seedlings, of each species, were randomly chosen. The mean number of days necessary to harvest all the plants for the two species of the same pair was 2 ± 0.7 days.

Roots were carefully separated from the media, washed with water and dried with filter paper. After this, roots, stems, and leaves were separated and weighed. Petioles were separated from the leaf and the weight was included in the stem fraction (as in Antúnez et al., 2001) since they have similar functions in the plant. Leaf area was calculated using the image analysis program *Imago* (Universidad de Córdoba). All plant parts were dried at 70°C in an oven for a minimum of 48 h and then weighed to determine their dry weight.

Table 1 Families and species utilized in the study, code for the phylogenetic independent contrasts (PICs), code for the species and some ecological characteristics of the species: functional group (D: deciduous; E: evergreen), growth form, soil type preference, water and temperature requirements, and distribution on the Iberian Peninsula (plant community in which it is normally found). Nomenclature and ecological characteristics are from Galán-Cela et al. (2000)

Family	PIC code	Species code	Species	Functional group	Growth form	Soil type preference	Water requirements	Temperature requirements	Distribution in the Iberian peninsula
Caprifoliaceae	1	A	<i>Lonicera xylosteum</i> L.	D	shrub, up to 1–2 m	calcareous	associated with humid and shady habitats	tolerant to winter cold	mixed deciduous forests
		a	<i>Lonicera implexa</i> Aiton	E	climbing bush, up to 2 m	no preference	associated with humid and shady habitats	less tolerant to winter cold than <i>L. xylosteum</i>	dense shrub and hedge communities and protected shady slopes of evergreen forests
Fagaceae	2	B	<i>Quercus faginea</i> Lam.	D	tree, up to 20 m	calcareous	less tolerant to the drought than <i>Q. ilex</i> .	tolerant to winter cold	mixed deciduous forests
		b	<i>Quercus ilex</i> L. ssp. <i>ilex</i>	E	tree, up to 25 m	no preference	tolerant to drought and it does not resist water-logging	little tolerance to winter cold	evergreen forests of eastern littoral region
Oleaceae	3	C	<i>Fraxinus ornus</i> L.	D	tree, up to 10 m	calcareous or siliceous	very demanding for water	tolerant to winter cold	mixed deciduous forests and rocky habitats in mountains
		c	<i>Phillyrea angustifolia</i> L.	E	shrub, up to 3 m	no preference	tolerant to drought, a xerophytic species	little tolerance to winter cold	evergreen forests of <i>Quercus ilex</i> and <i>Q. suber</i>
Rhamnaceae	4	D	<i>Frangula alnus</i> Miller	D	shrub, up to 5 m	not in calcareous	very demanding for water	slightly tolerant to winter cold	riparian forest or deciduous forests of <i>Quercus pyrenaica</i> , <i>Q. robur</i> , and <i>Q. petraea</i>
		d	<i>Rhamnus alaternus</i> L.	E	shrub, up to 5 m	no preference	tolerant to drought, a xerophytic species	little tolerance to winter cold	evergreen forests of <i>Quercus ilex</i> , <i>Q. suber</i> , <i>Pinus halepensis</i> , and <i>P. nigra</i>
Rosaceae	5	E	<i>Prunus dulcis</i> (Miller) D. A. Webb.	D	shrub or small tree, up to 8 m	soil–deep, well-drained, loamy soils with pH 6–7	growth better in humid soils but slightly tolerant to drought	tolerant to winter cold	rocky soils, hedges in headlands, crop lands, and orchards; native of Asia and NE Africa; cultivated in the Mediterranean, becoming naturalized
		e	<i>Prunus lusitanica</i> L. ssp. <i>lusitanica</i>	E	shrub or small tree, up to 15 m	siliceous	associated with humid habitats	very sensitive to frost	associated with evergreen or deciduous forest (<i>Quercus pyrenaica</i> , <i>Frangula alnus</i>) or riparian forests (<i>Salix</i> spp. and <i>Alnus glutinosa</i>)

Calculations and statistical analysis

RGR was calculated by linear regression of \log_e (dry weight) with respect to time (Hunt, 1982). Mean RGR was calculated using the data of the three harvests (1, 2, and 3), whereas for RGR of the first and second growth period (RGR₁ and RGR₂) we used the data of harvests 1 and 2, and data of harvests 2 and 3, respectively. LAR, SLA, and the proportions of leaves, stems, and roots were calculated using the formulas of Hunt (1982). NAR was calculated as:

$$([W_2 - \bar{W}_1][\log_e LA_2 - \log_e \bar{LA}_1]) / ([LA_2 - \bar{LA}_1][t_2 - t_1])$$

where W_2 and LA_2 are the plant dry mass and the leaf area at time 2 (t_2) and W_1 and LA_1 are the mean plant dry mass and the mean leaf area at time 1 (t_1). Dry matter content (DM) was calculated as the ratio of dry weight/fresh weight.

The comparisons between deciduous and evergreen species and the correlations between variables were done using the combined data for the two periods because the results were nearly the same for each period independently. Differences in RGR between deciduous and evergreen species were analyzed by comparing the linear regression slopes using analysis of covariance. The comparison between deciduous and evergreen species for other growth variables was done using analysis of variance in two different ways: 1) all species considered as a group, i.e., the 10 species (5 deciduous and 5 evergreen) comparing both functional groups using the family as a random factor, and 2) comparing each pair of deciduous and evergreen species by an analysis of variance. Because size differences may influence allocation patterns, the ANOVAs to compare deciduous and evergreen species were done using log plant dry mass as a covariate for the following variables: SLA, biomass allocation (LMR, SMR, RMR), and DM.

Temporal changes in RGR, LAR, NAR were estimated for each species using the values for period 1 (harvest 1 and 2; RGR₁, LAR₁, NAR₁) and for period 2 (harvest 2 and 3; RGR₂, LAR₂, NAR₂). To identify the causes of the temporal changes in RGR between period 2 and 1, the difference between RGR (RGR₂ - RGR₁) was correlated with the differences in LAR (LAR₂ - LAR₁) and NAR (NAR₂ - NAR₁) for the same periods.

Percentage variables (biomass allocation and dry matter content) were transformed to arcsine before ANOVA. When normal distribution and variance homogeneity were not met, a nonparametric test was used (U-Mann-Whitney). Statistical analyses were done using Statistica (StatSoft, 1996).

Results

Differences between deciduous and evergreen species

No significant differences were found between seed mass for deciduous and evergreen species ($p = 0.9$, Table 2). Deciduous species had a higher final dry mass (dry mass of harvest 3), RGR, and NAR than evergreen species ($p < 0.05$, Table 2). LAR of deciduous species was slightly higher than those of evergreen species, but not statistically significant. The higher SLA of deciduous species ($p < 0.01$, Table 2) was in part cancelled by a lower LMR ($p < 0.05$, Table 2), as LAR is equal to SLA \times LMR. There was no difference in stem (SMR) and root allocation

(RMR), and dry matter content (DM) between deciduous and evergreen species ($p > 0.25$, Table 2).

Differences between deciduous and evergreen species in phylogenetic independent contrasts (PICs)

Final dry mass (dry mass of harvest 3) was significantly higher for deciduous species within the five different pairs (Fig. 1A). Deciduous species presented a significantly higher RGR than evergreen species in four pairs of species (Fig. 1B). In four of the five pairs, deciduous species presented a higher NAR (Fig. 1C). In three pairs, LAR was higher in deciduous species, whereas in the *Q. faginea* and *Q. ilex* ssp. *ilex* pair the evergreen species presented a slightly higher LAR (Fig. 1D). Deciduous species presented a significantly higher SLA in all five pairs (Fig. 1E).

Regarding biomass allocation, four of the five pairs studied had a lower LMR for deciduous species (Fig. 1F). Three of the five pairs had a similar SMR for deciduous and evergreen species (Fig. 1G). For RMR, in three pairs deciduous species had significantly higher values and in two there was no difference (Fig. 1H). For dry matter content, three pairs of deciduous species had a lower value (Table 2).

Correlations between different variables

Seed mass was not correlated with RGR, but was negatively correlated with LAR ($p < 0.05$) and with SLA ($p = 0.06$) and positively correlated with RMR ($p < 0.05$) and DM ($p < 0.001$) (data not shown). The final dry mass and RGR were not correlated positively ($p > 0.1$, Fig. 2A). Nonetheless, for each pair of deciduous-evergreen species a higher final dry mass was associated with a higher RGR (Fig. 2A).

RGR was nearly positively correlated with LAR ($p = 0.06$, Fig. 2B). If we take into account phylogeny, we observe that for pairs A-a, C-c, and D-d, in which there are significant differences in RGR and LAR, an increase in LAR determines an increase in RGR (Fig. 2B). In pairs B-b and E-e, the increase in RGR for deciduous was not determined by higher LAR.

Furthermore, RGR was nearly positively correlated with NAR ($p = 0.08$, Fig. 2C). Taking into account phylogeny in all of the pairs except C-c, the deciduous species show a higher NAR and these differences in NAR are related to a greater RGR. The importance of NAR in determining RGR is clear for pairs B-b and E-e, where the higher RGR was not related to higher LAR, but to the higher NAR of deciduous species (Figs. 2B, C).

The variable that was the most highly correlated with RGR was SLA ($p < 0.01$, Fig. 2D). This tendency was seen in all of the pairs of species studied, in which deciduous species are characterized by having higher SLA and RGR.

There was no correlation between RGR and LMR (Fig. 2E). However, when phylogeny was taken into account, most of the pairs of species studied (4 out of 5) show higher LMR associated with lower RGR (Fig. 2E). RGR was not correlated with either SMR or RMR and there was no trend for each pair (Figs. 2F, G). Dry matter content (DM) was not correlated with RGR ($p > 0.1$, Fig. 2H). In three of five pairs (pairs A-a, B-b, and D-d) species with a higher DM have a lower RGR, but for the other two pairs (pairs C-c and E-e) it was the opposite.

Table 2 Mean values (\pm S.D.) of some variables for each pair of species (deciduous-evergreen) and for both functional groups, considering all species. Between the mean values of each pair the level of significance of differences between mean values of deciduous and evergreen species is shown. Final dry mass (at final harvest); relative growth rate (RGR); net assimilation rate (NAR); leaf area ratio (LAR); specific leaf area (SLA); leaf mass ratio, considering only the limbs (LMR); stems and petiole mass ratio (SMR); root mass ratio (RMR); and plant dry matter content (DM). Asterisks show the level of significance of differences between mean values of deciduous and evergreen species. n.s.: not significant; (-): $0.1 > p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. RGR was calculated as the slope of \ln dry mass against time (\pm S.D.) for each functional group. Under the species column the functional group: (D) deciduous, (E) evergreen is shown

Species	Seed fresh mass (g)	Final dry mass (g)	RGR ($\text{mg g}^{-1} \text{ day}^{-1}$)	NAR ($\text{g m}^{-2} \text{ day}^{-1}$)	LAR ($\text{m}^2 \text{ kg}^{-1}$)	SLA ($\text{m}^2 \text{ kg}^{-1}$)	LMR	SMR	RMR	DM (%)
<i>Lonicera xylosteum</i> L. (D)	0.003	8.62 \pm 6.59	35.34 \pm 2.72	2.24 \pm 1.79	14.44 \pm 4.44	36.65 \pm 10.33	0.41 \pm 0.09	0.34 \pm 0.04	0.24 \pm 0.08	26.27 \pm 2.50
		**	***	***	*	***	***	n.s.	**	**
<i>Lonicera implexa</i> Aiton (E)	0.013	0.39 \pm 0.21	8.66 \pm 3.34	0.32 \pm 1.51	12.28 \pm 3.36	25.01 \pm 6.38	0.50 \pm 0.10	0.29 \pm 0.09	0.19 \pm 0.08	28.37 \pm 4.99
<i>Quercus faginea</i> Lam. (D)	2.163	8.59 \pm 2.94	12.8 \pm 1.11	3.41 \pm 2.09	3.62 \pm 0.90	12.48 \pm 1.21	0.29 \pm 0.06	0.20 \pm 0.05	0.51 \pm 0.08	38.75 \pm 3.34
		***	**	***	~	**	***	***	***	***
<i>Quercus ilex</i> L. ssp. <i>ilex</i> (E)	2.938	4.37 \pm 0.85	8.36 \pm 0.76	1.89 \pm 1.13	3.95 \pm 0.70	9.40 \pm 1.64	0.42 \pm 0.05	0.25 \pm 0.06	0.32 \pm 0.08	42.10 \pm 3.50
<i>Fraxinus ornus</i> L. (D)	0.023	6.54 \pm 3.79	22.76 \pm 1.94	2.27 \pm 1.63	9.59 \pm 3.38	22.25 \pm 4.81	0.42 \pm 0.09	0.30 \pm 0.05	0.23 \pm 0.07	32.78 \pm 4.28
		**	n.s.	n.s.	**	**	n.s.	n.s.	n.s.	n.s.
<i>Phillyrea angustifolia</i> L. (E)	0.016	0.62 \pm 0.36	17.09 \pm 2.00	2.49 \pm 1.34	6.46 \pm 1.77	14.87 \pm 2.83	0.43 \pm 0.06	0.25 \pm 0.05	0.30 \pm 0.08	28.79 \pm 3.48
<i>Frangula alnus</i> Miller (D)	0.065	12.39 \pm 5.65	44.77 \pm 3.69	3.27 \pm 2.60	10.70 \pm 4.82	32.88 \pm 6.58	0.32 \pm 0.11	0.34 \pm 0.09	0.31 \pm 0.09	28.02 \pm 5.91
		***	***	*	**	***	***	n.s.	**	***
<i>Rhamnus alaternus</i> L. (E)	0.010	3.64 \pm 1.67	20.95 \pm 1.48	2.32 \pm 1.06	7.85 \pm 2.77	18.17 \pm 4.51	0.43 \pm 0.09	0.33 \pm 0.07	0.23 \pm 0.14	31.07 \pm 2.54
<i>Prunus dulcis</i> (Miller) D. A. Webb. (D)	1.085	16.04 \pm 5.82	18.11 \pm 2.54	2.87 \pm 2.95	5.31 \pm 3.19	24.61 \pm 5.74	0.21 \pm 0.11	0.47 \pm 0.09	0.30 \pm 0.10	41.66 \pm 5.77
		***	***	***	n.s.	***	***	***	n.s.	***
<i>Prunus lusitanica</i> L. ssp. <i>lusitanica</i> (E)	0.490	4.93 \pm 1.86	4.14 \pm 1.21	0.55 \pm 1.45	4.95 \pm 1.48	11.75 \pm 1.92	0.41 \pm 0.07	0.25 \pm 0.05	0.31 \pm 0.05	31.41 \pm 4.85
Deciduous	0.6 \pm 0.9	9.7 \pm 5.7	26.6 \pm 1.6	2.8 \pm 2.2	9.0 \pm 5.4	25.5 \pm 11.1	0.34 \pm 0.10	0.31 \pm 0.10	0.32 \pm 0.14	32.9 \pm 7.1
	n.s.	*	***	*	n.s.	**	*	n.s.	n.s.	n.s.
Evergreen	0.7 \pm 1.2	3.4 \pm 2.2	11.9 \pm 2.0	1.5 \pm 1.5	6.6 \pm 3.4	14.9 \pm 6.2	0.43 \pm 0.08	0.27 \pm 0.07	0.27 \pm 0.10	33.3 \pm 6.5

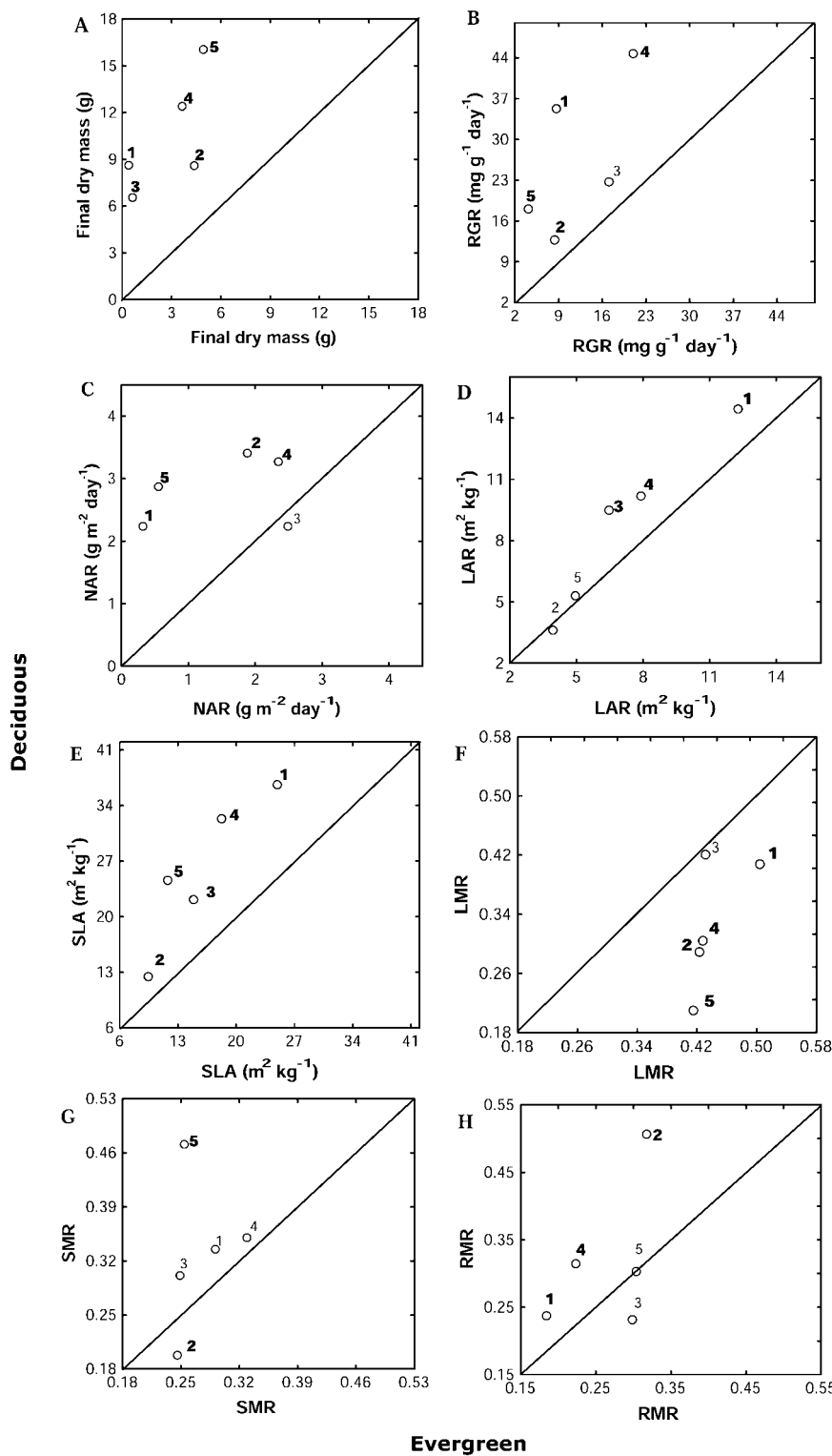


Fig. 1 Comparison between the mean values of the variables in deciduous and evergreen species of each phylogenetic independent contrast (PIC) for dry mass (at final harvest) (A), relative growth rate (RGR) (B), net assimilation rate (NAR) (C), leaf area ratio (LAR) (D), specific leaf area (SLA) (E), leaf mass ratio (LMR) (F), stem mass ratio (SMR) (G), and root mass ratio (RMR) (H). For PIC codes see Table 1. The straight line represents the 1:1 ratio between deciduous and evergreen species. Numbers in bold and larger font represent significant differences between the values for deciduous and evergreen species within each PIC.

Causes of temporal variations in RGR

Mean values of RGR for period 1 (day 0–69) for all species was $26.98 \pm 19.41 \text{ mg g}^{-1} \text{ day}^{-1}$; whereas for period 2 (day 69–124) the mean values were reduced to $8.04 \pm 9.23 \text{ mg g}^{-1} \text{ day}^{-1}$. This represents a reduction of more than a third.

The differences in relative growth rate ($\text{RGR}_2 - \text{RGR}_1$) between period 2 and 1 were mainly because of differences in NAR ($\text{NAR}_2 - \text{NAR}_1$, $r = 0.79$, $p < 0.01$, Fig. 3A) although differences in LAR ($\text{LAR}_2 - \text{LAR}_1$) were also important ($r = 0.69$, $p < 0.05$, Fig. 3B). When phylogeny was considered, it was observed that for the majority of PICs, the differences in RGR are also due to NAR and LAR (Figs. 3A, B).

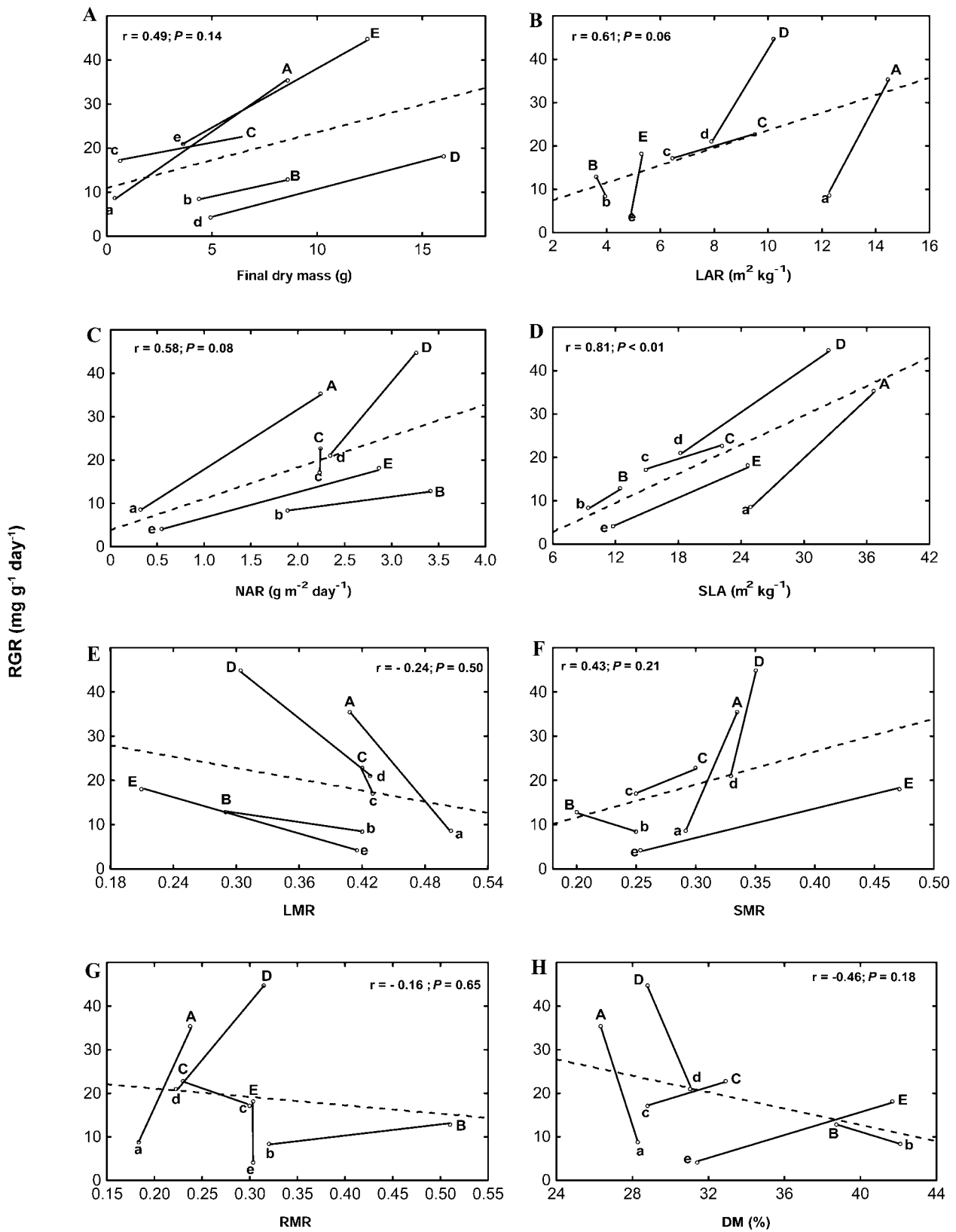


Fig. 2 Relationship between RGR and dry mass at final harvest (A), leaf area ratio (LAR) (B), net assimilation rate (NAR) (C), specific leaf area (SLA) (D), leaf mass ratio (LMR) (E), stem mass ratio (SMR) (F), root mass ratio (RMR) (G), and plant dry matter content (DM) (H). Up-

per- and lowercase letters represent the deciduous and evergreen species, respectively, of each PIC, linked by a solid line. The dashed line represents the regression line calculated for all data (r and p are shown). For species codes see Table 1.

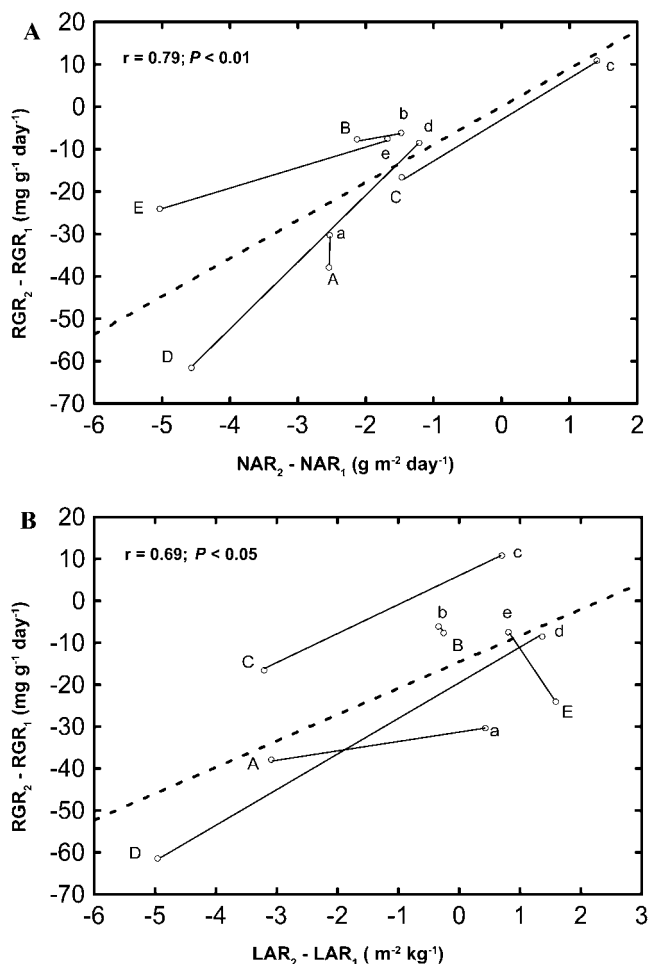


Fig. 3 Relationship between the temporal variation in RGR ($RGR_2 - RGR_1$) and the temporal variation in NAR ($NAR_2 - NAR_1$) (A) and LAR ($LAR_2 - LAR_1$) (B). Upper- and lowercase letters represent the deciduous and evergreen species, respectively, of each PIC, linked by a solid line. The dashed line represents the regression line calculated for all data (r and p are shown). For species codes see Table 1.

Discussion

RGR, seed, and final mass and use of PICs

From the results, one could conclude that a higher RGR is not important for final mass (mass in harvest 3) (Fig. 2A). However, considering each PIC, we observe that in everyone of them a positive relationship between RGR and final mass exists. Therefore, we can conclude that the plant dry mass in the first stages of development is mainly determined by RGR, but differences in seed size are also important. Hence, including phylogeny can be important to reveal patterns that one would not otherwise observe. A similar result occurs in the relation between RGR and LMR in which there is no significant correlation but for each PIC there is a negative relationship (Fig. 2E). Different studies (see Freckleton, 2000) have shown the usefulness of phylogenetic relationships in ecological studies.

We did not find the general negative correlation between RGR and seed mass found in other studies (Marañón and Grubb, 1993; Swanborough and Westoby, 1996; Huante et al., 1995;

Castro-Díez et al., 2003), although this relationship has not been clear in some studies (Shipley and Peters, 1990; Paz and Martínez-Ramos, 2003). However, we found negative correlations between seed mass, LAR, and SLA, which are variables closely associated with RGR, as also found by Hoffmann and Franco (2003). One explanation for these relationships is that species with smaller seeds have less resources and need a greater leaf area to ensure rapid growth. On the other hand, we found seed mass positively correlated with root allocation, which may have implications for survival. Lloret et al. (1999) suggested that species with bigger seeds dedicate more biomass to roots to guarantee resource acquisition (nutrients and water) to favour survival in the field, where water and nutrients are limited. Also, Paz and Martínez-Ramos (2003) in a study with eight woody species of the same genus, found that species with bigger seeds had a higher probability of survival.

Relative growth rate and specific leaf area

What are the causes of RGR differences between woody species? In this study we found that NAR and LAR were nearly positively correlated with RGR ($0.10 > p > 0.05$). Although most studies on growth in woody species have found that the main factor is LAR (Cornelissen et al., 1996, 1998; Reich et al., 1997, 1998; Antúnez et al., 2001), some have suggested that NAR is the main determinant (Shipley, 2002; Reich et al., 2003). One reason that might explain why NAR is as important as LAR is the high daily mean radiation throughout the experiment ($31.6 \pm 11.7 \text{ mol m}^{-2} \text{ day}^{-1}$; mean values of maximum photosynthetic active radiation: $928 \pm 265 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Shipley (2002) suggests that, in studies where daily mean radiation is higher than $20 \text{ mol m}^{-2} \text{ day}^{-1}$, the RGR differences between species are generally caused by NAR.

Differences in LAR between species that could be due to SLA and LMR ($LAR = SLA \times LMR$) are determined primarily by SLA (80%), with no significant correlation with LMR. For that, differences in RGR are determined mainly by differences in SLA. Many authors have found the same results, both in herbaceous and woody species (Poorter and Remkes, 1990; Garnier, 1992; Lambers and Poorter, 1992; Reich et al., 1992; Huante et al., 1995; Cornelissen et al., 1996; Poorter and Van der Werf, 1998; Wright and Westoby, 2000; Antúnez et al., 2001). Therefore, our results suggest that morphological parameters such as SLA determine differences in RGR in woody species.

In contrast, other morphological variables, such as biomass allocation to different organs, do not seem to be associated with a higher RGR. In other studies, a positive correlation between RGR and leaf allocation (Poorter and Remkes, 1990; Cornelissen et al., 1996; Wright and Westoby, 2000) or a negative correlation between RGR and root allocation has been found (Antúnez et al., 2001). In this study, we found the opposite because, by using the phylogenetic contrasts, RGR and LMR are negatively associated in all pairs. This could be due to the fact that, although LMR is lower in deciduous species, this is partially compensated by a higher SLA which, in most cases, determines a higher LAR and subsequently a higher RGR.

Causes of temporal variation in RGR

Species did not present a constant RGR during the period of this study; on the contrary, there is a generalized mean reduction. This RGR reduction over time is a common aspect of growth studies, and could be caused by a change in biomass allocation (increasing the inversion in structural tissue), SLA, or NAR (Poorter and Pothman, 1992; Hoffman and Franco, 2003). In this study, the temporal variations in RGR are more due to variations in NAR than to LAR. These reductions in NAR could also be determined by a reduction in daily mean solar radiation between periods 1 and 2 (from $40.0 \pm 8.96 \text{ mol m}^{-2} \text{ day}^{-1}$ to $21.4 \pm 7.2 \text{ mol m}^{-2} \text{ day}^{-1}$). It is interesting to note that in another study (Antúnez et al., 2001) temporal variations in RGR were also due to NAR but in this case increments in RGR were caused by increments in NAR and were associated with an increase in solar radiation.

Deciduous vs. evergreen

Clear differences have been found between deciduous and evergreen species in most of the variables studied, differences that are generally, with some exceptions, consistent within each PIC. For example, there are no differences between deciduous and evergreen species in RGR in all of the PICs (there were no differences in RGR in one of the five pairs studied, which represents 20%). Similarly, as in Antúnez et al. (2001), there were no significant differences in RGR in three of the eight pairs studied, which represents 37%.

In contrast with other studies, in this experiment the differences in RGR between deciduous and evergreen species are determined by NAR as well as LAR. If we use phylogeny, we observe that differences between RGR are due to NAR in four of the five PICs and to LAR in three of the five PICs (Figs. 2B,C), indicating a slightly greater importance of the effect of NAR on RGR.

It is interesting that the only variable associated indisputably with the deciduous-evergreen character is the specific leaf area (SLA), where significant differences exist in all the PICs studied. This tight association between SLA and deciduous-evergreen character may suggest that SLA could be of adaptive significance. Hence, species with a lower SLA (evergreens) would have an advantage in predefined habitats over species with a higher SLA (deciduous) (Cornelissen et al., 1996, 1998; Antúnez et al., 2001). In Mediterranean environments, evergreens are found in habitats with low water availability and with no frost in winter, whereas deciduous species are found in habitats with high water availability and/or with cold temperatures in winter (Table 1; Blanco et al., 1997). Evergreen species have sclerophyllous leaves, an adaptation to dry environments and/or to low nutrient availability (Loveless, 1962; Monk, 1966; Turner, 1994; Aerts, 1995; Nielsen et al., 1996; Lusk et al., 1997; Salleo and Nardini, 2000; Montserrat-Martí et al., 2004). The sclerophyllous leaves are characterized by presenting one or several of the following characteristics: thick cell walls, thick protective cuticle, hairy or waxy cuticle, and other mechanisms to diminish transpiration when water availability is low (Larcher, 1995). These characteristics of evergreen leaves determine their thickness and a low area/dry mass ratio (SLA) (Reich et al., 1992; Eamus et al., 1999; Castro-Díez et al., 2000; Villar and Merino, 2001; Wright et al., 2004).

However, adaptations to reduce water loss have the disadvantage of reducing the absorption of atmospheric CO_2 and hence photosynthesis, this would imply that leaves should persist longer to compensate for their construction costs (Villar and Merino, 2001; Wright et al., 2004).

We suggest that the genetic change of an evergreen ancestor to a deciduous species would be determined primarily by a change in SLA, with all other growth variables (RGR, LAR, NAR, etc.) being a consequence of the change in SLA. Similarly, Lambers and Poorter (1992) pointed out that the ecological consequences of having different RGRs between plant species could be due to the effect of SLA on the growth variables (RGR, LAR, and NAR). On the other hand, in many genera (for example: *Quercus*, *Pistacia*, *Rhamnus*, etc.) there are species with either deciduous or evergreen habits. This suggests that the change from the evergreen state (the old state of this character) to the deciduous state must be easy and then should involve only a few genes.

Conclusions

The variable that is more highly associated with RGR is SLA, a variable tightly associated with the deciduous or evergreen character. This highlights the importance of SLA, a variable that could provide adaptive advantages to these two functional groups in different Mediterranean habitats (evergreen species in drier, nutrient-poor, and warmer habitats; and deciduous species in wetter, nutrient-rich, and colder habitats).

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R. Villar

Area de Ecología
Campus de Rabanales
Universidad de Córdoba, 14071
Spain

E-mail: bv1vimor@uco.es

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