



Differences in the environmental control of leaf senescence of four *Quercus* species coexisting in a Mediterranean environment

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Abstract

Aims of study: Our aim is to check the effect of different environmental factors on the leaf senescence of four *Quercus* species with different leaf longevities, to help us better understand the implications of climate change on leaf demography.

Area of study: The study was carried out in two sites of the province of Salamanca (central-western Spain), both sites showing differences in their temperatures and soil water availability.

Material and Methods: Over four years (2007-2010) we monitored the number of leaves of the different cohorts labelled on five specimens of each species at both sites to elaborate life-tables and calculate mortality rates. Mortality rates were then related to several other variables measured during the same period: air temperature, soil water availability, precipitation, predawn water potentials (Ψ_{pd}) and leaf N resorption.

Main results: In the two deciduous species maximum daily temperatures and the time during which their values remain above a certain threshold (between 11 and 12°C of maximum daily temperature) are the main factors controlling the timing of leaf abscission. In the evergreen species abscission of old leaves showed no relationship with the environmental factors analyzed. By contrast, mortality rates of old leaves were related to seasonal N resorption values, with the maximum mortality of old leaves coinciding in time with the maximum withdrawal of N from shed leaves and also with the emergence of the new leaf cohort.

Research highlights: The increase in the duration of the leaves of the two deciduous species, as a result of the delayed senescence by warmer autumnal temperatures, could contribute to reducing the differences in the length of the productive leaf life with respect to the evergreen species. This could improve the competitive capacity of deciduous species as opposed to that of evergreen species, and thus alter their respective distribution patterns.

Keywords: Climate change; deciduous; evergreen; leaf abscission; temperature; water availability.

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Introduction

Leaf phenology and longevity are traits linked to a large number of essential processes for plants and hence for the biosphere in general, making them priority objects of study in research in ecology. The exact moment when different events such as unfolding and senescence occur in the life of leaves affects, for example, the photosynthetic yield of different species and, through this, their growth and survival rates, their competition relationships, and hence the characteristics of the community to which they belong (Sherry *et al.*, 2007; Willis *et al.*, 2008). Thus, phenology plays a crucial role in the carbon cycle at global scale (Chapin

et al., 2002; Piao *et al.*, 2007). The patterns of emergence, abscission and leaf duration are evidently governed by genetic factors, but they are also affected by environmental ones, to which these patterns may be highly sensitive. This has led phenology to become an integral part of studies on climate change and its effects on the vegetation (Ibáñez *et al.*, 2010; Chen & Xu, 2012; Clark *et al.*, 2014).

Most of the studies on the effect of environmental variables on phenology have focused on leaf emergence and its relationship with water availability and temperature. Water availability has shown contradictory results; while some authors have reported effects of increased precipitation on leaf emergence (Eamus &

Prior, 2001; Jolly & Running, 2004), in other cases these effects were not significant (Sherry *et al.*, 2007, Seghieri *et al.*, 2012). In contrast, temperature is revealed in most cases as one of the most important factors affecting phenology, higher temperatures contributing to advance the date of leaf emergence (Morin *et al.*, 2010; Wielgolaski *et al.*, 2011; Cook *et al.*, 2012). This has led to predict an increase in the length of the growing season due to global warming, with a consequent increase in forest productivity (Gordo & Sanz, 2005, 2009; Richardson *et al.*, 2009; Kolářová *et al.*, 2014). Less conclusive is, however, the analysis of the effects of different environmental factors on leaf senescence; therefore the possible changes of this phenological event in response to global warming are less predictable. Some authors suggest that changes in water status are the main determinants of the phenology of abscission in different woody species, with significant losses of leaf area coinciding with dry periods (Borchert *et al.*, 2002; Valdez-Hernández *et al.*, 2010). Therefore, the expected fall in precipitation levels (IPCC, 2007) could advance senescence in these species counteracting the advancement of leaf emergence. Other studies, however, particularly with deciduous species, rather point to the temperature as the factor that determines leaf abscission, showing that temperature increases contribute to delay leaf senescence, thereby increasing growing season length, although with some differences between species and environments (Vitasse *et al.*, 2009; Chen & Xu, 2012; Clark *et al.*, 2014). Finally, some authors also suggest that in many cases senescence does not depend on environmental conditions, but is likely governed by internal regulatory factors (Killmann & Thong, 1995; Olivares, 1997). In Mediterranean woody species, for example, it is common to observe a period of intense abscission coinciding with the formation of a new cohort of leaves (de Lillis & Fontanella, 1992; Castro-Diez & Montserrat-Martí, 1998). This has led several authors to suggest that the abscission of old leaves responds to the development of new resource sinks, which imposes an intense mobilization of nutrients from the old leaves to the new growing ones (Shaver, 1981; Chabot & Hicks 1982; Sa *et al.*, 2001). So, it is essential to provide information about the factors that determine senescence because, if the responses of different species are indeed different, this could lead to changes of different magnitude in the duration of the growing season for the different species as a consequence of climate change.

Mediterranean-climate regions are among the environments for which information about the potential effects of climate change is most crucial (Spano *et al.*, 2003; Gordo & Sanz, 2009, 2010). In these areas, due to the low temperatures recorded towards the end of

autumn and in winter, the growth season extends only from mid spring to summer. A considerable part of the growing season coincides therefore with periods of reduced soil water availability, which is the most important limitation imposed by the Mediterranean climate on plant growth (Mitrakos, 1980). The increase in temperatures and decrease in rainfall predicted for the new climate scenario will inevitably exacerbate the limitations imposed by the summer drought typical of the Mediterranean areas, rendering them especially vulnerable (Sánchez *et al.*, 2004; Solomon *et al.*, 2007). It is therefore indispensable to collect new data that will allow us to understand and predict the implications of global climate change on species inhabiting these areas.

The aim of the present study is to explore and compare the patterns of leaf phenology in four tree species that coexist across broad areas with Mediterranean climate, but show clear differences in leaf habit (deciduous and evergreens) and longevity, with leaves of different average duration between the evergreen species. We analyze the leaf mortality rates observed in both deciduous and evergreen species and the possible relationships with environmental differences between two sites. Our intention is to determine whether the two climatic factors most expected to change in the future – temperature and water availability – govern, and to what extent, leaf senescence in each of the four species. Alternatively, we will test the hypothesis that senescence is rather conditioned by internal regulatory factors, acting old leaves as nutrient sources for the production of new tissues (Shaver, 1981; Chabot & Hicks, 1982), with little dependence on environmental factors. In view of the climatic changes that occur along the year and the short duration of the growth period in these Mediterranean-climate regions, the adjustment of phenology is crucial for determining the final productivity of these species. If the different species do exhibit different phenological responses to changing temperatures and water availability, this may alter the competition relationships among them in the future, which will necessarily have repercussions on the species composition of Mediterranean forests.

Materials and methods

Study species and sites

This study included two deciduous (*Quercus faginea* Lam. and *Q. pyrenaica* Willd.) and two evergreen *Quercus* species, one of which has a mean leaf longevity of about one year (*Quercus suber* L.) and the other

one (*Q. ilex* L. subsp. *ballota* (Desf.) Samp.) has a mean leaf longevity of slightly more than two years (Mediavilla & Escudero, 2003 a). These species are typically Mediterranean species and they occupy large areas of open woodlands in the interior of the Iberian Peninsula.

The species were studied over four years (2007-2010) at two sites (“Garcirrey” and “Valdelosa”) located in the province of Salamanca (central-western Spain) at latitudes 40° 55′ N and 41° 08′ N and longitudes 6° 07′ W and 5° 47′ W respectively. All species were present at each of the selected sites, although *Q. suber* was monitored only during three years (2007-2009). The sites have sparse populations of isolated mature trees (about 50 specimens ha⁻¹) over 100 years old, with open pasture areas among them. The soils are Cambisols, poor in organic matter and nutrient contents in both cases. However, both sites show differences in both temperature and water-holding capacity (Dorronsor, 1992), allowing us to study the effects of these factors on possible differences in the timing of abscission of the same species between sites.

Temperature data for each site were obtained by means of data loggers (Hobo Pendant temperature/light datalogger, Part UA-002-08, Onset Computer Corporation, Pocasset, MA, USA) placed in the shade under the canopy of one tree next to the specimens selected for phenological measurements. The sensors, programmed to obtain a measurement every 10 minutes, were monitored at each site throughout the study period. Annual rainfall data from the station nearest to each site were provided by the National Meteorological Institute of Spain (AEMET). Two sensors (HOBO soil moisture smart sensor ECH2O-10 Dielectric Aquameter) buried at different depths (between 50-80 cm the first and between 1-1.5 m the second) allowed us to study the seasonal changes in soil moisture. The depths were selected according to those suggested in the literature to obtain estimates of soil water availability for tree species (Lloret *et al.*, 2004; Bader & Körner 2010). We are aware that the maximum depth reached by the sensors is probably insufficient to record the total absolute water amount that is available to the roots of the surveyed trees. However, it can be assumed that most of their root mass is concentrated in the selected range of depth, so that the data supplied by the sensors can be used to record the changes in water availability over time and the differences between sites, allowing us to identify possible relationships between leaf mortality and decreases in water availability.

The data provided by these sensors, programmed to obtain a measurement every 30 minutes, were then used to estimate the soil water reserve. First, we estimated the monthly soil water balance for each site,

applying the following input-output equation (Landsberg, 1986):

$$\Delta\theta_s = P - AET - q,$$

where $\Delta\theta_s$ = monthly variation in the soil water reserve, P = precipitation, AET = actual evapotranspiration and q = surplus (surface runoff plus deep percolation). All variables were measured in mm. We assumed soil water reserve was zero at the end of summer. From this time we calculated the cumulative water balance until the time when the electric signal from the soil moisture sensors reached saturation (usually at the end of autumn). The water balance accumulated up to this point was taken as an estimate of the maximum amount of water retained by the soil. Soil water reserve at each time interval was then calculated as a function of the electric signal from the sensors as compared with the signal at saturation. These data were used only for comparative purposes, and were not meant to represent true soil water reserve available for plants because of the difficulty of knowing the total volume of soil occupied by roots. Potential evapotranspiration was calculated applying the equation of Jensen & Haise (1963). Solar radiation data from the station nearest to each site were provided by the National Meteorological Institute of Spain (AEMET).

Field measurements of trees

Five specimens of each species were selected in each of the two study sites. Selected individuals were mature trees (over 100 years old) of similar size in both cases. Trunk diameter at 1.3 m height ranged from 20 to 60 cm and mean heights were 6-10 m.

To study leaf phenology, we monitored the number of leaves of the different age classes found in the crown. On each specimen two branches situated at mid-height in the sun-lit portion of the crown were selected. The different leaf age classes were labelled with wires of different colors at the beginning of the study. The number of surviving leaves of each age class, counted at intervals of 15 days (one week during the time of the year with more intense growth or abscission rates) was used to elaborate life-tables, according to standard methods (Begon & Mortimer, 1986). Relative mortality rates were calculated for the leaves of each age class as the difference in the number of leaves between two consecutive dates with respect to the number of leaves present at the beginning of that interval, all divided by the number of days elapsed between both dates. The mortality rates were used to identify the typical abscission period of each species and to detect possible differences at the level of a single species that could be related to changes in environmental variables between sites and years.

On all sampling dates, predawn water potentials (Ψ_{pd}) were measured as an estimate of plant water availability, using a pressure chamber (PMS Instruments Co., Mod. 1002, Corvallis, OR, USA) in twigs sampled from the same specimens and from the same position in the crown as those used for the leaf phenology measurements. The data were analyzed separately for each of the two periods in which the growth season can normally be divided as a function of water availability (the most favorable period was termed “spring” and that with the highest stress level “summer”). The seasonal variation in the predawn water potential was estimated as the difference between the mean maximum and mean minimum predawn water potentials recorded for each study year.

To test whether the mortality of old leaves occurs after these leaves have acted as nutrient sources for the production of new tissues, we analyzed the seasonal changes in the nitrogen resorption efficiency values for the two evergreen species. At each site we placed five 0.25 m² leaf-litter collection traps below the crowns of five specimens of the two evergreen species; the contents of these traps were collected at 15-day intervals. On each sampling date, green leaves of the different leaf age classes present on the crown were also collected from the trees used for litter sampling. All samples collected were taken to the laboratory, where the total projected leaf area was determined by image analysis (Delta-T Devices Ltd, Cambridge, UK). The samples were then oven-dried at 70° C to constant mass and the total dry mass was determined. From the data thus obtained, we calculated the leaf mass per unit area (LMA, g m⁻²). Leaf N concentrations were determined with a CE-Instruments NA-2100 autoanalyser (ThermoQuest, Milan, Italy). The N content was expressed per unit leaf area (N_{area} , g m⁻²), obtained as the nutrient concentration (mg g⁻¹) multiplied by the LMA. For each individual tree we obtained a value of the resorption efficiency for each date in which the leaf litter was collected as $[100 * (N_{area} \text{ in green leaves} - N_{area} \text{ in shed leaves}) / N_{area} \text{ in green leaves}]$. For the N content in living leaves, we took the maximum N_{area} registered for the green leaves collected throughout the year in each tree, which was compared with that of the shed leaves collected from the same tree in each sampling date. Resorption efficiency of each species was obtained as an average of the five specimens studied at each site.

Data analysis

One-way Analysis of Variance was used to establish significant differences among site means (temperature, precipitation, predawn water potentials and soil water

reserve) for $P \leq 0.05$ after applying the Levene test to check for homogeneity of variances.

To analyze the effect of environmental factors on abscission, we used the values of the mortality rates obtained at each site and year for each species (and the age class of the leaves on the evergreen species). Having identified the dates with significant mortality values, we calculated the average mean daily temperature, mean maximum and minimum daily temperatures, absolute maximum and minimum temperatures, precipitation and soil moisture data for different time intervals prior to each mortality peak. Then, for the leaves of each species and, in the case of evergreen species, the leaves of each age class, we used a linear regression (after transforming the data to a logarithmic scale) to analyze the relationships between mortality rate and environmental factors, repeating the analysis with the values of the environmental variables obtained for each of the different time intervals. The most significant relationships were found for a period of 15 days before each mortality peak, similarly to previous studies (Wielgolaski *et al.*, 2011). We took this interval as the optimum one to identify the effect of the environmental factors on leaf abscission. In the case of leaf water potential we used data from the dates closest to those of the mortality peaks. Multiple regression (after collinearity analysis, where necessary) was used to separate the effect of two independent variables, as applied in previous studies (Doi & Katano, 2008; Gordo & Sanz, 2010). All statistical analyses were carried out using the SPSS program (SPSS Inc., Chicago, IL, USA).

Results

Temperature and water availability: differences between sites

Table 1 shows the seasonal temperatures at each site for the four years of study. Garcirrey always showed higher temperatures than Valdelosa, with significant differences between sites especially during the summer and in the maximum temperatures (Table 1). Rainfall displayed similar levels and seasonal distribution between the stands. In all cases, rainfall was particularly low in the summer period, when less than 10% of the annual rainfall was recorded (Table 1). However, although there were no differences in the levels of precipitation, differences between sites in soil water content were apparent, significantly higher in Valdelosa for almost the entire study period (Table 1), due to higher water-holding capacity of the soil in this site.

Table 1. Climatic data of the two sites (V = Valdelosa, G = Garcirrey) along the study period. Mean m T^a = mean monthly temperature (°C); mean m max T^a = mean monthly maximum daily temperature (°C); mean m min T^a = mean monthly minimum daily temperature (°C); max T^a = maximum absolute temperature (°C); min T^a = minimum absolute temperature (°C); precipitation (mm); soil water reserve (l m⁻²). For each year, season and parameter, means with different letters indicate significant differences between plots at P = 0.05

Season	Year	mean m T ^a		mean m max T ^a		mean m min T ^a		max T ^a		min T ^a		Precipitation		Soil water reserve	
		V	G	V	G	V	G	V	G	V	G	V	G	V	G
Summer	2007	18.7 (0.97) b	20.6 (0.91) a	26.4 (1.01) b	32.4 (0.60) a	9.52 (0.95) b	12.5 (0.51) a	36.7 41.3	3.01 4.41	41 (8.03) a	34 (6.72) a	34.0 (4.32) a	18.6 (3.63) b		
Autumn	2007	6.60 (1.72) a	8.16 (1.38) a	13.3 (0.51) b	16.5 (0.98) a	1.86 (0.39) a	2.54 (0.23) a	21.7 29.8	-7.61 -4.51	162 (9.09) a	175 (10.3) a	110 (6.65) a	116 (4.85) a		
Winter	2008	6.38 (0.54) a	7.37 (0.63) a	12.4 (0.95) b	15.8 (1.44) a	1.42 (0.25) a	2.00 (0.39) a	21.6 24.9	-4.96 -3.97	48 (5.32) a	49 (4.26) a	143 (0.76) a	126 (1.15) b		
Spring	2008	13.4 (1.72) a	14.0 (1.44) a	20.1 (1.53) a	22.3 (1.67) a	7.03 (0.49) a	8.13 (0.31) a	32.0 34.8	-1.04 0.67	155 (4.64) a	171 (8.62) a	141 (3.63) a	115 (7.03) b		
Summer	2008	18.8 (1.32) b	20.4 (0.84) a	27.0 (1.30) b	30.2 (1.38) a	10.8 (0.55) a	11.2 (0.49) a	36.7 43.8	3.72 4.00	22 (1.21) a	22 (0.92) a	44.6 (6.54) a	17.3 (4.31) b		
Autumn	2008	6.18 (1.42) a	6.00 (1.48) a	11.6 (1.52) a	12.6 (1.00) a	1.54 (0.25) a	0.44 (0.14) a	23.7 28.6	-9.82 -12.6	109 (5.00) a	97 (4.32) a	75.7 (4.13) a	60.9 (2.81) b		
Winter	2009	5.68 (1.17) a	6.03 (0.90) a	11.6 (1.97) a	13.4 (1.71) a	0.28 (0.08) a	0.67 (0.17) a	24.6 29.2	-8.53 -9.73	109 (10.1) a	106 (8.78) a	142 (4.32) a	119 (5.14) b		
Spring	2009	14.5 (1.61) a	15.5 (1.96) a	22.1 (2.32) b	25.9 (2.74) a	7.71 (0.53) a	8.27 (1.00) a	34.9 37.9	-3.15 -0.44	54 (2.87) a	38 (2.31) a	100 (5.69) a	37.1 (4.04) b		
Summer	2009	20.7 (1.22) b	22.3 (1.00) a	29.2 (1.76) b	35.1 (1.04) a	11.0 (0.33) b	13.9 (0.60) a	36.1 45.0	4.14 5.96	47 (1.26) a	32 (0.92) a	16.7 (2.44) a	35.8 (0.78) b		
Autumn	2009	9.14 (1.58) a	9.79 (0.70) a	14.4 (1.79) a	17.0 (1.54) a	4.82 (0.45) a	5.21 (0.81) a	27.6 33.0	-10.3 -5.87	53 (2.65) a	71 (3.34) a	63.0 (6.11) a	78.0 (5.29) a		
Winter	2010	4.62 (0.86) a	4.92 (0.98) a	9.05 (0.21) a	10.2 (0.84) a	0.74 (0.23) a	0.98 (0.28) a	19.4 24.4	-8.17 -6.62	195 (9.97) b	257 (10.2) a	149 (0.34) a	129 (0.35) b		
Spring	2010	14.1 (1.75) a	15.0 (0.75) a	21.3 (1.19) a	23.9 (2.26) a	7.80 (0.90) a	8.34 (0.54) a	33.2 41.3	-2.48 -1.98	101 (6.98) b	140 (9.98) a	127 (7.73) a	82.7 (5.98) b		
Summer	2010	21.1 (1.88) b	23.7 (1.18) a	29.5 (0.98) b	35.3 (0.72) a	12.9 (0.72) b	15.8 (0.54) a	36.6 44.1	4.19 5.55	37 (3.31) a	30 (1.46) a	32.1 (3.72) a	9.12 (2.38) b		
Autumn	2010	6.72 (1.15) a	7.87 (0.81) a	12.1 (0.91) a	15.8 (1.23) a	2.30 (0.21) a	3.27 (0.66) a	23.7 32.1	-7.67 -6.92	85 (6.54) b	121 (7.63) a	76.0 (2.33) a	64.2 (2.31) a		

Table 2. Mean (\pm SE in parenthesis, n = 24) predawn leaf water potential values (Ψ_{pd} , MPa) during spring and summer, and seasonal range of Ψ_{pd} for each species in each plot (V = Valdelosa, G = Garcirrey), along the four years of study. For each species and parameter, means with different letters indicate significant differences between plots at P<0.05

Species	Ψ_{pd} Spring		Ψ_{pd} Summer		Ψ_{pd} seasonal range	
	V	G	V	G	V	G
<i>Q. pyrenaica</i>	-0.44 (0.06) a	-0.83 (0.10) a	-0.89 (0.13) a	-2.04 (0.20) b	0.54 (0.06) a	1.49 (0.11) b
<i>Q. faginea</i>	-0.40 (0.05) a	-0.83 (0.09) a	-0.87 (0.13) a	-1.92 (0.20) b	0.52 (0.07) a	1.32 (0.11) b
<i>Q. suber</i>	-0.53 (0.07) a	-0.70 (0.11) a	-0.79 (0.11) a	-1.68 (0.12) b	0.43 (0.03) a	1.11 (0.06) b
<i>Q. ilex</i>	-0.54 (0.07) a	-0.63 (0.06) a	-0.83 (0.09) a	-1.92 (0.12) b	0.50 (0.02) a	1.38 (0.08) b

For all species, predawn water potentials underwent a decline along the growth season and this was always more marked at Garcirrey, for which significantly lower Ψ_{pd} summer values and higher seasonal Ψ_{pd} range than at Valdelosa were found. However, differences between sites were already present in the favorable period, with mean Ψ_{pd} values higher at Valdelosa, although in this case the differences were not significant (Table 2).

Leaf senescence: effects of environmental parameters

In the two deciduous species, leaf emergence occurred between the second fortnight in April and the

first fortnight in May, the leaves appearing earlier at Garcirrey than at Valdelosa (Fig. 1). Both species retained around 80% of their leaves until November when senescence began, although with some differences between species and sites. In *Q. pyrenaica*, leaf abscission started during early November at Valdelosa, and two weeks later at Garcirrey (Fig. 1). Similar trends were seen for *Q. faginea*, in which the onset of senescence occurred around one month later in Garcirrey with respect to Valdelosa, and, in both sites, around two weeks later compared to *Q. pyrenaica* (Fig. 1). Similar results were observed with little differences between years of study.

Leaf mortality rates in the deciduous species increased as mean, maximum and minimum tempera-

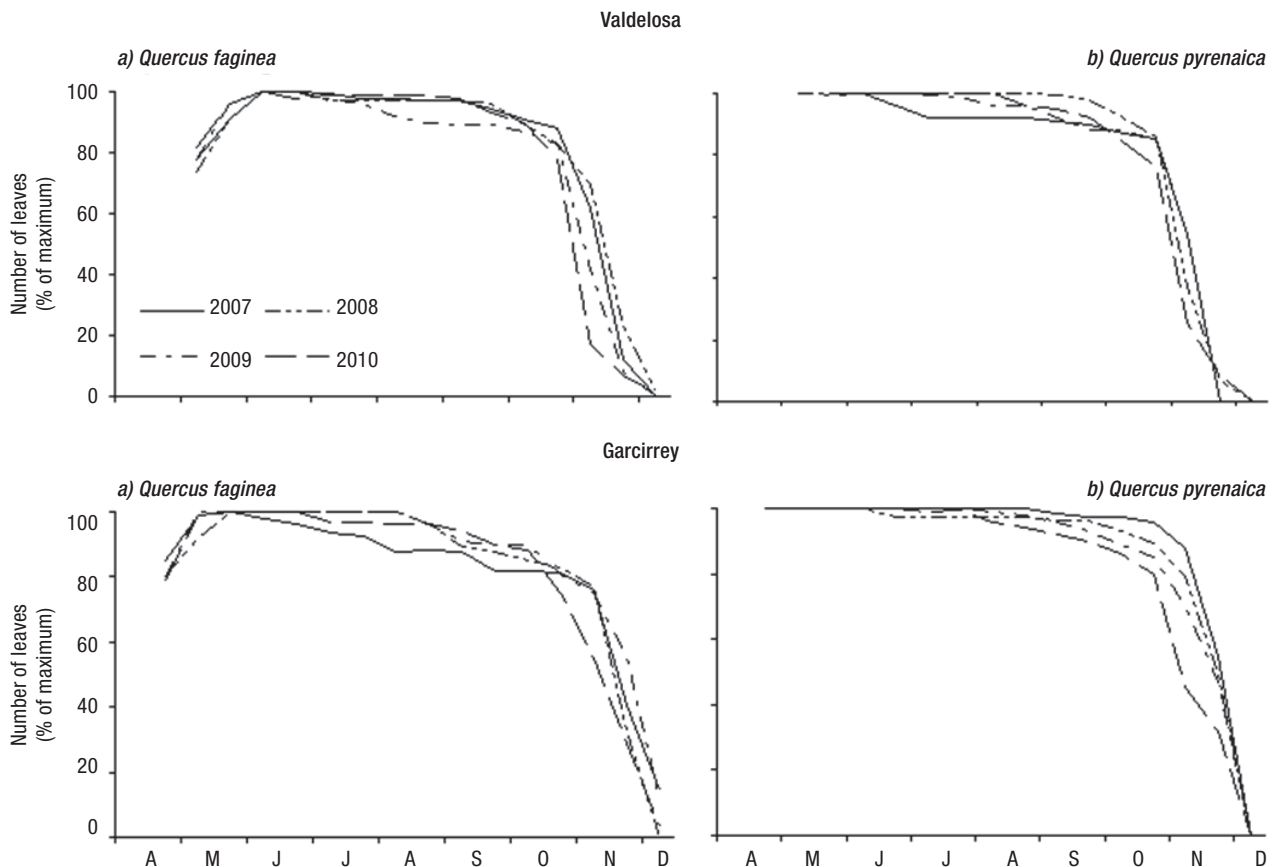


Figure 1. Leaf survivorship curves in *Q. faginea* (a) and *Q. pyrenaica* (b) for each site and year of study (n = 5 trees per species and site). Error bars are omitted for clarity.

tures declined and the number of days with frosts increased. The highest percentage of the variation in leaf mortality during a specific period was explained by the maximum temperatures in the two weeks preceding that period (data not shown). In multiple regressions, the effects of mean temperatures, minimum temperatures and days with frost on leaf mortality tended to be non-significant, unlike the effect of maximum temperatures (in particular absolute maximum temperatures during the two previous weeks, Table 3).

Quercus suber had a later leaf emergence than the other three species (between the end of May and the beginning of June), with no appreciable differences between sites (Fig. 2). Most of the leaves survived their first year, with losses of around 15-20% at the end of their first year of life. During the second year, the surviving leaves underwent massive mortality that coincided with the appearance of the new cohort (Fig. 2). The same trends, with few variations, were observed at both sites. There were no relationships between the different environmental factors (temperature and water availability) and the mortality

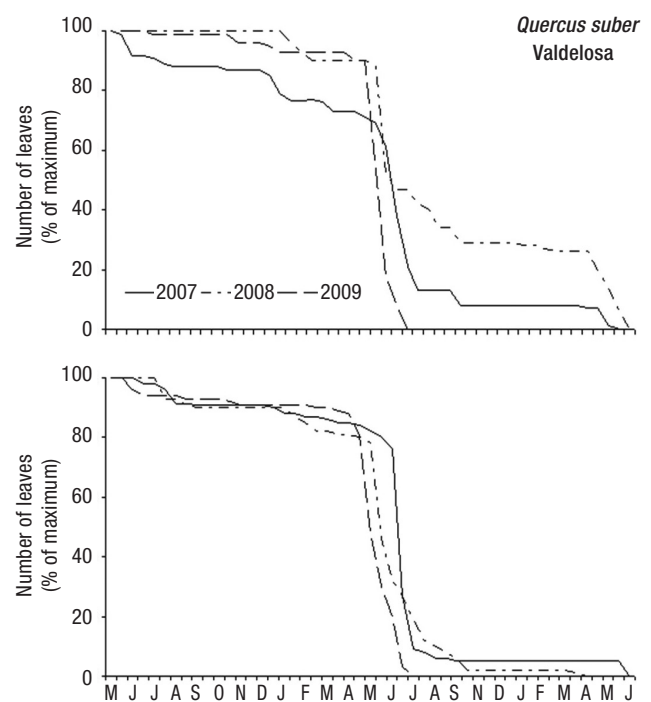


Figure 2. Leaf survivorship curves in *Q. suber* for each plot and year of study (n = 5 trees). Error bars are omitted for clarity.

Table 3. Multiple regression of the mortality rates (days^{-1} , $n = 24$) obtained for the two deciduous species, as dependent variable, against different estimates of temperature corresponding to the two weeks prior to each value of the rate of mortality as independent variables (all the data were transformed to a logarithmic scale). Temperature abbreviations as in Table 1. Significant relationships are highlighted in bold

Independent variables	<i>Q. faginea</i>					<i>Q. pyrenaica</i>				
	y-intercept	Slope	P	Multiple		y-intercept	Slope	P	Multiple	
				R ²	P				R ²	P
mean m T ^a	3.75	-0.56	0.4450	0.62	< 0.0001	5.50	-0.11	0.8662	0.69	< 0.0001
max T ^a		-1.66	0.0056				-3.22	0.0134		
mean m max T ^a	4.18	-1.11	0.1978	0.64	< 0.0001	5.38	-0.70	0.3438	0.71	< 0.0001
max T ^a		-1.37	0.0218				-2.57	0.0194		
mean m min T ^a	4.42	0.36	0.7180	0.61	< 0.0001	5.86	-0.86	0.1466	0.73	< 0.0001
max T ^a		-2.87	0.0029				-2.84	0.0003		
max T ^a	4.34	-2.27	0.0021	0.61	< 0.0001	5.47	-3.17	0.0022	0.69	< 0.0001
min T ^a		-0.29	0.4725				-0.14	0.7543		
max T ^a	1.32	-1.47	0.0155	0.63	< 0.0001	4.56	-2.99	0.0135	0.70	0.0998
days with frost		1.52	0.2511				0.50	0.6759		

rates observed for the old leaves in *Q. suber* (data not shown). However, although abscission of the older leaves extended along several weeks, the maximum mortality rates were observed during the dates closest to the emergence of the new leaves (Fig. 3). The maximum N resorption value recorded at each site and year was always obtained on the date closest to that of maximum mortality rate of the old leaves (Fig. 4).

The other evergreen species, *Q. ilex*, unfurled its leaves during the last days of April or beginning of May, one month earlier than *Q. suber* (Fig. 5). Leaf mortality was almost negligible during their first year

(less than 10%), and became significant coinciding with the emergence of a new cohort the following year, thereafter extending along the summer. Leaf mortality rates during the second year of life were higher at Garcirrey, where only about 55% of the leaves survived as compared to the 60-65% observed at Valdelosa (Fig. 5). For the leaves lost during the second year after leaf development, the maximum mortality was recorded in the pre-summer period in Valdelosa (around 60%, as opposed to 14% during the summer), whereas in Garcirrey mortality was more gradual (around 40% of the leaves that died in their second year was lost between May and June and 25% in the summer months). Similar results were obtained for the leaves during their 3rd year: the maximum mortality rates were obtained in the pre-summer period in Valdelosa, whereas in Garcirrey mortality was more gradual (Fig. 5)

The regression analysis failed to reveal any significant relationship between the mortality rates of leaves and the different environmental parameters in *Q. ilex* (temperature and water availability) (data not shown). Analysis of the temporal evolution of the N resorption indicated that the highest resorption values were always recorded in the dates closest to the emergence of a new cohort of leaves (Fig. 4). The only exception was obtained in 2010 at Valdelosa, where the highest N resorption values were recorded in late July, well after the emergence of the new cohort.

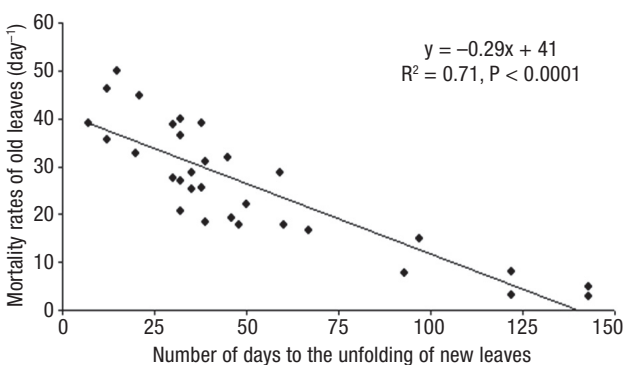


Figure 3. Relationship between per capita mortality rates ($\times 1000$) of the old leaves of *Q. suber* and the number of days between each mortality peak and the date of emergence of the new leaves on the same specimens.

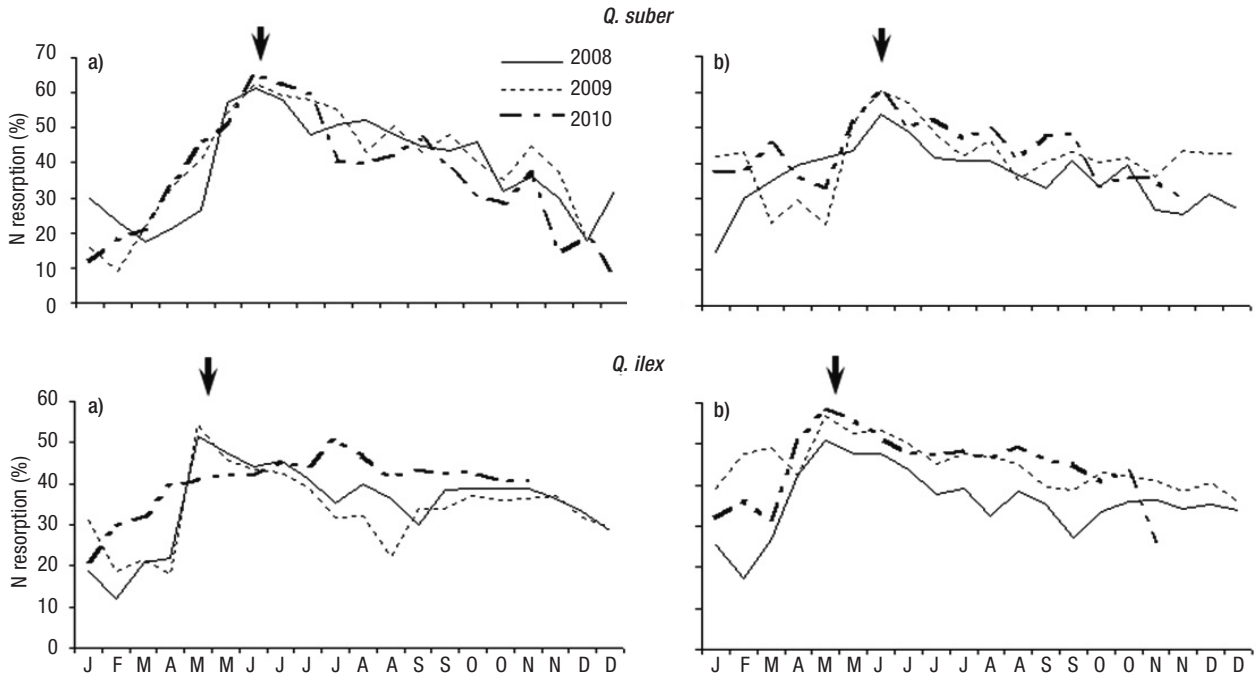


Figure 4. Temporal evolution of the N resorption values (%) recorded for the two evergreen species along each of the years of study in each site (n = 5 trees per species, year and site). Error bars are omitted for clarity. Arrows indicate the times at which maximum leaf mortality were recorded in each species and site.

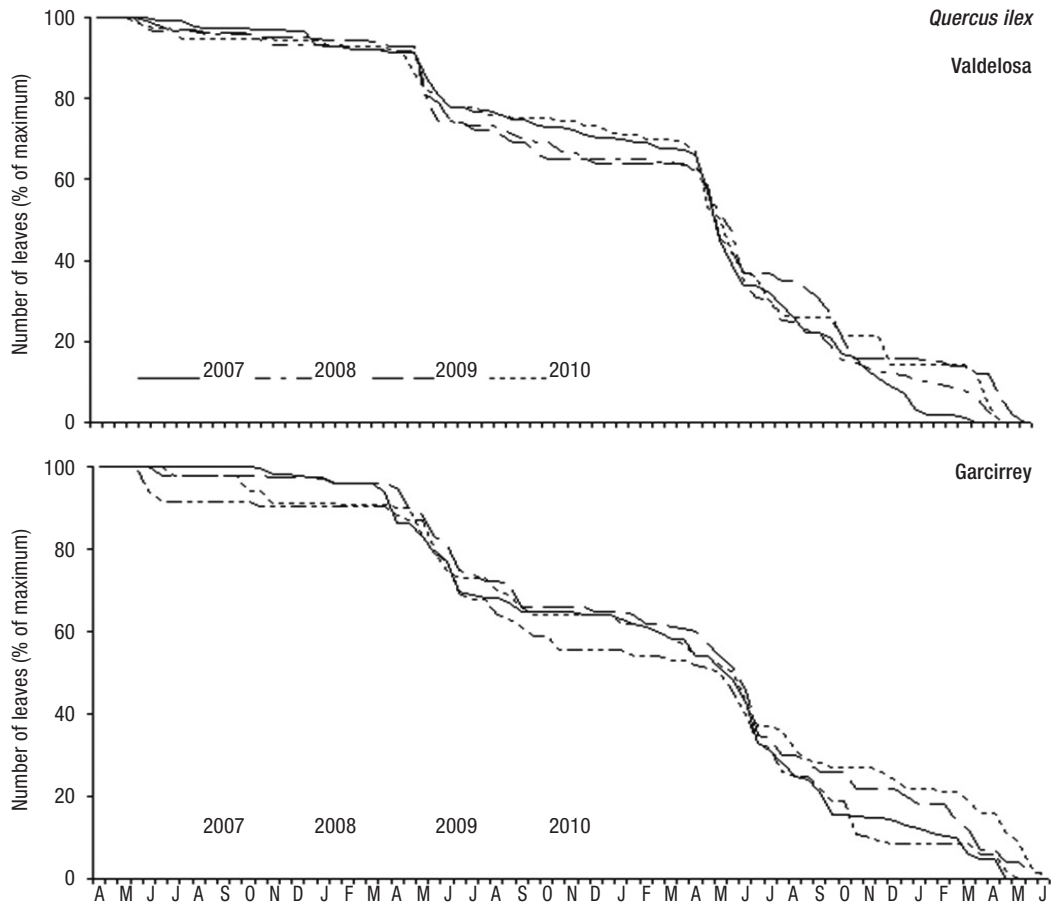


Figure 5. Leaf survivorship curves in *Q. ilex* obtained in each plot and year of study (n = 5 trees in each case). Error bars are omitted for clarity.

Discussion

Our results confirm the variety of leaf habits among tree species coexisting and belonging to the same genus in Mediterranean areas, as has already been discussed by several authors (Escudero *et al.*, 1987; De Lillis & Fontanella, 1992; Montserrat-Martí *et al.*, 2004). The different *Quercus* species studied here not only deploy different phenological strategies against the conditions they face at the same site, but also exhibit different responses to the same changes in environmental factors.

Some differences among the species under survey in this study were found regarding the effects of the different environmental factors on leaf senescence, which can have important repercussions on the total C assimilation of deciduous and perennial leaves in a new climatic scenario. In the two deciduous *Quercus* species, leaf death occurs in response to the decrease in temperature that accompanies the end of the growth season. This has been interpreted as an adaptation aimed at avoiding cold stress in winter (Chabot & Hicks, 1982). On analyzing the relationship between leaf mortality rates and different environmental patterns, we observed that leaves are apparently shed when maximum daily temperatures fall below a certain threshold (between 11 and 12°C of maximum daily temperature). The photosynthetic capacity in deciduous leaves remains more or less constant until their senescence (Reich *et al.*, 1991; Morecroft & Roberts, 1999). Since the deciduous habit imposes a short productive period, it may be expected that deciduous leaves will be retained as long as environmental conditions allow them to carry out a certain amount of C assimilation that will contribute to increasing total production per leaf. According to our data, the time of leaf abscission is determined by the length of time during which temperatures remain each day above a certain threshold that permits significant assimilation values to be reached. Thus, during October, most leaves are retained because, although minimum temperatures begin to fall sufficiently to be limiting for photosynthesis, maximum daily temperatures are still high enough to allow a certain amount of assimilation (Mediavilla, 2000). Stronger effects of maximum than minimum temperatures for plant phenology have been also observed for different species in Mediterranean ecosystems (Gordo & Sanz, 2010). Accordingly, warmer autumnal temperatures would trigger a delay in senescence in these two deciduous species, which suggests that climate change will increase the C assimilation period and therefore could enhance tree growth and productivity, as proposed for other species (Piao *et al.*, 2007; Vitasse *et al.*, 2009).

In *Q. suber* and *Q. ilex* leaf mortality mainly affects leaves more than one year old. In both cases, mainly in *Q. suber*, the most pronounced peaks are recorded at the end of spring, coinciding with the development of a new cohort, in agreement with other studies on the same species (Caritat *et al.*, 2006; Andivia *et al.*, 2010). Later leaf losses, although less significant, were also seen for *Q. ilex* along the dry season. Leaf abscission has often been associated with decreases in soil humidity, and the gradual shedding of leaves along the summer period has been interpreted as a means for the trees to face up to the gradual appearance of water stress (Kramer *et al.*, 2000; Peñuelas *et al.*, 2004). In our case, the mortality recorded during the summer for *Q. ilex*, and the fact that it was always more marked at Garcirrey (with less soil water availability and lower leaf water potentials), suggest that drought stress determines the rates of summer leaf mortality in this species. However, maximum abscission rates of the leaves of these two evergreen species were concentrated towards the end of spring, when water stress is still not manifest, such that none of the different indicators of water stress revealed any significant relationship with mortality rates. This coincides with the findings of other authors, who also failed to draw conclusive results with respect to the relationship between leaf senescence and environmental factors such as temperature or water availability (Gordo & Sanz, 2005; Morin *et al.*, 2010; Seghieri *et al.*, 2012).

The fact that this pattern of leaf abscission (maximum mortality rates of the old leaves coinciding with the emergence of the new cohort) is frequent in woody species from other biomes has led some authors to suggest that leaf mortality would not depend on the environmental conditions. These authors propose that leaf mortality is rather conditioned by factors of internal regulation (Killmann & Thong, 1995; Olivares 1997) and would respond to the development of new resource sinks, which impose an intense mobilization of nutrients from the old leaves to the new growing ones (Shaver, 1981; Chabot & Hicks 1982; Sa *et al.*, 2001). In the species studied here, N resorption from old leaves did not occur until the end of their life-spans, coinciding with their senescence, such that nutrient depletion in a leaf coincides with its shedding (Escudero & Mediavilla, 2003). Accordingly, if the abscission of old leaves were governed by the withdrawal of nutrients for the formation of new leaves, the lowest amounts of nutrients in the leaf litter should always be reached on dates close to the emergence of a new leaf cohort. This is indeed confirmed in most cases for the evergreen species studied here. It is therefore likely that the resources resorbed from the senescent leaves will be indispensable to meet the strong demand imposed by the synchronized production of new leaves, helping to decrease the dependence

on soil resources and to increase the amount of new biomass produced if the old leaves are discarded. In these evergreen species a marked decline in leaf photosynthetic capacity can be observed along leaf life (Mediavilla & Escudero, 2003c), such that the decrease in C assimilation, together with the marked stomatal sensitivity to water stress shown by these species (Mediavilla & Escudero, 2003b), significantly reduces the carbon gain by the older leaf cohorts. An early development of a new cohort of more productive leaves may help to improve the carbon balance of these species. In both species, senescence thus seems to show less dependence on environmental factors in comparison with emergence, in agreement with reports made by other authors (Gordo & Sanz, 2005; Vitasse *et al.*, 2009; Wielgolaski *et al.*, 2011).

The strong climatic variability characteristic of regions with a Mediterranean climate makes it difficult to extrapolate our results to a new climate scenario for which the predictions suggest warmer and drier conditions (IPCC, 2007). Nevertheless, bearing in mind the observed differences, it is clear that the possible implications of climate change will differ from one species to another. According to the present results, the foreseen rise in temperatures would lead to advances in emergence and delays in abscission of deciduous leaves (Gordo & Sanz, 2009; García-Mozo *et al.*, 2010; Kolářová *et al.*, 2014) thereby increasing the duration of the growth season in deciduous species. Additionally, if drought boosts leaf mortality, as seem to be apparent from the present study, at least in *Q. ilex*, we can expect to see a more marked summer leaf abscission in this species in response to the decrease in water availability. This greater mortality during the summer, together with the increase in the duration of the leaves of deciduous species, could contribute to reducing the differences in the length of the productive period between species with contrasting leaf habits, in favor of the competitive capacity of deciduous species. The distribution patterns of species with different leaf habits could be altered, in detriment to the evergreen species. It is clear that more prolonged studies should be conducted, covering a greater variability in the environmental conditions and seeking more reliable conclusions. Nevertheless, it is essential that studies such as the present one offer field observations that can be incorporated into models designed to predict the future implications of climate change for plant species.

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