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RESEARCH PAPER

Differential and interactive effects of temperature and photoperiod on budburst and carbon reserves in two co-occurring Mediterranean oaks

V. Sanz-Pérez[1], P. Castro-Díez[1] & F. Valladares[2,3]

1 Departamento de Ecología, Universidad de Alcalá, Madrid, Spain
2 Instituto de Recursos Naturales, Centro de Ciencias Medioambientales, Madrid, Spain
3 Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnológicas, Universidad Rey Juan Carlos, Móstoles, Spain

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Correspondence
V. Sanz Pérez, Departamento de Ecología, Universidad de Alcalá, Alcalá de Henares 28871 Madrid, Spain.
E-mail: virginia.sanz@uah.es

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ABSTRACT

Effects of temperature and photoperiod and their interactions on budburst and on the use of carbon reserves were examined in two Mediterranean oaks differing in wood anatomy and leaf habit. Seedlings of *Quercus ilex* subsp. *ballota* (evergreen and diffuse-porous wood) and *Q. faginea* (semi-deciduous and ring-porous wood) were grown under two temperatures (12 and 19 °C) and two photoperiods (10 and 16 h) in a factorial experiment. In the 16 h photoperiod at 19 °C, photosynthesis was suppressed in half of the seedlings by covering leaves with aluminium foil. The concentration of soluble sugars, starch and lipids in leaves, stems and roots was assessed before and after budburst. Under the 12 °C treatment (mean current temperature in early spring in the Iberian Peninsula), budburst in *Q. faginea* occurred earlier than in *Q. ilex*. Higher temperature promoted earlier budburst in both species, mostly under the 16 h photoperiod. This response was less pronounced in *Q. faginea* because its budburst was also controlled by photoperiod, and because this species needs to construct a new ring of xylem before budburst to supply its growth demands. Therefore, dates of budburst of the two species became closer to each other in the warmer treatment, which might alter competitive relations between the species with changing climate. While *Q. ilex* relied on carbon reserves for budburst, *Q. faginea* relied on both carbon reserves and current photoassimilates. The different responses of the two *Quercus* species to temperature and photoperiod related more to xylem structure than to the source of carbon used for budburst.

INTRODUCTION

Increasing temperatures in Europe and North America during recent decades have been related to earlier budburst in many plant species (Chmielewski & Rötzer 2002). Because this advance in budburst may modify important ecosystem processes, such as plant–animal interactions and water and carbon cycles (Myneni *et al.* 1997; Churkina *et al.* 2005; Parmesan 2006), many temperature-based models have been developed to predict the timing of budburst (Cannell & Smith 1986; Murray *et al.* 1989; Kramer 1994; Hänninen 1995). These models assume that the rate of development of buds is essentially determined by temperature, and that buds break when a certain number of 'thermal units' above a threshold temperature has been accumulated, after chilling requirements have been fulfilled in winter (Hunter & Lechowicz 1992). Consequently, the higher the mean daily temperatures in spring, the faster the thermal units accumulate. Despite this general tendency, budburst responsiveness to temperature varies significantly across species due to different threshold temperatures and thermal unit requirements (Heide 1993a; Lennartsson & Ögren 2004). These species-specific responses may alter competitive relationships of coexisting plants, changing population densities and eventually changing their geographic distributions (Kramer *et al.* 1996; Peñuelas & Boada 2003). Different sensitivity to temperature across species may also be explained by the fact that budburst can be concurrently controlled by other factors, such as resource availability and/or photoperiod (Murray *et al.* 1989; Häkkinen *et al.* 1998; Wielgolaski 2001). For instance, budburst of *Fagus sylvatica* is mainly controlled by photoperiod (photothermal model; Wareing 1953; Falusi & Calamassi 1990; Heide 1993b), so it is unlikely that an increase in spring temperatures will have a significant effect on its timing of budburst. Other species need the co-occurrence of long days and warm temperatures to trigger budburst (Partanen *et al.* 1998, 2001; Linkosalo *et al.* 2000), so that photoperiod can reduce the effect that temperature would have on budburst date under a warming scenario.

Some studies have compared budburst sensitivity to temperature and photoperiod across species in boreal and temperate regions, finding that temperature was the main controlling factor and that photoperiod only had an effect when winter temperatures were mild (Campbell & Sugano 1975; Heide 1993a; Myking & Heide 1995). However, this sensitivity varies across species, either due to genetic or to environmental factors (Lechowicz 1984; Myking & Heide 1995; Bertero *et al.* 2000; del Pozo *et al.* 2000). Wood anatomy has been suggested to be one relevant genetic factor (Hunter & Lechowicz 1992). In cold winter regions, diffuse-porous trees are able to retain part of their hydraulic conductivity after winter, while ring-porous trees lose most of this ability, so they must build a new ring of xylem before budburst to supply water to the growing shoots (Lechowicz 1984). Hence, budbreak time is expected to be more constrained in ring-porous than in diffuse-porous species. In addition, the carbon source used to supply budburst demands (current photoassimilates *versus* reserves, either carbohydrates or lipids) may also explain different budburst responses across species. Therefore, detecting and understanding differential budburst responses to the environment among co-occurring species, together with the mechanisms involved, can shed light on community responses to environmental changes.

We studied the effects of temperature and photoperiod on the timing of budburst and on the use of different carbon sources in seedlings of two Mediterranean oaks (*Quercus ilex* L. subsp. *ballota* and *Q. faginea* Lam.). These two species coexist in many continental regions of the Iberian Peninsula, but differ in wood anatomy and leaf habit. A difference in response of their seedlings to a warming scenario would affect their regeneration potential and therefore the future composition of the forests. Our aims were (i) to explore the sensitivity to temperature of budburst date of seedlings of two co-existing oaks, and the role of photoperiod on this sensitivity and (ii) to search for the main carbon source supplying budburst (carbohydrate or lipid reserves *versus* current photoassimilates). We tested the following hypotheses: (i) budburst sensitivity to temperature and photoperiod differs between coexisting species with different wood anatomy (ring-porous *versus* diffuse-porous xylem) and (ii) temperature and photoperiod in late winter/early spring modify both the amount and the type of carbon source supplying budburst.

MATERIALS AND METHODS

Plant material

One-year-old plants of *Q. ilex* subsp. *ballota* and *Q. faginea* were used for the experiments. While *Q. ilex* subsp. *ballota* is an evergreen tree with diffuse-porous xylem, *Q. faginea* is a semi-deciduous, ring-porous xylem tree (Villar-Salvador *et al.* 1997). Seedlings of the latter species can keep some of their leaves for up to 14 months, behaving like an evergreen tree (Silla & Escudero 2004). *Q. faginea* generally flushes 2–3 weeks earlier than *Q. ilex*, and both species quickly expand highly pre-formed primordia (Castro-Díez & Monserrat-Martí 1998; Castro-Díez *et al.* 2003).

Experimental design

Acorns of the two species were collected in autumn 2003 from Alcarria – Serranía de Cuenca (Spanish forest region ES9) and were grown in Forest Pot 300 containers with 50 cavities, each filled with 300 cm^3 peat (pH = 4) and vermiculite (3:1 v:v). The containers were placed outdoors (exposed to chilling and freezing temperatures) in the nursery of *Centro de Capacitación Agraria TRAGSA* in San Fernando de Henares (40º24′N, 3º29′W), Madrid, central Spain until the 31st January 2005. During this period, weed control, fertilisation and irrigation were carried out regularly. In February 2005, 200 seedlings of each species were randomly placed in four growth chambers (two large walking-in chambers and two ASL phytotron H-900, all from Ibercex-ASL S.A., Madrid, Spain), and left under different treatments until all apical buds had opened. The four treatments resulted from a factorial combination of two temperatures (12 and 19 °C) and two photoperiods (10 and 16 h, hereafter, abbreviations SD and LD to denote short and long day, respectively). The 12 °C SD and the 19 °C LD treatments correspond to the average day temperatures and day lengths in central Spain in early spring and in late spring–early summer, respectively, (estimated from official long term records (>40 years) in a representative sample of meteorological stations in central Spain obtained from the National Meteorological Institute, Spanish Ministry of Environment). The 19 °C SD treatment aims to mimic global warming scenarios for the Mediterranean region, where temperature may increase by 7 °C, advancing spring in terms of temperature, while photoperiod remains unchanged (Christensen *et al.* 2007).

In order to assess the role of current photoassimilates acquired from previous-year leaves on budburst, 50 additional seedlings per species were placed in the 19 °C LD chamber and the leaves were covered with aluminium foil to prevent photosynthesis (19 °C LD* treatment).

Irradiance in all growth chambers was kept at a PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by fluorescents tubes (Sylvania Gro-Lux 18W). This irradiance was well above the light compensation point for net photosynthesis for these Mediterranean oaks (Valladares *et al.* 2000) and minimised the warming effect of the artificial illumination (Zhang *et al.* 2003). The relative humidity was maintained above 70%. Plants were watered each week, and were not fertilised during the experimental period.

Budburst data and temperature sum requirements

The most apical bud of each seedling was checked for budburst on a weekly basis from February to July 2005. A bud was considered to be open when young leaves protruded beyond the bud scales. The temperature sum required for budburst (ºC per day) was calculated for each plant in the different treatments from February 1 to the date of budburst. These temperature sum requirements were calculated from different temperature thresholds (0, 3 and 5 °C) according to the literature (Heide 1993a; Myking 1997; Wesolowski & Rowinski 2006). As these previous studies focused on species from more northern latitudes, we also included 7 °C, which is the base temperature to start heat accumulation for flowering in Iberian *Quercus* (Jato *et al.* 2002). The best value of the temperature sum requirements was that which gave the minimum standard error of the temperature sum prediction within a treatment, and which gave the lowest difference between predictions for the two temperature treatments (Hunter & Lechowicz 1992; Myking 1997; Wesolowski & Rowinski 2006). The best temperature threshold was one which minimised the standard error of prediction.

Carbon reserve analysis

At the beginning of the experiment (February 2005), four seedlings per species were harvested to assess the pre-budburst carbon status. Five additional seedlings per species and treatment were harvested in the week when budburst was observed (post-budburst harvest). Samples were separated into leaves, stems and roots and gently washed. Then, all fractions were dried at 60 °C for 48 h, weighed, and the parts ground separately in a mixer mill (MM200, Retsch, Haan, Germany). Soluble sugar (SS), starch (St) and lipid (Lp) concentrations were determined for each plant fraction by near-infrared spectrophotometry (NIR Systems 6500; Foss NIR Systems, Raamsdonksveer, The Netherlands), according to the method described by Joffre *et al.* (1992) and Gillon *et al.* (1999). Given that the biomass of each fraction did not change between pre- and post-budbreak harvests in any treatment, we estimated the consumption of carbon reserves for each seedling fraction as:

$$\text{Carbon reserves use} = [X]_{\text{post-budbreak}} - [X]_{\text{pre-budbreak}} \tag{1}$$

where [X] was the concentration of SS, St or Lp either before ($[X]_{\text{pre-budbreak}}$) or after ($[X]_{\text{post-budbreak}}$) budburst. Pre-budbreak concentration values were based on a single composite sample taken from all four seedlings, while post-budbreak samples were taken from each individual seedling.

Statistics

Effects of treatments on plant responses were assessed for each species independently because variances differed between species, violating the homoscedasticity assumption of ANOVA, and this could not be solved with any transformation. The effects of temperature and photoperiod on the number of days for budburst were assessed by two-way ANOVA. The effect of photoperiod on temperature sum requirements for each species was tested by one-way ANOVA. Plant biomass and concentration of SS, St and Lp in each plant fraction were compared between pre- and post-budbreak with one-way ANOVA followed by the Dunnet test, using pre-budbreak values as control. When carbon reserve concentration changed significantly between dates, a two-way ANOVA analysis (temperature and photoperiod as factors) followed by a Bonferroni test were conducted to check for differences in carbon reserve consumption across treatments. The effect of suppression of photosynthesis on the same response variables was assessed by means of a one-way ANOVA or by a Mann–Whitney *U*-test if the variances were unequal. Prior to all comparisons, data were checked for outliers (Hair *et al.* 1998). To satisfy normality and homoscedasticity assumptions, some variables were root square- or log-transformed (Zar 1984). Statistical analysis was performed with SPSS 14.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Days to budburst and thermal requirements

The 7 °C increase in temperature led to earlier budburst in both oak species, the difference in days to budburst between treatments being higher in *Q. ilex* (Fig. 1, Table 1). At 12 °C, *Q. faginea* opened 20 days earlier than *Q. ilex*, while at 19 °C this difference was reduced to 5 days (Fig. 1). Photoperiod only affected days to budburst in *Q. faginea*, where the longer the daylength, the earlier the date of budburst at all temperatures (an advance of 8 ± 1 days) (Fig. 1, Table 1). There was a temperature by photoperiod interaction for the two species (Table 1); the advance of budburst by temperature being greater at longer than at shorter daylengths, especially in *Q. ilex*. The temperature thresholds necessary to

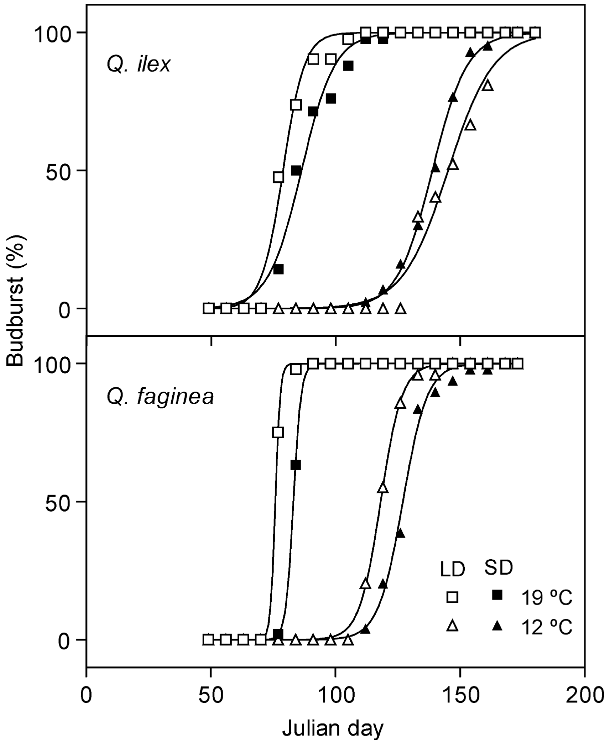

Fig. 1. Percentage of accumulated budburst through time for the two oak species under different daylengths and temperatures. LD, long days (16 h light per day); SD, short days, (10 h light per day).

Table 1. Effects of temperature and daylength on the number of days to budburst in the two oak species.

source of variation	df	*Quercus ilex*	*Quercus faginea*
temperature (T)	1	1048.38***	2910.80***
daylength (D)	1	0.76 n.s.	100.57***
T × D	1	15.75***	6.14*

F-values and their level of significance are given for temperature, daylength and their interaction.
***P < 0.001; **P < 0.01; *P < 0.05; n.s., not significant.

Table 2. Sum of degree-days accumulated to budburst (mean ± SE) for each species and photoperiod treatment.

species	short day	long day	P-value
Quercus ilex	628 ± 15	601 ± 10	0.193
Quercus faginea	725 ± 7	641 ± 6	0.000

P-values from one-way ANOVA.

start accumulating thermal units that gave better predictions were 7 °C for *Q. ilex* and 5 °C for *Q. faginea*. Photoperiod did not affect the temperature sum requirements for budburst in *Q. ilex* but reduced them in *Q. faginea* (Table 2).

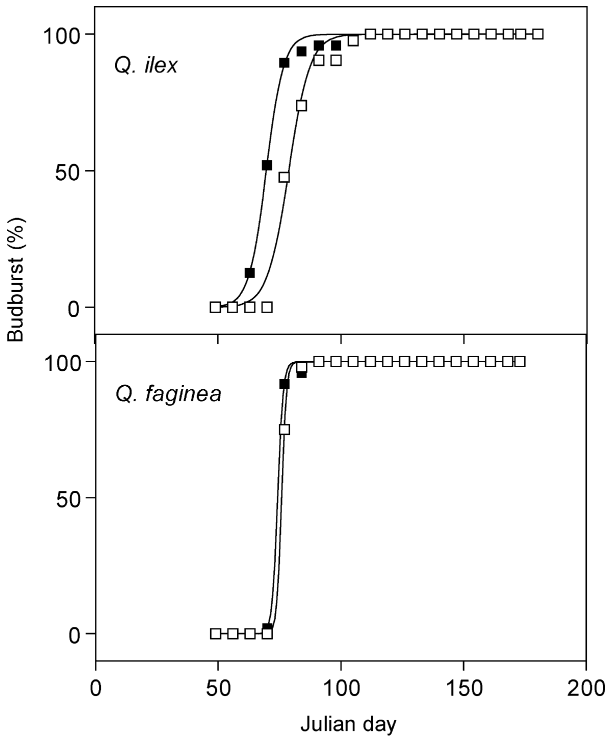

Fig. 2. Percentage of accumulated budburst through time for the two oak species with (closed symbols) or without (open symbols) leaves covered with aluminium foil to prevent current photosynthesis. All plants were grown at 19 °C and 16 h light per day.

Suppression of current photoassimilates by covering leaves with aluminium foil advanced budburst by 10 in *Q. ilex* ($F = 23.6$, $P < 0.001$) and 2 days in *Q. faginea* (Mann–Whitney $U = 988$, $P < 0.05$) (Fig. 2).

Reserve shifts between pre- and post-budburst

The biomass of each plant fraction and the whole plant biomass did not change between pre- and post-budburst harvests in either species or any treatment (Dunnet test, $P > 0.05$); hence, changes in carbohydrate concentrations will reflect changes in carbohydrate pools.

Plants with covered leaves did not use either the carbon reserves of roots or the starch in leaves in either species. Suppression of photosynthesis by leaf covering promoted the consumption of SS_{stem} and Lp_{leaf} in *Q. ilex* and of St_{stem} in *Q. faginea* ($F = 19.5$, $P < 0.002$). However, *Q. ilex* seedlings with covered leaves did not use SS_{leaf} unlike plants in which the leaves were uncovered ($F = 19.7$, $P < 0.002$) (Fig. 3).

Neither $[St]_{leaf}$ nor any of the three reserve compounds studied in roots changed from February (pre-budburst) to post-budburst in either species (Fig. 4), indicating that they were neither mobilised nor accumulated during the studied period. Changes in soluble sugars and lipids of both leaves and stems followed similar trends across

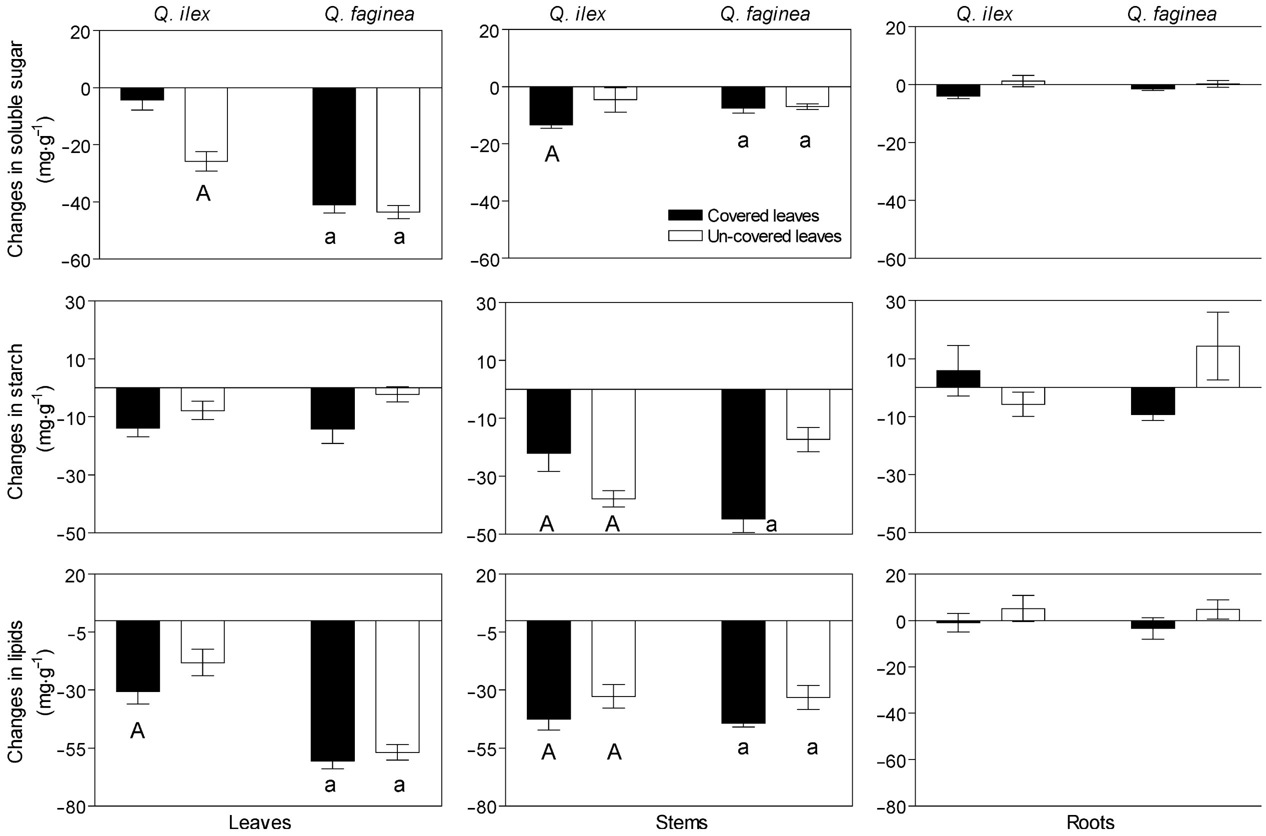

Fig. 3. Changes in the concentration of soluble sugars, starch and lipids in leaves, stems and roots of the two oak species from February to budburst in seedlings with covered and uncovered leaves. Different capital and small letters indicate differences in the reduction of compound concentrations at budburst across treatments in *Q. ilex* and *Q. faginea*, respectively (one-way ANOVA, Mann–Whitney *U*-test, $P < 0.05$) in cases where post-budburst concentration significantly differed from February concentrations (Dunnet *post hoc* test, $P < 0.05$). All seedlings were grown at 19 °C and 16 h daylength. Values are means ± SE ($n = 5$).

treatments in *Q. faginea* (Fig. 4). In *Q. ilex*, 12 °C SD led to the highest SS_{leaf} reduction, and this was the only treatment that promoted Lp_{leaf} consumption. Low temperature increased the consumption of SS_{stem} in both species and also of Lp_{stem} in *Q. faginea* (Table 3, Fig. 4). The same effect was found for St_{stem} in *Q. faginea* under LD (Table 3, Fig. 4). Mobilisation of starch from stems differed between treatments according to species (Fig. 4): $[St]_{stem}$ of *Q. ilex* was most highly reduced at 19 °C while there was no consumption at 12 °C LD. The reverse trend was found in *Q. faginea* (Fig. 4).

DISCUSSION

Budburst in response to temperature

Budburst of *Q. ilex* and *Q. faginea* was mainly controlled by temperature, as in most boreal and temperate plants (Hunter & Lechowicz 1992; Hänninen 1995; Badeck *et al.* 2004). Both co-occurring oak species displayed similar thermal time requirements, but these were higher than those of more northern species (Heide 1993a; Myking & Heide 1995). However, *Q. faginea* started to accumulate degree-days from a lower temperature threshold and, consequently, under 12 °C (mean temperature in early spring in the Iberian Peninsula for the last 40 years), the buds opened earlier than those of *Q. ilex*. This earlier budburst was additionally enhanced by long days because of the reduction in the thermal time requirements shown by *Q. faginea* under these conditions (photothermic model *sensu* Hunter & Lechowicz 1992). The earlier budburst of *Q. faginea* compared with *Q. ilex* has been observed in seedlings under field conditions when both species coexist (Mediavilla & Escudero 2003; Castro-Díez *et al.* 2006). *Q. ilex* can assimilate more photosynthates during favourable days in winter than the semi-deciduous oak, as it retains a higher proportion of foliage in this season. However, early budbreak, and thereby earlier leaf expansion, could be advantageous for *Q. faginea*, mainly growing in the understorey, given that new leaves have higher photosynthetic potential than older leaves (Niinemets *et al.* 2005). Early leaf expansion represents a phenological escape strategy exhibited by many understorey plants in deciduous forests (Uemura 1994) that is also

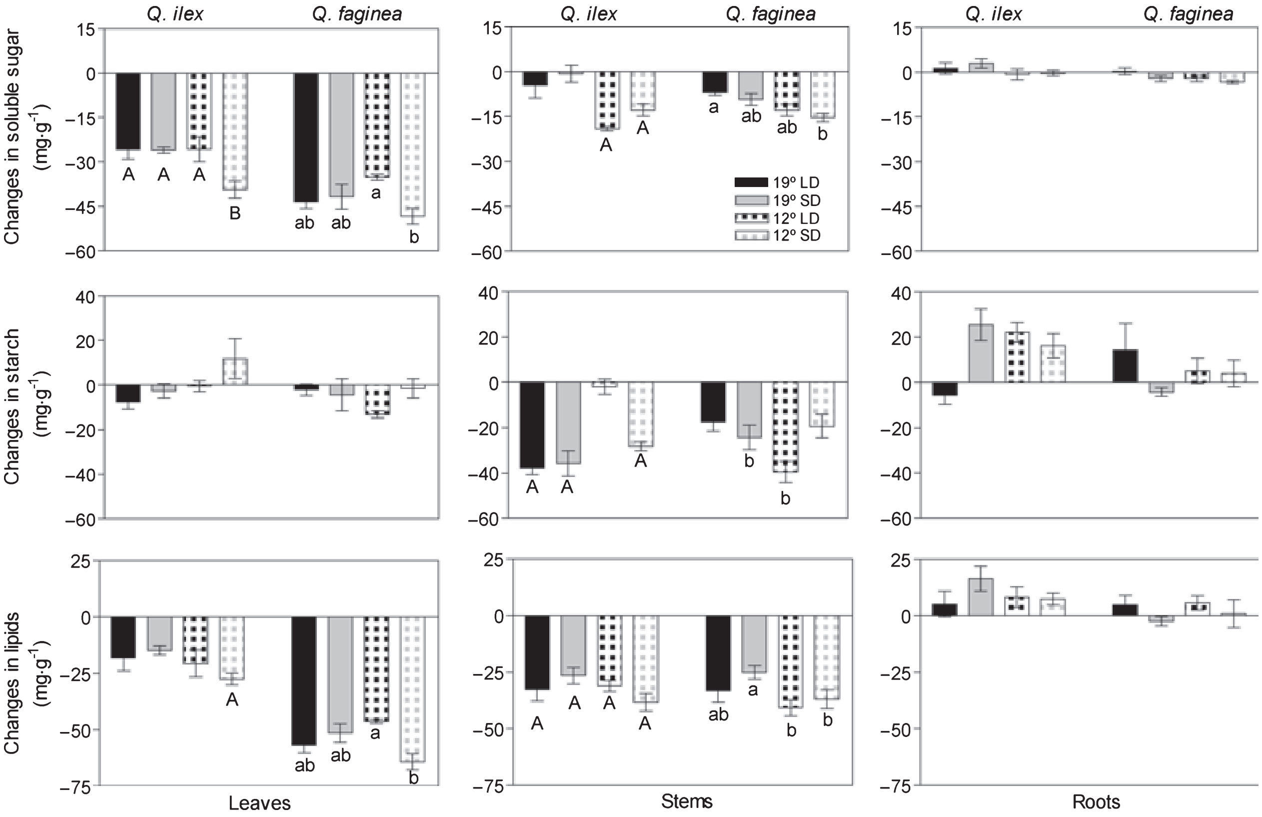

Fig. 4. Changes in the concentration of soluble sugars, starch and lipids in leaves, stems and roots of the two oak species from February to budburst among the different temperature–photoperiod combinations. Different capital and small letters indicate differences in the reduction of compound concentrations at budburst across treatments in *Q. ilex* and *Q. faginea*, respectively (Bonferroni *post hoc* test, $P < 0.05$) in cases where post-budburst concentration differed significantly from February concentration (Dunnet *post hoc* test, $P < 0.05$). Values are means ± SE ($n = 5$).

Table 3. Effects of temperature, photoperiod and their interaction on changes in soluble sugars, starch and lipids from February to budburst, for the two oak species.

	source of variation	leaf	*Quercus ilex* stem	root	leaf	*Quercus faginea* stem	root
soluble sugars	Temperature (T)	4.52*	22.54***	–	0.11 n.s.	13.01**	–
	Photoperiod (P)	4.93*	3.28 n.s.	–	4.68*	2.13 n.s	–
	T × P	4.61*	0.19 n.s.	–	8.14*	0.00 n.s.	–
starch	Temperature (T)	–	19.6***	–	–	4.53 *	–
	Photoperiod (P)	–	10.4**	–	–	1.89 n.s.	–
	T × P	–	14.4**	–	–	7.74*	–
lipids	Temperature (T)	2.86 n.s.	1.72 n.s.	–	0.13 n.s.	5.43*	–
	Photoperiod (P)	0.16 n.s.	0.02 n.s.	–	3.99 n.s.	1.99 n.s.	–
	T × P	1.30 n.s.	2.99 n.s.	–	14.05**	0.27 n.s.	–

Analysis was not performed in roots because root reserves did not change significantly between pre- and post-budbreak harvests. *F*-values and level of significance.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s., not significant.

exhibited by juveniles of the very same tree species making up the canopy (Augpurger & Bartlett 2003). Similarly, deciduous species from temperate forest of the southern hemisphere were found to leaf out earlier than coexisting evergreen species (Dungan *et al.* 2003).

Regional climate predictions for the Mediterranean region indicate a mean annual temperature rise of 2.2–5.5 °C, with some models and emission scenarios suggesting rises of up to 6.5 °C by 2100 (Christensen *et al.* 2007). Thus, the increase in spring temperature with respect to the current mean spring temperatures used here represents a possible, albeit extreme, thermal scenario for the end of the 21st century in Mediterranean ecosystems. Our results suggests that both species may advanced budburst by up to 6–10 days per degree spring temperature increase, which agrees with other advances in phenological events reported during recent decades for many plant species (Walkovszky 1998; Sparks *et al.* 2000; Chmielewski & Rötzer 2002). However, the advance was remarkably higher in *Q. ilex,* resulting in similar dates of budburst for the two species at 19 °C. Thus, early spring resources, otherwise mainly exploited by the newly produced leaves of *Q. faginea*, will be equally available for new leaves of both species under this warmer climate scenario. Therefore, this differential response to warming might alter biomass production and the competitive relationships between seedlings of these two *Quercus* species, as reported for other temperate species (Kramer *et al.* 1996). It has been demonstrated that long periods of warm conditions in tundra ecosystems alter the relative dominance across species (Chapin *et al.* 1995). Therefore, the budburst response to temperature found here for these two *Quercus* species should be further studied in field conditions in long-term experiments.

Interactive effects of temperature and photoperiod on budburst

Although photoperiod did not significantly affect the date of budburst in *Q. ilex*, it had a stabilizing effect on budbreak in both species, because the advance in the date of budburst promoted by higher temperatures was shorter under shorter days. This effect has also been found in many non-Mediterranean species (Hänninen 1990; Heide 1993b; Häkkinen *et al.* 1998). In contrast, budbreak of *Q. faginea* was directly affected by photoperiod, partly explaining its lower budburst responsiveness to increasing temperatures. We postulate that this differential response may be related to the wood anatomy of the two species. Photoperiod determines the start of secondary growth in many temperate species (Rossi *et al.* 2006). Given that *Q. faginea* is a ring-porous species (Villar-Salvador *et al.* 1997) that loses most of its hydraulic conductivity in winter through embolisms (Tyree *et al.* 1993; Sperry *et al.* 1994), it cannot leaf out until a specific daylength promotes the production of a new ring of xylem to supply the budburst water requirements. In contrast, *Quercus ilex* showed greater budburst sensitivity to temperature because, as a diffuse-porous tree (Villar-Salvador *et al.* 1997), its previous-year xylem may still supply water for budburst.

Water availability, which is predicted to decrease in the Mediterranean region, might also have an effect on the sensitivity of budburst to changing environmental conditions. Plants from arid zones show higher responsiveness to temperature than plants from more mesic habitats (Bertero *et al.* 2000). These authors suggested that this sensitivity allows species from drier zones to advance, and even extend, the growing period before summer drought. Indeed, although both *Quercus* species co-exist in many Iberian forests, *Q. ilex* occupies drier sites than *Q. faginea* (Blanco Castro *et al.* 1997). These observations are consistent with the results of a previous study where water stress led to an earlier budburst in *Q. ilex*, whereas no change was observed in *Q. fagine*a (V. Sanz-Pérez & P. Castro-Díez, unpublished results).

Use of carbon reserves *versus* current photoassimilates during budburst

Some studies have suggested that budburst, stem elongation and leaf expansion in many temperate species is mainly based on current available photoassimilates (Dickson *et al.* 2000; Barbaroux & Bréda 2002; Hoch *et al.* 2002). In our study, *Q. faginea*, the semi-deciduous species, showed a greater reduction in starch reserves in the stem when the leaves were covered, reflecting that the previous-year leaves supply part of the carbon requirement for budburst. Therefore, the retention of part of the leaf photoassimilate pool by *Q. faginea* seedlings during winter contributes to keep high carbon stores available to face other adversities, which may then be overcome at a later date. In contrast, photosynthesis suppression in *Q. ilex* seedlings did not increase reserve consumption, suggesting that budburst is based on carbon reserves rather than on current photoassimilates.

There was some consumption of carbon reserves (soluble sugars, lipids and starch) from February to budburst in the aboveground parts of both *Q. ilex* and *Q. faginea*. However, there was no change in reserve concentration in the roots of either species. This might be partly explained by the role of acorn reserves, which might still supply carbon for the second-year seedling budburst (Hewitt 1998). However, this result is also consistent with the finding that saplings and adult trees mobilise reserves from organs closer to sinks (Barbaroux & Bréda 2002; Landhäusser & Lieffers 2003; Cerasoli *et al.* 2004). In a previous report, adults of *Q. ilex* were found to mobilise soluble sugars and lipids from leaves and stems for spring growth, but not starch (Cherbuy *et al.* 2001). In our study, changes in the concentrations of soluble sugars, starch and lipids were similar in magnitude, but starch was only mobilised from stems. Because these seedlings had less biomass in stems than in other organs (data not shown), the starch pool appeared to contribute less than the other carbon reserves to budburst. Soluble sugars may act as osmolites to enhance water movement towards the budburst points (de Faÿ *et al.* 2000), while lipids may supply carbon skeletons and energy for shoot elongation (Jordy 2004). In contrast, other authors have observed a reduction in starch from leaves and roots during spring (Fischer & Höll 1991; Egger *et al.* 1996; Cerasoli *et al.* 2004). A possible explanation is that starch was consumed later than soluble sugars and lipids in those plant parts (Meletiou-Christou *et al.* 1994; Jouve *et al.* 2007) because it supports phenological events that occur after budburst, such as leaf expansion (Egger *et al.* 1996; Jordy 2004) or shoot elongation.

According to our second hypothesis, temperature and photoperiod modified the use of carbon reserves, both species exhibiting similar patterns. High temperature, particularly when combined with a long photoperiod, reduced the consumption of soluble sugars, starch and lipids in leaves and/or stems, probably due to photosynthetic enhancement through the temperature increase, as reported for Mediterranean oaks (photosynthesis is greatly enhanced by temperatures in the 5–20 °C range in Mediterranean oaks, see Corcuera *et al.* 2005). This may be beneficial for the seedlings, as their reserve pool will be available to face other adversities, such as to resprout after herbivore attack or fire, and also to cope with respiratory demands during stressful periods.

Although our results are derived from seedlings and we cannot extrapolate them to adult trees, this study suggests the importance of global warming for budburst during the regeneration phase of Mediterranean forests. Besides modelling work and field observations, controlled experiments can help us to understand the mechanisms that explain different budburst responsiveness to the environment. In conclusion, the present study demonstrates that the effects of the same increase in spring temperature differ between seedlings of coexisting species due to different responsiveness of budburst to temperature and photoperiod. In the case of *Q. ilex* and *Q. faginea*, different xylem anatomy, rather than different patterns of carbon reserve use, may explain their different budburst responsiveness to temperature. Our results suggest that the current differences in the timing of the budburst (earlier in *Q. faginea* than in *Q. ilex*) would be reduced in a global warming scenario, which could modify the competitive relationships between seedlings of these two species in the regeneration phase of mixed forests.

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