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Changes in leaf phenology of three European oak species in response to experimental climate change

Xavier Morin[1,2], Jacques Roy[1], Laurette Sonié[1] and Isabelle Chuine[1]

[1]Centre d'Ecologie Fonctionnelle et Evolutive, Equipe BIOFLUX, CNRS, 1919, route de Mende, F–34293 Montpellier Cedex 5, France; [2]Present address: Forest Ecology, Institute of Terrestrial Ecosystems, Department of Environmental Sciences, Swiss Federal Institute of Technology ETH, Universitätstrasse 22, CH–8092 Zürich, Switzerland

Author for correspondence:
Xavier Morin
Tel: +41 4 46320765
Email: xavier.morin@cefe.cnrs.fr

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Summary

- Because the phenology of trees is strongly driven by environmental factors such as temperature, climate change has already altered the vegetative and reproductive phenology of many species, especially in the temperate zone. Here, we aimed to determine whether projected levels of warming for the upcoming decades will lead to linear changes in the phenology of trees or to more complex responses.
- We report the results of a 3-yr common garden experiment designed to study the phenological response to artificial climate change, obtained through experimental warming and reduced precipitation, of several populations of three European oaks, two deciduous species (*Quercus robur*, *Quercus pubescens*) and one evergreen species (*Quercus ilex*), in a Mediterranean site.
- Experimental warming advanced the seedlings' vegetative phenology, causing a longer growing season and higher mortality. However, the rate of advancement of leaf unfolding date was decreased with increasing temperature. Conversely, soil water content did not affect the phenology of the seedlings or their survival.
- Our results show that the phenological response of trees to climate change may be nonlinear, and suggest that predictions of phenological changes in the future should not be built on extrapolations of current observed trends.

Introduction

Temperature is widely considered to be a major factor controlling the phenology of boreal and temperate tree species (Sarvas, 1972, 1974; Schwartz, 2003). Several studies have documented changes in the phenology of trees such as advancement of leaf unfolding and flowering of 2–3 d per decade on average during the last 50 yr (Keeling *et al.*, 1996; Menzel, 2000; Penuelas *et al.*, 2002; Walther *et al.*, 2002; and see the meta-analysis of Root *et al.*, 2003; Gordo & Sanz, 2005; Menzel *et al.*, 2006; Richardson *et al.*, 2006), and delay of leaf senescence of 1 or 2 d during the same period (Myneni *et al.*, 1997; Menzel & Fabian, 1999; Menzel *et al.*, 2001). These changes have been attributed to the temperature increase that many regions have experienced in the last decades because of global warming (Menzel *et al.*, 2006). Such changes in the growing period of trees have important consequences on carbon cycling (Chapin *et al.*, 2002) and on the Earth's climate system because of the feedback between vegetation and atmosphere (Betts *et al.*, 1997; de Noblet, 2000). Changes in the plant reproductive period also have important consequences on the reproductive success of populations, and thus on their dynamics (Sherry *et al.*, 2007). Moreover, changes in growth or reproductive phenology have major consequences on species interactions, either positive or negative which affect the dynamics of communities (Edwards & Richardson, 2004; Cattadori *et al.*, 2005; Sherry *et al.*, 2007). Therefore, it is imperative to precisely assess the impact of the on-going and future climate change on species phenology. However, to achieve this task one crucial question arises: Can we use the trends observed in the last decades as a predictive tool to forecast phenological changes for the future? (Primack *et al.*, 2009). So far, these trends appear linear with an average global warming of 0.6°C, but will they be conserved with a mean global warming of 3°C as projected for the middle of this century in some climate scenarios? (IPCC, 2007). To answer this question we need to consider the response of

individuals to warming through an experimental approach, using various levels of warming to explore how their phenology will respond in the long term.

Pioneer studies have consisted of transfer experiments, showing that phenology – and especially leaf bud burst – will be affected by future climate change (Beuker, 1994). However, the temperature increase in these experiments was often not realistic regarding the climate predictions for the future. During the last decade, several experimental works have been conducted to study the impact of global climate change on species phenology. Most of these studies have been conducted on herbaceous species (Sherry *et al.*, 2007) and the intraspecific variability of response has rarely been studied (Franks *et al.*, 2007; Doi *et al.*, 2010). Some other experiments tested the effect of an increasing CO_2 concentration or an increasing temperature, or both, on tree species phenology. In most cases, phenology was not affected by increasing CO_2 concentration (Guak *et al.*, 1998; Norby *et al.*, 2003; Korner *et al.*, 2005; Asshoff *et al.*, 2006; Kilpelainen *et al.*, 2006), but it was affected by increasing temperature. Leaf unfolding date was often advanced by increasing temperature (Repo *et al.*, 1996; Guak *et al.*, 1998; Arft *et al.*, 1999; Hollister *et al.*, 2005; Kilpelainen *et al.*, 2006), but some studies showed contrasting effects (Norby *et al.*, 2003) or no effect (Jones *et al.*, 1997). However, the response of leaf senescence was highly variable, from strong delay (Norby *et al.*, 2003) to advancement (Kilpelainen *et al.*, 2006), or absence of response (Jones *et al.*, 1997; Arft *et al.*, 1999). Recently, some authors have also tested the effect of increasing rainfall on herbaceous plant reproductive phenology (Sherry *et al.*, 2007) and found no significant effect.

Field studies that experimentally simulated warming have mainly used open-top chambers, either closed (Kennedy, 1995b; Kilpelainen *et al.*, 2006; Walker *et al.*, 2006), or not (Repo *et al.*, 1996; Jones *et al.*, 1997; Arft *et al.*, 1999; Hollister *et al.*, 2005), and night screens (Van Wijk *et al.*, 2003; Prieto *et al.*, 2009). In open-top chambers warming is achieved through high transmittance of solar radiation into the chamber. In night-screen systems, temperature is increased only during the night by reflectance of the infrared radiation emitted by the surface. However, in such passive temperature-enhancing systems, unwanted confounding ecological effects may occur, such as strong changes in moisture, nonconstant warming, different diurnal and night warming, site disturbance, wind exclusion and downregulation of photosynthesis (Kennedy, 1995a; Marion *et al.*, 1997; Ainsworth & Long, 2005; Kimball, 2005). Much fewer studies have used free-air temperature increase (FATI) systems, to overcome these problems (Price & Waser, 1998; Saavedra *et al.*, 2003; Loveys *et al.*, 2005). Such experimental efforts remain scarce, have focused only on temperature increase (except Sherry *et al.*, 2007), and were not designed to study vegetative phenology. Furthermore, these experiments usually concern annual plants, and thus do not explore the long-term impact of changing climatic conditions on plants.

In this study we aimed to determine whether projected levels of warming for the coming decades will lead to linear changes in the phenology of tree species or if more complex responses can emerge. To this end, we focused on three European oak species: common oak (*Quercus robur*), pubescent oak (*Quercus pubescens*) and holm oak (*Quercus ilex*). These species were chosen because they have contrasting geographical ranges: common oak is a temperate–boreal species, holm oak is a Mediterranean species and pubescent oak has an intermediate distribution (see the Supporting Information, Fig. S1). They have different leaf habit (common oak and pubescent oak are deciduous while holm oak is evergreen) and different tolerance to drought stress (Rameau *et al.*, 1989, 2008), are congeneric and they have a high economic importance in Europe. To our knowledge, of these three species, long-term phenological records are only available for common oak (Cannell *et al.*, 1999; Menzel *et al.*, 2001; Ahas *et al.*, 2002), which showed a mean advancement of 3.3 d per decade in leaf unfolding date.

We monitored the phenology (leaf unfolding and leaf colouring dates) of individuals from different populations of each species in a nonintrusive climate change field experiment using FATI systems and precipitation reduction systems. We sought to answer the following questions: Are the measured changes in phenology linear with temperature increase and consistent with those currently recorded in natural populations? Do the changes in phenology vary among species and populations (i.e. at both interspecific and intraspecific levels)? Is the survival of individuals affected by changes in temperature and precipitation?

Materials and Methods

Material sampling

C. 200–300 acorns from three populations of *Q. robur* L. and *Q. ilex* L., and two populations of *Q. pubescens* Willd. were sampled in October 2002 (Table 1, Fig. S1). The populations were chosen so as to sample contrasted climatic conditions within the species' range. The latitudinal amplitude of the sampled populations ranged from 2° to 22° depending on the species and the longitudinal amplitude ranged from 4° to 10°. Acorns were stored for a few days in moist sand at 5°C before sowing. In November, acorns were sown directly in the experimental field.

Experimental setup

The seedlings were cultivated in an experimental field site in Montpellier, France (43°64′N, 3°86′E, 57 m above sea level (a.s.l.)) for 3 yr (2003–2005), in a 50-cm deep clay-loam soil

Table 1 Location of the populations sampled for each oak species

Species	Population number	Site	Country	Location
Quercus robur	1	Tartu	Estonia	58.4°N; 26.7°E
	2	České Budějovice	Czech Republic	48.6°N; 14.3°E
	3	Lille	France	50.6°N; 3.1°E.
Quercus pubescens	1	Ménars	France	47.4°N; 1.2°E
	2	Montpellier	France	43.6°N; 3.9°E
Quercus ilex	1	Montpellier	France	43.6°N; 3.9°E
	2	Mitra	Portugal	38.4°N; 8.5°W
	3	Oléron Island	France	45.8°N; 1.3°W

and with a mean pH of 8. The experimental field was divided into 30 1.25 × 1.25 m plots grouped into five blocks. In each block, six plots received either ambient temperature conditions or increased temperature treatment of +1.5°C or +3°C compared with ambient temperature, and either ambient precipitation or reduced precipitation by 30% compared with ambient precipitation during spring and summer. Six different treatments (three temperature levels × two precipitation levels) were thus obtained. Treatments' levels were chosen according to climate predictions for the French Mediterranean region for 2100 (IPCC, 2001). The experimental design was a factorial split plot on a randomized complete block design. The treatments with a warming of +1.5°C and +3°C compared with ambient will be referred as 'T+' and 'T++' respectively in the following text.

Elevated temperature treatments were achieved by two infrared heaters (80 cm, 800 W, Vulcanic; Paris, France) equipped with reflectors that were installed at 1 m height laterally at the eastern and western border of the plot to avoid shading. It has been rigorously shown that the infrared heaters do not generate any visible light to influence phenology (Kimball, 2005). Moreover, such a design allows warming of plots during night and day. At each plot air temperature was monitored by infrared sensors (Raytek; Thermalert MID MIC, Berlin, Germany) at a height of 1 m, which were protected within a plastic cap. As these sensors were revealed to be poorly robust in outdoor conditions, supplementary thermocouples and PT100 sensors were installed in two of the five repetitions, and periodic measurements with an infrared manual sensor (Raytek; Thermalert MID MIC, Berlin, Germany) were made.

The plots of the reduced water supply treatment were equipped with an automatic, extensible shelter system composed of six plastic gutters. The shelter was activated by a rain sensor 12V (Kemo, Langen, Germany) and reduced incoming rainwater by *c.* 30% during spring and summer. During the summer 2003, which was particularly hot and dry, all plots were occasionally watered with 15 l and 10 l of water for normal and reduced water supply treatments, respectively, in order to attenuate drought stress, but in respect to summer precipitation norms usually recorded in Montpellier. Soil water content (volumetric water content in % of soil volume) of each plot was measured by Time Domain Reflectrometry (Trase Model 6050X1, Soilmoisture; Santa Barbara, VA, USA) at 0–15 cm and 0–30 cm depth. The waveguides (15 cm and 30 cm) were permanently installed.

All instruments were piloted by Datascans V4.52E 1998, which were themselves piloted by a DasyLab 6.0 2001 program (MeasXGmbH & Co. KG; Moenchengladbach, Germany) running on a delocalized computer.

Each plot was sown with a community of local annual and perennial herbaceous plant species and the three *Quercus* species. Oak seedlings were thus cultivated in competition with the local herbaceous community. Each plot was composed of a central plot of 0.75 m × 0.75 m within which the individuals were recorded, surrounded by a border of 0.25 m planted with individuals of the same species. Acorns were sown directly on the plots with a homogeneous density, each individual separated from its oak neighbours by 31.5 cm and from its herbaceous neighbours by 10.5 cm, and each individual surrounded by one individual of each of the other species in order to have homogeneous competition conditions within each plot. The sowing design was identical on each plot, within which three individual acorns of each provenance of each oak species were sown, but only one individual was selected to be monitored. Thus, nine oak individuals were present on each central plot, with the third *Q. pubescens* individual, not measured, being one individual of either the two provenances sampled. Thus, 240 seedlings were monitored during the experiment.

Measurements

The phenology of each individual was monitored for 3 yr. We recorded the date of first leaf unfolding (i.e. date when the first leaf of the terminal shoot was unfolded) and the date of leaf colouring of the two deciduous species, *Q. robur* and *Q. pubescens* (i.e. the date when half of the leaves of the individual had changed colour – turned from green to yellow). We then obtained the length of the growing season by calculating the difference between the date of leaf unfolding and the date of leaf colouring for each individual (note that this was only possible for the two deciduous species). Survival was also monitored all along the experiment. We also recorded the total leaf area and the total specific leaf area (SLA) of the seedlings of the two deciduous species. Leaf area was measured on senescent leaves each year with a leaf area meter.

Statistical analyses

We tested for a differentiation among species, among provenances within species and among treatments of the

different traits measured. We analysed the variance of leaf unfolding and senescence dates, and length of the growing season as well as the total leaf area and SLA with a linear mixed-effects model using the *lme* procedure of the R 2.4 2006 statistical software (Ihaka & Gentleman, 1996). The phenological variables satisfied the homoscedasticity requirement for the analysis. Both total leaf area and SLA were log-transformed to satisfy the homoscedasticity requirement. Because both temperature increase and precipitation reduction affected soil water content, we used the annual mean soil water content at 30 cm depth of each plot as a covariable in the model instead of using the precipitation reduction treatment as a fixed factor. This choice was made because the soil water content of the plots with reduced precipitation was significantly lower than that of the controls at 30 cm depth. The repetitions and the plots nested within the repetitions were declared as random effects. As soil depth varied linearly along the repetitions from 64 cm to 80 cm, a covariable 'soil depth' was added into the model. The variable 'year' was declared as a random effect in the model to take interannual variability into account. All other factors were declared as fixed factors. The normality of the residuals was checked by the use of Q–Q plots and the homogeneity of variances by a nonparametric Fligner's test. We also performed *post hoc* Tukey HSD tests to test pairwise comparisons. For each dependent variable, the best model was selected according to AIC (Aikaike's Information Criterion) comparisons.

We also tested the effect of species, provenances within species and treatments on the survival of the 240 individuals at the end of the experiment with a generalized linear mixed-model (*lmer* procedure in R 2.4 2006) with a binomial distribution of errors and a logit link function. Effects were tested with χ^2 statistics, by comparing the ratio of the residual log-likelihood of the model to a χ^2 statistic with a number of degrees of freedom corresponding to the difference of parameters between models. The best model was selected according to AIC comparisons.

Results

Effect of treatments on plots temperature and soil water content

The monthly climate conditions (temperature and precipitation) during the whole duration of the experiment are shown in Fig. 1. Leaf surface temperature measures showed that the surface temperature of warming treatments was increased with respect to ambient temperature by 1.54 ± 0.39°C (n = 190) and 3.06 ± 0.39°C (n = 190) and that the temperature difference was maintained all day long and all year. Plots with reduced precipitation had significantly a lower soil water content than the controls

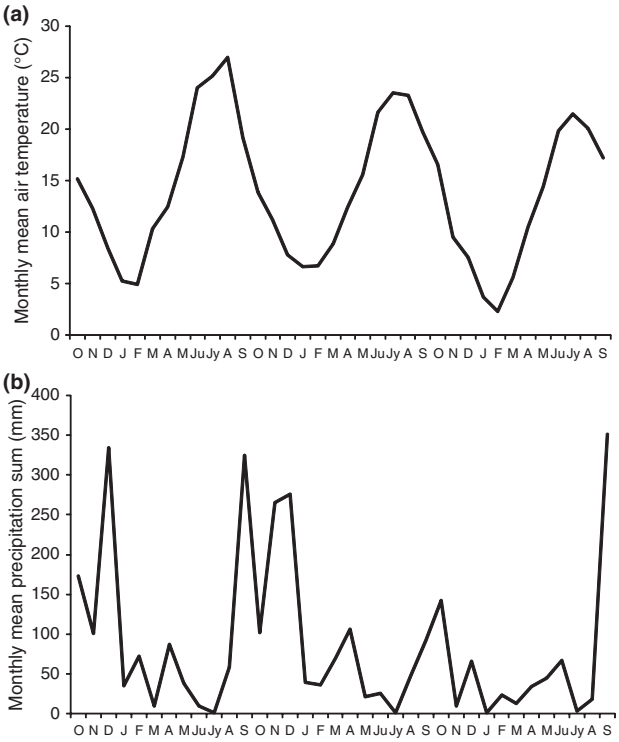

Fig. 1 Climatic conditions of the site during the duration of the experiment (Oct 2002–Oct 2005): monthly variation of mean air temperature (a) and sum of precipitation (b).

(−4.6%, $P < 0.01$ at 15 cm depth, and −3.6%, $P < 0.01$ at 30 cm depth) (see Fig. S2). The soil water content was also significantly affected by temperature (−1.0% $°C^{-1}$, $P < 0.001$ at 15 cm depth, and −0.9% $°C^{-1}$ at 30 cm depth, $P < 0.001$).

Selected models

The best models selected using AIC are shown in Tables 2–3. For the leaf unfolding date, leaf senescence date, and length of growth season, only the variable year was selected as a significant random effect. For leaf traits and survival analysis, no random effect was selected. Raw data are presented in Table S1.

Leaf unfolding

Species differed for their leaf unfolding date (Table 2). *Quercus ilex* had an earlier leaf unfolding date than the two other species by 22 d, on average, and *Q. pubescens* and *Q. robur* did not differ significantly in their leaf unfolding date (P = 0.98, Tukey HSD test). At the intraspecific level, leaf unfolding dates did not differ among populations of *Q. ilex* and *Q. robur*, while they did among populations of *Q. pubescens* (Table 2, P = 0.04, Tukey HSD Test).

Temperature increase had a strong effect on leaf unfolding date whatever the species or population (Table 2,

Table 2 Analysis of variance of the leaf unfolding date, the senescence date, and the delay between leaf unfolding and senescence dates

	Leaf unfolding date	Leaf senescence date	Growing season length
Source of variation	*F*	*F*	*F*
Species (S)	$65.92_{(2,540)}$***	$113.60_{(1,248)}$***	$97.73_{(1,243)}$***
Population (Pop)	$6.89_{(3,540)}$***	$61.80_{(2,248)}$***	$71.23_{(2,243)}$***
Temperature (T)	$19.72_{(2,540)}$***	$0.50_{(2,248)}$	$6.97_{(2,243)}$**
Water content (WC)	–	$0.47_{(1,248)}$	$0.69_{(1,243)}$
S × T	$1.99_{(4,540)}$†	$2.98_{(2,248)}$†	$3.77_{(2,243)}$*
S × WC	–	$6.68_{(1,248)}$*	$6.57_{(1,243)}$*
T × WC	–	$0.98_{(2,248)}$	$0.06_{(2,243)}$
Pop × T	$0.94_{(6,540)}$	$0.92_{(4,248)}$	$0.16_{(4,243)}$
Pop × WC	–	$0.60_{(2,2548)}$	$0.38_{(2,243)}$

The table shows type III *F*-statistics, followed by the degrees of freedom in parentheses. For the covariable Water content, the estimate of the slope is given.
†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3 Analysis of variance of survival of individuals at the end of the common garden experiment (i.e. after 3 yr)

Source of variation	Survival
Species (S)	$0.99_{(2)}$
Population (Pop)	$0.90_{(5)}$***
Temperature (T)	$0.91_{(2)}$***
Soil depth	$0.98_{(1)}$*

The table shows log-likelihood ratio (χ^2), followed by the degrees of freedom in parentheses.
*, $P < 0.05$; ***, $P < 0.001$.

Fig. 2a). Leaves appeared significantly earlier by 8.1 d, on average, in the heated plots T+ compared with control, and by 13.08 d, on average, in the heated plots T++ compared with control (Fig. 2a). The interaction between temperature treatment and species was marginally significant (Table 2), mostly because *Q. ilex* showed a much stronger response to warming (16.6 d advance between warming treatments and control) than *Q. pubescens* and *Q. robur* (9.8 d and 5.9 d advance between warming treatments and control, respectively; Fig. 2a). The leaf unfolding date was not significantly affected by variation in water content.

Leaf unfolding dates varied significantly with the year, and this is especially true when comparing 2003 with 2004 and 2005, with much earlier mean leaf unfolding in 2003 than 2004 or 2005 across species (not shown). The summer in 2003 was very warm in comparison with normal years throughout Western Europe (Schär & Jendritzky, 2004), and this was verified in our experimental site (Fig. 1). At the species level, mean leaf unfolding occurred much earlier in 2003 in *Q. ilex*, with a mean difference of 50.1 (± 2.02) d between 2003 and 2004, and of 41.2 (± 1.54) d between 2003 and 2005. Deciduous species showed an opposite trend, with mean differences of −37.7 (± 2.03) d for *Q. pubescens* and −39.8 (± 1.12) d for *Q. robur* between 2003 and 2004, of −32.8 (± 1.75) d for *Q. robur* and −34.8 (± 1.09) d for *Q. pubescens* between 2003 and 2005.

Leaf senescence

Leaf colouring date differed significantly between the two deciduous species (Table 2). *Quercus pubescens* had a later leaf colouring date than *Q. robur* (37.5 d later). As for leaf unfolding date, leaf colouring dates did not differ among populations of *Q. robur* (Tukey HSD Tests, Table 2) but it did differ between the two populations of *Q. pubescens* ($P < 0.001$, Tukey HSD Test, Table 2) with the Montpellier population having a later colouring date than the Ménars population.

The temperature treatment and the water content did not have a significant effect on the leaf colouring date (Table 2). However, species did respond differently to water content (Table 2). Leaf colouring dates varied significantly among years.

Growing season length

Deciduous species showed different lengths of growing season (Table 2). *Quercus pubescens* had a longer length of growing season than *Q. robur* (36.1 d later). As for leaf colouring and leaf unfolding, the length of growing season did not differ among populations of *Q. robur* ($P > 0.1$ for each comparison between populations, Tukey HSD Tests) but it did between the two populations of *Q. pubescens*, the Montpellier population had a longer growing season than the Ménars population ($P < 0.001$, Tukey HSD Test).

Temperature increase had a significant effect on the growing season but not the water content (Table 2). Individuals kept leaves, on average, 9.1 d more in the T+ heated plots compared with control, and 7.1 d more in the T+ heated plots compared with T++ heated plots (Fig. 2c). The response to warming and water content of the soil varied among species (Table 2, Fig. 2c). *Quercus pubescens* seedlings exhibited a longer growing seasons in the heated plots than in the controls ($P < 0.05$ Tukey HSD Test), while no difference was detected in *Q. robur* seedlings ($P = 0.71$ Tukey HSD Test).

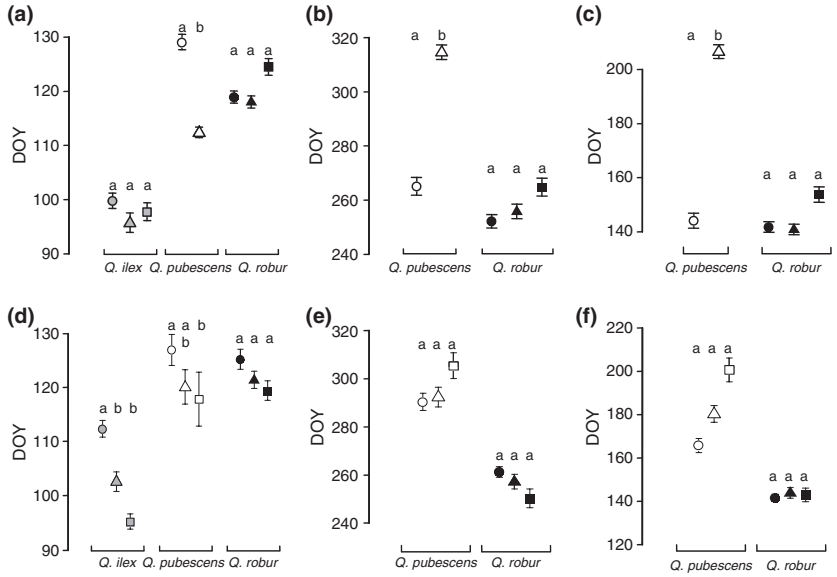

Fig. 2 (a–c) Mean date of leaf unfolding date (a), mean date of leaf colouring date (b) and mean length of growing season (c) among populations for each species. Lower-case letters show significant differences among populations (after *post hoc* Tukey comparisons). For each species: circles, population 1; triangles, population 2; squares, population 3 (according to Table 1). (d–f) Response of leaf unfolding date (d), leaf colouring date (e), and length of growing season (f) among warming treatments for each species. Lower case letters show significant differences among treatments (after *post hoc* Tukey comparisons). For each species: circles, control treatment; triangles, level of warming *c.* +1.5°C; squares, level of warming *c.* +3°C. Tinted symbols, *Quercus ilex*; open symbols, *Quercus pubescens*; closed symbols, *Quercus robur*. DOY, day of the year (days after January 1).

The length of the growing season of the two deciduous species varied significantly among years (Table 2) and was 27 d longer in 2003 than in 2004, and 22 d longer in 2004 than in 2005 (not shown).

Survival analysis

Although no difference was detected among species (Table 3), survival varied significantly among populations in *Q. pubescens* and *Q. robur*, but not in *Q. ilex* (Table 3, Fig. 3a). The southernmost population of *Q. pubescens* (Montpellier, France) had a lower survival than the northernmost population (Ménars, France). The westernmost population of *Q. robur* (Lille, France) had a lower survival than the easternmost population (Ceske Budejovice, Czech Republic).

The warming treatments had a significant effect on survival (Table 3), with greater mortality on warmer plots (Fig. 3b), and it was the case for each species studied. The survival of individuals during the whole experiment did not vary significantly with water content (Table 3).

Total leaf surface and SLA

These two traits showed differences across species, but no response to experimental warming (Table S2). No effect was detected for the water content of the soil for both traits.

Discussion

Impact of climate change on species leaf phenology

Our experimental warming of 1.5°C and 3°C significantly hastened the leaf unfolding date and tended to lengthen the growing season whatever the oak species and population. Conversely, the decreasing of the soil water content caused by the experimental warming and decreased precipitation did not affect the leaf unfolding and senescence dates, and did not change the length of the growth season. Both of these results are consistent with the many studies that have shown a strong effect of temperature on plant phenology (Sarvas, 1972; Hänninen, 1987; Cannell, 1989) and the few studies which have tested whether change in water availability has an effect on plant phenology (Sherry *et al.*, 2007). Furthermore, our results showed that the strength of the response can vary greatly among species.

The most important result of this study is the negative covariation observed between the leaf unfolding advancement and the level of experimental warming. Indeed, the advancement rate of the leaf unfolding date (calculated as number of days per degree Celsius of warming) was -5.6 d °C^{-1} in treatment T+ and -4.4 d °C^{-1} in treatment T++ across all species, and the difference between the two mean rates of warming is significant ($P = 0.02$, *t*-test). Reported observed changes in leaf unfolding date of *Q. robur* in the last decades in natural populations vary between

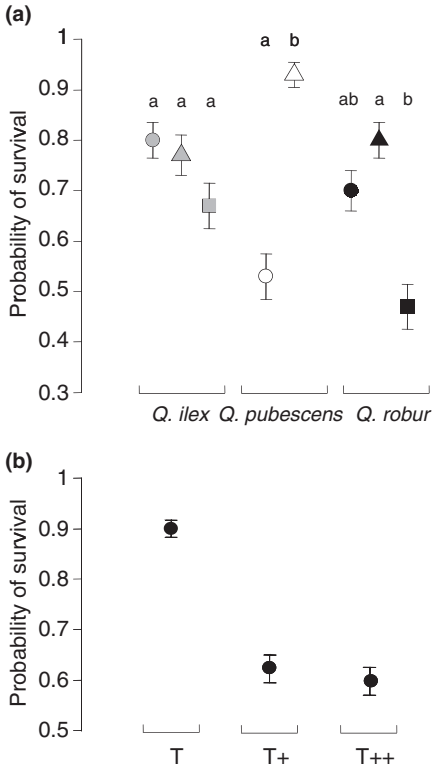

Fig. 3 Response of individuals' survival to different sources of variation. (a) Mean probability of survival of individuals among populations. Lower-case letters show significant differences among populations (after *post hoc* Tukey comparisons) for each species. Circles, population 1; triangles, population 2; squares, population 3 (see Table 1 for population identification). Tinted symbols, *Quercus ilex*; open symbols, *Quercus pubescens*; closed symbols, *Quercus robur*. (b) Mean probability of survival of individuals among warming treatments. T, control treatment; T+, level of warming *c*. +1.5°C; T++, level of warming *c*. +3°C. Letters show significant differences among populations (after *post hoc* Tukey comparisons).

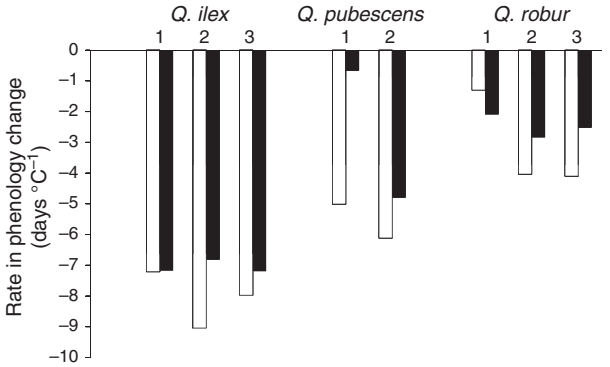

Fig. 4 Mean advancement rates between the warming treatments (T+, open bars; T++, closed bars) and the control treatment (Rate+, advancement rate in days °C^{-1} for the T+ treatment; Rate++, advancement rate in days °C^{-1} for the T++ treatment) for each sampled population (*Quercus ilex, Quercus pubescens* and *Quercus robur*). The population number corresponds to the number given in Table 1. T+, level of warming *c*. +1.5°C; T++, level of warming *c*. +3°C.

−2.8 d °C^{-1} and −9.6 d °C^{-1} for *Q. robur* (Cannell *et al.*, 1999; Ahas *et al.*, 2000; Menzel *et al.*, 2001), while here we measured a mean rate between 2.4 d °C^{-1} and 3.1 d °C^{-1} for this species (Fig. 4). No such specific records exist for *Q. ilex* and *Q. pubescens*. Our results thus suggest first, that the rate of advancement of spring events such as leaf unfolding or flowering might decrease in the next decades, and second, that the trend in the advancement of phenology is likely to be nonlinear if warming continues to increase.

Although this result is, to our knowledge, original and unprecedented, it is nevertheless consistent with a modelling study that predicted a decelerating rate of leaf unfolding advancement with global warming for adult North American temperate and boreal trees (Morin *et al.*, 2009). The results are also consistent with another experimental study on two North American deciduous temperate tree species, *Acer saccharum* and *Acer rubrum*, showing very weak changes in leaf unfolding dates with a simulated warming of 4°C (Norby *et al.*, 2003). More generally, our findings are consistent with the results of Murray *et al.* (1989), who were the first to suggest that the timing of budburst could be delayed owing to climatic change.

This slowing down in the advancement rate of leaf unfolding date may be caused by two different effects. First, as warming progresses, winter temperatures in some regions no longer drop below 5°C, on average (e.g. Mediterranean basin in winter 2006–2007), resulting in a lack of chilling temperatures required to break bud dormancy (Morin *et al.*, 2009). The delay of dormancy break causes a delay in leaf unfolding or flowering even if the period between the two development stages is accelerated by warmer temperatures during the spring. Second, a saturation of the effect of increasing temperature on cell growth during the quiescence phase may appear as this effect has been shown to be nonlinear for several tree species (e.g. logistic type response, Sarvas, 1972).

However an alternative hypothesis to explain our results may be a possible effect of daylength in constraining the advancement of spring phenology. Photoperiod has indeed been shown to affect the phenology of some species, especially boreal ones (Myking & Heide, 1995; Linkosalo *et al.*, 2006). Thus it is possible that under strong warming conditions (e.g. +3°C), the limiting factor in phenological advancement has shifted from temperature requirements to light requirements. Unfortunately, precise knowledge about this effect of day length on leaf phenology is presently too scarce to assess the plausibility of such hypothesis.

Furthermore, the results related to *Q. robur* should also be tempered according to the location of the sample populations studied here. Indeed, the experimental site is located in the southern part of the species distribution (Jalas & Suominen, 1972–1999), while the populations

of Lille (Northern France) and České Budějovice (Czech Republic) are located *c.* 1000 km north of the species southern range limit. We can thus hypothesize that the difference among the thermal conditions of the experimental site (Montpellier 3.9°E, 43.6°N) and those of the sites of the sampled populations was too large in comparison with the experimental additional warming to detect any difference in the phenological response of the individual seedlings among populations of *Q. robur*. Furthermore, according to our results, the more southerly a species was distributed, the stronger the rate of advancement of leaf unfolding was found. It is also noticeable that for both *Q. pubescens* and *Q. ilex*, a population was sampled from a site very close to Montpellier. Thus, at least for these two populations, there is no bias caused by the geographical position of the experimental site, and the *Q. ilex* population showed a stronger rate of advancement than the *Q. pubescens* one (7.7 d per degree vs 5.2 d per degree, Fig. 4).

Although our calculated rates of advancement of phenology may be underestimated for some populations, especially for *Q. robur*, we believe that the trend shown here (i.e. under strong warming the rate of advancement in leaf unfolding date will stop increasing or even decline) is likely to be correct, and that the interspecific differences in the rate of advancement we measured are not merely caused by the transplantation of the populations either to a northern or a southern site compared with their current range.

Impact of climate change on seedling's survival

Our results also show that the survival of seedlings of oak species will be sensitive to change in climatic conditions. Changes in soil moisture, related to both temperature, precipitation change and soil depth appear to have strong negative impact on seedlings survival under the Southern France climate. Although our estimates of survival probability were calculated only on 3 yr and although seedlings were exposed to an abrupt warming (but still much below the typical interannual variability), our results suggest that survival in the first life stages of temperate oak species will be strongly affected by change in temperature. It is especially noticeable that this trend is observed in the three species, even for the Mediterranean species *Q. ilex*.

During the next century, we can thus expect that this strong selection induced by gradual climate change at the early stages will favour the recruitment of preadapted individuals in each cohort if the intrapopulation adaptive genetic variability is sufficiently high (Kelly *et al.*, 2003). However, the intensity of the warming will be so large that some authors predict selection will not proceed sufficiently rapidly (Jump & Penuelas, 2005), causing local extinctions, especially at range margins (Hampe & Petit, 2005).

Differential responses at the intraspecific level

Although an experiment conducted at each population's point of origin would likely have allowed stronger conclusions, differences in phenological stages at the intraspecific level were only detected in *Q. pubescens*, and not for *Q. ilex* and *Q. robur*. If previous studies have failed to report a phenological differentiation among populations (Howe *et al.*, 2003; Vitasse *et al.*, 2009), most studies using provenance tests have already shown a strong differentiation among populations for tree species (Ducousso *et al.*, 1996; Jensen & Hansen, 2008). The experiment site is closely located to the southern edge of the distribution of *Q. robur*, which may have prevented any detection of phenological differences among populations. However, the relevance of our estimation of local adaptation is probably not affected, as the experimental warming is still much below the typical interannual variability in temperature. More generally our analysis may suffer from a lack of power to properly address that question, as we had only one individual per population in each plot. The fact that our results show intraspecific differentiation for *Q. pubescens* with this potential lack of power suggests that differentiation levels should be strong in this species.

Limits

Caution should be taken when comparing experimental results on seedlings phenology with natural observations of adult trees. But to our knowledge there is no study monitoring the leaf phenology of individuals from the seedling stage to the adult tree stage. Regarding survival, it is likely that adult trees show greater resilience to water stress than seedlings (Nakashizuka, 2001). However, experimental results for the response of seedlings to climate change are important because seedlings are a critical tree life-stage and are likely to be among the most sensitive to extreme conditions, such as heat and drought (Svensson *et al.*, 2005). A decrease in the recruitment may threaten the regeneration of populations as effects on seedlings can drive demographic patterns for years to come, and lead to change in geographic distribution.

Regarding the nonsensitivity of leaf phenology to reduced rainfall, there are caveats to our conclusion. First, although the rainfall exclusion treatment significantly decreased the soil water content at both 15 cm and 30 cm depth, this change, which was chosen according to IPCC scenarios (IPCC, 2001), might have been too mild to produce a significant stress on the seedlings. Second, although this experiment showed that increased water stress had no effect on the vegetative phenology of oak seedlings for 3 yr, we cannot exclude that such stress may affect seedlings or saplings after several more years. Furthermore, incoming rainfalls were only reduced during the current growing

season in this experiment following IPCC's scenarios for the Mediterranean Basin (IPCC, 2001). Increased drought during the previous winter may induce a downward change in phenological stages the following year, through after-effects.

Consequences of our findings

Using experimental results, we showed here that future warming should continue to advance tree species leaf unfolding as it is currently observed in natural populations (Menzel, 2000; Parmesan & Yohe, 2003; Root *et al.*, 2003; Menzel *et al.*, 2006), but our results also suggest that the phenological response of species to increasing temperature should be nonlinear, and can vary strongly across species. Our results are for seedlings, which necessarily restricts the extrapolation of this experimental work to other tree life-stages. However, the fact that tree species respond differently can affect the competitive relationships between species in the long term (Post *et al.*, 2001). For example, numerous ecophysiological studies show that sessile oak (*Q. petraea*) is more drought-resistant than common oak. Thus, in the context of global warming, many researchers estimate that sessile oaks should be relatively less affected than common oaks. Differential change in species phenology may thus affect the composition of forest communities and the geographical ranges of species (Morin *et al.*, 2008). More generally, these findings highlight the necessity to monitor and follow several species to better assess the effect of climate change on phenology, and suggest that predictions of phenological changes in the future should not be built on extrapolations of current observed trends.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Geographical distribution of *Quercus robur*, *Quercus pubescens* and *Quercus ilex*, and the locations of the populations sampled.

Fig. S2 Variation of soil water content at 15 cm and 30 cm depth in the plots in 2002–2004, according to rainfall exclusion and warming treatments.

Table S1 Mean date of leafing and leaf senescence for each year and species, according to populations, rainfall treatment and warming treatment.

Table S2 Analysis of variance of the total leaf area and of the specific leaf area.

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