

Quantifying phenological plasticity to temperature in two temperate tree species

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Summary

1. Phenotypic plasticity allows large shifts in the timing of phenology within one single generation and drives phenotypic variability under environmental changes, thus it will enhance the inherent adaptive capacities of plants against future changes of climate.

2. Using five common gardens set along an altitudinal gradient (100–1600 m asl.), we experimentally examined the phenotypic plasticity of leaf phenology in response to temperature increase for two temperate tree species (*Fagus sylvatica* and *Quercus petraea*). We used seedlings from three populations of each species inhabiting different altitudes (400, 800 and 1200 m asl.). Leaf unfolding in spring and leaf senescence in autumn were monitored on seedlings for 2 years.

3. Overall, a high phenological plasticity was found for both species. The reaction norms of leaf unfolding date to temperature linearly accelerated for both species with an average shift of –5.7 days per degree increase. Timing of leaf senescence exhibited hyperbolic trends for beech due to earlier senescence at the lowest elevation garden and no or slight trends for oak. There was no difference in the magnitude of phenological plasticity among populations from different elevations. For both species, the growing season length increased to reach maximum values at about 10–13 °C of annual temperature according to the population.

4. Since the magnitude of phenological plasticity is high for all the tested populations, they are likely to respond immediately to temperature variations in terms of leaf phenology. Consequently the mid- to high-elevation populations are likely to experience a longer growing season with climate warming. The results suggest that climate warming could lengthen the growing season of all populations over the altitudinal gradient, although the low-elevation populations, especially of beech, may experience accelerated senescence and shorter growing season due to drought and other climate changes associated with warming.

Key-words: altitudinal gradient, common garden, *Fagus sylvatica*, phenology, phenotypic plasticity, *Quercus petraea*, reaction norm

Introduction

During the postglacial period, species populations responded to global warming by migrating toward higher latitudes or altitudes, resulting in local extinctions and modifications in species distributions (Davis & Shaw 2001; Petit *et al.* 2003). Indeed, pollen records, chloroplast DNA analyses (Brewer *et al.* 2002; Petit *et al.* 2002; Magri *et al.* 2006)

and field observations (Walther *et al.* 2002) demonstrate that species migration is highly correlated with global climatic cycles. Although species migrations have been reported over the last few decades (Parmesan & Yohe 2003; Bertin 2008), in many cases the current rate of global warming might be too rapid for natural migration to successfully deliver species to suitable habitats (Rice & Emery 2003; Aitken *et al.* 2008). Some recently observed population extinctions seem to have been driven by current climate warming (Penuelas & Boada 2003; Lavergne *et al.* 2005; Lavergne, Molina & Debussche 2006).

However, populations could persist in their current location and withstand environmental changes if they have

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adaptive capacities (Lindner *et al.* 2009). Non-neutral genetic diversity and phenotypic plasticity are the two key processes that allow plant survival and development in different environments (Pigliucci, Murren & Schlichting 2006). First, a high genetic diversity among and within populations would improve opportunities of rapid adaptation to a new environment (Hamrick 2004). Common garden experiments have shown clinal variation in adaptive traits according to the climate of the tree populations (Morgenstern 1996; Howe *et al.* 2003; Premoli, Raffaele & Mathiasen 2007; Vitasse *et al.* 2009a; Viveros-Viveros *et al.* 2009). Secondly, short-term phenotypic plasticity is one of the most significant ways in which plants can react and cope with rapid environmental change (Sultan 2004; Pigliucci, Murren & Schlichting 2006; Valladares, Sanchez-Gomez & Zavala 2006; Ghalambor *et al.* 2007). Hence, under rapid climate change phenotypic plasticity rather than genetic diversity will likely play a crucial role in allowing plants to persist in their environment. At the population scale, low phenotypic plasticity in crucial characters for fitness might result in a high probability of extinction (Rehfeldt, Wykoff & Ying 2001). However, little is known about the phenotypic plasticity of many plants, particularly those with a long lifespan such as trees which may experience large changes in climate conditions during their life times (Rehfeldt, Wykoff & Ying 2001; Valladares *et al.* 2005). Therefore, to assess population responses to climate change, it is crucial to quantify both the magnitude of phenotypic plasticity and the rate of genetic evolution.

Phenotypic plasticity driven by temperature significantly influences species distributions. Traits that are highly significance for tree fitness, such as phenology, growth and frost resistance, seem to be widely implicated in these distributional patterns (Chaine & Beaubien 2001). To assess phenotypic plasticity of these functional traits along environmental gradients, we need to obtain reaction norms. Here, we conducted a reciprocal transplants experiment with multiple common gardens, in which individuals were planted in their native environment and the environments of other populations. This experimental design allows us to characterize local adaptation and to estimate the optimum conditions for the population by comparing traits in native and non-native climates (Rehfeldt *et al.* 2002; Kawecki & Ebert 2004; Savolainen, Pyhäjärvi & Knurr 2007). 'Home vs. away' comparisons are particularly interesting in the context of current global warming (Savolainen, Pyhäjärvi & Knurr 2007).

Altitudinal gradients are particularly relevant in order to study plants phenological responses to temperature because they provide a wide temperature range over a very short distance. Phenological plasticity should be of special importance for species located in mountain habitats since it is much more likely that the offspring will experience a very different climate than the parents, if seed dispersal occurs at relatively short distances up or down the mountain. This current study complements two previous studies that focused on phenological variations of temperate tree species along the same altitudinal gradient (Vitasse *et al.* 2009c) and on genetic differentiation among these tree populations (Vitasse *et al.*

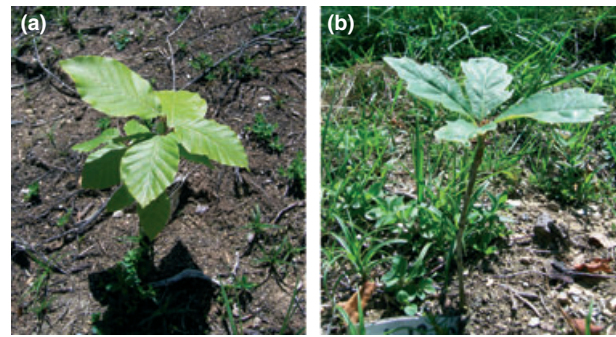


Fig. 1. Two-year-old seedlings of European beech (*Fagus sylvatica* L.) (a) and sessile oak (*Quercus petraea* (Matt.) Liebl.) (b) after transplantation at the high elevation site.

2009a). In this latter study, the authors pointed out genetic differentiation in leaf phenology among populations from different elevations. This present paper aims to focus on phenological plasticity, highlighting another aspect of the adaptive capacities of trees.

Here, through reciprocal transplant experiments along an altitudinal gradient in Pyrénées mountains, we assessed plasticity of leaf phenology of two dominant European tree species also largely used for timber industry, sessile oak (*Quercus petraea* (Matt.) Liebl.) and European beech (*Fagus sylvatica* L.) (Fig. 1). The objectives of this study were (i) to characterize reaction norms for leaf phenology to temperature in populations originating from different elevations, (ii) to test if these populations exhibit different patterns or magnitude of plasticity and (iii) to examine if these populations were currently located in their optimum climate according to their growing season length.

Materials and methods

STUDY SPECIES

Based on their large distribution in Europe, we selected two broadleaf species occurring over a large altitude range in the Pyrénées mountains: sessile oak (*Quercus petraea* (Matt.) Liebl.) and European beech (*Fagus sylvatica* L.). These two species have contrasting phenological patterns over altitudinal gradients; beech exhibits a low variation in leaf unfolding dates over the gradient and a high variation in leaf senescence dates whereas oak has a high sensitivity for both phenological events (Vitasse *et al.* 2009c).

PLANTING SITES

We set up five common gardens at different elevations: 131, 488, 833, 1190 and 1533 m in the Gave valley in Pyrénées Mountains (south-western France). This region is characterized by a temperate oceanic climate, with mean annual temperature of 12 °C and mean annual precipitation of 1079 mm at low elevation (for 1946–2001 in Tarbes, 43°11'N, 00°00'W, 360 m ASL, Météo France). The planting all occurred on north-facing slopes in open areas close to a beech forest. Locations and environmental conditions of planting sites are described in Table 1. At all sites, soils are deep and well-drained with a silty surface texture. In each planting site, the soil was tilled in

Table 1. Location and climate of the common gardens in the Pyrénées Mountains

Site	Altitude	Latitude	Longitude	Slope aspect	2007				2008			
					T_a	T_{3-5}	T_{9-11}	VPD _{max}	T_a	T_{3-5}	T_{9-11}	VPD _{max}
Laveyron	131	43°45'N	00°13'W	Flat	12.6	13.0	11.4	4.44	12.7	12.2	12.1	5.59
Lourdes	488	43°05'N	00°05'W	North	11.0	10.8	10.3	2.07	11.0	10.3	10.6	1.22
Arras-Sireix	833	42°58'N	00°08'W	North	9.6	9.3	8.5	2.26	9.5	8.5	8.8	1.36
Haugarou	1190	43°00'N	00°12'W	North	7.6	7.1	7.0	3.69	7.4	6.2	6.9	2.75
Lienz	1533	42°53'N	00°04'E	North	6.1	5.3	5.6	2.73	5.8	4.5	5.4	2.21

Altitude corresponds to the exact elevation above sea level (m); T_a is the mean annual temperature (°C); T_{3-5} is the mean temperature from 1 March to 31 May; T_{9-12} is the mean temperature from 1 September to 30 November. VPD_{max} is the average of the 10 maximum daily values of air vapour pressure deficit of August (kPa).

Table 2. Location and climate of beech and oak populations used for transplant experiments

Fagus sylvatica					Quercus petraea					
Altitude	Exact altitude	Latitude	Longitude	Slope aspect	T_a	Exact altitude	Latitude	Longitude	Slope aspect	T_a
400	488	43°05'N	00°05'W	North	11.4	427	43°08'N	00°00'W	South	12.3
800	773	42°55'N	00°02'W	North	10.3	803	42°55'N	00°02'W	South	11.0
1200	1190	43°00'N	00°12'W	North	7.8	1235	42°47'N	00°02'E	South	9.7

Altitude corresponds to the exact elevation above sea level (m); T_a is the mean annual temperature (°C) averaged over 3 years (2005–2006–2007).

September 2006 prior to planting. Herbicide was applied to remove existing weeds 2 weeks before tilling in each common garden.

POPULATION ORIGINS AND DESIGN

To set up the reciprocal transplant experiments, we used three populations of beech and oak originating from 400, 800 and 1200 m (± 50 m) of elevation in the Gave valley (Table 2). In November 2006, 2- or 3-year-old seedlings of each population were transplanted within 4 days in the five planting sites. Each common garden was divided into four separate blocks, with four seedlings from each population assigned to each block, for a total of 96 seedlings per garden (2 species \times 3 populations \times 16 replicates). Seedlings were transplanted in each block with a systematic design at spacing of 50 cm within and between rows. To minimize the impact of transplant shock, the seedling roots were covered with nutritional and protective gel. Seedlings were watered on planting date, and then received only ambient rainfall. Each common garden was treated with pesticide (deltamethrin, Pyrethroid) and molluscicide (5% metaldehyde, Metarex, to prevent slug damage) in spring 2007 and 2008. A fence was installed to prevent herbivore attack.

Survival rate was homogeneous across the five common gardens, with 62% and 81% surviving after the second growing season for oak and beech respectively. Phenological observations were therefore monitored on 149 and 194 seedlings of oak and beech respectively.

METEOROLOGICAL MEASUREMENTS

Air temperature and relative humidity were recorded every hour in each common garden (2006–2008) and sites of population origin (2005–2008), using data loggers (HOBO Pro RH/Temp; Onset Computer Corporation, Bourne, MA 02532, USA). Sensors were situated 1.5 m-high above the ground on a pole in one of the four replicate blocks. Sensors were protected by a white plastic shelter to prevent

any exposure to rain or to direct sunlight. Meteorological data of planting and population sites are summarized in Tables 1 and 2 respectively. Average annual temperatures decreased linearly with increasing elevation: temperature lapse rate was about 0.42 °C for every 100 m increase in elevation (average from 2007 to 2008). Therefore, the altitudinal gradient results in sharply contrasting climatic conditions among planting sites (amplitude of 6.3 and 7.7 °C between the lowest and the highest common gardens in autumn and spring respectively). Furthermore, we calculated the maximum air vapour pressure deficit (VPD_{max}, kPa) as the mean of the ten highest daily VPD values recorded in August (Table 1). At low elevation, values of VPD were particularly high in comparison to the other planting sites (more than 4.4 kPa), and values were overall higher in 2008 than in 2007 (3.18 and 1.71 kPa respectively).

PHENOLOGICAL OBSERVATIONS

The timing of leaf unfolding and leaf senescence were monitored from spring 2007 to autumn 2008 (2 years) by two observers. We examined each seedling every 10 days in spring from March to May and in autumn from September to December. Observations were standardized between observers throughout the first year of measurements. In spring, we recorded the stage of the apical buds from dormant winter-bud to leaf unfolding, using a 3 or 4 intermediate grading scale for beech and oak respectively (see Vitasse *et al.* 2009b). We considered that leaf unfolding date was reached for each seedling when at least one leaf was fully unfolded on the apical bud. Some buds were damaged in spring 2007 and 2008 by phytophagous insects. These damaged seedlings were removed before analyses. In autumn, we combined observations of coloration and leaf fall to more accurately estimate the end of growing season length. Percentage of missing or coloured leaves was assessed visually on each seedling. We considered that senescence date was reached for each seedling when 50% of its leaves were not functional, i.e. either coloured or fallen, following the

method provided in Vitasse *et al.* (2009c). Then, for each seedling, the dates of senescence were estimated by linear regression between two measurement campaigns.

Averages of leaf unfolding and senescence dates were calculated for each population per block and common garden as the average of the dates for all the individuals of the same population. Finally, average dates of leaf unfolding and senescence for each population at each common garden were calculated as the average date estimated for the four blocks. Growing season length was obtained by computing the difference between the senescence and the leaf unfolding date of each individual and the mean was calculated for each population per block and common garden.

STATISTICAL AND DATA ANALYSIS

An analysis of variance was made using the GLM procedure with the RANDOM statement considering the five common gardens together. The statistical model treats blocks as the fixed effect and common gardens and populations as random effects. In this paper, we are considering plasticity at the population level, as an average across individuals from each population (Richards *et al.* 2006; Valladares, Sanchez-Gomez & Zavala 2006) rather than in strict sense, at the genotype level. Since each common garden represented a different environment (different altitude), a significant main effect of altitude indicates environmentally induced phenotypic plasticity for the studied trait. A significant interaction between altitude and population indicates that the magnitude of the plastic response is dependent on the altitude of the population of origin which is the result of genetically induced phenotypic plasticity.

The relationship between phenological traits and altitude or climate of the planting sites was assessed with linear regressions and quadratic functions. Linear regressions were rejected if the probability of statistical significance (P) was >0.05 , whereas quadratic functions were rejected if P was >0.25 (according to Rehfeldt *et al.* 2002). Several temperature variables were tested to explain phenological variations: mean annual temperature, mean spring temperature and degree-days $>5^{\circ}\text{C}$ calculated from 1 March to 31 May, mean autumn temperature and degree-days $<0^{\circ}\text{C}$ calculated from 1 September to 30 November. In agreement with Vitasse *et al.* (2009c), we found that mean annual temperature was the most effective variable for explaining the growing season length and mean spring and mean autumn temperature for explaining the date of leaf unfolding and leaf senescence respectively. We therefore used these three variables

throughout the paper. A covariance analysis was used to compare slopes of the linear regression between leaf unfolding date and spring temperature.

We calculated the discrepancy between the inhabited (T_{pop}) and optimal (T_{opt}) temperature as the difference between these two values (ΔT), with T_{pop} corresponding to the mean annual temperature (2005–2007) of the origin sites of populations, T_{opt} refers to the optimal temperature at which growing season length is maximized within the environmental range examined. All the analyses were performed using SAS 9.1 software (SAS, version 9.2; SAS Institute, Cary, NC, USA).

Results

LEAF UNFOLDING

We found a strong significant effect of altitude on leaf unfolding date in 2007 and 2008 for both species, amounting to more than 76% of the total variance (Table 3). Timing of leaf unfolding differed among populations for beech both in 2007 and 2008, occurring earlier for the high elevation population (originating from 1200 m) whatever the elevation of the common garden (Fig. 2). In contrast, no population effect was found for oak. For beech, a weakly significant interaction between population and altitude was found only in 2007, amounting to 2.5% of the total variance (Table 3).

Mean leaf unfolding timing occurred around day 125 (5 May) and day 130 (10 May) for beech and oak respectively. According to temperature, reaction norms were linear ($R^2 > 0.80$, $P < 0.001$) but the magnitude of phenological plasticity was slightly higher for oak populations (ranging from -5.7 ± 0.8 SD to -6.3 ± 0.5 days degree $^{-1}$) compared to beech populations (ranging from 4.9 ± 0.6 to 5.8 ± 0.6 days degree $^{-1}$; Fig. 2). At low elevation (100 m), leaves of the two species unfolded at the same date, around day 110 (20 April), whereas at high elevation (1600 m), seedlings flushed about days 148 (28 May) and 155 (4 June) for beech and oak respectively (Fig. 2). For both species, no difference in slopes between leaf unfolding and spring temperatures was found among the three populations when the phenological measurements of the 2 years were pooled (Fig. 2, P -value from 0.80 to 0.92).

Table 3. Analysis of variance of leaf unfolding (LU) and leaf senescence dates (LS) for oak and beech seedlings, to examine the effects of altitude of common gardens, population origin and blocks within gardens

	Fagus sylvatica								Quercus petraea							
	LU ₂₀₀₇				LU ₂₀₀₈				LS ₂₀₀₇				LS ₂₀₀₈			
	VC		F		VC		F		VC		F		VC		F	
	VC	F	VC	F	VC	F	VC	F	VC	F	VC	F	VC	F	VC	F
Altitude	77.5	70***	85	285***	19.6	5.7*	11.7	2.1 ^{ns}	81.1	133***	76.3	47***	5.2	1.8 ^{ns}	8.5	9.7**
Population	2.4	5.6*	3.5	23.9***	0	0.5 ^{ns}	0	0.7 ^{ns}	0	0.02 ^{ns}	0.2	0.8 ^{ns}	3.6	2.8 ^{ns}	0	3.6 ^{ns}
Block	—	3.7***	—	5.3***	—	1.5 ^{ns}	—	0.3 ^{ns}	—	2.4**	—	1.7 ^{ns}	—	2.3*	—	8.5***
Population \times Altitude	1.1	2.5*	0.03	0.9 ^{ns}	3.3	1.6 ^{ns}	0.05	0.3 ^{ns}	0	1.3 ^{ns}	2.3	2.1 ^{ns}	6.3	1.6 ^{ns}	0	0.4 ^{ns}

VC, ratio (in %) of variance component of each random effect to total variance estimated; F, Fisher value; ns, non-significant.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

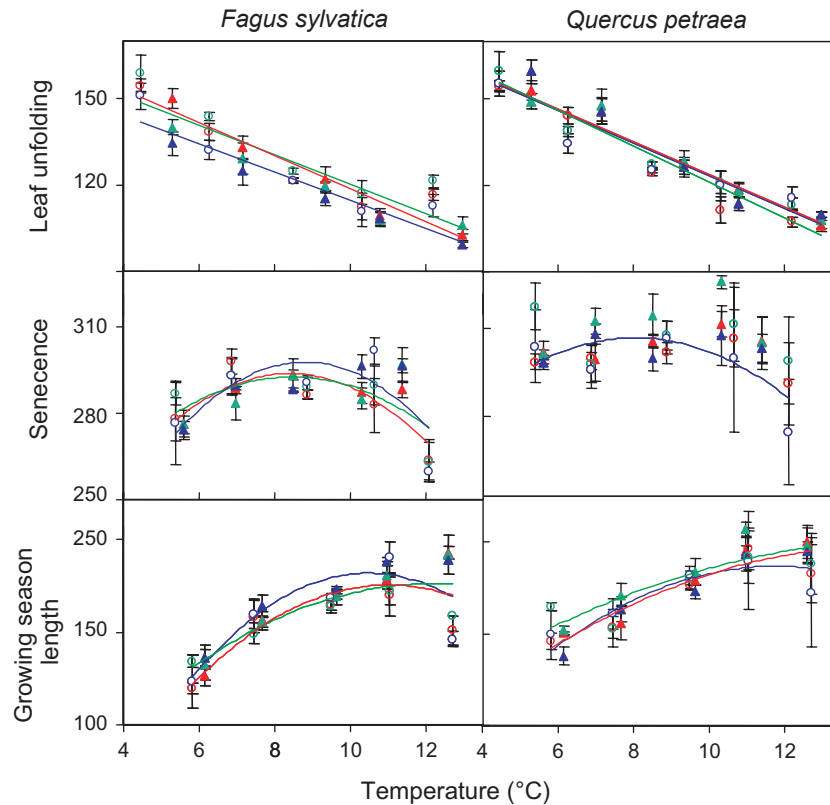


Fig. 2. Dates of leaf unfolding, leaf senescence (DOY, Day of the Year) and growing season length (number of day) according to temperature variables of common gardens for the three studied populations of each species. Mean spring, autumn and annual temperature were used for leaf unfolding, senescence and growing season, respectively. Colours correspond to the selected populations: red, population from 400 m, green, 800 m and blue, 1200 m. Full triangles and open circles represent the phenological observations in 2007 and 2008 respectively. Values correspond to the mean of all individuals per common garden and per population and bars to the standard errors. Linear and quadratic regressions have been done per population for each species.

LEAF SENESCENCE

For beech, we found a significant effect of altitude on leaf senescence timing only in 2007 (explaining 20% of total variance), whereas only a slight effect was detected for oak in 2008 (8.5%, Table 3). Finally, for both species, timing of leaf senescence did not vary among populations and no significant interaction between population and altitude was found (Table 3).

Mean senescence timing occurred later for oak than for beech, around day 303 (30 October) and day 285 (12 October) respectively. For the three beech populations, senescence follows hyperbolic trends: in 2008 and to a lesser extent in 2007, leaf senescence occurred the earliest both in the coldest common garden (at 1600 m of elevation) and the warmest one (at 100 m of elevation). For oak, a similar hyperbolic response was found for the population originating from the highest elevation (1200 m) but no significant trend was observed for the two other populations.

GROWING SEASON LENGTH

We found a significant effect of altitude on growing season length in 2007 explaining 78% and 63% of total variance for

beech and oak respectively (Table 4). In 2008, this altitude effect was also significant but explained less variability than in 2007 (48% and 27% of total variance for beech and oak respectively). For both species, growing season length did not differ among populations and no significant interaction between population and altitude has been found (Table 4). The length of the growing season increased with increasing temperature for both species but was lower for beech than for oak whatever the elevation (Fig. 2): at high elevation, growing season length was about 129 and 150 days for beech and oak, respectively, whereas at low elevation the season length increased to 172 and 192 days respectively (average of the 2 years at 100 and 1600 m asl. respectively).

For both species, optimal values of growing season length were found between 10 and 13 °C of annual temperature (Fig. 2). These optimal temperature values were quite different among beech populations (from 10.5 to 12.2 °C) but quite similar among oak populations (from 11.8 to 12.7 °C). For both species the difference between current temperature at the origin of population site and their optimal values were strongly negative for populations from 800 and 1200 m ($\Delta_T < -1$ °C) whereas these differences were close to zero for low elevation populations and even positive for beech (Fig. 3).

Table 4. Analysis of variance of growing season length (GSL) for oak and beech

	Fagus sylvatica				Quercus petraea			
	GSL ₂₀₀₇		GSL ₂₀₀₈		GSL ₂₀₀₇		GSL ₂₀₀₈	
	VC	F	VC	F	VC	F	VC	F
Altitude	77.8	156***	47.5	20***	63.1	58***	27.2	32***
Population	1.5	1.1 ^{ns}	1.2	1.7 ^{ns}	1.2	3.3 ^{ns}	0	1.9 ^{ns}
Block	—	1.8*	—	2.0*	—	2.1*	—	8.2***
Population × Altitude	0	0.3 ^{ns}	0.9	0.8 ^{ns}	0	0.7 ^{ns}	0	0.5 ^{ns}

VC, ratio (in %) of variance component of each random effect to total variance estimated; F, Fisher value; ns, non-significant.

* $P < 0.05$; *** $P < 0.001$.

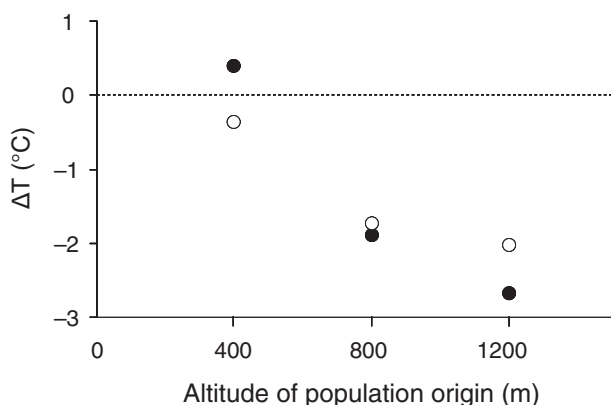


Fig. 3. Relationships of population altitude with the difference between optimal climate and inhabited climate for the growing season length (ΔT , °C) for each species. Annual mean temperature was used for assessing inhabited temperature and minimum derivative of the curve on Fig. 2 for each transplanted populations for assessing optimal temperature. Dark circles correspond to beech populations and open circles to oak populations.

Discussion

The altitudinal gradient was valuable for assessing the populations' response to climate change. Reaction norms of phenological traits in response to temperature revealed high magnitudes of phenological plasticity for both species. These magnitudes were not significantly different among populations within species. Ultimately, when considering growing season length, populations at mid to high elevations appeared to inhabit climates colder than their optimums, whereas low elevations populations of beech already endure climates warmer than their optimum.

SHAPE AND MAGNITUDE OF REACTION NORMS

The magnitude of plasticity for leaf unfolding timing was high for both species following linear clinal trends with an advance of more than 5 days degree⁻¹ of increase. Few studies have quantified phenological plasticity along altitudinal gradients. Worrall (1983) found, with a similar experiment, a linear reaction norm of leaf unfolding timing for populations of *Abies amabilis* and *Abies lasiocarpa* of about 8.3 days degree⁻¹.

Vitasse *et al.* (2009b) also reported a high phenotypic plasticity of leaf unfolding for adult sessile oak monitored over 22 years in Northern France (−6.5 days degree⁻¹). To our knowledge, it appears that leaf unfolding plasticity in response to temperature is high in temperate trees (Kramer 1995). However, for beech, a lower plasticity has been found in previous studies: between −2.0 and −2.5 days degree⁻¹ (Kramer 1995; Menzel, Estrella & Fabian 2001). The magnitude of plasticity found here is twice as high compared to these values, and to the phenotypic variability (phenological shifts) recorded in adult trees along the same altitudinal gradient (−1.9 days degree⁻¹; Vitasse *et al.* 2009b). This discrepancy could be due to the age difference, as the present study was conducted on 3-year-old seedlings. For oak, the phenological plasticity to temperature (6.0 days degree⁻¹) was comparable to the phenotypic variability monitored *in situ* (−6.5 days degree⁻¹; Vitasse *et al.* 2009b).

For leaf senescence, our results suggest that reaction norms may be hyperbolic across the temperature range due to early senescence at both low and high elevations, in particular for beech. This result contrasts to other studies in which timing of leaf senescence exhibited linear clinal variation in response to temperature: an increase in temperature lead to delayed leaf senescence (Matsumoto *et al.* 2003; Migliavacca *et al.* 2008; Vitasse *et al.* 2009c). At low elevation, this early senescence in 2008 may have been caused by the high temperature and low air humidity measured (see VPD_{max} in Table 1). Indeed, air water stress could prematurely trigger leaf discolouration and leaf fall (Breda *et al.* 2006), and in particular for VPD-sensitive species at seedling stage such as beech (Lendzion & Leuschner 2008). Oak seedlings were less affected by dry atmosphere than beech, although the high variability of senescence timing observed in 2008 at low elevations could indicate an abnormal early senescence in some individuals. Thus, the earlier senescence observed in this study could be explained by the greater vulnerability of seedlings to withstand water stress than mature tree, due to their shallow roots.

As a consequence for both species, the growing season lengthened with increasing temperature up to a certain low-to mid-elevation. The amplitude of growing season length variation along the elevation was consistently lower than the observations in other studies using adult trees *in situ* (Matsumoto *et al.* 2003; Migliavacca *et al.* 2008; Vitasse *et al.*

2009c). The lower amplitude found here is mainly the result of an earlier senescence due to a drought effect at low elevation, especially for beech.

One current interest in ecology is to distinguish for a given species whether the phenotypic variation along an environmental gradient is the result of genetic differentiation between populations or a purely environmental effect. Here, we found a high phenological variation across the common gardens (plasticity) compared to the weak but significant genetic differentiation reported in a previous study among these populations (around $-0.5 \text{ days}^\circ\text{degree}^{-1}$ for leaf unfolding; Vitasse *et al.* 2009a). Our results suggest therefore that environmental induced phenotypic plasticity rather than genetic variation explains the main part of phenotypic variations of leaf phenology observed *in situ*.

COMPARISON OF PLASTICITY MAGNITUDE AMONG POPULATIONS

Within species, we did not detect significant difference in phenological plasticity among populations of origin both for leaf unfolding and senescence events. As populations were markedly invariant in their response to environmental variation along the altitudinal gradient, we suggest that the phenological response to temperature might be similar among populations within species. Our results are in agreement with Berg, Becker & Matthies (2005) who found no evidence that central or marginal populations of *Carlina vulgaris* differed in overall plasticity. Therefore, climate change might affect the phenology of populations growing in different climates to the same extent, as suggested by other studies (Chuine, Belmonte & Mignot 2000; Berg, Becker & Matthies 2005; Vitasse *et al.* 2009b). These results are in conflict with the hypothesis that plasticity may differ among populations from different climates due to local adaptation (Sultan 1995; Baliuckas & Pliura 2003). However, more data collected along environmental gradients are needed to draw a firm conclusion. It was possible that differences in phenotypic plasticity among populations could not be detected with the small number of populations and the low frequency of phenological measurements in the current study.

POSSIBLE IMPACT OF CLIMATE CHANGE ON POPULATION FITNESS

Leaf phenology is particularly important in the assessment of fitness, due to its strong relationship with growth rate (Churkina *et al.* 2005). Indeed, an early flush has been related to a longer carbon uptake period and to an increased net annual carbon flux (Delpierre *et al.* 2009). Conversely, senescence timing is assumed to modulate to a lesser extent carbon assimilation due to both weaker photosynthesis capacity and shorter day length during the autumn season. However, senescence timing may affect growth because it is associated with nutrient remobilization, especially of nitrogen and storage of photosynthates (Norby, Hartz-Rubin & Verbrugge 2003; Keskitalo *et al.* 2005). Thus, our study highlights that

tree species have a high level of plasticity for phenological traits that could allow populations to immediately respond to new environmental conditions and cope with climatic warming (Matyas 1993; Pigliucci, Murren & Schlichting 2006; Ghalambor *et al.* 2007).

In addition, our results demonstrated that mid- and high-elevation populations should experience a longer growing season with climate warming. For beech, we found that climate change could reduce population fitness at low elevation by shortening the period of growing season mainly due to earlier leaf senescence, whereas it could increase population fitness at high elevation by increasing the growing season length and consequently growth. For oak, all populations tend to inhabit climates colder than the one corresponding to the optimum in their growing season length even though the population observed at the lowest elevation is currently close to its optimum. This study is therefore in line with previous studies showing that at high elevation, current changes in climate could drive conditions towards the optimal range for population growth (Persson 1998; Rweyongeza *et al.* 2007) while negatively affecting fitness in the southern part of the species range and at low elevation (Rehfeldt *et al.* 1999, 2002).

Author contribution

YV and CCB equally contributed to data extraction, statistical analysis and preparation of manuscript, therefore are considered as co-first authors.

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