Diversity of leaf unfolding dynamics among tree species: New insights from a study along an altitudinal gradient

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HIGHLIGHTS

- We focused on budburst dynamics (and not only on budburst date) to improve current knowledge on tree functioning.
- We highlighted the existence of thresholds of temperature effects on budburst.
- We showed that altitudinal and year effects differ.
- We identified two main species strategies in bud burst variability: leaf lifespan and shade tolerance.
- We provided new avenues to revisit budburst modelling.

ABSTRACT

Leaf unfolding is a key ecosystem parameter controlling carbon and water fluxes and affecting forest dynamics. This parameter is highly sensitive to temperature and, consequently, often used as an indicator of global change. In this paper, we analyzed weekly leaf unfolding dynamics for seven temperate species (*Fagus sylvatica* L., *Acer opalus* Mill., *Sorbus aria* L., *Quercus pubescens* Willd. *Abies alba* Mill., *Pinus sylvestris* L., *Pinus nigra* Arnold). The effects of temperature on leaf unfolding were studied *in situ* using several methods and proxies. First, in a spatial approach, leaf unfolding dates were measured along two altitudinal gradients situated on the north and south face of the Mont Ventoux to test altitudinal and slope effects. Second, in a temporal approach, the year effect was analyzed on the north face by comparing two contrasted years (2006 and 2007). Finally, the role of temperature was investigated directly by linking leaf unfolding patterns and temperatures recorded during the leaf unfolding process itself.

Two major conclusions were obtained. First, three distinct leaf unfolding patterns were revealed: a rapid sigmoid pattern for the deciduous species group, a slow kinetic pattern for the pines and an intermediate pattern for *A. alba*. Second, we found an unexpected pattern of variation in the response to spatial or temporal variation of temperature. The more sensitive a species was to temperature variations between years, the less sensitive it was to temperature variations due to altitude. Finally, we discuss that these results can be correlated to two major life history traits: evergreen vs. deciduous and shade tolerant vs. shade intolerant.

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1. Introduction

1.1. Leaf unfolding is a major plant trait

Phenology is the scientific study of cyclical biological events, such as (in plants) flowering, leaf unfolding (or budburst), seed set, and dispersal in relation to climatic conditions. These are important adaptive traits for vegetation since they determine the duration and timing of both the growing season and the reproduction period (Chuine et al., 2000) and are also a major determinant of plant species range (Chuine and Beaubien, 2001). Moreover, phenology has been shown to be related to individual plant fitness (see review by Rathcke and Lacey, 1985).

Plant phenological change is one of the most easily observed plastic responses to climatic change (Badeck et al., 2004; Parmesan and Yohe, 2003). Many studies have shown significant alterations in the timing of leaf unfolding as a response to climatic warming (Schwartz, 1998; Bradley et al., 1999; Menzel and Fabian, 1999; Beaubien and Freeland, 2000; Peñuelas and Filella, 2001; Peñuelas et al., 2002; Walther et al., 2002; Badeck et al., 2004; Bailey and Harrington, 2006; Richardson et al., 2006; Cleland et al., 2007). Peñuelas et al. (2004) also showed that changes in rainfall and water availability, another important effect of climate change,

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can cause complex phenological changes interfering with the temperature effect.

Budburst is a key factor determining the duration of the growing season for deciduous species. Davi et al. (2006) showed that the length of the leafy period may increase by 38 days from 1960 to 2100 due to climate change (early leaf unfolding contributes to 55% of this increase and delayed leaf fall to 45%). Moreover, since springtime in temperate deciduous forests corresponds to a period with a relatively high solar angle, abundant moisture due to snow fall, and/or reduced evaporation during winter, along with a flush of soil nutrients, while the corresponding autumn tends to be much darker and drier (Lopez et al., 2008), leaf unfolding date is an important feature for carbon budget. Therefore, a 15-day advanced budburst, such as the one which occurred in spring 2007 in Europe, could cause a 42% increase in Gross Primary Production (Delpierre et al., 2009). However, carbon gains obtained from advancement in leaf unfolding could potentially be offset by temperature-induced respiratory losses in autumn (Piao et al., 2008). These changes could also strongly impact wood growth. Kramer et al. (2000) in a study coupling growth models and phenological models, have showed that phenology significantly affects growth response for a given climate change scenario.

1.2. Determinants of budburst

The timing of leaf unfolding is mainly regulated by temperature in cold winter environments. Chilling temperatures break winter dormancy and subsequent warm temperatures induce leaf unfolding, which is why the phenological onset of spring correlates very well with the air temperature of the preceding months (Menzel, 2002). The leaf unfolding date results from a trade-off between increasing the vegetation length and minimizing the risk of damage by either late spring frosts or early autumn frost (Kramer et al., 2000). Several factors other than temperature can affect leaf unfolding phenology. Water availability is also an important factor, as it can regulate budburst, thus avoiding damages caused by drought, such as embolism in the xylem of woody plants (Magnani and Borghetti, 1995; Kramer et al., 2000). Lastly, photoperiod can be important for some species, such as Fagus sylvatica (Falusi and Calamassi, 1996). The importance of these factors on leaf unfolding depends on the species and the study site. For example, Wareing (1956) suggested that the controlling factor for leaf unfolding in F. sylvatica was photoperiod for southern populations and thermic conditions for the northernmost populations. Budburst, leaf fall and leaf duration of trees do not appear to be directly affected by atmospheric CO_2 concentration (Asshoff et al., 2006; Norby et al., 2003). However, CO_2 can play an indirect role through stomatal closure via higher leaf temperatures, with possible consequences under global change and related increased CO_2 levels.

Leaf unfolding date has been shown to be under strong genetic control for several species (Worral, 1983; Billington and Pelham, 1991; von Wuehlisch et al., 1995). Consequently this trait could rapidly evolve in response to local or global changes. Moreover, few studies have considered the phenology strategy jointly with other plant traits such as leaf lifespan (deciduous vs. evergreen) or shade tolerance. Leaf unfolding phenology is a key life trait that conditions both carbon and water budgets. Even within a single forest, leaf emergence during springtime varies between species over several weeks among coexisting native trees. The adaptive significance of such contrasting responses has been little addressed since Lechowicz (1984), who concluded that interspecific variations can only be explained by the well-known trade off between gains from early leafing and the danger of leaf damage caused by late frosts. This author also predicted that leaf phenology could be related to xylem structure and volume of vessels.

1.3. Shortcomings of current approaches

First, as phenology is directly linked to temperature and water condition in dry cold region, the recording of a date alone is inappropriate; temperature should also be recorded. However, using the state of chilling or forcing (i.e. the sum of temperature respectively for chilling during winter and leaf unfolding during springtime) for modelling phenology could also be misleading. In fact the sum of temperature could interact with other environmental variables (such as rainfall). Moreover, a temperature gradient caused by altitude or latitude is not equivalent to temperature variations in time because (1) variation in space affects different sets of individuals, whereas temporal changes affect each tree individually (2) variation in space combines different environmental variable changes than temporal variation.

Many studies have focused on the general pattern of phenology shifts. However, some gaps in our knowledge remain concerning: (i) the exact mechanisms involved in phenology spatial patterns; (ii) interspecific variation of leaf unfolding; (iii) variation of leaf unfolding between trees. Currently, temperature effect on leaf unfolding is often studied using either interannual data or latitudinal/altitudinal variations, without questioning whether temperature effects generated by different years, or altitudinal and latitudinal spatial variations, are similar. Consequently, the direct effect of temperature on the onset and speed of the leaf unfolding process or the possible local adaptation of budburst to one particular site have often been confused.

Finally, most studies have focused on one date to characterize the leaf unfolding process (e.g. when 80% of buds are open), which makes it impossible to accurately analyze the dynamics of this process from the first stages of leaf unfolding to the later ones, when leaves are totally expanded.

In this paper, we analyzed the weekly dynamics of leaf unfolding for seven tree species (F. sylvatica L., Acer opalus Mill., Sorbus aria L., Quercus pubescens Willd. Abies alba Mill., Pinus sylvestris L., Pinus nigra Arnold) for two altitudinal gradients, one on the north face and one on the south face of the Mont Ventoux in south-eastern France. This work made it possible to determine interspecific variation of leaf unfolding under the same climatic conditions and to clarify the role of temperature. The temperature effect was studied in the following ways: (i) by analyzing two climatically contrasted years, (ii) by determining the effect of altitude on leaf unfolding and (iii) by determining the exposure effect (north and south faces) on leaf unfolding, and by (iv) relating leaf unfolding dynamics to spring temperatures. The variation of leaf unfolding date between the trees of a species was also quantified and explained for each studied species. Finally, we concluded by drawing an overall scheme of species behaviors in the discussion.

2. Material and methods

2.1. Site

The study was conducted on Mont Ventoux (44°11′N; 5°17′E), a mountain located in the southwestern Alps (1909 m). In 1850, it was almost entirely deforested due to pastoral and forest over-exploitation, but a decrease in grazing combined with reforestation efforts undertaken in the 19th century, using pines, made it possible for post-pioneers (S. aria L, A. opalus Mill.) and shade-tolerant species (A. alba Mill. and F. sylvatica L.) to gradually colonize the planted pines which include P. nigra subsp. Nigra Arnold, and P. sylvestris L.. Plant material used and their main ecophysiological traits are given Table 1.

Climate in this area is typical of low altitude mountains with Mediterranean influences, with a 9.25 °C mean annual temperature and 1068 mm mean annual rainfall at 1000 m from years 2000 to

Table 1
Synthesis of sampled trees and species traits.

	Sample size in North	Sample size in South	Leaf life span (years)	Xylem structure	Vessel/tracheides diameter
Acer opalus	56		0.46[a, b]	Diffuse porous[c]	50–90 μm[c]
Fagus sylvatica	65	90	0.45[a]	Semi-ring porous[c]	>50–90 μm[c]
Sorbus aria	31			Semi-ring-porous[c]	30–40 μm[c]
Pinus sylvestris	45	20	2.44[a]	Ring porous	10 μm[d]
Abies alba	65		8.22[a]	Ring porous	9 μm[d]
Quercus pubescens		20	0.47[a]	Ring porous[c]	>200 μm[c]
Pinus nigra		60	3.84[a]	Ring porous	10.4 μm

[a] Withington et al. (2006).
[b] For Acer and quercus genus.
[c] WSL (Forschungsanstalt für Wald, Schnee und Landschaft) database (http://www.wsl.ch/dendro/xylemdb/index.php).
[d] Mayr and Zublasing (2010).

2005. The substrates range from calcareous lithosol to colluvium in average of one meter depth (Du Merle and Guende, 1978). The available water is low and spatially variable (between 30 and 60 mm), with a high degree of coarse elements (between 50 and 80%) and high water infiltration rate in the calcareous mother rock. Two altitudinal gradients were studied; the first was on the north face and is continuous from 961 to 1528 m, with an average slope of 30%: here, 263 trees were studied in 2006 and 2007. On the south face, the slope is moderate (<10%) and a continuous gradient cannot be followed. Consequently, 5 stands were studied in 2007, respectively at 890 m, 1115 m, 1410 m and 1530 m for a total of 190 trees.

2.2. Measurements

Timing of bud development was studied by monitoring bud dynamics weekly for every tree. Two determinations of leaf unfolding stage were conducted; one for the top (first 1/3 of the crown) and one for the bottom (last 1/3 of the crown) of the canopy and their average was used in later analyses. Six stages were used to accurately quantify leaf unfolding dynamics (Appendix A). 1: buds are dormant; 2: buds are swelling; 3: bud scales are broken; 4: leaves are emerging; 5: leaves are spread out; 6: leaves have reached their mature size. The social status (3 categories), tree height, and diameter at breast height (130 cm) were also recorded. All trees used for measurements were mapped (X, Y and altitude) using GPS equipment (Trimble Ranger Pro XR).

Temperature and relative humidity were recorded using eight Prosensor V2 air temperature/relative humidity sensors (U23-001 ± 0.2 °C; ± 2.5% RH), five located on the north face (995 m, 1117 m, 1225 m, 1340 m, 1485 m), and three on the south face (890 m, 1115 m, 1525 m) of Mont Ventoux. All sensors were placed in a clearing at 1.5 m above ground, inside a solar radiation shield (HOBO0039 RS1) protecting the sensors against direct radiation and overheating. A linear relationship between altitude, aspect (north and south) and temperature was determined and then used to calculate the daily temperature for each tree at a given altitude. The altitude effect on precipitation was determined using 7 meteorological stations on Ventoux recording monthly data from 2000 to 2005.

3. Calculation methods

Leaf unfolding was first studied by drawing the average curve of stage evolution over time for the different species. The same method was used to graphically show the altitude effect on the north face by grouping the trees into altitudinal classes (<1150; 1150 < 1400; > 1400) corresponding to three bioclimatic levels. This graphic analysis made it possible to reveal possible threshold effect.

The average date corresponding to the change from stage 4 to stage 5, the step where the measurements were the most accurate, was calculated by linear interpolation. The significance of the differences in leaf unfolding date between species and year were tested

by ANOVA and Tukey–Kramer tests using the MATLAB statistical toolbox. This test compares the means of each group for all pairwise comparisons and identifies where the difference between two means is greater than the standard error. Then, for each species and each year a linear regression model was built between leaf unfolding date ($D_{4\to5}$ the date of passage from stage 4 to stage 5) and altitude (alt).

$$D_{4\to5} = D_0 + a \times \text{alt} + \varepsilon \tag{1}$$

D_0 is the y-intercept and ε is the residuals. The value and significance of the slope (a) were used to quantify and compare the altitudinal effect between species. This was not performed for Q. pubescens because this species only exists at the lower altitude class.

The variation in leaf unfolding date among trees not due to altitude was also assessed for each species, year and face (North and South). Therefore, the sum of differences between the measured leaf unfolding date and the simulated ones calculated using the linear model was also calculated:

$$\text{std}_{\text{inter tree}} = \frac{1}{N}\sqrt{\sum_{1,N}(D_{4\to5}(\text{measured}) - D_{4\to5}(\text{calculated}))^2} \tag{2}$$

N is the number of sampled trees for one species, whereas $\text{std}_{\text{inter tree}}$ stands for the standard deviation of leaf unfolding date between trees.

The year and altitude effects were then normalized by temperature using Eq. (3)–(5). The thermal gradient during spring was quantified on the north and south faces and a linear regression was fitted:

$$T_{\text{March–April}} = T_0 + b \times \text{alt} \tag{3}$$

T_0 is the y-intercept and b is the slope of the relationship between average temperature over time (T) and altitude (alt). Leaf unfolding sensitivity to temperature (TS) can be assessed either through altitudinal variation ($\text{TS}_{\text{altitudinal}}$) or by using the between year variation ($\text{TS}_{\text{between year}}$) and was quantified as follows:

$$\text{TS}_{\text{altitudinal}} = \frac{a}{b} \tag{4}$$

$$\text{TS}_{\text{between year}} = \frac{D_{4\to5}(2007) - D_{4\to5}(2006)}{T_{\text{March–April}}(2007) - T_{\text{March–April}}(2006)} \tag{5}$$

Finally, a general linear model (type III) following a stepwise regression method (Venables and Ripley, 2002) was used on north slopes to confirm and statistically evaluate the previous results. We used the drop1 function from R software (Crawley, 2005) to determine the best model to explain $D_{4\to5}$. For this analysis, all variables at our disposal were used as independent variables. The significance of each effect was tested using ANOVA.
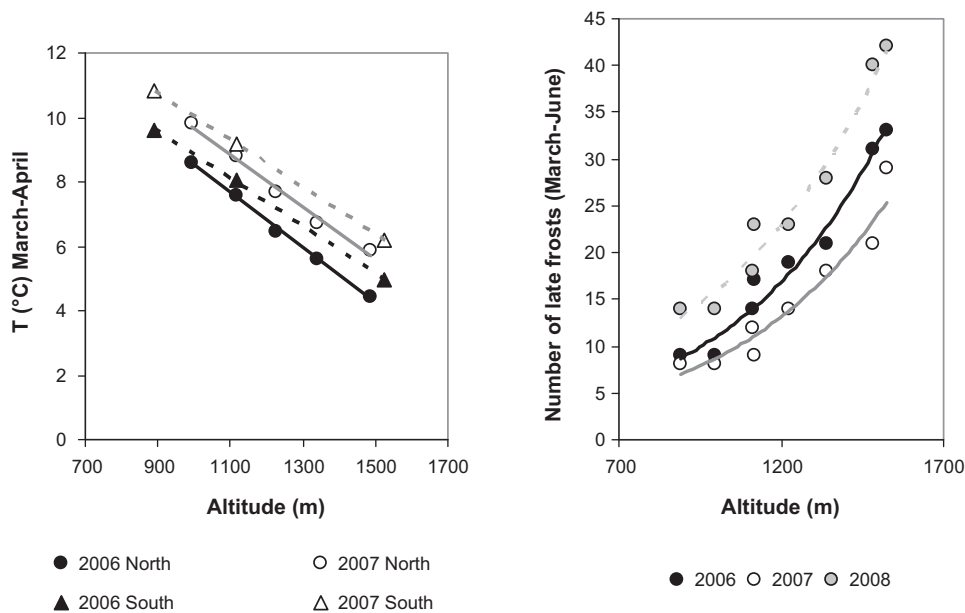
Fig. 1. Relationships between altitude and average temperature of March–April (left) or number of late frosts from 1st March to June (right).

4. Results

4.1. Meteorological data

Spring was warmer in 2007 (+1.1 °C) than in 2006 and late frost days were reduced from 20 to 16 days on average. The altitude effect on temperature during spring was linear, with slopes of 0.84 °C per 100 m respectively on the North and 0.73 °C per 100 m on the South facing slope (Fig. 1a). In both cases, there was little change in the altitude effect between 2006 and 2007 (less than 0.02 °C per 100 m). The number of frost days (temperature below zero) from the beginning of March to the end of June (after the leaves or needles are fully expanded and less sensitive to late frost) exponentially increased with altitude (Fig. 1b). Finally, as expected temperatures measured on the South face were higher than on the North face, by 1 °C in 2006 and 0.95 °C in 2007, but with more frost days in the south (+1.35 in 2006 and +4.25 in 2007), due to lower relative humidity and a higher thermal range (difference between minimum and maximum temperature).

Spring precipitation (from the beginning of March to the end of May) showed a linear increase with altitude ($r^2 = 0.97$) with a slope of 10 mm per 100 m. Spring 2006 was dry with 105 mm precipitation on the South face (1100 m) and 117 mm on the North face (1440 m), respectively, which was less than the average precipitation from 2000 to 2005. In comparison, 2007 was a wet year with a rainfall of 100 and 160 mm more than in spring 2006 for the South and North face, respectively. Overall, 2006 had a cold dry spring, while 2007 exhibited a warm wet spring.

4.2. Species effect

Three distinct types of leaf unfolding curves were found in 2007. First, deciduous trees showed a quick sigmoid development in less than 20 days and can be considered as "early species" (Fig. 2). Leaf unfolding of *A. opalus, F. sylvatica,* and *Q. pubescens,* occurred at the same period and with similar speed. Consequently, there was no significant difference between these three species on average when comparing $D_{4\rightarrow5}$ (Table 2). Leaf unfolding of *S. aria* occurred later ($p < 0.05$ using Tukey's test) but with the same dynamics. On the other hand, "late species" such as *Pinus* species had a delayed leaf unfolding with a much slower dynamics (Fig. 2) and took more

than 50 days to go from stage 1 to stage 6 in 2007. *A. alba* showed an intermediate strategy between deciduous (early) and *Pinus* (late) strategies.

4.3. Altitudinal effect

The effect of altitude was quantified by the slope of the linear regression between leaf unfolding dates (date of passage from stage 4 to 5) and altitude (Table 2). The coefficient of correlation between budburst date and the altitude was always very high ($r > 0.98$), when species, years and exposure were considered separately. The altitudinal effect was high for *Pinus* species (4.4 day delay per 100 m), low for *F. sylvatica* (1.8 day delay per 100 m) and intermediate for *A. alba* (2.8 day delay per 100 m). For these species, the slope value was found to be almost stable between the two years. However, for the two other deciduous species, *A. opalus* and *S. aria,* the altitudinal effect was not the same between the two years of measurements:

Table 2
Effect of species and altitude on leaf unfolding date (here passage from stage 4 to stage 5). Mean value by species, and respectively the slope and the correlation coefficient (r) of the relationship between leaf unfolding date and altitude.

	Average date stage 5 days of year	Altitudinal effect days/100 m	Altitudinal effect Model (r value)
2006 North			
Sorbus aria	131.4	2.56	−0.9907
Acer opalus	127.2[a]	1.10	−0.9982
Fagus sylvatica	124.0[a]	2.44	−0.9922
Pinus sylvestris	160.5	4.82	−0.9925
Abies alba	143.7	2.38	−0.9906
2007 North			
Sorbus aria	126.4	4.44	−0.9907
Acer opalus	116.8[b]	5.26	−0.9982
Fagus sylvatica	116.3[b]	1.60	−0.9922
Pinus sylvestris	156.9	4.66	−0.9925
Abies alba	135.9	3.23	−0.9907
2007 South			
Quercus pubescens	118[c]		
Fagus sylvatica	118[c]	1.54	−0.9816
Pinus nigra	152	3.85	−0.9829
Pinus sylvestris	144	2.11	−0.9999

a, b, c are groups of species whose difference are non-significant differences at $p < 0.05$.
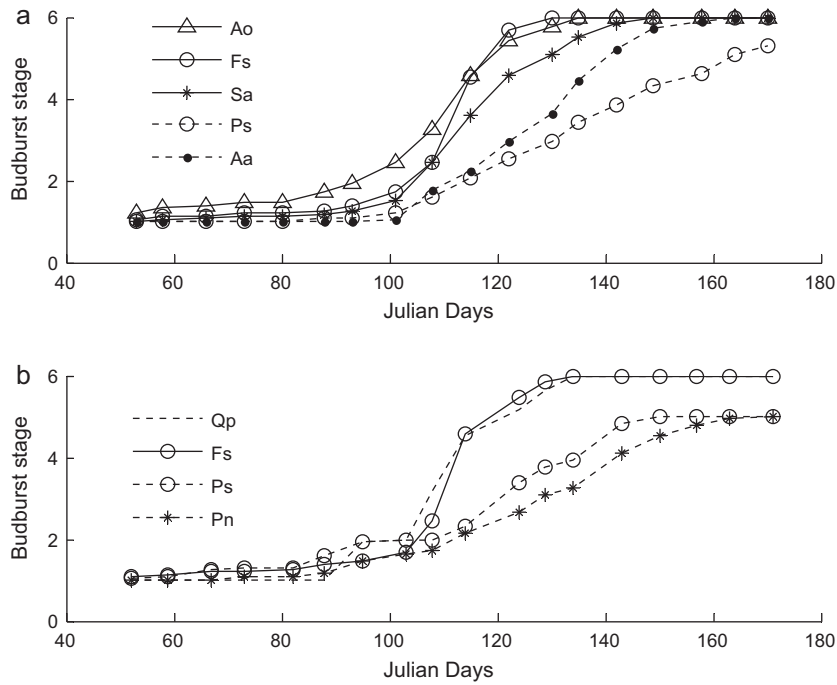
Fig. 2. Dynamics of average stage of leaf unfolding for each species in 2007 on (a) north and (b) south face. Ao: *Acer opalus*, Fs: *Fagus sylvatica*, Sa: *Sorbus aria*, Ps: *Pinus sylvestris*., Aa: *Abies alba*, Qp: *Quercus pubescens*, and Pn: *Pinus nigra*.

the altitudinal effect was very low in 2007 (1.8 days delay per 100 m on average) and much higher in 2008 (4.8 days delay per 100 m).

The altitude effect was graphically analyzed in 2007 by grouping together trees of the North face into three altitudinal classes (Fig. 3a). The periods where curves diverge were indicated in the graph using vertical lines. Fig. 3 indicates the existence of a threshold for the altitude effect for the four considered species. Trees at low altitudes (below 1150 m) showed an earlier leaf unfolding, whereas there were only small differences between trees situated at intermediate altitudes (1150–1400 m) and those situated at higher altitudes (>1400 m).
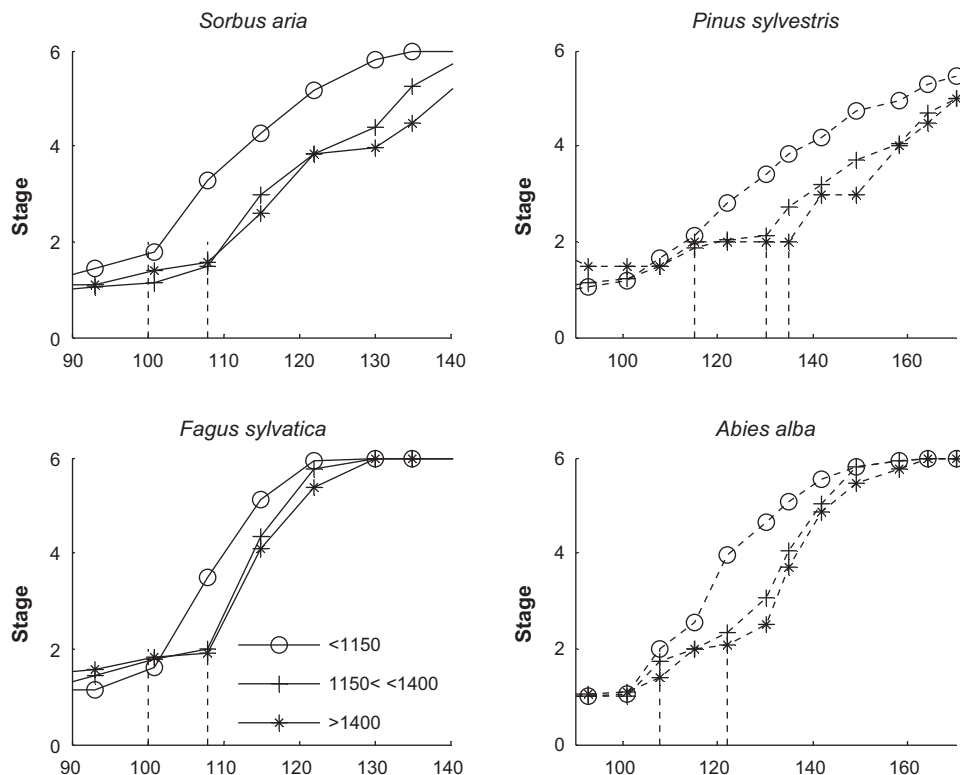

Fig. 3. Leaf unfolding dynamics of four species at three elevational classes in 2007. *Acer opalus* is not shown here.
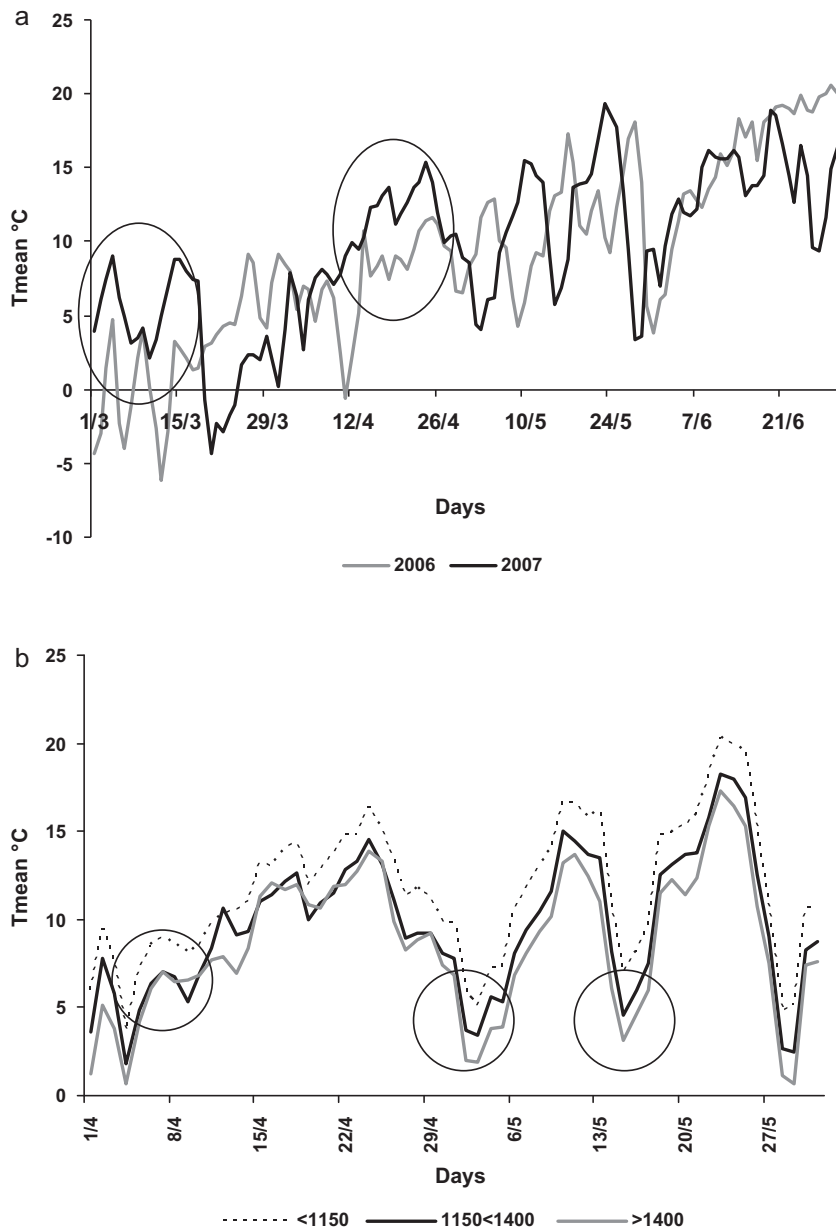
Fig. 4. (a) Evolution of the average temperature on the North slope in 2006 and 2007 from 1st March to 30 June. (b) Evolution of the average temperature in 2007 for three altitudinal classes. The encircled regions stand for the period of dissociation period in leaf unfolding dynamics between years (a) and between species (b).

4.4. Effect of exposure

The effect of exposure on leaf unfolding was only tested on *P. sylvestris* (late species) and *F. sylvatica* (early) in 2007. Leaf unfolding of *F. sylvatica* did not differ significantly between northern and southern faces (Fig. 2 and Table 2). As already shown, average temperatures were higher on the South face (Fig. 1), whereas exposure had no impact on the number of frost days in springtime. On the other hand, leaf unfolding occurred quickly on the south face in *P. sylvestris* (Fig. 2). On average, $D_{4\to5}$ was 13 days earlier on the South than on the North face (Table 2). The two dynamics were initially quite similar, the difference came from an earlier passage to stage 2 on the South face around day 90 (Fig. 2).

4.5. Year effect

Inter-annual variation was only studied on the north face by comparing leaf unfolding in 2006 and 2007. For all the species,

leaf unfolding occurred on average 6.9 days earlier in spring 2007 (Table 2). This earlier leaf unfolding was due to two warmer periods in 2007 as compared with 2006 (Fig. 4a): the first half of March, and from the 10th to the end of April. However, this year effect was not similar between species. The leaf unfolding occurred 10 days earlier in *A. opalus* in 2007, almost 8 days earlier in *A. alba* and *F. sylvatica*, 5 days earlier in *S. aria* and 3.5 days earlier in *P. sylvestris*. The advance of leaf unfolding in 2007 is due to an earlier start of the leaf unfolding process (from stage 1 to stage 2) or a quicker dynamics from stage 3 to stage 5. For *A. opalus* and *S. aria*, the process began earlier in 2007 (respectively 8 and 5 days earlier), whereas the change from stage 3 to stage 5 remained the same (respectively 2.3 and 0.2 days earlier, not significant). The three other species showed the exact opposite pattern: no difference concerning the beginning of the process between the two years, but more rapid dynamics: 6.5 days to reach stage 5 for *F. sylvatica*, 8 days less for *A. alba* and 11 days less for *P. sylvestris*. The first type of response is linked with warming in March and

Table 3
Results from analysis of variance/covariance model using stepwise method from the GLM procedure.

Variables	Df	Mean Sq	F value	Pr (>F)
Year	1	5832	244.9	<0.001
Altitude	1	3645	153.0	<0.001
Species	4	25,743	1081.0	<0.001
Year × species	4	149	6.3	<0.001
Altitude:species	4	231	9.7	<0.001
Species × status	8	79	3.3	<0.001
Status	2	93	3.9	0.02
Altitude × diameter	1	155	6.5	0.01
Diameter	1	71	3.0	0.08
Year × altitude	1	77	3.2	0.07
Altitude × status	2	21	0.9	0.42

the second one with other warming periods during April and May (Fig. 4a).

4.6. Results from general linear models

The general linear model (type III) was applied to the north face, since only one year was available for the south. Five variables played a significant role in determining $D_{4 \to 5}$ (Table 3). Species, altitude and year played co-dominant roles, indicating that both species strategy and temperature are decisive factors in leaf unfolding date prediction. Second, social status also had a significant impact on $D_{4 \to 5}$: the dominant trees (trees with crowns receiving full light from above) showed an earlier leaf unfolding. Finally, there were also four significant interactive effects. The first one was between species and year, since the year effect was not the same between species (see above). The interaction between altitude and species effects was also confirmed, since the different species had different sensitivity to altitude. The two other effects, however, were new: there was an interaction between tree status and species and between tree diameter and altitude. These two last results illustrated the importance, admittedly small compared with other major effects, but nonetheless valid, of tree size on phenology.

No interaction between altitude and year was found because the positive effect of altitude (delayed leaf unfolding at high altitudes) did not change between years despite a magnitude change for some species (*A. opalus* and *S. ari*a) as shown above.

4.7. Sensitivity to temperature assessed through year or altitude effect

As year effect was only assessed on the North slope, only five species were included in this part of the analysis. We found for the 5 species on the North slope a negative relationship between sensitivity of leaf unfolding to temperature assessed through year or altitude effect (Fig. 5a). Except for *A. opalus*, whose altitude effect changes between 2006 and 2007, the conclusion drawn was that the more sensitive a species was to altitude, the less sensitive it was to temperature variation between years.

4.8. Variance between trees

To assess variation between trees, the altitudinal effect was first removed using a linear regression. The residual variation between trees after this correction corresponds only to genetic and soil variability. We found that this greatly varied between species: from less than 0.5 day on average for *F. sylvatica* to more than 1 day for *P. sylvestris*. The variation of leaf unfolding variance between individual trees was found to be related to the sensitivity of the species to altitude effect (Fig. 5b). The more a species was found to be sensitive to altitude effect, the more it also showed higher residual variation of leaf unfolding between trees.
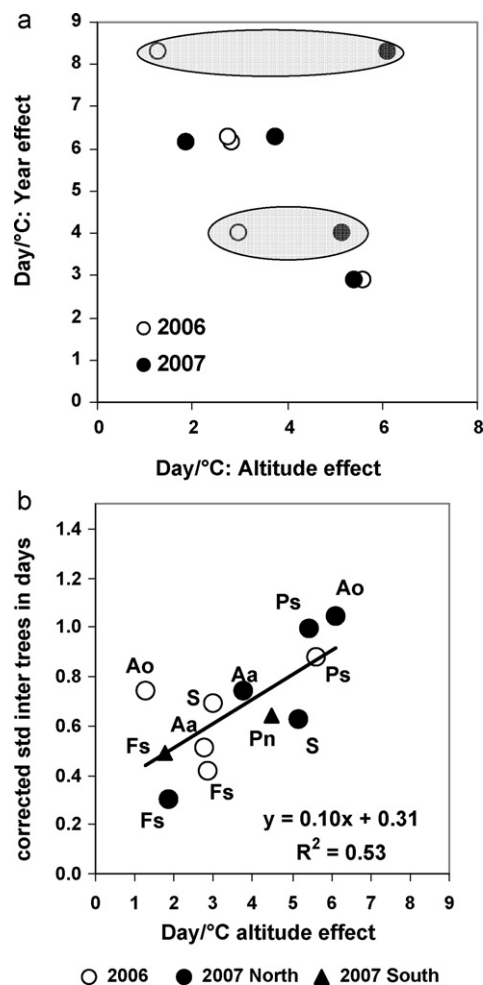

Fig. 5. (a) On the North aspect, relationships between the sensitivity to temperature assessed through interannual (named year effect) or elevational effect (named altitude effect) in Days/°C (b) Relationships between variance between trees independent of altitude effect.

5. Discussion

5.1. Summary of species strategy for leaf unfolding

Although based on a subset of species, our work reveals that there are at least four essential leaf unfolding strategies in the studied tree species. First, *early shade intolerant species* (*A. opalus*, *S. aria*) showed a year effect whose plasticity (i.e. variability between years) concerns the beginning of the process of leaf unfolding. These species were also probably sensitive to water availability, which explains a variable altitudinal effect that was more pronounced in a wet year than in a dry year. These characteristics are consistent with the autecology of shade intolerant deciduous species that need a very quick leaf unfolding strategy to avoid shade.

Second, *early shade tolerant species* (*F. sylvatica*) were characterized by plasticity between years affecting the speed of the process. This result agrees with another study in the U.S., where *Fagus* sp. was the fastest species to achieve full leaf expansion (Lopez et al., 2008). In our study, this species showed a low altitudinal effect, in spite of quite a high year effect. This contradictory result might be due (i) mechanistically to the short period of the leaf unfolding dynamic meaning there is little time for differences in accumulated daily temperatures between altitudes but potentially high differentiation between years or (ii) to a local adaptation of *F. sylvatica* at higher altitudes which means less forcing requirements (i.e. sum

of temperatures for leaf unfolding) than at low altitudes. This local adaptation was shown in common garden experiments for *F. sylvatica* by von Wuehlisch et al. (1995) where provenances from higher elevations tended to flush earlier than those from lower elevations.

Third, *late shade tolerant species* (*A. alba*) had the same characteristics as *F. sylvatica* but with a delayed leaf unfolding and a slightly higher altitude effect. In fact, the slower the process speed, the higher the differentiation between altitudes can be, since there is a higher cumulative difference between temperatures.

Fourth, *late shade intolerant species* (*Pinus* sp.) also showed a high plasticity of process speed during unfolding, but had an opposite behavior with altitude playing an important role, whereas the year effect was low. The slower leaf unfolding speed (up to more than two months) can explain this pattern. Increasing the duration of the leaf unfolding process certainly accentuated the altitude effect but also eliminated the year effect: between two years there was statistically less difference between two long periods than between two short periods.

As opposed to Lechowicz' (1984) hypothesis, differences in the vascular system do not explain the observed differences. In fact, vessel diameters of *F. sylvatica* and *A. opalus* are similar (between 50 and 80 μm, Table 1), whereas vessel diameter *of S. aria* are smaller (around 30 μm, Table 1).

The first axis of adaptation is quite obvious, late species (*Pinus* species and *A. alba*) are evergreens with long leaf lifespan (8–9 years for *A. alba*, 4–5 years for *P. nigra* and 2–3 years for *P. sylvestris*). Consequently, photosynthesis can occur before new leaf unfolding, and an early leaf unfolding with a related risk of frost damage is therefore not necessary. For deciduous species, the vegetation's growing season length is shorter and strongly depends on the date of leaf unfolding. The necessity of an early development of shoot explains both the earliness and the speed of the budburst process.

The second axis of adaptation seems to be shade tolerance. A link between shade tolerance and phenological strategy has already been assessed (Kikuzawa, 2003; Lopez et al., 2008). In our study, this tolerance has no impact on the earliness of the leaf unfolding process, while one could think that leaf unfolding in shade intolerant species would be earlier, to take advantage of light in early spring (Lopez et al., 2008). The differentiation between the two types of species arises from (i) the plasticity of the response to altitude and (ii) the origin of the plasticity between years. Shade tolerant species exhibit a higher sensitivity to April and May temperatures and avoid frost risks by speeding up the leaf unfolding process, with little time spent in stages where frost risk is maximal when leaves are not fully expanded (Cox and Levitt, 1969). Shade intolerant species, on the other hand, are more sensitive to March temperatures and perhaps water availability. These species probably avoid frost by delaying the beginning of the process, in particular when there is less precipitation, because it increases the risk of embolism (Borghetti et al., 1993), especially in open sites where evapotranspiration is higher. For the evergreens, *A. alba* and *Pinus* species differ only in the speed of leaf unfolding, which is quicker for shade tolerant *A. alba*. This can be explained by a trade off in carbon allocation. Shade tolerant species invest in new needles, which are more efficient than older ones (Niinemets and Lukjanova, 2003), to fully use the available light during spring. On the contrary, shade intolerant species do better in allocating energy to roots or height growth. Withington et al. (2006) found that root production of *Pinus nigra* in Poland was almost double that of *A. alba*.

5.2. Revisiting temperature effect: consequences on phenology modelling

Our average temperature effect agrees with other studies. For instance, Vitasse et al. (2009) found a leaf unfolding date variation between 2.03 and 7.48 days per degrees for similar species, with

results close to ours for *F. sylvatica* and *A. alba*. Leaf unfolding is known to be driven by chilling requirements followed by warming in spring to force bud break. However, our study confirms that the effect of temperature on leaf unfolding is not so simple.

5.2.1. The threshold effect

First, the temperature effect is confirmed to be non linear because of existing complex thresholds and these different thresholds need to be determined for the different species. Indeed, the differentiation of unfolding dynamics between altitude classes was probably due to the time to reach the threshold temperatures (Fig. 3b). The first example of altitudinal differentiation concerned an early warming period. For example, at low altitudes (<1150 m), *F. sylvatica* buds moved from stage 2 to stage 4 between days (of year) 100 and 110, whereas at higher altitudes they remained at stage 2 during the same period (Fig. 3). This effect was probably due to higher temperatures around day 100, where a threshold, due to instantaneous or accumulated temperatures, seems to be reached (Fig. 3b). A second example was a late cooling period that stopped the bud development of pine species at stage 2 for intermediate and high altitude trees between days 120 and 130. A last example was a cooling period at the beginning of June which only affected pine species at high altitude by stopping leaf unfolding at stage 3. To conclude, the non-linearity of the altitude effect can probably be explained by the existence of various temperature thresholds, with warming periods speeding up leaf unfolding dynamics and cooling periods stopping bud development. These thresholds appeared to be different for the different species and methodologies should be developed to assess them.

5.2.2. The probable role of minimal temperatures

We found that exposure had no impact on leaf unfolding of *F. sylvatica* even though average temperatures were higher on the south face. As exposure had no impact on the number of late frosts, this result shows that minimal temperatures can be better related to leaf unfolding than average temperature for *F. sylvatica*. This is consistent with the high sensitivity of this deciduous tree to the number and the intensity of late frosts. Minimal temperatures should thus be used for modelling phenology in *F. sylvatica*, as already done for some other species and sites (Jolly et al., 2005).

5.2.3. Others spatial factors thwarting temperature effect

We found that the more sensitive a species was to altitude, the less sensitive it was to temperature variation between years. This result implies either local adaptation to altitude or that the mechanisms induced by a warmer year and a lower altitude did not have the same effect on leaf unfolding.

Precipitation also needs to be taken into consideration. In our study, 2007 was a warmer year but also with a rainy spring. Consequently, if species respond differently to precipitation, the response to the altitudinal effect will be different between years, as found between 2006 and 2007 for *A. opalus* and *S. aria*. These two species responded strongly to altitudinal temperature variations only when spring was relatively wet. However, spatial variation of soil moisture conditions can also counteract variations of precipitation with altitude. The fact that the role of rainfall affects the spatial relationship between leaf unfolding and temperature was already shown by Peñuelas et al. (2004).

Finally, in the case *of A. alba* and *F. sylvatica.*, the plasticity in response to altitude might be buffered by local adaptation (von Wuehlisch et al., 1995; Sagnard et al., 2002). In fact, if there was local adaptation with earlier leaf unfolding at a higher altitude than expected, forcing requirements would change with altitude. This can explain the lower sensitivity of leaf unfolding to altitude for these two species.

5.3. Some perspectives

It appears necessary to study the effect of chilling and forcing processes as well as the direct effect of current spring temperature on bud development. Most studies focus on one leaf unfolding date without regarding what the temperature effects are between bud swelling and when leaves or needles reach their mature size.

As temperature affects leaf unfolding in different ways, we demonstrated that altitudinal and year effects are not simply interchangeable. For these reasons phenology parameters used in models should be determined using continuous long term time series, obtained on the same site, without mixing temporal data and spatial (latitudinal and altitudinal) data. Moreover, to improve phenology models leaf unfolding dynamics must be explicitly integrated and effects of local adaptation or other environmental variables on chilling or forcing requirement should be further studied.
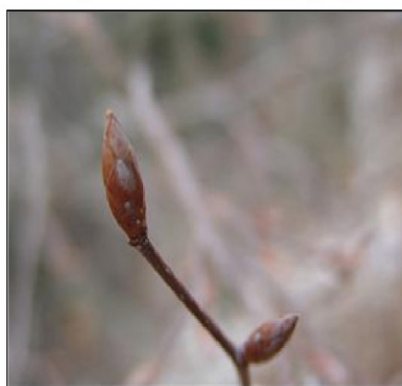
Appendix A.

The six phenological stages used in this study: an example for *Fagus sylvatica*.

Stage 1: buds are dormant

Stage 2: buds are swelling up

Stage 3: buds scales are broken

Stage 4: leaves are emerging

Stage 5: leaves are spread out

Stage 6: leaves reach their mature size

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