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# Tree Physiology

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**Cover image:** Phenological and water-use patterns underlie maximum growing season length at the highest elevations. Linares et al. (this issue, pages 161–170) report on the ecophysiological basis of the ongoing altitudinal shifts of the relict *Abies pinsapo* trees (the southern European fir) under changing climate. Compared to lower elevations, the delay in budburst date due to lower spring temperatures at higher elevations was overcome by a longer delay in growth cessation date due to the later onset of strong water-deficit conditions in the summer. Photo: Juan Carlos Linares Calderon.

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# Tree Physiology

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Research paper

# Phenological and water-use patterns underlying maximum growing season length at the highest elevations: implications under climate change

Juan Carlos Linares[1,3], Felisa Covelo[1], José Antonio Carreira[2] and José Ángel Merino[1]

[1]Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Ctra. Utrera km. 1, 41002 Sevilla, Spain; [2]Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Ed. B3, Paraje las Lagunillas s/n, 23071 Jaén, Spain; [3]Corresponding author (jclincal@upo.es)

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**Consequences of climate change on tree phenology are readily observable, but little is known about the variations in phenological sensitivity to drought between populations within a species. In this study, we compare the phenological sensitivity to temperature and water availability in *Abies pinsapo* Boiss., a drought-sensitive Mediterranean fir, across its altitudinal distribution gradient. Twig growth and needle fall were related to temperature, precipitation and plant water status on a daily scale. Stands located at the top edge of the distributional range showed the most favourable water balance, maximum growth rates and little summer defoliation. Towards higher elevations, the observed delay in budburst date due to lower spring temperatures was overcome by a stronger delay in growth cessation date due to the later onset of strong water-deficit conditions in the summer. This explains an extended growing season and the greatest mean growth at the highest elevation. Conversely, lower predawn xylem water potentials and early partial stomatal closure and growth cessation were found in low-elevation *A. pinsapo* trees. An earlier and higher summer peak of *A. pinsapo* litterfall was also observed at these water-limited sites. Our results illustrate the ecophysiological background of the ongoing altitudinal shifts reported for this relict tree species under current climatic conditions.**

**Keywords**: *Abies pinsapo*, altitudinal gradient, growing degree days, litterfall, Mediterranean firs, phenology, stomatal conductance, twig growth, xylem water potential.

## Introduction

With evidence of climate change and related biological responses accumulating rapidly, considerable attention has turned to predicting the fate of trees and forests (Polgar and Primack 2011). Understanding how climate affects tree phenology and how these changes in phenology will affect tree growth and survival is an important component for predicting how species will respond to global warming and increasing drought stress (Pigliucci et al. 2006, Aitken et al. 2008).

Populations would persist at their current location and withstand environmental changes if their adaptive capacities match the changes (Ghalambor et al. 2007, IPCC 2007). Phenological adjustments could be one of the most significant ways by which trees can react and cope with rapid environmental change (Kramer 1995, Pigliucci et al. 2006, Valladares et al. 2006), allowing trees to persist in their environment.

In temperate forests, the annual sequence of woody plant phenological phases is closely linked to temperature and photoperiod (Kramer 1995, Morin et al. 2009, Polgar and Primack 2011), suggesting a fine adjustment of tree physiology in response to climate seasonality (Chuine and Beaubien 2001, Gordo and Sanz 2010). However, this regulation should be

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more complex, and has been less investigated, in mountainous areas under Mediterranean-type climates. Mediterranean seasonality splits the favourable growing season into two periods separated by summer drought, spring and autumn, where water availability is subject to a high variance and unpredictability (Martínez et al. 1998, Linares et al. 2009, Pinto et al. 2011).

The southernmost European mountain forests are perhaps among the most vulnerable areas for the loss of tree species due to climate change (IPCC 2007). Indeed, several recent studies focusing on tree growth in the Iberian Mediterranean mountains have reported forest decline related to temperature rise and increasing drought (Macias et al. 2006, Andreu et al. 2007, Linares and Tíscar 2010, Camarero et al. 2011). Past climate-warming events seem to have promoted shifts in plant species and biomes towards the poles or higher elevations (Taberlet and Cheddadi 2002). However, the extent of current forest vulnerability to climate change presents large uncertainties; particularly, within-range tree responses to regional warming and extreme drought events remain poorly understood.

Relict Mediterranean mountain tree species typically show a relatively poor adaptation to local conditions and a high sensitivity to climatic stress (Linares and Tíscar 2010). They may thus serve as a model case to assess general mechanisms and patterns for forest tree response to temperature increases and changing regional rainfall patterns. We focus here on the southernmost European fir (*Abies pinsapo* Boiss.) to explore potential changes in tree phenology in response to global warming and the extent to which those changes will be dependent on the inherent drought sensitivity and phenological plasticity of the species. *Abies pinsapo* is a relic species, endemic to South Spain and North Morocco, and belongs to the group of circum-Mediterranean firs (Linares 2011). It is considered highly vulnerable to climatic warming and the related decrease in soil-water availability (Guehl et al. 1991). Since the middle 1990s, severe drought spells have been related to *A. pinsapo* growth decline, mainly in the lower-elevation limits of its altitudinal distribution (Linares et al. 2009).

As the starting point, we hypothesized that climate warming involves increase of both temperature and water stresses on water-limited populations, while water stress increase would be negligible if the populations received elevation-related higher precipitation amounts. For these non-water-limited populations, rising temperature could potentially lead to enhanced tree growth. We also hypothesized that offsetting effects of drought-induced growth limitations will be related to highly sensitive *A. pinsapo* drought-avoidance mechanisms. To test this, we have assessed the budburst date, the dynamics of twig growth and the litterfall production patterns in *A. pinsapo* populations across its altitudinal distribution range during two consecutive years. Our specific aims were (i) to describe intra- and inter-annual twig growth and litterfall production patterns, and (ii) to quantify the within- and among-population variations in drought-avoidance mechanisms (stomatal closure, growth cessation and needle withdrawal), in response to contrasting climatic conditions.

## Materials and methods

### Study area

The study was carried out over 2 years (2004–2005) in natural stands of *A. pinsapo* along an altitudinal gradient (~1200–1700 m a.s.l.) in the Sierra de las Nieves Natural Park (southern Spain; see Supplementary Figure S1, available as Supplementary Data at *Tree Physiology* Online). All studied stands are located on calcareous parent materials. In each stand (~0.1 ha), all trees >3 cm in dbh (diameter at breast height, 1.3 m from the ground) were tagged, mapped and identified, and their dbh was measured. The lowest elevation study stand (hereafter designated as the L stand; 36° 43′ 18″N, 4° 57′ 53″W, 1226 m a.s.l.) consisted of firs that were mainly 15–20 cm in dbh, scattered old firs (~80 cm dbh) and some *Pinus halepensis* Mill. individuals, totalling a stand density of ~40 $m^2$ $ha^{-1}$. A second stand was selected near the upper elevation of the same catchment area (U stand; 36° 42′ 37″N, 4° 59′ 00″W, 1557 m a.s.l.). This stand showed a tree density similar to that in the L stand, but the mean diameter was slightly smaller (10–15 cm dbh); *A. pinsapo* was the only tree species. In 2005, we added a higher elevation stand, located at the top edge of the altitudinal distribution range of *A. pinsapo* in the area (T; 36° 41′ 33″N, 5° 01′ 10″W, 1749 m a.s.l.). This stand had a lower tree density (~15 $m^2$ $ha^{-1}$); the mean tree diameter was 15–20 cm dbh with scattered old firs (~90 cm dbh) and young *Quercus alpestris* Boiss., *Acer granatense* Boiss., *Taxus baccata* L. and *Sorbus aria* (L.) Crantz. individuals (~10 cm dbh).

### Meteorological measurements

Air temperature and relative humidity were recorded every 6 h (6:00, 12:00, 18:00 and 00:00) in each population, using two data loggers per plot (HOBO Pro RH/Temp; Onset Computer Corporation, Bourne, MA, USA). Sensors were situated 1 m above the ground on a pole and were protected by a polyvinyl chloride shelter covered with polyethylene foam to prevent any exposure to rain or to direct sunlight. The vapour pressure deficit (VPD) was estimated using data logger air temperature and relative humidity data. Monthly precipitation data were also measured by rain gauges at the L and U stands between 2003 and 2005 to validate the rainfall altitudinal gradient estimated from nearby meteorological stations (see also Linares et al. 2009).

We used growing degree days (GDD) as a measure of heat accumulation. Growing degree days were calculated for each stand using datalogger temperature data. Based on the field data of budburst date, a basic temperature ($T_b$) of 10 °C was chosen

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as the threshold above which, after a certain amount of temperature is cumulated, budburst occurs and primary growth resumes after the winter. To compute GDD, we first determined the mean daily temperature ($T_m$); $T_b$ was then subtracted from $T_m$ to obtain daily GDD (°C day$^{-1}$) figures. If the daily GDD was a negative number, it was set equal to zero. Daily GDD data from each stand were then summed to get accumulated GDD (Eq. (1)).

$$\mathrm{GDD} = \begin{cases} T_m > T_b \rightarrow \sum_{i=1}^{n} (T_m - T_b) \\ T_m \leq T_b \rightarrow 0 \end{cases} \quad (1)$$

### Soil water content

Soil water content was measured by time domain reflectometry (TDR) in the upper 50 cm of the soil profile. For the 0–12 cm soil layer we used a portable TDR device (TDR100, Campbell Scientific, Inc., North Logan, UT, USA); for deeper horizons we used permanent probes (ECH2O, Decagon Devices, Inc., Pullman, WA, USA) buried at 25 and 50 cm depth. We performed a calibration curve to convert soil dielectric measurements to estimates of volumetric water content.

### Plant water status and stomatal conductance

To estimate plant water status we used a pressure chamber (PMS Instruments, Corvallis, OR, USA). Stomatal conductance to water vapour ($g_s$) was measured using a portable null-balance porometer (model 9810; Data Design Group, La Jolla, CA, USA). L and U stands were sampled in 2004; L, U and T stands were sampled in 2005. Xylem water potential and stomatal conductance were evaluated monthly from late autumn to early spring and biweekly between late spring to early autumn by taking measurements at ~2-h intervals between predawn and predusk. Measurements were performed on two twigs randomly selected within five dominant trees per stand (in the same five dominant trees always). Twigs were 2 years old (current and previous year growth), fully expanded and sunlight-exposed, located 1.5–2 m above the ground and separated by at least 2 m. Following each stomatal conductance measurement, each twig was cut and transported in a plastic bag to the laboratory. Data were expressed per unit leaf area using an empirical needle mass/needle surface area relationship. This relationship was obtained by scanning needles from each stand, carefully detached from the twigs, and measuring the needle's surface by image analysis software ImageJ (http://rsbweb.nih.gov/ij/). The needles were then oven dried (70 °C, 48 h) and weighed to obtain needle weight per unit of needle surface.

### Twig growth

Twig growth phenology was monitored, matching with plant water status and stomatal conductance samplings, by measuring the elongation of the renewals with a ruler at a 1-mm resolution. The initial and final point for length measures is easily distinguishable in *A. pinsapo* due to the presence of a single segment per year. We selected 20 trees per stand (elevation) and one sunlight-exposed twig per tree; the twigs were examined and those with bud malformations or disease symptoms were avoided (Orshan 1989).

### Litter production

Litter production in the L and U stands was sampled from bimonthly to quarterly from May 2002 to October 2006. The materials collected from seven randomly located litterfall pits (28 cm × 38 cm base, 25 cm height) per stand were oven dried (70 °C, 48 h) and carefully sorted to separate *A. pinsapo* needles from other materials such as seeds, cones, twigs, bark and leaves from other species.

### Statistical analyses

The twig length and litterfall data sets were tested for homogeneity of variance and for the assumption of compound symmetry of the variance–covariance matrix using the Bartlett–Box *F*-test and the Mauchly criterion, respectively. A repeated-measures analysis of variance (ANOVA) was used to compare twig length and litterfall (von Ende 2001). We tested differences within populations (i.e., within L and T sites across 2004 and 2005) and the effects of elevation (i.e., among L, U and T for 2005); the factor 'stand' was included in the repeated-measures ANOVA as a between-subjects factor (L, U and T), while the sampling date was regarded as a within-subjects factor. We used the Greenhouse–Geisser statistic to adjust the *F*-tests. Paired comparisons were corrected using the Bonferroni adjustment. The comparisons between years (2004 versus 2005) within a given stand were done by one-way ANOVA, testing differences between the means, for instance, for total litterfall production, total renewals length, etc., without including intra-subject factors. We used SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA). Average values of twig growth rate and GDD, $g_s$ and growth rate, and predawn water potential and $g_s$ were correlated by least squares linear regression. The differences among trends (slopes) for a given variable were assessed by the two-slope comparison test, which compares the slopes and intercepts of two regression lines (see Zar 1999). Twig elongation was fitted to a sigmoid function model (Eq. (2)).

$$y = \frac{a}{1 + e^{-((x - x_0)/b)}} \quad (2)$$

where $y$ is the twig length (mm), $x$ is the time (Julian calendar days) and $x_0$ represents the estimated onset of growth; $a$ and $b$ are estimated parameters related to (i) the asymptote and (ii) the slope of the sigmoid function, respectively (see Camarero et al. 1998).

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## Results

### *Climatic conditions, soil water content and plant water status*

In 2004, the mean temperature in the lowest elevation stand (L) was 1.1 °C higher than that in the U stand, which led to higher values of accumulated GDD in the former (Table 1, see Supplementary Figure S2 available as Supplementary Data at *Tree Physiology* Online). Early spring temperatures were below average relative to the previous decade. Spring precipitation was 142 mm greater than average, and rainfall was recorded until early summer (Table 1). In 2004, the late summer and early autumn were hot and dry, thus the drought period lasted until October. The year 2005 was one of the driest years in the last century. The annual rainfall was near to 50% lower than the average for the last decade. The differences in mean temperature among the stands increased in 2005. The winter was relatively cold; however, from April to the end of 2005, all months recorded above-average temperatures.

Vapour pressure deficit (see Supplementary Figure S3 available as Supplementary Data at *Tree Physiology* Online) became maximum (~1 kPa) and similar among stands at mid-July 2004; from September and onward the VPD was significantly higher at the lower elevation. In 2005, June to July VPD was significantly lower at the top elevation stand (T 1700 m); maximum VPD (~1.5 kPa) was reached at mid-August, when significant differences among stands disappeared. Overall, soil water content decayed from May to early July in all stands; and then was nearly steady so that minimum soil water contents (~10% v/v) were recorded until October (see Supplementary Figure S3 available as Supplementary Data at *Tree Physiology* Online).

Predawn xylem water potential (Figure 1) was significantly lower at the L stand from mid-July to mid-September 2004; minimum values (~ –1.5 MPa) were reached at the middle of October. In 2005, predawn xylem water potential was significantly lower at the L stand from June to mid-August, among-plot differences narrowed for the rest of the dry period, and minimum values did not fall below –1.2 MPa.

Table 1. Meteorological measurements for *A. pinsapo* populations along an altitudinal gradient (L, 1200 m; T, 1550 m; U, 1700 m). $T_{max}$ is the mean of the maximum daily temperatures; $T_{min}$ is the mean of the minimum daily temperatures; $T_m$ is the mean temperature (all expressed in °C). GDD, accumulated growing degree days; *P*, monthly precipitation (mm). All the variables were measured in situ by data loggers and rain gauges. Mean annual values for temperatures and total precipitation are also shown.

| | | $T_{max}$ (°C) | | | $T_{min}$ (°C) | | | $T_m$ (°C) | | | GDD (°C) | | | *P* (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | Month | L | U | T | L | U | T | L | U | T | L | U | T | L | U | T |
| 2004 | January | 8.57 | 7.13 | | 2.19 | 0.18 | | 4.61 | 3.48 | | – | – | | 35 | 40 | |
| | February | 9.24 | 7.89 | | 2.52 | 0.49 | | 5.12 | 3.99 | | 0.5 | – | | 25 | 29 | |
| | March | 10.32 | 9.11 | | 2.56 | 0.54 | | 5.69 | 4.58 | | 2 | 1 | | 161 | 188 | |
| | April | 12.01 | 11.03 | | 4.44 | 2.40 | | 7.51 | 6.65 | | 18 | 12 | | 172 | 201 | |
| | May | 12.96 | 11.81 | | 5.98 | 4.42 | | 8.84 | 8.01 | | 50 | 33 | | 78 | 91 | |
| | June | 24.38 | 23.70 | | 13.42 | 13.03 | | 18.58 | 17.63 | | 307 | 261 | | 20 | 23 | |
| | July | 24.09 | 24.17 | | 15.90 | 15.70 | | 20.63 | 19.54 | | 637 | 557 | | 0 | 0 | |
| | August | 22.91 | 22.84 | | 15.29 | 15.09 | | 19.72 | 18.61 | | 929 | 816 | | 0 | 0 | |
| | September | 20.60 | 20.21 | | 13.04 | 12.86 | | 17.39 | 16.22 | | – | – | | 0 | 0 | |
| | October | 13.39 | 12.05 | | 9.48 | 9.33 | | 11.90 | 10.60 | | – | – | | 78 | 94 | |
| | November | 9.10 | 7.20 | | 3.41 | 3.32 | | 6.61 | 5.18 | | – | – | | 131 | 157 | |
| | December | 4.41 | 1.88 | | 1.39 | 1.31 | | 3.19 | 1.67 | | – | – | | 90 | 108 | |
| Mean/ sum | | 14.33 | 13.25 | | 7.47 | 6.56 | | 10.81 | 9.68 | | | | | 789 | 930 | |
| 2005 | January | 4.73 | 4.73 | 3.02 | –0.67 | –0.67 | –1.34 | 1.82 | 1.82 | 1.14 | – | – | – | 0 | 0 | 0 |
| | February | 3.30 | 1.77 | –0.08 | –0.65 | –1.85 | –2.50 | 1.33 | –0.04 | –0.80 | – | – | – | 0 | 0 | 0 |
| | March | 8.16 | 5.66 | 3.47 | 3.57 | 2.79 | 1.02 | 5.77 | 4.17 | 2.81 | 10 | 1 | – | 166 | 199 | 210 |
| | April | 14.78 | 11.05 | 9.76 | 7.86 | 5.00 | 3.50 | 11.50 | 7.94 | 6.89 | 73 | 22 | 13 | 49 | 59 | 62 |
| | May | 17.49 | 16.64 | 15.36 | 10.72 | 9.61 | 8.54 | 14.25 | 13.05 | 11.85 | 207 | 128 | 93 | 0 | 0 | 0 |
| | June | 22.17 | 22.27 | 19.79 | 14.53 | 13.51 | 12.38 | 18.37 | 17.60 | 16.01 | 458 | 356 | 273 | 0 | 0 | 0 |
| | July | 24.54 | 24.38 | 22.63 | 17.39 | 16.16 | 14.37 | 21.07 | 19.99 | 18.43 | 801 | 665 | 535 | 0 | 0 | 0 |
| | August | 23.96 | 24.16 | 22.81 | 17.13 | 16.19 | 14.61 | 20.61 | 19.71 | 18.57 | 1130 | 966 | 801 | 0 | 0 | 0 |
| | September | 18.69 | 17.47 | 16.32 | 13.09 | 11.07 | 10.15 | 15.91 | 13.55 | 13.06 | – | – | – | 0 | 0 | 0 |
| | October | 13.67 | 12.86 | 12.23 | 9.73 | 7.61 | 7.44 | 11.75 | 9.97 | 9.94 | – | – | – | 77 | 81 | 84 |
| | November | 7.09 | 5.78 | 4.35 | 4.05 | 2.00 | 1.15 | 5.59 | 4.07 | 2.71 | – | – | – | 91 | 95 | 99 |
| | December | 5.41 | 4.61 | 2.83 | 3.13 | –0.73 | 0.28 | 4.21 | 2.36 | 1.57 | – | – | – | 34 | 36 | 37 |
| Mean/ sum | | 13.66 | 12.61 | 11.04 | 8.32 | 6.72 | 5.80 | 11.01 | 9.52 | 8.52 | | | | 418 | 470 | 492 |

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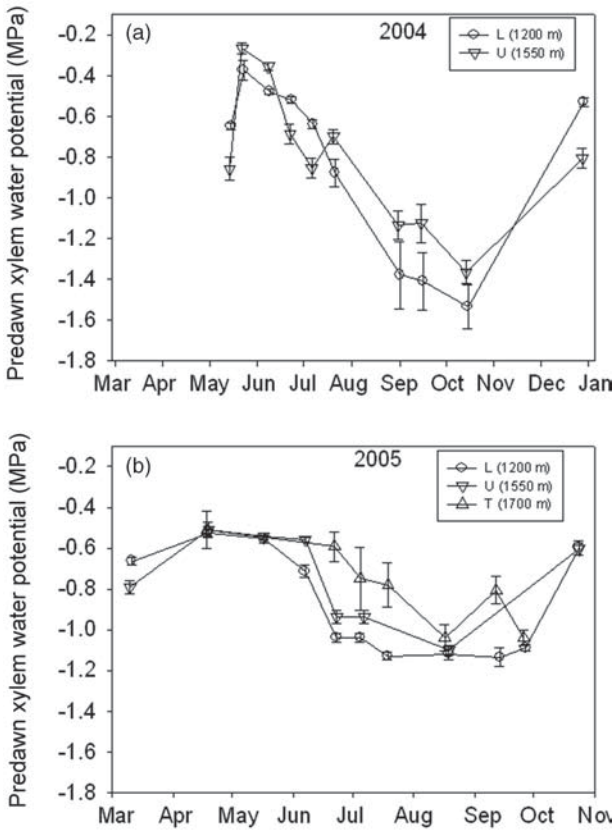


Figure 1. Predawn xylem water potential measured in *A. pinsapo* trees ($n = 5$) along an altitudinal gradient during the growing periods of 2004 (a) and 2005 (b). Errors bars represent the standard error.

### *Twig growth phenology and stomatal conductance*

There were no significant differences between 2004 and 2005 for cumulative twig length increment within either the L or the U stand (Figure 2). The average renewal growth values in 2004 were statistically different between the L (lower average length) and U stands from early July onward. Based on the sigmoid parameters fitted to the average renewal length values (see Supplementary Table S1 available as Supplementary Data at *Tree Physiology* Online), bud break occurred around 28 May in the L stand and, on average, 9 days later in the U stand. However, estimated growth cessation (the *a* parameter of the sigmoid function, see Eq. (2)) occurred significantly later for the U stand (1550 m) than for the L stand (1200 m).

The mean renewal length values were in 2005 statistically different between the L and the two upper elevation stands (U and T) from the beginning of June (Figure 2). Bud break occurred later in the top elevation stand (T); however, it showed the highest growth values and the longest growing period. The bud break in 2005, as estimated by sigmoid functions, took place on 15 May for the L stand (13 days earlier than in 2004), 9 days later for the U stand

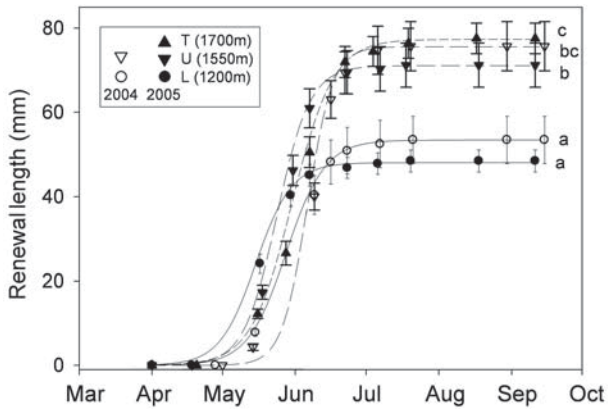


Figure 2. Renewal length measured in *A. pinsapo* stands along an altitudinal gradient (L, 1200 m; U, 1550 m; and T, 1700 m) during the growing periods of 2004 (empty symbols) and 2005 (filled symbols). Error bars represent the standard error; different letters denote significant differences for total renewal length (repeated measures ANOVA, $n = 20$ trees per elevation; $P < 0.05$). Lines represent the fitted sigmoid function.

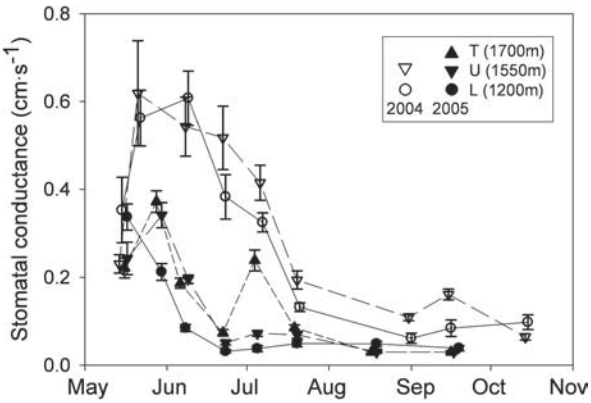


Figure 3. Midday stomatal conductance measured in *A. pinsapo* stands along an altitudinal gradient (L, 1200 m; U, 1550 m; and T, 1700 m) during the growing periods of 2004 (empty symbols) and 2005 (filled symbols). Error bars represent the standard error ($n = 5$ trees per elevation).

(8 days earlier than in 2004) and 15 days later in the T stand. Growth cessation was estimated to occur significantly later at the T stand (Figure 2; Supplementary Table S1 available as Supplementary Data at *Tree Physiology* Online).

Midday stomatal conductance within stands was significantly higher in 2004 than in 2005 for both L and U stands (Figure 3). Among stands, from late June in 2004 and from late May in 2005 and onward, the average stomatal conductance was significantly lower in the L stand than in the U and T stands.

Twig growth rate and GDD (Figure 4a) were significantly correlated only during the first part of the growing period. The slope of this relationship was similar at the higher

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elevations stands (U and T), but higher than that yielded by the L stand. Since the onset of the dry season (about late June), the twig growth rate versus GDD correlation disappeared, indicating a shift to driving factors other than temperature in growth rate control. However, the relationship between midday stomatal conductance and renewal elongation rate was significant over the entire growing season for the lower elevation sites (L and U), but not for the T stand (Figure 4b). A between-stand comparison showed that L and U stands have similar slopes, although within-stand comparison yielded a significantly higher slope for both stands in 2005 (Figure 4b). Midday stomatal conductance and predawn xylem water potential were also significantly related over the entire growing season (Figure 5), showing similar relationships within and among stands in the two studied years.

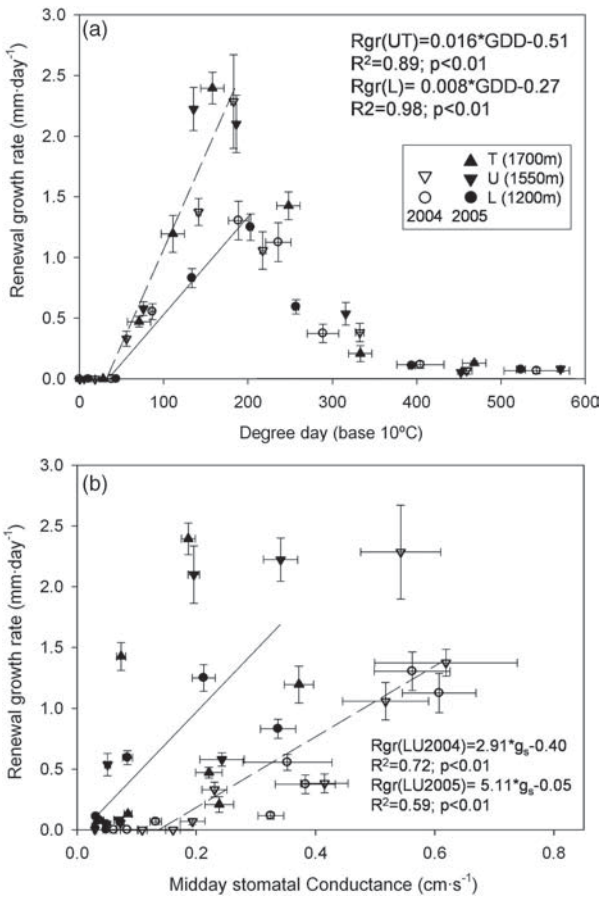


Figure 4. Relationships between renewal elongation rate (Rgr) and (a) the growing degree days (GDD) or (b) the midday stomatal conductance ($g_s$). Data are for the growing periods of 2004 (empty symbols) and 2005 (filled symbols). Error bars represent the standard error. Regressions were computed separately on each stand; when regression slopes were not significantly different ($P > 0.05$), the data were grouped; letters between parentheses denote the stands and the year used for the corresponding regression. Note that a correlation existed between both variables only during the first phase of the growing period.

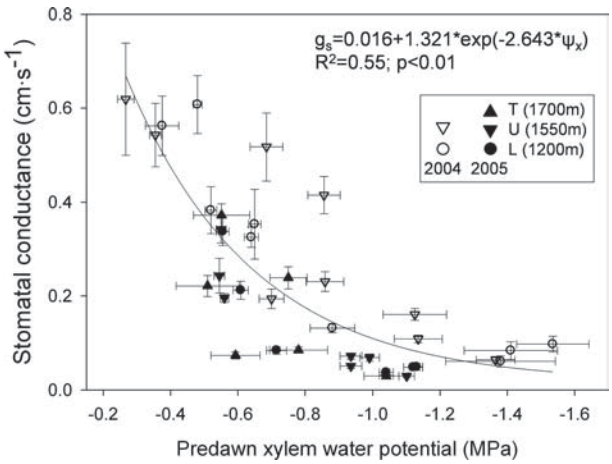


Figure 5. Relationships between midday stomatal conductance ($g_s$) and predawn xylem water potential ($\psi_x$). Data are for the growing periods of 2004 (empty symbols) and 2005 (filled symbols). Error bars represent the standard error. Regressions were computed separately on each stand; since none of the regression slopes were significantly different ($P > 0.05$), the data were grouped.

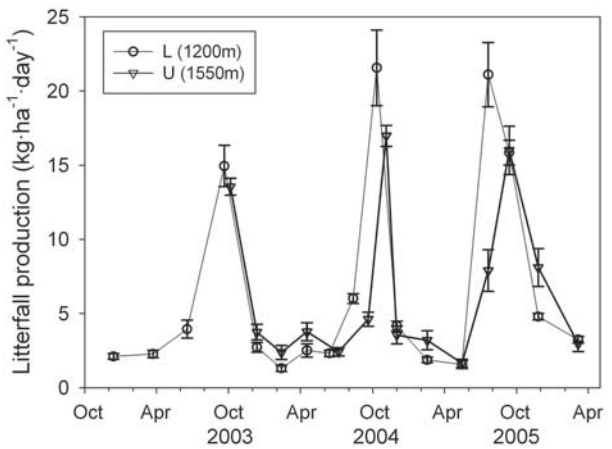


Figure 6. *Abies pinsapo* needle litterfall production from May 2002 to October 2006 in the L (1200 m) and T (1550 m) stands.

### *Litterfall production*

In 2004, the total litterfall production was 3003 kg ha$^{-1}$ in the L stand and 2449 kg ha$^{-1}$ in the U stand, while in 2005 these values increased to 3596 kg ha$^{-1}$ and 2500 kg ha$^{-1}$, respectively (Figure 6). During 2005 (drier than 2004), litterfall production peaked significantly earlier in the L than in the T stand. The L stand showed a mean value of 1204 kg ha$^{-1}$ for the July–August 2005 period, whereas only 508 kg ha$^{-1}$ was registered in U.

## Discussion

We hypothesized that, despite a longer growing period being expected with rising global mean temperatures as a



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consequence of earlier bud break date, worsening drought might offset or even reverse the temperature-based predicted growth in water-constrained forests. Such deviations from the expected growth enhancement, where growth onset occurs early, provide valuable information to understand the underlying ecophysiological mechanisms leading to tree-growth decline in a global warming scenario. Our results support that primary growth in a relict drought-sensitive fir is currently better, and the length of the growing season is longer, at the highest elevations.

Our results provide different information about intra- and inter-population variation on the species' ability to cope with climatic change. Intra-population variation in response to contrasting precipitation among years informs about adaptive capacity by phenological plasticity, while inter-population variation along an altitudinal gradient may be attributed to both phenological plasticity and to local adaptation. Phenological plasticity is a key trait for the inherent adaptive capacity of plants against future changes of climate, since it allows adjustment of the timing of phenological phases under environmental changes (Menzel et al. 2006, Cleland et al. 2007, Ghalambor et al. 2007, Peñuelas et al. 2009, Polgar and Primack 2011). The altitudinal gradient and the 2 years studied here were strong enough sources of variation for assessing *A. pinsapo* populations' response to contrasting temperature and water availability conditions. The high variability observed for the timing and extent of twig growth and litterfall reveal a significant phenological variability for this relict drought-sensitive Mediterranean fir.

Bud break in *A. pinsapo* showed 2.6–3 days of advance for each 100 m decrease in elevation. For *Abies amabilis* Douglas ex J. Forbes growing in a 1000 m altitudinal gradient, Worrall (1983) reported higher budburst delays among populations (5 days per ~100 m rise in elevation). The clinal trend for leaf unfolding date we observed for *A. pinsapo* is more similar to that reported for *Abies alba* Mill. (on average, 3.2 days 100 $m^{-1}$; Vitasse et al. 2009*a*), as well as for some temperate deciduous tree species such as *Fagus sylvatica* L. and *Quercus petraea* (Matt) Liebl. (on average, 2.7 days 100 $m^{-1}$; Vitasse et al. 2009*b*).

Since the rate of adiabatic temperature change in our study area is on average 0.67 °C 100 $m^{-1}$ (Linares et al. 2009), *A. pinsapo* shows an advance in budburst date of 3.9–4.5 days $degree^{-1}$. Worrall (1983) found a higher linear reaction norm of leaf unfolding timing for populations of *A. amabilis* and *Abies lasiocarpa* (Hook.) Nutt. of about 8.3 days $degree^{-1}$. Vitasse et al. (2009*a*) also reported a higher slope in leaf unfolding for adult sessile oak (*Q. petraea*; 6.5 days $degree^{-1}$). These results suggest that leaf unfolding plasticity in response to temperature is higher in temperate forests than in Mediterranean mountain forests (see also Kramer 1995). However, if we consider the spring temperature (2–3 months before the growth onset) directly measured in each site, to calculate the lapse rate occurring before and during bud break, we obtain an average of 0.33 °C 100 $m^{-1}$, and therefore, we obtain an advance in budburst date of about 8 days $°C^{-1}$ of increase.

The temperature effect on plant phenology markedly surpassed the effects of precipitation in recent investigations performed on Mediterranean ecosystems (Gordo and Sanz 2010). However, we have shown here a fine-tuning response of reduced leaf conductance in *A. pinsapo* as water stress increases, which compromised the growth rate and the time of needle retention (Bond and Kavanagh 1999). Our results suggest that increasing temperature may potentially extend the available growing period only upward (see also Kramer 1995, Menzel and Fabian 1999, Peñuelas and Filella 2001, Peñuelas et al. 2009). Twig growth at the top elevation stand (T, 1700 m) was less dependent on stomatal conductance and appeared to be more limited by cumulated growing degree days. These results suggest a positive effect of global warming on *A. pinsapo* forests at higher elevations. Similar results were also found in *F. sylvatica* (in the southern part of its distribution), where the maximal growing season was not found at the lowest site, as expected, but rather at mid elevation because low-elevation populations were affected by a drought effect, leading to an earlier senescence (Vitasse et al. 2010).

Indeed, water balance is more favourable for trees at the highest elevations, thus allowing the highest growth rates and reduced needle withdrawal in the summer. In contrast, earlier and more intense summer drought stress towards low elevations had the effect of shortening the time of renewal elongation and needle retention (Guehl et al. 1991). All these results support the idea that phenological adjustment to increasing temperature will not lead to greater tree growth in the case of water-limited forests (Rehfeldt et al. 2002).

We observed a similar relationship between midday stomatal conductance and renewal growth rate in the mid- and low-elevation sites, but it did not appear at the highest elevation. Such a relationship is linked to a relatively low water-availability threshold for *A. pinsapo* partial stomatal closure (~ −0.8 MPa for predawn xylem water potential; see also Guehl et al. 1991), which strongly determines the length of the growing period for the species under the conditions prevailing in its distribution area. Physiological adaptation for maintaining a certain growth rate at low stomatal conductance is expected in populations where average water availability is low (Mohren et al. 1997, Bond and Kavanagh 1999).

A recent investigation reported higher hydraulic conductivity at the shoot and branch levels in *A. pinsapo* compared with *A. alba*, a closely related congeneric species which does not withstand so intense summer drought periods (Peguero-Pina et al. 2011). A high efficiency of water transport will reduce the soil-to-leaf water potential gradient, which would allow *A. pinsapo*

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to cope with a high evaporative demand during summer while maintaining its xylem tension below the threshold for rapidly increasing cavitation. In that respect, it is interesting to note that the relationship between twig growth rate and stomatal conductance showed intra-population plasticity, since its slope did increase in response to the sharp decline in 2005 precipitation.

Our results suggest that a longer growing period could also be related to a longer carbon uptake period and to an increased net annual carbon flux (Churkina et al. 2005, Delpierre et al. 2009). Since photosynthetic rate is reduced by stomatal closure, carbon uptake will be also reduced by drought. Furthermore, carbohydrate consumption rates required to maintain cellular metabolism (respiration) will increase due to rising temperatures, potentially increasing negative effects of drought (McDowell et al. 2008). Co-existing tolerant species such as *Quercus*, or better drought-adapted species such as *Pinus* or *Juniperus*, have higher carbon uptake under drought conditions than *A. pinsapo* and, therefore, are able to maintain higher growth rates under low water availability (Martínez-Ferri et al. 2000). For instance, Mediterranean oaks can support significant carbon uptake even at low water potentials (below –2.0 MPa; Borghetti et al. 1998, Baquedano and Castillo 2007).

Changes in leaf area appear to be a very plastic mechanism by which the transpiring surface is adjusted in response to water stress. The leaf turnover rate, which critically depends on the needle lifetime, appears to be closely linked to both temperature and water limitation. It has been noted that evergreen trees, such as oaks and pines, can accelerate leaf dynamics by decreasing the lifetime of leaves in the canopy as temperatures rise (Kramer 1995, Sabaté et al. 2002, Peñuelas et al. 2009). For evergreen species, annual leaf production represents a small amount of total leaf biomass. Increasing litterfall may be a short-term response to increasing drought in drought-avoiding tree species, but leaf area recovery would require much more than one growing season (Kozlowski and Pallardy 1997). Additionally, leaf area development will be limited by the acquisition of water and nutrients by roots, but root growth will be limited by the availability of photosynthates coming from leaves (Guehl et al. 1991, Merino et al. 1995, Martínez et al. 1998).

Increasing foliar dynamics require more mobile carbon reserves to meet needle renewal. If climatic changes decrease needle lifetimes, more carbon will be required to maintain twig growth (McDowell et al. 2008). In addition, needle lifespan is associated with nutrient remobilization, especially of nitrogen, and storage of photosynthates (Kozlowski and Pallardy 1997). Then, reduced twig growth may limit carbohydrate allocation to root production, which, in turn, would enhance water and nutrient limitations as less soil volume is efficiently exploited. Moreover, decreasing root production enhances water deficit and increases needle loss, thereby limiting further carbon uptake. Therefore, the high sensitivity to water stress shown by *A. pinsapo* both in terms of reducing needle lifetime and early growth cessation may provide this species with short-term adaptive capacity to drought spells, but it will likely result in positive feed-back leading to tree decline and mortality if drying conditions prevail in the long term.

## Concluding remarks

We have illustrated here, for the case of *A. pinsapo*, that warming may not lead to extended growing seasons in the case of water-stressed forests, since non-linear responses to water availability regarding growth cessation will offset the linear effects of increasing temperature on advancing budburst date. This study is therefore in line with previous studies showing that, at high elevation, current climate change could drive conditions towards the optimal range for population growth while negatively affecting tree growth and water balance in the drier part of the species range.

The high sensitivity to drought observed in drought avoidance mechanisms of *A. pinsapo* may be benefiting this species by preventing xylem damage, but they also seem to strongly compromise tree growth. Our results suggest that mid- to high-elevation *A. pinsapo* populations will experience a longer growing season with climate warming, while for the lower elevation limit of this species, we found that climate change could reduce population fitness by shortening the growing season, mainly due to earlier stomatal closure.

## Supplementary data

Supplementary data for this article are available at *Tree Physiology* online.

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# Supplementary Material

**Phenological and water-use patterns underlying maximum growing season length at the highest elevations: implications under climate change.**

Juan Carlos LINARES [1,*], Felisa COVELO [1], José Antonio CARREIRA[2] and José Ángel MERINO[1]

[1]Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide. Ctra. Utrera km. 1, 41002 Sevilla, Spain.

[2] Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Ed. B3, Paraje las Lagunillas s/n, 23071, Jaén, Spain.

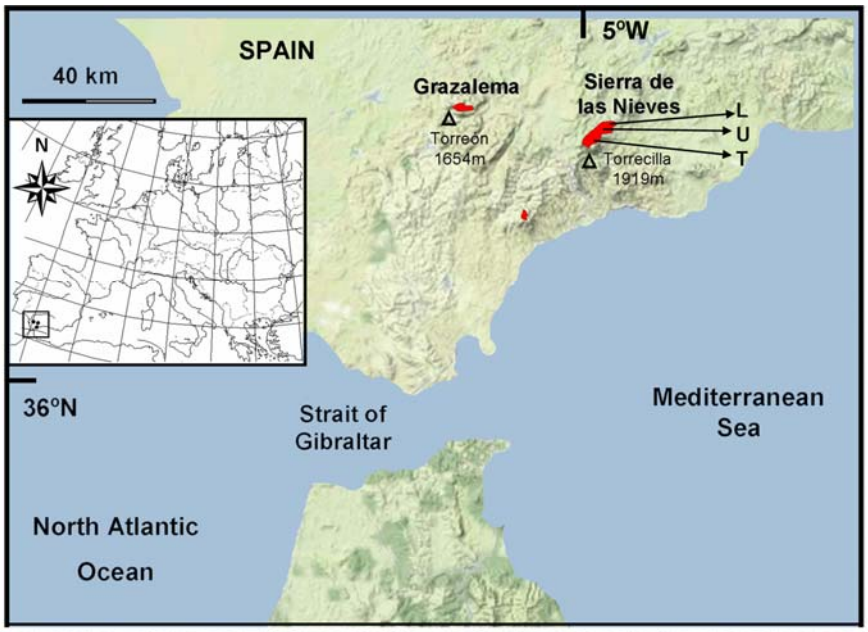
*Corresponding author: E-mail: jclincal@upo.es

**Supplementary Material**

Table A1. Sigmoid-fitted (eq. 2) parameters derived from average twig length values obtained throughout the 2004 and 2005 growing seasons in *Abies pinsapo* stands at different elevations. $x_0$ represents the estimated onset of growth; the *a* parameter is related to the asymptote of the sigmoid function (growth cessation), while *b* is related to the slope (see eq. 2).

| | Parameter | L (1200 m) | | T (1550 m) | | U (1700 m) | |
|---|---|---|---|---|---|---|---|
| 2004 | a | 53.47 | ± 0.34 | 75.57 | ± 0.86 | | |
| | b | 8.21 | ± 0.31 | 5.35 | ± 0.60 | | |
| | x0 | 28-May-04 | ± 0.51 | 6-Jun-04 | ± 0.53 | | |
| 2005 | a | 48.01 | ± 0.35 | 71.05 | ± 0.39 | 77.27 | ± 1.00 |
| | b | 7.61 | ± 0.47 | 7.05 | ± 0.23 | 8.70 | ± 0.59 |
| | x0 | 15-May-05 | ± 0.43 | 24-May-05 | ± 0.26 | 30-May-05 | ± 0.61 |

Figure A1. Top panel shows the distribution of *A. pinsapo* forests in Europe (inset) and the three study sites along an altitudinal gradient in Sierra de las Nieves Natural Park (T, 1700m; U, 1550m; and L, 1200m). Shaded areas indicate the major *A. pinsapo* forests distribution in S Spain. The photograph (a) shows a landscape picture of lower elevations stands, near to the L study site; photograph (b) shows the study site T (1700m).



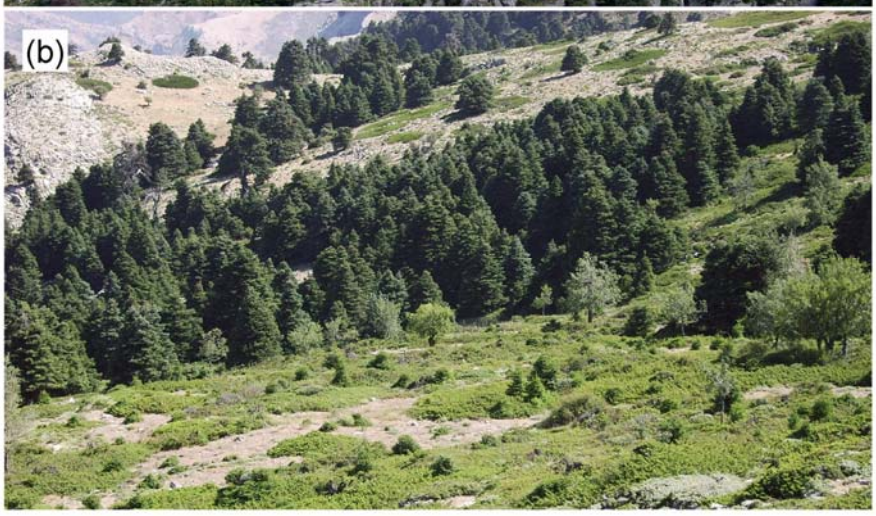


Figure A2. Daily values of temperature and accumulated growing degree days for the 2004 (a) and 2005 (b) growing seasons in stands of *Abies pinsapo* located along an altitudinal gradient.

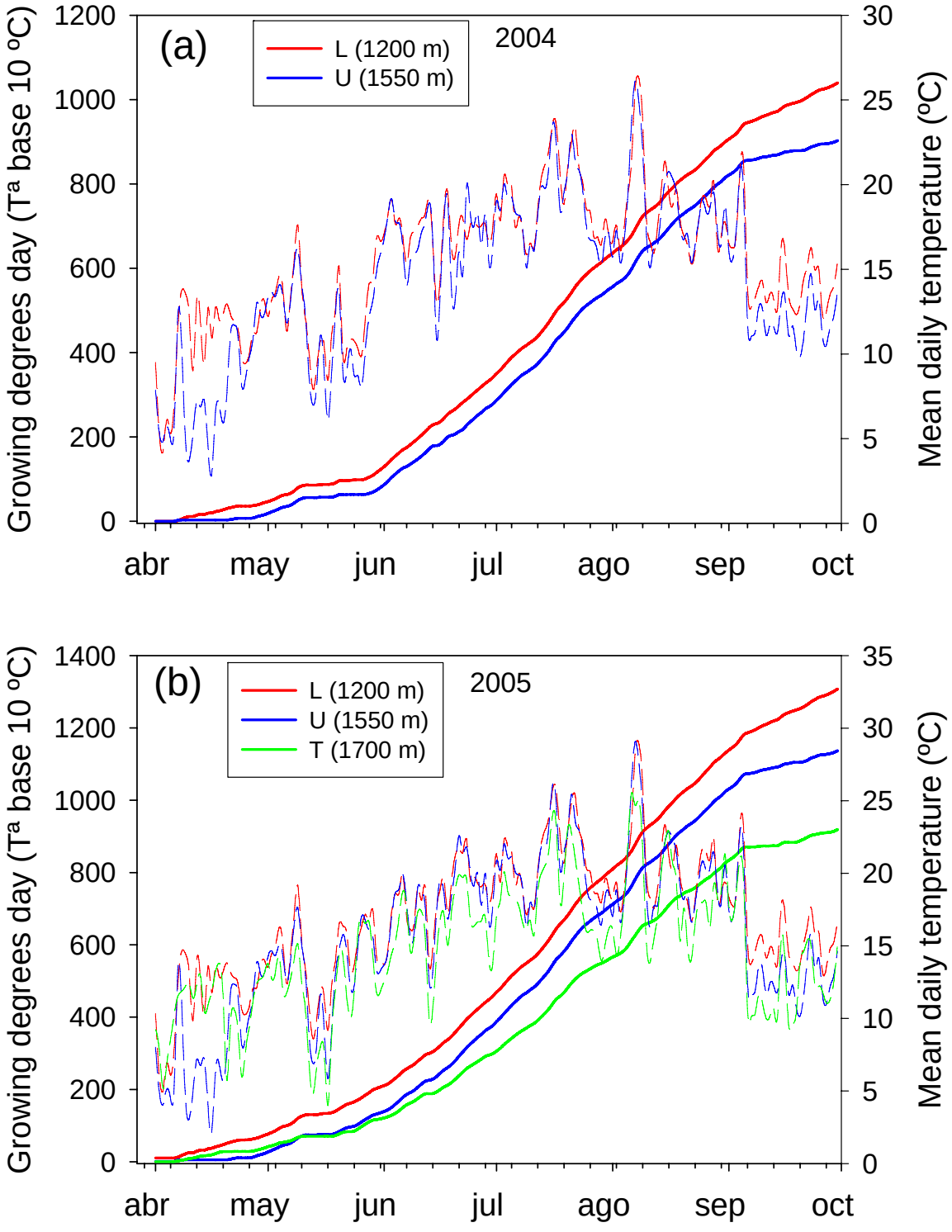
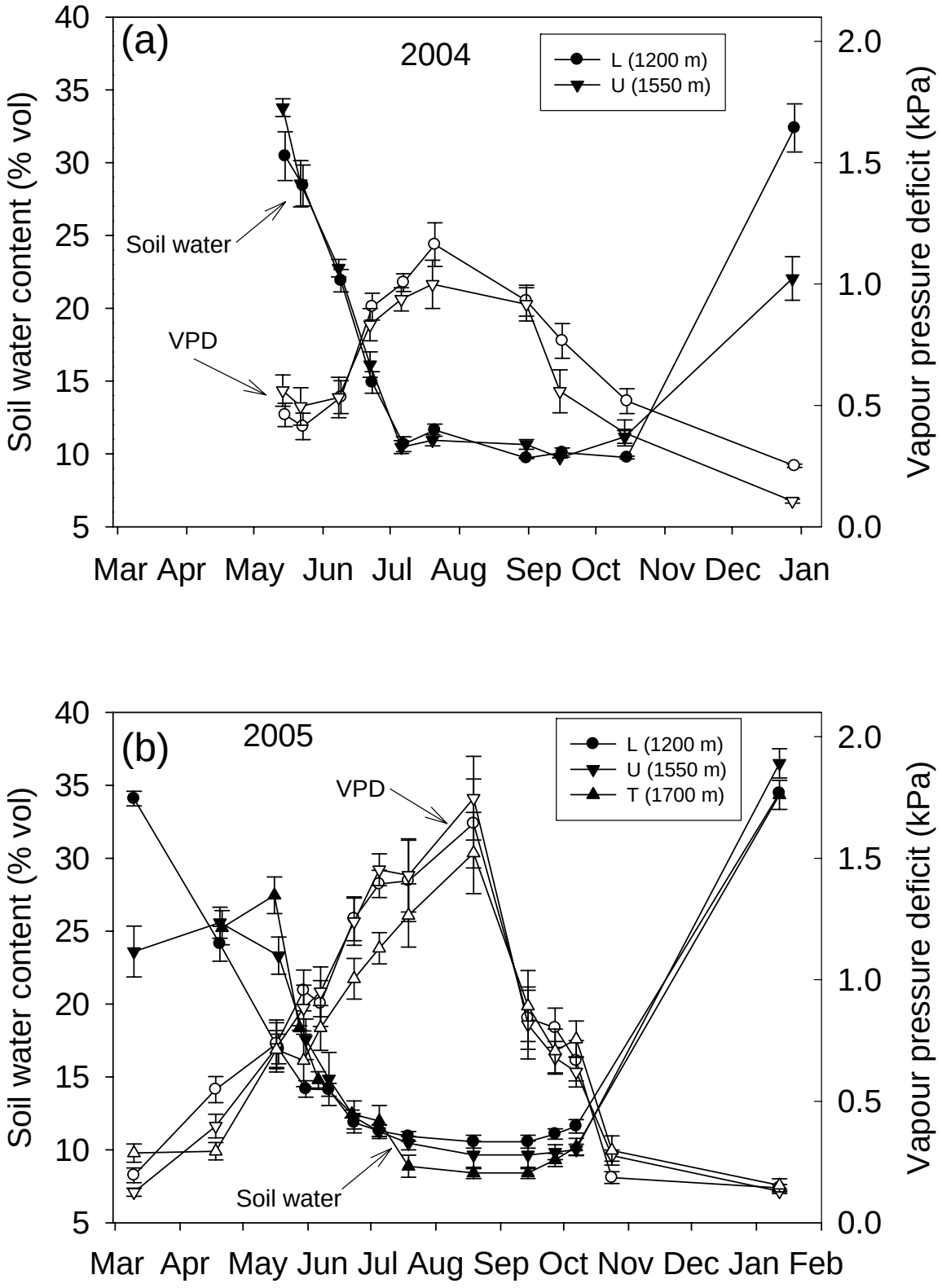



Figure A3. Soil water content (black symbols) and vapour pressure deficit (VPD, empty symbols) measured in *Abies pinsapo* stands along an altitudinal gradient during years 2004 (a) and 2005 (b). Error bars represent the standard error.