Cambium phenology, wood formation and temperature thresholds in two contrasting years at high altitude in Southern Italy

Running head: Effect of temperature on cambium phenology

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Summary

Xylogenesis was monitored during 2003 and 2004 in a timberline environment in Southern Italy in order to assess links between temperature, cambium phenology and wood formation at a short-time scale. Wood microcores were collected weekly from May to October on ten trees of *Pinus leucodermis* Ant., histological sections were cut with a rotary microtome and anatomical features of the developing and mature tracheids were observed and measured along the growing tree-ring. Spring 2003 was hotter than in 2004, with temperatures up to 2.6 ºC above the historical averages. These conditions determined an anticipation in the beginning of cambium activity and all the differentiation phases of about 20 days, resulting in an increased duration of xylogenesis of about 23 days. Air and stem temperatures at which xylogenesis had a 0.5 probability of being active were calculated using logistic regressions fitted on binary responses. In both years, similar thresholds were estimated with daily mean values of 8.2 and 9.5 ºC for air and stem temperatures respectively. The observed convergent responses of cambium phenology to temperature during the two contrasting springs confirm the key role of this environmental factor in determining onset and duration of wood formation in timberline areas. The intra-annual dynamics of ring-width increase differed between the two years, with significantly smaller ring-widths formed in 2004. These differences were mainly related to cell size since larger earlywood tracheids were produced in 2003. This study shows the plasticity of tree-ring formation in response to high temperatures by modifying the onset and duration of differentiation.

Keywords: Cambium activity, cell differentiation, cell production, *Pinus leucodermis*, temperature, tree-ring width

Introduction

An important aspect of climate change concerns the effects of temperature increase on tree growth and wood production and the evolution of forest ecosystems. Some changes in forest productivity can be attributed to longer growing seasons due to changes in tree phenology (McCarty 2001). At ecosystem level, increased activity of vegetation has been detected by changes in the CO_2 cycle reflecting a combination of increased plant growth and respiration (Keeling et al. 1996). According to Badeck et al. (2004), there is evidence that trends in spring phenology changed during the 20^{th} century because of higher temperatures, leading to a 5.4-day earlier start of the vegetation period over Europe. Quantification of the effects of changing plant physiology and related growth with its phenology are therefore required.

High latitude and altitude ecosystems are crucial environments that are considered biological pointers of climate change (Pisaric et al. 2003, Kullman 2007). Within a global perspective, treeline position seems to coincide with a mean temperature during the growing season of 6-7 °C (Körner 2003, Körner and Paulsen 2004), suggesting that growth processes might be strongly limited below this threshold. At the Alpine timberline, Rossi et al. (2007) found that xylogenesis was active with a daily air temperature of 5.6-8.5 °C and stem temperature of 7.2-9.0 °C. A temperature increase in early spring or late autumn would therefore lead to an increase in the duration of wood formation. During the growing season, a certain variability exists in growth onset and duration, which is caused by different intra-annual weather conditions (Deslauriers and Morin 2005, Rossi et al. 2006c). Moreover, cambium reactivation in spring is highly dependent on temperature (Oribe et al. 2001, 2003, Gričar et al. 2006, 2007). Wood formation takes place from cambium division and cell expansion to secondary wall production. These processes are regulated by several intrinsic factors, such as gene expression (Schrader et al. 2004) and

hormonal signals (Schrader et al. 2003), and environmental factors such as temperature and precipitation (Gorsuch and Oberbauer 2002, Deslauriers and Morin 2005, Gričar et al. 2007, Zweifel et al. 2006). However, the question arises as to whether there is a link between changes in cambium phenology (onset and duration) and intra-annual wood formation. For example, changes in duration of wood formation induced by a change in the cambium phenology might result in a modification to ring-width formation.

The ongoing climate change is an added source of stress for those species already threatened by local environmental modification and anthropogenic activity (McCarty 2001). In the Pollino massif (Southern Italy), the treeline is formed by *Pinus leucodermis* Ant., a low-endangered species at the limit of its geographical range. Todaro et al. (2007) recently found a positive influence of temperature on growth of *P. leucodermis* during the period 1953-2000, although gaps in age structure and growth decrease indicated a strong effect of human pressures. An annual temperature increase, from 10.1 to 10.9 °C between 1925 and 2000, was recorded in this area (Todaro et al. 2007), indicating a changing environment that might affect tree-growth. Eleven of the last twelve years (1995-2006) rank among the 12 warmest years in the instrumental records of global surface temperature (IPCC 2007). Among these years, 2003 was characterized by exceptionally hot conditions in most of Europe and represents quite closely the possible pattern of summer in the latter part of the 21st century (Beniston 2004). High temperature and a prolonged drought in 2003 have affected several forest areas from northern to southern Europe (Rebetez et al. 2006). During that year, only trees growing above a certain altitude benefited from the higher temperatures, while growth decreases were observed at lower altitude (Jolly et al. 2005).

We studied cambium phenology in multi-century trees of *P. leucodermis*, growing at a tree-line in Southern Italy with the aim of describing xylem cell formation with divergent spring temperatures at weekly scale. Intra-annual growth during 2003 and 2004 was monitored by assessing (1) differences in the phenological phases and (2) their effects on tree-ring formation.

Material and Methods

Site of study

The study was conducted on the Pollino massif in Southern Italy (Serra di Crispo, 39° 56’ N, 16°12’ E). The site was located at 2100 m a.s.l. with west-facing slopes varying between 40% and 60%, mean annual temperature of 4 °C and annual precipitation of 1557 mm, mainly concentrated in autumn and winter. Dry summer soil conditions that could affect tree growth are mitigated by fog and low clouds, frequent in this area influenced by the Tyrrhenian sea (Todaro et al. 2007). On the highest cliff peaks of the massif grow multi-century trees of *Pinus leucodermis* Ant., a relict of Tertiary flora and a Balkan endemic surviving only in the mountain regions of south-eastern Europe. The species distribution in Italy is narrow and fragmented between the regions of Basilicata and Calabria (Avolio 1996) and located only on the upper parts of the mountains. The timberline of this species is formed by low density stands with isolated *P. leucodermis* above the closed forest of *Fagus sylvatica*.

Data collection

Meteorological data

A two-meter tall weather station was installed in an open area at the centre of the site. Air temperature was measured at 2 m above the ground. Stem temperature was measured at 1.3 m height with stem sensors facing south, inserted beneath the bark close to the cambial zone and protected by insulating shields. Data were measured each minute and recorded as an average every hour by means of a CR10X datalogger (Campbell Scientific Corporation). Precipitations

were collected from two other weather stations, Campotenese (965 m a.s.l., 11 km from the site) and Teana (806 m a.s.l., 25 km from the site). The mean monthly precipitations were then calculated from these two stations.

Xylem formation

Ten trees of *P. leucodermis* were selected after a preliminary investigation performed on 20 trees before beginning the sampling in 2003. Cells of the last three rings formed were measured and trees with similar radial growths were chosen in order to ensure comparable growth rates (Deslauriers et al. 2003a).

Wood microcores (2.5 mm in diameter x 25 mm long) were collected around the stems at a height of 1.3 m from May to October using Trapsystem® surgical sampling needles (Rossi et al. 2006a). The microcores were placed in Eppendorf microtubes containing ethanol (50% in water) and stored at 5 °C. Samples usually contained the previous 3-6 tree rings and the developing annual layer with the cambial zone and adjacent phloematic tissues. Microcores were oriented by marking the transverse side with a pencil under a stereo-microscope at 10-20 magnifications, dehydrated with successive immersions in ethanol and D-limonene and embedded in paraffin (Rossi et al. 2006a). Transverse sections of 10-12 µm thickness were cut from the samples with a rotary microtome. Sections were stained with cresyl violet acetate (0.16% in water) and observed with visible and polarized light at 400-500 magnifications to differentiate the developing xylem cells. The cambial zone and cells in radial enlargement showed only primary walls that did not shine under polarized light compared with secondary walls. In cross section, cambial cells were characterized by thin cell walls and small radial diameters (Rossi et al. 2006b). During cell enlargement, the tracheids were composed of a protoplast still enclosed in the thin primary wall

but with radial diameter at least twice that of a cambial cell. The colour of cells in wall thickening changed from light violet at the beginning of the process to deep violet close to the mature cell state. Lignification was characterized by the appearance of blue, initiating in the cell corners and middle lamella and spreading into the secondary walls of differentiating tracheids. Xylem cells were considered lignified and mature when they were completely blue (Rossi et al. 2006b). For each sample, total xylem cell number was found by counting the number of cells in radial enlargement, in cell wall thickening and mature cells along three radial rows (Deslauriers et al. 2003a, Rossi et al. 2006b) and averaged for each site, species and year. In spring, when at least one horizontal row of cells was observed in the enlarging phase, xylem formation was considered to have begun. In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete.

Cell measurements

In both years, 3 microcores per tree from the last sampling date were processed as described above and sections stained with safranin (1% in water) and permanently fixed with Canadian balsam (Eukitt®) to measure the cell features using Wincell™. A camera fixed on an optical microscope was used for numerical image analysis. The parameters measured were single cell wall thickness (µm), lumen diameter (µm), lumen area (µm^2) and cell diameter (µm). On each section, 3 radial files were measured by selecting files with larger tracheids to ensure that the cell sections represented the middle part of their length (Deslauriers et al. 2003a). As these cores were taken and measured when tree-ring growth had finished, the tree-ring width increase over time, representing the cumulated cell diameter, was reconstructed for the growing season based on the cell number increase according to Deslauriers et al. (2003b).

Xylem phenology

The phenology of xylem development was assessed for each tree. Four phenophases were considered, including onset and ending of both cell enlargement and cell wall thickening. Normal probability plots were used to compare the ordered date values (one date per tree) with the percentiles of a normal distribution. The points on the plot adapt along a line when the distribution matches the normal pattern. For each phenophase, the median date of the population corresponded with the 50^{th} percentile of the normal distribution (Waggoner 1974). Phenophase differences between 2003 and 2004 were calculated with median tests.

Temperature threshold

Logistic regressions were used to calculate the probability of xylogenesis being active at a given temperature where binary response was coded as non-active (value zero) or active (value 1) (Rossi et al. 2007). The logistic regression takes the general form:

$$Logit(\pi_x) = \ln(\frac{\pi_x}{1-\pi_x}) = \beta_0 + \beta_1 x_j \qquad \text{(Eqn 1)}$$

where π_x is the probability of xylogenesis being active, x_j is the temperature on a given day j, β_0 and β_1 are intercept and slope of the logit regression (Quinn and Keough 2002). Temperature thresholds (x) were calculated when the probability of xylogenesis being active was 0.5, i.e. when $Logit(\pi)=0$ and then when $x=-\beta_0/\beta_1$. Therefore, for a temperature above x, the wood formation was more likely to be active than non-active. Model verification included χ^2 of the likelihood ratio, Wald's χ^2 for regression parameter and goodness of fit, Hosmer-Lemeshow $\hat{C}$ for eventual

lack of fit (Quinn and Keough 2002). For each tree and year, the model was fitted with the respective temperature series (mean, minimum and maximum air and stem temperatures). The estimated thresholds were compared between 2003 and 2004 with paired t-tests.

Comparison between growth in 2003 and 2004

A nonlinear mixed model (Proc NLMIXED procedure in SAS) was used to assess differences in the growth curves of cell number or tree-ring width between years. This model combines nonlinear response curve with mixed model analysis, allowing repeated observations (*j* sampling dates) on the same *i*th tree (Lindstrom and Bates 1990, Peek et al. 2002). The nonlinear mixed model had the form:

$$y = f(x_{ij}, \nu, u_i) + e_{ij} \qquad \text{(Eqn 2)}$$

where *f* is the growth curve of known vector covariates (x_{ij}), unknown fixed effect parameters (v), unknown vector of random effect parameters (u_i), and unknown random errors (e_{ij}). Incorporating u_i in the model, the assumption of independent error terms was respected, which is necessary when taking repeated measurements on the same trees at different sampling dates (Lindstrom and Bates 1990). The Gompertz equation was employed as growth curve (Deslauriers et al. 2003a) defined as:

$$y = A\exp(-e^{(\beta-\kappa t)}) \qquad \text{(Eqn 3)}$$

where *y* is the weekly cumulative sum of growth (expressed in number of cells or ring width increase), *t* the time computed in day of the year, *A* the upper asymptote, β the *x*-axis placement

parameter, and κ the rate of change parameter. The fitted curves were compared with one-way ANOVA, analysing the fixed effect *v* by means of dummy variables (Peek et al. 2002).

Cell measurements

Curves of cell size variation along radial files of xylem, called tracheidograms, were constructed for the 3 sampling points (microcores) of each tree and year. Standardization was required to compare tree-ring structures as different numbers of cells were found. The standardization method decreases or increases the initial tracheidogram, modifying the number of cells but leaving the overall cell dimensions unchanged (Vaganov 1990). The total number of cells deriving from the nonlinear mixed model was used to standardize the tree rings within each tree. Tracheids were classified as latewood when single wall thickness was four time higher than lumen diameter (Mork's formula described in Denne 1988). Cell lumen area, diameter and wall thickness were compared between years with ANOVA for both earlywood and latewood. The effect of year [degree of freedom (df)=1] was tested based on a factorial model crossed with trees (df=8) with the term year*tree (df=8) considered as the error term. Differences between years were found using LS-means with Scheffe's test. Verification of the ANOVA assumptions was performed by testing for evidence of non-normality and equality of variance of the data (Quinn and Keough, 2002).

Results

Temperature and precipitation

At the site, the temperature in 2003 was 0.5 °C higher than 2004, with an annual mean of 3.8 °C. Compared with historical series (years 1924-2003), 2003 and 2004 were 0.3 °C above and 0.2 °C below the mean annual temperature (3.6 °C) respectively. The deviation of 2003 was mainly due to the warmer March-August temperatures (figure 1a,b). The highest differences from the historical means were found in May and June. In May 2003, the temperature was 5.0 °C above 2004 and 2.6 °C above the long-term average (figure 1b). During spring and summer, only May temperature in 2004 was below the historical average (-2.3 °C), while the rest of the summer temperatures were close to that average (figure 1b).

In the Pollino mountain area, annual precipitation in 2003 and 2004 was 1160 and 1492 mm respectively. However, from March to May 2003, total precipitation was only 132 mm, compared with 478 mm in 2004 (figure 1c). During the summer and early-autumn, the patterns of precipitation in 2003 and 2004 were similar with a slightly higher sum in 2003 (figure 1c). Summarizing the observations of temperature and precipitation in the Pollino area, 2003 was mainly characterized by a hot dry spring, particularly in May, and higher summer temperatures.

Cambium and xylem phenology

The cambium phenology in 2004 showed similar but delayed dynamics to 2003. In 2003, the cambium was already active (9 cells in the cambial zone) when the first sampling was performed on May 17 [day of the year (DOY) 137] (figure 2). The number of cells in the cambial zone further increased until June 14, with a maximum of 11 cells. On July 12, the cambial zone

decreased to 6-8 cells, already indicating reduced division activity. Cambium activity started later in 2004, which postponed almost all the other processes of xylem formation. The activation of cambium was observed around June 8 and reached the maximum at the end of June (figure 2). The number of cells in the cambial zone returned to quiescence value (6-7) at the beginning of August (DOY 225), 2-3 weeks later than in 2003.

To compare the phenology between years, onset and ending of cell enlargement and wall thickening were computed in DOY and represented by normal probability plots (figure 3). In all cases, the ordered values of the trees matched the normal distribution ($P>0.05$). Significant differences were observed in the onset of cell enlargement ($P<0.0001$), with median dates of DOY 151 and 173 in 2003 and 2004 respectively. The variability in the onset was higher in 2003, with trees starting cell enlargement around DOY 138 until DOY 158 (figure 3). As for the onset, significant differences were observed in the median date of the end of cell enlargement ($P<0.0001$). In 2003, the 25-75 percentiles of tree distribution were located around DOY 200, compared with DOY 225 in 2004 (figure 3). Cell wall formation began earlier in 2003 ($P<0.0001$), with a median date of DOY 165, compared with DOY 187 in 2004 (figure 3). Xylogenesis was considered concluded when no further cell was observed in the phase of secondary cell wall thickening. The range of variation was similar, with the 25-75 percentiles of tree distribution located between DOY 240-270 (figure 3) indicating no difference between years ($P=0.08$) for the conclusion of cell differentiation.

The duration of a phenophase was calculated as the difference between its onset and ending. The period in which enlarging tracheids were observed was 10 days shorter in 2003 ($P=0.01$), lasting 54.8±8.8 days against 65.0±10.0 days in 2004. The period in which xylem cells were observed in

secondary wall formation was longer in 2003 (89.9±23.3 days) than 2004 (65.6±14.4 days) (P=0.02). The overall duration of xylogenesis, the time required to complete cell differentiation, was obtained by subtracting the ending of wall thickening from the onset of enlargement. Longer duration of xylogenesis was found in 2003 with 110.9±19.6 days compared with 87.7±15.3 days in 2004 (P=0.02).

Threshold temperatures

The threshold temperature at which xylogenesis had a 0.5 probability of being active was calculated for each tree and reported as average per year (table 1). Between the two years, the thresholds for all air temperatures were not significantly different (P>0.05). By considering both years, the calculated thresholds were about 5.5, 8.2 and 11.5 ºC for minimum, mean and maximum air temperature respectively. The minimum, mean and maximum stem temperature thresholds were higher than air thresholds, being 7.6, 9.5 and 12.1 ºC respectively for both years (table 1). No significant difference was found in the stem temperature thresholds between the two years (P>0.25). However, higher standard deviations between trees were estimated in 2003 (table 1) because of the wider variability in xylem phenology (figure 3).

Growth comparison

The prediction curves f of the nonlinear mixed model are illustrated in figure 4, while the one-way ANOVA, analysing the fixed effect v is reported in table 2. The number of cells and ring-width over time were characterized by a sharp increase, beginning around DOY 145 in 2003 and DOY 165 in 2004, followed by a plateau indicating the end of radial growth, reached at similar times of the year, around DOY 240 (figure 4). For cell number increase, mixed model analysis

showed that the population of trees had similar dynamic in 2003 and 2004 (figure 4, table 2). Although the cell number started to increase later in 2004, the confidence intervals of both years crisscrossed around DOY 210. No difference was found in the total number of cells produced (asymptote *A*), which varied between 29.3 and 24.8 cells for 2003 and 2004 respectively (table 2). However, for ring-width increase, the model indicated the existence of a year effect on the growth response curves (figure 4, table 2). The gap between the onset of ring-width formation was not filled, as shown by the separate confidence intervals throughout the growing period. Significantly smaller ring-widths were therefore formed in 2004 (425±62 µm) than in 2003 (608±30 µm), although a similar number of cells were formed.

In 2003, earlywood cells were formed until July 14 (DOY 195), while in 2004 earlywood production lasted until July 26 (DOY 208). On average, trees formed 17.5 earlywood tracheids in 2003, against 14.8 in 2004. In 2003, earlywood cells were larger in diameter (P=0.012), with wider lumen area than in 2004 (table 3). However, no difference was observed in the earlywood wall thickness (P=0.17). Larger cell walls were formed (P=0.019) in 2003 latewood, but tracheids showed similar diameter (P>0.05) (table 3).

Discussion

Cambium phenology and temperature

The spring temperature increase is important from an ecological perspective because of the potential effect on the timing of thaw and initiation of the growing season (Keyser et al. 2000). As expected, several phenological traits of xylem formation differed between 2003 and 2004, suggesting a strong effect of May and June temperatures on the early processes of xylem formation. Compared with 2004, onset of cambium activity, cell enlargement and cell wall thickening occurred earlier in 2003, which increased the duration of xylogenesis processes by 23 days. Cambial cells of evergreen conifers at the quiescent stage can re-initiate cell division independently of the growth of new shoots and development of buds in spring (Oribe et al. 2001, 2003). In early spring (e.g. March), heating experiments induced localized reactivation of the cambium (Oribe et al. 2001, Gričar et al. 2006), demonstrating that cambial cells are highly receptive to an increase in temperature. The early start in 2003 is in agreement with other results found in timberline areas. Compared with 2002 and 2004, the warmer spring of 2003 induced an earlier resumption of cell production in the cambium and a consequent earlier onset of xylem cell differentiation in several conifers (Rossi et al. 2007).

In 2004, the mean temperature threshold of 8 ºC was reached only around June 10 (DOY 162), significantly delaying the growth activity compared with 2003. Application of cooling during an entire growing season in *Picea abies* at temperatures of 9-11 ºC (Gričar et al. 2007), slightly above the threshold of this species (Rossi et al. 2007), shortened regular cambial activity and delayed fully mature cells by about one month. The convergent responses of cambium phenology to temperature thresholds in contrasting springs strengthen the effect of temperature in

determining onset, end and duration of wood formation. Recently, Seo et al. (2008) modelled the onset of wood formation of *Pinus sylvestris* in Northern Finland by using heat sum models (degree days) with a threshold of 5 ºC. However, the variability of degree days was too high for estimating the onset of wood formation so its value had to be transformed as percentage of long-term mean of degree days. According to the results of this study and to Rossi et al. (2007), temperature threshold calculated when trees have 50% probability of being active could be efficiently used for modelling the onset of wood formation and duration of wood growth.

Cambium phenology and wood formation

Temperature increase in timberline zones can have different effects, such as increasing height growth of trees (Gamache and Payette 2005) or enhancing the reproductive success (Kullman 2007). Plants growing at higher altitudes may benefit from warmer summer temperatures because their growth is primarily temperature-limited (Körner and Paulsen 2004, Rossi et al. 2007). In 2003, only trees growing above a certain altitude benefited from the higher temperatures, while growth decreases were observed at lower altitudes (Jolly et al. 2005). In comparison with previous and successive growth years (1996-2004), a 10-20% increase in circumference was observed during 2003 in *P. abies* and *Abies alba* in subalpine areas of the Swiss Alps (Jolly et al. 2005). In the same year, an increase in growth was also observed in Bavarian *Larix decidua* over 1500 m a.s.l. (Bavarian State Institute of Forestry, 2004). By contrast, in the inner Alpine valley (Tyrol Austria), a 35% reduction in growth was observed in *P. sylvestris* and *P. abies* due to an early ending of cambial activity (Pichler and Oberhuber 2007). In the present study, an increase of 11% in ring-width and 30% in radial cell number was measured in 2003 as compared to the two previous years of growth (2001 and 2002, data not shown). For 2004, contrasting results

were found, with a decrease of 14% in ring width and an increase of 17% in the total cell number as compared to 2001-2002.

In this study, a similar dynamic of cell production was observed in the two contrasting years, with an equal number of cells constituting the rings (29.3 cells in 2003 compared with 24.8 in 2004). Two combined causes could explain these results. First, the period in which tracheids were observed in the phase of radial enlargement was 10 days longer in 2004. Second, in 2004 the increase in cell number could reflect the climatic conditions of the previous year (2003), which favoured the C-storage used to build the tree-ring for the next year (Hansen and Beck 1990, Hansen et al. 1997). Even though the phenophases were delayed in 2004, the time elapse between onset of cell enlargement and onset of wall thickening was two weeks, indicating a similar duration necessary to enlarge and form the primary walls of the first xylem cells. In high altitude conifer species, the persistence of the cells in radial enlargement gradually decreased along the radial file from 14–25 days in the first earlywood tracheids to a very few days in the last cells (Rossi et al. 2006b). According to the model proposed by Fritts et al. (1999), radial growth depends on the intensity rather than the duration of the process, especially at the beginning of tracheid enlargement. According to the results of this study, the higher temperature in 2003 did not affect the duration of tracheid enlargement in the first cells.

Important differences emerged between the two years in the dynamics of ring-width formation, which resulted in wider tree-rings in 2003. These differences were more related to cell size than the number of cells constituting the rings, since larger earlywood cells were formed in 2003. Strong evidence of the effects of temperature on xylem elements has already emerged from several studies (Denne 1971, Antonova and Stasova 1993, Panyushkina et al. 2003, Fonti et al.

2006). In the arctic shrub *Salix pulchra* Cham., plants growing at +5 °C above the Alaskan summer temperatures had larger vessel diameters (Gorsuch and Oberbauer 2002). Near the treeline, the higher 2003 temperatures could have favoured carbohydrate metabolism and water osmotic potentials (Kontunen-Soppela et al. 2002), as temperature allows the available sucrose to be allocated (Hoch and Körner 2003) and metabolic processes of growth to be completed (Begum et al. 2007). Cell size is also dependent on tree water status (Steppe et al. 2006), but from June to August 2003 (i.e. during the highest period of cell production and radial growth) there were no extremely dry conditions in the Pollino massif (figure 2d), in contrast with other parts of Europe (Rebetez et al. 2006) and suggesting no water limiting conditions for growth in 2003. Moreover, anatomical signals of water stress in the tree-ring (abrupt decreases in radial diameter of a group of cells or reduced percentages of latewood) were lacking in both years.

Latewood formation, which involves a reduction in radial expansion and an increase in wall thickness, is strongly influenced by the duration of cell wall thickening (Denne 1976, Uggla et al. 2001, Deslauriers et al. 2003a, Rossi et al. 2006b). Compared with 2004, the period during which xylem cells were found in secondary cell wall formation was about 25 days longer and, as a result, latewood tracheids had thicker walls in 2003. Moreover, earlywood cell diameter was larger in 2003, which directly led to a greater volume of wall material produced. Onset, duration and end of cell wall formation is important for the C-balance of a tree as it represents a large sink for carbon. During cell maturation, trees assign a large amount of carbon directly obtained from photosynthesis to the production of cellulose microfibrils that contribute towards building the secondary wall (Hansen et al. 1997). According to these results, the modification of phenology in 2003 increased stem biomass production of *P. leucodermis* in terms of both ring width and in the amount of carbon fixed in the cell walls.

At high altitude in Southern Italy, the effect of contrasting spring temperatures significantly modified the duration of xylogenesis, which was controlled by the reaching of a daily mean air temperature threshold of 8 ºC. Compared with 2004, ring width, earlywood cell size and latewood wall thickness of *P. leucodermis* increased in 2003. This study shows the plasticity of tree-ring formation in response to high temperatures by modifying the onset and duration of differentiation. These results have important implication for trees growing near their limits. Larger xylem elements conduct a more than proportionally higher amount of resources, as a small increase in tracheid diameter leads to large increases in hydraulic conductance, which represents an advantage in terms of height growth potential for treeline species (Anfodillo et al. 2006). In conclusion, the warmer temperatures in 2003 at the timberline in Southern Italy promoted the growth of *P. leucodermis*, which currently seems to be more threatened by anthropogenic activity than by climate change.

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Caption list

Table 1. Threshold minimum, mean and maximum temperatures corresponding with the 0.5-probability of active xylogenesis for *P. leucodermis* estimated during 2003-2004. Error indicates standard deviation among 10 trees.

Table 2. Comparisons of growth response curves between 2003 and 2004, fitted to the cumulative number of cells or tree-ring widths (μm).

Table 3. Comparisons of cell diameter (μm) and single cell wall thickness (μm) between 2003 and 2004 for earlywood and latewood cells. LS-means and their 95% interval are shown representing the combination of 9 trees and 3 sampling positions per tree.

Figure 1. Temperature and precipitation patterns at the study site in the Pollino mountain area. A, mean monthly temperatures (°C) recorded in 2003 (black dots) and 2004 (grey dots) and mean monthly air temperature of historical series recorded from 1924-2003 (white dots); B, difference between the historical series and 2003 (black dots) and 2004 (grey dots); C, sum of monthly precipitation (mm) in 2003 (black bars) and 2004 (grey bars) from two weather stations.

Figure 2. Numbers of cells in the cambial zone during 2003 (black dots) and 2004 (white dots). Error bars and horizontal dotted line indicate the standard deviations among trees.

Figure 3. Distribution of the dates of onset and ending of cell enlargement and cell wall thickening for 10 *P. leucodermis* trees in 2003 (left) and 2004 (right).

Figure 4. Cumulative cell number and tree-ring width (µm) for both 2004 (black lines) and 2004 (grey lines). Dotted lines show 95% confidence interval. Differences between curves are reported in table 2.

Table 1

	Air temperature (°C)			Stem temperature (°C)		
	Year 2003	Year 2004	*F*-value(P)	Year 2003	Year 2004	*F*-value(P)
Minimum	5.73±1.01	5.40±0.29	0.85(0.42)	7.85±0.95	7.42±0.27	1.20(0.26)
Mean	8.44±1.08	7.95±0.31	1.18(0.27)	9.72±1.00	9.28±0.29	1.14(0.29)
Maximum	11.66±1.17	11.24±0.34	0.93(0.38)	12.35±1.13	11.84±0.33	1.17(0.27)

Table 2

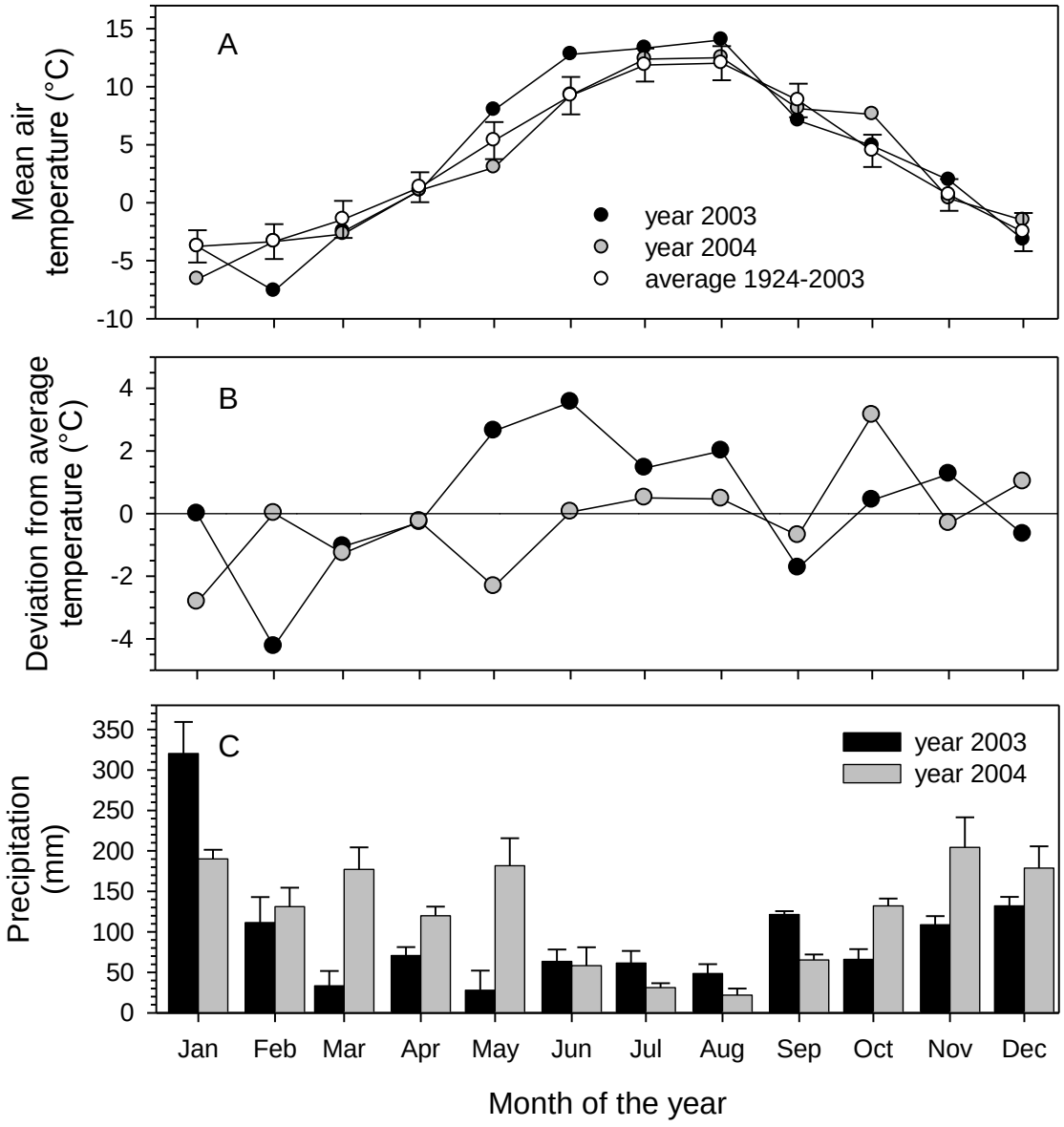
Year	A		β		$\kappa * 10^{-2}$	
	Cells	Tree-ring	Cells	Tree-ring	Cells	Tree-ring
2003	29.27 ± 2.3	608.1 ± 29.8	6.77 ± 0.7	8.09 ± 0.3	3.82 ± 0.4	4.83 ± 0.2
2004	24.82 ± 3.9	425.5 ± 61.7	8.41 ± 1.5	10.99 ± 0.9	4.35 ± 0.8	5.97 ± 0.5
F-value	1.32	8.71	1.12	9.64	0.38	3.95
P	0.2700	0.0105	0.2000	0.0077	0.5500	0.0667

Table 3

	Cell diameter (μm)			Single cell wall thickness (μm)		
	Year 2003	Year 2004	*F*-value(P)	Year 2003	Year 2004	*F*-value(P)
Earlywood	30.14±0.99	28.25±0.97	9.85(0.012)	3.27.±0.16	3.12±0.16	2.32(0.16)
Latewood	15.60±0.545	15.13±0.59	1.81(0.22)	3.92±0.19	3.57±0.20	8.69(0.019)

1

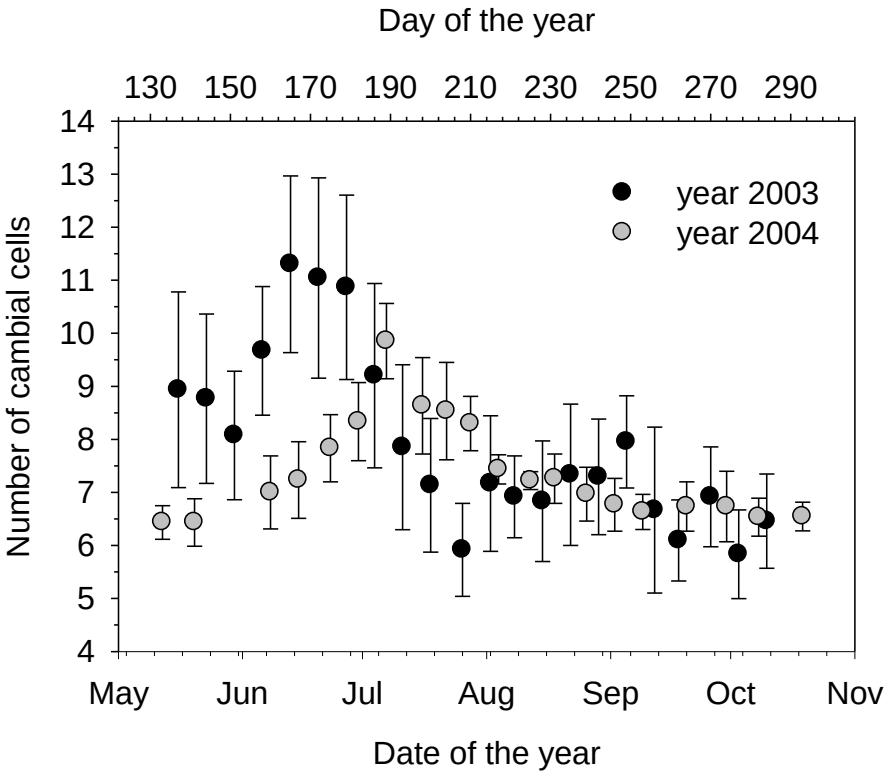
Figure 1


A
Mean air temperature (°C)
15
10
5
0
-5
-10
year 2003
year 2004
average 1924-2003
B
Deviation from average temperature (°C)
4
2
0
-2
-4
C
Precipitation (mm)
350
300
250
200
150
100
50
0
year 2003
year 2004
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month of the year

2

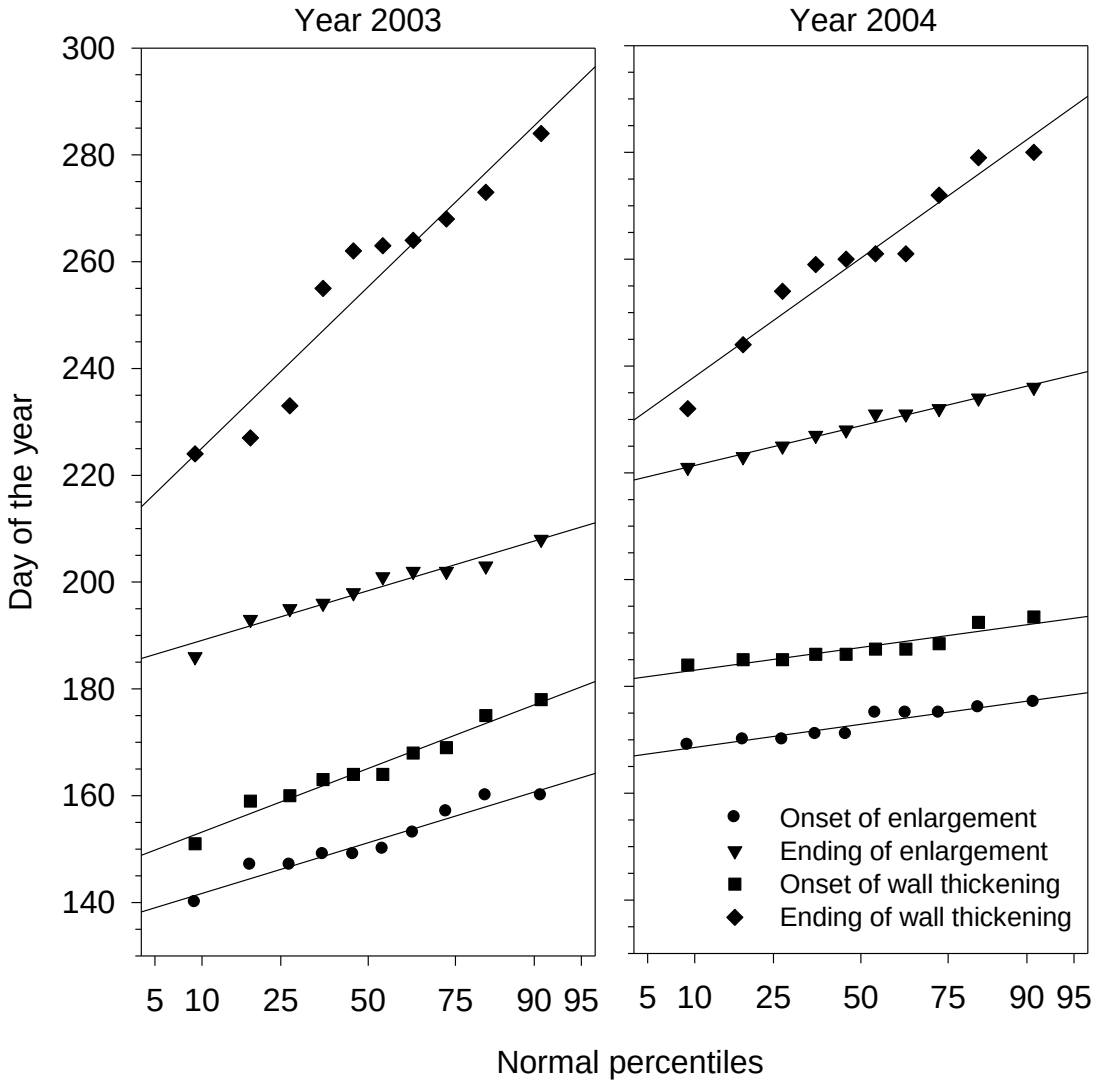
1

figure 2


Day of the year
130
150
170
190
210
230
250
270
290
14
13
12
11
10
9
8
7
6
5
4
Number of cambial cells
year 2003
year 2004
May
Jun
Jul
Aug
Sep
Oct
Nov
Date of the year

2

1 **Figure 3**

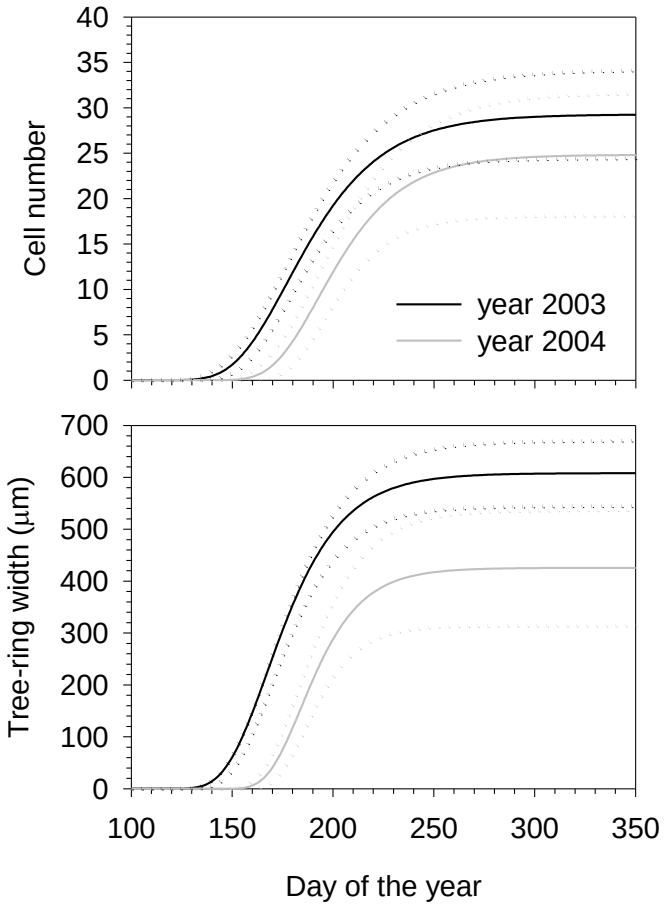

Year 2003
Year 2004
Day of the year
300
280
260
240
220
200
180
160
140
5 10 25 50 75 90 95
5 10 25 50 75 90 95
Normal percentiles
Onset of enlargement
Ending of enlargement
Onset of wall thickening
Ending of wall thickening

2

3

1

Figure 4


Cell number
Tree-ring width (µm)
Day of the year
year 2003
year 2004
0
5
10
15
20
25
30
35
40
100
200
300
400
500
600
700
100
150
200
250
300
350

2