

Using simple causal modelling to understand how water and temperature affect daily stem radial variation in trees

Running head: Climate influence on intra-annual growth

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Summary

Variations in stem diameter include reversible shrinking and swelling movements and irreversible radial growth, both influenced by the water status of the tree. In order to assess the causal effect of water and temperature on stem radial variation (ΔR) and maximum daily shrinkage (MDS), the diurnal cycle was divided into the three phases of contraction, expansion and stem radius increment. Diurnal cycles were measured during 1996-2004 in *Picea abies* (L.) Karst. *Pinus cembra* L. and *Larix decidua* Mill. in a timberline ecotone to understand the links between stem variation v (defined as MDS or ΔR), phase duration h , and weather or sap flow descriptors d . We demonstrated that a high proportion of MDS and ΔR was explained by h because of the non-linearity of the physiological responses to weather d . By causal modelling, we tested whether the relationship between d and v was due to h (lack of causal relationship between d and v) or both d and h (double cause). The results of this modelling added new physiological interpretations for daily growth-climate relationships. Negative correlations were found between ΔR and air temperature due to the negative effect of temperature on d only and didn't correspond to a direct physiological effect on tree growth in terms of metabolic activities. It was demonstrated that precipitation had two main effects: a direct effect on ΔR and an indirect effect caused by phase duration (h). A reduction in sap flow at night leads to an increase in ΔR for *P. abies* and *L. decidua*, but not for *P. cembra*. It would be worth taking phase duration into account in the models to establish the correct link with environmental factors.

Keywords: Conifers, growth-climate relationships, stem contraction, radial growth, tree-water relations, causal modelling

Introduction

Daily stem diameter variations in trees are due to water storage depletion and replenishment of the stem tissues and radial growth (Kozlowski and Winget 1964, Herzog et al. 1995, Irvine and Grace 1997, Génard et al. 2001, Daudet et al. 2005, Steppe et al. 2006). Several papers have been published that aimed to link stem radial diameter variations with climate (Downes et al. 1999, Tardif et al. 2001, Deslauriers et al. 2003, Mäkinen et al. 2003, Bouriaud et al. 2005, Zweifel et al. 2006), but interpretation of the relationships found appeared to be uncertain due to the superimposed effects of both water status and cambial activity.

Growth processes involve the production of xylem and phloem elements during the phases of cell division, extension of primary cell wall, secondary wall formation and lignification, precisely regulated by gene expression (Schrader et al. 2004), hormonal signals (Schrader et al. 2003) and environmental factors (Savidge 1996, Deslauriers and Morin 2005, Rossi et al. 2006b). At cellular level, enlargement processes are basically water-dependent, as turgor pressure drives irreversible cell expansion and deposition of wall polymers (Proseus and Boyer 2005). The two main processes measured by dendrometers, reversible changes and radial growth, are consequently both water-dependent. Mathematical models and experimental data both support the concept that daily variations in stem diameter are caused by replenishing of storage compartments in addition to stem growth (Steppe et al. 2006) and that the current tree water status plays a greater role than the C-balance (Daudet et al. 2005, Zweifel et al. 2006).

Considering the strong impact of tree water status on cell division and enlargement (Abe et al. 2003, Proseus and Boyer 2005), when using dendrometer measurements, the effects of water on stem variation are more readily explainable. A positive influence of water on radial stem growth has been observed for a wide range of species, such as *Eucalyptus* spp. (Downes et al. 1999), *Pinus hartwegii* Lindl. (Biondi et al. 2005), *Abies balsamea* (L.) Mill. (Deslauriers et al. 2003) and *Picea abies* (L.) Karst. (Bouriaud et al. 2005, Zweifel et al. 2006), *Quercus pubescens* and *Pinus sylvestris* (Zweifel et al. 2006). Other effects remain poorly understood: for example the negative relationship between temperature and growth rate in *P. abies* in France (Bouriaud et al. 2005) and in several boreal conifer species (Tardif et al. 2001). Temperature is a primary determinant of growth and has been found to influence cell production in several European conifer species (Antonova and Stasova 1997, Rossi 2003). However, the question remains as to how closely the relationships reflect a direct causal effect on stem growth. It is thus of interest to unambiguously distinguish the causal effect of water and temperature on stem radial variation to better interpret the growth and climate relationship found.

The *stem cycle approach* introduced by Downes et al. (1999) might be a tool for better understanding the effect of the different factors on stem radial variations. It divides the typical stem circadian cycle into three phases of contraction, recovery and stem radius increment (i.e. estimated radial growth). By following the periodic sap flow variations, this approach provides an accurate assessment of both components of the stem cycle: duration and stem radial variation defined as stem radius variation (ΔR) and maximum daily shrinkage (MDS). To date, the

components of the stem cycle have been analysed separately and the effect of climate on duration ignored. Downes et al. (1999) assumed these components to be physiologically independent. However, phase duration could be highly dependent on climate variation, as stem shrinking and swelling are driven by tree transpiration and sap flow (Herzog et al. 1995, Perämäki et al. 2001).

In this study, two components of the stem circadian cycle, duration of the different phases and the corresponding stem radial variation (MDS or ΔR), were analysed in order to assess the role of water and temperature on a daily basis. The hypotheses of causal relationships between descriptors (water, temperature variables), phase duration and stem radial variation (MDS or ΔR) were tested by means of two models in which the correlation between descriptor and stem radial variation is caused by (1) phase duration only or (2) both descriptor and phase duration. Both models are based on two initial hypotheses: (i) correlation between descriptors and phase duration and (ii) between phase duration and radial variation. This approach should allow us to better explain the effect of temperature on stem radial variation, which is particularly important in high-altitude forests. To verify the models, the dynamics of stem radial variation were recorded for 9 years using automatic band dendrometers on the three main conifer species at the Italian Alpine timberline.

Material and Methods

Study area

The study was conducted in the north-eastern Italian Alps [Dolomites, Cortina d'Ampezzo (BL), 46°27'N 12°08'E]. The site was located at 2080 m a.s.l. near the Cinque Torri mountain group on a south-facing slope of approx. 30%, within a mixed open forest with groups of 5-15 trees of larch (*Larix decidua* Mill.) and stone pine (*Pinus cembra* L.) and rare Norway spruce [*Picea abies* (L.) Karst.], corresponding to the timberline ecotone definition (Körner 1998). The timberline originated from abandoned pasture lands with tree establishment ranging from 1943 to 1971. The climate of the site is typical of the south-eastern Alps. The winters are dry with most precipitation occurring during summer and early autumn. Two plants of *P. abies* (91±11.3 cm dbh, 10.4±0.6 m height, 45.0±8.7 old), *L. decidua* (79±1.2 cm dbh, 11.6±1.0 m height, 60.6±18.7 old) and *P. cembra* (103±14.1 cm dbh, 10.1±0.7 m height, 60.4±7.9 old) were monitored from 1996 to 2004.

Data collection

Stem radius variation, climate and sap flow were monitored from 1996 to 2004 and recorded by dataloggers (CR10X, Campbell Scientific Corporation). Automatic band dendrometers [Tecno Penta, Teolo (PD), Italy] were used to continuously monitor tree growth. These instruments measure linear displacement of a stainless-steel band wrapped around the bark on the trunk. The operating principle is based on the use of a linear potentiometer. Displacement is resolved to six µm over an unadjusted range of 20,000 µm. As the stem expands and contracts, the band transmits the signal to the potentiometer. The band had a thermal coefficient

of linear expansion of $11 \mu\text{m.m}^{-1}.\text{°C}^{-1}$, representing an error of about 1% on the calculated value of MDS and ΔR . Teflon foils were placed between bark and band to reduce friction. Dendrometers were installed on two trees per species at a height of about 1.3 m. Readings were taken every 15 minutes and the hourly averages calculated. The circumference data were divided by 2π , giving radial measurements.

A two-meter tall weather station was installed in an open area in the middle of the site. Air temperature (°C) and relative humidity (%) at a height of 2 m were measured each minute and recorded as an average every 15 minutes. Other variables were measured each minute and stored every hour. Soil water content (%) was measured with a time domain reflectometer (TDR method) at 15-20 cm depth. The rainfall sum (mm) was recorded each hour from the one minute measurements. Vapour pressure deficit (VPD, KPa) was calculated from the hourly average of air temperature and relative humidity (Jones 1992). Sap flow density ($\text{dm}^3 \text{ dm}^{-2} \text{ h}^{-1}$) was measured in each tree using a continuously-heated thermal dissipation sensor (2 cm long needle) (Granier 1985). The sensors were inserted into the xylem at about 1.5 m, on the north-west facing side of the stem. Protection from high solar radiation was ensured by insulating shields placed over the sensors and, for *P. abies* and *P. cembra*, by the dense tree branches reaching the ground. Measurements were taken each minute and stored as an average every 15 minutes.

Extraction of stem variation and association with climate

For each tree and year, the extraction of stem variation was performed by dividing the stem cycle into three distinct phases (Downes et al. 1999, Deslauriers et al.

2003) as follows: contraction phase (1), the period between the morning maximum and afternoon minimum; expansion phase (2), the total period from the minimum to the next morning maximum; stem radius increment phase (3), part of the expansion phase from the time when the stem radius exceeds the morning maximum until the subsequent maximum (figure 1). The definition of the expansion phase is slightly different from the one used by Downes et al. (1999) (recovery phase) as the whole expansion process (recovery and stem increment) was considered (Deslauriers et al. 2003). The difference between the expansion maximum and the onset of the third phase represents the positive stem radius change estimate ($\Delta R+$, μm). When the previous cycle maximum was not reached, a negative stem radius change ($\Delta R-$, μm) was calculated but no phase 3 was defined (figure 1). Maximum daily shrinkage (MDS, μm) was calculated as the difference between the morning maximum and afternoon minimum. The duration (h , hours) of each phase was also calculated. Any additional small effect of temperature on wood expansion was not relevant to the cycle amplitude and not considered in the analysis. Similarly, variations caused by dead bark included in the dendrometer signal couldn't be removed. However, plants of the same species were of the same age with approximately analogous bark thickness.

From May to September, the stem cycle generally lasted about 24 hours, but heavy precipitation can cause longer cycles, because of a longer expansion phase (figure 1). Autocorrelation and trend in the series were assessed with the ARIMA procedure (SASTM). T-test and paired t-test were used to compare the duration of contraction and expansion phases within and between the days with positive and negative ΔR . The t-tests included all nine years. Relationships between phase

duration and stem radial variation (MDS or ΔR) were assessed by linear regressions.

Climate and flow data were coupled with the respective daily MDS and ΔR (Downes et al. 1999, Deslauriers et al. 2003) with special routines written in SASTM. For example, for each cycle the mean temperature occurring during the contraction phase was coupled with the corresponding value of MDS. The association was first made for each tree and averages were then calculated for each species and year.

Model description

The causal modelling used simple and partial correlations (r) to explain the relationships among descriptor (d), duration of the phases (h) and stem radial variations (v) based on two initial hypotheses. The descriptors d consisted of sap flow, VPD, precipitation, soil water content and air temperature. For v corresponding with MDS, the duration of the contraction phase was used. In that case, the descriptors represent the weather conditions or the sum of sap flow occurring in that phase. For v corresponding with $\Delta R+$, the duration of the stem radius increment phase was used. In that case, the descriptors represent the weather conditions or the sum of sap flow occurring in that phase.

Initial hypotheses The hypotheses define the causality links between d , h and v leading to the two models described below and correspond to the following correlations:

$$r_{dh} \neq 0 \quad (1)$$

$$r_{hv} \neq 0 \quad (2)$$

In the absence of significant correlations in formula 1 and 2, neither model can be formulated as causal links are missing.

Models Two models of causal relationships, deriving from the initial hypotheses, were defined as:

Model 1

$$d_i \rightarrow h_i \rightarrow v_i$$

$$\begin{array}{ccc} d_i & \rightarrow & h_i \\ & \searrow & \swarrow \\ & v_i & \end{array}$$

Model 2

where i represents a given phase. The arrows describe the possible interactions between d and v (representing MDS or ΔR) in the presence of possible interactions caused by h . In model 1 the correlation between d and v is caused by h (lack of causal relationship between d and v) while in model 2 the correlation between d and v is caused by both d and h (double cause).

Expectations of the models Simple and partial correlations were performed between d , h and v . A partial correlation coefficient measured the connection between two variables (e.g. d and v) controlling for the effect of a third (e.g. h) being constant at its mean and noted as $r_{dv.h}$. According to the results of the correlation, in terms of the expected values for the simple and partial coefficients, model 1 or 2 was formulated for a given descriptor (d). These expectations

followed Legendre and Legendre (1998) and are described in table 1. After verifying the initial hypotheses, model 1 or 2 was selected by controlling each expectation and rejecting one or both models when the correlation values did not correspond to the expectations.

The bootstrap procedure was used to test the significance of the correlation coefficients and estimate the stability of the results by producing confidence intervals (Efron and Tibshirani 1993). Median correlations were considered significant after 1000 bootstrapped iterations when the 95% confidence interval for each variable did not pass zero. The 5% and 95% percentiles were calculated based on the normal distribution of the correlation coefficients generated by bootstrap replications (Efron and Tibshirani 1993). Before performing the analyses, linearity of the correlations was verified and log-transformations performed when required. Annual trends in T and SW were removed by using spline functions. The correlations were performed with the circadian cycles (24 ± 3 hours) but long cycles (due to lengthy precipitation) were excluded to only analyse the daily stem movement. Only the main stem growth period, during June and July, was included in this analysis because the variability in stem variation measured by dendrometer in August is higher than the amount of stem growth in terms of latewood cell production and enlargement (Deslauriers et al. in press, Rossi et al. 2006a).

Comparison between ΔR , ring width and tree ring formation

From each tree, cores were extracted in the four cardinal directions (north, south, east and west) with a Presler borer. All cores were mounted, planed with progressively finer grade sanding paper and ring widths measured to the nearest

0.01 mm with a semiautomatic measuring system connected to a computer (Aniol 1987). For each tree, a paired t-test ($P=0.05$), including 1996 to 2004, was used to compare the cumulated ΔR with the measured tree-rings.

The cumulated ΔR was also matched with tree-ring growth during the course of the growing season. Tree-ring growth was calculated on five trees per species growing in the site (Rossi et al. 2007). Micro-cores were extracted from April to October, fixed in paraffin and cut in transversal sections, stained with 0.16% water solution of cresyl violet acetate in order to count the cambial cells, xylem cells undergoing differentiation and mature xylem cells (Rossi et al. 2006a). Cell width (μm) was measured on micro-cores collected from the 15 trees in October with WinCell™. The tree-ring growth was then reconstructed for the growing season according to Deslauriers et al. (2003) by using both measurements.

Results

Stem radial variations

The three species had very similar series of ΔR variations from May to October 2001 (figure 2c, 2001 was taken as an example) with alternating periods of positive and negative variations. During June and July (growth period), the amplitude of ΔR ranged from -50 to 250 μm for all species. In August, ΔR - occurred more frequently when radial growth was almost over (figure 2cd). These series were stationary (homoscedastic), with no probability of autocorrelation within the previous six cycles (lag-6, $P < 0.01$). The time series characterizing MDS were less similar between species and were frequently non-stationary as the range of variations changed from 0 to 50 μm in June-July, to 0 to 100 μm in August when stem growth was close to ending (figure 2e).

Annual and intra-annual radial growth

To evaluate the accuracy of dendrometer measurements, the total radial increment calculated with ΔR was compared with the tree-ring width formed from 1996 to 2004 (figure 3 a-c). For each tree, no significant difference was found between the cumulated ΔR and tree-ring width (paired t-test, $P > 0.1$). Measurement of ring-width differs according to the position around the stem, but the cumulated ΔR was generally within the range of variation of the ring width for *P. abies* and *L. decidua* (figure 3 ab). For *P. cembra* however, in some years, the cumulated ΔR was outside the range of variation of tree-ring measurement even if no significant difference was found in either tree (paired t-test, $P = 0.67$ and $P = 0.26$ for tree 1 and 2 respectively). When associated with measurement of tree-ring formation, the cumulated sum of ΔR detects similar intra-annual radial growth (figure 3 d-e). In

2004 for example, the ΔR rate of increase in June and July was similar to that of 5 other trees of the same age and dimensions.

Phase duration

The times when each phase began were calculated and averaged over nine years (table 2). For both *P. abies* and *L. decidua*, the contraction phase started on average at 10:30 (± 3 h) and continued until 18:00-19:00 (± 2 h) when the trees started stem expansion. *P. cembra* showed a delay of about one hour in expansion onset compared to the other species. When occurring, the third phase started between 22:00 and 24:00 (± 3 h). The stem cycles were divided in two groups: cycles < 28 h were considered as regular circadian diurnal and cycles > 28 h were considered as long (figure 1, table 2). The main difference characterising the long cycles was the longer expansion phase lasting 34-40 h, about two days up to four days. There were about five (± 2) long cycles per month from May to September, corresponding with rainfall events (figure 2a,c-d). The highest $\Delta R+$ were calculated during long cycles as stem expansion proceeded constantly for 34-40 h.

To understand the difference between the diurnal cycles with $\Delta R+$ and those with $\Delta R-$, the length in hours of contraction and expansion phases were compared (figure 4). When a cycle with $\Delta R-$ occurred, both contraction and expansion phases lasted about 12 h, with no significant difference between the two phases for all species (paired *t*-test, $P > 0.05$). In 1999 and 2003, the higher differences were due to a very low number of $\Delta R-$ ($n < 5$). In contrast, when a $\Delta R+$ occurred, the hours of contraction were significantly lower than the expansion phase in all species (paired *t*-test, $P < 0.001$). Compared to the cycle with $\Delta R+$, the hours of contraction were

significantly higher and hours of expansion were significantly lower for the ΔR^- (t -test, $P < 0.001$). The cycle with ΔR^+ therefore more likely occurred when there were unbalanced cycles with 6-8 h of contraction and 16-18 h of expansion (figure 4).

Relationship between duration and amplitude

The duration of the contraction and expansion phases influenced MDS and ΔR respectively (figures 5 and 6). In all species, the MDS (absolute values) linearly increased with duration ($p < 0.001$, figure 4). Log transformations were performed as variation in MDS increased together with duration. When the hours of stem contraction increased, MDS was observed to increase proportionally more in *P. abies* compared with *L. decidua* and *P. cembra*, as shown by the regression slopes (figure 5). The expansion phase duration influenced the ΔR for all species. Linear regressions showed positive slopes ($P < 0.01$), with higher rates of change in *L. decidua* and lower in *P. cembra* (figure 6).

Models of causal relationship

MDS The simple and partial correlation results demonstrated that descriptors sap flow and VPD supported the causal model 2 (table 3, figure 7). MDS of all species were positively correlated with sap flow (r_{dv}), with high coefficients varying between 0.65 for *P. abies* and 0.78 for *P. cembra* and, according to model 2, these correlations were caused by both duration of the contraction phase (h) and sap flow. The simple correlations between MDS and VPD were significant and varied between 0.48 and 0.69, depending on species. Except for *L. decidua*, the partial correlations ($r_{dv,h}$ and $r_{dh,v}$) indicated that this correlation was caused by both VPD

and phase duration. For *L. decidua*, the partial correlation $r_{dh.v}$ was not significant, which did not fulfil the conditions for model 2.

The initial hypotheses were not fulfilled for precipitation and soil water content as the simple correlations r_{dh} were not significant (figure 7). During a rainy period, the contraction phase normally lasted few hours or was completely skipped, leading to a long cycle. For air temperature, the partial correlations $r_{dh.v}$ were not significant, which did not fulfil the expectations of model 1 or 2 (figure 7). Neither model could therefore be formulated between these descriptors, contraction phase duration and MDS.

$\Delta R+$ According to the results of the model, sap flow or precipitation fulfilled the prerequisite and expectation of model 2, while air temperature and soil water content fulfilled the prerequisite and expectation of model 1 (table 3, figure 7). The correlations and partial correlations between VPD, phase duration and $\Delta R+$ supported neither model (table 2, figure 7). Non-significant or very low simple correlations (r_{dv}) were found between sap flow and $\Delta R+$. The partial correlations for *L. decidua* and *P. abies* indicated that this correlation (r_{dv}) corresponds to two opposing effects: a positive effect of sap flow on the duration of the stem radius increment phase ($r_{dh.v}$) and a negative effect of sap flow on $\Delta R+$ ($r_{dv.h}$). For *P. cembra* the expectations of model 2 were not fulfilled as $r_{dv.h}$ was not significant. For precipitation, all partial correlations were significant, fulfilling the expectations of model 2 for all species. The partial correlation $r_{dh.v}$ and $r_{dv.h}$ corresponded to two positive effects of precipitation: a positive effect on phase duration ($r_{dh.v}$) and also on $\Delta R+$ ($r_{dv.h}$). The simple correlation (r_{dv}) varying from 0.36 for *P. cembra* to 0.44

for *L. decidua* was therefore caused by both precipitation and duration of the stem radius increment phase.

The simple correlation (r_{dv}) between ΔR_+ and air temperature varied between -0.27 for *P. cembra* and -0.38 for *P. abies*. However, these correlations were caused by phase duration only (i.e. lack of causal relationship). Therefore, an increase in temperature shortened the duration, which in turn decreased ΔR_+ . The simple correlation (r_{dv}) between soil water content and ΔR_+ was also caused only by phase duration: an increase in soil water content increased the phase duration and in turn, ΔR_+ .

Discussion

The amplitude of stem radius variation, calculated as MDS and ΔR , was found to be highly dependent on phase duration. From spring to autumn, the timing of the diurnal dynamics of storage depletion and replenishment is subject to constant changes and varies as a function of environmental conditions, transpiration and sap flow (Perämäki et al. 2001, Zweifel and Häsler 2001). Despite this variability, the possibility of measuring a positive or negative ΔR was associated with the timing and duration of the diurnal cycles resulting in balanced (~12/12 h) or unbalanced (~7/17 h) cycles. Given that both components are dependent, a climatic or physiological factor acting on one component can influence the other.

Causal effect of water and temperature on MDS

In June and July, the contraction phase started on average from 10:00 to 11:00 (\pm 3 hours) with the latest onset observed for *P. cembra*. This onset was later (4-5 hours) than in very young trees (Steppe et al. 2006), suggesting that conditions for cell expansion in large trees are met not only during the night but also early in the day. For a subalpine *Picea abies*, Zweifel and Häsler (2001) reported an onset of the contraction phase at 09:00 at the base of the stem, on a sunny July day. The onset of stem contraction and sap flow at the base both show a time-lag in response to the onset of transpiration at the top of the tree creating a peristaltic wave of contraction along the stem (Wronski et al. 1985, Wullschlegel et al. 1998, Perämäki et al. 2001) varying as a function of the stored water used (Ford et al. 2004). The diameter changes along the stem correspond to a depletion of the stored water in response to transpiration (Zweifel and Häsler 2001). At the base of the stem, the MDS rate of change was lower for *L. decidua* and *P. cembra*

compared to *P. abies*. Although having a higher transpiration rate and sap flow than *P. cembra* and *P. abies*, *L. decidua* can support water losses by a higher water uptake capacity (Anfodillo et al. 1998) achieved by osmoregulation (Badalotti et al. 2000) leading to reduced stem de-hydration. Both *P. abies* and *P. cembra* have a general water saving strategy with higher minimum water potential than *L. decidua* (Anfodillo et al. 1998). However, *P. abies* showed a higher rate of change than *P. cembra* because of a relatively higher sap flow. Thus, the changes in MDS confirmed that, at the alpine treeline, *P. cembra* is the most water-saving species (Tranquillini, 1979).

Only sap flow and VPD had a direct effect on the contraction phase duration. For all species, these results are in agreement with the finding that sap flow is coupled with transpiration (Steppe et al. 2006). Sap flow, as well as VPD, also influences MDS, which agrees with other results on *P. abies* (Herzog et al. 1995). However, these high correlations were also caused by the phase duration (i.e. model 2) and not just by sap flow or VPD. In the morning, sap flow increases together with increasing VPD, but reaches maximum value at 4-5 hPa, for all studied species, and then remains stable (saturation effect) (Anfodillo et al. 1998). This maximum sap flow is related to the hydraulic resistance of the whole path (roots-leaves) and the capacity to lower the minimum water potential. The non-linearity of the relationship sap flow versus VPD explains the importance of considering the duration of the contraction phase in the relationship with MDS because the rate of increase in sap flow slows down when the processes of stem contraction (including duration) are still ongoing.

Causal effect of water and temperature on ΔR

In all species, $\Delta R+$ started around midnight ($\pm 3h$) and lasted for 11h ($\pm 5h$) in June and July, which is close to the timing found for *A. balsamea* in Canada (Deslauriers et al. 2003). In very young *Fagus sylvatica* L., radial stem growth is modelled to occur mainly during the night when the pressure potential in the stem storage compartment (outside xylem) exceeds a wall-yielding threshold value (Steppe et al. 2006). In mature conifers swelling usually lasts longer, hence radial growth during the morning cannot be excluded. In the studied species, *P. cembra* had the lowest change in ΔR because of higher minimum water potential and lower stem re-hydration (Anfodillo et al. 1998). Although having lower changes in MDS, the change in ΔR was higher for *L. decidua* because of its higher water uptake capacity and overall growth.

The results of causal relationships suggest a new physiological interpretation of the growth-climate relationship obtained when using daily dendrometer measurements. The variation in stem diameter includes several components (Daudet et al. 2005), with the main ones being irreversible radial growth and reversible living-cell dehydration/rehydration. In conifers, the water-related stem radius variations (i.e. when the growth trend is removed) are largely determined by soil and air conditions (Zweifel et al. 2005). However, air and soil conditions explain both the water-related change in stem radius and ΔR , considered as the estimated stem growth. Phase duration, taken as an indicator of the reversible changes in stem radius, depends mainly on transpiration and sap flow (Herzog et al. 1995) and as higher increments are found in longer expansion and stem radial increment

phases, this situation leads to a lack of causal relationship or both direct - indirect effects on ΔR changes.

Our results demonstrate that precipitation has two main effects: a direct effect on ΔR and an indirect effect caused by phase duration (h) due to the non-linearity of the physiological responses to climatic factors. The main effect of precipitation is to enhance the water conditions in the stem, creating favourable pressure conditions for cell enlargement (Proseus and Boyer 2005, Steppe et al. 2006). It is therefore not surprising that positive correlations with precipitation are found in different species and environments (Downes et al. 1999, Tardif et al. 2001, Deslauriers et al. 2003, Bouriaud et al. 2005). When including long cycles in the analysis, the simple correlations between ΔR and precipitation increase from about 0.40 (figure 7) to 0.60 (data not shown) as long cycles correspond to a high ΔR value occurring during a heavy rainfall event (figure 2).

Opposite effects of sap flow were found on the stem radius increment phase duration and ΔR : sap flow was positively correlated with duration at constant ΔR ($r_{dh.v}$), whilst it was negatively correlated with ΔR at constant duration ($r_{dv.h}$). The ΔR , considered as stem radial growth, is partly a result of replenishing of the internal storage in addition to irreversible stem growth as cell division and enlargement. However, the longest durations were those where total sap flow was the highest (i.e. cumulated sum). Therefore, when the variation explained by the duration is removed, a reduction in sap flow at night leads to an increase in ΔR for *P. abies* and *L. decidua* because sap flow can be predictive of water potential of meristems: if sap flow is higher a lower water potential should occur within the

living cells. Steppe et al. (2006) observed an increase in stem diameter in *F. sylvatica* after a decrease in the transpiration rate and a subsequent decrease in sap flow at the stem base. Irreversible changes in cell walls are expected to occur when the water around the cambium is replenished (Abe et al. 2003) and when the water potential in xylem increases at night and reaches higher values than the water potential of the storage compartment (Herzog et al. 1995, Zweifel and Häslar 2001). *P. cembra* showed no partial correlation with sap flow probably because the variation in water potential of cambial cells is much less than in the other two species.

The negative correlation with air temperature was caused by the effect of temperature on phase duration and doesn't correspond, considering the daily scale, with a direct physiological effect on tree growth in terms of metabolic activities. These results support the hypothesis that ΔR mainly reflect radial growth, in terms of cell enlargement, principally influenced by the tree water status and in a minor way, by the C-metabolism (Daudet et al. 2005, Zweifel et al. 2006). As suggested by Tardif et al. (2001), the negative relationship between ΔR and temperature is probably connected with a negative effect of transpiration on radial expansion: via transpiration, higher day and night temperatures lead to a balanced cycle which reduces ΔR .

Conclusion

This study demonstrated that the duration of refilling explains a high proportion of ΔR that needs to be considered in growth-climate relationships. When considered in correlation with sap flux and climate, duration removes a proportion of reversible

stem reaction effects and helps to discriminate better between the factors influencing ΔR . The growth and climate relationships found by using dendrometer measurements are not always in agreement with other results. In the Alps for example, a positive correlation between ring width and June-July temperature was found (Carrer and Urbinati 2004, Oberhuber 2004), while in this study, negative temperature effects, connected to phase duration, were observed. Therefore, at a short time-scale, interpretation of the relationships should be explained in function of the process measured (ΔR which mainly reflect radial growth in terms of cell enlargement), which is highly water-dependent.

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Figure captions

Figure 1. The stem cycle divided into three distinct phases for *P. abies* in 2001. Each circle represents an hourly measurement. The $\Delta R+$ was calculated when the stem radius exceeded the morning maximum until the subsequent maximum. The $\Delta R-$ was calculated when the previous cycle maximum was not reached. The MDS was calculated as the difference between the morning maximum and afternoon minimum. A diurnal cycle lasts about 24 hours and a long cycle more than one day.

Figure 2. Time-series of meteorological data and stem radial variation of *P. abies*, *L. decidua* and *P. cembra* from May to October 2001. Meteorological data included (a) air temperature and precipitation and (b) soil water content (%). Stem radial variation was expressed as (c) cumulative ΔR (μm), (d) ΔR (μm) and (e) MDS (μm , absolute values). The analysed period, i.e. the months of June and July, is highlighted in grey.

Figure 3. Left: Tree-ring width (mm) measured in four cardinal directions for tree1 (black dots) and tree2 (white circle) of each species associated with the corresponding cumulated ΔR (mm, black and white star) from 1996 to 2004. Right: Cumulated increase in ΔR (black line) and in tree-ring growth (black dots) during the 2004 growing season.

Figure 4. Duration of contraction and expansion phases for the diurnal cycles leading to negative (in grey) or positive (in black) ΔR . The standard errors (not reported) varied between 2-3 h. Paired t-tests were used to compare the duration

of contraction and expansion within the diurnal cycle with ΔR^- (grey capital letters) or ΔR^+ (black capital letters). T-tests were used to compare the duration of contraction (a and b) or the duration of expansion between positive and negative ΔR (y and z). Different letters indicate significant differences ($P < 0.05$) between series.

Figure 5. Relationship between MDS [$\log(\mu\text{m})$] and duration [$\log(h)$] for *P. cembra*, *L. decidua* and *P. abies* in June-July 1996-2004. Only diurnal cycles are illustrated. All regressions were significant at $P < 0.01$. Thick, thin and broken lines represent the regression, 95% confidence limits and 95% confidence bands, respectively.

Figure 6. Relationship between ΔR (μm) and duration (h) of the expansion phases of *P. cembra*, *L. decidua* and *P. abies*. Only diurnal cycles in June-July 1996-2004 are illustrated. All regressions were significant at $P < 0.01$. Thick, thin and broken lines represent the regression, 95% confidence limits and 95% confidence bands, respectively.

Figure 7. Correlation matrix representing simple (white background) and partial (grey background) coefficients among descriptors (d_i), phase duration (h_i) and radial variation (v_i) for *L. decidua* (white), *P. abies* (light grey) and *P. cembra* (grey). At left, v_i represent MDS and at right v_i represent ΔR^+ . The vertical bars indicate the 95% distribution of the correlation coefficients. Correlations are not significant when bars cross zero.

Table 1. Model expectation involving the variables d , h and v , in terms of the results for the simple and partial correlation coefficients (Legendre and Legendre 1998). d , descriptor; h , phase duration; v , stem radial variations.

Both Models	Model 1	Model 2
r_{dh} signif. $\neq 0$	$ r_{dh} \geq r_{dv} $	r_{dv} signif. $\neq 0^*$
r_{hv} signif. $\neq 0^*$	$ r_{hv} \geq r_{dv} $	$r_{dv.h}$ signif. $\neq 0$
$r_{dh.v}$ signif. $\neq 0$	$r_{dv.h}$ not signif. **	
$r_{hv.d}$ signif. $\neq 0$	$ r_{dh.v} \leq r_{dh} $ $ r_{hv.d} \leq r_{hv} $ $r_{dh} \times r_{hv} \approx r_{dv}$	

* For model 2, model holds even if *only one* of these two simple correlations is not significant. $^{**}r_{dv.h}$ not signif.' means that the correlation is not necessarily significantly different from zero.

Table 2. Timing characteristics and standard deviation (in brackets) of the contraction (1), expansion (2), stem radius increment (3) and regular diurnal or long cycles for *P. abies*, *L. decidua* and *P. cembra*. The times of phase onset and mean duration (hours) were calculated for the months of June and July 1996-2004.

Species	Phase	Time of onset	Mean duration of diurnal cycles	Mean duration of long cycles
<i>P. abies</i>	1	10:18 (3.48)	7.59 (3.29)	7.35 (3.24)
	2	18:35 (2.10)	14.55 (4.00)	40.18 (19.50)
	3	22:52 (3.11)	11.23 (5.26)	35.11 (20.18)
	Cycle	---	22.49 (3.18)	47.45 (18.43)
<i>L. decidua</i>	1	10:44 (3.51)	8.13 (3.06)	7.47 (4.04)
	2	19:06 (2.18)	14.54 (3.43)	36.26 (19.29)
	3	22:20 (3.10)	11.01 (4.55)	32.95 (20.56)
	Cycle	---	23.02 (3.16)	44.05 (18.10)
<i>P. cembra</i>	1	10:27 (3.33)	9.19 (3.41)	9.40 (5.55)
	2	20:26 (2.43)	13.28 (3.52)	34.44 (17.32)
	3	00:08 (3.00)	10.22 (5.05)	31.31 (17.16)
	Cycle	---	22.39 (3.17)	44.23 (15.57)

Table 3. Verifications of the initial hypotheses and model predictions for MDS and ΔR in the three studied species. VPD, vapour pressure deficit.

Descriptors	MDS			ΔR		
	$r_{dh} \neq 0$	$r_{hv} \neq 0$	Model fit	$r_{dh} \neq 0$	$r_{hv} \neq 0$	Model fit
Sap flow	Yes	Yes	2	Yes	Yes	2*
VPD	Yes	Yes	2**	Yes	Yes	No fit
Precipitation	No	Yes	-	Yes	Yes	2
Soil water content	No	Yes	-	Yes	Yes	1
Air temperature	Yes	Yes	No fit	Yes	Yes	1

No fit for **P. cembra* and ***L. decidua*

Figure 1

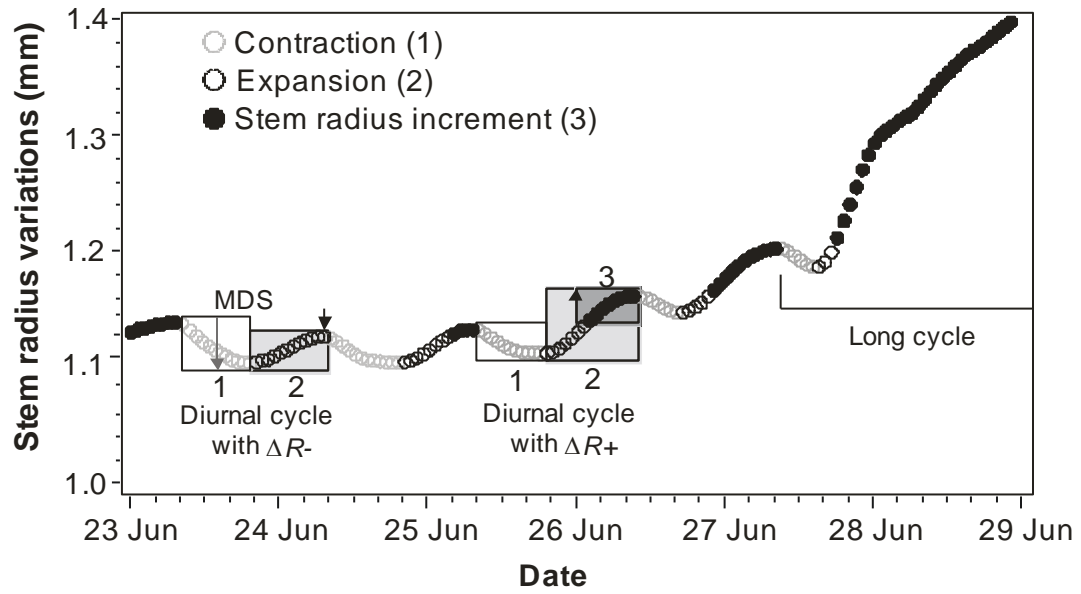


Figure 2

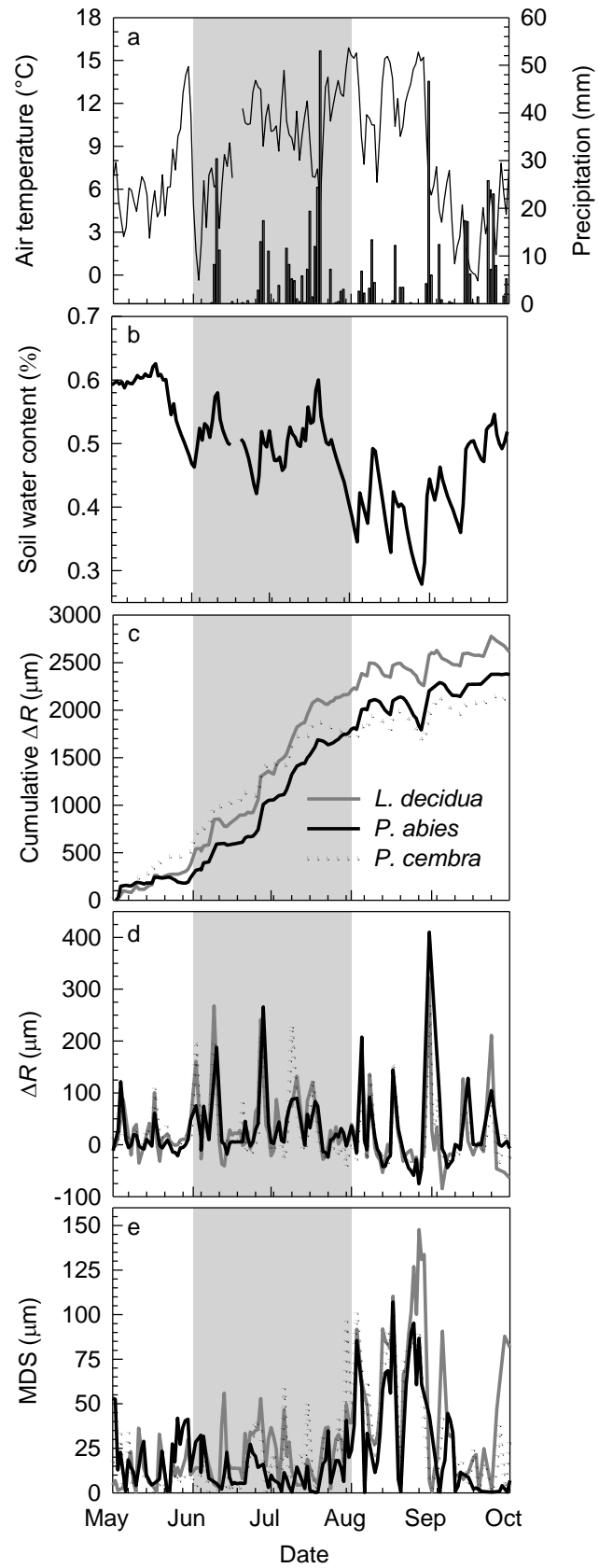


Figure 3

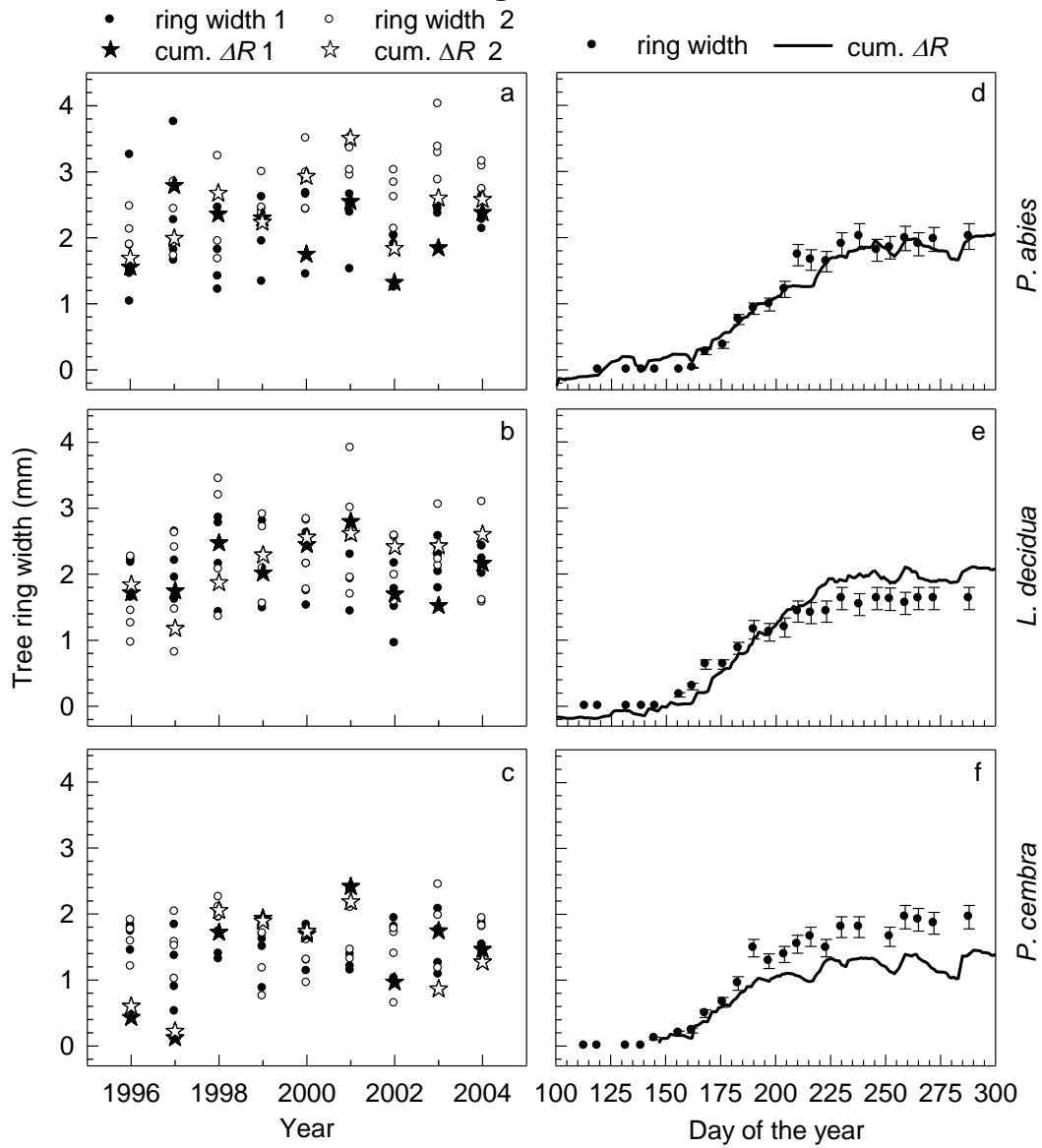


Figure 4

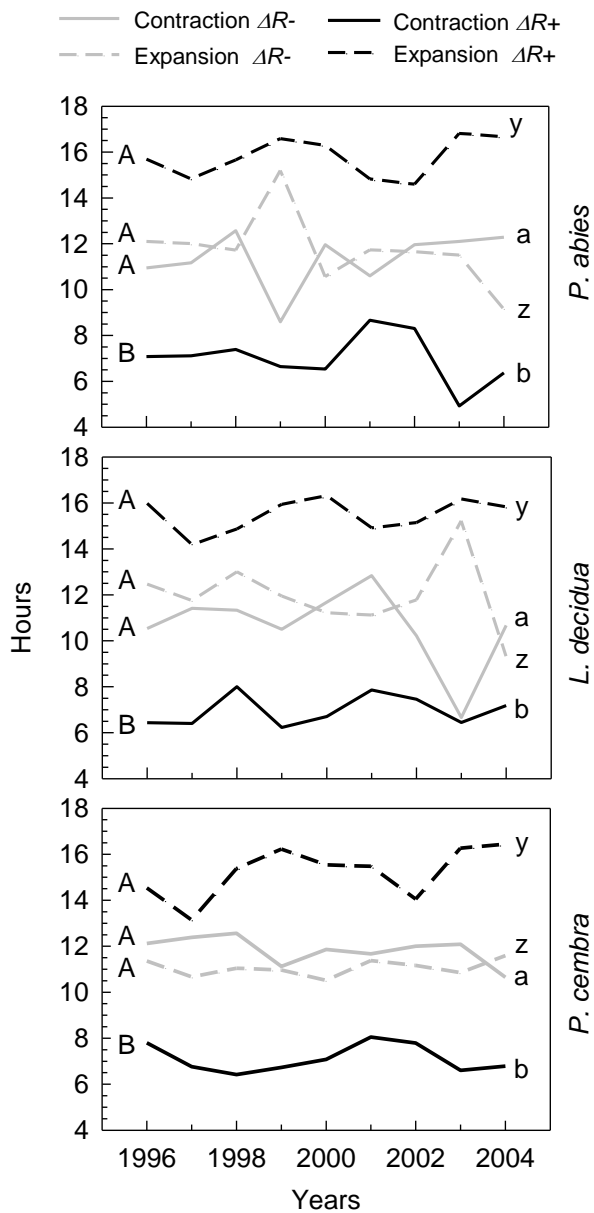


Figure 5

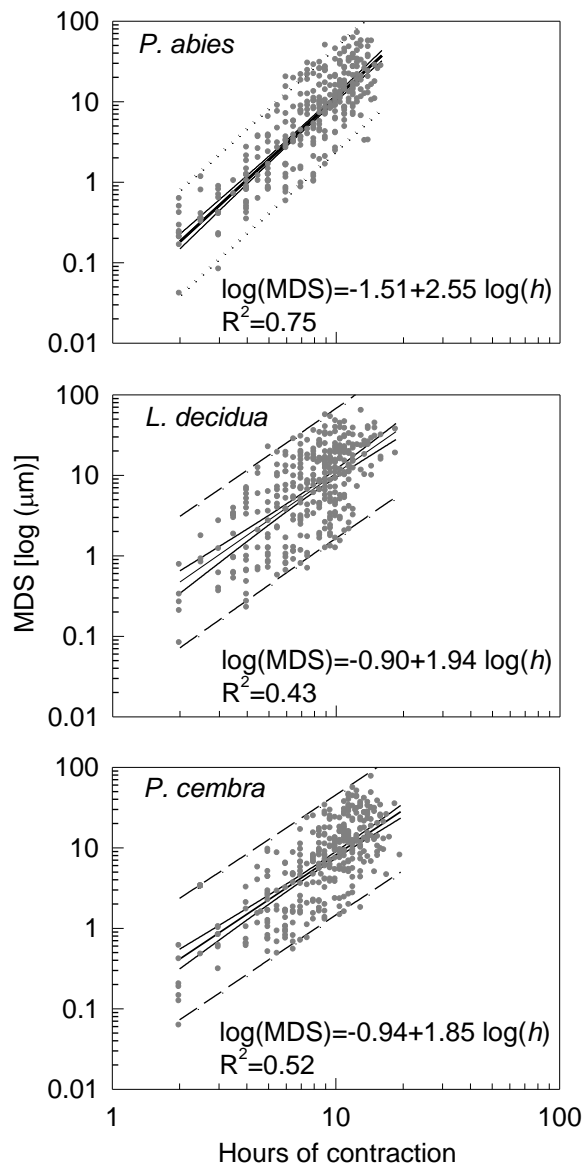


Figure 6

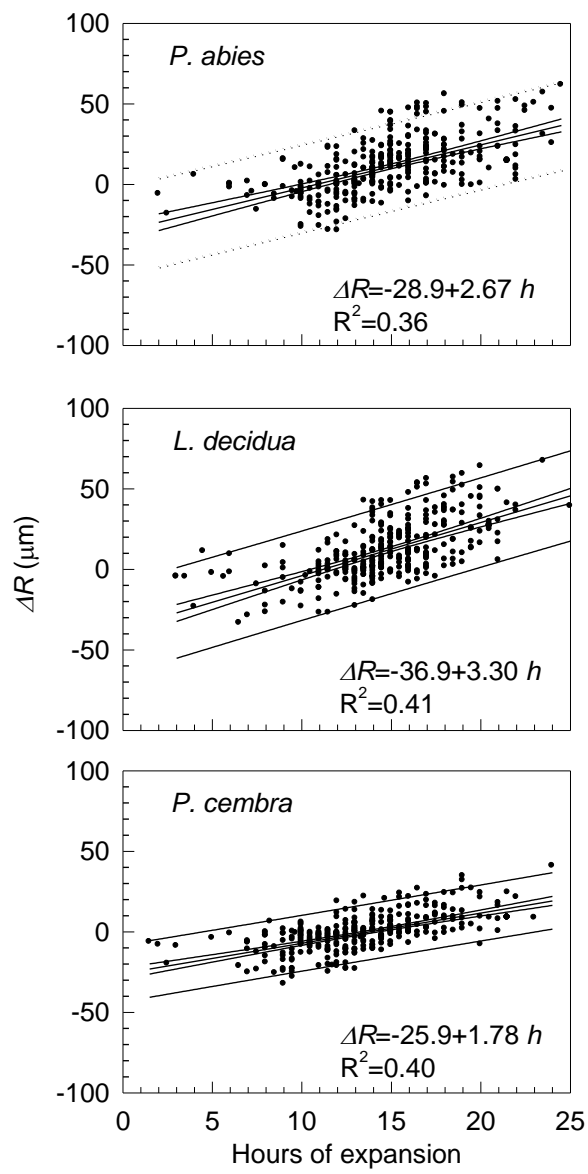


Figure 7

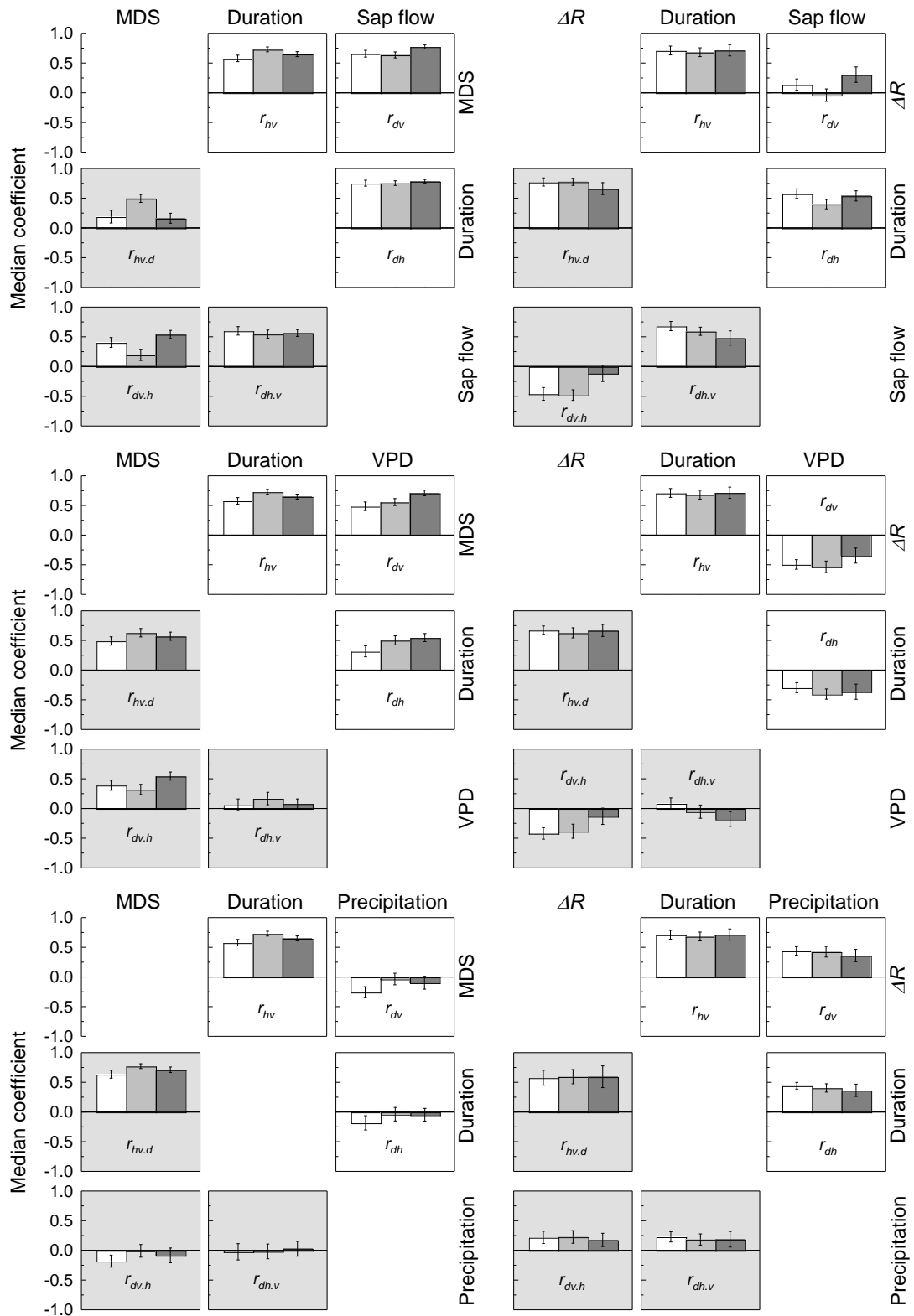


Figure 7 continued

