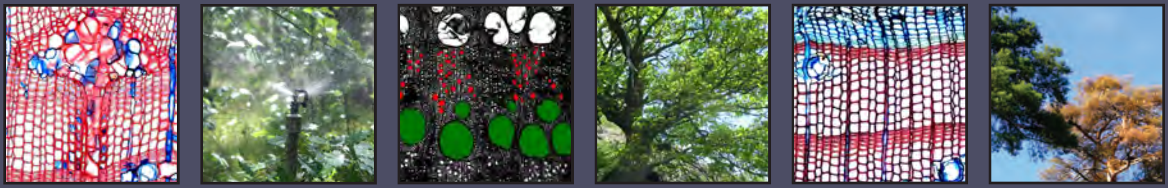
Doctoral thesis (Diss. ETH No. 18106)

Britta Eilmann

Water availability and tree growth of Scots pine and pubescent oak - a multiproxy analysis



Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

DISS. ETH NO. 18106

WATER AVAILABILITY AND TREE GROWTH OF SCOTS PINE AND PUBESCENT OAK - A MULTIPROXY ANALYSIS

A Dissertation submitted to

ETH ZURICH

for the degree of
Doctor of Sciences

presented by

BRITTA EILMANN

Dipl. Holzwirtin (University of Hamburg)
born July 01, 1976 in Walsrode (Germany)
citizen of Germany

Accepted on the recommendation of

Prof. Dr. N. Buchmann, examiner
Dr. A. Rigling, co-examiner
Prof. Dr. H. Bugmann, co-examiner

2008

Table of contents

Summary

Drought induced forest decline will gain in importance as the frequency and severity of drought events is expected to increase during the 21st century. Drought has also been discussed as a major cause for the decline of Scots pine (*Pinus sylvestris* L.) in the dry inner-Alpine valleys.

The impact of drought on tree performance, meaning the successful growth of trees, was examined in this thesis. The project aimed 1) to analyse the impact of severe drought on tree performance in the dry, low-elevated forest belt of the Swiss Rhone valley and 2) to evaluate the potential of Scots pine to maintain its dominance in these forests even under a projected hotter and drier climate. Tree growth under drought was analysed using different proxies (radial increment, wood anatomical parameters and stable carbon isotopes) on two time scales (inter- and intra-annual) using two different approaches (survey and experiment). The specific objectives of this thesis were 1) to identify the growth adaptation of Scots pine and pubescent oak (*Quercus pubescens* Willd.) to drought focussing on the inner-tree water conducting system, 2) to quantify the impact of drought and crown density on timing, quality and amount of wood formation in Scots pine and 3) to assess the process of storage depletion in Scots pine and estimate species potential to perform in a future climate.

Chapter I analyzed the adaptation of the water conducting system to drought for Scots pine and pubescent oak. Therefore tree growth at a passively irrigated site along an open water channel was compared with growth under naturally dry conditions. Under drought, the water conducting system of Scots pine consisted of fewer conduits with thinner cell walls but bigger lumen diameters. In contrast, the conduits were smaller in pubescent oak under drought. In addition, latewood width and therefore also the number of latewood vessels, was reduced. Tree growth of Scots pine was more dependant on water availability than tree growth of pubescent oak. Thus pubescent oak might have gradual advantages over Scots pine. However, also the growth of pubescent oak was retarded by drought, as seen by the frequent absence of latewood. Thus its success as a substitute species for Scots pine in a drier and hotter climate is doubtful.

In *chapter II* the synergetic impact of drought and crown density, as an estimator for tree vigour, on tree growth of Scots pine was analysed on an inter- and intra-annual scale within an irrigation experiment. The results showed that even trees of low crown density are able to recover from drought under improved environmental conditions, as all trees profited from

irrigation, as seen for example, by the increasing tree-ring width. Irrigation also led to a prolonging of the growth period by up to five weeks. Low crown density in combination with drought had a strong negative synergistic effect on tree growth as seen for example, by the long-lasting growth reduction after the drought year 2003 in trees of mid and low crown density. This might be due to progressing storage depletion; therefore the resistance against stressors might be rather low. As a result, increasing mortality, especially in tress of low crown density, should be expected under ongoing climate change.

In *chapter III* the analysis focused on the carbon metabolism and in particular on the process of storage depletion in Scots pine of diverse crown density classes, studied by $\delta^{13}C$ analysis on an inter- and intra-annual time scale. Results on the intra-annual scale showed that the assimilates produced at the beginning of the growth period were not sufficient to cover the carbon demand for tree growth. Thus stored carbohydrates were used for tree growth, even more in trees growing under low water supply. This continuous consumption of carbon reserves leads to storage depletion if the storages cannot be replenished within the growth period. On the annual scale, a high correlation between earlywood $\delta^{13}C$ and the current drought index and the immediate response of $\delta^{13}C$ to irrigation indicates high turnover rates. If high turnover rates were due to a reduced storage-pool, then all trees on dry sites, even if no or only slight signs of decline were observable in the crowns, would increasingly suffer under drought.

The results of the thesis contribute to a better understanding of the mechanistic role of drought in Scots pine decline, with implications not only for the future landscape development in Valais, but also for other dry regions in the inner-Alps. Under the assumption of a hotter and drier future climate, increasing mortality rates of Scots pines should be expected. Pubescent oak might act as a substitute species for Scots pine in today’s climate but our results also indicate that in a hotter and drier climate pubescent oak might also soon reach the physiological limits.

Zusammenfassung

Durch Trockenheit verursachte Waldschäden werden an Bedeutung gewinnen, da für das 21. Jahrhundert eine Zunahme der Frequenz und Stärke von Trockenheit erwartet wird. Auch für die Schädigung der Waldföhre (*Pinus sylvestris* L.) in inneralpinen Trockentälern wird Trockenheit als Ursache diskutiert.

In der vorliegenden Studie wird der Einfluss von Trockenheit auf die Baumperformance, d.h. das erfolgreiche Wachstum der Bäume, untersucht. Die Projektziele waren 1) zu analysieren, wie starke Trockenheit die Performance der Bäume in dem trockenen Waldgürtel des Schweizer Rhonetals beeinflusst und 2) das Potential der Waldföhre abzuschätzen, auch unter den prognostizierten wärmeren und trockeneren Bedingungen eine dominante Stellung in diesen Wäldern zu behalten.

Für die Untersuchung des Baumwachstums bei Trockenheit wurden verschiedene Proxys (radialer Zuwachs, holzanatomischer Parameter und stabile Kohlenstoffisotope) auf zwei Zeitskalen (inter- und intraannuell) mit Hilfe von zwei verschiedenen Ansätzen (Erhebung und Experiment) analysiert. Von besonderem Interesse war 1) die Identifizierung der Wachstumsanpassung von Waldföhre und Flaumeiche (*Quercus pubescens* Willd.) an Trockenheit unter besonderer Berücksichtigung des Wasserleitungsgewebes, 2) die Bestimmung des gemeinsamen Einflusses von Trockenheit und Kronendichte auf Timing, Qualität und Quantität der Holzbildung bei der Waldföhre, 3) die Abschätzung der fortschreitenden Leerung der Kohlenstoffspeicher bei der Waldföhre und ihr Potential, unter zukünftigen Klimabedingungen zu performen.

Die Anpassung des Wasserleitungssystems bei Waldföhren und Flaumeichen an Trockenheit ist Gegenstand von *Kapitel I.* Dazu wurde das Wachstum von passiv bewässerten Bäumen, die entlang offener Wasserkanäle wachsen, verglichen mit dem derjenigen Bäume, die unter natürlich trockenen Bedingungen wachsen. Unter trockenen Bedingungen bestand das Wasserleitungssystem der Waldföhre aus weniger Zellen, mit dünnere Zellwand aber grösserem Lumen. Im Gegensatz dazu waren die Wasser leitenden Zellen der Flaumeiche bei Trockenheit kleiner. Zusätzlich war die Spätholzbreite und damit die Anzahl der Spätholzgefässe bei der Flaumeiche stark reduziert. Das Wachstum der Waldföhre hing stärker von der Wasserverfügbarkeit ab als das der Flaumeiche. Daher mag die Flaumeiche zwar graduelle Vorteile gegenüber der Waldföhre haben, doch auch ihr Wachstum wurde

durch die Trockenheit stark behindert, wie z.B. das häufig fehlende Spätholz zeigt. Somit ist ihr Erfolg als Ersatz-Spezies in einem trockeneren und wärmeren Klima fragwürdig.

In *Kapitel II* wird der synergetische Effekt von Trockenheit und Kronendichte (als Schätzer der Baumvitalität) auf das inter- und intraanuelle Wachstum der Waldföhre in einem Bewässerungsexperiment analysiert. Die Ergebnisse zeigen, dass selbst Bäume mit geringer Kronendichte sich bei verbesserten Wachstumsbedingungen von der Trockenheit erholen konnten, da alle Bäume von der Bewässerung profitierten, wie an der zunehmenden Jahrringbreite zu erkennen war. Die Bewässerung hat auch die Wachstumsperiode um bis zu fünf Wochen verlängert. Trockenheit in Verbindung mit geringer Kronendichte hat einen negativ-synergetischen Effekt auf das Wachstum, wie am Beispiel der langfristigen Wachstumsdepression nach dem Trockenjahr 2003 bei Bäumen mittlerer und geringer Kronendichte erkennbar war. Ein Grund dafür könnte die fortgeschrittene Leerung der Kohlenstoffspeicher sein, die auch die Resistenz gegenüber Stressfaktoren herabsetzen würde. Somit ist im Zuge des derzeitigen Klimawandels eine weitere Zunahme der Mortalität, insbesondere bei Bäumen mit geringer Kronendichte, zu erwarten.

Kapitel III konzentriert sich auf den Kohlenstoffmetabolismus, insbesondere auf die Leerung der Kohlenstoffspeicher bei Waldföhren verschiedener Kronendichte, die mithilfe inter- und intraanueller $\delta^{13}C$ Analyse untersucht wurden. Die Ergebnisse auf intraanueller Ebene deuten darauf hin, dass die Assimilatproduktion zu Beginn der Vegetationsperiode nicht ausreichte, um den Kohlenstoffbedarf des Wachstums zu decken. Daher mussten gespeicherte Kohlenstoffe für das Wachstum herangezogen werden, insbesondere bei Bäumen mit geringer Wasserversorgung. Dieser kontinuierliche Verbrauch der Reserven führt zu einer Leerung des Speichers, wenn dieser bis zum Ende der Wachstumsperiode nicht aufgefüllt werden kann. Auf interanueller Ebene deutet die hohe Korrelation zwischen $\delta^{13}C$ im Frühholz und dem saisonalen Trockenheitsindex sowie die sofortige Reaktion des $\delta^{13}C$ auf Bewässerung auf hohe Turnover-Raten hin. Falls diese hohen Turnover-Raten auf reduzierte Speicherpools zurückzuführen sind, dann leiden alle Bäume auf Trockenstandorten zunehmend unter der Trockenheit, auch wenn sie keine oder nur geringe Anzeichen einer Kronenschädigung zeigen.

Die Resultate dieser Studie tragen dazu bei, den Mechanismus der Waldföhrenschädigung besser zu verstehen, mit Auswirkungen nicht nur für die zukünftige Landschaftsentwicklung im Wallis, sondern auch für andere inneralpine Trockentäler. Unter der Annahme eines wärmeren und trockeneren zukünftigen Klimas ist von einer weiteren Zunahme der

Waldföhrenmortalität auszugehen. Unter heutigen Klimabedingungen scheint die Flaumeiche die Waldföhre ersetzen zu können, jedoch weisen unsere Resultate darauf hin, dass auch die Flaumeiche unter trockeneren und wärmeren Bedingungen bald ihre physiologischen Grenzen erreicht.

General introduction

Forest ecosystems generate important goods, such as timber, food, and services of public interest, such as recreation, protection against natural hazards or biotopes for flora and fauna. The long-term sustainability of these goods and services depends on the forest stability. Changing climate conditions may destabilize forests by inhibiting regeneration, reducing growth and triggering tree mortality. As a result, future provisions of ecosystems goods and services are jeopardized. In mountain regions, where the protection of the settlements against rock fall, debris flow or avalanche is a highly important forest service, instability of forest ecosystems might have catastrophic consequences for the security of inhabitants but also e.g. for the international transport corridors.

Disturbances like drought, fire, storms or insect outbreaks are key factors reducing forest stability by introducing tree mortality (Dale *et al.*, 2001). Especially drought has been frequently discussed as an important trigger for tree mortality, forest decline and decline-induced vegetation shifts (e.g., Allen & Breshears, 1998; Penuelas & Boada, 2003; Breshears *et al.*, 2005; Gitlin *et al.*, 2006). The theory of storage depletion was discussed as one main mechanism leading to drought induced forest decline (Breda *et al.*, 2006; McDowell *et al.*, 2008). This theory based on the fact that drought reduces the primary production of trees (Ciais *et al.*, 2005; Granier *et al.*, 2007; Reichstein *et al.*, 2007) and the amount of stored carbohydrates (Breda *et al.*, 2006). Due to the continued metabolic demand for carbohydrates (e.g., for plant respiration) reduced photosynthetic activity during drought might lead to a negative carbon balance. As a result stored carbohydrates have to be used to cover the carbon need, which may, in the long run, lead to tree death by storage depletion. In addition to storage depletion, which is likely to occur after several dry years, hydraulic and symplastic failure was discussed as a second mechanism leading to drought induced tree mortality, which is most likely to occur during drought sufficiently intense to cause whole-system cavitation (Breda *et al.*, 2006; McDowell *et al.*, 2008). Therefore forest ecosystems exposed to drought are at risk of increasing tree mortality, which may result in decreasing forest stability and a reduction of forest goods and services. In the future, drought induced forest decline will gain in importance, since climate change is expected to increase not only temperature but also the frequency and severity of drought in Central Europe (Schär *et al.*, 2004; IPCC, 2007).

Landscape change in Valais

The Swiss Rhone valley (canton Valais) is one of the driest regions of the inner-Alps (Ozenda, 1985). The forests at low elevation are naturally dominated by the sub-Boreal Scots pine (*Pinus sylvestris* L.). Since 1900, high rates of Scots pine mortality have been observed in these forests, which was ascribed to the high fluorine emission of the nearby aluminium factories (Flühler, 1981). After the installation of filters in the factories the conspicuous symptoms, such as burnings or necrosis on the leaves and needles of trees in the forest, as well as in the vineyards and orchards, disappeared (Rigling & Cherubini, 1999).

But since the 1990s, a second mortality wave was observed, concerning not only Scots pine in Valais (Dobbertin *et al.*, 2005; Bigler *et al.*, 2006; Dobbertin & Rigling, 2006) but also in the inner-Alpine valleys of neighbouring countries (Vertui & Tagliaferro, 1998; Cech & Perny, 2000; Oberhuber, 2001). Since 1995, locally almost half of the Scots pine population has died in Valais (Rebetez & Dobbertin, 2004) while deciduous species, especially pubescent oak (*Quercus pubescens* Willd.) are becoming more abundant and are increasingly competing with pine on dry sites (Weber *et al.*, 2008). Not a single causal agent but an interacting set of factors, like pathogens, insect pests, drought and increasing competition, seemed to be responsible for these high pine mortality rates (Bigler *et al.*, 2006; Dobbertin & Rigling, 2006; Weber *et al.*, 2007; Wermelinger *et al.*, 2008)

In Valais, like in all inner-Alpine valleys, precipitation is typically low due to the rain shadow of the surrounding high mountain ranges (Rebetez & Dobbertin, 2004) suggesting drought as an important limiting growth factor. Recently, growth conditions changed as the water availability further decreased: Even though the total amount of precipitation decreased only slightly since 1980, temperature and evapotranspiration significantly increased (Rebetez & Dobbertin, 2004) what at least seasonally led to a more negative water balance of the trees (Zweifel *et al.*, 2006). In addition, an increase in water availability in winter but a significant decrease in summer was found by Weber et al. (2007) when analysing the change in seasonality of a monthly drought index between 1940 and 2000. The importance of drought for Scots pine decline was demonstrated by the fact that mortality rates were highest on dry sites and after drought years (Dobbertin *et al.*, 2005). In contrast the mortality rates of deciduous species like pubescent oak were much lower on these sites. Thus the critical question is: What is the mechanism leading to high mortality rates in Scots pines and why are other species like pubescent oak less affected by increasingly dry conditions?

Scots pine vs. pubescent oak

The sub-Boreal Scots pine has the widest distribution range of all pine species and can be found from northern Siberia to the mountain sites of the Sierra Nevada (Fig. 1; Meusel, 1992). In Valais at the low elevation sites, Scots pine is situated in the area of southern distribution. Further south Scots pine occurrence are restricted to high altitudes (Boratynski, 1991). Scots pine can be found on a variety of soils and in extreme climates. As a typical pioneer species, Scots pine is fast colonising and light demanding (Ellenberg, 1988). Due to its low competitiveness, Scots pine is typically restricted to extreme sites like nutrient poor or dry sites (see Bigler *et al.*, 2006).

Fig. 1: Distribution of Scots pine (black) and pubescent oak (grey) in Europe according to Meusel (1992). Dot marks the Valais.

The co-occurring pubescent oak has a much smaller range and is typically found in southern Europe (Fig. 1; Meusel, 1992). As a typical sub-Mediterranean species, pubescent oak is accustomed to periods of low rainfall in the summer. Anyway, if site conditions are getting too dry, pubescent oak is replaced by evergreen species like holm oak (*Quercus ilex* L.; Sayer, 2000). Pubescent oak is mainly found on basic sites rich in nutrients (Ellenberg, 1988). Due to its thermophilous behaviour, pubescent oak favours warm sites (Timbal & Aussenac, 1996).

Scots pine and pubescent oak are regarded as well drought adapted species (e.g., Ellenberg, 1988; Timbal & Aussenac, 1996; Nardini & Pitt, 1999). Thus it is difficult to estimate how increasing aridity will affect the long term performance, i.e., the successful growth of the species, in these dry low elevation forests in Valais. But this estimation is crucial to assess if these forests with important protective functions can sustainably produce its goods and services in the future. Scots pine and pubescent oak differ not only in their origin and the site requirements. They also have different kinds of hydrosystems, which means that different sorts of cells build their inner-tree water transport system (Fig. 2). These differences might be important for the question of drought survival, since an effective water transport system is essential for keeping leaf water potential above cavitation threshold (Breda *et al.*, 2006).

In Scots pine, like in all conifers, the xylem has a very homogenous structure as it consists mainly of tracheids with some living parenchyma cells. The tracheids are unspecialised as they cover both, water transport and stabilisation functions (Braun, 1970). In pubescent oak, the xylem is more heterogenic as various cell types such as tracheids, vessels, parenchyma cells and fibres are present. The cells are more specialised; the vessel-tracheid complex is responsible for the conduction of water, while fibres are in charge of stabilisation (Braun, 1970). Compared to tracheids in conifers, vessels in oaks have the advantage of greater potential water conductivity due to their greater diameter and length (Tyree & Zimmermann, 2002; Sperry, 2003). Thus a given conductivity can be packed in a smaller stem area in a vessel-based water conducting system due to its greater efficiency. As a result, the costs of water transport are lower in oak compared to pine, as less tissue has to be invested in water transport (Sperry, 2003).

All in all, pubescent oak might have an advantage over Scots pine: 1) due to its sub-Mediterranean origin, pubescent oak is accustomed to periods of low rainfall in summer; 2) pubescent oak has a specialized and effective water transport system. However, to estimate the future performance of oak and pine in Valais, not only the given structure of the water conducting system but rather its adaptation to drought should be further investigated.

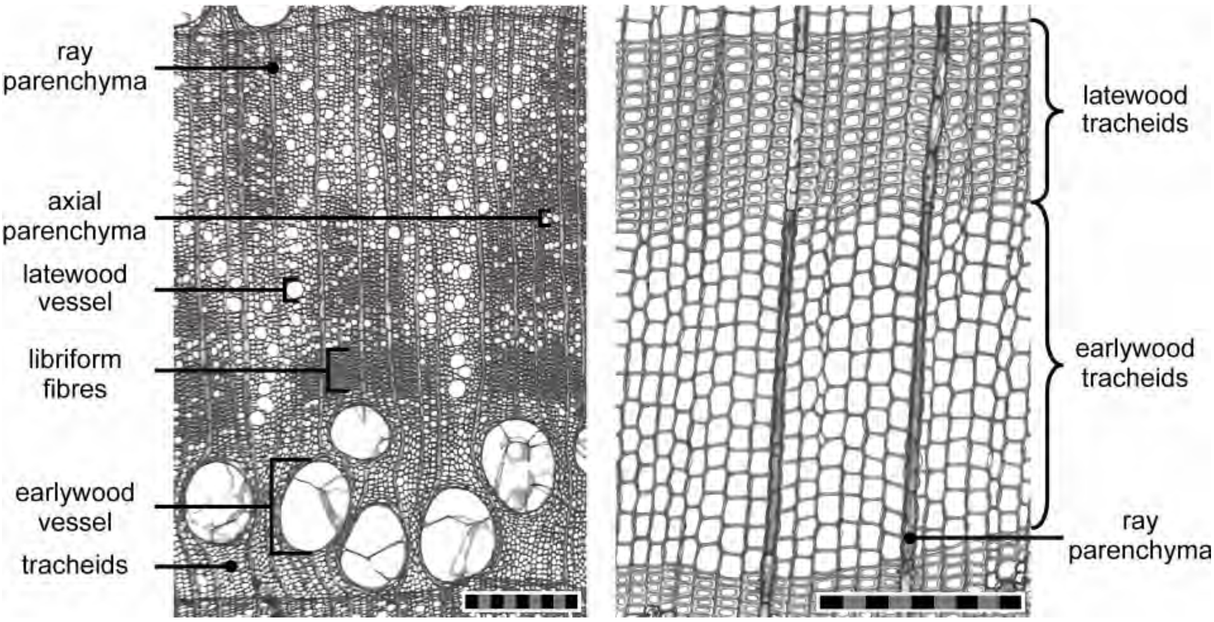


Fig. 2: Wood structure of pubescent oak (left) and Scots pine (right). Bar marks 1 mm.

Analysing environmental impact on tree growth

Trees archive information about past growth conditions in their tree rings. This archive can be used on different scales from the macro scale (e.g., analysing tree-ring width) to the atomic scale (e.g., studying element content or stable isotopic ratio).

Tree-ring width has been proven as a powerful approach to study climate impact on tree growth (e.g., Fritts, 2001; Briffa *et al.*, 2004). Tree-ring width (or earlywood and latewood width) is quickly and easily measurable and allows the construction of long chronologies consisting of many trees. Hence the influence of climate conditions on tree growth can be analysed over a long period of time leading to statistically reliable results. However, there is often insufficient knowledge about the physiological mechanisms and processes behind tree-ring formation to explain the observed correlations between climate and tree-ring width (Kagawa *et al.*, 2006). Thus the interpretation of tree-ring width has its limitation.

Cellular parameters, such as cell size or number of cells, have proven useful to analyse the impact of environmental conditions on tree growth (e.g., Wimmer, 2002; Vaganov *et al.*, 2006). Studies on a cellular level allow additional insights into environmental control on tree growth and other climatic signals are recorded in cellular parameters compared to tree-ring width (García-González & Eckstein, 2003; Panyushkina *et al.*, 2003; Fonti & García-González, 2004). In addition the consequences of growth limitations on the mechanical strengthening (Bodig & Jayne, 1993) or the potential water conductivity (Tyree &

Zimmermann, 2002) of the tree rings can be estimated by analysing cell wall thickness and cell size, respectively. But since more time is needed for sample preparation and measurement of cellular parameters than for tree-ring width, the formation of long cell-chronologies is more time-consuming. However, under the assumption that the resolution of scanners or cameras used for image preparation will continue to improve (taking into account that automated image analysis software is under construction (von Arx & Dietz, 2005)), then the disadvantages of cellular studies will continue to diminish making the usage of cellular parameters as proxy more attractive.

Stable carbon isotopes in tree rings have been used as a climate proxy (e.g., Farquhar *et al.*, 1989; McCarroll & Loader, 2004). Their advantage over tree-ring width is that the physiological mechanisms behind carbon fixation are well known and therefore interpretation is more evident. Isotopes are atoms of the same element, with the same number of electrons and protons but a different number of neutrons. As a result, the chemical properties of isotopes are similar but the physical properties differ due to the difference in mass. In the case of stable carbon isotopes, we distinguish between the lighter ^{12}C and the heavier ^{13}C. During the transition from external CO_2 to a carbon atom within the leaf sugar the lighter ^{12}C is preferred over ^{13}C due to fractionation processes along the pathway (Farquhar *et al.*, 1989). There are two points of fractionation during photosynthesis 1) during CO_2 diffusion, as the lighter ^{12}C diffuses easier (fractionation factor a ≈ -4.4‰); 2) during carboxylation, as the enzyme Rubisco tends to use ^{12}C in preference to ^{13}C (fractionation factor a ≈ -27‰). According to Farquhar *et al.* (1982), the overall discrimination against ^{13}C (Δ) during photosynthesis can be described using the simplified equation as

$$\Delta \cong a + (b - a)\frac{c_i}{c_a},$$

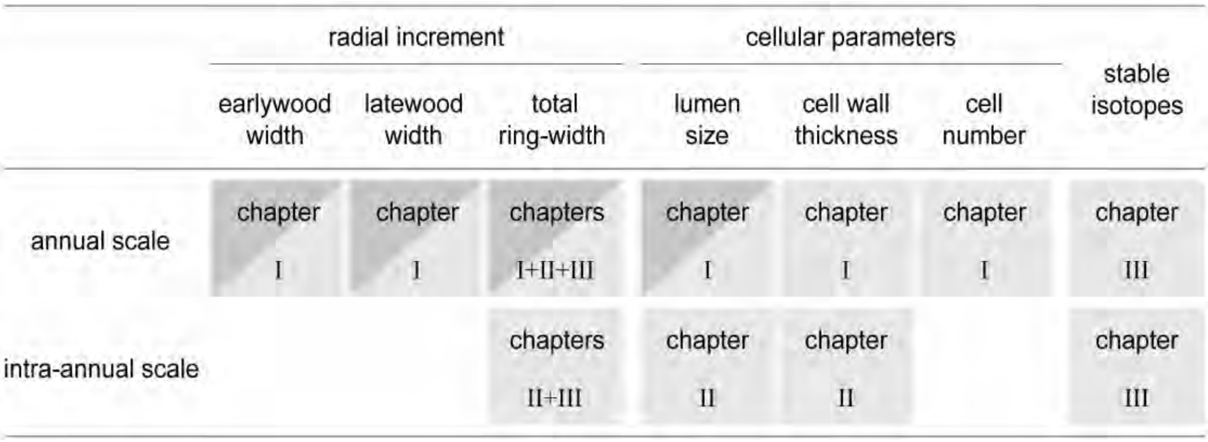
with c_i/c_a being the ratio of intercellular to ambient CO_2 concentration. As *a* and *b* are constants, variation in the discrimination mainly depend on changes in $c_i/c_{a.}$ The higher c_i, the more selective is the carboxylation process. Thus under high c_i the discrimination against ^{13}C is higher and the $\delta^{13}C$ ($^{13}C/^{12}C$ ratio of the sample (R_{sample}) expressed relative to the international standard VPDB (R_{VPDB} ; Vienna Pee Dee Belemnite): $\delta^{13}C = (R_{sample}/R_{VPDB} - 1) \times 1000$, measured in ‰) measured in the wood is lower.

Due to the strong dependence of Δ or $\delta^{13}C$ on c_i/c_a, the intrinsic water use efficiency (= ratio of assimilation to stomatal conductance) in C3 plants can be calculated based on $\delta^{13}C$ as also intrinsic water use efficiency changes with changing c_i/c_a (Farquhar *et al.*, 1989). Thus using stable isotopes in tree rings retrospective statements about tree physiology seem to be possible. However, there is still a lack of information on the impact of post-carboxylation fractionation on the $\delta^{13}C$ signal in the tree rings (Gessler *et al.*, 2004; Brandes *et al.*, 2006; Brandes *et al.*, 2007). Therefore the intrinsic water use efficiency calculated based on tree ring $\delta^{13}C$ might be underestimated if the effects of post-carboxylation processes are not taken into account (Duquesnay *et al.*, 1998). Anyway at least a rough estimation of the intrinsic water use efficiency based on $\delta^{13}C$ is possible.

Main objectives

The overall objectives of the study were 1) to analyse the impact of severe drought on tree performance in the dry, low-elevated forest belt of the Swiss Rhone valley and 2) to estimate the potential of Scots pine (for pubescent oak see chapter I) to maintain its dominance in these forests.

Table 1: Growth parameters analyzed in the three chapters. Light grey marks parameters analyzed for Scots pine, dark grey marks parameters analyzed for pubescent oak.



	radial increment			cellular parameters			stable isotopes
	earlywood width	latewood width	total ring-width	lumen size	cell wall thickness	cell number	
annual scale	chapter I	chapter I	chapters I+II+III	chapter I	chapter I	chapter I	chapter III
intra-annual scale			chapters II+III	chapter II	chapter II		chapter III

The thesis is organised in three chapters addressing the following specific research aims:

- Identifying adaptation strategies of Scots pine and pubescent oak to drought, focusing on the water conducting system
- Quantifying the impact of drought and low crown density on the timing and the amount of wood formation in Scots pine.
- Assessing the process of storage depletion in Scots pine.

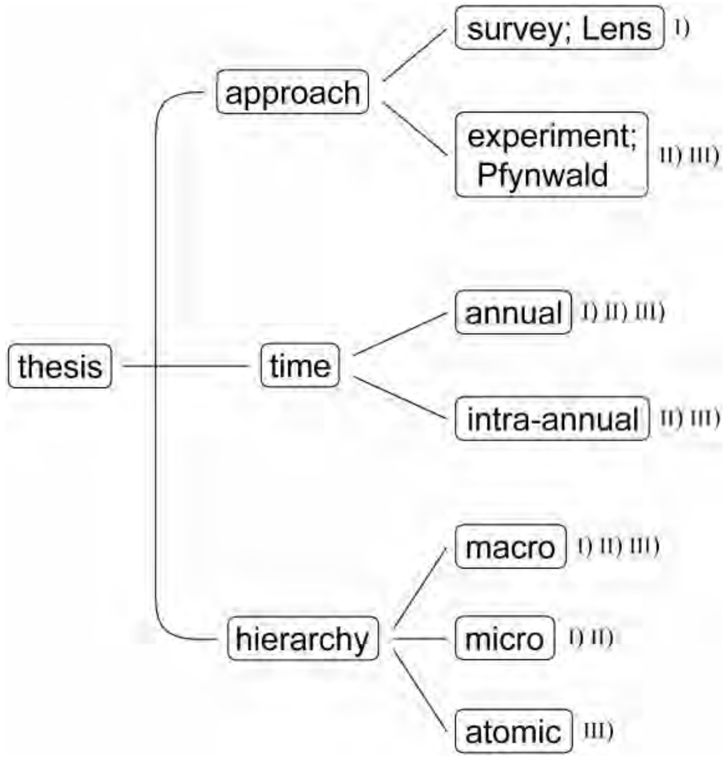


Fig 3: Structure of the thesis highlighting the two different approaches (survey and experiment) of the study and the different scales (time and hierarchic scale) of analysis. The chapters (I), II) or III)) dealing with the different scales or approaches are indicated as foot notes behind each point.

We studied tree performance under contrasting water supply by analysing different growth parameters (details see Table 1) on three different hierarchic scales (macro scale = radial increment, micro scale = cellular parameters, atomic scale = stable isotope ratio) and two temporal scales (annual and intra annual scale; thesis structure see Fig. 3). From the methodological point of view, the thesis covers two approaches: 1) a survey where tree growth under contrasting water supply was analysed retrospectively for 100 years (chapter I, study site Lens, Fig. 4) and 2) an irrigation experiment where wood formation and stable carbon isotope composition under contrasting water supply was analysed for two years under controlled conditions (chapters II and III, experimental site Pfynwald).

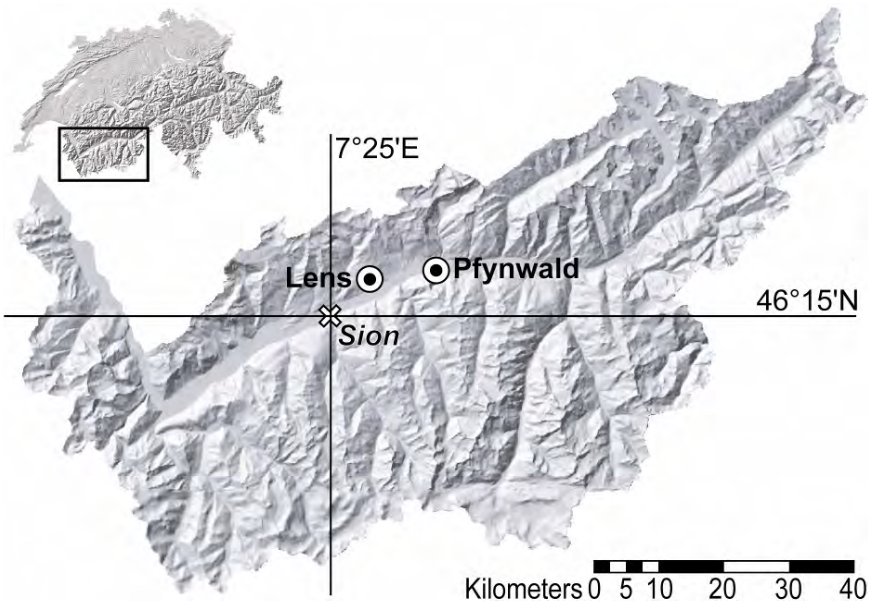


Fig 4: Location of the study sites (= dots; Lens and Pfynwald) and the weather station (= cross, Meteo Swiss, Sion).

Specific research questions of the thesis

Retrospective survey of tree growth on the annual scale (Chapter I)

Drought is an important factor limiting plant growth (Flexas *et al.*, 2006). Ample literature exists on physiological responses to drought (e.g., Rennenberg *et al.*, 2006; Granier *et al.*, 2007). During periods of drought the maintenance of an efficient water transport is of great importance to avoid xylem dysfunction by cavitation (Breda *et al.*, 2006) and a down-regulation of the photosynthetic activity (Rennenberg *et al.*, 2006). However detailed knowledge about the growth response of water conducting tissue to severe drought is lacking.

In this study, we focused on growth adaptation of the water conducting tissue in the stem of Scots pine and pubescent oak to severe drought. Growth adaptation to drought was evaluated, over the last decades (tree-ring width analysed for the last 100 years cellular parameter analysed for the last 45 years), by comparing tree growth under naturally dry conditions with trees growing along an open water channel. In addition, the differences in climate impact on tree growth of Scots pine and pubescent oak were studied by comparing the species-specific climate growth relationship.

The following research questions were addressed:

- Are there species-specific responses of the wood anatomy to drought and
- Does a change in water availability change the tree-species' sensitivity to drought and temperature?

The results are discussed in the context of the potential future performance of Scots pine and pubescent oak in a predicted hotter and drier future.

Inter and intra-annual analysis of tree growth within an irrigation experiment (Chapter II)

Drought was often discussed as a trigger in vegetation shifts and forest decline (e.g., Allen & Breshears, 1998; Penuelas & Boada, 2003; Breshears *et al.*, 2005; Gitlin *et al.*, 2006). Recent studies provide storage depletion and xylem dysfunction due to cavitation as important mechanisms leading to drought induced forest decline (Breda *et al.*, 2006; McDowell *et al.*, 2008). However, it is still unclear if trees of different crown density, representing different states along the pathway from high-vigour trees to dead trees, react differently to drought. Moreover the reversibility of the forest decline process is still under discussion.

This study aimed to quantify the impact of drought and crown density on the intra-annual timing and on the amount of wood formation in Scots pine. Therefore an irrigation experiment was installed on a xeric site in Valais and Scots pines growing under naturally dry conditions were compared with Scots pines growth under improved water supply. To estimate the potential of the pines to recover from the limitation by drought, trees of three different crown density classes (low, middle and high, estimated from the crown transparency) were selected. This study aimed to verify the following hypotheses:

- Trees with a low crown density are more affected by drought than trees with denser crowns, resulting in longer growth depression after severe drought events.
- Irrigation alters timing, quantity and quality of wood formation.
- Trees with a low crown density are not able to profit from irrigation, since they passed the "point of no return" and thus are irretrievably predisposed to die.

The results should contribute to a better understanding of growth limitations and the adaptive strategy of Scots pine under drought and should improve the understanding of the pine decline processes in Valais.

Inter and intra-annual stable isotope analysis within an irrigation experiment (Chapter III)

Drought leads to a negative carbon balance due to the down-regulation of photosynthetic activity while metabolic demands for carbon in plants are ongoing (Flexas *et al.*, 2006; Rennenberg *et al.*, 2006). According to the theory of storage depletion this may result, in the long run, in tree death (Breda *et al.*, 2006; McDowell *et al.*, 2008). It remains unclear if the amount of stored carbohydrates is reduced in all trees growing on a dry site or if symptoms of storage depletion can be detect only in trees showing clear signs of decline in their crowns.

In this study, we aimed to determine the impact of low soil water availability on tree growth and tree-ring $\delta^{13}C$ within an irrigation experiment. In addition the change in the intrinsic water-use efficiency (WUE) in response to irrigation was calculated based on tree-ring $\delta^{13}C$ to get estimation on water cost per unit carbon gain. Trees of different crown density classes (used as an indicator of tree vigour) were separately analysed for each treatment (watered and control), to ascertain if differences existed in the growth reaction indicating varying amounts of available carbohydrates.

The research questions addressed here were:

- Does tree-ring $\delta^{13}C$ of Scots pine respond to water availability and if so are there any differences in the response between earlywood and latewood?
- How fast is the irrigation signal recorded in $\delta^{13}C$; is there a delay, indicating a progressive mixing of old and new carbon sources?
- Does $\delta^{13}C$ differ in trees of different crown density classes and, if so, is it possible to predict a higher probability to survive a severe drought for trees with a higher foliage density?

The inclusion of stable carbon isotopes in studies on decline processes should improve the estimation of the impact of drought on future tree growth even though the fractionation processes at leave level might not be directly reproduced by tree-ring $\delta^{13}C$. In particular the importance of storage processes for tree growth was emphasised.

References

Allen CD, Breshears DD. 1998. Drought induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences of the United States of America* **95**(25): 14839-14842.

Bigler C, Bräker OU, Bugmann H, Dobbertin M, Rigling A. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* **9**: 330-343.

Bodig J, Jayne BA. 1993. *Mechanics of wood and wood composites*. Malabar, Florida: Krieger Publishing Company.

Boratynski A 1991. Range of natural distribution. In: M. Giertych C. Matyas eds. *Genetics of Scots pine*: Elsevier, 19-30.

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A. 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. *Global Change Biology* **12**(10): 1922-1939.

Brandes E, Wenninger J, Koeniger P, Schindler D, Rennenberg H, Leibundgut C, Mayer H, Gessler A. 2007. Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. *Plant Cell and Environment* **30**(1): 113-127.

Braun HJ. 1970. *Funktionelle Histologie der sekundären Sprossachse*. Berlin etc.: Borntraeger.

Breda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* **63**(6): 625-644.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **102**(42): 15144-15148.

Briffa KR, Osborn TJ, Schweingruber FH. 2004. Large-scale temperature inferences from tree rings: a review. *Global and Planetary Change* **40**(1-2): 11-26.

Cech T, Perny B. 2000. Kiefernsterben im Tirol. *Forstschutz aktuell* **22**: 12-15.

Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grunwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**(7058): 529-533.

Dale VH, Joyce LA, McNulty S, Neilson RP, Ayres MP, Flannigan MD, Hanson PJ, Irland LC, Lugo AE, Peterson CJ, Simberloff D, Swanson FJ, Stocks BJ, Wotton BM. 2001. Climate change and forest disturbances. *Bioscience* **51**(9): 723-734.

Dobbertin M, Mayer P, Wohlgemuth T, Feldmeyer-Christe E, Graf U, Zimmermann NE, Rigling A. 2005. The decline of *Pinus sylvestris* L. forests in the Swiss Rhone valley - a result of drought stress? *Phyton* **45**: 153-156.

Dobbertin M, Rigling A. 2006. Pine mistletoe (*Viscum album* ssp *austriacum*) contributes to Scots pine (*Pinus sylvestris*) mortality in the Rhone valley of Switzerland. *Forest Pathology* **36**(5): 309-322.

Duquesnay A, Breda N, Stievenard M, Dupouey JL. 1998. Changes of tree-ring delta C-13 and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant Cell and Environment* **21**(6): 565-572.

Ellenberg H. 1988. *Vegetation ecology of Central Europe*. Cambridge etc.: Cambridge University Press.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Reviews of Plant Physiology and Plant Molecular Biology* **40**: 503-537.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121-137.

Flexas J, Bota J, Galmes J, Medrano H, Ribas-Carbo M. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiologia Plantarum* **127**(3): 343-352.

Flühler H. 1981. *Waldschäden im Walliser Rhonetal (Schweiz).* Birmensdorf: Eidgenössische Anstalt für das forstliche Versuchswesen.

Fonti P, García-González I. 2004. Suitability of chestnut earlywood vessel chronologies for ecological studies. *New Phytologist* **163**: 77-86.

Fritts HC. 2001. *Tree rings and climate*. London: Academic Press.

García-González I, Eckstein D. 2003. Climatic signal of earlywood vessels of oak on a maritime site. *Tree Physiology* **23**(7): 497-504.

Gessler A, Rennenberg H, Keitel C. 2004. Stable isotope composition of organic compounds transported in the phloem of European beech - Evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport. *Plant Biology* **6**(6): 721-729.

Gitlin AR, Sthultz CM, Bowker MA, Stumpf S, Paxton KL, Kennedy K, Munoz A, Bailey JA, Whitham TG. 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. *Conservation Biology* **20** (5): 1477-1486.

Granier A, Reichstein M, Breda N, Janssens IA, Falge E, Ciais P, Grunwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Kostner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala I, Wang Q. 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology* **143**(1-2): 123-145.

IPCC 2007. Technical report. In: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Mille ed. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*.

Kagawa A, Sugimoto A, Maximov TC. 2006. Seasonal course of translocation, storage and remobilization of C-13 pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. *New Phytologist* **171**(4): 793-804.

McCarroll D, Loader NJ. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**(7-8): 771-801.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**(4): 719-739.

Meusel H. 1992. *Vergleichende Chorologie der zentraleuropäischen Flora III.* Jena: Gustav Fischer.

Nardini A, Pitt F. 1999. Drought resistance of *Quercus pubescens* as a function of root hydraulic conductance, xylem embolism and hydraulic architecture. *New Phytologist* **143**(3): 485-493.

Oberhuber W. 2001. The role of climate in the mortality of Scots pine (*Pinus sylvestris* L.) exposed to soil dryness. *Dendrochronologia* **19**(1): 45-55.

Ozenda P. 1985. *La végétation de la chaîne alpine.* Paris: Masson.

Panyushkina IP, Hughes MK, Vaganov EA, Munro MAR. 2003. Summer temperature in northeastern Siberia since 1642 reconstructed from tracheid dimensions and cell numbers of *Larix cajanderi*. *Canadian Journal of Forest Research* **33**(10): 1905-1914.

Penuelas J, Boada M. 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology* **9**(2): 131-140.

Rebetez M, Dobbertin M. 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and applied climatology* **79**(1-2): 1-9.

Reichstein M, Ciais P, Papale D, Valentini R, Running S, Viovy N, Cramer W, Granier A, Ogee J, Allard V, Aubinet M, Bernhofer C, Buchmann N, Carrara A, Grunwald T, Heimann M, Heinesch B, Knohl A, Kutsch W, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Pilegaard K, Pumpanen J, Rambal S, Schaphoff S, Seufert G, Soussana JF, Sanz MJ, Vesala T, Zhao M. 2007. Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biology* **13**(3): 634-651.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* **8**(5): 556-571.

Rigling A, Cherubini P. 1999. Wieso sterben die Waldföhrenwälder im "Tellwald" bei Visp? . *Schweizerische Zeitschrift für Forstwesen* **150**: 113-131.

Sayer U. 2000. *Die Ökologie der Flaumeiche (Quercus pubescens Willd.) und ihrer Hybriden auf Kalkstandorten an ihrer nördlichen Arealgrenze Untersuchungen zu Boden, Klima und Vegetation*. Berlin: J. Cramer.

Schär C, Vidale PL, Luethi D, Frei C, Häberli C, Liniger MA, Appenzeller C. 2004. The role in increasing temperature variability in European summer heatwaves. *Nature* **427**: 332-336.

Sperry JS. 2003. Evolution of water transport and xylem structure. *International Journal of Plant Sciences* **164**(3): S115-S127.

Timbal J, Aussenac G. 1996. An overview of ecology and silviculture of indigenous oaks in France. *Annales Des Sciences Forestieres* **53**(2-3): 649-661.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*. Berlin, Heidelberg, New York: Springer Verlag.

Vaganov EA, Hughes MK, Shashkin AV. 2006. *Growth dynamics of conifer tree rings images of past and future environments*. Berlin: Springer.

Vertui F, Tagliaferro F. 1998. Scots pine (*Pinus sylvestris* L.) die-back by unknown causes in the Aosta Valley, Italy. *Chemosphere* **36**(4): 1061-1065.

von Arx G, Dietz H. 2005. Automated image analysis of annual rings in the roots of perennial forbs. *International Journal of Plant Sciences* **166**(5): 723-732.

Weber P, Bugmann H, Fonti P, Rigling A. 2008. Using a retrospective dynamic competition index to reconstruct forest succession. *Forest Ecology and Management* **254**: 96-106.

Weber P, Bugmann H, Rigling A. 2007. Radial growth responses to drought of *Pinus sylvestris* and *Quercus pubescens* in an inner-Alpine dry valley. *Journal of Vegetation Science* **18**(6): 777-792.

Wermelinger B, Rigling A, Schneider Mathis D, Dobbertin M. 2008. Assessing the role of bark- and wood-boring insects in the decline of Scots pine (*Pinus silvestris*) in the Swiss Rhone valley. *Ecological Entomology* **33**: 239-249.

Wimmer R. 2002. Wood anatomical features in tree-rings as indicators of environmental change *Dendrochronologia* **20**(1-2): 21-36.

Zweifel R, Zimmermann L, Zeugin F, Newbery DM. 2006. Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. *Journal of Experimental Botany* **57**(6): 1445-1459.

Chapter I: Drought-induced adaptation of the xylem in Scots pine and pubescent oak

Britta Eilmann [1], Roman Zweifel [1], Nina Buchmann [2], Patrick Fonti [1], Andreas Rigling [1]

[1]Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
[2]Institute of Plant Sciences, ETH Zurich, Universitätsstrasse 2, 8092 Zurich, Switzerland

Summary

Drought impairs tree growth in the inner-Alpine valleys of Central Europe. We investigated species-specific responses to contrasting water supply, with Scots pine (*Pinus sylvestris* L.) threatened by drought-induced mortality, and pubescent oak (*Quercus pubescens* Willd.), showing no connection between drought events and mortality.

The two co-occurring tree species were compared growing either along an open water channel or at a site with naturally dry conditions. In addition, the growth response of Scots pine to a draining of a water channel was studied. We analysed radial increment of the last 100 years and wood anatomical parameters of the last 45 years.

Drought reduced the conduit area of pubescent oak, but increased the radial lumen diameter of the conduits in Scots pine. Both species decreased their radial increment under drought. In Scots pine radial increment was generally more dependent on water availability than that in pubescent oak. Irrigated trees responded less negative to high temperature as seen in the increase in the conduit area in pubescent oak and the removal of the limitation of cell division by high temperatures. After irrigation stopped, tree-ring width for Scots pine decreased within one year delay whereas lumen diameter and cell-wall thickness responded with a four year delay.

Scots pine seemed to optimize the carbon-per-conduit-costs under drought by increasing conduits diameter while decreasing cell numbers. This strategy might lead to a complete loss of tree rings under severe drought and thus to an impairment of water transport. In contrast, in pubescent oak tree-ring width is less affected by summer drought because parts of the earlywood are built in early spring. Thus pubescent oak might have gradual advantages over pine in today's climate of the inner-Alpine valley.

Introduction

Climate change is expected to increase the frequency and severity of drought events in Central Europe (Schär et al. 2004; IPCC 2007) and this will strongly affect the physiology, growth and survival of trees of different species. Drought reduces primary production (Ciais et al. 2005; Granier et al. 2007; Reichstein et al. 2007), stem growth (e.g. Schweingruber 1993; Fritts 2001) and the storage of carbohydrates (Bréda et al. 2006). According to the theory of storage depletion, shortage of carbohydrates might be a main cause for drought-induced tree death (Bréda et al. 2006; McDowell et al. 2008), leading to a negative carbon balance as more carbon is needed (e.g. for plant respiration) than the tree is able to take up. As a result more storedcarbohydrates have to be invested, which may, in the long run, lead to tree death by storage depletion (McDowell et al. 2008). Consequently, drought has been frequently discussed as a trigger for forest decline and decline-induced vegetation shifts (Allen and Breshears 1998; Penuelas and Boada 2003; Breshears et al. 2005). But how severely drought affects wood formation has, so far, only been rudimentarily demonstrated (Zweifel et al. 2006, 2007; Sterck et al. 2008).

Inner-Alpine forest ecosystems are regularly exposed to drought, as precipitation is generally low (≈ 600 mm per year), due to the rain shadow of the surrounding high mountain ranges (Rebetez and Dobbertin 2004). During past decades, climatic conditions have changed in these regions towards increasing summer droughts, exemplarily is the Swiss Rhone valley illustrated by Rebetez and Dobbertin (2004) and Weber et al. (2007). Even though the total amount of precipitation decreased only slightly since 1980, temperature and evapotranspiration significantly increased (Rebetez and Dobbertin 2004), leading, at least seasonally, to a more negative water balance of the trees (Zweifel et al. 2006). In addition the seasonality of water availability has changed: while water availability has increased in winter it has decreased in summer (Weber et al. 2007). The consequences of these climatic changes for species composition and species survival remain unclear.

The sub-boreal species Scots pine (*Pinus sylvestris* L., in the following pine) dominates the low elevation forests of the inner-Alpine valleys. In recent decades, unusually high mortality rates of pine have been reported from the Swiss Rhone valley (Dobbertin et al. 2005; Bigler et al. 2006; Dobbertin and Rigling 2006), the Italian Aosta valley (Vertui and Tagliaferro 1998) and the Austrian Inn valley (Oberhuber 2001). In the Swiss Rhone valley, locally almost half of the population of pine has died since 1995 (Rebetez and Dobbertin 2004), with the highest mortality rates at dry sites after drought years (Dobbertin et al. 2005). In the same period, the sub-Mediterranean species pubescent oak (*Quercus pubescens* Willd., in the following oak) has increased its abundance (Weber et al. 2008) and has not shown an increase in mortality (Dobbertin et al. 2005).

In this study, we examine tree growth of pine and oak by analysing tree-ring width over 100 years and a variety of wood anatomical properties for the last 45 years (oak: earlywood vessel size; pine: number, radial lumen diameter and cell-wall thickness of tracheids in earlywood and latewood). This combined approach of dendrochronological and wood anatomical methods enabled us to describe growth limitations by drought more precisely. Particularly, we focused on the growth of oak and pine under contrasting water availability, including temporal dynamics in growth response to climate. The situation that a water channel was falling dry after hundreds of years in use, enabled us to analyse how growth parameters developed after this sudden change in water availability. Our research addressed two main questions: (1) are there species-specific responses of wood anatomy to drought in oak and pine and (2) does a change in water availability change the tree-species' growth response to drought and temperature?

Material and Methods

Study area and sampling method

The study sites (46°16'N, 07°26'E) are located near the village Lens within the inner-Alpine Swiss Rhone valley (canton Valais). The climate is continental with a mean annual temperature of 9.2°C and an annual precipitation sum of 599 mm for the period 1961-1990 (MeteoSwiss, weather station Sion, 492 m a.s.l., 10 km distance from the study sites).

The forest with southeast exposition is dominated by oak and pine (*Erico-Pinetum sylvestris*). The soil type can be described as Rendzic, Leptosol with limestone as parent material. At about 1000 m a.s.l. an open irrigation channel runs through the forest. This channel was created in 1450 for the irrigation of lower agricultural areas (Crock and Jones 1999). It was carved out of stone or runs in the consolidated soil. The channel is fed from the end of April to the end of October, during winter no water runs through. Due to extensive water loss, a section of the water channel was replaced by a tunnel in 1983, and the former water channel section dried out.

To study adult trees growing under contrasting water supply, oak and pine were chosen at three different sites: 1) an un-irrigated control site, 50 m above the irrigation channel (altitude

1050 m.a.s.l., slope 90%; in the following “control”), 2) an irrigated site along the functioning section of the water channel (altitude 1000 m.a.s.l., slope 20-90%; in the following called “irrigation”) and 3) a formerly irrigated, but now dry site, where irrigation stopped in 1983 (altitude 1020 m.a.s.l., slope 20-90%; in the following called “irrigation stop”). As oak was nonexistent at the “irrigation stop” site only pines were analysed. Apart from water supply, all sites were similar with regard to site conditions.

Sampling and sample preparation

Two cores (diameter: 5 or 10 mm, length: from the bark to the pith) of fifteen dominant trees per species were sampled at each of the three sites. To avoid the confounding factor of young cambial age (Vysotskaya and Vaganov 1989; Lei et al. 1996) only trees were sampled that were at least 40 years old in 1960, the first year of cellular analysis.

The cores were planed using a custom-made core-microtome (WSL, Switzerland) to obtain clean surfaces for the analysis of radial increments. Since the cellular measurement in oak was carried out directly on the core surfaces, tyloses had to be removed using a high pressure water blast (Fonti and García-González 2004). To increase the contrast between cell wall and cell lumen, in a first step, the cell wall was darkened by applying sodium hydroxide (NaOH, 30%) twice with a brush on the core surface. In a second step, the cell lumen was filled by pressing white plasticine (M.creativ Plastilin, Switzerland) from the surface into the lumina. For cellular analysis in pine, the cores were subdivided into pieces (length approx. 5 cm) and thin sections (thickness of 10 µm) were cut using a sliding microtome (Reichert, Germany). For a better contrast between cell wall and cell lumen, the thin sections were stained with safranin (1% solution) and astrablue (2% solution), dehydrated with ethanol (70%, 95% and 100%) and xylol (>98%) and fixed with Canada balsam.

Measurements

Tree-ring widths, separated into earlywood and latewood widths, over the last 100 years were measured using a combination of a Lintab digital positioning table and the software TSAP (both Rinntech, Germany). Individual tree-ring series were cross-dated visually and detrended using the software ARSTAN (Holmes 1994) to remove the age-related trend. The tree-ring series were power-transformed to stabilize the variance (Cook and Peters 1997) and then fitted with a negative exponential curve (Fritts 2001).

Cellular characteristics (earlywood vessel area in oak; radial lumen diameter, cell-wall thickness and cell number in pine) were analysed along a 45-year sequence (1960 to 2004) on four randomly selected trees per species and site. Due to the disparity in xylem composition between oak, with a ring-porous structure, and pine, with a tracheidal structure, no parameters comparable to cell number or cell wall thickness were measurable in oak. However, conduit size, a key-factor for tree performance under drought, was measured in both species (vessel area in oak and radial lumen diameter in pine).

For the cellular analysis in oak, the cores were scanned with a distortion-free scanner (Color Scanner Expression 1000 XL, 12’000 dpi, Epson, CA) and earlywood vessels (minimum area 0.005 mm²) were measured on the whole core diameter (5 mm) using the software IMAGE PRO PLUS (Media Cybernetics, MD). For the cellular analysis in pine, micro-pictures were taken (100 times magnification, microscope: Olympus BX41; camera: ColorView III, Soft Imaging system, Germany) and radial lumen diameter, cell wall thickness, cell number in five radial cell rows per tree ring were analysed with the software WINCELL (Regents Instruments Inc, Canada). These measurements were separated into earlywood and latewood based on the Mork index, defining a latewood cell as a cell where the double of the cell-wall thickness exceeds the lumen diameter (Mork 1928) and mean values were calculated. To strengthen the common climate signal in earlywood among the years, only the first 10% of the earlywood tracheids were averaged to a mean, expecting these cells to be quite equal in the time of their formation. In latewood, no further separation was made, due to the generally small number of latewood cells.

Climate-growth correlation

To analyse the climate-growth relationships, Pearson’s correlations between climate data (precipitation and temperature, both recorded at the Sion weather station, self-calibrating Palmer drought severity index (PDSI) by van der Schrier et al. (2007)) and the growth parameters for oak and pine at irrigated and control sites were calculated separately. The parameters of the radial increment (earlywood, latewood and tree-ring widths) were correlated with monthly data on precipitation, temperature and the PDSI for the period 1900-2004. PDSI is a measure of regional soil moisture content, based on soil characteristics and records of precipitation and temperature. With the PDSI climatic conditions are classifying into 11 categories between extremely wet (PDSI ≥ 4.0) to extremely dry (PDSI ≤ -4.0).

The cellular parameters were correlated with daily climate data (precipitation and temperature) averaged by a moving window of 10 days for the period 1960-2004. To assure a reliable climate-growth analysis for wood anatomical parameters, characterized by a generally low common signal (correlation between trees r_{bt}) (e.g. Yasue et al. 2000; Fonti and García-González 2004), only growth parameters, having a r_{bt} greater than 0.05 for both treatments (irrigated and control) were included. Hence climate correlation were only calculated for earlywood vessel area in oak and cell number in pine To further diminish the risk of statistical artefacts, only climate signals above the 99% significance threshold were considered. The narrow moving window of only 10 days was chosen due to the rapid cell enlargement of earlywood cells (Zasada and Zahner 1969).

Results

Growth responses to irrigation

Irrigated oak and pine showed significantly larger radial increments (width of earlywood, latewood and entire tree ring) than the control trees (Table 1a). The average tree-ring width was almost doubled by the irrigation. In the control trees, the tree rings in oak were smaller due to the narrow latewood, in pine earlywood and latewood were evenly reduced (Table 1a and Fig. 1). In extremely dry years (e.g. years 1921, 1976), the latewood of oak or the entire tree ring of pine were often missing.

The conduit size in oak (area of earlywood vessel) and pine (radial lumen diameter of earlywood tracheids) showed opposite responses to irrigation (Table 1b). Irrigation increased the average vessel area of oak, whereas it decreased the radial lumen diameter of the tracheids of pine. The cell-wall thickness increased in earlywood but showed no significant change in latewood, the number of cells increased significantly in earlywood and latewood (Table 1b, pine only).

The sudden stop of irrigation in 1983 caused significant changes in nearly all growth parameters studied in pine (Table 2). The additional multiple comparison (Tukey HDS test) between the ‘control’, ‘irrigation’ and ‘irrigation stop’ sites showed that after the ‘irrigation stop’ all growth parameters significantly differed from those of the still irrigated pine ($p < 0.001$) and were statistically inseparable from those of the control pine. The tree-ring widths of pine at the ‘irrigation stop’ site were even smaller than the ones at the ‘control’ site which was never irrigated (Fig. 2a). The time lag in growth response to the irrigation stop differed among the growth parameters: Tree-ring width (Fig. 2a) and cell number (Fig. 3) immediately decreased whereas lumen diameter and cell-wall thickness responded with a delay of four years.

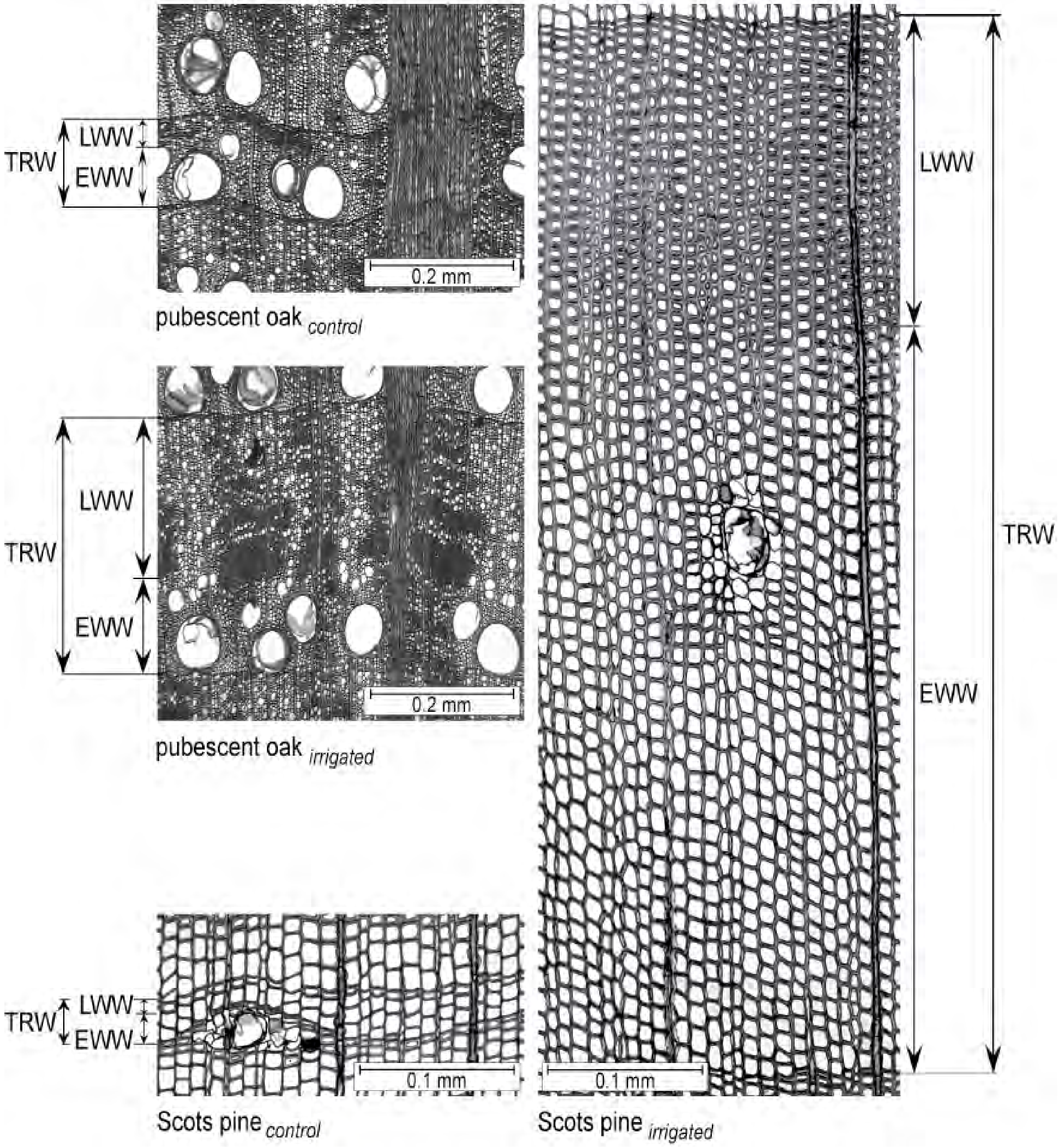
Table 1 a) Response of the radial increment (earlywood, latewood and tree-ring widths; n = 15 trees) to water status of oak and pine calculated for the period 1900 to 2004. P-values were calculated with a t-test between irrigated and control trees.
b) Response of the cellular parameters (earlywood vessel area for oak, lumen diameter and cell-wall thickness for pine; n = 4 trees) to water status of oak and pine calculated for the period 1960 to 2004. P-values were calculated with a t-test between irrigated and control trees. Units of measurements: radial increment = [1/100mm], VA = [mm²], LD and CWT = [µm]; * = mean lumen diameter and cell-wall thickness of earlywood cells were calculated for the first 10% of earlywood cells only. Abbreviations: SD= standard deviation, r_{bt} = common signal (correlation between trees); radial increment: TRW = tree-ring width, EWW= earlywood width, LWW = latewood width; cellular parameters: VA = vessel area, LD = lumen diameter, CWT = cell-wall thickness, #cell = number of cells; indices: ew=earlywood, lw=latewood.

a)

Parameter	Treatment	Mean	SD	r_{bt}	p-value control vs. irrigated
pubescent oak					
TRW	control	64	21	0.210	< 0.001
	irrigated	145	42	0.372	
EWW	control	25	3	0.092	< 0.001
	irrigated	46	6	0.164	
LWW	control	38	19	0.234	< 0.001
	irrigated	99	38	0.456	
Scots pine					
TRW	control	76	23	0.469	< 0.001
	irrigated	133	42	0.234	
EWW	control	53	16	0.431	< 0.001
	irrigated	89	29	0.413	
LWW	control	20	8	0.435	< 0.001
	irrigated	43	14	0.330	

b)

Parameter	Treatment	Mean	SD	r_{bt}	p-value control vs. irrigated
pubescent oak					
VA_{ew}	control	0.03	0.004	0.078	< 0.001
	irrigated	0.04	0.003	0.121	
Scots pine					
LD^{*}_{ew}	control	11.4	0.6	0.023	< 0.001
	irrigated	10.4	0.5	-0.050	
LD_{lw}	control	5.9	0.5	0.037	ns
	irrigated	5.8	0.4	0.042	
CWT^{*}_{ew}	control	1.0	0.2	0.043	< 0.001
	irrigated	1.2	0.2	-0.064	
CWT_{lw}	control	4.7	1.1	0.035	ns
	irrigated	4.9	1.2	0.024	
$\#\mathrm{cell}_{ew}$	control	9	3	0.225	< 0.001
	irrigated	19	4	0.234	
$\#\mathrm{cell}_{lw}$	control	6	2	0.202	< 0.001
	irrigated	20	5	0.241	



pubescent oak $_{control}$

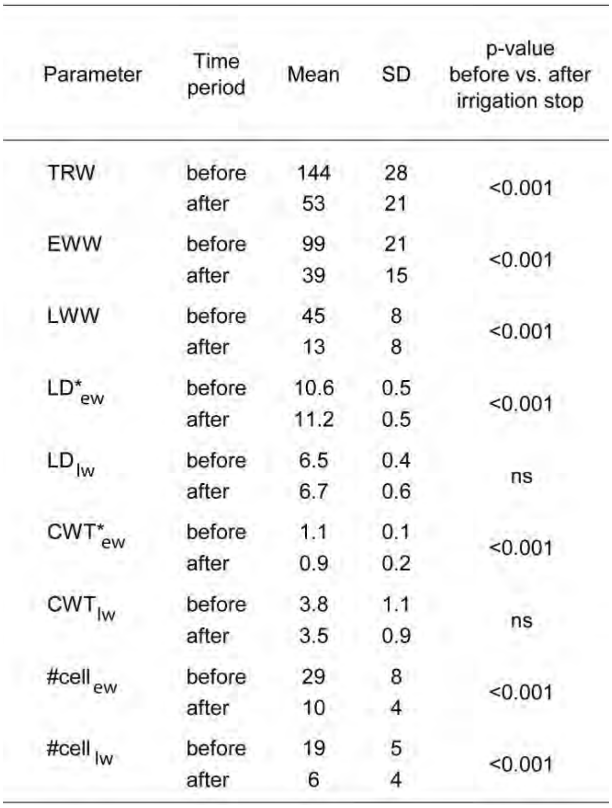
pubescent oak $_{irrigated}$

Scots pine $_{control}$

Scots pine $_{irrigated}$

Fig. 1 Tree rings of the drought year 1976 in control and irrigated pubescent oak and Scots pine Abbreviations: TRW= tree-ring width, EWW = earlywood width, LWW = latewood width.

Table 2 Growth parameters of pine before (1961-1982) and after (1983-2004) irrigation cessation (given as "time period"). P-values were calculated with a t-test between the growth parameters before and after irrigation stop. Unit of measurements: radial increment = [1/100mm], VA = [mm²] LD and CWT = [µm]; * = mean lumen diameter and cell-wall thickness of earlywood cells were calculated for the first 10% of earlywood cells only. Abbreviations: SD= standard deviation; radial increment: TRW = tree-ring width, EWW= earlywood width, LWW = latewood width; cellular parameters: LD= lumen diameter, CWT= cell-wall thickness, #cell = number of cells; indices: ew=earlywood, lw=latewood (radial increment: n = 15 trees; cellular parameters: n=4 trees).



Parameter	Time period	Mean	SD	p-value before vs. after irrigation stop
TRW	before	144	28	<0.001
	after	53	21	
EWW	before	99	21	<0.001
	after	39	15	
LWW	before	45	8	<0.001
	after	13	8	
LD^*_{ew}	before	10.6	0.5	<0.001
	after	11.2	0.5	
LD_{lw}	before	6.5	0.4	ns
	after	6.7	0.6	
CWT^*_{ew}	before	1.1	0.1	<0.001
	after	0.9	0.2	
CWT_{lw}	before	3.8	1.1	ns
	after	3.5	0.9	
$\#cell_{ew}$	before	29	8	<0.001
	after	10	4	
$\#cell_{lw}$	before	19	5	<0.001
	after	6	4	

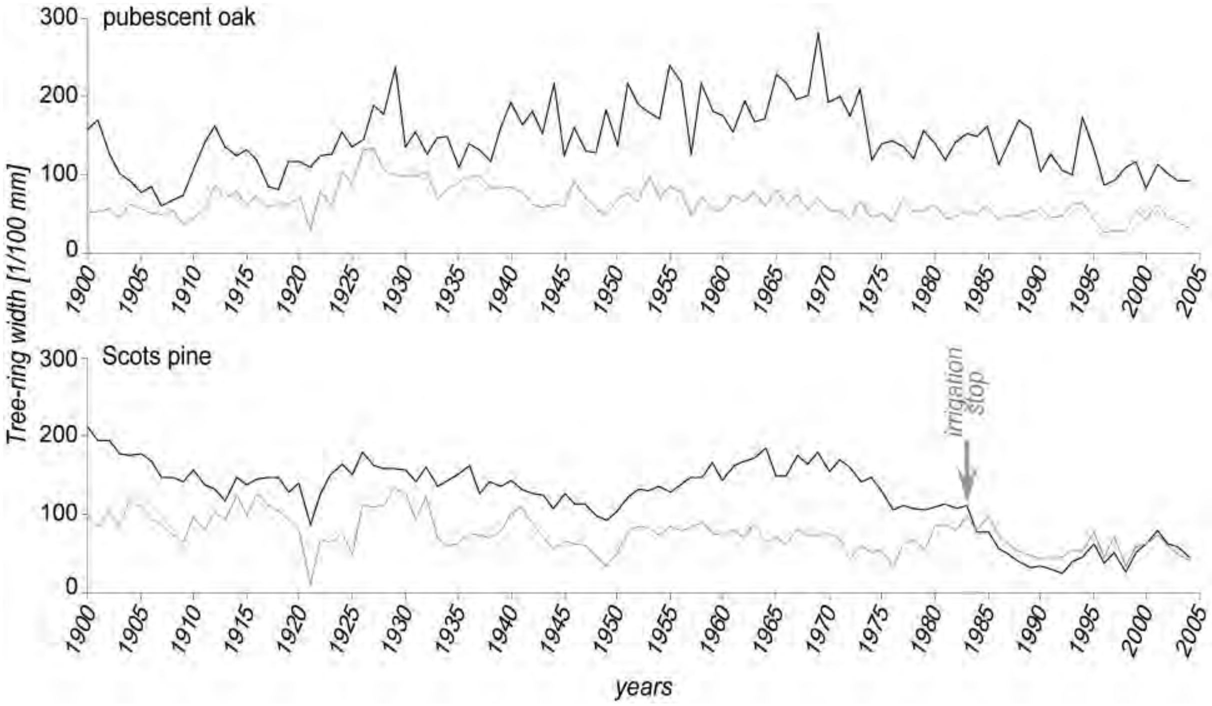


Fig. 2 Tree-ring width chronologies (n = 15) of control and (at least temporarily) irrigated oak and pine. black = trees of the irrigation or irrigation stop site, grey = trees of the control site; arrow= the year irrigation stopped.

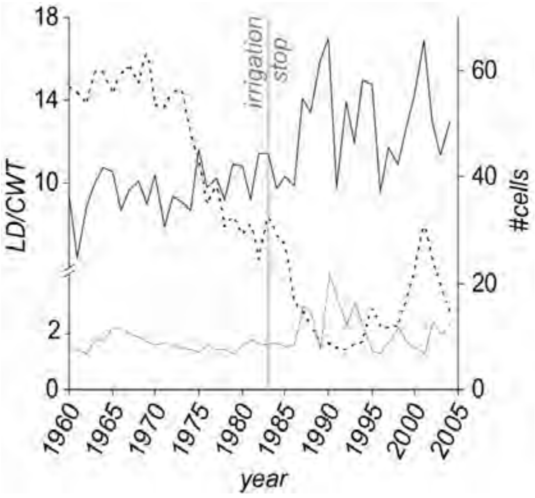


Fig. 3 Cellular growth reactions of pine to irrigation stop in 1983. Solid lines = ratio lumen diameter to cell-wall thickness, black = earlywood, grey = latewood. Dashed line = annual number of cells. Vertical line = the year irrigation stopped. Abbreviations: LD/CWT = lumen diameter to cell-wall thickness ratio, #cell = number of cells.

Response to climate

Independent of the treatment, tree-ring width of pine showed a stronger growth dependence on water availability (i.e. precipitation and PDSI) than of oak. In other words, to produce a wide tree ring in pine more months with high water availability are needed than in oak (Table 3).

Table 3 Pearson's correlation between the radial increment (earlywood, latewood and tree-ring widths) and monthly data of precipitation, temperature and the Palmer drought severity index (PDSI). Climate correlations with the earlywood width of oak were only calculated for the period August of the previous year to May of the current year. For the earlywood width in pine, climate correlations were only calculated for the period August of the previous year to July of the current year. Positive correlation: + $p < 0.01$, ++ $p < 0.001$. No negative correlation was found. Abbreviations: TRW = tree-ring width, EWW = earlywood width, LWW = latewood width.

	Quercus pubescens														*Pinus sylvestris*													
Precipitation	A	S	O	N	D	J	F	M	A	M	J	J	A	S	A	S	O	N	D	J	F	M	A	M	J	J	A	S
$\text{TRW}_{control}$					+										+							+		++				
$\text{EWW}_{control}$					+										+							+		++				
$\text{LWW}_{control}$					++							+										+		++		++	+	
$\text{TRW}_{irrigated}$																								+				
$\text{EWW}_{irrigated}$															+									+				
$\text{LWW}_{irrigated}$																												

PDSI	A	S	O	N	D	J	F	M	A	M	J	J	A	S	A	S	O	N	D	J	F	M	A	M	J	J	A	S
$\text{TRW}_{control}$						+	+	++	+	++	++	+	+				+	++	++	+		+	+	++	++	+		
$\text{EWW}_{control}$										+							+	+	+					+	+			
$\text{LWW}_{control}$					+	+	+	++	++	++	++	+					++	+	++	++	+	++	++	++	++	++	++	++
$\text{TRW}_{irrigated}$																												
$\text{EWW}_{irrigated}$																												
$\text{LWW}_{irrigated}$																												

At the control site radial increment of oak and pine responded differently to water availability (i.e. precipitation and PDSI) over time (Table 3). Radial increment of oak increased with high winter precipitation and latewood width benefited from high precipitation in summer. In contrast, the radial increment of pine growing on the control site showed no significant correlations with winter precipitation. But high precipitation at the beginning of the growth period had a positive effect on radial increment. Furthermore, earlywood and tree-ring width profited from high precipitation in the previous fall. High summer precipitation also promoted the latewood growth. Temperature, taken as an isolated factor, did not significantly determine tree-ring width at any time of the year (except for latewood of pine and July temperature, data not shown). However, radial increment of control trees strongly response to the water availability index PDSI, linking precipitation and temperature. The radial increment of oak increased with high water availability (high values of the PDSI) in the previous December until August. Radial increment in pine even responded to high water availability from the previous October to September.

In contrast to the control trees, the radial increment of irrigated trees was less affected by climatic conditions (Table 3). Growth response to precipitation, temperature and PDSI, was reduced, as seen in the earlywood and tree-ring width of pine or even non-existent, like in oak and the latewood width of pine.

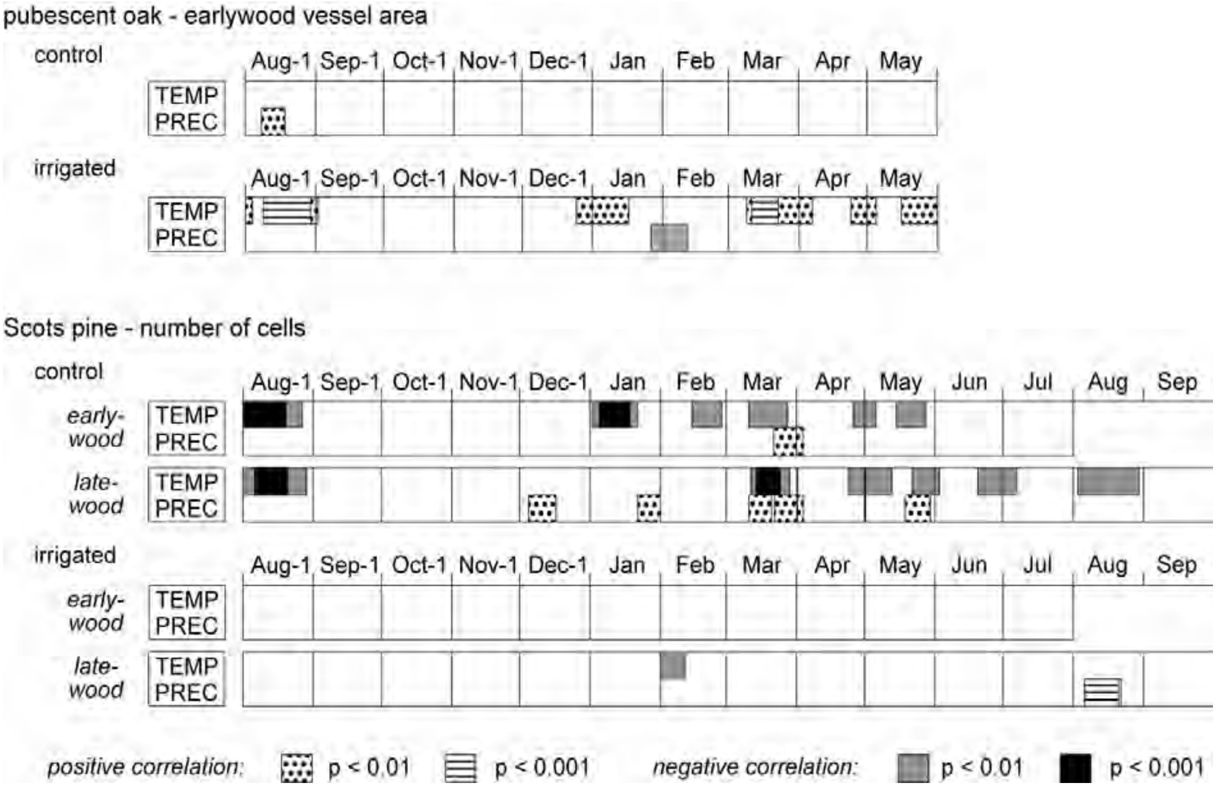


Fig. 4 Moving-window correlation between climate data and earlywood vessel area of oak (top), and cell number of pine (bottom). Climate data were averaged by a moving window of 10 days. Earlywood-vessel formation in pubescent oak takes place in April and May, thus climate correlations for this parameter were only calculated for the period August of the previous year to the end of May of the current year. As earlywood in Scots pine is built from April to July, the climate correlations with the number of earlywood cells were calculated for the period August of the previous year to the end of July of the current year. The number of latewood cells were correlated with climate data from August of the previous year to September of the current year, as latewood formation takes place from July to September. Months of the previous year are labelled with the suffix -1. Different fillings represent positive and negative correlations at two significance levels. Abbreviations: TEMP = correlation with temperature, PREC = correlation with precipitation EW = earlywood, LW = latewood.

On the cellular level, in contrast to the tree-ring level, the growth dependence on climate of the earlywood vessel area of oak increased with irrigation (Fig. 4). The irrigated oak trees responded positively to the temperature in August of the previous year and in January, March, April and May of the current year. Precipitation in January and February had a negative effect on earlywood vessel area in irrigated oak trees. The earlywood vessel area in control oak trees only responded to precipitation in August of the previous year.

Pine showed the same general response to irrigation on the cellular level as on the tree-ring level, as the growth dependence on climate was lower in irrigated than in control pine trees (Fig. 4). Although the cell number in pine was highly correlated with radial increment ($r_{\text{irrigated}} = 0.77$, $r_{\text{control}} = 0.84$), the cell number showed unlike radial increment, a high negative correlation with temperature in addition to precipitation signals. High temperatures during the previous August, in late winter (January to March) and in the months before and during their formation (April to June) led to a decline in the number of earlywood cells. The number of latewood cells decreased with high temperatures during the previous August and during the months before and during their formation (March to August). In addition, low precipitation in winter (December and January) and spring (March and May) reduced the number of latewood cells in pine.

Discussion

Growth adaptation to drought – tree-ring level

Under drought both species need to economize carbon in short supply as the photosynthesis and thus assimilate availability are low (Ciais et al. 2005; Granier et al. 2007; Reichstein et al. 2007). Both species reduced their radial increments (Table 1a) supporting the “carbon allocation hierarchy” theory postulated by Waring (1987), with stem growth being of lower priority than bud formation and root growth. Other studies from regions with a similar environment also found that tree-ring width was reduced under drought (Tessier et al. 1994; Oberhuber et al. 1998; Rigling et al. 2002 and 2003; Bigler et al. 2006; Weber et al. 2007).

Oak mainly reduced latewood width under drought while pine evenly reduced earlywood and latewood. These trends culminated in missing latewood in oak and even entirely missing tree-rings in pine in severe drought years. Due to these species-specific differences the impact of multiple drought years should be quite different for oak vs. pine. In ring-porous oak, the bulk water transport takes place in the big earlywood vessels of the youngest outmost tree rings

(Ellmore and Ewers 1985). Each spring at least one new tangential row of earlywood vessels is added (e.g. Eckstein and Schmidt 1974; Nola 1996). Thus oak has the chance to regenerate its maximal conductivity every spring, which is a successful strategy in summer-dry climates. However, latewood vessels are also important for water transport in oak. They represent the emergency system in water conduction of ring-porous species as they provide the water transport together with the tracheids in case of cavitation of the earlywood vessels (Granier et al. 1994). Therefore, a repeated missing or strong reduction of latewood under severe drought, as shown by our data, might in the long run hinder efficient water transport after cavitation of earlywood vessels.

Compared to oak, water transport in pine takes place in many more tree rings. Therefore, a single missing tree ring might be compensated. But multiple narrow tree rings due to severe drought years would significantly reduce water transport as pine relies on individual tree rings much longer than oak.

Growth adaptation to drought – cellular level

Focussing on the conduit sizes diverging species-specific adaptation patterns to drought were found (Table 1b). In oak, significantly smaller earlywood vessels were formed in control trees than in the irrigated ones. This is in accordance with other studies (Sass and Eckstein 1995; Steppe and Lemeur 2007; Sterck et al. 2008) and might be a strategy to decrease vessels vulnerability to cavitation (Hacke and Sperry 2001). In contrast, pine trees significantly increased their earlywood conduits under drought (Table 1b). Increasing lumen diameter might be an adaptation to compensate for the reduction in conducting area (reduced tree-ring widths) under drought, as hydraulic conduction is proportional to the 4th power of the conduit diameter (Hagen Poiseuille law according to Tyree and Zimmermann (2002)). Hence, with larger lumen diameter less tissue has to be invested to reach a given water conductivity and higher rates of transpiration can be tolerated (Sperry 2003). Larger lumen diameters under drought are also found by Maherali and DeLucia (2000) reporting higher specific hydraulic conductivity (K_s) due to larger lumen diameter in ponderosa pine growing at a semi-arid site than in those of moderate mountain site. They discussed the increase in K_s as a way to improve whole-tree hydraulic conductivity without increasing carbon costs. However, other studies reported decreasing lumen diameters under warm and dry conditions (Jenkings 1974; Nicholls and Waring 1977; Sheriff and Whitehead 1984; Sterck et al. 2008). It is important to realize that the lumen diameters we observed in were among the smallest (mean earlywood lumen diameter; irrigated = 10.4 µm, control = 11.4 µm) compared to other studies (range of earlywood lumen diameter = 14.4 to 40.3 µm), indicating a very strong restriction of cell enlargement under the prevailing site conditions at our site in Valais.

Beside the efficiency, the safety of the conducting system is important for tree survival. As mentioned before, larger conduits are more vulnerable to cavitation than smaller ones (Sperry 2003). In addition, the vulnerability to cavitation also depends on the mechanical strength of the conduits as in wood with a greater density more negative pressure is needed to induce 50% loss of hydraulic conductivity. Thus, especially in dry environment with more negative sap pressure, strong tracheids, having a thicker double cell wall relative to its span, are required (Hacke and Sperry 2001). However, our data showed that pine reduced the thickness to span ratio under drought, as cell wall thickness significantly decreased while lumen diameter increased (Table 1b). Thus, pine built a more effective water conducting system with lower construction costs in terms of carbon investment under drought, but at the expense of safety.

Growth response of pine to irrigation stop

After irrigation stop (Table 2), tree growth approached that of the control pine trees (Table 1). Interestingly, the time lag between irrigation stop and the growth reaction varied among growth parameters, indicating differences in their dependence on water availability. The immediate decrease in radial increment (Fig. 2) and cell number (Fig. 3) pointed to a direct control of cambial activity by water availability, as suggested by Zweifel *et al.* (2006) and Steppe and Lemeur (2007). In contrast, the delayed reaction of lumen diameter and cell-wall thickness (Fig. 3) indicates an indirect impact of drought on cell differentiation, probably via the amount of assimilates. Assimilates are crucial for the synthesis of cell-wall products but also for maintaining turgor pressure (via osmotic potentials) in the enlarging cells (e.g. Ray et al. 1972; Larcher 2003). The drop in photosynthesis due to reduced water availability might have been buffered by a mobilisation of stored carbohydrates as demonstrated by Högberg et al. (2001) and Bhupinderpal-Singh et al. (2003). These authors measured still high soil respiration rates after girdling pine trees, due to the usage of mobilized carbohydrates until storage depletion one year after. The four years' delay in the growth response to the irrigation stop we observed, might be due to reduced but still ongoing photosynthesis, providing assimilates for tree growth, and to the accessibility of the above-ground carbohydrate pools via xylem and phloem.

Climatic control of tree growth under naturally dry conditions

The analysis of climate impact on the radial increment of the control trees growing under naturally dry conditions revealed both clear species-specific but also common growth responses of oak and pine to climate. The radial increment in pine showed a stronger growth dependence on water availability than in oak, as seen in the strong positive precipitation signals in spring and summer as well as in the PDSI signals (Table 3). This is inline with the observation that pine closed its stomata sooner than oak when drought increased (Zweifel et al. 2007). Thus, pine might be more strongly affected by changing climate conditions in Valais, especially since the seasonality of water availability changed towards increasing summer drought (Weber et al. 2007), which will strongly reduce radial increment in pine but will affect that of oak less.

Beside the species-specific differences, strong common positive responses of radial increments of oak and pine to precipitation and the PDSI were observed (Table 3). Radial increments in both species did not show any relevant temperature signals, indicating no temperature limitation at this site.

Irrigation alters climate dependence of tree growth

Irrigated trees of oak and pine responded less negative to high temperatures compared to the control trees as seen in the increase in conduit area in oak (Fig. 4, top)and the elimination of the limitation of cell division by high temperatures (see cell number; Fig. 4, bottom). This might be due to the fact that water is no longer the limiting factor and therefore, higher transpiration rates under high temperatures can be tolerated. In irrigated oak trees, the earlywood vessel area increased under higher temperatures (Fig. 4) while earlywood vessels of the control oak trees showed hardly any climate signal at all. This low growth dependence on climate of the control oak trees might be evidence that not only the formation (Eckstein and Schmidt 1974; Nola 1996) but also the enlargement of earlywood vessels is endogenously controlled if drought was sufficiently severe.

In both species, growth dependence on climate in terms of the radial increment was pronouncedly reduced with irrigation (Table 3). However, species-specific differences in climate dependence exist as radial increments in irrigated oak showed no climate dependence at all, while irrigated pine still depended on high precipitation in May and in the previous August. In addition, the cell formation in pine depended on additional water supply by precipitation during August (see cell number; Fig. 4, bottom), even though irrigation was still ongoing. Thus, irrigation was obviously not sufficient to completely decouple radial growth from precipitation.

Conclusions

Species-specific differences between pine and oak became apparent after analysing adaptation of the water conducting system to drought. Oak showed a stress avoidance strategy with decreasing conduit size under drought leading to a reduction in water-conducting capacity but also a lower risk of cavitation. But the additional reduction in latewood width and therefore in the number of latewood vessels might create higher susceptibility to drought. Thus, even oak might soon reach the limits of its physiological capacity in this area if the frequency of drought years increases, leading to frequently missing latewood. In contrast, pine reduced carbon costs for the water conducting system under drought by decreasing the number and the cell-wall thickness of conduits, while increasing their lumen diameter. As a result the efficiency of water conduction might increased, but at the expense of decreasing safety. Climate-growth analysis revealed a stronger need for water of pine compared to oak. Assuming a hotter and drier climate in the future, a further decrease in cell numbers in pine must be expected with negative effects on water transport in the stem. Due to this, also the future efficiency of the water conducting system is at risk, which might together with the low safety of water transport further amplify the risk of pine mortality in Valais, at the dry distribution limit of pine.

Acknowledgements

We like to thank S. Osenstetter for support in the field, S. Paget for English corrections to the manuscript, the Dendro science unit of the WSL for support in the lab and H. Gärtner, W. Schoch and P. Weber for helpful discussions on the topic. We are grateful to the forest services of the canton Valais, the municipality of Lens and the local forest service for infrastructure and permission. This study was conducted in the frame of the CCES-project MOUNTLAND funded by the Velux foundation and the Canton Valais.

References

Allen, C.D. and D.D. Breshears 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences of the United States of America.* 95:14839-14842.

Bhupinderpal-Singh, A. Nordgren, M.O. Löfvenius, M.N. Högberg, P.E. Mellander and P. Högberg 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell and Environment.* 26:1287-1296.

Bigler, C., O.U. Bräker, H. Bugmann, M. Dobbertin and A. Rigling 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems.* 9:330-343.

Bréda, N., R. Huc, A. Granier and E. Dreyer 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science.* 63:625-644.

Breshears, D.D., N.S. Cobb, P.M. Rich, K.P. Price, C.D. Allen, R.G. Balice, W.H. Romme, J.H. Kastens, M.L. Floyd, J. Belnap, J.J. Anderson, O.B. Myers and C.W. Meyer 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America.* 102:15144-15148.

Ciais, P., M. Reichstein, N. Viovy, A. Granier, J. Ogée, V. Allard, M. Aubinet, N. Buchmann, C. Bernhofer, A. Carrara, F. Chevallier, N. De Noblet, A.D. Friend, P. Friedlingstein, T. Grünwald, B. Heinesch, P. Keronen, A. Knohl, G. Krinner, D. Loustau, G. Manca, G. Matteucci, F. Miglietta, J.M. Ourcival, D. Papale, K. Pilegaard, S. Rambal, G. Seufert, J.F. Soussana, M.J. Sanz, E.D. Schulze, T. Vesala and R. Valentini 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature.* 437:529-533.

Cook, E.R. and K. Peters 1997. Calculating unbiased tree-ring indices for the study of climatic and environmental change. *Holocene.* 7:361-370.

Crock, D.S. and A.M. Jones 1999. Traditional irrigation and its importance to the tourist landscape of Valais, Switzerland. *Landscape Reseach*:49-65.

Dobbertin, M. and A. Rigling 2006. Pine mistletoe (*Viscum album* ssp *austriacum*) contributes to Scots pine (*Pinus sylvestris*) mortality in the Rhone valley of Switzerland. *Forest Pathology.* 36:309-322.

Dobbertin, M., P. Mayer, T. Wohlgemuth, E. Feldmeyer-Christe, U. Graf, N.E. Zimmermann and A. Rigling 2005. The decline of *Pinus sylvestris* L. forests in the Swiss Rhone valley - a result of drought stress? *Phyton* 45:153-156.

Eckstein, D. and B. Schmidt 1974. Dendroklimatologische Untersuchungen an Stieleichen aus dem maritimen Klimagebiet Schleswig-Holsteins. *Angewandte Botanik.* 48:371-383.

Ellmore, G.S. and F.W. Ewers 1985. Hydraulic conductivity in trunk xylem of elm, *Ulmus americana*. *American Journal of Botany.* 72:814-814.

Fonti, P. and I. García-González 2004. Suitability of chestnut earlywood vessel chronologies for ecological studies. *New Phytologist.* 163:77-86.

Fritts, H.C. 2001. Tree rings and climate. *Academic Press, London.* 584 p.

Granier, A., T. Anfodillo, M. Sabatti, H. Cochard, E. Dreyer, M. Tomasi, R. Valentini and N. Bréda 1994. Axial and radial water-flow in the trunks of oak trees - a quantitative and qualitative analysis. *Tree Physiology.* 14:1383-1396.

Granier, A., M. Reichstein, N. Bréda, I.A. Janssens, E. Falge, P. Ciais, T. Grünwald, M. Aubinet, P. Berbigier, C. Bernhofer, N. Buchmann, O. Facini, G. Grassi, B. Heinesch, H. Ilvesniemi, P. Keronen, A. Knohl, B. Kostner, F. Lagergren, A. Lindroth, B. Longdoz, D. Loustau, J. Mateus, L. Montagnani, C. Nys, E. Moors, D. Papale, M. Peiffer, K. Pilegaard, G. Pita, J. Pumpanen, S. Rambal, C. Rebmann, A. Rodrigues, G. Seufert, J. Tenhunen, I. Vesala and Q. Wang 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology.* 143:123-145.

Hacke, U.G. and J.S. Sperry 2001. Functional and ecological xylem anatomy. *Perspectives in Plant Ecology Evolution and Systematics.* 4:97-115.

Högberg, P., A. Nordgren, N. Buchmann, A.F.S. Taylor, A. Ekblad, M.N. Högberg, G. Nyberg, M. Ottosson-Löfvenius and D.J. Read 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature.* 411:789-792.

Holmes, R.L. 1994. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bulletin.* 43:69-78.

IPCC 2007. Technical report. *In* Climate Change 2007: The Physical Science Basis. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* Ed. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Mille.

Jenkings, P.A. 1974. Influence of temperature change on wood formation in *Pinus radiata* grown in controlled environments. *New Zealand Journal of Botany.* 13:579-592.

Larcher, W. 2003. Physiological plant ecology. Ecophysiology and stress physiology of functional groups. *Springer Verlag, Berlin, Heidelberg, New York.* 506 p.

Lei, H., M.R. Milota and B.L. Gartner 1996. Between - and within -tree variation in the anatomy and specific gravity of wood in oregon white oak (Quercus garryana Dougl.). *IAWA Journal.* 17:445-461.

Maherali, H. and E.H. DeLucia 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology.* 20:859-867.

McDowell, N., W.T. Pockman, C.D. Allen, D.D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D.G. Williams and E.A. Yepez 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist.* 178:719-739.

Mork, E. 1928. Die Qualität des Fichtenholzes unter besonderer Rücksichtnahme auf Schleif-und Papierholz. *Papierfabrikant.* 28:741-747.

Nicholls, J.W.P. and H.D. Waring 1977. The effect of environmental factors on wood characteristics. IV. Irrigation and partial drouthing of *Pinus radiata*. *Silvae Genetica* 26:107-111.

Nola, P. 1996. Climatic signal in earlywood and latewood of deciduous oaks from northern Italy. *In Tree Rings, Environmental and Humanity* Eds. J.S. Dean, D.M. Meko and T.R. Swetnam. University of Arizona, Tucson, pp. 249-258.

Oberhuber, W. 2001. The role of climate in the mortality of Scots pine (*Pinus sylvestris* L.) exposed to soil dryness. *Dendrochronologia.* 19:45-55.

Oberhuber, W., M. Stumbock and W. Kofler 1998. Climate tree-growth relationships of Scots pine stands (*Pinus sylvestris* L.) exposed to soil dryness. *Trees - Structure and Function.* 13:19-27.

Penuelas, J. and M. Boada 2003. A global change-induced biome shift in the Montseny mountains (NE Spain). *Global Change Biology.* 9:131-140.

Ray, P.M., P.B. Green and R. Cleland 1972. Role of turgor in plant-cell growth. *Nature.* 239:163-164.

Rebetez, M. and M. Dobbertin 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and applied climatology.* 79:1-9.

Reichstein, M., P. Ciais, D. Papale, R. Valentini, S. Running, N. Viovy, W. Cramer, A. Granier, J. Ogée, V. Allard, M. Aubinet, C. Bernhofer, N. Buchmann, A. Carrara, T. Grünwald, M. Heimann, B. Heinesch, A. Knohl, W. Kutsch, D. Loustau, G. Manca, G. Matteucci, F. Miglietta, J.M. Ourcival, K. Pilegaard, J. Pumpanen, S. Rambal, S. Schaphoff, G. Seufert, J.F. Soussana, M.J. Sanz, T. Vesala and M. Zhao 2007. Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biology.* 13:634-651.

Rigling, A., O. Bräker, G. Schneiter and F. Schweingruber 2002. Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within Erico-Pinion in Valais (Switzerland). *Plant Ecology.* 163:105-121.

Rigling, A., H. Brühlhart, O.U. Bräker, T. Forster and F.H. Schweingruber 2003. Effects of irrigation on diameter growth and vertical resin duct production in *Pinus sylvestris* L. on dry sites in the central Alps, Switzerland. *Forest Ecology and Management.* 175:285-296.

Sass, U. and D. Eckstein 1995. The variability of vessel size in beech (*Fagus sylvatica* L.) and its ecophysiological interpretation. *Trees - Structure and Function.* 9:247-252.

Schär, C., P.L. Vidale, D. Luethi, C. Frei, C. Häberli, M.A. Liniger and C. Appenzeller 2004. The role in increasing temperature variability in European summer heatwaves. Nature 427:332-336.

Schweingruber, F.H. 1993. Trees and wood in dendrochronology morphological, anatomical, and tree-ring analytical characteristics of trees frequently used in dendrochronology. *Springer Verlag, Berlin, Heidelberg, New York.* VI, 402 pp.

Sheriff, D.W. and D. Whitehead 1984. Photosynthesis and wood structure in *Pinus radiata* D. Don during dehydration and immediately after rewatering. *Plant Cell and Environment.* 7:53-62.

Sperry, J.S. 2003. Evolution of water transport and xylem structure. *International Journal of Plant Sciences.* 164:S115-S127.

Steppe, K. and R. Lemeur 2007. Effects of ring-porous and diffuse-porous stem wood anatomy on the hydraulic parameters used in a water flow and storage model. *Tree Physiology.* 27:43-52.

Sterck, F.J., R. Zweifel, U. Sass-Klaassen and Q. Chowdhury 2008. Persisting soil drought reduces leaf specific conductivity in Scots pines (*Pinus sylvestris*) and pubescent oak (*Quercus pubescens*). *Tree Physiology.* 28:528-536.

Tessier, L., P. Nola and F. Serre Bachet 1994. Deciduous *Quercus* in the Mediterranean region - Tree-ring/climate relationships. *New Phytologist.* 126:355-367.

Tyree, M.T. and M.H. Zimmermann 2002. Xylem structure and the ascent of sap. *Springer Verlag, Berlin, Heidelberg, New York.* 283 p.

van der Schrier, G., D. Efthymiadis, K.R. Briffa and P.D. Jones 2007. European Alpine moisture variability for 1800-2003. *International Journal of Climatology.* 27:415-427.

Vertui, F. and F. Tagliaferro 1998. Scots pine (*Pinus sylvestris* L.) die-back by unknown causes in the Aosta Valley, Italy. *Chemosphere.* 36:1061-1065.

Vysotskaya, L.G. and E.A. Vaganov 1989. Components of the variability of radial cell-size in tree rings of conifers. *Iawa Bulletin.* 10:417-428.

Waring, R.H. 1987. Characteristics of trees predisposed to die. *Bioscience.* 37:569-574.

Weber, P., H. Bugmann and A. Rigling 2007. Radial growth responses to drought of *Pinus sylvestris* and *Quercus pubescens* in an inner-Alpine dry valley. *Journal of Vegetation Science.* 18:777-792.

Weber, P., H. Bugmann, P. Fonti and A. Rigling 2008. Using a retrospective dynamic competition index to reconstruct forest succession. *Forest Ecology and Management* 254:96-106.

Yasue, K., R. Funada, O. Kobayashi and J. Ohtani 2000. The effects of tracheid dimensions on variations in maximum density of *Picea glehnii* and relationships to climatic factors. *Trees-Structure and Function.* 14:223-229.

Zasada, J.C. and R. Zahner 1969. Vessel element development in earlywood of red oak (*Quercus rubra*). *Canadian Journal of Botany.* 47:1965-1971.

Zweifel, R., K. Steppe and F.J. Sterck 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. *Journal of Experimental Botany.* 58:2113-2131.

Zweifel, R., L. Zimmermann, F. Zeugin and D.M. Newbery 2006. Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. *Journal of Experimental Botany.* 57:1445-1459.

Chapter II: Drought alters timing, quantity and quality of wood formation in Scots pine

Britta Eilmann [1], Roman Zweifel [1], Nina Buchmann [2], Elisabeth Graf Pannatier [1], Andreas Rigling [1]

[1]Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
[2]Institute of Plant Sciences, ETH Zürich, Universitätsstrasse 2, 8092 Zürich, Switzerland

Summary

- Drought has been frequently discussed as a trigger for forest decline. Today, large-scale Scots pine decline is observed in many dry inner-Alpine valleys with drought discussed as a main factor. This study aimed to analyse the combined impact of drought and defoliation on tree growth.
- The intra-annual wood formation of mature Scots pine growing under contrasting water supply was analysed within an irrigation experiment installed in a dry inner-Alpine valley. Pines of three different foliage-density classes were separately studied and cellular parameters as well as increment analysed.
- In non-irrigated trees the period of wood formation was noticeably shorter, increment was significantly reduced and the water-conducting cells had significantly thinner cell walls but larger lumen diameters compared to irrigated trees. Drought and low foliage density had a negative cumulative effect on tree growth. However, all trees even those of low foliage density profited from irrigation.
- Pine trees built a more effective water-conducting system (larger cells), but which is, concurrently, more vulnerable to cavitation (larger cells in combination with thinner cell walls). The significant shortening of the growth period under drought indicates a decoupling of the actually realized growing season from the potential (=phenological) growing season under drought.

Introduction

The hot and dry summer of 2003 in Europe strongly impacted terrestrial ecosystems, in 2003 but also the year after (Ciais et al., 2005; Granier et al., 2007; Reichstein et al., 2007). Since frequency and severity of drought are expected to further increase (Schär et al., 2004; IPCC, 2007), such impacts of drought on plant growth and plant survival needs to be better understood. In forest ecosystems, drought was shown to decrease primary production (Ciais et al., 2005; Granier et al., 2007; Reichstein et al., 2007), reduce stem growth (Schweingruber, 1993; Fritts, 2001; Rigling et al., 2002, Zweifel et al 2006), and lower the amount of stored carbohydrates (Bréda et al., 2006). Beside the amount also the timing in which carbon is fixed or wood is built might be affected. But up to what extent remains unclear.

According to the theory of storage depletion (Bréda et al., 2006; McDowell et al., 2008), the reduced primary production under drought may lead to a negative carbon balance due to the continuous respiratory need of plants for carbohydrates. Although this demand might be buffered by the usage of stored carbohydrates, after exhaustion of this storage, trees might die from the imbalance between carbon need and supply. Consequently, drought has been frequently discussed as a trigger of vegetation shifts and forest decline (Breshears et al., 2005; Dobbertin et al., 2005; Bréda et al., 2006; McDowell et al., 2008).

Drought is also the main limiting growth factor in inner-Alpine valleys, where precipitation is generally low due to the rain shadow of the surrounding high mountain ranges. During the last decades, growth limitation by drought has become more severe, since climatic conditions have changed towards more severe drought during the growth period (Rebetez & Dobbertin, 2004; Weber et al., 2007). Forests at low elevation are naturally dominated by Scots pine (Pinus sylvestris L., in the following pine), but recently high rates of pine mortality have been observed in many inner-Alpine valleys (e.g., the Aosta valley (Vertui & Tagliaferro, 1998), the Inn valley (Oberhuber, 2001), or the Swiss Rhone valley (Dobbertin et al., 2005). Mortality rates were highest on dry sites after drought years (Dobbertin et al., 2005). Locally almost half of the pine population has died since 1995 (Rebetez & Dobbertin, 2004) demonstrating the importance of drought in pine decline. But even though a clear relationship between drought and pine mortality has been shown at a stand level, the processes leading to drought-induced pine decline remain unclear.

According to the decline spiral of Manion (2003), forest decline is a result of the interaction of predisposing, inciting and contributing factors. A long-term increase of drought can be described as a predisposing factor and an extreme drought event can be regarded as an inciting factor. Along the decline spiral, tree vigour and foliage density decrease which typically ends in tree death. If trees can recover at every point along the spiral if environmental conditions improve is still debated.

The maintenance of an efficient water-transport system is of particular importance for tree survival at dry sites (Bréda et al., 2006), to avoid drought-induced down-regulation of the photosynthetic activity (Rennenberg et al., 2006; Zweifel et al., 2007) and to keep xylem water potential above cavitation thresholds (Bréda et al., 2006). But how water conducting cells (conduits) in the xylem adjust to severe drought has so far only rudimentarily been demonstrated (Sterck et al., 2008)

An irrigation experiment in a mature pine-forest (average tree age: 90 years) at a xeric site in the Swiss Rhone valley (Valais) was used to study the effect of contrasting water availability on the growth of pine trees. Pine trees growing under naturally dry conditions were compared with irrigated pines. To study different points along the tree decline spiral, the growth respond to drought and irrigation was examined separately for trees of different foliage densities. Analyses focused on inter- and intra-annual radial increments as well as on intra-annual variation in wood structure (lumen diameter and cell wall thickness).

We hypothesized that (i) trees adjust their water-conducing system to resource limitation by drought, (ii) not only the amount and structure of wood but also the timing of its formation is altered by drought, (iii) trees of low foliage density are more affected by drought and are less able to profit from improved site conditions by irrigation.

Material & Methods

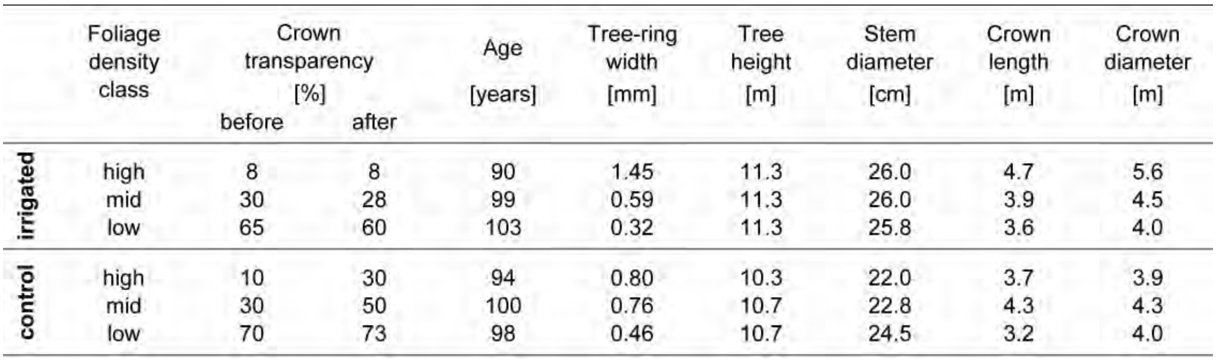
Experimental site and sample trees

The experimental site is located at the valley floor, in the driest central part of Valais close to the village Susten, (46°18’ N, 7°36’ E, elevation 615 m). The climate is continental-dry with a mean annual temperature of 9.2 °C and a mean annual precipitation sum of 599 mm (norm period 1961-1990, MeteoSwiss, weather station Sion, 20 km distance to experimental site).

The forest at the experimental site can be described as Erico-Pinetum sylvestris. The shallow, skeletal soil (soil depth 60 cm) is a pararendzina characterized by low water retention. The experimental site (about 1 hectare) was split up into eight plots of 1000 m2 each, separated by a 5 m buffering strip. Trees on four randomly selected plots were irrigated since June 2003 (referred to in the following as irrigated trees); trees in the other four plots were growing under naturally dry conditions (referred to in the following as control trees). Irrigation water was taken from an adjacent water channel, which is fed by the river Rhone. During the growth period (April to October), irrigation was applied using sprinklers of about 1m height. To minimise the water loss and the cooling by evapotranspiration, irrigation took place at night only. Irrigating earlier than April was not possible, due to the risk of equipment damage by late frost. The amount of irrigation corresponded to a supplementary rainfall of about 700 mm per year. The two years studied were drier than the long-term mean of precipitation (2004: 477 mm; 2005: 500 mm), leading to a pronounced difference between the control and the irrigated plots.

We analysed the growth of 6 irrigated and 6 control pine trees annually for the years 1993 to 2005 and intra-annually for the years 2004 and 2005. The analysed trees were ranked into three different foliage density classes (high, mid and low, see Table 1), allowing paired tests between the treatments and the analysis of the impact of foliage density on tree growth. The classification was based on visual rating of crown transparency in 5% steps ranging from 0%, i.e. fully foliated tree, to 100%, i.e., dead tree (foliage density classes high, mid, low correspond to < 25%, 25 to 50% and > 50% crown transparency, respectively, for details see Dobbertin et al., 2004). Rating and classification took place in March 2003 before the new shoots emerged. After 3 years of irrigation, the assessment was repeated in March 2006, to assess the developing of foliage density due to irrigation.

Table 1: Principal tree characteristics within the three productivity classes (mean of two trees). Tree-ring width was calculated for the 10 years before irrigation started (1993-2002). Crown transparency value shows the estimates of March 2003, before the start of irrigation (= before) and March 2006 (= after), after two and a half years of irrigation. Tree height, steam diameter, crown length and crown diameter were measured during sampling (spring 2006).



	Foliage density class	Crown transparency [%] before	Crown transparency [%] after	Age [years]	Tree-ring width [mm]	Tree height [m]	Stem diameter [cm]	Crown length [m]	Crown diameter [m]
irrigated	high	8	8	90	1.45	11.3	26.0	4.7	5.6
	mid	30	28	99	0.59	11.3	26.0	3.9	4.5
	low	65	60	103	0.32	11.3	25.8	3.6	4.0
control	high	10	30	94	0.80	10.3	22.0	3.7	3.9
	mid	30	50	100	0.76	10.7	22.8	4.3	4.3
	low	70	73	98	0.46	10.7	24.5	3.2	4.0

Sampling and sample preparation

The pinning method was applied on a weekly to biweekly basis to date the timing of wood formation during the vegetation period 2004 (April, 14th to October, 27th) and 2005 (April, 13th to October, 13th). Pinning is the marking of the cambium by micro-injury using a small needle (diameter 0.6 mm). The resulting wound reaction is used for the exact dating of the wood formation (Wolter, 1968; Yoshimura et al., 1981; Nobuchi et al., 1993), as the wound tissue separates cells formed before and after the pinning. At every pinning date, each sample-tree was wounded three times at breast height (for details see Schmitt et al., 2004).

In spring 2006, after two years of pinning, the trees were cut. The stem section including the pinning wounds and an additional stem disc from above the stem section were cut and stored at minus 20 °C to avoid fungal infestation.

Tissue including the pinning wound and tissue unaffected by the wound reaction was extracted from the stem section using a keyhole saw (19 mm) and stored at minus 20 °C in Strasburger solution (one third ethanol (95%), one third Glycerol (99%) and one third distilled water). Using a sliding microtome (Reichert, Germany) thin sections (thickness of 10 µm) were made i) of the tissue containing the pinning wound, for intra-annual increment measurements, and ii) of unaffected tissue, for the analysis of the wood structure. For a better contrast between cell wall and cell lumen, thin sections were stained with safranin (1% solution) and astrablue (2% solution). Subsequently the sections were dehydrated in an alcohol concentration gradient (70%, 95% and absolute ethanol), then in xylol (> 98%) and permanently fixed with Canada balsam. Pictures of the thin sections were taken for image analysis (100 times magnification, microscope: Olympus BX41, Japan; camera: ColorView III, Soft Imaging system, Germany).

To analyse tree-ring width, the stem disks were sanded (35 µm particle diameter). Tree-ring width was measured on two radii using a combination of a Lintab digital positioning table and the software TSAP (both Rinntech, Germany). These two measurements per tree were averaged to a mean per tree.

Measurement on the intra-annual scale

The intra-annual increment was measured (image analysis software IMAGE PRO PLUS, Media Cybernetics USA) as the distance between the previous tree-ring border and the last cells with a secondary wall below the wound (Fig. 1, white arrow), determined by polarised light on both sides of the needle entry and summed up to an average value. The relative increment, giving the percentage of the entire growth ring built until the pinning date, was calculated by relating the measured intra-annual increment to the total ring width (increment relative = increment intra-annual / tree-ring width). Therefore, tree-ring width was measured outside the tissue of wound reaction. Measurements of the cellular parameters (lumen diameter and cell wall thickness) were made on the samples of unaffected tissue to avoid potential bias by the wound reaction. Measurements were made along 10 cell rows per tree ring using the software WinCell (Regents Instruments Inc, Canada).

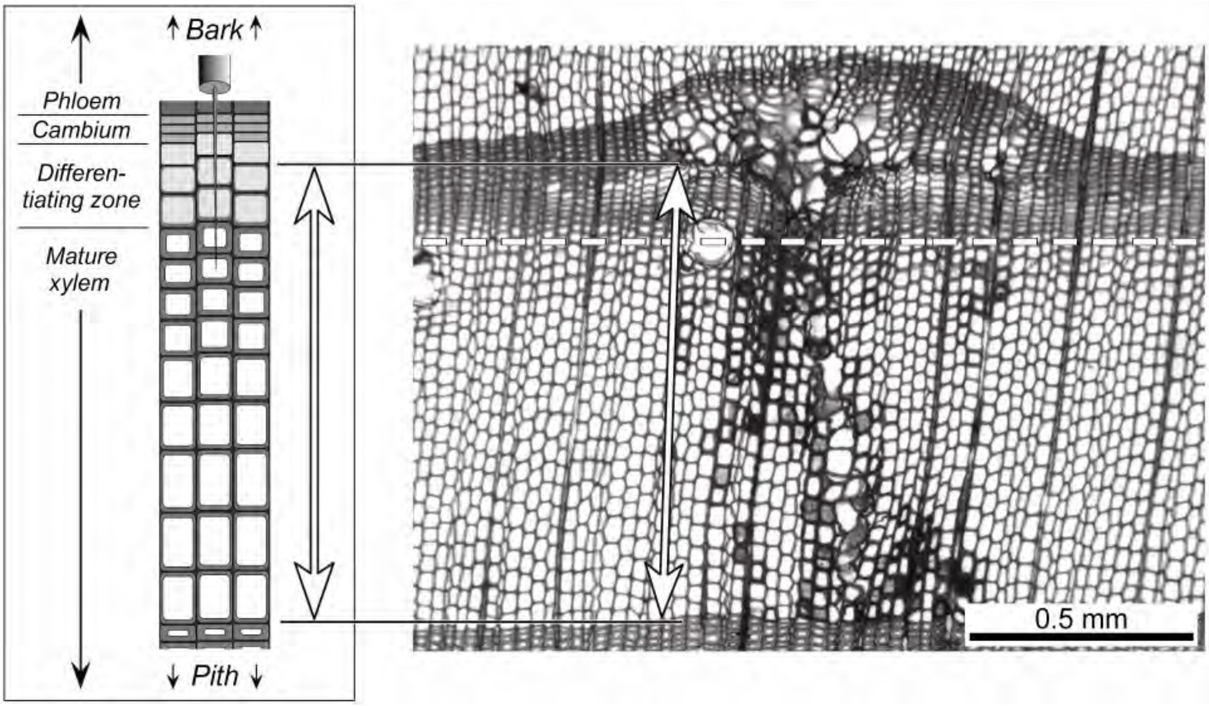


Fig. 1: Measurement of intra-annual increment. The scheme (left) shows the tissue at the time of the pinning. The thin section (right) shows the wound reaction found in the tissue one year after the pinning. The white arrow marks the distance between the previous tree-ring border and the last cell with secondary wall, which was measured as the absolute intra-annual increment at the time of the pinning. The wound tissue in the thin section corresponds to cambial and enlarging cells in the scheme (first 4 to 5 cells). The cells above the wound tissue in the thin section were built after the pinning. The horizontal broken line in the thin section shows the earlywood/latewood border.

Data processing and data analysis

The assignment of measurements to the pinning dates was done in two steps: First, the raw data (Fig. 2a) were standardized according to Vaganov (1990) to normalize the cell rows to a common length (100 cells, standardized data see Fig. 2b). This procedure was necessary (i) to avoid a distortion of the mean value at the end of the dataset due to varying cell numbers per measurements (see circles in Fig. 2a and b), and (ii) to prevent a rounding error in the following step. Second, we averaged all data belonging to the same pinning time frame according to the relative increment (giving the increment in percent), i.e., if 10% of the tree ring was built at the date of the first pinning, the lumen diameter of the first 10 cells of the standardized dataset were averaged, giving the mean lumen diameter for the first pinning date (chronological data = grey dots in Fig. 2c). The treatment-related differences between the parameters were tested using the paired Wilcoxon sign rank test.

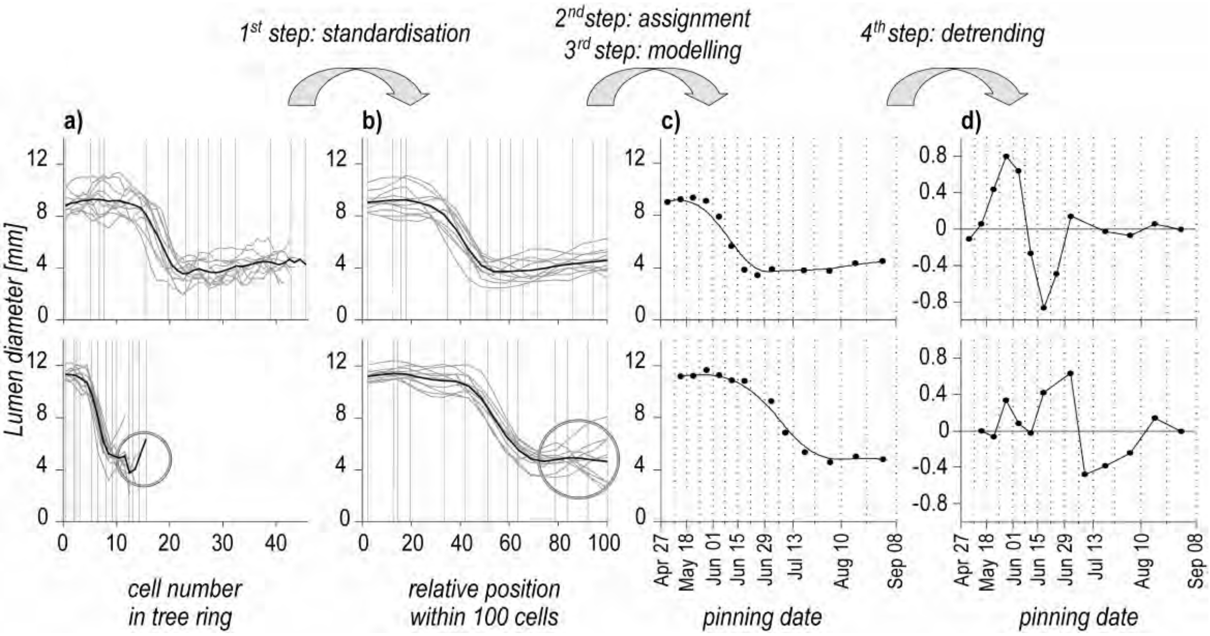


Fig. 2: Procedure for assigning and detrending data, exemplarily shown for the lumen diameter of a large (upper row) and a narrow tree ring (lower row). 1st step: Raw data (a) were standardized to a common length of 100 cells (b, see Vaganov (1990) for details). 2nd step: The relative position within 100 cells to which the standardized data are related to were assigned to the pinning dates by transferring the results of the relative increment [%] to the relative position of the standardized data set (increment in percent = relative position within 100 cells). The relative increments at the time of the pinning are shown in a) and b) as grey grid lines. The grid lines in a) were referred to the mean curve (black). All standardized data between two lines were averaged and referred to the pinning date of the posterior grid line (chronological data = grey dots in c). 3rd step: A non-linear, non-parametric regression model was fitted to the chronological data (modelled data = solid line in c). 4th step: The inverse sigmoid trend of the chronological data was eliminated by calculating the residuals (d) between the actual data and the model data, which were used as detrended data set for climate growth analysis. The broken grid line in c and d show the pinning dates.

Qualitative analysis of wood formation

The timing of wood formation was analysed by identifying different phases of tree-ring formation: The beginning of wood formation was defined as point, where the first tracheids with a secondary cell wall occurred below the wound. Earlywood/latewood transition was reached if the last cells with secondary wall were situated above a tangential line separating earlywood from latewood (Fig. 1 line c). The tree ring was complete, if a continuous band of flattened latewood cells was visible.

Intra-annual climate-growth analysis

For the intra-annual climate-growth analysis, datasets were detrended to remove the seasonal growth trend (Fig. 2c to d). The trend in the fortnightly increment was eliminated by trimming the first 5% and the last 10% of the data, where the increment curve usually flats out. In case of the lumen diameter, a nonlinear regression model (3rd step Fig. 2) was adapted to the chronological data (modelled data = solid line in Fig. 2c). The residuals calculated between the chronological and the modelled data (4th Step, Fig. 2) were used as detrended data (Fig. 2d). For detrending the cell wall thickness data, a similar procedure was used as described for lumen diameter but a linear regression model was applied.

The date of earlywood/latewood transition found in the qualitative analysis of wood formation was also used to separate earlywood from latewood in the detrended data-set

Daily temperature data were monitored at the weather station Sion. Soil moisture was measured every hour at 10, 40 and 60 cm depths on the experimental site using a Time Domain Reflectometry sensor (1502B cable tester, Tektronix, USA)) and averaged to a daily mean (see Fig. 3). For climate-growth analysis we focused on soil moisture at 10 cm depth as variation was maximal at this depth (Fig. 3). Daily temperature and soil moisture values were averaged using a moving window of 7 days and correlated with detrended parameters (lumen diameter and cell wall thickness separated in earlywood and latewood, and the fortnightly increment). The degrees of freedom were corrected depending on the autocorrelation of growth (rgrowth) and climate (rclimate) parameters (df = n * ((1-rgrowth * rclimate)/(1-rgrowth * rclimate)). The significance threshold based on the mean degrees of freedom was calculated for each growth parameter.

Since the climate impact on cell growth was studied for two years only, we focus on general trends. Therefore, mainly the direction of significant responses (i.e. positive or negative) was interpreted but not the timing of the individual correlation peaks. Correlations with growth parameters of irrigated trees were not performed for soil moisture but for non-manipulated temperature only as i) soil moisture was drastically manipulated by our irrigation treatment leading to a more balanced soil moisture regime (Fig. 3) and ii) trees need several years to adjust to changing water supply (Eilmann et al., 2009).

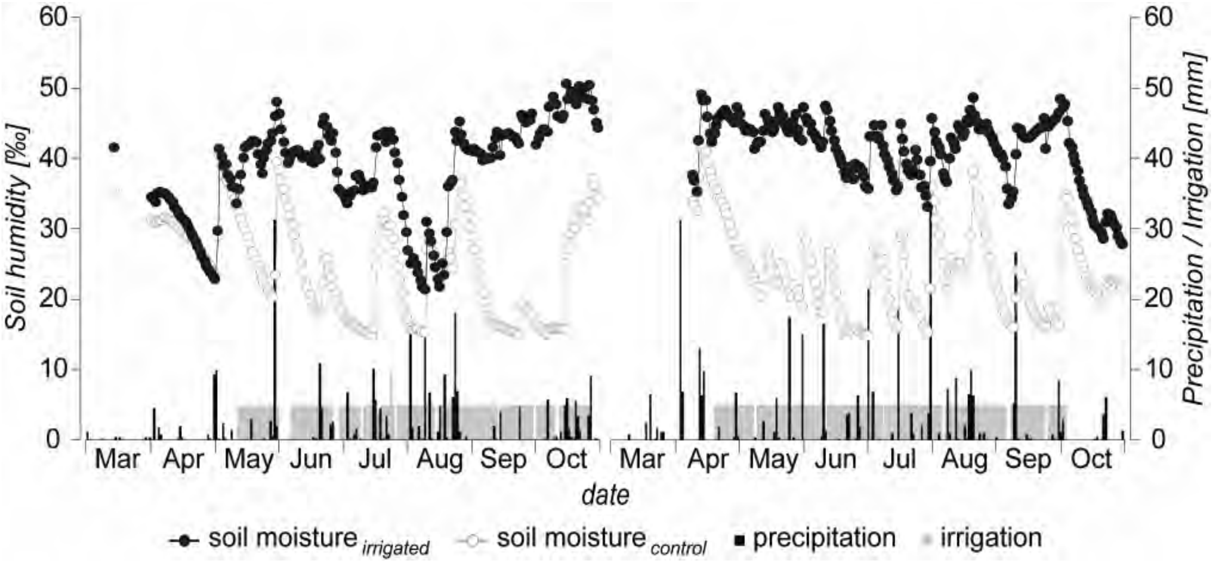


Fig. 3: Soil moisture on the irrigated and the control plot measured in 10 cm depth, together with the amount of precipitation and irrigation for the years 2004 (left) and 2005 (right).

Results

Effects of water availability on pines

During the drought year 2003, irrigation started in June, when most of the growth of the earlywood was already completed. Therefore a drought-induced decrease of annual tree-ring widths was found in control trees but also in irrigated trees (Fig. 4) in 2003. In 2004 and 2005, all irrigated trees recovered from this drought year and increased tree-ring widths, independent of foliage density. In contrast, radial increments of control trees of mid and low foliage densities remained low in 2004 and 2005, even though both years were less dry than 2003 (annual precipitation 2003: 426 mm; 2004: 477 mm; 2005: 500 mm).

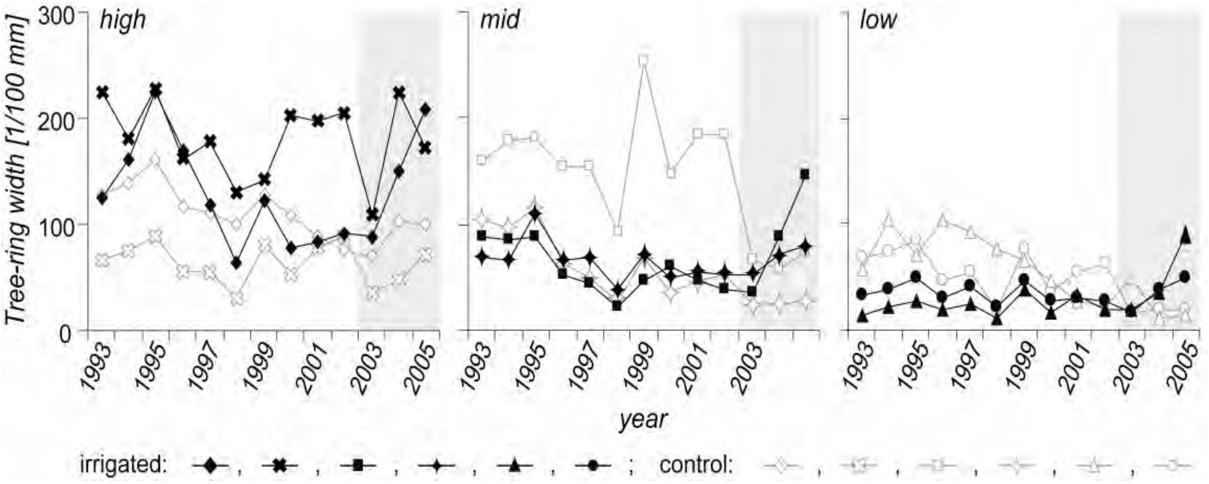


Fig. 4: Development of tree-ring width of the years 1993 to 2002 in irrigated (black) and control trees (grey) for the three crown density classes (high, mid, low). The diagrams show the two trees per crown density class. The irrigation period (2003 to 2005) is marked by the shaded area.

At the intra-annual scale, improved water supply by irrigation led to significant changes in all growth parameters: absolute radial increment (Fig. 5a) and cell wall thickness (Fig. 6b) increased ($p < 0.001$), while lumen diameter decreased ($p < 0.001$, Fig. 6a). Irrigated trees showed a constant increment rate over the years 2004 and 2005, as indicated by the constant slope and the late flattening of the relative intra-annual increment curves all irrigated trees irrespective of foliage density class (Fig. 5b). In contrast, the relative increment-rates of the control trees were less constant and varied among the different foliage density classes. But towards the end of the growth period, the increment curves of all control trees flattened about one month earlier than those of the irrigated trees.

The development of crown transparency from March 2003 to March 2006 differed between the treatments. In irrigated trees, crown transparency stayed more or less constant over the three years (Table 1). However, crown transparency in control trees clearly increased for trees with high and mid crown transparency during this period.

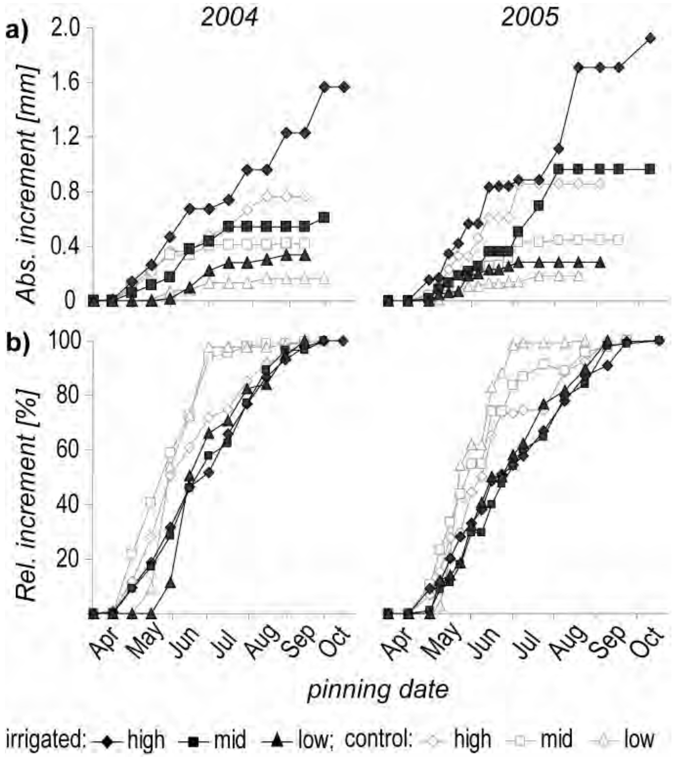


Fig. 5 a) Absolute and ***b)*** relative radial increment of irrigated and control trees at the pinning dates, separated for the three crown density class high, mid and low (= mean curves of two trees).

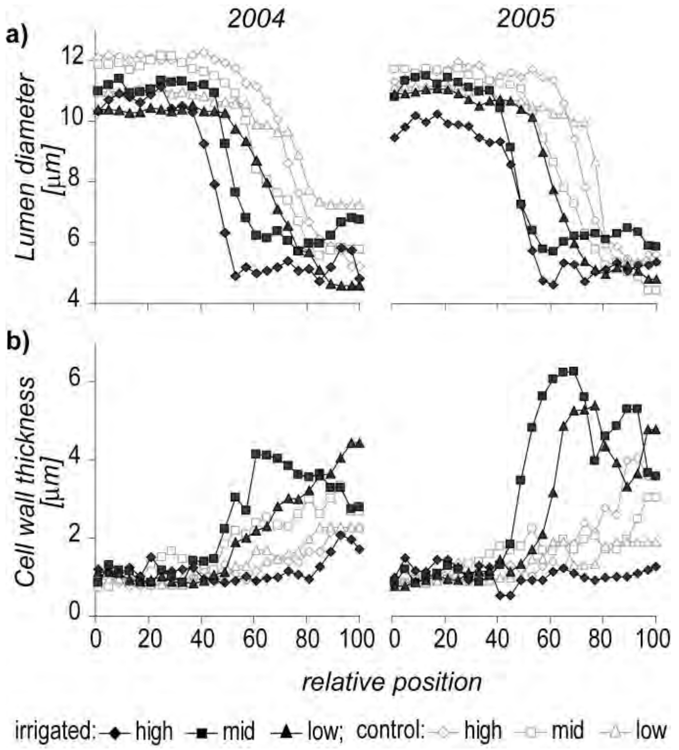


Fig 6a) Lumen diameter and ***b)*** cell wall thickness of irrigated and control trees standardized to a common length of 100 cells (= relative position), separately shown for the three crown density class high, mid and low (= mean curves of two trees).

Timing of wood formation

Water availability influenced the timing of wood formation (Fig. 7). Irrigation led to a prolongation of the growth period (p = 0.058) as the cessation of cell production occurred up to five weeks later in irrigated trees compared to control trees. This was related to a longer duration of both earlywood and latewood formations (p = 0.056). Thus, the earlywood/latewood transition occurred about two to four weeks later in irrigated trees than in control trees. Regarding the beginning of wood formation, no irrigation effect was observed.

The beginning of wood formation began about 4 weeks later in trees with low foliage density compared to trees with high foliage density. Thus, the shortest growth period was observed for trees with low foliage density in the non-irrigated control plots (15 weeks in 2005), while the longest growth period occurred in trees with high foliage density in the irrigated plots (26 weeks in 2005). One of the control trees with low foliage density showed an increment that was so low during both years studied that a clear detection of intra-annual growth was impossible.

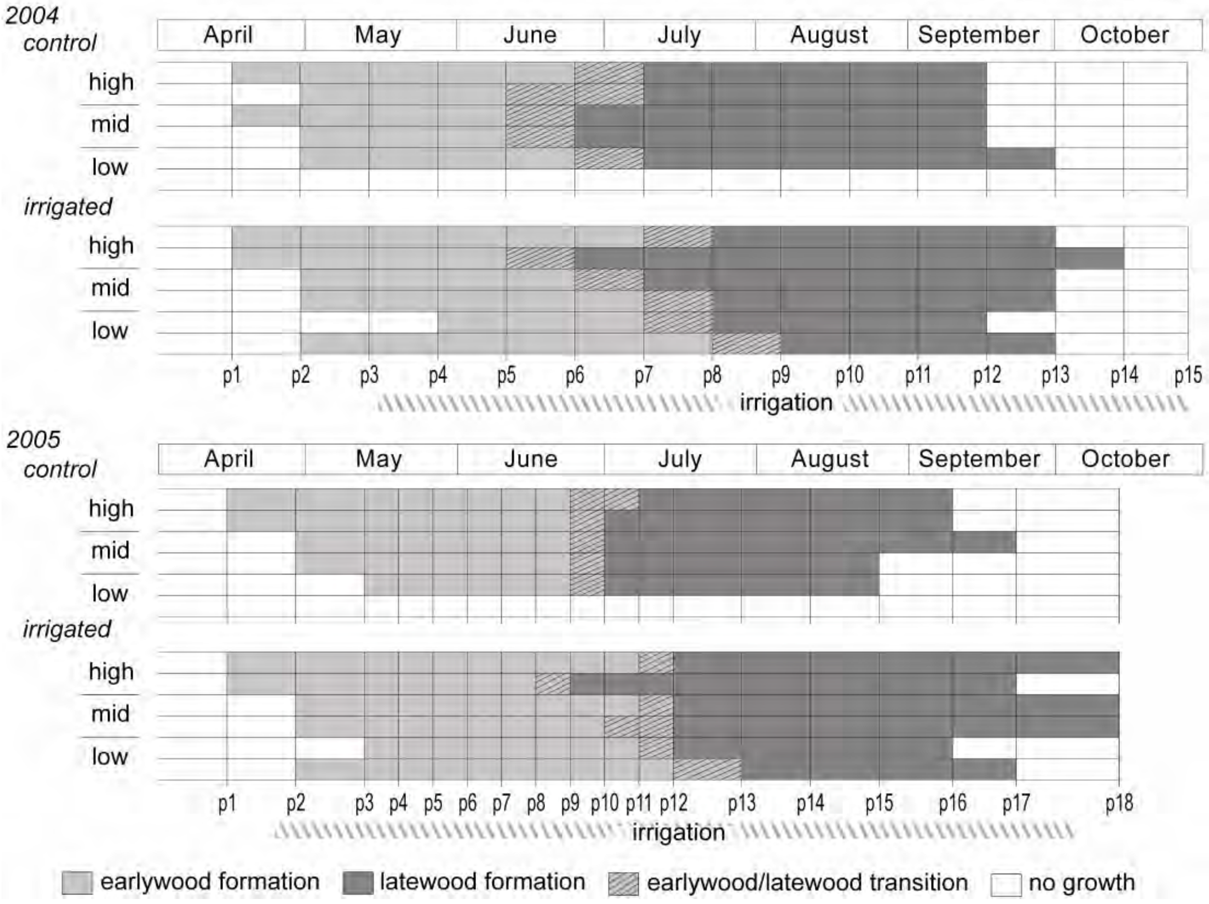


Fig. 7: Timing of wood formation for irrigated and control trees with different crown density classes (high, mid and low). p1 to p18 mark the 15 (2004) or 18 (2005) pinning dates per year, respectively. The hatched bar marks the time period where irrigation takes place.

Intra-annual climate-growth correlations

High temperature und low soil moisture led to decreasing increment and cell wall thickness but increasing lumen diameter in control trees (Fig.8).

While the fortnightly radial increments of control trees decreased under high temperature and low soil moisture, the limiting effect of high temperature on radial increment disappeared in irrigated trees. High temperature and low soil moisture led to an increase in the lumen diameter in earlywood and latewood of control trees. For irrigated trees, the response of lumen diameter to temperature was more pronounced than for control trees. Cell wall thickness in earlywood of irrigated and control trees showed no response to climate conditions at all, while in latewood, cell was thickness responded inversely to temperature as it decreased in control trees but increased in irrigated trees under high temperatures.

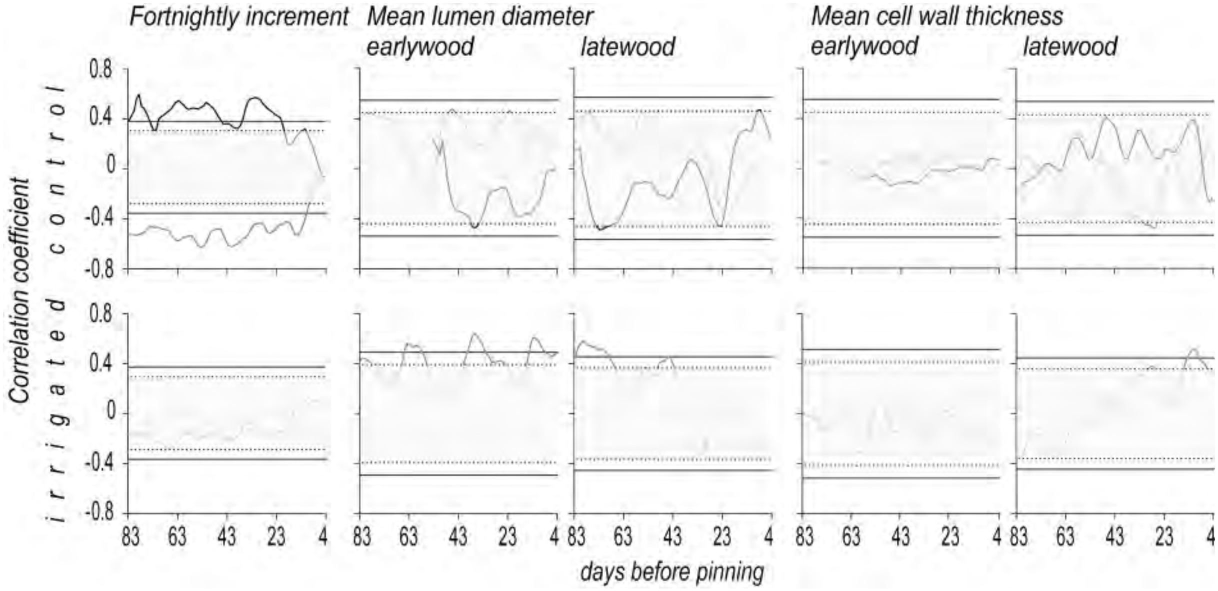


Fig. 8: Moving window correlations between the intra-annual growth parameters (fortnightly increment, lumen diameter and cell wall thickness, the later two separately for earlywood and latewood cells) and temperature (grey curve) or soil moisture (black curve; calculated for growth parameters of the control plot only) calculated for the years 2004 and 2005 for trees of all crown density classes. Data on the x-axis give the central number around which the 7 day mean of climatic data for the moving window correlation was calculated. The horizontal lines mark the significance thresholds (broken line for $p < 0.01$, solid line for $p < 0.001$) for an autocorrelation corrected mean degree of freedom. Non-significant correlations are located in the shaded area. The correlation between earlywood growth parameters in control trees and soil moisture was calculated for a period shorter than 83 days due to missing soil moisture data during the winter.

Discussion

Growth responses to drought

Irrigation significantly altered all of the examined growth parameters, emphasising the importance of drought as a limiting growth factor under natural conditions in Valais.

Many studies reported larger lumen diameters with increasing water availability (Jenkings, 1974; Nicholls & Waring, 1977; Sheriff & Whitehead, 1984; Sterck et al., 2008). We found an opposite pattern, as lumen diameters were significantly smaller in irrigated trees compared to control trees (Fig. 6a). However the lumen diameter observed in our study were at the lower end of the overall range (our study: mean lumen diametercontrol = 11.5µm; mean lumen diameterirrigated = 10.8 µm; other studies: range of earlywood lumen diameter = 14.4 to 40.3 µm) indicating a strong restriction of cell enlargement under the prevailing site conditions in Valais.

The observation of smaller lumen diameter under better water supply is in correspondence with the findings of Maherali & DeLucia (2000) on ponderosa pine at semi-arid (Great Basin) vs. moderate mountain site (Sierra Nevada) in the USA and also with the results of Eilmann et al. (2009) on Scots pine along an open water channel vs. naturally dry conditions in Valais. Increasing lumen diameters under dry conditions are thought to compensate for the disadvantage of decreasing conductive area (decreasing ring increment, Fig. 4) for water transport, as an increase in lumen diameter increases water conductivity by the power of four (Hagen Poiseuille law; Tyree and Zimmermann 2002). Hence, trees are able to reach a given water conductivity with less carbon invested (Sperry 2003). However, concurrently, vulnerability of conduits to cavitation increases with increasing lumen diameter under dry conditions. At the same time control trees built thinner cell walls than irrigated trees (Fig. 6b), lowering wood density (Bodig & Jayne, 1993). This leads to a reduction in bending strength of the conduits with again negative consequences on the resistance against drought-induced cavitation (Hacke et al., 2001; Pittermann et al., 2006). Hence, applied to our results this means that pine trees built a “cheaper” (in terms of carbon investment) and more effective but at the same time more vulnerable water conducting system under naturally dry conditions than under good water supply.

Radial increment was significant lower in control pine trees compared to the irrigated pines (Fig. 5a). This might be explained by the direct control of cambial activity by water availability, as suggested by Zweifel et al. (2006) and Steppe & Lemeur (2007). In addition lower primary production under drought (Larcher, 2003; Ciais et al., 2005; Reichstein et al., 2007) might also lead to low carbon availability for cell division.

Impact of drought on the timing of wood formation

The phenological growing season (PGS), determining the beginning and end of the growing season based of phenophases, prolonged in the last decades mainly due to an earlier occurrence of spring events (Menzel & Fabian, 1999; Defila & Clot, 2001; Menzel et al., 2006). However, net carbon uptake and therefore annual increment of trees do not necessarily increase due to PGS lengthening (Dunn et al., 2007). On the contrary, the ecophysiological growing season, the period of carbon uptake, tended to shorten in the last 20 years, due to an earlier stop in autumn (Piao et al., 2008). Concurrently, carbon uptake in summer was observed to decrease, which was probably caused by the hotter an drier summers (Angert et al., 2005). This decoupling of the actually realized growing season, where carbon fixation or wood formation takes place, from PGS due to drought was supported by our results as control trees showed an earlier stop of wood formation compared to irrigated trees (Fig. 7). Both, the period of earlywood and latewood formation was about two to five weeks earlier in the dry control plots than in irrigated trees (Fig. 7). This is in agreement with results for premature earlywood/latewood transition of Zahner et al. (1964), Whitmore & Zahner (1966) and Dünisch & Bauch (1994) and with the results for the early cessation of cell formation under drought of Dünisch & Bauch (1994). The decoupling of PGS and actually realized growth period might become even more pronounced in the future when frequency and severity of drought events are expected to increase (Schär et al., 2004; IPCC, 2007).

Effect of foliage density on tree growth

Drought together with low foliage density had a negative cumulative effect on tree performance since the shortest growth period was found for control trees with low foliage density (Fig. 7). In addition, the drought year 2003 had a long-lasting effect on control trees of mid and low foliage densities showing low growth rates until 2005 (Fig. 4), while control trees with high foliage density recovered immediately. This long-lasting effect of the dry summer 2003 might be the result of proceeding carbon storage depletion as discussed by Breda et al. (2006) and McDowell et al. (2008). This might also explain why trees with lower growth rates are at higher mortality risk than trees with high increment growth (Pedersen, 1998; Dobbertin & Brang, 2001; Wyckoff & Clark, 2002; Bigler & Bugmann, 2004).

However, all pine trees in this study benefited from the irrigation and exhibited increasing growth rates (Fig. 4) and no further loss of foliage (Table 1). We therefore conclude that the process of drought-induced tree decline is reversible for all trees once additional water lowers the drought stress. Consequently, when modelling drought-induced tree decline not only the stress by drought years must be taken into account (Bigler et al. 2006), but also the possibility of trees to recover from drought stress if conditions improve should be considered.

Climate impact on intra-annual growth

The results of the intra-annual analysis of climate-growth relationships correspond to the comparison of intra-annual tree growth under contrasting water supply (Fig. 5 & 6). Dry soil conditions were shown to lead to reduced intra-annual increment and cell wall thickness (latewood) but to increased lumen diameter (Fig. 8) for the control trees. Here, the effect of high temperature was always opposite compared to the effect of soil moisture, either due to the amplification of drought under high temperature by increasing evapotranspiration or due to direct reduction of cambial activity under high temperatures (Jenkings, 1974; Antonova & Stasova, 1993).

Irrigation reduced the climate dependence of intra-annual wood formation, as no growth response to temperature was found in the fortnightly increments of irrigated trees. This observation corresponds to the tree-ring studies by Rigling et al. (2003) and Eilmann et al. (2009) on passively irrigated trees nearby our study plots, where irrigation pronouncedly reduced the growth dependence on climate. In contrast, the response of lumen diameters to temperature increased due to irrigation. Thus, removing growth limitation caused by drought, causes temperature to gain in importance in regard to cell enlargement.

Conclusion

According to our first hypothesis, pine trees adjusted their water conducting system to drought by building a more effective water-conducting system with larger conduits. As a result, trees have to invest less carbon to reach a given conductivity. However, the increasing lumen diameter in combination with the concurrent thinning of the cell walls under drought makes the water-conducting system more vulnerable against cavitation. Hence, pine trees built a more effective and economic water conducting system under natural dry conditions but at the expense of safety.

Beside the negative effect on the amount and structure of wood built, drought also shortened the period of wood formation noticeably as assumed in our second hypothesis. This indicates that the actual realized growing season is decoupled from PGS as the limitation of water supply led to a premature cessation of wood formation in autumn.

Drought indeed had a stronger negative effect on trees of low foliage density as hypothesised. This might be due to carbon storage depletion in these trees, leading to a low resistance against biotic and abiotic stressors, including drought events. However, in contrast to our third hypothesis,all trees, even those with low foliage density, profited from improved water supply. Thus, the process of drought-induced forest decline was reversible all along the decline spiral when environmental conditions improved.

Acknowledgements

We like to thank M. Burger, M. Dobbertin, R. Köchli, P. Truniger U. Wasem, the Dendro science unit form the WSL and K. Egger and his team from the forest service Leuk for support in the field or in the lab. We are grateful to the forest service of the canton Valais and the Burgergemeinde Leuk for permission of the experiment, HYDRO Exploitation SA for technical support and to W. Landolt und P. Bleuler for managing the irrigation system. We also like to thank D. Frank, N. Zimmermann and P. Weber (WSL) as well as J. Seo and U. Schmitt (Johann Heinrich von Thünen Institute Hamburg, Germany) for valuable discussions concerning the methods and S. Paget for English corrections to the manuscript. This study was conducted in the frame of the CCES-project MOUNTLAND funded by the Velux foundation and the Canton Valais.

References

Angert A, Biraud S, Bonfils C, Henning CC, Buermann W, Pinzon J, Tucker CJ, Fung I. 2005. Drier summers cancel out the CO2 uptake enhancement induced by warmer springs. *Proceedings of the National Academy of Sciences of the United States of America* **102**(31): 10823-10827.

Antonova GF, Stasova VV. 1993. Effects of environmental factors on wood formation in Scots pine stems. *Trees - Structure and Function* **7**: 214-219.

Bigler C, Bräker OU, Bugmann H, Dobbertin M, Rigling A. 2006. Drought as an inciting mortality factor in Scots pine stands of the Valais, Switzerland. *Ecosystems* **9**: 330-343.

Bigler C, Bugmann H. 2004. Predicting the time of tree death using dendrochronological data. *Ecological Applications* **14**(3): 902–914.

Black BA, Abrams MD. 2003. Use of boundary-line growth patterns as a basis for dendroecological release criteria. *Ecological Applications* **13**(6): 1733-1749.

Bodig J, Jayne BA. 1993. *Mechanics of wood and wood composites*. Malabar, Florida: Krieger Publishing Company.

Bréda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* **63**(6): 625-644.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **102**(42): 15144-15148.

Castro-Diez P, Puyravaud JP, Cornelissen JHC, Villar-Salvador P. 1998. Stem anatomy and relative growth rate in seedlings of a wide range of woody plant species and types. *Oecologia* **116**(1-2): 57-66.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**(7058): 529-533.

Decoux V, Varcin E, Leban JM. 2004. Relationships between the intra-ring wood density assessed by X-ray densitometry and optical anatomical measurements in conifers. Consequences for the cell wall apparent density determination. *Annals of Forest Science* **61**(3): 251-262.

Defila C, Clot B. 2001. Phytophenological trends in Switzerland. *International Journal of Biometeorology* **45**(4): 203-207.

Dobbertin M. 2005. Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. *European Journal of Forest Research* **124**(4): 319-333.

Dobbertin M, Brang P. 2001. Crown defoliation improves tree mortality models. *Forest Ecology and Management* **141**: 271-284.

Dobbertin M, Hug C, Mizoue N. 2004. Using slides to test for changes in crown defoliation assessment methods. Part I: Visual assessment of slides. *Environmental Monitoring and Assessment* **98**(1-3): 295-306.

Dobbertin M, Mayer P, Wohlgemuth T, Feldmeyer-Christe E, Graf U, Zimmermann NE, Rigling A. 2005. The decline of *Pinus sylvestris* L. forests in the Swiss Rhone valley - a result of drought stress? *Phyton* **45**: 153-156.

Dunn AL, Barford CC, Wofsy SC, Goulden ML, Daube BC. 2007. A long-term record of carbon exchange in a boreal black spruce forest: means, responses to interannual variability, and decadal trends. *Global Change Biology* **13**(3): 577-590.

Dünisch O, Bauch J. 1994. Influence of soil substrate and drought on wood formation of spruce (*Picea abies* L. Karst.) under controlled conditions. *Holzforschung* **48**: 447-457.

Eilmann B, Zweifel R, Buchmann N, Fonti P, Rigling A. 2009. Drought-induced adaptation of the xylem in Scots pine and pubescent oak. *Tree Physiology*: doi:10.1093/treephys/tpp1035.

Fritts HC. 2001. *Tree rings and climate*. London: Academic Press.

Granier A, Reichstein M, Bréda N, Janssens IA, Falge E, Ciais P, Grünwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Kostner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala I, Wang Q. 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology* **143**(1-2): 123-145.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh K. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**: 457-461.

Heimann M, Reichstein M. 2008. Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature* **451**: 289-292.

IPCC 2007. Technical report. In: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Mille ed. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*.

Jenkings PA. 1974. Influence of temperature change on wood formation in *Pinus radiata* grown in controlled environments. *New Zealand Journal of Botany* **13**: 579-592.

Kilpelainen A, Gerendiain AZ, Luostarinen K, Peltola H, Kellomaki S. 2007. Elevated temperature and CO_2 concentration effects on xylem anatomy of Scots pine. *Tree Physiology* **27**(9): 1329-1338.

Larcher W. 2003. *Physiological plant ecology. Ecophysiology and stress physiology of functional groups*. Berlin, Heidelberg, New York: Springer Verlag.

Maherali H, DeLucia EH. 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology* **20**(13): 859-867.

Manion PD. 2003. Evolution of concepts in forest pathology. *Phytopathology* **93**(8): 1052-1055.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**(4): 719-739.

Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* **397**(6721): 659-659.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kubler K, Bissolli P, Braslavska O, Briede A, Chmielewski FM, Crepinsek Z, Curnel Y, Dahl A, Defila C, Donnelly A, Filella Y, Jatcza K, Mage F, Mestre A, Nordli O, Penuelas J, Pirinen P, Remisova V, Scheifinger H, Striz M, Susnik A, Van Vliet AJH, Wielgolaski FE, Zach S, Zust A. 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**(10): 1969-1976.

Müller E, Bosshard W. 1986. *Kronenbilder mit Nadel- und Blattverlustprozenten Sanasilva*. Birmensdorf: Eidgenössische Anstalt für das Forstliche Versuchswesen.

Nicholls JWP, Waring HD. 1977. The effect of environmental factors on wood characteristics. IV. Irrigation and partial drouthing of *Pinus radiata*. *Silvae Genetica* **26**: 107-111.

Nobuchi T, Fujisawa T, Saiki H. 1993. An application of the pinning method to the marking of the differentiation zone and to the estimation of the time course of annual ring formation in sugi (*Cryptomeria japonica*). *Mokuzai Gakkaishi (Journal of the Japan Wood Research Society)* **39**(6): 716-723.

Oberhuber W. 2001. The role of climate in the mortality of Scots pine (*Pinus sylvestris* L.) exposed to soil dryness. *Dendrochronologia* **19**(1): 45-55.

Pedersen BS. 1998. The role of stress in the mortality of midwestern oaks as indicated by growth prior to death. *Ecology* **79**(1): 79-93.

Piao SL, Ciais P, Friedlingstein P, Peylin P, Reichstein M, Luyssaert S, Margolis H, Fang JY, Barr A, Chen AP, Grelle A, Hollinger DY, Laurila T, Lindroth A, Richardson AD, Vesala T. 2008. Net carbon dioxide losses of northern ecosystems in response to autumn warming. *Nature* **451**(7174): 49-U43.

Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH. 2006. Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant Cell and Environment* **29**(8): 1618-1628.

Rebetez M, Dobbertin M. 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and applied climatology* **79**(1-2): 1-9.

Reichstein M, Ciais P, Papale D, Valentini R, Running S, Viovy N, Cramer W, Granier A, Ogée J, Allard V, Aubinet M, Bernhofer C, Buchmann N, Carrara A, Grünwald T, Heimann M, Heinesch B, Knohl A, Kutsch W, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Pilegaard K, Pumpanen J, Rambal S, Schaphoff S, Seufert G, Soussana JF, Sanz MJ, Vesala T, Zhao M. 2007. Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biology* **13**(3): 634-651.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* **8**(5): 556-571.

Rigling A, Bräker O, Schneiter G, Schweingruber F. 2002. Intra-annual tree-ring parameters indicating differences in drought stress of *Pinus sylvestris* forests within Erico-Pinion in Valais (Switzerland). *Plant Ecology* **163**: 105-121.

Rigling A, Brühlhart H, Bräker OU, Forster T, Schweingruber FH. 2003. Effects of irrigation on diameter growth and vertical resin duct production in *Pinus sylvestris* L. on dry sites in the central Alps, Switzerland. *Forest Ecology and Management* **175**: 285-296.

Schär C, Vidale PL, Luethi D, Frei C, Häberli C, Liniger MA, Appenzeller C. 2004. The role in increasing temperature variability in European summer heatwaves. *Nature* **427**: 332-336.

Schmitt U, Jalkanen R, Eckstein D. 2004. Cambium dynamics of *Pinus sylvestris* and *Betula spp.* in the northern boreal forest in Finland. *Silva Fennica* **38**(2): 167-178.

Schweingruber FH. 1993. *Trees and wood in dendrochronology morphological, anatomical, and tree-ring analytical characteristics of trees frequently used in dendrochronology*. Berlin, Heidelberg, New York: Springer Verlag.

Sheriff DW, Whitehead D. 1984. Photosynthesis and wood structure in *Pinus radiata* D. Don during dehydration and immediately after rewatering. *Plant Cell and Environment* **7**(1): 53-62.

Steppe K, Lemeur R. 2007. Effects of ring-porous and diffuse-porous stem wood anatomy on the hydraulic parameters used in a water flow and storage model. *Tree Physiology* **27**: 43-52.

Sterck FJ, Zweifel R, Sass-Klaassen U, Chowdhury Q. 2008. Persisting soil drought reduces leaf specific conductivity in Scots pines (*Pinus sylvestris*) and pubescent oak (*Quercus pubescens*). *Tree Physiology* **28**: 528-536.

Tyree MT, Zimmermann MH. 2002. *Xylem structure and the ascent of sap*. Berlin, Heidelberg, New York: Springer Verlag.

Vaganov EA 1990. The tracheidogramm method in tree-ring analysis and its application. In: E. R. CookL. A. Kairiukstis eds. *Methods of dendrochronology. Applications in the environmental sciences*. Dordrecht, Boston, London: Kluwer Academic Publishers, 63-76.

Vertui F, Tagliaferro F. 1998. Scots pine (*Pinus sylvestris* L.) die-back by unknown causes in the Aosta Valley, Italy. *Chemosphere* **36**(4): 1061-1065.

Weber P, Bugmann H, Rigling A. 2007. Radial growth responses to drought of *Pinus sylvestris* and *Quercus pubescens* in an inner-Alpine dry valley. *Journal of Vegetation Science* **18**(6): 777-792.

Whitmore FW, Zahner R. 1966. Development of xylem ring in stems of young red pine trees. *Forest Science* **12**(2): 198-210.

Wolter KE. 1968. A new method for marking xylem growth. *Forest Science* **14**(1): 102-104.

Wyckoff PH, Clark JS. 2002. The relationship between growth and mortality for seven co-occurring tree species in the southern Appalachian Mountains. *Journal of Ecology* **90**(4): 604-615.

Yoshimura K, Hayashi S, Itoh T, Shimaji K. 1981. Studies on the improvement of the pinning method for marking xylem growth. I. Minute examination of pin marks in taeda pine and other species. *Wood Research* **67**: 1-16.

Zahner R, Lotan JE, Baughman WD. 1964. Earlywood-latewood features of red pine grown under simulated drought and irrigation. *Forest Science* **10**(3): 361-370.

Zweifel R, Steppe K, Sterck FJ. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. *Journal of Experimental Botany* **58**(8): 2113-2131.

Zweifel R, Zeugin F, Zimmermann L, Newbery DM. 2006. Intra-annual radial growth and water relations of trees – implications towards a growth mechanism. *Journal of Experimental Botany* **57**(6): 1445-1459.

Chapter III: Inter- and intra-annual variation in tree-ring δ^{13}C indicates drought-induced storage depletion in Scots pine

Britta Eilmann [1], Nina Buchmann [2], Rolf Siegwolf [3], Matthias Saurer [3], Paolo Cherubini [1], Andreas Rigling [1]

[1]Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
[2]Institute of Plant Sciences, ETH Zürich, Universitätsstrasse 2, 8092 Zürich, Switzerland
[3]Laboratory of Atmospheric Chemistry, Paul Scherrer Institute, 5232 Villigen, Switzerland

Summary

- Drought-induced forest decline will gain in importance as frequency and severity of drought events are expected to increase. Already today, high rates of drought-induced Scots pine mortality can be observed in inner-Alpine valleys.
- To determine how drought affects tree growth, tree-ring δ^{13}C values and water-use-efficiency (WUE) of mature Scots pine, we installed an irrigation experiment on a xeric site in an inner-Alpine valley. We analysed trees at the inter- and intra-annual scale.
- At the inter-annual scale, irrigation decreased δ^{13}C and WUE, whereas tree-ring width increased. Correlation analysis between a drought index and the tree-ring parameters showed a strong influence of current years' growth conditions on early and latewood δ^{13}C values under naturally dry conditions, indicating rapid assimilate turnover and low amounts of stored carbohydrates. At the intra-annual scale, the seasonal maximum in d13C was reached earlier in irrigated trees suggesting an early switch to autotrophic metabolism.
- Our results indicate that a low amount of stored carbohydrates for control trees but its demand was high, especially in spring suggesting progressive depletion of stored carbohydrates. Thus, when stressors increase the need for carbohydrate for defence or regeneration a further increase in pine mortality should be expected in Valais.

Introduction

Tree mortality in response to extreme climate events can lead to rapid vegetation shifts and forest decline (McDowell *et al.*, 2008). Drought in particular has been discussed as an important trigger for forest decline and tree mortality (e.g., Allen & Breshears, 1998; Breshears *et al.*, 2005; Bréda *et al.*, 2006; Gitlin *et al.*, 2006), and is expected to gain in importance in the future. Increasing frequency and severity of drought are suggested by most climate-change scenarios for Central Europe (e.g., Schär *et al.*, 2004; IPCC, 2007), with still unknown consequences for tree performance and ecosystem dynamics.

Storage depletion was discussed as one mechanism leading to drought induced tree mortality (Bréda *et al.*, 2006; McDowell *et al.*, 2008). The theory is based on the fact that drought reduces primary production of forests (Ciais *et al.*, 2005; Granier *et al.*, 2007; Reichstein *et al.*, 2007) but also the amount of stored carbohydrates (Bréda *et al.*, 2006). Reduced primary production under drought may lead to a negative carbon balance for trees, due to the continued metabolic demand for carbohydrates, e.g., for tree respiration. Thus, more stored carbohydrates are needed to cover a tree's carbon needs, which may, in the long run, lead to tree death by storage depletion if the frequency and/or severity of drought years increase. However, it remains unclear if all trees growing on a dry site are affected equally by storage depletion or if only trees are affected, which initially were not able to proliferate their roots to deeper and more sustainable water sources.

Ecosystems such as the dry forests at low elevation in the inner-Alpine valleys are excellent case studies to analyze the role of drought on tree performance. Here, precipitation is generally low due to the rain shadow of the surrounding high mountain ranges and frequent drought years, with below average precipitation (Rebetez & Dobbertin, 2004). During the last three decades climatic conditions have continually changed towards increasing summer droughts due to a changing seasonality of precipitation (Weber *et al.*, 2007) and increasing evapotranspiration (Rebetez & Dobbertin, 2004) leading temporarily to a more negative water balance of the trees(Zweifel *et al.*, 2006).

Recently, the sub-boreal Scots pine (*Pinus sylvestris* L.), naturally dominating the dry forest at low elevation, show high mortality rates in the main inner-Alpine dry valleys (e.g., in the Aosta valley (Vertui & Tagliaferro, 1998), in the Inn valley (Oberhuber, 2001) or in the Swiss Rhone valley (Dobbertin *et al.*, 2005)). Locally in the Swiss Rhone valley, almost half of the Scots pine population has died since 1995 (Rebetez & Dobbertin, 2004), with the highest mortality rates on dry sites following drought years (Dobbertin *et al.*, 2005). Therefore drought seems to play an important role in pine decline in the inner-Alpine valleys, and the mechanism of a drought induced pine decline needs a more in depth investigation.

Tree-ring width is often used as powerful indicator to analyse the influence of drought or more generally, of climate impact on tree growth (e.g., Jacoby *et al.*, 1996; Fritts, 2001; Briffa *et al.*, 2004) , also allowing retrospective analyses. However, this method also has its limitations, since the observed climate-growth correlation and therefore the reasons for tree ring variations are often difficult to understand as the knowledge on the physiological mechanisms and processes behind tree-ring formation is limited (Kagawa *et al.*, 2006).

Stable carbon isotopes in tree rings have additional value as climate proxy (e.g., Francey & Farquhar, 1982; McCarroll & Loader, 2004), particularly on xeric sites (e.g., Ferrio *et al.*, 2003; Kagawa *et al.*, 2006). The advantage of stable isotope analysis over tree-ring width analysis is that the physiological mechanisms for carbon fixation, including ^{13}C fractionation, are well known. Thus retrospective statements about tree physiology are possible. Due to the strong relationship between discrimination against ^{13}C and intrinsic water-use efficiency (WUE_i = assimilation to stomatal conductance) on c_i/c_a (the ratio between the intercellular to ambient CO_2 concentrations in C3 plant), WUE_i can be estimated based on $\delta^{13}C$ (Farquhar *et al.*, 1989; but see Seibt *et al.*, 2008).

$\delta^{13}C$ values in earlywood and latewood show different climate signals. While latewood showed a clear response to current climate condition (Porte & Loustau, 2001; Jäggi *et al.*, 2002) $\delta^{13}C$ values in earlywood reflects more to previous year growth conditions, since it is built to a large part from previously stored carbohydrates (Smith & Paul, 1988; Kagawa *et al.*, 2006). Therefore, $\delta^{13}C$ in earlywood is expected to respond delayed to changing climate conditions, due to the mixing of old and new assimilates (Keel *et al.*, 2007; von Felten *et al.*, 2007). But whether this is true for trees on dry sites as well with eventually exhausted carbohydrate reserves due to proceeding storage depletion (Bréda *et al.*, 2006; McDowell *et al.*, 2008) remains unclear.

Studies on $\delta^{13}C$ at the intra-annual scale provide more detailed isotopic information, thus allowing for higher temporal resolution of the impact of environmental conditions on $\delta^{13}C$ (e.g., Barbour *et al.*, 2002). A prerequisite is, however, that the amount of produced wood is dated within the season. In some studies, $\delta^{13}C$ analysis was combined with dendrometer measurement for dating of wood formation (e.g., Walcroft *et al.*, 1997; Porte & Loustau, 2001; Barbour *et al.*, 2002). But dendrometers measure not only the actual increment but also changes in stem diameter due to variation in stem water content (Zweifel *et al.*, 2005)), thus measurement uncertainties should be expected. A more precise technique to determine the intra-annual increment is the pinning method (Wolter, 1968) as only xylem increment is detected. Pinning is the marking of the cambium by micro-injury, using the resulting wound reactions for the dating of wood formation. However, until now no combination of intra-annual $\delta^{13}C$ analysis and the pinning method has been undertaken.

To determine the impact of low soil water availability on tree growth, earlywood and latewood $\delta^{13}C$ values and water-use efficiency (WUE_i), we installed an irrigation experiment and compared trees growing under contrasting water supply for two growing seasons. Trees of different foliage density classes (used as indicators of tree vigour) were separately analyzed for each treatment (irrigated and control), to ascertain if any differences exist in the growth reaction of these trees, indicating differences in the ability to cope with drought events. Beside the inter-annual analysis of tree growth and tree-ring widths, $\delta^{13}C$ values were analyzed intra-annually for one season using thin-section analysis of the tree rings and combined with intra-annual increment analysis done with the pinning method.

In this study we aimed to answer the following questions: (i) Does earlywood and latewood $\delta^{13}C$ values in Scots pine responded differently to water availability? (ii) Is the irrigation signal recorded with a delay in earlywood $\delta^{13}C$ indicating a progressive mixing of old and new carbon sources? (iii) Does foliage density affect the $\delta^{13}C$ signal?

Material and Methods

Experimental site

The experimental site (46°18’ N, 7°36’ E, 615 m a.s.l.) is located in a xeric mature pine-forest (average age 90 years) at the valley floor in the driest central part of the Swiss Rhone valley (Valais). The climate is continental with a mean annual temperature of 9.2 °C and a mean precipitation sum of 599 mm (norm period 1961-1990, Meteo Swiss, weather station Sion, at 20 km distance from the experimental site).

The forest of the experimental site can be described as *Erico-Pinetum sylvestris*. The soil type is a pararendzina, which is shallow and skeletal (soil depth 60 cm) characterized by low water retention. The experimental site (about 1 ha) was split up into eight plots of 1000 m^2 each, separated by a 5 m buffering strip. Four randomly selected plots were irrigated since June

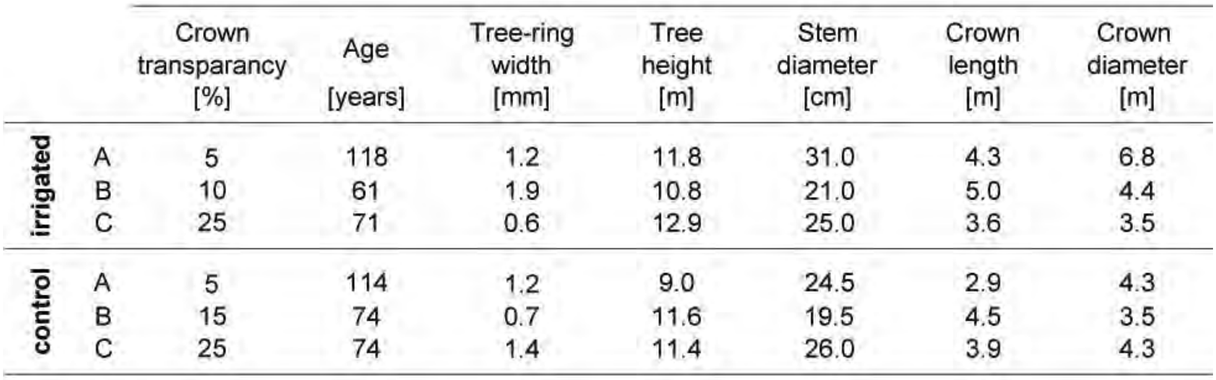
2003, using water from an adjacent water channel, fed by the river Rhone. Irrigation, corresponding to a supplementary rainfall of about 700 mm per year, was applied at night during the vegetation period (April to October) using sprinklers of about 1m height. Irrigation earlier than April was not possible, due to the risk of equipment damage by late frost. Site conditions differ only in soil humidity, since other factors such as vapour pressure deficit (VPD), radiation or soil properties were comparable on all plots.

Pinning technique and sample preparation

Three dominant trees per treatment (irrigated and control) were included in this study. The selected trees were classified by foliage density estimated by visual rating of the crown transparency in 5% steps using reference photographs ranging from 0% (a fully foliated tree) and 100% (a dead tree)). However, in this study we concentrated on trees with low defoliation rates (very high, high or mid foliage density, corresponding to A, B and C in Table 1) to assure that the tree rings were wide enough for intra-annual analysis of $\delta^{13}C$.

The pinning method was used for dating the intra-annual tree-ring formation. Pinning is a technique using the wound reaction resulting from regularly marking the cambium by inflicting micro-injury to date wood formation (e.g., Wolter, 1968; Yoshimura *et al.*, 1981; Nobuchi *et al.*, 1993). Trees were wounded at breast height according to Schmitt *et al.* (2004) on a biweekly basis during the growth period 2005 (April, 13th to October, 13th) using a small needle (diameter 0.6 mm).

Table 1 Principal characteristics of the six analyzed pine trees. Tree-ring width was calculated for the 10 years before irrigation started (1993-2002). Crown transparency value shows the estimates of March 2003, before the start of irrigation. Tree height, steam diameter, crown length and crown diameter were measured during sampling (spring 2006).



		Crown transparancy [%]	Age [years]	Tree-ring width [mm]	Tree height [m]	Stem diameter [cm]	Crown length [m]	Crown diameter [m]
irrigated	A	5	118	1.2	11.8	31.0	4.3	6.8
	B	10	61	1.9	10.8	21.0	5.0	4.4
	C	25	71	0.6	12.9	25.0	3.6	3.5
control	A	5	114	1.2	9.0	24.5	2.9	4.3
	B	15	74	0.7	11.6	19.5	4.5	3.5
	C	25	74	1.4	11.4	26.0	3.9	4.3

In spring 2006, the trees were cut and the stem sections with the pinning wounds and two further stem discs from above the pinning stem-sections were taken and stored until further processing at low temperature (- 20 °C) to avoid fungal infestation of the samples.

Intra- and inter-annual increments

To assess variation in annual increment, one stem disc per tree was sanded (35 µm particle diameter) and tree-ring width was measured on two radii using a Lintab digital positioning table and the software TSAP (both Rinntech, Heidelberg, Germany). The two measurements per disc were averaged to obtain a mean value per tree.

Cores (19 mm) including the pinning wound were extracted from the stem section using a keyhole saw. The extracted cores were stored in Strasburger solution (one third ethanol (95 %), one third Glycerol (99 %) and one third distilled water) at -20 °C. Using a sliding microtome (Reichert, Germany), thin sections (thickness of 10 µm) were cut of the cores including the pinning wound. For a better contrast between cell wall and cell lumen, thin sections were stained with safranin (1 % solution) and astrablue (2 % solution). Subsequently, the sections were dehydrated with an alcohol concentration gradient (70 %, 95 % and absolute ethanol), then in xylol (> 98 %) and permanently embedded in Canada balsam.

To study the intra-annual increment pictures of the thin sections were taken (100 times magnification, microscope: Olympus BX41, Japan; camera: ColorView III, Soft Imaging system, Muenster, Germany) and analysed using the software IMAGE PRO PLUS (Media Cybernetics, Silver Spring, USA). The intra-annual increment was measured as the distance between the last tree-ring border (between year 2004 and 2005) and the last cell below the pinning wound with a secondary wall (determined by polarized light) designating the last cell entering the maturation phase of cell formation before the pinning took place. The relative increment, the percentage share of annual increment until the pinning date, was calculated by relating the intra-annual increment to the tree-ring width measured near the wound ($\text{increment}_{\text{relative}} = \text{increment}_{\text{intra-annual}} / \text{tree-ring width}$).

The timing of wood formation was analysed, by identifying three different phases of tree-ring formation: (1) The first tracheid with a secondary cell wall found below the wound tissue, was regarded as a sign for the beginning of wood formation. (2) Earlywood/latewood transition was reached if the last cells with secondary wall were already situated above a tangential line separating earlywood from latewood. (3) The tree ring was regarded as finalized, if a continuous band of flattened latewood cells was visible completing the current tree ring.

Sample preparation for isotope analysis

The second stem disk from each tree was dried in the laboratory and a radial bar, including the last 10 tree rings, was cut from each disk using a band saw. Earlywood and latewood between the years 1996 to 2005 were separated with a scalpel using a stereo microscope (Leica Wild M3B, Germany; 40x magnification)and homogenized using a mill (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany)

To analyse the intra-annual variation of $\delta^{13}C$ values, an additional bar was cut from the disk and the last tree ring (2005) was sliced by cutting tangential thin sections (thickness of 15 µm) of the specimen using a sliding microtome. Depending on the tree ring width (0.7 to 2.1 mm), between 43 and 138 thin sections per tree ring were obtained. Based on the results of the relative intra-annual increment, the thin sections were assigned to the time scale between two pinning dates: The total numbers of thin sections per tree ring was regarded as 100% and all sections representing the tissue built between two pinning dates were pooled. For example, if 5% of the tree ring developed since the date of the first pinning, then the first 5% of thin sections were pooled as the first sample. If 20% of the tree ring developed until the second pinning, then the next 1 % of thin sections were pooled, and so on. . All thin sections belonging to the same pinning date were crushed with an ultrasonic processor (UP100S, Hielscher, Teltow Germany) since milling was not possible due to small sample size.

The wood powder (about 0.6 mg) of the earlywood and latewood samples and the thin sections was weighed into tin cups, combusted to CO_2 in an elemental analyser (EA-1110, Carlo Erba Thermoquest, Milan, Italy) and analyzed for $\delta^{13}C$ with an isotope ratio mass spectrometer (Delta-S Finnigan MAT, Bremen, Germany). All $\delta^{13}C$ values were expressed relative to the international standard VPDB (Vienna Pee Dee Belemnite): $\delta^{13}C = (R_{sample} / R_{VPDB} - 1)$, with R being the $^{13}C/^{12}C$ ratio of the sample or the VPDB standard and $\delta^{13}C$ being the measured isotope ratio in ‰. To quote tree-ring $\delta^{13}C$ relative to the pre-industrial standard, data were corrected by adding the difference between the atmospheric value and the standard value (per year) to the $\delta^{13}C$ of each tree-ring (McCarroll & Loader, 2004).

Water-use efficiency

The intrinsic water-use efficiency (*WUE_i*) can be calculated based on $\delta^{13}C$ since the isotopic discrimination in plants (Δ) is linearly linked with c_i/c_a , the ratio of intercellular to ambient CO_2 concentration (Farquhar *et al.*, 1982). The isotopic discrimination is defined as:

$$\Delta = \frac{\delta^{13}C_{atm} - \delta^{13}C_{tree}}{1 - \delta^{13}C_{tree}/1000} \approx \delta^{13}C_{atm} - \delta^{13}C_{tree}, \qquad (1)$$

where $\delta^{13}C_{atm}$ is the isotopic value of the atmospheric CO_2 and $\delta^{13}C_{tree}$ is the measured isotope ratio of the tree ring. In a simplified model according to Farquhar *et al.* (1982), Δ was shown to be dependent on c_i/c_a:

$$\Delta \cong a + (b - a)\frac{c_i}{c_a}, \qquad (2)$$

where *a* is the fractionation during CO_2 diffusion (≈4.4‰) and *b* is the enzymatic fractionation during carboxylation (≈27‰).

The intrinsic water-use efficiency is the ratio of net photosynthesis (*A*) to conductance for water vapour (g_{H2O}):

$$WUE_i = \frac{A}{g_{H2O}} \qquad (3)$$

Using the equation for net photosynthesis ($A = g_{CO2}(c_a - c_i)$) and the relationship between the conductance for CO_2 and H_2O ($g_{H2O} = 1.6 g_{CO2}$), WUE_i can be described as:

$$WUE_i = \frac{(c_a - c_i)}{1.6} \qquad (4)$$

By solving the combination of equation (1) and (2) for c_i and inserting the result in equation (4), we find:

$$WUE_i = \frac{c_a}{1.6}\frac{(b - \delta^{13}C_{atm} + \delta^{13}C_{tree})}{(b - a)} \qquad (5)$$

This equation was used to calculate WUE_i in this study.

Climatic data

A drought index (DI = $_{pot}$ET-P) was calculated using monthly data of precipitation sums (P) and potential evapotranspiration ($_{pot}$ET) according to Thornthwaite (1948), based on climate data from the weather station in Sion. For the climate correlation of earlywood and latewood $\delta^{13}C$, the monthly drought index was summed up for the three months before and the month of earlywood or latewood formation (earlywood = February to June; latewood = March to September) as climatic conditions in these month influence tissue formation. In addition, daily climate data of degree days (temperature sum above 5.5 °C of the last 10 days) and soil humidity, surveyed on the experimental site (soil humidity, measured at 10 cm depth using Time Domain Reflectometry (1502B cable tester, Tektronix, Beaverton, USA)) or monitored at the weather station in Sion (degree days), were smoothed by a moving window of 15 days and used for the visual comparison with the seasonal variation of the δ^{13}C values.

Results

Inter-annual δ^{13}C signal

In the pre-irrigation period (until 2002), δ^{13}C values in earlywood and latewood of all trees showed a similar trend, in accordance to the seasonal drought index (Fig. 1). Consequently, the correlation between δ^{13}C and the seasonal drought index was quite high for earlywood and latewood, even higher than the correlation between δ^{13}C in earlywood and previous growth (Table 2). According to the concordant strong growth response to the seasonal drought index in earlywood and latewood, the linear regression between δ^{13}C in earlywood and those in latewood was highly significant ($p < 0.001$, $R^2 = 0.81$), under naturally dry conditions (see "non-irrigated" in Fig. 2). In response to the drought year 2003, δ^{13}C values increased in control trees and stayed high during the following years, even though the drought index decreased.

Although irrigation did not start until June 2003 (the first year of the irrigation experiment), δ^{13}C decreased in the latewood of irrigated trees. Earlywood δ^{13}C values also responded immediately to irrigation, as detected by a decrease of the isotopic signature in 2004, the first earlywood formation under improved water supply.

Table 2 Coefficients of autocorrelation of δ^{13}C values in earlywood and latewood, of correlation between earlywood δ^{13}C and the δ^{13}C value of the current or the previous latewood δ^{13}C and the correlation between earlywood or latewood δ^{13}C and the seasonal drought index. δ^{13}C in earlywood was correlated with the drought index summed up for the month February to June. δ^{13}C in latewood was correlated with the drought index summed up for the month March to September. (** = $p < 0.01$).

	autocorrelation	latewood$_{current}$	latewood$_{previous}$	drought index$_{seasonal}$
earlywood	0.07	0.84**	0.11	0.76**
latewood	0.09			0.71**

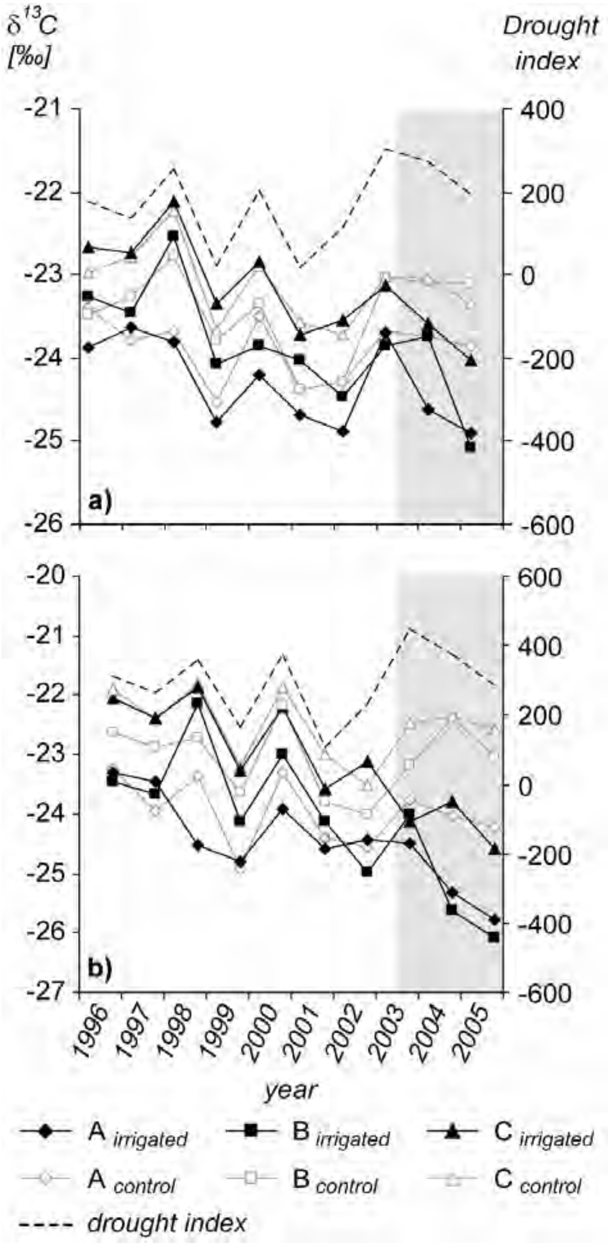


Fig. 1 $\delta^{13}C$ values in early and latewood of irrigated and control Scots pine. **a)** $\delta^{13}C$ in earlywood together with the drought index (summed up for February to June) **b)** $\delta^{13}C$ in latewood together with the drought index (summed up for March to September) separately for the three different productivity classes. $\delta^{13}C$ in earlywood and latewood are shifted against each other in the graph and plotted with the respective time period in which their were built. The irrigation period (June 2003 to 2005) is highlighted as shaded area.

A high foliage density in the pre irrigation period corresponded to lower $\delta^{13}C$ values (Fig. 1). However, the tree response to irrigation was not affected by foliage density, as the $\delta^{13}C$ values of all irrigated trees decreased after irrigation started.

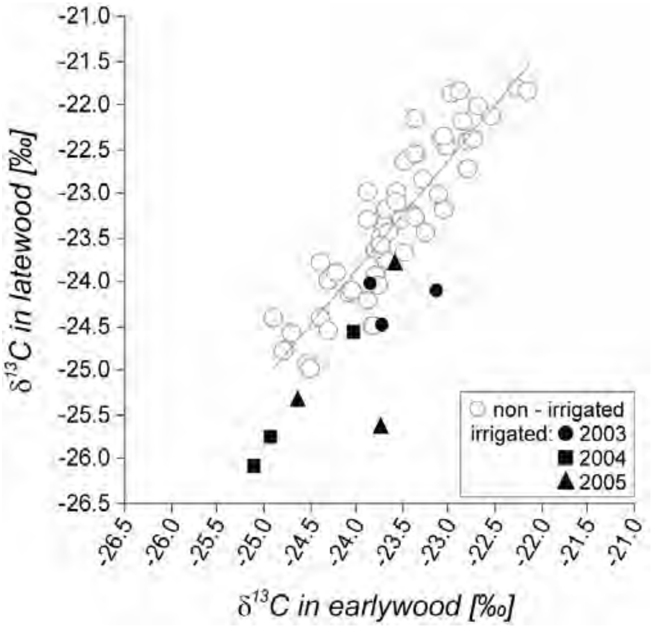


Fig. 2 $\delta^{13}C$ values in latewood vs. earlywood. The open symbols comprise $\delta^{13}C$ values of the pre-irrigation period (1996 to 2002) and control trees for the years 2003 to 2005. The regression for these pre-irrigated and control trees was $y = 1.2438x + 6$, $R^2 = 0.81$, $p < 0.001$, $n = 51$; regression for the irrigated period (2004 and 2005, only, as during earlywood formation in 2003 no irrigation took place) was not significant.

Water-use efficiency and tree-ring width

In response to irrigation, the calculated WUE_i (see equation 5) decreased (Fig. 3a), while tree-ring width increased (Fig. 3b). In the pre-irrigation period, WUE_i was high in the dry years 1998 and 2000. Similar values were reached also during the drought year 2003, when WUE_i in the control trees strongly increased. However, unlike the behaviour in 1998 and 2000, WUE_i stayed high over the next two years. Tree-ring width strongly decreased in drought years especially in 2003. However, in contrast to WUE_i and $\delta^{13}C$, no long-term effect of the drought was observed in tree-ring width as it increased again in 2004 and 2005, although at a low level.

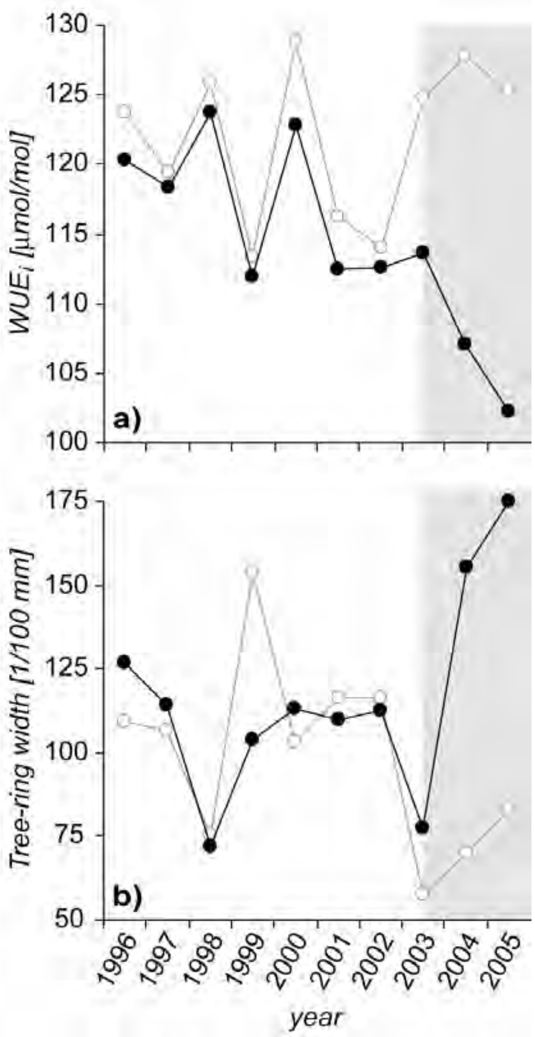


Fig. 3 a) Intrinsic water use efficiency (WUE$_i$ based on the $\delta^{13}C$ values in latewood) and **b)** mean tree-ring width of irrigated (black) and control pine trees (gray).

Intra-annual variation in $\delta^{13}C$

We found a clear seasonal signal in $\delta^{13}C$ with increasing values until a maximum was reached in mid summer and decreasing values until the end of the growth period (Fig. 4 a). The seasonal curve of $\delta^{13}C$ was rather smooth, with hardly any variation at the sub-seasonal (bi-weekly) scale.

$\delta^{13}C$ was significantly lower over the entire growth period in irrigated trees (Wilcoxon signed rank test, $p < 0.001$). In addition, the seasonal maximum in $\delta^{13}C$ was shifted by irrigation and occurred two weeks earlier in irrigated compared to control trees.

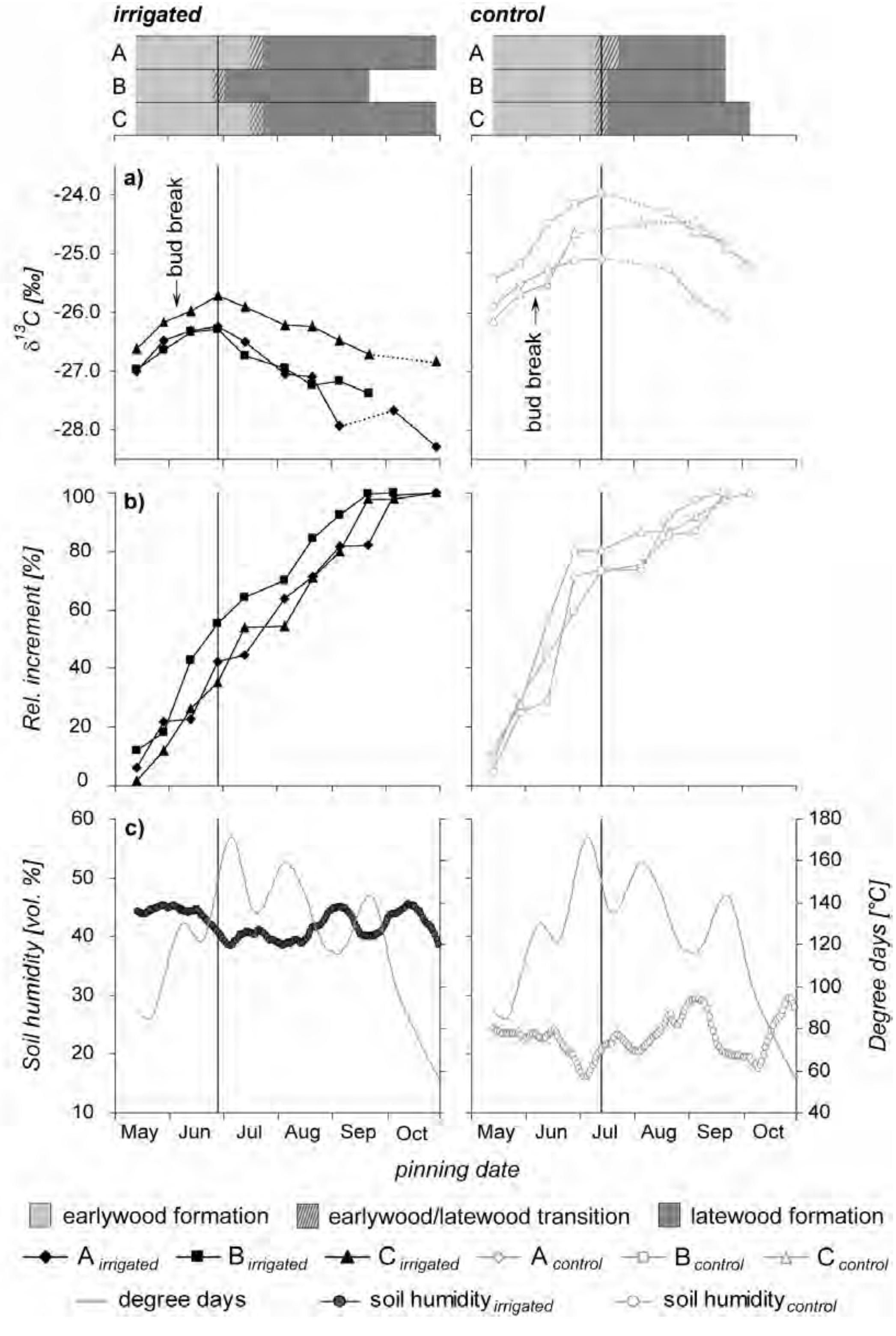


Fig. 4 Timing of earlywood and latewood formation and **a)** the intra-annual variations of the seasonal $\delta^{13}C$ signal, and **b)** the relative increment in irrigated and control Scots pines together with **c)** a running mean (15 days) of degree days and soil humidity on irrigated and control sites within the growth period in the year 2005. If data were missing in the $\delta^{13}C$ data set, adjacent points were jointed with dashed lines. The maximum in the $\delta^{13}C$ signal was highlighted in each diagram with the black vertical line. Bud break occurred between the second and the third pinning and was marked with an arrow in diagram a).

Comparing the seasonal development of $\delta^{13}C$ with the one of other parameters (earlywood-latewood transition, relative increment, soil humidity, degree days) no strict conformity was found between the occurrence of the seasonal maximum in $\delta^{13}C$ in irrigated and control trees with the other parameters (Fig. 4). Even though the seasonal maximum in $\delta^{13}C$ and earlywood/latewood transition occurred simultaneously in all control trees, this was not the case in irrigated trees. The growth rate in control trees (see slope in Fig. 4b) decreased just before the seasonal maximum of $\delta^{13}C$ was reached. But in the irrigated trees, however, the growth rate developed evenly over the year and decreased only at the end of the growth period. Soil water content was clearly higher in the irrigated than in the control plot throughout the season (Fig. 4c), as reflected in lower $\delta^{13}C$ values. But the difference between the treatments in the development of the seasonal $\delta^{13}C$ signal cannot be explained with variations in climatic conditions, since the minimum in soil humidity and the maximum in degree days appeared simultaneously for the irrigated and the control plot; a week after the maximum in $\delta^{13}C$ was observed for the irrigated trees and a week before the $\delta^{13}C$ maximum occurred in the control trees (Fig. 4).

Discussion

$\delta^{13}C$ values reflect variations in the seasonal drought index

The high $\delta^{13}C$ values in trees growing under naturally dry conditions probably reverberate a highly reduced stomatal conductance as a consequence of a chronic drought situation in Valais. Regarding the inter-annual variation, $\delta^{13}C$ was highly sensitive to water availability (see Fig.1, Table 2, high correlation with the drought index), which was in accordance to other studies (e.g., Leavitt, 1993; Livingston & Spittlehouse, 1993; McNulty & Swank, 1995; Warren *et al.*, 2001; Ferrio & Voltas, 2005). This was true for both earlywood and latewood ($p < 0.01$, see Fig. 1 and 2, Table 2), which was quite surprising since earlywood was expected to form mainly from previously stored carbohydrates (Smith & Paul, 1988; Kagawa *et al.*, 2006). Thus a stronger response to the previous year than to current year growth conditions (as shown by Porte & Loustau (2001) for pine) would have been assumed. But the opposite was shown by our data, as correlations with previous latewood $\delta^{13}C$ and the autocorrelation was not significant ($r = 0.11$ and 0.07, respectively, see Table 2). The unusually strong relationship of earlywood $\delta^{13}C$ to the current-year climatic conditions in our study points to a tight coupling of photosynthesis and tree ring formation using current photosynthetic products. This seems to indicate that only low amounts of stored carbohydrates were available for the formation of earlywood. Due to these low carbon pools trees' resistance against stressors should be rather low (Bréda *et al.*, 2006). Thus, increasing mortality should be expected, when a stressor either increases the need (parasitism) or decreases the production (drought) of the carbon reserves.

Under natural dry conditions, WUE_i tended to be in the upper range of the values (range of control trees = 113 to 129) that were observed in other studies in Switzerland and France (e.g. Bert *et al.*, 1997, found values between 80 and 120 mmol/mol; Saurer & Siegwolf, 2007, reported mean values for Abies alba = 99.4 mmol/mol and for Picea abies = 101 mmol/mol), or ranging all over Eurasia (Saurer *et al.*, 2004, mean: Larix spp. = 74 mmol/mol, Pinus spp. = 76 mmol/mol, Picea spp. = 84 mmol/mol). This indicated higher chronic drought stress at our sites compared with the other sites.

Fast response of $\delta^{13}C$ values and water-use efficiency to increasing water supply

Under improved water availability due to irrigation, annual values of $\delta^{13}C$ and WUE_i decreased while tree-ring width increased (Fig. 3). Remarkably there was no time lag between the irrigation start and the isotopic response in earlywood and latewood, as $\delta^{13}C$ decreased immediately after irrigation started (Fig. 1). Therefore, an intensive mixing of currently produced assimilates with the previous years' carbohydrate pools is highly unlikely much in contrast to deciduous trees (Keel *et al.*, 2006; Keel *et al.*, 2007) but also to conifers at the upper tree line (von Felten *et al.*, 2007). This observation is another clear indication of very small pools of stored carbohydrates.

WUE_i reflects the balance of changes between stomatal conductance and photosynthesis (Farquhar *et al.*, 1989). Decreasing WUE_i under increasing productivity (indicated by increasing tree-ring width) in response to irrigation points to disproportionately high increase of stomatal conductance and thus, transpiration rates under improved water supply. The water costs for tree-ring production increased compared to the pre-irrigation period as trees became wasteful with water in response to irrigation (see Korol *et al.* (1999), Ferrio *et al* .(2003) and Adams & Kolb (2004) showing the same reaction: increasing WUE_i under increasing drought).

While we used a linear model for the calculation of intrinsic water-use efficiency (Eq. 5), Seibt *et al.* (2008) recently suggested a more complex approach. The difference between intrinsic (= ratio assimilation to stomatal conductance) and instantaneous water-use efficiency (= ratio assimilation to transpiration) was particularly emphasized in this study due to the fact that transpiration depends not only on stomatal conductance but also on the vapour pressure deficit (VPD). Thus intrinsic and instantaneous water-use efficiency can be quite different if VPD is an important factor. Consequently, our interpretation that the transpiration rate strongly increases in response to irrigation would be inaccurate if the changes in WUE were predominantly a result of different VPD values between the differently treated plots. But in our experiment VPD should be identical between the treatments. There are no differences between the pots in soil conditions and in the microrelief. Therefore the soil hydrologic balance should be comparable. The diffusion equilibrium on the experimental site should be rather fast due to the very small size of the plots. In addition irrigation took place at night and the surface water evaporates fast in the morning. The rapid evaporation and the diffusion equilibrium over the plots are reinforced by the consistent occurrence of heavy winds. Due to these facts the differences in intrinsic and instantaneous WUE should be minute.

Another point of criticism on the calculation of WUE_i based on $\delta^{13}C$ is that tree ring $\delta^{13}C$ might not directly reflect physiological processes on leave level due to the blurring of the leave sugar signal by post-carboxylation fractionation. Post-carboxylative fractionation occurs during the loading of sugar in the sieve tubes as ^{13}C enriched components are preferred (Gessler *et al.*, 2004; Brandes *et al.*, 2006; Brandes *et al.*, 2007). Therefore the intrinsic water-use efficiency derived from tree ring $\delta^{13}C$ might be slightly underestimated as it is difficult to account for the effects of post-carboxylation processes (Duquesnay *et al.*, 1998). Nevertheless, we can accept WUE_i based on $\delta^{13}C$ from tree rings as a good integrative estimation for the entire vegetation period of trees.

Foliage density and variations in $\delta^{13}C$

Discrimination against ^{13}C was higher in trees with a high foliage density compared to trees with a lower foliage density as shown by decreasing $\delta^{13}C$ with increasing foliage density (Fig. 3). Since all trees studied were dominant trees and did not differ in micro-site condition, other factors must be responsible for a high foliage density and a lower $\delta^{13}C$. One possible reason for this difference might be found in the rooting depth: It is known that drought may lead to changes in carbon allocation in favour of the root system, triggering a downward shift of the rooting zone for maximal water uptake (e.g., Ericsson *et al.*, 1996; Puhe, 2003). Since more assimilates can be produced in trees with a higher foliage density and sufficient water supply more carbon might be invested in root growth. Consequently deeper water pools in the soil can be reached by the roots of these trees, leading to a better water supply compared to trees with a lower foliage density. Thus the more negative $\delta^{13}C$ values might be an indicator of a better water supply. Consequently, the probability of surviving a severe drought would be higher in high-foliaged trees, due to their deeper rooting system, but also as a consequence of a higher carbohydrate pool. This might explain why trees that proliferated their roots into deeper soil layers have a higher probability of surviving drought as they have access to deeper water sources (Oberhuber, 2001). N.b. mortality mortality models yielded better results if foliage density was taken into account (Dobbertin & Brang, 2001).

Intra-annual variation in $\delta^{13}C$ reflects the use of carbon storage

On the intra-annual time scale, clear differences between the treatments were found, as irrigation led to significantly lower $\delta^{13}C$ values and an earlier achievement of the seasonal maximum of $\delta^{13}C$ values (Fig. 4). Lower $\delta^{13}C$ values in response to irrigation corresponded with the results we found at the annual scale and with previous findings (e.g., Livingston & Spittlehouse, 1993; Warren *et al.*, 2001; Ferrio & Voltas, 2005). Throughout the year 2005, the $\delta^{13}C$ was lower in irrigated trees compared to control trees. This indicates that the main part of carbon utilized for wood formation in 2005 was fixed during the irrigation period. Seasonal changes in micrometeorology (e.g., Leavitt, 1993; Barbour *et al.*, 2002; Schulze *et al.*, 2004) or in carbon metabolism (autotrophic vs. heterotrophic metabolism, see Helle & Schleser, 2004) were discussed as triggers for the seasonal variation in $\delta^{13}C$ for conifers and deciduous trees, respectively.

Regarding climatic conditions, only soil humidity differed between the treatments. But these differences were only found in the total amount of soil water content and not in the intra-annual curve shapes as soil humidity reacted to rainfall events on both, the irrigated and the control plot. Thus, it is unlikely that this factor caused differences between the treatments in the seasonal development of $\delta^{13}C$ values.

Following the assumption of (Helle & Schleser (2004) that differences in the carbon source use for wood formation (autotrophic vs. heterotrophic metabolism) are responsible for the intra-annual variation in $\delta^{13}C$, the growth period can be divided into a first part with predominantly heterotrophic carbon-usage (carbon source: old and recent assimilates) and a second part with mostly autotrophic carbon-usage (carbon source: recent assimilates). The maximum $\delta^{13}C$ reached represents the transition from heterotrophism to autotrophism. Consequently the delayed seasonal maximum in $\delta^{13}C$ of the control trees might be explained by the fact that the amount of currently produced assimilates was not sufficient to maintain the total carbon demand by autotrophism alone. Thus tree growth in control trees compared to irrigated trees had to rely, for a longer period, on the supplementary usage of old carbohydrates. This longer reliance on old carbohydrates might be explained by the earlier closure of stomata in control trees due to lower water supply (see Zweifel *et al.*, 2007), resulting in a down regulation of photosynthetic activity (e.g., Rennenberg *et al.*, 2006). This is supported by the less negative $\delta^{13}C$ values and higher WUE_i in control trees compared to irrigated trees, indicating a lower c_i due to stomatal closure. Thus even though the amount of stored carbohydrates is low in the drought exposed trees as indicated by the results on the annual scale, the intra-annual variation in $\delta^{13}C$ is likely to reflect different carbon sources used for the formation of wood.

Irrigation delayed the transition from earlywood to latewood formation (Fig. 4, see also Eilmann in prep.) which is in contrast to the earlier achievement of the intra-annual maximum in $\delta^{13}C$. Thus the transition from earlywood to latewood formation is not necessarily linked to the switching between the different carbon sources. However, the occurrence of the intra-annual maximum in $\delta^{13}C$ seemed to be connected with the growth rate, as the curve of the relative increment in the control trees flattened just before reaching their $\delta^{13}C$ maximum (Fig. 4b). Thus, high growth rates, such as those at the beginning of the growth period, could not be maintained when only recent assimilates are invested in tree growth,

Conclusion

The results at the intra-annual scale indicated that at the beginning of the growth period trees growing under low water supply depended on the additional investment of stored carbohydrates for a longer time to cover the carbon need for wood formation. . As a result storage depletion is most likely intensified in drought exposed trees. On an annual scale high turnover rates due to shrunken carbon reserve pools were indicated by the unusually high correlation between $\delta^{13}C$ in earlywood and the current drought index and the immediate response to irrigation. Also the unusually high WUE_i showed that Scots pine in Valais is under considerable drought-stress. Since we studied only trees of the three highest foliage density classes showing only modest signs of decline, the situation must be even more exaggerated for less vital trees having a shallower root system and smaller storage pools. Thus an ongoing or even increasing mortality for the drought stressed trees should be expected if drought or other stress factors persist in the future.

Acknowledgements

We like to thank M. Dobbertin for valuable discussions and his assistance in the field, E. Graf Pannatier for surveying and providing soil humidity data, R. Köchli, U. Wasem and M. Burger for their help in sample preparation, S. Paget for English corrections to the manuscript and A. Kress for support in the lab. We are grateful to the forest services of the canton Valais, Burgergemeinde Leuk and HYDRO Exploitation SA for infrastructure, to W. Landolt und P. Bleuler for the managing of the irrigation system, and to K. Egger and his team from the forest service Leuk for their help in the field and their support on the experimental site. This study was conducted in the frame of the CCES-project MOUNTLAND funded by the Velux foundation and the Canton Valais.

References

Adams HD, Kolb TE. 2004. Drought responses of conifers in ecotone forests of northern Arizona: tree ring growth and leaf δ^{13}C. *Oecologia* **140**(2): 217-225.

Allen CD, Breshears DD. 1998. Drought-induced shift of a forest-woodland ecotone: Rapid landscape response to climate variation. *Proceedings of the National Academy of Sciences of the United States of America* **95**(25): 14839-14842.

Barbour MM, Walcroft AS, Farquhar GD. 2002. Seasonal variation in δ^{13}C and δ^{18}O of cellulose from growth rings of *Pinus radiata*. *Plant Cell and Environment* **25**(11): 1483-1499.

Bert D, Leavitt SW, Dupouey JL. 1997. Variations of wood δ^{13}C and water-use efficiency of *Abies alba* during the last century. *Ecology* **78**(5): 1588-1596.

Brandes E, Kodama N, Whittaker K, Weston C, Rennenberg H, Keitel C, Adams MA, Gessler A. 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. *Global Change Biology* **12**(10): 1922-1939.

Brandes E, Wenninger J, Koeniger P, Schindler D, Rennenberg H, Leibundgut C, Mayer H, Gessler A. 2007. Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.) stand through analyses of stable isotope composition of water and organic matter. *Plant Cell and Environment* **30**(1): 113-127.

Bréda N, Huc R, Granier A, Dreyer E. 2006. Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science* **63**(6): 625-644.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **102**(42): 15144-15148.

Briffa KR, Osborn TJ, Schweingruber FH. 2004. Large-scale temperature inferences from tree rings: a review. *Global and Planetary Change* **40**(1-2): 11-26.

Ciais P, Reichstein M, Viovy N, Granier A, Ogée J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, De Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* **437**(7058): 529-533.

Dobbertin M, Brang P. 2001. Crown defoliation improves tree mortality models. *Forest Ecology and Management* **141**: 271-284.

Dobbertin M, Mayer P, Wohlgemuth T, Feldmeyer-Christe E, Graf U, Zimmermann NE, Rigling A. 2005. The decline of *Pinus sylvestris* L. forests in the Swiss Rhone valley - a result of drought stress? *Phyton* **45**: 153-156.

Duquesnay A, Bréda N, Stievenard M, Dupouey JL. 1998. Changes of tree-ring δ^{13}C and water-use efficiency of beech (*Fagus sylvatica* L.) in north-eastern France during the past century. *Plant Cell and Environment* **21**(6): 565-572.

Ericsson T, Rytter L, Vapaavuori E. 1996. Physiology of carbon allocation in trees. *Biomass and Bioenergy* **11**: 115-127.

Farquhar GD, Ehleringer JR, Hubick KT. 1989. Carbon isotope discrimination and photosynthesis. *Annual Reviews of Plant Physiology and Plant Molecular Biology* **40**: 503-537.

Farquhar GD, O'Leary MH, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**: 121-137.

Ferrio JP, Florit A, Vega A, Serrano L, Voltas J. 2003. δ^{13}C and tree-ring width reflect different drought responses in *Quercus ilex* and *Pinus halepensis*. *Oecologia* **137**(4): 512-518.

Ferrio JP, Voltas J. 2005. Carbon and oxygen isotope ratios in wood constituents of *Pinus halepensis* as indicators of precipitation, temperature and vapour pressure deficit. *Tellus Series B-Chemical and Physical Meteorology* **57**(2): 164-173.

Francey RJ, Farquhar GD. 1982. An explanation of C-13/C-12 variations in tree rings. *Nature* **297**(5861): 28-31.

Fritts HC. 2001. *Tree rings and climate*. London: Academic Press.

Gessler A, Rennenberg H, Keitel C. 2004. Stable isotope composition of organic compounds transported in the phloem of European beech - Evaluation of different methods of phloem sap collection and assessment of gradients in carbon isotope composition during leaf-to-stem transport. *Plant Biology* **6**(6): 721-729.

Gitlin AR, Sthultz CM, Bowker MA, Stumpf S, Paxton KL, Kennedy K, Munoz A, Bailey JA, Whitham TG. 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. *Conservation Biology* **20** (5): 1477-1486.

Granier A, Reichstein M, Bréda N, Janssens IA, Falge E, Ciais P, Grünwald T, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Facini O, Grassi G, Heinesch B, Ilvesniemi H, Keronen P, Knohl A, Kostner B, Lagergren F, Lindroth A, Longdoz B, Loustau D, Mateus J, Montagnani L, Nys C, Moors E, Papale D, Peiffer M, Pilegaard K, Pita G, Pumpanen J, Rambal S, Rebmann C, Rodrigues A, Seufert G, Tenhunen J, Vesala I, Wang Q. 2007. Evidence for soil water control on carbon and water dynamics in European forests during the extremely dry year: 2003. *Agricultural and Forest Meteorology* **143**(1-2): 123-145.

Helle G, Schleser GH. 2004. Beyond CO_2-fixation by Rubisco - an interpretation of $^{13}C/^{12}C$ variations in tree rings from novel intra-seasonal studies on broad-leaf trees. *Plant Cell and Environment* **27**(3): 367-380.

IPCC 2007. Technical report. In: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Mille ed. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*.

Jacoby GC, DArrigo RD, Davaajamts T. 1996. Mongolian tree rings and 20th-century warming. *Science* **273**(5276): 771-773.

Jäggi M, Saurer M, Fuhrer J, Siegwolf R. 2002. The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*. *Oecologia* **131**(3): 325-332.

Kagawa A, Sugimoto A, Maximov TC. 2006. Seasonal course of translocation, storage and remobilization of C^{13} pulse-labeled photoassimilate in naturally growing *Larix gmelinii* saplings. *New Phytologist* **171**(4): 793-804.

Keel SG, Siegwolf RTW, Jaggi M, Korner C. 2007. Rapid mixing between old and new C pools in the canopy of mature forest trees. *Plant Cell and Environment* **30**(8): 963-972.

Keel SG, Siegwolf RTW, Korner C. 2006. Canopy CO2 enrichment permits tracing the fate of recently assimilated carbon in a mature deciduous forest. *New Phytologist* **172**(2): 319-329.

Korol RL, Kirschbaum MUF, Farquhar GD, Jeffreys M. 1999. Effects of water status and soil fertility on the C-isotope signature in *Pinus radiata*. *Tree Physiology* **19**(9): 551-562.

Leavitt SW. 1993. Seasonal $^{13}C/^{12}C$ changes in tree rings: species and site coherence and a possible drought influence. *Canadian Journal of Forest Research* **23**: 210-218.

Livingston NJ, Spittlehouse DL 1993. Carbon isotope fractionation in tree rings in relation to the growing season water balance. In: J. R. Ehleringer, A. E. Hall, G. D. Farquhar eds. *Stable isotopes and plant carbon-water relations*. San Diego: Academic Press, 141-154.

McCarroll D, Loader NJ. 2004. Stable isotopes in tree rings. *Quaternary Science Reviews* **23**(7-8): 771-801.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**(4): 719-739.

McNulty SG, Swank WT. 1995. Wood $\delta^{13}C$ as a measure of annual basal area growth and soil water stress in a *Pinus strobus* forest. *Ecology* **76**(1581-1586).

Nobuchi T, Fujisawa T, Saiki H. 1993. An application of the pinning method to the marking of the differentiation zone and to the estimation of the time course of annual ring formation in sugi

(*Cryptomeria japonica*). *Mokuzai Gakkaishi (Journal of the Japan Wood Research Society)* **39**(6): 716-723.

Oberhuber W. 2001. The role of climate in the mortality of Scots pine (*Pinus sylvestris* L.) exposed to soil dryness. *Dendrochronologia* **19**(1): 45-55.

Porte A, Loustau D. 2001. Seasonal and interannual variations in carbon isotope discrimination in a maritime pine (*Pinus pinaster*) stand assessed from the isotopic composition of cellulose in annual rings. *Tree Physiology* **21**(12-13): 861-868.

Puhe J. 2003. Growth and development of the root system of Norway spruce (*Picea abies*) in forest stands - a review. *Forest Ecology and Management* **175**: 253-273.

Rebetez M, Dobbertin M. 2004. Climate change may already threaten Scots pine stands in the Swiss Alps. *Theoretical and applied climatology* **79**(1-2): 1-9.

Reichstein M, Ciais P, Papale D, Valentini R, Running S, Viovy N, Cramer W, Granier A, Ogée J, Allard V, Aubinet M, Bernhofer C, Buchmann N, Carrara A, Grünwald T, Heimann M, Heinesch B, Knohl A, Kutsch W, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Pilegaard K, Pumpanen J, Rambal S, Schaphoff S, Seufert G, Soussana JF, Sanz MJ, Vesala T, Zhao M. 2007. Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biology* **13**(3): 634-651.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* **8**(5): 556-571.

Saurer M, Siegwolf R 2007. Human impacts on tree-ring growth reconstructed from stable isotopes. In: T. E. DawsonR. Siegwolf eds. *Stable isotopes as indicators of ecological changes*.

Saurer M, Siegwolf R, Schweingruber FH. 2004. Carbon isotope discrimination indicates improving water-use efficiency of trees in northern Eurasia over the last 100 years. *Global Change Biology* **10**: 2109-2120.

Schär C, Vidale PL, Luethi D, Frei C, Häberli C, Liniger MA, Appenzeller C. 2004. The role in increasing temperature variability in European summer heatwaves. *Nature* **427**: 332-336.

Schulze B, Wirth C, Linke P, Brand WA, Kuhlmann I, Horna V, Schulze ED. 2004. Laser ablation-combustion-GC-IRMS - a new method for online analysis of intra-annual variation of $\delta^{13}C$ in tree rings. *Tree Physiology* **24**(11): 1193-1201.

Seibt U, Rajabi A, Griffiths H, Berry JA. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* **155**(3): 441-454.

Smith JL, Paul EA. 1988. Use of an in situ labeling technique for the determination of seasonal ^{14}C distribution in Ponderosa pine. *Plant and Soil* **106**: 221-229.

Vertui F, Tagliaferro F. 1998. Scots pine (*Pinus sylvestris* L.) die-back by unknown causes in the Aosta Valley, Italy. *Chemosphere* **36**(4): 1061-1065.

von Felten S, Hattenschwiler S, Saurer M, Siegwolf R. 2007. Carbon allocation in shoots of alpine treeline conifers in a CO2 enriched environment. *Trees-Structure and Function* **21**(3): 283-294.

Walcroft AS, Silvester WB, Whiteheard D, Kelliher FM. 1997. Seasonal changes in stable isotope ratios within annual rings of *Pinus radiata* reflect environmental regulation of growth processes. *Australian Journal of Plant Physiology* **24**: 57-68.

Warren CR, McGrath JF, Adams MA. 2001. Water availability and carbon isotope discrimination in conifers. *Oecologia* **127**(4): 476-486.

Weber P, Bugmann H, Rigling A. 2007. Radial growth responses to drought of *Pinus sylvestris* and *Quercus pubescens* in an inner-Alpine dry valley. *Journal of Vegetation Science* **18**(6): 777-792.

Wolter KE. 1968. A new method for marking xylem growth. *Forest Science* **14**(1): 102-104.

Yoshimura K, Hayashi S, Itoh T, Shimaji K. 1981. Studies on the improvement of the pinning method for marking xylem growth. I. Minute examination of pin marks in taeda pine and other species. *Wood Research* **67**: 1-16.

Zweifel R, Steppe K, Sterck FJ. 2007. Stomatal regulation by microclimate and tree water relations: interpreting ecophysiological field data with a hydraulic plant model. *Journal of Experimental Botany* **58**(8): 2113-2131.

Zweifel R, Zimmermann L, Newbery DM. 2005. Modeling tree water deficit from microclimate: an approach to quantifying drought stress. *Tree Physiology* **25**: 147-156.

Zweifel R, Zimmermann L, Zeugin F, Newbery DM. 2006. Intra-annual radial growth and water relations of trees: implications towards a growth mechanism. *Journal of Experimental Botany* **57**(6): 1445-1459.

Synthesis

The overall aim of this project was to identify the impact of drought on the growth of Scots pine in the dry inner-Alpine Swiss Rhone valley. Therefore, the potential of Scots pine to maintain its dominance in the low elevation forest was estimated. In addition, xylem adaptation of pubescent oak, as a possible substitute species of Scots pine, to drought was analysed.

In this synthesis chapter, the methodological aspects and then the main findings of the thesis are discussed. Finally, the chapter concludes with an outlook on further research topics that contribute to completing the picture of landscape change in Valais.

Methodological aspects

Multiscale analysis

In this thesis, tree performance under contrasting water supply was analysed using several growth proxies of different *hierarchic scales*: The combination of tree-ring analysis (macro scale) with wood anatomical studies (micro scale) allowed a detailed view on growth strategies and on the consequences of growth limitations (chapters I and II). The additional analysis of stable carbon isotopes (atomic scale) in Scots pine gave new insights into the process of storage depletion.

The analysis on different *time scales* provided new information about environmental influences on tree growth, and contributed to a better understanding of the drought effect on the timing and the strategy of tree growth. For climate-growth analysis based on tree-ring data, climate correlation with monthly data gave a good overview of the impact of climatic conditions on tree growth. However, for cellular parameters, climate-growth analysis on a monthly scale was not sufficiently precise due to the shorter formation period of cells. Therefore, cellular parameters were correlated with daily climate data that were averaged by a moving window of 10 days.

Climate-growth analysis at the intra-annual scale further improved the preciseness of statements made about the climate impact on growth, as growth parameter could be correlated with climatic condition during the formation of the cells. But due to the time-consuming sample preparation, the study on the intra-annual scale was short-term, covering only two years. Thus, a generalization, especially about the timing of climate signals, might not always be possible. This is particularly true if hydric conditions changed shortly before the analysis period, as in the case of irrigated trees in the irrigation experiment. Thus, the climate-growth relationship might be out of balance for several years, as the trees adapt their root and crown system to the improved water supply.

Study design

In this thesis, two different *methodological approaches* (survey vs. experiment) were used to analyse the impact of drought on the growth of Scots pine at the macro and micro scale. Both approaches led to similar results. This was important especially in the case of lumen diameter in Scots pine, as corresponding results were only found on semi-arid sites (Maherali & DeLucia, 2000), while on more moderate sites, opposing growth adaptation to drought events was found (Vaganov, 1990; Dünisch & Bauch, 1994; Vaganov *et al.*, 2006). Thus, by presenting corresponding results from two sites, both affected by drought but differing in altitude, exposure or radiation, we could demonstrate the increase in lumen diameter in Scots pine as representative for the adaptation to severe drought in Valais.

Even though we were interested in the impact of drought on tree growth, we performed an irrigation experiment at a xeric site and not a drought experiment, as fewer side effects were expected. In greenhouse experiments, drought is easy to simulate by reducing the amount of irrigation water (e.g., Dünisch & Bauch, 1994), but in the field, drying is more complicated as mature trees have to be sheltered from precipitation by using permanently fixed covers (e.g., Whitmore & Zahner, 1966; Moehring *et al.*, 1975). The disadvantage of these rain shelters is that during periods of sunshine, temperature often increases under the cover, which might lead to a falsification of the results. In irrigation experiments, only the soil water content changes, other micrometeorological factors are not affected. Possible side effects in irrigation experiments are an altered pH or fertilisation by water constituents but on our experimental site no such changes were observed (M. Peters, WSL, personal communication).

The future performance of Scots pine and pubescent oak was estimated on the basis of climate-growth correlations. The validity of correlation analysis for future tree performance is limited as it is based on past climate impact on tree growth. Nonetheless, it is possible to derive the general trends of climatic control on tree growth, such as the negative impact of hotter and drier conditions on tree growth. Thus, a first estimate of future performance based on climate correlation is possible, but of course this estimate cannot make up for modelling analysis.

Tree growth under drought – Main findings of the thesis

This thesis gave new insights into the process of pine decline and was conducive to completing the picture of growth limitation by drought. The two different approaches (survey and experiment) lead to corresponding results. The multiproxy analyses helped to answer the overall research question (see General introduction, page 12):

- In Scots pine, increment and cell wall thickness was reduced under drought (chapters I and II; see Table 1) probably due to decreasing carbon supply (Rennenberg *et al.*, 2006). Narrowed latewood and thinner cell walls reduced the mechanical strength as a result of the reduced wood density (Fig. 1, Bodig & Jayne, 1993). Concurrently, conduit size increased under drought (chapter I and II), which might be an adaptation to increase the potential water conductivity despite the lower number of conduits (Fig. 1). But at the same time the valuability to cavitation increases due to the increase in cell size and the thinning of the cell walls. Thus, pine build a more effective water conducting system with lower carbon investments under drought, but at the expense of safety. In addition, $\delta^{13}C$ values were less negative under drought, pointing to a reduced CO_2 concentration in the respiratory cavity due to stomata closure. This indicates that water use efficiency increased (Fig. 1), meaning that at the leaf level, the unit water transpired per unit carbon gain, was reduced under drought. Thus, under these drought conditions, the photosynthetic CO_2 fixation might be more economic in terms of water costs but less intense due to low CO_2 concentration in the mesophyll.

  In pubescent oak, tree-ring width was reduced under drought due to a strong reduction in latewood width (chapter I), leading, as in Scots pine to decreased mechanical strength (Fig. 1). Water conduction might also be affected, as latewood vessels built the emergency system in water transport if earlywood vessels cavitated. Earlywood vessel size decreased under drought (chapter I), reducing the potential water conductivity (Fig. 1) but since the results of climate-growth analysis indicate an endogenous control of earlywood vessel enlargement (see chapter I, Fig. 4), a further significant decrease in vessel size under increasingly dry conditions is unlikely.

Table 1: Growth proxies were analysed using different methods for different species and at different time scales (cross). The arrows indicate the quality of the growth adaptation to drought (positive or negative). The Roman numerals indicate the chapter in which the growth proxy was studied.

growth proxy	method		species		time		drought-effect		chapter
	survey	experiment	pubescent oak	Scots pine	annual	intra-annual	pubescent oak	Scots pine	
radial increment	x	x	x	x	x	x	↓	↓	I, II, III
lumen size	x	x	x	x	x	x	↓	↑	I, II
cell number	x			x	x			↓	I
cell wall thickness	x	x		x	x	x		↓	I, II
$\delta^{13}C$		x		x	x	x		↑	III

↑: increase under drought; ↓: decrease under drought

- Drought and low tree crown density can shorten the growth period: Drought led to a premature cessation of wood formation in the autumn. In addition, pine with low crown density tended to a later onset of wood formation in spring compared to pine with a denser crown (chapter II). Thus, the general trend in phenology indicating a prolonged growth period due to climate warming (e.g. Menzel & Fabian, 1999; Defila & Clot, 2001; Menzel *et al.*, 2006) might be superimposed on dry sites by a drought induced shortening of the growth period.

- Storage depletion was discussed as one main reason for drought-induced Scots pine mortality (McDowell *et al.*, 2008). The analysis of $\delta^{13}C$ showed that on dry sites, even trees with no or only slight signs of decline, showed symptoms of storage depletion (chapter III). Anyway, trees with higher crown density might have the advantage of a better water supply due to deeper rooting system as indicated by more negative $\delta^{13}C$ values. Therefore, periods of low precipitation might have been buffered by using deeper water reserves. Conversely this means that the effect of drought should be more severe in trees of low foliage density due to shallower rooting. In addition, total primary production and therefore the amount of stored carbohydrates should be reduced in trees with a lower foliage density leading to early storage depletion.

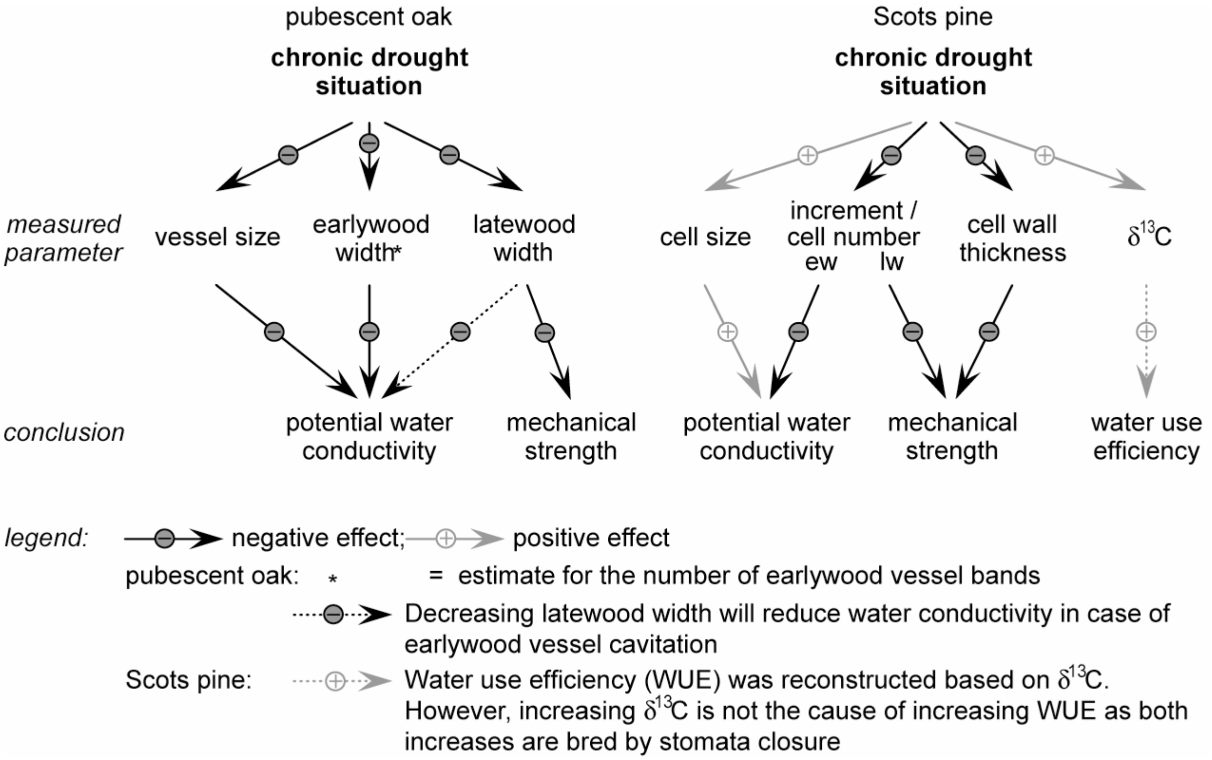


Fig. 1: Synthesis of the drought effect on the growth proxies and the derived functional consequences for oak and pine in Valais.

The results at the macro- and the micro-scale also indicated a stronger impact of drought on trees with a lower foliage density (chapter II). This became apparent when 1) the long-lasting effect of the drought year 2003 on the tree-ring width was observed, 2) the shortening of the growth period was detected, and 3) the inability to buffer the reduced potential water conductivity due to the low number of conduits by increasing conduit size in trees of low crown density was observed.

Although all irrigated trees, even those with low foliage density, profited from irrigation, the process of tree decline seemed to be reversible when site conditions improve. Thus, the existence of a “point of no return” on the trees’ pathway from high to low vigor ending in tree death can not be verified by our findings.

- Under a projected hotter and drier future climate, increment and cell wall thickness will further decrease while conduit size will further increase, as shown by the climate-growth analysis (chapters I and II). As a result, potential water conductivity per tree ring will further decrease, which may, in the long run, amplify tree death by storage depletion due to a down-regulation of photosynthetic activity under low water supply.

Even trees of high foliage density showed symptoms of progressed storage depletion (chapter III), leading to a higher susceptibility against biotic and abiotic stressors. Based on the findings of this thesis, we doubt that Scots pine, under a projected hotter and drier future climate, can maintain its dominance in the low elevation forests in the dry inner-Alpine valley. A further increase of Scots pine mortality at the dry sites in Valais is very likely.

In today's climate, pubescent oak might act as a substitute species for Scots pine on the driest sites but in a hotter and drier climate, pubescent oak might soon reach its physiological limit as well: Even though a further strong reduction in earlywood vessel size should not be expected, due to the endogenous control of earlywood vessel enlargement under severe drought, the frequency of missing latewood is expected to increase. Missing latewood might substantially reduce water conduction after cavitation of the big earlywood vessels, as with the latewood the emergency system for the water transport (= latewood vessels) is absent.

Prospects

This thesis contributed to a better understanding of Scots pine decline processes and helped to estimate the potential for the long-term performance of the two studied species. Filling the research gaps emerged from this thesis will allow for further comprehension of the landscape changes in the inner Alpine valleys.

- We analysed $\delta^{13}C$ values in the Pfynwald (chapter III) to obtain new insight into the tree physiology. Additional analysis of $\delta^{18}O$ values of the tree rings would provide further information on the source of the water used (precipitation water or ground water) and on relative humidity and would improve the interpretation of the intrinsic water use efficiency calculated based on $\delta^{13}C$ (e.g. Scheidegger *et al.*, 2000). In addition, it would be interesting to study how $\delta^{13}C$ and $\delta^{18}O$ of irrigated Scots pine responded to the irrigation stop at our survey site in Lens (chapter I).
- In pubescent oak, a strong reduction of latewood width in response to drought was found (chapter I), which might lead to a decrease in water transportation after the cavitation of earlywood vessels. To evaluate if latewood vessels as an emergency system are necessary, it is crucial to know whether or not earlywood vessels actually cavitate within the vegetation period. Thus, the pathways of water transport have to be visualized at various

dates during the year to evaluate the functioning of the earlywood vessels. One method might be to introduce a dye solution into the transpiration stream as accomplished by Sano *et al.* (2005).

- As pubescent oak might not have the potential to replace Scots pine under a projected hotter and drier future climate, other substitute species assuring ecosystem goods and services of the dry pine forests at low elevation should be found. Possible substitute species available in Valais are whitebeam (*Sorbus aria* Crantz) or juniper (*Juniperus communis* L.) but since both species stay relatively small in Valais, showing a shrublike habitus, their protective function regarding rockfall or avalanches will be rather low. Therefore, the potential of non-endemic species, like black pine (*Pinus nigra* Arnold), holm oak (*Quercus ilex* L.) or flowering ash (*Fraxinus ornus* L.), to replace Scots pine on the dry forest sites shall be evaluated.

Reference

Bodig J, Jayne BA. 1993. *Mechanics of wood and wood composites*. Malabar, Florida: Krieger Publishing Company.

Defila C, Clot B. 2001. Phytophenological trends in Switzerland. *International Journal of Biometeorology* **45**(4): 203-207.

Dünisch O, Bauch J. 1994. Influence of soil substrate and drought on wood formation of spruce (*Picea abies* L. Karst.) under controlled conditions. *Holzforschung* **48**: 447-457.

Maherali H, DeLucia EH. 2000. Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiology* **20**(13): 859-867.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez EA. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* **178**(4): 719-739.

Menzel A, Fabian P. 1999. Growing season extended in Europe. *Nature* **397**(6721): 659-659.

Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kubler K, Bissolli P, Braslavska O, Briede A, Chmielewski FM, Crepinsek Z, Curnel Y, Dahl A, Defila C, Donnelly A, Filella Y, Jatcza K, Mage F, Mestre A, Nordli O, Penuelas J, Pirinen P, Remisova V, Scheifinger H, Striz M, Susnik A, Van Vliet AJH, Wielgolaski FE, Zach S, Zust A. 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology* **12**(10): 1969-1976.

Moehring DM, Grano CX, Bassett JR. 1975. Xylem development of lolloby pine during irrigation and simulated drought. *USDA Forest Service Research Paper* **110**: 1-8.

Rennenberg H, Loreto F, Polle A, Brilli F, Fares S, Beniwal RS, Gessler A. 2006. Physiological responses of forest trees to heat and drought. *Plant Biology* **8**(5): 556-571.

Sano Y, Okamura Y, Utsumi Y. 2005. Visualizing water-conduction pathways of living trees: selection of dyes and tissue preparation methods. *Tree Physiology* **25**(3): 269-275.

Scheidegger Y, Saurer M, Bahn M, Siegwolf R. 2000. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **125**(3): 350-357.

Vaganov EA 1990. The tracheidogramm method in tree-ring analysis and its application. In: E. R. CookL. A. Kairiukstis eds. *Methods of dendrochronology. Applications in the environmental sciences*. Dordrecht, Boston, London: Kluwer Academic Publishers, 63-76.

Vaganov EA, Hughes MK, Shashkin AV. 2006. *Growth dynamics of conifer tree rings images of past and future environments*. Berlin: Springer.

Whitmore FW, Zahner R. 1966. Development of xylem ring in stems of young red pine trees. *Forest Science* **12**(2): 198-210.

Acknowledgements

It would not have been possible to write this thesis without the support of many people, to only some of whom it is possible to give particular mention here.

First of all I would like to express my gratitude to Dr. Andreas Rigling, for his constant support from the origin of the proposal to the completion of the final thesis. He provided an excellent working environment and left room for my individual preferences. His enthusiastic supervision during my research, the continued encouragement and the numerous discussions whenever necessary have been fundamental to the success of this work.

I wish to express my thanks to my supervisor Professor Dr. Nina Buchmann for her valuable advice and support during this work and and the careful reviewing of the manuscripts.

I also want to express my gratitude to Professor Dr. Harald Bugmann for making me feel always welcome.

I would like to thank the colleagues associated with the “pine-project” and with the irrigation experiment for creating a friendly and open working atmosphere.

I also want to thank the co-authors. Their constructive feedback and comments at various stages have been useful in shaping the chapters up to completion. In particular I wish to express my gratitude to Dr. Rolf Siewolf and Dr. Matthias Saurer from the PSI for analysing my samples in their lab.

I want to express my gratitude to the colleagues of the former section *Soil Ecology* and particularly Dr. Peter Blaser for his moral support during the first half of my PhD. I also would like to thank the colleagues of the newly formed research unit *Forest Dynamic*, especially the colleagues of the group *Disturbance Ecology* for their support during the second half of my work. Furthermore, I want to thank the colleagues of the research unit *Dendro Science* for their help in the lab and the valuable discussions.

In addition I would like to express my gratitude to Matthias Dobbertin, Roger Köchli, Ueli Wasem, Sebastion Osenstetter, Koni Egger and his team, Maria Burger and Patritzia Truniger for their help in the field or in the lab.

Furthermore I would like to thank the Velux foundation and the Canton Valais for funding this study, and the WSL for providing the infrastructure.

Finally I want to thank my family for their support during the last years, especially my mother for accepting my frequent absence in the north during the last year. And of course I would like to thank Daniel for his valuable comments on the manuscript, but even more for his constant support and encouragement during the last years, ensuring my moral wellbeing.

Curriculum vitae

Britta Eilmann

Born July 1, 1976, in Walsrode, Germany
Citizen of Germany

04/2005 /2008	Ph.D. thesis at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) and at the Swiss Federal Institute of Technology (ETH Zurich) “Water availability and tree growth of Scots pine and pubescent oak - a multiproxy analysis”, under the supervision of Prof. Dr. N. Buchmann (Grassland sciences, ETH Zurich), Dr. A. Rigling (Forest dynamic, WSL) and Prof. Dr. H. Bugmann (Forest ecology, ETH Zurich)
03/2004 – 09/2004	Trainee at the Swiss Federal Research Institute WSL.
05/2003 – 02/2004	Diploma thesis at University of Hamburg: “Growth reaction of Scots pine (*Pinus sylvestris* L.) and pubescent oak (*Quercus pubescens* Willd.) to drought years – a wood anatomical study.” (in German), under the supervision of Prof. Dr. D. Eckstein (Department of Wood Biology, University of Hamburg, Germany) and Dr. A. Rigling (Forest dynamic, WSL).
04/1997 – 04/2004	Diploma studies in Wood Science and Technology, University of Hamburg, Germany.
05/1996	Abitur (German university entrance qualification) at the Gymnasium Walsrode, Germany.