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Wood formation dynamics of Larch (*Larix decidua* Mill.) along a 1000 m elevation gradient in the French Southern Alps

Seyedehmasoumeh Sadari

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Le 21 Décembre 2017

Dynamiques de la formation du bois du Mélèze (*Larix decidua* Mill.) le long d'un gradient altitudinal de 1000 m dans les Alpes du sud Françaises

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Chapter 1

General Introduction

1.1 Importance of wood formation studies

1.1.1 The importance of wood

Wood is the most important natural and renewable source of energy and material. It has always had a crucial role in the history of human life. Nowadays it is still used as a privileged raw material in building, transport, furniture, art and sport. The global production of round wood was reported 3714 million m³ in 2015 with an increase of 19% compare to 1980 (FAO 2015). The rising demand for forest products will continue with growing world population and income. The majority of wood (80%) can be find in forest that cover one third of the land area on the earth (FAO 2010). Forests are considerable sources of food, energy and clean water for living organisms. Forests contribute to a wide range of environmental services such as supporting sustainable agriculture, stabilizing soil and climate and production of wooden and non-wooden materials (FAO 2016). The major planet's biodiversity depends on forest ecosystems. The broad variety in forest types including tropical, subtropical, mediterranean, temperate and boreal forests provide habitat, shelter and food for millions of plants, animals and insects species.

One of the most substantial roles of forests is participating in the carbon cycle, the backbone of life on the earth. Carbon is the fourth most abundant chemical element in the universe (Ren et al. 2013). The food, civilization, economy and transport system of human beings are based on carbon. Trees have the ability of sequestration of atmospheric carbon in their above and below-ground biomass structures through photosynthesis (Unwin and Kriedemann 2000). Carbon dioxide is absorbed through photosynthesis process by trees and convert into structural carbon in the form of cellulose, hemicellulose and lignin. The recent climatic changes highlighted the importance of carbon cycle in the last decades. Climate change has been defined as the statistically significant variation in the variables that define the climate of a region or it's variability persistent over an extended period of time (IPCC report 2014). The effects of climatic change due to increasing atmospheric CO₂ can be observed as changing temperature and modifying precipitation regimes.

Because of the all mentioned functions provided by forests, they need to be studied and protected. But disastrously, the extent of the world's forests has declined substantially in the recent decades. In this situation, it is crucial to use and preserve wood resources much more efficiently. One of the solution to provide woody

resources is to grow wood using silvicultural plantations. In order to have a plantation with maximum economic yield we need to have a good understanding of the mechanisms involved in wood formation process in different tree species and also finding the optimum environmental condition for their growth.

1.1.2 Wood function in trees

Tree has been defined as a form that a plant develops to become one of the tallest, free standing and perennial plants. All the plants like trees need to face the problem of gravity. Tree stem must be strong enough to support the crown under the wide range of stresses such as storms. The 4000 years old Bristlecone pine, is an example of a tree species that manage to survive against all the environmental stresses (Wilson 1984). Trees are able to cope with these problems through strengthening their stems via wood formation.

The woody cells composed of cellulose and lignin, the two materials conferring the strength of wood, originally produced by the photosynthetic process in the leaves. Cellulose is the most abundant biological compound on the earth. The vascular cambium is composed of a thin layer of meristematic cells located between the secondary phloem (i.e., the living bark) and secondary xylem (i.e., wood). It is the responsible of making a continuous envelope around the stems, branches and root of woody plants, producing xylem towards inside of the stem and the phloem outward (Wilson 1984) (figure 1.1). The woody structure plays four essential functions in trees: 1) supporting and distributing photosynthetic tissues; 2) transporting and uptaking the raw sap flow (nutrients and water) from the roots to leaves; 3) storing water and carbohydrates and other compounds; 4) storing and distributing defensive compounds in order to protect the trees against pathogens (Kozłowski and Pallardy 1997, Fournier et al. 2006).

1.1.3 Tree ring structure in conifers

In the microscopic observation of the transverse section in conifers, tracheids, parenchyma, rays and resin canals can be observed (figure 1.2). The basic structure of a conifer tree ring from pith toward the bark consists of earlywood, transition zone and latewood. The tracheids comprise 90 % or more of the total number of cells in conifers (Larson 1969). They are responsible of sap flow conduction and mechanical strength

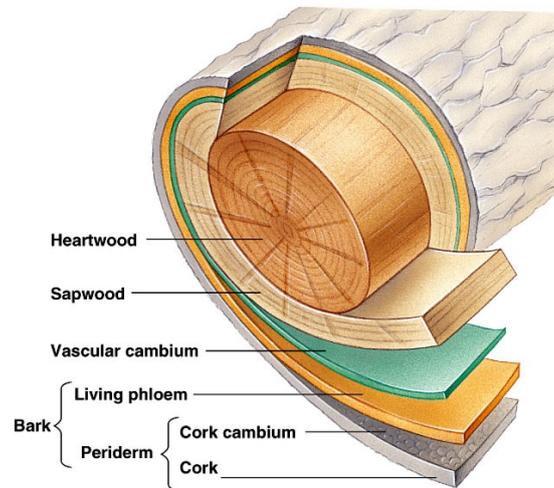


Figure 1.1: Structure of tree stem layers

in trees. Tracheids are elongated, spindle shaped cells of 3–6 mm in the length and 6–60 μm in diameter (Sperry et al. 2006) that are closed at their end (figure 1.3). The morphology of tracheids depends on the time in which they are produced within the growing season. The tracheids of early wood form at the beginning of growing season and have larger lumen area and thinner cell wall compare to late wood (Wilson 1984). Tracheids are connected through openings in their secondary lignified cell walls known as bordered pits (Carlquist 2013). The tracheid cells, die at the end of their development to fulfill their function. The parenchyma cells are the only cell types that stay alive for a couple of years.

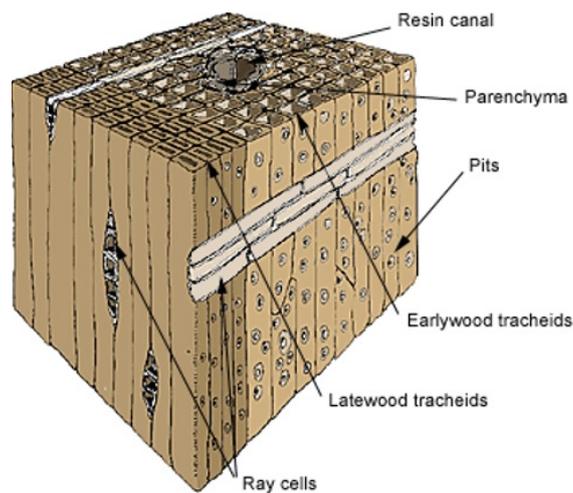


Figure 1.2: Xylem structure in conifers

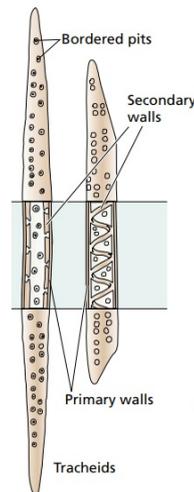


Figure 1.3: Structure of tracheid elements in conifers (Edited from [Taiz and Zeiger 2010](#)).

1.1.4 Cambium definition and function in trees

Cambium is responsible of wood formation in trees. It has been defined as the sheath of dividing meristematic cells between the bark and wood, producing both new wood and bark. Meristems are groups of undifferentiated cells that retain the ability to divide almost indefinitely ([Wilson 1984](#)). The origin of the word "cambium" was found in the Arabic language, where it was used in medicine and surgery ([Larson 1969](#)). Malpighi 1678 had the original idea of defining cambium as responsible of wood formation "the new layers of wood in stem and branches resulted from the periodic transformation of the inner layers of the bark" ([Sachs 1890](#)).

Nehemiah Grew used firstly the term of cambium in a botanical sense in edition of his book "The Anatomy of Plants" ([Grew 1965](#)). The formation of annual xylem rings occurs due to the production of new cells by cambium division, the growth of cambial derivatives by expansion, and secondary wall thickening. The former two processes determine the annual increment width and the latter one the accumulation of biomass in cell walls ([Antonova and Stasova 1993](#)). Trees also use the cambial activity for thickening the roots to make the trees strong enough to overcome the stresses of wind and gravity ([Wilson 1984](#)). Cambium is the main carbon sink in trees participating in the carbon allocation via photosynthesis ([Hansen and Back 1994](#)).

Toward the end of summer, in temperate and boreal zones, the cambial activity slows down and the number of cells in the cambial zone returns to their minimum number in the dormant tree. Cambium of trees is bigger in area compare to other

plants because of their bigger size, making trees a good choice for studying cambial activity (Wilson 1984).

Wood presents a variety of physical properties and chemical components. These properties are formed as the result of changes which took place during the differentiation process and conversion of cambium derivations into mature xylem elements (Barnet 1981). The onset of wood formation is not directly detectable through bud break or leaf phenology observation since it occurs in the inner part of the stems. Defining the dates of xylogenesis requires precise and direct measurement of cambial activity in a regular and short interval repetition along the growing season. Each cambial cell (the cambial initial), produces daughter cells to the inside and outside of the tree, along a radius of stem cross section. Derivations of cambial cells then go through the phases of enlarging, wall thickening and finally the programmed death of cells.

Cambial cell division

In the dormant season, the vascular cambium is inactive, and the cambial zone consisting in two to six layers of cells. These cells are rectangular in cross section, contain protoplasm in a viscous state and have clearly defined walls. The cambium cells are recognized by their thin primary walls, small radial size and rectangular shape compare to phloem cells. The formation of a xylem cell can be divided into five main steps. 1) the periclinal division in a mother cell that produces a new daughter cell; 2) the enlargement of the newly formed cell; 3) the secondary cell wall formation that is the deposition of cellulose and hemi-cellulose in cell wall; 4) the impregnation of cell walls with lignin and 5) the programmed death of cells (figure 1.4).

Enlarging phase

Cell enlargement is the first stage of cell differentiation in plants. During this phase an irreversible increase occurs in the cell volume. The visible result of cell enlargement is the great increase in the cell size that can multiplied the cell size by 10-100. (Vaganov et al. 2006). This change in the cell size are the result of relaxation of primary cell wall in order to create a passive inlet of water which is counter balanced by an active influx of solutes. These steps are crucial to maintain a high turgor pressure in the cells (Cosgrove 2005).

Under the normal condition, the hormonal regulation drives the cell enlarging phase

but turgor pressure can be considered as the engine of this phase. As a result any water shortage with changing turgor pressure can affect cell growth (Perrot-Rechenmann. 2010).

Secondary cell wall formation and lignification

The secondary wall which is the most important layer of cell is made of three main elements that are cellulose, hemicellulose and lignin (Barnet 1981). The cellulose in the secondary cell wall of tracheids and fibers is organised in three layers of S1, S2 and S3 from outside to inside of cell lumen which presents quite similar composition but different thickness and orientation of cellulose microfibrils (figure 1.5). The outer layer of (S1) has a crossed helical organization. It is the thinnest layer of secondary wall with 0.1 to 0,35 μm thickness.

The microfibril angle to the cell axis in this layer is 60° to 80° . The (S2) layer has a steeper helix than (S1) with a uniform helical organization. The thickness of this layer is 1 to 10 μm and the microfibril angle is 5° to 30° to the cell axis. The third internal layer of the secondary wall is (S3) that the structure of helix is similar to (S1). It is a thin layer with the thickness of 0.5 to 1,10 μm , with a microfibrils angle of 60° to 90° (Barnett 1981, Plomion et al 2001).

After the end of cell enlargement, the secondary cell wall formation starts with the deposition of S1 layer between the membrane and the primary wall, soon followed by formation of S2 and S3. The secondary cell wall covers whole the surface except pit membranes. This phase is known to be controlled by the expression of genes that activate the transport, deposition and biosynthesis of wall components.

Lignification is a process whereby the stem becomes woody. During this phase a cell with a thin and viscoelastic walls converts to an anisodiametric cell with a rigid and thick wall (Barnet 1981). Lignification begins at the corner of the cells in the primary cell wall at the same time as S1 layer deposition then goes toward the middle lamella. In this step lignin deposit inside the spaces left by the microfibrils to make chemical bonds with hemicelluloses and act as a cement that make the cells water proof. The lignification process was reported to be sensitive to temperature. Although the hormonal control on the lignification process is not clear but the effect of ethylen in inducing several enzymes that are involved in the biosynthesis of lignin has been found (Donaldson 2001).

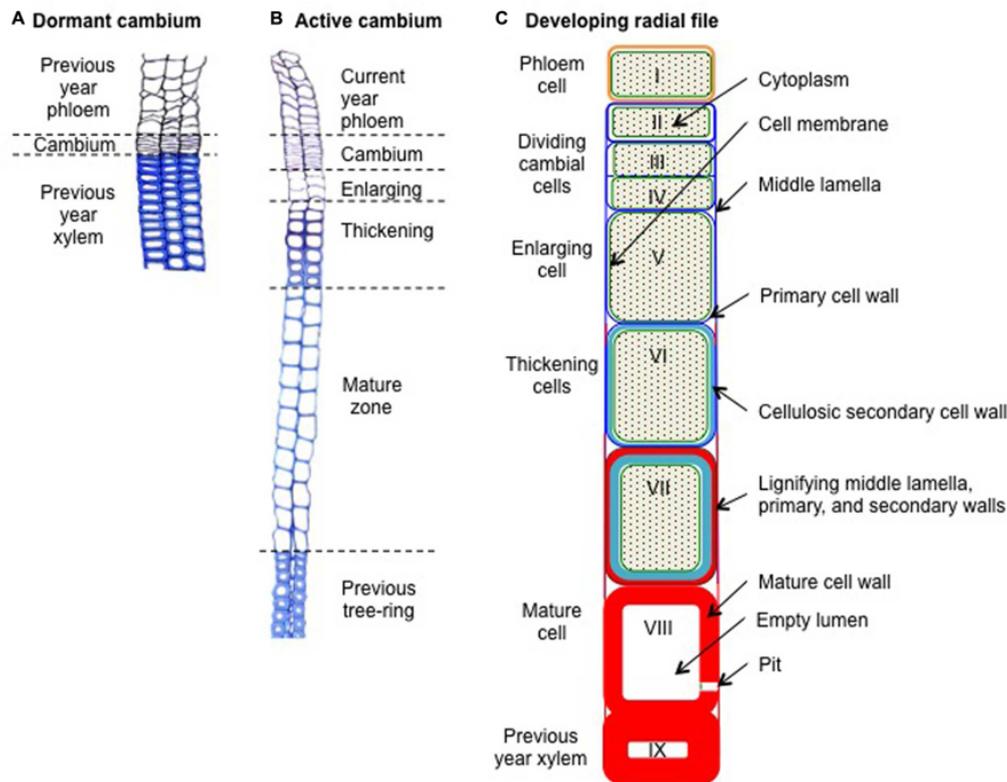


Figure 1.4: [

Schematic cross-sections of the resting and developing radial xylem cell files in conifers.](A) Dormant cambium during winter composed of a thin band of 4–6 reinforced cambial cells layers, (B) Active cambium and associated developing radial cell files at the beginning of summer. The active cambium, composed of a wide strip of 11–13 cambial cells layers, is located between the newly formed phloem and xylem. The developing xylem, which is composed of an enlarging zone (3–4 cell layers), a thickening zone (7 cell layers), and a mature zone (12–13 cell layers), is located between the cambium and the previous tree ring. (C) Schematic view of a developing xylem radial file. Cell I represents a newly formed phloem cell of the current year. Cells II–IV shows cambial cells, of which cells III and IV are dividing. Cell V shows an enlarging cell. Cells VI–VII show thickening cells. Cell VIII shows a mature xylem cell (there is no cytoplasm in it). Cell IX represents a latewood cell of the previous tree ring. Green backgrounds represent cytoplasm, green lines represent cell membranes, blue lines represent cellulosic cell walls (dark blue for primary walls, light blue for secondary walls), and red areas represent lignified cell walls (Rathgeber et al. 2016).

Programmed cell death

Maturation is the last phase of wood formation. The xylem cells die to become functional and the only cell type that escape the death and stay alive for several years is

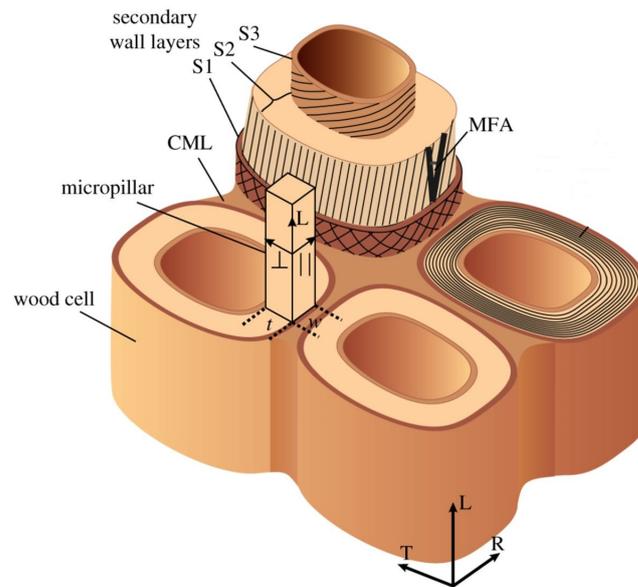


Figure 1.5: Microfibril orientation on the outer surface of primary wall and the secondary wall layers of S1, S2 and S3 of a conifer tracheid. CML shows the compound middle lamella. (Edited from [Rafsanjani et al. 2014](#))

parenchyma. The principal trigger of this phase is a massive influx of calcium ions (Ca^{2+}) to the vacuole through plasma membrane channels.

Then the vacuole breaks up rapidly (in few minutes) as a sudden and the cytoplasm is hydrolysed. After some days, the cell is formed as an empty space area with thick lignified wall with pits ([Bollhoner et al. 2012](#)). The mechanisms that control the death of cells were found to be connected to secondary wall formation. Programmed cell death is an essential step of xylem cell differentiation. It allows the mature xylem cells to perform their substantial function of mechanical support in the wood and conducting sap flows through their pits.

Cell cycle

Cell cycle, can be defined as the sequence of successive events that are highly controlled in all the cellular organisms that have nucleus. In the cell cycle, the meristematic mother cells undergo several developmental stages including division of the nucleus and separation of cytoplasm in order to produce two daughter cells ([Lachaud et al. 1999](#)). The cell division process in the cambium is slow and lasts about 10 to 50 days depending on different developmental stages, tree species and environmental conditions ([Larson 1969](#)).

1.2 Wood formation monitoring

1.2.1 Importance of wood formation studies

Monitoring the seasonal dynamics of wood formation in trees started more than 50 years ago with the aim of better understanding the influence of climatic factors on trees growth, phenology of cambial activity and wood formation dynamics. With the recent climatic changes, this question grabbed more attention and led to increase in the number of studies in the last decades. In conifers the diameter and wall thickness of tracheids along a radial file are divided and multiplied by factor 5 respectively (Vaganov et al. 2006, Cuny et al. 2014). It shows the variation in wood anatomy within a single tree ring. These transition depend on the species and the specific environmental condition along the wood formation period.

The aim of wood formation monitoring is finding to what extent these anatomical changes are related to climatic constraints. Cambial activity is affected by the interaction between endogenous and exogenous factors. As described recently by Cuny et al (2016), there are two main explanations to describe the tracheid cells development in conifer tree rings. One is the developmental theory that relates the tree ring formation mainly under the control of internal signals (Uggla et al 1996, 1998, Sundberg 2000, Oribe et al. 2003), according to that, the developmental stages of tracheid cell formation is affected by the concentration of phytohormones such as auxin (Uggla et al 1996). The second theory is the environmental theory describing the anatomical changes within a tree ring to be under the control of environmental factors (Larson 1969, Fritts et al 1999, Vaganov et al 2011). A lot of studies have been developed based on these two theories to understand the mechanisms that control the different response of cambial activity in different species and latitude/altitude.

Although the effect of environmental factors on the wood formation phenology (De Luis et al. 2007, Rossi et al. 2007, Gricar and Cufar 2008, Makinen et al. 2008, Gruber et al. 2009, Swidwark et al. 2011, Rossi et al. 2011, Balducci et al. 2013, Ziaco et al. 2016), along elevation gradients (Moser et al. 2010, Treml et al. 2015, Wang et al. 2015), maximum rate of cell production (Rossi et al. 2006), the dynamic of cell production (Rathgeber et al. 2011, Cuny et al. 2012) and its kinethic (Cuny et al. 2014), have been studied widely but there is still lack of knowledge in this field. The result of previous studies both on the external and internal factors

revealed that cambial activity is a complex dynamic system in trees. Without a clear understanding of the biological process that control each part of this system, it is not possible to define it's response to different environmental conditions and specially extreme events such as low temperature.

1.2.2 Method of wood formation monitoring

As a classical model of wood formation monitoring, samples are extracted along the growing season from tree stems. In order to avoid disturbance to trees, wood samples are repeatedly extracted as small cores or so called microcores (Deslauriers et al. 2003). Generally, sampling is done after removing bark, by means of a small cutting tube hammered into the stem. Although microcoring is known as one of the less destructive methods of wood formation, it can induce the wounding responses in trees. These reactions can be in the form of traumatic resin canals (Bollschweiler et al. 2008, Schneuwly et al. 2009) or callus tissue formation (Stobbe et al. 2002). Although microcoring method is widely used for wood formation studies but there is a gap to find the the effect of this method on the xylogenesis process and the final tree ring structure.

1.3 Characteristics of selected species, European Larch

1.3.1 Distribution of European Larch

Larch (*Larix* sp.) is a pioneer, very long-lived, fast-growing coniferous. It is one of the most abundant conifer species in the northern hemisphere. Larch belongs to the Pinaceae family (Matras and Pâques 2008) and the genus includes 10 species occurring in North America and Eurasia (Farjon 2010). It occurs naturally in the Alps, at the border of Czech Republic and Poland (the Sudeten Mountains), North Slovakia and southeastern Poland (Western Carpathians) with some residual stands in Central Poland and Romanian carpathians (figure 1.6). The upper limit of Larch in the Western Alps is 2300 m while it's lower limit can be observed at 1300 m in the south-western part of the Alps (Wagner 2013). Larch has a large ecological amplitude. In the Alps and Tatra Mountains it occurs in continental climates, with dry and very cold winters while in Poland and in the Sudeten Mountains it can grow at lower altitudes in sub-continental climates (Pâques et al. 2013).

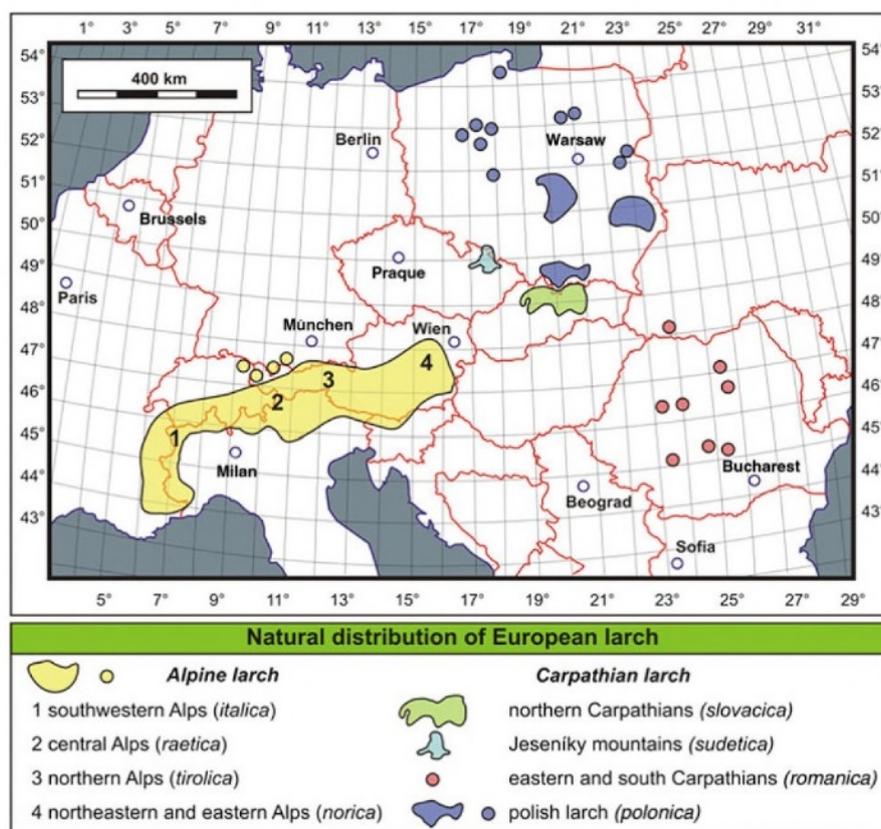


Figure 1.6: Distribution of Larch (Matras and Pâques 2008)

1.3.2 Characteristics of European Larch

European larch (*Larix decidua* Mill.) is one of the few deciduous conifer species. Its small wind-dispersed pollen doesn't have air sacs like the pollen of other conifers, let them to move in less extensive dispersal up to 300 m by wind (Sjögren et al. 2010). They reach to sexual maturity at the age of 15 in open stands but at the age of 35-40 years in close stands. They can produce valuable hybrids by crossing with some other larch species such as Japanese larch (*Larix kaempferi* (Lamb.)) (Matras and Pâques 2008). This typical pioneer conifer is a light-demanding species with a low competitive ability. Their close and enduring stands are established only when climatic conditions eliminate their competitors, such as mountain areas.

Larch can grow on a wide range of soil types, ranging from deep, well-structured and aerated to shallow stony soils, including calcareous soils, with a medium ground water level (Wagner 2013). The age of larch trees under optimal conditions and free standing can reach up to 600-850 years, while under forest conditions, their age will be limited to 150 years (Da Ronch et al. 2016). Larch has a high adaptation to

continental alpine climates, its tolerance to very low temperature can be related to the ability of losing needles and avoiding foliage desiccation (Wagner 2013).

1.3.3 Economically and ecological importance of French Alps and European Larch

The Alps, known as one of the major mountain ranges in Europe, have a significant role in providing water cycle resources. It is called as "water tower of Europe". This notion points to the climate of Alps with abundant precipitation and snow falls. It has also an important role in sorting fresh water and moderating the variations in river runoff (Isotta et al. 2014). But the occurrence of heavy precipitation and dry periods, can be a threat for the components of the Alpine ecosystem.

French Alps have valuable species both ecologically and economically. Using larch in historical building of ancient Rome was found by the archaeological studies (Matras and Pâques 2008). It is one of the fastest-growing conifer species in Western and central Europe and is planted even outside its natural range due to its silvicultural importance. The economical value of this species originates from both wood and resin production. Its wood is characterized by a high density and durability making it a valuable raw material in building industry, pulp for paper and also furniture making. The essential oil of Larch is used also in aromatherapy. High concentration of tannins (up to 10%) and resin content (about 2.6%) increases the durability of this species (Praciak et al. 2013). Under the optimal condition Larch can produce more than 10 m³ of wood per ha annually (Matras and Pâques 2008).

1.4 General context of study

1.4.1 Phenology as a life trait in trees

In plants, phenology is the trade-off between the environmental factors and the accessibility of required resources for their growth (Nord and Lynch, 2009). Changing temperature and precipitation pattern as a result of recent climatic changes are likely to drive significant modifications in all aspect of plants life such as phenology, distribution, mortality and interactions with other organisms (IPCC 2014). In temperate and boreal regions, this period shows the most favorable time for the tree-ring formation. It is the time window during which trees are open to receive the environmental signals and record them in the form of tree ring structures. Trees

growing in temperate regions experience short term environmental changes in different season. They need to protect themselves to be able to survive in occurrence of freezing temperature. Going to dormancy is one way of doing that.

Timing of onset and cessation of wood formation are the two critical factors identifying the limits of growing season period. Tree ring width at their range limits found to be mainly related to the growing season temperature. Bud break and leaf unfolding in adult trees are timed accurately to avoid the freezing damages. The range limits of tree species are set by the interaction between freezing resistance in spring and giving the enough time to the differentiation and autumnal maturation of xylem cells (Korner 2016). So monitoring the phenology of wood formation in trees growing in contrasting climate condition such as varying temperature reveals useful information about the response of tree species in a changing climate.

1.4.2 Elevation gradients in ecological studies

Elevation gradients provide useful natural experiments for ecological surveys because temperature, the main factor driving phenology of plants and animals decrease by increasing elevation. Mountain is defined as any elevation of land area from plains that is 300 m above the sea level (Kapos et al. 2000). Moving toward higher altitudes, the environmental condition will change and the species need to be well adapted into the local condition in order to be survived. Mountains provide valuable basis to test and develop the adaptation theories. In order to test the theories of species adaptation to elevation, it is crucial to understand the concept of elevation gradient to make the evaluation biological meaningful.

The global geophysiological change with altitude is decreasing land area and as a result the opportunity for life (Korner 2000). Four main atmospheric changes found to be associated with altitude. The first one is decreasing atmospheric pressure and also partial pressure of all atmospheric gases such as O_2 and CO_2 . These two gases are the vital components of life and are related to the respiration in animals and gas exchange and photosynthesis in plants. Second is decreasing temperature by increasing altitude. Another global atmospheric change with increasing altitude is solar radiation that increases under a clear sky and the last is higher fraction of UV-B radiation. Precipitation and wind velocity although are not globally related to altitude change (Korner 2007).

All the factors that are physically related to altitude can induce selective pressure in trees growing along different elevations (Barton 1999). Mountain forests, may

respond to the climatic changes in three basic ways: they persist in the modified climate, they migrate to more suitable climates or they will be extinct (Guisan et al. 1995). Their persistence can be in the form of phenotypic plasticity or gradual genetic adaptation (Davis and Shaw 2001, Aitken et al. 2008).

Phenological characteristics show significant phenotypic variability along elevation gradients and presence of phenotypic variability is a sign of local adaptation. Monitoring wood formation in trees is a way to assess the phenotypic response of trees to their environment. Different phases of wood formation (onset of cell enlargement, onset of cell wall thickening and the occurrence of the first mature cell) in larch were found previously to be highly correlated to elevation (Moser et al. 2010).

Recent rapid climatic changes raise uncertainty about the ability of tree species to keep pace with their changing environment. In elevation gradients, there are climatic trends that are not generally related to elevation such as precipitation. But the pattern for precipitation (water and snow) in the Alps was found to increase with increasing elevation due to local and regional effects (Sevruk 1998).

In addition to climate variables, there are other environmental factors that may not present altitudinal trend but may vary along elevation gradients and affect the phenotypic response of species. These factors can be biotic or abiotic. The amount of relative extractable water for trees along elevation gradients can change dependent on the factors such as soil type or the amount of precipitation. So in elevation related studies it is important to consider the environmental factors that can influence phenotypic variability in species.

The upper (northern) and lower (southern) limits of a species was found to be shaped generally by different ecological factors. The upper range of its distribution was found to be temperature limited and the lower limits by water availability (Scholes, 1994).

In this study European Larch trees (*Larix decidua* Mill.) growing along a gradient of 1000 m in elevation ranging from 1350 to 2300 m in French Alps were selected. Along this elevation gradient, trees are expected to experience contrasting climatic conditions. The temperature variation is likely to affect the phenology of wood formation in trees growing in different elevations. Different precipitation regimes may have also an effect on their phenotypic responses. Monitoring wood formation along this gradient of 1000 m in elevation can help us to understand how trees are able to adjust their phenology and dynamics in order to adapt themselves to contrasting climate variables. In this way it is possible to find the optimum elevation for the growth of larch trees in Southern Alps and also to predict the response of these trees

to the main climatic changes such as temperature increase in future.

1.5 Objective and plan of the thesis

The main objective of this thesis is to study is to understand how trees are able to grow in contrasting climate conditions by adjusting cambium phenology, wood formation dynamics and resulting tree-ring width and structure.

After chapter one which is the general introduction, in chapter 2 the material and methods that have been used for this study are presented in details.

In chapter 3, the objective to check that classical wood formation monitoring technics (e.g. weekly microcores sampling) do not influence xylogenesis, thus producing artifactual results such as wounding tissue or traumatic resin canals as defensive responses of studied trees was assessed.

The chapter 4 mainly focuses on the phenology of wood formation along the studied elevation gradient. The accurate timing of onset and cessation of cambial activity phases, as critical dates of cambial activity were extracted and compared in different sites. Then their trend along the gradient and relationship with the environmental factors were described in the selected gradient using simple and mixed linear models and non-parametric bootstrap tests.

Chapter 5 is based the comparison of Larch cambial activity dynamics in the Larch trees along elevation gradient. wood growth dynamics was describe fitting Gompertz models and growth rate as well as cell cycle length and the number of dividing cambial cells were computed. The contribution of rate and duration of cambial activity in the production of the total annual ring cell number was evaluated using sensitivity analysis of a basic physical model.

Chapter 6 is the general discussion where the main findings from the previous chapters are presented. The aim of this chapter is to draw a comprehensive picture of the relationship between the obtained results from the wood formation monitoring along an elevation gradient in the French Alps as a response to the environmental factors for drawing perspective on the long term effect of climate change in future.

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Chapter 2

Material and methods

Material and methods

2.1 Geographical location of study site

The study was conducted across an elevation gradient located in Villard St.Panrace close to Briançon (44° 53' 47" N, 6° 38' 08" E), in a west facing slope ranging from 1350 to 2300 m a.s.l in the French Alps (figure 2.1). This gradient is covered mostly by pure-uneven aged Larch (*Larix decidua* M.) trees with some patches of Silver fir (*Abies alba* M.), Scots pine (*Pinus sylvestris* L.), Stone pine (*Pinus cembra* L.) and mountain pine (*Pinus uncinata*). Four plots were selected within this elevation gradient at 1350, 1700, 2000, 2300 m a.s.l. (figure 2.2 and 2.3). The accurate geographical position of each site has been shown in the table 2.1.



Figure 2.1: Map of study site in Briançon, France.

Table 2.1: Geographical location of plots in Briançon.

Site (m)	latitude	longitude
1350	44° 50' 57.39"	6° 38' 12.23"
1700	44° 51' 4.58"	6° 37' 43.42"
2000	44° 51' 30.33"	6° 37' 30.04"
2300	44° 51' 15.63"	6° 35' 52.35"

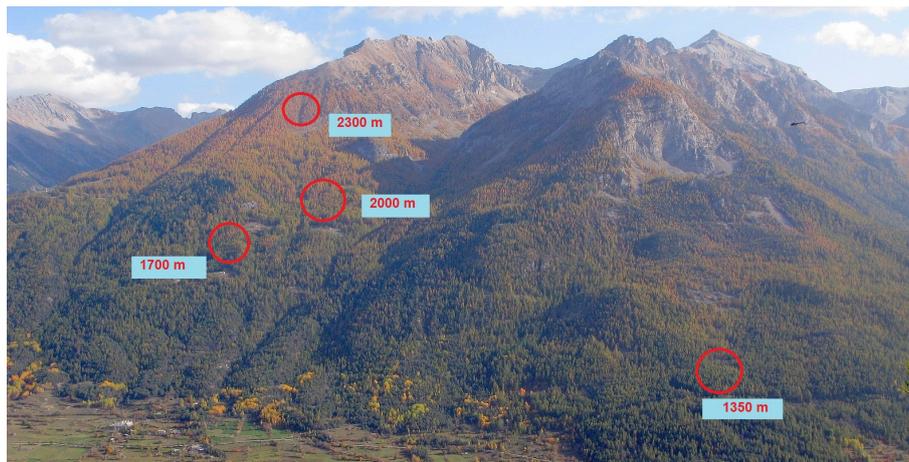


Figure 2.2: The study sites (red circles) along the elevation gradient in French Alps close to Briançon (Photo by P.Rozenberg).

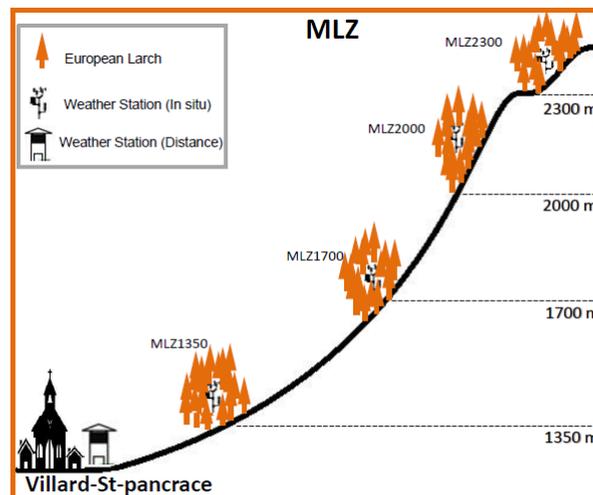


Figure 2.3: Schematic view of studied elevation gradient.

2.2 Characteristics of selected trees

At each study plot, 15 dominant Larch trees were selected for wood formation monitoring. The age, diameter at breast height (DBH) and height of each tree were measured. The mean age of trees was about 150 ± 5 years but a bit higher at 2000 m and a bit lower at 2300 m. The height of trees were about 25 ± 2 m but the trees at the top site were shorter (table in supplementary materials). At each site, a group of five trees were selected with almost the same age and diameter for further analysis (figure 2.4) (table 2.2).

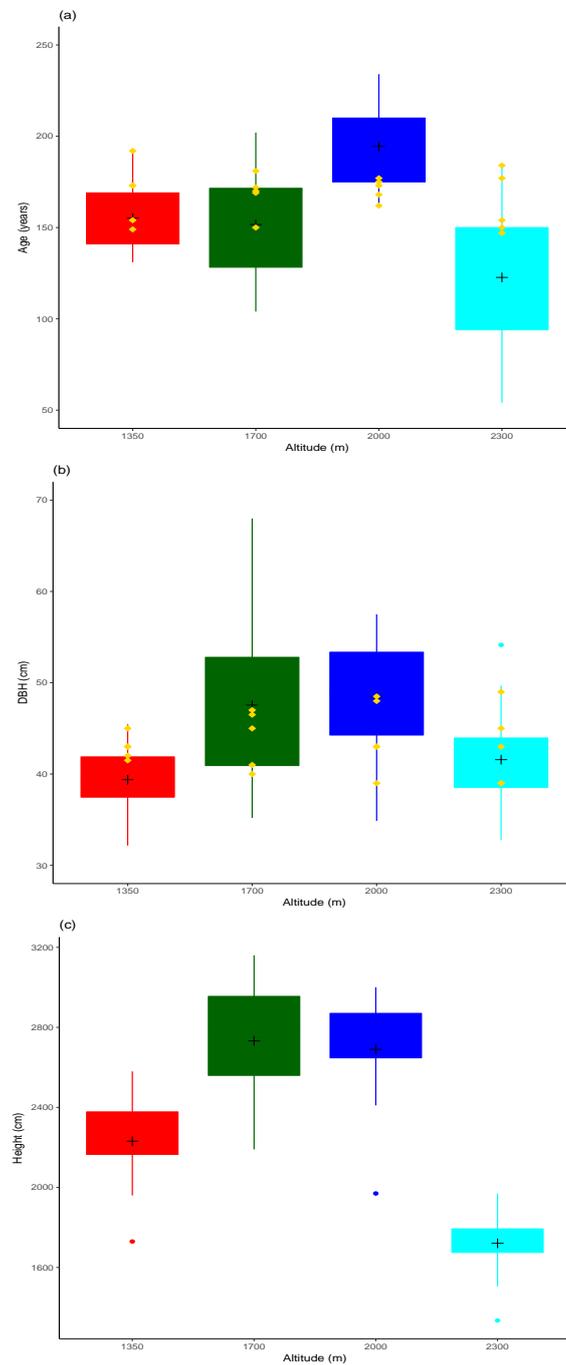


Figure 2.4: The plots of (a) the age (b) the diameter (DBH) and (c) the height of selected trees at each study site. The yellow points show the re-selected trees with the same range of age and diameter.

Table 2.2: Characteristic of selected trees with similar age and diameter

Site (m)	DBH (cm)	Age (years)
1350	43.0 ± 0.6	168.2 ± 7
1700	44.0 ± 1	168.4 ± 5
2000	44.3 ± 2	171.0 ± 2
2300	43.0 ± 2	162.4 ± 7

2.3 Environmental variables

In order to compare the climatic data in different study sites, it was tried to find the most accurate source of data such as temperature, precipitation, radiation. So the climatic data were obtained from local meteo stations in the study sites (Storm) and also the distance meteo stations (MeteoFrance) or Global data base (Worldclim). Other parameters such as soil relative water availability was then computed by a model using the climatic data such as precipitation, temperature, wind speed, radiation and soil layer characteristics

2.3.1 Temperature status along gradient

Temperature derived from "Storm" local meteo stations

The daily temperature data were recorded by the Storm data base center. Storm is the name of a local Meteo-research center belonging to the INRA in Avignon, France. https://w3.avignon.inra.fr/storm/Accueil_GRAAL.php. Climate stations have been installed by this center along the altitudinal gradient, in Briançon inside the stands. The daily maximum, minimum and mean temperature have been recorded by them. The daily mean temperature was compared along different altitudes. The comparison of monthly mean temperature along this gradient with regression analysis also shows the increasing trend of temperature with decreasing altitude ($T = -0.0054Alt + 14.3, R^2 = 0.97, P = 0.009.$) (figure 2.5). The observed trend was a decrease of 0.54 ° C per 100 m increase in altitude.

Temperature measured on tree stem

At each study sites, automatic dendrometers were installed on the stem of trees in order to record the stem size variation. They also measured temperature using thermometers. In order to evaluate the temperature recorded at tree stems, the mean

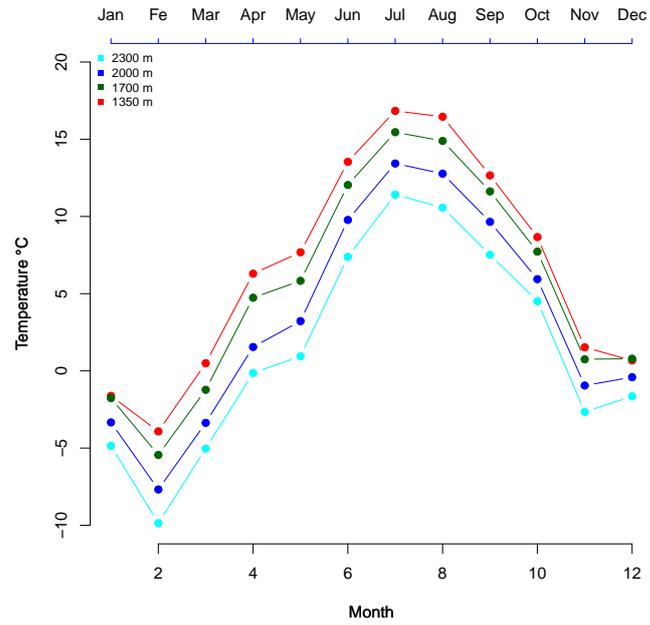


Figure 2.5: Monthly mean temperature derived from Storm meteo stations in four studied sites in 2013 (each points shows the monthly mean).

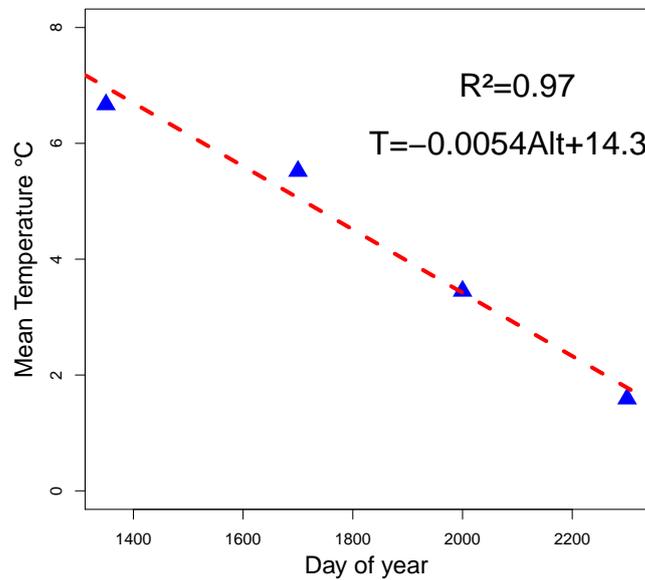


Figure 2.6: Plot of comparison the annual mean temperature derived from Storm local Meteo stations.

daily temperature was computed for each tree by averaging all the data recorded

within a day. The plots of daily mean temperature have been compared for each individual site and also along the gradient. The results of regression analysis for the mean temperature of the period in which the data were recorded by dendrometers (from January to November) showed a decrease in temperature with increasing altitude ($T = -0.0054Alt + 17$, $R^2 = 0.96$, $P = 0.002$). The observed trend can be stated as a decrease of 0.54° per 100 m increase in altitude (figure 2.7).

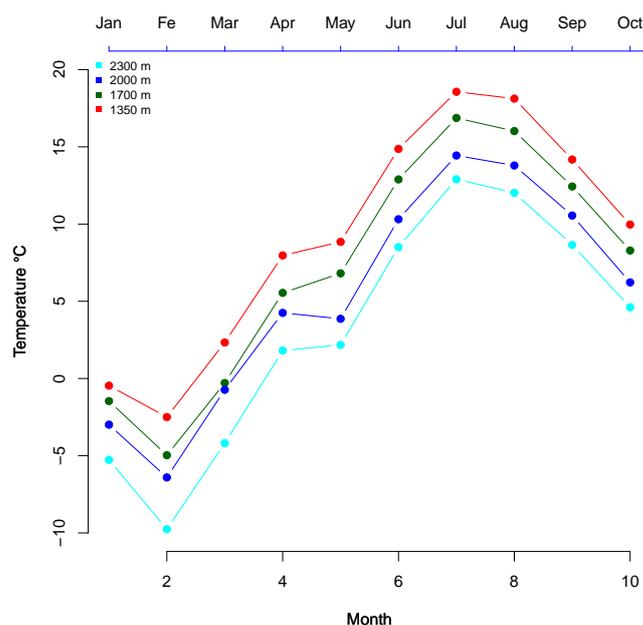


Figure 2.7: Monthly mean temperature recorded at tree stem in four studied sites in 2013 (each points shows the monthly mean).

Comparison of temperature data derived from different sources

The comparison of temperature data from Storm local climate stations and the data recorded at tree stem showed a strong accordance and significant regression analysis ($P < 0.0001$, ($R^2 > 0.90$) (figure 2.8). Since the data derived from Storm data set and the data at tree stem showed good accordance we used the Storm data base as the main resource of temperature.

Temperature derived from Meteo-France data base

Temperature data were also obtained from Meteo-France climate stations of “Villard St. Pancrace”, “LE MONETIER LES BAINS”, and “MONTGENEVRE” close

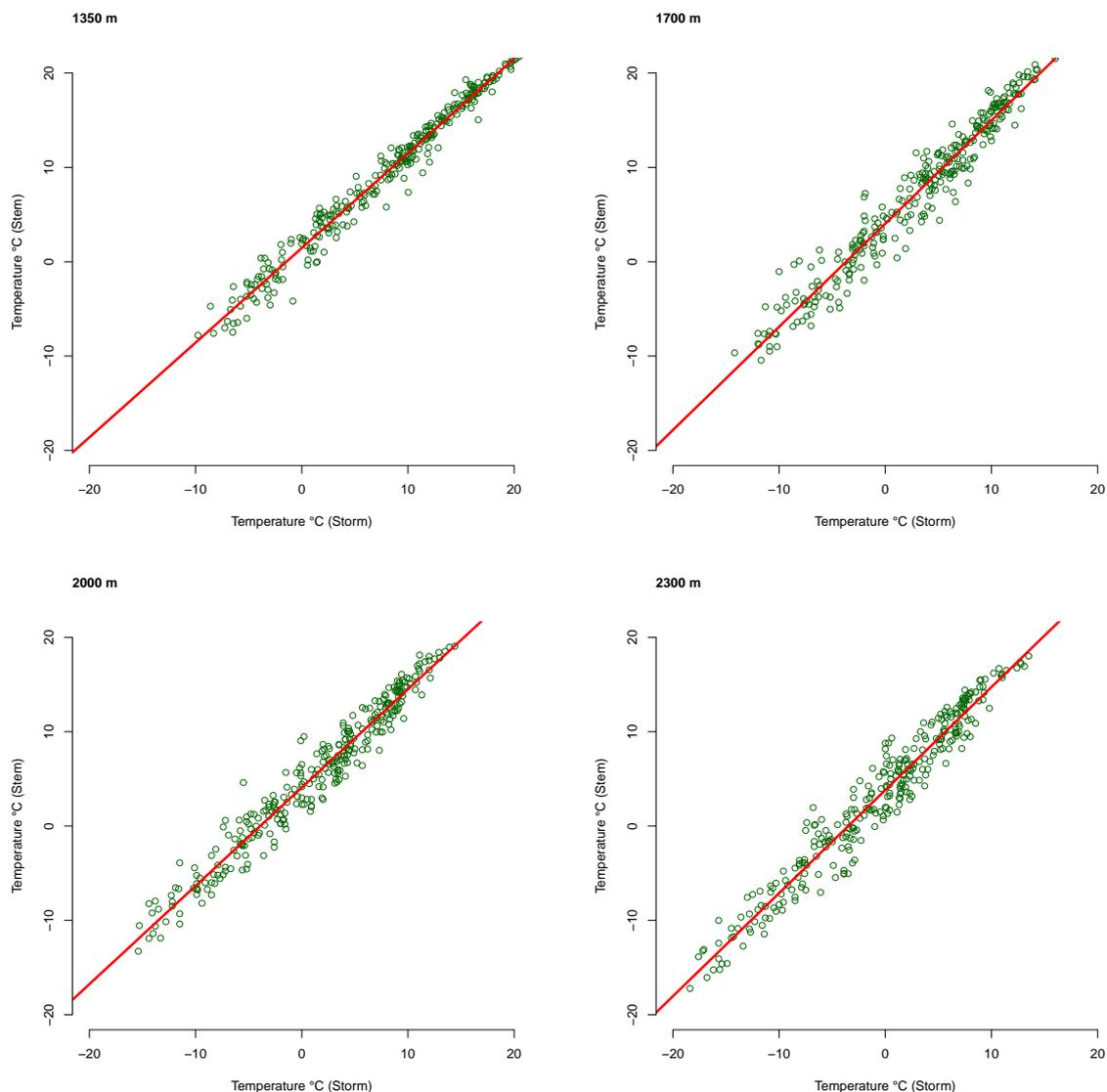


Figure 2.8: Plot of comparison the temperature data derived from dendrometer and Storm local station at the sites of 1350 m, 1700 m, 2000 m and 2300 m.

to our study site in Briançon (table 2.3). The daily minimum, maximum temperature were extracted from Meteo France stations. The daily mean temperature was computed by taking the average of minimum and maximum for each day (figure 2.9).

Table 2.3: Geographical position of Meteo-France Stations close to Briançon

Site	Latitude	Longitude	Altitude
Villard St.Pancrace	44°52'48"N	6°38'24"E	1310 m
LE MONETIER LES BAINS	44°58'12"N	6°30'48"E	1459 m
MONTGENEVRE	44°55'48"N	6°43'12"E	1848 m

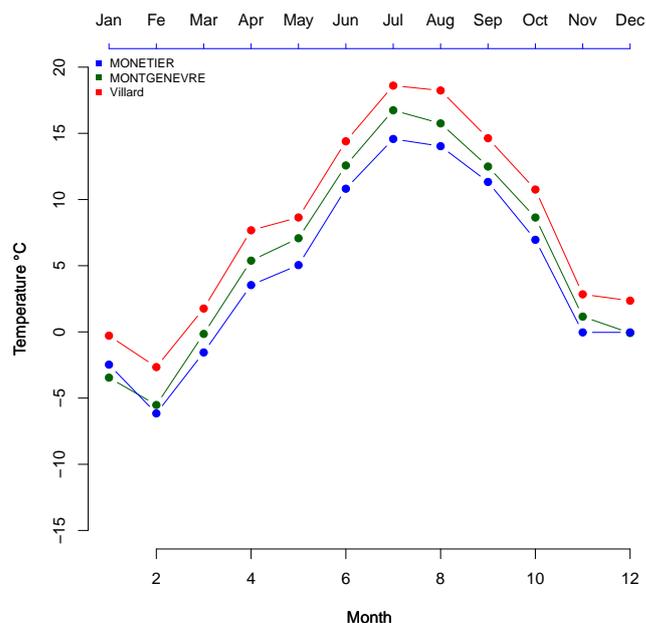


Figure 2.9: Plot of comparison the monthly mean temperature recorded by Meteo France stations.

Comparison of temperature data derived from Storm and Meteo France dataset

The comparison of Meteo-France stations also showed a good accordance with other resources of temperature so they were considered as good resources for obtaining other climatic data such as precipitation ($P < 0.001$, $R^2 > 0.90$) (figure 2.10).

Temperature status along gradient

In order to see if the study year (2013) had a significant different climate, the long term comparison of temperature and precipitation data (2004 to 2015) have been done. The results of this comparison revealed that the study year 2013 was a normal year (figure 2.11).

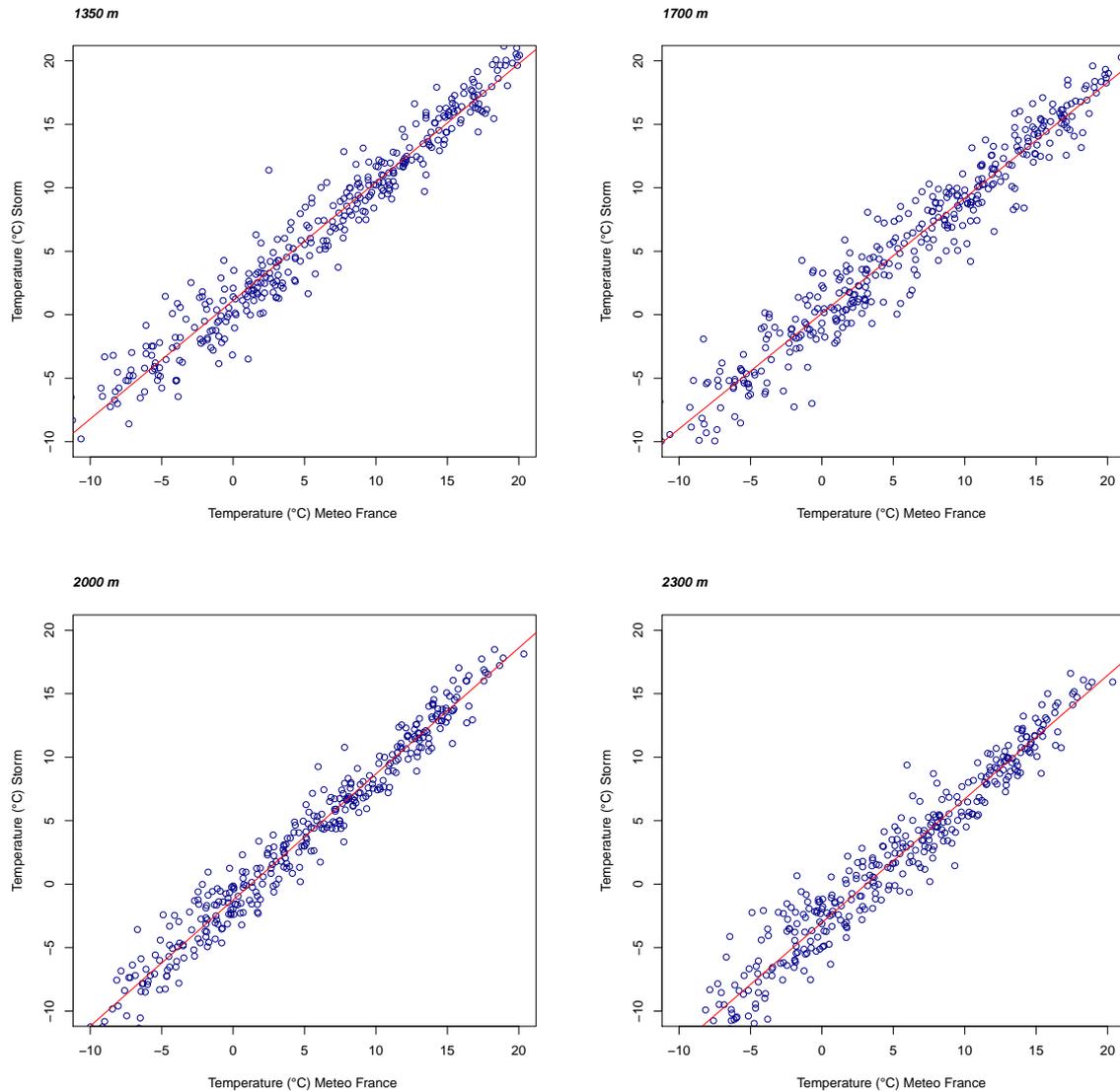


Figure 2.10: Plot of comparison the temperature data derived from MeteoFrance and Storm local station at the sites of 1350 m, 1700 m, 2000 m and 2300 m.

Computing growing degree days

Growing degree days (GDD) is the measurement of heat accumulation that is usually used to estimate the growth and development of plants during the growing season. The basic concept is that development will only occur if the temperature exceeds a minimum threshold known as T base. The GDD is usually computed using the base temperature of 5 ° C (Bonhomme 2000). The growing degree days (GDD) was computed for selected plot in 1350, 1700, 2000, 2300 m based on following formula 2.1) and base temperature of 5 ° C. The results of this comparison showed

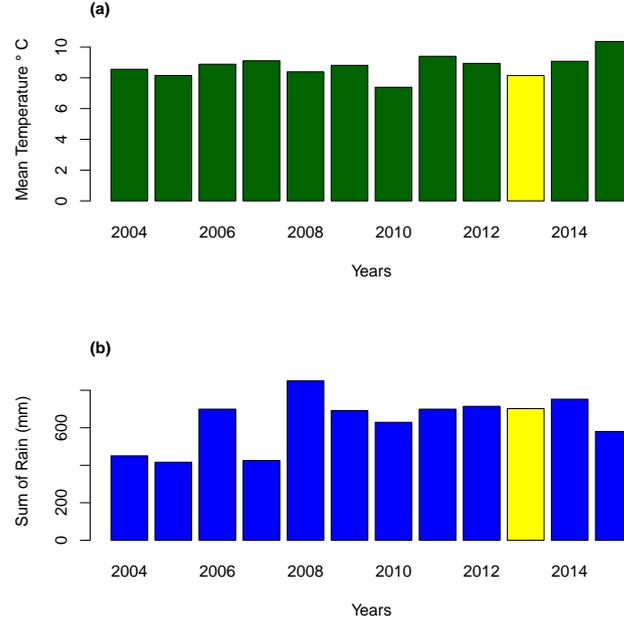


Figure 2.11: Inter-annual variation of (a) Mean temperature °C and (b) Sum of rain (mm) from Villard St. Pancrace station from 2004 to 2015. The yellow colour shows the studied year of 2013.

a decreasing trend for the amount of growing degree days with increasing altitude. The annual GDD₅ was (1714 °C at 1350 m), (1382 °C at 1700 m), (1026 °C at 2000 m), (700 °C at 2300 m). These value were found to increase by about three times from the high to the low elevation (figure 2.12).

$$GDD = \Sigma((T_{max} + T_{min})/2) - T_{base} \quad (2.1)$$

2.3.2 Precipitation status along gradient

The precipitation data were not available from Storm local Meteo station, so they were computed using the available data base. The daily precipitation data were only available from the nearby Meteo France station in Villard St.Pancrace located at 1310 m. The monthly precipitation data obtained from the "Worldclim" Global Climate data base, <http://www.worldclim.org>. These two resources were used to interpolate the daily precipitation data along the gradient. Then the slope of the monthly precipitation data derived from "Worldclim" precipitation data to interpolate the daily precipitation data (figure 2.13).

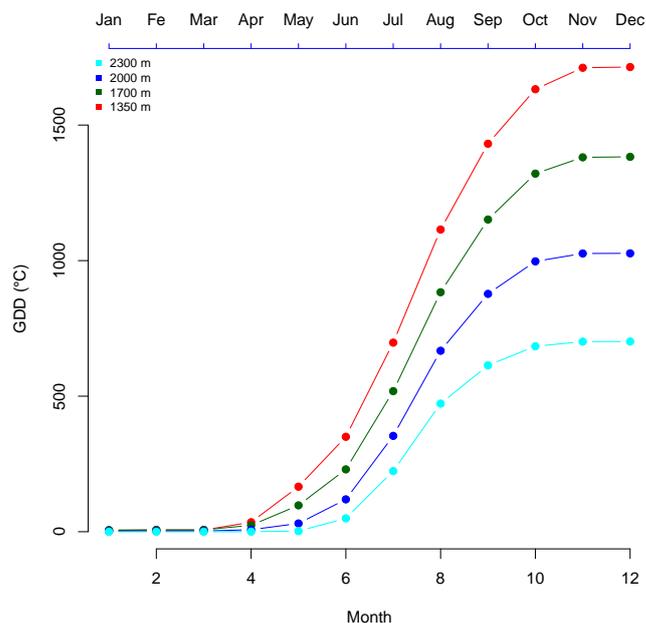


Figure 2.12: Plot of comparison the Growing degree days in Briançon.

2.3.3 Air humidity along gradient status

The air humidity was directly recorded by the "Storm" local climatic stations along this gradient of altitude. Comparing the monthly mean relative humidity in different study plots shows an uneven trend along altitude for example in March and April there is a big difference among high and low altitude in the amount of air humidity (figure 2.14).

2.3.4 Soil water status along the gradient

The soil water content along the gradient was quantified using Biljou model, <http://appgeodb.nancy.inra.fr/biljou/> (Granier et al. 1999). This model have been designed in order to estimate the water stress intensity and duration in forest stands. It can estimate the seasonal and annual water stress indices in order to characterise the drought stress that can affect growth of trees in long term. These indices are computed using daily relative extractable water (REW), that is an expression of the filling state of the soil. The REW is 100 % at field capacity and 0% at the permanent wilting point. When the soil REW drops below a threshold of 40 %, under which transpiration is gradually reduced due to stomata closure water stress is assumed to occur (Granier et al. 1999). The REW of 40 % is considered as critical REW.

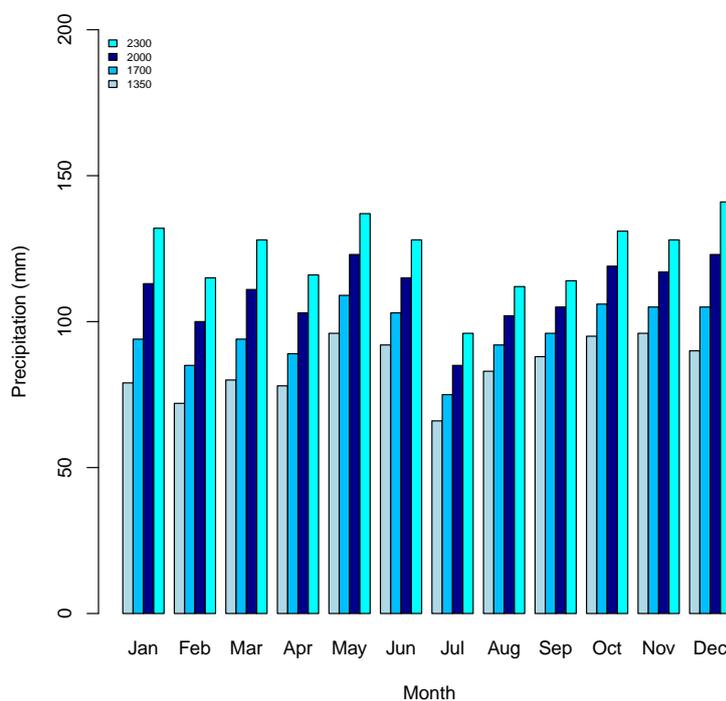


Figure 2.13: Bar plots showing the monthly amount of precipitation (mm) in the four study sites in the year of 2013.

According to that two stress indexes are computed by this model. The number of days of water stress and the water stress index (dimensionless) which cumulates the difference among REW and critical REW.

The input of this model are the meteorological data such as wind speed, rain, average air temperature, relative humidity and global radiation data. Moreover to meteorological data, the model takes soil characteristics in different layers (table 2.4 and 2.5). The soil characteristics were estimated in 2 layers using the soil manual books (Biljou data base) and specific water capacity of soil (mm) that was measured at each site by our project. The summary of climatic data that have been used is shown in the following table (table 2.6).

2.4 Measuring the stem diameter changes in trees

The stem diameter changes have been measured all over the the growing season

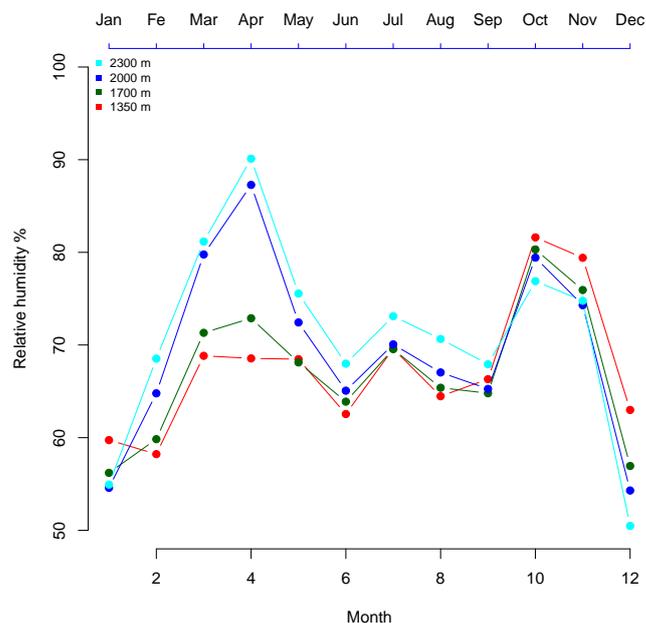


Figure 2.14: Plot of comparison the air humidity in the four study sites in the year of 2013.

Table 2.4: Soil characteristics at different study sites in Briançon

Site (m)	Soil type	Soil water Capacity (mm)
1350	Regosol/Colluviosol	46.5
1700	Colluviosol	66.7
2000	Eutric Brunisol/Calisol	53.2
2300	Calisol	38.7

Table 2.5: The soil characteristics at different sites

Altitude (m)	Layer number	Gravimetric water	Root pro-portion %	Depth (cm)	Water reserve (mm)	Bulk density
2300	1	0.05	0.6	40	25	1.2
2300	2	0.1	0.4	55	14	1.3
2000	1	0.05	0.6	20	30	1.2
2000	2	0.1	0.4	40	23.2	1.3
1700	1	0.05	0.6	40	40	1.2
1700	2	0.1	0.4	60	26.7	1.3
1350	1	0.05	0.6	15	20	1.2
1350	2	0.1	0.4	35	26.5	1.3

Table 2.6: Summary of meteorological data sources. Storm* is the name of local meteo station located at study site

Environmental variable	Source	Transformation/Type
GDD5	Local station (Storm*)	Equation
Global radiation	Distant station (Meteo France)	
Precipitation	Distant station (Meteo France), world clim	Correction
Relative humidity	Local station (Storm*)	
Temperature (Max, min, mean)	Local station (Storm*)	
REW	Local and distant stations	Biljou model
Drought index	Local and distant stations	Biljou model
Wind speed	Distant station (Meteo France)	

using two types of dendrometers: band and point dendrometers.

2.4.1 Manual band dendrometer

Manual band dendrometers were installed on the trees stem in order to measure their size variation (figure 2.15). Using this type of dendrometer makes it possible to measure the stem size with the resolution of 1/10 millimeter. The band dendrometers were installed on the 15 trees selected for the wood formation monitoring in each site. The stem size were recorded manually at the weekly intervals along the growing season (on the same dates that wood formation microcoring have been done). The stem size increment of each tree was computed with finding the difference of stem size at each sampling and the first measurement between the stem size at the beginning of growing season (figure 2.16) .



Figure 2.15: Manual band dendrometer on a tree stem

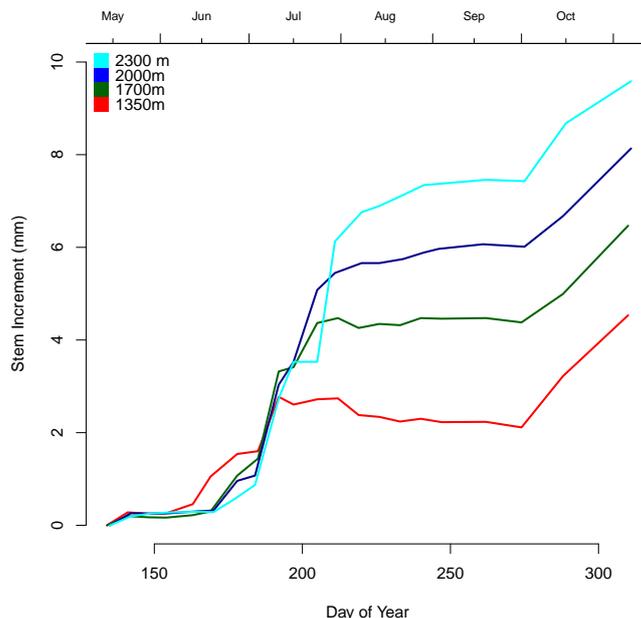


Figure 2.16: Mean stem size increment recorded by manual band dendrometers in the study sites

2.4.2 Automatic point dendrometer

Automatic point dendrometers have the ability of long term stem size variation recording. They provide useful data about the short time stem size changes such as daily or hourly scale. In this study, four automatic dendrometers were installed at each study sites at the beginning of the the studied year in 2013. In automatic dendrometers, the stem size variation were recorded on a data logger inside the dendrometer. The recorded data were extracted from data loggers in November of 2013 using a computer via bluetooth connection. The extracted data was in the form of an excel file with columns of date and time, stem size and also temperature data. There are two approaches to extract the stem size variation data. The daily approach consists of extracting one value per day from the average of total daily circumference measurements (Tardif et al. 2001) or taking the daily maximum value (Bouriaud et al. 2005). The other method is the stem cycle approach based on the patterns of stem shrinking and swelling (Downes et al, 1999). In this method, the amplitude and duration of three phases of contraction, expansion and stem size increase can be calculated (Deslauriers et al. 2003).

In this study the most common method in dendrometer data analysis that is the daily stem variation approach was used. In this method the maximum daily amount

of stem size at each day was extracted using R software and the stem size variation was monitored in all the trees at each site (figure 2.17). The first evaluation of data showed that all the dendrometers didn't work properly. In six trees among 16 no data was found. In these trees that the data were totally missed the problem was reported by technicians to be related to data loggers (electronical or software problems). The problematic dendrometers were replaced by new one but the data for the of 2013 was missed (table 2.7 to 2.10).

Four dendrometers worked properly only in some period of the year. In these trees there was not any problem for downloading the data but no data was recorded in some parts of the year. The problem in these trees was reported by technicians to be related to capturing the position (resulted from temperature or humidity) or because of problem in data loggers (electronical or software problems). The plots of stem increment changes in the studied sites have been shown in the following graphs (figure 2.17). The aim of using automatic dendrometers was to monitor the stem changes in the selected trees for wood formation monitoring in consecutive growing season but only one or two automatic dendrometers worked properly at each site. In the table 8 the number of automatic dendrometers that worked properly and the date of recording data have been summarized.

Table 2.7: Trees with automatic dendrometer at 1350 m

Site (m)	Tree	First day of record	Last day of record
1350	4385	06/01/2013	06/11/2013
1350	4389	28/12/2012	06/11/2013

Table 2.8: Trees with automatic dendrometer at 1700 m

Site (m)	Tree	First day of record	Last day of record
1700	4398	28/12/2012	06/11/2013
1700	4401	16/04/2013	06/11/2013
1700	4402	28/12/2012	06/11/2013

Table 2.9: Trees with automatic dendrometer at 2000 m

Site (m)	Tree	First day of record	Last day of record
2000	4418	29/12/2012	06/11/2013
2000	4421	17/04/2013	07/11/2013
2000	4425	13/04/2012	24/10/2013

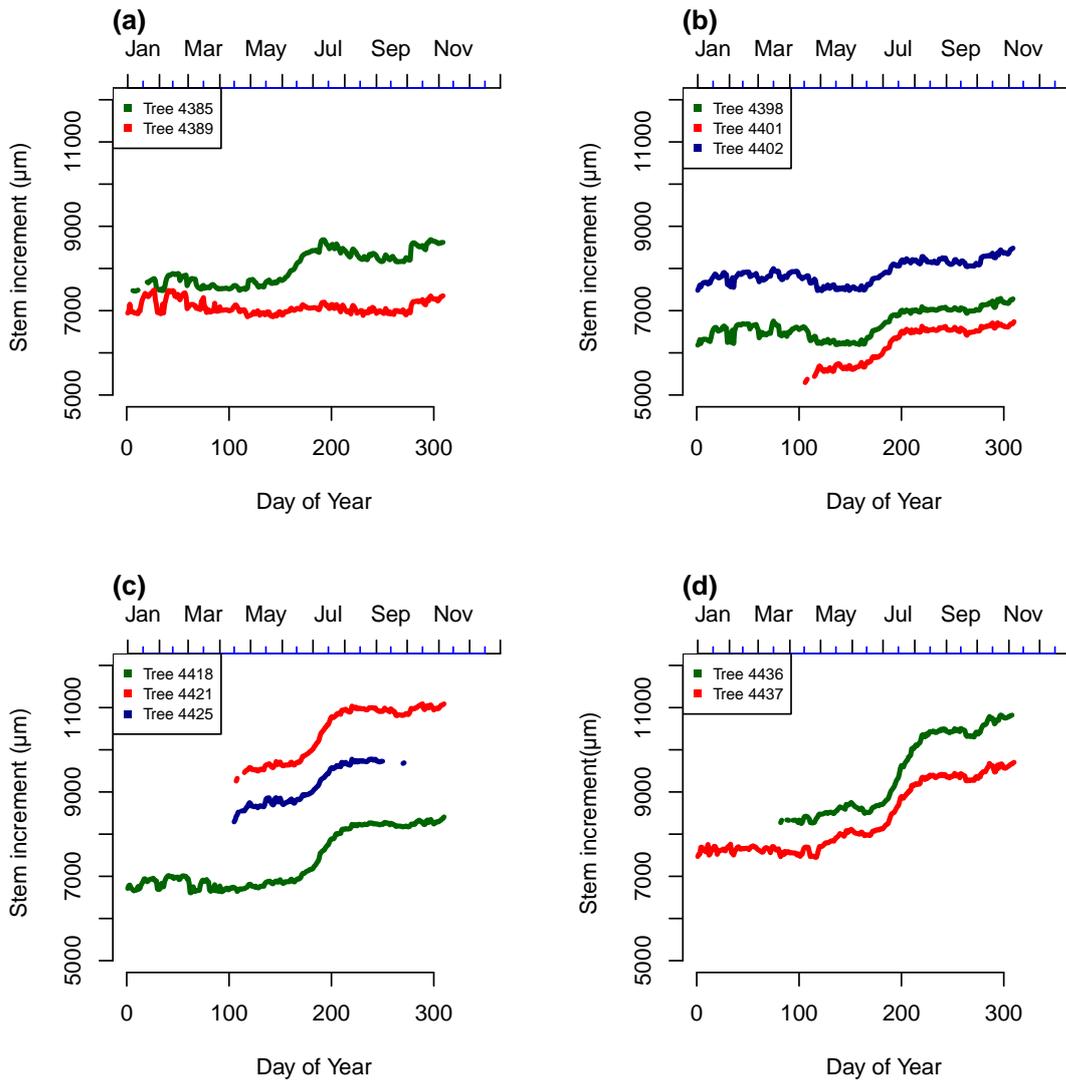


Figure 2.17: The mean stem increment recorded by automatic dendrometers in the four study sites located at (a) 1350,(b) 1700, (c) 2000 and (d) 2300 m.

Table 2.10: Trees with automatic dendrometer at 2300 m

Site (m)	Tree	First day of record	Last day of record
2300	4436	28/10/2012	07/11/2013
2300	4437	28/12/2012	07/11/2013

2.5 Wood formation variables

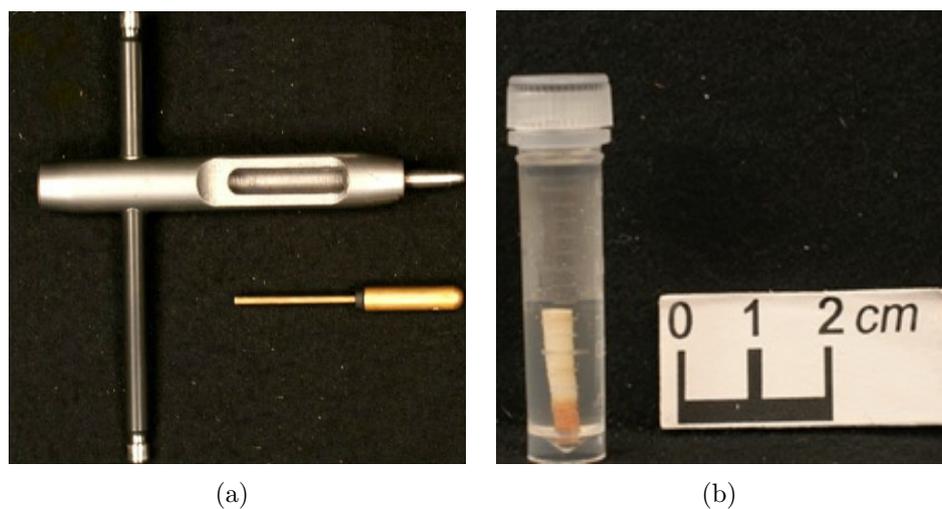


Figure 2.18: Microcoring tool (a) The trephor tool (b) a core extracted from tree preserved in a Eppendorf microtube

2.5.1 Microcoring method

In the growing season of 2013, from May to November micro-cores were extracted at about a weekly time step at breast height from each tree using a Trephor by LERFOB team (the sampling date in supplementary materials). Trephor is a tool specially designed for microcoring from trees by Centro Studi per l'Ambiente Alpino in San Vito di Cadore (Vitzani, Belluno, Italy) (Rossi et al. 2006 a). It consists of a handle and a drilling head made of steel stainless and two asymmetric coplanar arms. The piercing handle is composed of a cutting tube connected to the handle (figure 2.18 (a)).

The Trephor's cutting tube then is inserted in tree stem using a plastic hammer. It is crucial to put the Trephor perpendicularly on the stem (perpendicular to wood fibers) to take the samples in radial-tangential direction. It is better to tap the hammer as less as possible to avoid damaging the cambial zone. Then the head of trephor is removed by turning it to right and left. After taking the Trephor out from the stem, the microcore can be extracted from cutting tube by extractor tool. To avoid pathogens attack to trees, the head of Trephor should be cleaned regularly by alcohol.

2.5.2 Preserving samples

The extracted microcores then were placed in Eppendorf microtubes filled with 50 % diluted ethanol solution. The bark of samples were placed down into tubes. It will allow the operator to take the samples out from the wooden part and therefor the cambial area will be preserved (figure 2.18 (b)). The reference code of each microcore then was noted on the microtubes. In the laboratory, the microtubes were stored in a refrigerator at 5 ° C.

2.5.3 Preparing anatomical slides

The microcores are composed of two very different parts: one part that is hard and resistant and the fragile part composed of cambial zone, phloem and bark. This heterogeneity makes the preparing of anatomical sections difficult for example a common problem is breaking the cambial zone in two parts which makes the sample useless. Coating samples with paraffin, before cutting with microtome is a way to avoid breaking microcores. Under the binocular microscope, the microcores were oriented to put the wood fibers parallel to the vertical axis. Then the upper face of the core was marked with a pencil (Graphite HB) in order to point the vertical orientation which must be preserved during the coating (figure 2.19). This operation must be done quickly to avoid drying of microcores exposed to the lamps of the binocular ([Harroue et al. 2011](#)). The impregnation of the microcores was carried out by successive dipping of the samples in eight treatment baths using an impregnation machine (STP120, Microm, MM France, Francheville, France) (figure 2.20). The procedure of impregnation was described by [Anderson Bancroft \(2002\)](#) and used by [Rossi et al. \(2006 b\)](#). The microcores were dehydrated by immersing in five ethanol baths with concentration of 50% to 100%, cleaning with a bath of Histo-Clear, a low toxic natural solvent of paraffin. Finally, the microcores were immersed in two hot baths (65 ° C) of liquid paraffin to penetrate it in the wood and fill the lumen of cells. The complete procedure took about 20 hours. Each microcores were then placed at the bottom of a preheated aluminum mold according the direction of the fibers (figure 2.21). The paraffin solidified rapidly at the bottom of the mold with a coating machine. After about ten minutes the paraffin blocks were ready to be removed from the mold.



Figure 2.19: Marking the upper face of microcore using a pencil, (Photo by M.Harore).



Figure 2.20: Impregnation machine (STP120, Microm, MM France, Francheville, France), (Photo by M.Harore).

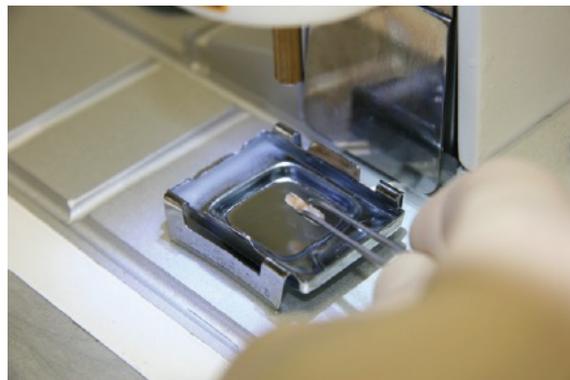


Figure 2.21: Coating microcores with paraffin, (Photo by M.Harore).

2.5.4 Sample cutting using microtom

The paraffin blocks were firmly attached to the sample holder of the rotary microtom (figure 2.22). The paraffin blocks were oriented perpendicular to the blade. The cutting speed was approximately 78 mm/s. The thickness of the cut must be

adjusted according to the species, from 1 to 5 μm . The rotary microtom produces a continuous paraffin cuts containing the anatomical sections (figure 2.23). Then the best sections were selected and placed on a thin lame in the same orientation and the lames were dried on a hot plate.



Figure 2.22: Rotary microtom, (Photo by M.Harore).

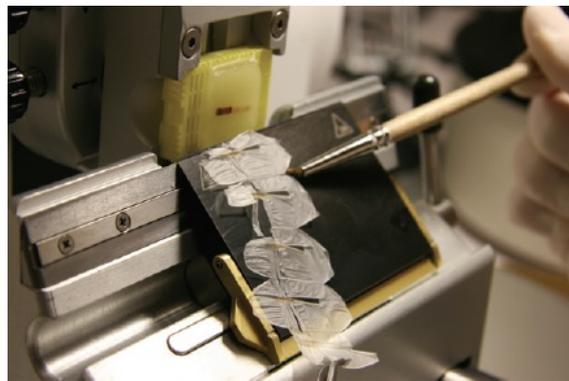


Figure 2.23: Cutting sample using a rotary microtom, (Photo by M.Harore).

2.5.5 Coloration

The slides were first dried for at least one hour at 50 ° C on a hot plate to increase the adhesion of cuts. Then the slides were put into two treatment baths successively: (1) Twenty minutes in a HistoClear bath to remove the paraffin residues; and (2)

Twenty minutes in a pure ethanol bath (100%) to clean the HistoClear residues. Then sections were ready to be colored with cresyl violet acetate (figure 2.24). To stain the sections, the thin cuts were to soaked for about 20 minutes in the dye. Then, the slides were rinsed for a few minutes in a bath of distilled water. After that, they were removed from the distilled water bath and put on an absorbent paper. The sections were then fixed immediately with histolac MRL. The cuts were then dried by exerting a slight pressure on them with absorbent paper(WYPALL L40, white paper). Immediately after, a few drops of histolac LMR were put on the lame, then a lamel was placed on it. Then the slides were placed in a flat surface to be fully dried (about one week).

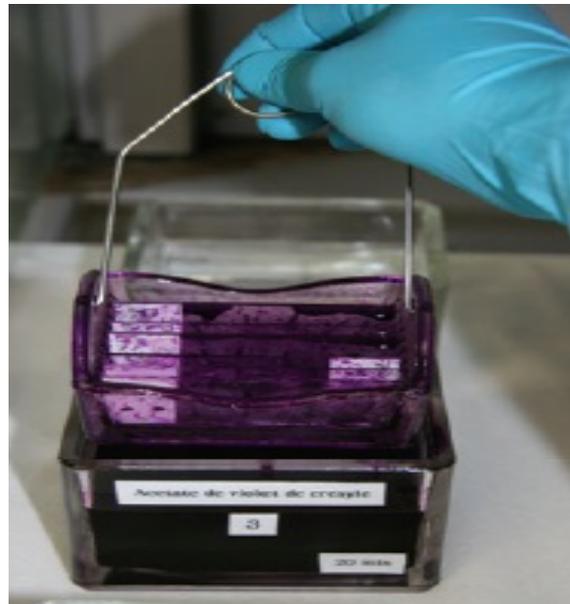


Figure 2.24: Coloration of the section with cresyl violet acetat (Photo by M.Harore).

2.5.6 Assessment of wood anatomy from anatomical slides

The observations of anatomical slides was done using an optical microscope (ZEISS, Germany) with objectives of 10, 20, 40, in normal and polarized light. In wood formation monitoring studies, the xylem formation divided into four main differentiation phase. (1) the dividing phase; during which the cambial mother cells divide to create the new daughter cells; (2) the enlarging phase, during which the size of newly formed xylem cells are expanded in the radial direction; (3) the thickening phase; during which the secondary cells walls formation and lignification occurs; (4) the maturation phase, the death of xylem cells occurs (Wilson, 1984) Previous year (P) was also counted in three radial files. Cambial cells can be distinguished by their thin primary wall and small radial diameter. In the enlarging phase cells were larger than cambial cells (at least two to three times) and had a thin primary wall that were not birefringent under polarized light.

The formation of the secondary wall begins with the deposition of microfibrils of cellulose and hemicellulose. Lignin acts as a cement and will be deposited between intercellular layers and microfibrils (Donaldson, 1991).

The secondary wall will form in addition to primary thin wall in wall thickening phase. Cells at the wall thickening and lignification phase, were birefringent under polarized light, due to the particular arrangement of cellulose microfibrils in the secondary wall they were shining under polarized light (Abe et al, 1997). They present also a gradient of pink-violet to blue color from inside to the outside of cell walls. Finally, the cells undergo programmed cell death. The mature tracheids have walls completely lignified and totally blue (figure 2.25). In each sample three radial files were chosen and the number of cambial cells (nC); enlarging (nE); wall thickening (nW) and mature cells (nM); and the number of cells in the previous ring (nP); were counted.

2.5.7 Computing crucial elaborated data

After counting the number of cells at each phase of developing xylem in three radial files at each tree, the data were recorded as an excel file. The data manipulation have been done using CAVIAR package in R software (Rathgeber et al, 2012). After formatting the excel file according to the format of CAVIAR package, the “readExcelCountTable” function was used to read the data. Then overview and visualisation of the raw data has been done using “overviewCellCountTable” function. The presence of all the required variable was checked. This function also provides

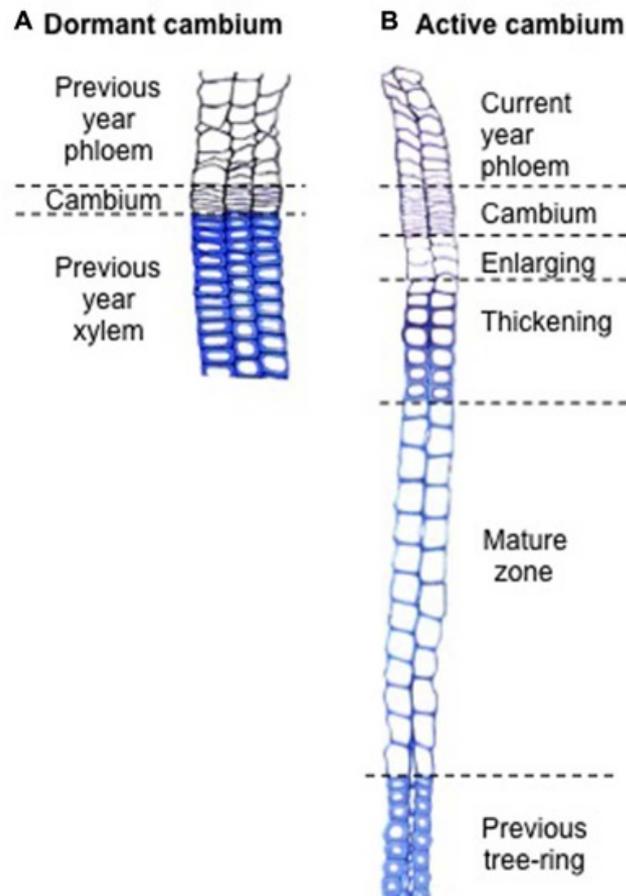


Figure 2.25: Schematic view of the cross-sections through resting and developing radial xylem cell files in conifer. (A) Dormant cambium during winter composed of thin cambial cells layers. (B) Active cambium and associated developing radial cell files at the beginning of summer. The active cambium, the developing xylem, which is composed of an enlarging zone, a thickening zone, and a mature zone, is embedded between the cambium and the previous tree ring (Rathgeber et al, 2016).

a short summary of the data set. After checking the imported raw data, the errors and outliers were detected to improve the overall quality of data using “plotClevelandDotchart” function. This function plots a revised Cleveland dot chart for each quantitative variable in cell count data frame and groups them per sites. In order to detect the outliers at the radial file level, the “plotWoodFormationDynamics” function was used. It allows checking of all cell count data at different levels and

plots the number of cells for each zone of differentiation (nC , nE , nW , nM , and nP). Then these days will be used to compute the critical dates of wood formation. Five main critical dates have been defined for wood formation in trees (1) onset of enlarging period (bE); (2) the onset of wall thickening period (bW); (3) the onset of occurrence of the first mature tracheids (bM); (4) the cessation of enlargement period (cE) and finally (5) the cessation of wall thickening and lignification period (cW) (Rathgeber et al, 2016). These five critical dates, along with their confidence intervals, were computed using the “computeCriticaldates” function. The visualisation of critical dates was done also using “plotWoodFormationcalender” function. The final ring cell number (RCN) were also computed using “computeRingCellNb” function in Caviar. This function automatically estimates RCN by pulling together the data from all the samples taken after cessation of cell enlargement. The dynamics of cell production was computed using “fitGompertz” function in CAVIAR. This function fits the data to the Gompertz model in order to estimate the daily cell production (Rossi et al, 2003):

$$nC(t) = A.e^{-e^{\beta-k.t}} \quad (2.2)$$

which nC is the cumulative number of cells, A is the upper horizontal asymptote parameter representing the final number of tracheid, β is the x-axis placement parameter that reflects the choice of origin time, and k is the growth rate parameter that determines the spread of the growth curve along the time axis and DY presents the day of year. This function will compute the model efficiency (R^2), and the corresponding maximal rate of tracheid production, as well as the mean rate for the period during which 90 percent of the tracheid cells were produced (Rathgeber et al, 2012).

2.5.8 Tracheidogram

The dimension of tracheids was measured for all the fifteen trees at each study site. In order to do the measurements, a good section of each tree containing the complete tree ring was selected. Digital images were taken from the section using a microscope equipped with a digital camera (Sony, XCDU100 CR), connected to a computer. The measurement was done manually using the software of “Videometer” on the recorded images. The radial and tangential dimensions of all the tracheids were measured in five radial files. In order to calculate the lumen area, the tracheids were assumed to have a rectangular shape (figure 2.26). The surface of lumen area

and mean value of each radial and tangential wall were calculated using the following formula:

$$\text{Lumen area} = b * d \quad (2.3)$$

$$\text{Mean value of radial wall} = (c - d)/2 \quad (2.4)$$

$$\text{Mean value of tangential wall} = (a - b)/2 \quad (2.5)$$

The comparison of radial wall thickness and lumen area in different sites have been shown (figure 2.27 and 2.28).

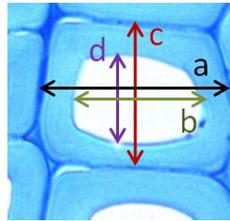


Figure 2.26: Transverse section of a tracheid. (a) and (b) show the tangential measurement, (c) and (d) show the radial measurement

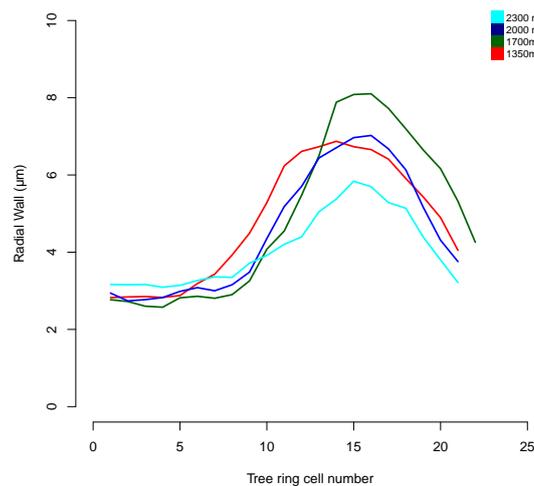


Figure 2.27: Comparison of radial wall thickness in four study sites.

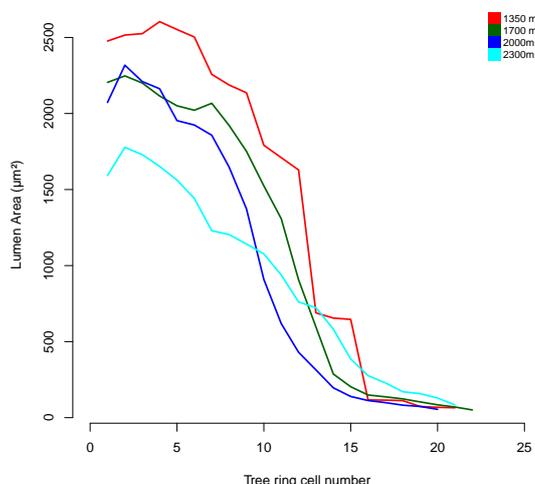


Figure 2.28: Plot of comparison the lumen area in the four study sites.

2.5.9 Traumatic resin canal analysis

1. Development of traumatic resin canal in *Larix decidua*

The observation of anatomical slides with microscope, showed that all the 60 selected trees produced traumatic resin canal (TRC) in 2013. They were formed usually as one or two continuous or non-continuous tangential bands. At each site five trees were selected to study TRC development. Three main phases were founded for the development of traumatic resin canals. (1) Anticlinal divisions in cambium to form the first epithelial cells; (2) Enlargement and division of newly formed thin wall epithelial cells as a circle and increasing the space among them; (3). Maturation of resin canals with wall thickening of Epithelial cells (figure 2.29). The placement of TRC was in the xylem part and non of them occurred among phloem. The phenology of different phases of TRC formation showed no evidence of it's occurrence in the dormancy of cambial zone.

The first sign of TRD formation in all the sites was observed between 12 to 20 of June, when the cambium was active. The newly formed resin canals passed to the second phase within a week and formed the cluster of thin-wall Epithelial cells with an enlarged space among them. Although the epithelial cells derived from cambial cell derivations formed very fast as a resin canal, their maturation lasted about two months. Around the middle of August, the epithelial cells surrounding the resin canals had thick walls.

At each tree, the placement of traumatic resin canals within the tree rings (early

wood, late wood and transition) was evaluated. The results showed that at lower altitudes, the traumatic resin canals mainly occurred in late wood (53%) while in top altitude it was mainly in early wood (80 %) (table 2.11).

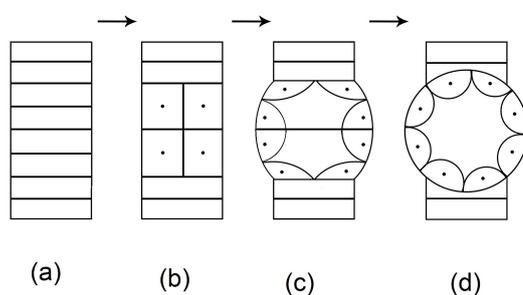


Figure 2.29: Development of traumatic resin canal in *larix decidua* (a) cambial cells (b) the squares show the first epithelial cells derived from cambial cells division (c) division of epithelial cells and increasing the resin canal size (d) A fully formed traumatic resin canal.

Table 2.11: Distribution of traumatic resin canals in tree ring

Site (m)	Late wood	Early wood	Transition
1350	53%	33 %	13 %
1700	40 %	60%	0%
2000	27%	53 %	20 %
2300	20%	80%	0 %

2.5.10 Data standardisation

Cambial activity and wood formation, can be different around the stem of trees at the same height (Wodzicki & Zajaczkowski, 1970). The methods of wood formation monitoring in trees, are usually based on the differences of number or size of cells between two consecutive sampling. So it is crucial to decrease the noises resulted from sample positioning in stem of trees. Rossi et al. (2003) build a method in order to standardize the wood formation data. It is a way to reduce the noises in the wood formation data set and can be done using the total number of cells in the tree ring of previous ring for each tree (Rossi et al, 2003). This method is based on the heterogeneity of growth in the previous formed tree ring which can also repeat for the following tree ring.

$$Xi_j = Xi_j * \frac{\bar{X}_{j-1}}{X_{j-1}}$$

Xi_j : The standardized number of tracheid for the sampling of i and the year of j

\bar{X}_{j-1} : The mean number of cells of tracheid cells for the sampling i and the year of j

Xi_{i-1} : The number of cells for the sampling i in the previous year

Methods of evaluating of the effect of standardization

In this study the `standardiseCellCounts` function of `CaviaR` package was used in order to standardise the recorded wood formation data (Rathgeber, 2012). The two estimators of the goodness-of-fit (EF and RMSD) were computed for each tree. The effect of standardisation on improving the wood formation data was evaluated using three methods. (1) comparing the signal to noise ratio SNR; (2) computing the efficiency of Gompertz model (EF); (3) comparing the RMSD (root mean square deviation) of Gompertz model.

2.5.11 Results of applying standardisation on the raw data

Signal to noise ratio SNR

The signal to noise ration in raw data aggregated by mean and the standardised data were compared in 15 selected trees at each site. The results of this comparison showed that at the lowest site located at 1350 m the SNR only increased in the standardised data of 8 trees among 15. This number was observed as 7, 10 and 10 trees at the sites located at 1700, 2000 and 2300 m respectively. It can be stated

that moving toward higher elevations the standardisation increased the SNR from 1/2 to 2/3 of data. It can be explained that at higher altitude the tree ring is more heterogeneous around the stem. Considering all the wood formation data set from gradient, standardisation had a positive effect on the signal to noise ratio of 35 trees among 60 (table in supplementary material).

Efficiency of Gompertz model analysis

The results of the efficiency of Gompertz model (EF) showed that the standardisation had different effect on the EF of the model in different sites. At 1350 m, the EF has increased in 8 trees among 15. For the other sites located at 1700, 2000 and 2300 m the model efficiency increased in 4, 8 and 7 trees respectively. This results show that standardisation has improved the quality of 1/2 data at 1350, 2000 and 2300 m, but it was not very efficient at 1700 m (table in supplementary material).

Comparing RMSD of Gompertz model

The comparison of RMSD showed that this value increased after standardisation. It means that the Gompertz model is less adapted to data only with considering only the RMSD value. But in conclusion it can be stated that the standardisation improved the quality of raw data by improving the signal to noise ratio and efficiency of Gompertz model although the RMSD didn't improved very much. So standardisation was applied to the whole data set before the wood formation analysis (table in supplementary material).

Supplementary materials

Table 2.12: Characteristics of selected trees (age, diameter and height) at each study site

Site (m)	Tree	DBH (cm)	Age	Height (cm)
	4382	45.44	131	2580
	4383	36.94	154	NA
	4384	42.19	149	2470
	4385	41.56	NA	2180
	4386	40.50	173	NA
	4387	37.99	139	2230
	4388	42.99	NA	2400

Site (m)	Tree	DBH (cm)	Age	Height (cm)
1350	4389	33.02	136	NA
	4390	39.04	NA	2155
	4391	38.59	192	2310
	4392	39.64	157	2175
	4393	41.40	NA	1730
	4394	36.30	NA	1960
	4395	42.86	173	2355
	4396	32.16	147	NA
	Mean		39.38± 3	155 ±19
1700	4397	55.41	107	2860
	4398	67.99	169	3160
	4399	46.49	150	2985
	4400	52.70	189	2800
	4401	61.78	172	2795
	4402	44.90	163	2955
	4403	42.03	170	3020
	4404	39.17	127	2450
	4405	35.19	120	NA
	4406	46.97	137	2720
	4407	41.24	NA	2685
	4408	36.78	202	NA
	4409	40.60	104	2190
	4410	52.86	132	2340
	4411	49.04	181	2560
Mean		47.54± 9	151± 30	2732± 283

Site (m)	Tree	DBH (cm)	Age	Height (cm)
2000	4412	43.31	NA	2495
	4413	43.31	189	3000
	4414	48.08	192	2990
	4415	51.75	162	NA
	4416	34.87	173	1970
	4417	49.36	190	2755
	4418	54.93	168	2870
	4419	43.31	175	2740
	4420	49.84	208	2870
	4421	56.05	233	2660
	4422	56.21	210	2900
	4423	57.48	217	2655
	4424	38.69	NA	2410
	4425	45.22	177	2700
	4426	49.20	234	2645
		Mean	48.5 ± 6	194 ± 24
2300	4427	42.67	154	1806
	4428	39.96	NA	1725
	4429	41.082	120	1775
	4430	49.36	90	1930
	4431	32.80	94	1710
	4432	36.62	74	1540
	4433	39.49	132	1335
	4434	40.12	147	1505
	4435	54.14	184	1710
	4436	39.49	122	1660
	4437	38.53	150	1690
	4438	38.53	97	1780
	4439	49.68	177	1950
	4440	35.82	54	1725
	4441	45.22	NA	1970
	Mean	41.57 ± 5	122 ± 39	1720 ± 170

Table 2.13: Wood formation sampling date at each site. The dates (DOY) are in Julian days from 1 to 365.

Sample	Site			
	1350 m	1700 m	2000 m	2300 m
1	134	135	135	135
2	141	142	142	142
3	148	149	149	149
4	154	154	155	155
5	163	164	164	164
6	169	169	170	170
7	178	178	178	177
8	185	184	184	184
9	192	192	192	192
10	197	197	197	197
11	205	205	205	NA
12	212	212	211	211
13	219	219	220	220
14	226	226	226	226
15	233	234	234	234
16	240	240	241	241
17	247	247	246	245
18	262	262	261	262
19	274	275	275	275
20	288	288	288	289
21	310	310	311	311

Table 2.14: Evaluation of SNR for the site located at 1350 m

Site	Year	Tree	Species	Raw	Mean	Sta	Dif	Pos	Neg	Best
MLZ1350	2013	4382	Larix decidua	3,41	3,14	6,13	2,99	1	0	6,13
MLZ1350	2013	4383	Larix decidua	4,56	4,24	4,67	0,43	1	0	4,67
MLZ1350	2013	4384	Larix decidua	13,61	17,64	15,31	-2,33	0	1	17,64
MLZ1350	2013	4385	Larix decidua	8,73	8,03	26,98	18,95	1	0	26,98
MLZ1350	2013	4386	Larix decidua	3,08	2,95	5,34	2,39	1	0	5,34
MLZ1350	2013	4387	Larix decidua	11,59	11,42	14,04	2,62	1	0	14,04
MLZ1350	2013	4388	Larix decidua	11,28	38,71	32,33	-6,38	0	1	38,71
MLZ1350	2013	4389	Larix decidua	6,21	15,5	13,96	-1,54	0	1	15,5
MLZ1350	2013	4390	Larix decidua	8,43	9,53	6,76	-2,77	0	1	9,53
MLZ1350	2013	4391	Larix decidua	8,27	9,6	8,11	-1,49	0	1	9,6
MLZ1350	2013	4392	Larix decidua	8,86	8,29	8,34	0,050	1	0	8,34
MLZ1350	2013	4393	Larix decidua	4	3,79	2,01	-1,78	0	1	3,79
MLZ1350	2013	4394	Larix decidua	10,25	11,1	12,22	1,12	1	0	12,22
MLZ1350	2013	4395	Larix decidua	4,74	4,12	9,74	5,62	1	0	9,74
MLZ1350	2013	4396	Larix decidua	11,08	63,33	25,95	-37,38	0	1	63,33

Table 2.15: Evaluation of SNR for the site located at 1700 m

Site	Year	Tree	Species	Raw	Mean	Sta	Dif	Pos	Neg	Best
MLZ1700	2013	4397	Larix decidua	19,77	36,67	23,3	-13,37	0	1	36,67
MLZ1700	2013	4398	Larix decidua	6,77	6,62	8,69	2,07	1	0	8,69
MLZ1700	2013	4399	Larix decidua	8,69	11,26	20,19	8,93	1	0	20,19
MLZ1700	2013	4400	Larix decidua	5,45	37,48	18,56	-18,92	0	1	37,48
MLZ1700	2013	4401	Larix decidua	13,56	18,1	223,29	205,19	1	0	223,29
MLZ1700	2013	4402	Larix decidua	11,31	11,54	6,52	-5,02	0	1	11,54
MLZ1700	2013	4403	Larix decidua	10,38	9,75	24,98	15,23	1	0	24,98
MLZ1700	2013	4404	Larix decidua	16,28	41,55	12,88	-28,67	0	1	41,55
MLZ1700	2013	4405	Larix decidua	12,23	9,69	7,04	-2,65	0	1	9,69
MLZ1700	2013	4406	Larix decidua	20,73	52,61	39,39	-13,22	0	1	52,61
MLZ1700	2013	4407	Larix decidua	9,59	9,47	5,88	-3,59	0	1	9,47
MLZ1700	2013	4408	Larix decidua	6,37	6,09	7,39	1,3	1	0	7,39
MLZ1700	2013	4409	Larix decidua	5,02	4,54	8,54	4	1	0	8,54
MLZ1700	2013	4410	Larix decidua	21,21	50	40,8	-9,2	0	1	50
MLZ1700	2013	4411	Larix decidua	5,87	5,57	9,6	4,03	1	0	9,6

Table 2.16: Evaluation of SNR for the site located at 2000 m

Site	Year	Tree	Species	Raw	Mean	Sta	Dif	Pos	Neg	Best
MLZ2000	2013	4412	Larix decidua	13,41	16,14	16,09	-0,050	0	1	16,14
MLZ2000	2013	4413	Larix decidua	18,68	24,8	23,06	-1,74	0	1	24,8
MLZ2000	2013	4414	Larix decidua	7,66	14,4	16,8	2,4	1	0	16,8
MLZ2000	2013	4415	Larix decidua	15,75	23,21	31,08	7,87	1	0	31,08
MLZ2000	2013	4416	Larix decidua	8,58	11,02	5,58	-5,44	0	1	11,02
MLZ2000	2013	4417	Larix decidua	4,81	4,01	19,58	15,57	1	0	19,58
MLZ2000	2013	4418	Larix decidua	10,35	12,28	9,01	-3,27	0	1	12,28
MLZ2000	2013	4419	Larix decidua	6,52	7,23	9,69	2,46	1	0	9,69
MLZ2000	2013	4420	Larix decidua	9,14	10,22	100,81	90,59	1	0	100,81
MLZ2000	2013	4421	Larix decidua	10,81	11,86	15,2	3,34	1	0	15,2
MLZ2000	2013	4422	Larix decidua	6,02	9,96	14,07	4,11	1	0	14,07
MLZ2000	2013	4423	Larix decidua	15,71	22,08	66,35	44,27	1	0	66,35
MLZ2000	2013	4424	Larix decidua	3,75	3,3	3,17	-0,13	0	1	3,3
MLZ2000	2013	4425	Larix decidua	8,6	9,18	23,84	14,66	1	0	23,84
MLZ2000	2013	4426	Larix decidua	8,45	8,54	12,99	4,45	1	0	12,99

Table 2.17: Evaluation of SNR for the site located at 2300 m

Site	Year	Tree	Species	Raw	Mean	Sta	Dif	Pos	Neg	Best
MLZ2300	2013	4427	Larix decidua	10,29	11,6	8,57	-3,03	0	1	11,6
MLZ2300	2013	4428	Larix decidua	11,97	12,5	8,25	-4,25	0	1	12,5
MLZ2300	2013	4429	Larix decidua	8,57	8,34	20,63	12,29	1	0	20,63
MLZ2300	2013	4430	Larix decidua	12,52	9,73	4,97	-4,76	0	1	9,73
MLZ2300	2013	4431	Larix decidua	13,29	13,29	9,32	-3,97	0	1	13,29
MLZ2300	2013	4432	Larix decidua	6,21	5,54	15,27	9,73	1	0	15,27
MLZ2300	2013	4433	Larix decidua	18,25	16,4	55,47	39,07	1	0	55,47
MLZ2300	2013	4434	Larix decidua	10,41	10,36	14,43	4,07	1	0	14,43
MLZ2300	2013	4435	Larix decidua	7,54	10,88	11,89	1,01	1	0	11,89
MLZ2300	2013	4436	Larix decidua	12,83	22,23	14,18	-8,05	0	1	22,23
MLZ2300	2013	4437	Larix decidua	8,96	24,63	57,75	33,12	1	0	57,75
MLZ2300	2013	4438	Larix decidua	2,95	2,76	17,48	14,72	1	0	17,48
MLZ2300	2013	4439	Larix decidua	9,15	13,44	13,77	0,33	1	0	13,77
MLZ2300	2013	4440	Larix decidua	7,3	7,66	13,82	6,16	1	0	13,82
MLZ2300	2013	4441	Larix decidua	9,35	10,95	11,16	0,21	1	0	11,16

Table 2.18: Efficiency of Gompertz model at the site located at 1350

Site	Tree	EF.mean.fit	EF.mean.fix	EF.sdd.fit	EF.sdd.fix	Dif	Pos	Equ	Neg	Best
MLZ1350	4382	0,83	0,82	0,83	0,83	0,01	1	0	0	0,83
MLZ1350	4383	0,83	0,54	0,79	0,53	-0,01	0	0	1	0,54
MLZ1350	4384	0,98	0,97	0,91	0,87	-0,1	0	0	1	0,97
MLZ1350	4385	0,97	0,97	0,94	0,94	-0,03	0	0	1	0,97
MLZ1350	4386	0,72	0,46	0,75	0,69	0,23	1	0	0	0,69
MLZ1350	4387	0,96	0,96	0,61	0,57	-0,39	0	0	1	0,96
MLZ1350	4388	0,77	0,63	0,78	0,64	0,01	1	0	0	0,64
MLZ1350	4389	0,94	0,94	0,94	0,92	-0,019	0	0	1	0,94
MLZ1350	4390	0,97	0,96	0,93	0,92	-0,039	0	0	1	0,96
MLZ1350	4391	0,89	0,85	0,88	0,88	0,03	1	0	0	0,88
MLZ1350	4392	0,88	0,85	0,89	0,86	0,01	1	0	0	0,86
MLZ1350	4393	0,91	0,89	0,68	0,64	-0,25	0	0	1	0,89
MLZ1350	4394	0,93	0,88	0,95	0,94	0,059	1	0	0	0,94
MLZ1350	4395	0,92	0,92	0,93	0,93	0,01	1	0	0	0,93
MLZ1350	4396	0,95	0,94	0,85	0,83	-0,11	0	0	1	0,94

Table 2.19: Efficiency of Gompertz model at the site located at 1700

Site	Tree	EF.mean.fit	EF.mean.fix	EF.sdd.fit	EF.sdd.fix	Dif	Pos	Equ	Neg	Best
MLZ1700	4397	0,98	0,97	0,92	0,86	-0,11	0	0	1	0,97
MLZ1700	4398	0,85	0,82	0,81	0,78	-0,039	0	0	1	0,82
MLZ1700	4399	0,93	0,93	0,95	0,95	0,019	1	0	0	0,95
MLZ1700	4400	0,96	0,92	0,92	0,89	-0,03	0	0	1	0,92
MLZ1700	4401	0,85	0,82	0,82	0,79	-0,029	0	0	1	0,82
MLZ1700	4402	0,98	0,97	0,94	0,93	-0,039	0	0	1	0,97
MLZ1700	4403	0,98	0,98	0,99	0,98	0	0	1	0	0,98
MLZ1700	4404	0,91	0,91	0,93	0,92	0,01	1	0	0	0,92
MLZ1700	4405	0,96	0,94	0,94	0,93	-0,009	0	0	1	0,94
MLZ1700	4406	0,91	0,9	0,93	0,92	0,02	1	0	0	0,92
MLZ1700	4407	0,9	0,89	0,89	0,89	0	0	1	0	0,89
MLZ1700	4408	0,93	0,93	0,89	0,89	-0,04	0	0	1	0,93
MLZ1700	4409	0,84	0,77	0,83	0,81	0,04	1	0	0	0,81
MLZ1700	4410	0,81	0,58	0,8	0,54	-0,039	0	0	1	0,58
MLZ1700	4411	0,81	0,6	0,79	0,57	-0,03	0	0	1	0,6

Table 2.20: Efficiency of Gompertz model at the site located at 2000

Site	Tree	EF.mean.fit	EF.mean.fix	EF.sdd.fit	EF.sdd.fix	Dif	Pos	Equ	Neg	Best
MLZ2000	4412	0,94	0,94	0,94	0,93	-0,009	0	0	1	0,94
MLZ2000	4413	0,94	0,93	0,88	0,86	-0,070	0	0	1	0,93
MLZ2000	4414	0,96	0,96	0,92	0,91	-0,049	0	0	1	0,96
MLZ2000	4415	0,95	0,94	0,95	0,94	0	0	1	0	0,94
MLZ2000	4416	0,9	0,88	0,9	0,9	0,02	1	0	0	0,9
MLZ2000	4417	0,59	0,54	0,75	0,71	0,17	1	0	0	0,71
MLZ2000	4418	0,93	0,92	0,94	0,94	0,01	1	0	0	0,94
MLZ2000	4419	0,91	0,91	0,94	0,94	0,029	1	0	0	0,94
MLZ2000	4420	0,96	0,96	0,98	0,98	0,02	1	0	0	0,98
MLZ2000	4421	0,97	0,96	0,98	0,97	0,01	1	0	0	0,97
MLZ2000	4422	0,91	0,89	0,95	0,93	0,04	1	0	0	0,93
MLZ2000	4423	0,94	0,94	0,95	0,94	0	0	1	0	0,94
MLZ2000	4424	0,92	0,88	0,9	0,84	-0,04	0	0	1	0,88
MLZ2000	4425	0,98	0,98	0,97	0,96	-0,02	0	0	1	0,98
MLZ2000	4426	0,9	0,9	0,98	0,98	0,08	1	0	0	0,98

Table 2.21: Efficiency of Gompertz model at the site located at 2300

Site	Tree	EF.mean.fit	EF.mean.fix	EF.sdd.fit	EF.sdd.fix	Dif	Pos	Equ	Neg	Best
MLZ2300	4427	0,95	0,94	0,96	0,93	-0,009	0	0	1	0,94
MLZ2300	4428	0,96	0,95	0,93	0,93	-0,019	0	0	1	0,95
MLZ2300	4429	0,88	0,87	0,95	0,94	0,07	1	0	0	0,94
MLZ2300	4430	0,98	0,98	0,94	0,94	-0,04	0	0	1	0,98
MLZ2300	4431	0,98	0,97	0,96	0,96	-0,01	0	0	1	0,97
MLZ2300	4432	0,95	0,95	0,95	0,95	0	0	1	0	0,95
MLZ2300	4433	0,97	0,97	0,93	0,92	-0,049	0	0	1	0,97
MLZ2300	4434	0,93	0,9	0,96	0,96	0,059	1	0	0	0,96
MLZ2300	4435	0,96	0,95	0,95	0,95	0	0	1	0	0,95
MLZ2300	4436	0,92	0,87	0,97	0,96	0,09	1	0	0	0,96
MLZ2300	4437	0,92	0,87	0,97	0,96	0,09	1	0	0	0,96
MLZ2300	4438	0,97	0,84	0,95	0,94	0,1	1	0	0	0,94
MLZ2300	4439	0,98	0,98	0,97	0,97	-0,01	0	0	1	0,98
MLZ2300	4440	0,95	0,94	0,96	0,96	0,02	1	0	0	0,96
MLZ2300	4441	0,89	0,88	0,92	0,91	0,03	1	0	0	0,91

Table 2.22: Comparing RMSD of Gompertz model at the site located at 1350 m

Site	Year	Tree Species	RMSD.mean.fit	RMSD.mean.fix	RMSD.sdd.fit	RMSD.sdd.fix
MLZ1350	2013	4382 Larix decidua	3,85	3,92	3,74	3,81
MLZ1350	2013	4383 Larix decidua	3,14	5,08	3,92	5,96
MLZ1350	2013	4384 Larix decidua	1,09	1,35	2,44	2,87
MLZ1350	2013	4385 Larix decidua	1,45	1,5	1,9	2,01
MLZ1350	2013	4386 Larix decidua	3,77	5,2	3,27	3,68
MLZ1350	2013	4387 Larix decidua	1,42	1,45	6,66	7
MLZ1350	2013	4388 Larix decidua	6	7,55	5,69	7,19
MLZ1350	2013	4389 Larix decidua	2,29	2,45	2,3	2,66
MLZ1350	2013	4390 Larix decidua	1,35	1,4	1,71	1,81
MLZ1350	2013	4391 Larix decidua	3,09	3,52	3,1	3,13
MLZ1350	2013	4392 Larix decidua	3,34	3,74	3,16	3,67
MLZ1350	2013	4393 Larix decidua	2,27	2,5	5,07	5,36
MLZ1350	2013	4394 Larix decidua	1,2	1,53	1,25	1,33
MLZ1350	2013	4395 Larix decidua	3,92	3,96	3,87	3,87
MLZ1350	2013	4396 Larix decidua	1,61	1,78	3,23	3,41

Table 2.23: Comparing RMSD of Gompertz model at the site located at 1700 m

Site	Year	Tree Species	RMSD.mean.fit	RMSD.mean.fix	RMSD.sdd.fit	RMSD.sdd.fix
MLZ1700	2013	4397 Larix decidua	2,41	2,99	4,24	5,42
MLZ1700	2013	4398 Larix decidua	5,32	5,79	7,22	7,81
MLZ1700	2013	4399 Larix decidua	3,81	3,83	3,28	3,37
MLZ1700	2013	4400 Larix decidua	2,42	3,15	3,7	4,26
MLZ1700	2013	4401 Larix decidua	5,75	6,42	6,45	7,04
MLZ1700	2013	4402 Larix decidua	1,52	1,8	2,6	2,69
MLZ1700	2013	4403 Larix decidua	1,55	1,64	1,37	1,68
MLZ1700	2013	4404 Larix decidua	3,34	3,4	3,08	3,22
MLZ1700	2013	4405 Larix decidua	3,04	3,62	3,62	3,88
MLZ1700	2013	4406 Larix decidua	4,3	4,63	4,22	4,63
MLZ1700	2013	4407 Larix decidua	3,16	3,28	3,43	3,46
MLZ1700	2013	4408 Larix decidua	1,99	2	2,66	2,66
MLZ1700	2013	4409 Larix decidua	11,14	13,29	12,07	12,88
MLZ1700	2013	4410 Larix decidua	4,85	7,2	5,24	7,91
MLZ1700	2013	4411 Larix decidua	3,35	4,89	3,58	5,1

Table 2.24: Comparing RMSD of Gompertz model at the site located at 2000 m

Site	Year	Tree Species	RMSD.mean.fit	RMSD.mean.fix	RMSD.sdd.fit	RMSD.sdd.fix
MLZ2000	2013	4412 Larix decidua	1,73	1,85	1,85	2,03
MLZ2000	2013	4413 Larix decidua	5,15	5,66	6,78	7,34
MLZ2000	2013	4414 Larix decidua	2,19	2,2	2,89	3,1
MLZ2000	2013	4415 Larix decidua	2,95	3,02	3,01	3,06
MLZ2000	2013	4416 Larix decidua	3,67	3,99	3,2	3,22
MLZ2000	2013	4417 Larix decidua	12,14	12,78	8,53	9,15
MLZ2000	2013	4418 Larix decidua	3,12	3,33	3,29	3,34
MLZ2000	2013	4419 Larix decidua	4,29	4,4	3,71	3,73
MLZ2000	2013	4420 Larix decidua	2,22	2,27	1,48	1,58
MLZ2000	2013	4421 Larix decidua	1,95	2	1,69	1,87
MLZ2000	2013	4422 Larix decidua	4,54	5,06	3,3	3,66
MLZ2000	2013	4423 Larix decidua	3,26	3,37	2,81	2,96
MLZ2000	2013	4424 Larix decidua	3,14	3,99	3,94	5,05
MLZ2000	2013	4425 Larix decidua	2,67	2,68	3,19	3,45
MLZ2000	2013	4426 Larix decidua	3,66	3,78	1,75	1,85

Table 2.25: Comparing RMSD of Gompertz model at the site located at 2300 m

Site	Year	Tree Species	RMSD.mean.fit	RMSD.mean.fix	RMSD.sdd.fit	RMSD.sdd.fix
MLZ2300	2013	4427 Larix decidua	1,98	2,25	1,89	2,36
MLZ2300	2013	4428 Larix decidua	2,2	2,5	3,5	3,66
MLZ2300	2013	4429 Larix decidua	4,52	4,81	2,9	3,14
MLZ2300	2013	4430 Larix decidua	1,75	1,75	2,93	2,95
MLZ2300	2013	4431 Larix decidua	1,87	2,26	2,94	2,98
MLZ2300	2013	4432 Larix decidua	2,58	2,58	2,99	3
MLZ2300	2013	4433 Larix decidua	2,74	2,98	4,1	4,61
MLZ2300	2013	4434 Larix decidua	3,42	4,07	2,38	2,43
MLZ2300	2013	4435 Larix decidua	2,08	2,23	2,35	2,48
MLZ2300	2013	4436 Larix decidua	3,93	4,88	2,28	2,31
MLZ2300	2013	4437 Larix decidua	3,79	4,84	2,46	2,54
MLZ2300	2013	4438 Larix decidua	1,91	4,49	1,64	1,7
MLZ2300	2013	4439 Larix decidua	2,99	2,99	3,72	3,79
MLZ2300	2013	4440 Larix decidua	3,9	4,19	3,47	3,54
MLZ2300	2013	4441 Larix decidua	3,03	3,25	2,47	2,62

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Chapter 3

Does wood formation monitoring
method influence wood formation?

Does wood formation monitoring method influence wood formation?

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Abstract

Wood formation in trees is a complex phenomena starting with cambial cell divisions. The newly formed cells then go through differentiation phases making the final tree ring structure. Microcoring is one of the the non-destructive methods of studying wood formation that is based on sampling by regular punching from tree stem along the growing season. In this method the size or number of cells in each sample is measured. Although the samples that are extracted with this method are small in size, it may have a wounding effect on trees triggering the defensive responses by cambium such as producing traumatic resin canals. Wounding tree stem was reported previously to increase cell production around the wounded area. The objective of this study is to assess if wood formation monitoring method based on taking regular sample from tree stem has any effect on the normal tracheid cell production in trees. The first hypothesis of this study is that wood formation monitoring method will lead to production of traumatic tissue such as callus and resin canals, and the second hypothesis is that using this method can stimulate cambium to produce more tracheid cells. In this study, wood formation was monitored over a total of 60 dominant Larch trees growing along an elevation gradient in the southern Alps. Wood samples (15 x 2 mm microcores) were taken every week from the stem of the selected trees by a distance of about 2 cm between each sample. Microcores were then cut, stained and fixed to produce anatomical sections, which were observed under microscope. At each anatomical slide the number of tracheid cells at their differentiation stage and the occurrence of wounding tissues (callus) and also the number of traumatic resin canals were also recorded. The Gompertz function was fitted to the cell counting data in order to compute the fitting residuals to assess cambial stimulation. The results of this study showed that wood formation monitoring method didn't lead to production of callus tissue but numerous traumatic resin canals were observed in all the trees. The examination of Gompertz fitting residuals

showed that microcoring method slightly stimulated cambial activity. In conclusion it can be stated that microcoring method can lead to wounding responses in trees by producing traumatic resin canals and disturbing xylogenesis and final tree-ring structure.

Keywords: Cambial stimulation, traumatic resin canal, Gompertz, *Larix decidua*, microcoring

3.1 Introduction

Wood formation studies are based on a detailed record of cambial activity development along the growing season. With repeated sampling approach it is possible to follow the gradual accumulation of new xylem cells produced by cambium. In this method the number of cells is counted in the samples taken along the growing season. During the last decades there has been an increasing interest in wood formation studies as a results different tools and methods of wood sampling have been developed. Extracting samples from trees need to be done very precisely specially in spring or early summer when the cambium and enlarging cells have a thin and fragile primary cell wall. So special tools have been developed for extracting sample from tree stem (Rossi et al. 2006). Microcoring is known as one of the less invasive methods in wood formation monitoring in which small microcores with the size of about 15 x 2 mm are extracted from tree stem (Deslauriers et al. 2003, Rossi et al. 2006).

Cambium is a very sensitive tissue to mechanical and physical disturbances. Even a small damage to trees such as removing partially the bark (Nagy et al. 2000, Stobbe et al. 2002) or more intense as rock falls or derbies flow damage (Stoffel and Perret 2006, Bollschweiler et al. 2008, Gartner et al. 2009) was found to trigger defensive responses by cambium. The reaction of trees to wounding was found to be in the form of producing callus tissue (Stobbe et al. 2002) or tangential rows of traumatic resin canals (Bollschweiler et al. 2008, Schneuwly et al. 2009). While scatter resin canals are a common anatomical feature in some conifer species such as *larix decidua*, in the case of disturbance to tree stem they appear in tangential bands among tree ring known as traumatic type (Larson 1994). Wounding was reported previously to produce false ring within tree rings by causing differentiation of one or two layers of tracheid cells as the latewood tracheid type (Fahn et al. 1979). Such a responses by trees may become very important in wood formation studies since

they can modify the normal growth pattern in trees and produce abnormal wood at the vicinity of wound (Larson 1994). Higher activity of cambium and cell division around the wounded area by enhanced cambial activity was also observed in *cedrus libani* (Fahn et al. 1979) showing that wounding may trigger cambium to start their activity and have an effect on the phenological trend of wood formation. In normal growth condition, variation in the growth pattern along and around the tree stem can occur (Wodzicki 1971). In the wood formation studies it is critical to know if the variation presents the changes due to cambial activity or the random within-tree noises. So in order to remove the noises and extract the biological signal of cambial activity, data standardisation methods have been developed. One of the commonly used methods is based on this assumption that the heterogeneity of growth in tree ring formed in the previous ring can also be repeated for the following tree ring (Rossi et al. 2003).

The Gompertz model was reported to provide a suitable description of xylem growth pattern (Camarero et al. 1998, Rossi et al. 2003, Rathgeber et al. 2011). Fitting the Gompertz function to cell counting data will result in an S-shape growth curve showing the cumulative number of tracheid cells along the growing season. This function uses the final number of tracheid cells reached at the end of growing season as an asymptote to estimate the daily cell number along the growing season. Although wood formation monitoring studies have been developed in the last decades, but the impact of microcoring method on the xylogenesis process and the final tree-ring structure is not still clear. So the main objective of this study is to assess if the current wood formation monitoring method can affect the xylogenesis process in trees.

The first hypothesis of this study is that microcoring method by regular punching from tree stem can lead to wounding responses in trees and production of wounding tissue such as callus or traumatic resin canals. Our second hypothesis is that microcoring method can lead to cambial stimulation and production of more tracheid cells. In this study, 60 larch trees located along an elevation gradient of 1000 m in French Alps were selected in four study sites for wood formation monitoring during the growing season of 2013. Samples were taken from trees at weekly intervals from May to November with microcoring method. For each sample the number of tracheid cells at their differentiation phases was counted as well as the number of callus tissue and traumatic resin canals. The Gompertz function was fitted to tracheid cell counting data and the residual of fitting was computed in order to assess the effect of microcoring on stimulation of cambial activity.

3.2 Material and methods

3.2.1 Study site, xylem sampling and sample preparation

The number of 60 dominant health Larch (*Larix decidua* Mill.) trees were selected in four study sites (4 x 15 trees) along an elevation gradient located in Villard St. Pancrace close to Briançon (44° 53' 47" N, 6° 38' 08" E), France. Microcores were collected from the stems of selected trees at weekly intervals from May to November 2013. The bark was partially removed in all the trees to be able to extract the proper sample from the trees. A distance of 2 cm between sampling was considered in order to avoid resin duct formation in the adjacent cores as proposed by (Deslauriers et al. 2003). The sample preparation and cutting was done in the laboratory to obtain the anatomical sections (5 to 10 µm thick). The sections were stained with Cresyl violet acetate and permanently mounted and fixed on glass slides.

The anatomical slides were observed under visible and polarized light using an optical microscope (Zeiss, Germany) to distinguish the different developmental zones in tracheids. The cambial cells were characterized by their rectangular shape. They had a thin primary wall and small radial diameter, the cells in the enlargement phase had still the thin primary cell walls but greater radial diameter size compare to the cambial cells.

The cells in the wall thickening phase under the polarized light were bright due to the particular arrangement of cellulose microfibrils on their wall (Abe et al. 1997). Coloration with Cresyl violet acetate resulted in a gradient of purple-blue in the cells that were under the lignification and wall thickening process (Kutscha et al. 1975). The mature cells presented totally blue colour in their cell walls. At each sample, the number of cambial cells (C); enlarging (E); wall thickening (W); fully matured (M) and previous year (P) cells were counted along three radial files. The traumatic resin canals (TRC) were recognised as tube-shaped spaces usually formed in tangential bands among the tracheid cells of the tree ring.

3.2.2 Standardisation

Cambial activity and wood formation, can be different along or around the stem of trees (Van der Warf et al. 2007). The standardisation of cell counting data have been done using the method of Rossi et al. (2003). This method is based on the heterogeneity of growth in the previous formed tree ring which can also repeat for the following year. The standardisation of cell number was applied on the samples to

reduce within-tree growth variability (Rossi et al. 2003) using a dedicated function of the package CAVIAR (Rathgeber et al. 2017) in R software.

$$Xi_j = Xi_j * \frac{\bar{X}_{j-1}}{X_{i(j-1)}} \quad (3.1)$$

Xi_j : The standardized number of tracheid for the sampling of i and the year of j

\bar{X}_{j-1} : The mean number of cells of tracheid cells for the sampling i and the year of j

$X_{i(j-1)}$: The number of cells for the sampling i in the previous year

3.2.3 Applying Gompertz function

The Gompertz function was fitted to the tracheid cell numbers (Rossi et al. 2003) defined as:

$$nC(t) = A.e^{-e^{\beta-k.t}} \quad (3.2)$$

Which nC is the standardized total number of tracheids at time t , A is the upper horizontal asymptote parameter representing the final number of tracheid, β is the x-axis placement parameter showing the choice of origin time, and k , the growth rate parameter that reflects the spread of the curve along the time axis. The Gompertz parameters were estimated for each tree using a dedicated function of the R package CAVIAR (Rathgeber et al. 2012). In CAVIAR, the total ring cell number (RCN) is computed using a function that automatically estimate RCN by using the data from all the samples collected after cell enlargement cessation. After this date it is assumed that the final number of cells in tree ring has been obtained. This estimation will be used to fix the asymptote of the Gompertz model for fitting the cell counting data.

3.2.4 Assessment of callus tissue and traumatic resin canal formation

The response of trees to wounding can be seen in the form of callus tissue within the tree ring (figure 3.1). In our studied trees the occurrence of callus tissue within the transverse anatomical sections was assessed as well as traumatic resin canals (TRC)

formation in the samples taken along the growing season. The number of TRC was counted in each sample to assess TRC number variation along the growing season.

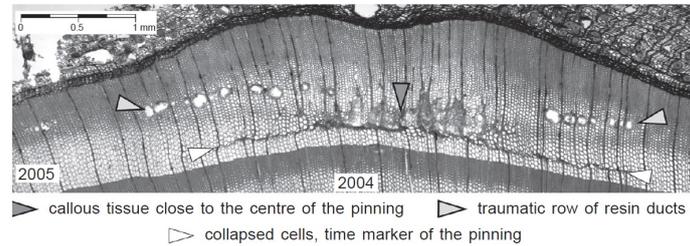


Figure 3.1. Occurrence of callus tissue and traumatic resin canals in *larix decidua* (Photo from Gartner et al. 2009)

3.2.5 Quantification of the problem

The cumulative number of cells produced by cambium during the growing season in a regular pattern (a) and an irregular pattern (b) where the total number of tracheid cells are higher at the middle of growing season have been shown in (figure 3.2).

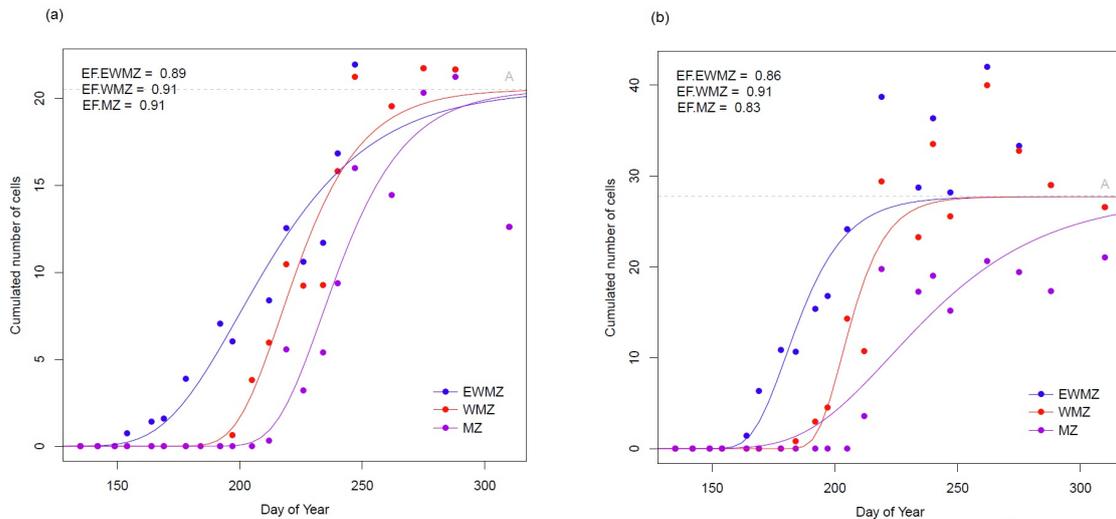


Figure 3.2. Example of verification plot produced from standard data by plotWood-Growth function in CAVIR package showing (a) cumulated number of cells in a normal pattern and (b) stimulated cell production. the lines showing the fitted Gompertz function and for the tree cumulated cell counts (MZ: mature zone, WMZ: wall thickening plus mature zone, EWMZ: enlarging plus wall thickening and mature zone). Modelling efficiency coefficient (EF) are given also in the top left corner for the three models.

In order to quantify the cambial stimulation, the variation in the total number of tracheid cells along the growing season was evaluated. In a normal pattern of

growth, the total number of tracheid cells will not change after cessation of cambial activity. The evaluation of the timing of cambial activity was based on the xylem cell enlargement phase rather than the cambial cell division phase as proposed by (Rathgeber et al. 2011a). Thus the onset and cessation of enlarging (cE) phase were used as proxies for the onset and cessation of cambial activity. So the total number of tracheid cells at the end enlarging and at the end of wall thickening phase should be the similar to the total number of cells at the end of growing season. So in order to quantify the cambial stimulation the total number of tracheid cells at the end of enlarging and wall thickening and the total number of fully mature tracheid cells were extracted then the slope between them was computed using mixed-effect models in all the 60 trees.

3.2.6 Evaluation of cambial stimulation

In order to test the hypothesis that microcoring method can stimulate cambium activity the number of total tracheid cells was assessed before and after cE. In the case of cambial stimulation, the total number of cells is expected to be higher before cE. In each tree the total number of tracheid cells were extracted from one sample before cE to the last sample and their slope was computed using mixed-effect models.

3.2.7 Evaluation of Gompertz function fitting residual

In other approach to evaluate the cambial stimulation, the residual of fitting Gompertz function to cell counting data was assessed. The difference between the estimated values by model and cell counting observation was computed at each tree using the following formula. The mean of standard residual was computed for each site. Then the pattern of standardized residual was assessed based on cE dates.

$$\text{Standardized residual } i = \frac{\text{Residual (i)}}{\text{Standard deviation of Residual (i)}} \quad (3.3)$$

3.3 Results

3.3.1 Evaluation of the traumatic tissue

The response of trees to wounding can be seen as formation of callus tissue, but in our studied trees such a response was not observed in the transverse sections. The only wounding tissue was traumatic resin canals that was produced in all the samples as tangential bands among tracheids (figure 3.3). The number of traumatic resin canals was evaluated in studied trees along the growing season. The results showed a variation in the TRC number formation along the growing season with an intensity before cE, following with a decrease toward the end of growing season in all the studied sites (figure 3.4).

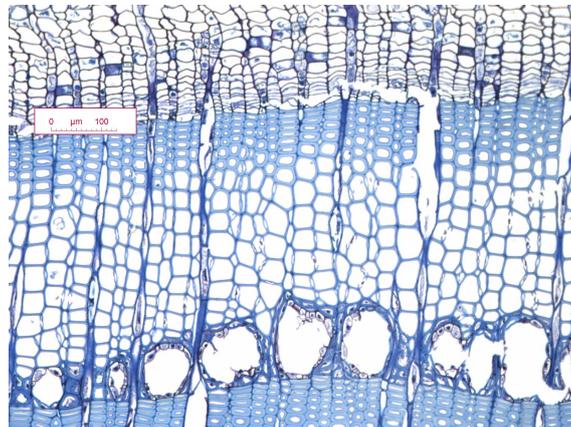


Figure 3.3. tangential band of traumatic resin canals in tree ring of *larix decidua* formed in the year of 2013.

3.3.2 Quantification of problem

The quantification of problem revealed a decrease in the total number of cells at the end of differentiation phases. The negative slope was observed in about 70 % of trees showing an irregular pattern of total tracheid cell number (supplementary material). Computing the slope using mixed effect model along the gradient showed a negative slope (Slope = -0.037) but the P value was not significant (figure 3.5).

3.3.3 Cambial stimulation

The results of evaluation tracheid cell numbers before and after cE showed a decreasing trend at all the studied sites however the regression was not significant

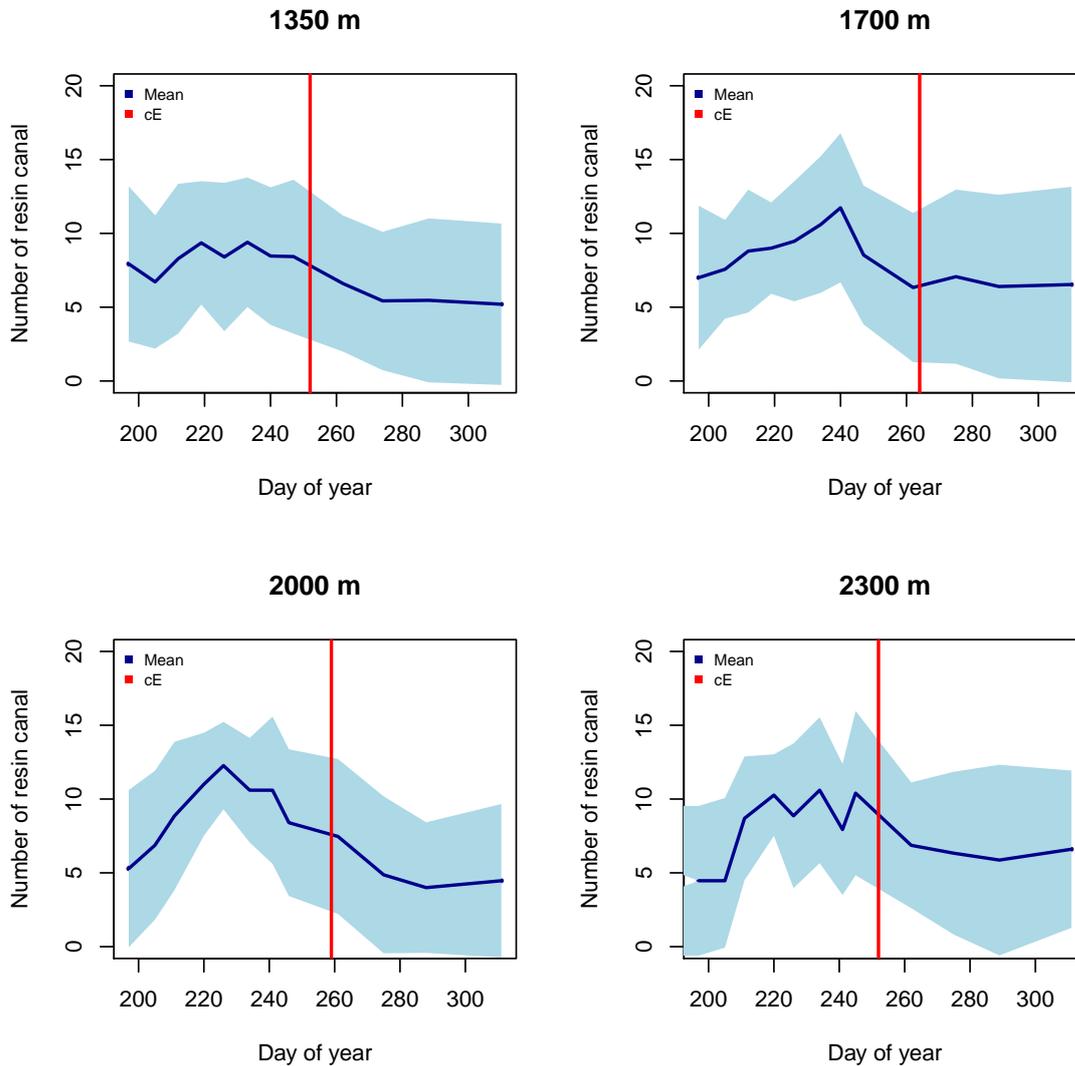


Figure 3.4. Number of resin canal along the growing season of 2013 in different sites.

(table 3.1). The general slope for the gradient computed with mixed-effect model was negative (slope= -0.017) (figure 3.6 and 3.7).

Table 3.1: Cell number evaluation and statistical analysis results

Site	P	Slope(b1)	Significancy
1350	0,84	0.006	Not significant
1700	0,16	-0,05	Not significant
2000	0,85	-0,007	Not significant
2300	0,76	-0,008	Not significant
All the sites	3.43	-0.017	Not significant

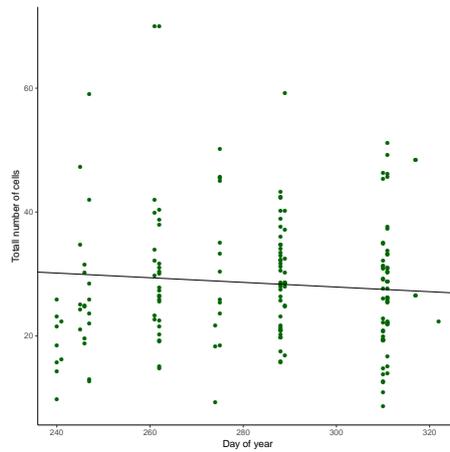


Figure 3.5. The regression line of mixed effect model analysis for all the sites for the number of cells at the end of each differentiation phase in 60 trees.

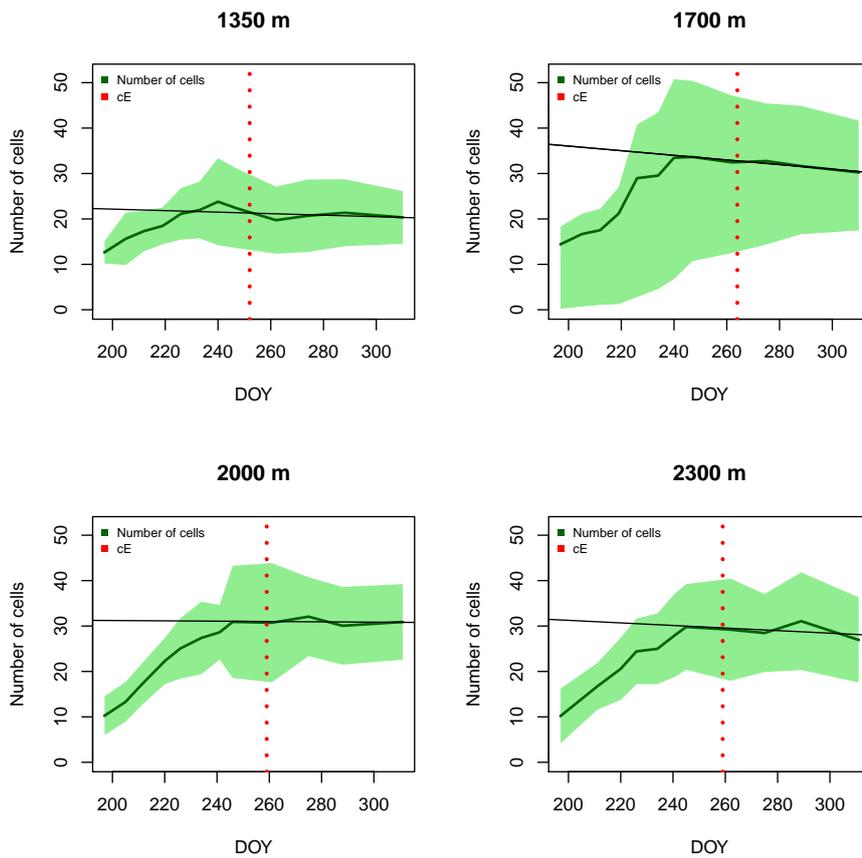


Figure 3.6. Total number of cells along the growing season in four study sites

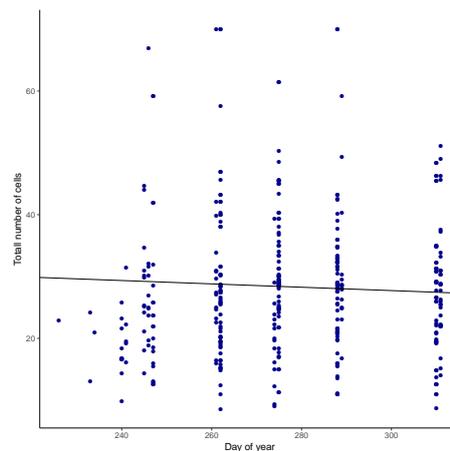


Figure 3.7. The regression line of mixed effect model for all the sites for the number of cells before and after cessation of cambial activity (cE).

3.3.4 Evaluation of Gompertz fitting residual

The standard residual between the Gompertz estimation and the cell counting data showed variation along the growing season. It can be observed from the pattern of Gompertz fitting residual analysis that before cessation of enlarging phases the number of cells are underestimated but after this date they are more overestimated (figure 3.8).

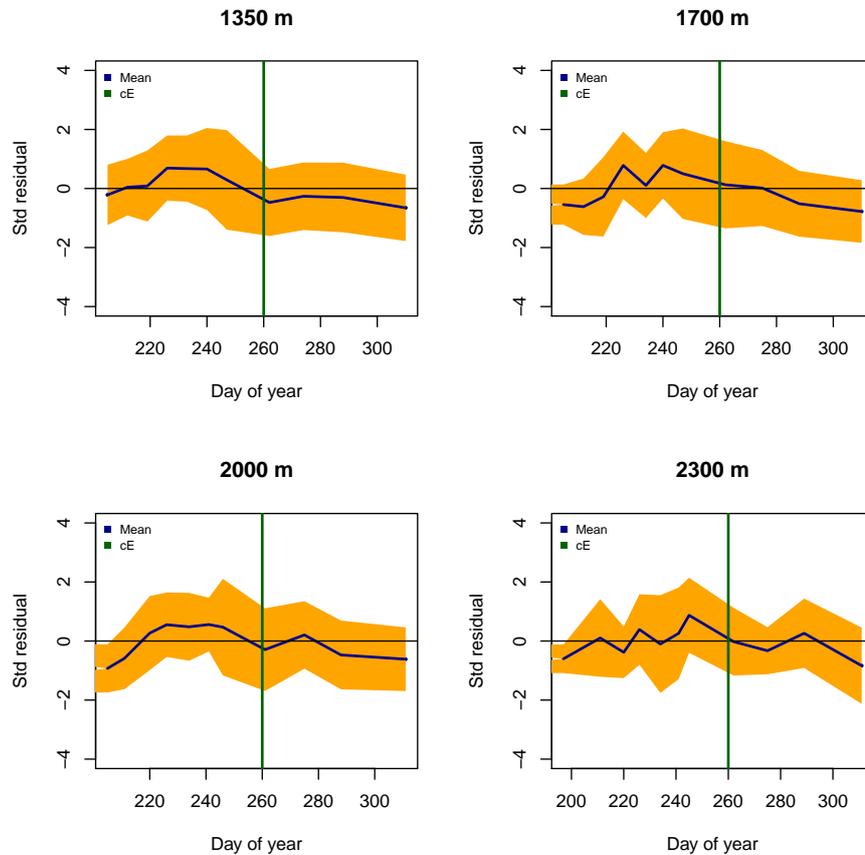


Figure 3.8. Standard residual between the tracheid cell observation and Gompertz estimation in four study sites

3.4 Discussion

The results of this study confirmed our hypothesis that wood formation monitoring using regular punching method will lead to wounding responses in trees. This responses observed in the form of procucing traumatic resin canal (TRC) among tracheid cells. We also found slight cambial stimulation effect in our studied trees by both tracheid cell number and residual of Gompertz evaluation.

Formation of traumatic resin canals

Trees belong to the Pineace family, in the case of mechanical injury or abiotic attacks were found to produce wounding tissue such as traumatic resin canals in their tree rings. They are considered as defensive mechanisms to protect and help trees to survive (Nagy et al. 2000, Bollschweiler et al. 2008, Gartner et al. 2009). Our stud-

ied trees produced TRC only at the year of 2013. Since no mechanical disturbance such as rock flow or abiotic attack were reported in the studied year, we related the occurrence of TRC to the wounding response in trees induced by microcoring method. [Deslauriers et al \(2003\)](#) observed the formation of TRC induced by microcoring method. They proposed a distance of 2 cm between sampling in order to avoid TRC formation in the adjacent cores ([Deslauriers et al. 2003](#)). However such a distance didn't prevent from TRC formation in our studied trees.

Cambial stimulation

The reaction of trees to wound was previously observed as cambial stimulation to produce parenchyma cells around the wounded area to protect the living tissue ([Schmitt and Leise. 1990](#)). The effect of wounding on cambial activity stimulation was also observed in *cedrus libani* where higher cell production was found around the wounded area leading to an eccentric tree ring structure. They observed that wounding in November lead to reactivation of cambium while wounding between December and March did not have any effect on it and a higher activity of cambium was found around the wounded area ([Fahn et al. 1979](#)). It shows that wounding may also have an effect on the phenology of wood formation.

The distribution of standard residual between the observed cell number and Gompertz prediction confirmed our hypothesis about cambial stimulation. Gompertz is an asymmetric sigmoid growth function that has been frequently used to describe the growth dynamics in animals and plants. The gradual accumulation of cells in xylem is classically described by Gompertz function. It was found to provide suitable description of xylem growth pattern ([Camarero et al. 1998](#), [Rossi et al. 2003](#), [Rathgeber et al. 2011b](#)). The biological trend of xylem cell development in trees was found to have an S-shape pattern that remains stable from year to year but with the small fluctuations due to environmental changes ([Deslauriers et al. 2003](#)). The Gompertz function uses the total number of tracheid cells at the end of growing season to fit the number of cells. So as we presented by the results of residual distribution, in the case of cambial stimulation using Gompertz function may add noises to the real wood formation data. The biological mechanism of cambial stimulation was explained by the effect of wounding on obstruction of growth hormones such as auxin and consequently increase its concentration in the wounded area ([Aloni and Zimmermann, 1983](#)). More intense response of cambium above the wounded area ([Fahn et al. 1979](#), [Gartner et al. 2009](#)) confirms higher accumulation of growth

hormones in the wounded area. However occurrence of TRC below the injuries is in contrast with this idea that wound can act as a physical barrier to the flow of growth hormones (Fahn et al. 1979).

Conclusion

The main results of this study showed that microcoring method from tree stem of *larix decidua* trees can induce formation of traumatic resin canals with a slight cambial stimulation in all the studied trees. In conclusion it can be stated that the current method of wood formation monitoring based on repeated punching method from tree stem can influence the xylogenesis and the final tree-ring structure. Although microcoring was observed to slightly stimulate cambial activity, but its impact on the phenology and dynamics of wood formation and may lead to a slight underestimation of phenological trend of wood formation along our studied gradient without invalidating our results. Wood formation studies in long term is a very time and labor demanding study. Any abnormality in the xylem tissue can decrease the quality of the anatomical section. So this study shows the demand to improve the methods of wood formation sampling, in order to obtain more accurate data in future.

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Supplementary materials

Table of slope computed for the cell number at the end of each phases

Tree	Slope	P	R2
4382	0,13	0,36	0,41
4383	-0,22	0,11	0,93
4384	-0,03	0,13	0,91
4385	-0,03	0,41	0,25
4386	-0,01	0,05	0,98
4387	0,01	0,61	0,3
4388	-0,01	0,66	0,5
4389	0,2	0,03	0,99
4390	0,05	0,17	0,84
4391	-0,03	0,74	0,74
4392	-0,08	0,52	0,06
4393	0,06	0,78	0,7
4394	-0,02	0,32	0,52
4395	-0,22	0,3	0,57
4396	-0,11	0,26	0,66
4397	-0,6	0,006	0,99
4398	-0,49	0,15	0,88
4399	-0,11	0,13	0,9
4400	-0,34	0,53	0,12
4401	-0,14	0,27	0,66
4402	0,06	0,55	0,16
4403	0,03	0,68	0,55
4404	0,15	0,31	0,54
4405	0,34	0,25	0,7
4406	0,01	0,91	0,96
4407	0,05	0,72	0,63
4408	-0,13	0,42	0,23
4409	-1,6	0,0006	0,99
4410	-0,2	0,25	0,69
4411	-0,04	0,55	0,15
4412	-0,07	0,0009	1

Table of slope computed for the cell number from cE to the last sample in Raw via Standard data

Tree	Slope	P	R2
4413	-0,5	0,37	0,39
4414	-0,02	0,7	0,61
4415	0,02	0,57	0,22
4416	0,08	0,25	0,68
4417	-0,28	0,09	0,95
4418	0,06	0,72	0,64
4419	-0,21	0,26	0,68
4420	0,007	0,69	0,5
4421	0,1	0,45	0,13
4422	-0,31	0,14	0,89
4423	-0,02	0,37	0,36
4424	0,45	0,4	0,3
4425	0,08	0,68	0,54
4426	-0,009	0,06	0,98
4427	-0,5	0,94	0,98
4428	-0,19	0,07	0,96
4429	0,02	0,61	0,34
4430	0,35	0,27	0,64
4431	-0,22	0,36	0,4
4432	-0,09	0,52	0,06
4433	-0,03	0,66	0,49
4434	-0,01	0,9	0,95
4435	-0,06	0,64	0,43
4436	0,04	0,61	0,35
4437	-0,02	0,45	0,14
4438	-0,01	0,7	0,6
4439	-0,008	0,97	0,99
4440	-0,008	0,97	0,99
4441	-0,06	0,11	0,93

Tree	Site	Raw/Sd	P	Slope(b1)	Significancy
4382	1350	Raw	0,19	0,18	No
4383	1350	Raw	0,8	-0,18	No
4384	1350	Raw	0,55	0,01	No
4385	1350	Raw	0,04	0,06	Significant at 0.05
4386	1350	Raw	0,02	-0,2	Significant at 0.01
4387	1350	Raw	0,71	-0,01	No
4388	1350	Raw	0,1	-0,05	No
4389	1350	Raw	0,61	0,07	No
4390	1350	Raw	0,69	0,01	No
4391	1350	Raw	0,26	-0,06	No
4392	1350	Raw	0,07	-0,15	No
4393	1350	Raw	0,22	0,1	No
4394	1350	Raw	0,13	-0,02	No
4395	1350	Raw	0,2	-0,28	No
4396	1350	Raw	0,09	-0,07	No
4397	1700	Raw	0,28	-0,1	No
4398	1700	Raw	0,04	-0,36	Significant at 0.01
4399	1700	Raw	0,57	-0,05	
4400	1700	Raw	0,35	-0,15	No
4401	1700	Raw	0,13	-0,21	No
4402	1700	Raw	0,14	0,07	No
4403	1700	Raw	0,71	0,03	No
4404	1700	Raw	0,16	0,04	No
4405	1700	Raw	0,81	0,03	No
4406	1700	Raw	0,22	-0,06	No
4407	1700	Raw	0,7	0,02	No
4408	1700	Raw	0,46	-0,03	No
4409	1700	Raw	0,33	-0,41	No
4410	1700	Raw	0,31	-0,2	No
4411	1700	Raw	0	-0,08	No
4412	2000	Raw	0	-0,04	Significant at 0.01
4413	2000	Raw	0,14	-0,29	No
4414	2000	Raw	0,1	0,13	No

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Tree	Site	Raw/Sd	P	Slope(b1)	Significancy
4415	2000	Raw	0,5	0,02	No
4416	2000	Raw	0	-0,1	Significant at 0.01
4417	2000	Raw	0,34	-0,5	No
4418	2000	Raw	0,27	0,12	No
4419	2000	Raw	0,02	-0,21	Significant at 0.05
4420	2000	Raw	0,03	-0,08	Significant at 0.05
4421	2000	Raw	0,18	-0,05	No
4422	2000	Raw	0,93	-0,02	No
4423	2000	Raw	0,62	-0,02	No
4424	2000	Raw	0,15	0,27	No
4425	2000	Raw	0,29	0,14	No
4426	2000	Raw	0,42	-0,12	No
4427	2300	Raw	0,64	0,015	No
4428	2300	Raw	0,58	-0,02	No
4429	2300	Raw	0,15	0,08	No
4430	2300	Raw	0,06	0,18	No
4431	2300	Raw	0,35	-0,09	No
4432	2300	Raw	0,21	-0,09	No
4433	2300	Raw	0,38	-0,07	No
4434	2300	Raw	0,02	-0,11	Significant at 0.01
4435	2300	Raw	0,67	-0,02	No
4436	2300	Raw	0,43	-0,12	No
4437	2300	Raw	0,07	-0,11	No
4438	2300	Raw	0	0,32	Significant at 0.001
4439	2300	Raw	0,71	-0,07	No
4440	2300	Raw	0,7	0,04	No
4441	2300	Raw	0	-0,08	Significant at 0.001
4382	1350	Sd	0,19	0,16	No
4383	1350	Sd	0,05	-0,2	significant at 0.05
4384	1350	Sd	0,66	-0,01	No
4385	1350	Sd	0,73	-0,01	No
4386	1350	Sd	0,27	-0,05	No
4387	1350	Sd	0,99	0	No

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Tree	Site	Raw/Sd	P	Slope(b1)	Significancy
4388	1350	Sd	0,64	-0,01	No
4389	1350	Sd	0,02	0,18	significant at 0.05
4390	1350	Sd	0,15	0,06	No
4391	1350	Sd	0,66	0,03	No
4392	1350	Sd	0,34	-0,11	No
4393	1350	Sd	0,29	0,2	No
4394	1350	Sd	0,61	-0,01	No
4395	1350	Sd	0,1	-0,23	No
4396	1350	Sd	0,02	-0,17	significant at 0.05
4397	1700	Sd	0,19	-0,18	No
4398	1700	Sd	0,19	-0,36	No
4399	1700	Sd	0,06	-0,01	No
4400	1700	Sd	0,82	-0,06	No
4401	1700	Sd	0,28	-0,11	No
4402	1700	Sd	0,28	0,11	No
4403	1700	Sd	0,48	0,03	No
4404	1700	Sd	0,12	0,14	No
4405	1700	Sd	0,91	0,02	No
4406	1700	Sd	0,91	0,01	No
4407	1700	Sd	0,41	0,11	No
4408	1700	Sd	0,15	-0,12	No
4409	1700	Sd	0,21	-0,3	No
4410	1700	Sd	0,28	-0,22	No
4411	1700	Sd	0,24	-0,04	No
4412	2000	Sd	0,01	-0,06	significant at 0.01
4413	2000	Sd	0,25	-0,4	No
4414	2000	Sd	0,3	-0,03	No
4415	2000	Sd	0,43	0,03	No
4416	2000	Sd	0,34	0,1	No
4417	2000	Sd	0,11	-0,24	No
4418	2000	Sd	0,94	0,01	No
4419	2000	Sd	0,91	0,01	No
4420	2000	Sd	0,34	0	No

Continued on next page

Tree	Site	Raw/Sd	P	Slope(b1)	Significancy
4421	2000	Sd	0,11	0,1	No
4422	2000	Sd	0,14	-0,31	No
4423	2000	Sd	0,48	-0,01	No
4424	2000	Sd	0,19	0,4	No
4425	2000	Sd	0,54	0,07	No
4426	2000	Sd	0,64	0,03	No
4427	2300	Sd	0,44	0,04	No
4428	2300	Sd	0,18	-0,14	No
4429	2300	Sd	0,5	0,02	No
4430	2300	Sd	0,27	0,35	No
4431	2300	Sd	0,22	-0,2	No
4432	2300	Sd	0,48	-0,08	No
4433	2300	Sd	0,15	-0,18	No
4434	2300	Sd	0,76	-0,016	No
4435	2300	Sd	0,53	-0,05	No
4436	2300	Sd	0,88	-0,009	No
4437	2300	Sd	0,23	-0,02	No
4438	2300	Sd	0,72	0,007	No
4439	2300	Sd	0,97	-0,008	No
4440	2300	Sd	0,86	0,03	No
4441	2300	Sd	0,55	-0,03	No

Chapter 4

Wood formation phenology

Wood formation phenology of Larch trees growing along a 1000 m elevation gradient in the Southern Alps

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Abstract

In a changing world, the survival of perennial plants depends on their ability to remain adapted to their ever changing environment. This adaptation in trees is amply implemented through the wood formation process driven by cambium. Wood formation monitoring should help to understand how wood formation is affected by environmental factors and to assess tree response to future climatic changes. Phenology, is a trade-off between the environmental factors and acquisition of necessary resources. The phenology of wood formation has been reported to be affected by environmental factors such as heat and water availability. The main objective of this study is to understand changes in wood formation phenology in relation to environmental factors variability. The xylogenesis of 60 larch trees, distributed in four stands (4 x 15 trees), and spread along an elevation gradient of 1000 m in the French Southern Alps was monitored all along the 2013 growing season. Wood samples were taken every week from the stem of the selected trees. Cambial activity started around mid-May at the lowest stand and around mid-June at the highest one, which correspond to a delay of 2.7 days per 100 m increase in elevation. The onset of the other phenophases of xylem formation followed the same trend with a delay of 2.5 and 1.5 days for onset of wall thickening and maturation respectively. The cessation of these phenophases followed a parabolic trend with trees from the lowest stand finishing first, followed by those from the highest stand, while those from the intermediate elevations finished the last. The duration of wood formation also exhibited parabolic trends along the gradient, but with the shortest occurring at the 2300 m site (about 4 months), and the longest ones at the 1700 m site (about 5 months). The delay in the wood formation onset with increasing elevation can be associated with the adiabatic temperature decrease and thus translated into a lengthening of the growing season about 5 days per °C. On the other hand, our results showed that the cessation of cambial activity is more related to photoperiod,

even if water shortage seems able to limit the termination of the growing season. In conclusion, our studied larch trees were able to adapt themselves to contrasting environmental changes through modifying their phenology. Shortening the length of growing season at the top and the bottom site makes the middle elevation (1700 to 2000 m) as the optimum site for the growth of Larch trees in Southern Alps.

Keywords : cambial activity, *larix decidua*, elevation gradient, phenology, French Alps.

4.1 Introduction

In a changing environment, perennial plants need to adapt themselves to their ever changing environment in order to survive. Trees, as long lived organisms are exposed to varying environmental condition during their life and need to show high levels of plasticity in their phenotype. Wood formation is the way that trees use to adapt themselves, so the phenological traits of wood formation need to be adapted to the environmental changes. Phenology is a term commonly used as the timing of the seasonal development in plants. The phenological studies in living organisms has gathered a considerable attention with the recent climatic changes since the modification in the phenological events can reflect the changes in the environment. For example the recent climatic changes such as temperature increase was found to increase the length of growing season in Europe (Menzel et al. 2006). Wood formation process has been defined as the production of new cells by cambium that undergo profound transformations through passing into successive differentiation phases of enlarging, wall thickening and maturation (Rathgeber et al. 2016). Regulating all this process is under the control of both endogenous factors such as hormones and exogenous factors such as climatic variables like temperature (Larson 1969).

Wood formation phenology can be affected by tree characters such age of cambium. Rossi et al (2008a) observed the effect of age on wood formation phenology, as older trees showed shorter period of wood formation compare to adult ones (Rossi 2008 a). In the boreal and temperate zones the activity of cambium specially its onset was found to be mainly under the control of temperature (Oribe et al. 2001, 2003, Gricar et al. 2006, Begum et al. 2007, Rossi et al. 2007). Temperature, is known to be the main factor controlling all the metabolic process in the living organisms of cold ecosystems such as trees (Korner 2003, 2007). It is also known as the main environmental factor changing along elevation gradients (Korner 2007). The effect

of temperature on the resumption of cambial activity was tested widely with artificial heating of cambium (Oribe et al. 2001, Gricar et al. 2007). Temperature with affecting growth hormones such as indole-acetic acid (IAA) can trigger cambial activity in early spring. Elevation gradients are one of the strong natural experiments to investigate the responses of species to the geophysical effects such as low temperatures (Korner 2007). Water availability is also an important factor in all tree physiological process. Any changes in the amount of available water for trees can affect their functioning from the leaf to whole tree level. The low amount of soil humidity can trigger an earlier conclusion of growth (Fritts 1956). Sever water stress will affect the meristematic activity in cambium such as cell division to save energy for maintaining the minimum methabolism and defense in trees (McDowell 2011). Decreasing soil water availability under higher temperature can put trees into water stress, following with a drop in stem and leaf water potential and could drop beyond the boundaries of hydraulic security (McDowell et al. 2008). In boreal and temperate regions, photoperiod found to be one of the most important factors triggering phenological phases in plants. Photoperiod was reported to have an effect on growth cessation as higher latitude species had a shorter growing season compare to the southern latitudes (Jackson 2009). Drop in air temperature in autumn also was found to have an effect on the length of wood formation and shortened it (Gricar et al. 2006). Trees growing at higher altitude and latitudes, experience less favorable climatic condition compare to lower ones (Chuine 2010). Previous studies showed that trees growing at higher elevations needs lower amount of heat requirement than lower sites (Gricar et al. 2014). But generally trees of temperate and boreal zone need to adjust the length of growing season in a way to avoid frost damages in winter. Adjusting the length of growing season in trees is an optimization between the frost damage and maximization of annual carbon assimilation (Chuine 2010). The main objective of this study is to understand how trees are able to adapt themselves to grow under contrasting climate conditions by adjusting their wood formation phenology. The hypothesis of this work is that firstly, with increasing elevation, a delay in the onset of wood formation phases (enlarging, wall thickening and maturation) will occur as a result of decreasing temperature. The second part of this hypothesis is that an earlier cessation of phenological phases of wood formation (enlarging, wall thickening) moving towards higher altitude is expected along elevation gradient.

In order to test these hypothesis, the xylogenesis of 60 larch trees, distributed in four stands (4 x 15 trees), and spread along an elevation gradient of 1000 m (1350, 1700,

2000 and 2300 m) in the French Southern Alps was monitored all along the 2013 growing season. Wood samples were taken every week from the stem of the selected trees. The critical dates of wood formation were computed for each study site. Meteorological data were obtained from nearby climate stations and global database. The relative extractable water was computed using a water balance model.

4.2 Materials and methods

4.2.1 Site description

The study was conducted across an elevation gradient located in Villard St.Pancrace close to Briançon (44° 53' 47" N, 6° 38' 08" E), in a west facing slope ranging from 1350 to 2300 m a.s.l in the French Alps. This gradient is covered mostly by pure-uneven aged Larch (*Larix decidua* M.) trees with some patches of Silver fir (*Abies alba* M.), Scots pine (*Pinus sylvestris* L.), Stone pine (*Pinus cembra* L.) and mountain pine (*Pinus uncinata*). Four plots were selected within this elevation gradient at 1350, 1700, 2000, 2300 m a.s.l.

4.2.2 Tree selection

In total, 60 dominant European Larch trees were selected and their total height and circumference were measured. Standard cores were taken at breast height to measure their age. The mean age of trees was about 150 years but a bit higher at 2000 m and a bit lower at 2300 m. The height of trees were about 25 m but the trees at the top site were shorter (Table 4.1)

Table 4.1: Characteristics of selected trees showing the mean \pm standard error for age, diameter at the breast height (DBH) and height of selected trees at each site

Site (m)	Age	DBH (cm)	Height (m)
2300	122 \pm 10	130 \pm 43	17.2 \pm 1
2000	194 \pm 6	152 \pm 71	27 \pm 2
1700	151 \pm 8	149 \pm 78	27.3 \pm 2
1350	155 \pm 6	123 \pm 71	22.3 \pm 1

4.2.3 Tree sampling and sample preparation

During the growing season of 2013, from May to November micro-cores were extracted at breast height from each tree in each plot. Microcoring has been done using Trephor (Vitzani, Belluno, Italy) (Rossi et al. 2006) with a distance of 2 cm between two samples. The collected microcores (with 2 mm diameter and 15 to 20 mm length) were placed in Eppendorf vials with a diluted solution of ethanol 50% and stored at 5° C to prevent the deterioration of living tissues. The microcores then were embedded in paraffin. The 5 to 10 µm thick transverse sections have been cut stained and fixed with a rotary microtome for further anatomical observations (Harroue et al. 2011).

4.2.4 Microscopic observation

The anatomical observations have been done using an optical microscope (ZEISS, Germany) with magnification between 10 to 40. Visible and polarized light were used for distinguishing different phases of cell development. In each sample, three radial files were chosen and the number of cambial (nC), enlarging (nE); wall thickening (nW); and fully mature (nM) and previous year (nP) cells were counted. The cambium cells were recognized by their thin primary walls, small radial size and rectangular shape compare to phloem cells. The enlarging cells had a greater lumen area with thin primary walls. Polarized light was used to distinguish the cells in the wall thickening phase because these cells were shining as a results of secondary wall formation. In wall thickening cells there was also a gradient of pink-violet to blue color from inside to the outside of cell walls while the fully mature cells were totally blue.

4.2.5 Timing of wood formation

The critical dates of wood formation phases were computed using CAVIAR, based on logistic regressions (Rathgeber 2011) which computes the dates of (1) the beginning of the dividing phase; (2) the beginning of the enlarging phase; (3) the beginning of the wall thickening phase; (4) the beginning of the mature phase; (5) the end of the dividing phase; (6) the end of the enlarging phase and (7) the end of the wall thickening phase. The critical dates correspond to 50% probability of a phenophase being active. The onset and cessation of cambial activity was based on the xylem cell

enlargement phase rather than the cambial cell division as proposed by (Rathgeber et al. 2011).

4.2.6 Meteorological data

Temperature

The daily minimum maximum and mean temperature and also air humidity, were directly obtained for the entire year of 2013 from local meteorological stations installed at each site. The growing degree days temperature GDD5 was computed according to the following equation based on T base of 5° C.

$$GDD = \Sigma((T_{max} + T_{min})/2) - T_{base} \quad (4.1)$$

Precipitation

The precipitation data were not measured at the local stations so the daily precipitation data were interpolated from the daily data derived from the close by Meteo France station in Villard St.Pancrace and Worldclim global data base.

Vapour pressure deficit

The vapor pressure deficit (VPD), the difference between saturation and actual air vapor pressure was computed using the following formula (Jones HG 1992).

$$VPD(kPa) = ((100 - Air.Humidity)/100) * (6.11 * exp(17.27 * T / (T + 273))) \quad (4.2)$$

Photoperiod

The day-length for the study site was computed using the "geosphere" package in R program. This package can compute the day-length in hours using the geographical position of the site.

4.2.7 Soil water content evaluation

In order to determine the soil water balance at each site, the meteorological data (mean air temperature, precipitation, global radiation, relative humidity, and wind speed) were used. The amount of daily water balance were computed for the year of

2013 in each study site using the Biljou model (<https://appgeodb.nancy.inra.fr/biljou/>) (Granier et al. 1999). In this model the mentioned meteorological data and the soil characteristics at each study site like the layer number, depth of layers and the proportion of fine roots per layer were used. The stand parameters such as forest type and the maximum leaf area index (LAI) were also considered in the model.

4.2.8 Data analysis

In order to assess if the wood phenology is related to elevation, the critical dates of wood formation were compared using bootstrap test. It was done on median of groups to check if their difference is statistically significant using the CAVIAR package in R program (Rathgeber et al. 2011). In order to find the main environmental factors affecting the cambial activity, the onset and cessation of enlarging phase were considered as the onset and end of cambial activity respectively. The climatic factors such as photoperiod, Growing degree days temperature at the onset and cessation of cambial activity were compared along the gradient. In order to assess the effect of temperature on the onset and cessation of cambial activity, first the mean daily temperature for the onset (from March to July) and the cessation (from August to November) were extracted to compare the temperature variation based on the dates of bE and cE at each site. Secondly the average of mean daily temperature in one week before the bE and cE for each tree was extracted and compared using bootstrap test analysis.

4.3 Results

4.3.1 Climatic variability along the elevation gradient

Long term comparison of temperature and precipitation

In order to see if the climate in the studied year (2013) was not an extreme condition, the mean annual temperature and sum of precipitation were compared from 2004 to 2015 using Villard St.Pancrace meteorological data. The results of this comparison showed that the climate at the study year was normal. The mean annual temperature was (8.1 ± 1) compare to the ten years mean (8.5 ± 0.5) . The total amount of precipitation was 702 mm while the long term amount was 634 mm (figure 4.1).

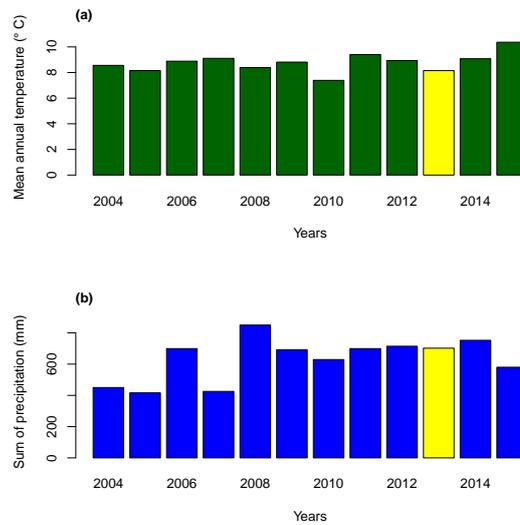


Figure 4.1. Villard St.Pancrace climatic data. (a) mean annual temperature ($^{\circ}\text{C}$), (b) the sum of precipitation (mm).

Temperature

Comparison of temperature along the gradient showed a decreasing trend of air temperature with increasing altitude in a significant linear trend ($T = -0.0054Alt + 14.3$, $R^2 = 0.06$, $P < 0.001$) (figure 4.2a). This trend in air temperature can be stated as a decrease of 0.54°C per 100 m increase in the elevation. The minimum and maximum temperature of 2013 also showed a significant decreasing trend with altitude (Figure 4.3a, b).

Precipitation

The comparison of precipitation along the gradient showed an increasing trend in the total amount of annual precipitation with increasing elevation (figure 4.2c). The amount of annual sum precipitation were (1015 mm at 1350m), (1153 mm at 1700 m), (1316 mm at 2000 m) and (1478 mm at 2300 m) .

Vapour pressure deficit

The results of vapour pressure deficit (VPD) comparison showed the lowest VPD at the top site that was 2.2 ± 0.09 (Kpa). Then there was an increasing trend toward the lower elevations with the observed amount of 2.6 ± 0.1 , 3.1 ± 0.1 , 3.2 ± 0.1 (Kpa) for the sites located at 2000, 1700 and 1350 m respectively (figure 4.3f).

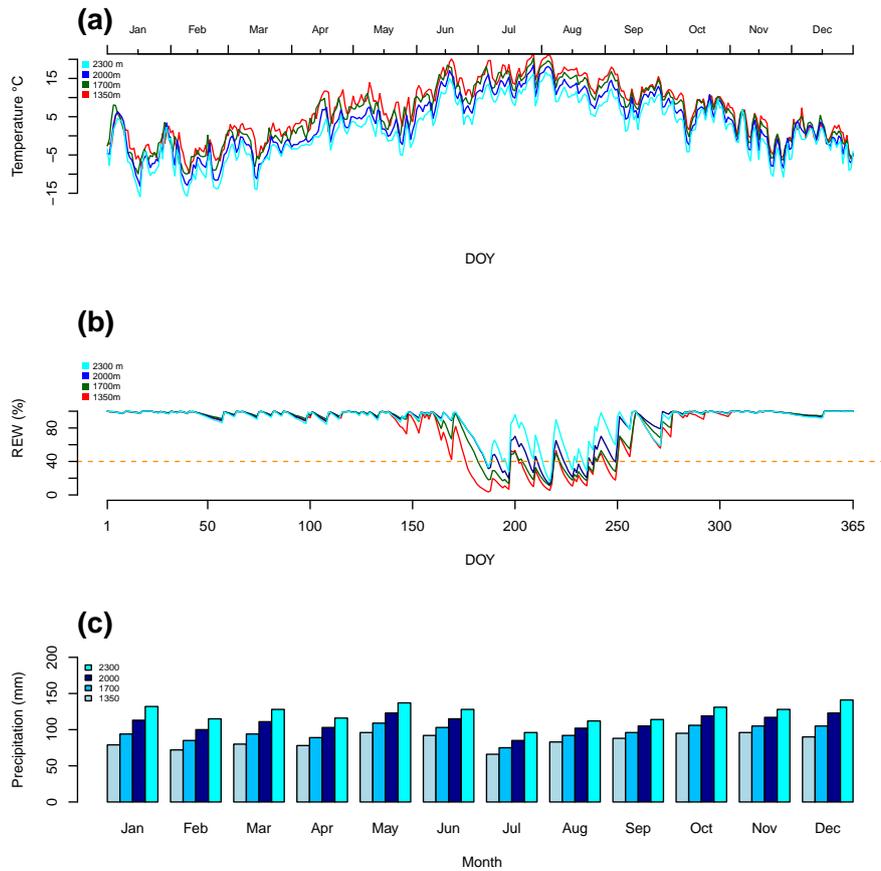


Figure 4.2. Climatic variable along the selected gradient in Briancon, (a) daily average temperature at the four study sites along the elevation gradient, (b) relative extractable water (%), (c) monthly sum of precipitation (mm) for the year of 2013.

4.3.2 Water balance status along gradient

The soil water content was computed at each study site as relative extractable water (REW). In this model the water stress is assumed to occur when the amount of REW drops below 40 % of maximum extractable water. Comparing the REW along selected elevation gradient showed the highest intensity of water deficit at the bottom site (1350 m) that was 33.3 (dimensionless). This value decreased with moving to higher elevation as (20.3 at 1700 m) , (15.5 at 2000) and (12.7 at 2300 m). The duration of water deficit was longer at the bottom site (66 days at 1350 m) with a decreasing trend toward the higher altitude as (54 days at 1700 m), (42 days at 2000 m) and (33 days 2300 m) (figure 4.2b).

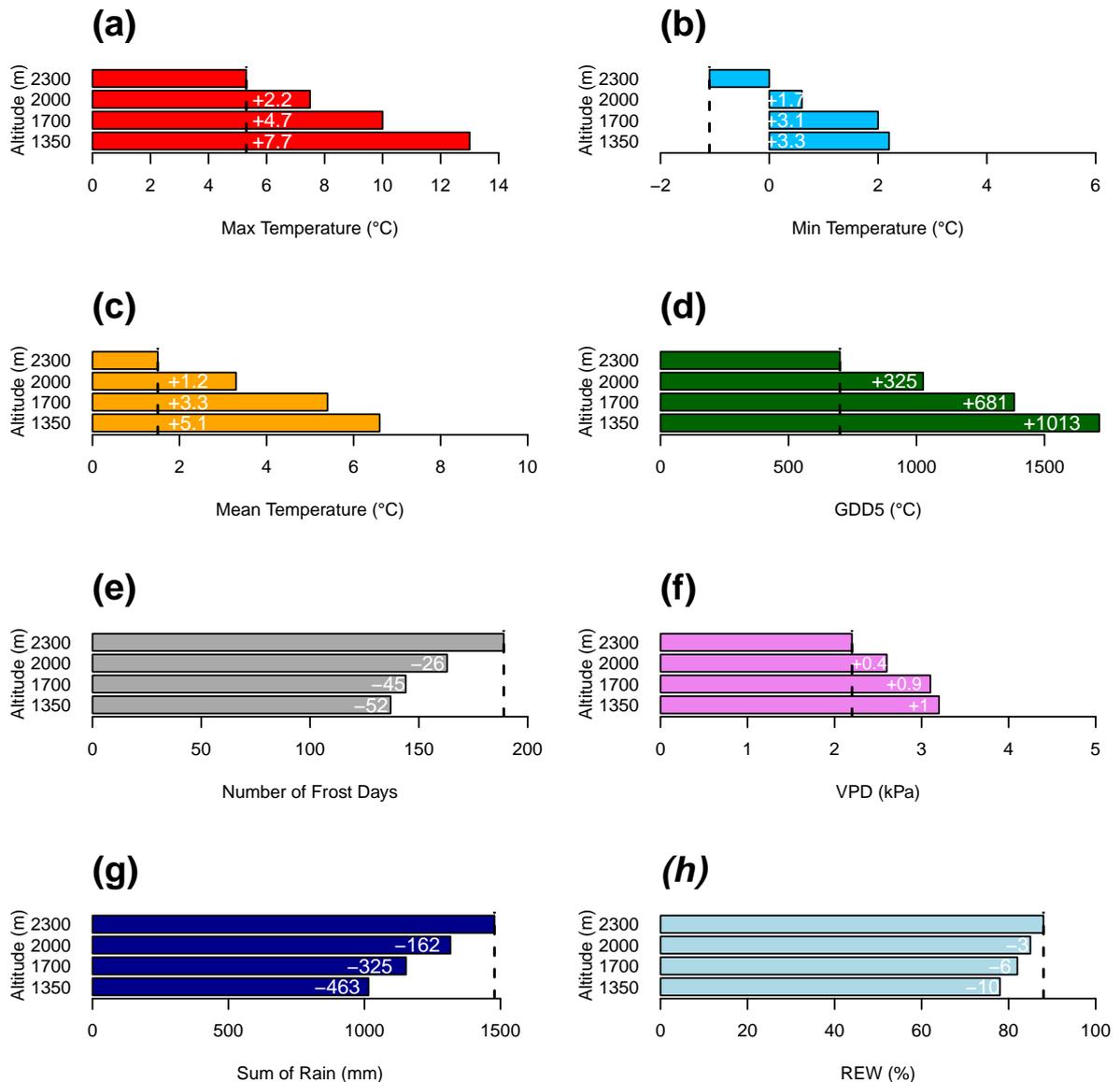


Figure 4.3. Climatic data comparison at the four study sites in Briançon; (a) annual mean of daily maximum temperature, (b) annual mean of daily minimum temperature, (c) annual mean of daily average temperature, (d) annual growing degree days (GDD5), (e) number of frost days (f) annual mean vapour pressure deficit (Kpa) (g) annual sum of rain (mm), (h) annual mean of the percentage of relative extractable water (%). The white numbers show the differences of a site relative to the value recorded at the highest sites (2300 m).

4.3.3 Phenology of wood formation

Onset of enlarging phase

The onset of enlarging phase occurred in the 22 of May, (DOY 143 ± 2) at the lowest elevation then observed with a delay in other sites when moving toward higher elevations. The enlarging phase occurred on 27 May (Doy 148 ± 3), 6 June (DOY 158 ± 1), and 17 June (DOY 169 ± 1) at 1700, 2000 and 2300 m respectively. The comparison of these dates for the onset of cambial activity phases revealed an altitudinal trend ($bE = 0.027Alt + 103$, $R^2 = 0.61$, $P = 0.01$). This trend can be translated into a delay of 2.7 days per 100 m increase in the altitude (figure 4.4a). The bootstrap t-test showed that the difference between sites was statistically significant for the onset of enlarging phase except for two sites of 1350 and 1700 m ($P = 0.15$ for 1350 and 1700 m).

Onset of wall thickening

The wall thickening period observed in 17 June (DOY 169 ± 1) in the bottom site located at 1350 m altitude. This phase started 27 June (DOY 179 ± 2), 5 July (DOY 187 ± 1) and 15 July (DOY 194 ± 1) at the sites of 1700, 2000 and 2300 m respectively. Comparison of these dates showed a delay of 2.6 days per 100 m increase in the altitude for the onset of wall thickening phase ($bW = 0.026Alt + 133$, $R^2 = 0.66$, $P < 0.001$) (figure 4.4b). The results of bootstrap test showed that the difference between the onset of wall thickening phase in different sites were statistically significant ($P = 0.004$ for 1350 and 1700 m), ($P = 0.0009$ for 1350 and 2000 m), ($P < 0.0001$ for 1350 and 2300 m), ($P = 0.03$ for 1700 and 2000 m), ($P = 0.003$ for 1700 and 2300 m), ($P = 0.018$ for 2000 and 2300 m).

Maturation

The first fully mature cells were first observed in 16 July (DOY 198 ± 1) at 1350 m. Then the maturation phase occurred in 19 July (DOY 201 ± 21), 25 July (DOY 206 ± 2), 1 August (DOY 213 ± 3) at 1700, 2000 and 2300 m respectively ($bM = 0.015Alt + 177$, $R^2 = 0.43$, $P < 0.001$) (figure 4.4c). Comparison of these dates showed a delay of 1.5 days per 100 m increase in the altitude for the fully mature cells. The bootstrap test also showed that the differences between each two site were statistically significant for the onset of enlarging phase except two sites of

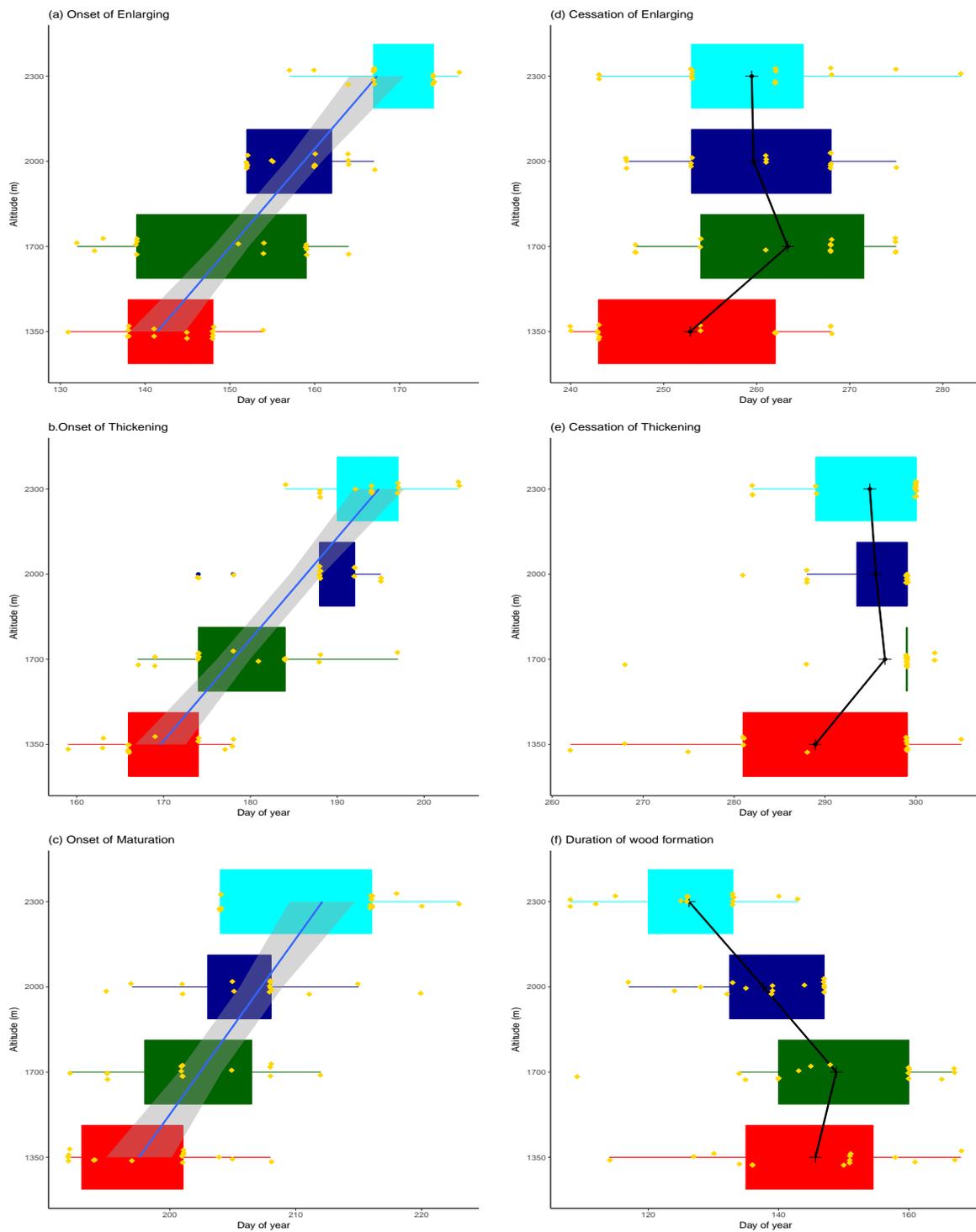


Figure 4.4. (a) onset of enlarging, (b) onset of wall thickening and (c) onset of maturation (d) cessation of enlarging (e) cessation of wall thickening phase (f) duration of wood formation period.

1350 and 1700 m ($P= 0.48$ for 1350 and 1700 m), ($P= 0.029$ for 1350 and 2000 m), ($P=0.002$ for 1350 and 2300 m), ($P= 0.01$ for 1700 and 2000 m), ($P= < 0.0001$ for 1700 and 2300 m), ($P= 0.04$ for 2000 and 2300 m).

Cessation of cambial activity

The cessation of cambial activity occurred in a parabolic trend along the gradient. It firstly observed at lowest site located at 1350 m in 10 September (DOY 253 ± 3), then in 16 September (DOY 259 ± 3) at 2300, in 17 September (DOY 260 ± 2) at 2000 m and finally in 20 September (DOY 263 ± 3) at 1700 m. The ending of wall thickening phase observed in 16 October (DOY 289 ± 3), 23 October (DOY 296 ± 2), 22 October (DOY 295 ± 1), 21 October (DOY 294 ± 2), at 1350, 1700, 2000 and 2300 m respectively (figure 4.4d,e).

Duration of wood formation

The total duration of wood formation computed from the beginning of enlarging to the end of wall thickening phase for each tree and then at each study site. The length of wood formation also showed a parabolic trend along the gradient. The shortest period of wood formation observed at the top site located at 2300 m that was about four month (126 ± 2 days) and the longest occurred at 1700 m that was about five month (149 ± 4 days). The duration of wood formation was less than five month (146 ± 3) and four month and 18 days (138 ± 2 days) at 1350 and 2000 m respectively (figure 4.4f).

Effect of age and diameter on the phenology of cambial activity

Comparing the age and diameter of trees showed significant difference between the trees growing at different sites (figure 4.5).

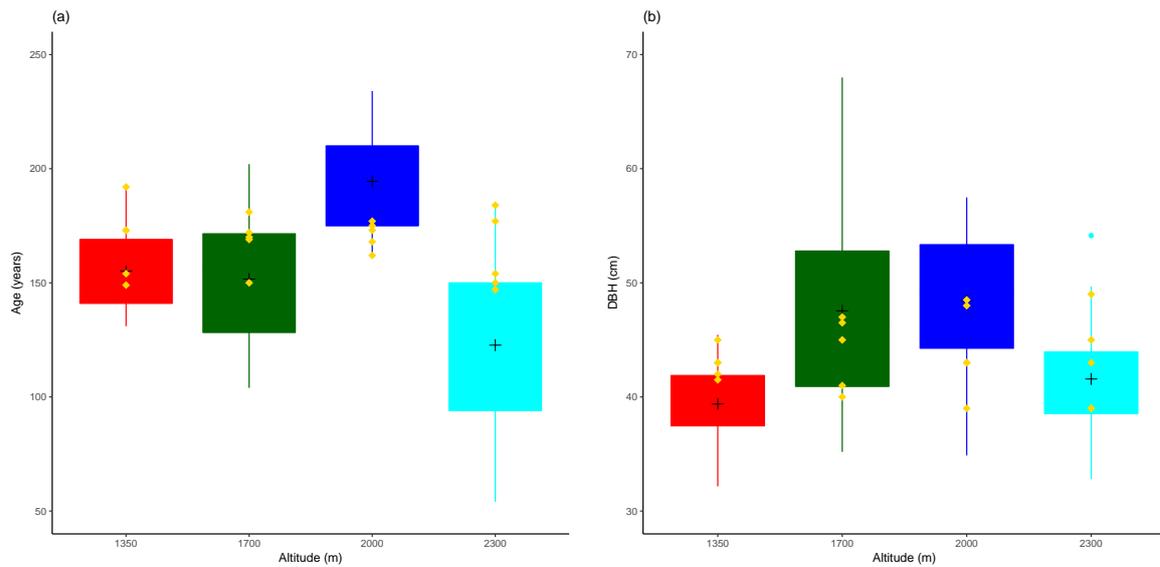


Figure 4.5. Comparison of (a) the age of trees (years), (b) the diameter of trees at the breast height (DBH) (cm). The yellow points show the selected five trees with the same age and diameter.

In order to evaluate the effect of age and diameter on the phenology of cambial activity, a group of five trees with the same age and diameter were selected among 15 trees and the critical dates were computed for each group separately. The results of this comparison showed also the linear trend for the onset of wood formation phases for the selected trees (figure 4.5). The result of regression was ($bE=0.02Alt+101$, $P<0.005$, $R^2 = 0.72$ for the onset of enlarging phase), ($bW=0.02Alt+128$, $P<0.0005$, $R^2 = 0.65$ for the onset of wall thickening) and ($bM=0.008Alt+188$, $P<0.04$, $R^2 = 0.15$ for the onset of maturation). The cessation of wood formation also didn't show any altitudinal trend for the selected trees. Comparison the phenology of wood formation in the selected trees with similar diameter also showed the delay for the onset of cambial activity phases with significant linear trend ($bE=0.01Alt+131$, $P<0.06$, $R^2 = 0.80$ for the onset of enlarging phase), ($bW=0.01Alt+160$, $P<0.01$, $R^2 = 0.95$ for the onset of wall thickening) and ($bM=0.005Alt+192$, $P<0.01$, $R^2 = 0.96$ for the onset of maturation).

4.3.4 Effect of temperature on the onset and cessation of cambial activity

Evaluation of mean daily temperature from March to July showed that at the two bottom sites (1350 and 1700 m) the temperature increase between April and May

didn't lead to onset of cambial activity. But at the two highest elevations (2000 and 2300 m), the onset of cambial activity was coincide with temperature increase in June (figure 4.6). However the cessation of cambial activity observed with a general temperature decrease in September in all the studied sites (figure 4.7).

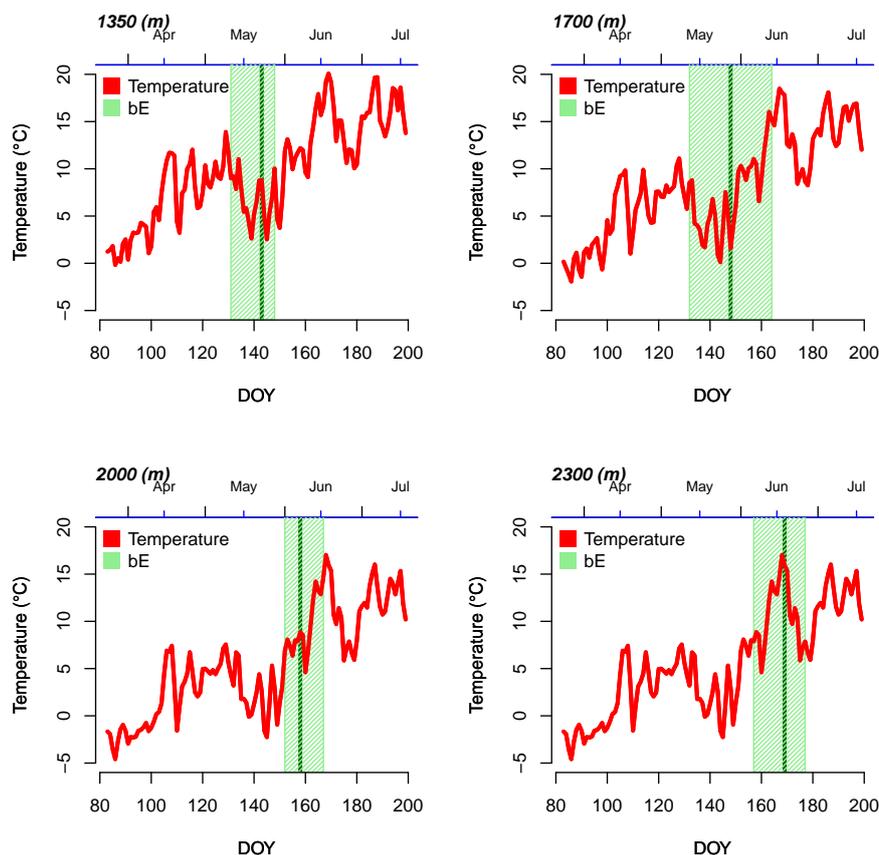


Figure 4.6. Plots of mean annual temperature from March to July (the light green area shows the dates of bE for all the trees and the darkgreen line shows the mean of bE for each site

The mean temperature for bE were (7.07 ± 0.3 °C at 1350 m), (7.3 ± 0.6 °C at 1700 m), (5.3 ± 0.8 °C at 2000 m), (8.6 ± 0.6 °C at 2300 m). The bootstrap test analysis revealed significant difference between the sites ($P= 0.005$) except two bottom elevations (1350 and 1700 m).

Comparison the temperature for the cessation of enlarging phase showed the values of (12.5 ± 0.3 °C at 1350 m), (12 ± 0.3 °C at 1700 m), (9.7 ± 0.14 °C at 2000 m), (7.2 ± 0.4 °C at 2300 m). The bootstrap test showed significant difference between the sites along the gradient ($P<0.1$).

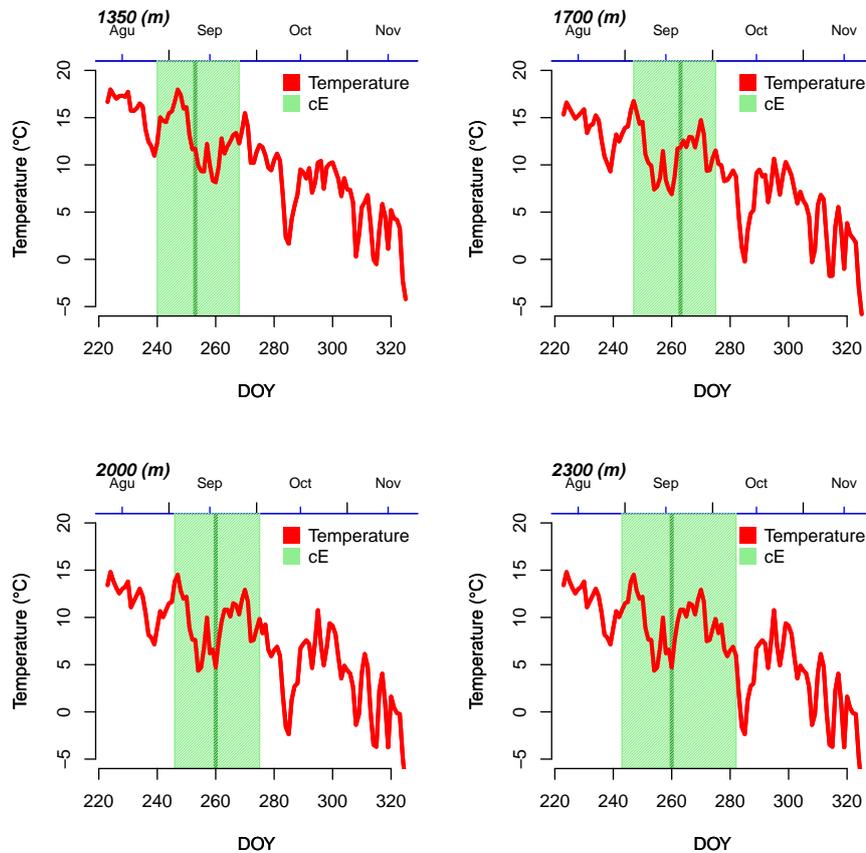


Figure 4.7. Plots of mean annual temperature from March to July (the light green area shows the dates of cE for all the trees and the darkgreen line shows the mean of cE for each site)

4.3.5 Effect of GDD on the onset and cessation of cambial activity

The GDD requirement for the onset and cessation of cambial activity was compared along the gradient. The results of this comparison showed a decrease the amount of GDD with increasing elevation for onset of cambial activity. The GDD of (194 ± 4 °D at 1350 m), (130 ± 8 °D at 1700 m), ($62.5 \pm$ at 2000 m), (60 ± 8 °D at 2300 m) were found for bE. According to the bootstrap test, the different sites required different GDD values for the onset of enlarging phase with significant P values (figure 4.9a).

Comparing the amount of GDD requirement for the cessation of cambial activity showed decreasing trend with increasing elevation (1374 ± 25 °D at 1350 m), (1186 ± 19 °D at 1700 m), (882 ± 12 at °D 2000 m), (614 ± 8 °D at 2300 m). The

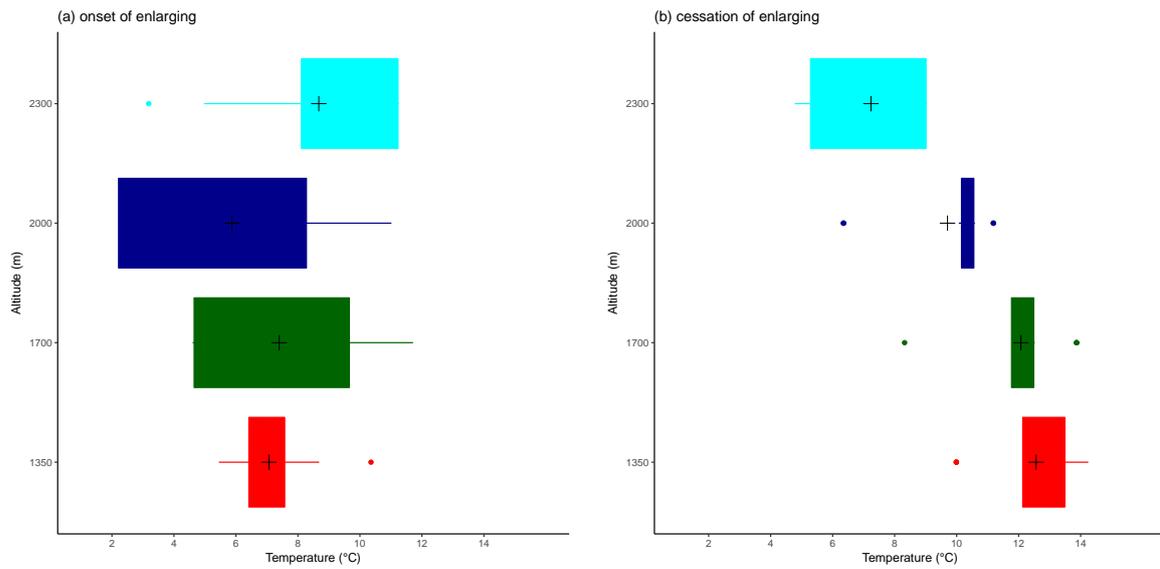


Figure 4.8. Comparison of mean temperature (a) onset of cambial activity (b) cessation of cambial activity.

bootstrap test showed significance difference of heat requirement for the cessation of cambial activity along elevation gradient (figure 4.9b).

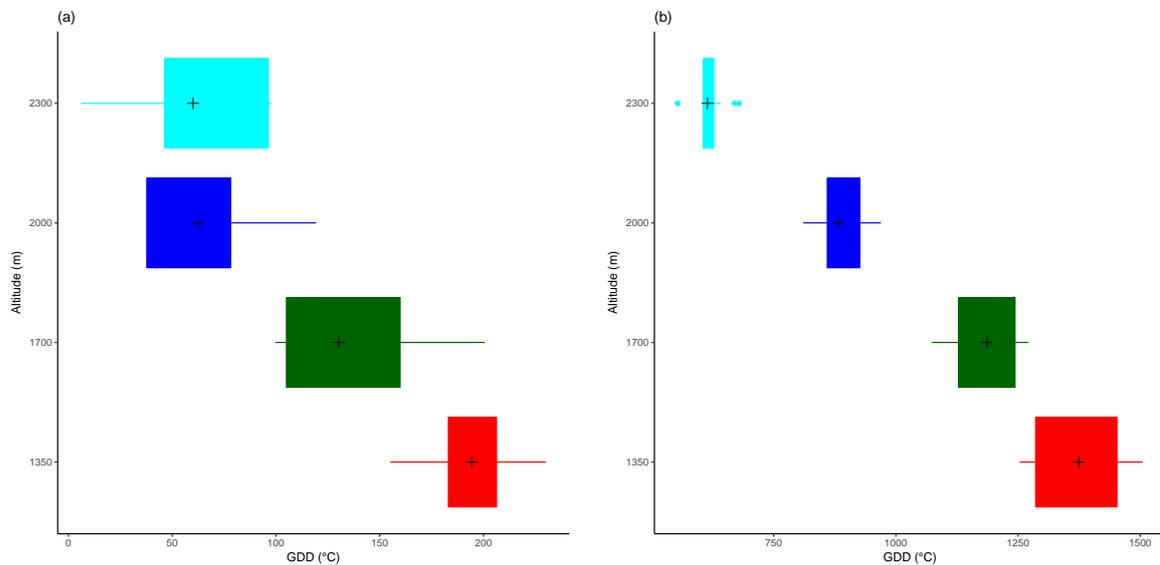


Figure 4.9. Comparison of growing degree days at the time of (a) onset of cambial activity (b) cessation of cambial activity.

Comparison of photoperiod at the onset and cessation of Enlarging phase

The photoperiod was compared at the onset and cessation of enlarging phase. The results of the mean length of photoperiod for the onset of enlarging was (15.13 ± 0.04 hours at 1350 m), (15.21 ± 0.07 hours at 1700 m), (15.46 ± 0.02 hours at 2000 m), and (15.56 ± 0.07 hours at 2300 m). The bootstrap test analysis revealed significant difference between the photoperiod at the onset of enlarging phase among studied sites except two site located at 1350 and 1700 m ($P=0.2$) (figure 4.10a).

Comparison the photoperiod length for the cessation of enlarging phase showed the values of (12.7 ± 0.14 hours at 1350 m), (12.20 ± 0.14 hours at 1700 m), (12.38 ± 0.12 hours at 2000 m), (12.40 ± 0.14 hours at 2300 m). The bootstrap test showed that the difference between the median of different sites was not significant except the two sites of 1350 and 1700 m ($P=0.03$) (figure 4.10b).

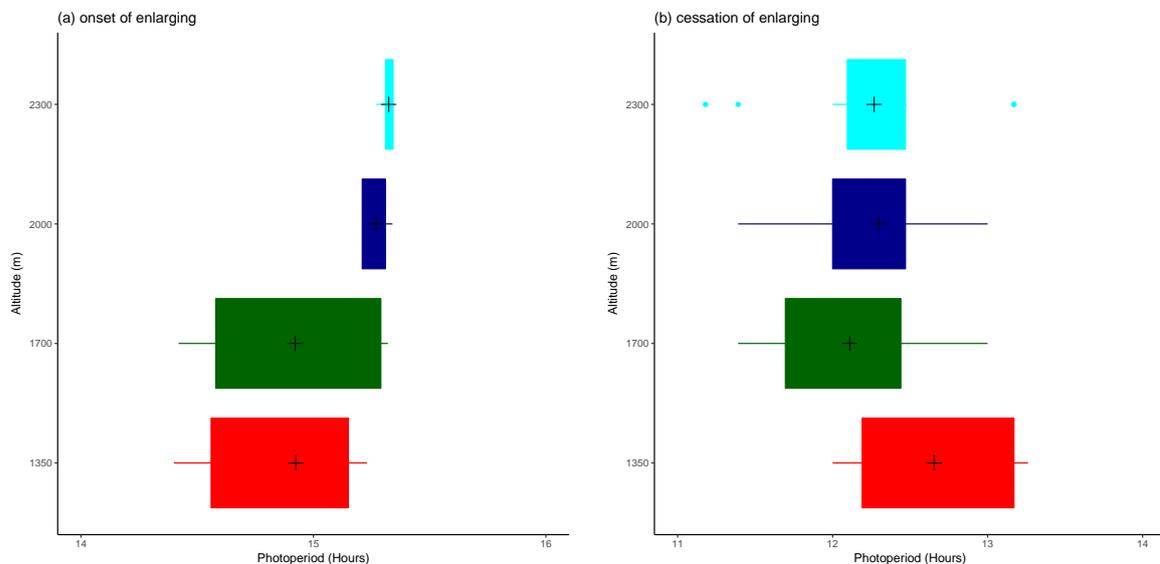


Figure 4.10. Comparison of photoperiod (Hours) at (a) onset of cambial activity (b) cessation of cambial activity.

4.4 Discussion

Elevation gradients are strong natural experimental design in ecological studies. They provide unique basis to perform space-for-time studies to look at the response of trees to the geophysical influences such as temperature in their natural habitat (Korner 2007). In this study the phenology and duration of cambial activity was quantified during 2013 growing season in larch trees along a gradient of 1000 m in

elevation in the southern Alps.

The present study showed a delay for the onset of wood formation phases by increasing elevation in a linear trend. The temperature analysis along the studied gradient showed a decrease of $-0.54\text{ }^{\circ}\text{C}/100\text{ m}$ increase in elevation. The observed delay for the onset of cambial activity regarding to temperature trend can be stated as $5\text{ days}/^{\circ}\text{C}$. The response of cambium to temperature was previously tested in natural and artificially heated stems. Applying heat to tree stem induced reactivation of cambium and cell divisions and led to xylem and phloem formation (Oribe and Kubo. 1997, Oribe et al. 2001, Gricar et al. 2006). Temperature can affect cambial activity through controlling the growth hormones such as indole-acetic acid (IAA). It allows the metabolic process of growth to be completed and can influence on biomass accumulation in cell walls (Antonova and Stasova 1993, Begum et al. 2007, Rossi et al. 2008).

This pattern of temperature induced cambial activity along elevation gradient was previously observed by Moser et al (2010) in a comparable gradient of about 800 m in the Swiss Alps with a delay of $7\text{ days}/^{\circ}\text{C}$ (Moser et al. 2010). Our finding was also consistent with the results obtained for Qilian Juniper trees along a gradient of about 700 m in China (Wang et al. 2014). Temperature is known as the main factor controlling onset of wood formation in trees. Rossi et al. (2008b) found a common critical temperature thresholds for xylogenesis in conifers at different latitudes and altitudes in Europe and Canada, with average values of 4–5, 8–9 and 13–14 $^{\circ}$ for daily minimum, mean and maximum temperature, respectively. However the critical temperatures calculated by Rossi et al. (2008b) for cessation of cambial activity in northern conifers were variable. In our study the temperature for the onset and cessation of cambial activity was found to be different both between trees growing at the same elevation and along gradient. The observed increase of temperature before onset of cambial activity in our study sites suggests that reaching to a certain air temperature is not enough for the onset of cambial activity and other factors such as soil temperature may also need to be increased (Treml et al. 2015). Comparing the GDD showed different heat requirement in trees for both onset and cessation of cambial activity with a decreasing trend toward higher elevations. Gricar et al (2014) found varying GDD values for the onset of cambial activity in *Picea abies* trees growing in different sites that were inversely related to the elevation (Gricar et al. 2014). The results of our study also confirms that lower amounts of heat accumulation is needed for both onset and cessation of cambial activity with increasing elevation. But in contrast Schmitt et al. (2004) found similar amount of

GDD values for the onset of cambial in *Pinus sylvestris* L. trees growing along an elevation gradient in the boreal forests of Finland.

The age of our studied trees were different along the gradient with younger trees at the top site (2300 m). However our results showed phenology of cambial activity is more related to elevation rather than specific tree characteristics such as age or diameter. The onset of wood formation occurred later in old trees compare to adults (Rossi et al. 2008a). In our study, the onset of all phenophases of xylem formation (enlarging, wall thickening and maturation) followed exactly the same trend showing that they are not separated from each other as reported in other studies (Rossi et al. 2012, 2013). Their successive occurrence shows the controlling role of the same factor on them relating to elevation.

Although a linear behavior observed for the onset, the cessation of wood formation phases occurred in a parabolic pattern with trees from the lowest stand finishing first, followed by those from the highest stand, while those from the intermediate elevations finished the last. Our results showed that the cessation of cambial activity is more related to photoperiod. Previous studies on the wood formation along elevation gradients reported also that the cessation of cambial activity was less related to elevation (Wang et al. 2015). Moser et al (2010) found a parallel cessation of growing season in *L. decidua* along different elevations in Swiss Alps (Moser et al. 2010) that can be explained by the effect of photoperiod providing signals for the cessation of wood formation phases. The growth cessation was found previously to be a photoperiodic response as trees at higher latitudes finished their growth earlier in autumn compare to Southern latitudes (Jackson 2008). So at it was observed in our study, photoperiod can be considered as the main factor providing signals for cambium to finish it's activity. However water shortage seems to be able to trigger an earlier termination of the growing season at the bottom site (1350 m). Low soil humidity can trigger an earlier conclusion of growth (Fritts 1956). Drought stress can modify plant physiology via affecting stomatal closure and photosynthesis activity (Breshears et al. 2013). In our studied gradient, the longer period of water deficit and higher drought intensity as well as greater amount of vapor pressure deficit was observed at the bottom site. Higher temperature and associated increase in VPD are the two most critical parameters in the senario of climate change (IPCC 2014). The soil water content will change along the growing season with increasing temperature that can intensify the water availability at the middle of growing season (Way and sage 2008).

In this study we observed also a parabolic trends for the wood formation duration,

with the shortest occurring at the 2300 m site (about four months), and the longest ones at the 1700 m site (about five month). Trees growing at higher altitude and latitudes, experienced less favorable climatic condition during their growth compare to temperate regions (Chuine 2010). They need to finish their wood formation in a limited time before the disturbances of very cold temperature. Adjusting length of growing season in trees is an optimization between frost damage and maximization of annual carbon assimilation (Chuine 2010). Regulating the phenological phases of xylem formation should guarantee enough time for latewood tracheids to finish their wall thickening phase. In colder environments the number of frost days is higher. In the frost condition, where the temperature is close to zero, the metabolism of trees will decrease as much as possible in order to maintain the minimum physiological activity and restrict the water uptake and root functioning. Low temperatures can affect the soil nutrient availability via limiting the decomposing and mineralizing organic materials (Korner 2003). Capturing nutrient from soil can be a constraint to growth in very cold environments so the cambial activity can be limited by low temperature, through controlling biogeochemical process of the soil and providing nutrient for tree growth.

A reduction in the length of wood formation period from 220 to 50 days was observed in the nine conifer species from Europe and Canada that was consistent to the decrease in the site temperature (Rossi et al. 2013). Moser et al (2010) also observed a decrease from 137 to 101 days in the length of growing season toward the higher altitude in Larch trees growing along a gradient of about 800 m in the Swiss Alps (Moser et al. 2010). Another example is decreasing the length of growing season from 5 to 2 months in *Picea abies* trees from higher elevation to the Alpine treeline (Rossi et al. 2008). In our study, shortening the length of growing season from the middle to top elevation from 150 to 126 days can be explained by low temperature limitation while an earlier cessation of wood formation at the bottom site (1350 m) can be related to water shortage.

The effect of higher tempertaure on earlier resumption of cambial activity can lead to lengthening of wood formation in future as a response to predicted temperature increase. The observed delay of 5 days/°C relating to the projected increases in temperature over France between 2.5 °C to 3.5 °C (Gosling et al, 2011), the onset of cambial activity can be predicted to start between 12.5 to 17.5 days earlier in 2100 compare to 1960.

4.5 Conclusion

This study on the intra-annual phenology of wood formation in larch trees growing along a gradient of 1000 m in elevation has shown that the timing of xylem formation phases, change as a relation to elevation. Wood formation started earlier at the bottom elevation and moved toward higher elevation with a linear trend. The onset of the other phenophases of xylem formation followed the same trend, but with a time lag of a few days up to one or two months respectively. The cessation of these phenophase followed a parabolic trend with trees from the lowest stand finishing first, followed by those from the highest stand, while those from the intermediate elevations finished the last. Wood formation duration also exhibited parabolic trends, with the shortest occurring at the 2300 m site (about 4 months), and the longest ones at the 1700 m site (about 5 month). The observed delay in the wood formation onset with increasing elevation can be associated with the adiabatic temperature decrease and thus translated into a lengthening of the growing season of about 5 days per °C. On the other hand, our results show that the cessation of cambial activity is more related to photoperiod, even if water shortage seems able to limit the termination of the growing season. In conclusion, the predicted climatic change and temperature increase in future can affect the larch trees growing along studied gradient in Southern Alps by lengthening their growing season. The temperature increase can limit water availability at lower elevations and in long term may lead to increase the optimum elevation for the growth of larch trees in Southern French Alps. However with the results of this study, it is not possible to explain how trees are able to adapt themselves to shortening of growing season at lower temperature. So further investigations on the dynamics of cambial activity is needed to answer this question.

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Supplementary materials

Table 4.2: Characteristics of selected trees at each study site

Site	Tree	DBH (cm)	Age (years)	Height (cm)
	4382	45.44	131	2580
	4383	36.94	154	NA
	4384	42.19	149	2470
	4385	41.56	NA	2180
	4386	40.50	173	NA
	4387	37.99	139	2230
	4388	42.99	NA	2400
1350	4389	33.02	136	NA
	4390	39.04	NA	2155
	4391	38.59	192	2310
	4392	39.64	157	2175
	4393	41.40	NA	1730
	4394	36.30	NA	1960
	4395	42.86	173	2355
	4396	32.16	147	NA
	Mean \pm Se	39.38\pm1	155 \pm6	2231\pm 72
	4397	55.41	107	2860
	4398	67.99	169	3160
	4399	46.49	150	2985
	4400	52.70	189	2800
	4401	61.78	172	2795
	4402	44.90	163	2955
	4403	42.03	170	3020
1700	4404	39.17	127	2450
	4405	35.19	120	NA
	4406	46.97	137	2720
	4407	41.24	NA	2685
	4408	36.78	202	NA
	4409	40.60	104	2190
	4410	52.86	132	2340
	4411	49.04	181	2560
	Mean \pm Se	47.54\pm2	151\pm 8	2732\pm78

Table 4.3: Characteristic of trees

Site	Tree	DBH (cm)	Age (years)	Height (cm)
	4412	43.31	NA	2495
	4413	43.31	189	3000
	4414	48.08	192	2990
	4415	51.75	162	NA
	4416	34.87	173	1970
	4417	49.36	190	2755
	4418	54.93	168	2870
	4419	43.31	175	2740
	4420	49.84	208	2870
2000	4421	56,05	233	2660
	4422	56,21	210	2900
	4423	57,48	217	2655
	4424	38,69	NA	2410
	4425	45,22	177	2700
	4426	49,20	234	2645
	Mean \pm Se	48.45 \pm 2	194 \pm 6	2690 \pm 71
	4427	42.67	154	1806
	4428	39.96	NA	1725
	4429	41.082	120	1775
	4430	49.36	90	1930
	4431	32.80	94	1710
	4432	36.62	74	1540
	4433	39.49	132	1335
2300	4434	40.12	147	1505
	4435	54.14	184	1710
	4436	39.49	122	1660
	4437	38.53	150	1690
	4438	38.53	97	1780
	4439	49.68	177	1950
	4440	35.82	54	1725
	4441	45.22	NA	1970
	Mean \pm Se	41.57 \pm 1	122 \pm 10	1720 \pm 43

Table 4.4: Characteristic of selected trees with similar age and diameter

Site (m)	DBH (cm)	Age (years)
1350	43 \pm 0.6	168.2 \pm 7
1700	44 \pm 1	168.4 \pm 5
2000	44.3 \pm 2	171 \pm 2
2300	43 \pm 2	162.4 \pm 7

Chapter 5

Wood formation dynamics

Seasonal dynamics of wood formation in Larch growing along an elevation gradient of 1000 m in the Southern Alps

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Abstract

Xylogenesis is a highly dynamic process that results from variations in the timing and rate of cell production by cambium. The result of this process is producing xylem cells within the period of wood formation in the form of tree ring structure. The rate of cell production was found to be related to both endogenous and exogenous factors such as size, age and species while the length of cambial activity was found to be mainly dependent to environmental factors such as temperature and water availability. Comparing wood formation dynamic along elevation gradients, will provide unique data about the adjustment of growth parameters such as rate by trees as a response to the main environmental factors changing with elevation such as temperature. The main objective of this study is to understand how the trees are able to grow in different climatic conditions by adjusting their seasonal dynamics of wood formation. To answer this question, the xylogenesis of 60 larch trees, distributed in four stands, and spread along an elevation gradient of 1000 m in the French Southern Alps was monitored all along the 2013-growing season. Wood samples were taken every week from the stem of the selected trees. Microcores were then cut, stained and fixed in the laboratory to produce anatomical sections, which were observed under microscope. Forming tracheids were classified and counted according to their differentiation stage. Meteorological data were obtained from nearby climate stations and global database. The relative extractable water was computed using a water balance model. Wood growth dynamics was described fitting Gompertz models to the cell counting data. The cell cycle duration of cambium was also computed using the growth parameters. The growth rates were then compared along the elevation gradient using bootstrap tests. The duration of cambial activity was computed based on the onset and cessation dates of enlarging phase. The relative contribution of rates vs. durations to the total annual production was assessed using a sensitivity analysis of a basic physical model.

The results showed an elevation related changes in the dynamic of wood formation along the gradient. The cambial activity (CA) durations exhibited parabolic trends along elevation, but with the shortest occurring at the 2300 m site (CA about 3 months), and the longest ones at the 1700 m site (CA about 4). However the shortening of the growing season was compensated by an increase in the cell production rate leading to comparable tree-ring widths in the three upper stands. The lowest stand, on the contrary, exhibited narrow tree rings due to both low production rates and short durations. The sensitivity analysis showed that the final tree-ring width was about 60% related to the rate of cell production and only 40 % to its duration. Moreover, our investigations showed that trees increased the number of dividing cells in their cambium in response to the elevation-related increase in cell cycle length, maintaining high production rate at high altitude. In conclusion it can be stated that with moving toward higher elevation where the length of growing season is limited by low temperature the dynamic of growth is more intense with higher rate of cell production helping trees to maintain their tree ring width, that can be considered as an adaptation strategy in trees to grow in colder environments. **Keywords :** cambial activity, *larix decidua*, rate, elevation gradient, French Alps.

5.1 Introduction

Xylem formation in trees is the result of interaction between the timing and duration of cambial activity. Understanding the dynamic of wood formation is a central question in tree ring based studies especially those focusing on tree growth and environmental factors relationship. The elements of growth dynamic such as rate and duration of xylem cell production was found to vary with species, environmental condition and sites. These variations in the timing and rate of cell production make xylogenesis a highly dynamic process ([Wodzicki 1971](#), [Deslauriers et al. 2003](#), [Rathgeber et al. 2011b](#), [Cuny et al. 2014](#)).

The stem size increment in trees starts with the periclinal divisions of cambium into daughter cells that then go through differentiation phases ([Wilson 1984](#)). The division of cambial cells was found to be affected by both endogenous and exogenous factors ([Uggla et al. 1996](#)). Cell division in all the cellular organisms containing nucleus, follows a highly controlled sequences of following events described as cell cycles ([Lachaud et al. 1999](#)). The meristematic activity of trees such as cell cycle was found to be affected by quantity of available resources such as sucrose around

dividing cell (Riou-Khamilchi et al. 2000). The higher concentration starch and sugar was observed in the cold treated seedling of *Larix decidua* and *Pinus mugo* trees (Hoch and Korner 2009). So the growth process such as cell divisions in colder environment is expected to be less affected by photosynthesis limitation, but other environmental factors changing with elevation such as temperature can have an impact. In boreal and temperate regions, the seasonal temperature variation has an influence on the timing of optimal growth condition in trees. The dynamic and length of growing season are not independent of each other as higher rate of cell production was observed in the trees with longer period of cambial activity (Rathgeber et al. 2011b). The rate of cell production can be affected also by age and size of trees as the cambial activity was reported to be less intense in smaller and older trees (Rossi et al. 2008, Rathgeber et al. 2011b). The differences between the number of cambial cells that was observed in different species (Larson 1994) also can affect the rate of cell production in trees (Vaganov et al. 2006).

The maximal growth rate defined as the timing of transition from growth acceleration to growth deceleration was found to occur before or very close to summer solstice as a response to the maximal day length (Rossi et al. 2006b, Rossi et al. 2011). Trees need to adjust their growing season length in a period to avoid the winter harmful events and also maximize the use of available resources for their growth. So the occurrence of maximal growth rate should guarantee the optimal temperature condition and full cell maturation.

Limited water availability is an environmental factor that can have an impact on tree growth. It was found to affect the size of tree ring as non-irrigated sapling exhibited a significant decrease in the number of cells within the newly formed ring (Balducci et al. 2013). A decrease in the annual ring width can occur due to both lower expansion rate of the cambial cell derivatives during the enlarging phase in the early stage of water deficit and decreasing cell division rate in the case of more severe water deficit (Abe et al 2003, Jyske et al. 2009, De Luis et al. 2011, Balducci et al. 2013). The tree ring size is the result of interaction between both rate and duration of cell production (Cuny et al. 2014). The final size of tree ring was found to be mainly affected by rate of xylem cell production rather than its duration (Rathgeber et al. 2011b). Monitoring the dynamic of wood formation has been started from several decades ago with the main objective of understanding the influence of climate on tree growth (Wilson 1984, Denne and Dodd 1981). Although there is a wide number of studies on the phenology of cambial activity (cell division, cell enlargement, wall thickening and maturation) in trees, its dynamic (rate and

duration of cell production) is not extensively explored. Elevational gradient, are strong natural experimental design to study the response of tree species to different environmental condition such as low temperature (Korner 2003a). One of the main questions in wood formation studies is to find if shortening in the length of growing season will limit tree ring width.

The main objective of this study is to compare the dynamic of wood formation in Larch trees along an elevation gradient of 1000 m as a response to environmental condition. The general hypothesis of this work is that the dynamic of cambial activity is under the control of environmental factors. The first part of this hypothesis is that the Larch trees growing at lower elevation present a higher rate of xylem production compare to higher elevation. The second hypothesis is that the number of tracheid cells is mainly under the control of cell production rate rather than its duration.

To answer this question, the xylogenesis of 60 larch trees, distributed in four stands, spread along an elevation gradient of 1000 m in the French Southern Alps was monitored all along the 2013-growing season. Wood growth dynamics was described fitting Gompertz models to the cell counting data. The cell cycle duration of cambium was also computed using the growth parameters. The growth rates were then compared along the elevation gradient using bootstrap tests. The duration of cambial activity was computed based on the onset and cessation dates of enlarging phase. The relative contribution of rates vs. durations to the total annual production was assessed using a sensitivity analysis of a basic physical model

5.2 Materials and methods

5.2.1 Study site and tree selection

The studied site is located along an altitudinal gradient located in Villard St.Pancrace close to Briançon ($44^{\circ} 53' 47''$ N, $6^{\circ} 38' 08''$ E), in a west facing slope. The 60 larch trees, distributed in four stands (4 x 15 trees), and spread along an elevation gradient of 1000 m (1350, 1700, 2000 and 2300 m) in the French Southern Alps was selected to study their wood formation dynamics all along the 2013-growing season.

5.2.2 Tree sampling and sample preparation

During the growing season of 2013, microcores (1.5 mm diameter and 15 to 20 mm length) were taken from the selected trees at the breast height from May to November using a Trephor tool (Rossi et al. 2006a). The microcores after extraction from the trees were immediately placed in Eppendorf vials with a diluted solution of ethanol 50 % and stored at 5° C to prevent the deterioration of the living tissues. Microcores then were dehydrated and immersed using an automatic tissue processor in the baths of ethanol, d-limonene and paraffin (STP121, MM France) (Rossi et al. 2006a). Then they were inserted in paraffin blocks with an embedding station (EC 350, MM France). A rotary microtome (HM 355S, MM France) was used to take the 5 to 10 µm thick transverse sections and then they were stained using cresyl violet acetate (0.16 % in water). After coloration the sections were permanently mounted on glass slides using Histolaque LMR for further anatomical observations.

5.2.3 Microscopic observation

An optical microscope (ZEISS, Germany) was used for anatomical observations of samples with magnification of 10 to 40 and visible and polarized light. The number of cells in each phases of cell differentiation were counted along three radial files. The cambium cells were characterized by their thin primary walls, small radial diameter and rectangular shape. The enlarging cells had a greater lumen area than cambial cells with thin primary walls and were not brilliant under polarized light. The cells in wall thickening phase were brilliant under polarized light with a gradient of pink-violet to blue color from inside to the outside of their cell wall. The tracheids were considered to be fully mature when they were totally blue. In each sample, the number of cambial (C); enlarging (E); wall thickening (W); fully mature (M); previous year (P) cells were counted along three radial files according the criteria described by (Rossi et al. 2006b).

5.2.4 Timing of wood formation

The critical dates of cambial activity: (1) the beginning of the dividing phase; (2) the beginning of the enlarging phase; (3) the beginning of the wall thickening phase; (4) the beginning of the mature phase; (5) the end of the dividing phase; (6) the end of the enlarging phase and (7) the end of the wall thickening phase were com-

puted based on anatomical observation data. A dedicated function of the R package, CAVIAR (Rathgeber 2011a) which computes the critical dates based on logistic regression. The evaluation of the timing of tracheid cell production was based on the xylem cell enlargement phase rather than the cambial cell dividing phase as proposed by (Rathgeber 2011a). Thus, the onset, cessation and duration of enlarging phase were used as proxies for the onset, cessation and duration of cambial activity, and the onset of enlarging and cessation of wall thickening phases were used as proxies for onset and cessation of xylogenesis respectively.

5.2.5 Rate of tracheid cell production

In order to evaluate the dynamics of tracheid cell production, the number of cells over time was fitted to the Gompertz function (Rossi et al. 2003). The Gompertz function defined as:

$$nC(t) = A.e^{-e^{\beta-k.t}} \quad (5.1)$$

which nC is the standardized total number of tracheids at time t , A is the upper horizontal asymptote parameter representing the final number of tracheid, β is the x-axis placement parameter showing the choice of origin time, and k , the growth rate parameter that reflects the spread of the curve along the time axis.

The Gompertz parameters were computed for each tree using a dedicated function of the R package in CAVIAR. This function will compute the model efficiency (R^2), and the corresponding maximal rate of tracheid production r_x , as well as the mean rate r_m for the period during which 90 percent of the tracheid cells were produced (Rathgeber et al. 2011b).

5.2.6 Cell cycle computation

The minimal cell cycle length CCL_n was calculated in order to assess if the rate of tracheid production depended more on the number of cambial cells or the division frequency of each cambial cell. The CCL_n was computed by dividing the number of cambial cells at time of maximal rate t_x by maximal rate of cell production r_x .

$$CCL_n = CZ(t_x)/r_x \quad (2)$$

5.2.7 A simple physical model of xylem cell production

In order to compute the total number of cells at the end of growing season (RCN) the median of the total number of cells from all the samples after the end of cell production was used. A simple model was applied to express the RCN as a function of the average rate (r_m) of cell production and the duration of cell production Δt_E computed from the onset and cessation of enlarging phase.

$$RCN = f(r_m * \Delta t_E) \quad (3)$$

In order to compute the specific contribution of the rate and duration to the total number of cells, a sensitivity analysis was performed. This analysis is based on the assessment of the relative variation of the input variables (using their standard deviation) on the output result of the model (Cariboni et al. 2007). The wood formation curves derived from Gompertz model showed variations in the total number of tracheid cells at the end of growing season in some trees, so in order to obtain the most accurate data, five trees at each site that showed the normal growth pattern were selected to test with physical model.

5.2.8 Meteorological data

The climatic data (mean air temperature, precipitation, global radiation, relative humidity, and wind speed) were extracted directly from local meteo stations installed at the study sites. The daily precipitation data were interpolated for the study gradient using the data from the nearby Meteo France station and "world clim" global data base.

Soil water content

The soil water balance in the studied sites and for the year of 2013 was computed using Biljou model (<https://appgeodb.nancy.inra.fr/biljou/>) (Granier et al. 1999). this model have been developed to quantify drought intensity and duration. It uses a set of meteorological data (mean air temperature, precipitation, global radiation, relative humidity, and wind speed) and other parameters such as forest type and the maximum leaf area index (LAI). This model can estimate the soil water content and the seasonal annual water stress indices. The soil characters such as

water reserve capacity (mm) were measured directly at each study sites. Then the other soil characteristics were estimated at two soil layers based on the Biljou water balance model manual books (table 5.1).

Table 5.1: The soil characteristics at different sites

Altitude (m)	Layer number	Gravimetric water	Root pro-portion %	Depth (cm)	Water reserve (mm)	Bulk density
2300	1	0.05	0.6	40	25	1.2
2300	2	0.1	0.4	55	14	1.3
2000	1	0.05	0.6	20	30	1.2
2000	2	0.1	0.4	40	23.2	1.3
1700	1	0.05	0.6	40	40	1.2
1700	2	0.1	0.4	60	26.7	1.3
1350	1	0.05	0.6	15	20	1.2
1350	2	0.1	0.4	35	26.5	1.3

5.2.9 Evaluation of temperature effect on cell cycle

In order to evaluate the impact of temperature on cell cycle length, the mean daily temperature at the time of maximal rate were extracted and compared among different sites.

5.2.10 Statistical analysis

The comparison of growth rate parameters as well as cell cycle and cambial cell number among different sites, was done by bootstrap test in order to see if the differences in the growth parameters is related to elevation. This test was done on median of groups using a CAVIAR package function in R ([Rathgeber et al. 2011b](#)).

5.3 Results

5.3.1 Ring cell number

The results of this study showed that the trees growing in the three upper stands produced about the same final tree-ring tracheid cell number. The lowest stand, on contrary exhibited narrow number. These values were (20 ± 1 in 1350 m), (30 ± 3 in 1700 m), (30 ± 2 in 2000 m) and (29 ± 3 in 2300 m) (figure 5.1 f).

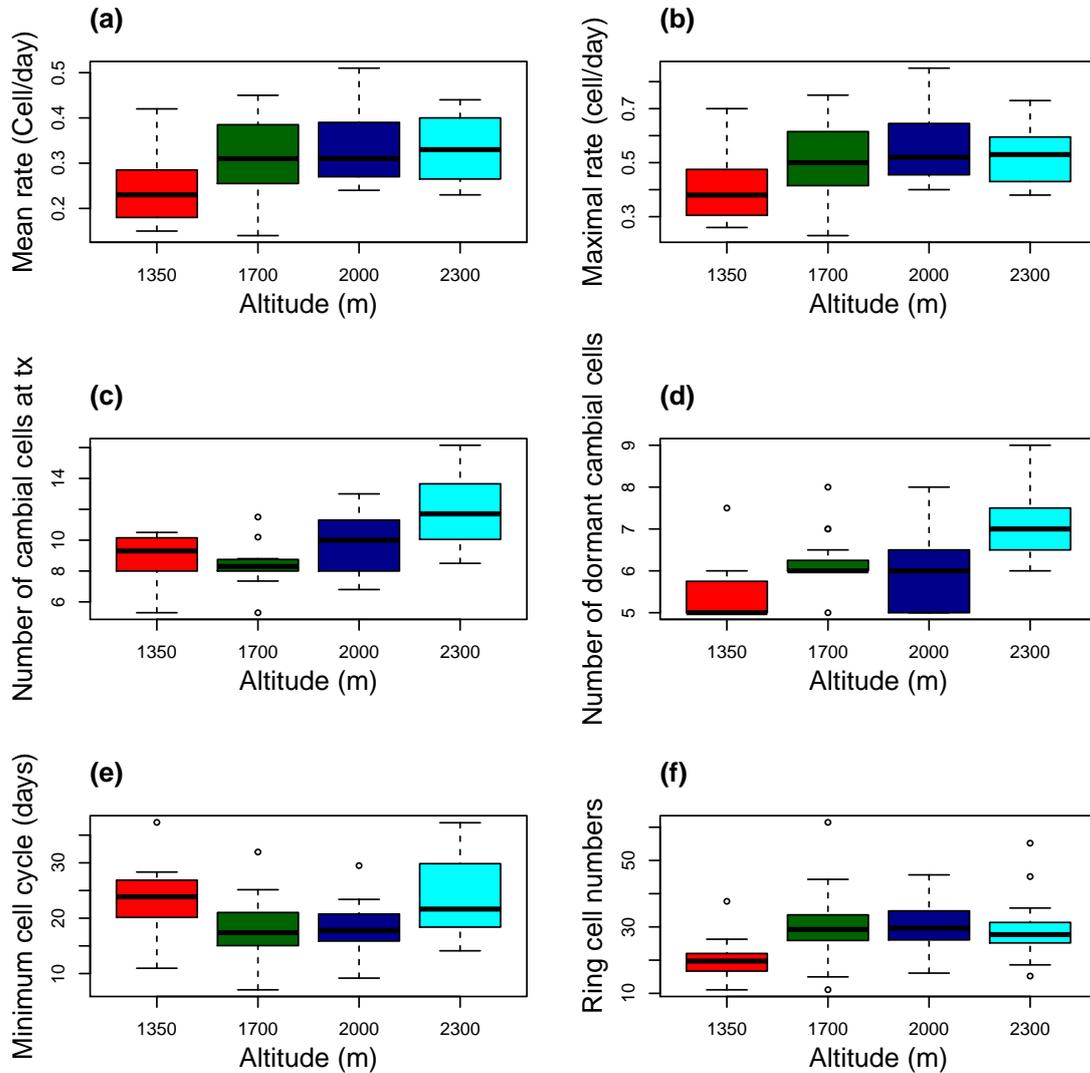


Figure 5.1. Comparison of (a) mean rate (cell/day), (b) Maximal rate of cell production (cell/day), (c) Number of cambial cells at tx, (d) Number of dormant cambial cells (the time of maximal rate), (e) The minimum cell cycle length (days) and (f) the ring cell number at different altitude

5.3.2 Duration of cambial activity

The shortest duration of cambial activity observed at 2300 m that was about three month (91 ± 3 days) and the longest was observed at 1700 m that was about four month (115 ± 4 days). This period was three month and 20 days (110 ± 3 days) and three month and 11 days (101 ± 3 days) at 1350 and 2000 m respectively (figure 5.2a).

5.3.3 Duration of wood formation

The total duration of wood formation computed from the beginning of enlarging to the end of wall thickening phase was found for each study site. The shortest period of wood formation observed at the top site located at 2300 m that was about four month (126 ± 2 days) and the longest occurred at 1700 m that was about five month (149 ± 4 days). The duration of wood formation was less than five month (146 ± 3) and four month and 18 days (138 ± 2 days) at 1350 and 2000 m respectively (figure 5.2b).

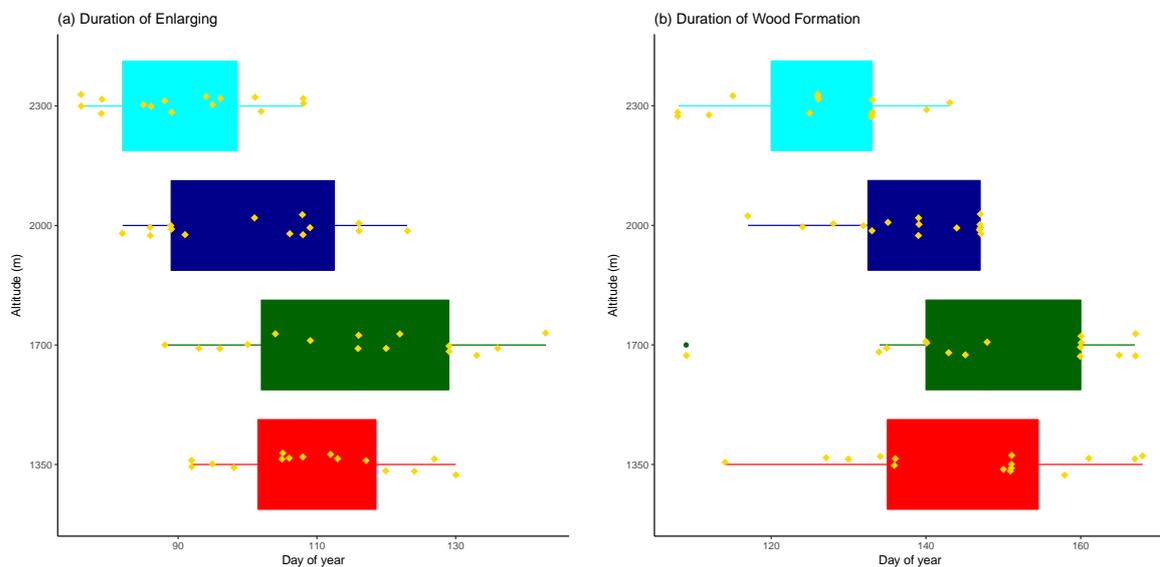


Figure 5.2. (a) Duration of Enlarging, (b) Duration of wood formation in different sites. The points show the duration for each individual tree).

5.3.4 Rate of xylem cell production

Fitting Gompertz model to the tracheid cell counting data yielded good fitness results in the studied sites (figure 5.3) with (R^2) ranging from 0.60 to 0.98 (Supplementary material table 1). The general shape of Gompertz function was found to be the same in the three upper elevation but different at the lowest site. As it can be observed in the plots of cumulated number of cells derived from Gompertz function, the increasing trend in the tracheid cell production occurred with a delay moving toward higher elevation but the three top sites reached the same tree-ring cell number. Although trees growing at the lowest elevation produced higher number of tracheid cells at the beginning of growing season but they reached also earlier to their maximum tracheid cell number.

The rates of tracheid cells production obtained differed between different sites. The mean rate of xylem cell production was (0.24 ± 0.01 (cell/day) in 1350 m), (0.30 ± 0.02 (cell/day) in 1700 m), (0.33 ± 0.02 (cell/day) in 2000 m) and (0.33 ± 0.01 (cell/day) in 2300 m). The maximal rate of xylem cell production was (0.40 ± 0.03 (cell/day) in 1350 m), (0.49 ± 0.03 (cell/day) in 1700 m), (0.55 ± 0.03 (cell/day) in 2000 m) and (0.52 ± 0.02 (cell/day) in 2300 m) (figure 5.3a, b). The regression analysis showed significant linear trend with elevation both for the maximal rate ($r_{max} = 0.09Alt + 0.0002$, $P = 0.009$, $R^2 = 0.54$) and mean rate of cell production ($r_{mean} = 0.12Alt + 0.00002$, $P = 0.001$, $R^2 = 0.65$)

5.3.5 The number of cambial cells

Comparison the number of cambial cells at the time of maximal rate and cambial dormancy (at the end of growing season) showed an increasing trend toward higher elevation. The number of cambial cells was (8.8 ± 0.4 in 1350 m), (8.4 ± 0.3 in 1700 m), (9.6 ± 0.5 in 2000 m) and (11.9 ± 0.5 in 2300 m). The number of dormant cambial cells was (5.4 ± 0.18 in 1350 m), (6.2 ± 0.17 in 1700 m), (6.0 ± 0.2 in 2000 m) and (7.6 ± 0.22 in 2300 m). The bootstrap test among different sites showed that the observed difference was statistically significant ($P < 0.05$) and the regression analysis also showed a significant trend with elevation ($P < 0.001$).

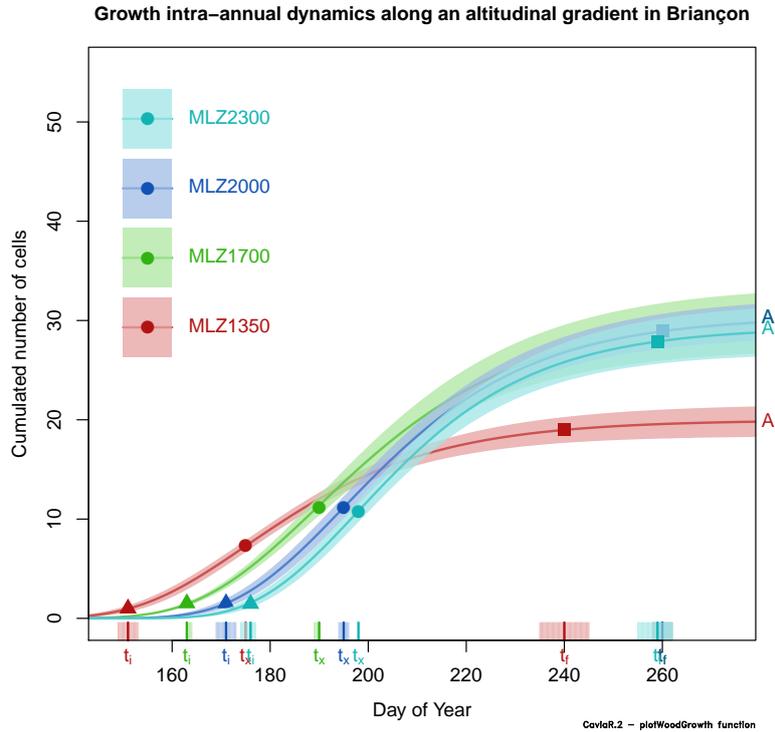


Figure 5.3. Cumulative number of tracheid cells in Larch trees over the 2013 growing season, computed by Gompertz function. t_i and t_f shows the date at which 5 % and 95 % of the cells are produced respectively, t_x shows the timing of maximal rate of cell production.

5.3.6 Cell cycle evaluation

The minimum cell cycle lengths presented different pattern of variation compare to the mean and maximal rates. The shorter cell cycle was observed at the middle elevations while it was longer at the bottom and top sites (23 ± 1 in 1350 m), (17.1 ± 1 in 1700 m), (17.5 ± 1 in 2000 m) and (23 ± 2 in 2300 m). The results of bootstrap test showed that the difference between the bottom site and the sites located at the middle elevation (1700 and 2000 m) were statistically significant ($P < 0.05$) (figure 5.1e).

5.3.7 Time of maximal rate of cell production

The time of reaching to the maximum growth rate occurred at different days of year for different sites (175 ± 2 in 1350 m), (189 ± 2 in 1700 m), (195 ± 1 in 2000 m) and

(198 ± 1 in 2300 m) with statistically significant difference ($P < 0.05$) (figure 5.3).

5.3.8 Duration versus rate of xylem cell production

The results of simple physical model showed significant regression between the observed and predicted value ($P < 0.01$, $n=20$) ($R^2 = 0.76$) (figure 5.4). The results of sensitivity analysis showed that the average RCN was mainly affected by r_m than duration. The simulated RCN found to be more sensitive to the variation in r_m than Δt_E . When r_m was kept constant to its mean value and the Δt_E varied around its mean within a range of twice of its standard deviation, the resulting RCN changed from 32 to 42 cells (a range of variation of 10 cells). When the Δt_E was kept constant to its mean value and r_m changed around its mean within a range of twice its standard deviation, the resulted RCN changed from 23 to 38 cells (a range of variation of 15 cells). Thus it can be stated that on average the number of simulated RCN was three times more sensitive to r_m than Δt_E . and about 40 % of RCN variability was related to Δt_E while 60 % was related to r_m (figure 5.5).

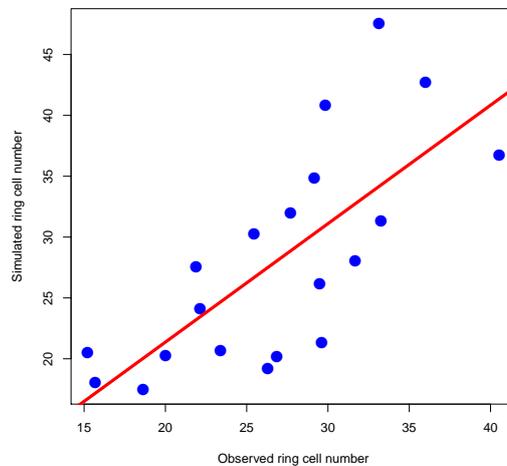


Figure 5.4. Relationship between the observed and simulated ring cell number.

5.3.9 Meteorological data

Temperature

Comparison of temperature along the gradient showed a decreasing trend for air temperature with increasing altitude in a significant linear trend ($T = -0.0054Alt +$

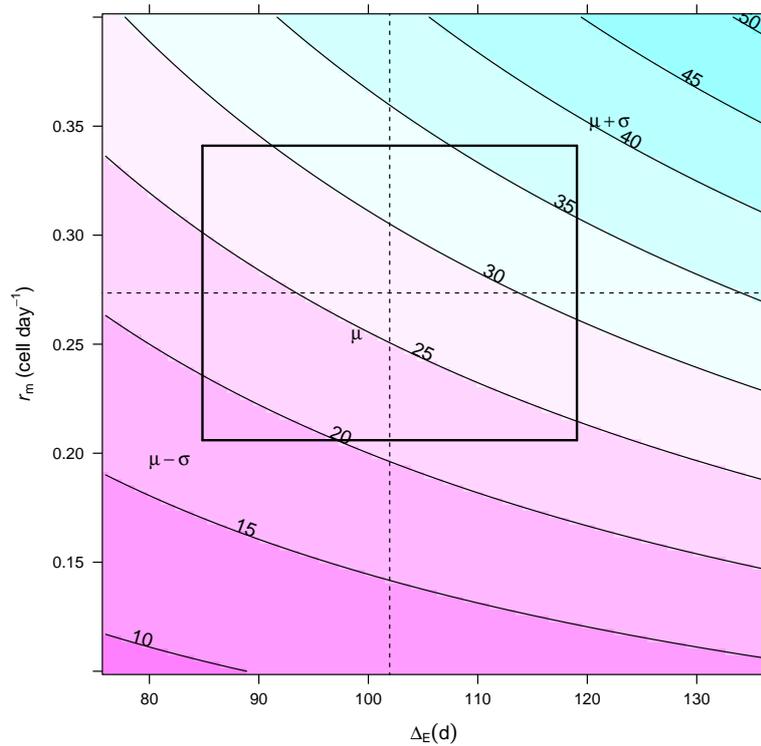


Figure 5.5. Simulated total cell number (RCN) when the average rate (r_m) and the duration Δt_E of cell production varied around their mean within a range of two times their standard deviation. The dashed lines represent the means and the frame shows the area of the mean \pm sd

14.3, $R^2 = 0.06$, $P < 0.001$). This trend in air temperature can be stated as a decrease of 0.54 °C per 100 m increase in elevation.

5.3.10 Relative soil extractable water

The soil water content was computed at each study site as relative extractable water (REW). In this model the water stress is assumed to occur when the amount of REW drops below 40% of maximum extractable water. This water stress index which cumulates the difference between REW and the critical REW of 40% showed the highest soil water stress index at the bottom site that was 33.3 (dimensionless). This value decreased with moving to higher elevation as 20.3, 15.5 and 12.7 at 1700, 2000 and 2300 m respectively. The duration of water deficit was also computed by the model. It was longer at the bottom site (66 days) with a decreasing trend toward the higher altitude as 54, 42 and 33 days at 1700, 2000 and 2300 m respectively (figure 5.6).

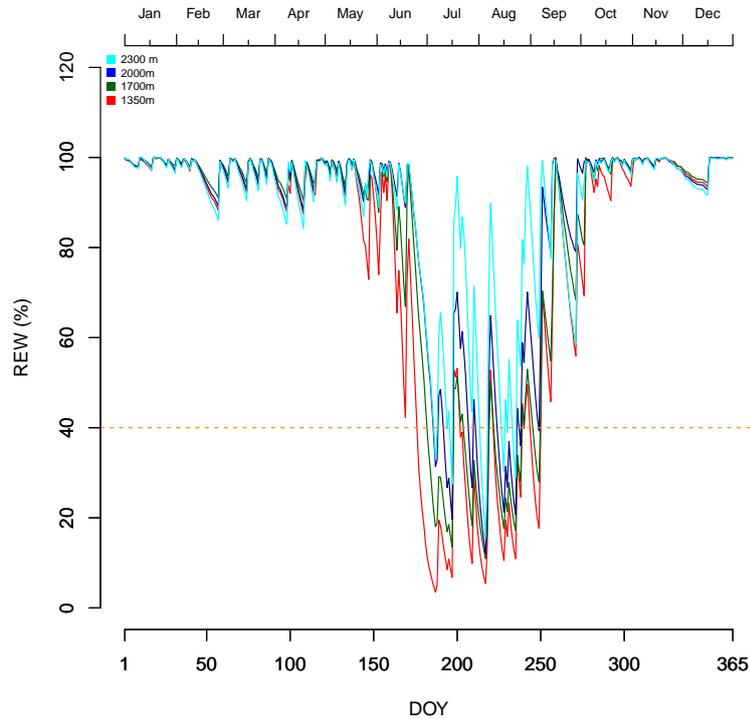


Figure 5.6. Plot of comparison the relative extractable water (REW) at four study sites computed by BILJOU model.

5.3.11 Evaluation of temperature effect on cell cycle

The results of comparing mean daily temperature at the time of maximal rate showed higher temperature at the two middle sites (1700 and 2000 m) along the gradient. The mean temperature at the time of maximal rate were found as (14.8 ± 0.6 ° C in 1350 m), (15.8 ± 0.3 ° C in 1700 m), (16.14 ± 0.4 ° C in 2000 m), (14.7 ± 0.4 ° C in 2300 m) (figure 5.7). The bootstrap test among different sites showed that the observed difference was statistically significant ($P < 0.05$) except among the two middle elevation (1700 and 2000 m)

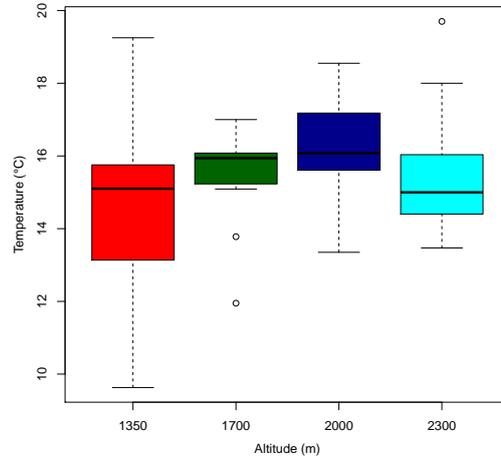


Figure 5.7. Plots comparing mean daily temperature at the time of maximal rate

5.4 Discussion

The results of this study, showed an elevation-related shortening of the growing season, however about the same number of tracheid cells were observed in three upper stands. The final number of tracheid cells is known to be affected by both rate and duration of cambial activity (Rathgeber et al. 2011b, Cuny et al. 2012, Rossi et al. 2014). Previous studies found narrow growth ring related to short growth period (Vaganov et al. 1999, Deslauriers et al. 2003). Such a reduction in the amount of xylem cell production was also observed along elevation gradients with shortening the wood formation period due to later onset of wood formation in spring and earlier cessation in autumn (Rossi et al. 2007, Moser et al. 2010). Rossi et al (2012) explained the relationship between cell production and length in this way that longer length of growing season, due to an earlier onset of cambial activity in warmer sites will provide more time to the cambial cells to divide and produce xylem cells (Rossi et al. 2012). However our results didn't confirm this explanation. But in another study the short period of wood formation in *Juniperus* didn't lead to lower number of tracheid in tree-ring. (Ren et al. 2015). So using only the growing season length to define the amount of tracheid cell production is not enough and other growth parameters such as rate also need to be considered. The relationship between rate with the age and size was observed previously as the dominant silver fir trees presented intense growth compare to suppressed and intermediate trees. The dominant trees with bigger size and larger crown area have more photosynthe-

sis ability. These advantages enable them to provide more carbohydrate for their cambium by increasing the concentration of growth assimilates in their phloem part (Rathgeber et al. 2011b). Differences in growth rate was founded due to the specific strategy of pioneer species in using growth assimilates (Cuny et al. 2012). Higher rate of cell production was reported to be controlled by the carbohydrate availability as higher sugar content was found in the more productive poplar clones (Deslauriers et al. 2009). Lower rate of cell production observed in *Lebanan cedar* trees growing at this site with lower available water (Guney et al. 2017).

The growth rate was reported also to be controlled by both the number of dividing cambial cells and their division rate (Vaganov et al. 2006). Number of cambial cells was found to be different in different tree species (Larson 1994) or vitality (Gricar et al. 2009). In our studied trees comparing the number of cambium cells both at the time of maximal rate of cell production and also at the dormancy of cambium at the end of growing season showed an increase with increasing elevation. Comparison of temperature at the time of maximal rate showed higher amount for the middle elevation where the cell cycle observed to be shorter. Cell division is known to be a slow process in cambium (Larson 1994). So in our study enhancing the rate of cell production can be explained by increasing number of dividing cambial cells.

Comparing the minimum cell cycle along the gradient showed longer length of cell cycle at the bottom and top site compare to the middle elevations. The different cell cycle duration between Firs and Spruces was explained by the difference in the assimilates. Early-successional species were found to be less efficient in converting the available resources to growth (Cuny et al. 2012). The meristematic activity of cambium such as cell division was found to be related to the quantity of available sucrose around the dividing cells (Rio-khamlichi et al. 2000).

Different sugar and starch concentration was observed in the cold treated seedling of *larix decidua* showing that low temperature can not be a limitation for photosynthesis (Hoch and Korner 2008). In cold environments, if low temperature limits the meristematic activity such as cell division, while photosynthesis still provide enough amount of photo-assimilates, the additional assimilates will be stored as non-structural carbon (Korner 2003a). Shorter cell cycle at the middle elevation shows trees have more ability to use the resources to assimilate carbon that can be related to higher ambient temperature.

The annual radial increment was estimated to be attributed about 60 % to the rate of cell production and about 40% to its duration. It is in accordance with the previous results for silver fir trees belonging to different social status (Rathgeber et al.

2011b) and three conifer species in a mixed forest (Cuny et al. 2012). It is also in accordance with Ren et al (2015) for *Juniperus* trees. All of these results confirm the main role of rate in the total annual ring cell number prediction. However Rossi et al (2014) reported that the most variability in xylem growth of black spruce trees to be related to duration of cambial activity rather than rate (Rossi et al. 2014). Our findings showed the more significant role of rate in shaping tree ring width. The maximal rate of tracheid culminated at different time showing that the most wood production occurred at different time along the gradient pointing to the different strategy of trees in allocating great amount of carbon in their structure. Previous studies reported that the maximal growth rate culmination close to the summer solstice previously proposed the effect of day length as the main environmental factor putting a safety for finishing the secondary wall formation before winter (Rossi et al. 2006b). The same results were also observed for Silver fir trees belonging to different social status (Rathgeber et al. 2011b). But the results of the inter-annual timing of maximal growth rate in a mixed stand of tree conifer species showed asynchronous time related to the controlling role of other environmental factors rather than day length (Cuny et al. 2012) that is in accordance with our finding. In our study, an earlier culmination of maximal growth rate was observed at the bottom site where the total REW was lower along the gradient. Decreasing soil moisture content below the defined thresholds of water deficit will lead to stomatal closure in trees. Consequently the water and C_{o_2} flux will decrease leading to reduction in photosynthesis efficiency and radial growth in trees (McDowell et al. 2013). Earlier timing of maximal rate at the bottom site where the water availability is limited can be consider as the management strategy of trees to finish earlier the cell production before severe water deficit.

5.5 Conclusion

The results of this study on the inter-annual dynamics of wood formation has shown that the rate, cell cycle, number of cambial cells and time of maximal rate are related to elevation along the gradient. In summary it can be stated that trees growing at higher elevation, expressed an intensive growth dynamic with a short period of cambial activity and higher rate of cell production resulting the same annual tracheid cell numbers at the three top sites. The lowest stand, on the contrary, exhibited narrow tree rings due to both low production rates and short durations. The sensitivity

analysis showed that the final tree-ring width was about 60% related to the rate of cell production and only 40 % to its duration. Moreover, our investigations showed that trees increased the number of dividing cells in their cambium in response to the elevation-related increase in cell cycle length, maintaining high production rate at high altitude. In conclusion it can be stated that with moving toward higher elevation where the length of growing season is limited by low temperature the dynamic of growth is more intense with higher rate of cell production helping trees to maintain their tree ring width, that can be considered as an adaptation strategy in trees to grow in colder environments.

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Chapter 6

General Discussion

General discussion

Inducing wounding response in trees as a response to microcoring method

Wood formation monitoring in this study was done using the classical method of punching tree stem at about weekly intervals (Rossi 2006a). In this way it is possible to observe the xylem development along the growing season, but since the number of sampling is high the size of samples need to be small.

The mechanical and physical disturbances to the cambium was previously reported to affect the defensive mechanism in trees. Even a small damage to trees such as removing partially the bark (Nagy et al. 2000, Stobbe et al. 2002) or small wounding (Gartner et al. 2009) or more intensive as rock falls or debris flow damage (Stoffel and Perret 2006, Bollschweiler et al. 2008) was found to trigger defensive responses by cambium. Such a wounding reaction by trees may become very significant in wood formation studies because it can affect the normal growth pattern in trees leading to production of abnormal tissue at the vicinity of wound (Larson 1994). Trees in the case of mechanical disturbance produce a new tissue known as callus (Stobbe et al. 2002, Gartner et al. 2009).

They also can produce tangential rows of traumatic resin canals (Bollschweiler et al. 2008, Schneuwly et al. 2009). While the normal scatter resin canals are known as an anatomical feature in conifers, the traumatic ones forming in tangential bands are considered as traumatic and abnormal tissue.

In most cases, wounding responses in the form of traumatic resin canals formation were greater in size and number above the wound (Fahn et al. 1979, Gartner et al. 2009) that can be explained by the higher accumulation of growth hormones at the top of wounding area. So the biological explanation of traumatic resin canals formation can be that wounds act as a physical barrier to the flow of hormones (Fahn et al. 1979). In our studied trees no callus tissue was observed but numerous traumatic resin canals occurred among the tracheid cells in all the study sites. Since the tangential rows of resin canals were formed only in the last tree ring formed in 2013 and no other mechanical disturbance such as rock fall or avalanches was recorded in the study year, punching was considered as the main reason of their occurrence.

Wodzicki and Zajaczkowski (1970) observed an increase in the rate of cell production due to the wounding effects on wood formation monitoring. They suggested the intervals up to 21 days at a 1 cm distance from two following samples as the optimum to avoid wounding effects (Wodzicki and Zajaczkowski 1970). Previous studies reported higher activity of cambium and cell division around the wounded area by enhanced cambial activity in *cedrus libani* (Fahn et al. 1979). This result suggest that microcoring may lead to an increase the final number of cells in tree-ring by stimulating cambial activity. The higher number of tracheid cells will need more time to finish their wood formation process and become fully mature, so cambial stimulation can lead to a delay for the cessation of wood formation.

In summary it can be stated that microcoring can have an impact on wood formation by both affecting rate of cell production by cambial stimulation and also changing the phenology of wood formation. In our study the accurate evaluation of Gompertz fitting residuals and comparing the total number of tracheid cells in the samples taken before and after cessation of cambial activity showed that microcoring method can slightly stimulate the cambial activity. Although microcoring is known as one of the less invasive methods in wood formation monitoring but the results of this study showed that it can have an affect on xylogenesis by disturbing normal growth and producing traumatic resin canals and also slightly affect the cell production.

Adjustment of wood formation phenology along elevation gradient

The results of this study showed differences in the phenological phases of wood formation in trees growing along elevation gradient of 1000 m. The onset of cambial activity first observed at the bottom site then occurred with a delay of 2.5 days/100 m toward higher elevation. Other phenological phases of wood formation (enlarging, wall thickening and mature) also occurred with a delay moving to higher elevations. The observed delay for the onset of wood formation phases can be related to the controlling role of temperature. Temperature is widely known as a key factor controlling resumption of cambial activity at the beginning of growing season (Oribe and Kubo. 1997, Oribe et al. 2001, Gricar et al. 2006).

Cambial cell division and differentiation of xylem cells both are source demanding process that need adequate amounts of sucrose (Oribe et al. 2003). Temperature

allows the allocation of available sucrose (Hoch and Korner 2003) and the metabolic process of growth to be completed in trees (Begum et al. 2007). Adjustment of wood formation phenology to different climatic conditions was previously reported along elevation gradients (Moser et al. 2010, Oladi et al. 2011, Prislan et al. 2013). Moser et al (2010) found a delay for the onset of cambial activity along the comparable elevation gradient in the Swiss Alps (Moser et al. 2010).

The survival of trees species in different elevations was found to be the results of interaction between three main components; freezing resistance in spring, adjusting phenology of bud break and leaving enough time for maturation of newly formed tissues (Korner et al. 2016). Tree phenology should be regulated in a way that put the freezing damages almost to zero (Vitasse et al. 2014).

The observed delay for the onset of cambial activity with increasing elevation in our study can be explained by the strategy of trees to start their activity in a time to avoid early spring freezing damages. later flushing date at higher elevation was found as an strategy to minimize the probability of freezing damages during flushing. The freezing resistance found to be different along elevation gradient with trees growing at lower elevations were reported to be more at the risk of freezing. So the interaction between the flushing date and freezing tolerance during flushing can provide a safety for tree survival across elevation gradients (Korner 2016).

Temperature and water availability are known as two dominant global climatic drivers of plant distribution that both act as a gradual and threshold effect. (Rossi et al. 2007). The effect of low temperature on tree growth and development can be observed as threshold and direct effect via tissue survival. Temperature can also have an indirect impact through affecting tissue maturation by adjusting length of growing season (Korner 2006). In our studied gradient, the response of wood formation phenology to temperature was observed as delaying onset of cambial activity. It can be explained as an strategy to protect tissues from extreme low temperature at early spring and can be considered as a threshold effect of temperature. The gradual effect of temperature can be observed through adjusting length of growing season to provide enough time for maturation of newly formed cells.

Previous studies on the phenology of wood formation and climate-growth relationship mainly focused on the onset of cambial activity while its cessation still remained unexplained (Gricar et al. 2007, Rossi et al. 2007). Among the environmental factors, the cessation of cambial activity was mainly found to be related to photoperiod in our studied trees in the three top sites. Moser et al (2010) found the simultaneous cessation of cambial activity along a comparable gradient in the Swiss Alps

pointing the controlling role of photoperiod on its termination. In other study the shoot growth of *larix decidua* trees observed with a delay toward higher elevations from 700 to 2000 m, while its termination observed approximately at the same time (Tranquillini et al. 1979). At the end of growing season, the living organs and tissues must enter the winter dormancy in a mature status to be able to survive in winter known as freezing resistance. So the tissue hardening occurs in autumn due to escape from late season freezing temperature damages (Vitasse et al. 2014).

The first stage of cold acclimation in trees was found to be triggered by shorter day length in autumn leading to accumulation of starch and lipid reserves in the overwintering tissues (Vitasse et al. 2014). The onset of hardening in autumn was found to be less dependent on elevation and to be controlled mostly by photoperiod leading to high freezing resistance before occurrence of very low temperatures (Korner et al. 2016). In our study a common photoperiod length at the cessation of cambial activity in the three top sites shows the effect of day length in providing signals that control end of growing season in trees.

Our results demonstrated the role of photoperiod on controlling cessation of cambial activity to provide signals for trees to finish their maturation before occurrence low temperature in autumn. This can be considered an indirect role of temperature on regulating maturation of tissue. However water availability limitation probably has a gradual effect on cambial activity via tracheid cell formation. The earlier cessation of cambial activity at the bottom site due to water availability limitation made such a parabolic trend for the cessation of enlarging phase along our studied elevation gradient.

Adjustment of wood formation dynamics along elevation gradient

The phenology of wood formation showed variability along studied elevation gradient. The wood formation dynamics also was compared to have a better understanding of the mechanisms in which trees are able to adjust their phenology along the studied gradient.

The results of this study on xylem cell production showed that the studied trees produced the similar number of annual tracheid cells at the three top elevations although different phenology of cambial activity resulted in different length of growing

season. The causal link between the length of growing season and tree ring size is not still well clear. Some of previous studies reported reduction in the period and amount of xylem production along altitudinal and latitudinal gradients explained by both later onset and earlier cessation of cambial activity in spring and autumn respectively (Rossi et al. 2008, 2010, Moser et al. 2010). Rossi et al (2011b) related the longer period of wood formation to greater tree ring size. The earlier resumption of cambial activity in spring was found to provide more time for the cambial cells to divide and consequently produce more xylem cells (Rossi et al. 2011b). However other studies found that shorter period of wood formation didn't lead to lower number of tracheid cell production (Ren et al. 2015). In a study carried out on the larch trees growing in our studied elevation gradient showed significant phenotypic variability for all the annual-ring microdensity variables except tree ring width (Nardin et al. 2013) that is in accordance with our results on tracheid cell numbers.

Xylem cell production is known as the result of both duration and rate of cell production. In order to explain how trees growing at higher elevation with shorter length of growing season are able to produce the same number of tracheid cells compare to lower elevations, we compared the rate of cell production along elevation gradient. In our study comparison rate of cell production showed an increasing trend with moving to higher elevation. Rate of cell production was also found previously to be related to tree characteristics such as age and size (Rathgeber et al. 2011) and also the species related ability of trees to use the growth assimilates (Cuny et al. 2012). But the rate of cell production was rarely compared along elevation gradient. Increasing growth rate toward higher altitude was previously reported in some animals such as Atlantic silversides fish and *Rana clamitans* green frog. This mechanism was explained by the selection of rapid growth in the shorter growing season environments that large body size is needed for long winters (Berven et al. 1979, Conover and Present 1990). This pattern that is known as countergradient theory leads to minimum phenotype variation along elevation gradients (Levins 1969). Although this theory have never been reported for plants and to be tested needs sapling control experiments but increasing rate of cell production in our studied trees can show the same mechanism in reducing phenotypic variation as an adaptation along elevation gradient.

In our studied gradient although the final tree ring cell number was similar in three top elevation, but the lowest stand, on the contrary, exhibited narrow tree rings due to both low production rates and short durations. The response of trees to water deficit is complex. Under severe drought, tree growth may slow down or even the

cell division can be stopped (Guney et al. 2017). Rate of cell production was found also to be related to the number of dividing cambial cells and their division rate (Vaganov et al. 2006). The results of this study showed an increase in the number of dividing cambial cells with increasing elevation. The cell division is known as a slow process in the cambium (Larson 1994) so higher number of dividing cambial cells can help the trees to reach higher rate of cell production within a shorter period. It can help them to make a balance between the shortening of growing season and tracheid cell production, that can be considered as the adaptation strategy of this species to keep their productivity in limited growing season length.

Comparison the cell cycle along the gradient showed a parabolic trend along the gradient with shorter cell cycle at the two middle elevations. The differences between cell cycle duration in firs and spruce trees was observed previously and explained by the different strategy of these trees to use the growth assimilates (Cuny et al 2012). The meristematic activity in trees is known to be dependent to the quantity of available sucrose around the stem (Rio-khamlichi et al. 2000). Temperature allows the allocation of available sucrose (Hoch and Korner 2003) and the metabolic process of growth in trees (Begum et al. 2007). But unlike temperature, photosynthesis activity was found to be less related to temperature and even high rate of assimilation was observed at the temperatures below 5 °C (Korner 1998, Hoch and Korner 2003, Begum et al. 2007). So shortening cell cycle length with decreasing elevation in our studied elevation gradient can be explained by the effect of higher temperature on trees that enable them to use more efficiently the growth assimilates however at the bottom site water access limitation can make it longer.

It is always difficult to define if the variation in the radial growth among different trees resulted from different phenology or dynamics of cell production. So in wood formation studies it is a critical question to find the contribution of rate and duration of cambial activity in the annual xylem cell production. Several studies demonstrated the that the final number of produced xylem cells is mainly affected by the rate of cell production and partially related to duration of growing season (Rathgeber et al. 2011, Cuny et al. 2012, Ren et al. 2015. while few studies pointed to the main effect of duration (Rossi et al. 2013). However we found the more significant role of rate rather than duration in the final tree ring cell number. It can be concluded that in wood formation studies, when comparing the phenology of cambial activity, it's dynamics need also to be taken into account in order to have a comprehensive comparison.

Response larch trees to the climate change in future

With the recent climatic change and temperature increase, a lengthening in the average annual growing season was observed in Europe (Menzel and Fabian 1999). The effect of temperature increase on the phenology of wood formation in our studied larch trees can be through increasing length of growing season. As we demonstrated by our results there was a delay for the onset of cambial activity phases moving from low to high elevations. This delay relating to the decrease of temperature by 0.54 /°C per 100 m increase in elevation can be stated as 5 days/°C. The observed delay of wood formation onset relating to the projected increases in temperature over France between 2.5 °C to 3.5 °C (Gosling et al, 2011), can be estimated to start between 12.5 to 17.5 days earlier in 2100 compare to 1960.

Comparing the length of growing season along our studied gradient showed shortening the length of growing season by 4 days/°C decrease in temperature. It is also very important to find the impact of this lengthening of the growing season on the final number of tree-ring tracheid cells in future. The question is if the lengthening of growing season will increase the size of tree-ring in future. We found the final tree-ring cell number to be about 60 % dependent to the rate of cell production by cambium and only 40 % to the duration of cambial activity. So lengthening the wood formation period need to be coincide with increasing rate of cell production in order to produce greater number of tracheid cells.

In the studied gradient there was an increasing trend for the rate of cell production with moving toward higher elevation. Increasing rate was found to be related to the interaction between longer cell cycle and lower temperature at higher elevations. Although we expect lengthening of growing season due to higher temperature in future, but the rate of cell production will not necessarily increase. Each of our studied sites in future will experience higher temperature so the rate of cell production will be similar to what was observed at their lower elevations, for example the rate of cell production at the site located at 2300 m in future will be the same as what we observed for the site of 2000 m. As a result the lengthening of growing season will not be coincide with higher rate of cell production. In summary it can be stated that increasing the length of growing season will not affect significantly the final number of tracheid cells.

At the bottom site (1350 m), we observed an earlier cessation of cambium activity that limited both the length of wood formation period and the the final number of

tracheid cells due to lower amount of water. The predicted climatic change in future can intensify the drought impact at this site.

Increasing temperature in future can lead to an earlier onset of cambial activity at the bottom site and consequently production of higher number of tracheid cells. However the cessation of cambial activity may occur even earlier than before due to drought impact. So the trees will have shorter time for thickening their secondary wall, therefore it can lead to production of tracheid cells with thinner cell wall and lower density.

Within the scenario of climate change, the drought stress condition may also happen for the middle elevation and trees growing at this range may show limited growth length and tracheid cell production like what we observed at the bottom site. This point is very important since the optimum site for the growth of larch trees can move to higher elevation. This movement will have an impact on the forest ecosystem and need to be considered in the forest management policies in future.

Conclusion

This study provides insight to the intra annual phenology and dynamics of wood formation in European larch trees growing along an elevation gradient in the French Alps. Accurate weekly microcoring method during the growing season allowed assessing the development of xylogenesis process. The results presented in this thesis, will help us to have a better understanding of the adjustment of the phenology and dynamics of cell production in trees as an adaptation to different environmental conditions. Moreover the results of this work will help us to predict the response of this species to the climatic change effect such as temperature increase in future.

In chapter one and two the introduction and material and methods have been presented. Chapter three mainly focuses on the phenology of cambial activity in studied trees. In this study, we monitored during the whole 2013 growing season, the intra-annual dynamics of tree-ring formation of 60 dominant Larch trees, equally distributed in four stand (4 x 15 trees), spread along an elevation gradient of 1000 m in the southern part of the French Alps. The main results of this chapter revealed that the observed trend in the phenological phases of wood formation in the studied larch trees was related to elevation. The earlier onset of wood formation observed at the bottom site that then occurred with a delay toward higher elevations with a linear trend. However the cessation of cambial activity phases was occurred in a parabolic trend along the gradient.

There was a decrease in the growing season length by increasing elevation. Among the environmental factors, temperature and photoperiod were found to be the main driver of the phenological phases of cambial activity with the effect of temperature mainly at the beginning and photoperiod on its cessation. However at the bottom site it seems that water availability is the main factor affecting earlier cessation of cambial activity. So shortening the growing season at the bottom site was related to less amount of extractable water by trees. In chapter four, the results on the dynamic of wood formation of larch trees along the gradient were presented. The main results of this chapter showed differences in the dynamics of cambial activity along the gradient. Comparing the annual number of tracheid cells showed that studied trees produced the same number of tracheid cells along the gradient. The only exception was the bottom site (1350 m) where the number of tracheid cells were limited. In order to explain the observed tracheid cell number along the gradient the rate of cell production were also compared. The results of this comparison showed an increasing trend for the rate of cell production moving toward higher elevation. Comparison the number of cambial cells revealed number of cambial cells with increasing elevation. Comparing the cell cycle length showed a parabolic trend along the gradient with shorter length at the middle elevation. The annual radial increment variability was estimated to be about 60% related to the rate of cell production and only 40 % to its duration. Although increasing the number of cambial cells can be considered as an adaptation in trees to colder environments.

In chapter five the effect of wood formation method on cambial stimulation was assessed. The results of this study showed that microcoring method induced tree response in the form of traumatic resin canals formation and slight cambial stimulation in the forming tree ring structure. The results of this chapter showed the need to improve the current wood formation monitoring methods in order to enhance both the quality of wood samples and the final results. Although the observed stimulation didn't have a significant impact on our final results of wood phenology and dynamics along studied gradient but this point need to be investigated deeply in future studies. Further studies with the main focus on the impact of microcoring on cambial stimulation and triggering cambial activity may provide useful information in this field.

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Résumé :

A. Introduction

1. Les forêts et le bois, ressources naturelles du passé et du futur :

Le bois est une ressource naturelle majeure pour l'énergie et les matériaux. Il a toujours eu de ce point de vue un rôle crucial dans l'histoire de la vie humaine. De nos jours, il est toujours de plus en plus utilisé comme matière première privilégiée dans la construction, le transport, le mobilier, l'art ou les articles de sport. Cette demande croissante en produits forestiers se poursuivra avec la croissance de la population mondiale et de son niveau de vie, et avec la transition écologique vers une dépendance plus faible aux produits pétroliers. La majorité de la matière ligneuse (80%) est produite par les forêts, qui couvrent un tiers de la superficie terrestre (FAO 2010). Les forêts sont des sources importantes de nourriture, d'énergie et d'eau de qualité pour les organismes vivants, et elles contribuent à un large éventail de services environnementaux tels que l'agriculture durable, la stabilisation des sols et du climat, la production de produits forestiers ligneux et non ligneux. La biodiversité de la planète dépend des écosystèmes forestiers.

2. Mieux comprendre le rôle des facteurs climatiques dans la formation du bois

L'un des rôles les plus importants des forêts est alors de participer à la régulation du cycle du carbone, un des cycles vitaux de notre Terre. Le carbone est le quatrième élément chimique le plus abondant de l'univers (Ren et al., 2013). Les civilisations humaines sont basées sur le carbone pour leur nourriture, leur transport, leur habitat ... La photosynthèse (Unwin et Kriedemann 2000) permet aux arbres de piéger le carbone atmosphérique par la production de biomasse aérienne et souterraine. Le dioxyde de carbone est ainsi absorbé par les arbres et transformé en carbone structural sous forme de cellulose, d'hémicelluloses et de lignines.

Les changements climatiques récents ont souligné l'importance du cycle du carbone au cours des dernières décennies. Les changements climatiques sont définis comme les changements statistiquement significatifs des paramètres qui définissent le climat d'une région, ou la variabilité persistante sur une longue période de ces paramètres (rapport du GIEC 2014). Les effets du changement climatique dû à l'augmentation du CO₂ atmosphérique tels que l'augmentation de température et la modification des régimes de précipitations sont observés. Les variations de température et de précipitations dues aux changements climatiques récents sont susceptibles de conduire à des modifications significatives de tous les aspects de la vie des plantes tels que la phénologie, la distribution, la mortalité et les interactions avec d'autres organismes (GIEC 2014). Les écosystèmes forestiers qui ont de nombreuses fonctions dans le cycle du carbone et la régulation du climat doivent être étudiés et protégés, d'autant que de manière désastreuse, la surface mondiale des forêts a considérablement diminué au cours des dernières décennies. Dans cette situation, il est crucial de cultiver, d'utiliser et de préserver les ressources en bois beaucoup plus efficacement. Pour cela, au-delà de la compréhension globale du cycle du carbone et des flux de carbone entre l'atmosphère et les écosystèmes, une bonne compréhension des mécanismes impliqués dans le processus de formation du bois et du rôle des facteurs climatiques dans ces mécanismes est cruciale. La formation du bois est le principal puits de carbone dans les arbres (Hansen et Back, 1994).

3. Les fonctions du bois dans l'arbre

L'arbre est un type biologique végétal qui se définit par le gigantisme, l'auto-portance et la pérennité (longévité). L'auto-portance et le gigantisme posent les problèmes de la conduction hydraulique sur de grandes hauteurs, et de la tenue mécanique face aux tempêtes ou simplement face aux poids supportés dans le champ de gravité. La pérennité pose le problème de la survie aux stress environnementaux sur de très longues périodes. Wilson (1984) évoque le pin « bristlecone » comme un exemple d'espèce d'arbre qui parvient à survivre plus de 4000 ans. Les arbres sont capables de faire

face à ces problèmes en épaississant progressivement leurs tiges grâce au cambium bifacial qui forme du phloème et du bois par couches successives, en répondant en permanence aux signaux environnementaux variables, dont les facteurs climatiques. Le bois a quatre fonctions essentielles dans les arbres: 1) la fonction mécanique de soutien, de maintien et d'orientation de la structure porteuse; 2) la fonction hydraulique de transport du flux de sève brute (nutriments et eau) des racines vers les feuilles; 3) la fonction de stockage d'eau et d'hydrates de carbone et autres composés; 4) la fonction de production et transport de composés de défenses afin de protéger les arbres contre les agents pathogènes (Kozłowski et Pallardy 1997, Fournier et al. 2006).

4. Anatomie et variations de propriétés du bois

Les cellules ligneuses sont composées de celluloses, d'hémicelluloses et de lignines, les composants structuraux carbonés qui confèrent la rigidité suffisante aux parois cellulaires, bio-synthétisés à partir des assimilats produits par la photosynthèse depuis les surfaces foliaires. Dans l'observation microscopique du bois, on peut observer différents types cellulaires. Chez les conifères, ces types sont les trachéides, le parenchyme axial et le parenchyme radial des rayons ligneux, les canaux résinifères. Les trachéides constituent 90% ou plus du nombre total de cellules dans les conifères (Larson 1969). Elles assurent à la fois les fonctions mécaniques et hydrauliques. Les trachéides meurent rapidement à la fin de leur développement et remplissent leurs fonctions en tant que cellules mortes. Le bois présente une grande variété de propriétés physiques et de concentrations en composants chimiques structuraux. Ces propriétés sont le résultat de modifications quantitatives dans les processus de formation du bois, processus de différenciation des types cellulaires (trachéide, parenchyme) et processus de formation de la cellule de xylème mature dans chaque type (Barnett, 1981). Chez les conifères, le diamètre et l'épaisseur de la paroi des trachéides le long d'une file radiale sont par exemple divisés et multipliés par un facteur 5 au cours de la saison de végétation depuis le bois dit de printemps ou initial, constitué de cellules larges aux parois minces, au bois dit d'été ou final, constitué de cellules plus petites aux parois épaisses (Vaganov et al., 2006, Cuny et al., 2014). Cela montre la variation de l'anatomie du bois dans un seul arbre en une seule saison. La cinétique de transition du bois initial au bois final dépend de l'espèce et des conditions environnementales tout au long de la période de formation du bois.

5. Les processus de la formation du bois issu du cambium

Le cambium vasculaire est le méristème fondateur du bois, composé d'une fine couche de cellules en croissance et division. Le cambium constitue donc une enveloppe continue autour des tiges, des branches et des racines des plantes ligneuses, produisant du xylème vers l'intérieur de la tige et du phloème vers l'extérieur (Wilson 1984). Les cellules du cambium sont reconnues par leurs parois primaires minces, leur petite taille radiale et leur forme rectangulaire par rapport aux cellules du phloème. Beaucoup de plantes ont un cambium, mais celui des arbres occupe une grande place et fonctionne en continu, ce qui fait des arbres un modèle privilégié pour l'étude des régulations climatiques *in situ* de l'activité cambiale (Wilson, 1984). Les différents processus de la formation d'une cellule de xylème en file radiale peuvent être divisés en cinq étapes principales. 1) la division périclinale dans une cellule mère qui produit une nouvelle cellule fille; 2) l'agrandissement de la cellule nouvellement formée; 3) la formation de paroi cellulaire secondaire qui est le dépôt de cellulose et d'hémi-celluloses dans la paroi cellulaire; 4) l'imprégnation des parois cellulaires par les lignines et 5) la mort des cellules. Le cycle cellulaire peut être défini comme la séquence de ces événements successifs hautement contrôlés dans tous les organismes cellulaires ayant un noyau. Dans le cycle cellulaire, les cellules mères méristématiques passent par plusieurs stades de développement incluant la division du noyau et la séparation du cytoplasme afin de produire deux cellules filles (Lachaud et al., 1999). Les processus (1) et (2) déterminent la largeur d'incrément annuel tandis que les processus (3) et (4) correspondent à l'accumulation de biomasse dans les parois cellulaires (Antonova et Stasova 1993).

6. La phénologie de la formation du bois

La phénologie du cambium est un sujet complexe au sein de l'étude générale de la phénologie des arbres. Chez les plantes, la phénologie est le compromis entre les facteurs environnementaux et l'accessibilité des ressources nécessaires à leur croissance (Nord et Lynch, 2009). Les études phénologiques dans les organismes vivants ont attiré une attention considérable avec les changements climatiques récents puisque la modification des événements phénologiques peut refléter les changements environnementaux. Par exemple, l'augmentation de la température a augmenté la durée de la saison de croissance en Europe (Menzel et al., 2006). La phénologie du cambium vise donc à déterminer la fenêtre temporelle pendant laquelle les arbres sont ouverts pour croître, pour recevoir les signaux environnementaux et les enregistrer dans leurs cernes de bois. Cela permet ensuite d'observer les changements interannuels de cette fenêtre et de formuler des hypothèses sur comment elle va évoluer avec les changements climatiques en cours. Les arbres qui poussent dans les régions tempérées ont besoin de se protéger pour être en mesure de résister aux basses températures en entrant en dormance. La phénologie foliaire et de la croissance primaire sont plus largement étudiées car les observations sont plus faciles. Par exemple, les rythmes du débourrement des bourgeons et du dépliage des feuilles sont bien connus pour être régulés par la température, permettant d'éviter les dommages dus au gel.

Le moment de l'apparition et de la cessation de la formation du bois déterminent les limites de la saison de croissance pour le cambium. Vers la fin de l'été, dans les zones tempérées et boréales où les saisons sont bien marquées par des chutes de température, l'activité cambiale ralentit et le nombre de cellules dans la zone cambiale diminue et se stabilise au nombre minimum de la période de dormance pendant laquelle le cambium est inactif. Dans la saison dormante, le cambium vasculaire est inactif, et la zone cambiale consiste en deux à six couches de cellules. Ces cellules ont une section transversale rectangulaire, contiennent du protoplasma à l'état visqueux et ont des parois bien définies. Le début de la formation du bois n'est pas directement détectable par l'observation de la rupture des bourgeons ou de la phénologie des feuilles, car il se produit dans la partie interne des tiges et il a ses rythmes propres. Le processus de division cellulaire dans le cambium est lent et dure environ 10 à 50 jours selon les différents stades de développement, les espèces d'arbres et les conditions environnementales (Larson, 1969).

Le monitoring de la dynamique saisonnière de la formation du bois dans les arbres a commencé il y a plus de 50 ans dans le but de mieux comprendre l'influence des facteurs climatiques sur la croissance des arbres, la phénologie de l'activité cambiale et la dynamique de formation du bois. Avec les récents changements climatiques, cette question a attiré plus d'attention et a conduit à un nombre d'études en forte augmentation au cours des dernières décennies. Le but du monitoring de la formation du bois est de déterminer dans quelle mesure les changements de structure anatomique, de composition chimique et de propriétés du bois, changements impliqués dans le fonctionnement des écosystèmes et le cycle du carbone, sont liés aux facteurs climatiques. La régulation des processus de formation du bois est étudiée du point de vue de sa dépendance à des facteurs endogènes (processus internes, hormonaux ou génétiques) ou exogènes tels que les variables climatiques (telles que la température) ou les stress de toutes natures (Larson 1969). Toutes les études antérieures sur les facteurs externes et internes révèlent que l'activité cambiale est un système dynamique complexe dans les arbres, et que savoir distinguer chaque processus de la formation du bois, sa cinétique et son contrôle, apporte des éléments importants pour comprendre comment la formation du bois répond aux différents facteurs de l'environnement (Cuny et al 2014).

7. Les techniques de monitoring de la formation du bois

Définir les dates clés de démarrage et fin des processus de xylogénèse et le nombre de cellules dans chaque processus nécessite une mesure précise et directe de l'activité cambiale, à des temps répétés à intervalle court et régulier le long de la saison de croissance. Il est jusqu'à présent impossible

d'observer le bois en cours de formation de façon non invasive et non destructive. Le monitoring de la formation du bois passe donc par le carottage régulier d'échantillons dans les tiges des arbres tout au long de la saison de croissance. Afin d'éviter de trop perturber les arbres, ces échantillons de bois sont extraits sous la forme de micro-carottes (Deslauriers et al., 2003), après avoir retiré l'écorce, au moyen d'un petit emporte-pièce. Bien que le micro-carottage soit connu comme l'une des méthodes les moins destructrices de la formation du bois, il peut néanmoins induire des réactions traumatiques. Ces réactions peuvent prendre la forme de canaux résineux traumatiques (Bollschweiler et al., 2008, Schneuwly et al., 2009) ou de tissu calleux (Stobbe et al., 2002). Le prélèvement de micro-carottes est largement utilisé pour étudier la formation du bois. Des protocoles d'observation et de mesure complexes ont été mis au point pour tirer de ces micro-carottes les informations pertinentes sur les processus de xylogénèse (e.g. Rathgeber et al. 2011).

8. Observer la formation du bois sur des gradients altitudinaux

Pour étudier l'effet de la température et pour imaginer l'impact du réchauffement climatique sur la formation du bois, observer le fonctionnement des écosystèmes et des plantes le long de gradients d'altitude en montagne est une méthode expérimentale classique. Quatre changements atmosphériques principaux sont associés à l'altitude. Le premier est la diminution de la pression atmosphérique et de la pression partielle de tous les gaz atmosphériques tels que l'O₂ et le CO₂. La deuxième est la diminution de la température en augmentant l'altitude. Un autre changement atmosphérique global avec l'altitude croissante est le rayonnement solaire qui augmente sous un ciel clair et le dernier est une fraction plus élevée du rayonnement UV-B. Les précipitations et la vitesse du vent ne sont pas globalement liées au changement d'altitude (Korner 2007).

La température est connue pour être le principal facteur contrôlant tout le processus métabolique dans les organismes vivants des écosystèmes tempérés (Korner 2003, 2007). L'effet direct de la température sur la reprise de l'activité cambiale a été largement testé par des expériences de chauffage du cambium (Oribe et al., 2001, Gricar et al., 2007). La température affecte les hormones de croissance telles que l'acide indole-acétique (AIA) impliquées dans les mécanismes de l'activité cambiale. La température est donc un des principaux facteurs qui affecte fortement le fonctionnement des plantes et qui change régulièrement et fortement lorsqu'on monte en altitude. Lorsqu'on parcourt un gradient d'altitude, on peut ainsi espérer observer comment les arbres répondent et s'adaptent à des températures changeantes.

Une variabilité phénotypique significative est toujours observée le long des gradients d'élévation. Concernant plus particulièrement le monitoring de la formation du bois, les différentes phases de xylogénèse (début de l'élargissement des cellules, début de l'épaississement des parois cellulaires et apparition de la première cellule mature) se sont révélées fortement corrélées à l'altitude (Moser et al., 2010).

Il existe néanmoins d'autres facteurs environnementaux qui affectent la réponse phénotypique des arbres, qui sont variables et même parfois variables de façon non indépendante du gradient d'altitude. Ces facteurs peuvent être biotiques ou abiotiques.

La disponibilité de l'eau affecte par exemple fortement le fonctionnement des arbres. La diminution de la disponibilité de l'eau du sol à des températures plus élevées peut mettre les arbres en état de stress hydrique, entraînant une baisse du potentiel hydrique des tiges et des feuilles et pourrait dépasser les limites de la sécurité hydraulique (McDowell et al., 2008). Le stress hydrique sévère affectera l'activité méristématique du cambium, comme la division cellulaire, pour économiser de l'énergie afin de maintenir le minimum de métabolisme et de défense dans les arbres (McDowell 2011). La faible quantité d'humidité du sol peut déclencher une fin de croissance plus précoce (Fritts 1956). La quantité d'eau extractible relative pour les arbres le long des gradients d'élévation varie en fonction de facteurs tels que le type de sol ou la quantité de précipitations.

Dans les régions boréales et tempérées, la photopériode est considérée comme l'un des facteurs les plus importants pouvant déclencher des phases phénologiques chez les plantes. La photopériode a été signalée comme ayant un effet sur l'arrêt de la croissance, car les espèces des latitudes plus élevées avaient une saison de croissance plus courte que les latitudes du sud (Jackson, 2009). La chute de la température de l'air à l'automne a également eu un effet sur la longueur de la formation du bois et l'a raccourcie (Gricar et al., 2006). La photopériode ne varie pas selon les gradients d'élévation. L'observation de caractères phénologiques stables le long de ces gradients peuvent corroborer l'hypothèse que ces caractères dépendent de la photopériode, hypothèse qui doit alors être vérifiée par des observations sur des gradients latitudinaux.

Enfin, la phénologie de la formation du bois peut être également affectée par des caractères ontogéniques tels que l'âge du cambium. Rossi et al. (2008a) ont montré que les arbres plus âgés présentaient une période de formation du bois plus courte que celle des arbres adultes (Rossi, 2008a).

Ainsi, dans les études qui utilisent des gradients d'altitude pour expérimenter l'effet de la température sur le fonctionnement des arbres, il est important de considérer les autres facteurs environnementaux ou ontogéniques qui affectent la croissance des arbres.

B. Objectifs de la thèse

L'objectif principal de ce travail de thèse est de comprendre comment les arbres sont capables de s'adapter à des conditions climatiques contrastées en ajustant leur phénologie et leur dynamique de formation du bois. Dans la continuité des travaux conduits dans l'équipe du Dr C.B.K. Rathgeber, le travail utilisera l'observation de la formation du bois le long d'un gradient d'altitude. La localisation de ce gradient, dans les Alpes du Sud (Briançonnais), suggère que le stress hydrique pourra perturber le contrôle de la phénologie du cambium par la température le long de ce gradient. L'espèce choisie est un conifère, *Larix decidua* Mill., très courant en Europe. Ce choix demande des adaptations des méthodes d'observations couramment pratiquées dans le laboratoire sur d'autres espèces de conifères (*Abies alba*, *Picea abies* and *Pinus sylvestris*).

C. Matériels et méthodes

1. Le site et les arbres

Dans cette étude, des mélèzes d'Europe (*Larix decidua* Mill) le long d'un dénivelé de 1000 m allant de 1350 à 2300 m d'altitude dans les Alpes françaises du sud ont été sélectionnés. Le mélèze est une espèce pionnière de conifères à croissance rapide et à aiguilles caduques. C'est l'une des espèces de conifères les plus abondantes de l'hémisphère nord. Le mélèze appartient à la famille des Pinacées (Matras et Pâques, 2008) et le genre comprend 10 espèces présentes en Amérique du Nord et en Eurasie (Farjon 2010).

Le site d'étude est situé à Villard St.Pancrace près de Briançon sur une pente orientée ouest. Ce gradient est couvert principalement par des mélèzes (*Larix decidua*) en peuplement pur et non équienne, avec quelques bouquets ou arbres isolés de sapin (*Abies alba* M.), de pin sylvestre (*Pinus sylvestris*), de pin cembro (*Pinus cembra*) et de pin à crochets (*Pinus uncinata*). Quatre parcelles ont été sélectionnées dans ce gradient d'élévation à 1350, 1700, 2000, 2300 m d'altitude. Au total, 60 arbres dominants ont été sélectionnés (15 dans chaque parcelle).

2. Carottage et préparation des échantillons

Les prélèvements et la préparation des échantillons ont été réalisés en 2013 par les équipes techniques de l'UMR LERFoB dans le cadre d'un projet de recherche « GRAAL » conduit avec l'UMR AGPF d'Orléans. Au cours de la saison de croissance de 2013, de mai à novembre, des micro-carottes ont été extraites à hauteur de poitrine de chaque arbre. Le carottage a été effectuée en utilisant un Trephor (Vitzani, Belluno, Italie) (Rossi et al., 2006) avec une distance de 2 cm entre deux échantillons. Les micro-carottes collectées (de diamètre de 2 mm et de longueur de 15 à 20 mm) ont été placés dans des flacons Eppendorf avec une solution diluée d'éthanol à 50%, puis stockés à 5 ° C pour empêcher la détérioration des tissus vivants. Les micro-carottes ont ensuite été incorporés dans de la paraffine. Les sections transversales de 5 à 10 μm d'épaisseur ont été coupées, colorées et fixées à l'aide d'un microtome rotatif pour d'autres observations anatomiques (Harroué et al., 2011).

3. Observations et comptages de cellules dans les différentes phases de la formation du bois

Les observations anatomiques ont été réalisées à l'aide d'un microscope optique (ZEISS, Allemagne). La lumière visible et polarisée a été utilisée pour distinguer différentes phases du développement cellulaire. Dans chaque échantillon, trois files radiales ont été choisies pour compter le nombre de cellules dans le cambium (nC), dans les phases d'élargissement (nE), d'épaississement de la paroi (nW) ainsi que le nombre de cellules complètement matures (nM) et celles l'année précédente (nP). Les cellules du cambium ont été reconnues par leurs parois primaires minces, leur petite taille radiale et leur forme rectangulaire par rapport aux cellules du phloème. Les cellules en élargissement avaient une plus grande surface de lumen avec des parois primaires minces. La lumière polarisée a été utilisée pour distinguer les cellules dans la phase d'épaississement de la paroi : la paroi secondaire en lignification apparaissait brillante alors qu'elle n'existait pas dans les cellules seulement en élargissement. Enfin les colorations permettaient de distinguer les cellules complètement matures, totalement bleues quand les cellules en épaississement montraient un gradient de couleur rose-violet à bleu de l'intérieur vers l'extérieur des parois cellulaires.

4. Traitement des données

A partir de ces comptages de trachéïdes, les durées de séjour dans chacune des phases de formation du bois ont été calculées en utilisant le logiciel CAVIAR, basé sur les régressions logistiques (Rathgeber 2011). CAVIAR calcule les dates de (1) le début de la phase de division; (2) le début de la phase d'agrandissement; (3) le début de la phase d'épaississement de la paroi; (4) le début de la phase mature; (5) la fin de la phase de division; (6) la fin de la phase d'agrandissement et (7) la fin de la phase d'épaississement de la paroi. Les dates critiques correspondent à une probabilité de 50% d'une phénophasse active. L'apparition et la cessation de l'activité cambiale étaient basées sur la phase d'agrandissement des cellules du xylème plutôt que sur la division cellulaire cambiale proposée par (Rathgeber et al., 2011).

Afin d'évaluer la dynamique de la production de trachéïdes, le nombre de cellules au cours du temps a été ajusté à la fonction de Gompertz (Rossi et al., 2003). La longueur minimale du cycle cellulaire CCLn a été calculée en divisant le nombre de cellules cambiales à la date tx où le taux de production de cellules est maximal par le taux maximum de production de cellules rx. Afin de calibrer le nombre total de cellules à la fin de la saison de croissance (RCN), la médiane du nombre total de cellules de tous les échantillons après la fin de la production de cellules a été calculée. Un modèle simple a été appliqué pour exprimer ce nombre total RCN en fonction du taux moyen (rm) de production de cellules et de la durée de production de cellules dtE calculée à partir du début et de la fin de la phase d'agrandissement. Afin de discuter comment les variations du taux et de la durée contribuent chacune indépendamment à la variation du nombre total de cellules, une analyse de sensibilité a été effectuée. Cette analyse est basée sur l'évaluation de la variation relative des variables d'entrée (en utilisant leur déviation standard) sur le résultat de sortie du modèle (Cariboni et al., 2007).

5. Etude des tissus traumatiques

Le micro-carottage est supposé peu perturbant. Néanmoins, nous avons observé qu'il provoquait des traumatismes visibles sur la formation du bois. L'apparition de tissus traumatiques a donc été enregistrée. Sur chaque coupe anatomique, l'apparition de calls de cicatrisation ainsi que le nombre de canaux résinifères traumatiques ont été enregistrés. Pour évaluer l'effet de la stimulation de l'activité cambiale par la perturbation du micro-carottage, nous avons ajusté des fonctions de Gompertz et calculé des résidus d'ajustement.

6. Données complémentaires

Concernant les données environnementales, les températures moyennes, minimum et maximum quotidiennes ainsi que l'humidité de l'air ont été directement obtenus pour toute l'année 2013 auprès de stations météorologiques locales installées sur chaque site. Les degrés jours de croissance GDD5 ont été calculés sur la base de T de 5 ° C.

Les données de précipitations n'ont pas pu être mesurées dans les stations locales, mais elles ont été interpolées à partir des données journalières issues de la station Météo France de Villard St.Pancrace et de la base de données mondiale Worldclim. La longueur du jour pour le site d'étude a été calculée en utilisant le package "géosphère" dans le programme R qui donne la longueur du jour en heures à partir des coordonnées géographiques du site. Afin de calculer le bilan hydrique quotidien du sol sur chaque parcelle, les données météorologiques (température moyenne de l'air, précipitations, rayonnement global, humidité relative et vitesse du vent) ont été injectées dans le modèle Biljou®(Granier et al., 1999). Le modèle a utilisé outre les données météorologiques, les caractéristiques du sol à chaque site d'étude (nombre, profondeur des couches et proportion de racines fines par couche) et les paramètres du peuplement - type de forêt et indice de surface foliaire maximale (LAI) -.

D. Résultats

Le micro-carottage n'a quasiment jamais conduit à la production de tissu calleux mais par contre, il a produit de nombreux canaux résineux traumatiques, assez systématiquement dans tous les arbres. L'examen des résidus d'ajustement de Gompertz a montré que la perturbation stimule légèrement l'activité cambiale, perturbant la xylogénèse et la structure terminale des cernes. Nous pensons cependant que l'impact global de cette perturbation reste limité sur la phénologie et la dynamique de la formation du bois et donc sur les résultats qui suivent.

L'activité cambiale a débuté vers la mi-mai au niveau le plus bas et vers la mi-juin au plus haut niveau, ce qui correspond à un retard de 3 jours par 100 m. Les débuts des autres phases de formation du bois suivaient exactement la même tendance d'élévation, mais étaient retardés de quelques jours jusqu'à un ou deux mois. La cessation de l'activité cambiale et la formation du bois suivent une tendance parabolique le long du gradient étudié : les arbres du peuplement le plus bas finissent en premier, suivis par ceux du peuplement le plus élevé, tandis que ceux des altitudes intermédiaires terminent les derniers.

Les durées de l'activité cambiale et de la formation du bois ont également montré des tendances paraboliques le long du gradient d'élévation, avec la saison la plus courte à 2300 m et la plus longue à 1700 m. Les analyses des effets de taux et de durée montrent que le raccourcissement de la durée de la croissance lié à l'élévation est compensé par une augmentation du taux de production cellulaire, conduisant à des largeurs de cernes similaires dans les trois sites supérieurs. Le peuplement le plus bas, au contraire, présente des cernes plus fins en raison de faibles taux de production et de durées courtes.

L'analyse de sensibilité a montré que le nombre final de cellules est plus lié au taux de production (60%) qu'à sa durée (40%). De plus, nos résultats ont montré que le nombre de cellules cambiales

augmente avec l'élévation, ce qui compense l'allongement du cycle cellulaire, et permet de maintenir des taux de production élevés, même à haute altitude.

E. Discussion

1. Les méthodes classiques de suivi de la formation du bois par microcarottage sont perturbantes, il faut rester vigilant sur l'impact de ces perturbations.

Le monitoring de la formation de bois dans cette étude a été effectué en utilisant la méthode classique de micro-carottage à intervalles d'environ une semaine (Rossi 2006a). De cette façon, il est possible d'observer le développement du xylème au cours de la saison de croissance, mais comme le nombre d'échantillons est élevé (la saison dure de nombreuses semaines), la taille des échantillons doit être aussi petite que possible.

Les perturbations mécaniques et physiques du cambium stimulent les mécanismes de défenses. De nombreux auteurs mentionnent que même un petit dommage aux arbres tel que l'enlèvement partiel de l'écorce (Nagy et autres 2000, Stobbe et al., 2002) ou de petites blessures (Gartner et al., 2009) voire les carottages plus agressifs (Perret 2006, Bollschweiler et al. 2008) déclenchent des réponses de défense par le cambium. Une telle réaction des arbres peut devenir très perturbante dans les études sur la formation du bois, car elle affecte la croissance normale des arbres menant à la production de tissus anormaux à proximité de la plaie (Larson, 1994), là où sont prélevées les carottes des semaines suivantes. Le cambium dans le cas d'une perturbation mécanique intense produit un nouveau tissu appelé cal de cicatrisation (Stobbe et al., 2002, Gartner et al., 2009). Il produit aussi des bandes tangentielles de canaux résinifères traumatiques (Bollschweiler et al., 2008, Schneuwly et al., 2009). Ces canaux sont connus comme une caractéristique anatomique normale chez certaines espèces de conifères, mais lorsqu'ils apparaissent en bandes tangentielles consécutivement à un événement datable, ils sont considérés comme des tissus traumatiques et anormaux.

Dans la plupart des cas, la formation de canaux résinifères traumatiques semble plus élevée au-dessus de la blessure (Fahn et al., 1979, Gartner et al., 2009), ce qui peut s'expliquer par l'accumulation plus élevée d'hormones de croissance au sommet de la zone de la blessure. Biologiquement, la formation de canaux résinifères traumatiques viendrait d'une barrière physique au flux d'hormones provoquée par la blessure (Fahn et al., 1979). Dans nos arbres étudiés, aucun tissu de cal n'a été observé mais de nombreux canaux résinifères traumatiques sont apparus. Puisque les bandes tangentielles de canaux de résine ont été formées seulement dans le dernier cerne formé en 2013 et qu'aucune autre perturbation telle que des chutes de pierres ou des avalanches n'a été enregistrée durant l'année d'étude, le micro-carottage a été considéré comme la raison principale de leur apparition. Par ailleurs, il est connu que les perturbations mécaniques stimulent l'activité cambiale. Wodzicki et Zajaczkowski (1970) ont déjà observé une augmentation du taux de production de cellules en raison des effets de la blessure lors du monitoring de la formation du bois. Ils ont montré que l'augmentation des intervalles de temps entre deux micro-carottages (allant jusqu'à 21 jours) et le maintien d'une distance d'au moins 1 cm entre deux micro-carottages diminuaient les effets de blessure (Wodzicki et Zajaczkowski 1970). D'autres études ont rapporté une activité plus élevée du cambium et de la division cellulaire autour de la zone blessée dans *Cedrus libani* (Fahn et al., 1979). Le micro-carottage peut donc conduire à une augmentation du nombre final de cellules dans l'arbre en stimulant l'activité cambiale. Ce plus grand nombre de cellules trachéides aura besoin de plus de temps pour achever le processus de formation du bois et devenir complètement mature, de sorte qu'une stimulation cambiale peut conduire à surestimer la date d'arrêt de la formation du bois.

En résumé, on peut affirmer que le micro-carottage peut avoir un impact sur la formation du bois en affectant non seulement les types cellulaires (formation de tissus traumatiques) mais aussi le taux

de production de cellules et la phénologie de la formation du bois. Cet impact ne pouvant être réduit *a posteriori* dans notre étude, nous avons supposé qu'il restait faible.

2. L'altitude retarde le démarrage des phases de formation du bois et modifie les durées des saisons de végétation, et cela peut être interprété comme une réponse physiologique ou adaptative à la diminution de température

Les résultats de cette étude ont montré des différences dans les phases phénologiques de la formation du bois le long d'un gradient d'altitude de 1 000 m. Le début de l'activité cambiale observée pour la première fois au site le plus bas s'est ensuite produit avec un retard de 2,5 jours tous les 100 m de dénivelé lorsqu'on monte en altitude. D'autres phases phénologiques de la formation du bois (agrandissement, épaississement de la paroi et maturité) se sont aussi produites avec un retard se déplaçant vers des altitudes plus élevées. Ce retard s'interprète par le rôle déterminant de la température.

La température est largement reconnue comme un facteur clé contrôlant la reprise de l'activité cambiale au début de la saison de croissance (Oribe et Kubo, 1997, Oribe et al., 2001, Gricar et al., 2006). La division cellulaire cambiale et la différenciation des cellules du xylème sont toutes deux des processus qui nécessitent une source de carbone importante pour satisfaire la demande en quantités adéquates de saccharose (Oribe et al., 2003). La température permet l'allocation du saccharose disponible (Hoch et Korner 2003) et le processus métabolique de la croissance (Begum et al., 2007).

L'adaptation de la phénologie de la formation du bois aux gradients de température a été précédemment rapportée le long des gradients d'altitude (Moser et al 2010, Oladi et al 2011, Prislán et al., 2013). Moser et al. (2010) ont trouvé un retard pour le début de l'activité cambiale d'un ordre de grandeur comparable au nôtre dans les Alpes suisses (Moser et al., 2010). La survie des espèces d'arbres aux hautes altitudes est supposée le résultat de l'interaction entre trois composantes principales : résistance au gel au printemps, ajustement de la phénologie du débourrement et temps suffisant pour la maturation des tissus nouvellement formés (Korner et al., 2016). La phénologie des arbres doit alors être régulée de manière à réduire presque à néant les dommages causés le gel (Vitasse et al., 2014). Le retard observé pour le début de l'activité cambiale avec l'augmentation de la température dans notre étude peut s'expliquer par la stratégie des arbres pour éviter les dommages de gel au début du printemps. La contrainte des basses températures sur la croissance et le développement des arbres s'expriment donc par des effets directs et indirects, et par des effets graduels ou de seuil.

Outre ses effets directs (les températures sont incompatibles avec la vie en dehors d'une niche bien définie), la température a un effet indirect car elle affecte la durée de la maturation des tissus et donc la longueur de la saison de croissance (Korner 2006). Dans notre gradient étudié, l'ajustement du démarrage de la formation du bois aux basses températures est un effet de seuil. Mais l'allongement progressif de la longueur de la saison de croissance pour fournir suffisamment de temps pour la maturation des cellules nouvellement formées est un effet graduel. Les modifications de longueur de saison de végétation observées avec l'altitude sont donc impactées par ces effets graduels indirects de la température. Néanmoins elles sont plus complexes qu'un simple allongement régulier de la saison le long du gradient.

3. D'autres facteurs perturbent la dépendance simple des processus de formation du bois à la diminution linéaire de température avec l'altitude

Des études antérieures sur la phénologie de la formation du bois et la relation climat-croissance se sont principalement concentrées sur le début de l'activité cambiale alors que son arrêt demeurerait inexplicé (Gricar et al., 2007, Rossi et al., 2007). Parmi les facteurs environnementaux, la cessation de l'activité cambiale s'est principalement révélée être liée à la photopériode dans nos arbres étudiés dans les trois

sites supérieurs. Moser et al. (2010) ont trouvé la cessation simultanée de l'activité cambiale le long d'un gradient comparable dans les Alpes suisses, soulignant le rôle déterminant de la photopériode sur sa terminaison. Dans d'autres études, la croissance des tiges de *Larix decidua* a été observée avec un retard de démarrage vers des altitudes plus élevées de 700 à 2000 m, tandis que son arrêt a été observée approximativement au même moment (Tranquillini et al., 1979). La première étape de l'acclimatation au froid chez les arbres serait déclenchée par une diminution de la longueur du jour en automne, qui entraîne l'accumulation de réserves d'amidon et de lipides dans les tissus (Vitasse et al., 2014). Le début de la dormance en automne a été parfois jugé moins dépendant de la température et surtout contrôlé par la photopériode, conduisant à une forte résistance au gel avant l'apparition de basses températures (Korner et al., 2016). Dans notre étude, une longueur de photopériode commune à la cessation de l'activité cambiale dans les trois sites supérieurs confirme l'effet de la durée du jour qui fournirait les signaux qui contrôlent la fin de la saison de croissance dans les arbres. Nos résultats ont donc démontré le rôle de la photopériode sur le contrôle de l'arrêt de l'activité cambiale.

La limitation de la disponibilité de l'eau a également des effets sur l'activité cambiale et la formation des trachéides. La réponse des arbres au déficit hydrique est complexe. En cas de sécheresse grave, la croissance des arbres peut ralentir ou même la division cellulaire peut être arrêtée (Guney et al., 2017). Dans notre gradient étudié, le nombre final de cellules produites était similaire dans trois altitudes supérieures, mais le peuplement le plus bas, au contraire, présentait des cernes étroits à la fois du fait de faibles taux de production cellulaire et d'une durée courte de la saison de croissance. L'arrêt de l'activité cambiale était plus précoce au site inférieur ainsi que l'arrêt des processus de division et d'élargissement. Cela peut s'expliquer par une plus faible disponibilité en eau sur le site inférieur malgré des conditions de température plus favorables.

Enfin l'ajustement des arbres au froid ne concerne pas seulement l'ajustement phénologique des dates de croissance aux périodes où la température est optimale, mais aussi l'ajustement physiologique des capacités à croître à des températures froides. Concernant le processus de croissance primaire, une date de démarrage plus tardive à plus haute altitude est une stratégie connue pour minimiser la probabilité des dommages dus au gel pendant le débourrement. Néanmoins, la résistance au gel s'est également révélée différente le long du gradient d'altitude avec des arbres plus vulnérables aux altitudes plus basses. Ainsi, c'est bien l'interaction entre la date du débourrement et la tolérance au gel pendant le débourrement qui définit la mise en sécurité des arbres le long du gradient d'altitude (Korner 2016).

4. La longueur de la saison de croissance n'explique pas la croissance en diamètre des arbres car un taux de production de cellules plus élevé compense la plus faible longueur de la saison de végétation en haute altitude

Nos résultats sur la production de cellules de xylème ont montré que les arbres étudiés produisaient le même nombre de cellules trachéides annuelles aux trois altitudes supérieures bien que la phénologie différente de l'activité cambiale ait entraîné une durée de croissance bien différente. Le lien de causalité entre la longueur de la saison de croissance et la taille des cernes n'est donc pas encore bien clair. Certaines études antérieures ont rapporté une réduction de la période et de la quantité de xylème le long des gradients altitudinaux et latitudinaux, expliqués par l'apparition tardive et l'arrêt précoce de l'activité cambiale au printemps et en automne respectivement (Rossi et al., 2010; Moser et al. 2010). Rossi et al. (2011b) ont associé une période plus longue de formation du bois à une plus largeur de cerne plus grande. La reprise plus précoce de l'activité cambiale au printemps s'est avérée donner plus de temps aux cellules du cambium pour se diviser et produire par conséquent plus de cellules de xylème (Rossi et al., 2011b). Cependant, d'autres études ont montré qu'une période plus courte de formation du bois n'entraînait pas une diminution du nombre de cellules trachéides (Ren et al., 2015)

car le taux de production de cellules peut augmenter et réguler ainsi la largeur de cerne. Sur le même matériel et le même gradient d'altitude, Nardin 2013 a montré une variabilité phénotypique significative avec l'altitude pour toutes les variables liées à la microdensité du cerne, excepté la largeur des cernes. Cela va dans le même sens que nos propres résultats sur le nombre de cellules peu variable aux trois altitudes supérieures. La production de cellules de xylème est le résultat de la durée et du taux de production de cellules. Afin d'expliquer comment les arbres qui poussent à une altitude plus élevée et dont la durée de croissance est plus courte peuvent produire le même nombre de cellules trachéïdes que ceux des altitudes inférieures, nous avons comparé le taux de production cellulaire le long du gradient d'altitude. Le taux de production cellulaire est alors logiquement croissant avec l'altitude. Ce taux de production de cellules a toujours été supposé lié aux caractéristiques des arbres telles que l'âge et la taille (Rathgeber et al., 2011) et à la capacité des arbres à produire des assimilats (Cuny et al., 2012). Mais ses variations le long de gradients d'altitude ont rarement été étudiées. Des taux de croissance croissants vers des altitudes plus élevées ont déjà été signalés chez certains animaux comme (poissons de l'Atlantique et la grenouille verte *Rana clamitans*). Ce mécanisme s'expliquait par la sélection d'une croissance rapide dans les environnements de saison de croissance plus courts, où une grande taille corporelle est nécessaire pour les longs hivers (Berven et al., 1979, Conover et Present, 1990). Ce modèle connu sous le nom de « countergradient theory » entraîne une variation phénotypique minimale le long des gradients d'altitude (Levins 1969). Cette théorie n'a jamais été testée expérimentalement chez les plantes mais le taux croissant de production cellulaire dans nos arbres étudiés lorsque la longueur de la saison de croissance diminue, associé à une largeur de cernes stable, peut s'interpréter par la même « countergradient theory ».

Le taux de production de cellules est lié au nombre de cellules cambiales en division et à leur taux de division (Vaganov et al., 2006). Les résultats de cette étude ont montré une augmentation du nombre de cellules cambiales en division avec l'altitude. La division cellulaire est connue comme un processus lent dans le cambium (Larson 1994), donc un nombre plus élevé de cellules cambiales en division peut aider les arbres à atteindre un taux plus élevé de production de cellules sur une période plus courte, et contribuer à équilibrer le raccourcissement de la saison de croissance et la production de trachéïdes.

Il est toujours difficile en l'absence d'études précises sur la formation du bois telles que la nôtre de définir si les variations de la largeur de cerne résultent d'une phénologie ou d'une dynamique différente de la production cellulaire. Les études sur la formation du bois permettent d'établir les contributions respectives du taux de production et de la durée de l'activité cambiale dans la production annuelle de cellules de xylème. Certaines ont conclu comme la nôtre que le nombre final de cellules de xylème produites est principalement influencé par le taux de production cellulaire et partiellement lié à la durée de la saison de croissance (Rathgeber et al., 2011, Cuny et al., 2012, Ren et al. 2015). D'autres ont montré un effet principal de la durée (Rossi et al., 2014).

5. Que pourrait être l'impact des changements climatiques sur la croissance radiale du Mélèze dans des conditions similaires?

Avec le récent changement climatique, une augmentation de la température et un allongement de la saison de croissance annuelle moyenne ont été observés en Europe (Menzel et Fabian 1999). La question est donc d'anticiper comment cela impacte précisément la formation du bois.

D'après nos résultats sur le Mélèze dans le Briançonnais, l'avancement du démarrage de l'activité cambiale serait de l'ordre de 5 jours/ ° C de réchauffement (le changement de température moyenne est de 0,54 / ° C par 100 m de dénivelé en altitude). Ainsi, la formation de bois démarrerait entre 12,5 et 17,5 jours plus tôt en 2100 qu'en 1960 du fait des augmentations prévues de la température entre 2,5 ° C et 3,5 ° C en France (Gosling et al, 2011).

La comparaison de la longueur de la saison de croissance le long de notre gradient étudié a alors montré une réduction de la durée de la saison de croissance de 4 jours/ ° C.

Outre ces changements phénologiques, Il est également très important de prédire la croissance des arbres et le nombre final de trachéides formées dans le cerne. La question est de savoir si l'allongement de la saison de croissance augmentera la largeur des cernes. Nous avons trouvé que le nombre final de cellules arborescentes dépendait d'environ 60% du taux de production de cellules par le cambium et de seulement 40% de la durée de l'activité cambiale. L'allongement de la période de formation du bois doit donc coïncider avec une augmentation ou au moins une stabilité du taux de production de cellules pour avoir un effet positif sur la largeur de cernes. Or, dans le gradient altitudinal étudié, le taux de production de cellules augmente lorsque la saison de croissance se raccourcit aux hautes altitudes. Le taux de production de cellules peut donc diminuer pour s'ajuster à l'allongement de la saison et réguler la largeur de cerne. Une saison de croissance plus longue ne se traduira pas nécessairement par une croissance en diamètre plus grande.

Sur le site le plus bas (1350 m), nous avons observé une cessation précoce de l'activité du cambium ce qui a limité à la fois la durée de la saison de formation du bois et le nombre final de trachéides formées. Cela est imputable à une contrainte hydrique. Le changement climatique prévoit une intensification des sécheresses pendant la saison de végétation. Il y a donc compétition entre ces effets négatifs sur la formation du bois et les effets stimulants de l'augmentation de température. densité plus faible. Le site optimal pour la croissance des mélèzes se déplacera très certainement vers des altitudes plus élevées. Ce mouvement devra être pris en compte dans les mesures de gestion forestière.

F. Synthèse et perspectives

Cette étude est une contribution à l'étude de la phénologie et de la dynamique intra-annuelles de la formation du bois et de la croissance en diamètre des arbres. Nous avons travaillé sur une essence de conifère, le mélèze d'Europe, et à partir d'observations sur une année le long d'un gradient d'altitude dans les Alpes du Sud françaises. Des méthodes de micro-carottage hebdomadaire durant la saison de croissance, et d'observation de coupes minces microscopiques, alliées à des packages performants d'analyse des données sous le logiciel R, ont permis d'évaluer les phases de développement du processus de xylogénèse avec leurs caractéristiques de vitesses et de durée. Les résultats portent sur l'observation des variations de ces caractéristiques le long du gradient d'altitude. La discussion aborde plus généralement l'effet de la température et de la durée de la saison de végétation sur la formation du bois, en tenant compte d'autres facteurs tels que la disponibilité en eau. Les perspectives de ce travail s'inscrivent d'une part dans des travaux d'écologie générale autour de la « countergradient theory » rarement discutée sur les plantes, et d'autre part dans des recherches appliquées pour la gestion forestière, sur l'adaptation de cette espèce au changement climatique.

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Title : Wood formation dynamics of Larch (*Larix decidua* Mill.) along a 1000 m elevation gradient in the French Southern Alps

Keywords : Wood formation, cambial activity, elevation gradient, (*Larix decidua* Mill), French Alps

Abstract :

The survival of perennial plants depends on their ability to remain adapted to their ever-changing environment. Vegetation model predictions indicate that climate change will have profound effects on forest production and distribution. Tree response to environmental factors amply relies on cambium ability to produce efficient wood. The main objective of this thesis is to understand which modifications of the wood formation (WF) processes allow trees to grow under contrasted conditions. The xylogenesis of 60 larch trees, in four stands along an elevation gradient of 1000 m in the French Southern Alps was monitored in the 2013. Wood samples were taken every week from tree stem.. Forming wood cells counted according to their differentiation stage (dividing, enlarging, and wall thickening and mature). Moreover, the occurrence of wounding tissues was recorded. Meteorological data were obtained from nearby climate stations and relative extractable water was computed using a water balance model. The critical dates of wood formation were computed based on logistic regression, while wood growth dynamics was described using Gompertz models. Dates and rates were then compared along the elevation gradient using bootstrap tests and mixed-effects models. The relative contribution to the total annual production of rates vs. durations was assessed using a sensitivity analysis of a basic physical model. Cambial activity (CA) started around mid-May at the lowest stand and around mid-June at the highest one, which correspond to a delay of 3 days per 100 m. The onsets of the other periods of wood formation followed exactly the same elevation trend, but were lagged in time of a few days up to one or two months. The cessation of CA and WF followed a parabolic trend with trees from the lowest stand finishing first, followed by those from the highest stand, while those from the intermediate elevations finished the last. CA and WF durations also exhibited parabolic trends, but with the shortest season occurring at 2300 m and the longest ones at 1700 m. Nevertheless, we discovered that the elevation-related shortening of the growing season, was compensated by an increase in the cell production rate, leading to comparable tree-ring widths in the three upper stands. The lowest stand, on the contrary, exhibited narrow tree rings due to both low production rates and short durations. The sensitivity analysis showed that the final cell number was more related to the rate of production than to its duration. Moreover, our results showed that the number of cambial cell increased with elevation, which compensated the lengthening of the cell cycle, and thus maintained high production rates, even at high elevation. Lastly, we didn't observe any callus tissue in our anatomical sections, but we counted numerous traumatic resin canals. Moreover, a careful examination of Gompertz fitting residuals led us to think that microcoring monitoring slightly stimulated cambial activity, disturbing xylogenesis and final tree-ring structure. We believe, however, that the overall impact of cambial stimulation on wood formation phenology and dynamics remains limited. So the delay in the wood formation CA and WF onsets with increasing elevation can be associated with the adiabatic temperature decrease and thus translated into a lengthening of the growing season of about 5 days per °C. On the other hand, our results show that the cessation of cambial activity is more related to photoperiod, even with water shortage being able to hasten growing season termination. However, even if phenology is a key component of forest functioning, our results underlined the pivotal role of the rate of cambial activity in tree growth. Structural investment to sustain cell division rates while elevation increase, thanks to increased number of cambial cells, and despite temperature-related increased of cell cycle length, unveil a strong adaptation mechanism of trees to high elevations.

Titre : Dynamiques de la formation du bois du Mélèze (*Larix decidua* Mill.) le long d'un gradient altitudinal de 1000 m dans les Alpes du sud Françaises

Mots clés : Formation de bois, activité cambiale, gradient d'altitude, larix decidua Mill, Alpes française

Résumé :

La survie des plantes pérennes dépend de leur capacité à s'adapter à leur environnement. Les modèles de végétation prédisent que les changements climatiques vont avoir un effet profond sur la production et la distribution des forêts. La réponse des arbres aux changements climatiques dépend de la capacité du cambium à produire un bois performant. L'objectif est de comprendre quelles modifications des processus de formation du bois permettent aux arbres de croître dans des conditions contrastées. Nous avons suivi la formation du bois de 60 mélèzes, en 2013, dans quatre peuplements, le long d'un gradient altitudinal de 1000 m dans les Alpes du Sud. Des échantillons ont été prélevés chaque semaine. Les cellules en formation ont été classées et comptées en fonction de leur stade de différenciation (division, élargissement, épaississement et maturité). De plus, l'occurrence de tissus cicatriciels a été notée. Le comptage cellulaire a ensuite servi à calculer les dates critiques de la formation du bois à l'aide de régressions logistiques, alors que la croissance du bois était décrite par l'ajustement de modèle de Gompertz. Les dates et taux obtenus ont été comparés au gradient altitudinal grâce à des tests bootstrap et des modèles linéaires mixtes. La contribution relative à la production annuelle totale du taux par rapport à la durée a été estimée à l'aide d'une analyse de sensibilité. L'activité cambiale (AC) a commencé vers mi-mai pour le site le plus bas du gradient et vers mi-juin pour le site le plus élevé, ce qui correspond à un retard de 3 jours par 100 m. Le début des autres périodes de la formation du bois (FB) suivaient la même tendance altitudinale, mais avec un décalage de quelques jours à un ou deux mois. Les fins de l'AC et de la FB suivaient une tendance parabolique avec les arbres du peuplement le plus bas finissant en premier, ceux du peuplement le plus haut en second, et ceux des peuplements intermédiaires en dernier. Les durées totales de l'AC et de la FB présentaient également des tendances paraboliques avec les durées les plus courtes à 2300 m, et les plus longues à 1700 m. Néanmoins, nous avons découvert que le raccourcissement de la saison de végétation lié à l'altitude était compensé par une augmentation des taux de production cellulaire aboutissant à des largeurs de cernes finales comparables pour les trois peuplements du haut. Le peuplement du bas, au contraire, présentait des cernes plus étroits, en raison de saisons de végétation plus courtes et de taux de production plus bas. L'analyse de sensibilité a montré que le nombre de cellule final était plus influencé par le taux de production que par sa durée. Nous avons constaté une augmentation du nombre de cellules cambiale avec l'altitude, qui contrebalançait l'allongement du cycle cellulaire, et maintenait un taux de production important. Enfin, nous n'avons pas observé de tissus cicatriciels mais un nombre important de canaux résinifères. Un examen détaillé des résidus des ajustements des Gompertz a conduit à penser que le microcarottage stimule légèrement l'AC, perturbant la xylogénèse et la structure finale des cernes de croissance. Les délais observés dans le démarrage de l'AC et de la FB peuvent être associés au gradient adiabatique de température et être ainsi convertis en un allongement de la saison de végétation de 5 jours par °C. D'un autre côté, nos résultats montrent que c'est plutôt la photopériode qui détermine la fin de l'AC, bien qu'une restriction hydrique soit capable de précipiter cette fin. Cependant, même si la phénologie est une composante importante du fonctionnement des forêts, nos résultats soulignent le rôle majeur du taux d'activité cambiale en ce qui concerne la croissance. L'investissement structural consistant à augmenter le nombre de cellules cambiales pour soutenir le taux de division quand l'altitude augmente, que la température baisse, et que le cycle cellulaire s'allonge, révèle un mécanisme d'adaptation puissant.

