

# Phenology of wood formation and its genetic variability in larch : A step towards a better understanding of wood formation in relation to climate

María Elena Gauchat Funes Drewes

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**María Elena GAUCHAT**

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**PHENOLOGY OF WOOD FORMATION AND ITS GENETIC VARIABILITY IN LARCH.  
A step towards a better understanding of wood formation in relation to  
climate**

Directeur de thèse : **Luc PAQUES**

Co-encadrement de la thèse : **Philippe ROZENBERG**

**Jury**

M. Sergio ROSSI, **professeur associé**, Université du Québec à Chicoutimi

M. Leonardo GALLO, **Chercheur**, INTA

Mme Nathalie FRASCARIA-LACOSTE, **Professeur**, AgroParisTech

M. Luc PAQUES, **CR1**, INRA

M. Philippe ROZENBERG, **DR2**, INRA

M. Hendrik DAVI, **CR1**, INRA

Rapporteur

Rapporteur

Examinatrice

Examineur

Examineur

Examineur

AgroParisTech

**INRA, UR588 Unite Amelioration, Genetique et Physiologie Forestieres  
2163 Av. Pomme de Pin, CS 40001, ARDON, 45075 ORLEANS, Cedex 2, France**

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**“After climbing a great hill, one only finds that there are many more hills to  
climb.”**

*Nelson Mandela*

**“Logic will get you from A to B. Imagination will take you everywhere.”**

*Albert Einstein*

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## THESIS PRESENTATION

In the context of climatic change, increase of temperature, changes in precipitation regimes and increase of the frequency and severity of extreme events like heat-drought waves are projected. If changes in climate occur as predicted, planning will be essential because trees planted today will grow in a climate highly different from the one experienced by the trees currently harvested. A valid strategy is to gain a better understanding of basic physiological and genetic mechanisms and use it to more efficiently predict tree reaction in front of the climate change. Then it will be possible to adjust the selection criteria used in tree-breeding programs.

European larch (*Larix decidua* Mill.) is the model species of the thesis. It naturally occurs in medium to high mountainous ranges across Europe. Because of the high potential of that species both in terms of production and of wood quality, larch forests have been extended well-beyond European larch native range, both northwards and westwards. Results from species and provenance trials revealed on one side the interest of Japanese larch (*Larix kaempferi* (Lamb.) Carr.) in strictly oceanic climates and on the other side, the excellent behaviour of Central Poland (*polonica*) and Sudetan Mountains (*sudetica*) populations of European larch over a broad range of ecological conditions (Schober 1985).

In France, both ecotypes are used in reforestation outside the Alps either from seed directly imported from Central Europe or from local seed orchards. Besides their vigorous growth, both populations are appreciated for their better resistance to canker (Pâques et al. 1999) but there are also more and more evidences of the superior wood properties of some provenances valued for their high wood density and high mechanical properties (Pâques and Rozenberg 1995) and for several properties of their heartwood in terms of size (Pâques 2001b), extractives content (Gierlinger et al. 2002) and natural durability (Curnel et al. 2008).

However, long-distance transfer of forest reproductive material from Central Europe with a more continental climate to French oceanic regions might face adaptive problems not necessarily observed and observable during the life-time of a provenance test. As an example, the high

susceptibility of *polonica* larch to *Meria laricis* Vuill. in western low Massif Central Mountains has been revealed only recently and highlighted by more frequent mild and humid springs. Even if Central Europe larch has shown a broad site plasticity (Giertych 1979, Schober 1985) and *polonica* larch in particular is considered by Kral (1966, 1967) as one of the most drought resistant ecotypes of European larch, question of its adaptation to regions marked by a much more variable (temperature and rainfall) and drier climate is raised.

Response of cambial activity (cell division, elongation and thickening of cell walls) to environmental factors (climate, soil fertility, competition, etc), physiological states and genetic component are reflected in variations of width and the tissue structures of annual rings.

Wood has been used in dendrochronological studies for many years as a record of past climatic variation. Apart from supporting trees, wood takes part in several physiological processes related to water transport and nutrient storage. Wood density is a comprehensive measure of annual rings characteristics, and its variation can be interpreted as tree response to environmental changes. The most inclusive data on wood density are computed from microdensity profiles (Polge 1966). Weather is probably the most important factor affecting microdensity profiles if we assume that there is not substantial change in global fertility during a growing season (Lebourgeois et al. 2005). Then, within-ring variation revealed by microdensity profiles is a valuable tool for study of tree reaction to weather.

Different approaches have been used in the study of inter and intra-annual variation of wood density that interpret this variation as a tree response to climatic variation, between and within growing seasons. In this sense, within-ring variation revealed by microdensity profiles is a valuable tool for the study of tree reaction to weather. This method offers the possibility to retrospective analysis of cambial activity in terms of wood tissue characteristics (lengths, densities).

The microdensity approach is used in tree improvement programs to improve wood quality components. The variation of density within ring not only permits to study the pattern variation between trees. One major limit of the use of microdensity profile to study tree response to

environmental conditions is the lack of relationship with the timing of ring formation. These profiles can be called static profiles. In a set of studies using wood as a marker of tree adaptability to environmental stress, the so-called “dendroplasticity approach” (Martinez et al. 2009a, Sanchez-Vargas et al. 2007) assumes that all trees start and end their annual radial growth at the same time. The within-ring density increase is additionally assumed to be an immediate response to weather variation. But phenology (bud burst and bud set) and timing of cambial activity (mostly during the last part of growing season) have been shown to be genetically and environmentally variable (Vargas-Hernández and Adams 1994). It would be more relevant to work with a dynamic profile, relating within-ring density variation to environmental (weather) variation along the corresponding growing season. For that we need a microdensity profile expressing the wood density variation as a function of time. Taking the time factor into consideration enables the generation of this new version of the microdensity profile, so-called dynamic microdensity profile. The dynamic microdensity profiles of this study were obtained directly applying a technique called the pinning method. Regrettably, the use of the pinning method to transform the static profile into a dynamic microdensity profile is very time-consuming and thus expensive, reducing its application potential in breeding programs. However, easy-to-observe vegetative phenology could reflect the dynamic of wood formation and the way in which tree reacts to weather variation. Such phenological events could act as easy-to-obtain external markers of cambial activity. These markers would be useful for early mass-screening of large numbers of genotypes.

Among the possible external markers of cambial activity, flushing seems a key character. The potential of flushing as a marker of the start of cambium activity justifies the study presented in the article “Indirect prediction of bud flushing from *ex situ* observation in hybrid Larch (*Larix decidua* x *L. kaempferi*) and their parents”. The accumulation of degree-days seems to be a good predictor of flushing avoiding the effect of year to year variation.

We took advantage of an experiment in which the same genetic entity underwent different irrigation levels to study the dynamic of wood formation for different amounts of soil water. Part of

these results is presented in the manuscript “Impact of soil water shortage on growth, heartwood development and wood density in Larch”, another part is presented in the manuscript “Dynamics of annual ring wood formation in Larch: links with growth and phenological events”. Complementary results about the variation of wood formation and the variation of tree response to environmental conditions are presented in the thesis.

Dynamic microdensity profiles can be also obtained indirectly by synchronizing a weather index of a given growing season with wood density variation of the corresponding annual ring. Different length of growing season and different types of weather indices were tested. Some complementary results concerning a first attempt to use this methodology are presented in the thesis.

Therefore all efforts in the thesis have been oriented,

- ✓ to establish basic knowledge on the phenology of wood formation in larch, attending to the major steps of cambial activity: initiation of growth (formation of early-wood), transition from early- to late-wood and end of growth (formation of late-wood). For each phase we focused on the importance of genetic variability for tissues extension (size) and on the timing, duration and speed of their formation and their potential in selection.

- ✓ to develop alternatives to direct observation of cambial activity and wood formation phenology, which could help us to facilitate studies on genetic variability of wood formation phenology. Two types of alternative were tested, on one side, the possibility to find external - phenological and morphological or climatic -markers of major steps of wood formation. On another side, the development of an indirect statistical synchronization method: combining information from microdensity profile and climatic data.

- ✓ to assess the reaction of cambial activity and wood properties to some environmental stress.

Variation of the phenology of cambial activity can be seen as a mechanism to have trees always better fitted to their environment and the tree anatomical response (tissue characteristics) can be

seen as an adaptive response. The major interest of adaptive traits is in case of higher frequency of climatic stressing events as it is projected in climate change context. Other significance is the impact of cambial phenology on wood quality properties. For both issues, if some genetic variability and high heritability of these phenological traits can be shown, selection of trees better adapted to climate stress and/or with higher wood quality properties may be expected.

Summarizing, this thesis is based on articles. Three articles are included:

**Article 1:** Dynamic of annual ring wood formation in Larch: links with phenological events. Gauchat, M. E., Pâques, L. E., 2011. Submitted to TREES – STRUCTURE AND FUNCTIONS.

**Article 2:** Impact of soil water shortages on growth, heartwood development and wood density in Larch. Gauchat, M. E., Pâques, L. E., 2011. Submitted to TREES – STRUCTURE AND FUNCTIONS.

**Article 3:** Indirect prediction of bud flushing from *ex situ* observation in hybrid Larch (*Larix decidua* x *L. kaempferi*) and their parents Gauchat, M. E., Pâques, L. E., 2011. Environmental and Experimental Botany 70, 121-130.

Before these three articles a general introduction is presented. It is followed by a material and method section where descriptions of genetic material, climate data and main methodologies used are provided. After that, four chapters present the results of the thesis. Three of them are composed by the three over-mentioned articles. The fourth chapter presents complementary results related to chapters 1 and 2. In Chapter 4 are presented three parts. The first part is about the genetic variability of phenological parameters of ring wood formation. The second part presents results about wood response to environmental conditions: study-case of formation of a false ring. The third part is about the statistical synchronization of microdensity profiles with climate variables. Finally, these chapters are followed by a discussion and some conclusions and perspectives.

## **INTRODUCTION**

### **1. Relevant aspects related to climatic change**

#### **1.1. Perspectives of climate change**

During almost the last two decades the topic of “climatic change” has attracted attention of the most varied public. Politicians, scientists, policymakers and society in general are concerned and involved, in some way, in activities related to the subject. Since the pre-industrial period, anthropogenic interference was responsible among others of many changes on the earth provoking great impacts on natural ecosystems and human life (IPCC 2001, COPA-COCEGA 2003, UNEP 2004, IPCC 2007).

There evidence of human and natural factors have led to alterations of energy balance of the climate system causing changes in the atmospheric abundance of greenhouse gases and aerosols, in solar radiation and in land surface properties (IPCC 2007). One the most remarkable thing is the inertia inherent characteristic of the interacting climate, ecological, and socio-economic systems. Thus some impacts of climate changes due to anthropogenic actions may be slow to become apparent, and some could be irreversible if climate change is not limited in both rate and magnitude before associated thresholds,- whose positions may be poorly known-, are crossed (IPCC 2001).

In this context, efforts were put on analysis of future possible scenarios, figuring out combinations of different levels of economic growth, global population and introduction of new and more efficient technologies (IPCC 2001). Proposed models considering the concentrations of greenhouse and aerosols kept constant at year 2000 levels, predict 0.1°C of temperature increase per decade. But more complicated scenarios involving increases of global population, use of fossil fuel resources and CO<sub>2</sub> emissions predict that global average surface air warming reach 2.4 – 6.4 °C in the next century (IPCC 2007).

Besides global warming, nearly a double of CO<sub>2</sub> concentration level recorded for year 2000 (368 ppm) would be expected at the end of the next century. During the same period of time, average sea

level is projected to increase between 0.09 and 0.88 meters and glaciers would continue their widespread retreat (IPCC 2001). Average annual precipitations and stressing events, as the 2003-heat and drought wave in Western Europe, would be more intense and more frequent, but also they will be longer lasting (Easterling et al. 2000, Frich et al. 2002, Meehl and Tebaldi 2004, Stott et al. 2004).

Concerning precipitations and extreme events, prospects are greater for changes in the extremes of floods and drought rather than in total precipitation amount. However changes in intensity, frequency, duration of precipitation are very probable at local scales (Trenberth et al. 2003). Accelerated surface drying increases the incidence and severity of droughts, whereas additional atmospheric water vapor increases the risk of extreme precipitation events (Karl and Trenberth 2003, Trenberth et al. 2003).

In general, evaporation at the surface cools and acts as “air conditioning” of the planet and its increase would be expected with an increase of temperature. A higher level of potential evapotranspiration is projected (IPCC 2001). This fact, in absence of precipitations, could lead to increased risk of drought and heat-waves because of once the soil moisture is consumed; all heating goes into raising temperatures making plants wither. Projections, obviously temperate by regional effects, could be fewer but more intense rainfall or snowfall events (Frich et al. 2002, Trenberth et al. 2003, Schär et al., 2004).

## **1.2. Impacts of climatic change on vegetation**

The impacts of change in climate system could be severe on all living organisms, animal, plants and obviously human being. However, plants are the first step in the food chain of many animal species and the basis of productive activities of human population. Then, impacts on plants of predicted changes could have greater consequences on life and production of superior organisms.

Several observed climatic changes have been in the direction of reducing climatic constraints to plant growth such that net primary productivity (NPP) increased globally (Boisvenue and Running 2006). From modeling, between 1982 and 1999, NPP increased significantly over 25% of the global

vegetative area, mostly in water- and radiation-limited regions, followed by temperature- and radiation-limited regions and temperature- and water-limited regions. And significantly decreases of NPP are showed over only 7% of the area (Nemani et al. 2003).

Impacts of drought events affect the water transfer along soil-tree-atmosphere continuum delimiting tree growth and individual tree survival may become complicated in extreme cases. Drought induces short term physiological disorders, consequently the amount of carbohydrates is reduced and the storage compartments are not fully refilled at the end of the growing season. Trees must assign stored reserves among demands for tissue reparation, maintenance, growth and defense against stress (Bréda et al. 2004, 2006). Any additional demand on already limited reserves could delay the recovery of the growth potential: as an example tree ring width is smaller during years following a severe drought (Martinez-Meier et al. 2008).

There is evidence of gradual increases of temperatures minimum and maximum temperatures and heat wave events (Easterling et al. 2000, Frich et al. 2002). Increases in minimum temperature could result in fewer days with frost and increases in maximum temperature would produce a larger number of days that inhibit physiological mechanisms. But also, the period with favorable conditions in which plants can growth could be extended. Consequently, modification in natural distribution of species and even worst the disappearance of some species is possible. (Esteve-Zelma et al. 2010, Ledig et al. 2010)

Heat waves and droughts, separately or combined as in 2003, have hard consequences on forests. Reduction of CO<sub>2</sub> assimilation from trees causes a decrease of primary productivity. High intensity and duration of environmental stressing conditions, as 2003 year, provoke a high atmospheric demand triggering irreversible damages in the process of water conduction through the xylem of trees. And it compromises in some cases the survival of trees (Bréda et al. 2006).

From a more regional point of view, consequences of climate disturbances on forest ecosystems would include regression of northern species and expansion of others; modification in



the distribution of pathogens and pests; changes in tree stand productivity; and increased abiotic risks such as storms, water stress or fires (Riou-Nivert 2005).

### **1.3. Consequences for foresters and breeders**

If changes in climate occur as it is projected, natural or artificial regeneration of forests with local seed sources will become gradually more complex. Taking into account rotation length used in temperate forest, trees planted now will grow in a climate different from the one enjoyed by trees currently harvested. The long-term nature of forestry means that planning will be essential if foresters are to practice sustainable forest management during and beyond this period of change. Although rising temperatures and CO<sub>2</sub> concentrations will promote increased growth rates, it is uncertain to what extent this growth will be limited by water and nutrient availability, greater difficulties in forest establishment.

In terms of stand management, foresters must therefore take great care in planting species adapted to each plot; the concern of water availability will have to be addressed, with low density planting, increased clearings, and control of herbaceous and shrub-type vegetation which is also a protection against fire (Riou-Nivert 2005). Commercial plantations established on sites deemed either unsuitable or marginal for agriculture may therefore respond differently to climate change than native or semi-natural forests.

Facing uncertainty about intensity and frequency of changes and extreme event occurrence, reforestation strategies should emphasize conservation, diversification, and broader exploration of species variability and seed sources: that represents great challenges for breeders. Planting programs may have to deploy non-local seed sources, imported from further south or from lower elevations, which demands a system for conserving native gene pools in seed or clone banks. Planting a diverse array of species and seed sources is a guarantee against the uncertainty inherent in current projections of warming. Most tree improvement programs already stress the need of genetic

diversity management and conservation and deployment of multi-progeny mixes; it may better prepare for climate change by testing selections in an even wider set of environments than is now the case (Ledig and Kitzmiller 1992).

Global warming and higher frequency of extremes events during coming years is predicted and a valid path to avoid greater loss could be a better knowledge of basic mechanisms (physiological and genetic) that permit more efficiency in reactions to predicted changes. Consequently, it is necessary to estimate at species, population and individual tree level the variability of adaptive characters. Assuming that a response to environmental variation is only adaptive if it represents a mechanism by which relative fitness is maintained in the face of environmental variation (Thompson 1991).

## **2. Phenology of forest trees: a major criteria of adaptation**

Conceptually, phenology expresses the dynamic or timing of periodic development events throughout tree life. Then, we could distinguish the phenology of 'external' events (i.e. vegetative bud, reproductive organ, needles development, shoot and root extension), rather easily visible and identifiable; but also phenology of 'internal' process of trees (cambial activity and xylogenesis, flux of endogenous hormones into trees), more difficult to follow up due to within tree hidden condition.

Trees are finely tuned to the seasonality of their environment, and shifts in the timing of tree activity (i.e. phenology) provide some of the most compelling evidence that species are being influenced by global environmental changes (Cleland et al. 2007).

### **2.1. Vegetative and reproductive phenology**

Phenological observations are one of the most sensitive and visible character in response to environmental changes. Annual timing of spring events (budding, leafing, flowering) is mainly driven by temperature after dormancy is realized. Many studies have shown the relationship between

spring phenology and air temperature. The temperature increase associated with the global climate change has a demonstrated effect on tree phenology, especially at the beginning of the growing season (Chmielewski and Rötzer 2001, 2002, Walther et al. 2002, Root et al. 2003, Chmielewski et al. 2004, Chuine et al. 2004)

At least two types of interests on vegetative and reproductive phenology could be stressed. One of them aims from an ecological point of view to predict the effects of climatic changes and the resulting changes in the distribution of species. Researchers started to take into account phenology (Lechowicz and Koike 1995) because of its vegetative and reproductive routines are synchronized with climate and, consequently, its impact on competitive relationships in the forest ecosystem. As well, phenology plays a role not less significant in plant productivity; length of the growing season (determined by the onset/termination of growth) affects, among other aspects, the risk of frost damage to trees and the yield potential (Persson and Beuker 1997, Cesaraccio et al. 2004, Rötzer et al. 2004). A second interest in phenology of spring events is from a breeding point of view: indeed breeders aim to improve global tree adaptation, in particular to climate and more specifically to reduce risks from frost damage when transferring genetic materials. They have also an increasing interest in the effect of phenology on growth and thereby indirectly on wood quality parameters (for example, in the study of early-latewood proportion as a consequence of the growing season length).

The most studied event in spring phenology is bud burst, which could be related to many subjacent mechanisms. Physiological processes are involved, but also environmental factors especially in temperate regions, where leaf bud burst for forest species depends mainly on spring air temperature and/or on its variation during the previous winter (Sarvas 1974, Campbell and Sugano 1975, Murray et al. 1989, Hänninen 1995, Chmielewski and Rötzer 2001, Migliavacca et al. 2008).

Because of year to year variation in budburst, application of phenological models has become an interesting tool for prediction at several spatial scales. The majority of models described in the literature, at the end, are related to the concept of summation of thermal units or heat-sum (Bloomberg 1978, Woodbridge et al. 1995, Persson and Beuker 1997, Seo et al. 2008). Some of them

take into account only forcing effect of temperature (Cannell and Smith 1983, Migliavacca et al. 2008), while others consider also chilling temperatures (Campbell and Sugano 1975, Murray et al. 1989, Hänninen 1990, Kramer 1994, Chuine et al. 1998, 1999, Chuine 2000, Linkosalo et al. 2006b). Besides air temperature, the photoperiod seems also to play a role on bud burst in some species: the accumulated duration of the light period can modify bud burst date (Campbell and Sugano 1975, Partanen et al. 2001). More importantly, Linkosalo and Lechowicz (2006a) showed that once chilling requirements are satisfied, the light environment (duration and quality) acts as a signal for the start of temperature accumulation leading to bud burst.

Whatever the chosen model, all of them link the number of days needed for phenological developments or growth to a threshold or base temperatures, a key parameter to determine the necessary heat unit accumulation for a given progress of an event. For this reason the choice of threshold temperature should be done correctly on the base of appropriate criteria. The thresholds over which the temperatures have an accumulative effect are often fixed on a putative value of 0°C (e.g. Spano et al. 1999, Rötzer et al. 2004) or 5°C (e.g. Cannell and Smith 1983, Murray et al. 1989, Alfaro et al. 2000, Ghelardini et al. 2006). The genetic variation of temperature thresholds and heat-sums for spring phenological events in forest trees was rarely studied (e.g. Worrall 1983) in contrast to the numerous studies demonstrating the moderate to high genetic control of bud burst phenology in conifers (El-Kassaby and Park 1993, Li and Adams 1993, 1994, Hannerz et al. 1999, Alfaro et al. 2000, O'Neil et al. 2000).

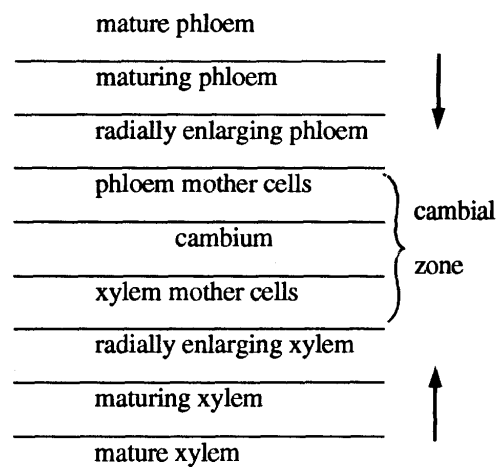
## **2.2. Phenology of wood formation**

Phenology of wood formation at the intra-annual ring scale has become necessary in particular to investigate the influence of climate in ring wood formation and tree reaction against extreme climatic events or environmental stresses. Knowledge about onset and end of cambium activity as well as of the transition from early- to latewood during the growing season gives information to

manage tree plasticity of anatomical and adaptive-related properties, from one side, and to improve wood quality characters, from other.

### 2.2.1. Wood formation process

In a general way, it is accepted that wood formation is a consequence of cambium activity. The process is initiated from meristematic single cells called cambial initials or cambium that are part of the cambial zone, the current term to denominate undifferentiated mother cells of either phloem or xylem (figure 1). The number of cell layers of cambial zone is from 1-3 up to 20 cells depending on the species, age and season of the year. For example, Douglas-fir has 4 cell layers in the cambial zone (Rensing and Owens 1994). In larch, it fluctuates from 4 to 9 along the growing season (Rossi et al. 2009a). The different steps in wood formation are represented in Figure 1.



**Figure 1: cambial zone organization and production of secondary conducting tissues. The arrows indicate the centripetal and centrifugal differentiation of phloem and wood. Edited from Lachaud et al. 1999.**

The cambium consists of a few layers of two types of cells (radial and fusiforme initials) that originate from 2 types of divisions: a) anticlinal (=radial) to produce daughter cells similar to the mother cells and ensure the increase in girth of the cambium and b) periclinal permitting the diameter growth of trees to generate new cells of xylem and phloem.

After divisions, cells undergo a period of radial enlargement or expansion and then, differentiation and maturation processes take place by deposition of cellulose and lignin compounds in the different layers of cell walls (cell wall thickening); at the same time their protoplasm is lost forming an empty element that will participate to water transport within the tree.

### **2.2.2. Wood structure as a response to environmental factors**

In a transverse section of a trunk of a tree, it is possible to identify the growth layers as concentric rings. Wood in conifers of temperate zone is an outcome of the seasonal periodicity of growth processes. Rings are constituted by tracheids that are vertically oriented cells with relatively thick lignified walls which die to become functional. In a radial direction (from pith to the bark), width and wall thickness of these cells can vary markedly within any one annual ring. Then, in the inner part of a ring we can first differentiate large cells with a wide lumen and thin wall, called early-wood cells. They are followed by a transition area (transition-wood cells) where cells have thicker walls than early-wood cells. And finally, in the outer portion of the ring, we can distinguish cells with the smallest lumen and thicker walls (Fritts 1976). This particular variation of cell morphologies is a consequence of internal (e.i. genetic, physiological) and external factors and of their interactions. External factors are made of a combination of effects: climate, soil moisture, availability of nutrients, physiographic conditions, and silviculture and management of stand (Wodzicki 2001, Vaganov et al. 2006). Because they give the physical conditions for all biological processes, it is probable that there is no environmental factor affecting tree growth which does not have some effect on wood structure.

Climate factors at the intra-seasonal level may be summarized as the seasonal course of temperature and precipitation. With less importance radiation and day length (photoperiod) also have effects on wood formation process. All these climatic factors in conjunction with soil properties regulate the initiation, ending, rate and extent of growth, as well as developmental process in trees (Vaganov et al. 2006).

Although temperature may be considered as the most important single factor in the initiation of meristem growth activity (Larson 1994), lacks of soil water could cause an earlier ending of growth (Fritts 1976). Combination of temperature and precipitations could result in increase or decrease of rate growth determining size and internal structure of the annual ring formed in that year (Wimmer and Grabner 2000, Park and Spiecker 2005). Differences in morphology of tracheids within a ring (e.i. lumen size, thickness of cell wall) produce variations in wood density that could affect stem hydraulic properties such as conductivity and cavitation resistance (Bréda 2004, Dalla-Salda et al. 2009).

### **2.2.3. Why are we interested in wood structure?**

There are many reasons why it is important to understand the nature and causes of wood formation and its properties. Wood have key basic functions: from one side, it provides support to tree through its structure, and from other side, it is involved in the transportation of water and of nutrients to whole tree, and it also have functions of storage. Moreover, wood is the result of radial increment and the knowledge of its dynamics could help identifying how limiting or critical factors affect it development and modifying wood production with silviculture and management objectives. The study of intra-annual growth dynamics is a necessary step to adequately interpret site and climate effects, directly related to possible future changes in the environment. Trees with similar ring width could have different intra-annual growth pattern and this would help interpretation of wood properties variations.

Despite economic values of wood and its importance in the worldwide market, variation in wood anatomy is the major determinant of the quality properties of products made from it. One

consequence of tracheid morphology variation is on wood density, a character directly related to wood quality, and also used as predictor of many other traits (Jones 2005) involved in product quality demanded by worldwide wood market.

#### **2.2.4. Wood: a potential recorder of cambial activity**

Direct observations of long-term environmental changes in natural ecosystems are extremely rare; it is thereby necessary to use indirect indicators (i.e. proxies). Tree ring can be used to identify years during which a given abiotic (i.e. drought) or biotic (i.e. insect attacks) event took place. This kind of dating can be possible using ring width as a reference because tree growth is frequently affected by environmental variations. In a broad sense, this field of research is called dendrochronology. Despite of some controversial results (Wimmer and Grabner 2000, Briffa et al. 1998), the techniques employed in this discipline are applied to understand several environmental aspects.

While tree rings are integrated records of the influence of environmental conditions, their anatomical characteristics record growth rate changes produced by these changing conditions (Yatsenko-Khmelevsky 1954, Vaganov et al. 1985, 1996b, Schweingruber 1988, 1996 cited in Vaganov et al. 2006). Tree rings not only integrate the outcomes of the growth process, but also register the process itself. Consequently, the internal structure of a tree ring contains information on environmental conditions at seasonal or even finer time-scales.

In this sense, concepts of dendrochronology are used for plasticity and adaptation studies in which wood is used as a record of stressing events (Martinez-Meier et al. 2010, Sanchez-Vargas et al. 2007). These studies relate within-ring density variation to weather variation within a growing season through application of different techniques of indirect synchronization. They have demonstrated the potential of wood properties variation along rings as a marker of environmental factors. Restricted to



a given annual ring, it escapes from ontogenic effects and other disturbing factors such as competition.

### **3. Incidence on tree breeding**

Commonly, drought, temperature and/or another factors limit tree species productivity in many different environments around the world. There are several strategies on hand of plant breeders for improving the productivity of the species under limiting factors. Among others, we can mention: a) analysis of genotype x environment interaction to improve parental selection and identify key evaluation and deployment sites, b) physiological characterization of genotypes to identify parents with complementary traits that will improve the heritability of selection, c) development of consistent and repeatable screening methods for a given limiting factor; d) broad genetic variation to select for adaptive traits for given limiting factor, e) identification and conservation of genomic regions that are associated with performance under given stress across environments and time.

In many parts of the world for several species, tree improvement programs have completed the first cycle of selection and they are now entering a new phase of advanced-generation breeding (second or third cycle). The first generation was relatively easy and without complications. Growth, stem form and wood density focusing in wood quality were improved managing large populations that could serve as basis for later selections. In some cases, mainly for temperate species, vegetative phenology as budburst was assessed to select against frost damages. For a next step, the challenge is to handle and to manipulate populations, minimizing inbreeding yet maximizing annual genetic gain. It is a more complex task compared with that carried out in the first cycle, especially in a context of predicted climate change. For second and third cycle of breeding in the climatic change context, there is a probable tendency of breeders to move away from the approach of breeding genotypes which grow well across a broad range of different sites or genotypes with ability to interact with

climate. To achieve further gains, breeders are trying to use genotype x environment interactions for selecting for specific adaptations attending to studies of tree reaction norms and phenotypic plasticity.

More frequently during the last decade, the reaction norm concept was included into tree studies attending to understand the range of responses of species or populations within species to environment. Another concept under large development is phenotypic plasticity which measures environment-dependent phenotype expression of single individuals (clones) (Dewitt and Scheiner 2004, Sánchez-Vargas et al. 2007, Martínez-Meier et al. 2008, Sánchez-Gómez et al. 2008). Plasticity represents an individual level response in a shorter time compared to adaptation. Tree breeders and gene-resource managers attempt to integrate these concepts searching for genetically inherited traits of acclimatization and adaptation to face climatic change (Johnsen and Skroppa 1996, Martínez Meier et. al. 2008, García Esteban et al. 2010, Pastorino et al. 2010). From other part, molecular genetics is a rapidly developing discipline, largely due to technological advances in sequencing and other methods. In this research field, adaptive traits in charge of local climatic adaptation are studied. Cold-hardiness, timing of bud flushing and bud set are investigated with the help of Quantitative Trait Loci studies (QTL). Ambiguous results have been produced so far (Karhu et al. 1996, Hurme et al. 2000, Jermstad et al. 2001a, 2001b, 2003, Neale and Savolainen 2004). The significance of particular genes for the adaptation to local conditions and the impact of such genes on adaptive traits and traits of economic importance are currently priority fields of research as illustrated in many temperate forest species. Meanwhile, traditional methodologies as selection by quantitative method are still successful tools to face the new challenges of breeding and conservation of species.

Adaptation and productivity are both complexly inherited traits and affected by environmental condition in agricultural crops as consequence of difficulties to identify the genetic mechanisms that have led to improvements in adaptation (Allard 1996). The same argument is applied to trees when

we trait to find a convincing argument to explain the genetic basis of adaptive traits. A lack of information about adaptive traits is noticeable for many species, but recently some genetic studies were carried out (Sánchez-Vargas et al. 2007, Martínez-Meier et al. 2009a). The longevity of trees suggests that the dynamics of genetic changes (fixation of characters) is slow in forest ecosystems. However, adaptation processes are needed in response to globally changing environmental conditions, in particular climate change in Europe. A better understanding of genetic inheritance process of forest trees and its temporal and spatial variation is of greatest importance for the enhancement of adaptation processes or at least the maintenance of adaptive potentials. Adaptation of forest ecosystems will be reached by complex processes, which require interdisciplinary research efforts involving not only forest genetic approaches, but also other advanced methods used in basic sciences such as tree physiology and more applied sciences as silviculture. Recent developments in forest genetic research aimed to a better knowledge of the functional importance of genetic processes by a sound understanding of physiological and genetic adaptation processes. These kinds of results are needed to develop strategies for the sustainable management of forests under changing climatic conditions. The conservation of genetic variation is not automatically guaranteed by means of natural regeneration in case of drastic environmental change. Thus, mid- and long-term environmental monitoring should also include observations of the temporal dynamics of genetic diversity within forest tree species. Despite of availability of innovative tools (molecular biology, computer facilities), development of new varieties for a given species take time and breeders have a great challenge need to match tree improvement

In temperate and cool temperate zones, many species have, during their domestication and breeding process, been transferred far from their original habitats (in Europe usually south to north, that is towards cooler more humid regions) and been dispersed over a wide range of climates demonstrating their potential to climatic adaptation.

Arguing that climate change will be so hurried and that species will not be able to adapt in parallel, different authors (i.e. Koski 1996) justify nowadays development of breeding programs for

global warming focusing on adaptive traits. However this is not an easy task and firstly it is necessary to discuss and define new climatic or meteorological threats to which adaptability is needed and to delimitate geographical zones because of perspectives of change are so variable. Synchronisation between growth of trees and changing seasons needs more than ever to be matched. Apparently, the main concern of this matching seems to be on summer: indeed spring growth initiation depends mostly on temperature and could be projected by the use of thermal time models. In contrast, the ending of growth is regulated by both thermal time and increasing night length (Koski and Sievänen 1985, Hänninen et al. 1990). For the large year to year variation and the uncertainty on the extent and speed of changes wide phenotypic plasticity is needed on individual level. But it is impossible to set precise thresholds to select for and aims to breeding. However, a reasonable method is to run in parallel several lines with different patterns of adaptive traits and to support breeding population on genetic diversity (Koski 1996). Even though trees naturally display potential of acclimatization, selection and breeding are appropriate tools to accelerate progress in adaptation and productivity traits and accompany assisted migration of species if needed. Thus, genetic variation within population in adaptive traits and plasticity of individuals are nowadays key issues in breeding.

### **3.1. Incidence on larch breeding**

*Larix* is a northern hemisphere conifer with a widespread extension in Eurasia and North America. Larches are fast-growing conifers, valued for their high quality timber (wood physical and mechanical properties, natural durability), their wind firmness and their susceptibility to only a limited number of pests. One of their main original biological features is that they are deciduous in a world of evergreen conifers. Each year, they have to rebuild their foliage and for this reason, they have a shorter period for photosynthesis than evergreen conifers in which the needles are replaced permanently (Rossi et al. 2009a).

In Europe while larch naturally occurs in mountainous ranges (Alps, Tatras, Carpath, Sudetan Mts, etc), the main cultivated area is focused on European low and middle lands of France, Belgium, Germany, Denmark, Great Britain and Ireland and involves European larch (*Larix decidua*), Japanese larch (*Larix kaempferi*) and their hybrid (*Larix x eurolepis*). In France, larch –ever as a pure or hybrid taxa- is considered as an alternative to Douglas-fir for plantations in several regions.

At INRA, a general objective of the tree breeding program for larch is to develop improved genetic materials for lowlands sites. It seems clear that a faster initial growth with a consequently shorter rotation, resulting in a greater rate of wood production, and a better resistance to larch canker (*Lachenellula willkommii*) justify the hybridization program led by the institution.

Since 1957, INRA was involved in tree breeding programs of Japanese (*L. kaempferi*) and European (*L. decidua*) larches and its interest in hybrid between them (*L. x eurolepis*) did not begin before 1979 (Pâques 2001a). In more than fifty years of work, the main goals achieved were: the study and consequently organization of genetic populations of pure species (Pâques 1996a, 1996b), the estimation and prediction of hybrid vigor and trait inheritances of hybrid populations (Pâques 2000, 2004), the determinism of stem form (Contrain 1993, Bastien and Pâques 2002), the genetic relationship between growth and several wood quality parameters (Pâques 2001b) and canker resistance (Sylvestre et al. 1994, 1999).

A challenge for these species is now also to provide adapted materials to the present and future changeable environmental conditions. Phenology of wood formation is probably one of the major entry-doors to tackle this problem as shown in chapter 2.

Some few published results exist for larch species on the phenology of wood formation, environmental factors affecting this process and physiological aspects related to wood formation. Rossi et al. (2006a) studied the dynamic of cambial activity and cell differentiation of European larch trees in the eastern Italian Alps at high altitude. They determined that dormant cambium of European larch is constituted by a layer of 6-8 cells. This number increased in mid-May, reaching 10-14 during May- June (the period of maximum growth activity) and the stop of cambial division

occurred in mid-August. Annual ring wood formation took 131 days. Moser et al. (2010) also reported that duration of ring formation lasted from mid-May to the end of October, with the length of the growing season decreasing along elevation (2150 to 1350 m a.s.l.) from 137 to 101 days. According to Moser et al. (2010), the onset of growth appeared minimally related to altitude.

Other studies focused on environmental factors affecting wood formation. Rossi et al. (2007, 2008a) determined the critical temperature for wood formation of European larch: 6, 11 and 16 °C on average for daily minimum, mean and maximum temperature, respectively.

In *L. sibirica* Ldb, Antonova and Stasova (1999, 2002) showed the higher influence of temperature than precipitation on cambial initial division throughout the season, especially on xylem cell production. And they determined the optimum temperature and precipitation for maximum cambial activity (Table I).

Parameters of larch annual ring	Mean day temperature °C	Mean maximal diurnal temperature °C	Mean minimal nocturnal temperature °C	Precipitation mm
Number of tracheid formed	21 - 22	25 - 26	11 - 12	1.8 - 1.9*
Tracheid radial diameter	21 - 22	26 - 27	8 - 9	1.9*
Cell wall cross section area	16 - 17	21 - 22	8 - 9	90 - 105**

\*precipitation, mm/day    \*\*sum of precipitation for whole period of tracheid development in differentiation zone, mm

**Table I.- Optimum of temperature and precipitation for larch tracheid formation. Edited from Antonova and Stasova 2002.**

Also, changes in the levels of plant hormones in the cambial region were studied on *L. kaempferi* by Funada et al (2002). The study demonstrated that the first divisions of cambial cells were not associated with changes in the total amounts of endogenous plant hormones, suggesting extrinsic factors triggering cambial activity resumption.

Although certain knowledge about wood formation on larch is available, no literature has been published attending to wood formation and environmental effects on this process with a genetic

structure (progenies). The most found studies are referred to pure species (*L. decidua*, *L. kaempferi*, *L. sibirica* Ldb.) but it would be very important to dispose of knowledge in hybrid genetic material compared with parental species. As result, this information could be helpful supporting new selection process in current breeding programs.

#### **4. Major Bottleneck: Synchronization of wood density and climatic condition**

Evaluation of phenotypic plasticity in trees or “dendroplasticity”, as mention Martinez-Meier et al (2009a), requires the interpretation of norms of reaction. Wood microdensity profiles (Guay et al. 1992) express the intra-annual variation of wood density as function of a distance. However, to compare reaction of trees to environmental conditions, it would be necessary to express wood density as a function of time in which the given wood density variation was produced. For this reason, the effective synchronization between wood density variation and climatic conditions became an important step in the process of production of reaction norms.

##### **4.1. Direct synchronization**

To track out the radial growth or indirectly the cambial activity over a given growing season, at least two basic possibilities exist. One of them tracks at regular time intervals the radial growth through **dendrometers**. Based on measurements of changes in a single radius through measurements of distance between a fixed plane attached in the wood and a point on the surface of the bark (Bormann and Kozlowski 1962, Clark et al. 2000), dendrometers provide an easy and convenient way for measuring changes in the diameter of tree trunks over time. However, they have several disadvantages when growth needs to be followed upon a great number of trees (e.g. in genetic trial) because cost of equipments is high and safety of equipment in field tests is not guaranteed. Other inconvenient is the variation of the water content of the bark along a day and through the growing season. Despite of the mentioned disadvantages, they allow construction of

growth curves and supply valuable information on growth responses of trees to environment, and indirectly on cambial activity (Label et al. 2000, Morabito et al. 2006, Deslauriers et al. 2007, Giovanelli et al. 2007, Berta et al. 2009).

An alternative to follow up cambial activity through time is to take advantage of techniques like the '**pinning method**', and the '**microcoring**' or '**microsampling**' method. The first one is an old technique that became more frequently applied in the last 10-15 years (Wolter 1968, Yoshimura et al. 1981, Antonova and Stasova 1997, Grotta et al. 2005, Griçar 2007a, Seo et al. 2007). It consists in inserting a mounting insect pin, into the tree trunk periodically, during all the growing season so to provoke an injury at the cambial zone level. As a reaction, the cambial cells produce deformed and distinctive cells than can be identified on microtome cuts with the help of a refraction microscope (Wolter 1968). Pinning method proved to be suitable for studies of seasonal dynamics of wood formation. However a thorough understanding of the cambial wound response is needed when pinning technique is applied. The cessation of regular activity of cambium could be masked with wound-induced reactivation of meristem. This is a problem of the samples pinned in the last part of the growing season (Griçar 2007a).

'Microcoring' consists in extracting a microcore of 1,2 mm in diameter and 1,5-2 cm long containing current and previous rings. A specific tool was developed to obtain such wood samples (Rossi et al. 2006b). In many studies, this technique was applied to follow up radial growth and its relation with climate, to study anatomical aspects of wood formation, etc (Deslauriers et al. 2003, 2008, Rossi et al. 2006b, 2008a, 2009a). This kind of method is much appropriated to study wood formation from an anatomical point of view and it could also be successfully used to study phenology of wood formation, the level of detail is excellent. It is possible to detect different states of cell development. As for pinning, microcoring has the major disadvantage to be very much time consuming and difficult to be applied on high number of trees because each wood sample need afterwards to be dehydrated, cut by microtome and stained before anatomical analysis.



Both techniques allow the construction of radial growth curves and anatomical characteristics curves over a growing season, and thus they offer the possibility to study in details the relationships between growth and some abiotic factors (climatic parameters, Antonova and Stasova 2002), silviculture (Grotta et al. 2005) and probably biotic factors as well (insect attacks).

#### **4.2. Indirect synchronization**

To avoid the mentioned constraints and limitations of direct synchronization methods, indirect synchronization has been attempted using several methodologies associating wood density changes (obtained from microdensity profiles) with some climatic variations observed during the growing season.

One method of synchronization (breakpoint method) consists in taking a few breakpoints from microdensity profiles of a ring showing a 'false-ring' and from climatic profiles (expressed by the variation of an index, i.e. De Martonne's aridity index, during a fixed growing season. The chosen points in the microdensity profile have to represent marked changes of wood density (increasing or decreasing). The same quantity of points selected in the wood density profiles have to be identified on the climatic profiles of the vegetative period searching the major variations presented in both profiles. The two groups of points from each genotype are connected by a scatterplot giving as a result the norm of reaction (DeWitt and Scheiner 2004). Details of this methodology can be found in Martinez-Meier et al. (2009b). This method proved to be appropriate to study a high number of trees; however, it is restricted by the existence of false rings within rings along the wood density profile. That seriously limits years which can be evaluated.

An alternative to skip this limiting factor is to apply a wavelet decomposition of the ring density profile (Daubechies 1992, Rozenberg and Cahalan 1997, Rozenberg et al. 2004, Martinez-Meier 2009b), where variation in wood density is compressed by steps. Each step represents the variation of a given portion of the microdensity profile. The same concept is applied on a chosen climatic data profile. Both profiles need to be described by the same number of steps. Relating the average values

of successive steps from both profiles we can obtain the reaction norms. Despite the wavelet decomposition does not request any special shape of microdensity profiles, their use still imposes a fixed growing season for all individuals or sample trees. A fixed duration of the growing season supposes that all trees start and end at the same time, omitting the variability demonstrated in length of growing season among individuals (Rossi et al. 2006b).

Some studies have demonstrated that the reaction norms could be a good tool to study the phenotypic plasticity in trees (Sanchez-Vargas et al. 2007, Martinez-Meier et al. 2009a). Indirect synchronization supposes to interpret the microdensity profile of a given ring as the record of the cambial activity variation in response to a climatic index during the growing season and to consider this response as an immediate reaction. Better indirect synchronization methods still need development to avoid negative points mentioned above. One important aspect to be taken into account is the variability of the growing season length of individuals, and the possibility that not all trees begin and finish their growing season exactly on the same days.

## **OBJECTIVES OF THE STUDY**

### **What do we expect from this research?**

Phenology of terminal bud flushing (and of leafing) is a key factor for breeders and it is currently assessed for many species. It allows them to select adapted genotypes avoiding frost damages. Phenology of apical growth but also of radial growth and wood formation could also potentially help breeders and genetic resource managers to select faster growing genotypes with an optimized season of vegetation, genotypes with outstanding parameters of wood quality and/or with a better capacity to react against (extreme) climatic events.

A better understanding of the phenology of wood formation and of its genetic variation would permit us to assess its effects on two different areas. On one side, variation in phenology parameters

of xylem formation could alter some adaptive characters, through alteration of anatomical properties like the size of tracheids and their wall thickness, change in sapwood-heartwood ratio, etc. and consequently through modification of wood cells hydraulic conductivity, water storage capacity, resistance to cavitation, etc. On the other side, our interest as breeders is also to evaluate how phenology could modify wood quality parameters, mostly through a change in the early-latewood ratio.

Therefore this thesis aims

- to establish basic knowledge on the xylem phenology in larch, attending to the major steps of cambial activity: initiation of growth (formation of early-wood), transition from early- to late-wood and end of growth (formation of late-wood). For each phase we will focus on the importance of genetic variability for tissues extension (size) and on the timing, duration and speed of their formation (chapters 1 and 4).

- to develop alternatives to direct observation of cambial activity and wood formation phenology, which could help us to facilitate studies on genetic variability of wood formation phenology:

  - By investigating the possibility to find external -phenological and morphological or climatic - markers of major steps of wood formation, (chapters 1 and 3).

  - By developing an indirect statistical synchronisation method: combining information from microdensity profile and climatic data, (chapter 4).

- to assess the reaction of cambial activity and wood properties to some environmental stress, (chapters 1 and 2 and 4).

- to evaluate the genetic variability of wood formation phenology parameters and their potential in selection, (chapter 4).

## MATERIAL AND METHODS

### 1. Description of the experimentation (genetic material, climatic and soil conditions)

The material used to reach the proposed objectives was sampled from 3 progeny tests of larch (*Larix* sp.) in France. For details about sites and experimental designs, see Table II.

- The first one is a lowland farm-field test, located at INRA-nursery in Orléans (ORL) and planted in 1995: it has been established with 20 half-sib progenies of European Larch (EL: *L. decidua* 'polonica', provenance Swinia Gora) and one hybrid larch (HL) open-pollinated progeny (FP201 seed orchard). The initial stand density was reduced by a thinning during winter 1999. In 2000, different fertilization and irrigation regimes were implemented in order to test the effect of soil water shortage on tree growth and wood properties. In the irrigated treatment, drop-irrigation was applied daily and regularly during the vegetation periods of years 2000-2006. Irrigation was started when frost risks were considered low enough and when most trees were fully flushed, that is between mid-May to mid-June; it was stopped arbitrarily by the end of September each year. Thirty-five trees were selected for this thesis work: 25 trees from 5 EL progenies in the non-irrigated treatment and 10 from the HL progeny (5 trees from irrigated and 5 from non-irrigated plot).
- The second one was established in a State nursery at Peyrat-le-Château (PEY) in 2003: it is a higher elevation farm-field test including full-sib progenies of hybrid larch (HL: *Larix decidua* x *L. kaempferi*) and one commercial variety of Japanese Larch (JL). In this test, 99 trees were sampled: 88 corresponded to 8 progenies of HL (11 trees per progeny) and 11 trees were from JL.
- The third trial was planted on an INRA-estate at Béchadergue (BECH) in 2004 and was composed of full-sib progenies of EL, JL, and HL. Altogether, 293 trees were chosen from 10 EL families, 16 HL, and 6 JL.

These trials were chosen based on several needs and constraints to reach our objectives. Each trial contributed to parts of results presented in this thesis. They were in fact complementary in terms of taxa. Some of them were easier to use than others for a detailed assessment of trees because of the smaller size of the trees (Peyrat-Le-Château and Béchadergue) and/or their proximity to headquarters (Orléans and Peyrat-Le-Château) facilitating daily-type of observations.

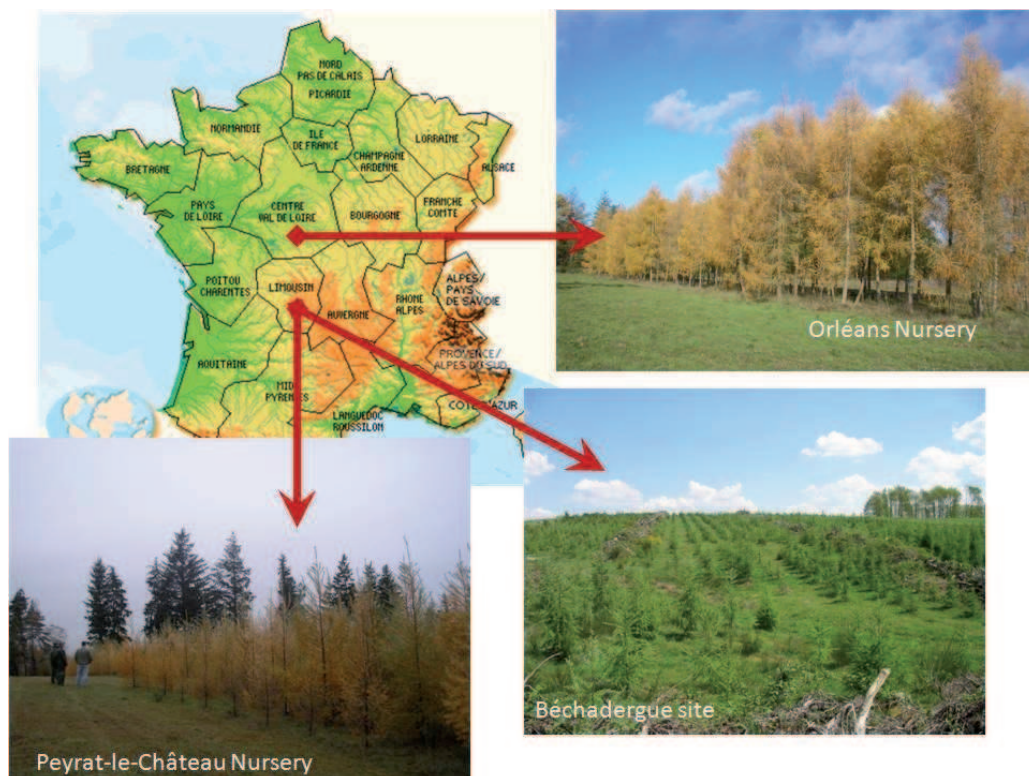
Site	Geographical position	Min./max. annual temperatures (°C) Year 2006	Mean annual precipitation (mm) Year 2006	Soil description	Plantation year	Field trial experimental design <sup>1)</sup>	Total height (year 2006) (meters)	Taxum <sup>2)</sup>	Number of progenies <sup>3)</sup>
Peyrat-le-Château (PEY) Limousin (West Massif central Mts)	Lat N 45° 46' 5''	3.4/14.6	1260	Deep arenitic soil, with poor water capacity and low fertility	2003	IRBD with 33 blocks, STP	4.2	HL	54 FS
	Long E 1° 45'							Altitude 455 m	JL
Orléans (ORL) Centre	Lat N 47° 49' 1'	6.0/15.0	620	Coarse sandy soil with gravels; low water retention	1995	CRBD with 20 blocks, STP	10.6	HL	1 OP
	Long E 1° 54'							Altitude 106m a.s.l.	EL
Béchedergue (BECH) Limousin (West Massif central Mts)	Lat N 45° 49' 1'	3.4/14.6	1260	Shallow arenitic soil with poor water capacity and low fertility	2004	IRBD with 44 blocks, STP	2.7	HL	63 FS
	Long E 1° 43' 5''							EL	17 FS
	Altitude 455 m a.s.l.							JL	33 FS

**Table II.- Description of experimental tests (Genetic material, climatic parameters and soil characterisation).** <sup>1)</sup> CRBD / IRBD: complete/incomplete randomized blocks design, STP: single-tree plots; <sup>2)</sup> HL: *Larix decidua x L.kaempferi*, JL: *L. kaempferi* and EL: *L. decidua 'polonica'*; <sup>3)</sup> HS = half-sib, FS = full-sib, OP = open pollinated

For our study corresponding to chapter 1 of our thesis, the sampled trees belonged to the two progeny farm-field trials installed at Peyrat-le-Château and at Orléans (Figure 2). These trials were selected because of their proximity to headquarters and rather small tree size, allowing close measurements at least every 7-10 days.

For the study presented in chapter 2, we only used the test located at Orléans to study the impact of irrigation vs non-irrigation on tree growth and wood properties. The implemented irrigation treatment on the test demanded more controls and infrastructure which was only available at Orléans nursery.

Results shown in chapter 3 were obtained from twig samples of trees collected in 3 tests (PEY, ORL and BECH), covering the three larch taxa. They were used in experimentations conducted in growth chambers located at INRA Orléans.



**Figure 2: Localization of progeny farm-field trials. Orléans Nursery located in region Centre and Peyrat-le-Château and Béchardegue in Limousin region.**

## 2. Main methodologies and/or techniques employed

### 2.1. Climatic parameters assessment of soil water deficit and climatic indexes. (Chapters 1, 2, 3 and 4)

Mean temperature, precipitation and soil water deficit are presented in Figure 3 for both sites, ORL and PEY. We collected ourselves daily data of temperatures and rainfall at both sites, while evapotranspiration values were provided by METEO FRANCE (Centre Départemental du Loiret and Limoges).

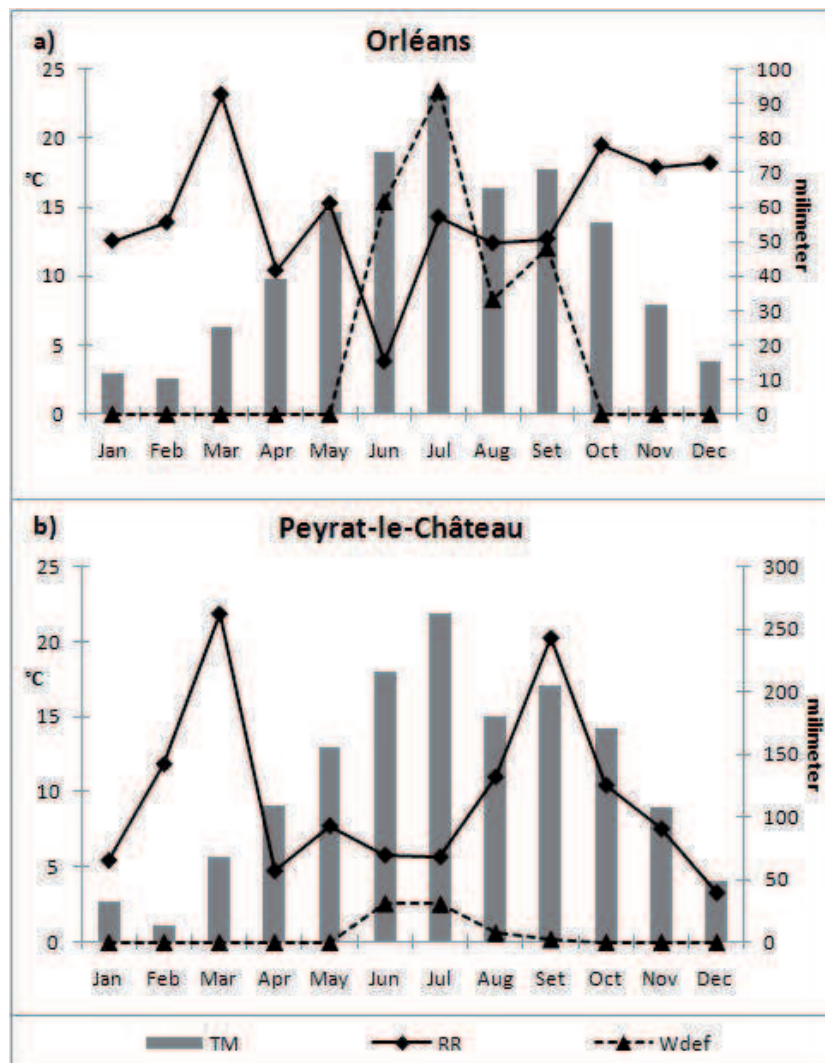


Figure 3: Monthly values of mean temperature (T M), precipitations (RR) and soil water deficit (Wdef) during 2006 at ORL (a) and PEY (b).



In soil water balance, to calculate all soil water availability and consequently the deficit, we used the methodology employed by METEO FRANCE. This model requires daily potential evapotranspiration (ETP) calculated by Penman method and rainfall (RR) as input climatic data. Additional parameters include a coefficient of crop cover (Kc) directly related to the type of vegetation covering the site and the maximal capacity of water retention by the cultivable soil profile (RU). The RU is divided into two types of reserve, superficial (Rsurf) and deep (Rprof). All these values (RU, Rsurf and Rprof) represent the theoretical-maximal capacities but for a given decade the actual water available for trees is summarized by the total soil water reserve (Rtot). The latter is the sum of Rsurf and Rprof for a given decade. Real evapotranspiration (ETR) is calculated in two steps. If there is enough water availability in Rsurf, Eq 2 was applied: ETR is equal to the maximal evapotranspiration (ETM) and Dj for this decade does not exist. But, if the Rsurf was exhausted, Eq. 3 was applied to obtain ETR and the soil water deficit is calculated by Eq. 1.

$$D_j = ETM - ETR \quad \text{Eq. 1}$$

$$\left\{ \begin{array}{l} \text{If } R_{surf} > 0 \\ ETR = ETM \quad \text{and} \quad ETM = K_c \times ETP \end{array} \right. \quad \text{Eq. 2}$$

$$\left\{ \begin{array}{l} \text{If } R_{surf} < 0 \\ ETR = \frac{R_{tot}}{(RU - R_{surf})} \times ETM \end{array} \right. \quad \text{Eq. 3}$$

Different types of calculations and indexes were performed for each particular study presented in the thesis. At ORL, cumulated soil water deficit were estimated by decade were calculated by decade for irrigated and non-irrigated plots during irrigation treatment (2000-2006) (Figure 4).

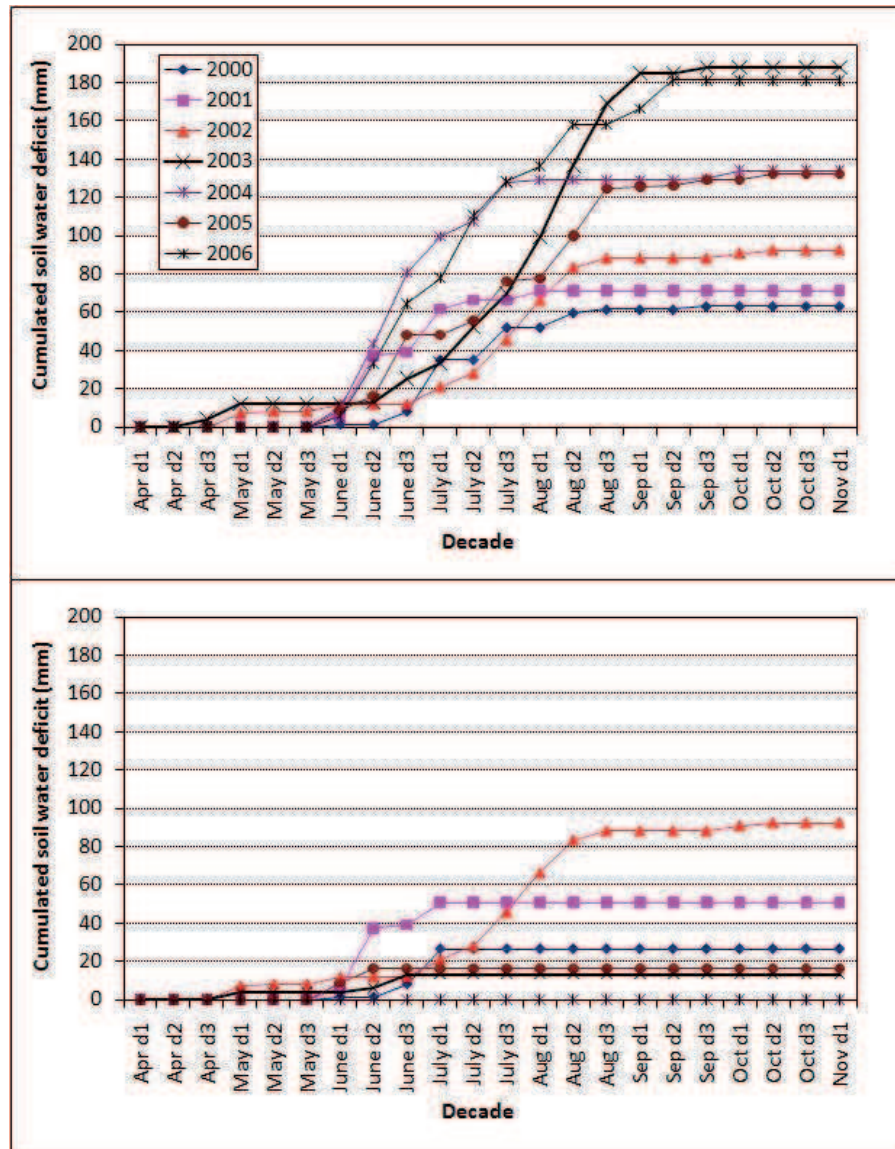


Figure 4.- Cumulated Soil water deficits (mm) estimated over decades in the non-irrigated (A) and irrigated (B) plots (for years 2000 to 2006) established at Orléans.

For PEY site, with climatic data, we built two climatic indexes: drought index (DI) showed in Figure 5-A and the soil water availability (WA) took from soil water balance (Figure 5 B). DI was based on the climatic index proposed by De Martonne (1926) modified by Martinez-Meier (2009) (Eq. 4).

$$DI = \frac{3TX + TN}{10 + RR} \quad \text{Eq. 4}$$

This index is simple to obtain because of required information is easily available. Daily maximum (TX) and minimum (TN) temperatures and precipitations (RR) are combined to calculate DI. From other site, we chose a more complex index, soil water reserve (WA) because of precipitations, potential evapotranspiration and soil texture is needed. This index is a direct result of soil water balance previously described and more realistic and complete index giving a measure of plant's environment.

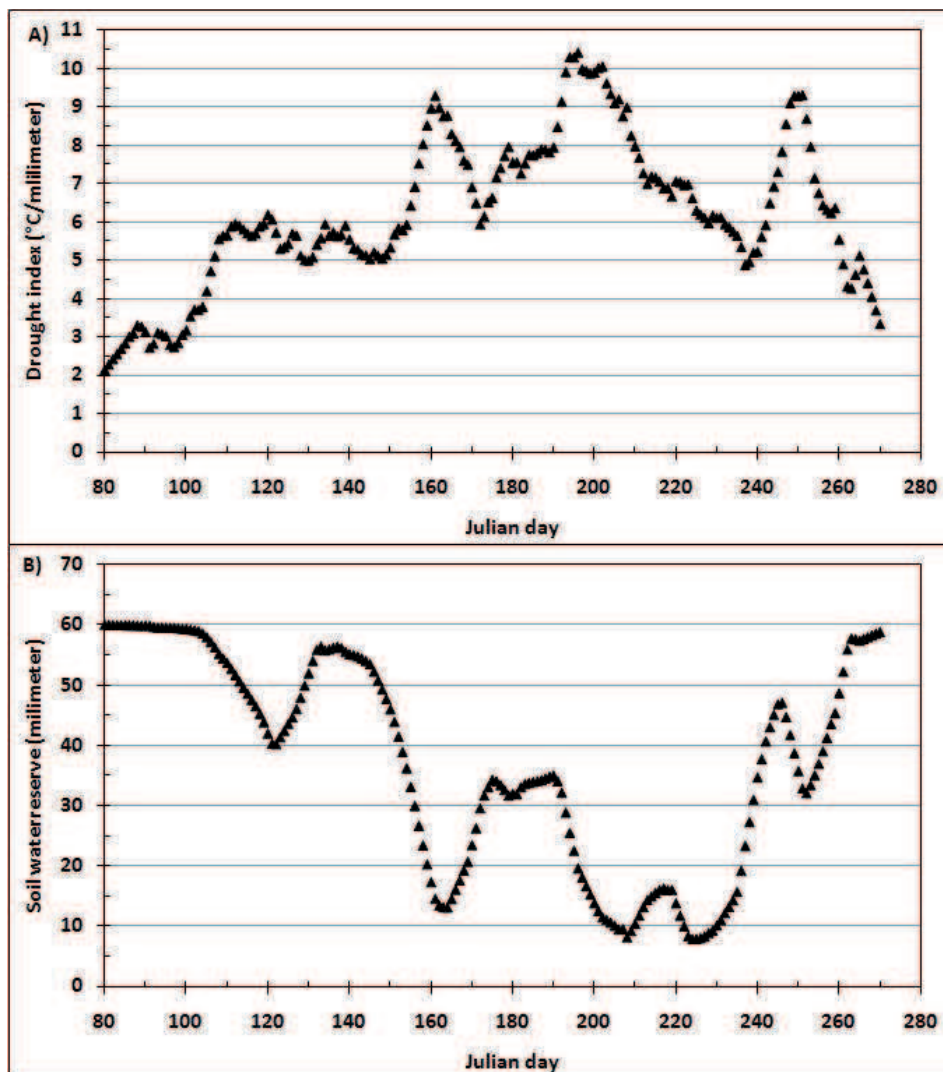


Figure 5.- Drought Index (A) and soil water availability (B) during 2006-growing season at Peyrat-le-Château.

## **2.2. Assessment of vegetative phenology and of growth traits (chapters 1 and 3)**

Except for budburst *ex situ* experience, all measurements were done every 7-10 days during year 2006 at ORL and PEY. Meanwhile, the measurements at ORL presented in chapter 2 were done annually.

### **2.2.1. Spring phenology**

In forest, the observation and record of phenological events are usually difficult to obtain, time-consuming and costly especially for genetic studies from aged genetic trials. Indeed, the large number of trees and genotypes needed for genetic studies together with the height of trees and plantation density make the bud observation laborious and imprecise; multi-sites trials dispersion and long distance from headquarters often limit surveys at the right time. The aim of this study was therefore to improve the observation procedure from both a practical and scientific aspect. More comfortable and robust indirect predictors of bud burst in the forest were searched for *ex situ* observations of bud flushing from tree explants in climatic chambers. The possibility to work in controlled indoor conditions looks indeed attractive from an operative point of view as well as the possibility to work with many replicates to reach an acceptable accuracy level. Then two types of experiences were carried out during years 2006 and 2007 to assess budburst in three tests: PEY, BECH and ORL. One of experiment evaluated the budburst *in situ*, and the other, in a *ex situ* way with the help of growth chambers.

#### **2.2.1.1. *Ex situ* bud burst assessment**

In early January 2006 and 2007, we collected 4-8 twigs per tree, each one with at least 30 buds, in Peyrat-le-Château and Orléans. Twigs were cut as far as possible from the same aspect of tree crowns and at the same relative tree height. The material was stored in a dark cold chamber at a temperature lower than 2°C. After a storage period of 25-30 days (2006) or 27 days (2007), twigs were put into jars filled with pure water, and the jars were immediately placed into two growth

chambers at 2 different conditions of temperature. The temperature was monitored continuously: chamber 1 was set at 16°C and chamber 2 at 11°C. A fixed photoperiod of 8 hours and a relative humidity above 80 % were used in both chambers.

In December 2007, we collected 6 twigs from another 148 trees from the Béchardegue trial: they were stored in the cold chamber up to February 4, 2008 and then twigs were handled as described above and placed into climatic chambers at 11 and 16°C with a longer photoperiod (16h).

Bud burst was daily recorded by counting buds along the twig, reaching stage 2 according to the scale used by Pâques for terminal bud flushing (Figure 6). The criterion to consider a twig “flushed” was the Julian day at which 50 % of the buds fulfilled the stage 2. Distinct values were obtained for chamber 1 (DCH1) and for chamber 2 (DCH2) and average values were calculated (DCH).

Heat-sums up to bud burst were then calculated using the Thermal Time (TT) model (Cannell and Smith 1983) also named the Spring Warming model (Hunter and Lechowicz 1992). This kind of models assumes a linear relationship between temperature and rate of development, and the occurrence of an event when a critical number of heat units are reached. The following expression corresponds to the model:

$$y \text{ such as } S_f = \sum_{t_0}^y R_f(x_t) = F^*$$

$$R_f(x_t) = \begin{cases} 0 & \text{if } x_t \leq Tb \\ x_t - Tb & \text{if } x_t > Tb \end{cases}$$

where  $y$  represents the date of flushing,  $x_t$  the daily mean temperature (°C),  $S_f$  the state of forcing or sum of degree-days,  $R_f(x_t)$  the forcing rate function,  $F^*$  critical value of state of forcing for transition from quiescence to flushing and  $Tb$  is the base temperature and  $t_0$  is the day when heat-sum starts to accumulate (Chuine et al. 2003).

The threshold over which heat units are accumulated was calculated using the linear regression method used by Arnold (1959) and Worrall (1993). To carry out this estimate, DCH1 and DCH2 were converted to heat-sums using the spring warming model; after that the threshold ( $T_b$ ) and the necessary number of heat units to bud burst (HS) were calculated for each individual tree. Then, progeny means were estimated by averaging individual trees values.

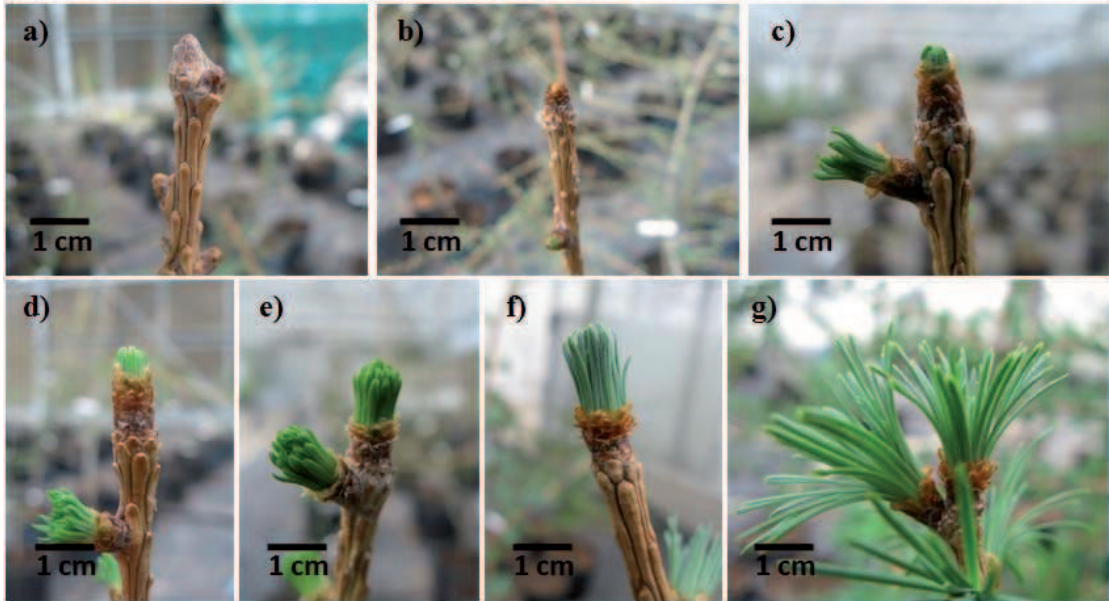
Heat-sums accumulated from a fixed arbitrary date (January 1<sup>st</sup>) up to bud burst were then calculated. They included degree-days accumulated in the plantation and in the cold room if any, and in the climatic chambers. Progeny means estimates of  $T_b$  were used as base temperatures. Using DCH1 and DCH2 and  $T_b$  progeny means, we calculated the average of heat-sums in the two chambers conditions ( $HS_{ch}$ ).

#### **2.2.1.2. *In situ* bud burst observations**

Bud burst was recorded in the field on the same trees on which we collected branches, every 7-10 days during 2006 and 2007 at Peyrat-le-Château and only during 2006 at Orléans. Flushing was assessed using two criteria:

a) Terminal bud flushing: using the subjective scoring system developed by Pâques (figure 6). The Julian day at which the terminal bud reached the stage 2 (TB2) was used to calculate the corresponding heat-sum accumulated from the 1<sup>st</sup> of January (HSBT2) using progeny means of  $T_b$  obtained from *ex situ* experiment.

b) Proportion of green crown: this measure was done looking for an alternative method (easier and less costly) to terminal bud observation. This looks particularly attractive for a deciduous species like larch. To carry out this measure, we considered 'green' the part of the crown with buds at stage 4 or more (figure 6). A tree was judged 'green' when more or less half of the crown height was 'green' assuming that this stage permits a better differentiation of genotypes due to a high variability of this phase. From that Julian day the crown was judged 'green' (G50), the corresponding heat-sum (HSG50) was calculated in the same way as for HSBT2.



**Figure 6.- Scoring used to evaluate terminal bud flushing and crown greening: 0: dormant bud (a); 1: bud swollen and whitish (b); 2: visible needles in the bud (c); 3: closed spindle-shaped needles (d and e); 4: needles completely unfolded, up to 1 cm elongated but still spindle-shaped (f); 5: elongated needles, 1-2cm in open rosette (g).**

In 2007, at Peyrat-le-Château, we modified this observation by following bud flushing on only 1 branch located at approximately half of the crown height. When one quarter up to half of the branch presented flushed buds, we assumed that this stage corresponded to the same reference stage as in 2006.

The observation of the proportion of green crown is considered easier than that of the terminal bud especially when trees exceed 6-7 meters of height and require the help of a binocular to observe the terminal bud.

At ORL, bud flushing was observed in April 29th, 2005 with a subjective scoring system (0= dormant to 5 = start of elongation, more details in chapter 3).

### 2.2.2. Sylleptic branch apparition, crown yellowing and bud set

The dates of apparition of sylleptic branches on the terminal shoot were also recorded using a scoring system: 0: absence of sylleptic branches, 0.5: small twigs at least 1 cm long, 1: twigs longer than 1 cm. Crown needle yellowing was observed in a global way following a subjective scoring showed in Figure 7.



Figure 7.- Scoring used to assess crown yellowing: 0: fully green crown (A), 1: some needles begin to turn yellowish (B), 2: some branches with needles completely yellow (C), 3: groups of branches with yellow needles localized randomly in the crown (D), 4: whole crown yellow except the branches along the terminal shoot (E) and 5: whole crown yellow (F). Photos at PEY site.



Bud set was assessed using also a subjective scoring: 0, no visible bud; 0.5, bud visible but still with some scales open; 1, bud set. Because of the too tall size of the trees at ORL, bud set, date of sylleptic branches apparition could not be assessed.

### **2.2.3. Radial and apical growth and terminal shoot lignification**

During 2006, radial growth of trees located at both sites (ORL and PEY) was derived from ring analysis based on the pinning method (see below 2.4). The elongation of the terminal shoot and the extension of lignification along the terminal shoot were also measured. We considered as 'lignified' the brown-brownish part of the shoot in contrast with the remaining soft green part towards the meristem. At ORL, lignification was assessed with a subjective scoring (scale, **1**: up to 25 % of the annual shoot length lignified, **2**: between 25 and 50 % of annual shoot length lignified, **3**: between 50 and 75 % of annual shoot length lignified, **4**: between 75 and 100 % of annual shoot length lignified). In contrast, trees at PEY were shorter than at Orléans and they were subjected to terminal shoot elongation and lignifications measurements with precision of 1mm.

At ORL, since plantation, total height and girth at breast height of trees have been annually measured until the trees were finally felled in March 2007. Girths were then recorded every meter from the stump and annual shoot increments were delimited (if necessary, with the help of available total height data) and measured. Oriented disks samples (3-4 cm thick) were collected every meter from stump to top for heartwood extension determination. Heartwood sizes along the stem were studied from disk analysis: a picture of each disk was first obtained and analyzed with Image-J software. Heartwood was distinguished from sapwood on the basis of color differentiation. From each disk, the total area (under bark) and heartwood area were measured. The total tree volume and heartwood volume were obtained by summing up the volumes of each 1 m-long log using Smalian's formula, plus the volume of the last log of the tree till the tip considered as a cone. A stem form factor calculated as the ratio of the total volume of the tree on the corresponding cylinder volume. The sapwood volume was calculated as the difference between total tree and heartwood volumes,

and relative proportions of heartwood extension were expressed in percentage of total BH area, of total tree height and of total tree volume. Combining these heartwood data with measurements already obtained from diametrical increment cores collected at breast height on the same trees in December 2004, the speed of heartwood extension was computed as the mean length and number of rings of heartwood formed during the 2004-2006 period.

### 2.3. Assessment of wood density profiles (chapters 1 and 4)

An orientated wood disk from each analyzed tree was sampled at 1 m or 1.30 m height above soil level according to age of trees. Details of number of sampled trees during 2006 can be seen in

Table III

Site	Age (years)	Taxa <sup>1)</sup>	Number of total sampled individuals	Number of progenies	Number of individuals sampled per progeny
PEY	3	HL	88	8	11
		JL	6	1	6
ORL	11	HL	10	1	10
		EL	25	5	5

<sup>1)</sup> HL: *Larix decidua* x *L. kaempferi*, JL: *L. kaempferi* and EL: *L. decidua* 'polonica'.

**Table III.- Details of quantity of sampled trees by taxa and progenies**

Disks were dried to moisture equilibrium and subsequently two millimeters-thick boards were extracted from pith to bark at the same orientation than the pins to be X-rayed following Polge (1966). The resulting X-ray films were scanned at a 1000 dpi resolution with 8 bits per pixel. The digital images were processed with the WinDENDRO software (Guay et al. 1992), obtaining a spatial resolution of 25 µm. The wood density profiles along the radius were then obtained, and the profiles of the 2006 ring extracted. These profiles were called "static" microdensity profiles as they describe the variation of wood density along the ring width (Figure 10 a). We obtained mean (MRD), maximum (MAD) and minimum (MID) wood densities. We distinguished three kinds of tissue inside profiles: early-, transition- and late-wood using the concept of MDP (mean density point) criteria calculated as (MAD – MID)/2. Namely, the transition from early- to late-wood was delimited by

MDPinf (MDP-MID)/2) and MDPup (MAD-MDP)/2) representing the inferior and superior limits (Figure 10a). Widths of early-(ewd), transition-(twd) and late-wood (lwd) and their proportions (ewp, twp and lwp) were obtained.

#### 2.4. Assessment of cambial activity and wood formation (chapters 1 and 4)

In our experiments, the pinning method was the technique selected to track cambial activity. An insect mounting pin (diameter 0.5 mm) was inserted every 7-10 days on each sampled tree at ORL and PEY. Dates in which pins were inserted are detailed in Table IV.

Pin code	ORL		PEY	
	Calendar date	Julian Days	Calendar date	Julian Days
1	31-Mar	90	30-Mar	89
2	7-Apr	97	12-Apr	102
3	11-Apr	101	19-Apr	109
4	18-Apr	108	24-Apr	114
5	28-Apr	118	4-May	124
6	9-May	129	16-May	136
7	19-May	139	23-May	143
8	29-May	149	6-Jun	157
9	8-Jun	159	19-Jun	170
10	19-Jun	170	28-Jun	179
11	29-Jun	180	7-Jul	188
12	10-Jul	191	17-Jul	198
13	20-Jul	201	27-Jul	208
14	27-Jul	208	7-Aug	219
15	4-Aug	216	16-Aug	228
16	14-Aug	226	28-Aug	240
17	25-Aug	237	6-Sep	249
18	5-Sep	248	13-Sep	256
19	16-Sep	259	26-Sep	269
20	25-Sep	268	3-Oct	276
21	5-Oct	278	12-Oct	285
22	25-Oct	298	26-Oct	299
23	6-Nov	310	7-Nov	311
24	13-Nov	317	14-Nov	318
25	17-Nov	321	21-Nov	325
26	23-Nov	327		

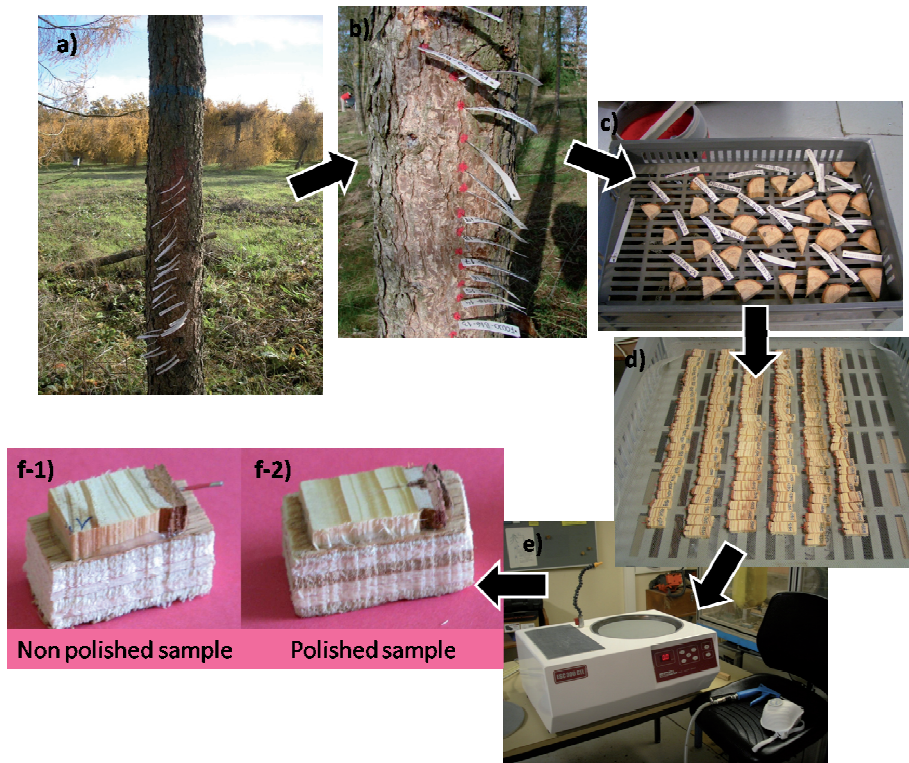
Table IV.- Detail of dates in which pins were inserted at PEY and ORL

First pins were inserted 60-80 cm above ground and the following ones were placed upwards along the stem as close as possible to each other (around two centimetres apart). Along the 2006 growing season, approximately 25-26 pins were installed and kept on the trees (figure 8a and 8b) until the collection of wood samples.

At the end of the growing season (December 2006 – February 2007), trees were felled and wood disks were obtained at the level of each pin. Small wood samples -about 0.5 cm thick and including at least the last two rings - were sawn off with the pins kept into the samples (Figures 8c and 8d).

Because of the enormous quantity of samples (129 trees x 25-26 pins=3260samples) to process, an adaptation of the traditional pinning method was necessary: indeed this method basically relies on the preparation of thin slices of wood obtained with a microtome that are staining and observed with a refraction light microscope (Wolter 1968, Yoshimura et al. 1981, Antonova and Stasova 1997, Grotta et al. 2005, Rossi et al. 2006b, Seo et al. 2007,). In our study, the upper face of each wood sample (Figure 8f-1) was carefully polished (Figure 8f-2) with the help of a precious stone polisher (Figure 8e) using grids from 600 to 1200 grains/square inch. The appearance of the pin injury was regularly checked with a binocular microscope during polishing.

Afterwards, we captured an anatomical image of the last complete ring from each sample with the help of a digital camera mounted on a reflected-light microscope Leica DM4000 M using an objective of 5x magnification.



**Figure 8.- Sample preparation. a) View of inserted pins on a sampled tree at the end of 2006 growing season. b) Detail of pins. c and d) reduction process of wood samples. e) Machine used to polish wood samples. f-1) view of wood sample ready to polish. f-2) view of polished sample**

With the Visilog 6.0 image analysis software, we measured two parameters on each image: the 2006-ring width (RW) and the cambial mark 'injury distance' (ID). For each image of wood sample at a given date, we obtained the proportion of radial growth up to a given date using the ratio ID/RW (Figure 9). This ratio was used instead of ID to avoid the effect of ring width variation along the stem. Proportions were then used to reconstruct absolute ring widths at the various dates using total ring width obtained from 'static' microdensity profile. Based on widths and the dates obtained through the pinning method, static profiles for the 2006-ring were synchronized so to express the variation of density through time. To do so, we assumed that growth was linear between 2 dates. So-called 'dynamic' microdensity profiles were constructed for each tree (Figure 10b).

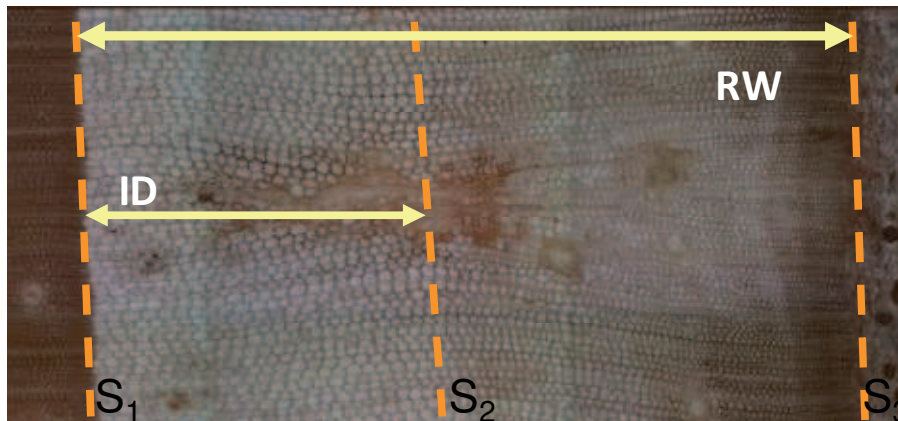


Figure 9.- Image from reflection microscope of annual ring growth 2006 with injury caused by inserted pin.  $S_1$ : limit between the last layer of latewood cells belonging to 2005-growing season and the first layer of earlywood cells of 2006-growing season,  $S_2$ : placed where the radial rows of tracheids changed their direction because of the injury,  $S_3$ : limit between the last layer of latewood cells of 2006-growing season and the phloem cells. Adapted from figure presented in manuscript of paper 1 (Chapter 1)

Taking as reference the 'static' profiles and key points MDPinf and MDPsup we identified these values over 'dynamic' profiles to determine starting and ending dates of formation of the different tissues (Sew, Stw, Etw, Elw) as well as their durations (Dew, Dtw, Dlw), the speed of their development (SFew, SFtw, SFlw) and the speed of change of density in the transition zone (SLOPEtw). They are presented in the chapter 1.

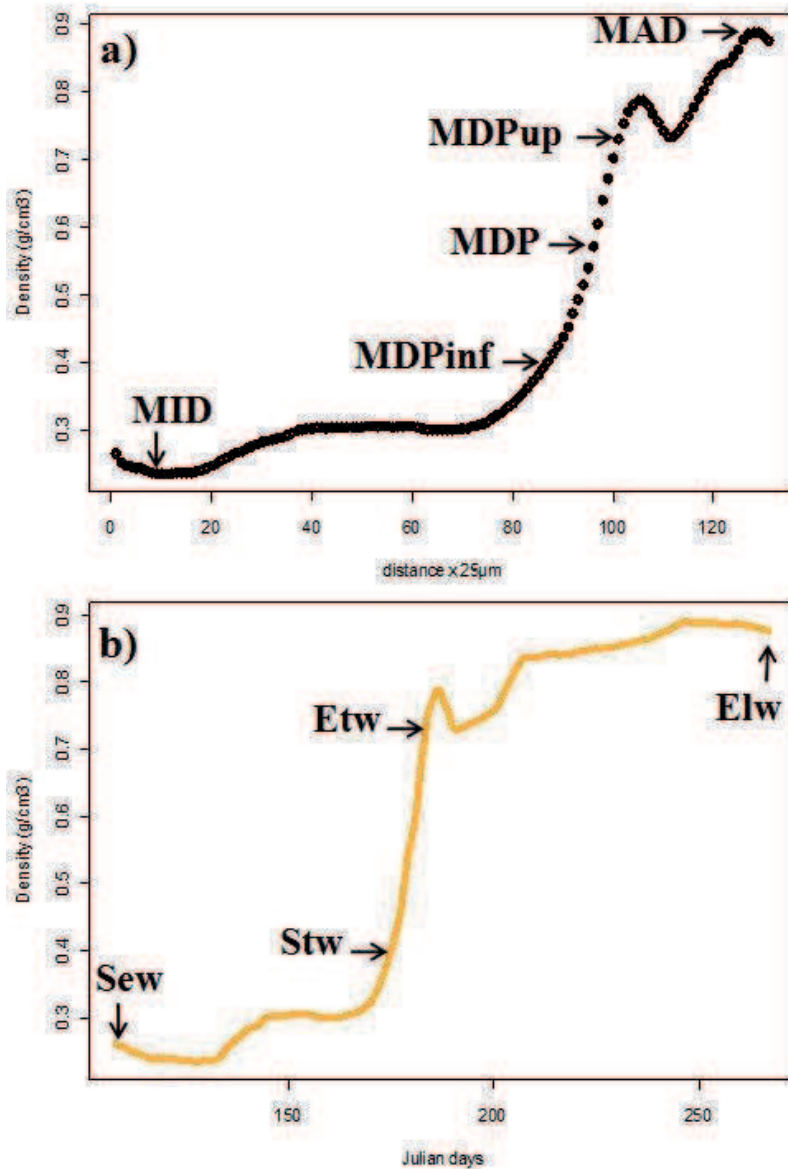


Figure 10.- Scheme of 'Static' and 'dynamic microdensity profile' from tree 128 (HL) at ORL . a) 'Static' microdensity profile, MID: minimum ring density, MAD: maximum ring density, MDP: mean density point, MDPinf: lower limit of transition-wood, MDPup: upper limit of transition-wood. b) 'Dynamic' microdensity profile, Sew: starting date of early-wood formation, Stw: starting date of transition-wood formation, Etw: ending date of transition-wood, Elw: ending date of late-wood formation

## 2.5.Data analysis

### 2.5.1. Models used in analysis

The analyses of variance (ANOVA) performed on variables to test the significance of differences among progenies based on the model expressed in eq. 5.

$$y_i = \mu + F_i + e_i \quad (\text{eq. 5})$$

Where  $\mu$ ,  $F_i$  and  $e_i$  are the general mean, the genetic effect due to progenies, and the error term, respectively.

For some variables, an additional model was also proposed to test differences among taxa (eq. 6), where  $\mu$ ,  $\tau_i$  and  $e_i$  are the general mean, the genetic effect due to taxa, and the error term, respectively.

$$y_i = \mu + \tau_i + e_i \quad (\text{eq. 6})$$

When variables differed significantly among progenies or taxa, multiple comparisons between progenies and/or taxa (HL, EL and JL) were performed using the Tukey's test.

### 2.5.2. Genetic parameters estimation: heritability- variance component-genetic correlations (chapters 3 and 4)

Heritabilities were estimated for parameters of wood formation (showed in chapter 4) and for bud flushing (chapter 3). Although, it was not possible to estimate narrow-sense heritabilities because of the genetic nature of the material used, heritabilities at the family level (eq.4) for *ex situ* and *in situ* bud flushing observations were estimated using the DIOGENE software, an extended version of the OPEP software (Baradat et al. 1995).



$$h_{fam}^2 = \frac{\sigma_{fam}^2}{\sigma_e^2 + \sigma_{fam}^2} \quad \text{eq. 4}$$

where  $h_{fam}^2$  is heritability at family level,  $\sigma_{fam}^2$  represents the variance component due to family and  $\sigma_e^2$  is the error variance component. Standard deviations of heritabilities were calculated using the Jackknife method with the same software (Baradat et al. 1995).

## **Chapter 1**

[Article 1]: Dynamic of annual ring wood formation in Larch: links with phenological events.

Gauchat, M. E., Pâques, L. E..

Manuscript submitted to TREES - STRUCTURE AND FUNCTION.

**DYNAMICS OF ANNUAL RING WOOD FORMATION IN LARCH: LINKS WITH GROWTH AND PHENOLOGICAL EVENTS.**

Gauchat, M. E. <sup>a\*</sup>, Pâques, L. E. <sup>b</sup>.

<sup>a</sup> INTA E.E.A. Montecarlo. Av. El Libertador 2472. CP3384. Montecarlo. Misiones. ARGENTINA. [mgauchat@montecarlo.inta.gov.ar](mailto:mgauchat@montecarlo.inta.gov.ar)

<sup>b</sup> INRA Orléans. Unité d'Amélioration, Génétique et Physiologie des Arbres Forestiers. 2163 Avenue de la Pomme de Pin. 45075. Orléans Cedex 2. FRANCE. [luc.paques@orleans.inra.fr](mailto:luc.paques@orleans.inra.fr)

\*Corresponding author:

Tel-fax: +54 3751480512 (extension 126).

Address: Av. El Libertador 2472. CP3384.

Montecarlo. Misiones.

ARGENTINA.

E-mail: [mgauchat@montecarlo.inta.gov.ar](mailto:mgauchat@montecarlo.inta.gov.ar)

**Key-words:** larch, apical and radial growth, phenology, pinning method, xylogenesis

## **Abstract**

In this study, we followed up weekly xylogenesis of 124 trees sampled from three *Larix* taxa (EL: European, JL: Japanese and HL: hybrid larches) across 2 sites in France. A method combining pinning and microdensitometry was developed to reconstruct dynamic microdensity profiles. From them, we derived dates (onset and completion), durations and speeds of formation of early-, transition- and late-wood. In parallel, bud phenology, apical and radial growths, as well as shoot lignification were recorded in search for easily observable 'external' signals of cambial activity. Early-wood started to form on average at Julian day 109, the transition-wood between Julian days 203 and 221 and the late-wood tissue was completed at Julian day 273. The main difference between taxa in the timing of xylogenesis was the dates of ending of late-wood and the length of the growing season (14-17 days longer for HL compared to JL and EL). The early-wood onset seems to be more temperature-dependant, while the transition from early- to late-wood seems to be related to water availability as shown in irrigated vs non-irrigated trees. Finally, we have been able to identify some 'external' traits synchronous to some wood formation parameters. Early-wood started when 30-60 % of crown was green and terminal bud was at an early stage of flushing. Transition-wood started and ended slightly before and after 90% of radial and apical growths were achieved and terminal shoot lignification reached its maximum rate of extension. Late-wood completion was concomitant with start of crown yellowing and 14 days later than terminal bud setting. The periods of tissues formation were well identified by 'external' markers over the growing season.

## **1 Introduction**

Productivity of trees is driven by basic factors such as radiation, water and carbon dioxide required for photosynthesis, and by more finer controls related to foliage, light-use efficiency, water availability, temperature conditions, availability of soil nutrients, the adaptation of species to extreme events and their use-efficiency of water and nutrients (Hopkins and Hüner, 2004; Schulze et al., 2002). Also, the length of the growing season is an important determinant of forest production (Nemani et al., 2003) and delays in apical meristem and cambium reactivation could reduce the period favorable for tree growth.

In the context of climatic change, the growing season length could be moderately affected by an increase of temperature (Boisvenue and Running, 2006; IPCC, 2007) and its duration will be probably more extended. But this is not the only change predicted for the coming years: regions including France, Germany and the Balkans area should suffer from an increase of frequency and intensity of heat and drought events, which could have even more serious impacts (Meehl and Thebaldi, 2004).

Tree productivity is results of ring wood formation. Such activity exhibits an annual periodicity in temperate regions and it is influenced by both external and internal factors: on one side, regularly changing seasons synchronized with alternation of favorable/unfavorable climatic conditions during the growing season; on the other side, internal factors related to age and physiological states (hormones and carbohydrate distribution and flow). Many processes are involved in wood formation and they can be tackled from different study areas like physiology (Aloni, 2001; Funada et al., 2002; Rossi et al., 2008a), dendroecology (Antonova and Satasova, 1997, 2002; Deslauriers et al., 2008, Rensing and Owens, 1994, Rossi et al., 2009a) or dendrochronology (Fritts, 1976, Schweingruber et al., 1993, Gartner et al., 2002).

Wood formation studies and in particular tree ring analysis, are well-adapted to answer questions from many disciplines. Studies of wood quality, competition among trees or genetic variability of wood properties consider wood in a “static” way or take into account the inter-annual ring variations. The key in this kind of works is to dispose of several rings to be analyzed. However, during the last 10 years, the study of wood formation at the intra-annual ring scale has become necessary in particular to investigate the influence of climate on ring wood formation and tree reaction against extreme climatic events or environmental stresses. Most studies tend to understand the development of tracheids from a physiological and anatomical point of view (Larson, 1994; Antonova and Satasova, 1997, 2002), the optimal environmental conditions for cambium reactivation or cell division (Begum et al., 2007; Gricar et al. 2007b; Rossi et al 2008a) and the effect of environmental conditions on intra-annual radial increment (Jenkins, 1975; Park and Spiecker, 2005; Rossi et al., 2006a, 2007; Linares et al., 2009).

The phenology of annual ring formation (onset and end of cambium activity as well as of the transition from early- to latewood during the growing season (Seo et al. 2007)) has become a key-topic in ecology (e.g. for tree plasticity studies) as well as in genetics and tree improvement (e.g. for improvement of wood properties and adaptability parameters). Phenology of annual ring formation has already been tackled in several studies but using a reduced quantity of sampled trees (Grotta et al., 2005; Cufar et al., 2008; Deslauriers et al., 2003, 2008; Seo et al., 2008; Gruber et al., 2009b). This is acceptable for physiological studies, but for genetic studies for which variability among trees is a critical parameter, it is necessary to significantly increase the amount of sampled trees. The methodologies applied so far, based on fresh tissue utilization, microtome cuts and staining, are difficult to carry out on high numbers of trees and thereby prevent the use of phenology of annual ring formation for example for breeding purposes.

Since the development of X-ray densitometry in the 70's (Polge, 1966; Schweingruber et al., 1993), wood density and size properties of annual rings have been intensively studied. This technique has also more recently proved to be useful to study adaptive traits and tree plasticity (Martinez-Meier et al., 2008, Sanchez-Vargas et al., 2007, Wimmer and Grabner, 2000).

Combination of methodologies and results coming from wood formation phenology on one side, and from X-ray densitometry, on the other side, could permit the development of a powerful analytical tool: it could provide at the same time a response to the sampling problem previously mentioned and a better insight into the genetic and environmental control of wood formation. Only scarce or null information is available tending to combine these two fields of research. A key application of time inclusion into intra-annual ring microdensity variation would be to compare patterns of tree response to environmental changes using variation of wood structure as a recorder of cambial activity over time.

Several authors have studied the relationships between wood formation and, bud phenology, needles development or shoot growing rhythm (Jayawickrama et al., 1997; O'Reilly and Owens, 1989, Rensing and Owens, 1994; Rossi et al, 2009a). This type of results helps better understanding the connection between physiological processes occurring at the same time in trees, but it could also help identifying external phenological or morphological markers of internal process of ring formation. These external markers, easier to observe, could be useful for example to characterize steps of cambial activity of ring wood formation.

External climatic factors during the growing season have a strong influence on cambial activity (Antonova and Stassova 1997, 2002), together with modification of concentrations of plant regulators within the tree (Reninger et al., 2006, Savidge, 2000, Uggla et al. 2001, Funada et al. 2002). Some particular regions of trees (terminal bud, new needles) are

suspected to act as receptors of environmental signals, thereby to be responsible for adjusting internal hormones gradients and, consequently for controlling wood formation (Reninger et al., 2006).

In Europe, larch (*Larix* sp.) is an alternative to Douglas-fir and spruces for plantation. A challenge for these species is to provide foresters with fast-growing, well adapted materials to the foreseen changeable environment and with suitable wood density properties. A better knowledge of the phenology of ring wood formation and of its genetic variability would help breeders to select material exploiting at best the growing season from an adaptive prospect as well as for wood production (quantitatively and qualitatively).

Within this scope, our aim in the present study is to identify the different phenological phases of ring wood formation in larch and to search for external signals of tree development (like phenological events, radial and terminal shoot growth, lignification) which could be related to annual ring formation. Therefore, this study aims to learn and compare the dynamics of growth and tree ring formation (onset, duration of growing season) for different larch taxa and to investigate links between some external morphological or developmental signals and internal wood formation process.

## **2 Materials and methods**

### **2.1 Trial description**

This study was carried out in two distinct farm-field progeny trials in France in order to benefit from the presence of pure European (EL) (*Larix decidua* Mill.) and Japanese (JL) (*L. kaempferi*) larches and of their hybrid (HL). The first one was established at Peyrat-le-Château (PEY) in 2003: it included 54 full-sib progenies of hybrid larch and one commercial



provenance of Japanese larch. A sample of 94 trees (88 HL from 9 progenies + 6 JL) was used for the study.

The second trial was located in Orléans (ORL) and planted in 1995: it has been established with 20 half-sib families of EL (*'polonica'*) and one HL progeny (seed orchard progeny). In 2000, a water-regime experimentation was carried out with a continuously irrigated treatment vs a non-irrigated treatment (Gauchat and Pâques, in revision). Thirty-five trees were sampled: 25 EL and 5 HL from the non-irrigated treatment and 5 HL from the irrigated treatment. More details on the trials are given in Table I.

## **2.2 Pinning method**

To track out the cambial activity over the growing season, we used the 'pinning method',. It consists in inserting a mounting insect pin into the tree trunk periodically, during the growing season, so to provoke an injury at the cambial zone level. As a reaction, the cambial cells produce deformed and distinctive cells than can be identified by microtome cuts with the help of refraction microscope (Wolter, 1968).

During 2006, we inserted a pin (diameter 0.5 mm) on each of the 129 sampled trees, every 7-10 days starting on 30<sup>th</sup> March and ending on 23<sup>rd</sup> November. The first pin was inserted at 60-80 centimeters above ground and the following ones were placed upwards along the stem as close as possible to each other (around two centimetres apart). Altogether, 25-26 pins were installed and kept on the trees (figure 1a and 1b) until the collection of wood samples.

At the end of the growing season, trees were felled and wood disks were obtained at the level of each pin. Small wood samples -about 0.5 cm thick and including at least the last two rings - were sawn off with the pins kept into the samples (figure 1c and 1d).

Because of the enormous quantity of samples (129 x 25-26 samples) to process, an adaptation of the traditional pinning method was necessary: indeed this method basically relies on the preparation of thin slices of wood obtained with a microtome that are staining and observed with a refraction light microscope (Wolter, 1968; Antonova and Stasova, 1997; Seo et al., 2007; Rossi et al., 2006b).

In our study, we used instead fine wood polishing: the upper face of each wood sample (figure 1f-1) –glued on a piece of wood for easier handling- was carefully polished (figure 1f-2) with the help of a precious stone polisher (figure 1e) using grids from 600 to 1200 grains/square inch. Regularly, the appearance of the pin injury was checked with a binocular microscope.

Afterwards, we captured an anatomical image of the last complete ring from each sample with the help of a digital camera mounted on a reflected-light microscope Leica DM4000 M using an objective of 50x magnification.

With the Visilog 6.0 image analysis software, we measured two parameters on each image: the 2006-ring width (RW) and the cambial mark ‘injury distance’ (ID). These parameters were obtained drawing three straight lines ( $S_1$ ,  $S_2$  and  $S_3$ ) on the image as it can be seen in figure 2. RW is equivalent to the distance between  $S_1$  and  $S_3$ . This parameter was obtained by an average of 2 measurements (RW' and RW'') made at both sides of the injury in order to avoid measurement errors caused by deformations (figure 2). ID was acquired measuring the distance from  $S_1$  up to  $S_2$ ; adopting the same criterion of averaging two measurements (ID' and ID'') on both sides of the injury due to possible different locations of the changes of tracheids rows.

For each image, that is for each sample at a given date, we obtained the proportion of radial growth up to a given date ( $P_{rg}$ ) using the ratio ID/RW. This ratio was used instead of ID to avoid the effect of ring width variation along the stem. Proportions were then used to

reconstruct absolute ring widths at the various dates from a reference ring used for the microdensitometry analysis (see below).

### **2.3 Static and Dynamic ring microdensity profiles**

An additional orientated wood disk from each tree was sampled at 1.30 m height in the trunk at Orléans and at approximately 1 m on younger trees at Peyrat-le-Château. Two millimeters-thick boards were extracted from pith to bark at the same orientation than the pins, and X-rayed following Polge (1966). With the help of WinDENDRO software (Guay et al., 1992), the wood density profiles along the radius were then obtained, and the profiles of the 2006 ring extracted. These profiles will be further on called “static” microdensity profiles as they describe the variation of wood density along the ring width (figure 3a). Mean (MRD), minimum (MID) and maximum (MAD) ring densities were extracted.

Classically in wood density studies, 2 tissues are distinguished along the ring: the early-wood and the late-wood. Sustained by anatomical properties, the delimitation is frequently done using MDP (mean density point) criteria calculated as  $(MAD - MID)/2$ . In this study, we included a third tissue, namely the transition wood that was delimited by  $MDP_{inf}$  (calculated as  $(MDP - MID)/2$ ) and  $MDP_{up}$  ( $(MAD - MDP)/2$ ) as represented in figure 3a. Early-(ewd), transition- (twd) and late-wood (lwd) widths and their proportions over the total ring width (ewp, twp and lwp) were obtained.

Based on  $P_{rg}$  and the dates obtained through the pinning method, static profiles for the 2006-ring were synchronized so to express the variation of density through time. To do so, we assumed that growth was linear between 2 dates. So-called ‘dynamic’ microdensity profiles were constructed for each tree (figure 3b). The start and the end of radial growth were determined by the first and the last dates in which cambial activity was detected by the microscope images.

From these ‘dynamic’ profiles, we identified 4 important dates delimiting the 3 types of tissues: the start of early-wood formation (Sew), the start and the end of transition-wood (Stw and Etw respectively), and the end of late-wood formation (Elw). From these, the duration of formation of the 3 tissues was calculated and expressed in absolute values (days) (Dew, Dtw, DIw) and as a percentage of the total duration (Dtot) of annual ring formation (%Dew, %Dtw, %DIw respectively). The timing of cell production (division) is different from that of cell maturation (mature tracheid) (Rossi et al. 2009a), this could lead to an error of 10-15 days in the analysis but it should not affect comparative analysis among taxum.

Based on the width of tissues and the duration of their formation, the average speed of formation of each tissue was estimated (SFew, SFtw, SFlw). As well, the change of wood density over time while the transition wood was produced was calculated as the slope between two points: Stw and Etw ( $SLOPE_{tw} = (\text{density at Stw} - \text{density at Etw}) / D_{tw}$ ).

#### **2.4 Phenological and growth data**

At each date when a pin was inserted, several phenological, morphological and growth parameters were assessed. Phenological observations included terminal bud flushing and crown greening during spring, and crown needles senescence and terminal bud setting at the end of the growing season. At both sites, terminal bud flushing was assessed with a subjective scoring system (0= dormant to 5 = start of elongation, more details in Gauchat and Pâques, 2011) and we took as reference the Julian day in which terminal bud was in stage 2 (TB Flushing), when green needles become visible. For crown greening, we measured the proportion of green crown, expressed in percentage of total crown (Gauchat and Pâques, 2011). We considered ‘green’ the part of the crown with needles completely unfolded, up to 1 cm long but still spindle-shaped and we took as a common indicator the Julian day in which between 30 and 60% of crown was “green” (Crown Greening). In contrast, bud set was only

observable at PEY on young trees; we applied a subjective scoring: 0, no bud visible; 0.5, bud visible but still open; 1, bud set. Crown needle yellowing (CY) was observed in a global way following a subjective scoring (0: green crown, 1: some needles that began to turn yellowish, 2: some branches with needles completely yellow, 3: groups of branches with yellow needles localized randomly in the crown, 4: whole crown yellow except the branches along the terminal shoot and 5: whole crown yellow).

Radial growth was derived from the ring analysis based on the pinning method for all trees. For apical growth, only trees at PEY could be followed because of the too tall height of trees at ORL. Every 7-10 days, we measured the elongation of the terminal shoot and the extension of lignification along the terminal shoot. Was considered 'lignified' the brown-brownish part of the shoot in contrast with the remaining soft green part towards the meristem. Finally, we noted the dates of apparition of sylleptic branches on the terminal shoot using a score of 0: absence of sylleptic branches, 0.5: small twigs at least 1 cm long, 1: twigs longer than 1 cm.

At ORL, lignification was assessed with a subjective scoring (scale, **1**: up to 25 % of the annual shoot length lignified, **2**: between 25 and 50 % of annual shoot length lignified, **3**: between 50 and 75 % of annual shoot length lignified, **4**: between 75 and 100 % of annual shoot length lignified).

## **2.5 Modeling and statistics**

In order to get a continuous expression over time, growth processes were modeled using a Weibull growth model (Yang et al., 1978). Well-fit to tree growth increment modeling, Weibull model has also the advantage to provide comprehensive synthetic biological parameters of growth process such as the dates of start (achievement of 10 % of growth, T10) and of end of growth (achievement of 90 % of growth; T90), and the date (Ti) of maximum

growth rate. Parameters were obtained for the radial growth (T10rg, T1rg and T90rg), the terminal shoot elongation (T10hg, T1hg and T90hg) and the lignification extension (T10lig, T1lig and T90lig).

Analyses of variance (ANOVA) were performed on all variables to test the significance of differences among progenies taxa based on the model expressed in eq. 1, where  $\mu$ ,  $\tau_i$  and  $e_i$  are the general mean, the genetic effect due to taxa, and the error term, respectively.

$$y_i = \mu + \tau_i + e_i \quad (\text{eq. 1})$$

For statistical comparisons between irrigated and non-irrigated groups of trees at ORL, we used a paired t-test.

Finally, Pearson's correlation coefficient was used to evaluate the relationships between external parameters (bud phenology, apical and radial growth and lignification) and key dates of ring wood formation.

### **3 Results**

#### **Bud phenology and dynamics of growth**

On average, the crown greening and the terminal bud flushing occurred simultaneously at both sites (Table II), that was on average after 105-107 Julian days (April 15-17<sup>th</sup>) for crown greening and a few days later for TB flushing (108-109 days). At individual tree level, crown greening usually occurred 7-10 days earlier than terminal bud flushing.

Period of needle senescence and dynamics of crown yellowing showed slight differences among sites. At ORL the whole process of needle yellowing was shorter and more abrupt, between Julian days 283 and 317 (10<sup>th</sup> Oct - 13<sup>th</sup> Nov) whereas at PEY crown yellowing took place over a longer period, between Julian days 269 and 325 (26<sup>th</sup> Sep – 21<sup>th</sup> Nov). Bud set

was only assessed at Peyrat and on average, it occurred on Julian day 266 (September 23<sup>th</sup>) (Table II): at individual tree level, that meant 16 days before crown yellowing.

Then radial growth started at PEY on average 3 days later than at ORL (110+/- 4 vs. 107+/- 4 Julian days). Differences were even greater for the termination of radial growth: trees at PEY finished their growth on average 30 days later than at ORL: 281+/- 16 vs. 251+/- 14 Julian days. The total growing season for the secondary meristem was 144 and 188 days at ORL and PEY, respectively.

Results from Weibull modeling (Table II) showed slight differences (2 days) among sites for dates in which 10% of radial growth was reached (T10rg), while differences were really pronounced (43 days) for dates in which 90% of radial growth (T90rg) was achieved. The maximum growth rate T<sub>irg</sub> was observed more lately at PEY on Julian day 180 (June 29<sup>th</sup>), that was 20 days later than at ORL.

Apical growth and terminal shoot lignification: they lasted 102+/- 14 and 100+/- 15 days, respectively. Shoot elongation (T10hg) began on Julian day 145 (May 25<sup>th</sup>), that was nearly one month later than the beginning of radial increment. Differences between the start of radial increment and the start of height growth (T10hg) at individual tree level ranged between 18 and 62 days. The maximum growth rate (T<sub>ihg</sub>) and the ending of elongation (T90hg) were produced on Julian day 195 (July 14<sup>th</sup>) and 225 (August 13<sup>th</sup>), respectively. The termination of shoot elongation (T90hg) was produced nearly two months (56 days) before the end of radial growth. Logically, lignification of terminal shoot started nearly one month later than the start of shoot elongation, and it finished also one month later than the end of shoot elongation (Table II).

### **Characterization of the 2006 annual ring**

Ring widths reached higher values at PEY than at ORL (Table III). However the proportions of each tissue were similar for both sites except for transition-wood. Early-wood represented at both sites the largest proportion of total ring width (71.8 and 79.1% at ORL and PEY respectively). Although the transition-wood width was higher at PEY, it represented a smaller proportion of total ring width compared to that in ORL (nearly half of it). In general, the individual tree variability was smaller for early-wood width than for late-wood width; the transition wood showed the highest variability among trees.

On average, mean ring (MRD), minimum (MID) and maximum (MAD) densities for both sites reached 0.42 (SD=0.04), 0.28 (SD=0.03) and 0.91 (SD=0.06)  $\text{gr}/\text{cm}^3$ , respectively. And densities values used to delimitate early-, transition- and late-wood were 0.43 (SD=0.03), 0.57 (SD=0.04) and 0.76 (SD=0.05)  $\text{gr}/\text{cm}^3$ , respectively for MDPinf, MDP, MDPsup.

### **Phenology of ring formation**

Parameters of the dynamics of ring formation during year 2006 are shown in Table IV. On average, early-wood started to form at Julian day 109 (19<sup>th</sup> April); it lasted 94 $\pm$  21 days and it showed the highest formation speed compared with other tissues (0.05 mm per day). To form early-wood, trees spent around 57% of total time employed to produce the total ring.

Transition from early- to late-wood took place, on average, between Julian days 203 (22<sup>th</sup> July) and 221 (9<sup>th</sup> August). Transition-wood was the tissue with the shortest period of formation (18 $\pm$  12 days) and its formation was proportionally less lasting than other tissues (~11%). Its speed of formation was intermediate (0.03 mm per day). During formation of the transition-wood, the density increased, on average, by 0.03  $\text{g}/\text{cm}^3$  per day.

The late-wood tissue was produced on average, between Julian days 221 and 273 (9<sup>th</sup> August and 30<sup>th</sup> September), respectively. To form late-wood trees spent ~32% of total time



invested to produce the complete ring, which meant 52 days. Late-wood formed at the slowest speed (0.02 mm/day).

Overall, the starting date of ring formation was much less variable than the ending date but the highest variability was observed for the transition-wood starting and ending dates.

#### *Differences among taxa*

Strict comparisons at taxa level between sites were not possible due to different genetic material of HL and age of trees, but analyses of trends within site are feasible. At both sites, dates of Sew, Stw and Etw did not show differences higher than 3 days among taxa (Figure 4 A). In contrast, for date of Elw at ORL, EL finished late-wood formation 21 days before HL (246 vs. 267 Julian days), and at PEY, JL completed late-wood formation 16 days before than HL (265 vs. 281).

Concerning the period to form the different tissues, early- and transition-wood showed at both sites differences among taxa not larger than 2-4 days but the duration of late-wood formation was markedly higher in HL than in pure species (EL and JL) (Figure 4 B). At ORL, formation of late-wood lasted 20 days more for HL compared to EL (74 and 54 days respectively), and 15 days at PEY in comparison with JL (36 for JL and 51 days for HL). Consequently, total duration of tissue formation was longer for HL than for the other taxa (17 and 14 days respectively at ORL and PEY). Results of proportions of duration of early-, transition- and late-wood compared to total duration are in correspondence with those of durations in absolute values (Figure 4 C).

Only slight differences among taxa were also found for the speeds of tissue formation (Figure 4 D) and there is no clear tendency of HL compared with its respective parental species at each site.

The change of density during production of transition-wood (SLOPE<sub>tw</sub>) was slightly steeper for EL than HL (0.023 vs. 0.016 g/cm<sup>3</sup>.day<sup>-1</sup>) at ORL while SLOPE<sub>tw</sub> of HL and JL were similar at PEY (0.032 g/cm<sup>3</sup>.day<sup>-1</sup> for both taxa).

#### **Environmental effects on wood formation: Soil water availability effects**

The mean ring width of trees in the well-watered conditions was nearly twice that of trees in stressed conditions (Table V). Early-, transition- and late-wood ring widths in irrigated-trees were also higher than in non-irrigated trees (superiority of 31, 131, 180 %, respectively over non-irrigated trees). For all variables, variability among trees was always higher in the irrigated treatment.

Radial growth started and ended at about the same time in irrigated and non-irrigated trees, and the total duration of overall ring formation was similar in both environments (difference of 3 days). However, the transitions from early- to transition-wood and from transition- to late-wood were delayed by about 11 and 14 respectively days in irrigated trees compared to non-irrigated trees (Figure 5).

Significant differences between well-watered trees and non-irrigated trees were found for the overall ring width, the proportion of early-wood width and the duration of its formation, and also for speeds of tissues formation: SF<sub>ew</sub>, SF<sub>tw</sub> and SF<sub>lw</sub> (Table V).

#### **Links between wood formation traits and, phenology of bud, growth and lignification**

Relating initiation of early-wood production and bud-burst (Figure 6), we observed that when the cambium began to produce early-wood cells (S<sub>ew</sub>), crown greening reached around 30-60 % of the crown height at ORL and PEY, and the terminal buds were on average at stage 3 at ORL and at stage 2 in PEY.

The first cells of early-wood (Sew) were detected at PEY about 7 days earlier than T10rg and 35 days earlier than T10hg. At ORL, on average, initiation of early-wood was 13 days earlier than T10rg (Table II and IV).

At PEY, transition-wood (Stw) started on average 18 days after the date of the maximum rate of shoot elongation increment (Tihg), nearly one month after the date of the maximum rate of radial growth (Tirg), and 10 days before the maximum rate of lignification (Tilig) (Figure 6 and Tables II and V). At ORL, transition-wood formation started, on average, 8 days later than the date of maximum rate of radial increment (Tirg). More precisely, at PEY, Stw was estimated to start forming when the terminal shoot reached around  $\frac{3}{4}$  of its total length and when a bit more than half (55%) of shoot length was lignified. Trees started the production of transition-wood about 26 days after the apparition of the first sylleptic branches on the terminal shoot (90% of the trees had sylleptic branches with an average length of about 1 cm). Although it was not possible at ORL to measure shoot elongation, lignification and to observe the sylleptic branch apparition due to tree size, we detected that start of transition-wood was produced more closely to Tirg than at PEY.

Late-wood (Etw) formation started at PEY nearly at the same time when radial and apical growth reached 90% (Figure 6). On average, Etw occurred 3 and 6 days later than T90rg and T90hg and Tilig, respectively. At ORL, the transition to late-wood formation (Etw) occurred on average 4 days later than T90rg. When late-wood started, most trees (72%) presented at least 25% of their terminal shoot lignified. At both sites, start to late-wood production seems to be related to the end of radial growth.

At PEY, the extension of late-wood (Elw) ended on Julian day 280 (7<sup>th</sup> October), that was nearly 56, 53, and 28 days later than the T90 of apical and radial growth and of lignification, respectively. It was more concomitant with terminal bud setting (14 days later) and especially with the start of early crown yellowing (stage 1): 2 days before (Figure 6). At ORL, the end of

the annual ring formation (Elw) took place, on average, 63 days after of radial growth reached 90%. At the time late-wood formation was achieved, the lignification of the terminal shoot reached stage 4 (75-100% lignified) in the 96% of the trees; Elw occurred on average 48 days before early crown yellowing (stage 1). The delays between Elw and T90rg at both sites may denote problems with a precise detection of the end of radial growth related to the method used to follow radial growth.

In Table VI, we presented the correlation coefficients between the dates of start and end of wood tissues formation obtained from the ‘dynamic’ profiles and needle phenology and key-dates for radial growth, annual shoot elongation and lignification extension (T10, Ti and T90) obtained from the Weibull growth modeling.

At PEY, weak but still positive and significant correlations were found between the date of start of early-wood formation and the starting date of radial growth (T10rg) and Crown greening (CG30-60):  $r=0.37$  ( $p$ -value:  $<0.001$ ) and  $r=0.24$  ( $p$ -value:  $0.02$ ), respectively. The initiation of transition-wood formation was closely linked to T1rg ( $r=0.44$ ;  $p$ -value:  $<0.001$ ). Although the transition to late-wood (Etw) was later in terms of timing, it was moderately correlated with maximum rate of radial growth ( $r=0.51$ ,  $p$ -value:  $<0.001$ ). The start of late-wood formation was also moderately linked to T90rg ( $r=0.56$ ,  $p$ -value:  $<0.001$ ). The best correlation was found between the end of late-wood formation and T90rg ( $r=0.81$ ,  $p$ -value:  $<0.001$ ). Links between Elw and the end of apical growth (T90hg) or lignification (T90lig) were always weaker than those for radial growth but they were still significant (Table VI).

At ORL, initiation of early-wood was moderately linked to T10rg ( $r=0.42$ ,  $p$ -value:  $0.021$ ). Although the delays in timing between the date of maximum rate of radial increment (T1rg) and start of transition-wood formation (11 days), these traits were significantly and tightly linked ( $r=0.61$ ,  $p$ -value:  $<0.001$ ). Also, a delay of 8 days was detected for transition to late-wood formation (Etw) and T90rg and their correlation was strong ( $r= 0.66$ ,  $p$ -value:

<0.001). Despite of the great difference in terms of timing between the end of ring formation (Elw) and T90rg, these parameters were significantly and moderately correlated (0.56, *p*-value: <0.001). Crown yellowing (CYstate 1 and 2) were weakly linked to the end of ring formation: 0.33 (*p*-value: 0.079) and 0.36 *p*-value: 0.051). Values of correlations found at ORL were always higher than PEY (Table VI).

## 4 Discussion

### Dynamics of ring formation

Unlike many other coniferous genera, *larches* are deciduous, and after turning yellow during autumn, they lose their needles every winter. This special feature puts larches in an unfavorable situation compared to evergreen conifers. Indeed, each year during spring, larches need to produce completely new foliage to start with photosynthesis process and consequently initiate their radial growth. Meanwhile, other evergreen conifers of temperate zone reactivate cambial activity and formation of wood ring as soon as their cold requirements are achieved to break dormancy and adequate temperature thresholds and heat-sums are reached for xylogenesis (Rossi et al., 2007, Seo et al. 2008).

The various combinations of external and internal factors result in the formation of wood tissues noticeably different (Linares et al., 2009). On the one side, cells (early-wood) produced during the first part of the growing season when the cambial meristem is highly active have a large diameter and lumen and thin-wall. On the other side, during the last part of the growing season when cell division in the cambial region declines, tracheids with a narrow diameter and a thick cell wall are then produced.

According to our results, the first cells of early-wood appeared by mid-April when trees had almost 30 to 60 % of their crown height with visible green needles; at that time their terminal bud was still at a very early stage of development. The terminal shoot started elongating almost one month later after radial growth started. The maximum rate of radial growth and the start of lignification of the terminal shoot were observed nearly one month before the start of the transition-wood. At PEY, transition from the early- to late-wood was produced between end-July and mid-August: during this period we registered the maximum rate of lignification of the terminal shoot. Finally the late-wood ended its formation on average, at the end of September nearly at the same time the terminal bud was formed and the first signals of yellowing of the crown were visible. However, at ORL on taller trees late-wood finished its formation more than one month before the start of yellowing of the crown.

Compared to other studies with larch, our results showed an earlier start of the radial growth. For example, Moser et al. (2010) and Rossi et al. (2009a) indicated mid-May and end of May as the starting dates of cell production for European Larch in the Alps. As well, Antonova and Satassova (2002) observed the resumption of cambium activity by mid-May and the development of xylem elements 10 days later than cambial activity reactivation for *Larix sibirica* Ldb. Besides differences in the species (or in genetic origin within species for European larch) studied, environmental differences might also explain our results: elevation (>1300-2100m) or continentality, and thus climate of sites from which trees were observed in these studies, were highly contrasted with our low elevation sites (<500m) under strong oceanic influences. If according to Moser et al. (2010), onset of the growing season is changed by 3-4 days/100 m elevation, then onset of growth in our study would be coherently predicted to be earlier. More precisely, Rossi et al. (2008a) showed that a mean temperature threshold of 8.4°C is requested for cambium reactivation: without any doubt, this threshold was reached much earlier in our two lowland sites, and a bit earlier at ORL than at PEY.

Methodological aspects may also explain in some respect these differences: it is clear that cambial activity resumes before newly differentiated xylem cells could be observed (Savidge and Wareing, 1981). But in our study, the dates of formation of tissues were identified retroactively from fully mature wood, without taking into account the stage of cell maturation. Consequently, dates of initiation and termination of tissue formation could be affected when compared with the cited studies that use anatomical cuts for date assessments.

Bud flushing in larch is acropetal and centrifugal and several days or even weeks usually separate flushing of buds on lower branches from flushing of the terminal bud and elongation of the main shoot. We showed in this study that early-wood cells formation started 6-12 days after buds started flushing at the base of the crown, but full greening of the crown was not necessary before reactivation. At least 30-60% of the crown needed to be green and the terminal bud at the initial stage of flushing before we observed resumption of radial growth. The terminal shoot elongation took place nearly one month later. This result is similar to Rossi et al. (2009a)'s observations on larch which indicated a reactivation of the cambium well in advance on shoot growth; but our results did not totally support their observation that cambium resumption was synchronous with the development (elongation) of needles. In agreement with Moser et al. (2010), we observed also that a large proportion (at least half) of buds of the crown should be flushed before radial growth could start, but in contrast with that study, we did not observe such a wide span of time (3-4 weeks) between needles apparition (50% of all buds broken) and the start of radial growth. In any case, in contrast with results for some other evergreen species (Rossi et al., 2009a), these 3 studies on larch confirm the existence of a link between cambial activity and bud flushing, but not with shoot elongation. It supports at least partly the hypothesis that hormones (auxins) produced by newly formed needles would -with some delay due to basipetal flow-, reactivate the vascular cambium and induce xylogenesis (Larson, 1969).

Transition from early- to late-wood is important in xylogenesis because it marks significant changes at the anatomical level with consequences on wood quality properties (e.g. increase of wood density) but also on physiological and adaptive properties (e.g. water storage). In this study, we considered independently the transition wood from other tissues: indeed we think that the high variability found around the formation of this tissue both in terms of delays or level of intensity corresponds to different cambial responses to factors triggering transition from early- to late-wood. Indeed transition to late-wood occurs each year but not at the same time (Deslauriers et al., 2003). Clearly differences in SLOPE<sub>tw</sub> may be interpreted as the capacity of trees to face and react to given environmental conditions (i.e. drought) and it could be used as an indicator of sensibility of trees to given stress.

Several factors have been suspected to act as possible triggers for transition from early- to late-wood. They include fixed environmental factors such as the photoperiod (Larson, 1962) but also variable ones like the soil water deficit and high temperatures (Kramer 1964; Antonova and Stasova, 1997; Lebourgeois, 2000). However, the high variability for dates of transition as observed in this study among trees and among sites did not comply with any single fixed or variable factors hypothesis; most probably a combination of them is needed. Internal factors connected either to the decrease in auxin concentration gradients as hypothesized by Sundberg et al. (2000) or to internal competition for carbohydrates between apical and cambial growth (Minchin and Lacoïnte, 2005) could also be involved in the transition towards late-wood. In the first case, it is hypothesized that transition to late-wood is synchronized with the cessation of needle or shoot growth whereas in the second, late-wood initiation occurs once shoot elongation is achieved.

While a different situation was described for *Picea* and *Pinus*, Rossi et al. 2009a observed on European larch that the first late-wood cells were produced when needles elongation was achieved but by that time, shoot elongation was just starting; however their



wall thickening occurred well when both needle and shoot growths were completed. In our study, the transition-wood tissue was formed over a period when 90% of terminal shoot growth was completed and its lignification was at its maximum speed rate. But typical late-wood tissue started to form well before the total cessation of shoot growth and bud setting. In concordance with Renninger et al. (2006) on Douglas-fir, we did not find any correlation between transition wood and cessation of terminal shoot growth, which led to suppose that even if late-wood is forming at about the same time the terminal shoot growth is ending, there is no causal relationship between these two phenomena. Instead, relationships were detected with the maximum rate of shoot elongation. Thus, changes in climatic variables may stimulate different organs (apical bud, roots, etc.) to change concentration or gradient of plant regulators in cambial zone controlling the differentiation of tracheids.

Our results showed an earlier transition to late-wood than that observed by Rossi et al. (2009a): in this latter study, first late-wood cells were indeed estimated to be produced at the end of June on European larches at high elevation in the Alps. In contrast for our trees, transition-wood started to form nearly one month later in our lowland sites and even more later (20 additional days) for typical late-wood tissue. This confirms once more that photoperiod alone is not sufficient to explain transition to late-wood. Temperature and water regime should also be considered.

The differences (more than 45 days) in the dates of formation of transition-wood tissue between ORL and PEY in our study could be attributed, following Antonova and Stasova (1997) and Lebougeois (2000), to different water regimes. The stronger soil water deficit recorded at ORL early-June and the higher mean and maximum temperatures would have enhanced the transition process from early- to late-wood. In contrast, at PEY with less stressful water regime conditions, the transition to late-wood started later, but its period of formation was also shorter.

More clearly, in our comparison of irrigated vs non-irrigated trees (same genotype) at ORL, the only major difference observed in ring phenology was the delayed date of transition from early- to transition-wood in irrigated trees. This is coherent with previous findings by Zahner et al. 1964 who observed that formation of early-wood was delayed in irrigated Douglas-firs compared to non-irrigated trees. While irrigated and non-irrigated trees started initiation of early-wood cells at about the same time, the duration of early-wood formation observed in our study was longer in non-limiting water regime conditions. So the start of early-wood formation looks more temperature-dependant in that period of the growing season where water is not limiting; in contrast, the completion of early-wood is obviously dependent on water availability. In the same way, Eilmann et al. (2006) found significant correlation ( $r=0.67$ ,  $p<0.05$ ) between the number of late-wood cells and drought index. Abe and Nakai (1999) showed that living cells around the cambial zone easily lose their turgor even with a small decrease in water potential in a tree stressed by water deficit. And the water stress inhibits cell expansion, producing cells with smaller diameter. In connection with soil deficits, water shortage could trigger an early change from early- to late-wood cells.

The end of xylem production observed in our study was strongly coincident with what is described in the literature. At ORL and PEY, the end of late-wood formation occurred respectively in early-September and early-October. Rossi et al. (2008b) in concordance with Moser et al. (2010) found as well that the end of xylem cell differentiation lasted from mid-September to mid-October in European larch but from high elevation sites. By that time, the terminal shoot growth was completed with setting of terminal buds, but trees were still at an early stage of crown needles yellowing. During that short period, twigs achieved their lignification and late-wood cells the thickening of their cell walls, benefiting then from a full allocation of carbohydrates resources (Renninger et al. 2006).

Differences in xylogenesis between ORL and PEY sites were noticeable for the timing of transition from early- to late-wood and for the end of late-wood formation, but not for the resumption of radial growth. At ORL, the transition-wood formation and the end of late-wood formation were produced at least one month before at PEY. Several factors –alone or in combination- could be advocated. Besides physiographic differences between sites and thereby related climates (differences of altitude of 350 m, 2° for latitude and soil conditions with stronger and longer soil water deficits at ORL), other factors such as the genetic origin (different taxa and populations) and age of trees might also be responsible for these differences.

Rossi et al. (2008b) found an effect of age on the timing and duration of xylogenesis and they observed a delay of 2-3 weeks of cambial activity in younger trees (40-70 yr-old) compared to adult trees (200-350 yr-old) in the same environmental conditions. Though not so important, age differences between PEY and ORL trees could partly explain why young trees at PEY with lower crown had a growing season one month longer than older trees located at ORL.

Although taxa did not differ in the timing of early- and transition-wood, noticeable differences were detected between pure species (JL and EL) and HL for the ending date of late-wood formation: the pure species finished their ring formation before HL. Consistently with a longer growing season, HL showed a moderately higher ring width than JL at PEY supported by a higher speed of tissue formation and finally a higher proportion of late-wood. In contrast, mean ring width in HL did not exceed that in EL at ORL. As proportions of early-, transition- and late-wood were similar between HL and EL, then the slightly higher speed of tissue formation demonstrated by EL resulted in a wider annual ring. According to our knowledge, this is the first study comparing dynamics of wood formation based on a high

number of sampled trees and involving three different genetic entities of larch: European, Japanese and hybrid larches.

Early-wood was consistently through sites wider both in absolute and in relative values than other tissues and on average its formation lasted also longer during the growing season. In any case, early-wood formed at a faster speed than other tissues: for example 3 and 5 times faster than late-wood, respectively at PEY and at ORL. Similarly to the less water stressful site of PEY, the irrigation treatment at ORL enhanced early-wood formation (wider and longer duration) at the expense of late-wood (less wide and shorter period of formation), even though late-wood remains larger in absolute value than in stressful conditions. As a consequence, better site conditions reduced also differences in speed of tissues formation (from 6 to 2 times when comparing early-wood over late-wood formation speeds in un-irrigated vs irrigated trees). But overall on sites with less limiting conditions late-wood proportion remains higher. The longer duration of early-wood formation directly benefits to the enlargement of cells which, according to Dodd and Fox (1990), depends more on the duration than on the rate of cell expansion; it should probably also benefit to the production of a larger number of cells as well. For example, in *Abies balsamea*, Rossi et al. (2009b) observed a higher final number of cells in the irrigated treatment than in the non-irrigated one.

Finally, the high variability observed among taxa and among individual trees for some parameters of radial growth (particularly in the transition zone: duration and speed of formation, density slope, etc) denotes a potential interest of these variables for a better understanding of the link between wood anatomical properties and tree adaptation to climatic factors as well as for selection for better wood properties.

### **Search of ‘external’ marker predictors of xylogenesis**

One of the main objectives of this study was to search for non-destructive, easily visible ‘external’ signs of cambial activity, and thereby of wood formation. Indeed, the alternatives to study wood formation (microsampling (Rossi et al., 2006b) or pinning (Wolter, 1968)) are much more time-consuming and costly. More precisely tree breeders are used to handle hundreds of microdensity profiles for their genetic diversity studies of wood properties but they are more and more interested by exploring these profiles in search of clues for adaptive traits (Martinez-Meier et al., 2008, 2009, Sanchez-Vargas et al., 2007, Wimmer and Grabner, 2000). Indeed density variation along the ring profile can be interpreted as a retroactive recorder of cambial response to environmental factors. Unfortunately, these profiles are static and one would like to date major cambial events. Synchronisation with some external markers would therefore be valuable.

As shown above and in Fig. 6, we have been able to identify some ‘external’ traits synchronous to some wood formation parameters. Early-wood initiation started when the terminal bud was at stage 2-3 of bud break and when 30-60% of the crown was green; transition wood started and ended slightly before and after 90% of radial and apical growths were completed and terminal shoot lignification reached its maximum rate of extension. Finally, late-wood formation was completed a few days after terminal bud setting and first sign of crown needle yellowing. But these relationships were only valid on traits means. At the individual tree level, the links between wood formation traits and external traits remained at best moderate ( $0.4 < r < 0.6$ ) but very often weak ( $r < 0.4$ ) especially for the easiest traits to assess like bud phenology.

So at best, we could situate in time the major steps of formation of the three wood tissues but without enough precision for exploring wood formation dynamics of individual trees. A closer observation of bud phenology, apical growth, terminal shoot elongation, terminal shoot branching but also of needle elongation at a higher frequency than in this study (less than

every 7-10 days, especially during the period identified above) would probably improve relationships. Other climatic markers triggering resumption of primary and secondary meristems, like temperature thresholds (Rossi et al., 2008a) or heat-sums (Gauchat and Pâques, 2011) would probably be more practical but they impose to know individual tree variation around these thresholds and heat-sums if to be used by geneticists and breeders (Gauchat and Pâques, 2011).

## **5 Conclusion**

The pinning technique combined with X-ray microdensity profiles and substitution of the traditional staining step used for “the pinning method” by surface polishing allowed us to efficiently reconstruct the dynamics of wood formation and it has permitted us to process a high number of wood samples, compatible with needs for genetic and adaptive studies.

The dynamics of wood formation in European, Japanese and hybrid larches and relationships between ‘external’ phenological and primary growth events, and wood formation were established. We detected among them some potential predictors of initiation and ending of annual ring wood formation, which could allow situating these events along the growing season. However, they looked not precise enough to date the main steps of wood formation at the individual tree level.

The genetic structure of the experimentation used in this study (progeny trials) will be exploited in another paper to estimate genetic parameters of phenology parameters of wood formation and their possible use in selection.

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Figure 1.- Wood sample preparation. a) View of inserted pins on a sampled tree at the end of 2006 growing season. b) Detail of pins. c and d) reduction process of wood samples by sawing. e) Machine used to polish wood samples. f-1) view of wood sample ready to polish. f-2) view of polished sample.

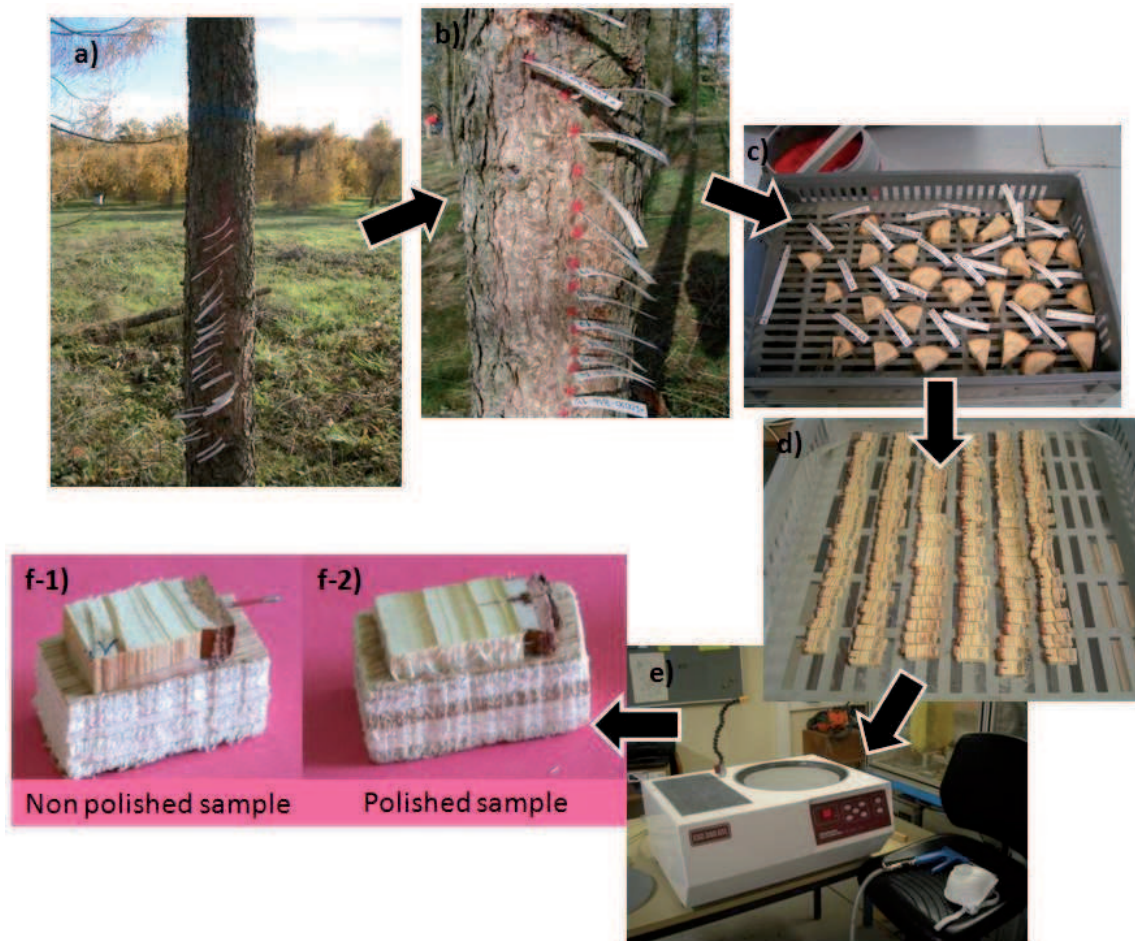


Figure 2.- Image from reflection microscope of 2006 annual ring with injury caused by the pin and its reaction.  $S_1$ : limit between the last layer of late-wood cells belonging to 2005-growing season and the first layer of early-wood cells of 2006-growing season,  $S_2$ : limit placed where the radial rows of tracheids changed their direction because of the injury,  $S_3$ : limit between the last layer of late-wood cells of 2006-growing season and the phloem cells.

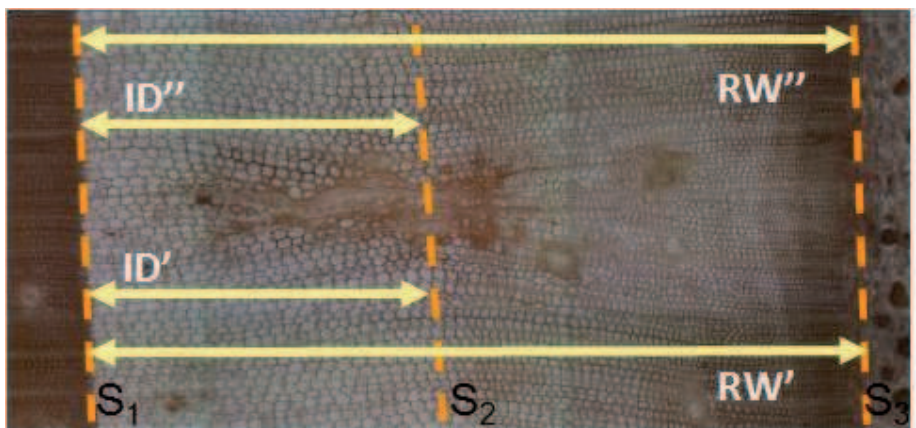
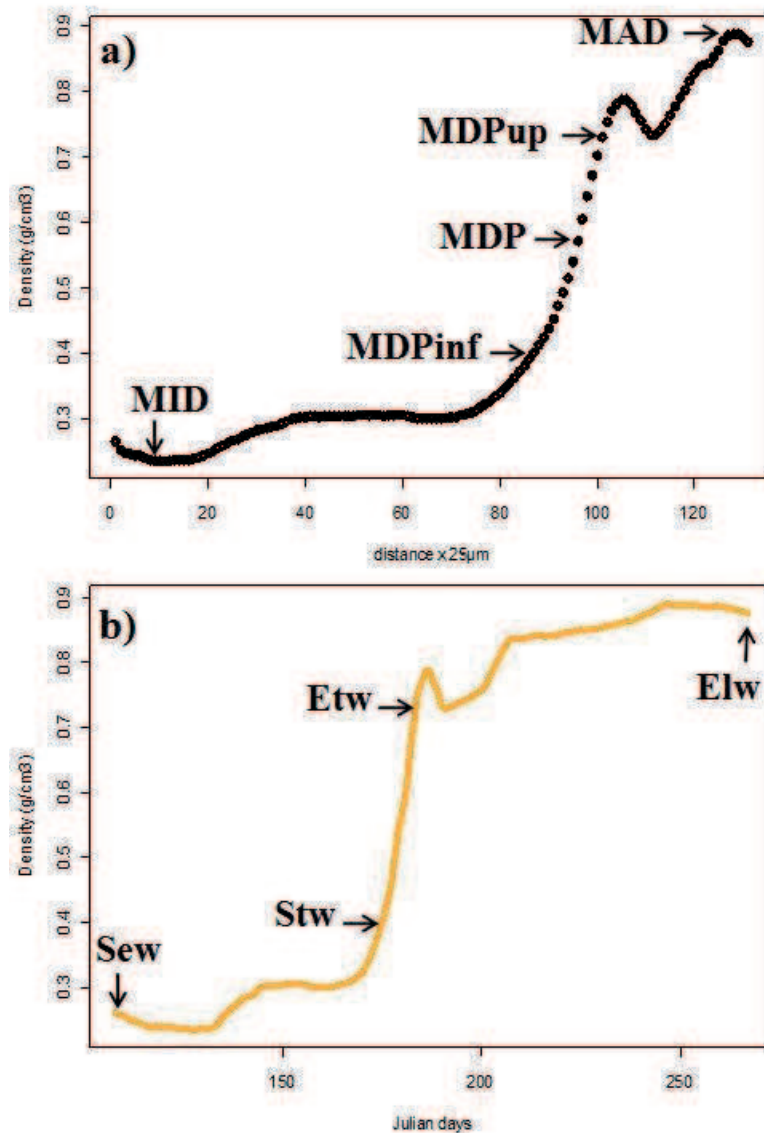
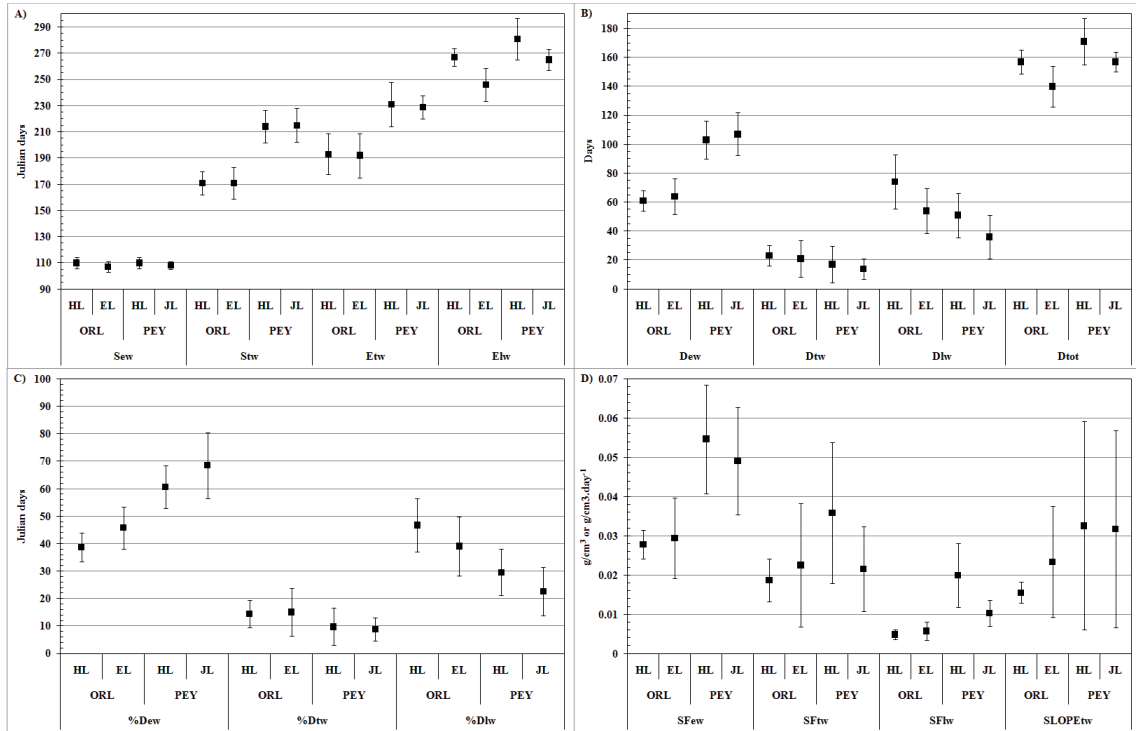


Figure 3.- Example of ‘static’ and ‘dynamic microdensity profile’. a) ‘Static’ microdensity profile, MID: minimum ring density, MAD: maximum ring density, MDP: mean density point, MDPinf: lower limit of transition-wood, MDPup: upper limit of transition-wood. b) ‘Dynamic’ microdensity profile, Sew: starting date of early-wood, Stw: starting date of transition-wood, Etw: ending date of transition-wood, Elw: ending date of late-wood.



**Figure 4.- Timing parameters of 2006-ring formation for EL, JL and HL at PEY and ORL.**





**Figure 5.- Box-plot of dynamics of wood formation in non-irrigated (NI) and in irrigated (I) trees at ORL. Sew: starting date of early-wood, Stw: starting date of transition-wood, Etw: ending date of transition-wood, Elw: ending date of late-wood. Whiskers indicate minimum and maximum values.**

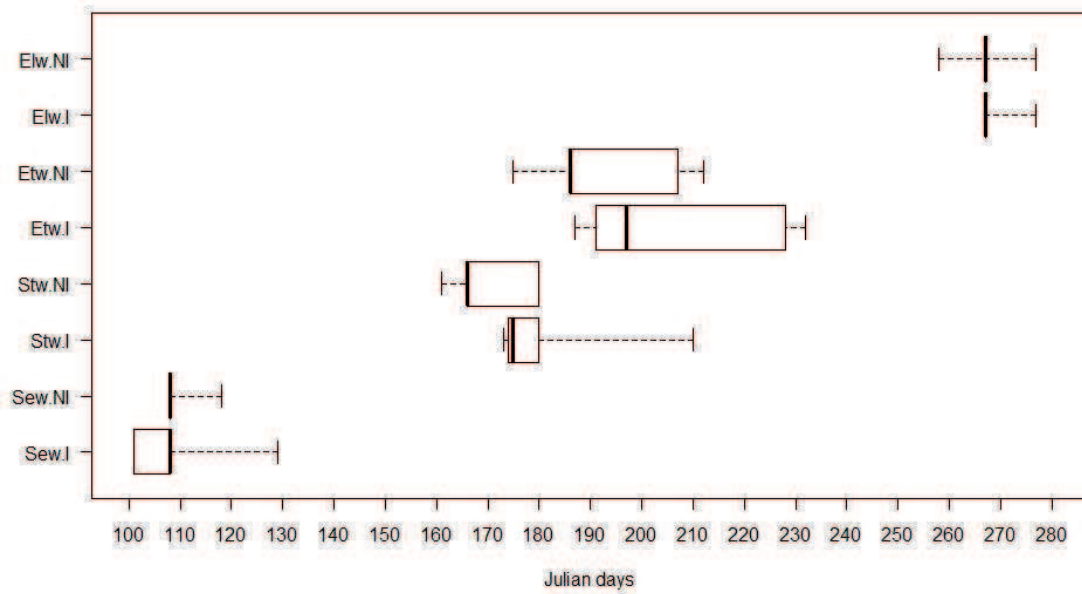
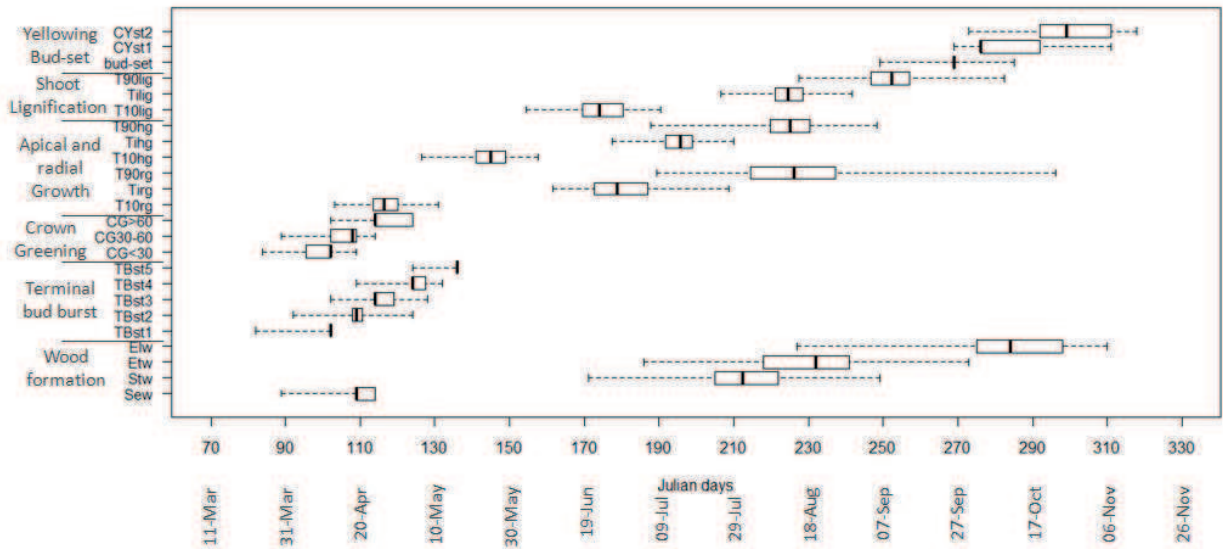


Figure 6.- Box-plot of phenology of ‘external’ parameters and key dates of wood formation process at PEY. Terminal bud burst at different states (TBst1, TBst2, TBst3, TBst4, TBst5), Crown greening proportions (CG<30: 0-30% of crown height green, CG30-60: 30-60% green, and CG>60: more than 60% of tree crown green), apical and radial growth (T10rg, Tirg, T90rg and T10hg, Tihg, T90hg), terminal shoot lignification (T10lig, Tilig, T90lig), bud-set and crown yellowing at initial stages (CYst1, CYst2). Whiskers indicate minimum and maximum values.



**Table I.- Material description.**

Site	Coordinates	Plantation year	DBH mean (year 2006) (centimeters) <sup>1)</sup>	Total height mean (year 2006) (meters)	Taxa <sup>2)</sup>	Number of sampled individuals
PEY (455 m a.s.l.)	Lat N 45° 46' 5''	2003	4.7	4.2	HL	88
	Long E 1° 45'				JL	6
ORL (106 m a.s.l.)	Lat N 47° 49' 1'	1995	12.7	10.6	HL	10
	Long E 1° 54'				EL	25

<sup>1)</sup> At Peyrat-le-château DBH was measured at 1 meter from ground.

<sup>2)</sup> HL: *Larix decidua* x *L. kaempferi*, JL: *L. kaempferi* and EL: *L. decidua* 'polonica'.

**Table II.- Mean, standard error (SE) and coefficient of variation (CV%) of Weibull parameters for radial growth (rg), Global crown greening (CG30-60), Terminal bud flushing (TB) and Crown yellowing (CYstate1 and 2) at Peyrat-le-Château and Orléans. Bud-set, shoot elongation (hg) and lignifications extension (lig) at Peyrat-le-Château. Julian day of 10 and 90 % (T10, T90)and maximal rate (Ti) of growth or lignification.**

variable code	Unit	Site	Mean	SE	CV (%)
CG30-60	Julian day	ORL	107	0.59	3.04
		PEY	105	0.66	6.06
TB	Julian day	ORL	108	1.70	8.59
		PEY	109	0.57	5.10
CYstate1	Julian day	ORL	298	1.51	2.78
		PEY	282	1.19	4.09
CYstate2	Julian day	ORL	305	1.53	2.75
		PEY	298	1.38	4.5
Bud-set	Julian day	PEY	266	0.77	2.81
T10rg	Julian day	ORL	119	0.62	2.88
		PEY	117	0.53	4.39
Tirg	Julian day	ORL	160	1.38	4.72
		PEY	180	1.05	5.56
T90rg	Julian day	ORL	184	2.34	6.97
		PEY	227	2.05	8.56
T10hg	Julian day	PEY	145	0.56	3.74
Tihg	Julian day	PEY	195	0.64	3.16
T90hg	Julian day	PEY	225	1.00	4.33
T10lig	Julian day	PEY	175	0.72	4.00
Tilig	Julian day	PEY	225	0.60	2.60
T90lig	Julian day	PEY	252	0.95	3.65

**Table III.- Comparison of 2006 annual ring components from 'static' microdensity profiles at PEY and ORL. Mean, standard deviation (SE) and percentage of variation coefficient (CV%) of ring width (l), early transition and late-wood distance (ewd, twd and lwd) and their proportions (ewp, twp and lwp).**

<b>Variable</b>	<b>Unit</b>	<b>Site</b>	<b>Mean</b>	<b>SE</b>	<b>CV (%)</b>
<b>l</b>	<b>mm</b>	<b>ORL</b>	<b>2.55</b>	<b>0.15</b>	<b>33</b>
		<b>PEY</b>	<b>7.10</b>	<b>0.20</b>	<b>27</b>
<b>ewd</b>	<b>mm</b>	<b>ORL</b>	<b>1.83</b>	<b>0.11</b>	<b>34</b>
		<b>PEY</b>	<b>5.55</b>	<b>0.14</b>	<b>25</b>
<b>ewp</b>	<b>percentage</b>	<b>ORL</b>	<b>71.76</b>	<b>1.06</b>	<b>8</b>
		<b>PEY</b>	<b>79.11</b>	<b>0.75</b>	<b>9</b>
<b>twd</b>	<b>mm</b>	<b>ORL</b>	<b>0.39</b>	<b>0.04</b>	<b>59</b>
		<b>PEY</b>	<b>0.56</b>	<b>0.05</b>	<b>93</b>
<b>twp</b>	<b>percentage</b>	<b>ORL</b>	<b>15.17</b>	<b>1.00</b>	<b>36</b>
		<b>PEY</b>	<b>7.46</b>	<b>0.63</b>	<b>84</b>
<b>lwd</b>	<b>mm</b>	<b>ORL</b>	<b>0.33</b>	<b>0.03</b>	<b>52</b>
		<b>PEY</b>	<b>0.99</b>	<b>0.06</b>	<b>58</b>
<b>lwp</b>	<b>percentage</b>	<b>ORL</b>	<b>13.07</b>	<b>1.03</b>	<b>43</b>
		<b>PEY</b>	<b>13.43</b>	<b>0.59</b>	<b>43</b>



**Table V.- Differences in timing of wood formation during 2006 growing season hybrid larch at two watering conditions (irrigated, IRR and non-irrigated, NON-IRR). General statistical parameters of Initiation, ending, proportional durations and speed formation of early, transition and late-wood and change of density from early to latewood per unit time (SLOPE<sub>tw</sub>). Numbers in italics indicate no significant differences among treatments,  $\alpha$ -level= 0.05.**

Variable	Unit	Irrigation	Mean	SE	CV (%)
l	mm	NON-IRR	2.45	0.17	15.93
		IRR	4.18	0.84	44.69
ewd	mm	NON-IRR	<i>1.69</i>	<i>0.12</i>	<i>16.24</i>
		IRR	<i>2.22</i>	<i>0.49</i>	<i>49.57</i>
ewp	%	NON-IRR	<i>68.79</i>	<i>2.27</i>	<i>7.37</i>
		IRR	<i>53.35</i>	<i>3.02</i>	<i>12.67</i>
twd	mm	NON-IRR	0.41	0.06	34.75
		IRR	0.95	0.27	64.27
twp	%	NON-IRR	16.53	1.89	25.63
		IRR	22.16	2.62	26.49
lwd	mm	NON-IRR	0.36	0.05	32.34
		IRR	1.01	0.24	53.28
lwp	%	NON-IRR	14.68	2.32	35.4
		IRR	24.49	4.35	39.73
Dew	day	NON-IRR	<i>61</i>	<i>3</i>	<i>11.76</i>
		IRR	<i>73</i>	<i>3</i>	<i>9.69</i>
Dtw	day	NON-IRR	23	3	30.91
		IRR	25	6	55.41
Dlw	day	NON-IRR	74	8	25.23
		IRR	62	11	38.69
Dtot	day	NON-IRR	157	4	5.18
		IRR	160	6	7.91
%Dew	%	NON-IRR	38.68	2.35	13.59
		IRR	46.17	3.46	16.76
%Dtw	%	NON-IRR	14.53	2.22	34.17
		IRR	15.41	3.64	52.78
%Dlw	%	NON-IRR	46.79	4.37	20.9
		IRR	38.41	5.98	34.82
SFew	mm.day <sup>-1</sup>	NON-IRR	0.02784	0.00161	12.96
		IRR	0.03083	0.00659	47.8
SFtw	mm.day <sup>-1</sup>	NON-IRR	<i>0.01865</i>	<i>0.00245</i>	<i>29.35</i>
		IRR	<i>0.03974</i>	<i>0.00706</i>	<i>39.7</i>
SFlw	mm.day <sup>-1</sup>	NON-IRR	<i>0.00483</i>	<i>0.00057</i>	<i>26.42</i>
		IRR	<i>0.01551</i>	<i>0.00233</i>	<i>33.59</i>
SLOPE <sub>tw</sub>	g/cm <sup>3</sup> .day <sup>-1</sup>	NON-IRR	0.01553	0.00121	17.46
		IRR	0.01817	0.00352	43.28

**Table VI.- Based on PEY (A) and ORL (B), correlation coefficients between dates of start of early- and transition-wood (Sew, Stw), end of transition- and late-wood (Etw, Elw) and dates of 10%, 90% and maximum rate of radial growth (T10rg, T90rg, Tirg), annual shoot elongation (T10hg, T90hg, Tihg), lignifications extension (T10lig, T90lig, Tilig) from Weibull model and phenology of needles.**

**A)**

	Sew	Stw	Etw	Elw
	0.37			
T10rg	(<0.001)			
T10hg	0.17 (0.112)			
CG30-60	0.24 (0.020)			
TB Flushing	-0.04 (0.693)			
Tirg		0.44 (<0.001)	0.51 (<0.001)	
Tihg		-0.05 (0.630)	0.02 (0.813)	0.27 (0.008)
T10lig		-0.14 (0.170)	-0.04 (0.708)	-0.10 (0.354)
Tilig		-0.07 (0.524)	0.01 (0.960)	0.17 (0.108)
			0.56 (<0.001)	0.81 (<0.001)
T90rg				
T90hg			0.01 (0.905)	0.23 (0.023)
T90lig			0.01(0.919)	0.22 (0.034)
Bud-set			0.07 (0.475)	0.10 (0.354)
CYstate 1			0.13 (0.229)	-0.23 (0.028)
CYstate 2			-0.09 (0.402)	-0.21 (0.043)

**B)**

	Sew	Stw	Etw	Elw
T10rg	0.42 (0.021)			
Crown Greening	-0.02 (0.908)			
TB Flushing	0.04 (0.824)			
Tirg		0.61 (<0.001)	0.64 (<0.001)	
T90rg			0.66 (<0.001)	0.56 (0.001)
CYstate 1				0.33 (0.079)
CYstate 2				0.36 (0.051)



## **Chapter 2**

[Article 2]: Impact of soil water shortages on growth, heartwood development and wood density in Larch.

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**TREES - STRUCTURE AND FUNCTION**

**IMPACT OF SOIL WATER SHORTAGES ON GROWTH, HEARTWOOD DEVELOPMENT AND WOOD DENSITY IN LARCH**

Gauchat, M. E.<sup>a\*</sup>, Pâques, L. E.<sup>b</sup>.

<sup>a</sup> INTA E.E.A. Montecarlo. Av. El Libertador 2472. CP3384. Montecarlo. Misiones. ARGENTINA. [mgauchat@montecarlo.inta.gov.ar](mailto:mgauchat@montecarlo.inta.gov.ar)

<sup>b</sup> INRA Orléans. Unité d'Amélioration, Génétique et Physiologie des Arbres Forestiers. 2163 Avenue de la Pomme de Pin. 45075. Orléans Cedex 2. FRANCE. [luc.paques@orleans.inra.fr](mailto:luc.paques@orleans.inra.fr)

\*Corresponding author:

Tel-fax: +54 3751 480512 (extension 126).

Address: Av. El Libertador 2472.

CP3384.

Montecarlo. Misiones.

ARGENTINA.

E-mail address: [mgauchat@montecarlo.inta.gov.ar](mailto:mgauchat@montecarlo.inta.gov.ar)

**Key-words:** *Larix*, irrigation, water stress, growth, wood density, heartwood, after-effect

## **ABSTRACT**

The impact of soil water shortage on growth and wood properties -including heartwood extension -was studied on European and hybrid larches. A fertilised-irrigated treatment was used as the reference against which effects of soil water shortages along growing seasons were measured in non-irrigated (fertilised or not) plots. The heat-drought wave which occurred during the experimentation in August 2003 allowed focussing on the direct impact of an exceptional stress and its after-effects.

After 6 years, larch growth was severely affected by these frequent soil water shortages: apical and radial growths were similarly impacted as well as earlywood and latewood ring widths. Trees flushed earlier; their form factor was higher. In addition, a severe reduction in heartwood and sapwood sizes was observed. But neither the proportion of heartwood nor the speed of heartwood formation were modified. As well, drought stress had no influence on ring wood density (and on its components) even if ring width characteristics were changed.

The 2003-heat-drought had a strong impact on tree growth and lasting effects. Height growth was less affected than radial growth but after-effects were more pronounced on height than on radial growth. The ‘temperature’ stress observed in the irrigated plot as opposed to the ‘global’ stress (including also drought) recorded in the non-irrigated plots had a similar severe impact (in relative values) on radial growth and earlywood width but it was much less harmful on height increment and latewood width. After-effects were less pronounced in irrigated vs non-irrigated plots for global radial growth and earlywood ring width, similar for height growth but more severe for latewood ring width.

European larch and hybrid larch reacted differently to soil water deficits: overall the experiment, European larch appeared globally more sensitive than the hybrid. During the 2003-heat-drought wave and after, European larch reacted similarly in both environments with a serious growth reduction. Hybrid larch had a more contrasted behaviour according to water regimes: irrigated trees had nearly no height growth reduction during 2003 compared to non-irrigated trees and showed also a positive recovery for radial growth whereas it remained negative for non-irrigated-trees.

This paper stresses the need for a study of cambial activity rhythms in relation to climatic events in order to better understand the impact of climatic stress on wood formation and their

consequences on anatomical and physical wood properties and thereby on related adaptive traits.

## 1. Introduction

European larch (*Larix decidua* Mill.) naturally occurs in medium to high mountainous ranges across Europe. Because of the high potential of that species both in terms of production and wood quality, larch forests have been extended well-beyond European larch native range, both northwards and westwards. Results from species and provenance trials revealed strong genetic variation in behaviour: on one side, the better survival and growth of Japanese larch (*Larix kaempferi* (Lamb.) Carr.) in strictly oceanic climates and, on the other side, the excellent behaviour of European larch ecotypes from Central Poland (*polonica*) and from the Sudetan Mountains (*sudetica*) over a broad range of ecological conditions (Schober, 1985).

In France, these two ecotypes of European larch are used in reforestation outside the Alps either from seed directly imported from Central Europe or from local seed orchards. Besides their vigorous growth, both ecotypes are appreciated for their better resistance to canker (Pâques et al., 1999). In addition, original wood properties of some provenances are becoming highlighted like their high wood density and mechanical properties (Pâques and Rozenberg, 1995) and several favourable properties of their heartwood: size (Pâques, 2001), extractives content (Gierlinger et al., 2002) and natural durability (Curnel et al., 2008).

However, long-distance transfer of forest reproductive material from Central Europe with a more continental climate to French oceanic regions might be faced with adaptive problems not necessarily observed and observable during the life-time of a provenance test. As an example, the high susceptibility of *polonica* larch to *Meria laricis* Vuill. in western low Massif Central Mountains has been revealed only recently and highlighted by more frequent mild and humid springs. Even if Central Europe larch has shown a broad site plasticity (Giertych, 1979; Schober, 1985) and *polonica* larch in particular is considered by Kral (1966, 1967) as one of the most drought resistant ecotypes of European larch, question of its adaptation to regions marked by a much more variable (temperature and rainfall) and drier climate is raised.

In fact, while the mean annual rainfall in Central Poland is usually much lower than in the reforestation zones in France (600 vs >1000 mm), rainfall distribution over the growing season in Poland is much different with a peak of precipitations concentrated in summer months. In French reforestation zones, summer droughts or soil water deficits are frequent

and because of climate change, even more frequent hot and dry summers are predicted with an increasing number of exceptional climatic events like the heat-drought wave of 2003 in Western Europe. Consequences on larch survival and growth are unknown. Part of the answer will come from long-term observations in a multi-site progeny trials network established across France and Poland in the mid-nineties. But some preliminary results could already be obtained from farm-test experimentations using contrasting water regimes.

Experimentation through field irrigation and/or more rarely through drought stress treatments have been conducted on several conifers (e.g. loblolly and radiata pines, Douglas-fir) and have shown the high sensitivity of these species to soil water availability or reversely deficiency. Strong and coherent negative impacts have been reported for growth traits (e.g. Albaugh et al. 2004; Brix, 1972; Hsu and Walters 1975; Nicholls and Waring, 1977; Waterworth et al. 2007; Wielinga et al., 2008) but also for stem form (Wielinga et al., 2008). More controversial results are found for wood properties: according to traits (anatomical, physical and mechanical properties) and species, effects could be positive, negative or null (e.g. Albaugh et al., 2004; Brix, 1972; Nicholls and Waring, 1977; Hsu and Walters, 1975; Wielinga et al., 2008). That is particularly the case for earlywood/ latewood widths, specific gravity and heartwood/sapwood extension.

The present study used different regimes of irrigation i) to study the possible impacts of annually and seasonally recurrent soil water shortages on growth traits, phenology, stem form, heartwood extension and wood density and, ii) to compare the behaviour of European larch versus European x Japanese hybrid larch. Finally, we took advantage of the exceptional heat-drought wave which occurred in Europe during summer 2003 (Bréda et al., 2004; Levinson and Waple, 2004; Meehl and Tebaldi, 2004) to evaluate the direct but also indirect (after-effects) impacts of such extreme events on growth of larch.

## **2. Material and methods**

### **2.1. Material and treatments**

A progeny farm-field trial of larch was the basis for the experimentation. It was established at the INRA- nursery in Orléans (Long. 1°54'43"E, Lat. 47°49'31"N, Alt. 106 m) during spring 1995. Twenty half-sib progenies of European larch (*polonica*, origin: Swinia Gora, PL),

coded 'EL', and one hybrid progeny (origin: hybridisation seed orchard FP201DK), coded 'HL', were evaluated under a complete randomized block design (20 blocks, single tree plots). The test was planted with 2-yr-old seedlings at a 1.20 x 1.20m-spacing with one border line surrounding the trial. The site is characterised by a deep coarse sandy soil (over 65%) with gravels and a low percentage of organic matters (less than 1.2%). Its fertility is also low due to deficiencies in P, K, and Mg and in several oligo-nutrients. Due to high soil drainage, trees were irrigated during summer time from plantation up to 1999: it is classically practised in this nursery on young plants to prevent any potential drought damage.

Five years after plantation, the stand density was reduced by thinning down to 1521 stems/ha: 89 trees were left and further evaluated: they included 73 EL (7 progenies) and 16 HL (1 progeny). The stand was then divided into 3 plots and 2 different treatments intending to speed up growth were applied. They included i) fertilisation and irrigation on 7 contiguous blocks, ii) fertilisation alone on 6 contiguous blocks. The remaining 7 blocks were reserved as control (no fertilisation and no irrigation). They were respectively coded 'FI', 'F' and 'C'.

Based on a soil chemical analysis, a fertilisation was carried out to correct deficiencies in some nutrients. Fertilisation was first applied in FI and F plots on June 6, 2000 with potassium sulphate (733 kg/ha), ammonium phosphate (283 kg/ha), and ammonium nitrate (95 kg/ha); a mixture of copper sulphate (136 gr), zinc sulphate (300 gr) and solubore boron (500 gr) was prepared in 55 litres of water and applied at the same time. A second fertilisation with ammonium nitrate (95 kg/ha) was applied one month later.

For the FI treatment, drop-irrigation was applied daily and regularly during the vegetation periods of years 2000-2006, except during 2002 because of a mistake in field operational watering schedule. Irrigation was started when frost risks were considered low enough and when most trees were fully flushed, that is between mid-May to mid-June; it was stopped arbitrarily by the end of September each year. This explains why water supply varied from year to year. [Table I](#) summarizes climatic conditions and artificial watering of the plots during the study. Soil water deficits were a posteriori estimated using information from soil physical analysis and rainfall records. To calculate deficits of a given period of time (i.e. decades) or  $D_j$  (Eq. 1) we applied the model used by Meteo-France. This model requires daily potential evapotranspiration (ETP) calculated by Penman method and rainfall (RR) as input climatic data. Additional parameters include a coefficient of crop cover ( $K_c$ ) directly related to the

type of vegetation covering the site and the maximal capacity of water retention by the cultivable soil profile (RU). The RU is divided into two types of reserve, superficial (Rsurf) and deep (Rprof). All these values (RU, Rsurf and Rprof) represent the theoretical-maximal capacities but for a given decade the true water available for trees is summarized by the total soil water reserve (Rtot). The latter is the sum of Rsurf and Rprof for a given decade. Real evapotranspiration (ETR) is calculated in two steps. If there is enough water availability in Rsurf, we applied Eq 2: ETR is equal to the maximal evapotranspiration (ETM) and Dj for this decade does not exist. But, if the Rsurf was exhausted, we applied Eq. 3 to obtain ETR and the soil water deficit is calculated by Eq. 1.

$$D_j = ETM - ETR \quad \text{Eq. 1}$$

$$\left\{ \begin{array}{l} \text{If } R_{surf} > 0 \\ ETR = ETM \end{array} \right. \quad \text{and} \quad ETM = K_c \times ETP \quad \text{Eq. 2}$$

$$\left\{ \begin{array}{l} \text{If } R_{surf} < 0 \\ ETR = \frac{R_{tot}}{(RU - R_{surf})} \times ETM \end{array} \right. \quad \text{Eq. 3}$$

Decade-based and cumulated deficits over the growing season are presented in [Figure 1](#) and in [Table I](#). Supply of water in the ferti-irrigated plot aimed to compensate soil water deficits. It proved to be efficient during most of the year excepted at the start of 2000 and 2001 growing seasons ([Figure 1](#)), because of a late start of irrigation.

## 2.2. Measurements

Since plantation, total height and girth at breast height of trees have been regularly measured before the trees were finally felled in March 2007. Girths were then recorded every meter from the stump and annual shoot increments were delimited (if needed with the help of available total height data) and measured. Oriented disks samples (3-4 cm thick) were collected every meter from stump to top for heartwood extension determination. An additional disk at 1.30 m was collected for microdensitometry analysis.



Heartwood sizes along the stem were studied from disk analysis: a picture of each disk was first obtained and analysed with Image-J software. Heartwood was distinguished from sapwood on the basis of colour differentiation. From each disk, the total area (under bark) and heartwood area were measured. The total tree volume and heartwood volume were obtained by summing up the volumes of each 1 m-long log using Smalian's formula, plus the volume of the last log of the tree till the tip considered as a cone. The sapwood volume was calculated as the difference between total tree and heartwood volumes, and relative proportions of heartwood extension were expressed in percentage of total BH area, of total tree height and of total tree volume.

Combining these heartwood data with measurements already obtained from diametrical increment cores collected at breast height on the same trees in December 2004, the speed of heartwood extension was computed as the mean length and number of rings of heartwood formed during the 2004-2006 period.

Finally, after preparation of a 2mm-thick board from each tree and their X-ray process, microdensitometry analysis allowed the determination of classical wood parameters. They included: ring width (RW), early (EW) and latewood (LW) widths, latewood proportion (LWP), overall density (D), density of EW (EWD) and LW (LWD) and density contrast (maximum minus minimum ring densities) for rings of years 2000 to 2006.

Additional traits included bud flushing, observed in April 29, 2005 with a subjective scoring system (0= dormant to 5 = start of elongation, more details in Gauchat and Pâques, 2011) and a stem form factor calculated as the ratio of the total volume of the tree on the corresponding cylinder volume.

### **2.3. Data analysis**

After validation of hypothesis, an analysis of variance was performed on individual tree data to test for treatments effects. The following model was used:

$$Y_{ijk} = \mu + T_i + B/T_{ij} + \epsilon_{ijk}$$

Where  $Y_{ijk}$  is the observation of tree  $k$  in block  $j$  nested in treatment  $i$ ,  
 $T_i$  is the effect of treatment  $i$ , (fixed)  
 $B/T_{ij}$  is the block  $j$  nested in treatment  $i$  effect, (fixed)  
 $\epsilon_{ijk}$  is the residual effect.

A separate analysis of variance was performed on data prior to year 2000 and after 2000 to test for the absence of differences among plots before the start of the experiment. The absence of autocorrelation effects between growth performances before and after the start of the experiment was confirmed by an analysis of covariance using 1999-data as covariates (results not shown).

For variables expressed as proportions (heartwood and latewood), transformed data using an arcsine transformation were also used, but similar ANOVA results were found. As well, for heartwood extension (expressed in number of rings), differences among treatments were tested using a chi-square test: the same results as those from the ANOVA were also found.

Due to large differences in numbers of individuals per taxa and heterogeneity of variances, it was not advisable to directly integrate the taxa effect into this analysis but the analysis used the whole set of data. When significant differences among treatments were observed, treatment means were compared using the Bonferroni test.

A second set of analysis was performed for each species separately on data adjusted to any significant block effects in order to compare treatments and derive trends of taxa reaction to treatments.

Despite drought stress on plants were not specifically measured, we assumed the growth reduction of non-irrigated trees compared to ferti-irrigated ones to be a direct consequence of soil water deficits experienced along this study. We quantified this stress by computing the differences between performances of the well-watered trees from the FI-treatment minus those of non-irrigated-trees for both HL and EL.

To evaluate the direct effects of the 2003 year-heat-drought wave and their after-effects on growth increments, 2003-results were compared to the average results over 2 previous years (2000-2001). We focused only on 'typical' (average) years prior 2003 to avoid 2002 watering

abnormalities. Tree recovery was considered by comparing performances during 2004, 2005 and 2006 with those of 2003.

### **3. Results**

#### **3.1. Soil water deficits during the growing seasons**

Each year during the experimentation, trees were submitted to soil water deficits, roughly extending from the last decade of May up to the last decade of September ([Figure 1 A](#)). However, their occurrence along the growing season, their duration and intensity were highly variable among years. During 2000, 2001 and 2002, cumulated water shortages were low compared to those of 2003 and 2006; 2004 and 2005 were intermediate ([Table I](#), [Figure 1 A](#)). In 2001 and 2004, water depletion occurred early in the season (2<sup>nd</sup> and 3<sup>rd</sup> decades of June) but more lately in 2002 and 2003 (1<sup>st</sup> and 2<sup>nd</sup> decades of August). Years 2003 and 2006 were also characterized by long, non-discontinuous periods of deficits (over 8 decades), coupled also to extreme temperatures in 2003 but delayed compared with 2006.

Supply of water in the FI-plot proved to be efficient to prevent water deficits during most of the year excepted at the start of 2000 and 2001 growing seasons ([Figure 1 B](#)), because of a later start of irrigation. Problems with watering during 2002 were previously mentioned.

#### **3.2. Growth, stem form and bud flushing**

Growth traits were not significantly different among the 3 plots before the application of the treatments in 2000 ([Table II](#), [Figure 2 A, B and C](#)). Afterwards, trees from the FI-treatment had a significantly higher mean BH girth, total height and stem volume. While the F-treatment produced slightly more vigorous trees, the difference with the C-treatment was never significant.

At the end of the experimentation, FI-trees showed superiority over control trees of 30.2% and 27.5% respectively for BH girth and total height, and of over 75% for stem volume. More precisely, if one considers only the period of application of the treatments, these figures reached 40.9% and 57.1% respectively for girth and height.

The FI-treatment boosted height and ring annual increments much more than the F-treatment alone and nearly all along the experiment (Figure 2 B and C). However, both height and ring growth in the FI-plot dropped down to the level of that observed in F-and C-plots as a consequence of the absence of irrigation in 2002.

The stem shape also differed among treatments (Table II). FI-stems had a smaller form factor (0.54 - closer to a 2<sup>nd</sup> degree paraboloid) than that of the control trees (0.61 – more like a 3<sup>rd</sup> degree paraboloid). Irrigation had also an influence on bud flushing as FI-trees flushed significantly later than trees from the other two treatments. F- and C-treatments did not differ significantly among themselves for bud flushing.

### **3.3. Heartwood size and extension**

Total heartwood but also sapwood volumes were significantly larger for FI-trees than for other trees (Table II): heartwood volume of FI-trees reached on average 31.7 dm<sup>3</sup> out of a total volume of around 80.8 dm<sup>3</sup> compared to less than 20.0 dm<sup>3</sup> for trees from the other treatments. FI-trees produced nearly 66% more heartwood in absolute value than other trees. Fertilisation alone had no effect. But, the proportion of total heartwood volume did not differ among the 3 treatments: it represented about 40% of total tree volume.

The radial extension of heartwood at breast height appeared larger in absolute value in FI-trees than in other trees while the proportion of heartwood over the total BH area was higher in control trees than in FI-trees. But in fact, the differences among treatments were not statistically significant ( $p > 0.05$ ). The vertical distribution of heartwood along the stem differed significantly among treatments. In absolute value, heartwood reached a higher height along the stem in FI- and F-trees than in C-trees. In relative value, it developed up to between 67 and 74% of total height but these proportions did not differ significantly among treatments.

At the end of the experimentation, trees had similar numbers of heartwood rings overall treatments with an average of 10.7 rings out of 14. Speed of heartwood extension along the radius did not seem to be influenced by the treatments during the period of observation. Indeed, no significant difference among treatments was observed neither for the number of heartwood rings formed during 2004-2006 (on average 1.4 ring per year) nor for the heartwood radius extension (on average 7 mm/yr).

### 3.4. Ring width and wood density

The mean annual ring width of FI-trees over the treatment period was significantly larger than that of F-trees, which was superior to that of C-trees (Table II). The same ranking was observed for EW and LW widths. The ring width components of FI-trees were on average 1.5 times larger than in control trees and these differences were significant. Fertilisation alone significantly improved EW width (1.4 times larger than in C-trees) but not LW width. Finally, the latewood proportion was similar for FI- and C- trees (over 26%) and significantly larger than for fertilised trees.

No treatment had a significant impact on wood density (Table II). Overall ring density but also EW and LW densities were not significantly different among treatments. On average, these parameters reached 455, 372 and 708 kg/m<sup>3</sup> respectively. In the same way, the within ring density contrast did not differ among treatments.

### 3.5. Effects of soil water deficits effects on EL and HL

Soil water deficits affected both taxa mostly in the same direction but globally less intensively hybrid larch than European larch (Figure 3 A, B and C). In fact, excepted for growth traits, differences between FI- and C- trees were not significant for HL whereas they were for many more traits for EL (Table III).

While the non-irrigated treatment affected both taxa by about the same intensity for total height (-36%), its impact on BH girth and above all on stem volume was more severe for EL (-29 and -55.2% respectively) than for HL (-20.2 and -18.9%) (Figure 3 A). Even more contrasted were the differential impacts on ring growth components (-40% vs -22% or less) and heartwood volume (-61.6% vs -2%) of EL and HL respectively. In HL, the mean annual LW width was more affected by the water deficits stress than EW width (-23% vs -6%); as well, sapwood volume was more reduced than heartwood volume (-30.4% vs -2%). By contrast, the impact of the non-irrigated treatment on EL was similarly as severe on EW as on LW widths and on heartwood as on sapwood volumes (Figure 3 B and C).

### 3.6. Impact of the year 2003-heat and drought stress on growth

Symptoms of the stress created by the year 2003-heat and drought wave were already visible on trees by mid-August 2003 with overall crowns of non-irrigated trees turning yellow-brown. The impact was strong on both height and radial growth (Table IV B, Figure 4) but it was much stronger on radial growth compared to height growth. Compared to increments of preceding years (2000-2001), the 2003-shoot length was reduced on average over treatments by 27% whereas 2003-ring width was reduced by as much as 51%. Both EW and LW ring widths were seriously affected by 2003-climatic conditions (Table IV B) with an overall mean reduction of 49% and 56% respectively.

Following 2003, trees reacted differently for height and radial growth: whereas height increments remained smaller than in 2003 up to 3 years after the 2003-stress, radial growth increased in 2004 by nearly 54% over that of 2003, but it had not yet recovered its level prior to 2003 at the end of the experiment (Table IV A and B, Figure 5 A). As well, both EW and LW widths increased but notably more LW (+139% in 2004) than EW (+38%) (Table IV B, Figure 5 B).

In general, for both apical and radial increments, FI-trees had maintained during and after the 2003-heat-drought wave a significantly superior growth over non-irrigated trees (Figure 2 B and C). It should also be noted the strong effect of the lack of irrigation in FI-treatment during 2002: both height increment (Figure 2 B) and ring width (Figure 2 C) dropped down to levels lower than for C-trees.

The relative impact of 2003-year on 2003-growth was overall similar for the FI and non-irrigated treatments for radial growth but not for apical growth (Figure 4): for this trait, the impact was more than twice larger for non-irrigated trees compared to FI-trees (-38% and -17% respectively). Differences among treatments were also observed for ring width components: while the same relative impact was noticed for EW width, LW width of the non-irrigated treatment was significantly more reduced (-73%) than that of the FI-treatment (-39%). As a consequence, latewood proportion increased in FI-trees whereas it decreased in non-irrigated trees. Post-2003 growth was also different among treatments: FI-trees recovered more slowly than non-irrigated trees for height increment and for LW width but the opposite was observed for radial growth and EW width (Figure 5 A and B).

Some major differences of reaction to the 2003-heat and drought wave were also noted among taxa. Whereas EL and HL relative loss of radial growth was similar among treatments, the importance of height increment decrease was dependant on both taxa and treatments. For EL, height increment reduction was moderate and similar across treatments (around 26%) while for HL, it was very strong for the control treatment (49%) but growth was not significantly affected in the FI-treatment ([Figure 4](#)). For ring components, the impact on irrigated and non-irrigated trees was similar among taxa excepted that LW width showed a relatively stronger decrease for HL compared to EL in the FI-treatment.

Post-2003 effects on height increments were strong and negative and of similar intensity over treatments for both taxa but they were quite different on radial growth ([Figure 5 A](#)). Ring widths kept on decreasing for HL in the non-irrigated treatments while they increased in the FI-treatment, but still less rapidly than in the irrigated EL. This decrease corresponded to a slower recovery of EW width in the non-irrigated trees ([Figure 5 B](#)). Neither for EL or HL, annual growth increments after 2003 reached the level of those prior to 2003.

## **4. DISCUSSION**

### **4.1. Treatment effects on growth**

In this experimentation, fertilisation and irrigation were used to boost growth of trees by correction of soil nutrient deficiencies and prevention of soil water deficits. Fertilisation effectively corrected most nutrient deficiencies as confirmed by foliar analysis (not shown) and irrigation prevented most soil water deficits along the growing seasons.

The combined fertilisation-irrigation treatment has been the most favourable treatment: growth was vigorous and it even exceeded the best yield class found in Western Europe for European larch (yield class 12 according to Hamilton and Christie (1971) and to Pauwels and Marenne (1999)). Such a positive synergy of fertilisation and irrigation treatments has been described by several authors (Albaugh et al. 2004; Brix, 1972; Waterworth et al., 2007).

Fertilisation alone did not really boost growth as expected. With the exception of average ring width, growth parameters never significantly differed between fertilised (F-trees) and non-

fertilised trees (C-trees). When applied only once at the start of such experiments, fertilisation alone proved to have no or only a limited impact on growth at the opposite of an on-going fertilisation along the experimentation (Albaugh et al., 2004; Waterworth et al., 2007). However as shown by Ewers et al. (1999), fertilisation could benefit to trees by other ways than the increase of fertility itself. In a study with loblolly pine, fertilisation not only improved soil fertility but also the water use efficiency of trees: it could thereby limit the impact of soil water shortage on tree growth. The low response of larch trees to fertilisation in our study and their low vigour -comparable to that of C-trees- let us hypothesise that water-use efficiency of F-trees was not improved. We suspect too that compared to other conifers, soil water shortages are more stressful to larches and predominant over soil fertility problems. Fertility is indeed usually not recognised as a major limiting factor for larch compared to soil physical properties and to water availability (Masson, 2005).

If fertilisation alone did not prove efficient in enhancing growth, this study does not unfortunately allow confirming that irrigation alone would be more efficient (no single irrigation treatment available). But irrigation and its interaction with fertilisation had a strong effect on growth. Considering that in the FI-treatment, water was not a limiting factor, we used for the rest of the discussion the FI-treatment as the new control against which the impact of more stressful conditions (i.e. F- and C-treatments) was evaluated for growth but also for heartwood development and wood density parameters. More stressful growth conditions due particularly to repeated soil water depletion within and over years are indeed more and more frequently expected in a context of climatic change.

#### **4.2. Impact of soil water shortages on growth**

Frequent soil water deficits as experienced along this study severely reduced larch vigour: apical as well as radial growth traits were similarly concerned by this decrease and led to an overall drop of individual mean tree volume of more than 40%. Mean annual ring width decreased as a result of an equal drop in size of its components: mean annual earlywood width was as severely affected as latewood width, so that latewood proportion was only slightly reduced in trees from the non-irrigated C-treatment. Such behaviour is not common in conifers. Nicholls and Waring (1977) observed in radiata pine that drought stress reduced earlywood size and increased latewood proportion. Similarly in several experimentation with irrigation (e.g. Kostianen et al. (2004) with Norway spruce, Hsu and Walters (1975) with



loblolly pine, Brix (1972) on Douglas fir), fertilisation-irrigation treatments compared to control usually increased earlywood width and decrease latewood proportion or said in another way, less favourable environments reduced earlywood size and increased the latewood proportion.

One of the main consequences of irrigation and fertilisation could be either to delay transition to latewood formation or to maintain a longer period of active radial growth or both. For example, Brix (1972) observed such a transition delay to latewood of 15-30 days on Douglas fir. Haasemann (1986) showed in an experience with different soil moisture regimes that the 'wet' regimes delayed the end of the vegetation period of larch by up to 7 days compared to the 'dry' regime. Larch which can probably be seen as an opportunistic species might take benefit of both advantages: an extended period for earlywood development and a longer period of active growth. In addition to a postponed transition to latewood as seen by Brix (1972), the extension of the period of earlywood formation could also be explained by a growth activity starting earlier in ferti-irrigated trees, as suggested in this study by bud flush results. Following this reasoning, the observation of a similar earlywood width in fertilized trees compared to ferti-irrigated trees but of a smaller latewood width similar to that of controls let us assume that fertilized trees benefited from an extended period for earlywood development as ferti-irrigated ones but suffered from a reduced period of active growth in absence of irrigation. A closer look at cambial activity over the growing season and ring formation dynamics is needed to better understand timing and extension of early vs latewood in relation to stress.

#### **4.3. Impact of soil water shortages on wood density traits**

At the opposite of growth traits, wood density parameters were not significantly modified under the contrasted water conditions. Overall ring density remained similar among treatments even while ring width was significantly changed. It is in contrast too with several published studies where the combined treatment of fertilisation and irrigation enhanced growth and thereby induced a wood density decrease (e.g. Albaugh et al., 2004 with loblolly pine but mostly because of fertilisation; Kostianen et al., 2004 with Norway spruce). Overall wood density decrease could be attributed either to a reduction in the density of its components (EW-LW) or to a change in latewood proportion or to both as observed in the

studies mentioned above but not in our study: neither earlywood or latewood densities nor latewood proportion were simultaneously modified by treatments.

More in agreement with our results, Wielinga et al. (2008) observed no significant decrease in the overall density for ferti-irrigated radiata pines. They supported this result by Beets (1997)'s findings, who noticed that irrigation would have a positive effect on ring density but it could be offset by the negative effect of fertilisation. But our study did not fully confirm this finding: indeed, fertilisation alone did not affect negatively wood density. Nicholls and Waring (1977) even found that irrigation and partial droughting treatments could produce a slight increase in wood density of radiata pine, due either to an increase in maximum density in the case of irrigation or to an increase in the minimum density and in latewood ratio in the case of partial droughting.

Finally, the apparent absence of link between vigour and density (i.e. the slow growing trees from the non-irrigated treatment do not produce a more dense wood than the more vigorous trees from the ferti-irrigated treatment) may seem in opposition with results from genetic trials of larch where a negative correlation is often found between diameter and density (Jacques, 2003, Pâques and Jacques, 2002). But, these results are usually obtained for a given population in a given site and are coherent with what was observed in this study within a given treatment (not shown). But when such studies are conducted over sites with different growth potential, site effect (and thus the growth rate effect) on wood density is not significant (Jacques, 2003).

#### **4.4. Impact of soil water shortages on heartwood size**

Overall size of heartwood was vertically (and radially) seriously affected in the non-irrigated plots with a resulting reduction of over 39% in absolute volume. In fact, its decrease rate paralleled that of total volume. This is to be connected to the good link found in other studies on larch between heartwood content and growth (Pâques, 2001). The sapwood volume decreased also in a similar proportion and this explains why finally the heartwood proportions (over BH area, total height and volume) did not differ significantly among treatments.

Impact of irrigation, or reversely of water stress, on heartwood extension is not well documented. Hillis (1987) concluded that studies on the 'effects of the level of humidity and

availability of water' have yielded contradictory results. Clearer was the positive relationship between sapwood width and rate of growth: the more rapidly growing trees require a greater uptake of water and hence larger volumes of sapwood to provide the crown with moisture. That is compatible with our results at least for radial dimensions where the sapwood proportion remained indeed smaller in the less vigorous and more stressed trees.

In an irrigation experimentation conducted over two years with Douglas fir, Polge (1982) showed that drought-stressed trees had about the same rate of growth than irrigated trees, but they had a significantly lower number of sapwood rings with a slight (but not significant) decrease in sapwood width. Examination of heartwood extension in our study 5-6 years after application of treatments showed a slight increase in the number of heartwood rings newly formed (or reversely decrease of sapwood rings) in the C treatment compared to the FI-treatment but differences were not significant. They were not statistically different too for radial extension.

#### **4.5. Impact of the 2003-heat-drought wave on growth traits**

A direct evaluation of 2003-stress impact on growth was not straightforward because of questionable reference years with which to compare 2003-growth. Indeed an apparently declining trend was observed for both apical and radial growth, which if not considered could bias the impact of 2003-stress. Although shortening of annual increments is normal with ageing (Hamilton and Christie, 1971; Pauwels and Marenne, 1999), this decline could look particularly rapid on this atypical site for larch. But this would be to forget the history of the management of the experimentation, the short span of years for observations and the naturally high inter-annual variation in growth of larch in oceanic conditions (Pâques, personal communication). The thinning of the stand in winter 1999 and the start of irrigation in 2000 enhanced height growth in 2000 of FI-trees because of irrigation and radial growth of all trees in irrigated and non-irrigated plots because of release of competition. Benefits of the latter were probably gradually attenuated by yearly recurrent drought stress in non-irrigated plots during 2001 and 2002 until the climatic event of 2003. In FI-trees, growth increments fluctuated over years with similar amplitude from the beginning of the plantation until 2002 when increments dropped due to a lack of irrigation. In these conditions, averages of 2000-2001 annual increments looked as reliable enough references to measure 2003-stress impact. The 2003-climatic event allowed us to evaluate the impact of the hot temperature stress more

or less independently from the more global stress including also water stress, and on the other side, it permitted to study the recovery of tree growth up to three years after that event.

While FI- trees had and kept both an apical and radial growth superior over non-irrigated trees before and at the end of 2003 ([Figure 2 B and C](#)), they suffered too from 2003-climatic events. If the water supply can be supposed to be not limiting in this treatment, the heat shock (together with a low atmospheric humidity) could then be considered as the main deleterious factor. Impact was more severe on radial growth (-57%) than on shoot growth (-17%). Waring (1987) ranked stem growth at a lower rank of importance among growth processes behind foliage growth, root growth and bud growth, and Dobbertin (2005) noted that under stress, with the alteration of photosynthesis and of carbon allocation, less important processes including stem growth are reduced first. More precisely, Dreyer et al. (2004) found on poplar that among stem growth components, cambial activity was first affected by drought stress before height growth, stomata conductance, new foliage production and photosynthesis.

One might have expected that growth reductions in irrigated trees would be -in relative values- smaller than those observed in non-irrigated treatments. But this was only true for latewood width (and thereby for latewood percentage): a reduction was observed in irrigated trees (-39%) but it represented only about half the level observed in trees submitted to the 'global' heat-drought stress (-73% for non-irrigated trees). The greater impact of the heat-drought event of 2003 on latewood vs earlywood increments was already noticed by Vennetier et al. (2004) and Rozenberg and Pâques (2004) for several conifers. In fact, the impact of the 2003-stress observed on FI-trees was probably exaggerated by the lack of irrigation in 2002; it suddenly placed FI-trees in the same water regime conditions than non-irrigated trees. But the drop of both apical and radial growth was more severe on FI-trees than on non-irrigated trees, suggesting that larches not used to some water constraints are more susceptible to drought stress than trees regularly faced to soil water deficits. As a result, the growth of these weakened trees might have been reduced in 2003, inflating the impact of 2003-stress.

A delayed start of growth (later flushing) probably coupled with a higher growth speed afterwards might also explain why irrigated trees were not advantaged over non-irrigated trees (in relative value) for shoot elongation and earlywood radial growth but well for latewood growth. Indeed the peak of the 2003-heat-drought wave occurred in mid-August, at a period

critical for latewood formation but without any more influence on earlywood formation and with a low impact on shoot elongation. According to Rossi et al. (2009), earlywood in European larch is in fact completely formed by the end of June in coincidence with results presented by Antonova and Stasova (2002) for *Larix sibirica* Ldb. and the maximum rate of shoot elongation occurs before mid-August (Pâques, 2009, Marin, 1994).

Non-irrigated trees heavily suffered from this combined hot temperature-drought stress: for the first time during the whole experimentation, their crown foliage was completely damaged by mid-August; their radial growth nearly stopped while it continued somehow with latewood development in irrigated and still green-foliated trees.

Obviously, the heat-drought stress had a lasting effect on tree growth and was more acute on apical than on radial growth: up to 3 years later, height growth kept on decreasing whereas radial growth restarted but without recovering its level prior to 2003. If according to Bréda and Badeau (2008), trees more vigorous prior to the stress are supposed to recover more rapidly, we were expecting that after-effects in irrigated trees would be less pronounced than in non-irrigated trees which were still submitted to some soil water depletion. This was in fact confirmed for global radial growth (+50-65% increase vs +28-43% for irrigated vs non-irrigated trees) and for EW width (+42-62% vs +14-26%) but neither for height growth (same level of decrease) nor for LW width (+64-132% increase vs +104-203%). Three years after the 2003 stress, non-irrigated but also irrigated trees had not yet fully recovered.

#### **4.6. Taxa behaviour**

For statistical reasons, it was not recommended to directly compare European versus hybrid larch reaction to irrigation. Nevertheless, this study could reasonably reveal some major trends in species response, besides the general superior vigour -including heartwood extension- of hybrids compared to European larches. This aspect is well-documented (Pâques, 1989).

At the end of the experimentation, the relative impact of non-irrigated treatments appeared to be much more severe on EL than on HL for a majority of traits among those showing a significant effect of treatments. They included BH girth increment, global stem volume,

heartwood and sapwood volumes, and ring width and its components but not total height increment.

Focussing now on 2003-climatic event we saw that taxa responded quite differently to the two types of stress ('heat stress' in irrigated plots and 'heat-and-drought stress' in non-irrigated plots). Direction and intensity of effects and after-effects were quite uniform for EL under the two types of stress while HL appeared to be much more sensitive to the type of stress. Its height growth was very much affected by the combined 'heat-and-drought' stress as seen through impacts in the non-irrigated treatments but not by the 'heat' stress alone observable in the irrigated treatment. As well, recovery of radial growth by HL was very much different according to treatments: it remained still negative in 2004, 2005 and 2006 for ring width and EW width in non-irrigated plots whereas recovery was observed in irrigated ones.

The differential impact of 2003-stress on taxa may be partly explained by the different phenology and growth rhythm observed between EL and HL. In a previous study in the same site (Pâques, 2009), we observed that apical growth started on average earlier in EL than in HL and that the maximum growth rate increment occurred 17-26 days earlier in EL than in HL, well before the peak of temperatures and water deficit. Most probably when climatic conditions became worst, HL had not yet or just reached its maximum rate of height increment whereas EL did already 2-3 weeks before. Unfortunately, we do not have the same information for radial growth. But we can suspect that EW could still be formed nearly normally in HL but that for both species, climatic conditions were too harsh for formation of LW.

Very few results are in fact available on the behaviour of the different larch species and ecotypes towards environmental conditions, and more particularly about their response to drought stress. Kral (1966) used amplitudes of osmotic pressures in needles to characterise drought resistance of several larch species and provenances. In accordance with ecological observations, Kral (1966, 1967) concluded that European larch populations from Interior Alps and *polonica* larch (as in our study) were more drought resistant than populations from Sudetan Mountains and Eastern Alps and than Japanese larch. But nothing is said about hybrid larch (European x Japanese). Knowing by experience the large range of possible responses of the hybrids relatively to their parents (similar to one parent, intermediate, or well-beyond them due to heterotic effects) (Pâques et al. 2006, Pâques, 2009), it would be

hazardous to conclude that HL is intermediate for drought resistance or less drought resistant than EL.

In nursery experimentation with different soil moisture regimes, Haasemann (1986) observed as in our study that HL was in absolute value superior to European and Japanese larches whatever the soil moisture. But he noticed that species expressed more optimally their height growth (2yr old) relative to other species in different soil moisture regimes: i.e. under the dry regime for EL, under the wet regime for JL and under the intermediate one for HL. From data reconstruction, it seemed too that the height decrease observed from the moist to the dry treatments was in relative value less severe for EL than for HL while we observed no difference in our study on much older trees.

#### **4.7. Impact on larch forest management**

The water regime used in this study in the irrigated treatment corresponded to some extent to that of the currently most suitable planting sites of larch in France; in particular in the West Massif Central range over 400 m. In contrast, the water regime in the non-irrigated plots mimic -but with some excess- what can be observed in lower land regions or what is expected on higher altitude sites in a context of climate change with more frequent and intense episodes of soil water shortages during summer.

In no case -even during the 2003-event with extreme drought and heat-, the vitality of larches was affected even in the non-irrigated plots. Whereas the foliage of trees was severely damaged in these plots, no additional top-dieback and increase of mortality were noticed. At the opposite, the impact on growth was severe and foresters may expect major drops in total tree volume (up to 40%) but also in total volume of heartwood, the most valuable part of larch timber.

Clearly even if some variability exists among ecotypes (Kral, 1966, 1967), European larches have considerable demands on water due to an intensive transpiration (Olaczek, 1986). Polster (1967) estimated that the daily transpiration rate in larch was 1.7-1.9 times higher than that of some *Pinus* species including Scots pine. Precipitation in May-July seems to be particularly critical in ring formation (Oleksyn and Fritts, 1991). Unless some water compensations from

the soil, the lack of precipitation during that period seriously depresses growth as observed in our study.

In any case, the proper choice of the larch species or taxa is critical to optimise reforestation success and yield. Because of its sensitivity to summer droughts, Japanese larch has already been removed from the French list of recommended Forest Reproductive Materials for reforestation (Anonymous, 2003). Over years -with their annual soil water shortages episodes during summer-, the hybrid between European and Japanese larches showed an overall better growth behaviour with a less pronounced tree volume depression than European larch. Nevertheless, the heat-drought wave of 2003 impacted more the growth of non-irrigated hybrid larches than that of European larches, but also their further recovery. The heat-drought wave of 2003 was an exceptional climatic event. Be it more frequent, it is not sure that hybrid larch could maintain over years its overall superiority.

Interestingly, soil water shortages do not seem to affect either wood density properties or tissue proportions (early vs latewood, sapwood vs heartwood) in larch even when radial growth is drastically reduced. Reversely, this means too that enhanced growth conditions (due for example to ferti-irrigation) do not degrade wood density in larch; this is seldom the case in conifers (Rozenberg et al., 2001).

In order to better understand the impact of climatic stresses on wood formation and their consequences on anatomical and physical wood properties and thereby on related adaptive traits, a study of cambial activity rhythms in relation to climatic events is definitely needed.

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**Figure 1. Cumulated soil water deficits (mm) estimated over decades in the non-irrigated (left) and irrigated (right) plots (for years 2000 to 2006).**

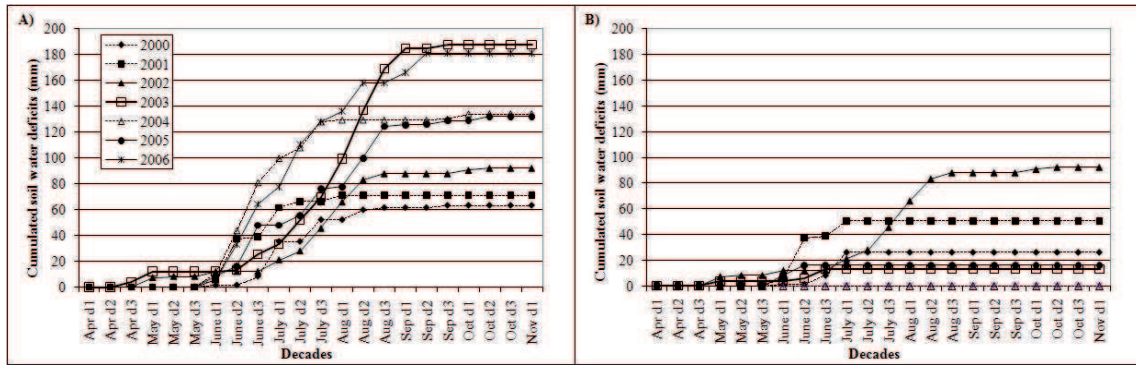
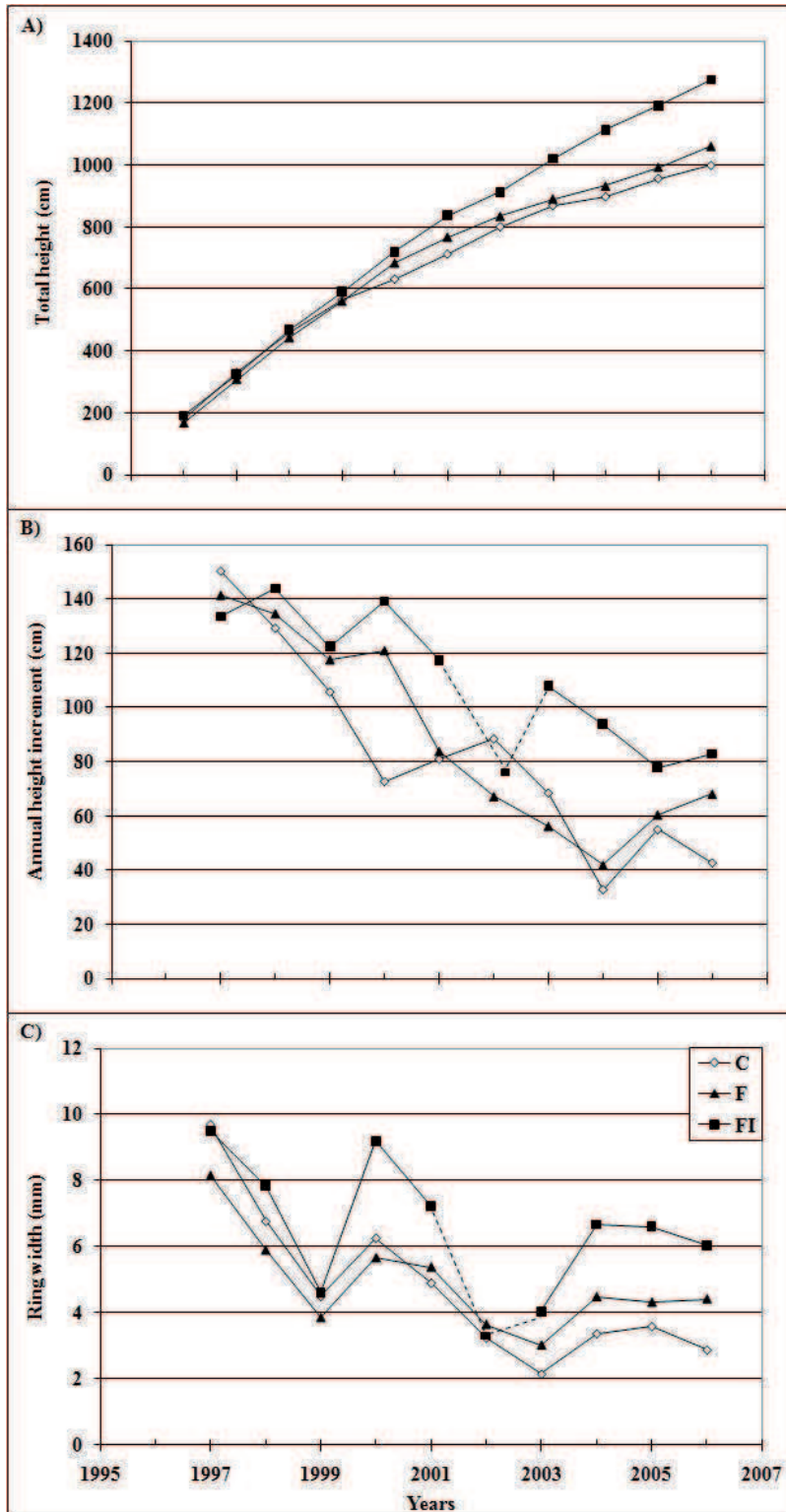


Figure 2. Total height and radial growth curves from start to the end of experiment. A) Total height, B) Annual height increment and C) Annual ring width



**Figure 3. Impact of the absence of irrigation (-fertilisation) on European (EL) and hybrid (HL) larches for A) growth, stem form and bud burst, B) heartwood size components and C) wood density traits. (Impact is expressed as the difference between ferti-irrigated and non-irrigated trees performances). Asterisks mean a significant difference among treatments,  $\alpha$ -level= 0.05).**

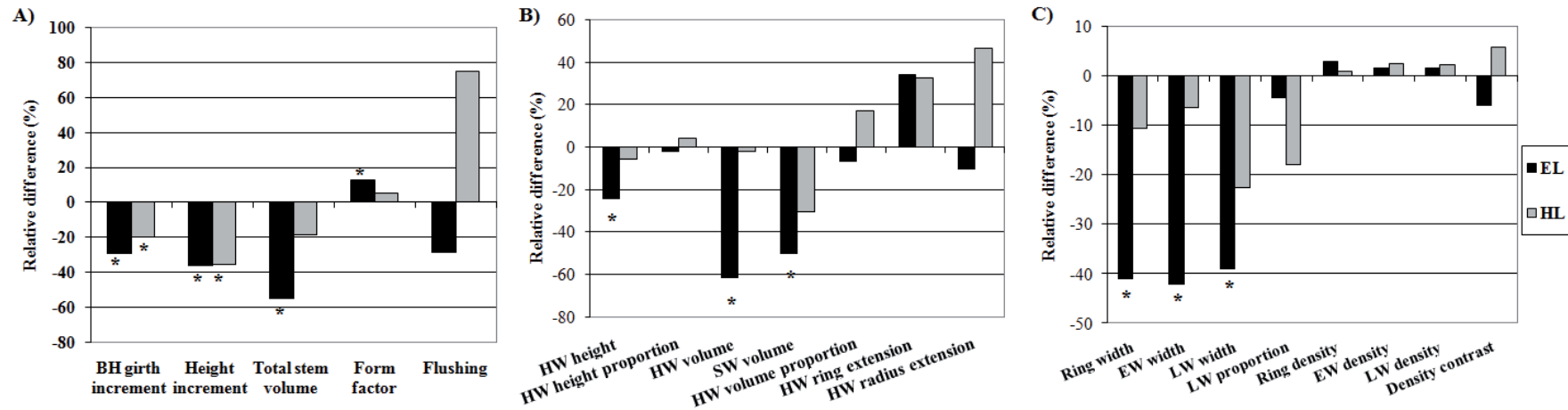


Figure 4. Direct-effects of year 2003-heat-drought stress on mean height increment (HI), ring width (RW), early(EW) and latewood (LW) width and latewood percentage (LW prop.) for European (EL) and hybrid (HL) larches in non-irrigated (gray box) vs irrigated (black box) treatments (Direct effects are expressed as the relative difference between 2000/2001 mean performances and those of 2003).

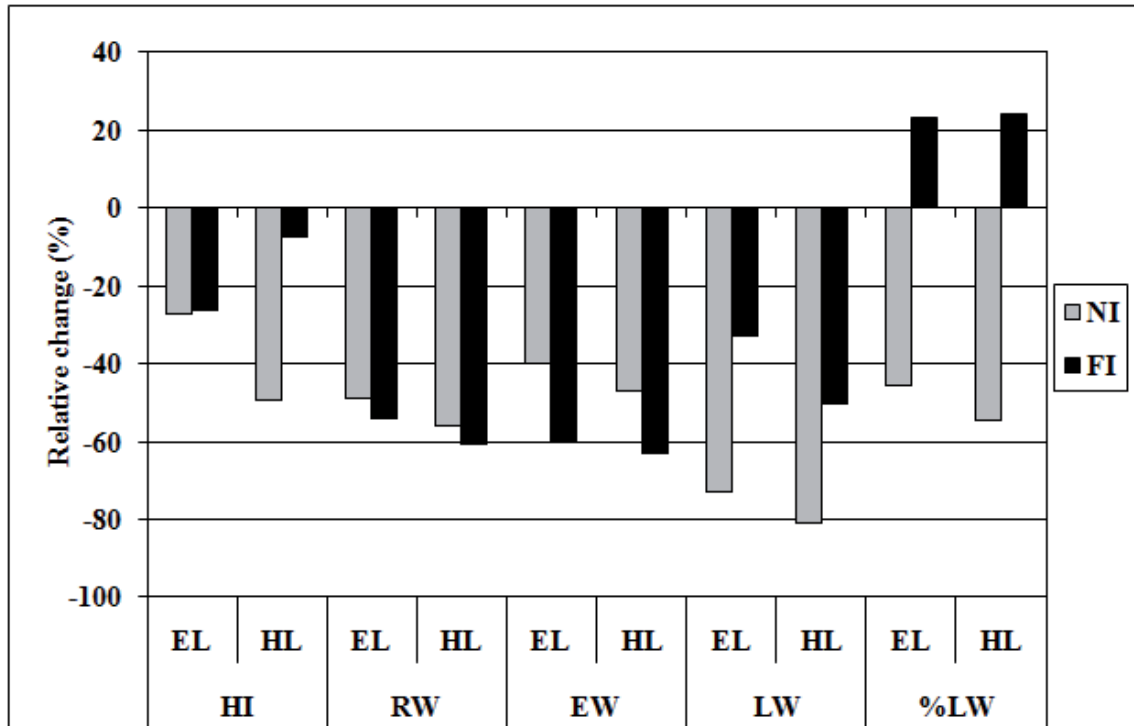
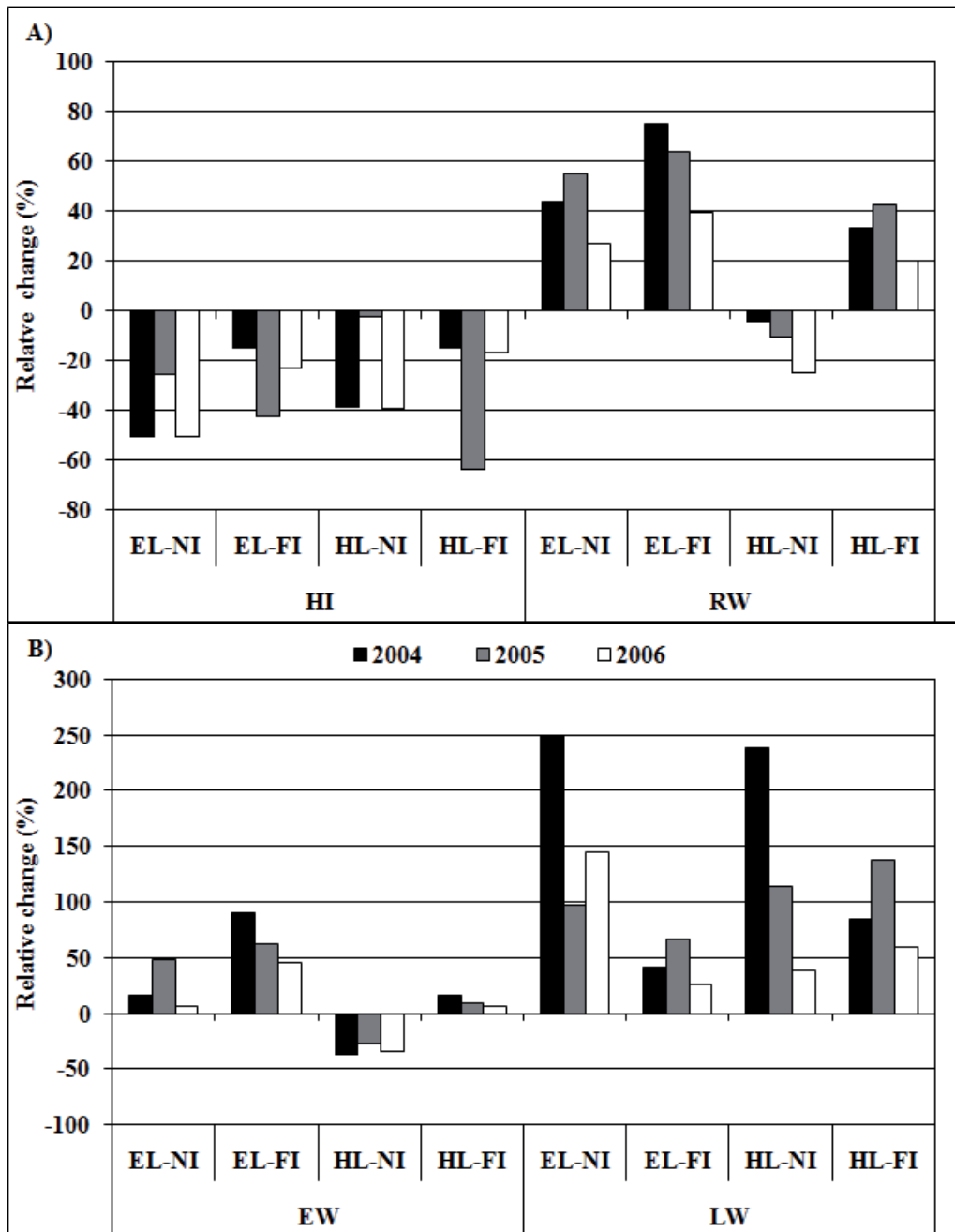




Figure 5. After-effects of year 2003-heat-drought stress on mean height increment (HI) and ring width (RW) for European (EL) and hybrid (HL) larches in non-irrigated (NI) vs irrigated (FI) treatments. After-effects are expressed as the relative difference between 2004, 2005 and 2006 mean performances and those of 2003.



**Table I. Mean annual temperature and precipitation (between brackets during the vegetation period May-September), water supply and estimated cumulated soil water deficit**

	Temperature (°C)	Precipitation (mm)	Water supply <sup>1)</sup> (mm)	Cumulated soil water deficit <sup>2)</sup> (mm)
2000	12.2 (17.8)	987 (389)	1031	63.2
2001	11.8 (17.6)	1053 (475)	802	71.1
2002	12.2 (17.0)	786 (293)	0	92.3
2003	12.5 (19.7)	685 (227)	836	187.7
2004	11.8 (18.0)	744 (272)	2235	134.1
2005	12.0 (18.5)	672 (271)	1784	132.1
2006	11.5 (18.2)	763 (212)	2540	181.2

<sup>1)</sup> in fertilised-irrigated plot only

<sup>2)</sup> in non-irrigated plots

**Table II. Comparison of treatments for growth traits, heartwood size and extension, ring width and wood density for EW and LW ring components: mean (and standard deviation) statistics and mean comparison test (different letters mean a significant difference among treatments); (ni = number of individuals per treatment).**

	Control (ni=33)	Fertilised (ni=22)	Irrigated + Fertilised (ni=34)
BH girth (mm)	400.8 (70.5) a	425.7 (72.8) a	522.2 (102.7) b
Increment up to 1999	162.9 (26.4) Ns	163.0 (27.3) ns	186.8 (32.5) ns
Increment 2000- 2006	237.9 (53.2) A	262.7 (53.5) a	335.3 (80.1) b
Total height (cm)	999.0 (113.2) A	1060.9 (106.3) a	1274.2 (145.8) b
Increment up to 1999	563.6 (46.8) Ns	559.1 (50.7) ns	590.0 (44.5) ns
Increment 2000- 2006	435.4 (95.1) A	501.8 (86.0) a	684.2 (108.1) b
Total stem volume (dm <sup>3</sup> )	46.1 (20.8) A	51.5 (19.0) a	80.8 (40.6) b
Bud flush 2005	3.1 (0.9) A	3.2 (1.2) a	2.3 (1.1) b
Form factor	0.61 (0.05) A	0.58 (0.04) ab	0.54 (0.07) b
Heartwood height (cm)	671.8 (125.0) A	780.5 (123.1) b	760.9 (172.7) b
Proportion of total height reached by heartwood (%)	67.2 (7.0) Ns	73.9 (6.6) ns	68.0 (6.8) ns
Heartwood BH area (cm <sup>2</sup> )	42.6 (20.3) Ns	43.9 (21.7) ns	56.0 (29.6) ns
Heartwood BH area proportion on total area (%)	55.9 (17.9) Ns	47.9 (14.8) ns	48.3 (16.1) ns
Heartwood volume (dm <sup>3</sup> )	19.1 (10.7) A	19.6 (11.6) a	31.7 (18.4) b
Sapwood volume (dm <sup>3</sup> )	27.0 (11.8) A	31.9 (12.0) a	49.0 (23.5) b
Heartwood proportion on total volume (%)	40.7 (9.6) Ns	37.7 (11.7) ns	40.4 (5.7) ns
Heartwood extension 2004-2006: number of rings	3.4 (1.1) Ns	2.7 (1.0) ns	2.5 (1.1) ns
Heartwood extension 2004-2006: along the radius (cm)	1.35 (0.59) Ns	1.48 (0.63) ns	1.36 (0.76) ns
Ring width (mm)	3.77 (1.00) A	4.87 (0.78) b	6.04 (1.59) c
EW width (mm)	2.76 (0.87) A	3.82 (0.64) b	4.48 (1.26) b
LW width (mm)	1.02 (0.25) A	1.05 (0.23) a	1.56 (0.37) b
LW proportion (%)	26.1 (4.8) A	21.5 (3.9) b	27.0 (5.4) a

Ring density (kg/m <sup>3</sup> )	464.7 (39.4)	448.3 (35.1)	451.7 (31.5)
	Ns	ns	ns
EW density (kg/m <sup>3</sup> )	373.6 (30.7)	374.3 (28.1)	366.2 (29.8)
	Ns	ns	ns
LW density (kg/m <sup>3</sup> )	706.9 (42.9)	713.9 (50.6)	703.2 (45.6)
	Ns	ns	ns
Density contrast (kg/m <sup>3</sup> )	574.6 (44.9)	584.4 (51.2)	596.2 (40.4)
	Ns	ns	ns

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**Table III. Mean performances of European larch and hybrid larch within treatments (standard deviations between brackets – figures in italics indicate no significant differences among treatments,  $\alpha$ -level= 0.05)**

	European larch		Hybrid larch	
	Non-irrigated ni=27	Irrigated ni=27	Non-irrigated ni=6	Irrigated ni=7
BH girth increment 2000- 2006 (mm)	237.9 (52.5)	335.3 (86.4)	264.7 (41.0)	331.6 (23.1)
Height increment 2000- 2006 (cm)	435.4 (93.5)	684.2 (111.1)	476.3 (107.8)	740.6 (65.5)
Total stem volume (dm <sup>3</sup> )	46.2 (16.6)	103.2 (39.6)	<i>69.0 (24.4)</i>	<i>85.1 (47.8)</i>
Heartwood height (cm)	719.7 (126.2)	949.3 (144.1)	<i>766.7 (103.3)</i>	<i>814.3 (267.3)</i>
Proportion of total height reached by heartwood (%)	<i>70.4 (7.5)</i>	<i>72.0 (6.2)</i>	<i>71.6 (2.4)</i>	<i>68.9 (9.4)</i>
Heartwood BH area (cm <sup>2</sup> )	42.8 (15.9)	71.0 (29.6)	<i>72.0 (19.6)</i>	<i>62.0 (32.0)</i>
Heartwood BH area proportion on total area (%)	<i>64.3 (18.8)</i>	<i>55.5 (17.8)</i>	67.2 (14.0)	47.9 (6.9)
Heartwood volume (dm <sup>3</sup> )	17.1 (7.5)	44.5 (18.5)	<i>33.9 (10.7)</i>	<i>34.6 (19.7)</i>
Sapwood volume (dm <sup>3</sup> )	29.1 (10.9)	58.7 (22.6)	<i>35.1 (14.0)</i>	<i>50.4 (28.4)</i>
Heartwood proportion on total volume (%)	<i>39.0 (9.0)</i>	<i>41.7 (5.3)</i>	<i>49.9 (3.8)</i>	<i>42.6 (5.8)</i>
Heartwood extension 2004-2006: number of rings	<i>3.40 (1.23)</i>	<i>2.53 (1.20)</i>	<i>2.75 (0.52)</i>	<i>2.07 (0.67)</i>
Heartwood extension 2004-2006 along the radius (cm)	<i>1.20 (0.57)</i>	<i>1.34 (0.79)</i>	<i>1.73 (0.48)</i>	<i>1.18 (0.65)</i>
Ring width (mm)	3.59 (0.89)	6.09 (1.53)	<i>4.81 (1.15)</i>	<i>5.38 (1.96)</i>
EW width (mm)	2.58 (0.76)	4.45 (0.62)	<i>3.67 (1.08)</i>	<i>3.92 (1.39)</i>
LW width (mm)	1.00 (0.28)	1.64 (0.49)	<i>1.13 (0.21)</i>	<i>1.46 (0.76)</i>
LW proportion (%)	26.7 (4.9)	27.9 (5.4)	<i>23.8 (4.6)</i>	<i>29.0 (5.6)</i>
Ring density (kg/m <sup>3</sup> )	<i>471.1 (41.9)</i>	<i>458.0 (31.1)</i>	<i>429.4 (21.5)</i>	<i>425.1 (17.0)</i>
EW density (kg/m <sup>3</sup> )	<i>378.0 (30.2)</i>	<i>371.9 (28.5)</i>	<i>345.5 (13.8)</i>	<i>336.9 (27.0)</i>
LW density (kg/m <sup>3</sup> )	<i>706.9 (42.1)</i>	<i>695.5 (42.0)</i>	<i>701.3 (42.1)</i>	<i>685.3 (61.7)</i>
Density contrast (kg/m <sup>3</sup> )	<i>560.5 (46.4)</i>	<i>595.6 (41.6)</i>	<i>605.8 (36.3)</i>	<i>572.4 (80.1)</i>

**Table IV. A) Mean annual height increment and ring width, early, latewood width and latewood proportion before (yrs 2000-2001), during (yr 2003) and after (yrs 2004, 2005 and 2006) the 2003-heat-drought wave in the different plots. (Standard deviations between brackets –figures in italics indicate no significant treatment effects,  $\alpha$ -level= 0.05) B) Direct and indirect impact of year 2003-heat-drought wave (%).**

<b>A)</b>	Height increment (cm)		Ring width (mm)		Early-wood width (mm)		Late-wood width (mm)		Late-wood proportion (%)	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated
2000-2001	97.8 (26.9)	128.2 (27.3)	6.0 (1.8)	8.2 (2.4)	4.4 (1.6)	6.5 (2.1)	<i>1.6 (0.7)</i>	<i>1.6 (0.9)</i>	27.8 (9.6)	21.4 (10.0)
2003	60.5 (19.8)	107.9 (34.9)	2.9 (1.1)	4.0 (1.8)	2.4 (1.0)	3.0 (1.4)	0.4 (0.4)	1.0 (0.7)	16.6 (11.7)	26.6 (11.9)
2004	38.2 (20.1)	93.8 (30.1)	4.1 (1.4)	6.7 (2.6)	2.8 (1.1)	4.9 (2.3)	1.3 (0.6)	1.8 (0.9)	33.5 (12.0)	28.2 (13.0)
2005	<i>57.0 (24.4)</i>	<i>77.9 (25.0)</i>	4.0 (1.6)	6.6 (2.4)	3.1 (1.4)	4.3 (2.2)	0.9 (0.5)	2.3 (1.3)	23.8 (9.4)	34.6 (16.3)
2006	52.4 (18.2)	82.7 (32.3)	3.7 (1.4)	6.0 (2.1)	2.8 (1.0)	4.4 (1.5)	0.9 (0.5)	1.6 (0.7)	24.0 (6.8)	27.1 (7.9)

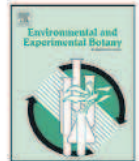
<b>B)</b>	Height increment		Ring width		Early-wood width		Late-wood width		Late-wood proportion	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Irrigated
direct effect	-38.2	-15.9	-52.0	-50.8	-44.2	-53.8	-72.7	-39.0	-40.4	24.0
post-effect										
2004	-36.8	-13.1	43.0	65.4	13.9	62.2	202.7	75.2	101.6	6.1
2005	-5.7	-27.8	38.9	63.9	26.4	41.5	107.3	131.6	43.1	30.4
2006	-13.3	-23.3	27.9	49.6	14.0	45.0	104.0	63.5	44.8	1.91

## Chapter 3

[Article 3]: Indirect prediction of bud flushing from ex situ observation in hybrid Larch (*Larix decidua* x *L. kaempferi*) and their parents.

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## Indirect prediction of bud flushing from *ex situ* observation in hybrid Larch (*Larix decidua* x *L. kaempferi*) and their parents

M.E. Gauchat<sup>a,\*</sup>, L.E. Pâques<sup>b</sup>

<sup>a</sup> INTA E.E.A. Montecarlo, Av. El Libertador 2472, CP3384, Montecarlo, Misiones, Argentina

<sup>b</sup> INRA Orléans, Unité d'Amélioration, Génétique et Physiologie des Arbres Forestiers, 2163 Avenue de la Pomme de 45075, Orléans Cedex 2, France

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### ABSTRACT

Phenology of bud burst was studied for larches (*Larix*) using *ex situ* and *in situ* observations. The main objective of the study was to test the possibility to predict flushing in the field (*in situ*) from *ex situ* parameters obtained in growth chambers. Several progenies of European (EL), Japanese (JL) and hybrid (HL) larches were assessed in progeny trials for dates of terminal bud flushing and of overall "greening" of the tree (proportion of green crown). Twigs collected on the same trees during the previous winter were observed under controlled environments in two growth chambers, one at 11 °C and the other at 16 °C. Dates of flushing were recorded and from these, threshold temperatures and heat-sum requirements to flush were estimated. Correlations between *in situ* and *ex situ* parameters were studied and several linear models were tested to predict *in situ* flushing parameters using flushing days and heat-sums from *ex situ* experimentation.

On average, HL and EL flushed after 61–63 days from January 1st against 56–61 days for JL in the growth chamber at 16 °C, while at 11 °C, HL needed 70–80 days, EL 70–78 days and JL 63–73 days. Estimated mean threshold temperatures were 1.7–3.0 °C for JL, 2.2–3.6 °C for HL and 2.3–3.3 °C for EL. Heat-sum requirements reached 250–320 degree-days for JL, 257–315 for HL and 307–326 for EL. In the field, terminal bud flushing took 98–109 days and crown greening of trees took around 101–102 days. In terms of heat-sums, 237–270 degree-days were needed for terminal bud flushing and between 186 vs 294 degree-days for crown greening.

Relationships between heat-sum requirements *ex situ* and *in situ* were stronger across progeny trials than those found for dates of flushing and also more stable for greening (0.71–0.83) than for terminal bud flushing (0.54–0.86). As well, for both *ex situ* and *in situ* flushing assessment, the heat-sum variables presented a higher genetic control ( $h^2_{fam} = 0.70 - 0.95$ ) than the dates of flushing ( $h^2_{fam} = 0.57 - 0.59$ ).

The two best predictive models involved only heat-sums parameters. Although they are simple, some of them looked successful enough in predicting field flushing parameters with an acceptable accuracy. Heat-sum estimated in growth chambers from twigs could be used as a reliable indirect predictor of bud burst or flushing in the forest.

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### 1. Introduction

Phenology has become a key topic in the last 20 years. An increasing number of studies are being published in which changes in phenology of woody plants during the last decades are revised to better predict response of forest trees in a context of global climatic change (Kramer, 1994, 1995a,b; Hänninen, 1997; Persson and Beuker, 1997; Kramer et al., 2000; Chuine and Beaubien, 2001; Cleland et al., 2007). These kinds of research have taken advantage from phenological data recorded *in situ*, and from the possibility

to simulate variations of temperature, photoperiod and other factors in controlled phenological experiments. Technology available today like cameras (Graham et al., 2009) could also help following phenological events in the field.

At least two types of interests can be distinguished about phenology studies. One of them aims from an ecological point of view to predict the effects of climatic changes and the resulting changes in the distribution of species. Ecologists started to take into account phenology (Lechowicz and Koike, 1995) because of its vegetative and reproductive routines are synchronized with climate and, consequently, because of its impact on competitive relationships in the forest. As well, phenology plays a role not less significant in plant productivity. For example, the length of the growing season (determined by the onset of growth) affects, among other aspects, the risk of frost damage to trees and the yield potential (Persson

\* Corresponding author. Tel.: +54 3751480512x126; fax: +54 3751480512x126.

E-mail addresses: [mgauchat@montecarlo.inta.gov.ar](mailto:mgauchat@montecarlo.inta.gov.ar) (M.E. Gauchat), [luc.paques@orleans.inra.fr](mailto:luc.paques@orleans.inra.fr) (L.E. Pâques).



and Beuker, 1997; Cesaraccio et al., 2004; Rötzer et al., 2004). Thus, a second interest in phenological studies is from a breeding point of view: indeed breeders aim to improve global tree adaptation, in particular to climate and more specifically to reduce risks from frost damage when transferring genetic materials. They have also an increasing interest in the effect of phenology on growth and thereby indirectly on wood quality parameters (for example, in the study of early-latewood proportion as a consequence of the growing season length).

An event frequently studied in phenological studies is bud burst, which could be related to many subjacent mechanisms. Physiological processes are involved, but also environmental factors especially in temperate regions, where leaf bud burst for forest species depends mainly on spring air temperature and/or on its variation during the previous winter (Sarvas, 1974; Campbell and Sugano, 1975; Cannell and Smith, 1983; Murray et al., 1989; Worrall, 1993; Hänninen, 1995; Chuine and Cour, 1999; Chmielewski and Rötzer, 2001; Rötzer et al., 2004; Migliavacca et al., 2008).

Air temperature has been known since a long time to influence a number of phenological events in crops and it has been used as an elementary factor in several agricultural models to predict these events. In forest trees, the interest for phenological studies (i.e. bud burst, flowering) is more recent but it gains more and more importance, especially in temperate regions.

With the help of phenological models, many studies have successfully predicted plant phenology at several spatial scales. The majority of models described in the literature are mechanistic (Chuine et al., 2003). At the end, all these models are related to the concept of summation of thermal units or heat-sum (Bloomberg, 1978; Woodbridge et al., 1995; Persson and Beuker, 1997; Seo et al., 2008). Some of them take into account only forcing effect of temperature (Cannell and Smith, 1983; Migliavacca et al., 2008), while others consider also chilling temperatures (Campbell and Sugano, 1975; Murray et al., 1989; Hänninen, 1990; Kramer, 1994; Chuine et al., 1998; Chuine and Cour, 1999; Chuine, 2000; Linkosalo et al., 2006b). Besides air temperature, the photoperiod seems also to play a role on bud burst in some species: the accumulated duration of the light period can modify bud burst date (Campbell and Sugano, 1975; Partanen et al., 2001). More importantly, Linkosalo and Lechowicz (2006a) showed that once chilling requirements are satisfied, the light environment (duration and quality) acts as a signal for the start of temperature accumulation leading to bud burst.

In this context, the number of days needed for phenological developments or growth is linked to threshold or base temperatures, a key parameter to determine the necessary heat unit accumulation for a given progress of an event. For this reason the choice of threshold temperature should be done correctly on the base of appropriate criteria. The thresholds over which the temperatures have an accumulative effect are well-known for different stages of flowering and fructification in agricultural or horticultural species. But this is not the case for forest tree species. Most often a fixed putative value of 0 °C (e.g. Spano et al., 1999; Rötzer et al., 2004) or 5 °C (e.g. Cannell and Smith, 1983; Murray et al., 1989; Alfaro et al., 2000; Ghelardini et al., 2006) is used.

Studies of the genetic variation of temperature thresholds and heat-sums for phenological events in forest trees are rare (e.g. Worrall, 1983) in contrast to the numerous studies demonstrating the moderate to high genetic control of bud burst phenology in conifers (El-Kassaby and Park, 1993; Li and Adams, 1993, 1994; Hannerz et al., 1999; Alfaro et al., 2000; O'Neil et al., 2000).

In forest, the observation and record of phenological events are usually difficult to obtain, time-consuming and costly especially for genetic studies from aged genetic trials. Indeed, the large number of trees and genotypes needed for genetic studies together with the height of trees and plantation density make the bud observa-

tion laborious and imprecise; multi-sites trials dispersion and long distance from headquarters often limit surveys at the right time. The aim of this study was therefore to improve the observation procedure from both a practical and scientific aspect. More comfortable and robust indirect predictors of bud burst in the forest were searched for *ex situ* observations of bud flushing from tree explants in climatic chambers. The possibility to work in controlled indoor conditions looks indeed attractive from an operative point of view as well as the possibility to work with many replicates to reach an acceptable accuracy level.

This study was conducted on larch, a fast growing conifer from the northern hemisphere. Because larch is one of the rare deciduous conifers, phenological studies look particularly attractive: indeed times for flushing and for needle shedding appear even more critical than for evergreens as they directly condition the length of the growing season and thereby the photosynthetic activity and the growth potential. A special focus was directed towards the hybrid *Larix x eurolepis*, the phenology of which is highly dependent on that of its pure species parents *Larix decidua* and *L. kaempferi* (Pâques, 2009). For breeders, phenology studies have become one of the main challenges. Indeed, through breeding, the combination of genitors with different phenology may lead to variously adapted hybrids (to late and early frost), with various growth potentials but also wood properties.

Specifically, this study conducted on larch aims to answer the following questions: (i) is it possible to predict *in situ* flushing from *ex situ* observation? (ii) could heat-sum be used as a predictor of bud burst? and (iii) what is the genetic variability for heat-sum traits?

## 2. Materials and methods

### 2.1. Plant and trial materials

The material used to carry out this experiment was sampled in France from 3 progeny tests of larch (*Larix* sp.). Altogether, 293 trees were chosen from 10 European larch (EL), 16 hybrid larch (HL), and 6 Japanese larch (JL) families. Families of a given taxa were different among the trials. More details about the selected material can be seen in Table 1.

Each trial contributed to parts of results presented in this paper. They were in fact complementary in terms of taxa and some were also easier to use than others for a detailed assessment of phenology because of the smaller size of the trees (Peyrat-Le-Château and Béchadargue) and/or their proximity to headquarters (Orléans and Peyrat-Le-Château). Therefore, progenies from the three trials were used for determination of threshold and heat-sum requirements and the study of their variation among taxa. Relationships between *ex situ* and *in situ* parameters were studied in Peyrat-Le-Château and Orléans trials. Genetic parameters could only be estimated and prediction models tested from Peyrat-Le-Château trial.

### 2.2. *Ex situ* bud burst experiment

In early January 2006 and 2007, we collected 4–8 twigs per tree, each one with at least 30 buds, in Peyrat-Le-Château and Orléans. Twigs were cut as far as possible from the same aspect of tree crowns and at the same relative tree height. The material was stored in a dark cold chamber at a temperature lower than 2 °C. After a storage period of 25–30 days (2006) or 27 days (2007), twigs were put into jars filled with pure water, and the jars were immediately placed into two growth chambers at 2 different conditions of temperature. The temperature was monitored continuously: chamber 1 was set at 16 °C and chamber 2 at 11 °C. A fixed photoperiod

**Table 1**  
Material description.

Site	Description	Min./max. annual temperatures (°C)	Mean annual precipitation (mm)	Plantation year	Field trial experimental design <sup>a</sup>	Total height means (year 2006) (m)	Taxa <sup>b</sup>	Number of progenies	Number of individuals sampled per progeny
Peyrat-le-Château	Lat N45°46'5" long E1°45' altitude 455 m	3.4/14.6	1260	2003	IRBD with 33 blocks, 54 HL and 1 JL progenies, single-tree plots	4.2	HL	9	11
Orléans	Lat N47°49'1" long E1°54' altitude 106 m	6.0/15.0	620	1995	CRBD with 20 blocks, 20 EL and 1 HL progenies, single-tree plots	10.6	JL HL	1 1	11 10
Béchardegue	Lat N45°49'1" long E1°43'5" Altitude 455 m	3.4/14.6	1260	2004	IRBD with 44 blocks, with 17 EL, 33 JL and 63 HL progenies, single-tree plots	2.7	EL HL	5 6	5 10
							EL JL	5 5	10 9

<sup>a</sup> CRBD/IRBD: complete/incomplete randomized blocks design<sup>b</sup> HL: *Larix decidua* x *L. kaempferi*, JL: *L. kaempferi* and EL: *L. decidua* 'polonica'.

of 8 hours and a relative humidity above 80% were used in both chambers.

In December 2007, we collected 6 twigs from another 148 trees from the Béchardegue trial: they were stored in the cold chamber up to February 4, 2008 and then twigs were handled as described above and placed into climatic chambers at 11 and 16 °C with a longer photoperiod (16 h).

Bud burst was daily recorded by counting buds along the twig, reaching stage 2 according to the scale used by Pâques for terminal bud flushing (Fig. 1). The criterion to consider a twig "flushed" was the Julian day at which 50% of the buds fulfilled the stage 2. Distinct values were obtained for chamber 1 (DCH1) and for chamber 2 (DCH2) and average values were calculated (DCH).

Heat-sums up to bud burst were then calculated using the Thermal Time (TT) model (Cannell and Smith, 1983) also named the Spring Warming model (Hunter and Lechowicz, 1992). This kind of models assumes a linear relationship between temperature and rate of development, and the occurrence of an event when a critical number of heat units are reached. The following expression corresponds to the model:

$$y \text{ such as } s_f = \sum_{t_0}^y R_f(x_t) = F^*$$

$$R_f(x_t) = \begin{cases} 0 & \text{if } x_t \leq T_b \\ x_t - T_b & \text{if } x_t > T_b \end{cases}$$

where  $y$  represents the date of flushing,  $x_t$  the daily mean temperature (°C),  $S_f$  the state of forcing or sum of degree-days,  $R_f(x_t)$  the forcing rate function,  $F^*$  critical value of state of forcing for transition from quiescence to flushing and  $T_b$  is the base temperature and  $t_0$  is the day when heat-sum starts to accumulate (Chuine et al., 2003).

The base temperature or threshold over which heat units are accumulated was calculated using the linear regression method used by Arnold (1959) and Worrall (1993). To carry out this estimate, DCH1 and DCH2 were converted to heat-sums using the spring warming model; after that the threshold ( $T_b$ ) and the necessary number of heat units to bud burst (HS) were calculated for each individual tree. Then, progeny means were estimated by averaging individual trees values.

Heat-sums accumulated from a fixed arbitrary date (January 1st) up to bud burst were then calculated. They included degree-days accumulated in the plantation and in the cold room if any, and in the climatic chambers. Progeny means estimates of  $T_b$  were used as base temperatures. Using DCH1 and DCH2 and  $T_b$  progeny means, we calculated the average of heat-sums in the two chambers conditions (HS<sub>ch</sub>).

### 2.3. In situ bud burst observations

Bud burst was recorded in the field on the same trees on which we collected branches, every 7–10 days during 2006 and 2007 at Peyrat-le-Château and only during 2006 at Orléans. Flushing was assessed using two criteria:

- Terminal bud flushing: using the subjective scoring system developed by Pâques (Fig. 1). The Julian day at which the terminal bud reached the stage 2 (TB2) was used to calculate the corresponding heat-sum accumulated from the 1st January (HSBT2) using progeny means of  $T_b$  obtained from *ex situ* experiment.
- Proportion of green crown: this measure was done looking for an alternative method (easier and less costly) to terminal bud observation. This looks particularly attractive for a deciduous



**Fig. 1.** Scale used for terminal bud flushing assessment: 0, dormant bud; 1, bud swollen and whitish; 2, visible needles in the bud; 3, closed spindle-shaped needles; 4, needles completely unfolded, up to 1 cm elongated but still spindle-shaped; 5, elongated needles, 1–2 cm in open rosette.

species like larch. To carry out this measure, we considered 'green' the part of the crown with buds at stage 4 or more (Fig. 1). A tree was judged 'green' when more or less half of the crown height was 'green' assuming that this stage permits a better differentiation of genotypes due to a high variability of this phase. From that Julian day the crown was judged 'green' (G50), the corresponding heat-sum (HSG50) was calculated in the same way as for HSBT2.

In 2007, at Peyrat-le-Château, we modified this observation by following bud flushing on only 1 branch located at approximately half of the crown height. When one quarter up to half of the branch presented flushed buds, we assumed that this stage corresponded to the same reference stage as in 2006.

The observation of the proportion of green crown is considered easier than that of the terminal bud especially when trees exceed 6–7 m of height and require the help of a binocular to observe the terminal bud.

#### 2.4. Data analysis

To analyze datasets within experiment, analyses of variance (ANOVA) were performed on all variables to test the significance of differences among progenies based on the model expressed in Eq. (1).

$$y_i = \mu + F_i + e_i \quad (1)$$

where  $\mu$ ,  $F_i$  and  $e_i$  are the general mean, the genetic effect due to progenies, and the error term, respectively.

For DCH1, DCH2, and  $T_b$ , an additional model was also proposed to test differences among taxa (Eq. (2)), where  $\mu$ ,  $\tau_i$  and  $e_i$  are the general mean, the genetic effect due to taxa, and the error term, respectively.

$$y_i = \mu + \tau_i + e_i \quad (2)$$

When variables differed significantly among progenies or taxa, multiple comparisons between progenies and/or taxa (HL, EL and JL) were performed using the Tukey's test.

As HS and  $T_b$  are not independent by construction, it was necessary to validate the existence of true differences among taxa or progenies for HS whatever the level of  $T_b$  using the following

model:

$$y_{ij} = \mu + Tb_i + G_j + TbG_{ij} + e_{ij} \quad (3)$$

where  $y_{ij}$ ,  $\mu$ ,  $Tb_i$ ,  $G_j$ ,  $TbG_{ij}$  and  $e_{ij}$  are HS of individual tree, the mean, the covariate effect of  $Tb$ , the genetic effect (taxa or progenies), the interaction term between them and the error term.

A fourth model combining progeny and flushing environment (chamber, field) factors was also used to test the importance of interaction effects in Peyrat-le-Château and Orléans experimentations. Relative ecovalences (Wricke, 1962) were also calculated in case of significant interaction to identify interactive progenies; as well Spearman rank correlations allowed studying eventual progeny rank changes.

#### 2.5. Prediction of heat-sum in the field using ex situ bud burst parameters

Using the data sets from 2006 for the Peyrat-le-Château and Orléans *ex situ* experimentations, several linear models were tested using all possible combinations of the following variables: DCH1, DCH2 and  $HS_{ch}$  as the independent variables and  $Tb_2$ , G50, HSTB2 and HSG50 as the dependent variables. In this article, the best and some worst models were presented. The models were compared using determinant coefficient of the model ( $r_{model}^2$ ), Spearman's correlation coefficient between predicted and observed values ( $r_{pred-obs}^2$ ) and the square root of the mean square error (RMSE) calculated by the following expression:

$$RMSE = \sqrt{\frac{\sum (P - O)^2}{n}}$$

where  $P$  is predicted and  $O$  is observed Julian dates of bud burst and  $n$  is the number of observations.

The best model was finally considered as the one which could successfully predict *in situ* flushing. This could only be tested for the Peyrat-le-Château experimentation with the *in situ* 2007-flushing dataset.

#### 2.6. Genetic parameters

Although, it was not possible to estimate narrow-sense heritabilities because of the genetic nature of the material used,

**Table 2**

Descriptive parameters of variables for the 3 *ex situ* experiments in 2006. DCH1, Julian day of bud flushing in chamber at 16–17 °C; DCH2, Julian day of bud flushing in chamber at 11 °C;  $T_b$ , base temperature or threshold in °C; HS, heat-sum accumulated from 1st January up to flushing day in degree-days.

Traits	Parameters	Peyrat-le-Château	Orléans	Bécharde
DCH1 (days)	Mean	62.5	63.1	59.5
	CV (%)	6.6	4.5	10.3
	Min-max	58.0–88.5	59.6–70.5	52.0–85.0
	CV <sub>fam</sub> (%)	39.6	130.7	122.4
	Min <sub>fam</sub> -max <sub>fam</sub>	61.0–65.6	60.6–67.4	54.3–69.9
	F-value Eq. (1) (p-value)	1.22 (0.293)	7.26 (<0.001)	9.30 (<0.001)
DCH2 (days)	F-value Eq. (2) (p-value)	1.51 (0.224)	0.08 (0.782)	13.64 (<0.001)
	Mean	79.5	78.6	67.9
	CV (%)	8.8	7.7	10.7
	Min-max	65.0–110.0	67.5–92.0	57.0–92.0
	CV <sub>fam</sub> (%)	63.3	84.7	112.3
	Min <sub>fam</sub> -max <sub>fam</sub>	73.0–85.4	71.8–84.4	61.1–78.4
$T_b$ (°C)	F-value Eq. (1) (p-value)	3.66 (<0.001)	3.39 (0.016)	9.18 (<0.001)
	F-value Eq. (2) (p-value)	5.78 (0.004)	0.14 (0.713)	15.70 (<0.001)
	Mean	3.01	2.25	3.29
	CV (%)	46.2	49.8	61.4
	Min-max	0.03–7.11	0.44–4.84	0.00–8.19
	CV <sub>fam</sub> (%)	29.4	19	14.5
HS (degree-days)	Min <sub>fam</sub> -max <sub>fam</sub>	1.71–4.67	1.60–2.78	1.95–4.10
	F-value Eq. (1) (p-value)	6.53 (<0.001)	0.69 (0.637)	0.49 (0.940)
	F-value Eq. (2) (p-value)	8.14 (<0.001)	0.03 (0.855)	1.20 (0.303)
	Mean	274.7	323	285
	CV (%)	24.7	18.6	37.3
	Min-max	168.7–530.9	220.3–489.8	129.3–727.9
	CV <sub>fam</sub> (%)	12.2	11.9	17.2
	Min <sub>fam</sub> -max <sub>fam</sub>	232.7–328.4	273.8–386.7	225.9–384.0
	F-value Eq. (3) family (p-value)	2.28 (0.024)	9.47 (<0.001)	7.66 (<0.001)
	F-value Eq. (3) taxa (p-value)	0.02 (0.890)	0.06 (0.809)	18.39 (<0.001)

heritabilities at the family level (Eq. (4)) were estimated for *ex situ* and *in situ* variables using the DIOGENE software, an extended version of the OPEP software (Baradat et al., 1995).

$$h_{fam}^2 = \frac{\sigma_{fam}^2}{\sigma_e^2 + \sigma_{fam}^2} \quad (4)$$

where  $h_{fam}^2$  is heritability at family level,  $\sigma_{fam}^2$  represents the variance component due to family and  $\sigma_e^2$  is the error variance component. Standard deviations of heritabilities were calculated using the Jackknife method with the same software (Baradat et al., 1995).

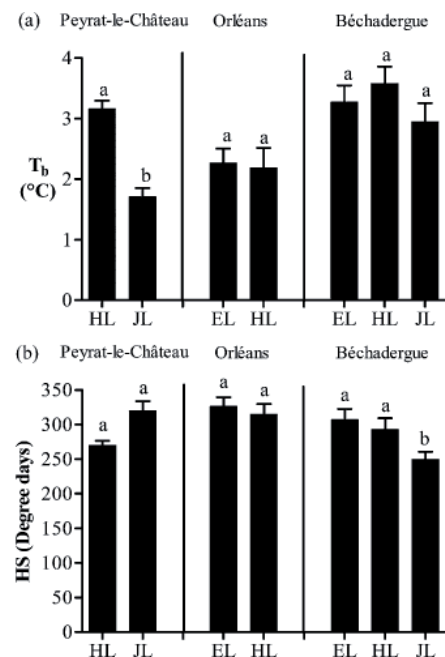
### 3. Results

#### 3.1. Flushing date, threshold temperature and heat-sum in bud burst *ex situ* experiment

General statistics for the flushing dates in the two temperature conditions, threshold temperatures and heat-sums are provided in Table 2. While at continuous mean temperatures of 16–17 °C bud burst took between 59.5 and 62.5 days from January 1, at 11 °C the means ranged from 68 up to 79.5 days according to experiments. Spearman's correlation coefficients between DCH1 and DCH2 were 0.60 (*p*-value: 0.0697) for Peyrat-le-Château, 0.94 (*p*-value: 0.0167) for Orléans and 0.95 (*p*-value: <0.001) for Bécharde.

Mean threshold temperatures ( $T_b$ ) ranged from 2.2 up to 3.3 °C and heat-sums (HS) from 275 up to 323 degree-days. Results were mostly consistent among the three experiments but with a slightly lower  $T_b$  and higher HS for Orléans. A higher variability for temperature thresholds was consistently observed compared to heat-sums at both individual and family levels.

In fact, these overall results may hide different realities connected to taxa representation in each experiment. Significant differences among taxa (HL, EL and JL) were found for temperature thresholds at Peyrat-le-Château but not at Orléans and Bécharde (Fig. 2(a)). At Peyrat-le-Château, a clear difference between JL



**Fig. 2.** Taxa means and standard errors in the 3 *ex situ* flushing experiments in 2006 (Peyrat-le-Château and Orléans) and 2008 (Bécharde). (a) Base temperature expressed in °C ( $T_b$ ). (b) Heat-sum accumulated from 1st January up to flushing day (HS) expressed in degree-days. (Different letters mean the existence of significant differences among taxa (*p* < 0.005).)

**Table 3**  
Statistics for phenological parameters observed in field plantations at Peyrat-le-Château and Orléans in 2006. TB2, Julian day by which the terminal bud reached stage 2; G50, Julian day by which 50% of the crown was flushed. HSTB2, heat-sum accumulated from 1st January up to TB2; HSG50, heat-sum accumulated from 1st January up to G50. F and p-value using model described in Eq. (1).

Site	Variable	Code	Unit	Mean	CV%	Min-max	CV% <sub>fam</sub>	Min <sub>fam</sub> -Max <sub>fam</sub>	F-value	p-Value
Peyrat-le-Château	Terminal bud flushing	TB2	Days	109	4.94	92–124	2.13	105.6–114.2	2.26	0.024
	Crown greening	G50	Days	101.1	6.21	89–114	3.38	93.4–106.6	4.07	<0.001
	Terminal bud flushing heat-sum	HSTB2	Degree-days	237.2	23.88	105.6–410.0	17.95	183.7–310.7	11.71	<0.001
	Crown greening heat-sum	HSG50	Degree-days	186.3	24.03	74.4–314.0	20.1	136.7–249.0	19.37	<0.001
Orléans	Terminal bud flushing	TB2	Days	98.1	2.39	93.5–101	1.42	97.5–100.2	2.07	0.097
	Crown greening	G50	Days	101.9	3.95	96–108	2.86	99.0–107.3	3.64	0.011
	Terminal bud flushing heat-sum	HSTB2	Degree-days	269.6	10.18	215.5–330.1	11.11	234.2–310.8	40.6	<0.001
	Crown greening heat-sum	HSG50	Degree-days	293.8	11.07	247.4–364.6	9.5	252.0–319.3	6.84	<0.001

and the hybrid material (HL) was detected. Mean values of  $T_b$  were 1.7 °C and 3.2 °C respectively for JL and HL. While at Orléans the means were 2.3 °C for EL and 2.2 °C for HL, at Béchadergue the mean values were higher than in the previous sites: 3.3 °C for EL, 3.6 °C for HL and 3 °C for JL. Analysis of variance (Eq. (2)) showed at Peyrat-le-Château, significant differences among progenies for  $T_b$  (Table 2), but only JL differed from HL progenies.

For heat-sums (Fig. 2(b)), significant differences among taxa were observed in Béchadergue where JL was significantly different from EL and HL. But significant differences were detected neither at Orléans among HL and EL nor at Peyrat-Le-Château among HL and JL. Mean HS values found at Peyrat-le-Château were 320 and 270 degree-days for JL and HL, respectively. While at Béchadergue HS reached 250, 293 and 307 degree-days for JL, HL and EL, the same parameter, reached 315 degree-days for HL and 326 °C days for EL at Orléans. When variability was examined at the progeny level using eq. 3 (Table 2), significant differences for HS were found within each experimentation but they concern only one HL progeny at Peyrat-le-Château and only one EL progeny at Orléans. At Béchadergue, differences among progenies were significant (F-value: 7.66 and p-value: <0.001).

### 3.2. In situ bud burst observation

Table 3 shows a summary of basic statistics for variables observed and measured in the field at Peyrat-le-Château and Orléans in 2006. F- and p-values come from analysis of variance using the model presented in Eq. (1).

It took more than 98 days on average since January 1st to see flushing of the terminal bud (stage 2, Fig. 1). Orléans was about 10 days earlier than Peyrat. Significant differences among progenies were found only in Peyrat but not in Orléans. Greening of trees took a bit longer: around 101 days. G50 mean values were very similar among the two sites and significant differences among progenies were found for this parameter in both sites.

In terms of heat-sums, 237 vs 270 degree-days were needed for terminal bud flushing respectively in Peyrat and Orléans. The difference among sites was much larger for greening: more than 100 additional degree-days were needed in Orléans to reach G50 (186 vs 294 degree-days).

As noticed in Table 3, one major difference between classical phenological parameters (TB2, G50) and heat-sums parameters was the very small variability observed both at the individual and family levels for classical parameters compared to heat-sums.

### 3.3. Correlations between ex situ and in situ phenology parameters

Combined analysis of variance for Peyrat-le-Château and Orléans experiments showed that progeny and environmental (chamber-field) effects are significant (results not presented), while interaction terms were not significant, except for Orléans experiment for heat-sum (Table 4). In this case, only one EL family strongly contributed to the interaction term as revealed by its high relative ecovalence (72.2%).

Spearman rank correlation coefficients between variables assessed *ex situ* (DCH1, DCH2, and  $HS_{ch}$ ) and their corresponding variables *in situ* (G50, TB2, HSG50 and HSTB2) are presented in Table 5 for Peyrat-le-Château and Orléans experimentations. Note that for heat-sums ( $HS_{ch}$ , HSG50 and HSTB2), the mean progeny temperature thresholds (obtained from *ex situ* experience) were used.

Positive relationships between flushing dates in the field and in the climatic chambers were observed but they were quite different according to sites. They were homogeneously high and significantly different from 0 for Orléans (0.83–0.94) but much more variable

**Table 4**

F and (p-values) for progeny x environment interaction terms from combined analysis. Environments include chambers (CH1, CH2) and field.

Environment tested	Variables tested	Peyrat-le-Château F (p-value)	Orléans F (p-value)
Chamber 1–chamber 2	DCH2 and DCH1	1.60 (0.126)	0.90 (0.494)
Chambers-field	DCH and G50	1.20 (0.298)	0.20 (0.058)
Chambers-field	DCH and TB2	1.00 (0.537)	1.80 (0.126)
Chambers-field	HS <sub>ch</sub> and HSG50	1.40 (0.209)	1.80 (0.128)
Chambers-field	HS <sub>ch</sub> and HSTB2	1.50 (0.160)	6.40 (< 0.001)

**Table 5**Spearman correlation coefficients (and p-values) at Peyrat-le-Château and Orléans between progeny means for *in situ* and *ex situ* parameters for year 2006: flushing dates expressed in Julian days (DCH1, DCH2, G50 and TB2) and corresponding heat-sums accumulated from 1st January (HS<sub>ch</sub>, HSG50 and HSTB2).

Site	Variable	DCH1	DCH2	HS <sub>ch</sub>
Peyrat-le-Château	TB2	0.38 (0.283)	0.82 (0.004)	-0.45 (0.192)
	G50	0.26 (0.475)	0.57 (0.083)	-0.46 (0.184)
	HSTB2	-0.03 (0.946)	-0.68 (0.035)	0.86 (0.003)
	HSG50	-0.006 (1.000)	-0.68 (0.035)	0.83 (0.005)
Orléans	TB2	0.89 (0.033)	0.83 (0.058)	0.71 (0.136)
	G50	0.89 (0.033)	0.94 (0.017)	0.54 (0.297)
	HSTB2	0.14 (0.803)	-0.09 (0.919)	0.54 (0.297)
	HSG50	0.26 (0.658)	0.029 (1.000)	0.71 (0.136)

and mostly non-significant for Peyrat (0.26–0.82). For the latter, the best link between *in situ* and *ex situ* flushing was with DCH2.

Relationships between flushing dates in the field and heat-sums in chambers were moderate, negative or positive but never significantly different from 0. Correlations between heat-sums calculated in the field and dates of flushing in the chambers were always very low or negative.

The best links between *in situ* and *ex situ* parameters were found for heat-sum variables (HS<sub>ch</sub> and HSG50 and HSTB2). Correlation coefficients were more consistent across sites and more stable for greening (0.71–0.83) than for terminal bud flushing (0.54–0.86).

#### 3.4. Linear model for prediction of *in situ* flushing from *ex situ* observation

We examined relationships between years for G50 and TB2. While correlation coefficients were low (0.24, *p*-value = 0.497) between progeny means for 2006- and 2007-'greening' dates (G50) at Peyrat-le-Château, they became much higher (0.68, *p*-value < 0.001) between 2006- and 2007-dates for terminal bud flushing (TB2). The highest correlations (0.93, *p*-value < 0.001) were found between 2006- and 2007-heat-sums (HSG50 and HSTB2). The lower link revealed between G50 variables could be explained by a modification of the methodology of observation described in Section 2.3.

Based on available information, we looked for the best linear models combining *in situ* and *ex situ* parameters to predict field flushing from *ex situ* flushing results. Some of these models are presented in Table 6. The two best models involved only heat-sums parameters. Heat-sum accumulated from 1st January up to

**Table 6**Linear models for prediction of terminal bud flushing (TB) and greening (G50) in plantation (Peyrat-Le-Château, 2007) based on *ex situ* flushing observations (Peyrat-Le-Château and Orléans, 2006): flushing is expressed either in Julian days (TB2 and G50) or in heat-sums (HSTB2 and HSG50).

Model	$r^2_{\text{model}}$	RMSE	$r^2_{\text{pred-obs}}$
HSTB2 = 0.6747503*HS <sub>ch</sub> + 60.1047456	0.52	188.05	0.88 (0.002)
HSG50 = 1.235871*HS <sub>ch</sub> - 122.210509	0.71	58.87	0.86 (0.003)
TB2 = -0.5291*DCH1 + 114.4242	-0.05	10.36	-0.58 (0.076)
TB2 = 0.4729*DCH2 + 86.7648	0.01	1.59	0.58 (0.076)
G50 = 1.2711*DCH1 + 77.8410	0.38	22.83	0.24 (0.503)
G50 = -0.7888*DCH2 + 71.6163	0.71	23.46	0.52 (0.118)
TB2 = -0.07938*HS <sub>ch</sub> + 127.37882	0.27	1.75	0.72 (0.018)
G50 = 0.0005208*HS <sub>ch</sub> + 01.2	-0.08	22.89	-0.42 (0.232)

TB2: in the field up to 50% greening of trees (HSG50) was better explained by *ex situ* HS<sub>ch</sub> than heat-sum for terminal bud flushing *in situ* (HSTB2). In terms of accuracy of prediction, the model proposed for greening showed a lower RMSE but also a lightly lower  $r^2_{\text{pred-obs}}$ .

Although, the model predicting the date of flushing (TB2) using HS<sub>ch</sub> like independent variable had acceptable values of RMSE and  $r^2_{\text{pred-obs}}$ , TB2 is weakly explained by HS<sub>ch</sub> ( $r^2_{\text{pred-obs}} = 0.27$ ). For this reason this model could not usefully achieve our objectives.

Focusing on models in which TB2 and G50 were involved, we saw that these two variables were always better explained by DCH2 than DCH1; the relationships between predicted and observed values were from low to moderate but never significantly different from 0.

#### 3.5. Heritability of phenological parameters

Heritability parameters at family level with their corresponding standard deviation are presented in Table 7 for *ex situ* and *in situ* variables. Results are presented only for Peyrat experiment for which enough trees per progeny were studied. These estimations concerned only hybrid families. In some cases (DCH1 and G50) due to the absence of significant differences among families (see *p*-value in Table 7), heritabilities were not estimated.

For both *ex situ* and *in situ* flushing assessment, the heat-sum variables (HS<sub>ch</sub>, HSG50, HSTB2) presented a higher genetic control ( $h^2_{\text{fam}} = 0.70 - 0.95$ ) than the dates of flushing ( $h^2_{\text{fam}} = 0.57 - 0.59$ ) themselves (DCH2, TB2) and also higher family coefficients of variation (10.6–20.5% vs less than 2.5%). The highest genetic control was

**Table 7**Heritability (SD= standard deviation), coefficient of variance at family level and ANOVA results for *ex situ* and *in situ* flushing observation at Peyrat-le-Château.

Trait	$h^2_{\text{fam(SD)}}$	CV% <sub>fam</sub>	F-value	p-Value
<i>Ex situ</i>				
DCH1	<sup>a</sup>	2.13	1.0679	0.393
DCH2	0.59 (0.24)	3.81	2.4295	0.02
HS <sub>ch</sub>	0.70 (0.15)	10.64	3.286	0.002
<i>In situ</i>				
G50	<sup>a</sup>	2.19	1.7533	0.097
TB2	0.57 (0.29)	2.22	2.3112	0.026
HSG50	0.95 (0.02)	20.45	19.395	<0.001
HSTB2	0.87 (0.04)	15.68	7.9328	<0.001

<sup>a</sup> Parameters not calculated because of the absence of significant differences among families.

detected for HSG50 ( $h_{fam}^2 = 0.95$ ) with the lowest standard deviation ( $SD h_{fam}^2 = 0.02$ ) and the highest variation ( $CV_{fam} = 20.45$ ).

#### 4. Discussion

##### 4.1. Temperature thresholds and heat-sums in *ex situ* experiments

Our results clearly showed higher values for temperature thresholds and heat-sums than those obtained by Worrall (1993) using a similar methodology and model to calculate thermal time. Our values rose around 2.85 °C (standard deviation = 1.5) for the temperature threshold, and 294 degree-days (standard deviation = 78.8) for the heat-sum. Worrall found for another larch species (namely the subalpine larch *Larix lyallii* Parl.), a threshold temperature very low, 1.46 °C (SD = 0.17) and a heat-sum requirement of less than 75 degree-days above this threshold.

While Worrall's results may be highly imprecise as they are based on very few trees (6), this difference could nevertheless be explained because subalpine larch is a high elevation species: often species from high altitude have either lower heat accumulation requirement to bud burst or lower base temperature, or both (Worrall, 1983). In our case, the genetic origin of the European larch material directly tested (at Orléans) or used as genitor of the hybrids (at Peyrat) came from much lower elevations (Central Europe, 400–600 m asl) in comparison to Worrall's material, which would entail greater quantities of heat necessary to bud burst. According to Worrall (1993), there are two broad groups of species depending on threshold values, a 'continental' one with low threshold temperatures, and a 'maritime' one with higher thresholds. We could infer that EL and HL are rather 'maritime' and JL is in the "continental" category.

Compared to other conifers, larch showed in our study similar temperature thresholds as *Abies lasiocarpa* (2.4–2.8 °C) but lower than for *Abies amabilis* (3.6–5.3 °C) (Worrall, 1983). Nevertheless, both of these species had globally lower heat-sum requirements (between 168 and 185 degree-days for *A. amabilis*; 115 and 122 for *A. lasiocarpa*). Compared to Douglas-fir (*Pseudotsuga menziesii*), larch showed lower values for temperature thresholds and lower or similar heat-sum requirements (Campbell and Sugano, 1975). These authors studied bud burst phenology using provenances of Douglas-fir under different combinations of photoperiod and temperature. They estimated threshold temperatures above 4 °C and heat-sum requirements for flushing between 210 and 400 degree-days, using the conventional temperature summation method (Arnold, 1959). We obtained 2.2–3.3 °C for  $T_b$  and 237–270 degree-days for terminal bud flushing.

If a fixed threshold of 5 °C was used in our *ex situ* experiment (results not shown), heat-sum requirements would have been around 200 degree-days with 51 chilling days (days with mean temperature <5), a result similar to that found by Murray et al. (1989) for *L. decidua*. In that study, heat-sums ranged from 25 to 150 degree-days with chilling days varying from 144 to 56. Murray et al. (1989) conducted their experience at sites within a latitude range of 55–57°N which was 10° more northerly than our sites. That could explain the lower requirement of degree-days to bud burst for a similar range of chilling days. They also found, for the same range of chilling days, that European larch had a lower requirement of heat units to bud burst than other conifers like *Tsuga heterophylla* and *Picea sitchensis* which required between 100 and 300 degree-days. In concordance, our results reported lower values than those found for *Tsuga* and *Picea* for the same range of chilling days.

The selected model we used to calculate thermal requirements to bud burst (Thermal Time model) was one of the simplest among many different models developed so far (Campbell and Sugano,

1975; Cannell and Smith, 1983; Murray et al., 1989; Hänninen, 1990; Kramer, 1994; Chuine et al., 1998; Chuine, 2000; Linkosalo et al., 2006b; Migliavacca et al., 2008). But, results obtained by application of TT-model proved to be acceptable in terms of accuracy (Arnold, 1959; Karlsson et al., 2003; Migliavacca et al., 2008).

This type of model only considers a forcing phase and does not take dormancy into account. It is assumed either that the chilling requirement of the species has been achieved or that plants do not have chilling requirements (Chuine et al., 2003). The advantages of TT-model are their simplicity of application and the low level of information required (daily mean temperature). However, the threshold temperature over which heat is accumulated must be carefully selected. It is known that there are differences for heat-sum requirements to bud burst for different genotypes within a given species (Nienstaedt, 1972; Alfaro et al., 2000). For this reason, in genetic studies, it would be more appropriate to use true thresholds estimated for each genetic entity.

##### 4.2. Variability and genetic control of bud burst date and of heat-sum requirements

An abundant literature exists on the genetic variability for bud burst phenology in terms of bud flushing date or flushing stage (e.g. Christophe and Birot, 1979; El-Kassaby and Park, 1993; Li and Adams, 1993, 1994; Matziris, 1994; Hannerz et al., 1999; Alfaro et al., 2000; Howe et al., 2000; O'Neil et al., 2000; Codesido and Fernandez-Lopez, 2009) and provides numerous estimates of genetic parameters for many species. Most studies reveal moderate to strong genetic control for these phenological traits as we found in this study.

In contrast, information is much scarcer on the genetic variability for heat-sum requirements connected to phenology. This study showed a broad phenotypic variability at the individual tree level for both  $T_b$  and HS (CV% in Table 2). As well, evidence of variability for  $T_b$  and HS was identified at the taxa level (Fig. 2). As for  $T_b$  of JL; it only differed from the other taxa at the Peyrat-le-Château, where JL had a consistently smaller threshold temperature than on the other sites, like Béchadargue, where JL did not differ from others taxa. For heat-sum requirements (HS), the situation differed on the sites, where no significant differences were found among taxa at Orléans (EL and HL) and Peyrat-le-Château (JL and HL) and significant differences were found at Béchadargue with JL differing from HL and EL. However, no significant differences were found among the last two taxa.

By other hand, the statistical analysis found significant differences among progenies for HS at all sites, confirming the existence of true differences among them, whatever the level of the covariation between HS and  $T_b$  variables was.

Such a genetic variability has been described by a few authors either at the species level or at the population level. For example, Worrall (1983) put into evidence significant differences for both heat-sums and temperature thresholds among two fir species (*Abies amabilis* and *A. lasiocarpa*). Significant differences among populations within both species could be found for thresholds but not for heat-sums. In contrast we found significant differences at progeny level for heat-sums (HS) but not for threshold temperatures, except for Peyrat-le-Château, where values for JL were strongly different from HL. In others studies, Campbell and Sugano (1975) found significant differences among provenances of Douglas-fir for both heat-sums and temperatures. As well, Chuine et al. (2001) showed provenance differences in temperature thresholds for shoot elongation for *Pinus contorta*.

However, estimates of heritability for heat-sum requirements are scarce. Nienstaedt (1972) reported narrow-sense heritabilities for heat-sum requirements ranging from 0.53 to 0.71 for progenies of white spruce (*Picea glauca*) in experimental indoor environ-

ments; Alfaro et al. (2000) found family heritability values of  $0.79 \pm 0.25$  for heat accumulation required for terminal bud burst for Sitka spruce (*Picea sitchensis*). Our estimates of heritability for  $HS_{ch}$ , HSTB2 and HSG50 (i.e. 0.70–0.95) were similar to the latter. This is an encouraging finding as it means that heat-sum variables are overall under strong genetic control but also that genotype with a given performance (i.e. early or late flushing) could be selected and genetically improved.

Another important finding to emphasize from our study is the higher genetic control observed for heat-sum variables than for dates of bud burst. We obtained heritability values of 0.57–0.59 for bud burst days *in situ* and *ex situ*, which are comparable to many published results: for example Li and Adams (1993) found for Douglas-fir heritabilities for bud burst expressed in days that reached 0.44–0.47.

#### 4.3. Indirect prediction of *in situ* flushing from *ex situ* observation

The stable performance of progenies (Table 4) and the high correlation coefficients shown in Table 5 between some traits observed in controlled conditions and those directly observed in the field (DCH1/DCH2 and TB2/G50; HS and HSTB2/HSG50) demonstrated that *ex situ* observations could be a reliable alternative to field observation. *Ex situ* experiments allowed indeed a proper ranking of genotypes for bud flushing. This type of indoor test could be particularly helpful for breeders, particularly when a large number of genotypes have to be assessed, from aged and far-away trials, situations where field observations become very difficult and time-consuming.

The possibility to use *ex situ* results to predict *in situ* flushing both forwards in time or backwards looks also particularly attractive in special studies: for example, interest could be to retroactively determine the start of the vegetation growth period or to plan time for some growth observation work in remote places or collection of flushed samples. In this context, the stability of phenology variables through years is another important condition. Significant positive (genetic) correlations among flushing dates over years have been reported by several authors (e.g. Christophe and Birot, 1979; Worrall, 1983; Danusevicius and Gabrilavicius, 2001; Codesido and Fernandez-Lopez, 2009). We found as well significant positive correlation for terminal bud flushing dates (TB2) at Peyrat-le-Château during 2 successive years.

In fact, for these studies, the knowledge itself of flushing dates would be of little use even if the ranking of genotypes over years was correct. In contrast, the knowledge of heat-sum requirements to reach a certain flushing stage looks much more useful in the perspective of prediction of flushing over years because heat-sums could be reconstructed from past climatic data or from current spring temperatures as long as temperature thresholds are known.

#### 4.4. Limits and perspectives of the study

Obviously, the objective of our study did not focus on the physiological mechanisms of bud burst. Instead our intent was to develop a simple methodology able to predict bud burst and compatible with genetic variability studies. For this reason we simplified the experimental protocol by assuming that the chilling requirements were met thanks to outdoor and cold chamber low temperatures, by using a fixed and short photoperiod which did not mimic the photoperiodic conditions in nature, by starting accumulating temperature from a fixed arbitrary date, as classically used in the literature (e.g. Cannell and Smith, 1983; Alfaro et al., 2000; Rötzer et al., 2004).

As shown previously, although the models tested for prediction of *in situ* 2007 – flushing from *ex situ* 2006 – observations are simple (Table 6), some of them looked successful enough in predicting field

flushing parameters such as HSG50 or HSTB2 with an acceptable accuracy.

However, it is clear that if we want to further improve the prediction of bud burst, more sophisticated models are needed. For many years, authors (Campbell and Sugano, 1975; Murray et al., 1989; Cannell and Smith, 1983; Chuine and Cour, 1999; Rötzer et al., 2004) have indicated that critical forcing temperature accumulations are in fact dependent on chilling requirements through some compensation. More recently, the photoperiod (accumulation and quality of light) has been shown to influence too bud burst date (Partanen et al., 2001; Linkosalo and Lechowicz, 2006a). Following Linkosalo and Lechowicz (2006a), the photoperiod would act as a triggering factor for initiation of bud development: while chilling requirements were met by the end of December, the latter authors showed on birch that the accumulation of temperature leading to bud burst started only 2 months later once plants received the proper photoperiodic signal. Much probably by neglecting these aspects in our study we may have overestimated the heat-sum requirements.

In a context of climatic change, bud burst looks as a critical developmental stage and the expectation of more frequent damages by late winter frosts is often advocated (Hänninen, 1991). But contradictory predictions have been published too (Linkosalo and Lechowicz, 2006a). Species-dependent requirements and use of different models may explain these differences. Advanced dates of bud burst are often foreseen by ST (sequential time)-type models. In contrast, TT-type models including the photoperiod effect as used by Linkosalo and Lechowicz (2006a) do not predict an earlier flushing and thus an increase of frost risks.

Precise chilling requirements on one side and the possible effect of the photoperiod on bud burst on the other look like priorities for our further investigation on larch phenology. More particularly the genetic variation of the responses to these parameters is our main interest.

## 5. Conclusion

Reliable indirect predictors of bud burst or flushing in the forest have been searched for. Observation of flushing dates *ex situ* – on twigs placed in climatic chambers – allowed the proper ranking of genotypes for flushing in the field. More interestingly, heat-sum requirements for flushing looked as an even more reliable but also more useful predictor, in particular when indirect prediction aims at predicting flushing events either retroactively or for future years. The proper knowledge of temperature thresholds above which heat-sum is accumulated is a pre-requisite. Our study showed that for both parameters (Tb and HS), some genetic variability exists either at the taxa or at the progeny level and that they are under strong genetic control.

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## **Chapter 4**

### **Complementary results related to chapter 1 and 2**

**First part:** Genetic variability of phenological parameters of ring wood formation

**Second part:** Wood response to environmental conditions:  
study-case of formation of a false ring

**Third part:** Statistical synchronization of microdensity profiles  
with climate variables

In this chapter, we present complementary results, not published so far, which contribute to the objectives of the thesis.

In a first part, a description of the genetic variability and heritability for all variables related to phenology of wood formation will investigate the potential use of these parameters in breeding and selection. They will complement results presented in *chapter 1*.

In a second part, we present additional results on the effect of environmental stimuli (soil water shortage) on tree response, namely the formation of false ring, in connection to *chapter 2*.

Finally, in a third part, we investigate an original methodology aiming to date microdensity profiles through a statistical synchronization with climatic data; results on phenology of wood formation are then compared with results obtained from the ‘pinning’ method (connected to *chapter 1*).

## **First part: Genetic variability of phenological parameters of ring wood formation**

### **Context and Objective**

Variability of characters and their probability to be transferred to offspring are key starting points for selection process in tree improvement. As we mentioned in the introduction of the thesis, variations in wood anatomy are translated to variations of wood density and consequently these changes determine the quality properties of products made from wood as raw material. The timing and the duration of formation of the different wood tissues through the growing season may influence the late-wood proportion and thus the overall wood density. A better knowledge of the genetic determinism of dynamics parameter of wood formation will contribute greatly to a better understanding of the genetics of wood density,

which will be essential for an efficient incorporation of this wood quality characteristic in tree breeding programs.

In chapter 1, we study the timing of xylogenesis in different taxa of larch. Trees of hybrids, European and Japanese larches (HL, El and JL) were sampled and the major differences were detected for the dates of ending of late-wood formation and for the length of the growing season (14-17 days longer for HL compared to JL and EL). Based on these results and taking advantages from the progeny structure of our experimental design (comparison of families test), we studied the genetic variability of phenology of wood formation and the relationships among phenology of wood formation characters at individual and family levels.

However, it is necessary to keep in mind some constraints related to the genetic material used in the thesis which limit the interpretation of our results. The plant material and the experimental design used in this study were probably far from optimal. Besides the heterogeneous nature of the inter-specific hybrids tested (F1, F2 with various genetic backgrounds), for feasibility reasons, we had first to limit the sample of trees studied (number of progenies and individuals per progeny) to a manageable size (handling of wood samples) and secondly to restrict the frequency of tree observations and measurements to every 7-10 days. Due to this diffuse sampling of trees across the progeny trial, we could not benefit from the initial experimental design (complete randomized block design) in subsequent data analysis and could not thereby adjust for some obvious environmental effects.

## **Material and Methods**

Data from the farm-field progeny trial in Peyrat-Le-Château (PEY) were used to study genetic parameters of phenology of wood formation. As a reminder, for this specific study, 9

full-sib hybrid families were tested, with 5-11 individual each. More details about the experiment and observations and measurements can be found in *chapter 1*.

## **Results**

### **Variability levels (individual and family)**

Table V presents phenotypic variability observed at individual and family (progeny) levels. Dates of start and completion of formation of the different tissues (Sew, Stw, Etw, Elw) presented a low variability across analyzed levels (individual and family): consequently, no significant differences were detected among families.

Variables corresponding to duration of tissue formation and proportion of these durations over the growing season showed higher level of variability at both individual and family levels. Duration of transition-wood formation and transition-wood proportion (Dtw and %Dtw) were particularly variable with coefficient of variation around 70% at individual tree level and 21% at family level. But differences among families were not significant.

Ring width and its components (l, ewd, twd, lwd) were highly variable both at individual and family levels, much more for transition- and late-wood than for early-wood. Significant differences among progenies were detected for total ring, early-wood and late-wood widths but not for transition-wood width.

In contrast, overall ring density and its components showed a low variability. Significant differences among families were detected for mean, minimum and maximum ring density and also for early and transition-wood densities, but not for late-wood density.

**Table V.- Basic statistics of wood formation and growth variables and F-test values from ANOVA analysis on the Progeny factor (figures in italics indicate significant differences among treatments,  $\alpha$ -level= 0.05).**

Variable	Unit	Mean (SD)	CV%	CV% <sub>fam</sub>	F-value (p-value)
Sew	julian days	110 (4.37)	4	1.63	0.90 (0.428)
Stw	julian days	214 (12.37)	6	1.60	0.70 (0.251)
Etw	julian days	230 (16.32)	7	1.73	0.50 (0.133)
Elw	julian days	280 (16.05)	6	2.51	1.60 (0.128)
Dew	days	104 (13.24)	13	3.52	0.70 (0.250)
Dtw	days	17 (12.28)	73	22.10	0.60 (0.158)
Dlw	days	50 (15.57)	31	12.05	1.10 (0.361)
Dtot	days	170 (15.82)	9	4.08	1.50 (0.137)
%Dew	percentage	61.19 (8.24)	13	4.95	0.90 (0.495)
%Dtw	percentage	9.70 (6.6)	68	20.38	0.60 (0.160)
%Dlw	percentage	29.11 (8.58)	29	9.84	0.80 (0.381)
l	mm	7.13 (1.93)	27	17.70	3.80 ( <i>&lt;0.001</i> )
ewd	mm	5.57 (1.37)	25	16.88	4.90 ( <i>&lt;0.001</i> )
ewp	percentage	79.17 (7.42)	9	4.31	1.50 (0.143)
twd	mm	0.56 (0.52)	94	32.95	0.80 (0.326)
twp	percentage	7.42 (6.29)	85	26.23	0.70 (0.293)
lwd	mm	1.00 (0.57)	57	31.14	2.40 ( <i>0.014</i> )
lwp	percentage	13.40 (5.85)	44	21.91	2.00 ( <i>0.046</i> )
SFew	mm.day <sup>-1</sup>	0.05 (0.01)	26	14.91	3.1 ( <i>0.002</i> )
SFtw	mm.day <sup>-1</sup>	0.03 (0.02)	51	26.35	1.60 (0.130)
SFlw	mm.day <sup>-1</sup>	0.02 (0.01)	43	33.17	2.60 ( <i>0.008</i> )
SLOPEtw	g/cm <sup>3</sup> .day <sup>-1</sup>	0.03 (0.03)	81	27.04	0.80 (0.343)
MRD	g/cm <sup>3</sup>	0.42 (0.04)	9	4.74	2.1 (0.0331)
MID	g/cm <sup>3</sup>	0.28 (0.03)	11	6.16	3.6 (0.0007)
MAD	g/cm <sup>3</sup>	0.91 (0.06)	7	3.12	2 (0.0421)
ew.density	g/cm <sup>3</sup>	0.33 (0.03)	8	5.36	3.5 (0.001)
tw.density	g/cm <sup>3</sup>	0.61 (0.05)	8	4.26	3.4 (0.0012)
lw.density	g/cm <sup>3</sup>	0.85 (0.06)	7	3.29	1.8 (0.0824)
T10rg	julian days	117 (5.14)	4	1.42	0.50 (0.143)
Tirg	julian days	180 (10.2)	6	2.68	1.9 ( <i>0.051</i> )
T90rg	julian days	227 (19.86)	9	4.17	2.1 ( <i>0.033</i> )
T10hg	julian days	145 (5.37)	4	1.74	1.6 (0.1108)
Tihg	julian days	195 (6.1)	3	1.11	1.1 (0.3963)
T90hg	julian days	225 (9.67)	4	1.37	0.9 (0.4846)
T10lig	julian days	175 (6.98)	4	2.00	2.5 ( <i>0.0128</i> )
Tilig	julian days	225 (5.85)	3	1.02	1.2 (0.3089)
T90lig	julian days	252 (9.21)	4	1.57	1.7 (0.1007)

Speed of tissue formation (SFew, SFtw, SFlw) and of density change per day during the transition-wood formation (SLOPEtw) showed moderate to high variability levels (CV, between 26 – 81 % at individual and 15 – 33 % at family level). The speed of early- and late-wood formation showed significant differences among families.

For some variables probably more sensitive to environmental factors (such as dates of tissues formation), the evaluation of progeny values might be biased and did not allow us to reveal statistically significant differences among progenies.

Heritabilities ( $h^2_{fam}$ ) at family level and their corresponding standard deviations (SD) were estimated for variables showing significant differences among families (Table VI).

**Table VI.- Heritability at family level (and Standard deviation (SD)) for traits showing significant differences among families.**

Variable	$h^2_{fam}$	SD
l	0.74	1.116
ewd	0.796	0.853
lwd	0.586	0.281
lwp	0.494	2.658
SFew	0.68	0.007
SFlw	0.62	0.004
MRD	0.523	0.018
MID	0.726	0.017
MAD	0.502	0.03
ew.density	0.711	0.016
tw.density	0.704	0.028

Moderate to high values of heritabilities (0.49 – 0.80) were estimated, but the precision level was very low in some cases. Although traits of dynamic of wood formation as dates of initiation and completion of tissues not showed significant differences among families, we obtained an estimation of heritability to observe trends. Values of heritability resulted negative and unreliable for Sew, Stw and Etw and positive for ending date of late-wood (0.36) with standard deviation equal to 6.59.

#### **Relationship between characters (individual and family)**

Breeders are usually interested in selecting fast growing trees (large l), together or not with a high mean density (MRD). It might be also of interest for them to select trees better

adapted to the conditions of the growing season. That means, on one hand, to avoid frost problem (not a major issue in larch), and, on the other hand, to optimise growth, overall wood density, and adaptive anatomy-related wood properties, separately or jointly.

It is for example well-known that overall ring density is directly related to late-wood proportion (lwp). Hence to increase late-wood proportion, several strategies could be proposed, such as to select genotypes with a later starting radial growth date. Other alternatives are a later ending growth period, or a faster early- to late-wood change.

Examination of correlations (at individual levels) between phenology of wood formation variables and ring widths and wood density parameters (Table VII) reveals interesting trends.

First of all, one could expect that trees with a later radial-growth initiation date would have a higher late-wood proportion and as a consequence a higher mean ring density. But this hypothesis was rejected because of the not significant relationship found between early-wood starting date and MRD, MID, MAD, ew.density, tw.density and lw.density ( $r$  between -0.09 and 0.13).

In contrast, a later ending late-wood formation is highly correlated with a higher total duration of annual ring formation ( $r= 0.96$ ,  $p\text{-value}=<0.001$ ) but also to a larger ring width ( $r= 0.63$ ,  $p\text{-value}=<0.001$ ) and of its components: early-, transition- and late-wood widths ( $r$  between 0.47 and 0.54). A later ending date of late-wood formation is positively associated with higher mean and maximum ring densities ( $r= 0.42$ ,  $p\text{-value}=<0.001$  for MRD and  $r= 0.23$ ,  $p\text{-value}=0.021$ , for MAD) and lower minimum ring wood density values ( $r= -0.22$ ,  $p\text{-value}=0.027$ ).

The date of transition from early- to late-wood affects the total duration of ring formation, (especially  $E_{tw}$ ) and ring width but less than the ending date of late-wood formation, and has nearly no impact on overall ring density ( $r<0.15$ ).



A larger ring width ( $l$ ) is strongly positively related with larger early-wood and late-wood widths ( $r= 0.94$ ,  $p\text{-value}=\leq 0.001$  for  $ewd$  and  $r= 0.70$ ,  $p\text{-value}=\leq 0.001$  for  $lwd$ ) and slightly less connected with transition-wood width ( $r= 0.46$ ,  $p\text{-value}=\leq 0.001$ ). Also a larger ring width is moderately to strongly associated to a higher speed of tissue formation ( $r$  between 0.45 and 0.85) and to a higher mean ring density ( $r= 0.37$ ,  $p\text{-value}=\leq 0.001$ ). Concerning mean ring density (MRD), higher MRD were connected with higher density of the tissues forming the annual ring ( $r$  between 0.36 and 0.69, results not shown).

Duration of tissue formation is strongly related to the widths of the corresponding tissue, except for transition-wood. It is also significantly and weakly related to their formation speed. Trees with a longer duration of early-wood formation had a slower speed of early-wood formation ( $r= -0.31$ ,  $p\text{-value}= 0.002$ ) and a later start of transition-wood formation ( $r= 0.94$ ,  $p\text{-value}=\leq 0.001$ ), while trees with a longer duration of late-wood formation showed a higher late-wood proportion ( $r=0.79$ ) and a higher speed of tissue formation ( $r= 0.26$ ,  $p\text{-value}= 0.011$ ) and a higher mean ring density ( $r= 0.27$ ,  $p\text{-value}= 0.007$ ). Duration of transition wood formation seems not to be associated to its formation speed, but it is positively related to transition-wood density ( $r= 0.29$ ,  $p\text{-value}= 0.004$ ).

At family level as well as at individual tree level, (Table VIII), annual ring width and its components are highly dependent on the total duration of ring formation ( $r=0.55\text{-}0.89$ ). The strongest correlations with the latter were also observed with the end-date of late-wood formation and the end-date of transition-wood formation. Ring width was also negatively correlated to the starting-date of early-wood formation ( $r=-0.48$ ): the earlier the start and the latter its completion, the larger the ring. Late-wood proportion was mostly linked to the duration of late-wood formation ( $r=0.89$ ) and mostly to the completion date of late-wood ( $r=0.76$ ).

**Table VII.- Coefficients of correlation at individual tree level between dates and duration of wood tissues formation and, ring characteristics and apical and radial growth parameters. P-values are shown between brackets.**

	Sew	Stw	Etw	Elw	Dew	Dtw	Dlw	Dtot
Stw	-0.03 (0.764)							
Etw	-0.07 (0.509)	0.67 (<0.001)						
Elw	0.19 (0.065)	0.34 (0.001)	0.54 (<0.001)					
Dew	-0.36 (<0.001)	0.94 (<0.001)	0.64 (<0.001)	0.25 (0.012)				
Dtw	-0.06 (0.566)	-0.12 (0.229)	0.66 (<0.001)	0.37 (<0.001)	-0.1 (0.352)			
Dlw	0.27 (0.009)	-0.35 (<0.001)	-0.49 (<0.001)	0.47 (<0.001)	-0.41 (<0.001)	-0.31 (0.002)		
Dtot	-0.08 (0.408)	0.35 (<0.001)	0.56 (<0.001)	0.96 (<0.001)	0.36 (<0.001)	0.39 (<0.001)	0.4 (<0.001)	
%Dew	-0.29 (0.004)	0.65 (<0.001)	0.22 (0.031)	-0.48 (<0.001)	0.71 (<0.001)	-0.37 (<0.001)	-0.72 (<0.001)	-0.4 (<0.001)
%Dtw	-0.05 (0.661)	-0.17 (0.089)	0.61 (<0.001)	0.28 (0.005)	-0.15 (0.15)	0.99 (<0.001)	-0.35 (<0.001)	0.3 (0.003)
%Dlw	0.31 (0.002)	-0.49 (<0.001)	-0.68 (<0.001)	0.24 (0.018)	-0.56 (<0.001)	-0.41 (<0.001)	0.96 (<0.001)	0.16 (0.126)
l	0.12 (0.229)	0.18 (0.083)	0.38 (<0.001)	0.63 (<0.001)	0.12 (0.223)	0.32 (0.001)	0.25 (0.012)	0.6 (<0.001)
ewd	0.07 (0.511)	0.21 (0.036)	0.31 (0.002)	0.47 (<0.001)	0.18 (0.084)	0.19 (0.056)	0.16 (0.115)	0.46 (<0.001)
ewp	-0.2 (0.047)	0.09 (0.398)	-0.26 (0.009)	-0.67 (<0.001)	0.15 (0.149)	-0.44 (<0.001)	-0.42 (<0.001)	-0.63 (<0.001)
twd	0.02 (0.863)	0.06 (0.562)	0.69 (<0.001)	0.50 (<0.001)	0.05 (0.628)	0.85 (<0.001)	-0.2 (0.047)	0.5 (<0.001)
twp	0.01 (0.89)	0.00 (0.993)	0.62 (<0.001)	0.40 (<0.001)	0.00 (0.97)	0.83 (<0.001)	-0.24 (0.016)	0.4 (<0.001)
lwd	0.24 (0.019)	0.04 (0.733)	-0.1 (0.353)	0.54 (<0.001)	-0.05 (0.655)	-0.16 (0.113)	0.66 (<0.001)	0.48 (<0.001)
lwp	0.24 (0.017)	-0.11 (0.278)	-0.34 (0.001)	0.42 (<0.001)	-0.18 (0.072)	-0.34 (0.001)	0.79 (<0.001)	0.36 (<0.001)
SFew	0.24 (0.019)	-0.25 (0.013)	0 (0.963)	0.33 (0.001)	-0.31 (0.002)	0.25 (0.015)	0.34 (0.001)	0.27 (0.008)
SFtw	0.08 (0.453)	0.16 (0.122)	0.01 (0.888)	0.3 (0.002)	0.12 (0.233)	-0.14 (0.172)	0.3 (0.003)	0.29 (0.004)
SFlw	0.11 (0.276)	0.22 (0.032)	0.21 (0.044)	0.46 (<0.001)	0.17 (0.102)	0.05 (0.606)	0.26 (0.011)	0.43 (<0.001)
MRD	0.13 (0.208)	0.04 (0.667)	0.15 (0.131)	0.42 (<0.001)	0.00 (0.991)	0.16 (0.116)	0.27 (0.007)	0.39 (<0.001)
MID	-0.06 (0.535)	0.09 (0.403)	0.05 (0.604)	-0.22 (0.027)	0.10 (0.324)	-0.02 (0.88)	-0.29 (0.004)	-0.21 (0.039)
MAD	0.08 (0.412)	0.17 (0.105)	0.15 (0.139)	0.23 (0.021)	0.13 (0.216)	0.03 (0.738)	0.08 (0.42)	0.21 (0.035)
ew.density	-0.05 (0.599)	0.14 (0.187)	0.11 (0.29)	-0.09 (0.371)	0.14 (0.159)	0.01 (0.937)	-0.21 (0.04)	-0.08 (0.446)
tw.density	-0.09 (0.402)	0.04 (0.685)	0.25 (0.015)	-0.01 (0.919)	0.07 (0.512)	0.29 (0.004)	-0.27 (0.007)	0.01 (0.898)
lw.density	0.08 (0.417)	0.2 (0.054)	0.18 (0.083)	0.12 (0.252)	0.16 (0.127)	0.04 (0.718)	-0.06 (0.531)	0.10 (0.349)

But at the family level, overall ring density was weakly related to any parameter of wood formation phenology ( $r < 0.48$ ). The duration of early-wood formation was much more linked to a later start of transition-wood formation (Stw,  $r = 0.86$ ) than to an early start of early-wood formation ( $r = -0.39$ ).

At family level as well as at individual level, a larger ring width (l) was found to be strongly related to larger early-, transition- and late-wood widths ( $r$  between 0.67 and 0.98) and to a higher speed of tissue formation ( $r$  between 0.6 and 0.97) (results not showed). On another hand, a longer duration of late-wood formation seems to be associated to a higher speed of late-wood formation, while early- and transition-wood duration were shown to be independent of their formation speed.

In conclusion, our results demonstrate that, to increase ring width and overall wood density, breeders must select trees with a longer growing season. The duration of the growing season is much more dependent on the final stages of radial growth than on its early stages. Thus trees with the longest growing season and with the earliest shift from transition- to late-wood will produce a larger ring with a bigger late-wood proportion, and thus with a higher density. If a larger late-wood proportion and a faster transition from early- to late-wood are also advantageous from an adaptive point of view (greater water storage, lower vulnerability to cavitation), then this strategy would be beneficial from all points of view as long as the trees do not suffer from early frost and cold.

**Table VIII.- Coefficients of correlation at family level between dates and duration of wood tissues formation and, ring characteristics and apical and radial growth parameters. P-values are shown between brackets.**

	Sew	Stw	Etw	Elw	Dew	Dtw	Dlw	Dtot
Stw	0.14 (0.686)							
Etw	-0.41 (0.212)	0.49 (0.130)						
Elw	0.08 (0.813)	0.27 (0.426)	0.59 (0.054)					
Dew	-0.39 (0.240)	0.86 (0.001)	0.66 (0.026)	0.21 (0.540)				
Dtw	-0.56 (0.074)	-0.34 (0.303)	0.66 (0.028)	0.41 (0.213)	-0.03 (0.928)			
Dlw	0.38 (0.244)	0.00 (0.993)	0.05 (0.890)	0.83 (0.002)	-0.2 (0.555)	0.05 (0.876)		
Dtot	-0.16 (0.629)	0.23 (0.494)	0.69 (0.019)	0.97 (<0.001)	0.30 (0.370)	0.54 (0.086)	0.73 (0.011)	
%Dew	-0.13 (0.71)	0.35 (0.287)	-0.2 (0.546)	-0.80 (0.003)	0.39 (0.231)	-0.52 (0.098)	-0.85 (0.001)	-0.76 (0.007)
%Dtw	-0.57 (0.067)	-0.39 (0.230)	0.6 (0.049)	0.32 (0.331)	-0.07 (0.829)	0.99 (<0.001)	-0.02 (0.964)	0.46 (0.155)
%Dlw	0.55 (0.081)	-0.12 (0.734)	-0.2 (0.557)	0.66 (0.026)	-0.39 (0.235)	-0.11 (0.738)	0.96 (<0.001)	0.52 (0.099)
l	-0.48 (0.131)	0.21 (0.539)	0.72 (0.012)	0.75 (0.008)	0.44 (0.172)	0.60 (0.052)	0.43 (0.189)	0.86 (0.001)
ewd	-0.59 (0.058)	0.19 (0.582)	0.71 (0.014)	0.64 (0.036)	0.48 (0.139)	0.60 (0.050)	0.30 (0.372)	0.77 (0.005)
ewp	-0.26 (0.437)	-0.10 (0.774)	-0.31 (0.347)	-0.73 (0.011)	0.04 (0.900)	-0.25 (0.453)	-0.69 (0.020)	-0.65 (0.029)
twd	-0.37 (0.262)	-0.06 (0.870)	0.7 (0.017)	0.47 (0.148)	0.14 (0.684)	0.80 (0.003)	0.1 (0.774)	0.55 (0.078)
twp	-0.16 (0.629)	-0.18 (0.603)	0.46 (0.157)	0.20 (0.564)	-0.08 (0.814)	0.64 (0.032)	-0.07 (0.831)	0.23 (0.489)
lwd	0.01 (0.971)	0.33 (0.321)	0.43 (0.192)	0.90 (<0.001)	0.30 (0.368)	0.17 (0.614)	0.82 (0.002)	0.89 (<0.001)
lwp	0.43 (0.184)	0.24 (0.477)	0.08 (0.820)	0.76 (0.006)	0.00 (0.998)	-0.12 (0.716)	0.89 (<0.001)	0.65 (0.031)
SFew	-0.59 (0.058)	0.03 (0.919)	0.64 (0.033)	0.62 (0.041)	0.33 (0.316)	0.66 (0.027)	0.33 (0.325)	0.76 (0.007)
SFtw	-0.33 (0.318)	-0.06 (0.860)	0.35 (0.296)	0.29 (0.386)	0.11 (0.737)	0.42 (0.193)	0.12 (0.722)	0.37 (0.264)
SFlw	-0.1 (0.776)	0.41 (0.208)	0.56 (0.072)	0.84 (0.001)	0.43 (0.183)	0.25 (0.462)	0.65 (0.029)	0.85 (0.001)
MRD	0.15 (0.660)	0.48 (0.133)	0.25 (0.463)	0.06 (0.861)	0.37 (0.259)	-0.15 (0.658)	-0.10 (0.778)	0.02 (0.947)
MID	-0.19 (0.571)	0.11 (0.754)	-0.09 (0.790)	-0.77 (0.006)	0.2 (0.558)	-0.19 (0.575)	-0.89 (<0.001)	-0.71 (0.014)
MAD	0.00 (0.993)	0.53 (0.091)	0.35 (0.291)	0.01 (0.969)	0.5 (0.121)	-0.08 (0.805)	-0.23 (0.505)	0.01 (0.971)
ew.density	-0.18 (0.601)	0.34 (0.309)	0.06 (0.854)	-0.62 (0.044)	0.41 (0.215)	-0.22 (0.508)	-0.81 (0.003)	-0.57 (0.070)
tw.density	-0.36 (0.280)	0.29 (0.386)	0.5 (0.118)	-0.15 (0.664)	0.45 (0.160)	0.29 (0.395)	-0.53 (0.094)	-0.06 (0.863)
lw.density	0.05 (0.879)	0.41 (0.207)	0.09 (0.783)	-0.35 (0.293)	0.36 (0.281)	-0.26 (0.449)	-0.5 (0.119)	-0.36 (0.280)

## **Second part: Wood response to environmental conditions: study-case of the formation of a false ring.**

### **Context and objectives**

In chapters 1 and 2, we showed through a retroactive approach how some environmental conditions, mainly soil water shortage and temperature, affected the cambial activity and consequently some properties (e.i.: heartwood, early-, transition- and late-wood widths) of the resulting wood. But this retroactive approach did not allow us to understand the way cambial activity reacts and in particular how fast is the response to environmental stimuli. When trees are facing a climatic stress, how fast is their cambium reaction and is there any variability among genotypes in the way they react?

Generally in the temperate zones radial growth is periodic. In an annual ring, early-wood is formed at the start of the growing season and composed of cells with thin wall and large lumen while late-wood is formed toward the end of the growing season and is made of cells with thicker wall and smaller lumen (Larson, 1994). We demonstrated in *chapter 1* how water stress influences ring characteristics (widths, early-wood proportion, duration and speed of tissue formation) of irrigated versus non-irrigated trees.

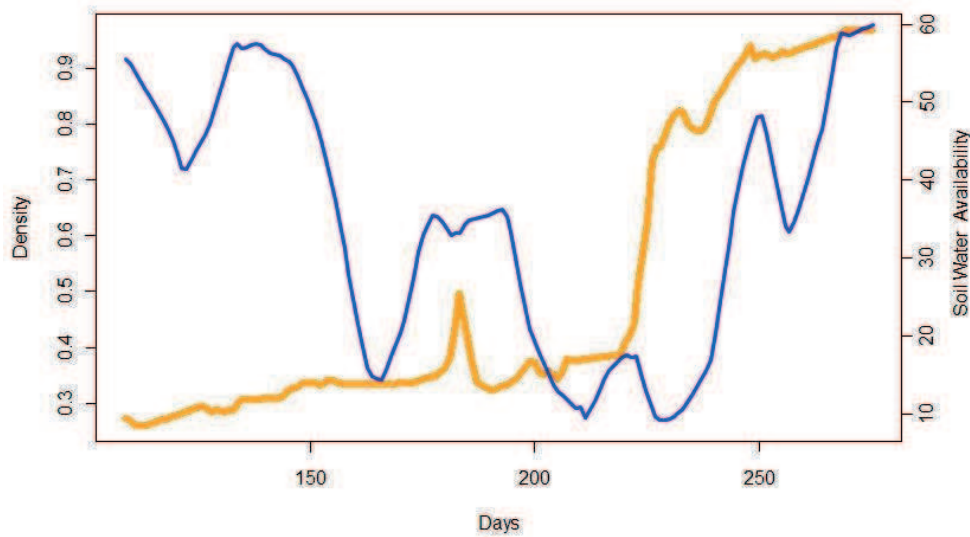
A drought event during the first part of the growing season can induce the formation of late-wood-like cells (Rozenberg et al. 2002). If soil water content returns to favorable conditions, the cambium starts again to form early-wood-like cells. The tissue band inside the early-wood and compounded of thicker-wall smaller-lumen cells is called a ‘false ring’ (Figure 11). In lowland oceanic climate sites like the ones used in this study, false rings are common in larch annual wood rings and in other conifers and can be generally related to water stress during the early part of the growing season.

These changes in wood anatomy can be interpreted, from an adaptive point of view, as a response to a stressful environment. Analysis of this response delay, severity of impact and

reactivity to the constraining conditions may allow us to select individuals with a favorable response according to our breeding objectives.

Thus, false rings are particularly suitable to study delay and speed of cambium reaction to a given stress.

**Figure 11.- False ring in hybrid larch at PEY. Microdensity profile (yellow) and soil water availability (blue) for the year 2006.**



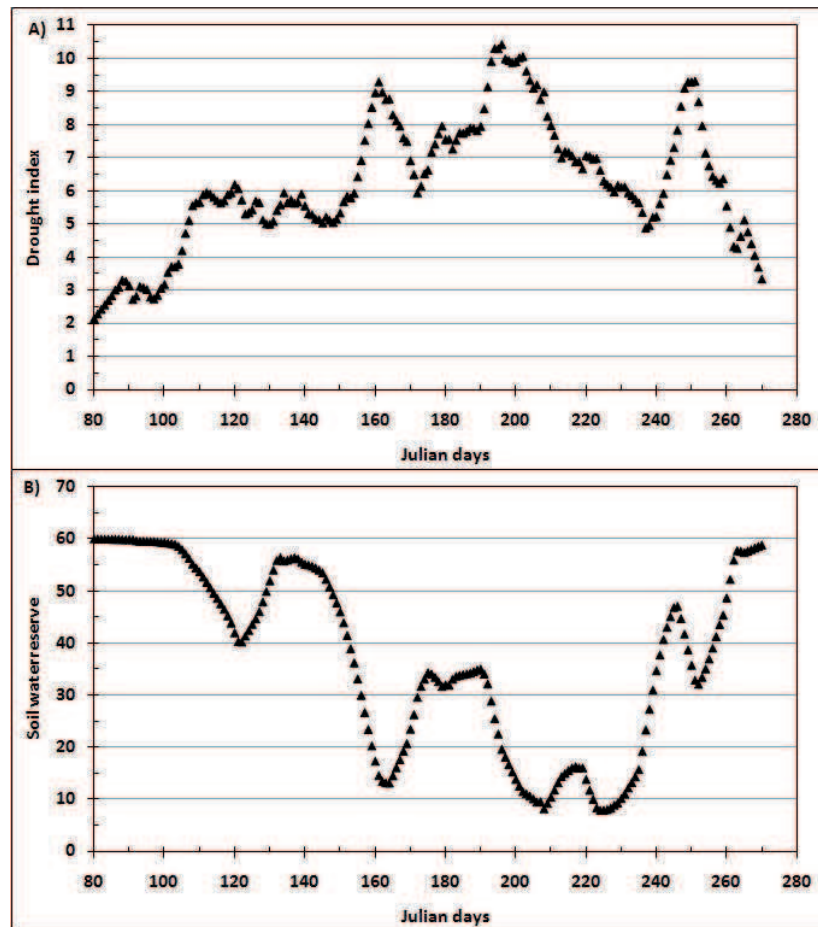
## Material and Methods

We took advantage of the weekly radial growth observations in our pinning experimentation at PEY (see *chapter 1*) during 2006 and of records of early drought events (suspected from soil water deficits) to conduct this study. Results are presented based on 94 trees from PEY.

The drought index (defined in the material and methods, section 2.1, page 37) and soil water availability variations over year 2006 are shown in Figure 12. We focused on a period spanning between Julian days 100 and 140; that is when the first decrease of water availability (or increase of drought index) was observed. The first strong increase of drought index (DI), nearly the double of its initial value, was reached in Julian day 112 and a marked

decrease (33% of total soil water reserve) of soil water availability (WA) occurred in Julian days 122.

**Figure 12.- Drought index (A) and soil water availability (B) during 2006-growing season for PEY site.**



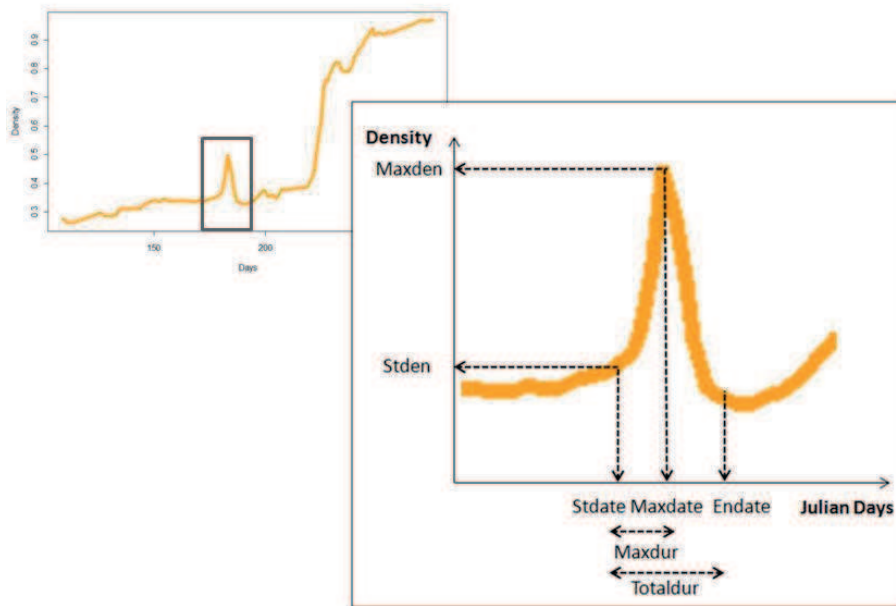
From the dynamic wood microdensity profiles, reconstructed from pinning and microdensity profiles information (see *chapter 1*), we extracted some key dates and wood densities (Figure 13) related to the false ring formed during the first part of the growing season. We calculated the dates corresponding to the initiation and completion of this density peak (Stdate and Endate), the date when the maximum density of the peak was reached

(Maxdate) and the two corresponding densities of Stdte and Maxdate (Stden and MAXden, respectively). In addition, we computed the total duration of the false ring event (number of days of the false ring formation; Totaldur) and the number of days up to the maximum density of the peak (Maxdur). Additional variables were generated by computing differences between Julian day in which climatic indexes showed the first increase or decrease (112 and 122 for DI and WA, respectively) and the Julian day in which the false ring started to be formed (DifDI and DifWA). Similarly as for the transition-wood (see *chapter 1*), we calculated the speed of wood density change (SLOPE-FR) during the constitution phase of the false ring as  $(MAXden - Stden) / (Maxdate - Stdte)$ .

We interpreted Stdte, Maxdate, Maxdur and DifDI and DifWA as different measures of the delay of cambial response to the climatic stress; MAXden and Totaldur as measures of the severity of the stress impact and finally SLOPE-FR as a measure of the reactivity of trees to stress.



**Figure 13.- Detailed measurements on a false ring extracted from a dynamic wood density profile (tree P008 at PEY): Sdate: Julian day when false ring peak started; Maxdate when it reached its maximum density and Endate when it ended; Stden, Maxden and Enden: corresponding wood densities; Totaldur: total duration of peak and Maxdur: number of days up to the reach of maximum peak density.**



## Results

On average, the false ring started to form in Julian day 164 +/- 9 days and ended in Julian day 173 +/- 9 days (Table IX). The time needed to reach the peak of maximum density was, on average, 9 +/- 4 days. Delays between the initiation of stressing conditions by an increase of DI or a decrease of WA and the observed start of false ring formation were between 42 and 52 days for DifDI and DifWA, respectively. Soil water availability started to decrease at Julian day 100 and continued to do up to Julian day 122; during this period, cells at the stage of differentiation reduced their enlargement process and precipitated the substance deposition to form their secondary cell wall resulting in cells with smaller lumen and thicker walls than

typical early-wood neighboring cells. And cells that were in enlargement stage at the time of the stress continued their development looking like typical early-wood cells.

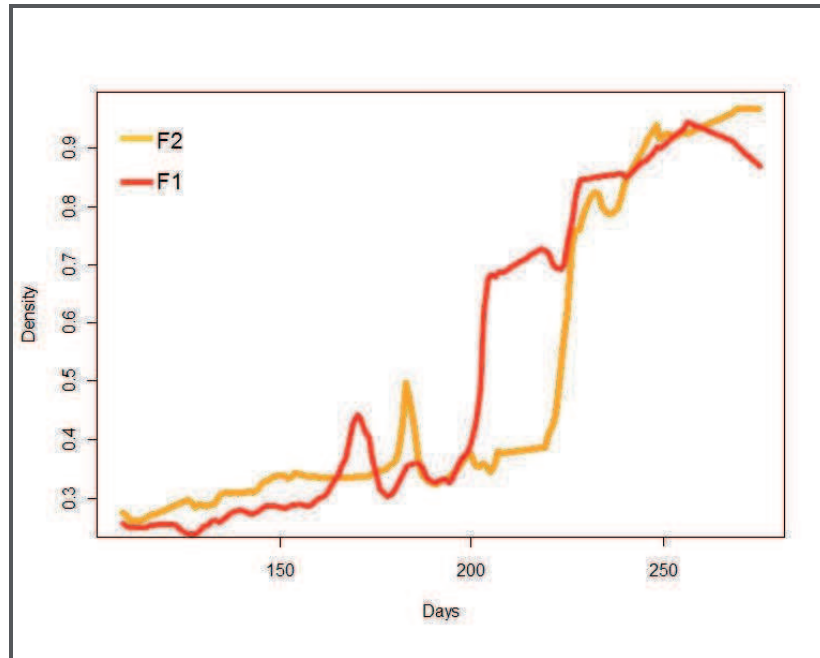
Unfavorable water conditions (Figure 12) must persist long enough to give to the tree enough time to react and produce late-wood like-cells. Here a water deficit event of 40 days long produced this false ring. Afterwards, trees returned to the production of typical early-wood cells. Overall stress reaction and post-stress reaction of trees to this spring water shortage event seems better correlated with soil water availability than to the drought index, which kept on increasing over spring and summer.

**Table IX.- Mean and standard deviation (SD) of 2006-false ring characteristics.**

Variable	Unit	Mean	Min - Max	CV (%)
Stdate	julian day	164	127 - 179	5.47
Maxdate	julian day	173	141 - 189	5.06
Maxden	g/cm <sup>3</sup>	0.43	0.32 - 0.61	11.63
Endate	julian day	181	147 - 207	4.63
Totaldur	Day	18	6 - 33	33.20
Maxdur	Day	9	3 - 25	43.40
DifDI	Day	52	15 - 67	17.30
DifWA	day	42	5 - 57	21.45
SLOPE-FR	g/cm <sup>3</sup> .day <sup>-1</sup>	1.22	0.09 - 4.06	60.66

While some trees showed contrasted timing of start and end of formation of the false ring, individual tree variability was rather low for these traits (CV less than 10%). Figure 14 illustrates the differential reaction of two trees (F1 and F2 hybrid larches) submitted to the same stress in the same environmental conditions.

**Figure 14.- False ring formation in two trees from F1 and F2 hybrid family at PEY (same growing season length).**



On average, the maximum density reached within the peak (Maxden) ranged from 0.32 to 0.61 g/cm<sup>3</sup>. The time needed to form the whole false ring (Totaldur) ranged from 6 to 33 days with an average of 18 days (Table IX). These variables (Maxden and Total dur) were interpreted as a measure of the severity of the impact of the environmental stress on cambial activity. A higher value of Maxden, corresponding to a higher density, seems to be related to more time spent in substance deposition on cell walls or to a more efficient deposition mechanism during the formation of late-wood like-tracheids. On another hand, the total duration of false ring formation represents the time needed for a tree to return to the production of early-wood-like cells after environment came back to be more favorable conditions. For both parameters, a high variability of the severity of the impact was observed among trees.

The reaction speed of the trees (their reactivity) assessed by SLOPE-FR was also highly variable among individuals: speed ranged from 0.09 to 4.06 g/cm<sup>3</sup>.day<sup>-1</sup> showing extreme tree behaviors.

In fact, it was not much the timing of responses (Stddate and Enddate) which differed much among trees but instead, the intensity, speed and duration of the response (Maxden, SLOPE-FR, Maxdur, Totaldur).

In Table X, we presented the relationships between false-ring characteristics at individual tree level. The delay of tree reaction (Stddate>, or Maxdate) is moderately correlated with tree reactivity and with severity of tree response to the stress: following a stress, a tree responding lately (Stddate>, or Maxdate>) reacts then faster (SLOPE>) and with more intensity (reaches a higher density maximum (Maxden)) but over a shorter time period (Totaldur) than trees reacting without delay.

**Table X.- Relationships at individual tree level among 2006-false ring parameters. Coefficients of correlation and p-value (between brackets).**

	Stddate	Maxdate	Maxdur	DifDI	DifWA	Totaldur	Maxden	Endate
Maxdate	0.89 (<0.001)					-0.18 (0.088)	0.28 (0.006)	0.88 (<0.001)
Maxdur	-0.29 (0.005)	0.11 (0.289)				0.74 (<0.001)	0.11 (0.31)	0.15 (0.143)
DifDI	1 (<0.001)	0.89 (<0.001)	-0.29 (0.005)			-0.49 (<0.001)	0.25 (0.017)	0.77 (<0.001)
DifWA	1 (<0.001)	0.89 (<0.001)	-0.29 (0.005)	1 (<0.001)		-0.49 (<0.001)	0.25 (0.017)	0.77 (<0.001)
Totaldur	-0.49 (<0.001)	-0.18 (0.088)	0.74 (<0.001)	-0.49 (<0.001)	-0.49 (<0.001)		0.01 (0.94)	0.14 (0.182)
Maxden	0.25 (0.017)	0.28 (0.006)	0.11 (0.31)	0.25 (0.017)	0.25 (0.017)	0.01 (0.94)		
SLOPE-FR	0.37 (<0.001)	0.11 (0.276)	-0.63 (<0.001)	0.37 (<0.001)	0.37 (<0.001)	-0.54 (<0.001)	0.51 (<0.001)	0.03 (0.798)

Obviously in larch, trees facing a major temporal pedo-climatic constraint are more or less heavily affected and have different reaction patterns, which can be summarized as an intense-reaction type and a weak reaction type related to Maxden. And according to SLOPE,

we identified two different patterns: fast- and slow-reaction. Combining delay, intensity and speed of reaction, two strategies were indentified in studied trees. One tree strategy was to react rapidly, intensively during a shorter period of time, while other trees reacted with a larger delay, less intensively during a longer period of time.

The formation of false ring as reaction to environmental constraints has consequences at different levels.

- 1) Consequences at anatomic and physiological level: These cells are obviously less conductive than early-wood type cells reducing the capacity of water transport within-tree. However, less conductive cells are sometimes assessed to be more drought resistant (Dalla-Salda et al. 2009) but contradictory results have been also published (Rosner et al. 2007) who failed to find relationships between hydraulic vulnerability and lumen diameter.
- 2) Consequences on radial growth: we could hypothesize: trees with rapid, intense and long reaction produce less early-wood or narrower ring width. However, non significant correlation between reaction features and early-wood and ring width proved that this reaction not penalized the radial growth.

What is favorable? A rapid, intensive and shorter reaction-type or the delayed, less intensive and longer reaction-type? The answer is not straightforward, since it depends on the timing during the growing season, the duration and the intensity of the water deficit event. It depends also on the physiological consequences of the false ring formation, which are not obvious.

### **Third part: Statistical synchronization of microdensity profiles with climate variables.**

#### **Context**

The combined pinning and microdensitometry methods developed in this thesis as well as the microcore methodology (Rossi et al. 2006) have proved to be efficient in precisely constructing or re-constructing precisely wood formation dynamics in annual rings. However, they are still limiting for studies requiring investigation of large numbers of individuals like in genetic studies or for breeding purposes. Other methods such as the breakpoints method used by Martinez et al. (2009) and by Sanchez Vargas et al. (2007), while less time-demanding and less costly, assume that there is an absence of individual tree variability for the growing season duration and of its components (onset-completion), and the consequences of this assumption have not been verified yet.

#### **Objective**

In order to overcome these limitations, our objective here was to test a novel synchronization procedure where variability in the growing season and dates for onset and completion are allowed to vary between individuals for a given set of weather records. This procedure searches for the optimal synchronization between the distance-based static microdensity profiles and the time-based records of climatic variables, and for this we developed an ad-hoc optimization algorithm. Unlike previous indirect synchronization methods, this optimization approach has no a priori assumptions other than the length of the climatic vector that is used for the set of individuals to be synchronized. Like previous indirect methods, this method assumes that wood density variation along the annual ring is an imprint of determinant climatic variables along the growing season on cambial activity, and that this reaction happens with no delay respect to the weather event for all individuals. This drawback in failing to take into account individual delays is the price to pay, however, for an

indirect synchronization method. The objective here is to compare dynamic profiles resulting from pinning and those resulting from synchronization by using this novel optimization approach and, ultimately, to assess to what extent the latter indirect method affects genetic estimates drawn from synchronized profiles.

## **Method**

A simulated annealing procedure was used as the optimization engine for the synchronization problem. Simulated annealing finds optima in a way analogous to the reaching of minimum energy configurations in metal annealing, and it is used for efficient and rapid search in combinatorial problems. This technique was applied to the allocation of subsequent segments of the microdensity record to the sequence of climatic records, in such a way that the correlation between the resulting arrangement of microdensity and weather records was maximized. The process proceeded with one tree at the time. This process involved a random search throughout the feasible space of solutions, without changing the initial sequence of records, to maximize the objective function given by the Pearson correlation coefficient between microdensity and weather records. In this study, we used drought index and soil water availability.

The procedure started with a random association of each value of wood density (obtained from microdensity profiles with a step of 25 microns) to one or several consecutive climatic values (obtained from climatic profiles for a given index over the growing season), in such a way that each climatic record is associated to one and only one microdensity record. This first random association was a seed to start the optimization process, and it was subsequently followed by other associations. Each association was then evaluated by the level of correlation between microdensity and weather variables. The routine evaluated  $>2 \times 10^6$  associations, always saving the association with the highest correlation. When the best

correlation is found, the process stops and associated density and climatic variables are then used to construct dynamic density profiles.

Trees are allowed to have no a priori fixed starting and ending dates of cambial activity, and thereby different growing season lengths between trees are possible, as observed in nature. However, and in order to avoid unnecessary computations, it was still necessary to define starting and ending bounds for the growing season length, delimitating the climatic profile. For instance, weather records from winter months were excluded from the synchronization with certainty of not being associated to seasonal growth.

For indirect synchronization, we used combinations of two climatic indexes: a drought index (denoted hereafter as DI) and soil water availability (denoted WA and detailed in point 2.1 in section Materials and Methods), and with three different growing season lengths (see Table XI for details). The two tested climatic indexes were selected based on multivariate analysis. DI was chosen for simplicity of calculation and WA because of it represents with more complexity the environmental conditions, taking into account soil water available for trees. Different season lengths were tested to assess their effect on synchronization. For each index we selected a short (120-130 days), a medium (145-190) and a long (225-245) growing season. The lengths of growing seasons are not exactly the same for both indexes, due to particular variation of each index.

Different growing season lengths, climatic index, and setting parameters of annealing procedure were tested in search of the closest association between density and climatic variables. Synchronization was attempted on year 2006 data sets for each of the 94 trees in PEY, and then compared to the dynamics of wood formation observed through the pinning method in 2006.



**Table XI.- Combination of climatic indexes and growing season lengths used in the statistical synchronization process**

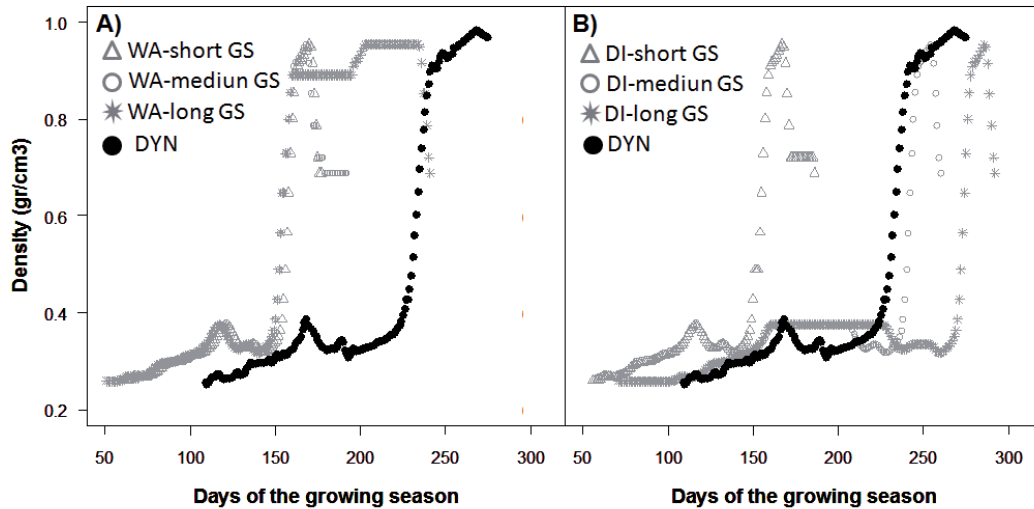
Climatic Index	Duration of growing season
WA (soil water availability)	short (growing season fixed from julian day 60 to 180)
	medium (growing season fixed from julian day 55 to 200)
	long (growing season fixed from julian day 50 to 295)
DI (drought index)	short (growing season fixed from julian day 55 to 185)
	medium (growing season fixed from julian day 80 to 270)
	long (growing season fixed from julian day 70 to 295)

As a result, 6 so-called ‘synchronized’ wood density profiles were obtained for each individual tree (WA-short, WA-medium, WA-long; DI-short, DI-medium, DI-long). From them, phenological parameters of wood formation (Sew, Stw, Etw, Elw, Dew, Dtw, Dlw, Dtot, SFew, SFtw, SFlw) were calculated applying the same criteria used in the ‘dynamic profiles’ that were obtained through the combined ‘pinning’-microdensity method (see *chapter 1*). These parameters from the so-called ‘synchronized’ profiles were then compared to those of the dynamic profiles obtained from the pinning method.

#### Results

Similarities between both profiles depended ultimately on the weather index and the growing season length that were implemented, with some scenarios resulting in coarse overlapping, while others showing reasonable matching. In figure 15, we show two examples of synchronized versus dynamic profiles for one particular tree. Absolute differences appear to be of relevance, at first sight, for WA, with a shift in synchronization towards early dates. At least one of the scenarios for DI matched reasonably well the dynamic profile.

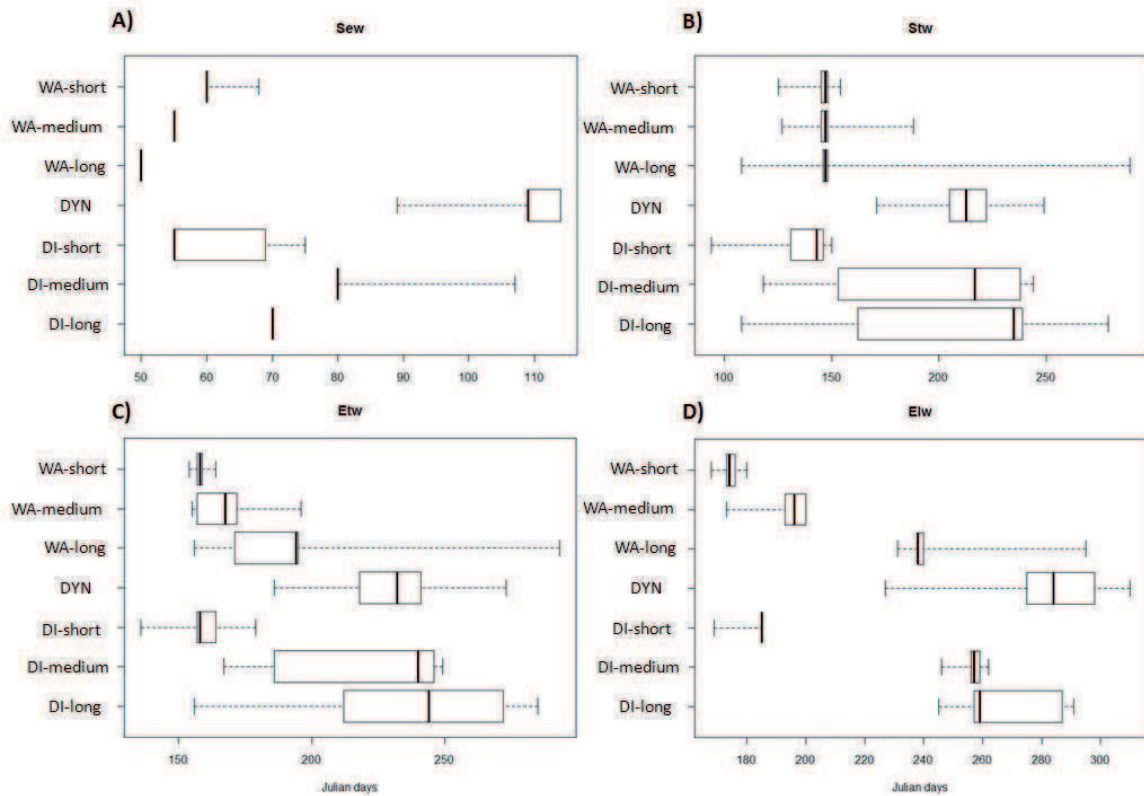
**Figure 15.- Example of reconstructed microdensity profiles: ‘dynamic’ profiles (in black) and ‘synchronised’ profiles (in gray) for tree P012 located at PEY. Synchronization using A) soil water availability (WA) and B) drought index (DI) as climatic index for different lengths of the growing season (GS).**



Phenological parameters estimated from synchronized profiles are given in Table XII (see in appendix A, at the end of the present chapter) and could be compared to those from dynamic profiles (see Table IV, in *chapter 1*). As a whole, the former differed from those obtained from ‘dynamic’ profiles (Figure 15).

In Figure 16 we show median, minimum and maximum values of key dates of tissue formation for the six synchronization and dynamic profile. The average date of onset of wood formation was the most critical to fix and it was also the most affected by the length of growing seasons.

**Figure 16.- Box-plot of key dates of kinetics of wood formation. A) Sew: starting date of early-wood, B) Stw: starting date of transition-wood, C) Etw: completion date of transition-wood, D) Elw: completion date of late-wood.**



The initiation and ending of transition wood (Figure 16 B and C) were affected by the selected index and the length of growing season. From other side the estimated date of late-wood completion (Figure 16 D) was underestimated in all cases whatever index and length of growing season were tested.

The optimization algorithm produces a better synchronization when variation exists along the two profiles (climatic and wood density), such as in the presence of false ring or in the transition from early- to late-wood. But, at the start of radial growth, variations in early-wood density are usually weak and the function had difficulties for finding variation through the records where to “anchor” the synchronization: as a result, the start of wood formation is

often associated to the first day available in the chosen growing season. Consequently, the timing of formation dates of other tissues is also disturbed (Table XII in appendix A).

Differences between values from the synchronized and dynamic profiles were computed for all variables mentioned above (Sew, Stw, Etw, Elw, Dew, Dtw, Dlw, Dtot, SFew, SFtw, SFlw) for the 94 trees used for this study. In order to construct a reference distribution for each of these variables, from which inferences over differences could be made, we used a Monte-Carlo randomization approach (Mainly, 1997, Martinez-Meier et al., 2008) written in R language (R Development Core Team, 2008). This randomization approach used all the pairs of synchronized and dynamic profiles from the 94 trees in the study as a pool from which new random pairs without replacement could be subsequently formed. The resulting new differences from these sampled pairs were computed and stored, and the process repeated over 1000 times in order to build up a distribution of differences. These distributions represented the expected difference between any two synchronized and dynamic profiles under a null hypothesis, thus due purely to chance.

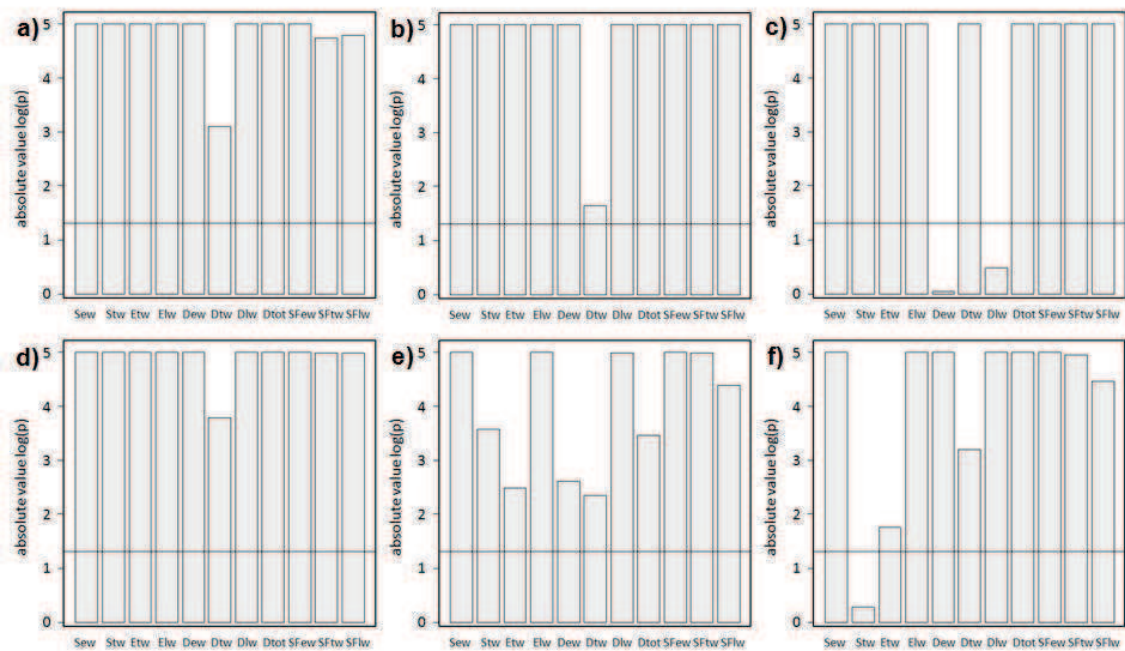
Each observed difference truncated its corresponding null hypothesis reference distribution, resulting in a probability of the observed difference among synchronization methods (synchronized and dynamic) being due by chance (Figure 17). We assumed a significance threshold of 0.05 for a one-tail test.

Results of the comparison between distributions of variables from the ‘dynamic’ and ‘synchronized’ profiles showed that differences for most of variables are related to the method rather than purely to chance (Figure 17). However, differences were dependent also on the trait and the season length being used for the synchronization (i.e. WA-long, DI-medium and DI-long). This leads to think that differences might be somehow reduced by an appropriate choice of traits and length of weather records.

Regarding the fact that synchronized profiles assumed no delay between weather events and microdensity responses, most of the differences between synchronized and dynamic profiles were a matter of sifted positions along the time axis. This is the result of the basic assumption that is made for the indirect synchronization, i.e. all trees react immediately to the weather stimuli. Commonly, trees do not react immediately to environmental stimuli (see part 2 of the present *chapter*) and the algorithm fail *by construction* to take that into account. Unfortunately, there seems to be no way to know retrospectively the “age” of a microdensity response to a given weather event by using indirect methods. The synchronization approach allows us to compare the magnitudes of responses to given weather events among trees, but we should neglect the differences in delays in response between those trees being compared.

According to obtained results, additional information such as the average date of budburst or temperature thresholds for onset of cambial activity, probably, need to be included in the function to orientate the local search of the starting date of radial growth.

Figure 17.- Differences between ‘synchronized’ and dynamic profiles for dates of initiation of early-wood (Sew), initiation of transition-wood (Stw), end of transition-wood (Etw), end of late-wood (Elw), duration of early-, transition- and late-wood (Dew, Dtw, Dlw), total duration of growing season (Dtot) and speed of early-, transition- and late-wood formation, using different climatic indexes and growing season length. Figures a, b and c correspond to soil water availability for short (a), medium (b) and long (c) growing seasons. Figures d, e, f correspond to drought Index combined with short (d), medium (e) and long (f) growing seasons. The grey bars show the log10 of the probability associated to differences among synchronized and dynamic profiles in the Monte-Carlo randomization process. The horizontal line represents the 5% significance level.



As an example, in Table XIII we present the variation and estimated heritability (when correspond) for variables from dynamic and synchronized profiles. Although marked differences showed in Figure 15 by synchronization using WA-long, variation and genetic

control of the most variables seems not suffer greater changes. That allows us to be optimistic with the used of results from synchronization to breeding objectives.

Table XIII.- Comparison of genetic variation and heritability estimates for dynamic (Dyn) and synchronized profiles (DI-medium and WA-long).

Variable	Dyn		DI-medium		WA-long	
	F-value (p-value)	$h^2_{fam}$ (SD)	F-value (p-value)	$h^2_{fam}$ (SD)	F-value (p-value)	$h^2_{fam}$ (SD)
l	3.8 (<0.001)	0.740 (1.116)	1.1 (0.386)	-	0.5 (0.150)	-
ewd	4.9 (<0.001)	0.796 (0.853)	0.9 (0.465)	-	2.7 (0.008)	0.63 (0.163)
lwd	2.4 (0.014)	0.586 (0.281)	0.9 (0.488)	-	2.2 (0.03)	0.545 (0.082)
lwp	2.0 (0.046)	0.494 (2.658)	0.9 (0.500)	-	2.3 (0.02)	0.571 (2.623)
SFew	3.1 (0.002)	0.680 (0.007)	0.10 (0.001)	-	0.5 (0.108)	-
SFlw	2.6 (0.008)	0.620 (0.004)	0.70 (0.264)	-	0.8 (0.369)	-
MRD	2.1 (0.033)	0.523 (0.018)	1.1 (0.351)	-	2.9 (0.006)	0.65 (0.018)
MID	3.6 (<0.001)	0.726 (0.017)	4.2 (<0.001)	0.765 (0.018)	2.3 (0.024)	0.561 (0.015)
MAD	2.0 (0.042)	0.502 (0.030)	1.9 (0.062)	0.475 (0.029)	1.8 (0.082)	0.441 (0.028)

APPENDIX A

Table XII.- Mean, standard deviation, standard error, coefficient of variation (CV%), and minimum and maximum of duration and speed of tissue formation from indirectly synchronized profile.

Variable	Unit	Synchronization	Mean (SD)	CV%	Min - Max
Dew	Days	DILong	144 (47.17)	32.81	38 - 209
		DImedium	116 (41.43)	35.57	38 - 164
		DIshort	77 (12.86)	16.66	39 - 95
		WAlong	110 (40.62)	36.99	58 - 239
		WAmmedium	93 (7.69)	8.30	72 - 133
		WAsshort	85 (4.14)	4.86	65 - 94
Dtw	Days	DILong	25 (21.78)	85.61	4 - 122
		DImedium	24 (20.63)	87.45	4 - 95
		DIshort	24 (12.14)	50.90	7 - 63
		WAlong	37 (16.88)	46.16	4 - 88
		WAmmedium	20 (11.41)	55.85	7 - 52
		WAsshort	13 (4.1)	32.70	5 - 33
Dlw	Days	DILong	28 (27.25)	98.27	2 - 105
		DImedium	30 (28.08)	92.37	2 - 80
		DIshort	24 (7.82)	32.95	6 - 48
		WAlong	49 (19.79)	40.78	2 - 88
		WAmmedium	27 (9.44)	34.52	4 - 41
		WAsshort	16 (3.47)	21.37	9 - 26
Dtot	Days	DILong	198 (14.51)	7.33	176 - 222
		DImedium	176 (5.27)	2.99	145 - 182
		DIshort	126 (8.18)	6.50	101 - 131
		WAlong	196 (17.09)	8.72	182 - 246
		WAmmedium	141 (5.46)	3.86	119 - 146
		WAsshort	115 (3.47)	3.02	104 - 121
SFew	mm.day <sup>-1</sup>	DILong	0.0256 (0.0001)	0.39	0.0255 - 0.0261
		DImedium	0.0257 (0.0001)	0.39	0.0256 - 0.0261
		DIshort	0.0257 (0.0001)	0.39	0.0257 - 0.0261
		WAlong	0.0256 (0.0001)	0.39	0.0255 - 0.0258
		WAmmedium	0.0257 (0)	0.00	0.0256 - 0.0258
		WAsshort	0.0257 (0)	0.00	0.0257 - 0.0258
SFTw	mm.day <sup>-1</sup>	DILong	0.0252 (0.0054)	21.43	0.0078 - 0.0318
		DImedium	0.0243 (0.0068)	27.98	0.0044 - 0.0318
		DIshort	0.025 (0.004)	16.00	0.0117 - 0.029
		WAlong	0.0153 (0.0089)	58.17	0.006 - 0.0318
		WAmmedium	0.021 (0.0071)	33.81	0.0062 - 0.029
		WAsshort	0.0265 (0.002)	7.55	0.02 - 0.0305
SFlw	mm.day <sup>-1</sup>	DILong	0.0242 (0.0088)	36.36	0 - 0.0643
		DImedium	0.0359 (0.0364)	101.39	0 - 0.2053
		DIshort	0.0275 (0.0122)	44.36	0.019 - 0.1101
		WAlong	0.0346 (0.0106)	30.64	0 - 0.0484
		WAmmedium	0.0345 (0.0236)	68.41	0.0152 - 0.182
		WAsshort	0.0231 (0.0017)	7.36	0.0203 - 0.0288



## **DISCUSSION**

### **1. Phenology of wood formation in larch and genetic variability**

Variation of wood anatomy during a growing season is well represented by the corresponding variation in wood density. Wood density variation can be difficult to understand due to the complexity of this trait. Mean ring density is based on early-wood and late-wood density and the late-wood proportion. Consequently, a particular value of mean wood density can result from different contributions from these two components. Analogously, ring characteristics are based on the density of their corresponding components, comprising a static dimension related to magnitude and a dynamic dimension related to the timing in which ring was formed. This in-depth knowledge of ring formation could benefit breeders by giving the possibility to evaluate individuals or families not only by their density and ring components, but also by the strategy developed by trees to form the ring. Moreover, the knowledge of the timing of wood formation could allow us to assess the consequences of individual or family selection based only on density values.

Estimations of genetic determinism of wood components in terms of density, tissue proportion and of the phenology of xylogenesis contribute greatly to a better comprehension of the genetics of wood formation dynamics, which will be essential for an efficient incorporation of wood characteristics (dynamic and static) as adaptive traits in tree breeding programs.

Most studies on the dynamics of wood formation aim at determining the starting and ending dates of annual ring formation, the timing of cell development (Deslauriers et al. 2003, Rossi et al. 2006b, Cufar et al. 2008) and the factors controlling cambial activity (Antonova and Stasova 1997, Moser et al. 2010). However, only a few studies consider the estimation of genetic parameters for wood formation variables (Worrall 1975, O'Reilly and Owens 1989, Li and Adams 1994, Vargas-Hernández and Adams 1994), and they are focused on evergreen conifers. In the first part of Chapter 4 we studied the parameters defining the variability of wood formation in larch taking advantage of the existence of a family structure in the PEY experiment. We showed that phenotypic variation for

variables related to the dates of initiation and completion of the formation of different tissues and to the total duration of ring formation is low in larch, both at individual and family levels. However, within-family variation was greater than between-family variation, suggesting that for these latter variables within-family selection is to be recommended. Variables linked to the duration and speed of tissue formation showed a higher variability both at individual and family levels. Speed of early- and late-wood formation showed differences among families. These differences were mostly related to differences (among family) of early- and late-wood widths. We can note here that traits associated to the last period of ring formation are slightly more variable than those connected to the formation of the first part of the ring. Similar results were showed by Worrall (1975) for provenances of Norway spruce and more recently by O'Reilly and Owens (1989).

A low genetic variation and a low heritability have been reported for wood formation variables (Vargas-Hernández and Adams 1994, Li and Adams 1994). In our case, we estimated heritability only for variables showing significant differences among families: timing traits, i.e. speed of formation of early- and late-wood. Despite the relatively heterogeneous material used here, the low number of individuals per family, the estimated heritabilities were higher (0.62-0.68 with SD 0.004-0.007) compared to those calculated by Vargas-Hernández and Adams (1994) (0.24-0.26 with SE 0.10-0.09) for speed of formation of early and late-wood. Significant relationships between speed of formation of early and late-wood, and initiation of early-wood and ending of late-wood, respectively, denote the possibility to select indirectly families with late initiation and ending of cambial activity and with high speed of tissue formation.

Ring density and its components were studied in many species. In many studies wood density was found to be highly heritable (Zobel and van Buijtenen 1989, Zobel and Jett 1995, Louzada and Fonseca 2002, Fujimoto et al. 2008), although contradictory results were found in the same studies for early and late-wood density. According to our results presented in Chapter 4, larch ring density and its components show a low phenotypic variability (at individual and family level), and a high heritability ( $h^2_{fam}$  between 0.50 and 0.73).

Due to the lack of heritability estimations for dates of initiation and completion of tissue, the high heritability found for density traits and the weak correlation between phenology traits and density traits, we can expect that selecting on density traits will not affect the starting and ending dates of secondary growth. Our results demonstrate also that sensibility to early frost damage would not be increased by such a selection.

Speeds of formation of early- and late-wood appear to be under high heritability. They are moderately phenotypically correlated with the starting date of early-wood formation and the ending date of late-wood formation. Selection of higher speed of early- and late-wood formation is linked to a late initiation and ending of cambial activity.

As the length of the growing season is strongly positively correlated to the total ring width and the duration of latewood formation, selection of families and individuals with a longer growing season should lead to larger rings with a higher late-wood proportion and consequently a higher density, but also to a greater risk of growth when climatic factors are not favorable.

From our results, the inclusion of the ring density variables, ring width and of some phenological variables as speed formation of tissue as selection criteria in larch breeding programs would modify other features like initiation and ending of cambial activity. A suitable selection would tend to avoid frost damage and to increase the length of the growing season, which is strongly correlated to a higher proportion of latewood.

### **Combining the pinning method with density profiles**

The modifications we applied to the pinning method in our study, aiming at simplifying and speeding up the procedures, had several consequences:

- Sample polishing enables the precise detection of cambium position at a given date.
- Our process allows the analysis of a higher number of trees than the methodologies employing cuts from microtome.

- No information is provided about the dynamic of cell development: developmental states of cells cannot be identified because samples were collected at the end of growing season, when ring formation is completed.

Wood formation is the result of cambial activity and the product of many interacting physiological processes. This could also clarify some differences in timing with other studies where the terms wood formation or xylogenesis mean cells at enlarging, wall thickening or mature stages (Antonova and Stasova, 1997; Deslauriers et al., 2003; Moser et al., 2010).

We present an approach that integrates anatomical changes through the use of microdensity profiles synchronized by pinning. This integrative approach is well suited to the study of tree reactions to chronological weather events.

## **2. Environmental constraints and their influence on cambial activity and wood properties**

Variation in wood anatomical characteristics represents adaptive structural solutions adopted by trees in order to achieve the optimal balance among competing needs of support, storage and transport under changing environmental conditions (Chave et al. 2009).

In Chapter 1 we showed the effect of soil water shortage on the phenology of wood formation by testing the same genetic material (one progeny of hybrid larch) in the same environmental conditions (soil, temperature) under two contrasted treatments, irrigated (non-limiting soil water availability) with fertilization and non- irrigated (more stressing water condition). Total radial growth and components of ring width were reduced in the trees growing under more stressing conditions despite the fact that dates of initiation and of completion of ring formation and total duration of overall ring formation were similar in both treatments. However, the transition from early- to late-wood was delayed in trees growing in non-limiting soil water conditions: these trees produced a wider early-wood at the expense of a narrower late-wood width and a shorter duration of formation of late-wood.

A number of factors have been suggested to be at the origin of the transition from early- to late-wood. They include fixed environmental factors as photoperiod (Larson 1962) and variable ones like soil water deficit and high temperatures (Kramer 1964; Antonova and Stasova, 1997; Lebourgeois, 2000). Most probably, it is an interacting combination of signals that triggers this transition-wood formation. However, based on our results, drought could initiate internal mechanisms that reduce the cell division and shorten the period of cell enlargement, thus more or less gradually changing cell anatomy. These internal mechanisms could be connected to changes in auxin concentration gradient (Sundberg et al. 2000) or to internal competition for carbohydrates between apical and radial growth (Minchin and Lacointe 2005).

From the results showed in the Chapter 2 and in connection with drought affecting wood properties, we showed that soil water shortage reduced significantly the heartwood size and its extension. Tree growth in the non-irrigated treatment was half the growth of the trees in the fertirrigated treatment, but the wood density was not affected. Heartwood extractives are formed at the sapwood-heartwood boundary from translocated carbohydrates or lipid substrates that infiltrate the cell walls (Magel 2000 cited in Gierlinger 2004). Soil water deficits could affect some of the mechanisms of substance deposition by restraining formation of heartwood extractives or their infiltration in cell walls.

From the results showed in Chapters 1 and 2 we assessed the influence of soil water shortage on growth, wood properties and wood formation dynamic through an irrigation experiment. These experiences do not allow us to be aware of tree reaction and how fast is the response to environmental stimuli. Then, we took advantage of a false ring formed during year 2006 to assess how trees reacted to soil water deficits. Then, in the second part of Chapter 4 we studied the delay of the response, the severity of the impact and the reactivity of the trees facing a soil water deficit. We detected delays of response between 5 and 57 days. Such delays could be explained in part by the complexity of the mechanism involved in the first steps of secondary wall formation. The environmental condition (water status) of the previous years (not investigated here) can be

suspected too of playing a role here. In this sense Martinez (2009b) showed that a strong heat-drought wave as the one that occurred in 2003 could affect the growth during the subsequent year.

The water status affects the cambial activity and consequently the resulting cell anatomy (Larson 1994). Also, the water availability in soil strongly affects the seasonal growth rate and the diameter of produced cells (Vaganov et al. 2006). A decrease in the frequency of cambial cell division and a reduction in the radial diameter of tracheids are the response of conifers to water deficits (Abe and Nakai 1999, Abe et al. 2003). In the second part of Chapter 4, we showed the influence of a soil water deficit on change of cell anatomy, through the study of density variation during early-wood formation (false ring).

Different strategies of response are associated to the intensity and persistence of water stress. At an early stage, the decrease in water potential directly affects the cell expansion during the early-wood formation, while the rate of cambial cell division declines only during the later stage of water deficit (Abe and Nakai 1999). Then, the response of trees facing a few days of water stress during the early part of the growing season or facing a more intense stress during the last part of the growing season is different. The latter probably triggers the formation of the transition from early- to late-wood while a water deficit at an early stage during the growth period activates the formation of a false ring.

Late-wood-like tracheids formed during a period of water stress are obviously less conductive than early-wood type cells, limiting water transport within the trees. Thicker cell walls and smaller diameter lumens (corresponding to higher density wood) increase resistance to drought-induced cavitation and help trees to maintain the safety of their hydraulic system (Hacke et al. 2001). Less conductive cells in Douglas-fir were assessed to be more drought resistant (Dalla-Salda et al. 2009). Then trees that produce more quickly a bigger quantity of late-wood-like cells type during stress period would present a higher minimum and mean density, and could be more resistant to cavitation. In this case, wood density could be used as an indirect selection criterion for resistance to drought. Stressing events like persistence of high temperature and water deficit lead to a high

atmospheric demand and the closure of stomata. The first observed reaction is a growth reduction (Bréda et al. 2004, 2006). We found no correlation at the individual level between ring width and the intensity and delay of tree response. This demonstrates that fast growing trees do not necessarily react faster than slow growing trees. However, moderate to high positive correlations were found between maximum false-ring-density and mean, minimum and maximum density of the whole ring. These relationships support the hypothesis that larch trees with a higher ring density suffer a more severe stress impact. They also seem to be more reactive. The latter could be a strategy to compensate the higher impact of drought.

### **3. Alternatives to direct observation of cambial activity**

The main constraint of direct observation of cambial activity, as we stated in the Introduction section (point 4.1), is that existing methods are very time-consuming. This is of particular relevance when the aim is to study genetic variation and subsequently to estimate genetic parameters of cambial activity components in the wood. These quantitative studies require often a large amount of genetic entities in a field test in order to get enough statistical power to detect genetic differences. These numbers are far beyond what is feasible for a direct observation of cambial activity. Consequently breeders have to search for non-destructive and easily visible signs of cambial activity.

Observable phenological changes can be often easily linked to weather variables, or at least recorded in a time scale. Microdensity profiles are expressed in a distance scale, while weather records are in a time scale. Vegetative phenology events are an option to relate microdensity and time avoiding high resolution wood tissue observation. Vegetative phenology events have demonstrated to be sensitive to environmental conditions (Chmielewski and Rötzer 2001, 2002, Chmielewski et al, 2004; Chuine et al, 2004). In Chapter 1, we study the connection between wood formation parameters and external and easily observable variables, looking for predictors of wood formation stages. We focused the search on phenological events of spring and summer, with some of

them being linked to some of the major steps in wood formation. We identified budburst and crown greening as possible predictors of initiation of early-wood formation, while bud-set and early stages of crown yellowing could be used to identify the completion of ring formation. Other weak connections were suggested between transition from early- to late-wood and radial and apical growth and maximum rate of terminal shoot lignification. Therefore, we could situate in time the major steps of wood formation. However, this did not have enough precision for investigation of wood formation dynamics at individual tree level. Closer and more frequent observations of bud phenology, terminal shoot, branch elongation and needle elongation would eventually be necessary to improve the relationships between external signals and cambial activity.

Other climatic markers prompting resumption of primary and secondary meristems like temperature thresholds or heat-sum would probably be more feasible. Although we did not study these features for wood formation, we started to study temperature threshold and heat-sum requirements of primary meristems. In Chapter 3 we demonstrated the high genetic variation and genetic determinism for threshold temperature and heat-sum necessary to bud flushing. Also, we studied the possibility of modeling heat-sum requirements based on individual thresholds calculated in an *ex situ* experiment. Results of Chapter 3 and bibliographic references (Rossi et al 2008a, Seo et al 2007) demonstrate that heat-sum models should be tested for the study of wood formation in larch.

Some of the most recent approaches for indirect synchronization of microdensity profiles onto weather records are based on breakpoints (Martinez et al. 2009a) or wavelets (Rozenberg et al. 2004, Martinez-Meier 2009b) methodologies. Under both approaches, intra-annual variation of wood is associated to the variation of a climatic index. In the third part of Chapter 4, we attempted to synchronize microdensity profiles using soil water availability and a modified De Martonne's aridity index. We tested a novel synchronization procedure based on maximizing microdensity to weather index correlations, and where variability in the growing season and dates for onset and completion are allowed to vary among individuals for a given set of weather records. We detected a



strong influence of the selected climatic indices and of the growing season length on the resulting synchronized profiles.

The major assumption of all indirect methods is that all trees react simultaneously to a given weather event and that differences between individuals are only observed in terms of magnitude of responses. This is a drawback of indirect methods, but there is no way of overcoming this problem retrospectively by looking at an increment cores. Differences in delayed response among individuals are then neglected and assumed to be zero, which comes to be the major divergence with the dynamic profiles. However, when we compared estimations of genetic variation and of heritability coming from dynamic and synchronized profiles, we conclude that the results allow us to use this type of statistical approach. Less costly and time-consuming methodologies are essential features for the evaluation of great numbers of trees as needed in tree breeding programs. Thus, statistical synchronization could be a reasonable tool to be implemented in programs where a very high number of genetic entities are evaluated.

#### **4. Breeding for climatic change making capital out of wood formation**

Basically, wood physical properties have been used in dendrochronology to reconstruct past climate and in studies of wood quality. Recently, the need for newer and more rapid techniques to assess the response to environment within wood encouraged some authors to point out the possibility to use wood density as an indicator of tree reaction to climate at the within-growing season time scale (Rozenberg 2002, Martinez-Meier et al. 2008) and also to estimate the genetic determinism of tree response to extreme stress events (Martinez-Meier et al. 2008).

For refining relationships between wood structure and environment it is necessary to understand which wood developmental processes are controlled by hormonal factors (Uggla et al. 2001, Funada et al. 2002), which ones are controlled by environmental factors such as temperature, water condition and day length (Denne and Dodd 1981), and to what extent both types of factors

interact. Such information would be extremely valuable to hypothesize alternative explanations for observed anatomy features.

Evidence of species-specific effect caused by drought is shown in Chapter 2, where soil water shortages affect differently hybrids and European larches. Also, confirmation of differential tree reaction is shown in the second part of Chapter 4. Then, incorporation of wood formation dynamic variables in hybrid larch breeding strategies is supported by these results. Moreover, thinking of the future climate in which trees will grow, more site-specific genetic material should be offered as outputs of tree improvement programs. For example, genetic material with a higher density (small lumen tracheids, thicker walls) and high speed of tissue formation is more appropriated for zones with sandy soils than clay soils. Probably material with lower wood density should be used in plantation zones with more loamy soils with a higher capacity of water retention and a lower probability of drought. However, the conservation of a broad genetic basis supporting improvement programs are a key point to avoid the loss of genetic variability and to give the possibility to change selection criteria through the time.

The positive correlation between phenology of radial growth and cambial activity allows us to select fast growing genotypes with a late start of cambial growth period, thus avoiding possible late frost.

Higher temperatures, changes in precipitation regimes and higher frequency of extreme events as the 2003-heat-drought wave are expected in the context of climate change (IPCC 2007). The uncertainty about the magnitude and the rate of mentioned changes make difficult the decision-making concerning the determination of selection criteria in tree breeding programs. If the speed of climatic changes is confirmed in the coming years, populations might become unable to adapt themselves at the same pace as that expected for the environmental changes. In that context, individual plasticity could be expected to play a more important role. Breeding for climatic change must be focused on adaptive traits. Firstly, it is necessary to discuss and to define the climatic and meteorological characteristics for which adaptation is needed. Secondly, because the world wide

perspective of climate change is very variable in terms of geographical space, it is necessary to limit the geographical zone (Koski 1996). Moreover, a detailed study of physiographic, soil and climatic conditions would help us to determine the possible limiting factors.

It would be appropriated to discuss which type of response we expect from trees facing stressing environmental conditions? What is more desirable as tree response? The answer to these questions is not simple because the efficiency of tree response is in strongly dependant of the intensity and duration of stress (Bréda et al. 2006). However, more reactive trees could have more chance to stop their whole physiological process before collapsing in case of persistence of extremely stressing conditions.

## CONCLUSIONS

We have demonstrated that a combination of methodologies coming from wood formation phenology on one side, and from X-ray densitometry, on the other side, provides at the same time a response to the sampling problem and a better insight into the genetic and environmental control of wood formation.

The existence of variability for timing in wood formation mostly during the last part of the growing season was demonstrated for larch. The phenology of wood formation has an impact on the tree adaptive value allowing us the study of tree adaptation to predicted climatic changes.

Unlike many other coniferous genera, larch is deciduous, and after turning yellow during autumn, it loses its needles each winter. This special feature puts larch in an unfavorable situation compared to evergreen conifers. Indeed, each spring, larch needs to produce completely new foliage to start the photosynthesis process and consequently to initiate radial growth. Thus, the initiation of wood formation process is strongly related to temperature and to bud dormancy break. The low variability for budburst dates could be related to the lack of variability at the start of wood formation process.

Phenology of vegetative events during spring and summer demonstrated to be connected with phenology of wood formation. At least, the start and the end of radial growth were denoted by external features. That could be an option to convert the distance axis of the of the microdensity profile into a time axis and then to study reaction of trees facing drought conditions. Also heat-sum models demonstrated more robustness to point out genetic differences compared to simple dates of vegetative event which include the effect of year to year variation.

Statistical synchronization of microdensity profile has demonstrated to be a powerful alternative to insert a time notion for studies of tree reaction. Although the synchronization did not show a perfect fit with dynamic profiles, the variables obtained from synchronized profiles demonstrated similar levels of variation and genetic control.

Inclusion of time into intra-annual ring microdensity variation allowed us to compare patterns of tree response to environmental changes using variation of wood structure as a recorder of cambial activity over time. Environmental conditions demonstrated to have effects on timing of wood formation, consequently on quantity of wood formed. Also, influences of environmental factors were showed for apical growth and heartwood volume. Moreover, the use of false ring as a measure of delay of response, intensity of stress impact and reactivity of tree demonstrated to be a valid approach to study the variation of the reaction in front of a stress. Marked changes in density demonstrated to be connected to water availability variation over a growing season. The magnitude of density variation is related to the moment, the intensity and the persistence of soil water shortage.

## PERSPECTIVES

As results of this thesis, original information was generated on wood formation process in different Larch species. Studies of ecophysiological traits related to water transport in the stem could be an interesting direction to continue the investigation and to confirm hypothesis postulated for other evergreen conifers. For example, hydraulic conductivity and vulnerability to cavitation, related to resistance to drought, were showed to be features connected to wood density (Hacke et al. 2001, Dalla-Salda et al. 2009).

The implementation of the pinning method during several successive years is necessary to capture the inter-annual variation in the timing of wood formation. The incorporation of heat-sum models for key dates in wood formation could avoid implementing costly and very much time-consuming technique commonly applied to the study of the dynamic of wood formation. Moreover, the heat-sum index should allow us to retrospectively determine the start and ending of past growing season and the timing of microdensity profiles. Incorporation of heat-sum models to determine the start and ending of secondary growth is possible. Heat-sum models have demonstrated (chapter 3) to be more robust than just dates of a given event or state.

### **Extrapolation to other species and environmental conditions**

In Argentina, commercial plantations with exotic genus mostly *Pinus*, *Eucalyptus*, *Populus* and *Salix* reach 1.2 million hectares. The northeast region so-called Mesopotamic region is compounded by three provinces (Corrientes, Misiones and Entre Ríos) and it concentrates approximately 60 % of the country tree plantation surface. *Pinus* is the preferred genus used to establish afforestations in the Mesopotamic region. From the early '70's, different *Pinus* species started to be genetically improved. At the moment, a second breeding generation is in development for *P. elliotii* and *P. taeda*, the most used species. During the first generation volume was the only improved trait. However traits related to wood density are planned to be included in the second improvement cycle. The incorporation of wood microdensity profile and of studies of the dynamic of wood formation will

benefit from a better understanding of the strategies used by individual trees to form the annual ring and of the estimation of genetic parameters.

During the last years, the observation of a signal of water stress in commercial plantation has attracted the attention of some breeders. The symptoms consist mainly of branches and apical shoots death after a period of drought stress of trees in plantations on soils with a low water retention capacity. Because of the low impact of possible fungi and insect attacks, these variables have been discarded. The conclusion was that observed signal was connected to water stress during the growing season. Although mesopotamic region has a positive water balance over its growing season, during the last ten years more and more frequently appeared short periods of drought that began to affect trees. The study of water stress consequences on wood formation in progenies evaluated in the second breeding cycle gives the possibility to incorporate adaptive traits as a selection criterion if drought is considered a constraint in the future climate of the region.

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## SUMMARY

*Larix* is a genus of high interest for plantation due to its fast growth rate and appreciated wood quality. Larch has a great potential to be introduced in middle and lowland afforestations as an alternative to Douglas-fir. One important question for breeders is adaptation to environment. It is particularly relevant in the context of climatic changes where a strong decrease of soil water availability during the growth period is predicted. Three larch species are used by foresters in France for plantation establishment, for which breeders must supply more adapted material: European and Japanese larch and their interspecific hybrid. The higher performance of hybrid larch in afforestations demonstrates its great potential.

The tissue structure of annual rings reflects the response of cambium (cell division, elongation and thickening of cell walls) to environmental factors (climate, soil fertility, competition, etc), as well as physiological states and genetics. Then, wood density can be interpreted as the result of cambial activity and its variation as the response of the tree to environment. However, to gain a better understanding of this response and of the variation of the xylogenesis process itself, it is important to spot the timing of wood cell production. There are different approaches to study the timing of wood formation. We used a combination of wood microdensitometry and of pinning method as a new approach to study the dynamic of wood formation from a tree improvement point of view. This new approach allowed us to go beyond the static character of the microdensity profile (where density variation is related to distance) and to transform it into a dynamic profile (where density variation is related to time).

Variation in phenology of cambial activity can be seen as a mechanism better adjusting trees to their environment. If this adjustment improves fitness, then the corresponding tree anatomical response to environment variation may be seen as an adaptive response. As breeders, our interest is twice: on one side, a better understanding of cambial phenology and of wood formation in relation to pedo-climatic factors and climatic stress is urgent for profiling genotypes better fitted to their environment. On the other side, exploitation through breeding of knowledge about phenology of wood formation will help to design trees with better wood properties. In larch, phenotypic variation of variables related to the dates of initiation and completion of the formation of different tissues and to the total duration of ring formation is low. Other variables linked to the duration and speed of tissue formation and to ring components showed a higher variability and a high heritability. Phenotypic relationships among variables can be used to select individuals with a desirable density as well as adapted to possible stressing climate conditions.

Thanks to the dynamic profiles, we estimated delay of response, severity of stress impact and reactivity of the trees. We found a moderate to high phenotypic variation for the traits related to the severity of impact (maximum density of false ring, total duration of false ring). Most variation was

concentrated at individual level and we did not find significant differences among families for the traits studied; however different strategies of reaction were identified at the individual level.

Methods commonly used to study wood formation are very much time-consuming and it is unrealistic to apply them in routine for the evaluation of breeding populations. One alternative could be to identify external phenological signals as markers of wood formation. We tested the potential of vegetative phenology events which have demonstrated to be sensitive to environmental conditions. We identified budburst and crown greening as possible predictors of initiation of early-wood formation. We also propose to use bud-set and early stages of crown yellowing to detect the completion of ring formation. Another promising option is the optimization of the synchronization of the microdensity profile with climate variable.

**Key-words:** wood formation, pinning method, larch, genetics, tree response, indirect markers.

## RESUME

Le genre *Larix* intéresse les reboiseurs en raison de sa croissance rapide et de son bois apprécié. En particulier, le mélèze est une alternative intéressante au douglas pour les reboisements à basse et moyenne altitude. L'adaptation à l'environnement est une question clé pour les améliorateurs d'arbres forestiers. Cette question est particulièrement importante dans le cadre du changement climatique, où une diminution de la disponibilité en eau durant la saison de végétation est prédite. Trois espèces principales de mélèzes sont utilisées en reboisement en France : le mélèze d'Europe, le mélèze du Japon et leur hybride. Les bonnes performances du mélèze hybride en plantation témoignent de son grand potentiel.

La structure cellulaire des cernes annuels reflète la réponse du cambium (division cellulaire, allongement et épaissement des parois) aux facteurs environnementaux (climat, fertilité, compétition, etc), ainsi que des stades physiologiques et l'effet de la génétique. La densité du bois peut être interprétée comme le produit de l'activité cambiale, et sa variation comme la réponse de l'arbre à son environnement. Toutefois, afin de bien comprendre cette réponse et les variations du processus de xylogénèse lui-même, il est important de repérer les événements qui se succèdent lors de la production des cellules de bois. Plusieurs approches permettent de dater la formation du bois. Nous avons combiné de façon originale microdensitométrie et blessures du cambium (pinning method) afin d'étudier la dynamique de la fabrication du bois. Nous avons analysé les données obtenues du point de vue de l'améliorateur d'arbres forestiers. Cette nouvelle approche nous a permis d'aller au-delà le caractère statique du profil microdensitométrique (où la densité varie en fonction de la distance) afin de le convertir en profil dynamique (où la densité varie en fonction du temps).

La variation de la phénologie de l'activité cambiale peut être vue comme un mécanisme ajustant les le fonctionnement des arbres à leur environnement. Si cet ajustement améliore leur valeur adaptative (fitness), alors les variations correspondantes de l'anatomie du bois peuvent être interprétées comme des caractères d'adaptation. L'intérêt pour les améliorateurs est double : d'un côté, il est urgent de mieux comprendre les liens entre phénologie cambiale, formation du bois et stress pédoclimatiques afin de concevoir des génotypes mieux adaptés à leur environnement. D'un autre côté, l'utilisation par les améliorateurs de nouvelles connaissances sur la phénologie de la formation du bois permettra de produire des arbres avec de meilleures propriétés du bois.

Chez le mélèze, nous avons mis en évidence que la variation phénotypique de variables liées aux dates d'initiation et de fin de la formation des différents tissus du cerne, et à la durée totale de formation du cerne, était faible. D'autres variables liées à la durée et à la vitesse de formation des tissus ainsi qu'aux caractéristiques des cernes sont plus variables et fortement héréditaires. Nous avons

mis en évidence des corrélations phénotypiques qui peuvent servir à sélectionner des individus ayant une densité améliorée et adaptés à des conditions stressantes.

Grâce aux profils dynamiques, nous avons pu estimer des délais de réponse, intensités de réaction au stress et réactivités des arbres. Nous avons trouvé une variation phénotypique modérée à forte pour des caractères liés à l'intensité de la réaction (densité maximum du faux-cerne, durée totale de formation du faux-cerne). La plus grande partie de la variation est concentrée au niveau intra-famille, nous n'avons pas trouvé de différences significatives entre familles pour ces caractères ; toutefois des types de réactions très différents ont été repérés au niveau individu.

Les méthodes courantes d'étude de la formation du bois sont consommatrices de temps et il n'est pas très réaliste de les utiliser en routine pour l'évaluation de populations d'amélioration. Une alternative est d'identifier des marqueurs externes de stades phénologiques comme marqueurs des étapes de la formation du bois. Nous avons testé le potentiel d'évènements de phénologie végétative connus pour être sensibles à l'environnement. Nous avons identifié le débourrement et l'apparition des feuilles comme prédicteurs possible du début de la formation du bois initial. Nous proposons également d'utiliser la formation des bourgeons et les premiers stades de la décoloration automnale des feuilles comme marqueurs de la fin de la formation du cerne. Une autre possibilité intéressante est la synchronisation du profil microdensitométrique par optimisation de l'ajustement d'une relation avec une variable climatique.

Mots clés: formation du bois, blessure du cambium, mélèze, génétique, réaction au climat, marqueurs indirects