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Phenological responses to climate in meristems of conifers along altitudinal and latitudinal gradients

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Tutte le favole hanno luogo nei boschi...
(Authorless)

ABSTRACT

Phenology is the study of recurring biological events that involve plants and animals and their relationships with the environmental factors. The phenological phases, such as flowering and growth reactivation, occurring in primary and secondary meristems, determine the annual plant development.

Climate change is one of the factors that may alter the vulnerability of trees in several aspects, including the phenology. A better understanding of the phenological events occurring in the two meristems, the relations between them and the environmental factors is necessary. Understanding the effect of the climatic factors, such as temperature and precipitation, on plant phenology is an essential step to establish a link between recent climate change and phenological responses. These approaches can produce reliable predictions about future plant responses in a long-term perspective.

The objective of this thesis was to analyse the phenology of meristems in conifers along latitudinal and altitudinal gradients, to deeper understand their responses to climatic drivers. Different methodological approaches were used to investigate the plant phenology: from anatomical analyses and direct observation in field to satellite observation. Moreover, the timing of phenological phases under future climatic scenarios were explored. A general hypothesis of this study was that a time variation in the phenological phases of growth meristems could be observed among different latitudes and altitudes; it was then verified if the variation hypothesized can be explained by site-specific climatic conditions and their changes in time.

In this thesis, 10 study sites located along a latitudinal and altitudinal gradients were selected. Seven of these are located in the boreal forest of Quebec (Canada), while other three sites are located in the Mediterranean area, along the Italian Peninsula. All the sites are characterized by forest stands dominated by coniferous: balsam fir [*Abies balsamea* (L.) Mill.] and black spruce [*Picea mariana* (Mill.) BPS] in the boreal forest, and silver fir (*Abies alba* Mill.) in the Mediterranean mountain forest. Micro-cores were collected in all the sites with the aim to analyse the xylem phenology, while the bud phenology was studied in the boreal forest, through direct and satellite observation.

The study correlated the phenological phases of bud development with the xylem differentiation. This relationship was also analysed between long-term chronologies of xylem growth and the timing of plant phenology detected by Normalized Difference Vegetation Index (NDVI). Furthermore, the relationships between xylem phenology with temperature and precipitation were explored.

The results obtained provided new knowledge on the dynamics of spring phenology and novel information on the synchronisms between the two meristems for boreal tree species.

Moreover, the realized study demonstrated the importance of precisely define the phases of bud development in order to correctly analyse the relationships with xylem phenology. The analyses of long-term chronology demonstrated that the timing of xylem formation could be suitably estimated at wide geographical scale using remote sensing data. Furthermore, temperature resulted the main driver on phenological phases of the two meristems in the boreal forest. Differently, the precipitation during the growing season together with minimum temperature were the most important variables that affected the duration of phenological phases in the Italian study sites.

Response of trees to future environmental conditions should be carefully studied locally, because this interaction can be used for planning adaptive forest management strategies.

RIASSUNTO

La fenologia studia le fasi biologiche ricorrenti nel ciclo vitale di piante ed animali e le loro relazioni con i fattori ambientali. Le fasi fenologiche, quali fioritura e riattivazione dei processi di crescita che avvengono nei meristemi primari e secondari, determinano l’accrescimento annuale delle piante.

Il cambiamento climatico è un fattore che condiziona la vulnerabilità delle piante in vari aspetti, compresa la fenologia. É indispensabile, quindi, migliorare ed integrare le attuali conoscenze degli eventi fenologici nei due meristemi, le relazioni tra essi ed con i fattori ambientali. Comprendere gli effetti dei fattori climatici, quali temperatura e precipitazioni, sulla fenologia delle piante è uno *step* essenziale per stabilire un link tra il cambiamento climatico in atto e le risposte fenologiche. Questi approcci possono produrre previsioni affidabili inerenti gli adattamenti fisiologici delle piante in una prospettiva di lungo termine.

In questa tesi si è proposto di analizzare la fenologia dei meristemi in conifere lungo gradienti latitudinali ed altitudinali, per comprendere più a fondo le loro risposte ed adattamenti ai cambiamenti climatici. Diverse metodologie sono state utilizzate per indagare la fenologia: dalle analisi anatomiche ed osservazione diretta in campo all'osservazione satellitare. Inoltre, è stata indagata la tempistica delle fasi fenologiche considerando futuri scenari climatici, attraverso un approccio modellistico. É stato ipotizzato che la variazione nelle fasi fenologiche dell’accrescimento meristematico verificatasi a diverse latitudini e altitudini, può essere spiegata dalle condizioni climatiche specifiche del sito e loro rispettive variazioni.

In questo lavoro sono stati selezionati 10 siti di studio lungo gradienti latitudinali ed altitudinali. Sette di questi sono situati in foresta boreale (Quebec, Canada), mentre gli altri tre siti si trovano nel bacino del Mediterraneo, lungo la penisola italiana. Tutti i siti sono caratterizzati da boschi di conifere, dominati da abete balsamico [*Abies balsamea* (L.) Mill.] pino nero del Canada [*Picea mariana* (Mill.) BPS] in foresta boreale, e dall’abete bianco (*Abies alba* Mill.) per le foreste montane mediterranee. In tutti i siti è stata effettuata la raccolta di microcarote al fine di analizzare la fenologia dello xilema, mentre in foresta boreale è stata studiata la fenologia dei getti attraverso sia osservazioni dirette in campo che da immagini telerilevate.

Lo studio ha correlato le fasi fenologiche di sviluppo dei germogli e la differenziazione dello xilema. Questo rapporto è stato, inoltre, analizzato tra cronologie a lungo termine della crescita dello xilema e la fenologia vegetale rilevata dal *Normalized Difference Vegetation Index* (NDVI). Infine, si è verificato quanto la fenologia dello xilema possa essere influenzata dalla temperatura e dalle precipitazioni.

I risultati ottenuti hanno fornito nuove conoscenze sulla dinamica della fenologia primaverile e preziose informazioni sui sincronismi tra i due meristemi per le specie boreali. Inoltre, lo studio ha dimostrato l'importanza di definire con precisione le fasi di sviluppo del germoglio per analizzare correttamente i rapporti con la fenologia dello xilema. Le analisi delle cronologie di lungo termine hanno dimostrato che la formazione dello xilema potrebbe essere opportunamente stimata su larga scala geografica utilizzando i dati telerilevati. Inoltre, la temperatura risulta essere il principale *driver* delle fasi fenologiche dei due meristemi nella foresta boreale. Diversamente, lungo la penisola italiana, le precipitazioni durante la stagione di crescita, insieme con la temperatura minima, sono le variabili più importanti nell'influenzare la durata delle fasi fenologiche.

La risposta delle piante alle future condizioni ambientali dovrebbe essere attentamente studiata localmente, perché questa interazione può essere utilizzata nella pianificazione delle strategie di gestione forestale adattiva.

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CHAPTER I

GENERAL INTRODUCTION

1.1. Current knowledge

The impact of the environmental factors, mainly weather, on the seasonal dynamics of organisms is studied also through the phenology (Forrest and Miller-Rushing 2010), which investigates the variations of periodic or recurrent events over time both in plants and animals. Plant phenology thus refers to the science of the growth phenomena and developmental processes of plants, also known as "*phenological phases*" or "*growth phases*". These phases determine the annual plant development and can be used to recognize the cycles of the individual plant growth.

Growth in plants takes place in specialised tissues, the meristems, which act as central control points for growth and development by receiving, integrating, responding to and broadcasting the growth-regulating signals (Risopatron et al. 2010). In particular, two meristems are responsible for tree growth: the apical meristem, determining the lengthening of the stem, and the lateral meristem, controlling the growth in diameter (Lachaud et al. 1999). The activity of meristems is related to genetic and hormonal regulation (Ursache et al. 2013) and it is influenced by the environment (Lachaud et al. 1999). In some climatic areas, such as the tropics, tree growth may continue throughout the year whereas, in temperate regions, the growth activity is usually periodical, influenced and regulated by multiple environmental variables such as temperature, precipitation, photoperiod and other biotic and abiotic factors (Evert 2006; Begum et al. 2013).

In the last decades, phenological events received a renewed attention because of the effect of global climate change that is considered to be the basis for the increased vulnerability of trees in several forest ecosystems. At the seasonal scale, climate warming often leads to earlier events in spring (flowering, bud break, onset of xylogenesis) and later events in autumn (senescence, leaf fall, end of xylogenesis), leading to an extended period of active plant growth under warmer conditions (Menzel et al., 2006). However, few studies have analysed the bud development in conifers (e.g.:Dhont et al. 2010; Sutinen et al. 2012; Rossi and Bousquet; 2014), while xylem phenology was generally more studied (e.g.: Deslauriers et al., 2003; Gričar et al., 2007; Rossi et al.; 2013). Further studies on bud and cambium phenology have revealed different patterns and links between primary and secondary meristems (Rossi et al. 2009; Moser et al., 2010; Swidrak et al., 2013; Cuny et al. 2012).

Phenological studies can be used to document and evaluate the effects of climate change, both at the individual and population level (Schwartz et al. 2006; Cleland et al. 2007). Furthermore, the study of changes occurring in tree phenology for various species could support efforts to reconstruct the past climates, giving also the opportunity to make predictions about biological responses to future climate scenarios (Primack et al. 2009). Climate change affects differently a wide range of ecosystems, inducing latitudinal and altitudinal variations, both in tree physiology and species shifiting (IPCC 2014). Therefore, studies are recommended at regional level (Giannakopoulos et al. 2009), since not all the geographical areas and biomes are impacted uniformly. Among the regions of the globe that are particularly vulnerable to climate changes, two prominent hotspots could be considered at severe risk: (1) the northern latitudes, which are predicted to experience the greatest temperature changes, and (2) the Mediterranean basin, where changes are still evident regarding the increase in average temperature, but also for the decline of the mean annual precipitation (Giorgi 2006).

Forests are one of the most important ecosystems for the terrestrial carbon budget (Pan et al., 2011), and the cyclic dynamics of wood formation represent the primary biological process through which the carbon is stored in plants. A better understanding of the variability in growth resumption and cessation of trees is necessary for identifying the period of wood formation and carbon sequestration in temperate and boreal ecosystems. There is, therefore, a growing need and interest in identifying the timing of wood formation by means of phenological investigations at wide geographical scale.

In this study is presented an original work that explores in detail, over several years and sites, the development of two plant meristems and their relationships. Furthermore, the phenological measurements of xylem at ground level were combined with remote sensed observations, on mature conifers trees, along a latitudinal and altitudinal gradient in the boreal forest. Moreover, the cambial phenology in the Mediterranean basin was investigated, applying a model for predicting changes in xylem growth under different climatic scenarios of temperature and precipitation.

1.1.1. Phenology and climatic drivers

The characterization of the relationships between environmental factors and phenological activity is crucial to understand the plant growth and their coordination under environmental stress. The timing of phenological phases depends on numerous environmental conditions: temperature, precipitation, soil type and moisture, and insolation (Bruns et al., 2003). However, at mid and high latitudes, according to the seasonal variations, it is well established that the timing of growth resumption after winter dormancy is driven principally by the temperature, besides photoperiod and length of chilling period (Begum et al., 2013; Hänninen and Tanino, 2011; Linkosalo et al., 2006; Körner and Basler, 2010). This is especially true for the phenological phases occurring in the early-spring period.

Sarvas (1972) affirmed that the bud burst takes place when the accumulated temperature exceeds a specific threshold. The air temperature is a good predictor of bud development and shoot elongation, once ontogenetic development begins (Schwalm and Ek 2001). Moreover, at the level of the secondary meristems, Rossi et al. (2008) observed that the onset of xylogenesis occurred with daily average temperature of 8–9 °C. Several authors were able to induce an earlier break of cambial dormancy during heating and a delay in cambial reactivation during cooling through artificially heated and cooled stems (Gričar et al. 2007, Begum et al., 2013). These experiments demonstrated the effect of temperature on the xylogenesis onset. Moreover, a "*temperature sum approach*" has been also used for modelling the onset of xylem formation (Seo et al., 2008; Swidrak et al., 2011; Jyske et al., 2014; de Lis et al., 2015).

Climatic factors driving the plant phenology could be significantly different among the various regional climates (Wolkovich et al., 2014). Most of the phenological studies have been conducted in wet and cold regions, where the precipitation is abundant throughout the year and moisture is rarely a limiting factor (Rossi et al., 2011); in these conditions, temperature is often the major driver of the phenology. In the Mediterranean regions, but also in the tropical dry forests, plant phenology is responsive to rainfall and water availability more than to the temperature (Bernal et al., 2011; Peñuelas et al., 2004). Moisture could be considered a critical driver of plant growth, especially in dry summer regions (Allen et al., 2010). Declines in water availability during the growing season could affect the plant growth in terms of quantity (xylem amount) and, consequently, on the wood production (Balducci et al., 2013, 2014). Understanding the effect of the climate on plant phenology is an essential step to establish a plausible link between recent climate change and plant phenological responses; these approaches can also produce reliable predictions about future plant responses in a long-term perspective.

1.2. Objective and structure of the thesis

The general hypothesis of this thesis is that variations in the phenological phases of growth meristems can be observed among different latitudes and altitudes; they could be explained by site-specific climatic conditions and their changes. The thesis is structured into 3 chapters, including two scientific papers published in a peer-reviewed journal and one manuscript ready to be submitted. In addition, a general introduction and final conclusion are also reported.

More in detail, the main objective of this study was to analyse the phenology of meristems in conifers along latitudinal and altitudinal gradients, to deeper understand their responses to climatic drivers. Three different studies were carried out in two diverging environments: Boreal and Mediterranean mountain forests at multiple spatial scales, from field data to satellite observations. Finally, the timing of wood formation under future climate scenarios was also modelled.

The first study monitored and compared the timings of bud and xylem development in two conifer species of the boreal forest of Quebec (Canada); the spring temperature thresholds and cumulated degree-days required for triggering the first phases of bud and cambium development were identified. The study was conducted during five growing seasons, investigating in detail all the phenological phases for the growth resumption of bud and cambium in an altitudinal transects of the boreal forest. The occurring climatic drivers were considered to improve the understanding of seasonal patterns and responses to inter-annual and long-term variation in climate.

In the second study, the relationships between plant phenology, detected by Normalized Difference Vegetation Index (NDVI), and long-term chronology of black spruce xylem formation were analysed, for understand the timing of wood formation in relation to the dynamics of bud phenology at wide geographical scale. The study was conducted along a latitudinal gradient, covering the entire closed boreal forest of Quebec (Canada). The methodological approach here used elucidated the interdependence of foliage and xylem phenological phases, but also their interrelationships with regional temperature trends through a remote sensed approach.

In the third study, the cambium phenology and the effects of temperature and precipitation on the period of xylem production were investigated for silver fir, along a latitudinal gradient in Italy. A model for predicting changes in xylem growth under different climatic scenarios (varying temperature and precipitation) was built and applied.

1.2.1. Study sites and methodological approach

In this thesis, 10 study sites located along a latitudinal and altitudinal gradient were selected. Particularly, 7 of them are located in the Saguenay-Lac-Saint-Jean region, QC, Canada. The other 3 sites are located in the Mediterranean basin along a latitudinal gradient in the Italian Peninsula.

All the sites are characterized by coniferous stands: balsam fir [*Abies balsamea* (L.) Mill.] and black spruce [*Picea mariana* (Mill.) BPS] in the boreal forest, and silver fir (*Abies alba* Mill.) in the Mediterranean mountain forest.

Xylem phenology was studied analysing microcores collected weekly in each study site, while the bud phenology was analysed through direct and satellite observations in the Canadian boreal forest. Details on sites and on the analyses realized are explained in each chapter.

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CHAPTER II

SYNCHRONISMS AND CORRELATIONS OF SPRING PHENOLOGY BETWEEN APICAL AND LATERAL MERISTEMS IN TWO BOREAL CONIFERS

RESEARCH PAPER

Title: Synchronisms and correlations of spring phenology between apical and lateral meristems in two boreal conifers

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ANNEX I: Antonucci S., Rossi S., Deslauriers A., Lombardi F., Marchetti M. and Tognetti R. (2015) Synchronisms and correlations of spring phenology between apical and lateral meristems in two boreal conifers. *Tree Physiology* 35(10): 1086-1094.

Abstract

Phenological synchronisms between apical and lateral meristems could clarify some aspects related to the physiological relationships among the different organs of trees. This study correlated the phenological phases of bud development and xylem differentiation during spring 2010-2014 in balsam fir (*Abies balsamea* Mill.) and black spruce [(*Picea mariana* Mill. (BSP)] of the Monts-Valin National Park (QC, Canada) by testing the hypothesis that bud development occurs after the reactivation of xylem growth. From May to September, we conducted weekly monitoring of xylem differentiation using microcores and bud development with direct observations on terminal branches. Synchronism between the beginning of bud development and xylem differentiation was found in both species with significant correlations between the phases of bud and xylem phenology. Degree-day sum was more appropriate in assessing the date of bud growth resumption, while thermal thresholds were more suitable for cambium phenology. Our results provide new knowledge on the dynamics of spring phenology and novel information on the synchronisms between two meristems in coniferous trees. The study demonstrates the importance of precisely defining the phases of bud development in order to correctly analyse the relationships with xylem phenology.

Keywords: Balsam fir, black spruce, bud break, cambium, temperature, xylogenesis

2.1. Introduction

Phenology is one of the most responsive plant traits driven by climate (Badeck et al. 2004), making trees reliable indicators of climate change (Donnelly et al. 2004). Phenological observations record earlier events in terms of bud break, shooting, leafing and flowering, which may be related to the recent spring warming and extended growing seasons (Chuine and Beaubien 2001, Menzel et al. 2006). In spring, bud development is mainly regulated by temperature (Sarvas 1972), although other interacting factors such as chilling and photoperiod can play an important role (Heide 1993, Häkkinen et al. 1998, Partanen et al. 2001, Rossi et al. 2007). Air temperature is a good predictor of bud development and shoot elongation, once ontogenetic development begins (Schwalm and Ek 2001).

Bud swelling is a phenological event of growth resumption composed of sequential events during which the embryonic shoots and leaves rapidly proliferate and emerge from the bud scales. Generally, studies on apical meristem have identified bud swelling with the phase of leaf unfolding (Takahashi and Koike 2014, Cuny et al. 2012, Moser et al., 2009), while few studies have taken into account all bud development phases (Dhont et al. 2010, Huang et al. 2014, Rossi and Bousquet 2014).

As with bud development, stem growth also occurs through different phases. Secondary xylem production is a complex process that derives from cell periclinal and cataclinal division in the vascular cambium. The differentiation of annual rings in conifers involves the formation of tracheids that pass through several phases before reaching their final form (Deslauriers et al. 2003). Several studies have revealed an early onset of cambium activity and xylem cell production as well as a longer duration of xylem formation and this has often been related to the influences of the on-going climate change

(Huang et al. 2011, Boulouf-Lugo et al. 2012). The activity of meristems involves a number of biochemical processes resulting in a sequence of phases of development and maturation. These phases are identified according to the morphological or anatomical changes and can last from a few days, in primary meristems, to several weeks, in the case of secondary meristems (Rossi et al. 2013, Rossi 2015).

Aloni (2015) showed how leaf development (related to the primary meristems) and biomass accumulation (related to the secondary, or lateral, meristems) are regulated by a number of environmental factors. These factors influence the wood production and its biochemical traits, but also the amount and differentiation of vascular cells. Moreover, physiological factors, such as carbohydrate availability and hormone distribution, could determine the dynamics of wood formation (Uggla 1998, 2001, Rossi et al. 2008). Studies on bud and cambium phenology have revealed different patterns and links between primary and secondary meristems. In conifers, the last phase of bud development, often identified as bud break, has been found to occur either before or after the onset of xylem cell production (Ladefoged 1952, O’ Reilly and Owens 1989, Rensing and Owens 1994, Rossi et al. 2009, Cuny et al. 2012), while in diffuse porous species, cambial reactivation takes place immediately after bud break (Schmitt et al. 2000, Čufar et al. 2008). Thus, the relationships between apical and lateral meristems are still unclear, at least in conifer species. Understanding these relationships is crucial for identifying the timings of C-uptake and seasonal exchanges of water, nutrients and gases between the land surface and atmosphere (Chen and Xu 2012). In order to elucidate the role of meristems as indicators of the effects of climate change, in particular warming temperature, the relationships between primary and secondary growth need to be defined for a mechanistic understanding of the growth dynamics in all tree organs (Huang et al. 2014).

There are still few ecophysiological studies aiming to explain the relationships between bud and cambial phenology and their synchronism over the growing season (Deslauriers et al. 2009, Huang et al. 2014). Characterisation of the relationships between environmental factors, particularly temperature, and meristem activity is crucial to understand the fate and differentiation of meristem cells and their coordination under environmental stresses. Heat accumulation above a threshold temperature, commonly expressed in degree-days, has often been used to predict or verify the effect of temperature on biological processes (Baskerville and Emin 1969). In this study, having monitored and compared the timings of bud and xylem development in two conifer species of the boreal forest, we identified the spring temperature thresholds and cumulated degree-days required for triggering the first phases of bud and cambium development. The study was conducted during five growing seasons, investigating in detail all the phenological phases for growth resumption of bud and cambium and their climatic drivers, to improve understanding of seasonal patterns and responses to inter-annual and long-term variation in climate. The hypothesis that the beginning of bud development occurs after the reactivation of xylem growth was tested.

2.2. Materials and methods

2.2.1. Study site and tree selection

This study was conducted in the Monts-Valin National Park (QC, Canada). Two sites at different altitudes were selected in the black spruce-feather moss bioclimatic domain, Gaspard (abbreviated as GP) and Lagacé (LA), located at 227 and 900 m a.s.l., respectively (Table 1). The climate in the region is dominated by dry and moderately polar air masses in winter and moist maritime air masses in summer (Sheridan 2002). Absolute minimum temperature reaches -41 °C, and mean air temperature ranges between -1.4 and 2.5 °C, with annual precipitation higher than 1000 mm. The sites experience between 204 and 256 frost days year^{-1}.

Primary and secondary growth was monitored weekly from May to September, during the 2010-2014 growing seasons, in balsam fir [*Abies balsamea* (L.) Mill.] and black spruce [*Picea mariana* (Mill.) BPS]. Three trees per species were randomly selected in each site, choosing healthy dominant or co-dominant individuals.

2.2.2. Bud phenology

Two north-facing and two south-facing branches per tree were selected in the bottom part of the canopy. On each branch, the phases of bud development were recorded on terminal buds according to Rossi and Bousquet (2014): (1) open bud, with the scales starting to separate and a pale spot visible at the tip; (2) elongated bud, with lengthening scales; (3) swollen bud, with smooth and pale coloured scales but no visible needle; (4) translucent bud, with needles visible through the scales; (5) split bud, with open scales but needles still clustered; and (6) exposed shoot, with needles completely emerged from the surrounding scales and spreading outwards. All data were computed in days of the year (DOY), by averaging the data observed in the field.

2.2.3. Cambial phenology

Wood microcores (2 mm in diameter) were extracted from the stem at breast height, using a Trephor tool (Rossi et al. 2006*a*). The samples contained the preceding 4-5 tree rings and the developing annual layer with the cambial zone and adjacent phloematic tissue. The microcores were placed in Eppendorf microtubes with an ethanol solution (50% water) and stored at 5 °C to avoid tissue deterioration. Microcores were dehydrated with successive immersions in ethanol and D-limonene and then embedded in paraffin according to Rossi et al. (2006*a*). In order to obtain thin slices, the samples were cut (7 μm in thickness) using a rotary microtome. The sections were stained with cresyl violet acetate (0.16% in water) and examined within 10-15 min under visible and polarized light at 400-500× magnification to differentiate the developing and mature xylem cells.

The radial number of cells was counted along three radial rows, according to the criteria described in Rossi et al. (2006*b*). Observations under polarized light discriminated the zones of enlarging and cell wall thickening of tracheids. The progress of cell wall lignification was detected with cresyl violet acetate reacting with lignin (Antonova and Shebeko 1981). Lignification was shown by a colour change from violet to blue. The colour change over the whole cell wall revealed the end of lignification and the tracheid reaching maturity (Gričar et al. 2005). The dormant cambium consisted of 3-4 cells. In spring, when the diameter of the new xylem cells was at least twice that of a cambial cell, the onset of cell division was considered to occur. Xylogenesis was considered to have started when at least one radial file of enlarging cells was observed (Rossi et al. 2008) (Figure 1). In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete.

2.2.4. Weather stations

In both sites, a standard weather station was installed in a forest gap to measure temperature and precipitation. Data were collected every 15 min during the five years of monitoring and recorded as hourly averages by means of CR10X data-loggers (Campbell Scientific Corporation, Logan, UT, USA). Daily mean values were calculated with the time series obtained from the 24 measurements per day.

2.2.5. Data analysis

Timings of bud phenology were compared between species, DOY and years with analysis of covariance (ANCOVA). Phases of bud and xylem phenology were compared using analysis of variance (ANOVA), while the Tukey test was applied for multiple comparisons. The correlations between phenological phases were tested through Pearson correlations.

Degree-days sum (DDS) at the onset of bud and cambium phenology were calculated as a sum of the positive differences between mean daily air temperature and the threshold value 0 °C (Man and Lu 2010). This threshold was used for calculating DDS according to Man and Lu (2010), who suggested 0 °C as an optimal base temperature for black spruce. Thermal thresholds (THR) for bud and xylem phenology were assessed using logistic regressions to calculate the probability of active meristem growth at a given measured daily temperature. Binary responses were coded as non-active (value 0) or active (value 1) growth, and temperature thresholds were calculated when the probability of active meristem growth was 0.5 (Rossi et al. 2008). Coefficient of variation was used for comparing DDS and THR, given that these measurements take non-negative values. All statistical analyses were conducted using JMP (Version 11, SAS Institute Inc., Cary, NC, USA).

2.3.Results

2.3.1. Bud phenology

The phases of bud phenology occurred earlier in fir than in spruce at both altitudes, though showing different trends in the investigated years. The period of bud development was shorter in fir than in spruce, and longer in 2010 and 2013 than in other years. In 2014, the trend of the two species was similar at lower elevation, and almost identical at higher elevation. Occasionally, some intermediate phases were not recorded, especially at low elevation, because they occurred within the same sampling week. The first bud development phase occurred in the second half of May. The two species showed a week of difference for phase 1, occurring on average on DOY 138 and 145 in fir and spruce, respectively. Phase 6 occurred approximately 4 and 5 weeks after phase 1 in fir and spruce, respectively (Figure 2). In LA (900 m a.s.l.), bud phenology started 2 weeks later than in GP (227 m a.s.l.). ANCOVA model was highly significant ($p<0.001$) for both altitudes, with F=435.77 and 431.51 for 227 m and 900 m a.s.l., respectively (Table 2). As expected, the bud development phases occurred on different DOY. The two species showed different phenological development behaviour and the years considered differed from each other. All the factors considered were statistically significant with $p<0.001$.

2.3.2. Xylem phenology

In the analysed cross section, cambial cells were characterized by thin cell walls and small radial diameters. At the onset of cambial activity, the cambial zone began to widen rapidly as the number of cells increased, revealing that cell division had started. During cell enlargement, the tracheids were composed of protoplast still enclosed in the thin primary wall, but with radial diameter at least twice that of a cambial cell.

The start of xylem cells production was similar and within the same week in the two species (Figure 3). As expected, xylem growth and differentiation started first at lower elevation, at the beginning of May. At higher elevation, cambial resumption occurred between the end of May and beginning of June. The first mature cells were recorded 22 days and 24 days after the start of enlargement in fir and spruce, respectively. The difference in days of mature cell production was greater in LA than in GP. In 2010 and 2013, too few micro-cores were available to determine the date of cell maturation with precision.

2.3.3. Comparing xylem and bud phenology

The correlations between the date of bud and xylem phenology were highly significant (Table 3). A strong association was detected between the open bud (phase one) and date of the first enlargement for both fir (r=0.91) and spruce (r=0.90) (p<0.001). The first mature xylem cell and the stage of exposed shoot showed a significant correlation in fir (r=0.98, p<0.01), but not in spruce (r=0.57, p>0.05), meaning that the variation between both stages is not always synchronous for spruce.

The multiple comparison of phenological events, performed with ANOVA (p<0.001), showed that the dates (DOY) of first cell enlargement and open bud phases were not statistically different in fir. In addition, DOY of the first mature cell and exposed shoot phases did not show statistical difference. In spruce, only the first phases of xylem differentiation and bud development at higher elevation showed no statistical differences. On the contrary, differences were observed at lower elevation between all the analysed phases (Figure 4).

2.3.4. Degree-days sum and thermal threshold

Open bud occurred in fir and spruce when 230±44 (mean ± standard deviation) and 284 ±33 degree-days had accumulated, respectively. The occurrence of the first enlargement cell was observed at lower DDS than the open bud phase, with 217±43 degree-days in spruce and 201 ±37 degree-days in fir. DDS in the exposed shoot phase were higher than DDS in the first mature cell phase (Figure 2.5). The mean THR were estimated at 7.9±1.2 °C for the first enlarging cell phase in spruce, and 7.4±1.5 °C in fir. The open bud phase had a THR of about 9.1±1.2 °C in spruce, and 7.7±1.8 °C in fir (Figure 5). The standard deviations of DDS and THR were similar between the first phases of xylem differentiation and the first phases of bud phenology, in both species. For spruce, the coefficient of variation showed higher values for the first enlarging cell phase than for the other phases, though the exposed shoot phase showed higher values than the open bud and first mature cell phases. The same trend was observed for the coefficient of variation of THR. In fir, the highest coefficient of variation was found in the open bud phase, which occurred for both DDS and THR. The coefficient of variation was smaller for DDS than for THR for the open bud phase, in both species. Finally, the first enlarging cell phase showed higher coefficient of variation for DDS than THR (Table 4).

2.4.Discussion

2.4.1. Synchronisms and correlations between meristems

In this study, we monitored all the phenological phases of bud development and xylem differentiation in black spruce and balsam fir during five growing seasons, investigating the possible relationships between bud development and xylem phenology. This is the first study, to our knowledge, that examines long-term chronologies of the two meristems in these boreal species. The hypothesis that the beginning of bud development in conifers occurs after xylem reactivation was only partially accepted. This result was related to the criteria defined a priori in this study. Generally, bud break has been represented by the last phases of bud development, leaf unfolding (Rossi et al. 2009, Moser et al. 2010, Cuny et al. 2012, Takahashi and Koike 2014). However, the bud growth resumption begins one month before or even earlier, when the scales start to separate following bud swelling. Although the phases of bud phenology are well known and documented (Dhont et al. 2010, Rossi and Bousquet 2014, Sutinen et al. 2012), we are not aware of any studies that considered their relationships with xylem phenology in detail. Indeed, according to the general definition of bud swelling, apical phenology can occur before or after cambial resumption. However, from a biological point of view, the phase of bud swelling, occurring when the scales start to separate and a pale spot is visible at the tip (Rossi and Bousquet 2014), and not that of leaf unfolding, seems to be the most appropriate to represent the break of bud dormancy, as also suggested by Sutinen et al. (2012). As a consequence, we are confident that the beginning of xylem differentiation was synchronous with the beginning of bud development in both the studied species, although no causal relationship between the two meristems can be deduced by this monitoring. Temperature is known to control the phenological events in spring, thus the synchronisms observed between meristems could be the results of a response of the meristems to a common driving factor (e.g., high temperature and available liquid water in early spring may promote photosynthetic activity and carbohydrate storage).

Recent studies have demonstrated that localized heating anticipated cambium reactivation and earlywood vessel formation in seedlings of a broadleaved deciduous species, in comparison with non-heated seedlings, and occurred before bud break (Kudo et al. 2014). Through artificially heated and cooled stems, several other authors were able to induce an earlier break of cambial dormancy and higher rates of cambial cell production (during heating) and a delay in cambial reactivation and lower rates of cambial cell production (during cooling) (Oribe and Kubo 1997, Oribe et al. 2001, 2003, Gričar et al. 2006, Begum et al. 2007, 2010, 2012, 2013). Nevertheless, our results showed a strong coupling between phases of bud development and those of xylem phenology in spring, under natural growing conditions in the boreal forest. This means that both phenological patterns varied in a similar manner along the time axis, i.e., when the phase of open bud occurred earlier, the first enlarging cell was also observed earlier. The synchronization between the last two phases, exposed shoot in bud phenology and mature cell in xylem differentiation was unclear, because significant correlations between the last phases of the two meristems were observed only in fir. Variability in xylem and phloem phenology among years and between species might be determined by exogenous factors (contrasting temperatures prevailing at the start of the growing season, water availability) and intrinsic factors (gene expression, hormonal signals) (Swidrak et al. 2014). Correlation between primary and secondary growth processes over the growing season would indicate optimal mechanisms to simultaneously allocate photosynthetic products and stored non-structural carbon for the growth of the different organs in trees (Huang et al. 2014).

2.4.2. Synchronisms between species

Phenological differences were observed between the two species. Bud phenology began between mid-May and late-June. Fir began earlier than spruce, which might be attributed to a greater sensitivity to spring temperatures, fir being a microthermal species in comparison with spruce. This behaviour could also be explained by genetic differences related to the species-specific capacity to perceive exogenous signals through hormones (Aloni 2001). Jones and Cregg (2006) studied the timings of bud break in different types of North American firs, showing that the date varied with locations, species and years. As expected, in all the years considered, open bud occurred first in trees at lower elevation. This was attributed to earlier spring warming at lower elevation (Moser et al. 2010). Several studies suggest that the accumulation of lipids in the cortex area of the buds would trigger a series of events connected to the onset of bud development (Sutinen et al. 2012, Alaudinova and Mironov 2010). However, studies on xylem phenology showed similar timing between species. Thibeault-Martel et al. (2008) showed no differences in onset of cell enlargement for *A. balsamea* and *P. mariana*, whereas the same cambium activity date was observed for fir and pine trees in temperate environments (Cuny et al. 2012, Gričar et al. 2014).

By comparing bud phenology, it was possible to disentangle the differential responses to climate patterns in these co-occurring species. While conservative responses (low sensitivity to warming temperature) may opportunistically minimize the risk of late (spring) or early (autumn) frost at little cost (Guy 2014), with greater climate warming, phenologically flexible species may advantageously occupy new early or late temporal niches within a short time (Wolkovich et al. 2013). Black spruce has a current competitive advantage over balsam fir in coniferous forests of the boreal shield due to a greater tolerance to cooler temperatures and soil waterlogging (Messaoud et al. 2014). However, warmer temperatures will potentially cause asynchrony in biological activities, disrupting ecological interactions (e.g., between these conifers and spruce budworm and/or fire). A longer period for primary and secondary tree growth with warming temperature could advance black spruce phenology, making this species a more suitable host for spruce budworm.

2.4.3. Temperature of growth

Accumulated temperature in spring is important for bud development and the onset of radial growth (Tadaki et al. 1994). DDS increased in the order of first cell enlargement, open bud, first mature cell and exposed shoot phases, as did the thermal threshold. In our study, we used the coefficient of variation for comparing the methods generally applied in agriculture and forestry to evaluate the influence of temperature on phenological phases. The coefficient of variation for the open bud phase was lower in DDS than in THR. The opposite occurred for the first cell enlargement phase. This coefficient indicated that DDS better explained the first phase of bud phenology, whereas thermal thresholds were more suitable for the first phase of xylem differentiation. The degree-days, therefore, appeared to be the more appropriate monitoring technique for apical meristems, while the thermal threshold was more advisable for cambium phenology. Rossi et al. (2008) determined common critical temperature thresholds for xylogenesis in conifers at different latitudes and altitudes in Europe and Canada, with average daily temperature values of 8–9 °C. Analyses at a wider geographical scale comparing several conifer species have confirmed convergence of the thermal threshold around specific critical values, demonstrating the existence of a precise thermal limit in radial growth and tree ring formation (Deslauriers et al. 2008, Rossi et al. 2008). Prislan et al. (2013) observed that the onset of cambial cell division in *Fagus sylvatica* L. was associated with an extended period of preconditioning that varied with site, rather than with a threshold temperature. Patterns of cambial activity and climate thresholds have been found to vary among sites in *Picea abies* (Gričar et al. 2014), suggesting that local adaptation may play a role in coupling processes of wood formation and foliage renewal within populations. Gričar et al. (2014) showed site-specific amounts of accumulated heat units at the onset of cambial cell production. In contrast, Schmitt et al. (2004) reported that growing degree-days for the onset of cambial activity in *Pinus sylvestris* were similar along a latitudinal gradient in the boreal forests of Finland. Instead, DDS have been used to predict the growth stages of major crops and trees, particularly in tracking bud development (Wang 1960, Førland et al. 2004, Jones and Cregg 2006). It should be pointed out that longer and warmer summers at higher elevations and northern latitudes might result in disproportional resource requirements for these species, namely water.

2.5. Conclusions

To our knowledge, this is the first study that examines the synchronisms between the phases of growth resumption in buds and xylem, using chronologies of 5 years of data collected from two species and two sites. Detailed phases of bud break and xylem cell differentiation showed a significant synchronism in the timings of activation between the two meristems. Considering the bud swelling as break of dormancy and not exposed shoot, we found that the beginning of bud development occurred in the same period as xylem differentiation in both species. This demonstrates the importance of considering all phases of bud break in order to correctly evaluate the relationships with xylem phenology. Data from the two meristems were strongly correlated to one another, although fir dormancy release was earlier than spruce. This study allowed the dynamics of spring phenology to be explored in detail, providing novel information about the potential physiological correlation between meristems and filling an important gap in the knowledge on the growth synchronisms in conifer species. A straightforward quantification of the dynamics of growth and response of phenology to climatic drivers is essential to link plant-level events of life cycles with the environmental factors occurring at short time scale. The presence of synchronism in leaf and cambial activity in these conifers may be conveniently used to relate field (leaf phenology and wood production) and satellite (vegetation indices) data and explain seasonal variability in boreal forest productivity.

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Conflict of interest

None declared

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Table 1

Species	Site	Stand age (yr)	Height (m)	Diameter at breast height (cm)
Spruce	LA	72.7 (20.3)	4.6 (1.04)	14.0 (0.03)
	GP	39.7 (3.7)	14.2 (1.6)	24.7 (1.4)
Fir	LA	25.3 (2.5)	7.6 (0.7)	16.8 (4.0)
	GP	49.7 (3.7)	17.6 (2.6)	32.2 (9.1)

Table 2

Altitude	Model		Factor	Statistics	
	F-value	R^2		*F*-value	*P*
900 m a.s.l.	431.51	0.67	Year	53.25	<.0001
			DOY	1281.49	<.0001
			Species	93.64	<.0001
227 m a.s.l	435.77	0.66	Year	49.44	<.0001
			DOY	1266.52	<.0001
			Species	156.93	<.0001

Table 3

	Open Bud	Exposed shoot	First enlarging xylem cell	First mature xylem cell
Open bud	-	0.82**	0.90***	0.81*
Exposed shoot	0.82**	-	0.77*	0.57
First enlarging xylem cell	0.91***	0.78**	-	0.88*
First mature xylem cell	0.93*	0.98**	0.89*	-

Table 4

		DDS	THR
Spruce	First enlarging cell	217±43 (19.9)	7.9±1.2 (14.9)
	Open bud	230±44 (11.7)	9.1±1.2 (12.9)
	First mature cell	468±50 (10.8)	11.8±1.1 (9.3)
	Exposed shoot	795±103 (13.0)	13±1.7 (13.1)
Fir	First enlarging cell	201±37 (18.4)	7.4±1.5 (18.7)
	Open bud	284±33 (19.2)	7.7±1.8 (22.8)
	First mature cell	420±68 (16.3)	11.3±1.3 (11.2)
	Exposed shoot	585±98 (16.8)	11.3±1.1 (9.4)

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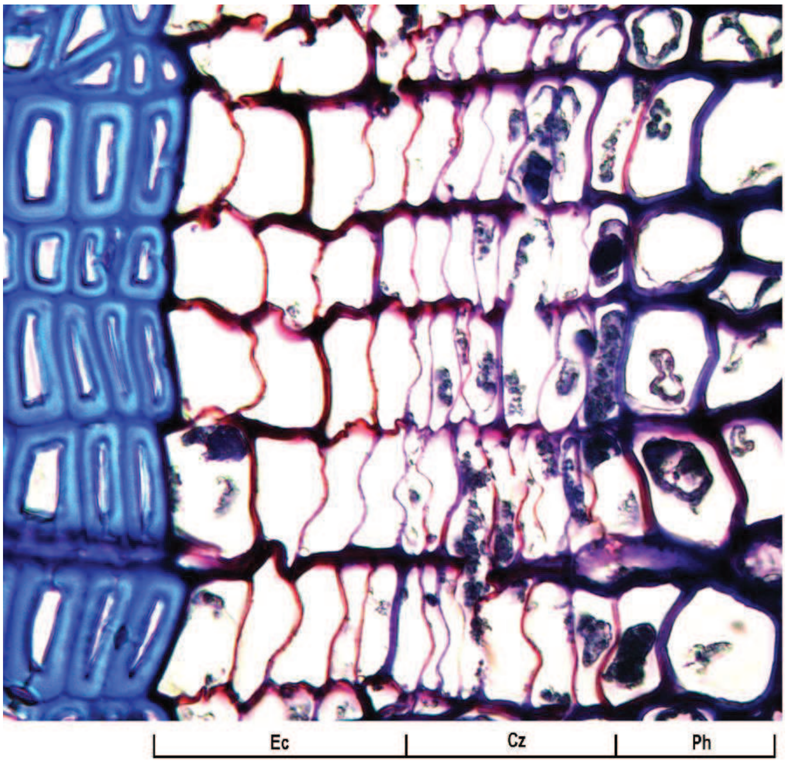

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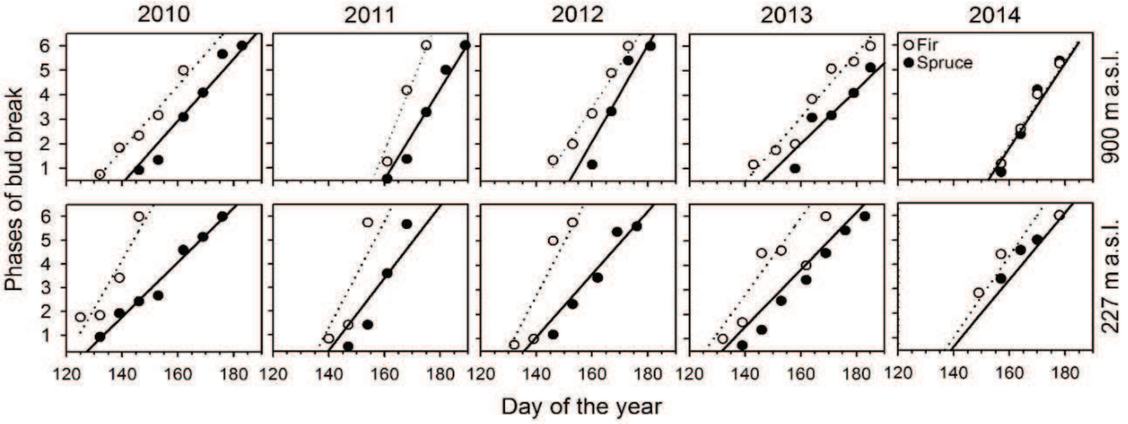

2010
2011
2012
2013
2014
○ Fir
● Spruce
900 m a.s.l.
227 m a.s.l.
Phases of bud break
1
2
3
4
5
6
Day of the year
120
140
160
180

Figure 3

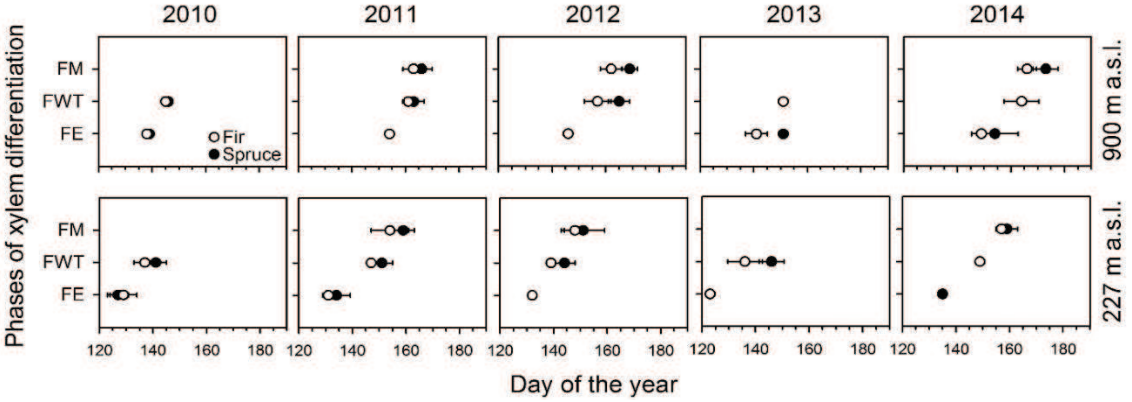

2010
2011
2012
2013
2014
Phases of xylem differentiation
FM
FWT
FE
○ Fir
● Spruce
900 m a.s.l.
227 m a.s.l.
120
140
160
180
Day of the year

Figure 4

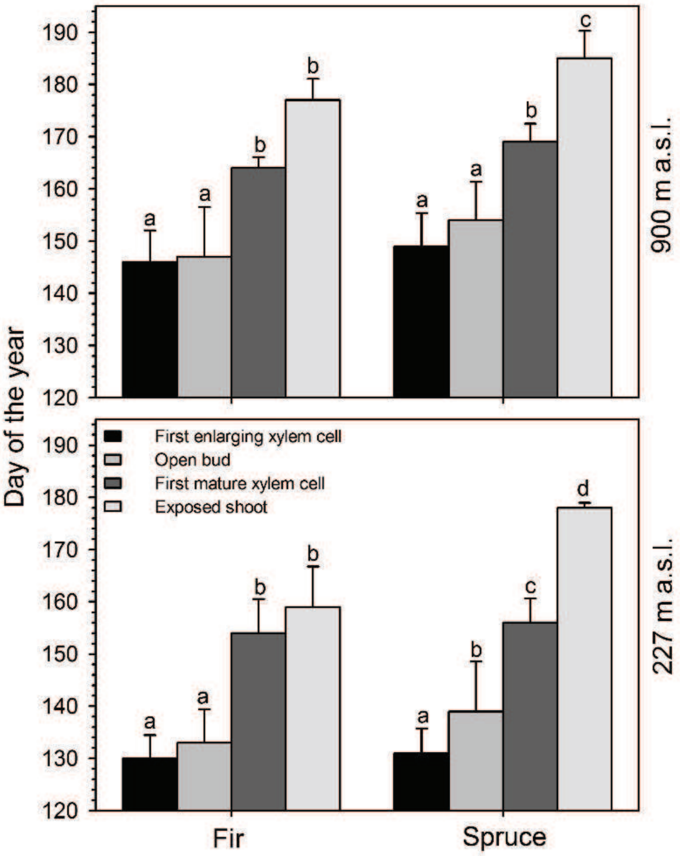
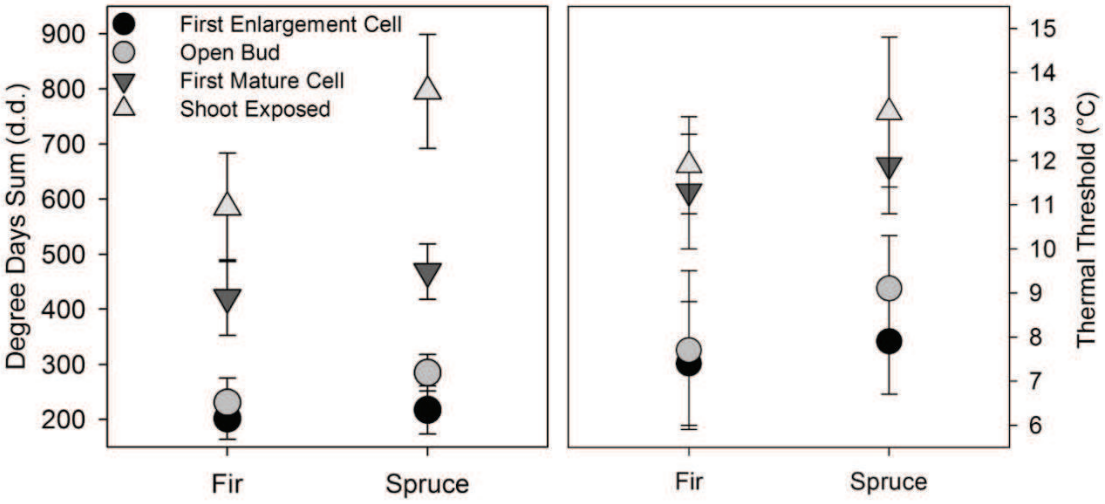

Day of the year
190
180
170
160
150
140
130
120
a
a
b
b
a
a
b
c
900 m a.s.l.
First enlarging xylem cell
Open bud
First mature xylem cell
Exposed shoot
a
a
b
b
a
b
c
d
227 m a.s.l.
Fir
Spruce

Figure 5


First Enlargement Cell
Open Bud
First Mature Cell
Shoot Exposed
Degree Days Sum (d.d.)
900
800
700
600
500
400
300
200
Fir
Spruce
Fir
Spruce
Thermal Threshold (°C)
15
14
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12
11
10
9
8
7
6

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CHAPTER III

LARGE SCALE ESTIMATION OF XYLEM PHENOLOGY IN BLACK SPRUCE THROUGH REMOTE SENSING

RESEARCH PAPER

Title: Large scale estimation of xylem phenology in black spruce through remote sensing

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ANNEX II: Antonucci S., Rossi S., Deslauriers A., Morin H., Lombardi F., Marchetti M. and Tognetti R. (2017) Large-scale estimation of xylem phenology in black spruce through remote sensing. *Agricultural and Forest Meteorology* 233: 92-100.

Abstract

There is a growing need for understanding the timing of wood formation in relation to the dynamics of bud phenology at wide geographical scale. This study analysed the relationships between long-term chronologies of xylem growth and the timing of plant phenology detected by–Normalized Difference Vegetation Index (NDVI) in five permanent stands across the latitudinal distribution of black spruce [*Picea mariana* (Mill.) BPS] in the boreal forest of Quebec, Canada. Xylogenesis was studied weekly from April to October for thirteen years (2002-2014) on anatomical sections derived by wood microcores. The timing of the growing season detected by remote sensing was extracted from MODerate resolution Imaging Spectroradiometer (MODIS) 250 m 16-days NDVI data. The NDVI time-series were fitted using a double-logistic curve. Phenological chronologies from remote sensing and xylem phenology showed a latitudinal trend. The models correlating the data inferred from satellite sensors and the spring observations of xylem phenology were significant ($p<0.0001$). The length of NDVI growing season and the duration of xylogenesis showed a close correlation. This study demonstrated that the timing of xylem formation could be suitably estimated at wide geographical scale using remote sensing data. However, the inter-annual phenological variability remained unexplained, which might limit the application of the models only to the years considered by our chronologies.

Keywords: black spruce, cell differentiation, phenology, remote sensing, vegetation index, xylogenesis.

3.1.Introduction

Phenology is the study of recurring biological events, such as growth reactivation or flowering, and their connection with environmental factors (Cleland et al., 2007). Forests are the most important ecosystems for the terrestrial carbon budget (Pan et al., 2011), and the cyclic dynamics of wood formation represent the primary biological process through which carbon is sequestered in plants. Wood formation, or xylogenesis, occurs annually in trees of temperate and cold climates according to the cycles of summer and winter, producing a distinct pattern in the wood conferred by the tree rings (Rossi et al., 2014). Xylogenesis lasts from a few weeks to several months, according to species and individual growth rate, and, more specifically, to climate (Schmitt et al., 2004; Rathgeber et al., 2011; Treml et al., 2015; Vieira et al., 2015). The wide variation in timing of wood formation observed across geographical regions demonstrates the high plasticity of trees in adapting their growth to local environmental conditions (Cuny et al., 2015; Rossi et al., 2016). In cold ecosystems, trees display conservative responses to temperature to minimize the risk of late (spring) or early (autumn) frost. A better understanding of variability in growth resumption and cessation of trees is necessary for identifying the period of wood formation and carbon sequestration in temperate and boreal ecosystems. Seasonal dynamics of wood formation is largely dependent on temperature (Cocozza et al., 2016). Understanding how the thermal conditions, which occur during spring and summer, affect the timing and dynamics of wood formation has great ecological and economic relevance in a region as the boreal forest (Balducci et al., 2014). Indeed, productivity and growth are directly mediated by phenology at scales from the cell to ecosystem. There is therefore a growing need to identify the timing of wood formation by means of phenological investigations at wide geographical scale.

Optical satellite observations of land surface reflectance and their combinations in the form of vegetation indices are associated with the biophysical and biochemical properties of vegetation (Wu et al., 2014). Nowadays, it is possible to study vegetation phenology from field measurements and through satellite observations (Piao et al., 2015), because remote sensing of phenology using time-series of vegetation indices is based on the intra-annual changes of canopy greenness. These methods generally use time-series of Normalized Difference Vegetation Index (NDVI) data from different sensors. The NDVI is an effective parameter for monitoring the spatio-temporal patterns of vegetation phenology because it is related to the amount of green-leaf biomass (Lillesand and Keifer, 2000). Due to its close relationship with plant activity, NDVI has been considered a good proxy for investigating the impact of climate change on tree phenology at regional to continental scale (Peng et al., 2013; Piao et al., 2015). NDVI correlates directly with vegetation productivity (Reed et al., 1994); there are numerous applications of this index for ecological purposes, and specifically for detecting changes in vegetation phenology, estimating biomass and net primary productivity, or assessing vegetation's response to climate (Wu et al., 2014; Balzarolo et al., 2016). Plant phenology is studied at different spatial scales, from plant to landscape (Antonucci et al., 2015; Richardson et al., 2013). Obviously, each scale of investigation produces results with specific resolution. How much can these different spatial scales be integrated? In particular, we raise the question whether and at what strength the phenological data inferred by remote sensing represents the dynamics of wood formation.

In this study, along a latitudinal gradient covering the entire closed boreal forest of Quebec (Canada), we analysed the relationships between phenology detected by NDVI and a long-term chronology of black spruce [*Picea mariana* (Mill.) BPS] xylem formation, divided into the different phases of xylem growth, ending in tree ring formation. Boreal forests are undergoing rapid northward shifts as a result of warming climate, which can influence vegetation patterns and growth responses by modifying the start and duration of the growing season. Although these shifts appear first at the biome's margins (Beck et al., 2011), it also clear that the effects of climate warming on growth dynamics vary across the distribution range of a species (Gazol et al., 2015). A hypothesis is proposed that different phases of xylem phenology are correlated with NDVI data. Linking landscape-level vegetation measurements of detailed time-series analysis derived from remotely sensed data to xylem phenology may provide an innovative tool to estimate the timing of xylem formation and carbon sequestration in wood at wide geographical scale. This approach may also elucidate the interdependence of foliage and xylem phenological phases and their interrelationships with regional temperature trends through a remote sensing approach.

3.2.Materials and methods

3.2.1. Study area

The study was conducted in the boreal forest of Quebec, Canada, at five permanent sites located between the 48th and 53rd parallels across the latitudinal distribution of black spruce: Simoncouche (abbreviated as SIM), Bernatchez (BER), Mistassibi (MIS), Camp Daniel (DAN) and Mirage (MIR) (Figure 1; Table 1). SIM and BER were in the balsam fir-white birch bioclimatic domain, MIS and DAN were in black spruce-moss bioclimatic domain, and MIR was in the black spruce-lichen bioclimatic domain. All sites consisted of coniferous stands dominated by mature and even-aged black spruce. Other coniferous or broadleaf species were rare or missing (Rossi et al., 2013). The climate of the area is typically boreal, with cool summers and very cold winters. The mean annual temperature varied between -1.6 and 4.1 °C, with the highest values recorded in the southern site. Winter temperatures attained a minimum ranging between -29.8 and -47.1 °C, while May-September mean temperature was 12.1 °C.

3.2.2. Assessing xylem phenology

Ten dominant or co-dominant trees were chosen in each site. Tree-ring formation was studied from April to October 2002-2014 in all sites, except for MIR, where the study lasted from 2012 to 2014. Wood microcores were collected weekly following a spiral trajectory on the stem from 30 cm below to 30 cm above breast height (1.3 m) using Trephor (Rossi et al., 2006*a*). Trephor is a chisel-shaped tool for the fast recovery of 2 mm diameter microcores. Its cutting tube is hammered into the wood, and the wood sample is separated from the xylem by rotating and extracting the tool like a corkscrew. The very small wounds inflicted by the thin piercing tubes of the tool and the consequently narrow areas of traumatized tissues around the sampling points allowed repeated samplings.

The samples contained the preceding four to five tree rings and the developing annual layer with the cambial zone and adjacent phloem tissue. The microcores were placed in Eppendorf microtubes with an ethanol solution (50% water) and stored at 5 °C to avoid tissue deterioration. Microcores were dehydrated with successive immersions in ethanol and d-limonene and then embedded in paraffin according to Rossi et al. (2006a). In order to obtain thin sections, the samples were cut (7 µm in thickness) using a rotary microtome. The sections were stained with cresyl violet acetate (0.16% in water) and examined within 10–15 min under visible and polarized light at 400–500× magnification to differentiate the developing and mature xylem cells.

The number of cells was counted along three radial rows, according to the criteria described in Rossi et al. (2006b). Observations under polarized light discriminated the zones of enlarging and cell wall thickening of tracheids. The progress of cell wall lignification was detected with cresyl violet acetate that reacts with lignin (Antonova and Shebeko, 1981). Lignification was shown by a colour change from violet to blue. The colour change over the whole cell wall revealed the end of lignification and the tracheid reaching maturity (Gričar et al., 2005). The dormant cambium consisted of three to four cells. In spring, when the diameter of the new xylem cells was at least twice that of a cambial cell, the onset of cell division was considered to occur. Xylogenesis was considered to have started when at least one radial file of enlarging cells was observed (Rossi et al., 2008). In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete. Xylem phenology was computed in day of the year (DOY) corresponding to the dates of (i) beginning of cell enlargement, (ii) beginning of cell wall thickening, (iii) first mature cell, (iv) ending of cell enlargement, and (v) ending of cell wall thickening. The duration of xylogenesis was assessed as the number of days between the onset of cell enlargement and the ending of cell wall lignification.

3.2.3. Assessing the NDVI growing season

Timing of the growing season detected by remote sensing was extracted for the five sites from MODerate resolution Imaging Spectroradiometer (MODIS). MODIS instrument is operating on the Terra and Aqua spacecraft. Its detectors measure 36 spectral bands and acquire data at different spatial and temporal resolutions. In our study, we used the 16-days MODIS 250 m NDVI maximum value composite product, where data are provided every 16 days at 250-meter of spatial resolution (MOD13Q1 product v 005). The study region is represented in two different MODIS datasets. Therefore, forty-six NDVI images per year from 2002 to 2014 were acquired from USGS EarthExplorer (www.earthexplorer.usgs.com), for a total of 598 MODIS images. MODIS data were re-projected from a sinusoidal projection to a NAD 1983 reference system, using a Nearest-Neighbor resampling routine using ArcGis version 10.3 (ESRI, Redlands, CA).

NDVI values were extracted for each pixel within a circular plot of 3.75 km radius centred on each site. The pixels including lakes and rivers were eliminated based on the hydrological maps of Quebec, Canada. The fortnightly NDVI time-series extracted were fitted using a double-logistic curve based on three pairs of coefficients: winter and maximum NDVI (*w*NDVI and *m*NDVI), the inflection points when the curve rises and falls (*S* and *A*), and the rate of increase or decrease (*mS* and *mA*) of the curve at the inflection points (Beck et al., 2006; Fisher et al., 2006). The time is represented by *t*, included as Day Of the Year (DOY).

$$NDVI\ (t) = wNDVI + (mNDVI - wNDVI) \times (\frac{1}{1 + \exp\left(-mS \times (t - S)\right)} + \frac{1}{1 + \exp\left(mA\ \times (t - A)\right)} - 1)$$

This function allows asymmetry in the NDVI temporal evolution of spring and autumn, leading to a precise estimation of the growing season (Beck et al., 2006). Beginning and ending of the NDVI growing season represented the date when the NDVI curve crossed the 50% threshold in upward and downward direction, which corresponded to the inflection points of the function (White et al., 1997).

3.2.4. Weather stations

At each site, a standard weather station was installed in 2001 in a forest gap to measure air temperature. The weather station in MIR was installed in 2012. Air temperature was recorded at 3 m above ground level every 15 min and stored as hourly average by means of CR10X dataloggers (Campbell Scientific Corporation, Logan, UT). Daily mean values were calculated from the time-series obtained from the 24 measurements per day.

3.2.5. Statistical analyses

Relationships between mean annual temperature at the site and phases of NDVI growing season were tested through linear regression. The effect of year and site on the variance of the dependent variable was estimated using the Restricted Maximum Likelihood (REML) method, considering the phases of xylem phenology, duration of xylogenesis and NDVI growing season as dependent variables. Generalized Linear Models (GLM) were used to estimate the timing of xylem phenology with the NDVI growing season using the variable year as a covariate. The type-III error was used for

GLM. All statistical analyses were conducted using SAS 9.2 (SAS Institute, Inc., Cary, NC).

3.3.Results

3.3.1. Xylem phenology

Enlargement of the xylem cells began first in SIM, on DOY 147, and was observed later at the higher latitudes (MIR), where xylem growth resumption occurred at the beginning of June, on average between years, on DOY 159 (Figure 2). On average, the beginning of cell wall thickening occurred on DOY 168, while the first mature cells were observed on DOY 182. The beginning of cell enlargement, wall thickening and the first mature cell were observed to occur synchronously in BER and DAN. The ending of cell enlargement occurred on DOY 213, while the ending of xylem differentiation was observed between the end of August and end of September (DOY 266 and 242), in SIM and MIR respectively. The period required for completing the process of xylogenesis decreased with the latitude, and lasted from 83 d in MIR to 118 d in SIM (Figure 2).

3.3.2. NDVI growing season

SIM, the site at the lowest latitude, showed the highest NDVI values and exhibited a beginning of the growing season ranging between DOY 71 and 117 (Figure 3). The site at the highest latitude (MIR) began the NDVI growing season later, between DOY 144 and 173. A delay of 10 d was observed in the beginning of the NDVI growing season, between BER and DAN (DOY 129 and 139, respectively). In 2010, the sites showed the earliest beginning of NDVI growing season. The NDVI growing season ended earlier in the north (DOY 269) than south of the gradient (DOY 318). On average, BER and MIS differed by only 4 d in the ending of the NDVI growing season (DOY 304 and 301, respectively). The years 2008 and 2014 were those with the later ending of the NDVI growing season, on DOY 300. Like xylem phenology, NDVI growing season was longer at the lower latitudes, on average 222 vs. 112 d in SIM and MIR, respectively. DAN and

BER showed similar durations of NDVI growing season (175-180 d). The longest NDVI growing season was observed in 2012 (Figure 3).

3.3.3. Temperature and NDVI

The timing of the NDVI growing season showed a strong relationship with the mean annual temperature at the sites. Mean annual temperature exhibited high linearity with NDVI growing season (R^2 0.85). The relationship between temperature and ending of NDVI growing season was less strong than the relationship with beginning of NDVI growing season (R^2 0.78) (Figure 4). The colder site showed shorter NDVI growing season than the hotter sites, as a consequence of a later beginning and earlier ending.

3.3.4. Variance partition

The factors “year” and “site” explained 40.4 and 39.9% of the variance of the beginning of cell enlargement, respectively (Table 2). The two factors had similar effects on the variance of the successive phases of xylem phenology. The effect of site on the variance of the duration of xylem phenology (cell production, wall thickening, and xylogenesis) ranged between 64.2 and 69.7%, while that of year explained a lower proportion of the variance, between 11.8 and 19.6%.

Most variance of the NDVI growing season was explained by the site, with proportions ranging between 69.9 and 86.5%. The factor “year” had a minor effect, ranging between 3.9 and 9.7%.

3.3.5. Generalized linear models

GLMs were highly significant ($p<0.0001$) for all the spring phases of xylem phenology, with a high R^2 that ranged between 0.85 and 0.94 (Table 3). The beginning of the NDVI growing season was significant ($p<0.001$), while no effect was detected for the factor “year” or the interaction (*p*>0.05). Also for beginning of lignification and first mature cell the year and interaction between factors were not significant (p>0.05).

GLMs were not significant for the ending of the enlargement phase (*p*>0.05), showing R^2 of 0.61, while highly significant for the ending of lignification (*p*<0.0001) (Table 4). The ending of the NDVI growing season was significant in both phases of ending of cell differentiation, while the “year” and the interaction were not significant (*p*>0.05).

All models involving the duration of xylem growth were significant with *p*<0.001, and showed R^2 ranging between 0.71 and 0.82 (Table 5). As for the other two factors of variation, also NVDI growing season was significant, while no significant effects were observed for the “year” or the interaction (*p*>0.05).

Overall, the relationships between xylem phenology and NDVI growing season were positive and linear (Figure 5). However, the slopes had low values, suggesting that a variation of the NDVI was associated to a smaller variation in the phases of xylem phenology. The beginning of growth exhibited higher slopes than the ending, which indicated stronger relationships between the phenological variables in spring. Strong relationships were also detected with duration of the NDVI growing season, which produced regressions with similar slopes (0.23-0.25) for the duration of cell production, wall thickening and xylogenesis. Consequently, according to the estimated regression, a lengthening of ten days in the NDVI growing season was associated to an increase of 2.3 days of the xylogenesis.

3.4. Discussion

3.4.1. Phenology across the latitude

This study compared the timing of the NDVI growing season detected by remote sensing with chronologies of xylem formation collected during a long-term monitoring of black spruce across its latitudinal distribution in the boreal forest of Quebec, Canada. As expected, the first enlarging, wall-thickening and mature xylem cells appeared earlier in the warmer than in the colder sites. The ending of xylogenesis occurred later in warmer sites, resulting in a longer growing season. This trend confirmed previous studies that analysed altitudinal and latitudinal gradients in different species (Camarero et al., 1998; Delsauriers et al., 2003; Rossi et al., 2007; Rossi et al., 2016 in press; Moser, 2010). The formation of xylem cells is the result of different biochemical processes underlying the sequential stages of cell division and maturation that are represented by the observed phenological traits (Prislan et al., 2009). Xylem phenology, in terms of beginning and ending of cell differentiation, varied gradually with temperature, represented here by the latitudinal gradient (Rossi et al., 2014; Rossi et al., 2016).

As observed for xylem formation, the phenological chronologies derived from remote sensing also showed a clear latitudinal trend between sites, with xylem production starting earlier and stopping later in the southern than northern sites. The length of the NDVI growing season ranged by approximately one hundred days across the black spruce distribution analysed, which covers more than 5° in latitude. Reed (2006) found trends in seasonality and integrated NDVI across North America, but other studies have reported contrasting findings for the northern latitudes. Different approaches with NDVI data could produce diverging results, with the start of the season ranging by four and two weeks in terms of average and variability, respectively (White et al., 2009).

3.4.2. Effect of the temperature

This study showed a close relationship between mean annual temperature at sites and the phenological chronologies detected by NDVI. The temperature monitored with weather stations installed at the sites showed high R^2 when correlated with the timing of NDVI growing season. Consequently, the site with warmer temperature exhibited the longer NDVI growing season. Mao et al. (2012) examined the environmental drivers behind the NDVI changes during spring in mid-high latitudes and revealed that temperature is an important factor explaining the variation in NDVI. Also Zhang et al. (2006) reported a strong correlation between the phenological traits estimated from MODIS data and the temperature patterns in ecosystems of mid and high latitudes. Temperature has been long recognized as a main driver of plant growth, and spring air temperature was found to trigger the reactivation of photosynthesis in most boreal and temperate ecosystems (Barr et al. 2009; Chen et al., 2003; Suni et al., 2003). Moreover, in temperate and boreal forests, xylogenesis is active from late spring to early autumn for a period ranging between 90 and 160 days, which exploits the thermally favourable period when the environmental conditions permit division and maturation of xylem cells to take place (Rossi et al., 2007). At the current rate of warming, temperature limitations on xylem growth of black spruce may soon be released in northern sites, while southern sites may shift beyond optimum for this species, increasing the risk of drought-induced tree mortality (Beck et al., 2011; D'Orangeville et al., 2016). However, actually water availability in the studied region can be hardly considered a limiting factor for tree growth due to the low temperatures and the frequent and abundant precipitation falling during the growing season (Belien et al., 2012, 2014). Establishing a link between xylem phenology, NDVI data, and air temperature would provide a comprehensive and spatially-explicit view of boreal forest productivity patterns and trends, particularly useful where systematic collections of phenological data covering different forest sites are lacking.

3.4.3. Relationships between xylem and NDVI phenology

The NDVI growing season was on average 10 d longer than the period of xylem formation. Studies on bud and cambium phenology have revealed different patterns and links between primary and secondary meristems (Rensing and Owens, 1994, Rossi et al., 2009; Schmitt et al., 2000; Čufar et al., 2008). Nevertheless, the models between the data inferred from satellite sensor and our observations of xylem phenology were significant and linear, showing also a close correlation between the length of NDVI growing season and duration of xylogenesis. Studies of canopy greenness affirm that it is easier to identify spring than autumn phenology (Richardson et al., 2013; White et al., 2014; Wu et al., 2014). Our study demonstrated that it is possible to estimate xylem phenology through remote sensing at wide spatial scale. The statistical models performed in our analysis represent valid tools to suitably spatialize the timing of xylem formation across the latitudinal distribution of black spruce for the studied years. In the studied region, and more in general in the boreal ecosystems, the gently rolling topography, associated to the homogenous stands dominated by few tree species, contributes to a reliable application of our models across wide spatial scales. However, most of the inter-annual variability remained unexplained, thus limiting application of the models to the period considered by our xylogenesis chronologies.

Needles of evergreen conifers can reactivate temporarily, showing photosynthetic activity and producing assimilates during the warmest days of winter and early spring if liquid water is available for roots (Bergh and Linder, 1999; Goodine et al., 2008).

Moreover, in boreal evergreen trees, photosynthesis occurs before snowmelt, since solar irradiance is close to the seasonal maximum and the dark-coloured needles of black spruce warm up easily when exposed to the solar rays. In contrast, xylem, which has synchronous activity in stem and roots (Thibeault-Martel et al., 2008), differentiates only after the snow disappears and soil temperature begins to increase (Rossi et al., 2007; Turcotte et al., 2009; Lupi et al., 2010). Rossi et al. (2011) showed that onset and duration of xylogenesis and cell production were strongly correlated with the dates of complete snowmelt.

Phenological data using time-series of vegetation indices are based on the intra-annual changes in canopy greenness. Data from remote sensing represent the greenness derived from chlorophyll abundance (Myneni et al., 1997), the absorption of photosynthetically active radiation, and canopy photosynthetic capacity in regions with high leaf area (Sellers, 1985, 1987). Studies on the relationships between bud and xylem phenology and their synchronism over the growing season in conifer species are still scarce (Antonucci et al., 2015; Huang et al., 2014; Deslauriers et al., 2009). In Antonucci et al. (2015), a spring synchronism was found between bud and xylem formation, with significant correlations between the two meristems. Huang et al. (2014), demonstrated that xylem formation after budburst was positively associated with bud phenology, shoot growth, needle growth and cambium activity. In this study, we have identified the existence of a relationship between the primary and secondary meristem at wide geographical scale by means of remote sensing data. These relationships may be applied for estimating xylem phenology in remote areas where direct observations or recurrent samplings are precluded or logistically unachievable.

3.4.4. Limitations of the study

Activity of the apical meristem involves a number of biochemical processes resulting in a sequence of development and maturation phases that can be identified in detail by direct visual inspection on individual organisms in the field (Sparks and Menzel, 2002). Growth resumption is composed by sequential events in which embryonic buds rapidly proliferate in shoots and needles emerge from the bud scales (Dhont et al., 2010). As the trees transit from dormancy to active growth, also needle growth in spring is relatively rapid, which provides a signal that can be detected through remote sensing. Our analysis is thus subjected to classical point-vs.-pixel comparison errors. These errors are produced by the analyses of single xylem phenology of a limited number of plants and correlated with data recorded on a surface that represents, at the finest scale, one pixel of 250 m in length, and, consequently, the phenology at stand level. On the contrary, the phases of xylem phenology were scaled at an individual level: the direct analyses occurred weekly and showed less intra-annual variation than the phenological patterns extracted by NDVI. Uncertainties in using NDVI for phenology studies arise from limitations such as contaminations by background reflectance from soil, leaf litter, dead branches, snow, snow thaw, soil thaw and shadows, which also have distinct seasonal dynamics, potentially misinterpreted as vegetation (Gonsamo et al., 2012). Other factors can play an important role in NDVI variation within a pixel: plant architectural arrangement, canopy cover, height, and vigour and stress of vegetation. Some of these factors can significantly affect the remotely sensed information **(**Tucker et al., 2005; Pettorelli et al., 2005)**.** Moreover, different methodologies are available for estimating phenological events from remote sensing data, each one with its limitation (De Beurs and Henebry, 2010). The most confounding issue related to phenological modelling to date is the limited potential of a vegetation index to estimate the start and end of the season in needleleaf forests because of small seasonal vegetation variations (Guyon et al., 2011; Hufkens et al., 2012; Melaas et al., 2013). For example, Hmimina et al. (2013) showed that MODIS is unable to accurately infer phenological patterns for needleleaf forests. By comparison, with clear variations in canopy greenness, changes in phenology of deciduous forests are much easier to detect (Garrity et al., 2011; Gonsamo et al. 2012; Luo et al., 2014; Melaas et al., 2013; Ryu et al., 2014; Wu et al., 2014). In this study, we used the better performing curves at our NDVI values, among the fitting curves tested in other studies, and added the threshold method for better estimating the phenological phases during the growing season. However, modelling phenology from remote sensing is still a challenging task, particularly when applied on a wide geographical scale (Liu et al., 2016).

3.5.Conclusions

In this study, we correlated xylem phenology with the length of the growing season detected by remote sensing and demonstrated that most relationships between data obtained from the satellite sensor and direct observations of the wood formation phases were significant. Our results highlighted the potential to integrate remote sensing and xylem phenology and describe wood formation at spatial level, although the factors driving inter-annual variation still remain undetermined. Nevertheless, remote sensing has the advantage of offering a broad scale and spatially-explicit method for monitoring indicators of ecosystem processes, and when combined with detailed tree-based data (e.g., xylem phenology) may provide answers to ecological questions across a range of scales. Large-scale estimations of the timing and dynamics of xylem formation can represent a suitable tool for accurately estimating the temporal variations of carbon sequestration using the remote-sensing phenology at wide geographical scale.

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Conflict of interest

None declared

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Table 1

ID	Site	Latitude (°N)	Longitude (°W)	Altitude (m a.s.l.)
SIM	Simoncouche	48° 22’	71° 25’	338
BER	Bernatchez	48° 86’	70° 34’	611
MIS	Mistassibi	49° 73’	71° 94’	342
DAN	Camp Daniel	50° 69’	72° 18’	487
MIR	Mirage	53° 79’	72° 86’	384

Table 2

	Variance partition		
	Year (%)	Site (%)	Residual (%)
Beginning of enlargement	40.4	39.9	19.6
Beginning of wall thickening	55.3	37.0	7.7
First mature cell	56.3	35.7	8.0
Ending of cell enlargement	23.9	49.1	26.9
Ending of lignification	45.6	42.3	12.1
Duration of xylem production	11.9	64.2	23.9
Duration of wall thickening	17.2	69.7	13.1
Duration of xylogenesis	19.7	64.4	15.9
Beginning of the NDVI growing season	9.8	83.7	6.5
Ending of the NDVI growing season	7.6	69.9	22.5
Duration of the NDVI growing season	3.9	86.5	9.7

Table 3

	Model			Factors of variation Beginning of NDVI growing season		Year		Interaction	
	F-value	*p*	R^2	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Beginning of enlargement	6.05	<.0001	0.85	17.94	0.0002	0.56	0.8508	0.76	0.681
Beginning of lignification	13.70	<.0001	0.93	53.31	<.0001	0.78	0.6611	0.58	0.838
First mature cell	15.86	<.0001	0.94	70.50	<.0001	0.85	0.6001	1.08	0.415

Table 4

	Model			Factors of variation Ending of NDVI growing season		Year		Interaction	
	F-value	*p*	R^2	*F*-value	*p*	*F*-value	*p*	*F*-value	*P*
Ending of enlargement	1.72	0.0847	0.61	5.24	0.0301	0.80	0.6461	0.79	0.659
Ending of lignification	5.16	<.0001	0.83	14.61	0.0007	1.27	0.2932	1.06	0.430

Table 5

	Model			Factors of variation					
				NDVI growing season		Year		Interaction	
	F-value	*p*	R^2	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Duration of cell production	2.63	0.0079	0.71	14.92	0.0006	0.80	0.6518	0.84	0.615
Duration of cell wall thickening	4.86	<.0001	0.82	32.66	<.0001	1.41	0.2223	0.94	0.528
Duration of xylogenesis	4.40	0.0001	0.80	26.21	<.0001	1.23	0.3110	0.80	0.651

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Figure 1

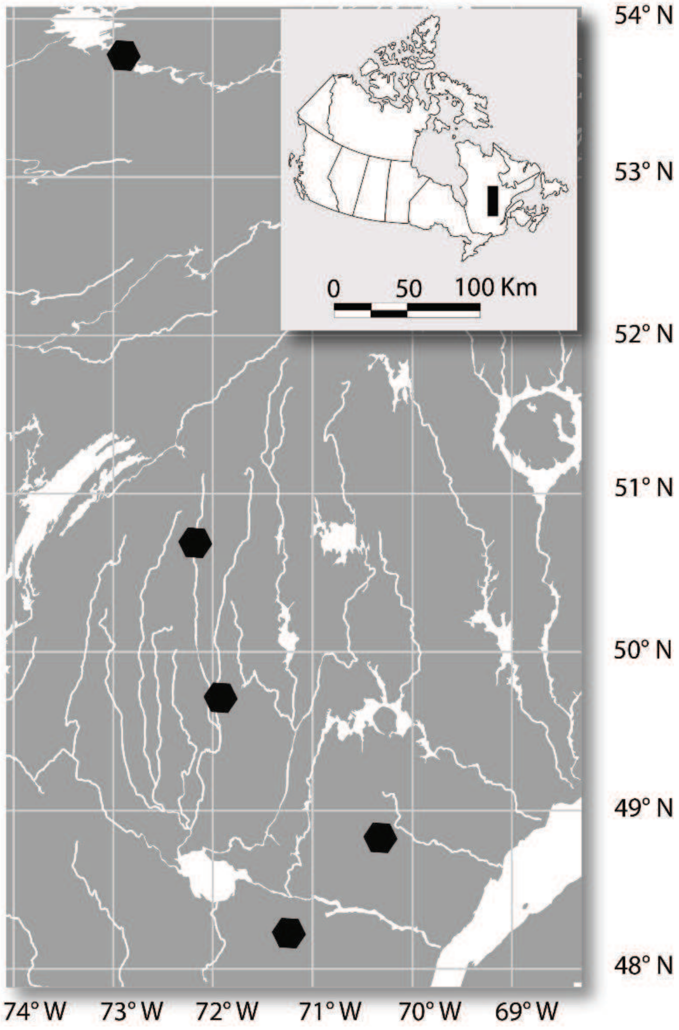


Figure 2

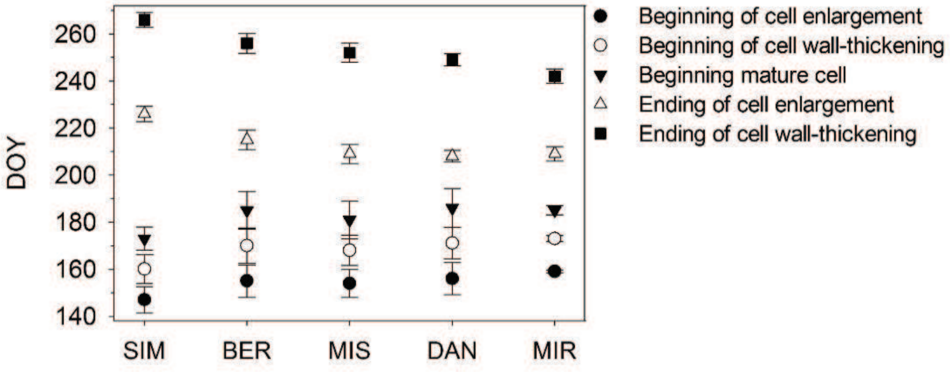

DOY
260
240
220
200
180
160
140
SIM
BER
MIS
DAN
MIR
● Beginning of cell enlargement
○ Beginning of cell wall-thickening
▼ Beginning mature cell
△ Ending of cell enlargement
■ Ending of cell wall-thickening

Figure 3

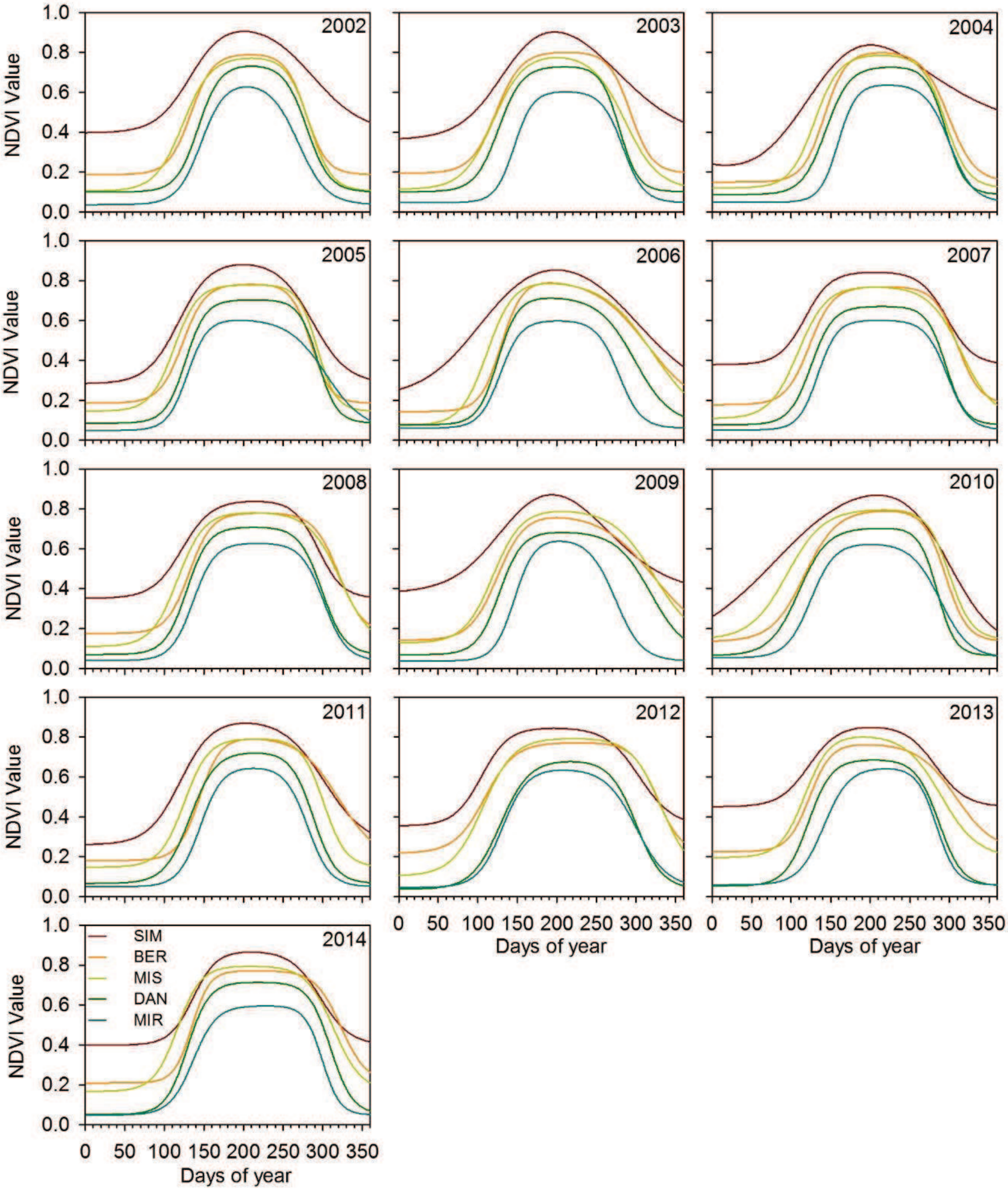

2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
NDVI Value
1.0
0.8
0.6
0.4
0.2
0.0
0 50 100 150 200 250 300 350
Days of year
SIM
BER
MIS
DAN
MIR

Figure 4

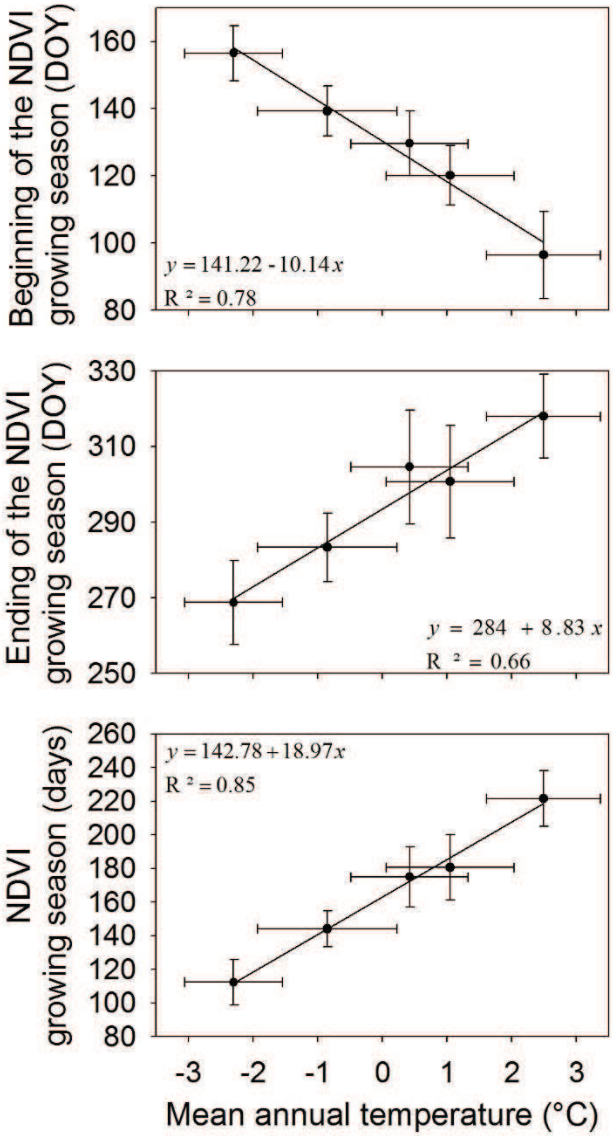
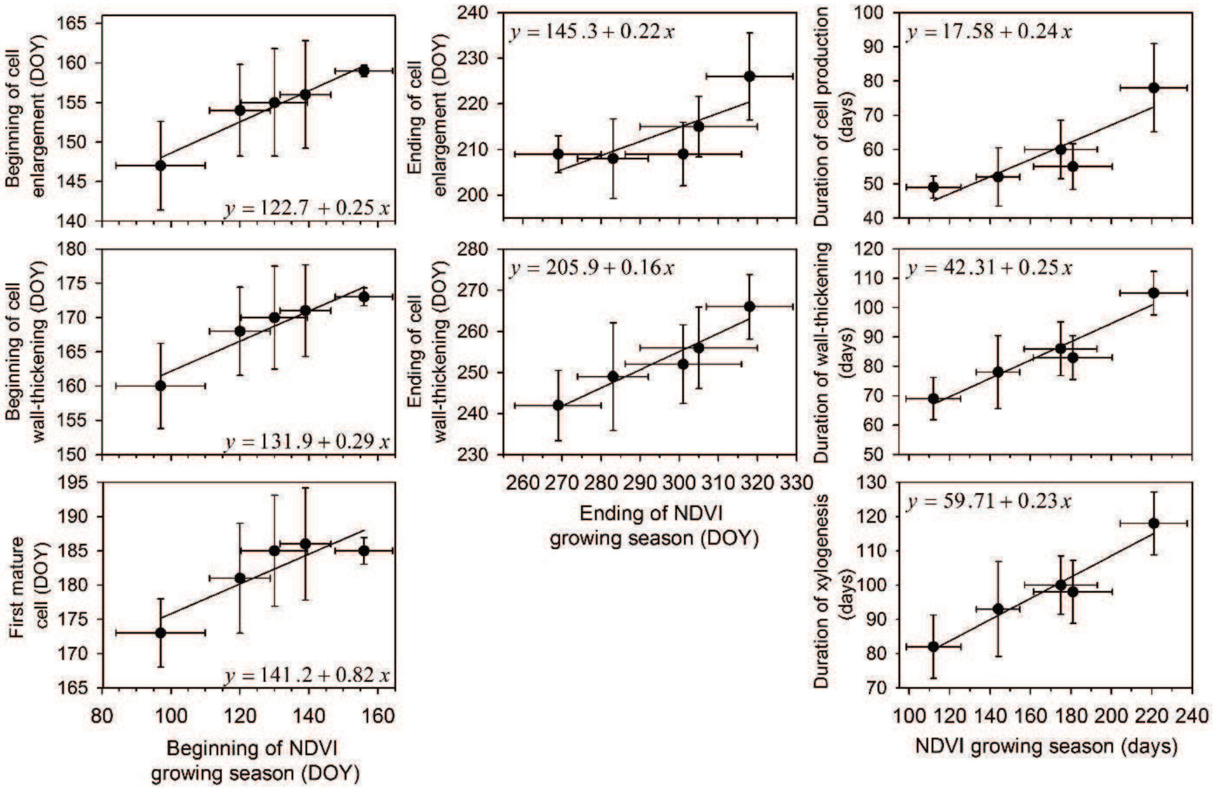

Beginning of the NDVI growing season (DOY)
160
140
120
100
80
y = 141.22 - 10.14x
R ² = 0.78
Ending of the NDVI growing season (DOY)
330
310
290
270
250
y = 284 + 8.83 x
R ² = 0.66
NDVI growing season (days)
260
240
220
200
180
160
140
120
100
80
y = 142.78 + 18.97x
R ² = 0.85
-3
-2
-1
0
1
2
3
Mean annual temperature (°C)

Figure 5


Beginning of cell enlargement (DOY)
165
160
155
150
145
140
y = 122.7 + 0.25 x
Beginning of cell wall-thickening (DOY)
180
175
170
165
160
155
150
y = 131.9 + 0.29 x
First mature cell (DOY)
195
190
185
180
175
170
165
y = 141.2 + 0.82 x
80
100
120
140
160
Beginning of NDVI growing season (DOY)
Ending of cell enlargement (DOY)
240
230
220
210
200
y = 145.3 + 0.22 x
Ending of cell wall-thickening (DOY)
280
270
260
250
240
230
y = 205.9 + 0.16 x
260
270
280
290
300
310
320
330
Ending of NDVI growing season (DOY)
Duration of cell production (days)
100
90
80
70
60
50
40
y = 17.58 + 0.24 x
Duration of wall-thickening (days)
120
110
100
90
80
70
60
50
y = 42.31 + 0.25 x
Duration of xylogenesis (days)
130
120
110
100
90
80
70
y = 59.71 + 0.23 x
100
120
140
160
180
200
220
240
NDVI growing season (days)

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CHAPTER IV

TIMINGS OF XYLEM GROWTH IN SILVER FIR AND THEIR CHANGES UNDER FUTURE CLIMATIC SCENARIOS

RESEARCH PAPER

Title: Timings of xylem growth in silver fir and their changes under future climatic scenarios

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Abstract

One of the effects of climate change is a shift in plant phenology, which could potentially influence forest growth and productivity. This study investigated xylem phenology of silver fir and the relationships with temperature and precipitation. Xylogenesis was studied weekly from March to November 2015 on microcores collected at three different sites along a latitudinal gradient between the 38th and 46th parallels in Italy. A longer growing season was detected in the southern site, which was related to the delayed completion of xylem differentiation in autumn rather than an earlier onset of cambium reactivation in spring. The duration of phenological phases was influenced by both precipitation and minimum temperature during the growing season, but the former had greater importance than the latter. Regression models performed with different climatic scenarios estimated an increase in the duration of xylem under warmer conditions. However, the expected reduction in precipitation could have a negative influence by shortening the growing season. Our study suggested the importance of considering both temperature and precipitation in order to address issues on the growth dynamics of silver fir stands under global change scenarios.

Keywords: *Abies alba*; xylogenesis; Mediterranean area; temperature; precipitation.

4.1.Introduction

Climate change involves variations in temperature, precipitation and the frequency of extreme climatic events (Meehl and Tebaldi 2004). These variations increase the likelihood of severe, pervasive and irreversible impacts on ecosystems that may implicate changes in distribution of plants, and shift in their phenological events. Climate change affects a wide range of ecosystems with different latitudinal variations (IPCC 2014) and studies are recommended at regional level (Giannakopoulos et al. 2009), because not all areas are impacted uniformly. The trajectories of forest tree species distributions under climate change scenarios still remain uncertain (Cheaib et al., 2012; Ruosch et al., 2016). Monitoring vegetation dynamics therefore constitutes a crucial effort for forest management and adaptation planning (Zhou et al., 2001; Garonna et al., 2014).

Guiot and Cramer (2016) observed that temperatures in the Mediterranean basin are actually 1.3 °C warmer than during 1880÷1920. Compared with the worldwide increase of 0.85 °C, only a warming scenario of 1.5 °C would permit the Mediterranean climate to remain within the variability of the past 10,000 years (Guiot and Cramer, 2016). Mediterranean regions are also sensitive to changes in water availability. For most scenarios, the changes in both precipitation and temperature will combine to reduce water availability and trigger losses of Mediterranean ecosystems in the decades to come (Keenan et al., 2011).

It is important to understand the capacity of forests in the Mediterranean basin to withstand, adapt or recover from a context of increasing temperatures and decreasing precipitation (Hoerling et al., 2012). One major concern is if and how plant growth changes over time and how it will be affected by climate change and modulated by site-specific conditions (Schelhaas et al., 2003; Seidl et al., 2011). Plants are finely synchronized to the seasonality of their environment and one of the effects of climate change on forests is a potential shift in the timing of plant phenology (Richardson et al., 2013). Shifting in plant phenology has received significant attention in the last decades because the sensitivity of plants to weather can be a proxy for the long-term biological impact of climate change on terrestrial ecosystems (Menzel and Fabian, 1999; Cleland et al., 2007).

In Europe, phenological responses to climate change have demonstrated the occurrence of earlier onsets of spring events, especially at mid-latitudes (Sparks and Menzel, 2002; Menzel et al., 2006), as well as delayed leaf senescence (Vitasse et al., 2011) and a lengthening of the growing season. These results mostly concern measurements on budding, leafing and flowering and represent the activity of the vegetative and reproductive meristems.

In extra-tropical ecosystems, cambium activity and wood production are usually seasonal, according to the cyclic variation in environmental factors (Evert, 2006; Begum et al., 2013). Most data on the relationships between radial growth and climate are obtained from dendrochronological studies. Tree-ring chronologies provide information on inter-annual to multi-centennial time scales (Esper et al., 2002), although when ring formation takes place and how specific environmental factors influence the cambial activity remain matters for discussion. A thorough understanding of the patterns and processes of xylem formation is thus necessary (Vaganov et al., 2006). The phenology and intra-annual dynamics of xylem production and maturation has already been accurately quantified, especially at the higher latitudes (Cuny et al., 2014; Rossi et al., 2013). However, trees at the lower latitudinal limits of the species distributions deserve similar attention, because of their particular sensitivity to climatic oscillations (Hampe and Petit, 2005). The phenology of a single species as silver fir (*Abies alba* Mill.) has been investigated in central Europe (Rathgeber et al. 2011; Gričar et al., 2009), but few studies have been conducted in the southern regions of Europe, at the core of the Mediterranean basin, where silver fir reaches the limit of its southern distribution (Marcias et al., 2006; Mauri et al., 2016). Ruosch et al. (2016) suggested that silver fir would continue to provide important ecosystem services under warmer conditions in central Europe and in the mountain areas of southern Europe. However, evidence of the beneficial effect of climate warming on the radial growth of silver fir, observed in central Europe during the past decades (Büntgen et al., 2014; Gazol et al., 2015), need to be confirmed in southern Europe.

Silver fir is considered an important ecological and functional balancer of European forests, a keystone species for maintaining high biodiversity in forest ecosystems (Tinner et al., 2013). It is the most sensitive species to frost and drought among the European conifers (Aussenac, 2002). Thus, silver fir stands often occur in fresh and humid environments, and avoid cold sites at the higher altitudes and latitudes. Because of its low tolerance to extreme events, this species shows a long history of decline in the southern part of its distribution (Oliva and Colinas, 2007; Potocic et al., 2005).

In Italy, silver fir is widespread across the Alps, and more scattered along the Apennines, where it forms mixed stands between 400 and 1800 m a.s.l., reaching the southernmost limit in Calabria (Aussenac, 2002; Mauri et al., 2016). This latitudinal gradient along the Italian peninsula creates the ideal conditions for investigating xylogenesis at wide geographical scale in a climatically complex area like the Mediterranean basin. In this work, cambial phenology was used as a marker of short-term adaptation to changing environment (Steppe et al., 2015).

We investigated the cambium phenology and how temperature and precipitation affected the period of xylem production in silver fir along a latitudinal gradient. We built and applied a model for predicting changes in xylem growth under different scenarios of temperature and precipitation. We hypothesized that: (1) a decrease in precipitation would reduce the growing season and, consequently, xylem growth in silver fir, and (2) the duration of xylem phenology would differ along the Italian Peninsula.

4.2.Materials and methods

4.2.1. Study area

The study was conducted in three sites along a thermal gradient represented by the latitudinal range covering the distribution of silver fir (*Abies alba* Mill.) in Italy. The three sites, located between the 38th and 46th parallels (Figure 1), were selected in Borca di Cadore (abbreviated as BOR; 46°44’N, 12°19’E, 1150 m a.s.l.), Pescopennataro (PES; 41°86’N, 14°30’E, 1380 m a.s.l.), and Serra San Bruno (SER; 38°46’N, 16°31’E, 1008 m a.s.l.). Northern and central sites are included in the temperate oceanic bioclimate, while the southern site is classified as temperate oceanic sub-Mediterranean (Rivas-Martìnez, 2004). Average annual temperature of the sites ranged between 7.3 and 11.5 °C, with total precipitation varying between 1124 and 1788 mm.

4.2.2. Xylem sampling and sample preparation

Five healthy, dominant or co-dominant silver fir trees with straight stems and no damages were chosen in each site. The height of the sampled trees ranged between 23.1 and 30.6 m, with an average diameter at breast height of 47.7 cm.

Cambial activity and tree-ring formation was studied from March to November 2015. Wood microcores of 2 mm in diameter were collected weekly following a spiral trajectory on the stem, from 30 cm below to 30 cm above breast height (1.3 m) using the Trephor instrument (Rossi et al., 2006*a*). Trephor is a chisel-shaped tool for the fast recovery of microcores. Its cutting tube is hammered into the wood, and the sample is separated from the xylem by rotating and extracting the tool like a corkscrew. The very small wounds inflicted by the thin piercing tubes of the tool and the consequently narrow areas of traumatized tissues around the sampling points allow repeated samplings on the same trees.

The samples contained the preceding four to five tree rings and the developing annual layer with the cambial zone and adjacent phloematic tissue. The microcores were placed in Eppendorf microtubes with an ethanol solution (50% water) and stored at 5 °C to avoid tissue deterioration. Microcores were dehydrated by successive immersions in ethanol and d-limonene and then embedded in paraffin according to Rossi et al. (2006a). In order to obtain thin sections, the samples were cut at 7 μm in thickness with a rotary microtome. The sections were stained with cresyl violet acetate (0.16% in water) and examined within 10–15 min under visible and polarized light at 400–500× magnification to distinguish the developing and mature xylem cells.

4.2.3. Microscope observations

The number of xylem cells was counted along three radial rows, according to the criteria described in Rossi et al. (2006b). Observations under polarized light discriminated the zones of cell enlarging and wall thickening. The progress of cell wall lignification was detected with cresyl violet acetate that reacts with lignin (Antonova and Shebeko, 1981). Lignification was shown by a colour change from violet to blue. The colour change over the whole cell wall revealed the end of lignification and the tracheid reaching maturity. The dormant cambium consisted of four to five cells. In spring, the onset of cell division was considered to occur when the diameter of the new xylem cells was at least twice that of a cambial cell. Xylogenesis was considered to have started when at least one radial file of enlarging cells was observed (Rossi et al., 2008). In late summer, when no further cell was observed in wall thickening and lignification, xylem formation was considered complete. Xylem phenology was computed in days of the year (DOY) corresponding to the dates of (i) onset of cell enlargement, (ii) onset of cell wall thickening, (iii) first mature cell, (iv) ending of cell enlargement, and (v) ending of cell wall thickening. The duration of xylem phenology was assessed as the number of days between the onset of cell enlargement and ending of cell wall thickening. The duration of cell production was assessed according to the phase of cell enlargement rather than of cell division in the cambial zone, because cambium can be active without necessarily increasing its cell numbers (Rathgeber et al., 2011). Cambium produces indistinct xylem and phloem cells, which are identical before undergoing differentiation; onset and ending of cell division and enlargement occur at approximately the same time, generally within one week (Gričar et al., 2009; Rathgeber et al., 2011). Rate of cell production was calculated as the ratio between number of cells and the duration of xylem production expressed in days.

4.2.4. Meteorological data

Temperature and precipitation data were collected from the weather stations nearest to the study sites belonging to the "Agenzia Regionale per la Protezione Ambientale" (ARPA) and the "Università degli Studi di Padova". Temperature and precipitation were recorded hourly and stored in data loggers. The daily average, minimum and maximum temperature and total precipitation were then calculated. Temperature and precipitation of the growing season were also calculated based on April-October data.

4.2.5. Model development and application

The Wilcoxon test was applied for comparing the phases of xylem phenology, durations of cell differentiation and total cell production between sites. Minimum and maximum temperature and total precipitation of the growing season were tested sequentially by stepwise regressions based a forward selection process of duration of cell production, cell wall thickening and complete xylogenesis as dependent variables. This method selects the best predictors explaining the dependent variable of the model. The specific contribution of the variables selected by the model was estimated by sensitivity analysis. This analysis consisted of computing the impact of the relative variation of each variable on the results of the model (Cariboni et al., 2007). The models were used to estimate the duration of each silver fir phenological phase under different temperature and precipitation conditions. Two scenarios with increasing temperature and decreasing precipitation were simulated: Scenario 1 simulated a warming of 1.5 °C associated with a 10% decrease in precipitation, and Scenario 2 a warming of 2 °C associated with a 20% decrease in precipitation.

All statistical analyses were conducted using JMP (Version 11, SAS Institute Inc., Cary, NC).

4.3.Results

4.3.1. Weather conditions

In 2015, mean annual temperature at the sites varied between 8.1 and 11.4 °C (Table 1). Precipitation occurred during the whole year, but mostly in spring and autumn in the central and southern sites. In BOR, precipitation was also frequent and abundant during the summer. The highest values of both temperature and precipitation were recorded in SER. All sites had a winter minimum temperatures close to or lower than 0 °C. The highest temperatures, ranging between 21 and 28 °C, were reached in July and August (Figure 2). During the growing season (April-October), average temperature varied between 12.6 and 15.4 °C, and precipitation varied between 601.0 and 842.8 mm (Table 1).

4.3.2. Xylem phenology

The first enlarging cell appeared in the same week in SER and BOR, on DOY 118 and 121, respectively (Figure 3). No significant differences were observed between northern and southern sites. Xylem growth resumed significantly later in PES than in the other two sites, on average on DOY 132. The onset of cell wall thickening occurred between mid-May (SER) and beginning of June (PES), with significant differences between sites. The first mature cells were observed in SER on DOY 139, significantly earlier (26 days) than in the other sites. Instead, no significant differences occurred between BOR and PES. Both the ending of cell enlargement and cell-wall thickening followed a latitudinal gradient, with significant differences among the three sites. The first site to complete growth at the end of August was BOR (Figure 3).

Duration of cell production was similar between BOR (117 days) and PES (119 days), while it was significantly longer in SER (147 days). The duration of the xylogenesis process lengthened southwards, from 120 days in BOR to 163 days in SER.

Cell production ranged between 50 and 53 days, with minor differences among sites (Figure 3).

4.3.3. Relationship between climate and xylogenesis

Stepwise regressions indicated minimum temperature and precipitation of the growing season as predictive variables, while maximum temperature was not included in the models (Table 2). Step 1 of all models included precipitation as variable. For the duration of cell production, an R^2 of 0.86 was estimated by step 1, which increased to 0.90 when minimum temperature was added to the model by step 2. Similarly, the model for cell wall thickening showed an initial R^2 of 0.70 that increased to 0.87 with minimum temperature. The R^2 of duration of xylogenesis increased from 0.62 (only precipitation) to 0.82 (precipitation and minimum temperature). Sensitivity analysis quantified the impact of precipitation and minimum temperature on the model results (Fig. 4). Precipitation explained the largest proportion of variance, while minimum temperature had a minor role, ranging between 24 and 33%. Among the three analysed variables, precipitation showed the highest proportion of variance for the duration of cell production.

4.3.4. Model application

Results of the model application revealed how the phenological phases increased in duration when both temperature and precipitation were higher. A different effect was observed under increasing temperature and decreasing precipitation (Figure 5): in this case, the duration of cell production was shorter than that observed at least during 2015. In addition, the duration of cell wall thickening and complete xylogenesis resulted as longer than that of cell production.

Phenological phases were longer when a small variation of temperature and precipitation occurred, as also highlighted in the scenarios detailed in Table 3. As confirmation, the duration of phenological phases was longer in Scenario 1 than in Scenario 2.

Moreover, considering the duration of cell production, Scenario 1 showed different effects among the three study sites, with PES reporting the highest variation (+ 3 days) when compared to the year 2015. The variation was slightly shorter in BOR and SER (+1 and -1 day, respectively). On the contrary, Scenario 2 highlighted a large variation in SER (-6 days), while this phenological phase resulted as slightly different in PES and BOR (+1 and -1 day, respectively).

As regards cell wall thickening, the results revealed a longer time lag for all study sites. Both prospected scenarios resulted in a lengthening of the duration, even though no differences between Scenario 1 and Scenario 2 were observed in PES.

Similar results were obtained for the duration of xylogenesis, where the lengthening appeared longer in Scenario 1 than in Scenario 2. In this case, PES also showed a similar response in both hypothesized scenarios.

Lastly, the duration of wood formation increased throughout the latitudinal distribution in Italy, revealing significant differences from north to south, with time lags between 133 and 169 days, respectively (Table 3).

4.4. Discussion

Cambium phenology and xylem cell production were investigated in silver fir during the 2015 growing season along its latitudinal distribution in Italy, with the aim of assessing how temperature and precipitation affect the timings of xylem phenology. We defined and applied a model to predict potential changes in the growing season under future climatic scenarios. We found a longer growing season in the southern site, which was related to the delayed completion of xylem differentiation in autumn rather than to the earlier onset of cambium reactivation in spring. Both precipitation and minimum temperature of the growing season influenced growth, but contributed differently to the duration of xylem differentiation, the former having greater importance than the latter.

4.4.1. Timing of xylogenesis

Wood formation lasted 4-5 months, revealing higher time-values in the southern site. As in this study, Cuny et al. (2012) showed that xylogenesis in silver fir started between mid-April and mid-May. However, we found that the end of xylogenesis occurred later in the southern site than in the other two. Similar results were obtained in other studies that analysed the effect of alti-latitudinal gradients. Ziaco and Biondi (2016) found significant differences in the end of xylogenesis in limber pines growing at different altitudes. Moser et al. (2010) showed delayed endings of stem growth from 1600 to 2150 m a.s.l.. Moreover, the duration of the xylem formation shortens at increasing latitudes in several species (Del Castillo et al., 2016; Jyske et al., 2014; Prislan et al., 2013).

In this study, both growth rate and cell production showed high variability. The southern site had a longer duration of phenological phases, but no difference occurred in cell production among the three sites. Cuny et al. (2012) indicated that silver fir growth is characterized by high rates of cell production. Rossi et al. (2012) found that longer periods of cell differentiation in black spruce are the result of a longer duration of cell division in cambium, which produces a larger number of xylem cells along the tree ring. However, the relationship between growth, duration and growth rate was not confirmed among ecotypes (Perrin et al., in press). Moreover, the number of cambial cells is known to depend on tree species (Larson, 1994) and vigour (Gričar et al., 2009), and differences between genotypes can not be ruled out. It is consequently difficult to separate the effect of timing from rate of cell differentiation on wood production, and the relationships between cambial phenology and cell production remain unclear.

4.4.2. Climatic drivers of xylem growth

Spring temperature is recognized as one of the dominant environmental signals triggering tree phenology, especially in cold climates (Körner, 2006; Rossi et al., 2008; Hanninen and Tanino, 2011). Gričar et al. (2014) found that mean temperature in April was strongly correlated with the onset of cell production in Norway spruce in central Europe. According to other studies (Deslauriers and Morin, 2005; Rossi et al., 2008; Li et al., 2012), the minimum, rather than maximum temperature, positively influenced wood formation and the duration of cambial cell production, as in silver fir of the present study. Again, an increase in temperature in autumn led to a delayed cessation of xylogenesis in black spruce and European larch (Girard et al., 2015; Moser et al., 2010).

Precipitation during the period of cell production proved to be the main factor that positively influenced the duration of cell production in Norway spruce in the Czech Republic (Giagli et al., 2016). Ren et al. (2015) found that precipitation plays a key role in the onset of xylogenesis and xylem cell production in Qilian junipers. Furthermore, the processes of cell division and expansion are sensitive to water potential (Abe et al., 2003; Fonti et al., 2010), and water deficit can represent a constraint during xylem formation (Balducci et al., 2013; Vieira et al., 2014). Water availability, especially in temperate environments, is important for cambial resumption and xylem differentiation (Camarero et al., 2010; Vieira et al., 2014). Soil water availability during the growing season, which depends on precipitation, is a major driver of wood formation and radial growth in inner areas at Mediterranean latitudes (Martin-Benito et al., 2013). Declines in water availability during growing season could affect the growth in terms of quantity (xylem amount), consequently influencing wood production (Balducci et al., 2013, 2014). Our results demonstrated the importance of precipitation in affecting the duration of wood formation in silver fir. Tinner et al. (2013) found that silver fir requires relatively high moisture conditions throughout the year, with annual precipitation between 700 and 1800 mm, and summer temperatures ranging from 14 to 19 °C. These values were confirmed in our sites.

4.4.3. Model application

The applied model showed that the duration of phenological phases in silver fir in its southern distribution is influenced primarily by precipitation and minimum temperature, but not by maximum temperature that occurs during the growing season. Rossi et al. (2011) also used minimum temperature for establishing a first spatial pattern of onset, cessation and duration of xylem growth in black spruce, in the boreal forests of Quebec (Canada). These authors showed that mild conditions close to lakes or the harsh climate at high altitudes affected xylem phenology irrespective of the latitude of the sites. In the case of the Italian Peninsula, it is possible that the orography and relative mesoclimatic conditions could modify the predictable relationships between geographical coordinates and timing of growth. This provides further evidence of the high plasticity of conifers in adapting their growth processes to local environmental conditions.

Model results highlighted an increase in the time for completion of xylogenesis according to future warmer scenarios. Different patterns emerged between northern and southern sites. Considering the effect of different climatic scenarios, a smaller increase in the length of growing season was observed for the southern site. The projected rise in temperature associated with the reduction in precipitation might have a negative impact on the timing of radial growth for adult silver fir in Mediterranean regions (Aussenac, 2002; Camarero et al., 2011; Linares and Camarero, 2012*a*, 2012*b*), as also observed in this study.

The effects of climate warming on species performance may vary across their distribution range (Vila et al., 2008; Carrer et al., 2010). Büntgen et al. (2014) showed that radial growth of silver fir increased considerably during the last 20 years across central Europe, whereas there was a noticeable growth decline in drought-prone Mediterranean sites. Moreover, different patterns of tree growth have been reported between northern and southern populations of silver fir in Italy. These findings suggest that silver fir does not provide a monotonic response at different sites to the many tree-ring growth sensitive environmental factors (Carrer et al., 2010). The growth decline is much more apparent in many Iberian sites than in the populations of southern Italy, despite the similar latitudes. These differences may be caused by the more mesic conditions of mountain areas in Italy than Spain (Linares, 2011), probably determined by the lower continentality of the Italian mountains.

The future responses of silver fir forests to climate warming are currently under debate (Tinner et al., 2013; Ruosch et al., 2016). Recently, several studies have hypothesized a growth reduction in response to the expected warming, while others have indicated stable conditions or expansions (Maiorano et al., 2013; Cailleret et al., 2014; Ruosch et al., 2016). There are several discrepancies on whether the recent rise in temperature alone or in combination with a reduction in the amount of precipitation will lead to a contraction of the silver fir distribution range (Alba-Sanchez et al., 2010;

Maiorano et al., 2013). These divergences arise between the projections of different models: whereas Maiorano et al. (2013) predicted a reduction in the species' distribution range due to a moderate warming, others suggested that this reduction will only occur if accompanied by a substantial decrease in precipitation (e.g., Di Pasquale et al., 2014). The millennia of human impacts have greatly diminished the geographical distribution of silver fir (Tinner et al., 2013; Pignatti, 1997). The human pressure markedly influenced the dimensions, structure, and species composition of European forests (Pyne 1997). Furthermore, time-series analysis suggest that increased fire activity caused a strong decline in silver fir, that it is also a highly fire-sensitive species (Colombaroli et al., 2007). Recent correlative bioclimatic and dynamic vegetation modelling studies support the hypothesis of a wider potential range of silver fir (Keller et al. 2002, Svenning and Skov 2004, Henne et al. 2013).

Our study highlights that silver fir, still with difference between north-central sites and the southern site, will be able to grow under warming climate in the mountain areas of southern Europe. However, a smaller increase of the growing season is expected under more negative climatic effects due to a combination of both increased temperature and decreased precipitation.

4.5.Conclusions

This study quantified the timings of xylem phenology in silver fir at the southern limit of its distribution. The precipitation amount and distribution during the growing season were the most important variables that influenced, positively, wood formation and duration of the phenological phases. Minimum temperatures concurred with precipitation in determining these xylem phenological traits. Our study suggested the importance of considering both temperature and precipitation in order to address issues on the growth dynamics of silver fir stand under global change scenarios.

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Conflict of interest

None declared

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Table 1

	Annual		Growing season			
Sites	Mean Temperature (°C)	Precipitation (mm)	Mean Maximum Temperature (°C)	Mean Temperature (°C)	Mean Minimum Temperature (°C)	Precipitation (mm)
BOR	8.1	753.4	18.9	12.6	7.8	601.0
PES	9.3	1057.6	17.7	13.5	9.3	521.8
SER	11.4	2037.6	22.1	15.4	8.5	842.8

Table 2

	Estimated coefficients			Model	
	Intercept	Precipitation	Minimum Temperature	R^2	p
Duration of cell production	-0.25	0.10**	6.95**	0.90	<.0001
Duration of cell wall thickening	-143.48**	0.18***	17.45***	0.87	<.0001
Duration of xylogenesis	-73.95	0.13***	14.77**	0.82	<.0001

One, two and three asterisks correspond to a $p<0.05$, 0.01, and 0.001, respectively

Table 3

		Temperature °C	Precipitation %	BOR	PES	SER
Duration of cell production	Actual	–	–	117	119	146
	Scenario 1	+1.5	-10	118	122	145
	Scenario 2	+2.0	-20	116	120	140
Duration of cell wall thickening	Actual	–	–	98	110	152
	Scenario 1	+1.5	-10	116	129	168
	Scenario 2	+2.0	-20	114	129	161
Duration of xylogenesis	Actual	–	–	120	132	163
	Scenario 1	+1.5	-10	134	147	172
	Scenario 2	+2.0	-20	133	147	169

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Figure 1

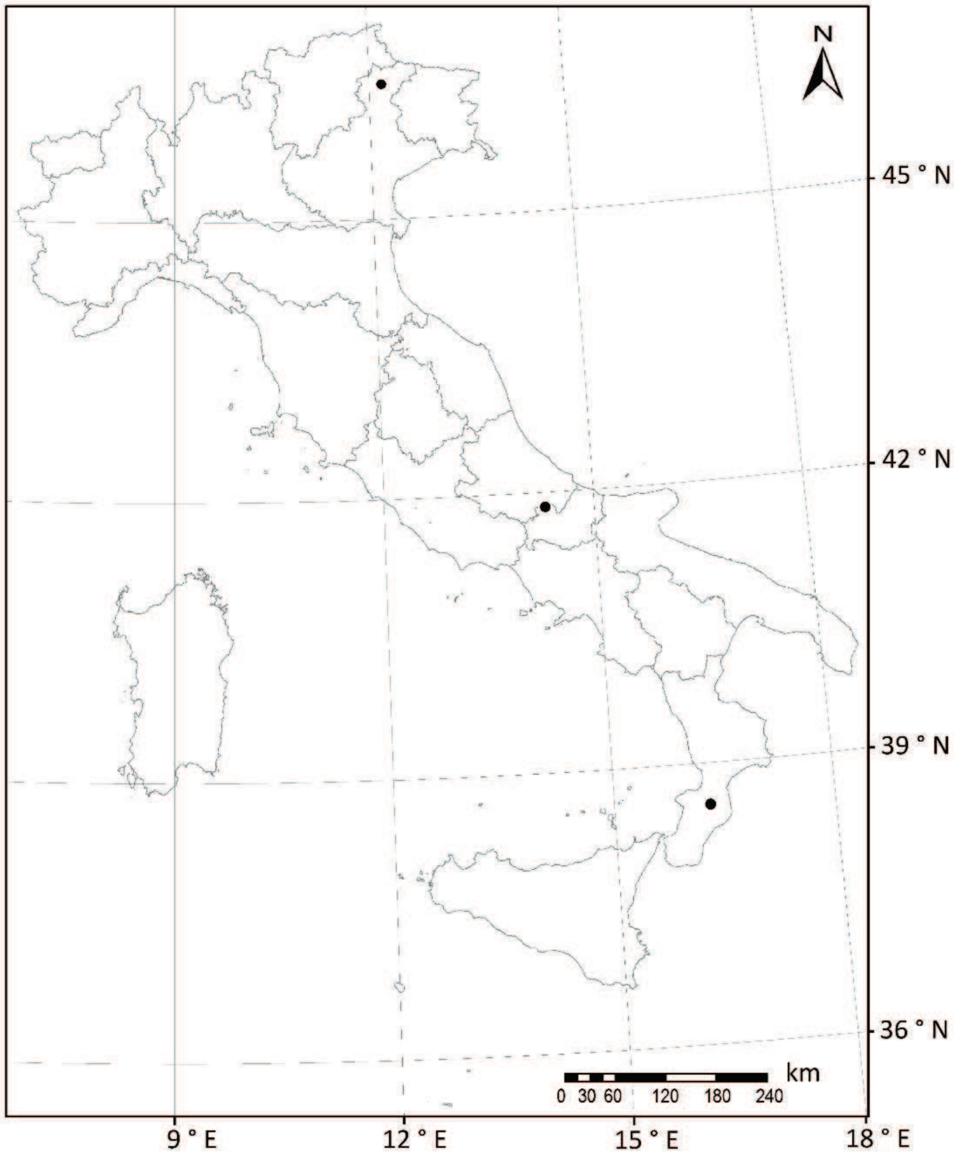


Figure 2

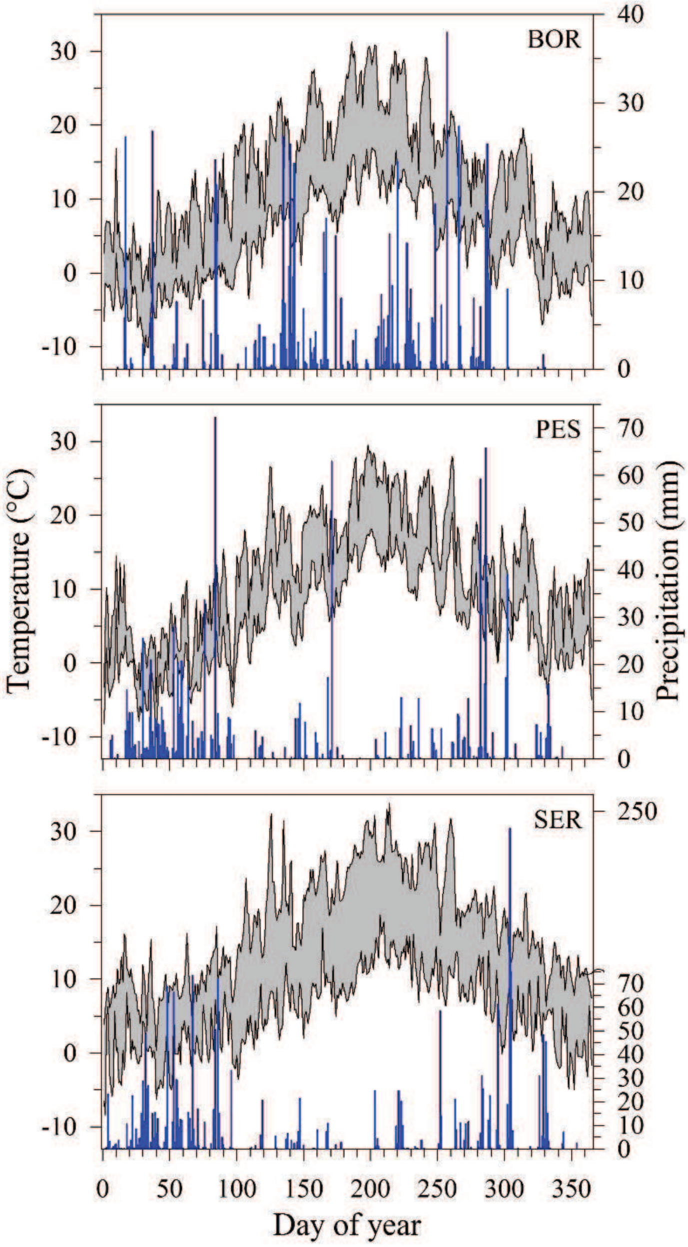

BOR
PES
SER
Temperature (°C)
Precipitation (mm)
Day of year
30
20
10
0
-10
40
30
20
10
0
70
60
50
40
30
20
10
0
250
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350

Figure 3

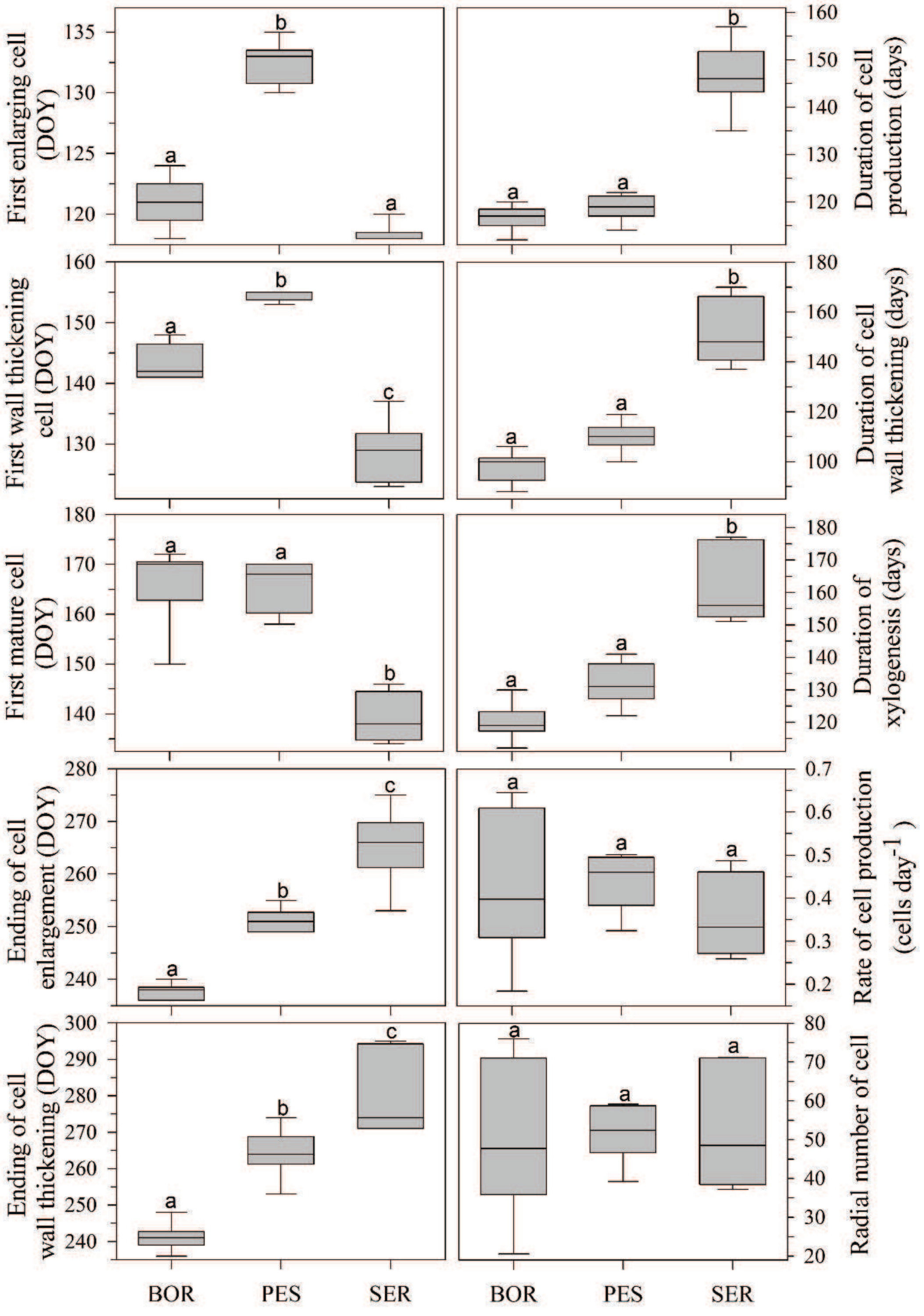

First enlarging cell (DOY)
135
130
125
120
a
b
a
Duration of cell production (days)
160
150
140
130
120
a
a
b
First wall thickening cell (DOY)
160
150
140
130
a
b
c
Duration of cell wall thickening (days)
180
160
140
120
100
a
a
b
First mature cell (DOY)
180
170
160
150
140
a
a
b
Duration of xylogenesis (days)
180
170
160
150
140
130
120
a
a
b
Ending of cell enlargement (DOY)
280
270
260
250
240
a
b
c
Rate of cell production (cells day^{-1})
0.7
0.6
0.5
0.4
0.3
0.2
a
a
a
Ending of cell wall thickening (DOY)
300
290
280
270
260
250
240
a
b
c
Radial number of cell
80
70
60
50
40
30
20
a
a
a
BOR
PES
SER
BOR
PES
SER

Figure 4

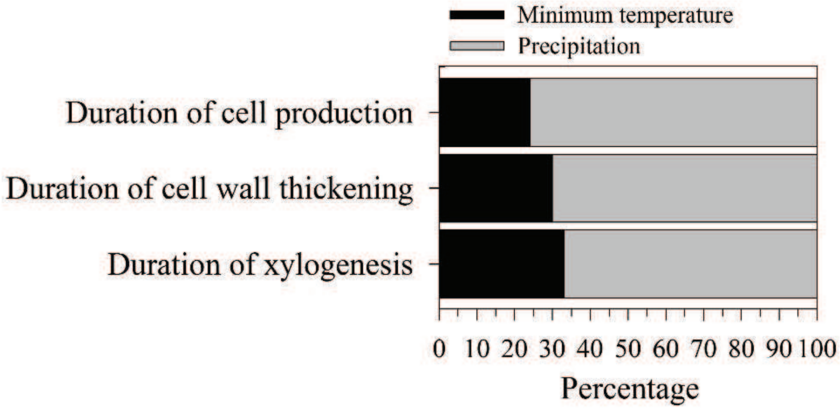
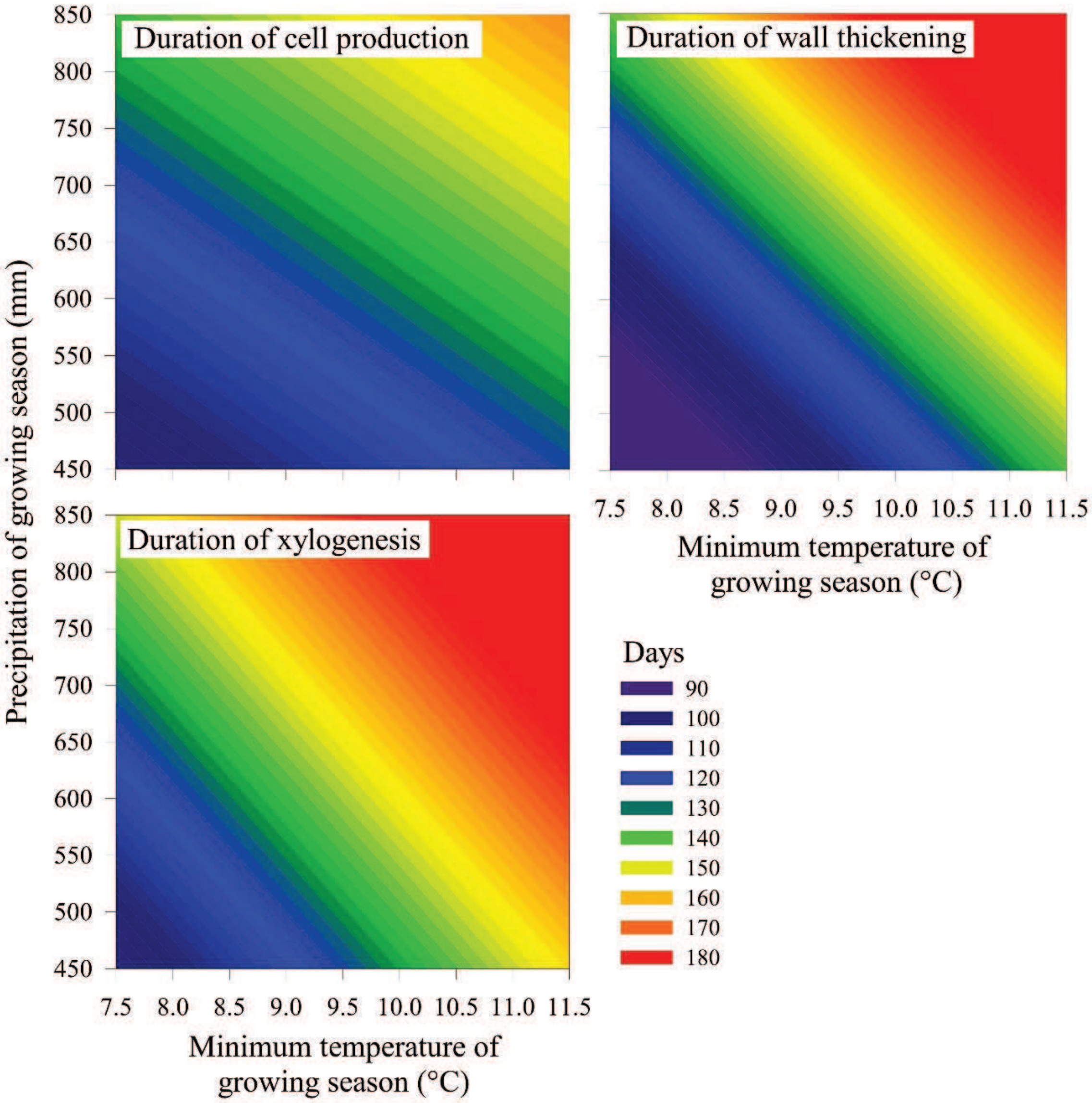

Minimum temperature
Precipitation
Duration of cell production
Duration of cell wall thickening
Duration of xylogenesis
0
10
20
30
40
50
60
70
80
90
100
Percentage

Figure 5


Duration of cell production
Duration of wall thickening
Duration of xylogenesis
Precipitation of growing season (mm)
850
800
750
700
650
600
550
500
450
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5
Minimum temperature of growing season (°C)
Days
90
100
110
120
130
140
150
160
170
180

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CHAPTER V

GENERAL CONCLUSION

5.1. Final remarks

The objective of this doctorate was to explore the phenology of meristems in conifers, along latitudinal and altitudinal gradients and how they respond to climatic drivers. The objective was attained through a multidisciplinary approach with different study scale of detail.

The first study provides new knowledge on the dynamics of spring phenology and novel information on the synchronisms between two meristems in black spruce and balsam fir. The study demonstrates the importance of precisely defining the phases of bud development in order to correctly analyse the relationships with xylem phenology. Furthermore, the study confirmed that temperature trigger the phenology in boreal environment, but degree-day sum was more appropriate in assessing the date of bud growth resumption, while thermal thresholds were more suitable for cambium phenology. The most relevant result of this study, the synchronism and correlation between the two meristems, may be used to explain the seasonal variability of boreal forest productivity, at wide geographical scales, through satellite observation.

Indeed, in the second study, the xylem phenology was correlated with the foliar phenology detected by remote sensed data. The result demonstrated that the relationships between data obtained from the satellite sensor and direct observations of the wood formation phases were significant, in most cases, as well as those between temperature and the phenological chronologies detected by NDVI. Moreover, the length of NDVI growing season and the duration of xylogenesis showed a close correlation. This study demonstrated that the timing of xylem formation could be suitably estimated at wide geographical scale using remote sensed data, although the inter-annual phenological variability remained unexplained. The study established a link between xylem phenology, NDVI data and air temperature, providing a comprehensive and spatial-explicit view of boreal forest productivity. The results here obtained highlighted that remote sensed data can be particularly useful where systematic observations or recurrent samplings are precluded or logistically unachievable.

Furthermore, in the third study, the timing of xylem phenology was quantified in silver fir at the southern limit of its distribution. Along the Italian Peninsula, the southern site showed a longer growing season than northern and central sites, which was related to the delayed completion of xylem differentiation in autumn rather than to the earlier onset of cambium reactivation in spring. Differently to the climatic drivers observed in the boreal forest, in these Mediterranean mountains, the precipitation during the growing season was the most important variable that affected the duration of phenological phases, together with minimum temperature. Moreover, the application of the model highlighted an increase of the timing required for the completion of xylogenesis according to the future warmer scenarios. A smaller increase of the growing season is expected under more negative climatic effects, due to a combination of both increased temperature and decreased precipitation.

Studies at regional level allowed us to highlight the different effects of environmental factors on meristems: the main drivers were the temperature in the boreal forest, and precipitation and temperature in the Mediterranean mountain forests.

Climate change and the response of species to future environmental conditions should be carefully studied locally, because this interaction can be used for planning adaptation management strategies. Direct observations and modelled trends, but also their associated uncertainties, will help the scientific community to develop suitable forest management options and a more sustainable forestry in a long term perspective.

5.2. Opportunities for further research

Research continuously improves and produces models that conjugate observation at ground level with proximal sensing (phenocam), but also with data derived from satellite observation. The results here obtained can give a precious support to better understand the mechanisms of development of bud and xylem in prominent areas of climate change, under actual and future climatic conditions. This thesis deepened some aspects of the impact of climate change on tree growth, in order to interpret the mechanisms and factors that affect the xylogenesis, and the links with wood production. Indeed, these aspects could play a role in understanding the dynamics of carbon storage in wood tissue, with possible impacts on wood quality. Certainly, the timing and dynamics of xylem formation estimated at large-scale can represent a suitable tool for accurately assessing the temporal variations of carbon sequestration. In addition, correctly defining the climatic factors that affect plant growth is important for modelling purposes. Finally, this approach could support the task for developing useful indicators for sustainable forest management linked to climate change scenarios.