IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 11 Ver. III (Nov. 2016), PP 34-42
www.iosrjournals.org

Intra-Annual Xylem Production in Aleppo Pine By Means of Periodic Micro-Cores Sampling in the Kroumirie

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Abstract: *Climatic factors may have an effect on the production of Mediterranean forests. The radial stem growth is an outstanding process to understand the ecology of a species and the study of the production of cambium cells provides a way to test this hypothesis in natural environment. Histological analysis of stem growth based on periodic wood micro-cores sampling will help building up a chronology of cell production during the growing season. The data used in this study come from four Aleppo pine stands located in the northwestern Tunisia (Kroumirie Forest). This study led to the establishment of a training calendar of the xylem's cells related with meso-climate of 2014 and 2015. This work allowed identifying the concordances between the dynamics of intra annual cambial activity, and the weather data. The construction of cambial cells of Aleppo pine is characterized by a spring growth phase, a summer rest period and winter dormancy. The reaction of a population to the climate and site conditions simulates a decrease in productivity of the radial cells linked to an increase in summer drought.*

Keywords: *Pinus halepensis Mill., Kroumirie, climate, intra-annual variations.*

I. Introduction

The migration of trees and changes in their relative abundance during the Holocene has been interpreted as responses to climate changes (Ritchie and Yarranton 1978; Huntley and Webb 1989; Huntley 1990; Pitelka *et al*., 1997). Tree-line fluctuations (Payette and Lavoie 1994; Kullman 1990, 2002) and changes in vitality (Kullman and Högberg 1989; Hofgaard *et al.*, 1999) are well known responses to changes in climate. Future climate changes are likely to include increases in mean temperature (about 2–4°C globally) with significant drying in some areas, as well as increases in frequency and severity of extreme droughts, hot extremes, and heat waves (Spekat *et al.,* 2007). Understanding and predicting the consequences of these climatic changes on ecosystems is one of the grand challenges for global change scientists, and forecasting the impacts on forests is of particular importance.

In the Mediterranean basin, summer drought is the main constraint on tree growth. Climate models predict in the Mediterranean basin a faster warming than in most other areas in the world, associated with reduced rainfall during the growth season (Beniston, 2004; Fuhrer *et al.,* 2006). The Mediterranean area is therefore an advanced field laboratory to study and model climate change impact on ecosystems. As considerable uncertainty remains in understanding and modelling how relevant processes will affect forest production and the risk of forest mortality under a changing climate, additional researches are required to fill existing gaps in scientific knowledge.

Stem growth is an important process to understand tree ecology. Intra-annual analysis of wood formation throughout the year is essential for understanding the tree reaction to short-term changes in environmental conditions (Forster *et al.,* 2000). Because *ca*. 60% of structural sugars are allocated in the branches and stems in young conifers and greater amounts are expected in older trees, the dynamics of biomass accumulation are strictly related to wood growth (Forster *et al*., 2000; Rossi *et al*., 2006). Understanding how and when trees produce their annual structures during the growing season and react to changing environmental factors in forest systems is therefore of great interest.

Given that the timing of changes in stem radius obtained by automatic dendrometers may be altered by stem internal water variation, the histological technique, based on periodic micro-cores sampling and producing a chronology of cell production during the growing season will be preferred (Chaffey *et al.,* 2002; Rossi *et al*., 2006). We concentrated on Aleppo pine (*Pinus halepensis* Mill.), a common thermophilous and heliophilous species in the Mediterranean areas with nearly 3.5 million ha, which shows a high plasticity, growing on any substrate and on poor soils. Its strategy towards drought is to avoid water stress by closing early its stomata. Despite their adaptation to drought, some of Aleppo pine growth processes may be negatively impacted by repeated dry and warm years, as shown after 2003 (IPCC, 2001a). These results are consistent with models and

long-term observations showing that Aleppo pine growth is expected to suffer from changes in timing and duration of drought, particularly in spring and summer. This study documents the intra-annual development of growth rings of four Aleppo pine stands located in the Kroumirie forest. The aim of this project is to understand the mechanisms and dynamics of wood and tree-ring formation, and to assess factors influencing stem radial growth. Intra-annual analysis of wood formation provides chronologies of cell numbers or stem radial variation at short time scale by taking into account the growth dynamics and trying to answer a key question: when are the xylem cells affected by climate? A study of the 2014 and 2015 growth year patterns was conducted; factors influencing wood cell production were studied and compared.

II. Materials and methods

Study sites and parameters

The study is carried out in four Aleppo pine stands located in the Kroumirie forest (NW Tunisia) (figure 1). The four populations are Bechater 'Bech' (37.31 N, 9.79 E, 178 m) belonging to the Bizerte governorate, Sod Bouhertma 'SB' (36.78 N, 8.69 E, 321 m), Fernana 'Fer'(37.04 N, 9.15 E, 363 m) and Sweni 'Sw' (36.48 N, 8.58 E, 121 m) belonging to the jendouba gouvernorate.

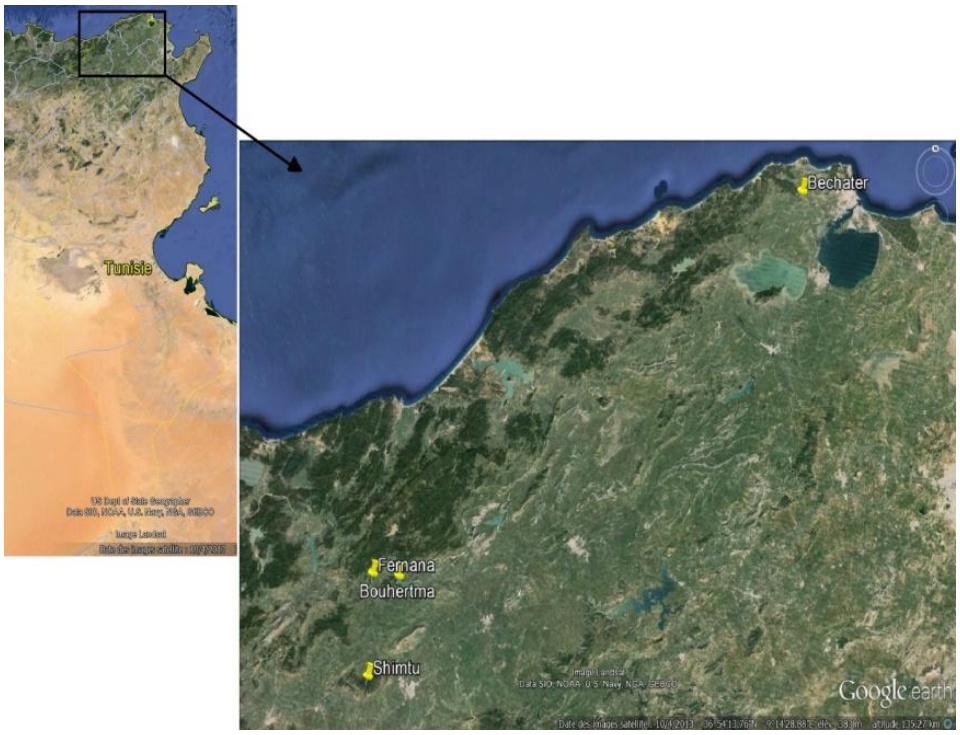

Figure 1: Study areas in the Kroumirie forest.

Variations in the microclimate in each site, mainly the temperature and humidity were recorded through Hobo recorders logger U23 Pro V2. The recorders were installed since 2010 (one measurement every two hours). Once the data are collected, the minimum daily temperatures, the maximum daily temperatures, average daily temperatures and daily relative humidity were determined.
Likewise, we measured per site "the diameter at breast height (DBH) and the height of all living.

Data sampling and chronology construction

Investigation of cambial activity by repeatedly sampling is done on 16 Aleppo pines per site from April to November every 8 to 15 days following the procedure carried out in 2014 and 2015. Dominant, undamaged pines of the same class age (ca. 70 years) in order to ensure comparable growth rates are cored, starting at breast height. After removal of most of the outer bark, micro-cores of wood and inner bark are collected by using the increment puncher developed at the Swiss Federal Research Institute Birmensdorf in order to be as little invasive as possible (figure 2).

Figure 2. Determing the seasonal dynamic of cambial activity: periodic micro-samplings in the outer part of the trunk.

Cores are 1.5 mm in diameter and 5 to 8 mm long; they are extracted following a spiral pattern, at a height of around 1.3 m, 3 cm apart and above the previous one. Such a distance guarantees minimal disturbance given that traumatic tissues are solely formed within a circumference of about 1 cm around the punching hole (figure 2). Microcores are then refreshed by using a razor blade in order to obtain a transverse plan on which the cells are visible. In all tree rings, six radial files are selected to count under a stereomicroscope the number of cells at each sampling date *n*t and to average the number of tracheids (figure 3). The cell formation rate (number of cells/day) are calculated for each tree by the difference between numbers of cells counted at two consecutive samplings *n*t – *n*t-1 divided by the number of days elapsed between two consecutive samplings. Relationships between cell production and *in situ* meteorological variables are calculated by cross-correlations.

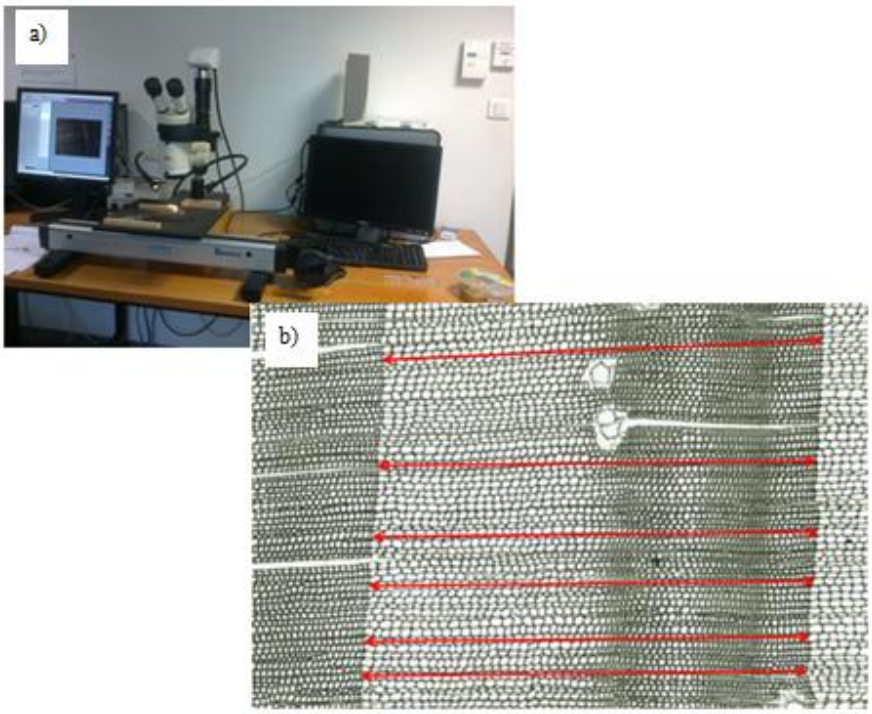

Figure 3. a) Cell counting instrument; b) Seasonal dynamics of cambial activity: counting of cells produced at different sampling dates during the growing season on 6 different rows.

III. Results

A regional climatic analysis was established for 2014 and 2015. The data was taken from button piles located near the populations studied. The average monthly precipitations of the four sites show a decrease in rainfall between 2014 and 2015 especially at Sweni. In parallel, the average monthly temperatures revealed an increase between 2014 and 2015 and the highest values are registered at Sweni followed by Bechater and finally by Fernana and Bouhertma (figure 4).

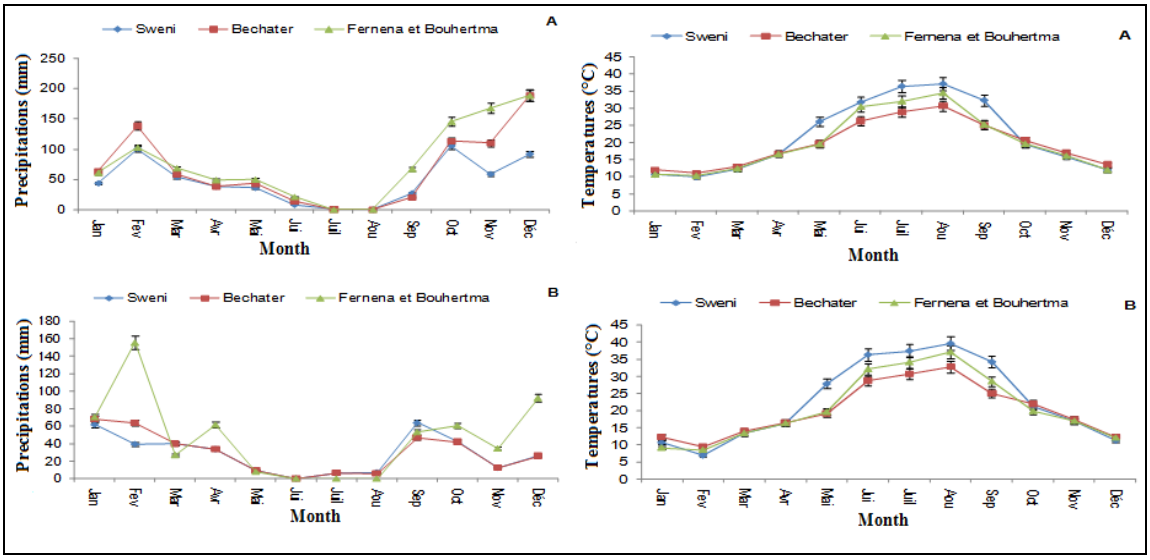

Figure. 4. Rainfall and temperatures variations in 2014 (A) and 2015(B) of the four sites.

Morphological features showed that trees were significantly larger and taller in Fernana (164.15 cm, 17.06 m) than the other sites (figure 5). Differences between Bechater, Bouhertma and Sweni are not significant.

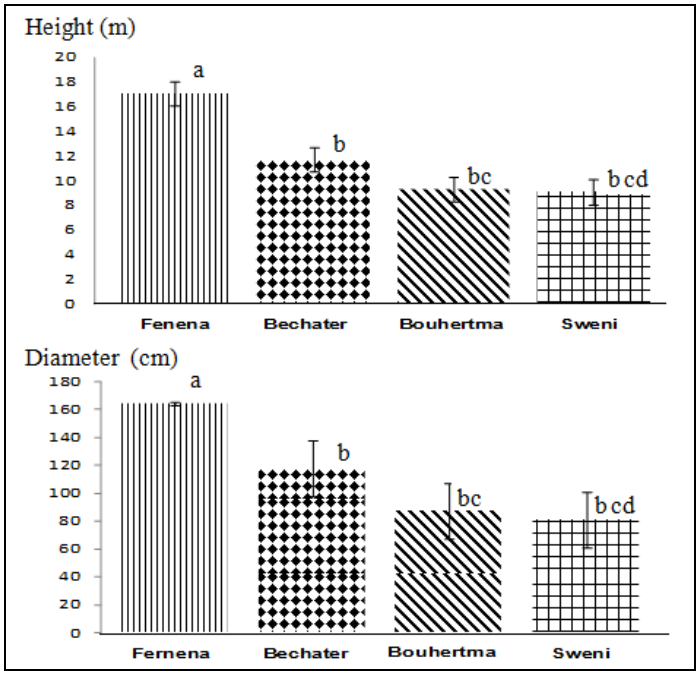

Figure 5. Morphological parameters measured in the four *Pinus halepensis* Mill. populations. Security intervals are calculated at 5% threshold.

Mechanisms of cell production and maturation and dynamics of xylem formation have been widely studied in trees in order to better characterize stem radial growth. Histological analyses have been used in this study to describe cambial activity in different *Pinus halepensis* trees. Wood microcores were collected to determine the number of cells in the cambial zone.

The total number of cells forming the growth ring in 2014 and 2015 for the four sites show homogeneity between the individuals of each population (figures 6, 7).

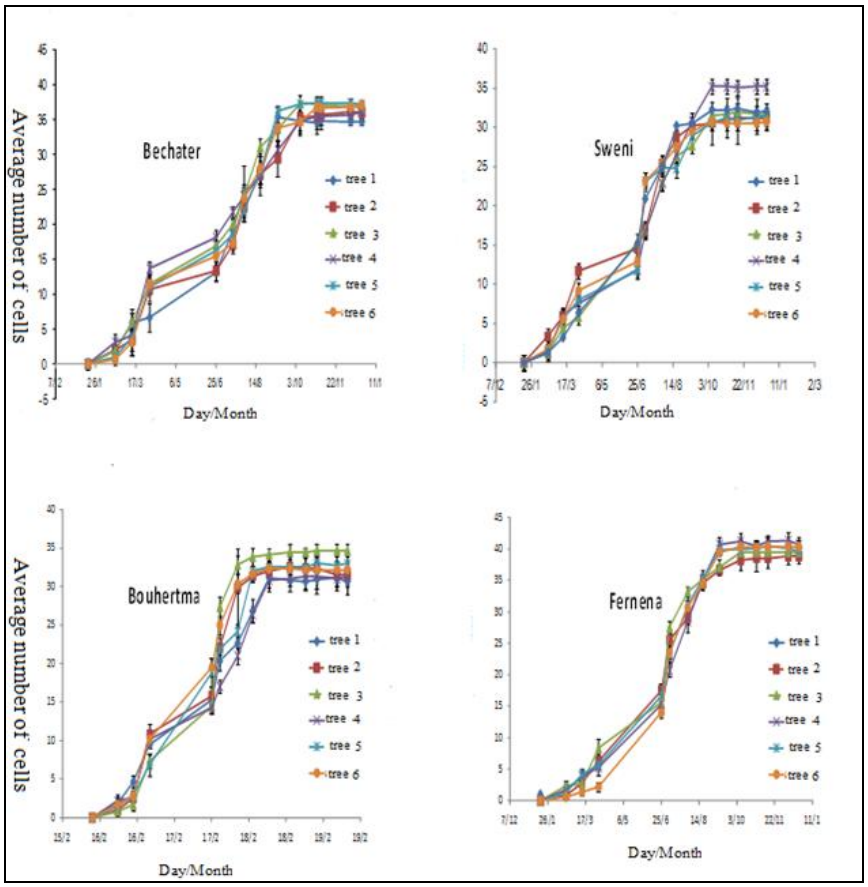

Figure 6. Average number of cells per tree in the four Aleppo pine sites in 2014(every 15 days).

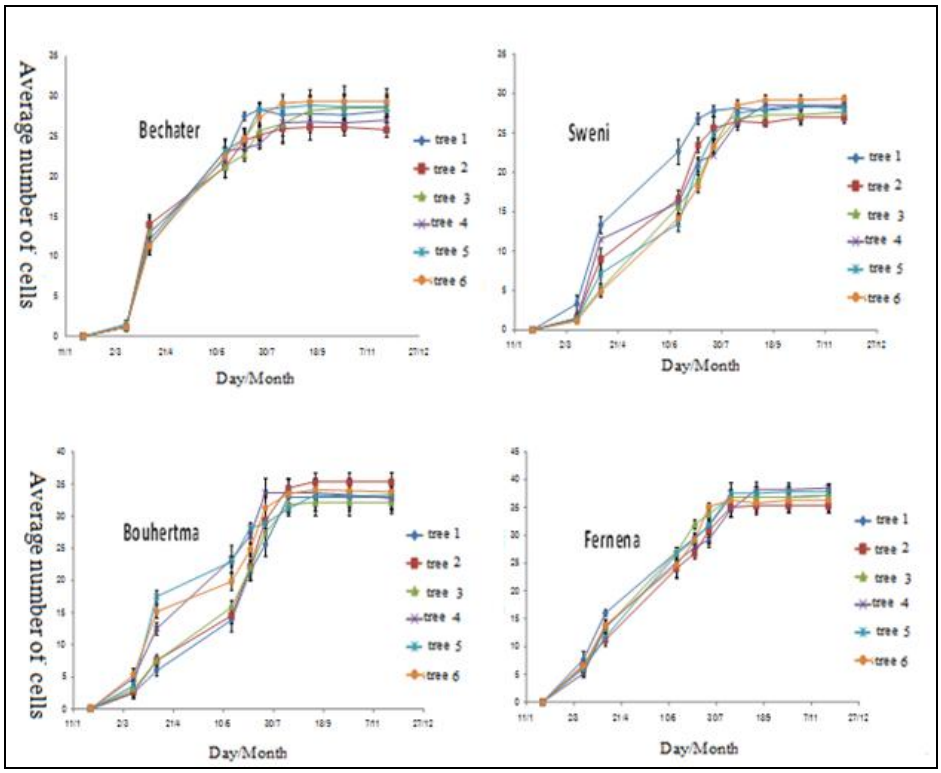

Figure 7. Average number of cells per tree in the four Aleppo pine sites in 2015 (every 15 days).

In terms of the total number of cells forming the growth ring 2014 and 2015 (figure 8), three main periods of cell activity were detected: period of winter pause from January to February, period of high cell production from February to September and a period of stopping growth that begins around September until December excepted the Bouhertma individuals who stopped their growth in August.

Furthermore, from 2014 to 2015, cells number counted varied significantly between the four sites (figure 8). In 2014, the highest rate of cells production was observed in Fernana site (≈40 cells) followed by Bechater (≈36 cells). The cell production rate in Bouhertma and Sweni was almost identical (≈32 cells). In 2015, Fernana was always qualified by the active site (≈37 cellules) followed by Bouhertma (≈34 cellules) then Bechater and Sweni (≈28 cellules). Also, it was found that the highest rate of cells production was observed in 2013 than 2014 (figure 8). Finally, it is important to note that we detected a shift of cells production between the four populations: Sweni starts first, followed Bechater then Bouhertma then Fernena (figure 8).

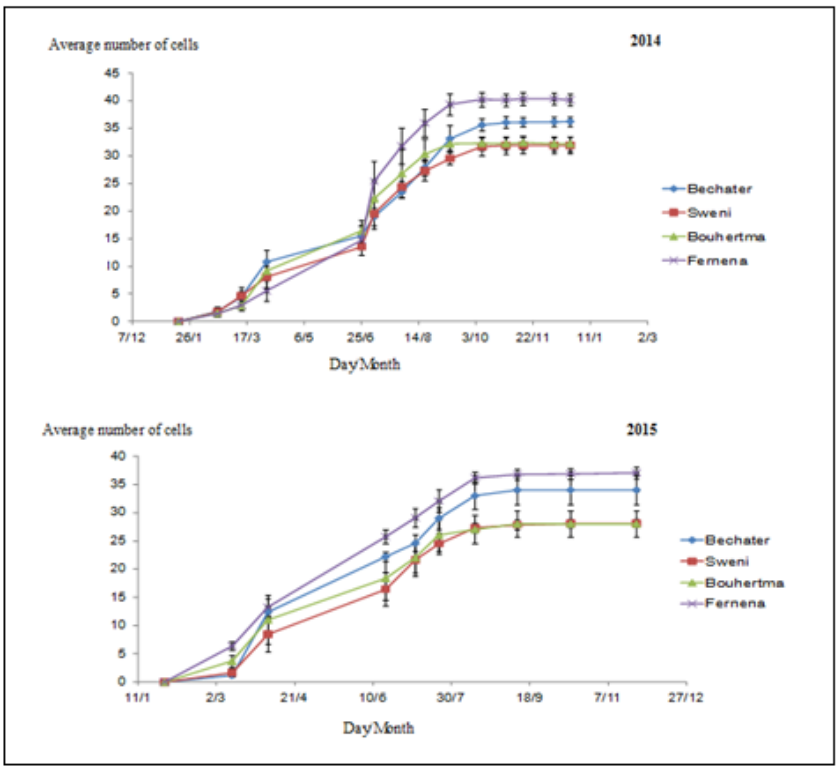

Figure 8. Average number of cells per population in 2014 and 2015 (every 15 days).

IV. Discussion

The aim of this research is to understand the mechanisms and dynamics of wood and tree-ring formation, timing of xylem production and differentiation, and to access factors influencing stem radial growth. In Mediterranean climate, characterized by mild and rainy winters and hot dry summers, the growth and development of trees is not always associated with regular dormancy in the cold season, as it is in temperate or boreal climatic zones (Cherubini *et al.*, 2003). Cambial activity of Mediterranean trees can vary from year to year presenting continuous radial growth, *i.e.* one or two interruptions in line with the variability of climatic conditions (De Luis *et al.*, 2007). *Pinus halepensis* has been characterized as a sensitive Mediterranean species based on the strong response of radial growth dynamics to changes in climate conditions (Lev-Yadun 2000). Cambial activity of Aleppo pine can slow down or even stop during summer drought (Gindel 1967; Liphschitz *et al.*, 1984) and later again when more favourable wet conditions appear in autumn (Liphschitz *et al.*, 1984).

During these two years (2014-2015), our study showed that the cambium activity in Aleppo pine can activate in spring (March, April), followed by a slower growth phase (June, July and August) and enter dormancy in autumn from September to January (figure 8). This result was observed in all pine trees of all populations. Cambial activity can stop during five months; it depends on the prevalence of high temperatures and low precipitations (Gindel 1967) which can considerably influence tree vigour (Oppenheimer 1945). Cell division in the cambium started in March and ended in the majority of trees in August, indicating that cambial activity lasted at least 6 months. Our results were different to those of Nicault *et al.* (2001) who report a growing period of eight months for *Pinus halepensis* in South-East France. His study showed that the cambium activity in Aleppo pine can activate in spring, remain active in summer and enter dormancy in winter from December to

March. Cambial activity and radial growth in Mediterranean forests are periodic, alternating periods of growth with precipitations. In winter, when there is no cell production, dormant cambium is constituted by a minimum number of cells (Rossi *et al*., 2006). In spring and autumn, cambial cells swell and expend radially, radial cell walls grow thin, cytoplasm assumes shinier and less density granulate features and the nuclei enlarge (Fahn and Werker, 1990). The cambial cell number decrease during summer. The number of cells decreases and corresponds to quiescence conditions.We also noted that there were no intra-annual significant variations between selected trees in the same population (figures 6, 7). This was caused by the choice of trees. Indeed, the study was conducted on representative trees of the population, the most similar on morphological and edaphic characters. In the same population ecological conditions are homogeneous and thus the radial growth is uniform between the individuals. These results confirm those obtained by geneticists (Arbez *et al*., 1990).

Climate changes can have significant influences on tree establishment and on forest structure and dynamics (Barbero *et al.,* 1990; Améztegui *et al.,* 2010; Chauchard *et al.,* 2010; Gimmi *et al.,* 2010). In the Mediterranean region, the availability of water is the main factor that limits tree growth (Ogaya *et al.,* 2003; Linares *et al.,* 2010) and forest regeneration (Pulido and Díaz, 2005). In addition, inter-annual variability in precipitation significantly affects annual tree recruitment (Urbieta *et al.,* 2008). However, differences in annual rainfall might not be the only climatic factor that influences forest regeneration, temperature and the amount of precipitation act together to dictate water availability (Larcher, 2000; Vicente- Serrano *et al.,* 2010).Indeed, observations made from 2014 to 2015 in the four sites and regional climate analysis (figure 4) led to conclude that there is a strong relationship between precipitation and cell division. The influence of spring and autumn precipitation on wood cell production without time lag was also identified. There has been also an apparent influence of temperature on the radial growth. In 2015, the decrease in cells number compared to the previous years (2014) is interpreted by a decrease in precipitations especially during autumn and the winter associated with higher temperatures compared to the normal season. Insufficient precipitation in summer (June, July and August) and high temperatures in 2015 have produced less cells than in 2014. The absence of rainfall in summer is accompanied by a slower growth during October, November and December whither the cells number is preserved; that was confirmed by Gindel (1967), Liphschitz et al., (1984) when they asserted that the cambial activity of Aleppo pine can decrease or even stop during the summer drought. Similarly, the important rains of November 2014 especially in Fernana and Bouhertma led to good start of the radial growth in 2015. Several authors including Papadopoulos (1992) reported the importance of precipitations from October to December and their effects on the cambial activity of the following year.

Summer 2014 was well watered which explains the high number of cells during this growth period. So, it can be concluded that summer drought plays an important role in cells division and radial growth (Kozlowski, 1971; Fritts, 1976). These interpretations should be confirmed by a climatic analysis that will allow us to identify possible correlations between cells number and climate variables. According to our observations, Fernana was identified as the most productive population (figure 5) (circumference 164.15 cm and height 17.06 m). This site is characterized by a cool climate and high water reserve (921.3 mm of annual rainfall and 20 °C of mean annual temperature); contrary to Sweni that has been identified as the least productive site (figure 5) with severe climatic conditions (southern exposure, 564.7 mm et 22 °C). Vennetier *et al*., 1999 argue that changes in the Aleppo pine growth can be linked with the multi-year variations in climate of the study area.

V. Conclusion

Wood formation is a complex phenomenon involving cell production and differentiation phases with specific development rates and durations. Observations of wood development during the growing season began more 100 years ago (Knudson, 1913). Understanding how and when plants produce their annual structures during the growing season and react to environmental stresses in natural, fast growing and urban systems is therefore of great interest (Downes *et al.,* 2002). This study provides news points of view for further research and other possible innovative approaches to histological method. In this study, tree growth in the stem is considered and analysed as a dynamic community of reproducing, developing and maturing cells from cambium to mature xylem (Rossi *et al.,* 2006). In this study, we found pronounced characteristics in cambial activity throughout years, and between populations. High variability marked between populations may be explained by a subsequent genetic study which is in progress. However, in the Mediterranean area, climate strongly differs from year to year, in such a way that it is necessary to carry on studies on cambial activity throughout several years in order to access the influence of climate on wood formation and to better understand the physiological processes behind these climate-growth relationships.

Using models for predicting ecosystem carbon and water budget is another important component for estimating carbon sequestration capacity and resilience to environmental changes. Models play also a major role in integrating knowledge of forest processes, help to bind together information obtained by distinct experiments, and provide a powerful base for establishing a comprehensive understanding of forest function. In that sense, data obtained in this research will help calibrating and validating models.

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