Forest Ecology and Management 424 (2018) 71–77

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Xylogenesis in the early life stages of maritime pine

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A B S T R A C T

Trees change throughout their life stages, and although age-dependent changes are reported in the literature, the early life stages of trees are often excluded from these studies. The sapling/pole life stage corresponds to the establishment phase of a tree in the forest. To understand how wood formation changes in the early growing stages we compared xylogenesis in saplings/pole (10–15 years old) and young trees (50–55 years old). Trees were selected from two adjacent areas in different regeneration phases from a maritime pine (*Pinus pinaster* Aiton) plantation located on the west coast of Portugal. The cambial and differentiating xylem cells were monitored from March 2015 to March 2017. The climatic conditions in these years were contrasting: 2015 was hot and dry and 2016 wet. Xylogenesis started around the same time in both age-classes but ended later in young trees in both years. Despite the shorter duration, sapling/pole trees formed more tracheids than young trees in both years, presenting an intensive growth strategy. Tracheids in young trees did not present differences between years, but sapling/pole trees presented fewer tracheids with a higher latewood proportion and smaller lumen area in the driest year (2015). Our results show that sapling/pole trees have an intensive growth strategy, whereas older trees (50–55 years) present a conservative growth strategy with a longer growing season. If the frequency and intensity of droughts increases, sapling/pole trees will reduce growth immediately, whereas older trees will reduce productivity in the following years. Thus, it is expected that forest productivity will decrease under a scenario of increasing drought.

1. Introduction

Trees are the dominant element in a forest. During their lifetime, trees process and store large quantities of carbon. As trees get older and taller physiological processes such as photosynthetic capacity, hydraulic conductivity and growth rate change (Martínez-Vilalta et al., 2007; McDowell et al., 2005). Changes in growth rate affect net carbon uptake and losses, which can have serious consequences for the global carbon cycle, since carbon is stored in trees during wood formation (Cuny et al., 2015). Wood formation or xylogenesis is a genetically controlled process dependent on the ontogenetic status of trees (Begum et al., 2013). Studies comparing xylogenesis between age groups have reported that the duration of xylogenesis was shorter on old trees (Rossi et al., 2008) and that young trees presented faster growth rates (Li et al., 2013). In both studies, the trees designated as young were older than 40 years. Thus, although the age-dependence of wood formation has been addressed in the literature, no study has investigated it in sapling/pole trees. During their lifetime, trees go through different phases: seedling, saplings, pole, young/mature and old trees. The sapling phase is characterized by individuals that have not yet reach sexual maturity and the pole phase by trees that although sexually mature, have not yet reached the full height for the species (Santos-del-Blanco et al., 2012).

It is well known that trees present a biological growth trend, by which trees form wider tree rings during their early years (Ivković et al., 2013). This biological growth trend is commonly removed by dendrochronological standardization. By doing so, the climate response is considered to be age-independent. However, several dendrochronological studies have shown that tree growth responses to climate are site-, age- and species-dependent (Campelo et al., 2018; Carrer and Urbinati, 2004; Szeicz and MacDonald, 1994; Vieira et al., 2009; Wang et al., 2009; Yu et al., 2008). For instance, it was found that young trees (65–75 years old) of *Pinus pinaster* Aiton started to grow earlier and thus responded to climatic conditions earlier in the growing season, whereas old trees (115–200 years old) presented a stronger climatic signal (Vieira et al., 2009). The xylogenesis of this species has also been studied in young trees (50–60 years) (Vieira et al., 2014a, 2014b), however xylogenesis has not yet been compared among different life stages. Studies reporting on the cambial activity and wood formation dynamics of maritime pine trees have observed that warmer

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https://doi.org/10.1016/j.foreco.2018.04.037
Received 1 February 2018; Received in revised form 23 March 2018; Accepted 18 April 2018

winters triggered an earlier onset of xylogenesis and that xylogenesis duration was dependent on water availability, stopping earlier in drier years (Vieira et al., 2015, 2014a). Although some studies have investigated the climatic response of xylogenesis in maritime pine, none of them have considered the most vulnerable life stage, the sapling/pole phase.

The age-dependent climatic response of trees presents an additional challenge: estimating the growth of trees and forests growth under the forecast climate change. Climate change is one of the greatest environmental challenges for mankind in the twenty-first century (Beniston et al., 2007). Temperatures are rising and the precipitation regime has become more irregular with extreme events such as dry spells and floods becoming more frequent (Coumou and Rahmstorf, 2012). The climatic scenarios for the Mediterranean region project a higher risk of summer drought associated with decreasing precipitation and increased temperature (Christensen et al., 2007). Understand how sapling/pole trees will respond to such conditions is critical to predict forest succession and tree establishment and productivity in the future. The two years reported in this study represent a very dry year (2015) and a wet year (2016). Comparing the response of xylogenesis in young and sapling/pole trees in two contrasting years will determine how this response is modulated by age and climate, shedding some light on the future response of maritime pine forests.

In the present study we compared the cambial activity and xylogenesis in saplings/pole (13 ± 2 years) and young (52 ± 2 years) maritime pine trees (*Pinus pinaster* Aiton) growing in the west coast of Portugal. The aim of this study is to determine (1) if cambial activity and wood formation are age-dependent; (2) if there are differences in the timings of xylogenesis between age-classes and (3) if the rates of cell production change with age. We determined that sapling/pole trees presented a higher rate of cell production than young trees. Regarding the climatic impact on wood formation, although young trees showed no differences in tracheid production between years, sapling/pole tress formed fewer tracheids in the drier year. Our results are relevant for forest management strategies as they report how trees in the early life stages respond to climate.

2. Materials and methods

2.1. Study site and tree selection

The study was performed in a permanent plot, located in Perímetro Florestal Dunas de Cantanhede, in the west coast of Portugal (40°21′35.15" N, 8°49′10.06" W; 15 m a.s.l.). The plot is a maritime pine (*Pinus pinaster* Aiton) plantation on sand dunes. For this study we selected trees located in two adjacent stands, in different regeneration phases: one with sapling/pole trees and another with young trees. Sapling/pole trees were 13 ± 2 years-old and young trees 52 ± 2. Tree age was determined by removing a core from each tree at breast height. Sapling/pole (young) trees presented a diameter at breast height (DBH) of 11.8 ± 1.4 (34.4 ± 5) cm and 5.4 ± 0.5 (15.2 ± 0.8) m of height.

The climate is typically Mediterranean with precipitation occurring mainly in the fall and winter months and the summer is dry and warm. The mean annual temperature for the last 30 years (1985 – 2014) was 16.3 °C, and the average total annual precipitation of 877 mm. Daily values of maximum and minimum temperature and precipitation were downloaded from the Royal Netherlands Meteorological Institute (http://www.climexp.knmi.nl/; v15.0).

A soil moisture probe PR2 from Delta-T-Devices was installed in the study site (in January 2014) and programed to record soil moisture values every 30 min at a depth of 100 cm. Data were stored in a DL2e data logger also from Delta-T-Devices.

2.2. Cambial activity and wood formation

Sampling was performed on five trees per age-group from March 9th 2015 to March 15th 2017 by collecting microcores from the tree stem using a Trephor (Rossi et al., 2006). Samples were taken every 10 days from 45 cm above and below breast height, on the south-facing side of the stem, in order to minimize growth variability around the stem (Lupi et al., 2014). Each sample was collected at approximately 5 cm from each other to prevent getting resin ducts formed in response to the previous samplings. Before collecting the microcore, the dead bark was carefully removed to reach the living tissues. Wood microcores were placed in eppendorfs filled with alcohol 50% (v/v) and stored in the refrigerator (5 °C) to prevent tissue deterioration. The samples were then processed by successive immersions in alcohol and D-limonene solutions of increasing concentration until dehydrated, and then embedded in paraffin. Transverse sections 7 µm thick were cut from the samples with a rotary microtome, stained with cresyl violet acetate (0.17%), and immediately observed with a microscope (Leica, DM4000B) under visible and polarized light to distinguish between the xylem cells in different stages of development. Cambial and enlarging cells only present primary wall, thus do not shine under polarized light. Cambial cells are characterized by a small radial diameter while enlarging cells present a diameter of at least twice that of a cambial cell. Wall thickening cells shine under polarized light and present a violet coloration changing to dark violet at the end of maturation. Mature cells present a blue coloration in the entire cell wall. In each sample, the number of cambial, enlarging, wall thickening and mature xylem cells were counted along three radial rows.

The total number of cambial, enlarging, cell-wall thickening and mature cells determined during the growing season were fitted with generalized additive models (GAMs) in order to compare the effect of early growth stage in xylogenesis using the *mgcv* package (Wood, 2006) in the R computing environment (R Development Core Team, 2016). For each year, differences between sapling/pole and young trees were considered significant when the point-wise confidence intervals of the fitted curves did not overlap.

A linear regression was calculated between the duration of xylogenesis and the number of tracheids formed in each year per group of trees.

2.3. Xylem phenology

The number of cells in each differentiation phase at each sampling point was used to assess the onset and end of the xylem differentiation phases. The onset of each phase was considered when at least one cell was observed per row. At the end of the growing season, the end of each differentiation phase was considered when no cells were observed in that phase. Xylem phenology was represented by the dates corresponding to: the first enlarging tracheid; the first cell wall thickening tracheid; the first mature tracheid; the last enlarging tracheid and the last wall thickening tracheid. The timings of xylogenesis were computed as day of year (DOY) and determined for each tree and year. The computation of the critical dates was performed using the R package CaviaR (Rathgeber et al., 2011).

The dynamics of tracheid production were assessed by fitting a Gompertz sigmoid curve to the total number of tracheids produced over the growing season (Rossi et al., 2003), defined as:

$$y = a \times e^{-e^{(b - k \times t)}}$$

where y is the cumulative number of tracheids; t is time; a is the upper asymptote corresponding to the maximum number of tracheids produced; b the x-axis placement parameter; and k is the growth rate parameter that determines the spread of the curve along the x-axis.

Gompertz functions were fitted for each group of trees and their parameters used to calculate the dates at which 5% and 95% of the cells