

XYLOGENESIS OF *PINUS HELDREICHII* AND *PINUS PEUCE* IN PIRIN MTS.

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Abstract

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Pinus heldreichii and *Pinus peuce* are tree species characterized by longevity. Previous studies have outlined the potential for constructing long tree ring chronologies, which may be used for studying the climatic variation in the past. A difficulty is the mixed climatic signal with negative effect of summer droughts, but also of extremely cold summers and the positive effect of warm winters. Therefore, for successful dendroclimatic analysis better understanding of the processes of tree ring production is necessary. Our aim within this project was to study the cambial activity and production of tracheids during the growth period. Old 100–250 years trees of *P. heldreichii* and *P. peuce* from natural forests in Pirin Mts. were chosen. Beginning from 2010 microcores was taken every 10 to 14 days. The samples were prepared with a sliding microtome GSL-1 and analyzed for the onset of cambial activity, the period of production of first cells and the period until cells were produced. In 2010 and 2011, the onset of cambial activity was delayed until the end of June – beginning of July. In 2012, it started at the beginning of June. The late start in 2010 and 2011 was probably due to cold periods in April and May. Despite this the trees produced tree rings with more rows of cells and higher width than all the rings in the period 2000–2009. This confirms our initial hypothesis that warmer winters promote the production of wider tree rings. The production of new cells continued until the end of September, while the differentiation – until the middle of October. In both years *P. heldreichii* trees produced more cells than *P. peuce* trees.

Key words: tree rings, xylogenesis, *Pinus heldreichii*, *Pinus peuce*, Pirin

Introduction

Annual tree-ring differentiation involves the production of cells through the phases of division, expansion, secondary wall thickening, lignification's and programmed cell death (Savidge, 1996; Plomion et al., 2001). Intra-annual radial growth rates and durations in trees are reported to differ greatly in relation to species, site and environmental conditions. Understanding of the pattern of response mechanisms is critical for predicting vegetation changes associated with projected future climate change, and fundamental for adopting appropriate and timely management measures. Radial patterns of wood structure reflect the changing demands placed upon woody plants as they grow and experience changing environmental conditions. The study of their variation over time offers the opportunity to evaluate how well plants will respond to predicted global changes.

Previous studies on tree ring width series of *Pinus heldreichii* Christ (Panayotov et al., 2010) and *Pinus peuce* Griseb (Panayotov and Yurukov, 2007) have shown strong inter-series correlation, but mixed climatic signal. Tree ring growth was found to be strongly dependent from both summer precipitation and temperature, but from winter temperatures (*P. heldreichii*). Thus, better knowledge of the relationship between tree-ring development and climate is needed to improve dendroclimatic studies. At the same time up to this moment, there are no detailed studies for the cambial activity and tree ring production of the Balkan endemic species *P. peuce*. The cambial activity of *P. heldreichii*, which is also a species under global protection, was studied only in a location in Italy (Rossi et al., 2006) but not for the Balkan Peninsula. Climate differences are present between these two regions (Panayotov et al., 2010) and this might be a reason for differences in the cambial activity phases.

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This paper presents the first results from a study about the timing of production of tracheids and tree ring formation in *P. peuce* and *P. heldreichii* in the Pirin Mountains. Our aim was to analyze the differences in the onset of cambial activity, the duration of cell production and number of cells produced between the two studied species and between sites with different exposures.

Material and Methods

The study area is situated in the Bunderitsa valley in the Pirin Mountains, Bulgaria. On the eastern slope of Vihren peak, 5 trees from *P. heldreichii* and 2 trees *P. peuce* situated from 1850 m to 2000 m a.s.l. were studied. On the north-western slope of Todorka peak 4 *P. peuce* and 2 *P. heldreichii* trees situated between 1750 and 1850 m a.s.l. were studied.

Wood microcores (1.2–2.4 mm in diameter) were collected at breast height (1.3 m) using increment punchers (Forster et al., 2000). The microcores were placed in ethanol-vinegar based solution in Eppendorf microtubes. Samples usually contain the previous five to ten tree rings, newly developing cambial zone and adjacent phloematic tissue. Micro sections were cut with 10–15 µm thickness with sledge microtome GSL-1 following procedures described by Schweingruber (1990). Micro-sections were stained with Shafranin and Astra-blue, observed with ZEISS Standart 20 microscope, and captured with Progres-CT3 camera. The number of cells each year was counted in five lines of cell rows.

Results and Discussion

In 2010 started the collection of the first data of this type in Bulgaria and the first for *P. peuce* worldwide. In 2010, the cambial activity of *P. peuce* started in early June, the first

formed cells being observed on June 11th (Figure 1). By mid-November the cambial activity and formation of new cells had completely stopped. In *P. heldreichii* trees from the same slope and altitude similar start and end of cambial activity was observed, but more cells were produced. That confirmed the data for total number of cells generated in the previous years (Todorova et al., 2010), which were more in *P. heldreichii* than *P. peuce* trees.

Although the data for cambial activity in 2010 were scarce, it set an interesting trend. The beginning of cell formation was in the middle of June, the most active period of cell formation in August and the end of the formation of new cells till October. This was a surprising result in comparison with data from other studies in the Alps (Rossi et al, 2003), showing the start of the division at the beginning of June, the most active phase in July and the end of the formation of new cells in September. Probably this was because 2010 was special in several aspects. First, excluding January and October, average monthly temperatures throughout the year were higher than the long-term average temperatures. With the exception of February the autumn-winter period (November 2009 – March 2010) was very dry and warm with lower than normal snowfalls. The relatively warmer beginning of May was followed by a sharp cooling in the second decade of the month followed by snow accumulation even in lower mountain areas (i.e. Petrohan area, Stara Planina Mts, aprox. 1300 m a.s.l.). Perhaps that played a role in delaying the start of cambial activity. Meanwhile the summer could be characterized by high temperatures in the presence of normal rainfalls. The relatively warm autumn probably contributed for the observed prolonged cambial activity, which lasted until the end of October, when there was a cold period with heavy snowfalls (30 October 2010).

In 2011 the cambial activity also started at the end of June (Figure 1). First formed cells (3 to 10 lines) were observed



Fig. 1. Duration of tracheid production period in 2010-2012

in the samples taken on July 2nd. This was also a surprising result, because for a second consecutive year the start of cambial activity was later than expected. In addition to literature data (Rossi et al., 2003), analysis of observed frost rings (Panayotov, 2007; Panayotov and Yurukov, 2007) formed in years with late frosts events in late May and early June in the studied species gave additional evidence that in other years cambial activity started earlier. It should be noted however that 2011 was also special in several aspects. The winter was unusually warm, with less than normal snow. In mid-April most of the eastern slope of Vihren peak, where most of the studied trees are situated, was without snow cover. Then similarly, to 2010 there were long-lasting cold period in May. This is the most likely reason for the delay in the start of cambial activity. Also similarly, to 2010 the summer was warm without the presence of long droughts. Autumn was extremely warm (September) followed by heavy snowfalls and very strong cooling ($-6 / -7^{\circ}$ C at an altitude of 1700-2000 meters on October 16th).

It is interesting, that despite the late start of cambial activity in 2011- 2012, the number of cells generated in all trees was higher than those in previous 5 to 10 years.

Although these data should be interpreted with caution as they are based on different samples, they clearly outline the trends that during those three years were formed larger than usual number of cells. Thus our findings for the cam-

bial activity in 2010 and 2011 confirm conclusions from the analysis of the correlations of chronologies from tree rings widths in *P. heldreichii* that in years with warmer winters and summers without long lasting droughts, wide annual rings were formed, which in turn contain larger number of cells (Panayotov et al., 2010; Todorova et al., 2010).

In 2012, cambial activity started at the beginning of June (Figure 1). First cells were formed shortly before 10th of June. In comparing with the observed previous years, winter of 2012 was much colder, with heavy snowfalls and long lasting snow cover. This is the main difference in winter climate conditions between these years. Yet, the spring was normal, without any cold spells and late snowfalls. Perhaps this contributes for start of cell formation in the expected period in 2012. The summer and autumn were extreme hot and dry. Despite this, the cambial activity in 2012 ended at the end of October as in earlier years. Perhaps this is an indication of lack of dependence of the termination of cell division on the temperature regime, but rather on light conditions. In general, plants synchronize their preparation for the winter season with the decreased light and reduced average temperatures.

Comparing the number of produced cells also showed that *P. heldreichii* trees generally produce more cells than those of *P. peuce* (Figure 2). Those finding must be verified by testing more *P. peuce* trees.

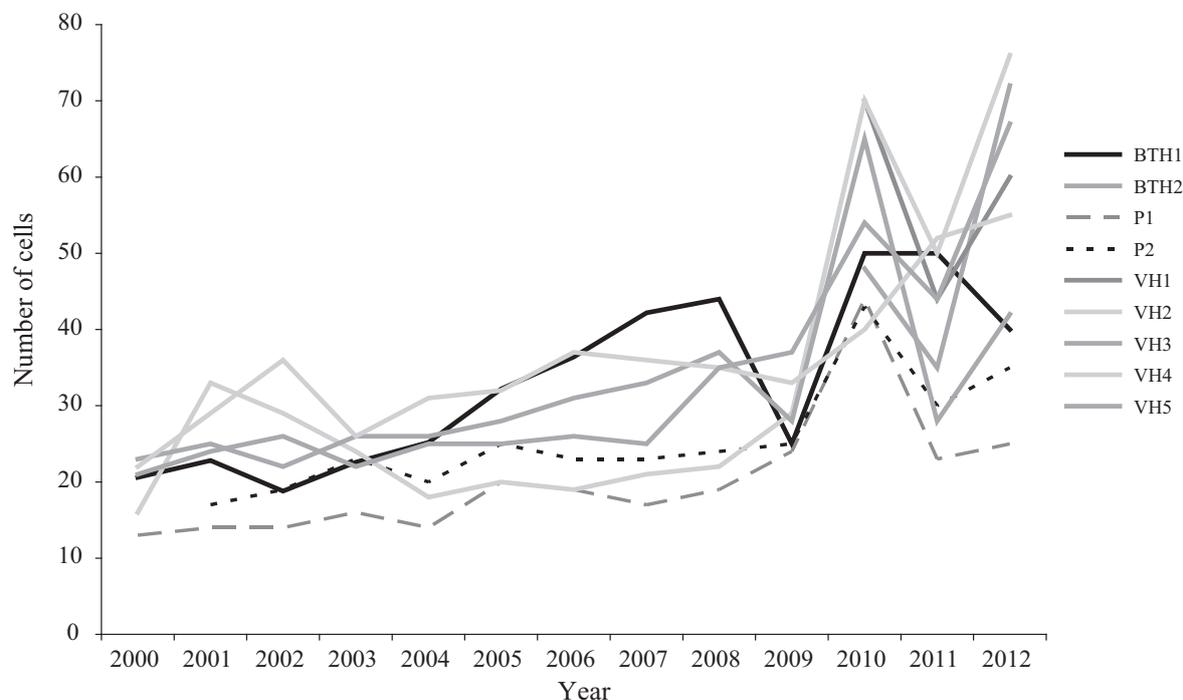


Fig. 2. Number of produced cells in *Pinus heldreichii* and *Pinus peuce* for the period 2000–2012, from the eastern slope of Vihren peak and the northwestern slope of Todorka peak in Pirin Mts.

Conclusions

Our data shows that the hypothesis based on the analysis of variation in tree rings widths that warmer winters contribute to the formation of wider annual rings is confirmed. This study suggests that this is due to the greater number of formed cells. Even late start of the cambial activity caused by spring frosts did not change the observed production of more cells in such years. Thus, our data confirm that chronologies of *P. heldreichii* can be used to analyze past climate extremes and outline unusually warm or cold winters for periods without available instrumental climate measurements.

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References

- Panayotov, M., Bebi, P., Trouet, V. and S. Yurukov**, 2010. Climate signal in tree-ring chronologies of *Pinus peuce* and *Pinus heldreichii* from the Pirin Mountains in Bulgaria. *Trees – Structure and Function*, **24**: 479–490.
- Forster, T., Schweingruber F. H. and B. Denneler**, 2000. Increment puncher: a tool for extracting small cores of wood and bark from living trees. *IAWA Journal*, **21**: 169–180.
- Panayotov, M. P. and S. Yurukov**, 2007. Tree ring chronology from *Pinus peuce* in Pirin Mts and the possibilities to use it for climate analysis. *Phytologia Balcanica*, **13**(3): 313–320.
- Plomion, C., G. Leprévost and A. Stokes**, 2001. Wood Formation in Trees. *Plant Physiology*, **127**: 1513–1523.
- Rossi, S., A. Deslauriers, T. Anfodillo, H. Morin, A. Saracino, R. Motta and M. Borghetti**, 2006. Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. *New Phytol.*, **170**: 301–310.
- Rossi, S., A. Deslauriers and H. Morin**, 2003. Application of the Gompertz equation for the study of xylem cell development. *Dendrochronologia*, **21**(1): 33–39.
- Savidge, R. A.**, 1996. Xylogenesis, genetic and environmental regulation, a review. *IAWA Journal*, **17**: 269–310.
- Todorova, Y., A. Ivanova and M. Panayotov**, 2011. Annual cell formation of *Pinus heldreichii* and *Pinus peuce* from Pirin Mountain in Bulgaria. Proceedings of “Klimentovi Dni” conference, Sofia, 22–23 Nov. 2010, pp. 66–68.