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TREE RING AND ANATOMICAL STUDIES IN *PINUS HELDREICHII* FORESTS IN PIRIN MOUNTAINS, BULGARIA

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Abstract

Pinus heldreichii forests in pristine condition can still be found in Pirin National Park in Bulgaria. Although they are of high conservational value still numerous questions for their structure, the physiological and genetic peculiarities of the species exist. Here we present results for several tree ring chronologies constructed along altitudinal gradient and different exposures. We also studied the variation of anatomical leaf parameters like number of resin ducts and stomata. We found that the oldest trees in the valley reach 800 years and are situated on isolated by rock bands sites close to the local treeline. Forests on the slopes at the bottom of the valley are 200–300 years old with single older trees. Many of them are probably shaped by fires in the past. We found similar variability in tree ring chronologies from different exposures. Only one chronology constructed from a mixed coniferous forest was found to differ at certain periods from the other chronologies. The most probable reason for this is exogenous disturbance like a fire. We did not find enough evidence that the anatomical traits of the needles are altitude dependent, although number of resin ducts and the number of stomata on the outer surface were found to increase with altitude.

Key words: *Pinus heldreichii*, tree rings, leaf anatomy.

Introduction

Pinus heldreichii Christ (*Pinus leucodermis* Antoine) is a tertiary relic species occurring in isolated subalpine and treeline locations in some mountains within the Balkan Peninsula and Southern Italy (Barbero et al. 1998). In Bulgaria, it grows only in the Pirin and Slavyanka Mountains on soils formed on marble and limestone bedrocks. All of its locations are under strict protection being included in nature reserves and Pirin National Park, which is in UNESCO's World natural heritage list. Especially in Pirin Mts. hardly accessible steep slopes helped the conservation of numerous forests in pristine state, which provides the chance to conduct studies in ecosystems that evolved under small or without any human influence. Although *Pinus heldreichii* is of high conservational value, still many topics related to its habitats are not well studied. Among them is morphological variation. Research was mostly locally based and focused on single parameters (Gudeski et al. 1975, Yurukov et al. 2005, Panayotov and Yurukov 2007, Todaro et al. 2007, Guerrieri et al. 2008, Panayotov et al. 2010a). Therefore further studies are needed to fill in some of these gaps. Moreover, besides pure conservational research needs the species additionally provides high potential for proxy climate studies due to its longevity (Panayotov et al. 2010a).

Here, we aim at studying the variation of tree ring and leaf morphology along altitudinal gradient within one valley. Additionally we compare the influence of exposure on these parameters.

Material and Methods

Study area

The study area was situated in the Banderitza valley in the Pirin Mountains, Bulgaria, 41°45′N, 23°26′E (Fig. 1). We selected 3 study sites at different elevations for collecting tree ring cores, 2 on the eastern slope of Vihren peak and one on the north-western slope of Todorka peak (Table 1). For leaf morphology we additionally collected samples from two

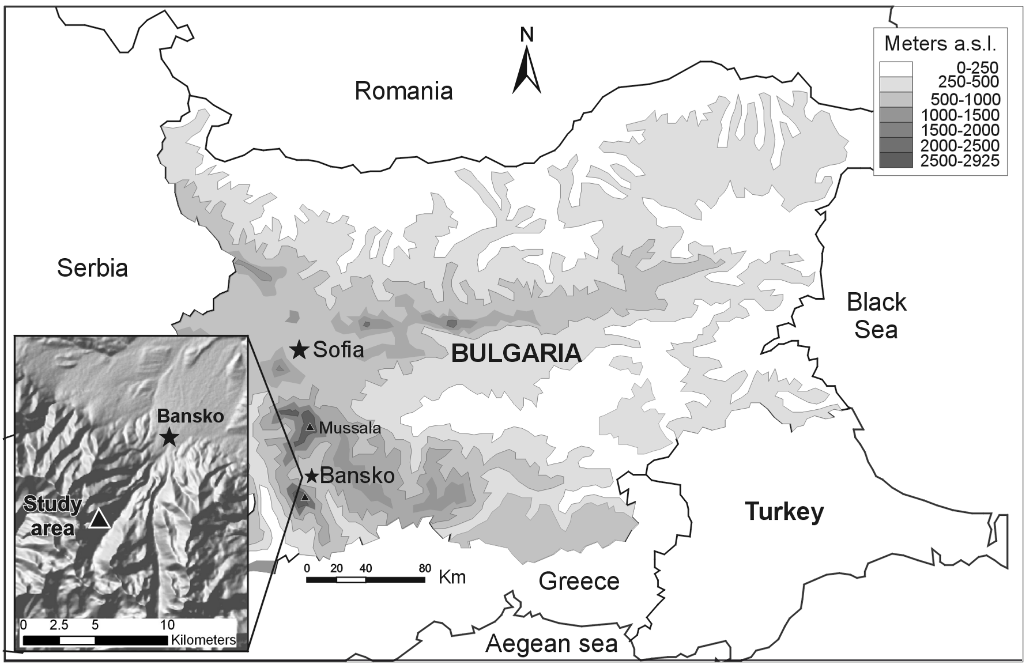

Fig. 1. Study area.

Table 1. Position of study plots.

Site name	Altitude	Exposure	Number of tree ring cores	Number of needles studied
SHADE-LOW	1800	NW	–	50
SHADE-MID	1950	NW	17	50
SUN-LOW	1750	E	15	50
SUN-MID-1	1900	E	29	50
SUN-MID-2	1950	E	–	50
HIGH-1	2000	E	–	50
HIGH-2	2100	E	55	–

more locations, one on Vihren slope and the other on Todorka slope.

The slopes were steep, with inclination of 20-50° and covered mostly with thin Rendzic Leptosols and Regosols formed on marble bedrock. Forests were pure *Pinus heldreichii* at higher elevations (i.e. sites "SUN-MID-1", "SUN-MID-2", "HIGH-1" and "HIGH-2") and mixed with *Picea abies, Pinus sylvestris*, and the Balkan endemic *Pinus peuce* at lower elevations.

The climate in the region is typically mountainous, with strong influence of the Mediterranean air masses. The mean annual temperature (Vihren chalet climate station, 1970 m a.s.l.) is 3.5°C. It ranges from a mean monthly temperature of -4.7°C in January to +12.2°C in August. The annual precipitation amounts to 1378 mm, with a maximum in autumn and winter. Deep snow covers are characteristic for the region. At the same time, the summer precipitation minimum combined with shallow soil profiles on steep rocky sites might cause local drought conditions on sites with eastern and southern exposure (Panayotov et al. 2010a).

Data collection and analysis

Tree ring cores were collected with increment borer at breast height (1.3 m) from dominant trees that were not affected by avalanches or rock-falls. They were mounted on wooden boards, air-dried and sanded. Tree ring widths were measured in the dendrochronology laboratory at the University of Forestry in Sofia following standard procedures with precision of up to 0.001 cm. Obtained tree ring width series were cross-dated with the use of visual clues (Stokes and Smiley 1968) and the computer program COFECHA (Holmes 1983). Then the data were standardized with the software package ARSTAN (Cook 1985) using negative exponential function. In cases with higher low-frequency tree ring variation, which could be due to non-climatic forcing (Fritts 1976) and was typical mostly for the lower altitude sites, we also used cubic splines to perform the standardization. The final chronologies were composed by calculating bi-weighted robust means of annual ring widths. The highest altitude chronology (HIGH-2) was previously published (Panayotov et al. 2010a) and thus we compare newer obtained tree ring series to it.

For the study of leaf morphology we collected 5 two-year old needles from the lower southern parts of the crowns of 10 trees at each site (e.g. 50 needles per site). We measured the number of resin ducts (NRD), number of stomata per unit length on the abaxial leaf surface (NSO) and the number of stomata per unit length on the adaxial leaf surface (NSI). Anatomical traits were observed and recorded on micro-sections obtained from the mid-length of the needle.

Results and Discussion

Tree ring chronologies

Oldest trees were found at site "HIGH-2" (Fig. 2). The longest series had its first tree ring in 1026 AD (i.e. 803 tree rings). Though this is below the expected maximum longevity for the species as the famous tree "Baikushevata mura" is believed to be about 1300 years old, our core produced the longest dated tree ring series available in Bulgaria. Other trees at that site were also close to the age of 800 years, which allowed the tree ring chronology to reach a replication of at least 5 cores at 1250 AD (Panayotov et al. 2010a). This tree ring chronology was found to be very well correlated with others available from *Pinus heldreichii*. The highest coefficients ($R = 0.66$, $p<0.001$, common period 1600–1981; GLK = 70%; $t = 16$) were found in a tree ring chronology produced by Schweingruber (1981) for Olympus Mts. in Greece (Panayotov et al. 2010a).

The second-oldest site was "SHADE-MID". There the longest series had 566 tree rings. Yet, because

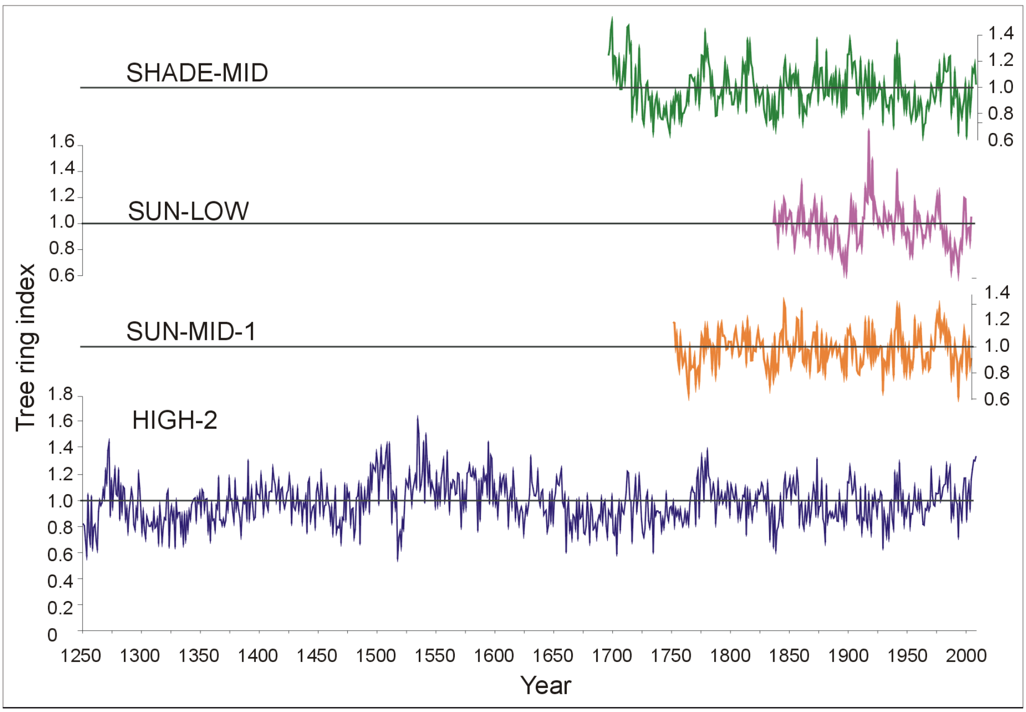

Fig. 2. Tree ring chronologies.

other trees at that site were younger the tree ring chronology was truncated at 1696. The trees at the other studied sites were found to be much younger. Few of them were older than 250 years. Youngest were the trees at site “SUN-LOW”, which was a mixed *Pinus heldreichii-Picea abies-Pinus peuce* forest found at the bottom of the valley close to the Banderitza river. We suggest two possible reasons for the found differences in the age of the trees at the studied sites. One is heavy human management in the past at the lower sites. This could explain the lack of older trees at the more accessible lower parts of the slopes (sites “SUN-LOW” and “SUN-MID-1”. Yet, 100 to 200 years ago mechanization and wider road was unavailable which would hinder attempts to transport numerous larger-sized trees. This would suggest that if the forests were influenced by cuttings, such could be rather more irregular selective ones. Other possible influence could be fires, either natural or set by local shepherds to clean up pastures. Such could easily escape the valley bottom and cause burning of forests on the slopes above. In that case only isolated by high rocks sites could remain intact. The position of site “HIGH-2” is exactly the same. It is completely isolated by a 30 to 100 meter high rock band from the valley below. One further fact that would support the hypothesis of a huge fire 250 to 300 years ago in Banderitza valley is the complete lack of older trees, besides the “Baikushevata mura”, below the rock band that isolates site “HIGH-2”. Such older trees would be expected on more inaccessible locations, which are present on the slopes, if the forest age was due only to cuttings.

High and low frequency variation was rather similar between the composed chronologies. This was expressed by the correlation coefficients, which were found to be higher than 0.6. An exception was chronology “SUN-LOW”, which had lower correlations ($R < 0.31$) with chronologies “HIGH-2” and “SHADE-MID-1”. This was probably due to differences in two periods in chronology “SUN-LOW”. It had “a peak” in tree ring indices starting from 1914 and finishing in 1923 and a decrease starting at the beginning of the 1980-s. They were not matched by other chronologies. The 1914 sharp increase of index values was due to the presence of numerous “releases” in the tree ring width series of the trees at site “SUN-LOW”. Such releases could be due to exogenous disturbances, which normally cause a sharp change in tree ring widths that is not matched by other regional tree ring chronologies (Fritts 1976). In our case several types of disturbances could be expected – fires, cuttings or avalanches. Because the location of site “SUN-LOW” is close to an avalanche couloir, a tourist path and former trade route, any of these reasons is possible. Numerous fire-scars in the region and age of much of the younger trees of about 90 years could be a clue for a fire about 100 years ago, but further studies are needed to confirm or reject such hypothesis.

We expected to find difference in the tree ring chronologies composed from trees situated at sun-exposed and shady slopes. Yet, chronology “SHADE-MID-1” is rather similar in variation to the chronologies from the sun-exposed eastern slope. A comparison of the correlation coefficients for the climate-growth relationship of these chronologies also demonstrated similar reactions to the general climate variability (Panayotov et al. 2010b).

Leaf anatomical features

Previous results for morphological parameters of *Pinus heldreichii* leaves published by Yurukov et al. (2005) and Panayotov et al. (2010b) demonstrate that basic leaf features like length, width and thickness vary much between sites, but do not necessarily demonstrate altitude or exposure dependent variability. Therefore we chose to present and comment results for anatomical parameters like number of resin ducts and stomata. Our data show that the number of resin ducts (NRD) in leaves from sites in Banderitza valley was higher on the sun-exposed eastern slope than on the shady western slope (Table 2). The mean and maximum values increased with increase of the altitude till 1950 m a.s.l. and then decreased slightly at sites close to the local treeline. Existence of differences between altitudes is also demonstrated by the results from the ANOVA test (Table 2). Yet, no clear trend could be observed, which raises the question whether the found differences were site or altitude dependent. The number of resin ducts was also much smaller (up to twice) than indicated by previous results from Bulgaria presented by Yurukov et al. (2005). This is probably due to differences in measurement equipment, which was with lower quality in the previous study. Moreover, at

Table 2. Anatomical leaf parameters.

Site name	Altitude, m	Exposure	NRD			NSO			NSI		
			Mean	min	max	Mean	min	max	Mean	min	max
SHADE-LOW	1800	NW	2.6	0	6	14.0	10	20	14.2	10	17
SHADE-MID	1950	NW	2.4	0	6	14.9	11	20	14.6	11	18
SUN-LOW	1750	E	2.8	1	6	13.9	10	16	13.5	9	16
SUN-MID-1	1900	E	2.9	2	7	14.5	10	20	13.9	10	16
SUN-MID-2	1950	E	3.9	1	10	14.9	12	19	15.1	12	18
HIGH-1	2000	E	3.0	0	8	14.4	8	18	14.5	10	18
	ANOVA		$p<0.0001$			$p<0.0001$			$p=0.477$ n.s.		
	Regression against altitude		$R^2=0.08$; $p=0.49$			$R^2=0.012$; $p=0.93$			$R^2=0.011$; $p=0.80$		

Legend: NRD – number of resin ducts; NSO – number of stomata per unit length on the abaxial surface; NSI – number of stomata per unit length on the adaxial leaf surface.

least one of the locations for collection of leaves for the previous study was close to our locations and therefore less difference is expected.

We found similar tendency for increase of the number of stomata on the abaxial leaf face per length unit (i.e. NSO) with the increase of altitude (Table 2). Highest mean values were found again for the sun-exposed slope at altitude of 1950 m a.s.l. Values were higher on the shady slope at comparable altitudes. No altitude or exposure – dependent variability was observed for the parameter number of stomata per unit length on the adaxial leaf surface (NSI). Because the results for stomata are novel for the country, we cannot comment them in respect to previous findings.

Conclusion

Old-growth *Pinus heldreichii* forests with age of the dominant trees of up to 800 years can be found in Banderitza valley. They allow construction of reliable tree-ring chronologies, which can be used for proxy climate reconstructions. The lower parts of the valley slopes are occupied by younger forest. Among the possible reasons for this are disturbances such as fires and avalanches. Our data provide a clue for a possible fire at the 1910-s. No differences were found between tree ring chronologies obtained from sun-exposed and shady slopes. This is a clue that the climate-related variability of *Pinus heldreichii* tree rings is rather stable and less dependent on factors like exposure.

We could not find evidence that leaf anatomical features are altitude or exposure dependent. Although the number of resin ducts and the number of stomata on the abaxial leaf surface were found to increase with the increase of altitude, further studies from other locations are needed to draw more sound conclusions.

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