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SEASONAL RADIAL GROWTH OF BLACK PINE (*Pinus nigra* Arnold) FROM BOSNIA AND HERZEGOVINA, MONITORED BY THE PINNING METHOD AND MANUAL BAND DENDROMETERS

SEZONSKA DEBELINSKA RAST ČRNEGA BORA (*Pinus nigra* Arnold) IZ BOSNE IN HERCEGOVINE, SPREMLJANA Z METODO PINING IN ROČNIMI DENDROMETRI

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ABSTRACT

Despite numerous dendroclimatological investigations into different tree species from Bosnia and Herzegovina, information is lacking on intra-annual wood formation patterns, which would help us to interpret the climate signal in tree rings better. Using the pinning method and manual band dendrometers, we investigated the seasonal dynamics of radial growth of black pine (*Pinus nigra* Arnold) trees in two successive growing seasons: 2011 and 2012. The up to 60-year-old trees grew in a stand at the base of a hill in the western, mountainous part of the Balkan Peninsula. The seasonal dynamics of wood formation and final number of cells differed between the studied years. Wood formation started in both years in early to mid-March. Differences were noticed in the wood production culmination; in 2011 it occurred at the end of May and beginning of June in 2012 and 2011, respectively. Xylem growth finished in 2012 in the middle of August and in 2011 in the middle of September. Based on the first derivative of the Gompertz function calculated rate of xylem growth was lowest in 2011. The dendrometers recorded a slow increment rate in spring, higher in summer and a decreasing rate again in the late summer in both growing seasons. In comparison with pinning, dendrometers showed a delay in the start of radial growth of up to 20 days in 2012. Additionally, dendrometers showed an increase in stem girth after the end of both growing seasons, when wood formation was already completed. Deviations between the two methods could be ascribed to the influence of water storage dynamics in the main stem and numerous structural processes in bark tissue, which are captured in dendrometer data. The influence of weather conditions on xylem phenology is also indicated by differences between the two studied years, although it is difficult to identify the influence of particular short-term weather events.

Key words: pinning, manual dendrometers, radial growth, *Pinus nigra*, cambium, Balkan Peninsula

IZVLEČEK

Kljub velikemu številu dendroklimatoloških raziskav o različnih drevesnih vrstah iz Bosne in Hercegovine primanjkuje informacij o sezonskem nastajanju lesa, ki bi pripomogle k boljši interpretaciji klimatskega signala v branikah. Z uporabo metode pining in ročnih dendrometrov smo raziskali sezonsko dinamiko debelinske rasti dreves črnega bora (*Pinus nigra* Arnold) v dveh zaporednih rastnih sezonah: 2011 in 2012. Do 60 let stara drevesa so rastla v sestoju ob vznožju planote v zahodnem, goratem predelu Balkanskega polotoka. Med obema letoma je bilo zaznati razlike v sezonski dinamiki nastajanja lesa in končnem številu celic. V obeh letih se je les pričel nastajati v začetku oziroma sredi marca. Razlike so bile opažene v najvišji stopnji nastajanja lesa: v letu 2011 je nastopila na dan v letu (DVL) 153 in na DVL 145 v letu 2012; prav tako pa tudi v koncu ksilemske rasti: končala se je okoli DVL 223 v letu 2012 in okoli DVL 255 v letu 2011. Na podlagi prvega odvoda Gompertzove funkcije je bila stopnja ocenjene ksilemske rasti nižja v letu 2011. Dendrometri so v obeh rastnih sezonah zabeležili nizko stopnjo priraščanja spomladi, višjo poleti in ponovno nižjo jeseni. V primerjavi z metodo pining so dendrometri zabeležili zamik v začetku debelinskega priraščanja do 20 dni v letu 2012. Poleg tega so dendrometri zabeležili povečan obseg debla po koncu rastne sezone v obeh rastnih sezonah, ko se je nastajanje lesa že zaključilo. Razlike med obema metodama lahko pripišemo vplivu gibanja vode v ksilemu in floemu ter številnim strukturnim procesom v skorji, ki so zabeleženi v meritvah dendrometrov. Vpliv vremenskih razmer na nastajanje ksilema je prav tako povezan z razlikami med obema rastnima sezonama, čeprav je težko opredeliti posledice kratkotrajnih vremenskih pojavov.

Ključne besede: metoda pining, ročni dendrometri, debelinska rast, *Pinus nigra*, kambij, Balkanski polotok

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1 UVOD

1 INTRODUCTION

Black pine (*Pinus nigra* Arnold) is an ecologically and economically important tree species that grows in the northern part of the Mediterranean region and is widespread on the Balkan Peninsula. It is often present on extreme sites, where conditions for other tree species are too extreme (Vidaković, 1991). In dendroclimatological terms, *P. nigra* is interesting since it often has a strong climate signal in tree-ring widths (e.g., Levanič et al., 2012; Szymczak et al., 2012; Levanič et al., 2015). Tree-ring chronologies of *P. nigra* were used in several studies to study climate-growth relationships or to reconstruct climate in the western (Martin-Benito et al., 2011) and eastern Mediterranean basin (Touchan et al., 2003; Sevgi and Akkemik, 2007), on the margins of its natural areal, for instance in Austria and Romania (Strumia et al., 1997; Leal et al., 2008; Levanič et al., 2012), and in its core natural distribution on the Balkan Peninsula (Poljanšek et al., 2013; Levanič et al., 2015).

In the western Balkan Peninsula, tree-ring widths are positively influenced by higher spring temperatures and negatively by higher summer temperatures (Poljanšek et al., 2013). Positive spring temperatures are undoubtedly connected to the earlier onset of cambial xylem cell production after winter dormancy, while summer droughts result in premature cessation of cambial cell divisions and reduction of the cambial cell production rate, which results in narrow rings. Levanič et al. (2012) established a statistical relationship between the tree-ring width of *P. nigra* from Romania and two meteorological parameters – monthly sum of precipitation and standardised precipitation index. Poljanšek and Levanič (2012) explored the possibility of the minimum blue intensity method in *P. nigra* from Slovenia. An overview of dendroclimatological studies in the Balkan Peninsula for *P. nigra* is given by Poljanšek and Levanič (2015).

Despite numerous dendroclimatological investigations into *P. nigra*, information on its intra-annual wood formation patterns is lacking with just a few wood-formation studies from Balkan Peninsula (Semeniuc et al., 2014). The seasonal dynamics of xylem growth is valuable information, since it helps in understanding growth patterns and interpreting the calculated climate signal from tree-ring widths in climate reconstructions (Rossi et al., 2014). It also helps to understand the mechanisms of wood formation and their relations to climate on a finer temporal scale. Based on wood formation monitoring, we can understand the impact of seasonal variation in environmental condition on wood formation phenology, xylem cell differentiation and rate of cell production. Intra-annual wood formation phenology monitoring was therefore performed, with repeated sampling of the developing xylem increment and with manual band dendrometers recording variations in stem diameter (e.g., Deslauriers et al., 2007; Mäkinen et al., 2008; Zweifel et al., 2016).

In order to understand the intra-seasonal dynamics of *P. nigra* radial growth better, also in the light of previous investigations of the climate signal embedded in tree-ring widths of *P. nigra*, we set our goals as: 1) to assess wood formation phenology (i.e., onset, maximum and cessation of wood formation) in *P. nigra* in two successive growing seasons, 2011 and 2012; 2) to compare cellular observations of wood formation dynamics with manual band dendrometer measurements, and 3) to compare xylem phenology and weather conditions.

2 MATERIALS AND METHODS

2 MATERIALI IN METODE

2.1 Sampling site

2.1 Opredelitev rastišča

Our site was located in Bosnia and Herzegovina at 390 m a.s.l. at the base of a hill in the upper part of the River Neretva valley (N 43.51, E 18.10). The site is characterized by its southern aspect, dolomite bedrock and shallow soil. The location in the Dinaric mountains causes winters to receive an extraordinary amount of precipitation with low temperatures. In late winter or early spring, snowfall can occur due to the vicinity of mountain peaks and the collision of northern cold air masses with humid air from the coast, resulting in orographic precipitation. In summer, the area receives only minimum precipitation which, in combination with high air temperatures, results in heat waves and a danger of forest fires.

2.2 Weather data

2.2 Vremenski podatki

From the first day of the pinning, weather data was recorded at the site every 15 minutes, including air temperature and relative air humidity (Table 1). Weather variables were measured using a VoltCraft DL-120 TH temperature and humidity data logger, fitted in a radiation shield and mounted on a 2 m high pole. The start of weather measurements in spring 2011 therefore corresponded to the first day of pinning. Mean daily temperature and relative humidity were calculated as the mean value of the daily minimum and maximum observations. In addition, we included gridded daily precipitation data provided by the Joint Research

Centre, Agri4Cast Data Resources Portal (http://agri4cast.jrc.ec.europa.eu/DataPortal/). To explain the effect of short-term weather events on radial growth, 10-day moving averages were calculated for all climate variables (Figure 1). Only in the 2012 season we had a complete temperature data set from 1st January on, which enabled calculation of growth degree days (GDD) (McMaster and Wilhelm, 1997). Based on earlier research of cambium activity on *P. sylvestris* (GDDs were also calculated by Schmitt et al., 2004), the T base was set to 5 °C.

Throughout a comparable period; DOY 70–270, periods of deviations in mean temperature could be observed between the two seasons, and periods with a similar decrease or increase in mean air temperatures (Figure 1). Relative humidity and precipitation data show similar patterns. There were also periods of similar decrease in mean air temperature, for example around DOY 110 but, at the same time, the seasons differed in relative humidity and precipitation measurements. Hot spells in summer were more frequent in 2012; from DOY 70–270 there were 82 days with a maximum air temperature higher than 30 °C, while in 2011 there were only 56 days.

2.3 Sampling, sample preparation and data analysis

2.3 Vzorčenje, priprava vzorcev in analiza podatkov

A homogeneous group of six *P. nigra* trees, growing in close proximity to each other was selected. Trees were on average up to 60 years old, 22 m high, 36 cm in diameter at breast height and with 35 cm of mean annual height increment (Poljanšek et al., 2015). The trees had no sign of crown or bark damage. On each selected tree, manual band dendrometers (D1 Permanent Tree Girth, UMS, Germany) were installed at breast height of the stem, about 10 cm above the pinning line, in order to avoid any possible wound reactions to the pinning experiment and contamination with resin. Each time the stems were pinned, measurements of stem girth were recorded with 0.1 mm accuracy. Pinning was performed using a needle, 1.75 mm wide at its thickest part. Two pinning holes were set in the stem of each tree on the same experimental date. The holes were at least 3 cm apart to avoid a wound response from neighbouring samples. The holes were marked and numbered. The pinning experiment started on 11th March in 2011 (DOY 70) and ended on October 5th (DOY 278), while in 2012 it started on March

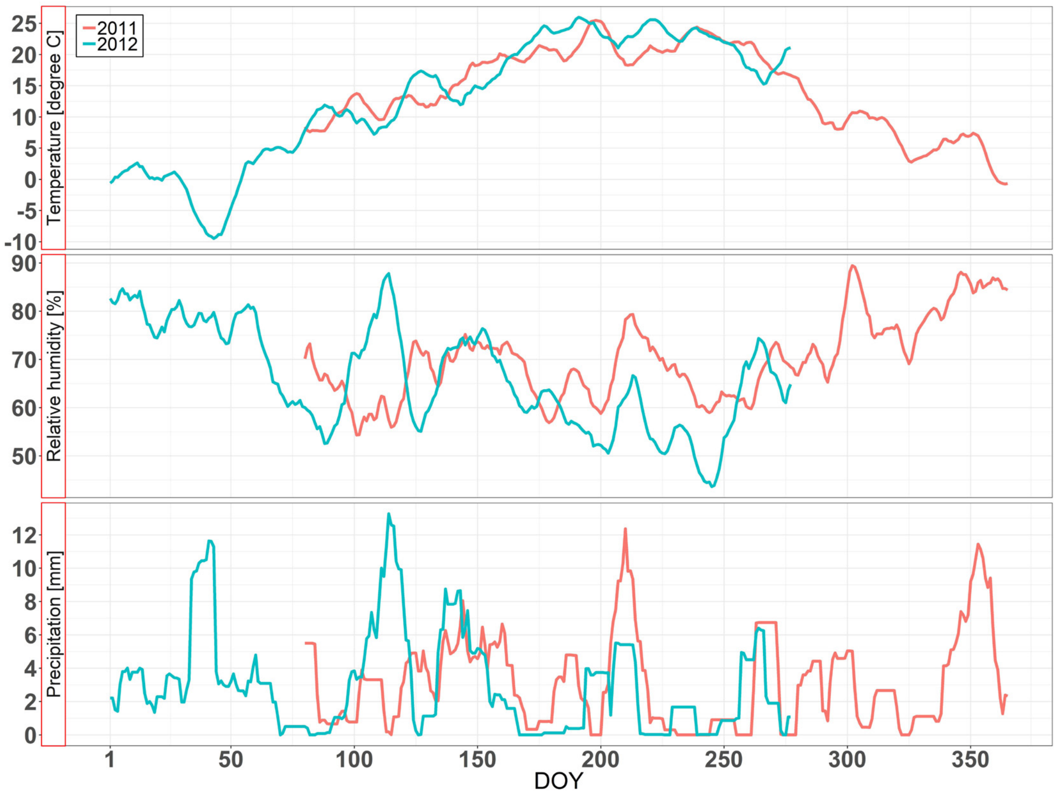

Fig. 1: Mean air temperature, mean relative air humidity and sum of precipitation calculated backward using a 10-day window for the 2011–2012 period

Slika 1: Povprečna temperatura zraka, povprečna relativna zračna vlažnost in vsota padavin, izračunane z uporabo 10-dnevnega vzvratnega okna za 2011–2012

Table 1: Data on monthly average air temperature, relative humidity and sums of precipitation in 2011 and 2012

Preglednica 1: Podatki o povprečnih mesečnih temperaturah zraka, relativni zračni vlažnosti in vsotah padavinah za leti 2011 in 2012

		Temperature / *temperature* [°C]						Relative humidity / *relativna zračna vlažnost* [%]						Precipitation *padavine* [mm]	
		min / *min*		mean / *povprečje*		max / *max*		min / *min*		mean / *povprečje*		max / *max*		sum / *vsota*	
Month / *mesec*	**DOY / *DVL***	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
March / *marec*	59–89	–2.3	–6.2	8.7	8.3	22.0	24.1	17	17	68	59	98	97	61.6	11.4
April / *april*	90–119	0.2	–3.9	12.1	10.5	25.8	31.2	15	11	60	74	97	100	72.5	190.2
May / *maj*	120–150	0	1.6	15.1	14.6	28.7	32.2	20	14	71	70	99	99	138.0	146.7
June / *junij*	151–180	8.9	8.5	20.0	21.7	35.7	38.5	23	19	66	62	100	97	90.3	17.1
July / *julij*	181–211	9	8.8	21.4	23.8	37.1	38.4	26	10	69	57	100	98	153.2	95.3
August / *avgust*	212–242	7.7	8.6	22.2	23.5	38.4	39.5	16	10	66	51	99	97	10.0	17.1
September / *september*	243–272	6	2.4	19.9	19.2	35	34	22	8	66	64	99	98	76.4	72.6

28th (DOY 88) and ended on October 2nd (DOY 276). Pinning was repeated periodically, on average every 11 days. Sampling and stem girth measurements were finished at the end of the second growing season with the trees being felled.

Samples containing wounded tissue were removed from the discs and processed for observations with a light microscope. The sample blocks (30 × 10 × 10 mm) consisted of the inner part of the living bark, the cambium and outer xylem, with at least three youngest increments. After removal, the tissues were fixed in formalin-ethanol-acetic acid solution and after one week dehydrated in a graded series of ethanol (30 %, 50 % and 70 %). Permanent cross-sections of tissues of approximately 25 μm in thickness were prepared using a "G.S.L. 1" Sledge microtome (©Gärtner and Schweingruber; design and production: Lucchinetti, Schenkung Dapples, Zürich, Switzerland) with disposable blades. Sections were stained with a water mixture of safranin (Merck, Darmstadt, Germany) (0.04 %) and astra blue (Sigma-Aldrich, Steinheim, Germany) (0.15 %), and finally mounted in Euparal (Waldeck, Münster, Germany). Histometric observations were performed under an Olympus BX51 (Tokyo, Japan) light microscope and analysed with the Nikon NIS-Elements Basic Research v.2.3 image analysis system (Tokyo, Japan).

Xylem increment formed until the moment of the needle injury was determined by counting the tracheids between the callus and the growth ring boundary between the xylem increments formed in the current and previous years. Cambial cells with small radial dimension, located in the callus, were not included in the analyses. We counted the number of newly formed xylem cells in at least three radial files and then averaged (Seo et al., 2007). In addition, we counted the cells of the final annual xylem increment to the left and to the right of the callus to calculate the relative weekly xylem increment, thus reducing the effect of variability in the number of cells, which is typical around the stem circumference. Using the R software (R Core Team, 2019), cell number increase and change in radial growth were fitted with a Gompertz function (Rossi et al., 2003). The first derivatives of each Gompertz function were calculated to identify the day on which the rate of cambium production culminated and to view differences in xylem increment during the growing season for each method and year (Rathgeber et al., 2011). For comparison, the number of newly developed cells per day was calculated, by dividing the difference in the number of cells by the number of days between two consecutive samplings. The onset of xylem formation was defined when the first earlywood tracheids were at least partially formed, while the end of wood formation was determined when the final number of fully lignified cells was recorded. The transition from early to latewood was determined visually, as cell lumen was smaller than twice the cell wall (e.g., DeSoto et al., 2011).

3 RESULTS

3 REZULTATI

3.1 Seasonal dynamics of wood formation

3.1 Sezonska dinamika formiranja lesa

At the beginning of the pinning experiment in both seasons, wood formation had already begun, as indicated by 2.1 and 2.4 cells produced in 2011 and 2012, respectively (Figure 2). Based on the number of days between the first and second sampling in each of the seasons and on the low number of counted newly produced xylem cells in the early stages of development (i.e., cell expansion), wood formation was estimated to have started 12–15 days earlier, around DOY 59–56 in 2011 and DOY 76–73 in 2012 (mid-March). Calculation of GDD in the 2012 season showed that days from

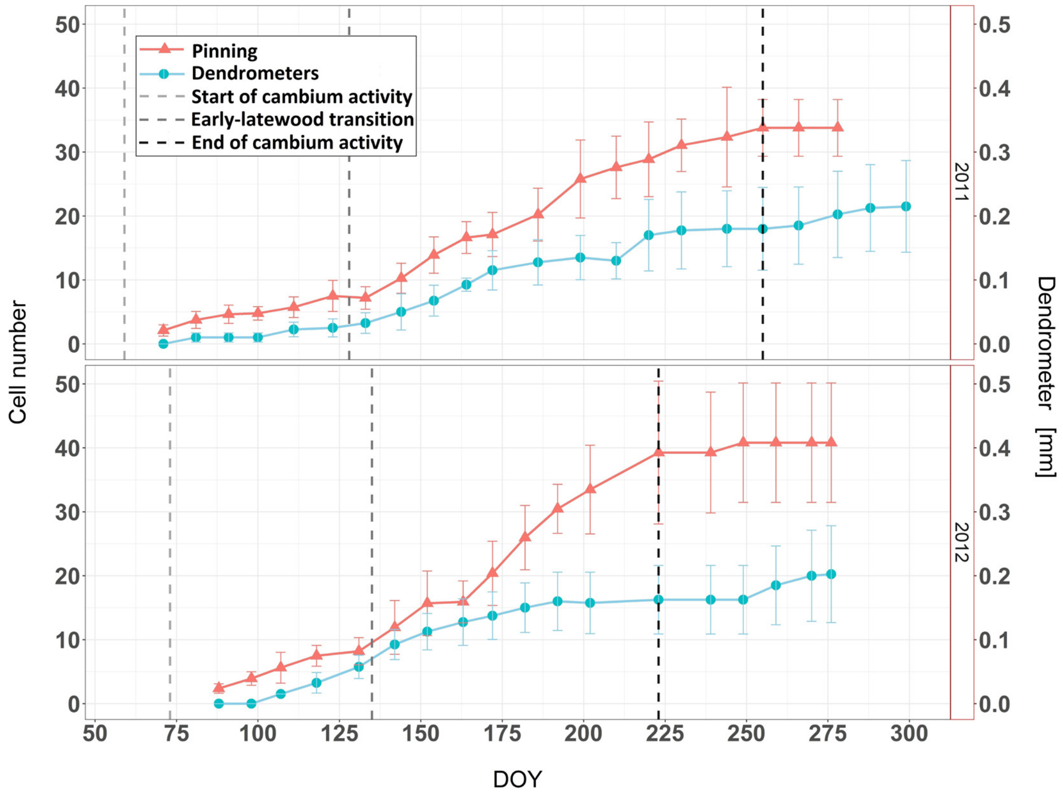

Fig. 2: Comparison of seasonal dynamics of xylem growth expressed in number of cells using pinning method (triangles) and radial increment increase expressed in millimetres using dendrometer data (dots) in 2011 (upper graph) and in 2012 (lower graph). Vertical dashed lines represent time of the onset of cambium activity, early-latewood transition and the end of cambium activityo.

Slika 2: Primerjava sezonske dinamike rasti ksilema, izražene kot število celic z uporabo pining metode (trikotniki), in debelinski prirastek, izražen v milimetrih, izmerjen z dendrometri (pike) za leto 2011 (slika zgoraj) in 2012 (slika spodaj). Navpične črtkane črte ponazarjajo čas začetka kambijeve aktivnosti, prehod iz ranega v kasni les in konec kambijeve aktivnosti.

DOY 68 to 73, adjacent to the calculated start of wood formation, had a value of 55 °D. The transition from earlywood to latewood occurred in the second quarter of May, on DOY 128 (2011) and DOY 135 (2012), when 22 % and 27 % of the annual xylem increment had been formed in 2011 and 2012, respectively. The transition therefore occurred around 59 (2011) and 62 (2012) days after the onset of xylem growth. In the time of transition, the 10-day averages of daily mean temperature in 2011 and 2012 were 12.0 °C and 14.8 °C, daily mean RH 71 % and 68 %, respectively. The culmination of daily radial stem growth occurred earlier in 2012 than in 2011, despite an earlier start of wood formation in 2011 (Figure 3). Based on the Gompertz function (Figure 3), the culmination of daily radial stem growth occurred in 2011 around DOY 153 (start of June) with 0.32 cells/day. This was 25 days after the earlywood to latewood transition and when 41 % of the 2011 xylem ring had been formed. The overall highest increase in cell number in 2011 was observed for DOY 199 (second half of July) with 0.38 cells/day. This increase was probable related to higher average temperatures prior to this culmination (Figure 4).

In 2012, the culmination of daily radial stem growth calculated by the Gompertz function, with 0.38 cells/day, was dated to DOY 145 (end of May), which is one week earlier than in the 2011 season, and 10 days after the earlywood to latewood transition in 2012, when 31 % of the annual xylem increment had been formed. However, the overall highest increase in 2012 in cell number was measured on DOY 172 (22nd June) with 0.36 cells/day. Finally, the 2011 growing season was longer and ceased one month later, by DOY 255 (middle of September), but had on average fewer cells produced; 34, compared to 40 cells formed by DOY 223 in the 2012 season.

3.2 Comparison of pinning and dendrometer measurements

3.2 Primerjava metode pining in meritev dendrometrov

The increase in stem diameter obtained by manual band dendrometers showed different temporal dynamics than wood formation in both growing seasons (Figures 2 and 3). In 2011, the increase in cell number, based on the pinning method and the tree's circumfer-

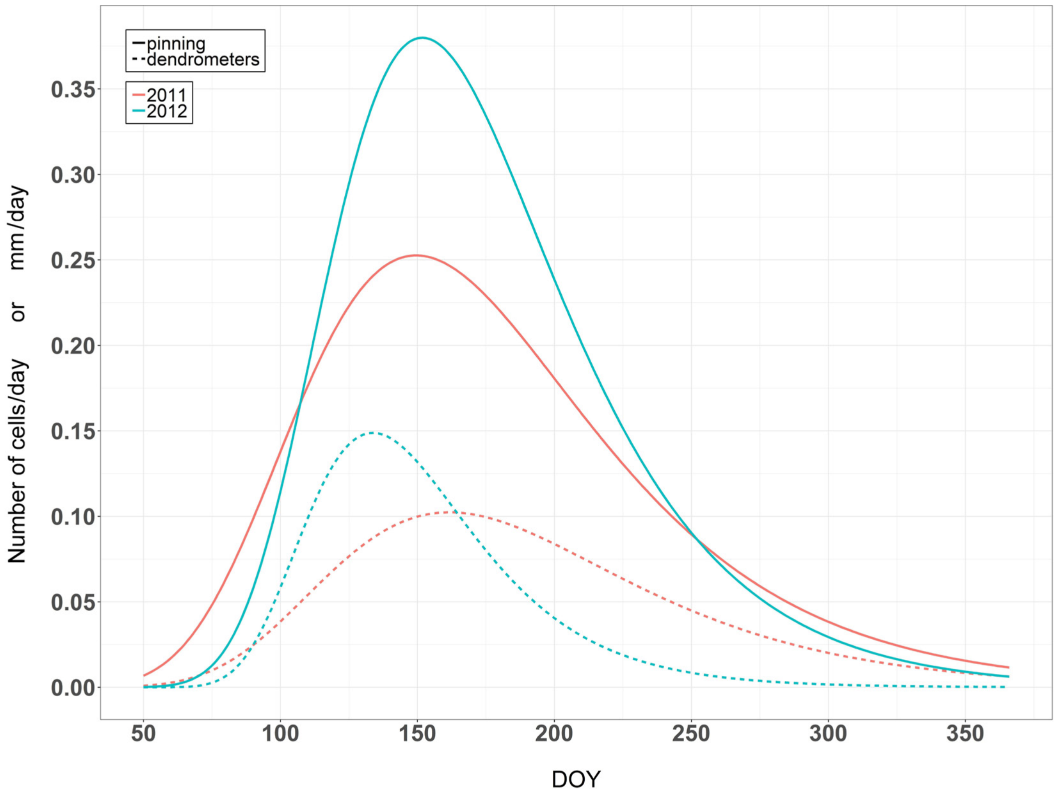

Fig. 3: First derivation of Gompertz function, based on pinning measurements and band dendrometers, shows the culmination of wood formation in 2011 and 2012

Slika 3: Prvi odvod Gompertzove funkcije na podlagi meritev metode pining in ročnih dendrometrov kaže najvišjo stopnjo nastajanja lesa v letih 2011 in 2012

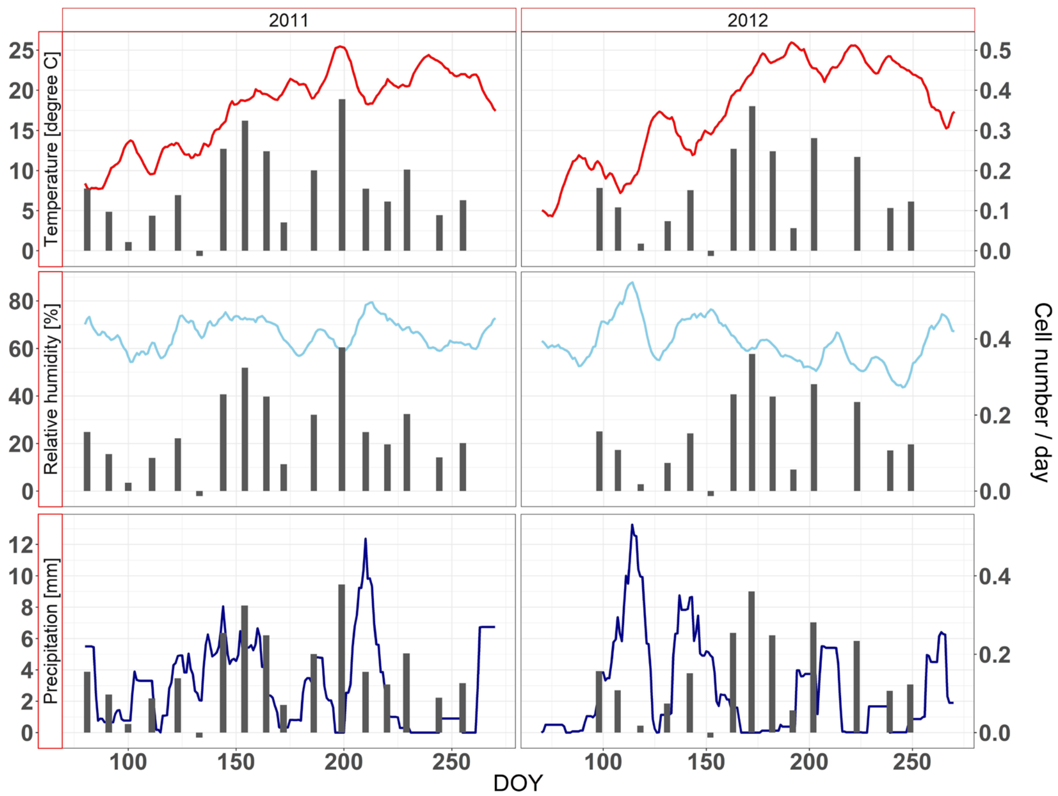

Fig. 4: Comparison of cell number increase (bars) in the period between two consecutive samplings and 10-days moving averages for mean daily temperatures, relative humidity and sum of precipitation (lines) for the 2011 and 2012 seasons

Slika 4: Primerjava števila novonastalih celic (stolpci) med dvema zaporednima vzorčenjema in 10-dnevne drseče sredine za povprečne temperature, relativno zračno vlažnost in vsote padavin (krivulje) v letih 2011 in 2012 ence, measured by band dendrometer in millimetres, was synchronous until DOY 100 (middle of April). From this day on, dendrometer data documented a higher rate of increase until DOY 200 (late July), when the dendrometers recorded a decrease in stem diameter (Figure 2). After DOY 220, an increment increase was recorded once more, which continued even after the production of the last xylem cells on DOY 250, as observed by the pinning technique. In 2012, a noticeable delay in increment increase obtained by dendrometers was found until DOY 110 (late April). The same as in 2012, the increase in band dendrometers continued after the end of xylem growth.

4 DISCUSSION

4 RAZPRAVA

4.1 Seasonal dynamics of wood formation

4.1 Sezonska dinamika nastajanja lesa

We investigated the phenology of wood formation (i.e., onset, culmination of daily radial stem growth and cessation of xylem ring growth) of *P. nigra* from Bosnia and Herzegovina in two successive growing seasons. In both studied seasons, wood formation had already begun before our first sampling. Nevertheless, based on the low number of formed xylem cells at the onset of the pinning experiment and data obtained for other conifers in different locations (e.g., de Luis et al., 2011; Gričar et al., 2014), we estimated that xylem growth had started 12–15 days before the start of the experiment. In 2011, wood formation start was assessed to be around DOY 59 (end of February) and in 2012, DOY 73 (early March), which is about two weeks later than in the previous year. The onset difference could be explained by the weather conditions at the beginning of the growing seasons. In 2012, cold winter temperatures (on 9th of February even –19.3 °C) and late snow seemed to delay onset of radial growth. The influence of cold weather in summer 2012 (between DOY 140 and 160) may also have influenced cambial cell production in terms of a reduced growth rate (Figure 1).

In 2012, we were able to calculate GDD at the start of xylem growth: days adjacent to the calculated start of wood formation had a value of 55 °D. In the case of *Fagus sylvatica* (Prislan et al., 2013) and *Picea abies* (Gričar et al., 2014) in Slovenia, higher values were recorded for the onset of xylem growth: *F. sylvatica*: 73–147 °D and *P. abies*: 85–217 °D. In both species, GDD values were inversely proportional to the elevation, demonstrating that lower amounts of heat accumulation were needed at higher (1,200 m a.s.l.) than at lower (400 m a.s.l.) elevations. In contrast, (Schmitt et al., 2004) detected similar GDD values (80–90 °D) in *Pinus sylvestris* and in *Betula pendula* (110–120 °D) along a latitudinal gradient in the boreal forests of Finland. The large variations in GDD values also indicate that other factors than temperature influence the start of xylem growth.

A pinning experiment performed during the only two growing seasons is not ideal for obtaining general information on intra-annual xylem growth of *P. nigra* at the selected site. Ideally, monitoring over several growing seasons, including extreme years and trees from different elevations, would give us much better insight into xylem development and its relation to environmental factors. However, the information gathered is valuable as despite the numerous dendroclimatological studies on the climate-growth relationship of *P. nigra*, knowledge of its intra-annual xylem growth patterns is generally lacking. We were able to determine the main period of xylem ring development, which will help us pinpoint months that are crucial for the radial growth of *P. nigra* and help us to improve tree-ring based climate reconstructions to reconstruct only months (or periods) that are the most important for the growth of *P. nigra* in the region. With an expected air temperature rise, and a decrease or changed pattern in precipitation in the *P. nigra* distribution area (IPCC, 2016), it is important to predict the impact of changing climate on tree growth in the future.

In this study, intra-annual wood formation data confirmed the start of cambial cell production in March (Figure 2). The calculated dependence on summer precipitation in connection with soil moisture and sunshine hours (Poljanšek et al., 2013), forecast *P. nigra* with smaller radial growth due to the expected reduction in precipitation throughout the growth season (IPCC, 2016). On the other hand, higher spring and autumn air temperatures could prolong the species' growth season. However, *P. nigra* could in future react similarly to some pine species from the Mediterranean, e.g., *Pinus halepensis* (Prislan et al., 2016), whereby in the middle of the summer moisture stress, species reduce cambium productivity due to unfavourable growth conditions. The reduction of xylem formation in 2012 between two consecutive samplings at the beginning of June (DOY 152–163), with only 0.2 cells/day produced on average (Figure 4) remains unexplained. More detailed investigation of this is needed in the future.

The transition from earlywood to latewood occurred in both years by mid-May. The morphology of tracheids in earlywood and latewood depends on factors regulating developmental patterns and factors that affect rates of cell division and differentiation (Cartenì et al., 2018). Concentration gradients of auxin and soluble sugars in the cambial region define the developmental patterns of xylem cells (Uggla et al., 2001). Latewood formation is associated with a slower rate of cell division, decreased rates and duration of cell expansion and a longer duration of secondary wall formation (Whitmore and Zahner, 1966; Skene, 1972). In addition, external factors such as soil water content greatly determine tracheid size through their effect on turgor pressure (Tyree and Sperry, 1989; Ryan et al., 1994).

The difference between the day of maximum growth and the end of cambial production of xylem cells between seasons was 8 and 32 days, respectively. In 2012, a slight shift of maximum growth rate towards the beginning of the growing season could be explained by the timing of xylem formation culmination to the most favourable weather factors. According to literature, maximum growth rate is strongly related with photoperiod. Therefore, this milestone of xylem formation usually occurs during the summer solstice (e.g., Rossi et al., 2006). Our records on weather show that hot spells in summer occurredin both seasons but were more frequent in 2012 and presumably coincided with the time of the most active wood formation. For illustration, in 2011, the highest recorded daily maximum air temperature was on DOY 194: 37.1 °C, and on DOY 240: 38.4 °C. Furthermore, in 2012 the maximum air temperature was even higher: 38.5 °C on DOY 182 and 39.5 °C on DOY 220 (Table 1).

4.2 Comparison of pinning and manual band dendrometer methods
4.2 Primerjava pining metode in meritev ročnih dendrometrov

The pinning technique is based on a very small injury of the cambium with a needle and its response to the injury (Wolter, 1968; Yoshimura et al., 1981) and is very common in wood formation studies (Schmitt et al., 2000; Seo et al., 2007). Manual band dendrometers precisely measure and monitor tree stem diameter radius changes (Dobbertin et al., 2016) and therefore offer complementary information to intra-annual wood formation data provided by the pinning method. Information provided by the pinning technique and manual band dendrometer data differ to some extent. This can be partially explained by different units compared (millimetres vs. cell numbers), but there are other factors that need to be considered as well. While pinning provides information on cell number, manual dendrometers measure changes in stem diameter, comprising xylem and phloem increments and secondary changes in phloem tissue as well as the activity of cork cambium. The difference can be ascribed to numerous other secondary changes that occur in older secondary phloem, as well as to phloem growth, cell collapse and shrinkage due to dry weather. In cases of very narrow xylem increments of trees with thick bark, inaccuracy of the data is fairly high (Gričar et al., 2015). In addition, water fluctuations in the secondary tissues greatly influence stem radial variations (e.g., Deslauriers et al., 2003; Deslauriers et al., 2007; Oberhuber and Gruber, 2010). Comparison of the dendrometer and pinning data revealed a noticeable delay in the start of 2012 xylem growth of up to three weeks, measured by dendrometers. A similar decrease in dendrometer readings at DOY 210 in the 2011 season may also indicate colder air temperatures and perhaps the physical contraction of bark, which influenced the circumference measurements. On the other hand, at DOY 200 in the 2012 season, a decrease in dendrometer readings and a slight reduction in cell number increase was timed to a heat wave and drought.

There was also an anomaly, recorded at the end, when the dendrometers showed an increase in circumference, although the cambium was no longer active. Based on the pinning results, xylem growth had stopped, which clearly demonstrates that the activity of the cambium is very difficult to follow solely with dendrometer measurements. The reasons for the anomaly in dendrometer readings may be related to stem water status and the bark being saturated with water, and/or the stem being thicker due to sap flow (Mäkinen et al., 2008). An influence of water storage fluctuations has previously been reported (Deslauriers et al., 2007). In *P. sylvestris* in Finland, for example, an increase in stem girth measured by dendrometers in the period when no wood formation occurred, was also associated with evapotranspiration and water uptake (Mäkinen et al., 2008). Measuring soil moisture would shed some light on this matter, since there is strong influence of available water on radial growth (Zweifel et al., 2006).

5 CONCLUSIONS
5 ZAKLJUČKI

Using the pinning method, we obtained data on wood formation phenology in *Pinus nigra*. It can be concluded that the seasonal dynamics of wood formation and increment widths differed between the studied years and that weather influences xylem growth, which was shown in differences at the start of cambium activity and, especially, in the culmination of daily radial stem growth and end of wood formation between the two seasons. However, it is difficult to identify the influence of particular short terms of hot or cold weather events, although the results suggest that both hotter and colder than usual summer temperatures can cause a temporary decrease in cell production. Dendrometers recorded the correct start of activity in the 2011 season but, for an unknown reason, were late in timing in 2012. Dendrometers failed to determine the right start or end of wood formation activity, because their data capture numerous other factors associated with the processes in bark tissue and water uptake. The results on the timing of cambium activity are analogous to the calculated influence of climate factors from previous investigations of *P. nigra* in this region.

6 SUMMARRY IN SLOVENIAN LANGUAGE

6 POVZETEK V SLOVENSKEM JEZIKU

Črni bor (*Pinus nigra* Arnold) je ekološko in ekonomsko pomembna drevesna vrsta, ki jo najdemo na območju severnega Mediterana in je pogosta na Balkanskem polotoku. Kljub velikemu številu dendrokronoloških raziskav je malo znanega o sezonskih značilnostih nastajanja lesa pri črnem boru, kar je še posebej pomembno za interpretacijo klimatskega signala v branikah. Z uporabo metode pining in ročnih dendrometrov smo raziskovali sezonsko dinamiko debelinske rasti pri šestih drevesih črnega bora v dveh zaporednih rastnih sezonah: 2011 in 2012. Izbrano rastišče leži ob vznožju planote v zahodnem, goratem predelu Balkanskega polotoka ob dolini reke Neretve (N 43.51, E 18.10). Sezonsko dinamiko debelinske rasti smo spremljali z metodo pining in ročnimi dendrometri. Metoda pining temelji na majhni poškodbi kambija in njegovem odzivu na vbod z iglo premera 1–2 mm, s čimer je označen ksilemski prirastek, ki je nastal do trenutka poškodbe. Poškodbe z iglo in popis ročnih dendrometrov smo opravili v približno 10-dnevnih intervalih. Na ploskvi smo prav tako spremljali temperaturo zraka in relativno zračno vlažnost, podatke o padavinah smo pridobili s podatkovnega portala Skupnega raziskovalnega središča Evropske komisije – Agri4Cast (http://agri4cast.jrc.ec.europa.eu/DataPortal/) (preglednica 1, slika 1). Sezonska dinamika nastajanja lesa in končno število celic sta se razlikovala med obema letoma. V obeh letih se je formiranje lesa pričelo v začetku oziroma sredi marca: leta 2011 na dan v letu (DVL) 59–56 in leta 2012 na DVL 76–73. Razlike so bile opažene v najvišji stopnji nastajanja lesa: v letu 2011 je nastopila na DVL 153 in na DVL 145 v letu 2012. Kambijeva aktivnost se je leta 2012 končala okoli DVL 223 in v letu 2011 okoli DVL 255 (slika 2). Na podlagi prvega odvoda Gompertzove funkcije je bila stopnja ksilemske rasti nižja v letu 2011 (slika 3). Dendrometri so v obeh rastnih sezonah zabeležili, z majhnimi zamiki, nizko stopnjo priraščanja spomladi, višjo v poletju in ponovno nižjo jeseni (slika 2). V primerjavi z metodo pining so dendrometri zabeležili zamik v začetku debelinskega priraščanja do 20 dni v letu 2012. Poleg tega so dendrometri zabeležili povečanje obsega debla po koncu rastne sezone v obeh rastnih sezonah, ko je bilo formiranje lesa že zaključeno. Vpliv vremenskih razmer na nastajanje ksilema je prav tako povezan z razlikami med obema rastnima sezonama (slika 4), čeprav je težko neposredno povezati spremembe dinamike debelinske rasti in kratkotrajne vremenske pojave. Ugotovili smo, da se meritve debelinske rasti na podlagi metode pining in z ročnimi dendrometri pomembno razlikujejo. Razlike smo zabeležili pri začetku in koncu kambijeve aktivnosti, kot tudi pri določanju točke najhitrejše rasti. Razlike med obema metodama lahko pripišemo številnim strukturnim procesom v skorji in vplivu dinamike zaloge vode v deblu, ki so zabeleženi v meritvah dendrometrov.

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