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WOOD FORMATION IN NORWAY SPRUCE ON A LOWLAND SITE IN SLOVENIA IN 2015 AND COMPARISON WITH OTHER CONIFERS ALL OVER EUROPE

NASTAJANJE LESA SMREKE NA NIŽINSKEM RASTIŠČU V SLOVENIJI V LETU 2015 IN PRIMERJAVA Z IGLAVCI PO EVROPI

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UDK 630*811.13:174.7 (*Picea abies* L. Karst.)

Original scientific article / Izvirni znanstveni članek

Abstract / Izvleček

Abstract: *We present xylem formation in Norway spruce (Picea abies (L.) Karst.) in 2015 at Panška reka, near Ljubljana, Slovenia (ca. 46°00'N, 14°40'E, 400 m a.s.l.) with a temperate climate. The research was a part of a long-term project, which involves different sites and tree species. We measured the widths of cambium and the formation of xylem growth rings with differentiating cells in postcambial (PC) and secondary wall formation (SW) phases, and mature cells (MT). The results for 2015 were compared with those for previous years (2009–2011) in the same species and site, and with the published data from wood formation studies in conifers from all over Europe. The latter were used in two-factor regression models which confirmed the effects of latitude and altitude on the critical dates of onset, end, and duration of xylem production. The models thus helped us to predict/reconstruct phenology of wood formation in conifers in Europe.*

Keywords: *Norway spruce (Picea abies (L.) Karst.), phenology, cambium, xylogenesis, model, conifers, Europe*

Izvleček: *Študija prikazuje nastajanje lesa smreke (Picea abies (L.) Karst.) v rastni sezoni 2015 na rastišču Panška reka v bližini Ljubljane (pribl. 46°00'N, 14°40'E, 400 m n.v.). Raziskava je del večletnega projekta spremljanja nastajanja lesa več drevesnih vrst na različnih rastiščih. V okviru raziskave smo spremljali širino kambijeve cone ter nastajajoče branike lesa, kjer smo ločili celice v fazah postkambialne rasti (PC) in nastajanja sekundarne celične stene (SW) ter zrele (MT) celice. Rezultate smo primerjali z ugotovitvami študije, opravljene na smreki z istega rastišča v rastnih sezonah 2009–2011 ter s podatki, objavljenimi za več vrst iglavcev z različnih rastišč po Evropi. Primerjave smo uporabili za izdelavo regresijskih modelov z dvema neodvisnima spremenljivkama. Modeli so potrdili vpliv zemljepisne širine in nadmorske višine na kritične datume začetka in konca ter na trajanje nastajanja lesa. Modeli so omogočili rekonstrukcijo fenologije nastajanja lesa iglavcev po Evropi.*

Ključne besede: *smreka (Picea abies (L.) Karst.), fenologija, kambij, ksilogeneza, modeliranje, iglavci, Evropa*

1 INTRODUCTION
1 UVOD

Wood production (xylogenesis) is closely related to the function and survival of trees and forest ecosystems (Cuny et al., 2012). It is also linked to long-term carbon sequestration, and has a great impact on terrestrial ecosystems (Cuny et al., 2015). Variability and potential changes in the wood formation process thus have ecological and economic implications. Wood, or secondary xylem, is an important tree tissue, with mechanical and physiological functions (Wilson & White, 1986; Cuny et al., 2014). It is produced by cell division in cambium, which is a secondary meristem that produces secondary xylem and secondary phloem. Axial tracheids are the main cells in secondary xylem of conifers. After their formation, tracheids undergo the process of differentiation with the phases of (I) cell enlargement, (II) wall deposition and lignification and (III) programmed cell death (Plomion et al., 2001).

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Secondary meristems are highly plastic. They can respond to environmental stimuli and allow modifications of the plant morphology during its lifecycle in order to adapt to the local conditions (Gričar et al., 2014). Plants try to maximize the use of resources while minimizing the effects of various harmful events. Growth occurs when the conditions are favourable (Cuny et al., 2012). In temperate and boreal biomes, cold temperatures are the main limiting factor of growth (Rossi et al., 2008), and cambium is usually active from spring until autumn, with a shorter growth period in boreal ones. Wood increment is deposited in the form of annual growth rings, with clear growth ring boundaries, which are studied in disciplines like dendrochronology (e.g., Novak, 2016; Wilson & White, 1986). Besides temperature, precipitation, photoperiod and other factors also affect wood formation. A lack of water is, for instance, the main factor impacting growth in xeric ecosystems, while fires, attacks by parasites or herbivores, air pollution or human activity are also important (Novak, 2016; de Luis et al., 2011). All these factors impact the physiological and structural properties of wood, which are important for its use (Humar et al., 2008; Wilson & White, 1986). Therefore, understanding the wood formation process and the factors influencing it can help us predict the performance and survival of trees as well as the properties of the related wood.

Many of the mentioned factors, especially the climatic ones, are influenced by the latitude and altitude of the sites where the trees grow. Martinez del Castillo et al. (2016) showed a clear negative relation between latitude and xylogenesis duration in *Fagus sylvatica* (shorter at lower latitudes) and *Pinus sylvestris* (higher at lower latitudes). Altitude also affects the growth, especially through duration of xylogenesis (Rossi et al., 2008; Cocozza et al., 2016). Understanding relationships between xylem formation phenology and intra-annual climatic/weather conditions could help us to better understand the potential of species to adapt to future climate conditions.

Norway spruce (*Picea abies* (L.) Karst.) is a species whose wood formation dynamics has been investigated on a great variety of sites in Europe. The natural area of this species is mainly in the NE boreal zone and mountainous areas of Europe (EUFORGEN, 2009). Since the 18th century it has been widely cultivated for timber production all over Europe, also outside its natural range, such as on the lowland sites in Slovenia (Zavod za gozdove Slovenije, 2017).

In Slovenia spruce has a great importance from both economic and ecological perspectives (Brus, 2012; Caudullo et al., 2016). Its phenology is affected by climatic change which is in Slovenia mainly characterized by increasingly mild winters which favour the early onset of spring phenology (Čufar et al., 2008, 2012; de Luis et al., 2014), frosts which often interrupt spring phenology (Sinjur, 2017), as well as increasing temperatures with hot spells and frequent drought periods during the vegetation period (Cegnar, 2015 a, b).

The objective of this study was to investigate the wood formation and dynamics of cambial activity in *Picea abies* on a site near Ljubljana, Slovenia in 2015. The second objective was to compare the obtained results with the published data from wood formation studies in *Picea abies* and other conifers from all over Europe, and to apply models to evaluate the effects of latitude and altitude and to predict/reconstruct phenology of wood formation in conifers in Europe.

2 MATERIALS AND METHODS
2 MATERIAL IN METODE

2.1 STUDY SITE, TREES, TISSUES AND ANALYSES
2.1 RASTIŠČA, DREVESA, TKIVA IN ANALIZE

The dynamics of xylem production were studied in six mature Norway spruce (*Picea abies* (L.) Karst.) trees growing at Panška reka, near Ljubljana (ca. 46°00'N, 14°40'E, 400 m a.s.l.). The site is a mixed forest of the Hacquetio-Fagetum typicum type, containing mainly *Fagus sylvatica*, *Acer pseudoplatanus* and *Picea abies*, with dolomites as the predominant rock type in the area (Gričar et al., 2014). The climate at the site is temperate humid. The site is included in the long-term monitoring of xylem and phloem formation and cambial activity in different species and sites which started in 2006 (Čufar et al., 2015).

The microcores were collected from the stems at 1.1–1.7 m above ground from March until October 2015. The methodology of sample collection and tissue preparation followed the procedure described by Prislan et al. (2014). According to this methodology, the micro cores were collected with a Trephor tool at weekly intervals, fixed in a solution of ethanol, formalin and acetic acid, and then stored in ethanol. Afterwards, the microcores were trimmed, dehydrated in a graded series of ethanol, infiltrated with D-limonene and paraffin, and embedded in paraffin blocks. The 9 µm thick transverse sections were cut, transferred to object glasses, stained with safranin and astra blue and embedded in Euparal. The tissues were observed with a Nikon Eclipse E800 light microscope, and analysed with the NIS Elements image analysis program.

We measured the width (in µm) and counted the number of cells in different tissue portions: cambium (CC), current xylem increment with postcambial cells (PC), cells in the phase of secondary cell wall deposition and lignification (SW) and mature (MT) xylem cells (Fig. 2). We also measured/counted the width and cell number in the three previous xylem rings.

2.2 DATA PROCESSING

2.2 OBDELAVA PODATKOV

For each tree and date, measurements were taken along three radial rows and then averaged. To study the behaviour of the trees as a whole population we performed quality control of the data, which showed that two of six trees contained numerous anomalies due to wounding, therefore we averaged the data of four trees and calculated the basic statistics.

The data were then processed by using the R package “CaviaR” (Rathgeber et al., 2011). The Gompertz function was fitted to the data to describe the production of new cells in the wood (Rossi et al., 2003):

$$N(t) = A \cdot e^{-e^{\beta-kt}}$$

where N(t) is the number of tracheids (all phases) at a given time t; A the upper asymptote, indicating the final number of tracheids; β the x-axis placement parameter, reflecting time 0; and k the rate of change parameter, which determines the spread of the curve.

We also used CaviaR for determining the critical dates of xylogenesis: onset, cessation and duration of PC, SW and MT phases of xylem formation (Rathgeber et al., 2011).

2.3 CLIMATIC DATA

2.3 KLIMATSKI PODATKI

Monthly sums of precipitation as well as minimum and maximum mean temperatures from 1974 until 2015 were obtained using KNMI Climate explorer (Trouet & Van Oldenborgh, 2013). The station found by the gridded system was situated at 45°30’-46°N and 14°30’-15°E. Comparing the data of 2015 with the average data of the previous 30 years, we noticed an increase in the average minimum and maximum temperatures during summer and winter months, and a noticeable decrease in the precipitation of March and April (Figure 4).

In addition, we also collected daily climatic data for 2015 from the Grosuplje meteorological station (45°58’N, 14°39’E, 350 m a.s.l.) located near the forest site using the website of the Slovenian Environment Agency (ARSO) (meteo.si), and checked the reports on climatic characteristics for the year 2015 in Slovenia (Cegnar, 2015 a, b).

2.4 EFFECTS OF LATITUDE AND ALTITUDE ON PHENOLOGY OF XYLOGENESIS

2.4 VPLIV ZEMLJEPISNE ŠIRINE IN NADMORSKE VIŠINE NA FENOLOGIJO NASTAJANJA LESA

To place the wood formation data of *Picea abies* at Panška reka in 2015 in a wider context, we compared them with the published data on conifers growing on a wide range of sites all over Europe. We collected the data on onset, cessation and duration of xylem production from 47 studies involving *Picea abies* and seven other coniferous species growing at latitudes ranging from 38.1 to 68.5°N and altitudes from 15 to 2156 m a.s.l. (Table 3).

Using the R package “lmtest” we used two-factor linear regression models to check the correlation of latitude, altitude and their interaction with the dates of onset, end, and duration of the cell production. Variables were selected using a stepwise process. Absence of significant autocorrelation in the residuals was checked with the Durbin-Watson test. We calculated the models with the dependent variable unchanged, transformed logarithmically, and square rooted, and then chose the one with better fitting (using AIC criteria). Finally, we applied the obtained models to the whole European continent and mapped the predicted values of onset, end and duration of the cell production (Rossi et al., 2011).

3 RESULTS AND DISCUSSION

3 REZULTATI IN RAZPRAVA

3.1 WOOD FORMATION IN SPRUCE AT PANŠKA REKA IN 2015

3.1 NASTAJANJE LESA SMREKE NA PANŠKI REKI LETA 2015

Assessment of cambial activity was based on number of cambial cells (CC) (Figure 1, Figure 2a). We recorded 5.11±0.69 CC on 10 April (DOY 100). Afterwards, the number of CC increased and showed a peak in the second week of May (8.22±3.09 cells) and another in the second week of July (7.55±0.60 cells). Thereafter the number of CC decreased (Figure 2a).

The first postcambial cells (PC) which indicate the onset of wood production were observed on 24 April (DOY 114) (Figures 2b, 3). The maximum number of PC cells was observed from the end of May to the first weeks of June. In most trees PC production finished between 24 August and 5 September (DOY 236-248). However, in some samples individual PC cells could be observed until the end of the sampling period in October 2015 (Figure 2b).

The first SW cells, undergoing secondary cell wall deposition and lignification, were observed between 12 and 23 May (DOY 132-143) (Figures 1, 2c). Between 28 May and 9 June (DOY 148-160) we observed the first fully mature MT cells (Figures 1, 2d).

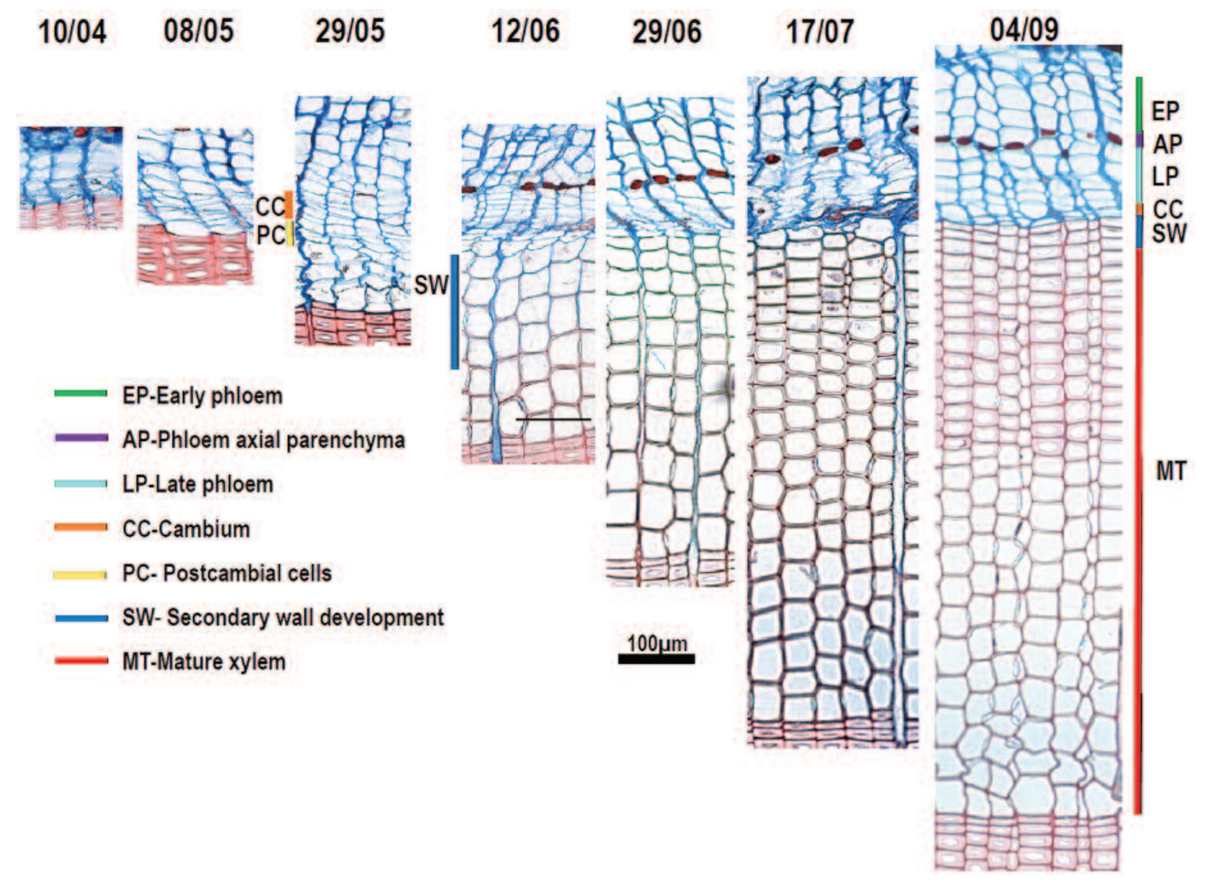


Figure 1. Cross-sections of Norway spruce (Picea abies) tissues on different dates of the year in 2015 at Panška reka site near Ljubljana with varying numbers of cambial cells (CC), cells of secondary xylem in various phases of differentiation (PC and SW), and mature cells (MT). The newly formed secondary phloem increment consists of varying portions of early (EP) and late (LP) phloem, divided by axial parenchyma (AP).

Slika 1. Prečni prerezi tkiv smreke (Picea abies) z rastišča Panška reka v okolici Ljubljane na različne datume v letu 2015. Opazujemo lahko različno število celic v kambiju (CC) in nastajajoči braniki lesa, kjer je mogoče ločiti celice v različnih fazah diferenciacije (PC in SW) in zrele celice (MT). Novo nastali floem vsebuje različne deleže ranega (EP) in kasnega (LP) floema, ki ju razmejuje aksialni parenhim (AP).

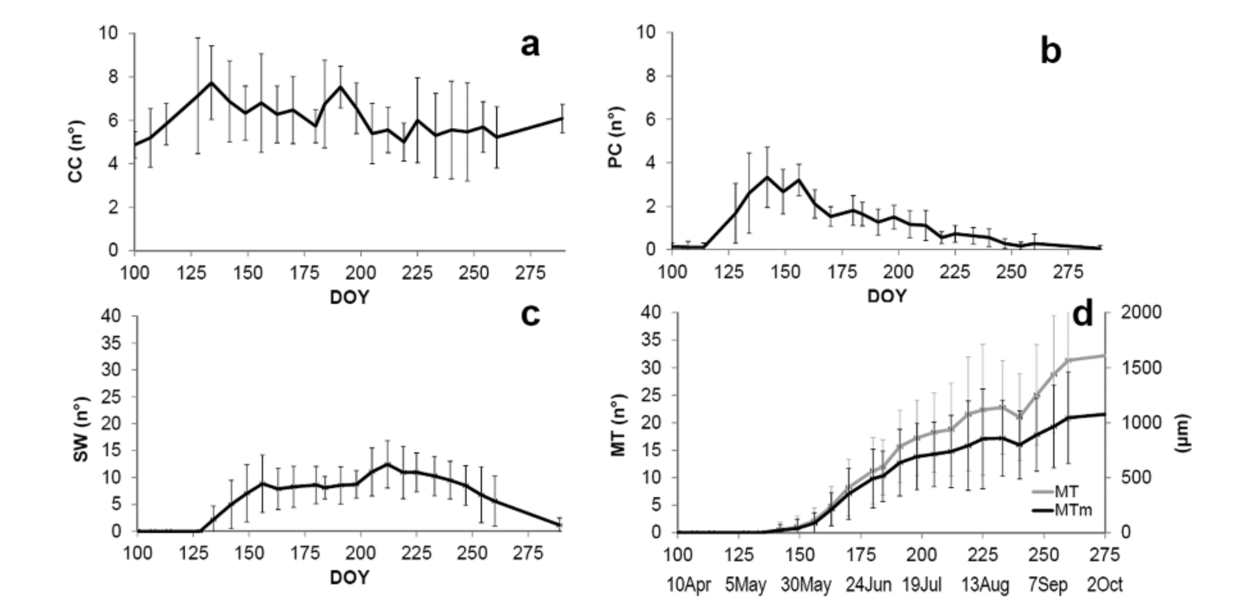


Figure 2. Average number of cells or widths of differentiating and mature xylem in Norway spruce (Picea abies) from Panška reka near Ljubljana on different days of the year (DOY) in 2015: (a) cambial cells (CC), (b) xylem cells in post-cambial growth (PC), (c) xylem cells in phase of secondary cell wall deposition and lignification (SW), and (d) mature xylem (MT) expressed in number of cells (MT-gray line) and micrometers (MTm-black line) and time expressed in DOY and calendar dates. Lines represent the average of four trees, and bars show standard deviations.

Slika 2. Povprečno število celic oz. širine diferencirajočega in zrelega lesa nastajajoče branike smrek (Picea abies) na Panški reki v bližini Ljubljane v letu 2015: (a) kambijeve celice (CC), (b) ksilemske celice v fazi post-kambialne rasti (PC), (c) ksilemske celice v fazi odlaganja sekundarne celične stene in lignifikacije (SW) in (d) zrele celice lesa (MT), izražene kot število celic (MT-siva črta) in širina v mikrometrih (MTm-črna črta). Grafi predstavljajo povprečne vrednosti podatkov za 4 drevesa, ročaji prikazujejo standardni odklon. Podatki so prikazani za različne dneve v letu (DOY), na (d) pa so pod DOY pripisani tudi koledarski datumi.

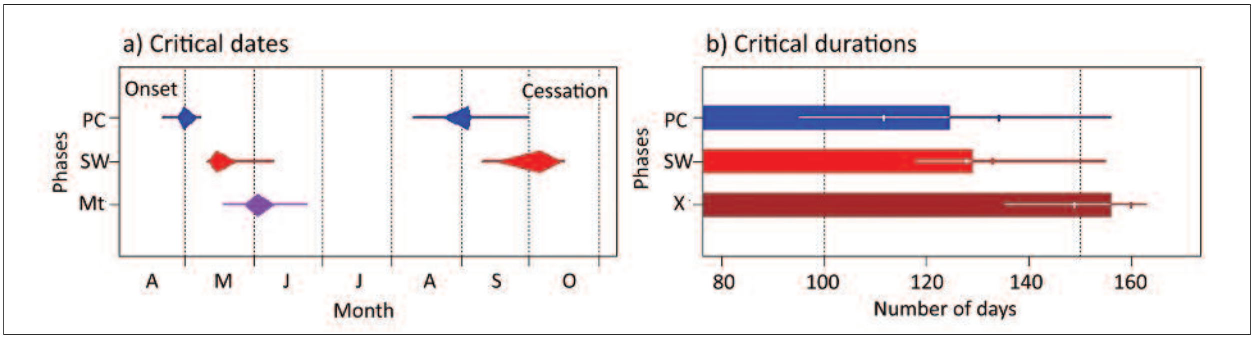


Figure 3. Critical dates for (a) onset and cessation of wood differentiation phases and (b) their duration. PC: xylem cells in post-cambial growth, SW: secondary cell wall deposition and lignification, MT mature cells, X: duration of xylogenesis. In (a) the ends of the line indicate maximum and minimum values, the corners of the diamond the first and third quartiles, and the middle of the diamond the median. In (b) different points on the line represent, minimum, Q1, median, Q3 and maximum duration.

Slika 3. Kritični datumi za (a) začetek in zaključek faz PC, SW in MT ter (b) njihovo trajanje. PC: celice lesa v postkambialni rasti, SW: razvoj sekundarne celične stene in lignifikacija, MT: zrele celice, X: trajanje nastajanja lesa. Na grafu (a) konca črt kažeta maksimalne in minimalne vrednosti, vogali romba predstavljajo prvi in tretji kvartil (Q1 in Q3), sredina romba pa mediano. V grafu (b) točke na črti prikazujejo minimalno, Q1, mediano, Q3 in maksimalno trajanje posamezne faze.

Table 1. Parameters calculated with the Gompertz function showing averages and standard deviation (SD) for four trees. Dt90 indicates the number of days needed to produce from 5% to 95% of the xylem ring. r90 is the average growth rate (cells/day) in that period and rmax the maximum growth rate.
Preglednica 1. Parametri, izračunani s pomočjo Gompertzove funkcije, ki kažejo povprečja in standardne odklone (SD) štirih dreves. Dt90 prikazuje število dni, potrebnih za nastanek od 5 % do 95 % celotne branike. r90 je povprečni prirastek (celic/dan) v tem obdobju in rmax je maksimalni prirastek.

	A	**β**	**k**	**tip**	**Dt90**	**r90**	**rmax**	**R2**
Average	43.59	5.32	0.035	154.75	124.75	0.32	0.53	0.913
SD	10.90	1.28	0.010	8.26	35.98	0.06	0.088	0.075

Differentiation of the cells was completed by mid-September to mid-October (Figures 2c, d, 3). The final xylem growth ring of 2015 was on average 1468 µm wide and composed of 37 cells (Figure 2d).

Additional parameters of wood formation dynamics in 2015, as calculated by the Gompertz function, showed that the trees on average needed 125 days to produce 90% of the growth ring, produced 0.32 cells per day, and that the maximum production was 0.53 cells per day (Table 1).

3.2 WOOD FORMATION IN 2015 COMPARED TO THAT OF PREVIOUS YEARS

3.2 NASTAJANJE LESA V LETU 2015 V PRIMERJAVI S PREDHODNIMI LETI

As the wood formation has been followed over a longer period in *Picea abies* at Panška reka (Gričar et al., 2014, 2015; Čufar et al., 2015), the results for 2015 could be compared to those available for 2009, 2010 and 2011, while the data for 2012, 2013, and 2014 are still being processed. The comparison showed (Table 2) that on average xylem cell production in 2015 started five-11 days later than in previous years (2009–2011). On the other hand, maximum cell production and cessation of cell production occurred at approximately the same time as in previous years. Consequently, the duration of cell production was shorter and the tree ring was narrower in 2015 than in 2009, 2010 and 2011.

Later onset of cell production in 2015 could possibly be due to extremely low precipitation in March and especially April 2015, which were below the average of the last 30 years and lower than in the years 2009, 2010, and 2011 (Figure 4). Although previous investigations mainly confirmed the importance of spring temperatures for the onset of cambial production in spruce (Gričar et al., 2006, 2014, 2015), the extremely dry conditions in March and April 2015 could be a reason for the later onset of wood production. According to published reports on the climatic characteristics of 2015, it should also be noticed that summer was particularly warm, with five heat waves with daily temperatures of above 30 °C (Cegnar, 2015 a, b). Due to this, more detailed studies are needed to clarify the effects of high temperatures on the phenology of wood formation.

Table 2. Parameters of xylogenesis for Norway spruce (Picea abies) at the forest site Panška reka, near Ljubljana, Slovenia in 2015 compared with the published data for previous years (Gričar et al., 2014).
Preglednica 2. Kazalniki nastajanja lesa smreke (Picea abies) na Panški reki v bližini Ljubljane (Slovenija) v letu 2015 in primerjava z razpoložljivimi podatki, objavljenimi za predhodna leta (Gričar et al., 2014).

	2009	2010	2011		2015
Onset of cell production (DOY)	106.17±4.58	109.00±0	103.17±2.86		114.00±0.00
Maximal cell production (DOY)	154.15±5.24	157.91±10.77	164.11±22.41		154.75±8.26
Cessation of cell production (DOY)	243.00±14.68	243.17±8.18	237.50±17		241.50±15.83
Duration of cell production (days)	136.83±15.38	134.17±8.18	134.33±19.63		124.75±35.98
Number of cells in final xylem ring	68.66±31.72	55.47±12.38	63.49±27.37		37.50±13.77

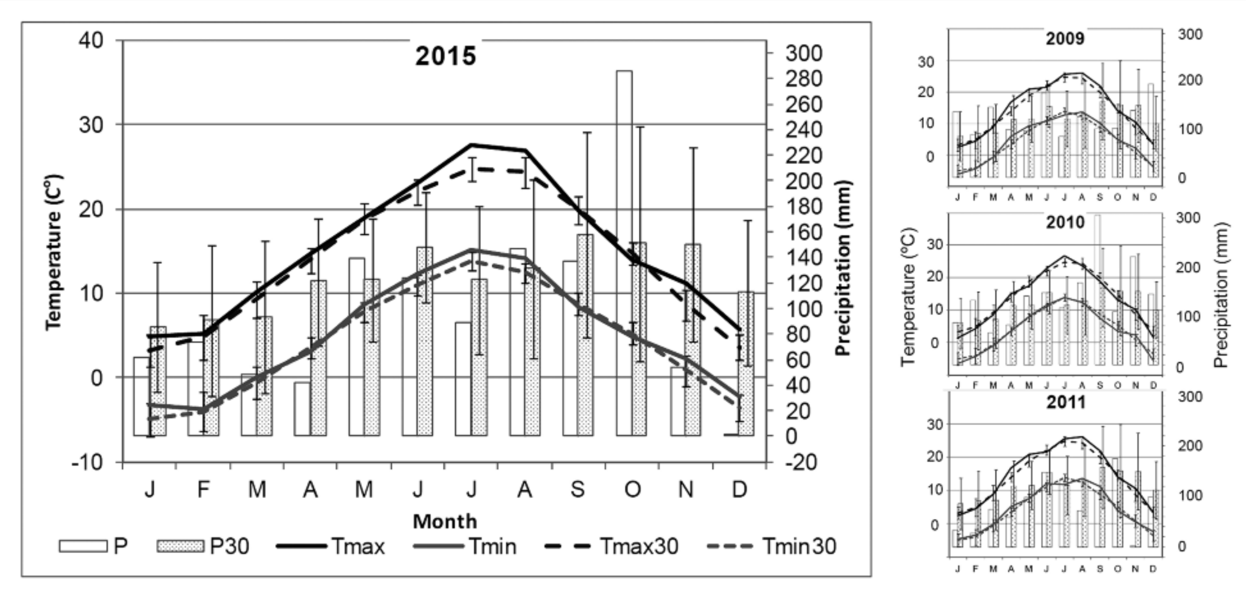


Figure 4. Monthly sum of precipitation (P) and average minimum and maximum temperatures (Tmin and Tmax) in 2015 and 2009–2011, compared with the averaged 1974–2015 data (P30, Tmin30, Tmax30), with the standard deviation represented by error bars. Source: KNMI Climate explorer (Trouet & Van Oldenborgh, 2013).
Slika 4. Mesečne padavine (P) in povprečne minimalne in maksimalne temperature (Tmin in Tmax) v letih 2015 in 2009–2011 v primerjavi s povprečnimi vrednostmi za obdobje 1974–2015 (P30, Tmin30, Tmax 30). Ročaji prikazujejo standardni odklon. Vir: KNMI Climate explorer (Trouet & Van Oldenborgh, 2013).

3.3 COMPARISON OF XYLEM PHENOLOGY ON DIFFERENT SITES - EFFECT OF LATITUDE AND ALTITUDE

3.3 PRIMERJAVE FENOLOGIJE NASTAJANJA LESA MED RASTIŠČI – VPLIV ZEMLJEPISNE ŠIRINE IN NADMORSKE VIŠINE

Although the results obtained for one year and location provide valuable insights into the wood formation process, we wanted to relate these findings to wood formation data for a larger sample from the published literature (Table 4). As *Picea abies* mainly grows on higher elevations and latitudes (EUFORGEN, 2009), and its wood formation data cover latitudes from 45.93 to 67.50°N, we collected also the data of other conifers (mainly *Pinus* sp.) to widen the interval to 38.10 – 68.5°N and altitudes from 15 to 2156 m a.s.l. The collected data showed high variability of onset (DOY 47 – 168, 16 February-17 June), cessation (DOY 198-334, 17 July-30 November) and duration (49–287 days) of wood production (Table 4).

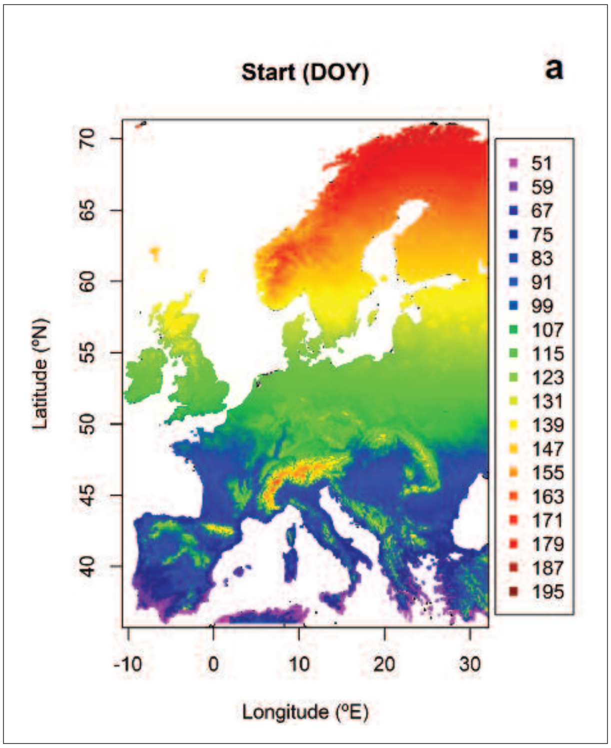
Table 3. Models showing the effects of latitude (l) and altitude (a) on phenology (onset, end and duration) of wood production.
Preglednica 3. Modeli, ki prikazujejo vpliv zemljepisne širine (l) in nadmorske višine (a) na fenologijo (začetek (onset), konec (end) in trajanje (duration)) kambijeve produkcije celic lesa.

Model	Onset DOY = $3{,}6447\, l + 0{,}0289\, a - 77{,}3589$	End DOY = $365{,}515 - 2{,}2280\, l - 0{,}0218a$	Log(Duration) = $1.008 \cdot 10^{-5}\, l * a - 2{,}574 \cdot 10^{-2}\, l - 6{,}525 \cdot 10^{-4}\, a + 3{,}428$
R2	0.8275	0.3810	0.803
p-value	2.2 · 10-16	9.84 · 10-6	7.1 · 10-16
F	111 on 2 and 44 DF	15.15 on 2 and 44 DF	63.8 on 3 and 43 DF

Table 4. Onset, cessation and duration of cambial cell production in Picea abies and other conifers from various sites in Europe used for the regression models.

Preglednica 4. Podatki o začetku, koncu in trajanju kambijeve celične produkcije smreke in drugih iglavcev na različnih rastiščih po Evropi, ki so bili uporabljeni v regresijskem modelu.

Species	**Country**	**Latitude (°N)**	**Longitude (°E)**	**Altitude (m a.s.l.)**	**Onset (DOY)**	**End (DOY)**	**Duration (days)**	**Reference**
Picea abies	**Slovenia**	**46.00**	**14.65**	**400**	**114**	**241**	**125**	**This study**
Picea abies	Italy	45.93	11.05	680	106	258	152	Cocozza et al., 2016
Picea abies	Slovenia	46.18	14.38	392	126	225	99	Rossi et al., 2008
Picea abies	Slovenia	46.27	16.80	1200	117	228	111	Gričar et al., 2014
Picea abies	Slovenia	46.35	13.97	1280	144	221	77	Rossi et al., 2008
Picea abies	Italy	46.35	11.48	1780	168	256	88	Cocozza et al., 2016
Picea abies	Italy	46.45	12.13	2156	157	225	68	Rossi et al., 2008
Picea abies	Italy	46.45	12.13	2085	155	233	78	Rossi et al., 2008
Picea abies	Austria	47.23	10.84	750	119	238	119	Swidrak et al., 2014
Picea abies	France	48.48	7.15	643	122	221	99	Cuny et al., 2012
Picea abies	Czech Rep.	49.29	16.67	650	112	232	120	Gričar et al., 2014
Picea abies	Finland	60.20	25.00	60	152	229	77	Jyske et al., 2014
Picea abies	Finland	60.20	25.00	60	142	232	90	Jyske et al., 2014
Picea abies	Finland	61.20	25.60	120	163	229	66	Jyske et al., 2014
Picea abies	Finland	61.20	25.60	120	163	237	74	Jyske et al., 2014
Picea abies	Finland	61.90	24.30	181	152	215	63	Jyske et al., 2014
Picea abies	Finland	61.90	24.30	181	147	217	70	Jyske et al., 2014
Picea abies	Finland	62.40	27.30	110	158	220	62	Jyske et al., 2014
Picea abies	Finland	62.40	29.40	84	159	217	58	Jyske et al., 2014
Picea abies	Finland	62.40	27.30	110	153	217	64	Jyske et al., 2014
Picea abies	Finland	63.00	29.50	183	166	216	50	Jyske et al., 2014
Picea abies	Finland	66.20	26.40	140	165	221	56	Jyske et al., 2014
Picea abies	Finland	66.20	29.50	140	168	226	58	Jyske et al., 2014
Picea abies	Finland	67.50	29.40	390	163	215	52	Jyske et al., 2014
Pinus halepensis	Spain	38.10	-0.65	15	47	334	287	de Luis et al., 2007
Pinus halepensis	Spain	38.52	-0.64	845	75	310	235	de Luis et al., 2011
Pinus halepensis	Spain	39.16	-1.15	850	93	198	105	de Luis et al., 2007
Pinus leucodermis	Italy	39.90	16.20	2053	155	220	65	Rossi et al., 2008
Pinus sylvestris	Spain	41.79	-1.82	1600	100	245	145	Martinez del Castillo et al., 2016
Pinus sylvestris	Spain	41.80	-1.81	1200	87	246	160	Martinez del Castillo et al., 2016
Pinus sylvestris	Austria	47.23	10.84	750	111	248	137	Gruber et al.,2010
Pinus sylvestris	Austria	47.23	10.84	750	119	279	160	Gruber et al.,2010
Pinus sylvestris	Austria	47.23	10.84	750	107	214	107	Oberhuber et al., 2011
Pinus sylvestris	Austria	47.23	10.84	750	108	236	128	Swidrak et al., 2014
Pinus sylvestris	France	48.40	6.32	270	111	259	148	Rathgeber et al., 2011
Pinus sylvestris	France	48.48	7.15	643	116	250	134	Cuny et al., 2014
Pinus sylvestris	Finland	66.37	26.72	150	160	219	59	Rossi et al., 2008
Pinus sylvestris	Finland	68.50	27.50	220	166	215	49	Rossi et al., 2008
Pinus uncinata	Italy	45.05	6.67	2030	143	204	61	Rossi et al., 2008
Abies alba	France	48.48	7.15	643	117	243	126	Cuny et al., 2012
Larix decidua	Italy	45.05	6.67	2030	155	208	53	Rossi et al., 2008
Larix decidua	Italy	46.45	12.13	2156	154	218	64	Rossi et al., 2008
Larix decidua	Italy	46.45	12.13	2085	150	226	76	Rossi et al., 2008
Larix decidua	Austria	47.23	10.84	750	120	217	98	Swidrak et al., 2014
Pinus cembra	Italy	45.05	6.67	2030	139	201	62	Rossi et al., 2008
Pinus cembra	Italy	46.45	12.13	2156	145	223	78	Rossi et al., 2008
Pinus cembra	Italy	46.45	12.13	2085	141	223	82	Rossi et al., 2008



The models applied to data obtained from 47 sites confirmed the statistically significant effects of latitude and altitude on the onset, cessation, and duration of xylem production (Table 3). Latitude and altitude both have a positive influence on the date of onset (higher latitude and altitude, later onset), and a negative one on the cessation date (higher latitude and altitude, earlier cessation). The correlations are higher for the onset than for the cessation of cell production.

Latitude proved to be the most important factor for explaining the variability in phenology of cell production in conifers on different sites. The effect of altitude proved to be important as well, but it only became evident after filtering the effect of latitude. Altitude therefore explained the residuals of the latitude model. As such, the model including both latitude and altitude gave the best predictions.

These results support the observation that temperature is likely the main determinant of the onset, cessation and duration of wood production in tem-

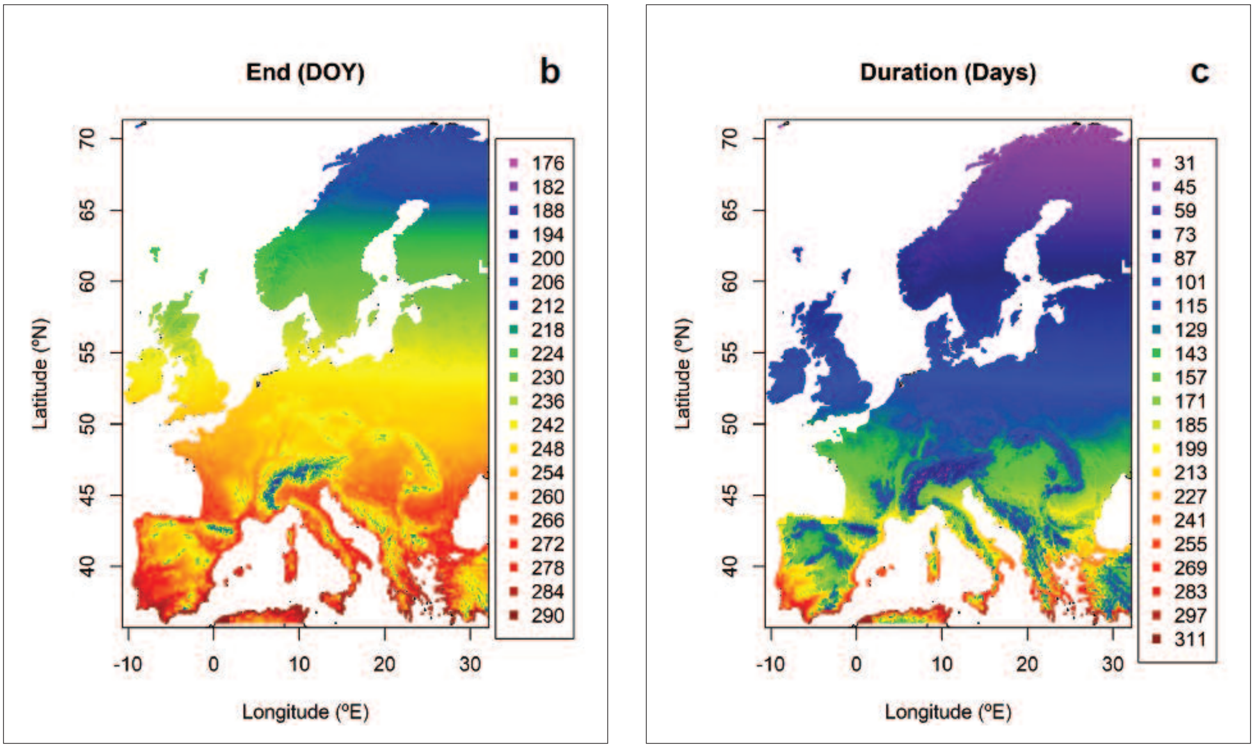


Figure 5. Maps of Europe showing predicted values for (a) onset - start, (b) cessation – end, and (c) duration of cell production in wood according to the two-factor, altitude and latitude, regression models (see Tables 3 and 4).
Slika 5. Zemljevidi Evrope in napoved vrednosti za (a) začetek, (b) konec in (c) trajanje kambijeve produkcije celic lesa, izračunanimi s pomočjo dvofaktorskega regresijskega modela (faktorja: zemljepisna širina in nadmorska višina) (glejte tudi preglednici 3 in 4).

perate ecosystems, although extremely dry conditions during the spring may also affect the onset of this. Trees at different latitudes are adapted to local conditions, and mainly to the duration of the warm period (Rossi et al., 2006; Cocozza et al., 2016). These relations with temperature are highest for the onset of cell production, whereas cessation of cell production seems to be more dependent on individual factors (e.g. tree vitality, social status, soil properties, topography) and therefore shows a weaker correlation in the model. Increasing latitude and altitude also decrease the duration of cell production by the cambium.

These results confirm the high plasticity of *Picea abies* and other conifers, which allows them to adapt to the environmental conditions of a wide range of sites (Gričar et al. 2014, 2015; Martinez del Castillo et al., 2016). An increase in latitude is generally accompanied by a decrease in temperature and shorter duration of the warm period that allows growth. Variation in latitude also entails differences in day length (photoperiod), whereas variation in altitude in a particular area does not include this effect.

As we worked with data from various publications, it should be noted that the predictions may be less reliable in some areas, for instance in the Mediterranean, where the data are scarce, and lack of water availability is generally more important for limitation of growth than temperature (Novak et al., 2013).

4 CONCLUSIONS
4 ZAKLJUČKI

Xylogenesis in *Picea abies* at Panška reka near Ljubljana during 2015 followed the pattern which could be fitted to the Gompertz function. The characteristics of wood formation in 2015 were a delayed onset of cell production, shorter duration of cell production and narrower tree ring width compared to the years 2009, 2010, and 2011. This can possibly be ascribed to the very dry March and April in 2015, whereas this study could not explain the effects of that year's hot summer on the xylogenesis phases, with five heat waves having occurred.

Maximal tracheid production occurred around 4 June 2015 (± ca. 1 week), which is comparable to the situation in 2009–2011 at the same site, and earlier than reported for conifers in colder climates, which generally show maximal tracheid production around the summer solstice, i.e. 21 June (Rossi, 2006). The factors involved in cessation of cell production seem to be less evident than those involved in its onset.

Comparison of wood formation in *Picea abies* on the site near Ljubljana with the phenology of xylem production in the same species and other conifers (mainly *Pinus* sp.) all over Europe showed a significant correlation with latitude and altitude. An increase in latitude and altitude as a rule resulted in a delayed onset, earlier cessation and shorter duration of cambial production. Latitude proved to be more important for explaining the variability in phenology of cell production than the altitude.

This study is part of long-term monitoring project of wood formation in Slovenia, and shows that long-term observations of the same species and site are necessary to predict the intra-annual and interannual growth of trees, which is crucial for their survival and productivity in changing climate as well as for wood quality.

5 SUMMARY
5 POVZETEK

Vaskularni kambij je sekundarni meristem, ki proizvaja sekundarni ksilem (les) in sekundarni floem. Nastajanje lesa in floema sta osnovna procesa, potrebna za rast in preživetje dreves, kar posledično omogoča obstoj gozdnih ekosistemov. Nastajanje lesa (ksilogeneza) vpliva tudi na kroženje ogljika in na kakovost lesa. Kambijeva aktivnost in produkcija celic lesa sta odvisna od okoljskih dejavnikov. V zmernem podnebnem pasu temperatura in padavine pomembno vplivajo na kambijevo produktivnost. Kambij praviloma deluje periodično in je aktiven v toplejšem ter mirujoč v hladnejšem obdobju leta, zato v enem koledarskem letu praviloma nastane ena prirastna plast oz. branika lesa. Periodično letno priraščanje je osnova za dendrokronološke študije.

Namen te študije je bil proučiti dinamiko nastajanja lesa smreke (*Picea abies* (L.) Karst.) na rastišču Panška reka v bližini Ljubljane v letu 2015. Cilj je bil tudi primerjati pridobljene rezultate s podatki o nastajanju lesa v isti vrsti na istem rastišču v preteklih letih (2009–2011), ter s podatki o nastajanju lesa v

smreki in drugih iglavcih iz študij za rastišča po Evropi. Namen primerjave je bil preveriti vpliv zemljepisne širine in nadmorske višine na nastajanje lesa ter pripraviti model za napovedovanje ali rekonstrukcijo fenologije nastanka lesa iglavcev v Evropi.

Proces nastajanja lesa smo spremljali na 6 smrekah v rastni sezoni 2015 na Panški reki v bližini Ljubljane (pribl. 46°00'N, 14°40'E, 400 m n.m.v.). V ta namen smo od aprila do oktobra 2015 tedensko odvzemali mikro izvrtke iz debel izbranih dreves na višini 1,1 m–1,7 m nad tlemi. Izvrtke smo fiksirali v raztopini formalina, ocetne kisline in etanola (FAA), dehidrirali in prepojili s parafinom. Z rotacijskim mikrotomom smo odrezali 9 µm debele preparate prečnega prereza tkiv, jih obarvali s kombinacijo barvil safranin ter astra modro in jih vklopili v euparal (Prislan et al., 2014). Na izdelanih anatomskih preparatih smo vzdolž treh radialnih nizov merili in šteli celice kambija (CC), lesa nastajajoče branike ter predhodnih branik. V nastajajoči braniki smo razlikovali celice v fazi postkambialne rasti (PC), odlaganja in lignifikacije sekundarne celične stene (SW) in zrele celice (MT) (slika 1). Po opravljenih meritvah smo podatke preverili in zaradi rastnih anomalij izločili 2 vzorčni drevesi. Podatke smo statistično obdelali, za izravnavo podatkov pa smo uporabili tudi Gompertzovo funkcijo.

Vremenske razmere (mesečne padavine, minimalne ter maksimalne temperature) v letu 2015 smo primerjali z razmerami v obdobju 1974–2014. Za leto 2015 so bile značilne podpovprečne padavine v marcu in še posebej v aprilu ter nadpovprečne temperature pozimi in poleti (slika 4). Po nižinah je bilo leto 2015 drugo ali tretje najtoplejše v Sloveniji, poleti pa se je zvrstilo 5 vročinskih valov, ko so temperature dosegle vsaj 30 °C (Cegnar, 2015 a, b).

Ocena aktivnosti kambija je temeljila na številu celic v kambijevi coni (CC) (slika 1, slika 2a). 10. aprila (na 100. dan v letu, DOY) je število CC znašalo 5,11 ± 0,69. Potem se je število CC povečalo z vrhom v drugem tednu maja (8,22 ± 3,09). Po drugem tednu julija, ko je število CC znašalo 7,55 ± 0,60, se je število začelo zmanjševati (slika 2a).

24. aprila (DOY 114) smo opazili prve celice v fazi postkambialne rasti (PC), kar je pomenilo, da se je začela kambijeva produkcija celic lesa (slika 2b, 3). Največje število PC celic smo opazili od sredine maja do prvih tednov junija. V večini dreves je bilo nastajanje PC končano med 24. avgustom in 5. septembrom (DOY 236–248). Prve SW celice, kjer sta potekala odlaganje sekundarne celične stene in lignifikacija, smo opazili med 12. in 23. majem (DOY 132–143) (slika 1, 2c). Med 28. majem in 9. junijem (DOY 148–160) smo opazili prve popolnoma zrele MT celice (slika 1, 2d). Diferenciacija celic je bila zaključena od sredine septembra do sredine oktobra (slika 2c, d, 3). Povprečna ksilemska branika smreke v letu 2015 je bila široka 1468 µm in je vsebovala 37 celic – traheid v radialnem nizu (slika 2d). Parametri, izračunani s pomočjo Gompertzove funkcije, so pokazali, da so drevesa v povprečju potrebovala 125 dni, da so proizvedla 5 %–95 % branike; v povprečju so proizvedla 0,32 celice na dan, največja proizvodnja je znašla 0,53 celice na dan in je bila zabeležena 4. junija (DOY 154.75±8.26) (preglednica 1 in 2).

Podatke nastajanja lesa smreke na Panški reki smo lahko primerjali z razpoložljivimi podatki iz predhodnih let za isto drevesno vrsto in rastišče (Gričar et al., 2014, 2015, Čufar et al., 2015). Primerjava je pokazala (preglednica 2), da se je proizvodnja ksilemskih celic v letu 2015 v povprečju začela 5–11 dni kasneje kot v predhodnih letih (2009–2011). Maksimum in zaključek produkcije celic smo v letu 2015 zabeležili ob približno istem času kot v predhodnih letih. Posledično je bilo trajanje produkcije celic krajše, branika 2015 pa je bila v splošnem ožja kot branike, nastale v letih 2009, 2010 in 2011.

Kasnejši začetek produkcije celic v letu 2015 bi bil lahko posledica suše v marcu in zlasti aprilu 2015, vpliva poletne vročine pa v tej študiji nismo mogli potrditi.

Modeli za obdelavo podatkov smreke in drugih iglavcev s 47 lokacij po Evropi so zajeli območje med 38.1° in 68.5° severne zemljepisne širine in nadmorske višine od 15 do 2156 m n.m.v. Potrdili so statistično značilen učinek zemljepisne širine in nadmorske višine na začetek, zaključek in trajanje kambijeve produkcije lesa (preglednica 3). Večja zemljepisna širina in višja nadmorska višina vplivata na poznejši začetek in bolj zgoden zaključek ter posledično na krajši čas kambijeve produkcije, kar nakazuje velik vpliv temperatur na omenjene fenološke faze. Rezultati kažejo, da so zveze bolj značilne za začetek kot za zaključek celične produkcije. Omenjeni rezultati dvofaktorskih linearnih regresijskih modelov za določanje datumov začetka in konca ter trajanja kambijeve produkcije lesa so prikazani na zemljevidih Evrope (slika 5 a, b, c).

Predstavljena študija je del dolgoročnega projekta spremljanja nastajanja lesa (in floema) v smreki in bukvi (*Fagus sylvatica*) na dveh rastiščih različnih nadmorskih višin v Sloveniji (Čufar et al., 2015). Rezultati kažejo, da je večletno spremljanje nastajanja lesa na isti drevesni vrsti in rastišču pomembno za razumevanje rasti dreves znotraj enega leta ter razlik med leti. Razlike so v veliki meri odvisne od letne in medletne variabilnosti klimatskih dejavnikov in izjemnih klimatskih razmer. Procesi nastajanja lesa in floema, na katere vplivajo tudi klimatske spremembe, so ključni za preživetje in produktivnost dreves ter nenazadnje za kakovost lesa.

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