DENDROBIOLOGY
2012, vol. 67, 15–24

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Monitoring of xylem formation in *Picea abies* under drought stress influence

Received: 29 March 2011; Accepted: 31 October 2011

Abstract: The effect of drought stress on regular cambium activity and wood formation in stems of two different clones of Norway spruce (*Picea abies* (L.) Karst.) was investigated. Tissue samples were taken during the growing season from May to September 2010. Artificial drought stress, induced by long-term sheltering of the soil, was significantly manifested in clone 15. In the stressed individual, the period of cambium activity was shorter, the total number of formed cells was lower and the resulting tree ring was narrower. The number of cells in the phases of postcambial growth and secondary cell wall formation was significantly lower in comparison to the control tree. The tracheid lignification process was slower in the tree stressed by water deficit and the first mature tracheids were observed later. On the other hand, in clone 18 probably genetic dispositions played an important role as no considerable deviations in the cambium activity and new wood cells production were observed. Fitting xylem increments to the Gompertz function showed that the period of the most intensive cell formation was at the turn of June and July and the maximum daily production of new cells was higher in non-stressed individuals than in the stressed ones. The results of the experiment lead us to the conclusion that drought stress can significantly affect the cambium activity of some clones, the differentiation process of anatomical elements, and thus also the resulting tree ring width.

Additional key words: Norway spruce, cambium, wood formation, drought stress, Gompertz function

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Indroduction

Conversion of unnatural spruce forests into more natural ones will certainly not be possible during one or two decades. It is highly probable that Norway spruce (*Picea abies* (L.) Karst.) will remain an economically significant tree species in many European countries (Mayer and Prins, 2003). One of the aims of sustainable forest management is the process of unstable spruce monoculture conversion into stands with more suitable tree species composition and structure. With respect to the danger of possible ecological and economic damage, these stands cannot be left to their own succession. The climatic change will be considerably manifested in the deviations of the hydrological cycle and the availability of water during the year will change in many world regions (Allen and Ingram 2002). In some areas a higher level of CO_2 will offer potential for a higher production but the risk of water deficit will strengthen the impact of several other stress factors. The requirements of vegetation for water will increase. Moreover, some levels of other

stress factors, especially the effects of O_3, NH_3 and NO_x tolerated so far, may become risky (Percy and Ferretti 2004).

Radial increment of wood species is a result of activity of two lateral meristematic tissues: cambium and phellogen. Phellogen produces new cells of the periderm; cambium divides new secondary xylem cells centripetally and secondary phloem cells centrifugally. The process of the formation of new xylem cells is highly complicated. It starts with the periclinal division of cells in the cambium which is followed by the differentiation process. The differentiation consists of a phase of postcambial growth and a phase of the synthesis of secondary cell wall formation and lignification. The process of xylem differentiation is finished by programmed cell death and tracheids then assume their normal function in long distance transport of water and solutes (Wilson et al. 1966; Panshin and de Zeeuw 1980; Larson 1994).

The cycle of seasons is marked by the periodicity of radiation, length of day, temperature, and soil moisture (precipitation); in each season there are favourable or unfavourable conditions for the cambium activity, i.e. for the growth of plants (Larcher 2003). Cambial activity is periodic and in temperate-zone trees occurs from spring to early autumn.

The activity of cambium depends on many internal (e.g. genetic properties of plants, their health, age and the position in the plant) and external factors (Panshin and de Zeeuw 1980). Wodzicki (1971) divided the external factors of the environment into (1) basic conditions for xylogenesis (temperature, moisture, nutrients in the soil, gravity, photoperiod) and (2) occasional factors (wind, fire, frost, floods, defoliation, forest management, air pollution).

The content of water in the soil is one of the main factors affecting the cambium activity and the formation of new xylem cells (Larson 1994; Horáček et al. 1999). If the water content in the soil is insufficient, the division of cells in the cambial zone is reduced and so is the radial dimension and the thickness of tracheid cell walls (Abe et al. 2003; Rigling et al. 2004; Schweingruber 2007). Abe and Nakai (1999) found that cell diameter decreased during the early stage of water deficit, followed by a decrease in the number of cells produced. The drought stress leads to the reduction of the cell turgor, affects the cell enlargement, leads to the decrease in synthesis of auxins and carboxyhydrates and reduces their storage in the cambium (Kramer 1983). In the conditions of stress trees usually respond by reducing the relatively less important processes at first, such as the growth of stem. Rossi et al. (2009) explored the response of apical and lateral meristems of young plants of *Abies balsamea* (L.) Mill., which were exposed to the drought stress during the growing season. The irrigated and the non-irrigated trees showed the same trend of xylem formation and timing of cell differentiation. In the case of the non-irrigated trees a reduction of tracheid lumina and tracheid dimensions was observed during the dry period.

The air temperature represents another key parameter for cambium activation at the beginning of the growing season. The renewal of cell activity in the cambial zone in the spring in temperate climates begins when the mean temperature rises above 4.4–8°C for about a week (Wilcox 1962; Matovič 1990; Horáček et al. 1999; Gričar 2007; Deslauriers et al. 2008). Rossi et al. (2007) reported that xylogenesis was active in conifers at high altitudes when the mean daily air temperature was 5.6°C.

While dendroclimatology is based on analysis of a completed annual ring width and its relation to climatic factors (Rybníček et al. 2010), to study the processes of xylogenesis, researchers need to use a repeated micro-sampling method (Wodzicki 1971; Horáček et al. 1999; Deslauriers et al. 2003; Gričar et al. 2006, Rossi et al. 2006) which requires repetitive sampling throughout the growing season. To calculate timing of tracheid differentiation an algorithm (Wodzicki 1971) is applied in a software tool (Vavrčík and Gryc 2011).

The presented study focused on the description of the cambium activity and the formation of wood in clones of Norway spruce (*Picea abies* (L.) Karst.) which have been exposed to long-term period of drought stress.

The drought period also included June and July, when the maximum radial increment of wood and thus also the need of soil moisture is expected in standard conditions (Mäkinen et al. 2003; Rossi et al. 2006).

Methods

Site description

Fifty seven Norway spruce clones were planted in a 1×1 m grid at Hoxmark (Norway, N 59°40', E 10° 43', 90 m a.s.l.) on a former agricultural land in 1990. Stand height was about 9 m, stand density in the time of planting was 10,000 trees per ha. No thinning was performed, however, the natural mortality reduced the stand density to about 9000 trees per ha. The basal area was about 50 m^2 per ha in 2009, i.e. at stand age of 19 years. Climatic conditions of Äs (the nearest location of meteorological station, 2 kilometres from Hoxmark) are given by Hansen and Grimenes (2003). The mean annual precipitation is 785 mm and the mean annual temperature is 5.3°C. The Norway spruce roots developed mainly in the upper horizons (30 cm) on an albeluvisol.

Experimental design

Four trees of the two different clones (clone 15 and 18) were selected in the stand, two individuals on the experimental plot (trees marked as 15D, 18D) and two corresponding ones on the control plot (15C, 18C). Dendrometric parameters of trees are in Table 1 (clone 15: mother: 2598, Krödsherad, Buskerud, 440 m, and father: 5262, Gol, Buskerud 395 m; clone 18: mother: 2644: Nordre Land, 400 m, and father: 2693: Äsnes, Hedmark, 345 m). Two tree lines surrounding the selected individuals were cut off in August 2008 on the experimental plot. In this way, stand density was reduced by 50% to 5000 trees per ha. The tree cutting was done one year before the sampling to avoid the sudden light shock (Tucker et al. 1987; Kozlowski et al. 1991). The same clones with approximately the same tree diameter were chosen in the control plot. A plastic roof was built on the experimental plot to evoke drought stress in May 2009 (Fig. 1). We also dug out a trench to the depth of 250 cm around the experimental plot to avoid horizontal water percolation through the soil.

Sampling and determination of wood formation

Tissue samples were taken at fourteen days intervals, from the beginning of May to the end of September 2010. Sampling was carried out by means of the Trephor tool (Rossi et al. 2006). Due to small diameters of stems (see Table 1) two microcores (1.8 mm in diameter) were taken 50 cm above a ground and around the stem perimeter, so that they contained phloem, cambium and xylem of the developing tree ring. The distance between two neighbouring microcores was 2 cm so that the samples did not contain traumatic tissue. Immediately after sampling, the microcores were immersed in FAA (formalin-ethanol-acetic acid), where they were left for a week; afterwards, they were stored in 30% ethanol.

Microcores were dehydrated in an alcohol series, then followed clearing in xylene. The actual paraffin infiltration of microcores was carried out in a laboratory drying oven at a temperature of 60°C for 4 hours. Paraffin was poured by means of the Leica EG1120 dispenser and the microcores were connected with histological cases to be mounted in the microtome. Then the Leica RM2235 rotation microtome was used to make cross sections 12 μm thin. An adhesive (egg white and glycerine, 1:1) was used for better adhesion of the sections on glass slides. The sections were dried in an oven at a temperature of 60°C for 30 minutes. Further steps were the removal of the paraffin (xylene), dehydration (ethanol) and staining of the sections by safranin and astra blue. The sections were mounted in Canada balsam. To monitor and scan the microsections we used the Leica DMLS microscope with the fluorescence light and Leica DFC280 digital camera. To measure the radial increment we used the ImageJ open source program.

Fig. 1. Hoxmark site

The cross sections were used to identify cambial cells and differentiating cells (tracheids). The cells in the cambial zone (CC) were identified based on the thin cell wall and small radial dimension. In the phase of postcambial growth (PC) the cells still had a thin cell wall, lumina included protoplast and the radial dimension was at least double in comparison with cambial cells. Application of polarized light enabled us to distinguish cells in the phase of secondary cell wall deposition (SW) from the phase of postcambial growth. During the phase of secondary cell wall deposition cellulose microfibrils are deposited in the cells, which causes glistening in the polarized light. The lignification process was detected based on cell wall colour change. Non-lignified cells were dyed blue with astra blue solution, safranin solution dyed the cells red in the reaction with lignin. Mature tracheids (MT) had red-stained cell walls and empty lumina without protoplast. The numbers of cells in individual phases were recorded for three randomly chosen files and then the wood radial increment was measured. Further, the average was calculated.

The xylem formation of the growth ring has been analysed with Gompertz function (Dufour and Morin 2007; Rossi et al. 2003) using equation:

$$y = Ae^{-e^{B-kt}}$$

y – weekly cumulative cells, t – day of year, A – upper asymptote, representing the maximum number of

Table 1. Dendrometric parameters of trees which were selected for experiement on Hoxmark site, Norway

		Clone 15	Clone 18
Control plot	diameter (cm)	9.1	7.8
	height (m)	10.8	8.8
Experimental plot	diameter (cm)	10.9	7.5
	height (m)	9.7	9.3

cells, *B* – place on *x* axis, estimating the beginning of cambial activity, *k* – inflection point on the curve.

Results

Cambial activity

At the beginning of the monitored period (7th May) the cambial zone had from 7 to 10 cells, the mean being 8.6 cells for both of experimental and control trees (Fig. 2). At last sampling (9th September) the mean number of cells in the cambial zone was 6.2, ranging from 5 to 8 cells. At the beginning of the growing season an increase in the number of cells could be observed in the cambial zone (with the exception of 18D), followed by a gradual decrease in the number of cells in the cambial zone during the growing season (Fig. 3). The maximum number of cells in the cambial zone was observed in the period from 21th May to 17th June. At sampling of 12th August no new cells in the postcambial growth were observed in sample trees 15D, 18C and 18D. This means that the cambium has finished its division activity in these sample trees at the beginning of August. Also the number of cells in the cambial zone corresponds to the number of cells found at the begining of the monitored period. In sample tree 15C there were cells observed in postcambial growth even on 27th August, i.e. the cambium in this control tree was active one month longer. The morphological appearance of the cells, their number and the structure of cambial zone in the trees exposed to drought stress was not different from the control sample trees at the end of the growing season.

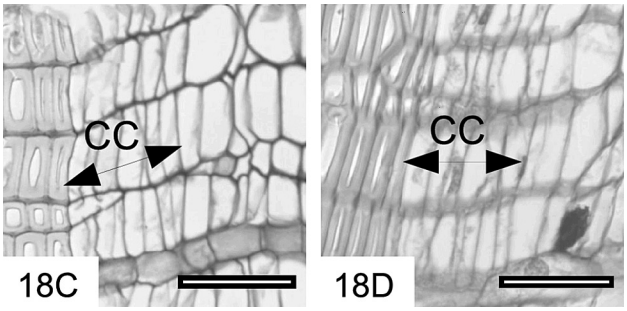

Fig. 2. Cambium (CC) in Norway spruce (*Picea abies* (L.) Karst.) at the beginning of the growing season 7th May; 18C control tree, 18D drought stressed tree. Scale bar = 50 μm

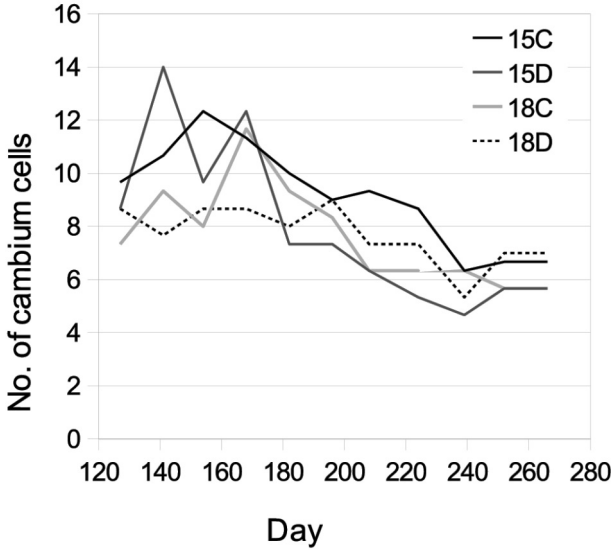

Fig. 3. Number of cambium cells in individual sample trees during growing season 2010 (day 140 = 20 May, 200 = 19 July, 260 = 17 August)

The mean daily temperature from 1st May until the first sampling (7th May) was 4.76°C, however in the last five days in April the mean daily temperature was 7°C. The short decrease in the mean daily temperature below 5°C in the first week of May slowed down the process of cambium activation which had already begun.

Tracheid differentiation

The process of differentiation and the number of tracheid cells in individual phases are presented in Figure 4. The first early wood cells, which appeared in the phase of postcambial growth, were observed in all trees on 21st May.

Clone 15 manifests different numbers of cells in individual phases of a ring formation between the plots. As regards the control tree (15C), Figure 4 shows that the number of cells in postcambial growth remains about the same during the entire growing season (4.5 cells on average). By contrast, the drought stressed tree (15D) has the maximum number of cells in postcambial growth on 21st May and then the number falls gradually during the following phases of the growing season. The earlier termination of the division activity in cambial zone in the stressed tree (15D) was manifested by the fact that the cells in postcambial growth were last observed on 12th August (not in all rows of tracheids); in contrast, in the control tree (15C) these were observed as late as on 27th August.

Also the number of cells in the phase of secondary cell wall formation is different in the two trees of clone 15. The first cells (8 cells 15C; 12 cells 15D) in this phase were observed at the beginning of June (3rd June). In the control tree (15C), the number of cells in this phase remained constant during the entire monitored period reaching the average value of 17 cells. On the other hand, in the stressed tree (15D) the number of cells reached its maximum (17th June, 17 cells) and then dropped gradually. The first fully lignified cells in the control tree (15C) appeared on 17th June; in the stressed tree (15D) it was later, on 1st July.

The process of tracheid differentiation (number of cells in individual phases ring formation) was different in clone 18 in comparison to clone 15. There were no differences between the control (18C) and the

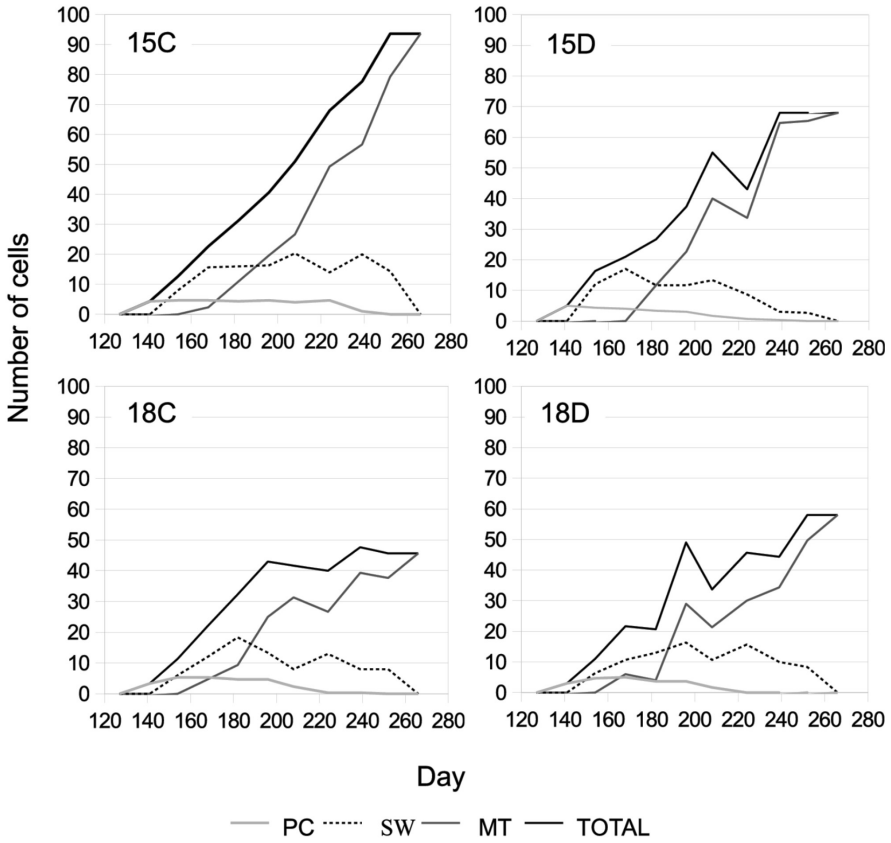

Fig 4. Average dynamics of xylem growth ring formation and individual phases of xylogenesis; PC postcambial growth, SW formation of secondary wall and lignification, MT mature tracheids, TOTAL total number of formed xylem cells. Trees 15D and 18D were influenced by drought stress, 15C and 18C control trees (day 140 = 20 May, 200 = 19 July, 260 = 17 August)

stressed tree (18D) in the number of cells in the phase of postcambial growth, also the beginning (21st May) and the end (27th July) of the period when these cells were observed in this phase are the same. The first cells in the phase of secondary cell wall formation in clone 18 were observed on 3rd June and their number was the same both for the stressed and the control tree: 6 cells on average. During the monitored growing season of 2010 the number of cells in this phase was very similar. The first fully lignified cells were observed on the same sampling date on 17th June and the number of cells in trees 18C and 18D was the same.

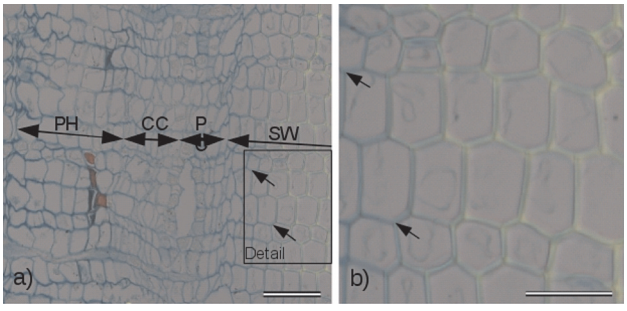

Fig. 5. a) Individual phases of xylem growth ring formation in Norway spruce (*Picea abies* (L.) Karst.) on the transverse section. Example of tree 15D on 17th June 2010. Fluorescence shows intensity of tracheid lignification. CC cambial cells, PC tracheids in the phase of postcambial growth, SW synthesis of secondary cell wall and lignification, PH phloem. Arrows indicate start of tracheid lignification. Scale bar = 100 μm; b) Detail of starting tracheid lignification. Scale bar = 50 μm

Figure 5 documents the process of tracheid lignification from the perspective of the cross section. In all trees lignification of tracheids starts in their corners and proceeds over the middle lamella into the primary and then the secondary cell wall.

The number of cells during the growing season, growth ring

The changes in the number of cells during the growing season are presented in Figure 4. We can see that the actual numbers of formed cells among individual sample trees differ; however, the dynamics of the increase in the cell numbers in all sample trees is very similar and corresponds to an S-shaped growth curve. The cambium in clone 15 formed more tracheids during the growing season than clone 18. The control tree of clone 15 (15C) formed 95 cells during the growing season on average; whereas in the stressed tree (15D) only 68 cells were formed. In

Table 2. Parameters of Gompertz function for 2010 xylem ring formation in Norway spruce trees

	15C	15D	18C	18D
Maximum number of cells	95.15	72.41	45.94	56.37
Maximum daily rate of wood formation (cells/day)	0.92	0.74	0.87	0.66
Date of maximum daily rate	15.7	1.7	17.6	1.7
Duration of wood formation (days)	150	142	89	123

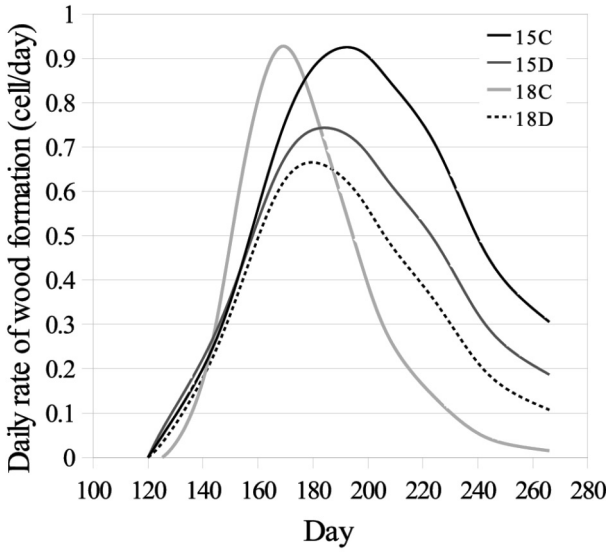

Fig. 6. Daily dynamics of xylem growth ring formation in the growing season of 2010 (day 140 = 20 May, 200 = 19 July, 260 = 17 August)

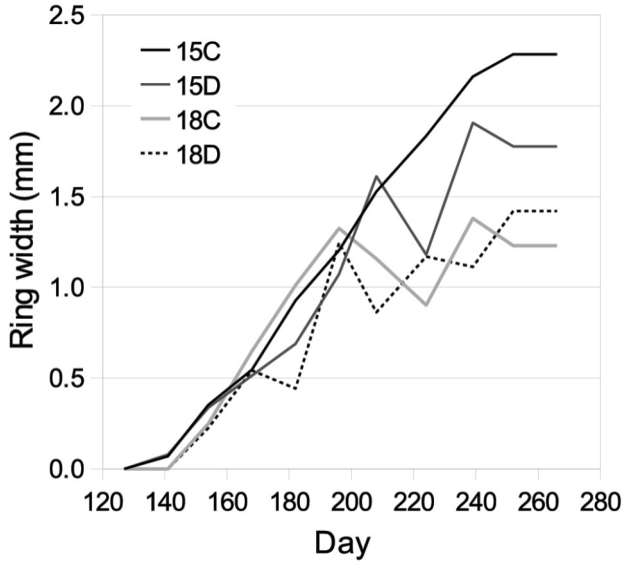

Fig. 7. Dynamics of xylem growth ring formation during the growing season of 2010 in individual trees

clone 18 the difference in the number of formed cells was not so large – 58 cells in the stressed tree and 46 cells in the control tree. The observed fluctuation in the number of cells during the growing season was caused by the fact that the samples were taken gradually during the growing season from various parts around the stem.

We created a model for the Hoxmark site for individual trees which describes the radial increment of spruce wood during the growing season of 2010 by means of the Gompertz function. The following values were calculated on its basis: (1) the maximum number of formed cells, which ranges from 46 to 95 in the individual sample trees; (2) the maximum daily increment – 0.92 (15th July) in tree 15C; and (3) the number of days necessary for the formation of most xylem cells – from 89 to 150 days (see Table 2). The time necessary for the formation of most cells calculated according to the Gompertz function differs from the results of monitoring by means of a microscope. Especially in sample tree 18C the time necessary for the division of most cells was considerably longer than assumed by the Gompertz model. The daily dynamics of cell increment calculated by the Gompertz function are presented in Figure 6. It follows from the model that the maximum daily number of newly formed cells is found in the sample trees which were not stressed by drought.

Figure 7 represents the wood increment in the examined trees. Clone 15 has a larger radial increment than clone 18. The final width of growth ring in clone 15 was 2.3 mm in the control tree (15C) and 1.8 mm only in the drought stressed tree (15D). As regards clone 18, the differences between the control and the stressed tree are not so pronounced. The resulting width of growth rings corresponds to the number of cells formed by cambium during the growing season (Fig. 4). In mid-July it is possible to see a reduction of the radial dimension of a tracheid in all sample trees; it means the cambium was already forming latewood.

Discussion

Norway spruce is a typical tree species with a relatively large range in Europe. Its artificial planting in unoriginal stands where the are climate and thus also the water regime unsuitable has brought many ecological and economic problems. In this research project, two clones of spruce were exposed to a long-term drought stress, which was induced by sheltering the soil surface. It was supposed that trees respond to the drought stress by a change in the cambial activity, formation of new cells of wood and phloem and dimensions of anatomical elements (Abe and Nakai 1999; Abe et al. 2003; Gebauer et al. 2011).

The results show that the monitored clones responded to a changed water regime of the site differently. Clone 15 manifested significant differences between the control tree and the stressed tree. The differences between the control tree and the stressed tree of clone 18 were not confirmed. It seems that the genetic disposition of clone 18 was probably more suitable and this clone adapted better to the changed site conditions. By covering the ground and digging a trench 25–30 cm deep we ensured the upper layers of soil (down to 30 cm) dried. However, in 30–40 cm depth it was possible to find soil moisture even after two years of covering. Spruce usually develops a shallow and flat root system (Nadezhdina et al. 2006) but it was able to use a considerable adaptability to the changed site conditions by directing its root system into deeper layers.

The determining factor of the beginning of cambium activity is an air temperature (Oribe et al. 2001, 2003; Gričar et al. 2006). In 2010 cambial activity at research site Hoxmark, Norway, probably started at the beginning of May. The increase in average daily temperatures at the end of April (above 7°C) was probably sufficient for the cambium to be activated and the drop in temperatures for several days at the beginning of May only caused a slowing down of the cambium reactivation which had already started. To establish the exact time of the beginning of cambium activation in mature trees is highly difficult in natural conditions. Due to the used interval of sampling (two weeks) it is very difficult to capture such a short moment. In 2010 the first cells in postcambial growth appeared in all sample trees on 21st May. The number of cells in postcambial growth was near the maximum, therefore, we can conclude that the cambium at the research site was already active in the first half of May. Matovič (1985, 1990) considered the appearance of the first cell in postcambial growth to be the beginning of cambium activity.

Rossi et al. (2009) observed by *Abies balsamea* very similar amount of cells in the cambial zone in both irrigated and non-irrigated plants. This corresponds with our observations. The number of cells in the cambial zone was highly variable and changed during the growing season. The established average number of cambium cells at last sampling (end of a growing season) was 6.2 and this corresponds to the results published before (Matovič 1985 and 1990; Gričar 2007; Rossi et al. 2007; Gričar and Čufar 2008). The cambial zone in trees exposed to drought stress and in control trees was not different, which is in agreement with Rossi et al. (2009). The number of cells in the cambial zone at the explored site in 2010 gradually increased; from mid-July there was a decrease in the number of cells in the cambial zone, which is connected with the gradual reduction of cambial activity and related to the overall procedure of tree ring formation.

The total time of cambium activity depends on the climate and soil conditions of the growing season and the social position of the tree in the stand (Larson 1994). Temperature (fall of average daily temperatures below 8°C) seems to be the determining factor as regards the end of cambium activity, in critical situations it is also a decrease in soil moisture (a decrease in water storage to the limit or below the limit of reduced availability), or a combination of both (Matovič 1985). The cambial activity in stressed trees ended earlier, which can be put in relation with the water deficit in the soil. In 2010 the cambium in sample trees was active for 4.2 up to 5.4 months (4.8 months on average).

The observations of Rossi et al. (2009) that irrigated and non-irrigated trees showed the same trend of xylem formation and timing of cell differentiation were only paralleled in clone 18 in our research. The stressed and the control tree of clone 18 manifested a very similar behaviour, i.e. very similar trends in the number of cells in particular phases of a newly forming ring, but also in the time concurrence of cell differentiation. By contrast, in clone 15 obvious differences were observed. The tree stressed by water deficit manifested: (1) a decrease in cambium activity, which resulted in a lower number of formed cells and consequently a narrower growth ring; (2) different progress – number of cells in individual phases of a newly forming ring; (3) a shorter activity of cambium; and (4) a slower lignification process at the beginning of the growing season.

The cumulative increment of cells was fitted by the Gompertz function, which had been used in the previous studies (Deslauriers et al. 2003; Rossi et al. 2003; Gričar 2007; Gričar et al. 2008; Gryc et al. 2011). The calculated number of cells formed a day during the time of active growth in Hoxmark in 2010 was from 0.66 to 0.92. This is the average of the entire growing season while the dynamics of the development of new cells were different at the beginning, during, and at the end of the growing season. Gryc et al. (2011) presented the maximum calculated daily increment of number of wood cells for Norway spruce to be 0.34. The lowest increment in Hoxmark site was seen in drought stressed tree 18D: 0.66 cells/day. This value is almost double in comparison with the above mentioned study. This could be explained by the time of photoperiod and the effect of day length and night shortening on the cambial growth.

The calculated final width of xylem growth ring in Hoxmark in 2010 was from 45 to 95 cells. The final average wood increment and the average percentage increment of cells during the growing season was expressed by an S-shaped growth curve, which corresponds to Mäkinen et al. (2003), Schmitt et al. (2004), Heinrichs et al. (2007) and Gryc et al. (2011).

Rossi et al. (2006) found out that the maximum increment of conifers (growing both in Europe and

North America) occurs at the time of the summer solstice, when the photoperiod is the longest. The maximum number of newly formed cells in Hoxmark, as calculated by means of the Gompertz function, agrees with this: the maximum speed of new cell formation occurred from the second half of June to mid-July. The maximum daily production of cells was modelled for the control and stressed trees and it was found that the number of newly formed cells during a day is lower in the stressed trees.

Deslauriers and Morin (2005) estimated, based on weekly sampling, that the daily cell production rate was higher in June or in July, while the transition from earlywood to latewood was observed later in the growing season. In Hoxmark the transition between early- and latewood was observed immediately when the cell production rate reached the maximum value.

We have not observed any intra-annual xylem density fluctuation. Schweingruber (2007) states that it can be a consequence of a water deficit, or other factors come into play. The density fluctuation is caused by a reduction in the tracheid radial dimension, which is brought about by the drop in water potential in xylem, which is unfavourable for cell enlargement (Larson 1994, Rossi et al. 2009). The stressed trees in the research site were exposed to a permanent long-term drought stress and this was probably the reason why no density fluctuation was observed.

Conclusion

The effect of drought stress on regular cambium activity and wood formation in the stem of two different clones of Norway spruce (*Picea abies* (L.) Karst.) was investigated.

The number, dynamics and appearance of cells in the cambial zone were not different in the stressed sample trees when compared with the control trees. The dormant cambium contained 5 to 8 (6.2 on average) cell layers, the active cambium contained 8 to 14 (10.3 on average) cell layers. The period of cambial activity was shorter in drought stressed trees.

The genetics probably caused that in the case of clone 18 no considerable differences in the number of cells in individual phases of tracheid differentiation were observed between the stressed and the control sample trees. Also the resulting number of cells and the ring width was not different in the explored sample tree in the case of clone 18.

The drought stress was most markedly manifested in clone 15. The stressed tree contained a lower number of cells in all phases of new ring formation, the lignification process started later and the resulting number of created tracheids was lower in comparison with the control tree.

The Gompertz function indicates that the drought stressed trees had a lower daily increment of cells than the control trees. Due to the long-term drought stress the intra-annual xylem density fluctuation was not observed.

Clones will probably be highly important for forestry in the future to maximize wood production, not only thanks to the faster production of wood but also their resistance to unfavourable biological and abiotic factors. Therefore, for the future it proves to be necessary to analyse and compare the wood formation of cloned trees with natural trees.

Acknowledgments

The authors wish to thank Toril D. Eldhuset for sampling and Tore Skrøppa for providing the information about the clone material. This work was funded by the Ministry of Education, Youth and Sports of the Czech Republic (grant no. 6215648902), by Iceland, Liechtenstein and Norway through the EEA Financial Mechanism together with the Norwegian Financial Mechanism (grant no. A/CZ0046/2/0009), by Mendel University in Brno (grant IGA 12/2010).

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