WOOD RESEARCH
53 (4): 2008
35-44

PARAMETERS OF GOMPERTZ FUNCTION FOR EVALUATION OF WOOD FORMATION DYNAMICS EXPRESSED AS NUMBER OF CELLS OR MEASURED WIDTHS IN NORWAY SPRUCE

Jožica Gričar, Tom Levanič
Slovenian Forestry Institute, Department of Yield and Silviculture,
Ljubljana, Slovenia

Primož Oven
University of Ljubljana, Biotechnical Faculty,
Department of Wood Science and Technology, Ljubljana, Slovenia

ABSTRACT

We evaluated the seasonal dynamics of wood formation in Norway spruce during 2002-2004 using the Gompertz function. We counted the cells and measured the widths of the xylem increments, calculated the parameters of the Gompertz function and compared the data to verify whether the obtained results are comparable. The number of xylem cells reflects cambial productivity, while xylem increment also includes radial expansion of the newly formed tracheids. Comparison of the parameters of the Gompertz function obtained from cell numbers and from measured widths revealed minor differences in the values, but they were not statistically significant. In cases of crushed or compressed developing tissue, measurement of the xylem increment is not reliable. Measurement of the xylem increment is faster than cell counting and might be more appropriate for studying deciduous trees, in which the xylem is more complex and composed of morphologically diverse types of elements that are not arranged in radial files.

KEY WORDS: Norway spruce *(Picea abies)*, wood formation, cambial activity, Gompertz function, cell counting, width measurements, pinning method, image analysis

INTRODUCTION

Cambium is meristematic tissue from which secondary growth occurs in stems, branches and roots of woody plants, producing xylem in the centripetal and phloem in the centrifugal direction (Panshin and de Zeeuw 1980). The cambium of temperate-zone tree species exhibits periods of activity and dormancy. It is normally inactive in winter, when the number of cambial cells in the dormant state is reduced (Lachaud et al. 1999, Larcher 2003). Resumption of cell division in the cambium in spring coincides with an increase in the number of cambial cells. A process of differentiation subsequently occurs, which can be divided into four major steps: cell expansion (post-cambial growth), secondary cell wall formation and lignification and, in the case of tracheids, also programmed cell death. A mature cell is finally capable of water conduction and mechanical support. Phases of cell differentiation temporally and spatially succeed cambial cell divisions (Fig. 1a). Cell divisions in the cambium and radial expansion of the xylem cells determine the width of the xylem growth rings. The processes of secondary wall formation and lignification do not contribute to an increase in the stem diameter, but only to an accumulation of the biomass in the cell wall (Panshin and de Zeeuw 1980, Larson 1994, Kozlowski and Pallardy 1997, Plomion et al. 2001, Wodzicki 2001).

The formation of the xylem growth ring of an individual tree or studied growth site during growth seasons has frequently been explained with different functions (logistic, Weibull, Korf, Richards etc.); however, the Gompertz function has proved to be most suitable because of its asymmetric shape (Zeide 1993, 2004, Camarrero et al. 1998, Deslauriers and Morin 2005, Gričar 2007, Rossi et al. 2003, 2006, 2007). The application of various functions that correspond to biological trends of seasonal tree growth attempts to eliminate the variability in the number of cells (increment width) within the same growth ring around the stem circumference. However, the xylem increment can be interpreted as the number of cells or measured widths, although these two biometrical values do not contain exactly the same information (Fig. 1b). The number of cells reflects the cell productivity of the cambium; whereas measured widths record also the extent of radial expansion of the newly formed tracheids.

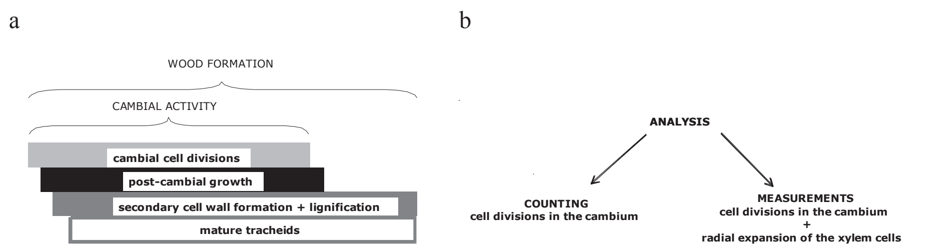

Fig. 1: a – Successive phases of wood formation. b – Two different methodological approaches for studying xylem growth ring formation.

The aim of the present research was to evaluate the seasonal dynamics of wood formation in Norway spruce at two elevations in Slovenia during 2002-2004 using the Gompertz function. Since various studies use different methodological approaches for such studies, it is sometimes difficult to compare findings with other groups. We therefore counted the cells and measured the widths of the xylem increments, calculated the parameters of the Gompertz function and evaluated the data to verify whether the obtained results are similar.

MATERIAL AND METHODS

We selected for the study Norway spruce trees (*Picea abies* (L.) Karst.) growing at Sorsko polje (SorP - 350 m a.s.l.) and at Pokljuka (Pok - 1250 m a.s.l.). SorP is a typical lowland plantation of Norway spruce. (Fig. 2). The average yearly temperature at SorP measured at the nearby meteorological station of Ljubljana is 9.8°C, with January being the coolest (-1.0°C) and July the warmest month (20.0°C). The distribution of precipitation should favour spruce growth, May-September are well supplied with precipitation (47% of annual precipitation falls in this period). However, despite being well supplied with precipitation during the growing period, the regular occurrence of drought is being observed at this site. This is mainly because there is a thick layer of gravel just below the organic horizon, which drains water into deeper layers, inaccessible to the spruce roots. Trees growing on such shallow, organic soils are therefore subjected to high seasonal oscillations in the soil water contents, resulting in regular summer water stress. Bark beetle attacks seem to be one of the secondary drought effects in this forest stand. Moreover, many of the trees are affected by root rot disease.

The Alpine site Pok is a typical, natural, sustainably managed sub-alpine spruce forest site *(Piceetum subalpinum)*. The site is characterized by low winter temperatures and high amounts of snow (Fig. 2). January and February are the coolest and driest months at the Pok (average monthly minimum -4.4 - -5.2°C and 117 and 115 mm precipitation). July and August are the warmest months, with long-term monthly average of 12.3 and 11.9°C, respectively. Precipitation is abundant throughout the year, with October (186 mm), November (194 mm) and December (227 mm) being the wettest months. Average yearly precipitation at the Pok site is 1978 mm, with an absolute maximum of 2604 mm and minimum 1351 mm. The site is moist and relatively cool in the summer and therefore optimal for Norway spruce growth. Ground vegetation at the site is scarce due to very dense tree crowns.

Fig. 2: Location of the research plots Pokljuka (Pok) and Sorško polje (SorP)

At each site, 5 trees with comparable biometric characteristics and age (about 70 years) were selected. The DBH was around 30 cm at SorP and 37 cm at Pok. Crowns of the sampled trees were in the stand canopy and normally developed (social position 2 by Kraft's classification), stems were without any visible mechanical damage and root systems were intact and at least 10 m from skidding trails. Both sites were visited weekly in 2002, 2003 and 2004, from the end of April until the end of October, when pinning experiments were carried out. The pinning method uses the ability of the cambium and its youngest derivatives to respond to a minute mechanical injury without affecting the integrity of the tree (Wolter 1968). Pin insertion into the cambium causes minute wound reactions which define the xylem increment formed up to the time of pinning. Six pinning holes were therefore set in a semi-helical pattern along the stem of each tree at the same experimental date, using a needle that was 1.75 mm at its thickest part. The holes were marked and numbered. After the end of each growing season, the pinned trees were felled, and samples containing wounded tissue were removed and processed for light microscopy. The sample blocks (30 x 10 x 10 mm) contained the inner part of the living bark, the cambium, the current xylem increment, and at least one previous fully formed xylem growth ring. Immediately after removal, the tissues were fixed in FAA (formalin-ethanol-acetic acid solution) and dehydrated in a graded series of ethanol (30%, 50% and 70%). Permanent transverse sections of approximately 25 μm in thickness were prepared using a Leica SM 2000R sliding microtome, stained with safranine and astra blue and finally mounted in Euparal. Microscopic observations and analysis were carried out with a Nikon Eclipse E800 light microscope (bright field or polarized light) and the Lucia G 4.8 image analysis system. We counted the number of cells and measured the width of the xylem increment formed from the onset of cambial growth to the time of pinning in at least 3 radial files of tracheids.

We used the Gompertz function to describe the seasonal dynamics of wood formation of the sampled trees at both sites in each year:

$$y = A \exp(-\exp(B - k t)) \tag{1}$$

y – weekly cumulative increment expressed in sum of cells or measured widths
t – time expressed as a day of the year
A – upper asymptote representing the maximum number of cells or xylem growth ring width
B – x-axis placement parameter
k – rate of change parameter

For all 3 sampling sites, we established the inflection points on individual model curves. The inflection point on the Gompertz function corresponds to the maximum value of the first derivative of function *y'*:

$$y' = A k \exp(-\exp(B - k t)) \exp(B - k t) \tag{2}$$

At the maximum value of the first derivative *y'*, the second derivative *y''* = 0:

$$y'' = y' k (\exp(B - k t) - 1) = 0 \tag{3}$$

The Gompertz function has an “S-type” shape and the inflection point is at the maximum of the first derivative, where *y''* changes from positive values into negative. The second derivative *y''* equals 0 when

$$T = B/k \tag{4}$$

T – inflection point on the horizontal axis representing the period of maximum growth rate
B – x-axis placement parameter
k – rate of change parameter

With the help of parameter *k* of the Gompertz function that defines the dynamics of growth, it is possible to calculate the time *d* required for the formation of the majority of the 2003 xylem growth ring in trees at individual growth sites, as already explained in detail by Deslauriers and Morin (2005):

$$d = 4/k \qquad (5)$$

k – rate of change parameter

With parameters *A* and *k,* we have calculated coefficient *r*, which represents the average weekly xylem increment (expressed as number of cells or measured widths) formed per day during the 2003 growth season (Deslauriers and Morin 2005):

$$r = A\,k/4 = A\,d^{-1} \qquad (6)$$

k – rate of change parameter

All statistic calculations were made in Microsoft Excel and SYSTAT v.11 and *R* (R Development Core Team 2006), and the graphs were drawn in software SigmaPLOT 10.

RESULTS AND DISCUSSION

Comparison of the curves of the Gompertz function revealed very similar patterns of wood formation dynamics in the case of cell counting and of width measurements, regardless of the investigated plot and year (Fig. 3a, b). At both sites, the process of wood formation was similar in 2002 and 2004, but completely different at SorP in 2003 due to a major drought in Europe.

a b

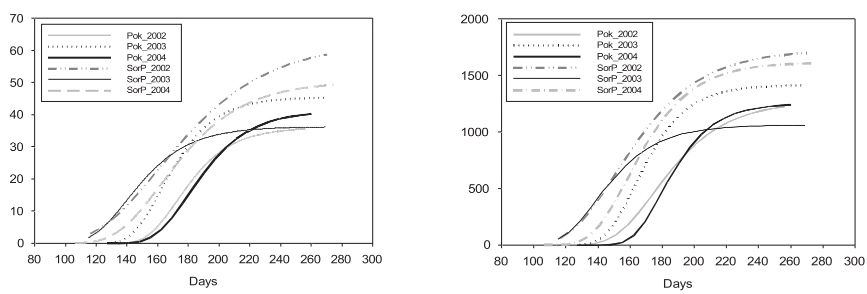

Fig. 3: Seasonal dynamics of wood formation in Norway spruce at the two sites using the Gompertz function expressed in number of cells (a) or measured widths (b) of the xylem increment achieved by the time of wounding.

Parameters of the Gompertz function shown in Tab. 1 and 2 indicate higher values of the coefficient *k* (estimating rate of change parameter) of the measured widths than of the counted cell number in all cases. This could be explained by a significant contribution of early wood tracheids with wide radial dimensions to the final widths of the accomplished xylem growth ring, comparing to late wood cells produced in the second part of the vegetation period. Coefficient *B* reflecting the placement on the x axis or onset of wood formation was always a little higher with measuring, very likely due to the relatively small share of the initial cells still undergoing radial cell expansion in the final xylem growth ring widths (Fig. 3, Tab. 1, 2). Shortly afterwards, when the cells achieve their final dimensions, the curve of the measured increments rapidly changes from a regressive shape into a progressive one. The values of coefficient *d* (time required for the formation of the majority of the xylem growth ring) were lower in the case of cell numbers. Since this coefficient only refers to the major part of the growing season, it eliminates the last couple of weeks, when only a few new cells are formed over a longer period of time. Understandably, with the measurements of the increment widths, which include also radial cell dimensions, more days are removed from the estimation of *d* because of the very narrow most recently formed layers of late wood cells.

The period of maximum growth rate (coefficient *T*) was judged to be higher in the case of cell numbers by up to 4 days or even 9 days at SorP in 2002 (Tab.1, 2). The intensity of xylem growth ring formation occurred earlier when the xylem increment was measured, on account of very wide early wood tracheids. Student's t-test revealed no statistically significant differences between the parameters of the Gompertz function *B* (t = -0.9602^{NS}, df=10), *k* (t = -1.2842^{NS}, df=10), *T* (t = 0.3809^{NS}, df=10) and *d* (t = 1.2012^{NS}, df=10) calculated from cell numbers and measured widths, suggesting that it is possible to compare the results obtained by cell counting and increment measurements. Rossi et al. (2006) similarly found no statistical differences between the slopes of regression with cell counting and dendrometer measurements, and coefficient *T* corresponded to the summer solstice on 21 June. However, it is necessary to stress that dendrometers measure total stem radial increment, including phloem growth ring formation, xylem formation, increase and decrease of cambial tissue, collapse of older phloem tissues etc.

Tab. 1: Parameters of Gompertz function of wood formation dynamics expressed in number of cells for spruce at SorP and Pok in 2003.

Site / Parameters	SorP 02	SorP 03	SorP 04	Pok 02	Pok 03	Pok 04
A	62.711	36.272	50.526	36.128	45.508	41.440
B	4.0219	6.2569	5.5191	8.8645	8.5228	8.2231
k	0.0251	0.0447	0.0340	0.0514	0.0525	0.0457
T	160.43	140.13	162.28	172.50	162.37	180.05
r	0.3935	0.4053	0.4295	0.4642	0.5973	0.4735
d	159.36	89.49	117.65	77.82	76.19	87.53

A – upper asymptote indicating maximum number of cells; B – placement on the x axis estimating onset of cambial activity; k – rate of change parameter; T – placement of the inflection point on the horizontal axis indicating the period of maximum growth rate; r – average number of cells per day produced during the growing season; d – time required for the formation of the majority of the xylem growth ring.

Tab. 2: Parameters of Gompertz function of wood formation dynamics expressed in widths of spruce at SorP and Pok in 2003.

Site / Parameters	SorP 02	SorP 03	SorP 04	Pok 02	Pok 03	Pok 04
A	1729.29	1059.81	1619.81	1227.58	1418.65	1254.91
B	5.2245	6.6470	7.1757	9.5431	9.0561	10.190
k	0.0346	0.0479	0.0453	0.0565	0.0557	0.0568
B/k	151.17	138.81	158.47	169.05	162.53	179.50
r	14.94	12.69	18.34	17.32	19.76	17.81
d	115.74	83.53	88.34	70.86	71.78	70.46

A – upper asymptote indicating maximum width of the xylem growth ring; B – placement on the x axis estimating onset of cambial activity; k – rate of change parameter; T – placement of the inflection point on the horizontal axis indicating the period of maximum growth rate; r – average xylem increment per day produced during the growing season; d – time required for the formation of the majority of the xylem growth ring.

Linear regression between counted number of weekly formed cells and measured widths of the increment showed a positive slope with high correlation (R^2 = 0.918) (Eqn. 7, Fig. 4). Because of the differences in cambial cell production around the stem circumference of an individual tree, xylem ring widths also vary. The xylem increment in a given week can therefore be smaller than in the preceding when samples taken at different locations around the stem are compared in a chronological order (Fig 4.). This can be presented as an artificial "negative increment" of the xylem growth ring (Rossi et al. 2003, Gričar 2007). "Negative increments" were mostly calculated in the second half of the growing season, when the rate of cell divisions in the cambium decreased and fewer cells were formed over a longer time period.

$$\textit{Cell number} = 0.489327 + 0.02389 * \textit{measured width} \quad (7)$$

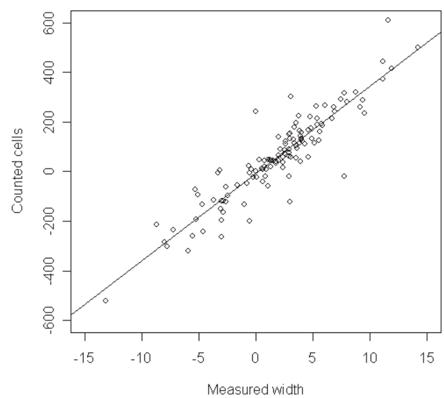

Fig. 4: Linear regression between the counted number of cells and the measured widths of the weekly xylem increments

We calculated the time required for the formation of 25%, 50%, 75% and 100% of the xylem growth ring for each site and year (Tab. 3,4). In all of the investigated samples, three quarters of the xylem growth ring was formed 2-4 times faster than the last quarter. This difference was more pronounced with increment measurements when the development of the latest late wood cells with narrow radial dimensions at a very slow rate negligibly increased xylem growth ring widths. Proportions in the final widths of the accomplished xylem growth rings expressed as either number of cells or measured widths stayed relatively unchanged among years and between sites (Fig. 5).

Tab. 3: Days required for the formation of 25%, 50%, 75% and 100% of the xylem growth ring (XGR) expressed in number of cells in Norway spruce at SorP and Pok during 2002-2004.

Site / XGR [%]	SorP 02	SorP 03	SorP 04	Pok 02	Pok 03	Pok 04
25	0	0	0	0	0	0
50	27.1	15.1	20	13.1	12.9	14.8
75	33.8	18.5	24.7	16.1	15.9	18.3
100	117.7	54.8	81.0	47.6	50.7	56.2

Tab. 4: Days required for the formation of 25%, 50%, 75% and 100% of the xylem growth ring (XGR) in expressed in widths Norway spruce at SorP and Pok during 2002-2004.

Site / XGR [%]	SorP 02	SorP 03	SorP 04	Pok 02	Pok 03	Pok 04
25	0	0	0	0	0	0
50	20.1	14.5	15.3	12.3	12.5	12.2
75	25.4	18.3	19.4	15.5	15.7	15.8
100	179.5	119.5	135.7	103.9	108.0	103.7

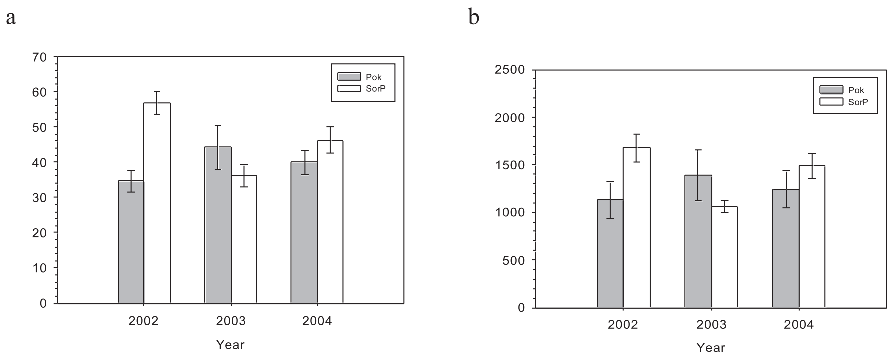

Fig. 5: Accomplished xylem growth ring 2002, 2003 and 2004 in trees at SorP and Pok expressed in number of cells (a) or measured widths (b). Error bars indicate standard deviation.

CONCLUSIONS

- The number of the xylem cells reflects cambial productivity; while xylem increment also includes radial expansion of the newly formed tracheids.
- Comparison of the parameters of the Gompertz function for evaluation of wood formation dynamics obtained by cell counting and by measurement of the xylem increment revealed

minor differences in the values of the parameters, but these were not statistically significant in Norway spruce. The results of the two methodological approaches could therefore be compared.

- Measurement of the xylem increment is faster than cell counting, but in cases of crushed or compressed developing tissue, measurements are not reliable.
- Measurement of the xylem increment might be more appropriate for the study of the wood formation dynamics of deciduous trees, where the xylem is more complex and composed of morphologically diverse types of elements that are not arranged in radial files.

ACKNOWLEDGEMENTS

We are grateful to Martin Zupančič and Peter Cunder from the Department of Wood Science and Technology, Biotechnical Faculty, University of Ljubljana, Slovenia and to Risto Jalkanen and Tarmo Aalto, METLA, Rovaniemi, Finland for their invaluable help in the field and laboratory. We are indebted to the Slovenian Forest Service, OE Bled and OE Kranj for enabling us experimental work in the field. The work was funded by the EU project PINE (EVK2-CT-2002-00136).

REFERENCES

1. Camarero, J.J., Guerrero-Campo, J., Gutiérrez, E., 1998: Tree-ring growth and structure of *Pinus uncinata* and *Pinus sylvestris* in the Central Spanish Pyrenees. Arctic and Alpine Research, 30: 1-10
2. Deslauriers, A., Morin, H., 2005: Intra-annual tracheid production in balsam fir stems and the effect of meteorological variables. Trees, 19: 402-408
3. Gričar, J., 2007: Xylo- and phloemogenesis in silver fir (*Abies alba* Mill.) and Norway spruce (*Picea abies* (L.) Karst.). Professional and Scientific Works. Slovenian Forestry Institute, Silva Slovenica. Ljubljana, 106 pp.
4. Kozlowsky, T.T., Pallardy, S.G., 1997: Growth control in woody plants. Academic Press Inc. San Diego, 641 pp.
5. Lachaud, S., Catesson, A.M., Bonnemain, J.L., 1999: Structure and functions of the vascular cambium. Life Sciences, 322: 633-650
6. Larcher, W., 2003. Physiological plant ecology. Ecophysiology and stress physiology of functional groups. Fourth edition. Springer. Berlin Heidelberg New York, 513 pp.
7. Larson, P.R., 1994: The vascular cambium: development and structure. Springer. Berlin Heidelberg New York, 725 pp.
8. Panshin, A.J., de Zeeuw, C., 1980: Textbook of wood technology. Fourth edition. McGraw-Hill. New York, 722 pp.
9. Plomion, C., Leprovost, G., Stokes, A., 2001: Wood formation in trees. Plant Physiology, 127: 1513-1523
10. R Development Core Team, 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org
11. Rossi, S., Deslauriers, A., Morin, H., 2003: Application of the Gompertz equation for the study of xylem cell development. Dendrochronologia, 21: 33-39

12. Rossi, S., Deslauriers, A., Anfodillo, T., Morin, H., Saracino, A., Motta, R., Borghetti, M., 2006: Conifers in cold environments synchronize maximum growth rate of tree-ring formation with day length. New Phytologist, 170: 301-310
13. Rossi, S., Deslauriers, A., Anfodillo, T., Carraro, V., 2007: Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia, 152: 1-12
14. Wodzicki, T.J., 2001: Natural factors affecting wood structure. Wood Science and Technology, 35: 5-26
15. Wolter, K.E., 1968: A new method for marking xylem growth. Forest Science, 14: 102-104
16. Zeide, B., 1993: Analysis of growth equations. Forest Science, 39: 591-616
17. Zeide, B., 2004: Intristic units in growth modeling. Ecological Modelling, 175: 249-259

Jožica Gričar
Slovenian Forestry Institute
Department of Yield and Silviculture
Večna pot 2
SI-1000 Ljubljana
Slovenia
E-mail: jozica.gricar@gozdis.si
Tel: +386 1 200 78 00
Fax: +386 1 257 35 89

Tom Levanič
Slovenian Forestry Institute
Department of Yield and Silviculture
Večna pot 2
SI-1000 Ljubljana
Slovenia
Tel: +386 1 200 78 00
Fax: +386 1 257 35 89

Primož Oven
University of Ljubljana
Biotechnical Faculty
Department of Wood Science and Technology
Rožna dolina
Cesta VIII/34
SI-1000 Ljubljana
Slovenia