Turkish Journal of Agriculture and Forestry

Volume 33 | Number 5 Article 4

1-1-2009

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Available at: https://journals.tubitak.gov.tr/agriculture/vol33/iss5/4

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This article is available in Turkish Journal of Agriculture and Forestry: https://journals.tubitak.gov.tr/agriculture/vol33/iss5/4

TÜBİTAK

Research Article

Turk J Agric For
33 (2009) 455-462
© TÜBİTAK
doi:10.3906/tar-0903-42

The relationship between the form of dead bark and lignin content in Scots pine (*Pinus sylvestris* L.)

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Received: 24.03.2009

Abstract: In this study the proportion of lignin in wood tissue was analysed in 36 Scots pines differing in their form of dead bark. Material for analyses came from pure pine stands aged 89-91 years located in northern Poland. Chemical analyses were performed in the mature wood zone comprising the last 10 diameter increments located at a height of 1.3 m (DBH). Results indicate differences in lignin content and growth rate in pines whose stems were characterized by different forms of dead bark. The study showed significant differences in lignin contents between trees with shell and scaly dead bark and trees with ropy bark form. Pines with ropy bark were characterized by the lowest lignin content in relation to trees having the other 2 forms of dead bark. Moreover, a relationship was observed between diameter growth rate and lignin content in wood tissue.

Key words: Form of dead bark, lignin content, Pinus sylvestris L.

Introduction

Variation in outer bark in Scots pine has been investigated by many botanists and foresters searching for a relationship between its form (thickness and appearance) and characteristics of trees. In 1820 Szubert, as one of the first researchers, reported that in poor sites pines are characterized by thinner outer bark, which is less regularly cracked than that of trees in more fertile sites.

In Latvia, variation in the form of bark in pines was investigated by Schwerin (1911), in turn in Germany Seitz (1926) identified 3 forms of dead bark, i.e. platy (German "Plattenkiefer"), scaly ("Schuppenkiefer"), and shell bark ("Muschelkiefer").

Bakos (1965) stated that there is a relationship between the type of bark and annual growth. Erteld (1966) in his investigations on 100-year old pines found that trees with shell type bark are characterized by larger annual growth compared to trees with the other bark forms.

A study concerning the relationship between the form of dead bark and other characteristics of *Pinus sylvestris* L. trees was published for the first time in the pre-WWII period by Seitz (1926). Further

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investigations on the relationship between the form of dead bark in pines and the age, growth rhythm, and diameter growth dynamics were conducted by Dengler (1937, 1938) and Wagenknecht (1939). In 1975 studies on the dependence between the type of dead bark on characteristics of trees and wood properties were systematized by Klon (1975).

The process of lignification of plant tissues is connected with the synthesis of lignin and according to Sjostrom (1993) its accumulation in plant tissues ranges from 16% to 30%; however, in trees lignin can account for 18%–36% of the dry weight of wood (Sarkanen and Hergert 1971). Not only the high proportion of lignin and its structure, but also its distribution in the secondary cell walls of xylem elements have a significant effect on technical properties of wood. The presence of lignin in wood tissue increases the resistance of cell walls to deformation and ensures the stability of woody plants (Boudet et al. 1995; Santos Abreu et al. 1999). In addition, lignin facilitates water transport and impedes the degradation of wall polysaccharides, thus acting as a major line of defence against pathogens, insects, and other herbivores (Monties 1989; Doke et al. 1994; Hatfield and Vermerris 2001).

Apart from cellulose, lignins are the most numerous group of organic substances in the world, accounting for 20%-30% total plant biomass (Lewis 1999). From the chemical point of view, lignin is a complex phenolic polymer, usually derived from various proportions of 3 cinnamyl alcohol precursors, i.e. *p*-coumaryl, coniferyl, and sinapyl alcohols, which are synthesized from phenylalanine ammonio-lyase (PAL) via the phenylpropanoid pathway (Campbell and Sederoff 1996). The aromatic rings of these alcohols are named p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), respectively, at the same time constituting the basis for the designation of types of lignins. The proportions of monomers participating in the structure of the discussed macromolecule depend on the type of wood: hardwood contains lignin composed primarily of coniferyl, and sinapyl alcohols (GS lignins), while softwood - of coniferyl alcohol (G lignin). Thus, in conifers, lignin composed of G units is found, while in deciduous species lignins contain both G and S units. The biological role of this variation in the composition and its significance in the structure–function relationship of lignin still remain unclear.

The chemical structure of wood and the proportions of individual components depend on many factors. One of the rather poorly understood such relationships is that between morphological characteristics, such as the form of dead bark and the proportions of individual cell wall components, including lignin. The study was an attempt to determine lignin contents in wood of Scots pines characterized by 3 basic types of dead bark,namely platy, shell, and scaly bark.

Materials and methods

Field studies

Sample plots were chosen in 4 locations of Scots pine (*Pinus sylvestris* L.) found within the limits of natural range of this species in Europe.

All plots were selected in northern Poland in the Tuchola Forest Division (53° 36’ N 17° 51’ E), found within the administrative boundaries of the Regional Directorate of State Forests in Toruń (Figure 1).

Stands in which sample plots were located are found in the temperate climate zone with a mean annual temperature of +7.1°C and mean annual precipitation of 550 mm.

Figure 1. Location of the study.

Investigations were conducted between October 2007 and May 2008. They were realized on 36 trees (*Pinus sylvestris* L.) aged 89 to 91 years, growing in fresh coniferous forest and fresh mixed coniferous forest. A detailed description of sample plots is given in Table 1.

In each stand an area of 1 ha sample plot was established, where diameters at breast height (DBH) of all trees were measured, at the same time dividing them into 3 groups differing in the form of dead bark, i.e. trees with ropy bark form (G), shell type bark (M), and scaly bark (L), respectively.

L - *scaly form* – outer bark in this form is deeply grooved, scales are thick and overlapping.

M - *shell form* – outer bark in this form is cracked into scales with involute margins.

G – *ropy form* – outer bark in this form is thick, smoothly flaking, it is also called mirror bark (Figure 2).

The most frequent form observed in those trees was ropy bark, followed by pines with shell bark, while the least numerous group comprised trees with the thinnest bark, i.e. scaly form (Figure 3). The range of individual bark forms, as presented in Figure 3, was analogous to that mentioned above.

In each group heights of all trees were measured in assumed 2 cm diameter subclasses.

Table 1. Characteristics of stands in which experimental plots were established.

Age [years]	Site	Quality class	Area [ha]	Closure	Stockin
91	MFCF	I	2.68	moderate	0.9
89	MFCF	I	1.28	moderate	0.9
89	FCF	II	23.62	moderate	0.9
89	FCF	II	19.9	moderate	0.9

Figure 2. Form of dead bark: scaly bark (L), ropy bark (G), and shell type bark (M) *(I. Niemiec)*.

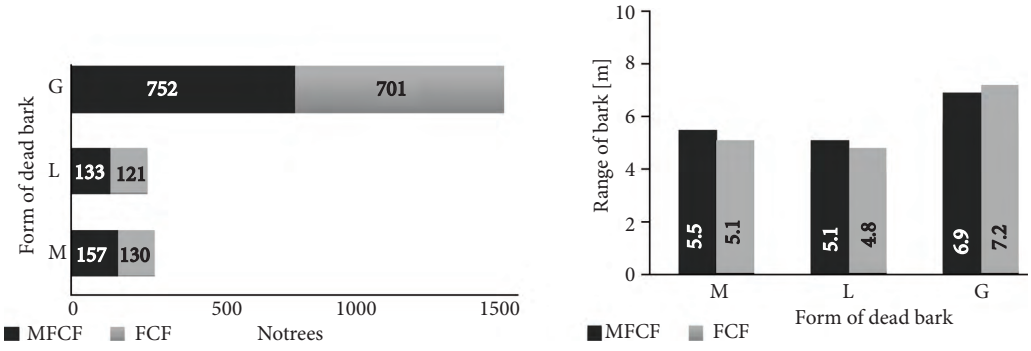

Figure 3. Frequencies of trees and the range of dead bark in trees depending on the form of dead bark and forest site type.

Model trees were selected using both the Urich II dendrometric method (Grochowski 1973) and the classification developed by Kraft (1884), including the main stand, namely predominant, dominant, and codominant trees.

Class I – predominant trees: trees dominate in height and have a strongly developed crown;

Class II – dominant trees: form the main canopy of the stand with well-developed crowns;

Class III – codominant trees: crowns still normally developed, but laterally narrowed, they are slightly inferior in height in comparison to dominant trees according to Kraft (1884).

Overall in each experimental plot nine model trees were identified, with 3 for each assumed forms of dead bark.

Experimental material was collected from each model tree from drillings with an accretion borer at a height of 1.3 m (DBH). Drill cores with a diameter of 1 cm were collected from 2 geographical directions – the north and the south.

Moreover, based on the width of annual increments in diameter, the juvenile and mature wood zones were distinguished while in the mature wood zone the dynamics of increment in diameter was determined. The width of annual rings was measured accurate to 0.01 mm with an increment meter with a mechanical guide and electronic recording of ring width. The *Grubecom* software (GRUBE KG, 29646 Bispingen OT Hützel, Germany) was used to operate the device and record measured values.

Material for lignin analyses was collected from the last 10 increments in diameter and thus they were the samples collected from the mature wood zone in the sapwood section.

Determination of lignin content

Lignin content was measured according to a modified method of Doster and Bostock (1988). Wood of the 10 last annual rings was treated for 48 h with double-replaced methanol, using 1 mL of methanol per 1 g of tissue. Next wood was dried in a desiccator. The amount of 20 mg dry tissue was mixed with 5 mL of 2N HCl and 0.5 mL of thioglycolic acid. Samples were heated at 95 °C for 4 h and centrifuged at 3000 × g for 20 min. The precipitates were washed twice with deionized water, extracted by 5 mL of 0.5 N NaOH for 18 h at room temperature and centrifuged at 15,000 × g. Next, the NaOH extract was collected and the precipitate was washed with 4 mL of deionized water and centrifuged at 15000 × g. The obtained supernatant was added to the NaOH extract. Then the extract was acidified with 1 mL of concentrated HCl, placed at the temperature of 5 °C overnight and centrifuged (15000 × g). The obtained precipitate was dissolved in 5 of 0.5N NaOH and centrifuged at 15000 × g. Absorbance was measured at 280 nm using a UV-1202 Shimadzu spectrophotometer. Calibration curves were generated by subjecting increasing amounts of 0.5–2.5 mg of commercial lignin (alkaline spruce lignin, Sigma-Aldrich) to the same procedure. Lignin content was expressed in mg per one gram of dry matter. Determinations were performed in 5 replications for each sample.

Microscopy

Samples for microscopy observations were embedded in epoxy-resin and ultrathin sections were cut on an ultramicrotome (Reichert Ultracut S). Sections were observed to detect the natural fluorescence of lignin with a Zeiss Axiovert 200M inverted microscope equipped with a confocal laser scanner (Zeiss LSM 510, Germany). The excitation wavelength applied was 488 nm, whilst emission spectrum was measured at 530 nm. Images were processed and analyzed by Zeiss LSM 510 software. Visible light autofluorescence is primarily due to the presence of lignin, thus shows the distribution of lignin across the tracheid cell wall and increased autofluorescence corresponds to an increased lignin content (Kutscha and McOrmond 1972; Donaldson et al. 1999).

All analyses were conducted using the STATISTICA 8.0 software package.

Results

Since the analysis of variance did not show the effect of site conditions on the proportion of lignin, site type was excluded from analyses in this study and only the form of dead bark was investigated in terms of modification of the analyzed characteristics.

Lignin contents in the wood of analyzed trees ranged from 180.5 mg g^{-1} in pines with the thickest form of bark (ropy bark) to 249.4 mg g^{-1} in trees with shell bark. The biggest standard error (4.51), standard deviation (15.61), and coefficient of variation for the analyzed variable (7.31%) were found in pines with ropy outer bark and they decreased gradually with the transition to trees with increasingly thin bark (shell – scaly) (Table 2). Mean lignin content was 240.7 mg g^{-1} in pines with scaly outer bark (L), followed by 235.43 mg g^{-1} in pines with shell bark (M) and 213.38 mg g^{-1} in trees with the thickest, ropy bark (G) (Table 2, Figure 4).

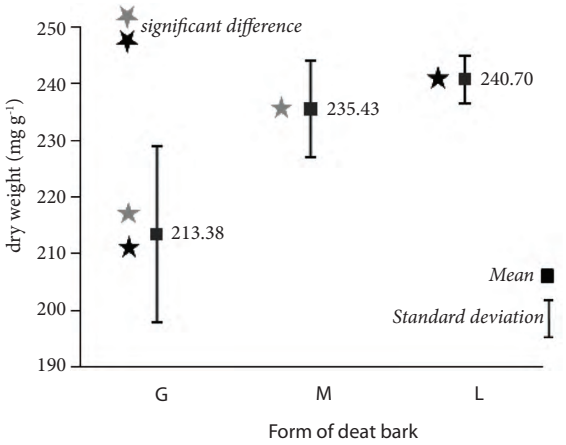

Figure 4. Lignin contents in wood of Scots pines depending on the form of dead bark.

The distribution of analyzed variables was similar to the normal distribution and the conducted analysis of variance indicates the occurrence of differences in lignin contents in pines depending on their form of dead bark.

Based on Tukey's LSD test it was found that statistically significant differences in lignin contents occurred between trees with ropy bark (G) and trees with shell bark (M) and scaly bark (L). The significantly lowest lignin content was recorded in trees with ropy bark. In turn, no significant differences were observed between lignin content in trees with shell bark (M) and scaly bark (L) (Table 3). In this study the dynamics of increment in diameter was also analyzed on the basis of width of annual increments in diameter. Results presented in Table 4 indicate that the statistically significant highest dynamics of increment in diameter was recorded in pines with ropy bark. Mean width of annual rings in the mature wood zone in those trees was 1.40 mm. These trees were also characterized by the biggest standard deviation (0.37) and the highest variation in increment in diameter (30.5%) among all the analyzed groups (Table 4).

The mean width of annual rings in the mature wood zone in pines with shell bark was 1.01 mm and ranged from 0.58 mm to 1.71 mm. In turn, in pines with scaly bark these values were comparable,

Table 3. Results of HSD test of lignin content of Scots pine (*Pinus sylvestris* L.).

		type of bark		
		G	M	L
type of bark	G		**0.000291**	**0.000127**
	M	**0.000291**		0.371722
	L	**0.000127**	0.371722	

Marked effects are significant at $P < 0.05$

Table 2. Lignin content in wood of Scots pines depending on the form of dead bark.

		Mean [mg g^{-1}]	Total [mg g^{-1}]	Standard deviation	Standard error	Minimum [mg g^{-1}]	Maximum [mg g^{-1}]	Coefficient of variation [%]
Type of bark	G	213.381	2560.570	15.607	4.505	180.520	231.100	7.31
	M	235.430	2825.160	8.581	2.477	218.190	249.360	3.64
	L	240.702	2888.420	4.191	1.210	232.580	246.670	1.74
Total for groups		229.838	8274.150	15.788	2.631	180.520	249.360	6.87

Table 4. Analysis of width of annual increment in diameter in Scots pines depending on the form of dead bark.

		Mean [mm]	Standard deviation	Minimum [mm]	Maximum [mm]	Coefficient of variation [%]
Type of bark	G	1.400	0.370	0.545	2.435	30.5
	M	1.005	0.260	0.580	1.710	25.5
	L	1.085	0.285	0.470	2.230	26.5
Total for groups		1.103	0.305	0.532	2.125	27.5

amounting analogously to 1.09 mm, as well as 0.47 mm and 2.23 mm, respectively.

No statistical differences were observed in the dynamics of increment in diameter between trees with shell bark (M) and those with scaly bark (L).

The material used for chemical analyses was also subjected to confocal scanning laser microscopy (CSLM). The green colour in the image represents the autofluorescence of lignin (Figure 5). The most marked lignin deposition was observed in the middle lamella, as well as in the outer layers of cell wall. However, obtained images reveal no differences in the lignification of secondary cell walls of analyzed xylem specimens. All comparisons were made with the same growth ring.

Discussion

The study was an attempt to analyze lignin contents in mature pines characterized by different forms of dead bark, using the spectrophotometric method, which is based on the decomposition of lignin into soluble degradation products and the determination of their absorbance in the UV. Analyses were conducted on wood samples coming from 36 pines (*Pinus sylvestris* L., aged 89 - 91 years), growing under optimal conditions for this geographical location (FCF and MFCF).

Lignin content in dry matter of wood tissue in Scots pines ranging from 180.52 to 249.36 mg g^{-1} is consistent with the results presented by White (1987) and Santos Abreu et al. (1999).

Recorded results indicate significant differences in lignin contents between trees with shell and scaly dead bark and trees with ropy bark form. Trees with the thickest bark form were characterized by the lowest content of lignin in wood tissue and at the same time by the biggest variation in the proportion of this component (7.3%). Perhaps in this group of trees the high variation in the dynamics of increment in diameter, in mature wood amounting to slightly over 30% in relation to pines with the other bark forms, was significantly affected by lignin contents. Factors undetermined in the course of this study, such as individual or genetic variation, may also have had an effect on recorded results. However, due to its complexity the problem requires further detailed studies.

We also need to focus on the observed dependence between the form of dead bark, lignin content, and the dynamics of increment in diameter in the mature wood zone. Pines with the thickest bark were characterized by the highest dynamics of increment in diameter with a mean of 1.40 mm for the annual ring in the mature wood zone. In coniferous species the width of annual rings is increased thanks to the increase in the width of the early wood zone in the increment in diameter (Kollmann and Côté 1968). Most probably it has a significant effect on lignin contents in wood tissue whose proportion in the annual ring differs between the early and late wood zones. Total lignin content in wood is, to a considerable extent, determined by lignin contents in secondary cell walls (Fukazawa 1974) and the lignin compounds of middle lamella (Fengel and Wegener

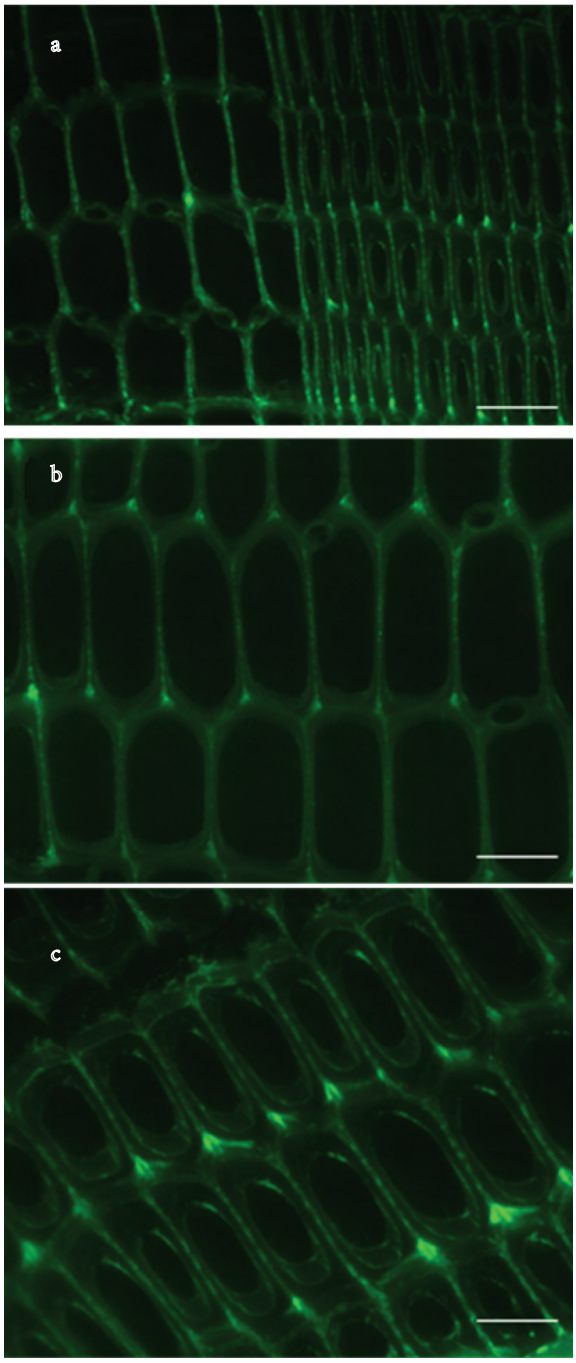

Figure 5. Autofluorescence of lignins in wood of Scots pine with scaly bark (L); a – annual ring; b – earlywood; c – latewood; Bars: 60 μm (a), 30 μm (b), 25 μm (c).

1984). The middle lamella retains its thickness throughout the growth ring, resulting in a higher lignin content in early wood (Gindl 2001); however, in late wood, each cell accumulates more lignin due to the thicker cell wall. Thus, in early wood, composed of thin-walled tracheids, lignin is found in lower amounts compared to late wood (Raiskila 2008), which was also observed in this study on confocal fluorescence micrographs of Scots pine wood. Moreover, in *Pinus taeda* variation was reported on the molecular level, i.e. the transcript level of 10 genes involved in the biosynthesis of lignin in the differentiating late wood was markedly higher than in early wood (Egertsdotter et al. 2004).

No differences were found either in lignin content or growth dynamics between trees with shell vs. scaly bark. A relatively high proportion of lignins in these trees indicates bigger rigidity of anatomical elements and resistance to biotic factors than in the group of trees characterized by the ropy type.

Since lignin is responsible, among other things, for protection of hydrophilic cellulose and hemicelluloses, which are mechanically weak when wet (Gindl 2001), as well as provide compressive strength of wood (Santos Abreu et al. 1999), it may be assumed that trees with shell and scaly bark are more wind-resistant than trees with ropy bark. In the opinion of Klon (1975), the form of dead bark to a certain limited degree may be an indicator suitable in the estimation of technical quality of standing timber. This is especially important when a high modulus of elasticity is required in case of wood exposed to external factors.

Moreover, wood harvested from trees with ropy bark, due to the lower proportion of lignins and thus higher affinity of cellulose to water, will tend to exhibit bigger shrinkage and unpredictable changes in linear dimensions (warp), and as a consequence crack along the grain and reduce strength properties of elements produced from such wood.

On the basis of conducted studies it may be assumed that wood of trees with various forms of dead bark has different lignin contents and dynamics of increment in diameter. Thus, further investigations, aiming at the confirmation of these results for trees growing under various conditions and in different geographical locations, seem necessary to reliably verify the hypotheses presented in this study.

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