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Genetic Studies of Wood Structure Using an Optical Fibre Analyser

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Abstract

An automatic fibre size analyser - KajaaniFiberLab was used in studies designed to examine and analyse the genetic basis of fibre size variability. Fast and accurate measurement in accordance with the standards may, however, give incorrect results due to errors associated with the multi-staged maceration of wood samples to obtain disintegrated fibre water suspension. Errors in fibre size estimation may occur throughout studies of the increment cores of living trees. Nevertheless the results obtained from the Kajaani analyser allow for the estimation of fibre sizes (length, width and cell wall thickness) with a precision comparable to that in studies using traditional measurement techniques, which is why these results may be used to estimate genetic parameters of the traits mentioned - heritability and genetic correlation. Such parameters are applied when making decisions concerning the management of forest stands and plantations of fast-growing trees. On the basis of the results of measurements of the biometric parameters of fibres, we obtained high values of genetic parameters (heritability and genetic correlations) for the traits of the European larch analysed. Those results allow for the achievement, with a high level of probability, of the breeding objective through selection directed at the improvement of the properties of timber produced in plantations of fast-growing trees.

Key words: optical analyzer, fibre length, genetic parameters, wood quality of European larch.

plantations of fast-growing trees. The diversification of wood structure, consisting of fibres and vessels, is determined by both environmental and genetic factors. The impact of genetic factors on the formation of the anatomical elements of wood is best characterised by two genetic parameters: heritability and genetic correlations. The selection of plant material intended to establish plantations of fast-growing trees for the purpose of the production of timber with strictly specified properties requires the determination of genetic parameters, the determination of which demands precise estimation of fibre sizes on the basis of a relatively large sample of research material, using non-destructive methods. Obtaining accurate and repeatable results of dimensions of the anatomical elements of which the conductive and reinforcement tissue is made is possible due to the use of optical analysers of water solution of disintegrated wood material. Analysers designed by Metso Automation can be divided into those dedicated for industrial purposes (Lab Metso Paper, Metso Pulp Analyser), laboratory and industrial purposes (Metso Fibre Image Lab) and scientific studies (KajaaniFiberLab). The KajaaniFiberLab 1.3 analyser, apart from typical studies on paper properties, has been used in studies on the genetic aspects of the wood structure of types valuable for the pulp industry [1, 2].

The KajaaniFiberLab 1.3 analyser uses polarised laser light for fibre visualisation with a high resolution CCD camera. Digital fibre visualisation and the use of

advanced technologies to analyse digital images enabled us to obtain measurements of wood anatomical elements. The technologies applied allow for measurement of the fibre length along the fibre line during the flow of the fibre suspension through the analyser's capillary tubes.

An innovative solution for the measurement process, carried out using a Kajaani-FiberLab 1.3 analyser, consists in the application of assumptions specified in the TAPPI271 standard, as well as in the ISO16065-1 and ISO16065-2 standards.

During tests on the variability of the European larch wood structure, an attempt was made to investigate genetic conditions related to the formation of fibre sizes. For that purpose the major genetic parameters of length and width of fibres was determined, along with the value of the cell wall thickness.

Material and methods

To measure the average fibre length of wood of the European larch we used the automatic fibre analyser KajaaniFiberLab 1.3. The studies were part of a larger research project aimed at determining genetic parameters (heritability and genetic correlation) for eight families of European larch growing as an experimental trail in the Młynary Forest District of northern Poland. Samples of wood in the form of increment cores were taken from

Introduction

The length of fibres obtained from the processing of timber for the pulp industry is one of the most important characteristics alongside the cellulose, lignin and hemicellulose content. The key term is the possibility to shape - at the stage of tree selection - the properties and uniformity of qualities of timber produced in

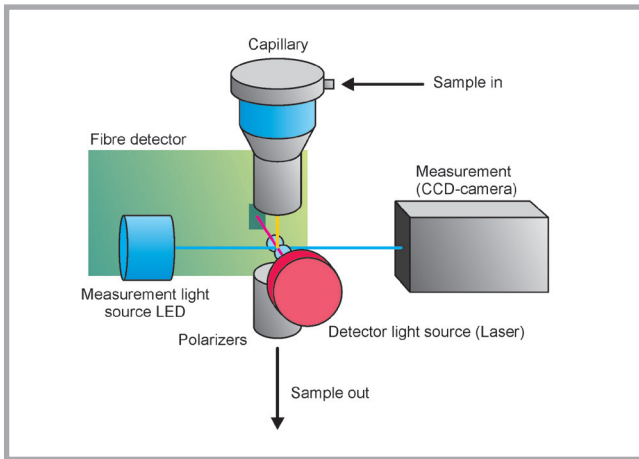


Figure 1. Construction of the measuring module of the Kajaani FiberLab 1.3 analyser [6].

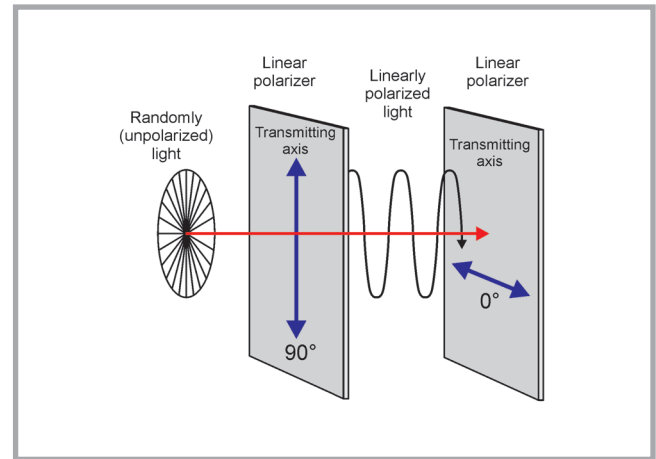


Figure 2. Fibre detection in polarised and unpolarised light [6].

161 living trees, at the height of 1.3 m above ground level. From the increment cores we isolated sections having the five youngest annual rings, representing juvenile wood.

To perform automatic analysis of tracheid sizes, the wood samples (increment cores) had to undergo maceration and the fragmentation process until we obtained a suspension of individual anatomical elements in distilled water. The suspension of disintegrated tracheids was analysed in three analytical runs using the KajaaniFiberLab 1.3 automatic analyser [3]. Maceration of the wood samples was carried out in a 25% solution of H₂O₂ and acetic acid CH₃COOH in a proportion of 1:1, and at a temperature of 90 - 100 °C for 20 - 24 hours [4]. After being rinsed with distilled water, the disintegrated wood samples were fragmented in order to obtain a homogeneous suspension of anatomical elements of wood. The precision at this stage of the wood sample preparation directly affects that of the fibre size analysis. The suspension obtained was replenished with distilled water to 5000 ml. 50 ml of the suspension obtained from the solution was taken via a pipette for measuring fibres [5]. Fibres contained in the 50 ml were subjected to automatic analysis using the Kajaani-FiberLab 1.3 analyser.

The dynamic the analysing process using a high resolution CCD camera, the semiconductor laser source and laser detector enabled us to obtain a current image of

the fibres' dimension distribution (Figure 1).

Fibre visualisation was performed in laser light. However, the measurement process using the image registered by the CCD camera uses unpolarised light. The appearance of fibre in the imaging area and recording of this image by the CCD camera triggers a series of processes of high-quality imaging (Figure 2). The background is eliminated, a fibre image recorded in binarised form, and measurement of the fibre length and width is carried out on the basis of the greyscale distribution of the fibre image using the subpixel technique [6].

During one analytical run, 4000 - 10000 fibres were measured. For 10% of these fibres, recording of a digital image of the fibre shape was possible. Measurement results contain a set of four fibre characteristics: fibre length, fibre length weighted, fibre width, and cell wall thickness - assigned to one fibre. Average fibre characteristics for each analytical run was estimated automatically according to the following formulas:

$$T_l = \frac{\sum n_i l_i}{\sum n_i}, \text{ mm}$$

$$T_{lw} = \frac{\sum n_i l_i^2}{\sum n_i}, \text{ mm}$$

$$AW = \frac{\sum n_i w_i}{\sum n_i}, \mu\text{m}$$

$$ACWT = \frac{\sum n_i CWT_i}{\sum n_i}, \mu\text{m}$$

where:

- T_l – fibre length,
- T_{lw} – fibre length weighted,
- l_i – mid-class length in class i ,
- n_i – fibre count in class i ,
- AW – average tracheid diameter,
- $ACWT$ – average cell wall thickness,
- w_i – average fibre width in width class i ,
- CWT_i – average width in cell wall class i .

Due to the presence of cut fibres, insufficiently fragmented or joined-together fibres in some of the samples analysed, for which digital images of the fibres were taken, were exported to a web database. Analysis of the fibre image allowed for individual classification of results as follows: acceptable, unacceptable due to cutting, unacceptable due to joined fibres and unacceptable due to incorrectly measured fibres associated with the shape thereof.

On the basis of the results obtained for “acceptable” fibres only, we estimated the average length, enabling the relating of the real values to estimated values average for all four types of fibres as well as calculation of the conversion factors determining the real values of the characteristics. According to Nilsson’s work [7], all fibres were classified into six classes of length (Table 1).

Wood samples in the form of increment cores with a diameter of 5 mm have a significant share of fibres cut during sampling. Such a large share of cut fibres affects the reliability of analyses of fibre length based on such types of samples. Therefore in further analyses data relat-

Table 1. Fibre length classes.

Fibre length classes	1	2	3	4	5	6
Length limit, mm	0.00 - 0.19	0.20 - 0.49	0.50 - 0.99	1.00 - 1.99	2.00 - 2.99	3.00 - 8.00

ing to the first and second length classes were omitted (0 - 0.49 mm). Characteristics of fibres for the remaining four length classes were verified using the indicators calculated.

The average values of fibre sizes obtained for each sample representing a tree from one of the eight families analysed were used at the appropriate stages of the analyses. For each trait the variant components were calculated using the SAS 9.2 PROC VARCOMP procedure and the covariant components between the traits analysed were determined using the MANOVA formula SAS. Coefficients of genetic and phenotypic correlations were calculated using the following formulas:

$$r_{G_{xy}} = \frac{Cov_{F_{xy}}}{(\sigma_{F_x^2} \times \sigma_{F_y^2})^{0.5}}$$

$$r_{P_{xy}} = \frac{Cov_{F_{xy}} + Cov_{E_{xy}}}{[(\sigma_{F_x^2} + \sigma_{E_x^2}) \times (\sigma_{F_y^2} + \sigma_{E_y^2})]^{0.5}}$$

where:

- $r_{G_{xy}}$ – genetic correlation,
- $r_{P_{xy}}$ – phenotypic correlation,
- $\sigma_{F_x^2}$ – family variance components for traits X and Y, respectively,
- $\sigma_{E_x^2}$ – error variance components for traits X and Y, respectively,
- $Cov_{F_{xy}}$ – family covariance components for traits X and Y,
- $Cov_{E_{xy}}$ – error covariance components for traits X and Y.

The narrow-sense heritability was calculated on an individual tree basis:

$$h^2 = \frac{4\sigma_F^2}{\sigma_F^2 + \sigma_{PF^2} + \sigma_{E_y^2}}$$

where:

- h^2 – narrow-sense heritability,
- σ_F^2 – family variance,
- $\sigma_{E_y^2}$ – residual variance,
- σ_{PF^2} – family × seed orchard variance.

Results

The mean fibre length proved to be up to 0.77 mm (SD = 0.09) for the 2864 family and up to 0.95 mm (SD = 0.09) for the 2549 family. The coefficients of variance for the families were low, fluctuating between 0.08 and 0.15 (for the 2860 and 2818 families, respectively). Similar coefficients of variance for fibre length in European larch are given by Fabisiak [9]. Variance analysis for the mean fibre length confirmed the significance of the family factor (F = 7.59; p = 0.000)

[10]. The 2549 family had a considerably higher mean fibre length in comparison to other families. The results obtained indicate that the mean fibre length depends on genetics. Similar conclusions were drawn by the authors of studies on the fibre length variance [11 - 15].

The mean weighed fibre length proved to be up to 2.18 mm (SD = 0.27) for the 2864 family and up to 2.53 mm (SD = 0.18) for the 2549 family. Similar mean length values (fibres 2.9 - 3.0 mm) for European and Japanese larch are given by Einspahr et al. [16]. The coefficients of variance for the families were low, fluctuating between 0.07 and 0.12 (for the 2549 and 2864 families, respectively). Two-factor variance analysis for the factors “fibre length class” and “family” confirmed the significance of both factors and their interaction (F = 519.4; p = 0.000; F = 6.88; p = 0.000; F = 1.76; p = 0.019). The weighed fibre length for the 2549 family was significantly higher than for the others and the value of this trait for the 2860 family was significantly higher only in comparison to the 2514, 2864, 3076 and 3078 families. Also in the case of the biometric characteristic of the fibres the genetic influence on the size of fibres could be observed. The largest class was the fourth class of fibre length (1.00 - 1.99). The frequency analysis of the fibre length classes of the European larch studied by Fabisiak [9] shows that the length class of 3.0 - 3.5 mm is the most common. The discrepancies in frequency results for the length classes obtained for larch are most probably due to the different sampling methods (increment cores with a diameter of 5 mm/strip of wood 20 mm thick), sampling height (1.3 m/¼ of the tree height) and the cambial age of increments from which the fibres were extracted (20 - 25 years/subsequent annual increments: 3, 6, 9, 12, 15, 20, 25, 30, 35 and 40).

The statistics presented for characteristic features of the fibres allowed estimation of the basic genetic parameters of fibres, narrow-sense and genetic correlations between the biometric traits of the fibres as well as genetic correlations with wood density traits, the annual ring width, earlywood and latewood. The narrow-sense of the mean length and weighed length fibres, according to expectations, reach high levels (0.86 and 0.85, respectively). Such observations are confirmed by the current studies of other authors [17, 18]. However, Hannrup and Wilhelmsson

[19], Hannrup and Ekberg [20] and Ericsson and Fries [1] obtained significantly lower values for the individual heritability of fibre length in Scots pine. In the investigations performed by the said authors the errors in estimating genetic parameters and the few samples for fibre length measurements may raise concerns as to the reliability of the results.

Genetic correlations between the biometric traits of fibres, in particular between the length and width thereof and the cell wall thickness, reached high values (0.90 and 0.89, respectively) [21]. Slightly lower values for genetic correlations between the said traits could be seen in the studies of Hannrup et al. [2] on clones of the Norway spruce. Surprising results of the said genetic parameters can be found in the above-mentioned work by Ericsson and Fries [1], who obtained a negative value (-0.72) for the correlation between the length and width of fibres. Although the authors state that the estimation error was high (0.2), the difference in the correlation between the traits is still intriguing. The works by Ericsson and Fries and the results presented for European larch were performed and obtained in the same Laboratory for Wood and Fibre Analysis at Umeå University in Sweden.

Genetic correlations between the fibre length and traits associated with the width growth rate, i.e. the width of an annual ring and wood density, give interesting information about the possibility to select trees for the quick production of pulp wood with the fibre length required by the customer. The relation between fibre length and wood density is shown by low positive values of genetic correlations between those traits [22]. Genetic correlations between the fibre length, annual ring width and components (earlywood and latewood) have moderate negative values, as expected. Hannrup et al. [23] indicate a reverse relation between the fibre length and ring width based on the results obtained for a 28-year-old Scots pine. A moderate positive genetic correlation between the fibre length and wood density is confirmed in the studies carried out on Norway spruce and Loblolly pine [2, 24].

Conclusions

Metso optical analysers - a series dedicated for scientific research - are used in research programs focused on the analy-

sis of genetic aspects of fibre variability. Measurements in laser and non-polarised light give results compliant with the TAPPI271, ISO16065-1 and ISO16065-2 standards very quickly and with a low workload. However, one should remember that wood is a material which requires special treatment and processing to obtain a water suspension with anatomical elements (vessels, fibres and tracheids). Accuracy during the preparation stage is crucial as it may affect the quality of samples. A content of fractions of fibres which are insufficiently macerated or damaged is essential. The KajaaniFiberLab cannot automatically distinguish between damaged and undamaged fibres, but the possibility to record digital images of individual fibres allows for evaluation of the quality of biometric dimension values of fibres obtained.

The use of a KajaaniFiberLab analyser for genetic analyses performed on trees requires the application of non-destructive wood sampling methods. The most popular are increment cores with a diameter of 5 mm. Such samples, however, contain a large share of cut fibres, which affects the fibre length measurement negatively [25]. This problem requires a specific approach to the analysis of the results in order to obtain high reliability, comparable to measurements performed using standard methods [26].

The measurement of biometric dimensions of fibres, including their length, for the purpose of estimating the genetic parameters of those traits on the basis of samples collected from living trees with various degrees of relationship requires proper planning of the number of trees to be used for sampling. Meeting the condition of the minimum number of trees for sampling to calculate the heritability or genetic correlations helps to obtain results with a low estimation error. Only such reliable results can be applied in practice to manage forests and take action associated with the control of tree stands and plantations of fast-growing trees, in particular in terms of the culture and selection of forest trees.

The high values of genetic parameters, heritability and genetic correlations of biometric traits of fibres of European larch obtained allow for the achievement of good results in the selection of appropriate families of this species to carry out intensive production of timber with the wood qualities required. The selection of

genotypes for controlled cross-breeding should enable the improvement of biometric properties of fibres in progenies with regards to plus trees. It should be remembered, though, that despite the high heritability of fibre traits, they are, to a serious extent, determined by the conditions of growth, some of which, such as the spacing, site fertility or competition from other species, may be controlled in plantations of fast-growing trees; however, the impact of the climate is still beyond the control of humans.

References

- Ericsson T, Fries A. Genetic analysis of fibre size in a full-sib Pinussylvestris L. progeny test. *Scand. Jour. For. Res.* 2004; 19: 7–13.
- Hannrup B, Cahalan C, Chantre G, Grabner M, Karlsson B, Le Bayon I, Jones GL, Müller U, Pereira H, Rodrigues JC, Rosner S, Rozenberg P, Wilhelmsson L, Wimmer R. Genetic parameters of growth and wood quality traits in Piceaabies. *Scand. Jour. For. Res.* 2004; 19: 14–29.
- Klisz M. Automatyczna metoda określania rozkładu parametrów cewek i włókien w oparciu o nie destrukcyjne metody pobierania prób z drzew. *Leśne Prace Badawcze* 2008; 3, 69: 270–273.
- Franklin GL. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for Wood. *Nature* 1945; 3924, 13: 51.
- Kauppinen M, Oikarinen M. FiberLab. Installation and operating manual W4230467 V1.3. Valmet Automation Kajaani Ltd., Finland. 1998.
- Ruonala J. Kajaani fiber portfolio – continuous technological development in fiber measurements since 1980's. Metso Automation Inc., Helsinki, 2010: 1–9.
- Nilsson O. Fibre length variation in Norway Spruce (*Piceaabies*) and Scots pine (*Pinussylvestris*). Institutionenförsökskötset. SLU, Umeå, 2004; 1–16.
- Bergqvist G, Bergsten U, Ahlqvist B. Effect of radial increment core diameter on tracheid length measurement in Norway spruce. *Wood Sci. Tech.* 1997; 31: 241–250.
- Fabisiak E. Zmienność podstawowych elementów anatomicznych i gęstości drewna wybranych gatunków drzew. *Roczniki Akademii Rolniczej w Poznaniu. Rozprawy Naukowe.* 2005; Zeszyt 369: 1–175.
- Klisz M, Michalska A. Genetic aspect of differences in tracheid length of European larch families from seedling seed orchards. *WULS – SGGW, For. and Wood Technol.* 2012; 78: 149–153.
- Ledig FT, Zobel BJ, Matthias MF. Geoclimatic patterns in specific gravity and tracheid length in wood of Pitch pine. *Can. Jour. For. Res.* 1975; 5: 318–329.
- Frimpong-Mensah K. Fibre length and basic density variation in the wood of

- Norway spruce (*Piceaabies* L. Karst) from northern Norway. *Norsk Institutt for Skogforskning* 1987; 40.1: 1–25.
- Jaakkola T, Mäkinen H, Sarén MP, Saranpää P. Does thinning intensity affect the tracheid dimensions of Norway spruce. *Can. Jour. For. Res.* 2005; 35: 2685–2697.
 - Mäkinen H, Jaakkola T, Piispanen R, Saranpää P. Predicting wood and tracheid properties of Norway spruce. *For. Ecol. Management* 2007; 241: 175–188.
 - Mäkinen H, Tuula J, Saranpää P. Variation of tracheid length within annual rings of Scots pine and Norway spruce. *Holzforchung* 2008; 62: 123–128.
 - Einspahr DW, Wyckoff GW, Fiscus M. Larch – A fast-growing fiber source for the Lake States and Northeast. *Jour. Forestry* 1984; 82: 104–106.
 - Goggans JF. Correlation and heritability of certain wood properties in Loblolly pine (*Pinustaeda* L.). *Tappi Journal* 1964; 47, 6: 318–322.
 - Burdon RD, Low CB. Genetic survey of Pinusradiata. 6: Wood properties: variation, heritabilities, and interrelationships with other traits. *New Zealand Journal of Forestry Science* 1992; 22: 228–245.
 - Hannrup B, Wilhelmsson L. Genetic parameter estimates of wood density and tracheid length of Pinussylvestris. In: *Timber management toward wood quality and end-product value. CTIA/IUFRO International Wood Quality Workshop.* Quebec, Canada, 1997; IX-15.
 - Hannrup B, Ekberg I. Age-age correlations for tracheid length and wood density in Pinussylvestris. *Can. Jour. For. Res.* 1998; 28: 1373–1379.
 - Klisz M. Genetic correlations between tracheid biometric traits in European Larch. *WULS – SGGW, For. and Wood Technol.* 2011; 74: 170–173.
 - Klisz M. Genetic correlations between wood density components and tracheid length in European larch. *WULS – SGGW, For. and Wood Technol.* 2011; 74: 166–169.
 - Hannrup B, Ekberg I, Persson A. Genetic correlations among wood, growth capacity and stem traits in Pinussylvestris. *Scand. Jour. For. Res.* 2000; 15: 161–170.
 - Sykes R, Isik F, Li B, Kadla J, Chang HM. Genetic variation of juvenile wood properties in a Loblolly pine progeny test. *Tappi Journal* 2003; 86: 3–8.
 - Mörling T, Sjöstedt-de Luna S, Svensson I, Fries A, Ericsson T. A method to estimate fibre length distribution in conifers based on wood samples from increment cores. *Holzforchung* 2003; 57: 248–254.
 - Svensson I, Sjöstedt-de Luna S, Mörling T, Fries A, Ericsson T. Adjusting for fibre length-biased sampling probability using increment cores from standing trees. *Holzforchung* 2007; 61: 101–103.

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