University of West Hungary

Theses of doctoral (Ph.D.) dissertation

The production, accumulation and distribution of beech (*Fagus sylvatica* L.) extractives

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1. Introduction and aims of research

Beech (*Fagus sylvatica* L.) is regaded as a valueable native hungarian hardwood species. Almost 7% of the hungarian forests are made up by beech trees, the contribution of beech to the total value of the forests in hungary is 20%.

Beech grows in the most developed ecosystems and prefers atlantical forest sites with humid climate. Across Hungary beech can mostly be found in the hills of Göcsej, Bakony, Pilis, Börzsöny, Mátra and Bükk.

Researchers have not investigated the extractives and chemical parameters of hungarian beech yet.

The primary aim of the author has been to track the distribution and accumulation of soluble carbohydrates. This group of compunds has distinct functions in the metabolism of plant tissues. These compounds can also be regarded as a carbon pool for plant polyphenol production.

The non-structural carbohydrates are important representatives of both primary and secondary metabolism: they interconnect the two processes.

A large fraction of the beech stands in Hungary is affected by the formation of red heartwood. Facultatively coloured heartwood formation is the most important structural and colour anomaly of living beech, degrading significantly the value of beech stands, therefore causing substantial economic loss. The phenonema seems to spread out more from year to year, the proportion of red-heartwooded stems grows in the stands. The investigation of the red heart in beech is also an accentuated research topic in the EU.

The author aims in her work to track the distribution and accumulation of soluble carbohydrates, organic acids and phenolic compounds in the healthy and red-heartwooded beech by:

- Measuring the given parameters radially from the bark towards the pith, at given heights.
- Measuring the total dissoluble carbohydrate content.
- Separating and indentifying the carbohydrate compounds and investigating the distribution of each sugar component.
- Measuring the distribution of dissoluble carbohydrates in the vegetation period with a special regard to the time of heartwood formation (September-March).
- The comparative investigation of the chemical environment where the processes of heartwood and red heartwood formation take place.
- Measuring of the distribution of the free- bound- and total acid content as well as the totalphenol content radially from the bark towards the pith.
- Comparing the extraction methods described in the literature.

1. Experimental methods

2.1 Sample collection and processing

For her measurements the author took a large number of disks which had been taken from the stems from a height of three meters. Three red-heartwooded and three non-red-heartwooded beech stems were chosen from each forest stand.
In order to track the vertical variation of the chemical parameters, disks were taken from one red-heartwooded and one white stem, from the felling surface up to the first branch (fork) by a sampling interval of one meter.
In order to investigate the radial variation of the measured chemical parameteres eight samples, ranging from the bark to the pith were taken from the red-heartwooded disks and five samples from the non-discoloured disks. The comparability of disks with different diameter was achieved by assigning the samples in a given ratio of the disk radius.
The sample disks originated from various forest stands of SEFAG Ltd. (Kaposvár district) and TAEG Ltd. (Sopron district). The time of sample disk collection was chosen in the time range between 2001-2007 considering the seasonal period of obligatory coloured heartwood formation (July-January).

Milled wood samples were extracted according to the current method. Extraction was carried out using water, sodium-acetate or 80% aqueous methanol solution.

2.2 Measured parameters and analytical methods

pH and acidity. The determination of the pH and acidity (free-, bound- and total acidity content) was achieved applying potentiometry (NÉMETH, 1987).

Determination of total dissoluble carbohydrate content. Measured spectrophotometrically according to the method of DUBOIS (1956).

Qualitative and quantitative analysis of sugars. Using optimum performance laminar chromatography (OPLC). Stationary phase: HPTLC silicagel; Mobile phase: 85:15 acetonitrile:water (SÁRDI et al., 1996); Development: in OPLC 50 overpressurized chamber with 2 × 6000 µl mobile phase (double development); Visualization: spraying with 4 g diphenyl-amin + 4 ml anilin + 20 ml 86% phosphoric-acid reagent. Quantitative evaluation: Camag TLC Scanner 3 densitometer, at 540 nm, absorbance mode.

Ionchromatography: Extraction was carried out in Dionex ASE 200 extractor. Evaluation using HPIC Dionex ionchromatograph.

Determination of total phenol content.
According to the method of Folin-Ciocâltău (SINGLETON and ROSSI, 1965), standard: quercetin.

Determination of (+)-catechin content.
The identification and quantitative analysis of catechins was achieved using thin layer chromatography.

Stationary phase: TLC silicagel; Mobil phase: 9:1 diisopropyl-ether:formic-acid (FECKA et al., 2001); Chamber: normal unsaturated twin trough chamber; Visualization: spraying with vanillin + sulphuric-acid reagent (STAHL, 1962). Qualitative and quantitative evaluation: Camag TLC Scanner 3 densitometer, absorbance mode.

3. Methods of data processing and evaluation

The author used Microsoft Excel software for the evaluation of her research data. Documentation was carried out using Microsoft Word word processor.
Statistica 6.1 software was used for carrying out statistical (ANOVA) analyses. The Tukey HSD calculation method was used, at p=0.05% significance level in all of the variance analyses.

4. New scientific results

4.1 The author has been the first to investigate the radial distribution of dissoluble carbohydrates in healty and red-heartwooded hungarian beech tissues.

4.2 The author has separated and identified the following sugars from the tissues of beech: succrose, glucose, fructose, raffinose, stachyose and maltose.

4.3 The author has proved with her measurements that the major sugar components in the non-red-heartwooded tissues are glucose and fructose, regadless of height, radial location or anatomical buildup.

4.4 She has established that the highest sugar concentrations can be measured in the young sapwood tissues at all heights investigated. In non-red-heartwooded beech a radial decrease in the concentration can be observed towards the pith. This finding is also true for succrose, glucose and fructose.

4.5 The distribution of the sugars in the red-heartwooded stem differs from the distribution measured in healthy beech. The author has established that the most important difference between the two stems can be experienced regarding the sharp decrease at red heartwood bondary. This has been proven in case of total dissoluble carbohydrate content and also for succrose, glucose and fructose concentrations.

4.6 The author has established that the dissoluble carbohydrate content varies with the height. The lowest values can be measured at the middle height of the stem, which can be in connection with the change of the water content.

4.7 The author has evidenced that the dissoluble carbohydrate content varies significantly during the vegetation period. She has experienced high succrose, glucose and fructose concentrations during January in the tissues of healthy beech stems. During March and July the values obtained have been low.

4.8 The author has measured low raffinose and stachyose levels which can be connected with the rapid metabolic

transformation of these compounds. The result are in good correlation with the data published in the literature: in other trees these compounds are also not reserve carbohydrates, they are in fact the most mobile sugars.

4.9 The author has evidenced maltose only in October from the sapwood tissues of both healthy and red-heartwooded stems.

5. Bibliography of personal publications considering the dissertation

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Research summaries

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