

University of West Hungary

Theses of doctoral (Ph.D.) dissertation

**Chemical investigation of abiotic effects during the
storage and early ontogenesis of the Pendunculate oak
(*Quercus robur* L.) acorn**

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1. Introduction

Pendunculate oak (*Quercus robur* L.) is a dominant native hungarian tree species, inhabiting 9.1% of the forest sites of Hungary. Pendunculate oak stands can be found at the best soil sites, they also respresent highest economic and natural value among the forests in Hungary.

A substantial condition for realizing large scale afforestation projects is the breeding of acorns and nursery trees. This requires the balanced supply of nursery trees, which in turn demands the storage of acorns without quality loss, the controlling of the quality of acorns and the constant monitoring the germination ability. In order to preserve the economic value of acorns is essential to develop new ways of storage. To be able to hoose resistant individuals, effective and reliable measurement techniques are needed. The realization of all these is a theoretical task which also has abilities for inventing applications for the industry, especially for the national afforestation programme. Mayor importance is regarded to the understanding of the connection between the plant and its environment, investigation of physiological processes and the understanding of how a plant reacts to the change in the environmental conditions. This requires the knowledge of how different plant species react to the stress caused by the sudden change in the environmental parameters. Under ambient conditions the germination and development of a plant during the early ontogenesis is influenced by the coupled effects of several parameters (eg. water supply, quality of soil, humidity, ventilation, other biotic and abiotic stress

effects). It is a task still to be resolved to find ways to choose the most resistant and adaptive species and also to enhance the adaptational-, stress resisting-, and productional abilities. Tracking the effects of ontogenesis, storage and stress the author has applied such physical and chemical indicators, which proved to be stress sensitive.

Aims of research:

- Characterisation of the ontogenesis with the relative weight-density parameter of the seed-leaves.
- Modelling cold-shock, a possible environmental stressor of stored acorns, using endogenous formaldehyde content, polyphenol concentration and catalase enzyme activity.
- Determining the totalphenol content and the endogenous formaldehyde content of various tissues (seed-leaves, roots, leaves) during the early ontogenesis of Pendunculate oak (*Quercus robur* L.).
- Investigation of the role of oxydoreductase enzymes (catalase, peroxydase, polyphenol-oxydase) during the different developmental stages of the plant.
- Investigating of the effect of the applied storage technique on the physiological processes of the acorns using chosen signal molecules and parameters.
- Characterisation of the ontogenesis and the storage via enzyme correlation.

- Investigating of the stress response of six-leaved-state plantlets by exposing them to cold-, dark- and drought stress conditions. Measuring of endogenous formaldehyde content and totalphenol content of the leaves.
- Mapping of the change in the enzyme (peroxydase, catalase, polyphenol-oxydase) activities by various environmental effects.
- Characterisation of stress events by enzyme correlations.

2. Experimental conditions

2.1 Sample collection and preparation

The acorns investigated originated from various hungarian plantation sites: Délalföldi Erdészeti Zrt. (in 2004), Gyulai Erdészet (in 2005) and from Vitnyéd from the Kisalföldi Erdőgazdaság Zrt. Rábaközi Erdészet (2006).

To track the ontogenesis extraction was carried out from the seed-leaf, root and the leaf. For the characterisation of the method of the storage the seed-leaf and the leaves of the plantlet were extracted.

For measuring endogenous formaldehyde content extraction was carried out using 0.01% methanolic dimedone solution. The extraction solvent for totalphenol content determination was 80% methanol. Enzyme extraction was carried out with phosphate buffer (pH: 5.5 and 6.0).

2.2 The parameters investigated and the respective analytical techniques

Endogenous formaldehyde content. The measurements are based on the reaction of dimedone with formaldehyde generators. The reaction yields formaldehyde. The endogenous formaldehyde content can be established by the qualitative analysis of formaldehyde after the sample make-up (NÉMETH, 2002).

Totalphenol content determination. According to the method of Folin and Ciocâltău (SINGLETON and ROSSI, 1965), using quercetin as standard.

Catalase enzyme activity. The activity can be established by the amount of oxygen produced by a given amount of tissue during a given time period. Determination was carried out using Frenyo gasvolumetric device (BOTZ *et al.*, 1995).

Peroxidase enzyme activity. The measurement was carried out using a spectrophotometer and 3,3'-diaminobenzidine as a substrate. Evaluation at 480 nm according to the method of SHANNON *et al.*, (1966).

Polyphenol-oxidase enzyme activity. The author has investigated the cresolase activity of the enzyme spectrophotometrically using pyrocatechine as a substrate, at 420 nm.

Protein content. Determined spectrophotometrically according to the method of BRADFORD (1976), at 595 nm. Standard: 92 % Bovine Serum Albumine.

3. Data processing and evaluation

For the evaluation and analysis of the measurement data Microsoft Excel software was applied. Statistical analysis was performed using StatsDirect v. 2.6.5 software. Calculation of analysis of variance was carried out with the multiple comparison method of Tukey-Kramer, at 95% probability level. Documentation was carried out using Microsoft Word word processor software.

4. New scientific results

The results included in the dissertation confirm the following statements and new scientific accomplishments:

4.1 The ontogenesis of Pendunculate oak (*Quercus robur* L.) acorns is similar to that of European Turkey oak (*Quercus cerris* L.), hence the following consequences can be drawn:

- the relative increase of weight correlates linearly to the decrease of density of the acorns. The slope of the equation is a general biological measure of germinating oak acorns,
- the deterministic changes in the endogenous formaldehyde content taking place in the early phase of the ontogenesis follow the same pathway in the case of both of the oak species,

- at the beginning of the storage the oak acorns are in a state of rest, which can be characterised with a maximal in the endogenous formaldehyde forming potential, which decreases as a result of decreasing acorn quality when the acorns are stored over the spring period,

- the stress response of the acorns to cold shock can be established in the form of an oscillating endogenous formaldehyde level, which reflects the disturbance of the equilibria of the transmethylation processes. This could give information about the stress-bearing ability of the acorns indirectly.

4.2. The parameters investigated can be utilized to indicate the differences between acorns originating from different forest sites:

- regarding the cold- and dark stress response significant differences could be established between the acorns originating from the Gyulai Erdészeti and the Rábaközi Erdészeti (Vitnyéd),

- acorns originating from the same forest site however resulted similar results regarding both the direction of the response given to a given stressor and also the extent of the change.

4.3. The author has applied an effective qualifying method for eliminating the differences between the characteristic properties of the individuals of a given species:

- the detection of the synchronized regulation of the peroxydase and polyphenol-oxidase activities is achieved by carrying out enzyme activity measurements at the same sampling time, from the same plant samples,
- the correlation equations reflect over the characteristic states of the ontogenesis also the changes in the quality of the acorns taking place during the storage.

4.4. The author has established that using enzyme correlational analysis, stress can be detected and its proceeding can be tracked:

- the R^2 value of the correlation provides information about the regulation of the metabolism,
- from the change in the slope of the equation information regarding the ratio of the enzyme activities can be obtained,
- the differences between the intensity of the metabolism of the different tissues and the heterogeneity of metabolism is reflected by the validity range of the regressions.

4.5. The author has proven that the parameters of the enzyme correlation are stress-sensitive. The correlations are also able to indicate significant changes, also when the mean values of the activities and the heterogeneity of the metabolism do not indicate any significant changes otherwise:

- the environmental impacts are able to change the validity range of the correlations significantly,
- the pathways drawn through the centres of gravity of the regression ranges are specific of the stressor,
- the differences between the slope of the correlation equations may indicate a general stress adaptation reaction resulting from the environmental impacts.

5. List of publications concerning the dissertation

Articles in cited journals

Németh, Zs. I., Pozsgai-Harsányi, M., Badáczy, D., Horváth, A. (2008) Characterization of plant developmental stages by enzyme correlation. *FEBS Journal* (manuscript submitted)

Németh, Zs. I., Pozsgai-Harsányi, M., Gálos, B., Albert, L. (2008) Stress sensitivity of a plant enzyme correlation. *Plant Science* (manuscript submitted)

Presentations at conferences (oral and poster)

Németh, Zs. I., Albert, L., Tyihák, E., Hofmann, T., Rétfalvi, T., **Pozsgai-Harsányi, M.** (2003) Alteration of measurable formaldehyde level of European Turkey Oak acorn in the function of parameters of sample preparation. 6th International Conference on Role of Formaldehyde in Biological Systems, Pécs.

Pozsgai-Harsányi, M., Németh, Zs. I., Gálos, B., Albert, L., Varga, Sz. (2005) Comparison of the alarm phases induced by cold shock in case of germinating acorns of *Quercus cerris* L. and *Quercus robur* L. International Conference on Non-linear Processes in Life Sciences. Lengyelország, Lublin.

Németh, Zs. I., **Pozsgainé Harsányi M.**, Albert, L. (2006) A környezeti hatásvizsgálatok módszertani fejlesztése. Szimpozion, Nyugat-Magyarországi Egyetem, Környezeti Erőforrásgazdálkodási és –védelmi Kooperációs Kutatási Központ, Sopron.

Németh, Zs. I., **Pozsgainé Harsányi, M.** (2008) Növényi enzimkorrelációk: a környezeti hatások indikátorai? III. Regionális Természettudományi Konferencia, Nyugat-Magyarországi Egyetem, Szombathely.

Németh, Zs. I., **Pozsgainé Harsányi, M.**, Gálos, B., Albert, L. (2007) Növényi enzimkorreláció stresszérzékenysége. Tudományos Konferencia, Nyugat-Magyarországi Egyetem, Erdőmérnöki Kar, Sopron.

Pozsgainé Harsányi, M., Németh, Zs. I., Badáczy, D., Horváth, A. (2007) Stressz-szindróma jellemzése enzimkorrelációval. Tudományos Konferencia, Nyugat-Magyarországi Egyetem, Erdőmérnöki Kar, Sopron.

Research reports

Tölgyfajok környezeti hatásokra adott válaszreakcióinak kémiai vizsgálata. OTKA T046408 (2004-2007)

Nyugat-magyarországi Egyetem, Környezeti
Erőforrásgazdálkodási és –védelmi Kooperációs Kutatási
Központ. A környezeti hatásvizsgálatok módszertani
fejlesztése.