

**Theses of Ph D Dissertation**

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**FORMALDEHYDE AND ITS NATURAL GENERATORS AS  
MARKER MOLECULES OF ENVIROMENTAL EFFECTS IN THE  
EARLY ONTOGENESIS OF EUROPEAN TURKEY OAK**

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**Sopron  
2002**

## **I. Project and aims of research**

In perspective development of Hungarian forest economy the projected and ongoing procedures of several silvicultural activities will play a significant role. In the forthcoming period of the next 50 years the importance of forestation of the abandoned farmlands and wastelands will be increased. The approximately 700 thousand hectare area, which is to be involved in the project of forestation will dynamically increase the demands of the propagation materials and in the course of the implementation the seedling production is to be favoured, which is controlled and able to provide the suitable quantity and quality.

The Department of Silviculture at University of West Hungary has been involved in the store of oak acorn and the investigation of germination since 1993. Having appraised the results obtained in the beginning of the investigations it became evident that the project can not be performed intensively without application of complex biological and biochemical knowledges. This realization gave reasons for a cooperation of departments at University and State Forest Agency.

European Turkey oak (*Quercus cerris* L.) as a tree species bearing acorns regularly has been chosen. Thus, fresh acorns have been gathered from the same district every year for investigations.

The aims of the investigations were to characterize early ontogenesis of European Turkey oak and to track the effects of environmental factors on endogenous formaldehyde content.

## **II. Material and method**

### Experiment of germination

Fresh acorns were made to germinate. District of their origin: Vitnyéd, Hungary, (1994-2001). Acorns were disinfected before germination with a plant protective (DITHANE M45 0.5 % and Chinoin Fundazol 0.1 %). Equipment for germination: Jacobsen desk QB-117/3 (LABORMIM Co., Esztergom, Hungary).

Temperature: 20-22 °C.; Illumination: 750 Lux/16 h every day.

### Analytical technique

The HPLC equipment applied for determination of endogenous formaldehyde consisted of a Gynkotek M 480 pump, TOSOH 6040 UV detector (260 nm), Rheodyne 8125 injector with a 20 µl loop. The column used was ChromSpher C-18 (150x4.6 mm; 5µm). The chromatograms were recorded through an EF 2102 ADDA converter (Elektroflex GM, Szeged, Hungary) by a personal computer. The applied mobile phase was methanol - 0.01 M HCl (76:24, v/v; pH=2.63). The mass spectrometer used in this work was a Finnigan LASERMAT 2000 (Finnigan MAT Ltd., Hemel Hempstead, UK).

## **III. Summary**

1. Reaction of formaldehyde and dimedone has been studied to make my experimental design. For quantitative analysis of the derivative compound, formaldemethone forming in the reaction a reserved phase liquid chromatography separation has been worked out. Regardless of

developmental conditions this new analytical method proved being suitable for determinations of formaldehyde contents of the acorns in germinating and resting states.

With the kinetic modelling of sample preparation reaction of dimedone and formaldehyde generators in methanolic solution, the reaction applied in sample clean-up has been established to follow second reaction order but with such rate constants differing from the ones of aqueous medium. As compared to aqueous medium presence of methanol does not modify the mechanism of the reaction.

**2.** For investigation of seedleaf extracts a method of MALDI analysis has been developed for detection of the components including formaldehyde generators.

**3.** In experiments of germination a linear relationship between mass and density of acorn has been experienced. In investigation of effect on the mass of the acorn by germination a specific water take-up has been established to be characteristic for early ontogenesis of the acorn independent of its mass. Linear correlation between the increase of mass and the decrease of density indicates that the decrease of density belonging to the unit of increase of relative mass is a general biological property of the germinating acorn. Determined alteration of physical parameters accompanying germination, European Turkey acorn has been proved to derive from the essence of biological system. On the bases of the findings mentioned before, the physiological conditions of ontogenesis has been defined with point pairs of the relative mass and the density. Their values are able to characterize the condition of germination. The acorns in the same

developmental condition can be chosen with the sampling carried out through the measurement of mass and density of germinating acorn. In such a way the experiments of germination can be compared to each other. Definition of developmental conditions by the values of relative mass and density has made verification and perception of periodical alteration resulted in endogenous formaldehyde level possible.

**4.** Formation of tissue structures of the seedling was characterized with an increase of the activity of demethylation because endogenous formaldehyde level had increased.

During imbibition of the acorn catalase activity and endogenous formaldehyde content alter in opposite directions. In the seedleaves the increase of endogenous formaldehyde content proceeds beside the decrease of catalase activity. Decreasing activity is coupled with increasing content of formaldehyde and, of course, the similar relation is experienced in the reversed direction. In spite of relationship observed in the tendency of the alterations a strong statistical correlation can not be interpreted between the parameters.

**5.** Endogenous formaldehyde level of the acorn in resting condition has been experienced to increase as far as beginning of vegetation period and then it decreases. In case of those acorns stored in different time intervals and made to germinate as far as a increase of 10% of their mass, significant alteration of endogenous formaldehyde level can not be observed with applied analytical method until March. Hereupon, its value decreases significantly by July. In case of the acorns in resting state, highest endogenous formaldehyde level has

been established as a tendency at the beginning of the vegetation. During the store over spring endogenous formaldehyde level decreases which can be related to the decrease of percentage of the germination experienced during longer period of the store.

6. For cold shock (-20 °C) endogenous formaldehyde content is oscillating in the alarm phase similar to the biotic stress but with a contrasting deflection and then reaches a higher steady state in resistance range than the control acorn has.

7. As a result of heat shock (40 °C) endogenous formaldehyde level of the acorns without their shells increase to a higher value than that of the acorns wrapped into foil. In this case endogenous formaldehyde content alters periodically in the alarm phase, too. The acorns without their shells has got two local minima of endogenous formaldehyde content in the alarm phase. These are independent of each other but they are induced by the same heat effect with a phase shift. It supposed that twofold alarm response is the result of increasing the temperature and afterwards, that of the withering which is becoming more and more significant during stress effect. Beside the alteration of biochemical factors, significant differences have also been experienced in the physical parameters. Before forming the stress syndrome germination of the acorn has got a strong linear correlation between relative mass and density of the seedleaf. Afterwards the linear relationship of mass and density has disappeared.

8. For the effect of dimedone, (chemical stress) endogenous formaldehyde level is being increased. As a result of dimedone dose applied *in vivo* way the rate of demethylation is speeded up. The stress by dimedone increases the potential forming endogenous formaldehyde.

9. In the stress response without retardation of time either the processes of demethylation or those of methylation become dominant. Disturbing this process by stress factors induces a periodical alteration in endogenous formaldehyde content. Oscillating formaldehyde content supplies some information about the ability of the stress tolerance in plant. The oscillation of formaldehyde level, which increases with higher and higher amplitude, indicates that the cells can govern heavier and heavier the enzymatic processes of methylation and demethylation with their control mechanisms.

#### **IV. Possibilities for utilization of the results**

It follows from the experimental results that some information can be obtained during imbibition about the viable of the acorn through measurement of chemical and physical parameters, too. Alteration of biochemical and physical markers in case of germinating acorn can be a basis of new and alternative method for investigation of the viable.

## V. Articles and lectures published in the theme of dissertation

### Articles:

1. Varga Sz., Albert L., Németh Zs.I. (1997): A formaldehid metabolizmus vizsgálata a cser makk csírázásának kezdeti szakaszában, *Erdő-, vad- és fagazdálkodás*, (szerk. Bondor A., Solymos R.), MTA Agrártudományok Osztálya, Budapest, pp. 79-86.
2. L. Albert, Zs. I. Németh, T. Barna, Sz. Varga, E. Tyihák, Measurement of endogenous formaldehyde in the early development stages of European Turkey oak (*Quercus cerris* L.), *Phytochemical Analysis*, **9**, (1998), 227.
3. L. Albert, Zs.I. Németh, Sz. Varga, The effect of heat shock on the formaldehyde cycle in germinating acorns of European Turkey oak, *Acta Biologica Hungarica*, **49 (2-4)**, (1998), 363.
4. L. Albert, Zs.I. Németh, Sz. Varga, Changes in formaldehyde contents of germinating acorns of *Quercus cerris* L. under low temperature stress conditions, *Acta Biologica Hungarica*, **49 (2-4)**, (1998), 369.
5. Németh Zs. I., Albert L., Varga Sz. (1999): A csírázó csertölgy makkok stressz jelenségeinek vizsgálata. Kutatói nap 1998-1999 - Tudományos eredmények a gyakorlatban, (szerk. Horváth B.), *Alföldi Erdőkért Egyesület*, Sopron, pp.81-90.

### Lectures:

6. L. Albert, T. Barna, Zs. I. Németh, Sz. Varga (1997): Change of formaldehyde and some betaines in parts of *Quercus cerris* L. at abiotic stress conditions, "*Stress of Life*". *International Congress of Stress*, Budapest, július 1-5.
7. L. Albert, Zs. I. Németh, Sz. Varga, T. Barna (1997): The cold shock in the early stage of European Turkey oak (*Quercus cerris* L.), "*Stress of Life*". *International Congress of Stress*, Budapest, július 1-5.

- 8.** Zs. I. Németh, L. Albert (1997): Identification of endogenous formaldehyde in different biological samples by MALDI-MS, *"Stress of Life"*. *International Congress of Stress*, Budapest, július 1-5.
- 9.** Zs. I. Németh, L. Albert, Sz. Varga (1998): Change of formaldehyde and some betaines in the germinating acorns of European Turkey oak (*Quercus cerris* L.) at low temperature stress conditions, *4<sup>th</sup> International Conference on ROLE OF FORMALDEHYDE IN BIOLOGICAL SYSTEMS*, Budapest, július 1-4.
- 10.** L. Albert, Zs.I. Németh, Sz. Varga (1998): Effect of the heat shock for formaldehyde cycle in germinating accorns of European Turkey oak (*Quercus cerris* L.), *4<sup>th</sup> International Conference on ROLE OF FORMALDEHYDE IN BIOLOGICAL SYSTEMS*, Budapest, július 1-4.
- 11.** E. Tyihák, Zs. I. Németh, L. Albert, Zs. Király-Véghely, Gy. Kátay : Advantages of combination of MALDI MS, HPLC and OPLC in Biochemical Analysis. *Balaton Symposium '99 on high-performance separation methods*, September 1-3, 1999, Siófok.
- 12.** Zs. I. Németh, T. Hofmann, L. Albert, E. Tyihák: Analogies and differences in the reactions of dimedone with formaldehyde and special fromaldehyde generators, *5<sup>th</sup> International, Jubilee Conference on ROLE OF FORMALDEHYDE IN BIOLOGICAL SYSTEMS*, October 9-13, 2000 Sopron, Hungary.
- 13.** Zs. I. Németh, L. Albert, Sz. Varga, M. Balaskó: Changes of catalase activity and endogenous formaldehyde level in the germinating acorns of *Quercus cerris* L., *5<sup>th</sup> International, Jubilee Conference on ROLE OF FORMALDEHYDE IN BIOLOGICAL SYSTEMS*, October 9-13, 2000 Sopron, Hungary.
- 14.** Zs. I. Németh, L. Albert, Sz. Varga: Relationship between dimedone shock and formaldehyde level in the germinating acorns of European Turkey oak, *5<sup>th</sup> International, Jubilee Conference on ROLE OF FORMALDEHYDE IN BIOLOGICAL SYSTEMS*, October 9-13, 2000 Sopron, Hungary.