Phyton (Austria) Special issue: "Eurosilva"	**Vol. 39**	Fasc. 4	(275)-(280)	15. 7. 1999

Reduced Germination is Associated with Loss of Phytic Acid in Stored Seeds of Sessile Oak (*Quercus Petraea* (Matt.) Liebl.)

By

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K e y w o r d s : Sessile oak, seed, storage, phytic acid, phosphorus.

S u m m a r y

ZITNIK S., HANKE D.E. & KRAIGHER H. 1999. Reduced germination is associated with loss of phytic acid in stored seeds of sessile oak (*Quercus Petraea* (Matt.) Liebl.). - Phyton (Horn, Austria) 39 (4): (275) - (280).

The aim of the research was to develop new, cheap and practical methods for storage of acorns of sessile oak. Currently it is only possible to store them for 6 months. The research is based on the hypothesis, that the germination of acorns after storage is positively correlated with the amount of phytic acid in them, which is positively correlated with the content of available P in soil. The experiment was established as a case study on two plots of sessile oak, which differed in the P content of the leaves, used as an indicator of available P in the soil. The seeds were collected in October 1997 and stored for 9 months until August 1998. Before storage and at three monthly intervals, the following observations at -1°C were made: germination and moisture, phytic acid and P content of acorns. The results show that the plot with a higher content of P in the leaves also has higher content of P and phytic acid in acorns, and germination is higher. The differences can be observed only at the beginning of storage. The results suggest that there is a positive correlation between the vitality of acorns in storage, the content of phytic acid in acorns and the content of P in soil. However, the data are not yet sufficient to prove such a correlation.

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Introduction

Acorns of sessile oak are recalcitrant seeds (ROBERTS 1973), and cannot be dried to less than 40% moisture content without loss of vitality or stored at temperatures lower than -3°C (SUSZKA & al. 1996). Because of this, it is almost impossible in practice to store them for more than one winter. Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is very important in the vitality of seeds including (GRAF & EATON 1990): antioxidant function, storage of cations, P, proteins and potential energy, the inositol component of phytic acid is important for cell wall formation. Higher contents of phytic acid in seeds could improve their vitality during storage. Phytic acid in seeds probably increases with the content of total P in seeds (RABOY & DICKINSON 1993). Furthemore, the P content of seeds has been positively correlated with available P in the soil (MILLER & al. 1980, RABOY & DICKINSON 1984, 1993, LAU & STEPHENSON 1994).

The research is based on the hypothesis, that the germination of acorns after storage is positively correlated with the acorn phytic acid content and available soil P. Leaf P was measured to estimate soil P levels (STEVENSON 1986, SIMONCIC 1995) and possible levels of P in seeds (RABOY & DICKINSON 1984, 1993). The experiment was established as a case study on two plots of sessile oak, which essentially differed in the content of total P in leaves. The seeds were collected in October 1997 and stored for 9 months until August 1998. Before the storage and every three months (4 times altogether) the following observations were made: germination, phytic acid, P and moisture content of acorns.

Material and Methods

Material

One thousand acorns of sessile oak were collected from each of two forest plots in Slovenia, each of 1 ha and a square form. The two plots were selected on the following three criteria: sessile oak trees represents more than 50% of all trees, difference in the content of total **P** in leaves of sessile oak between the plots, and a suitable crop for collecting of sessile oak. Other characteristics of the plots are shown in Table 1 (AZAROV 1995, KALAN 1995, SIMONCIC 1995, SMOLE 1995). Acorns were collected evenly under all trees of sessile oak. Only the healthy acorns were collected.

Table 1. The characteristics of the plots on which seeds were collected.

Characteristics	**Plot 1**	**Plot 2**
altitude	150 m	470 m
forest association	Carici umbrosae - Quercetum petraea	Haquetio-Fagetum - var. Ruscus hypoglossum
total P in leaves [dry weight]	0,056 %	0,096 %
average age	60 years	140 years
longitude	13°44'	15°43'
latitude	45°58'	46°01'

Methods

Storage of acorns. The collected acorns were treated with thermotherapy - 2 hours in a water bath at 41°C to destroy *Ciboria batschiana* (Zopf.) Buchwald and other pathogenic organisms (DELATOUR & MORELET 1979). Then the acorns were dried to a moisture content of 45 % and stored at a temperature of -1°C (SUSZKA & al. 1996). Stored acorns were held in a plastic box without medium and covered with a plastic sack, to regulate moisture loss and maintain exchange of air.

The germination of acorns was tested according to the ISTA International rules (ISTA 1993). The acorns were soaked for 48 hours in water at room temperature. Then they were peeled and one third of the endosperm opposite the radicle was cut away. Three samples of 50 prepared acorns from each plot were put on wet vermiculite and lightly covered. The germination took place in complete darkness at a constant temperature of 20°C. Every five days the germinated acorns (at least 5 mm of radicle visible) were counted and discarded. The last count was made after 25 days. The germinated acorns were expressed as a percentage of all 50 acorns.

The moisture content of acorns was determined gravimetrically according to the ISTA International rules (ISTA 1993). Three samples of 5 g of acorns, cut into pieces approx. 3 mm across, were dried for 17 hours at a temperature of 103 °C. The moisture content was expressed as a percentage of the fresh weight of acorns.

The content of phytic acid in acorns was determined by the m.d.d. (metal-dye detection) method (MAYR 1988) in combination with a spectrophotometer. The samples of acorns were extracted by the following procedure (CRANS & al. 1995): 18 ml of H_20 and 2 ml of 15% HCL (vol:vol) were added to 2 g ground acorns. The sample was stirred and after 30 min at room temperature, the sample was filtered. Three samples of 2 µl were mixed with 1ml of reagent [10 µM YCL_3 (Aldrich), 70 µM 4-(2-pyridylazo) resorcinol (Aldrich), 700 mM TRIS/HCL pH 8.5] and absorbance at 546 nm measured (LAUSSMANN & al. 1996). The content of phytic acid was expressed as a percentage of dry weight of acorns.

The total content of P in three samples of acorns was determined by wet digestion in an acid mixture $HNO_3/HCLO_4$ (5:1, vol:vol) (BLUM & al. 1989, ÖNORM L 1085 1989). Acorn P content was expressed as a % of acorn dry weight.

Data were analysed using the nested anova test (ZAR 1984). The difference between the two plots for each parameter (moisture content, germination, the content of phytic acid and P) was tested.

Results

The moisture content of the acorns during storage was constantly around 45 % and there was no difference between the two plots ($p>0.05$). Average final germination was greater in plot 2 (57.33%) compared with plot 1 (45.33%) ($p=0.0043$). Storage of acorns at -1°C reduced germination in both plots by approximately the same amount. Phytic acid content was greater in acorns collected from plot 2 (2.67%) ($p=0.0095$). Storage at -1°C resulted in a gradual depletion of phytic acid in acorns from both plots. But significant differences between samples from each plot were not detectable after 6 months storage. The results of the other analyses are shown in table 2. The results of the nested anova test are shown in table 3. They show that there was significant difference between the plots for the analyzed parameters. However, the LSD (Least Significant Difference) tests for the two plots show that the differences were detectable only at the beginning of storage. The plot that had a higher content of total P in the leaves

also had a higher content of total P in seeds. In this plot, the seeds also had a higher content of phytic acid and their germination was higher.

Table 2. The results of germination and the content of phytic acid and P in acorns, the interval estimation is given with the level of significance of 5%.

time of storage [months]	germination [%]		phytic acid [%]		P [%]	
	plot 1	plot 2	plot 1	plot 2	plot 1	plot 2
0	72.00	90.76	0.93	2.67	0.019	0.036
deviation	13.33	4.65	0.31	1.19	0.008	0.005
3	74.00	93.33	0.62	1.69	0.027	0.039
deviation	12.12	3.85	0.14	0.77	0.003	0.007
6	69.33	88.00	0.51	0.94	0.028	0.036
deviation	13.11	4.89	0.02	0.34	0.004	0.005
9	45.33	57.33	0.47	0.75	0.026	0.037
deviation	9.42	8.57	0.03	0.26	0.005	0.007

Table 3. The results (the level of significance - p: <5% - *, <1% - **, <0.1% - ***) of the nested anova test

parameter	nested anova test	LSD test for the two plots [months after storage]			
		0	3	6	9
germination	0.00432**	0.01760*	0.00861**	0.02539*	0.27421
phytic acid	0.00953**	0.00224**	0.04090*	0.37466	0.22703
P	0.00180**	0.00253**	0.02813*	0.07726	0.02600*

Discussion

Based on the results we can predict that there may be a positive correlation between the vitality of acorns, the content of phytic acid in them and the content of P in soil at the beginning of storage. However, there is no sign of such a correlation at the end of storage, when it would be most important, for new methods of improving the vitality of stored acorns. Also the between plot differences in the analyzed parameters at the beginning of storage were not very high and they could be a consequence of other factors which differ between the plots (local climate, soil, fructification, different stands of sessile oak - age, height, density) rather than the hypothetical correlation. To exclude other factors and to confirm or disprove the hypothesis, this study should be repeated on several other plots on different sites. In view of this we can conclude, that the results partly confirm the research hypothesis.

Acknowledgements

The results presented are part of the Master of Science Thesis of researcher S. ZITNIK, which is financed from the Slovenian Ministry of Science and Technology - project CRP-V9-6912 and is supervised by Doc. Dr. H. KRAIGHER. The phytic acid research methodology is developed in collaboration with the group of Prof. Dr. D.E. HANKE from the Department of Plant Sciences in Cambridge (UK) in the framework of COST E6 TREEPHYSIOLOGY (one month of scholarship for visiting Cambridge in 1997) under the supervision of Dr. Ch. BREARLEY.

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