Effects of moisture content, storage temperature and type of storage bag on the germination and viability of stored European beech (*Fagus sylvatica* L.) seeds

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ABSTRACT: Beechnuts from three *Fagus sylvatica* lots were dried at 20°C to 5–6% or 8–9% (fresh weight basis) moisture content and stored at –7°C or –22°C for 3.5 or 4.5 years in sealed polyethylene bags containing air and routinely used for the long-term storage of beechnuts or vacuumed (de-aerated) polyethylene bags with air removed before sealing. The germination and viability (tetrazolium test) of beechnuts were determined according to the Czech Technical Rules (1997) once a year during storage. The results were subjected to factorial ANOVA and the means were compared using the Scheffe test at $P > 0.05$. Compared to beechnuts dried to an 8–9% moisture content a significant decrease in both germination and viability occurred in beechnuts dried to a 5–6% moisture content regardless of how long the beechnuts were stored. However, the effects of the other treatments (type of storage bag, storage temperature) were not significant.

Keywords: beechnut storage; germination; tetrazolium viability; moisture content; storage temperature; bag

Seeds (beechnuts) of European beech (*Fagus sylvatica* L.) belong to the orthodox, strictly speaking to the sub-orthodox, category of seeds according to Bonner and Vozzo (1990 in Bonner, Leak, http://www.nsl.fs.fed.us/wpsm), who subdivide orthodox seeds into (a) true orthodox which "can be stored for long periods at seed moisture contents of 5–10% and subfreezing temperatures" and (b) sub-orthodox which "can be stored under the same conditions, but for shorter periods due to high lipid content or thin seed coats". Thus the moisture of beechnuts can be reduced significantly before storage. Drying should be done, without heat, at 18–20°C. For operational storage of up to 5–6 years it is recommended to reduce beechnut moisture content (mc) to 8–9% and store at –5°C to –10°C in hermetically sealed containers (Suszka et al. 1994). According to Poulsen (1993) drying to 5% mc significantly increases the storability of beechnuts. She reported that after drying at 15°C and 15% RH (relative humidity) the initial germination percentage of beechnuts was reduced, but after a few months of storage germination increased again. A similar initial reduction in germination of beechnuts dried to below 6% mc with subsequent improvement in germination after a few months of storage was also observed by Suszka et al. (1994). Consequently it is recommended to store beechnuts at 5% mc if 10 or more years of storage are required (Poulsen 1993).

To double the seed life span Harrington's Rules prescribe a 1% drop in water content or a 6°C decrease in temperature (Black et al. 2006). About –20°C is generally used for the long-term storage of orthodox seeds in seed banks. However, the expense, e.g. for

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refrigeration, of maintaining such low temperatures is sometimes greater than the cost of initially reducing seed moisture content with subsequent storage at –7°C. At the Tree Seed Centre in the Czech Republic –7°C is used for the long-term storage of beechnuts with 8–10% mc and packing in plastic, polyethylene bags that contain air. One disadvantage of such bags is that they can be broken by careless handling. These practices raised the questions how operational drying of beechnuts at 20°C to 5–6% mc would affect their storability and if beechnuts can be stored in vacuum-sealed (de-aerated) plastic bags. Specifically we wondered if the storage of dried beechnuts in thicker (and stronger), (hermetically) vacuum-sealed bags would adversely affect beechnut germination.

The goals of the work reported here were to determine the long-term (up to 3–4 years) storability of beechnuts having target moisture contents of 5–6 or 7–9% and stored at –7 or –22°C in two types of plastic bags.

MATERIALS AND METHODS

Seeds and experimental design

Three beechnut seedlots (83, 442 and 7,001) were harvested in forest stands in 2000 and 2001 (Table 1). The beechnuts were collected from the forest floor and then operationally processed at the Tree Seed Centre in Týniště nad Orlicí. After drying at 20°C using a Heindl dehydration line (Heindl GmbH, Mainburg, Germany) the empty and insect-damaged beechnuts were removed at an air-pressure cleaner and the seedlots were divided into subsamples that were stored as follows: (*i*) approximate target moisture contents 5–6% (hereafter 5%) or 7–9% (hereafter 8%), (*ii*) in two types of sealed bags: PE = polyethylene bags 0.11 mm thick (CZECHOBAL, s. r. o., Hradec Králové, Czech Republic) containing air as has been routinely used for the long-term storage of beechnuts and VAK = vacuumed (de-aerated) 0.18 mm thick polyethylene bags (FRIMARK CZ, s. r. o., Pardubice, Czech Republic) and (*iii*) at –7 or –22°C. The mc of the beechnuts in the 5% mc treatment varied from 4.7–5.7% and those in the 8% mc treatment varied from 7.3–8.9% (Table 2). The beechnuts were then stored at the Tree Seed Centre and once a year samples were transported to the laboratory in insulated boxes where they were stored at –5°C until tested for germination and viability. The total weight of the subsamples for each treatment – seedlot combination was 1.5–2.5 kg.

Moisture content, viability and germination determination

The moisture content, viability and germination capacity of each seedlot and each treatment were determined after drying at the start of storage and then each year of storage: for seedlot 442 which was harvested in 2000 after 12, 30, 45 and 54 months (1, 2.5, 3.75 and 4.5 years) and for seedlots 83 and 7,001 which were collected in 2001 after 18, 33 and 42 months (1.5, 2.75 and 3.5 years) of storage. The storage period is reported according to the beginning of germination tests. The initial mc of beechnut lot 442 was determined only for the treatments with 8% mc, not for 5% mc.

Moisture content (fresh weight basis) was determined on two replications of cut beechnuts (10 g each) dried at 103 ± 2°C for 1 hour in a Brabender apparatus (Czech Technical Rules 1997).

A tetrazolium test (Czech Technical Rules 1997) was used for determining the viability of four replications of 100 seeds each.

Germination tests were done using a peat-sand substrate (1:1 by volume) (Czech Technical Rules 2006) with 400 seeds of each seedlot being mixed with a peat-sand substrate (one volume of seed to two volumes of substrate, 28–30% mc) for germination in 17 × 12 cm boxes at 4 ± 1°C. The boxes were kept closed with translucent lids except when they were opened weekly to check germinants. Beechnuts with visible protruding radicles were considered as germinated and discarded after counting. Germination counts were done weekly from the first week after sowing until when no germinants were observed in two consecutive weeks.

Table 1. Details about *Fagus sylvatica* seeds used in the experiments

Seedlot No.	Year of collection	Natural forest region	Altitudinal zone (altitude)	Forest district
442	2000	21 Jizerské hory and Ještěd	5 (601–700 m)*	Frýdlant
83	2001	10 Středočeská pahorkatina	4 (551–600 m)	Vodňany
7,001	2001	30 Nízký Jeseník	4 (551–600 m)	Plumlov

*altitude above sea level

Table 2. Seedlots and initial pre-storage (after drying at 20°C) viability, germination and moisture content of beechnuts used in the various treatments*

Seedlot No.	Viability (%)	Germination (%)	Initial moisture content (%)			
			PE**		VAK***	
			–7°C	–22°C	–7°C	–22°C
442	85	71	7.4	7.3	7.7	7.3
	*n*****	*n*****	5.1	4.7	5.7	5.0
83	68	43	8.4	8.1	8.3	8.0
	66	14	5.6	5.6	5.4	5.5
7,001	83	66	8.9	8.8	8.9	8.8
	83	25	5.3	4.8	5.1	5.5

*The values are the means of four replications (100 seeds each) for viability and germination and two replications (10 g each) for moisture content, **PE – polyethylene bag with air; ***VAK – polyethylene vacuum-sealed bag, ****n – viability and germination not determined

Then, all the remaining (ungerminated) seeds were cut and the dead (rotten), empty and 'fresh' seeds were counted. Using the tetrazolium test the 'fresh' seeds were classified as being either viable or non-viable and the viable seeds were included when calculating the germination percentage.

Statistical analyses

Viability and germination (both based on full seeds in %) data were subjected to factorial ANOVA to determine the significance of individual factors and their interactions, and the means were compared using the Scheffe test at $P > 0.05$ (StatSoft 2005).

RESULTS

Germination of two seedlots (7,001 and 83) decreased (25 and 14%) significantly immediately after the seeds were dried to 4.8–5.6% mc compared to beechnuts with an 8–9% mc (66 and 43%). Viability, as determined by tetrazolium test, of both seedlots immediately after being dried to the target mc at the start of storage was nearly the same for both moisture contents (5 and 8%) while germination decreased significantly (Table 2). Initial viability and germination for beechnuts from the third lot (442) were determined only for treatments with the higher moisture content (above 7%) and the difference between viability (85%) and germination (71%) was the lowest compared to all the treatments in seedlots 83 and 7,001 (Table 2).

The factorial ANOVA analysis showed a significant ($P < 0.05$) effect of moisture content on the germination of beechnuts stored for 42–45 months (ca 3.5 years) while storage temperature (–7 or –22°C) and type of bag (polyethylene bags containing air or vacuum-sealed bags) used for storage did not

Table 3. Results of the multiple factor analysis of variance (ANOVA) showing the effects of individual factors: beechnut moisture content (5 and 8%), storage temperature (–7 and –22°C) and bag type (polyethylene bag containing air and polyethylene vacuum-sealed bag) and their interactions on the germination of European beech seeds stored for 42 to 45 months (3.5–3.75 years) ($P < 0.05$)

Factor	SS	DF	MS	*F*	*P*
Moisture content (MC)	22,478.8	1	22,478.80	6,328.33	0.000
Temperature (T)	3.8	1	226.10	1.06	0.307
Bag type (B)	10.0	1	29.62	2.82	0.098
MC × T	41.3	1	41.30	11.64	0.001
MC × B	3.8	1	3.80	1.06	0.307
T × B	17.5	1	17.50	4.93	0.030
MC × T × B	0.1	1	0.10	0.03	0.871

significantly affect germination (Table 3). The germination of beechnuts in all three seedlots was significantly lower in all the treatments where the mc was 5–6% regardless of the storage temperature or type of storage bag (Table 4, Figs. 1 to 3). This drop in germination occurred immediately after drying and persisted over the entire storage period with the largest decrease occurring in the germination of beechnuts from seedlot 442 dried to a mc of 8% (Figs. 1 to 3). Figs. 1 to 3 show that all three beechnut seedlots responded similarly to all treatments regardless of their initial germination.

DISCUSSION

Our results show that the operational drying of beechnuts at 20°C to a mc of 5–6% results in a significant decrease in germination compared to beechnuts dried to 8–9% mc and then stored at –7 or –22°C. These results differ from those of Poulsen (1993), who found that beechnuts with a 5% mc and stored at –8°C did not deteriorate over the 623 day (less than 2 years) test period or Suszka et al. (1994), who reported only a short-term drop in the germination of beechnuts dried below 6% mc with a subsequent rebound in germination. Poulsen estimated that a 1% reduction in moisture content increases longevity much more than a 1°C reduction in storage temperature and predicted that if beechnuts were stored at 5% mc and at –15°C for 95 years, germination would decrease from the initial 80 to 40% only. The overall quality of our experimental beechnuts was lower than the initial ca 80% germination of those beechnuts used by Poulsen (1993) and Suszka et

Table 4. The mean germination and viability (as determined by tetrazolium test) of three beechnut lots stored for 42 to 45 months (3.5–3.75 years) at 5 or 8% moisture content and at –7 or –22°C, in polyethylene bags containing air (PE) or in vacuum-sealed bags (VAK)*

Seedlot No.	Treatments			Germination (%)	Viability (%)
	temperature (°C)	moisture content (%)	bag type		
442	–22	5	VAK	23.0 a	53.3 bcd
	–22	5	PE	23.3 ab	55.8 bcd
	–22	8	VAK	53.3 de	52.8 bcd
	–22	8	PE	51.0 d	52.5 bcd
	–7	5	VAK	31.5 bc	50.0 abcd
	–7	5	PE	31.8 c	48.0 abc
	–7	8	VAK	59.8 def	63.0 cd
	–7	8	PE	57.3 def	71.8 d
83	–22	5	VAK	11.8 a	29.5 abc
	–22	5	PE	13.7 a	20.8 ab
	–22	8	VAK	36.0 c	41.5 bcd
	–22	8	PE	38.0 c	42.5 bcde
	–7	5	VAK	16.8 ab	43.5 cde
	–7	5	PE	13.5 a	37.5 abc
	–7	8	VAK	30.7 c	46.5 cde
	–7	8	PE	37.0 c	41.0 bcd
7,001	–22	5	VAK	17.5 ab	17.8 a
	–22	5	PE	21.3 b	50.5 bc
	–22	8	VAK	60.0 de	56.0 c
	–22	8	PE	63.8 e	50.5 bc
	–7	5	VAK	11.8 a	61.0 c
	–7	5	PE	15.5 ab	29.5 ab
	–7	8	VAK	58.8 cde	56.8 c
	–7	8	PE	53.0 cd	63.5 c

*The values are the means of four replications (100 seeds each), the values within the same column and each seedlot followed by the same letter(s) are not significantly different (Scheffe test, $P < 0.05$)

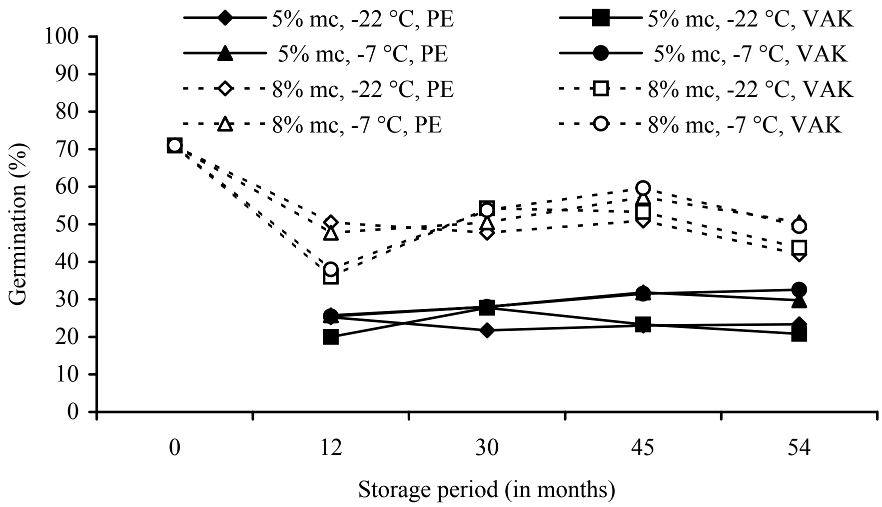

Fig. 1. Germination of beechnut seed lot 442 stored for 54 months (4.5 years) with 5 or 8% moisture content, and at –7 or –22°C in sealed polyethylene bags containing air (PE) or vacuum-sealed bags (VAK)

al. (1994). For example, the initial viability (according to the tetrazolium test) of our seedlots varied from 85 to 66% while the highest germination of seeds after drying to 8% mc was 71% (Table 2). The tetrazolium test gives higher values than do germination tests as less vigorous, viable seeds such as those with some permitted necrosis on the distal end of cotyledons (Czech Technical Rules 1997) can deteriorate, especially during long germination tests. Our results of initial viability and germination demonstrate that the tetrazolium test did not distinguish any significant differences between the 5 and 8% mc treatments in seedlots 7,001 and 83 after drying (Table 2).

The germination of our seedlots required up to 22 weeks and the peak germination occurred in 10–19 weeks (data not shown), indicating quite deep dormancy. In 2004 the 74 operationally-collected beechnut seedlots processed at the Tree Seed Centre had an overall average germination of 66% (range 29–86%) while average viability determined by tetrazolium staining was 73% (51–97%). Germination tests lasted for 20 weeks on average, indicating rather deep dormancy (Procházková et al. 2002). In 2001, when 146 beechnut seedlots were collected, the mean germination was 75% (5–93%) and viability was 71% (34–93%) (data from accredited laboratory L 1175). In the Czech Republic the germination of beechnuts varies greatly both among crops and provenances within crops. The assessment of germination usually requires 20–25 weeks while the tetrazolium determination of viability provides in 3 days (duration of the test) more or less reliable information about beechnut quality and their suitability for long-term storage. However, we do not recommend to store beechnuts at 5% mc if 10 or more years of storage are contemplated, i.e. as Poulsen (1993) did especially if the initial germination is less than 80%.

Our results (Table 4) showed that the germination of beechnuts with a 8–9% mc and stored at –22°C did not decrease significantly over 42 months (3.5 years) of storage in two seedlots (83 and 7,001) harvested in 2001(Figs. 2 and 3). Beechnuts in the same treat-

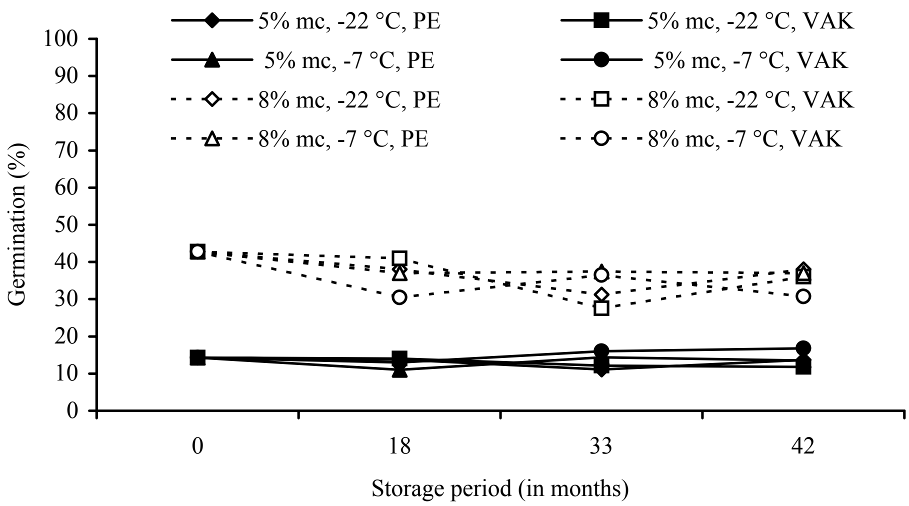

Fig. 2. Germination of beechnut seed lot 83 stored for 42 months (3.5 years) with 5 or 8% moisture content, and at –7 or –22°C in sealed polyethylene bags containing air (PE) or vacuum-sealed bags (VAK)

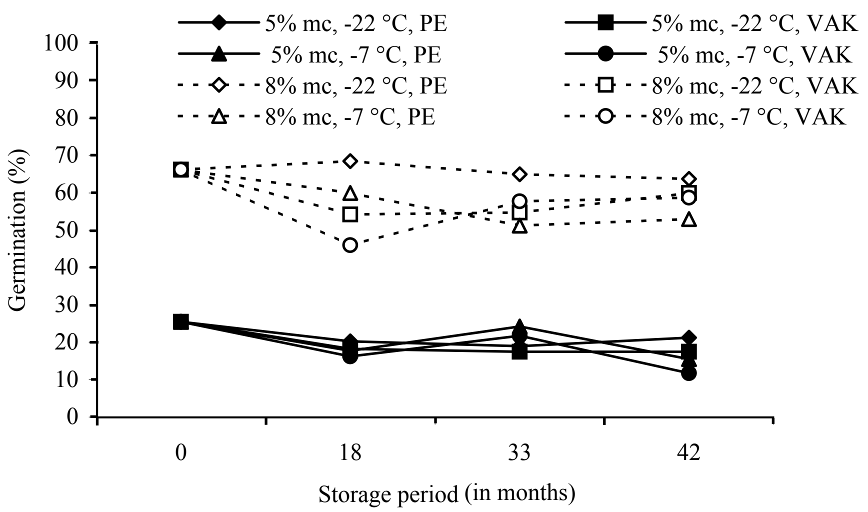

Fig. 3. Germination of beechnut seed lot 7,001 stored for 42 months (3.5 years) with 5 or 8% moisture content, and at –7 or –22°C in sealed polyethylene bags containing air (PE) or vacuum-sealed bags (VAK)

ments from the 2000 crop year (seedlot 442) lost from 20 to 30% of their initial germination after 1.5 year of storage, but the germination partly improved after 30 months (2.5 years) of storage (Fig. 1). These results do not fully support Gosling's finding (1991) that the germination percentage of stored beechnuts typically drops 10% per year. A decrease in the germination of stored beechnuts is largely affected by conditions during ripening (dormancy) as was determined for beechnuts from the 1995 mast year (Procházková et al. 2002).

A distinctive drop in germination after seed drying with subsequent (but only partial) improvement after 30 and 45 months (2.5 and 3.75 years) of storage was observed only in one seedlot (442) for beechnuts dried to 8–9% mc (Fig. 1). Beechnut lots 83 and 7,001 showed no significant change in germination during 42 months (3.5 years) of storage (Figs. 2 and 3). Our results do not support those of Suszka et al. (1994) or Poulsen (1993), who reported a sharp reduction in the initial germination of beechnuts that had a moisture content below 6% shortly after drying (from 85 to 65%) with a subsequent rebound after a few months of storage. However, Poulsen (1993) dried her beechnuts at a lower temperature (15°C) than we did when we used operational drying at 20°C, without any heating. This might explain why such operational machines cannot be used for drying beechnuts to moisture content below 8% for long-term storage in seed banks. Besides weather conditions during seed ripening drying temperatures seem to be crucial and a drying temperature below 20°C is mandatory for the good storability of beechnuts (Poulsen 1993).

Our results show that beechnuts with an 8–9% mc can be safely stored at temperatures down to –22°C with no significant difference occurring in germination between beechnuts stored at –7°C or –22°C for 3–4 years (Table 4, Figs. 1 to 3). This is consistent with the recommendation that European beech seeds can be stored for 10 years without loss of germination by drying the seeds (by blowing re-dried air through the seeds at 18–20°C) to a moisture content of 8–9% and keeping them in sealed containers at –5 to –10°C (Suszka et al. 1994). No difference was detected between the germination of beechnuts stored in sealed, plastic bags with some remnants of air versus vacuum-sealed bags. In the past the 0.11 mm thick plastic bags used for beechnut storage were sometimes ripped open during handling by the sharp seed coats of beechnuts. Consequently we tested to see if thicker plastic bags (0.18 mm) filled with dried beechnuts and then vacuum-sealed would adversely affect the germination of stored beechnuts. Our results showed that beechnuts could be stored in vacuum-sealed, plastic bags without negatively affecting the beechnut germination. However, the amount of torn thicker storage bags was greater than in the plastic bags that were routinely used in the past (Hlavová, personal communication).

CONCLUSIONS

Based on our results we conclude that:

(*i*) drying of beechnuts at 20°C to a moisture content below 6% has a significant negative effect on their germination immediately after drying and afterward during storage,

(*ii*) beechnuts operationally dried at 20°C to a moisture content of 8–9% can be stored in sealed, plastic bags for 3–4 years without any significant decrease in germination,

(*iii*) beechnuts can be stored either in plastic bags with ambient air or in vacuum-sealed plastic bags without any significant effect on their germination,

(*iv*) beechnuts with a moisture content of 8–9% in sealed plastic bags can be stored either at –7 or –22°C for 3–4 years without suffering a significant decrease in germination

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Vliv obsahu vody, teploty a obalu na klíčivost a životnost skladovaných bukvic (*Fagus sylvatica* L.)

ABSTRAKT: Bukvice (*Fagus sylvatica* L.) tří oddílů byly vysušeny při 20 °C na obsah vody 5–6 % nebo 8–9 % (čerstvé hmotnosti) a skladovány při –7 °C nebo –22 °C v zatavených polyetylenových sáčcích obsahujících vzduch a používaných provozně pro dlouhodobé skladování bukvic a polyetylenových sáčcích s odsátým vzduchem vakuováním při zatavení. Klíčivost a životnost bukvic byla hodnocena podle ČSN 48 1211 (1997) každý rok skladování. Výsledky byly zpracovány vícefaktorovou analýzou variance ANOVA a průměry porovnány Scheffeho testem ($P > 0,05$). Významný pokles klíčivosti byl zjištěn u bukvic vysušených na obsah vody 5–6 % bez ohledu na dobu skladování. Také životnost těchto bukvic byla nižší ve srovnání se semeny s obsahem vody 8–9 %, ale rozdíly mezi variantami nebyly signifikantní. Teplota skladování ani typ obalu neovlivnily významně ani klíčivost, ani životnost bukvic.

Klíčová slova: skladování bukvic; klíčivost; tetrazoliová zkouška životnosti; obsah vody; teplota skladování; obal

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