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Betula pendula seed storage and sowing pre-treatment: effect on germination and seedling emergence in container cultivation

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Abstract: Irregular seed crop in *Betula pendula* (Silver Birch) is a reason to create seed reserves, ensuring nursery production in years of crop failure. This study investigated the effects of seed moisture content and storage temperature on germinability and seedling emergence in container cultivation. Mature catkins were collected separately from 3 trees. The mixture of winged nuts and scales was dried to 3 levels of moisture content and stored at 3°C, –3°C and –10°C. After storage for 3, 5 and 6 years, the seeds were separated from scales and next dewinged and sorted in acetone into empty and filled seeds. Cleaned nuts were germinated in the Jacobsen germinator. Besides, to assess seedling emergence, seeds were sown on the surface of peat mixed with perlite, in multi-cell trays under a plastic tunnel. Significant differences in germinability were detected depending on the mother tree, seed moisture content and storage time. The viability of seeds stored at –10°C remained unchanged for 6 years regardless of moisture content (ca. 8–12%). Seeds stored at higher temperatures lost their germinability faster. An unexplained increase in seedling emergence was observed after extended seed storage at –10°C, in contrast to a gradual decrease in seedling emergence after extended storage at –3°C.

Additional key words: Silver Birch; liquid seed sorting; seed moisture content; storage temperature; container nursery; pH of imbibition water

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Introduction

Birches (*Betula pendula* and *B. pubescens*) in Poland account for 7.4% of the total forest area, i.e. they rank second, after *Pinus sylvestris* (GUS 2010). In respect of standing timber volume, birches rank sixth, after alder (*Alnus* sp.). In the northern part of its range, in Finland, birch is the third most important forest tree (Kowalczyk and Wojda 2005). As pioneer species, birches are often the first trees to colonize wasteland and abandoned farmland (Rebele 1992). *Betula pendula* (Silver Birch) is used in forests as one of the 6 major species for fast-growing forest plantations (Zajączkowski and Załęski 2007). Apart from a wide range of applications and valuable wood (especially the so-called Karelian birch, i.e. birch burr from Karelia), the decorative value of birch (e.g. shape of the tree or bark colour) make it very attractive, so it is often used for planting in parks and open landscapes.

Birch seeds are winged nuts, easily disseminated by wind over large distances. Before seed collection, their quality is usually assessed, as some seed lots are completely useless. Seed quality, to a large extent, depends on the abundance of flowers and the pattern of

weather conditions. Good seed crops are recorded every 2–3 years, and then their germination capacity is higher (Antosiewicz 1975, Bodył 2006). Such high-quality seed lots can be stored as reserves to be used in years of crop failure. However, according to Aniśko et al. (2006), they must be first dried to a moisture content of about 3.5%. After shedding, the seeds can remain ungerminated on the ground till spring. In the soil, as a rule, birch seeds are viable for up to 1–3 years (Hill and Stevens 1981, Granström and Fries 1985, Perala and Alm 1990), although in the north of Sweden, seeds in the soil are viable longer (Granström 1987).

In forest nurseries, the seeds are not separated from the scales before sowing. When sown in belts (so-called "partial sowing"), 0.6–0.9 kg of such a mixture is used per 100 m^2 (Rozwałka 2003), whereas when spaced evenly (so-called "full sowing"), 1.5–3.0 kg is used per 100 m^2, but seeds account for 35–38% of the total weight (Suszka et al. 2000). For container cultivation and "point sowing" (i.e. sowing of single or up to about a dozen seeds at widely spaced points), seeds should be separated from the scales, dewinged, and fully viable. PREVAC (Simak 1981) and IDS methods (Bergsten and Sundberg 1990), seed blowers, seed table sorting are common techniques for seed separation. Flotation of seed in some liquids with different specific gravity is used for separation also: pure water, carbon tetrachloride, hexane, ethanol, diethyl ether, petroleum ether, n-Pentane, linseed oil and other (Björkroth 1973; Bewley et al. 2006).

In *B. pendula*, 1000 nuts weigh about 0.1 g (Rozwałka 2003) or 0.15 g (Suszka et al. 2000). For production of potted seedlings, the sowing efficiency coefficient in plastic tunnels should reach 0.1, which corresponds to 250 seedlings per 1 m^2 (Rozwałka 2003).

The aims of this study were: (1) to develop an effective method for separation of filled seeds from empty seeds; (2) determine the effects of seed origin, storage time, temperature and moisture content on germination in the laboratory, as well as seedling emergence and height in multi cell trays.

Material and methods

Plant material

Mature catkins of *B. pendula* were collected on 20 July 2004 from 3 trees fallen the same day in the Konstantynowo Forest District (Uroczysko Woźniki). Seed lots from individual trees were marked with numbers 1920, 1921, and 1922.

Germination of fresh seeds

After collection, germination capacity was assessed for fresh seeds (without drying and dewinging) in 4 replicates of 100 seeds each, in the Jacobsen germinator, after 2 weeks at cyclically alternating temperatures of 20~30°C (i.e. for 16h at the lower temperature and 8h at the higher temperature in the diel cycle; ISTA 2008). In the periods of higher temperature, the seeds were illuminated with fluorescent light (Daylight Osram L 36W/950-1, irradiance 22 μmol m^{-2} s^{-1}).

Seed drying and storage

For further experiments, the mature catkins after collection were dried at room temperature and in a BCC cabinet drier (Björkemar Construction & Consulting AB, Landskrona, Sweden), to 3 levels of moisture content (mc, fresh weight basis): A, B, and C (Table 1). Seeds with scales and debris were dried first at room temperature to mc A, next in BCC for 1.5 hour at 25°C (mc B) and additionally 5 hours at 30°C (RH 25–30%) to mc C. Seed mc was estimated in 3 replicates. Next, the seeds with scales were separated into samples of 8–11 g each (depending on the total weight of the seed lot) and next placed in plastic bags, tightly sealed, and stored for several years at 3°C, –3°C, and –10°C.

Table 1. Moisture content (%, fresh weight basis) of seeds with scales after drying of mature catkins of 3 trees (seed lots 1920, 1921, 1922)

Moisture content level	Moisture content (%) of seed lot		
	1920	1921	1922
A	12.7 ±0.1	11.6 ±0.15	11.4 ±0.0
B	9.5 ±0.0	10.7 ±0.15	9.6 ±0.15
C	7.8 ±0.1	7.6 ±0.47	8.6 ±0.15

Germination and seedling emergence after seed storage

In spring, after storage, the individual bags were opened only once, and their germination was tested in the Jacobsen germinator in 4 replicates of 100 seeds each. Seeds after storage at –3°C, and –10°C, were also subjected to seedling emergence tests. They were sown in 4 replicates of 40 seeds each, on the surface of the germination substrate in plastic growing trays HIKO V-93 (BCC, Landskrona, Sweden), kept in a plastic tunnel. The growing trays were placed in plastic darkroom trays (55×40×7.5 cm) filled with lake water from Lake Kórnickie to the height of about 4 cm. The germination substrate was composed of *Sphagnum* peat (pH 5.5-6.5, Sterlux, Hollas Sp. z o.o.) with perlite No. 3 (at a ratio of 3:1, v/v) and the 2 kg/m^3 dose of Osmocote® Classic Standard fertilizer (which releases nutrients for 5–6 months). Water losses in the flow trays were replenished regularly, ensuring continuous sub-irrigation of the growing trays.

In the first spring after collection, winged seeds were sown, whereas after 3, 5 and 6 years of storage, the seeds were first separated from scales by sieving, and next the seeds were dewinged manually to separate the nuts from wings. Sieves were also used to separate the seeds infested by larvae of the genus Semudobia, most probably *S. betulae* (Roskam 1977).

Dewinged nuts were sorted in acetone (after earlier pilot trials, not published), to separate the empty seeds (which floated on the surface) from filled seeds (which sunk to the bottom). Filled seeds were strained and next dried on blotting paper under fan air flow. The separation of filled seeds from empty seeds lasted less than 1 min, whereas their drying after the floating in acetone lasted about 5 minutes. The dry weight of 3 replicates of 1000 filled nuts each was assessed by drying at 105°C for 24 h.

Filled seeds were placed in individual cells manually, by pushing them down from a glass plate with the use of a metal spatula. Seeds and the surface of the soil after sowing were sprinkled with water. The trays were next placed in a plastic tunnel.

To protect the sown seeds against exposure to direct sunlight, the southern side of the tunnel was sprinkled with a shading paint every year. To allow effective hardening of the seedlings before autumn frosts, the plastic cover of the tunnel was removed in early August.

Germination at various pH levels

An additional laboratory experiment was performed to determine the influence of pH on seed germination (after 14 days). Germination tests were performed on filter paper in Petri dishes (sealed with laboratory film Parafilm "M" ®) for seed lot 1921, at cyclically alternating temperatures of 20~30°C after storage for 6 years at 3°C. Water was acidified with citric acid (Sigma-Aldrich) to various levels of pH, ranging from 3.8 to 5.0 (measured with Metler Toledo, SevenCompact™ S230 Conductivity meter)

Weather data

To interpret some results, selected data from the weather station of our Institute were used: daily records of minimum and maximum temperature and insolation from May to September in 2007, 2009, and 2010. The weather station is located about 100 m away from the plastic tunnel.

Final measurements and statistical analysis

At the end of each growing season, all seedlings were counted and their height was measured. The results expressed as percentages (germinability and seedling emergence), after arcsin $\sqrt{\%}$ transformation, were subjected to analysis of variance (ANOVA) and the Tukey test, at $p = 0.05$, by means of Statistica software (1998).

Results

1000-seed weight

Clean seeds accounted for about 20% of the dry weight of mature catkins. The dry weight of 1000 dewinged filled seeds after the sorting in acetone varied from 0.147 g in lot 1921, through 0.180 g in lot 1920, to 0.218 g in lot 1922. The results differed significantly at $p<0.001$ (Tukey test).

The studied birch seed lots were infested with larvae to a varying extent. The infested seeds were nearly disc-shaped, in contrast to healthy seeds, which were spindle-shaped. After dewinging, the infested nuts could be easily separated by sieving. The mean weight of 1000 seeds infested by larvae of the genus Semudobia was about 0.835 g, so it was 4–5-fold higher than the weight of healthy seeds.

Germination tests

Soon after collection, the germination capacity of fresh seeds (which have not been dewinged) varied from 31% (lot 1922) and 35% (lot 1920) to 46% (lot 1921).

The ANOVA shows that all the analysed factors (seed origin, moisture content, temperature, and storage time) significantly affected the germination percentage and the interactions between them were significant (Table 2).

Generally, the studied seed lots differed significantly in germination percentage. It was significantly lower if: (a) the seeds were dried to a higher range of moisture content vs. lower mc; (b) storage temperature was higher vs. lower temperature; and (c) storage time was extended vs. short time of storage.

Depending on the moisture content of stored seeds (lots 1920 and 1922), their germination capacity significantly decreased already after 3 years of storage at 3°C, as compared to germination results after storage at –3°C, while in seed lot 1921, germination was 2 days delayed (Fig. 1). The separation of seeds in acetone resulted in a substantial increase in seed quality and germination percentage after 3 years of storage (Figs. 1 and 2).

The seeds stored at 3°C lost their viability most quickly. However, this partly depended on the mother tree, as in seed lot 1920, germination was close to zero as early as after 5 years, whereas in seed lot 1921, it was over 30% after 6 years. Because of the low germination of seeds after storage at 3°C, they were not subjected to seedling emergence tests in multi-cell trays.

The response of seed lots 1920 and 1922 to different temperature and moisture content during storage

Table 2. Results of ANOVA of germination (after arcsin $\sqrt{\%}$ transformation) of 3 seed lots, dried to 3 levels of moisture content and stored for 3, 5, and 6 years at 3°C, –3°C, and –10°C

	df	MS	df	MS	*F*	*P*
	effect	effect	error	error		
1 SEED LOT	2	10201.49	243	26.96118	378.377	0.000000
2 STORAGE	2	15536.33	243	26.96118	576.248	0.000000
3 TEMP	2	41463.69	243	26.96118	1537.903	0.000000
4 MOISTURE	2	1950.46	243	26.96118	72.343	0.000000
1×2	4	603.15	243	26.96118	22.371	0.000000
1×3	4	2646.21	243	26.96118	98.149	0.000000
2×3	4	5120.14	243	26.96118	189.908	0.000000
1×4	4	942.47	243	26.96118	34.957	0.000000
2×4	4	76.75	243	26.96118	2.847	0.024683
3×4	4	724.34	243	26.96118	26.866	0.000000
1×2×3	8	431.32	243	26.96118	15.998	0.000000
1×2×4	8	99.63	243	26.96118	3.695	0.000431
1×3×4	8	188.91	243	26.96118	7.007	0.000000
2×3×4	8	303.82	243	26.96118	11.269	0.000000
1×2×3×4	16	266.76	243	26.96118	9.894	0.000000

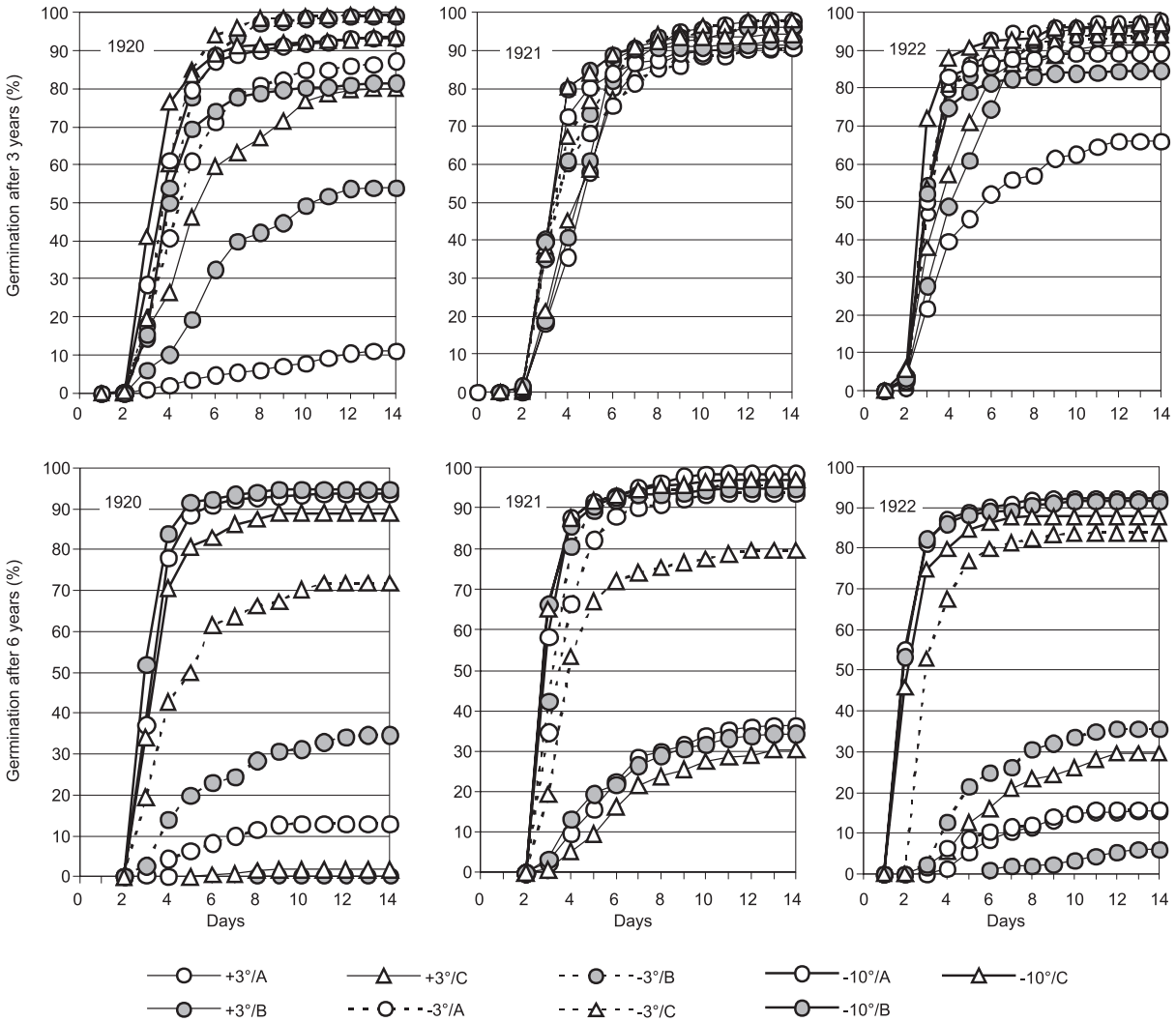

Fig. 1. Germination curves of 3 seed lots (1920, 1921, and 1922) in the Jacobsen germinator after 3 years (upper row) and 6 years of storage (lower row) at 3°C, –3°C, and –10°C, at moisture content levels A, B, and C (see Table 1)

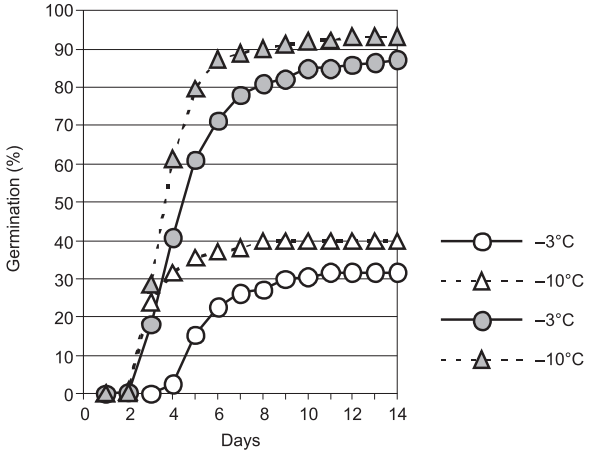

Fig. 2. Comparison of seed germination after 2 (white symbols) and 3 years (dark symbols) of storage at –3°C (solid line) and –10°C (dotted line), with a moisture content of 12.7%. After 2 years the seeds were not sorted in acetone, whereas after 3 years, filled seeds were separated in acetone before the germination tests. Seed lot 1920

for 6 years was similar. At –3°C, at the highest (A) and mean (B) moisture content, germination capacity was greatly reduced. No such a relationship was observed in seed lot 1921 (Fig. 1), as it maintained a high germinability irrespective of seed moisture content during storage. Its stable high germination capacity suggests that this is a special feature of this tree, characterized by an unusually high seed quality.

Irrespective of the moisture content of stored seeds, the germination generally decreased in the course of seed storage at –3°C. The decrease varied between seed lots.

At –10°C, when storage time was extended, the germination capacity was stable or even slightly increased (Table 3).

Any sound seeds were detected after germination test finished during 1–6 years of storage. All non-germinated seeds were dead. About 0.0–1.5% of seeds germinated abnormally and was not included to germination percentage.

Table 3. Germination (%) of 3 seed lots (1920, 1921, and 1922) dried to 3 levels of moisture content (A, B, and C) and stored for 3, 5, and 6 years at 3°C, –3°C, and –10°C. Significant differences are marked with different small letters ($P < 0.05$)

Storage temp.	Moisture content level*	Storage time (years)	Germination (%) of seed lot		
			1920	1921	1922
3°C	A	3	11.0 s-x	93.0 a-h	64.2 i-l
		5	0.75 y	53.0 l-o	22.2 o-t
		6	0 y	36.2 m-p	15.2 r-w
	B	3	54.7 j-m	94.2 a-h	94.5 a-h
		5	2.5 w-y	51.2 l-o	33.2 n-r
		6	0.2 y	34.2 n-s	6.0 t-x
	C	3	79.5 g-j	93.5 a-h	91.5 a-h
		5	4.7 u-y	40.7 l-p	46.5 i-o
		6	1.7 xy	30.2 n-s	28.2 n-t
–3°C	A	3	87.2 d-i	93.0 a-h	98.0 a-d
		5	39.2 n-t	94.2 a-g	83.5 f-j
		6	13.2 r-w	93.7 a-h	15.7 p-u
	B	3	98.7 ab	96.0 a-e	93.2 a-h
		5	53.2 k-n	98.7 a-c	83.2 e-j
		6	34.7 n-s	95.7 a-h	35.5 n-r
	C	3	99.5 a	98.7 ab	94.2 a-f
		5	90.5 a-i	95.7 a-g	89.0 c-i
		6	71.7 h-k	79.5 h-j	83.7 e-j
–10°C	A	3	93.0 a-h	93.2 a-h	89.5 a-h
		5	93.2 a-h	96.5 a-g	91.2 a-h
		6	93.2 a-h	98.5 a-d	92.5 a-h
	B	3	81.2 f-j	92.7 a-h	85.0 e-j
		5	92.7 a-h	94.0 a-h	85.2 d-i
		6	94.7 a-h	94.7 a-h	91.5 a-h
	C	3	93.2 a-h	92.5 a-h	96.7 a-e
		5	95.5 a-g	89.5 b-i	88.5 c-i
		6	89.0 c-i	97.2 a-e	87.2 c-i

*See Table 1.

Seedling emergence

The level of seedling emergence in multi-cell trays was lower than germination percentage in laboratory conditions (Table 4). The ANOVA shows that seedling emergence significantly depended on all the 4 studied factors (very much like germination capacity). Secondary, tertiary, and quaternary interactions between them were significant. An exception was the interaction between moisture content and storage time (Table 5).

Generally, seedling emergence was the highest in seed lot 1921, which maintained the initial, high seedling emergence for 6 years, in contrast to both the other seed lots (Table 6).

After 3 years of seed storage at –3°C, seedling emergence was significantly higher than after storage at –10°C. However, when storage time was extended to 5–6 years, inverse relationships were observed (Table 7).

Table 4. Seedling emergence (%) of 3 seed lots (1920, 1921, and 1922) dried to 3 levels of moisture content (A, B, and C) and stored for 3, 5, and 6 years at 3°C, –3°C, and –10°C. Significant differences are marked with different small letters ($P < 0.05$)

Storage temp.	Moisture content level	Storage time (years)	Seedling emergence (%) of seed lot		
			1920	1921	1922
–3°C	A	3	66.9 a-c	68.1 a-c	59.4 a-d
		5	21.9 d-i	66.2 a-d	45.0 b-f
		6	11.2 h-i	76.9 a	17.5 f-i
	B	3	55.6 a-d	70.6 a-c	55.6 a-d
		5	36.2 c-h	66.2 a-d	46.8 a-e
		6	10.0 i	50.6 a-e	17.5 g-i
	C	3	72.5 ab	67.5 a-c	50.6 a-e
		5	68.7 a-c	55.6 a-d	56.9 a-d
		6	47.5 a-e	52.5 a-e	56.2 a-d
–10°C	A	3	54.4 a-d	48.1a-e	48.7 a-e
		5	68.1 a-c	61.9 a-d	61.9 a-d
		6	70.0 ab	69.4 a-c	63.1 a-d
	B	3	51.2 a-e	56.9 a-d	45.0 b-f
		5	62.5 a-d	62.5 a-d	57.5 a-d
		6	68.7 a-c	65.6 a-d	72.5 ab
	C	3	69.4 a-c	61.9 a-d	41.2 b-g
		5	70.0 a-c	65.6 a-d	68.1 a-c
		6	54.4 a-d	66.2 a-d	70.6 a-c

Table 5. Results of ANOVA of seedling emergence (after arcsin $\sqrt{\%}$ transformation) of 3 seed lots dried to 3 levels of moisture content and stored for 3, 5, and 6 years at 3°C, –3°C, and –10°C

	df	MS	df	MS	*F*	*P*
	effect	effect	error	error		
1 SEED LOT	2	998.048	162	40.46352	24.66539	0.000000
2 STORAGE	2	335.367	162	40.46352	8.28814	0.000374
3 MOISTURE	2	527.807	162	40.46352	13.04402	0.000006
4 TEMP	1	2417.967	162	40.46352	59.75672	0.000000
1×2	4	307.856	162	40.46352	7.60823	0.000012
1×3	4	265.618	162	40.46352	6.56438	0.000064
2×3	4	48.273	162	40.46352	1.19301	0.315920
1×4	2	914.721	162	40.46352	22.60608	0.000000
2×4	2	2715.937	162	40.46352	67.12064	0.000000
3×4	2	229.629	162	40.46352	5.67497	0.004149
1×2×3	8	152.726	162	40.46352	3.77442	0.000439
1×2×4	4	187.637	162	40.46352	4.63718	0.001432
1×3×4	4	337.049	162	40.46352	8.32971	0.000004
2×3×4	4	269.430	162	40.46352	6.65859	0.000055
1×2×3×4	8	81.215	162	40.46352	2.00712	0.048639

Table 6. Mean seedling emergence (%) of 3 seed lots depending on their storage time. Significant differences are marked with different small letters ($P < 0.05$)

Storage time (years)	Seedling emergence (%) of seed lot		
	1920	1921	1922
3	61.7 a	62.2 a	50.1 bc
5	54.6 ab	63.0 a	56.0 ab
6	43.6 c	63.5 a	49.6 bc

Table 7. Mean seedling emergence (%) of all seed lots, depending on seed moisture content, temperature, and storage time. Significant differences are marked with different small letters ($P < 0.05$)

Moisture content level	Seedling emergence (%) after storage					
	3 years		5 years		6 years	
	–3°C	–10°C	–3°C	–10°C	–3°C	–10°C
A	64.8 ab	50.4 cd	44.4 de	63.9 a-c	35.2 ef	67.5 a
B	60.6 a-c	51.0 b-d	49.8 cd	60.8 a-c	26.0 f	68.9 a
C	63.5 a-c	57.5 a-d	60.4 a-c	67.9 a	52.1 b-d	63.7 a-c

In seed lots 1920 and 1922 dried to moisture content A and B and stored at –3°C, seedling emergence was low, whereas in those dried to moisture content C, seedling emergence was high. By contrast, in seed lot 1921, after storage at –10°C, seedling emergence was high regardless of seed moisture content.

Seedling emergence after storage at –3°C gradually decreased when storage time was extended, but the decrease varied depending on seed origin. When the seeds were stored at –10°C, seedling emergence was stable in spite of extension of storage time from 3 to 6 years. Generally, when the seeds were stored longer at –10°C, a higher percentage of seedlings survived until the end of the first growing season (Table 4).

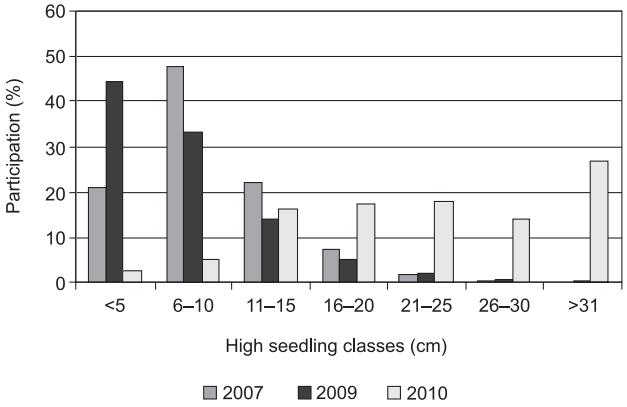

Fig. 3. Average (3 seed lots, 3 mc levels, 2 temperatures) frequency distribution of seedlings in seedling height classes in individual years

Seedling height

Mean seedling height after seed storage for 3 and 5 years (i.e. in 2007 and 2009, respectively) was relatively low (Table 8) and many seedlings were dwarf (Fig. 3). In 2007, the percentage of seedlings <5 cm high was over 20%, whereas in 2009, it was twice as high. Seedlings from seeds stored longest, i.e. for 6 years (in 2010), were much higher, on average 23.2 cm high, and as many as 59% of seedlings were >20 cm high (Fig. 3).

Table 8. Mean height (cm) of seedlings developed from 3 seed lots stored with various moisture content at –3°C and –10°C for 3, 5, and 6 years

Seed lot	Storage temperature	Moisture content level	Mean seedling height (cm) and standard deviation after seed storage		
			3 years	5 years	6 years
1920	–3°C	A	8.4 ±3.4	5.6 ±3.3	21.5 ±14.5
		B	7.9 ±3.5	4.4 ±2.8	17.5 ±12.7
		C	9.2 ±3.5	8.4 ±4.2	31.1 ±12.8
	–10°C	A	8.6 ±3.8	8.3 ±6.21	21.4 ±9.0
		B	8.9 ±3.3	11.5 ±4.4	20.1 ±8.7
		C	8.9 ±4.6	6.5 ±3.1	20.8 ±9.3
1921	–3°C	A	7.6 ±3.2	6.5 ±4.5	18.1 ±8.3
		B	10.5 ±4.0	5.8 ±4.6	29.0 ±12.7
		C	10.9 ±5.8	9.2 ±5.7	29.1 ±13.8
	–10°C	A	9.1 ±4.7	15.7 ±8.7	25.3 ±11.1
		B	9.6 ±4.7	4.8 ±2.6	21.0 ±10.1
		C	7.3 ±3.7	5.5 ±3.7	25.7 ±11.7
1922	–3°C	A	9.3 ±4.2	11.1 ±4.9	32.9 ±13.5
		B	10.5 ±5.2	11.6 ±4.8	25.2 ±12.8
		C	13.4 ±6.2	4.5 ±2.8	22.3 ±12.6
	–10°C	A	8.1 ±3.7	5.7 ±3.1	22.9 ±10.9
		B	7.5 ±4.2	5.6 ±2.6	19.0 ±9.8
		C	10.0 ±5.6	4.1 ±2.5	20.9 ±9.6
Mean			9.3	7.6	23.3

Discussion

The lower limit of 1000-seed weight in *Betula pendula* according to the Polish Rules of Silviculture (Rozwałka 2003), i.e. 0.1 g, is greatly underestimated in the light of results of this study. Its results indicate that 1000-seed weight varied from 0.161 to 0.237 g, considering that seed moisture content was about 8%. Tyszkiewicz (1949) reported that 1000-seed weight in this species is on average 0.12 g, and germination capacity is 30%. The above differences may result not only from individual variation but also from the lack of unambiguous procedures for seed weight assessment. Empty seeds should not be taken into account in the assessment.

According to long-term observations of birch seed germination in Poland (Bodył 2006) the mean germinability of birch seeds collected in 2004 was about 20%. This indicates that seed lots 1920 and 1922 were of medium quality, while the quality of seed lot 1921 was relatively high.

In conducted studies that intentionally missed the influence of temperature and chilling time of seeds and other light conditions of the methodology adopted in order not to obscure the effect of storage temperature and seed moisture content. High seed germination percentage (Fig. 1), at over 90% indicates that the tested batches of seeds were probably not as strongly dependent on chilling pretreatment and extended photoperiod as provide Vanhatalo et al. (1996), De Atrip and O'Reilly (2005, 2006), and others.

The separation of filled seeds from empty seeds by floating in acetone (density 0.792 g/cm^3) caused a remarkable improvement of seed quality. The short period of separation (<100 s) was not harmful for the seeds. The seed fraction heavier than acetone was characterized by an over 2–3-fold increase in germination (to over 90%), as compared with seeds that had not been sorted in acetone (Fig. 2). In the seed fraction lighter than acetone, less than 10% of seeds germinated. It seems that acetone as liquid for seed separation was used first time; I didn't meet any information about such uses of it.

In earlier research on birch seed storage, many authors have assessed only the germination percentage (Vincent 1958, Heit 1967, Chmielarz 2010). Sowing value assessment of various seed lots on the basis of germination capacity (Table 3) usually differs considerably from seedling emergence (Table 4). In forest nurseries, seedling emergence is frequently much lower than seed germination in laboratory conditions. This results mostly from their lower viability, due to infection of seeds and seedlings by various pathogens. After seed storage, the differences between the values of germination and seedling emergence vary depending on storage temperature and other factors.

Birch seed germination and seedling emergence can be significantly affected by soil acidity, as reported by Rudnicki (1953), who claimed that birch seeds did not germinate when soil pH was lower than 5. This view is difficult to accept because *B. pendula* is found on soils whose pH ranges from 3.7 to 6.5 (Niemiera 2009). In my additional laboratory experiment testing this hypothesis no significant differences were detected in seed germination within the pH range of 4.0–5.5. A pH decrease to 3.8 caused a significant decrease in germination, as compared with pH 5.5, but germination percentage within the pH range of 3.8–5.0 did not differ significantly (Fig. 4).

Mean seedling height in 2007 and 2009 was extremely low. Such a low height is reached by one-year-old seedlings in field conditions (Krüssmann 1964), whereas in container cultivation it should be much higher. It is difficult to determine unambiguously what caused seedling growth inhibition in multi-cell trays in those years.

Viherä-Aarnio et al. (2005) proved that seedling growth and height are strongly affected by sowing date. However, in this study, seeds were sown every year in late April, so this factor is unlikely to be the cause of the variation in seedling height, especially in 2010.

There are a few other possible causes. One of them, most probable, is the pattern of weather conditions, especially temperature at the beginning of the growing season. In early May in both 2007 and 2009, ground frost was recorded for a few days. In the plastic tunnel (with no heating), this could block the growth of young seedlings. In 2010, no such late frosts were recorded. Heide and Prestrud (2005) reported that in micropropagated apple and pear rootstocks the autumn syndrome was not caused by photoperiod but by low temperature. Thus, in container cultivation, especially in the initial period after sowing, birch seedlings apparently should be protected from very low temperatures.

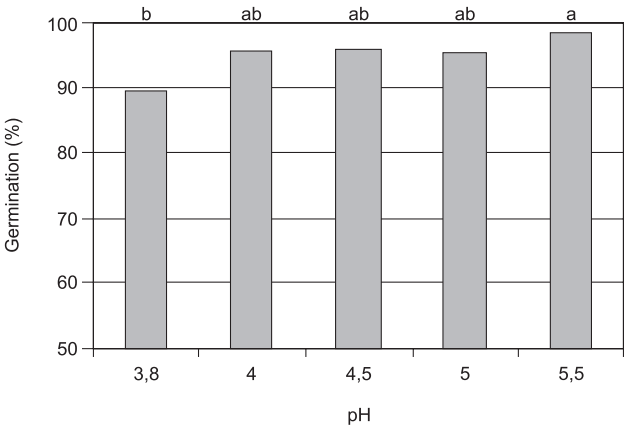

Fig. 4. Seed germination on filter paper (14 days) depending on medium pH. Significant differences are marked with different small letters ($P < 0.05$, Tukey test)

Seedling height seems to be negatively correlated with the sum of daily maximum temperatures in the first month of seedling growth (i.e. in May). In 2007 and 2009, i.e. after 3 and 5 years of seed storage, the sum amounted to 653.1°C and 601.3°C, respectively, whereas in 2010, it was only 485.2°C. Temperature was measured within the weather station (at the height of 2 m), while in the plastic tunnel the temperature was markedly higher. The greenhouse effect in the plastic tunnel was associated with high insolation in May (over 230 h) in 2007 and 2009, as compared to only 105 h in 2010. These observed differences seem to be consistent with the observations of Aphalo and Lehto (1997), who found that light quality affects most strongly the initial growth of seedlings of *B. pendula* and Wayne et al. (1998) who reported that birch seedling growth is limited by high temperature.

The excessively high temperature, and the drought associated with it, limits the distribution of *B. pendula*, as indicated by the natural range of this species (Vakkari 2009). In warmer parts of Europe (the Iberian Peninsula, Italian Peninsula, southern Balkans, southern parts of Bulgaria, Romania and Ukraine), this species does not occur.

Sub-irrigation, without any additional watering, could also be insufficient for normal seedling growth in 2007 and 2009.

It cannot be excluded that the germination substrate itself could have a negative effect on birch seedling growth and height in 2007 and 2009. Such negative effect of the substrate was observed in an earlier study (Tylkowski and Musiela 2003) on seedling growth in *Quercus robur*, both in laboratory conditions and in a forest nursery in the Jarocin.

Clean birch seeds account for about 35% of the weight of mature catkins (35.2–37.9% according to Antosiewicz 1975), so the 1.5–3.0 kg of mature catkins should contain 0.525–1.05 kg of clean seeds. Assuming that 1000 seeds weigh 0.15–0.20 g, the 0.525 kg of mature catkins contain 2.6–3.5 million seeds, while the 1.05 kg contain 5.2–7.0 million seeds. The production 30 000 seedlings (of class I and II) in an area of 100 m^2 in field conditions (Rozwałka 2003) is equivalent to sowing efficiency of 0.9–1.2% and 1.75–2.3%, respectively, so it is about 50-fold lower than sowing efficiency in container cultivation.

The optimized methods of seed storage, pretreatment and sowing of *B. pendula* can be applied in practice. Their implementation in forestry practice, on a rational scale, will allow to create valuable seed reserves (e.g. from seed orchards) for production of potted seedlings. Seed storage for 6 years is, from a practical standpoint, sufficiently long to replenish the reserves of sowing material collected in good crop years, although the Polish Principles of Forest Management (Rozwałka 2003) recommend that for birch, seed reserves should be made for 2 years.

Conclusions

1. Temperature of –10°C and seed moisture content of about 8% is the best for *Betula pendula* seeds storage during 6 years, ensuring the highest germination and seedling emergence.
2. After storage of mature catkins, seeds are separated from scales by sieving, and next dewinged (by rubbing). Dewinged seeds are next sorted in acetone (filled seeds sink to the bottom, while empty ones float on the surface).
3. For long-term seed storage, it is more favourable to store seed lots separately after collection from individual trees (because of high individual variation in seed quality). Whenever seed quality is greatly reduced in the individual seed lots, they should not be stored any longer.
4. The application of filled seeds for production of seedlings of *Betula pendula* in container cultivation increase about 50-fold the sowing efficiency and greatly limits the amount of sown seeds (from about 1.5–series to about 7.9–10.5 g of clean seeds per 100 m^2 in multi-cell trays).
5. Birch seedlings should be protected from low temperature events in the initial period after sowing, as its may contribute to inhibition of their further growth.

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