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RESEARCH PAPER

Effect of different conditions of storage on seed viability and seedling growth of six European wild fruit woody plants

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Abstract

• ***Key message*** **Malus sylvestris (L.) Mill., *Pyrus communis* (L.), *Sorbus aucuparia* (L.), *Prunus avium* (L.), *Prunus padus* (L.), and *Cornus sanguinea* (L.) are related, co-occurring species producing *orthodox* seeds. However, we observed differences in their response to storage conditions, such as storage at different seed moisture contents (5%, 8%, and 11%) and/or temperatures (− 3 °C, − 18 °C, and − 196 °C). Severe desiccation to ca. 5% of MC negatively affected seeds of *M. sylvestris*. Seeds of *P. avium* were sensitive to storage in LN or at − 18 °C. *S. aucuparia* seeds are best stored at − 3 °C, whereas *C. sanguinea* seeds tolerate desiccation and storage in LN. In general, species with deeper physiological dormancy (*S. aucuparia*, *P. padus*, and *C. sanguinea*) tended to be more tolerant to desiccation and low temperatures. For all species, storage conditions did not affect seedling growth.**

• ***Context*** Wild fruit woody species face many threats such as genetic loss, population fragmentation, and alien species; thus, their genetic variability should be preserved.

• ***Aims*** To examine the effect of storage conditions on seed viability and the initial growth of seedlings of six European wild fruit species: *Malus sylvestris* (L.) Mill., *Pyrus communis* (L.), *Sorbus aucuparia* (L.), *Prunus avium* (L.), *Prunus padus* (L.), and *Cornus sanguinea* (L.).

• ***Methods*** Seeds were desiccated to three different levels of moisture content (ca. 5, 8, or 11%) and stored at three different temperatures (− 3°, − 18°, or − 196 °C; liquid nitrogen, LN) for up to 3 years. Germination and seedling emergence tests were performed as well as measurement of seedling growth.

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Contribution of the co-authors
PC and MM conceived and designed the experimental work and collected material; PC, MM, and MW performed the experiments; MW collected data and made a statistical analysis of the results; MW, MM, and PC analyzed and interpreted data; MW drafted the article; PC and MM reviewed and revised the paper. All authors have approved the final article.

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• ***Results*** Desiccation of *M. sylvestris* seeds from 10.7 to 4.9% significantly lowered germination from 91 to 77% and seedling emergence from 88 to 74%. In *P. avium*, LN storage significantly inhibited seedling emergence, both in the laboratory and the greenhouse, but did not affect total seed germination. In *P. communis*, *P. padus*, and *C. sanguinea*, neither germination nor seedling emergence was affected by seed storage conditions. There were small or no differences in stem height and root collar diameter in the first year of seedling growth of stored seeds.
• ***Conclusion*** Species with deeper physiological dormancy (*S. aucuparia*, *P. padus*, *C. sanguinea*) tended to be more tolerant of various storage conditions. Seeds of *P. padus* and *C. sanguinea* can be stored long term at − 18 °C or in LN at 5–8% MC without losing viability. *M. sylvestris* and *P. avium* seeds are sensitive to desiccation below 6% MC or low temperature of storage at − 18 °C or − 196 °C, respectively. We observed that storage conditions had significant influence on germination and seedling emergence but had no effect on seedling growth after the first growing season.

Keywords Seeds storage · Cryopreservation · Seeds traits · Conservation · Seed banking

1 Introduction

Biodiversity is the multiplicity of life, including variation among genes, species, and functional traits, in an ecosystem and has a profound effect on the functioning of ecosystems (Cardinale et al. 2012). The benefits of biodiversity in forest ecosystems have been well recognized in recent years, indicating resistance to natural disturbances (drought, severe winds, etc.), greater ecosystem productivity, and higher resilience (Liang et al. 2016; Jactel et al. 2017; Isbell et al. 2015). However, Europe's forest biodiversity remains under pressure due to land-use changes, invasive species, pollution, habitat, and climate changes (EEA 2016). This results in declining species richness in European forests as well as in global forests (Hooper et al. 2005; Schulze et al. 2014; Forest Europe, 2015).

Some of the species that make the greatest contributions to broad-leaved forest ecosystem function are fruit-bearing trees and shrubs. They provide multiple ecosystem services as a source of nutrition, pollination, genetic variation for breeding programs, and even of valuable income for people living around them (Powell et al. 2013). Wild fruit trees are considered less economically important than timber species and thus have often been neglected in conservation programs. Although wild fruit species are usually not considered endangered by European countries, their genetic diversity is threatened (Dirlewanger et al. 2002; Larsen et al. 2006; Wünsch and Hormaza 2007; Urrestarazu et al. 2016). Wild fruit trees are usually pioneer or intermediate species with weak competitive ability (Stephan et al. 2003; Russell 2003). Even considering their usually broad natural distribution in Europe due to their rare occurrence and scattered distribution pattern, populations are disappearing at an alarming rate (Coart et al. 2003). Their high light and soil demands, as well as slow growth, often result in low natural regeneration restricted to forest ecotones (Stephan et al. 2003; Russell 2003). A number of wild species often hybridize with cultivated species, leading to loss of their true wild form and genetic swamping (Fellenberg et al. 2000; Bleeker et al. 2007). The plantation-based model of fruit collection, as well as the lack of a uniform plan for preserving genetic resources in most of these species in the EU, leads to a further decrease in these populations (Wolf et al. 1999). Nevertheless, a number of wild fruit tree populations registered in the EUFGIS database for forest genetic resources are underrepresented and restricted to few countries in comparison with timber species. Therefore, more efforts to conserve the genetic resources of those species must be made.

One very efficient method for conserving the genetic resources of temperate forest trees is seed banks (FAO 2014). All wild fruit tree species from temperate and boreal regions produce orthodox seeds that can be stored for long periods of time at a low seed moisture content, below 5%. Such desiccation tolerance allows the storage of seeds beyond average tree lifespan at a conventional temperature of − 20 °C (Pritchard et al. 2014) and later use in reintroduction programs. Although the total number of temperate tree species stored in seed banks is rather high, interspecies genetic diversity is generally not well conserved (FAO 2015). In addition to conventional methods, cryopreservation in liquid nitrogen (LN) is used as a secure backup for plant genetic resources. Cryopreservation is usually utilized for storing recalcitrant seeds, but due to its simple methodology, it is used for many orthodox seeds as well (Plitta et al. 2013). Effective seed cryopreservation has been proven for many temperate forest tree species, e.g., *Alnus glutinosa* L. (Chmielarz 2010), *Corylus avellana* L. (Michalak et al. 2013), *Malus sieversii* M.Roem. (Kushnarenko et al. 2010), *Fraxinus excelsior* L. (Chmielarz 2009a), *Prunus armeniaca* L. (Jaganathan et al. 2015), *Populus nigra* L. (Michalak et al. 2015a), and *Prunus padus* L. (Popova et al. 2016).

In our study, we examined seed storage and seedling growth in 6 wild fruit species: European crab apple (*Malus sylvestris* L.), European pear (*Pyrus communis* L.), mountain-ash (*Sorbus aucuparia* L.), wild cherry (*Prunus avium* L.), bird cherry (*Prunus padus* L.), and common dogwood (*Cornus sanguinea* L.). All described species suffer from fragmentation and disappearance of their natural habitats as

mentioned earlier. In the case of the endangered European crab apple, the hybridization between this species and the domesticated apple could lead to the extinction of its wild populations (Reim et al. 2012). On the other hand, populations of bird cherry have to compete for habitats with highly invasive species such as black cherry (*P. serotina*; Pairon et al. 2010). Nevertheless, apart from European crab apple and wild cherry, due to their usage in fruit production, information about seed storage behavior in the scientific literature is very limited and has not been properly examined. However, with the current climate and environmental changes, it is essential to assess the best possible storage conditions for effective conservation of these often-neglected species.

Seeds stored in gene banks must produce plants capable of reproduction in natural conditions, and high seedling quality is crucial for the initial success of any reforestation program (Grossnickle 2012). Often, seedling death is the result of environmental stress, animal grazing, disease, or insects. However, in many cases, survival can be significantly increased with good-quality planting stock (Campo et al. 2010). Seed storage conditions and practices have a strong influence on seed quality and therefore on seedlings. The effects of these practices on early seedling establishment need to be understood to produce high-quality seedlings that have a high probability of being "coupled" into forest ecosystems and reproducing (Grossnickle 2005). To our knowledge, studies examining seedling quality after storage are still rare. Recent publications assessing this subject confirm a possible effect on seedling quality both in annual plants (Marshall and Lewis 2004; Arguedas et al. 2018) and in woody species (Benamirouche et al. 2018).

The aim of this study was to examine the seed biology of 6 wild fruit tree species before and during storage under controlled conditions for 3 years. Our research questions were as follows: how do storage temperature and seed moisture content affect seed germination, both in controlled and field conditions? Do desiccation-tolerant seeds always tolerate storage at ultra-low temperatures (− 196 °C) and does it affect subsequent seedling growth? What are the possibilities for ex situ conservation in nurseries, seed, and cryobanks of examined species?

2 Material and methods

2.1 Plant material

Mature seeds of the six tested species, European crab apple (*Malus sylvestris* L.), European pear (*Pyrus communis* L.), mountain-ash (*Sorbus aucuparia* L.), wild cherry (*Prunus avium* L.), bird cherry (*P. padus* L.), and common dogwood (*Cornus sanguinea* L.), were collected between May and September 2012 (Table 1) in natural stands in Poland (Wawrzyniak et al. 2020). Seed lots of each species were collected from individual trees (2–3), with the exception of European crab apple, where seeds from three adjacent individuals were mixed together. All experiments were conducted on whole seeds, with *P. avium*, *P. padus*, and *C. sanguinea* seeds consisting of endocarp. All tested species produce fleshy fruit; in order to obtain the seeds, collected fruits were pressed and seeds were extracted from the pulp, as described by Suszka et al. (1996). Then, seeds were air-dried in ambient conditions (20 °C) until they reached approximately 11% moisture content (control). Seeds were stored at 3 °C until the start of the experiment (1–2 months depending on species).

2.2 The moisture content of the seeds

Seed moisture content (MC) refers to the seed-containing embryo and seed coat. MC was calculated on a fresh weight basis (%) using the formula:

$$\mathrm{MC1} = \frac{(\mathrm{FW1}-\mathrm{DW})100}{\mathrm{FW1}} \quad (1)$$

where MC_1 is the moisture content, FW_1 is the initial fresh weight, and DW is the dry weight. The MC of the seeds was adjusted prior to seed storage with desiccation over activated silica gel to obtain two levels of MC: 5% and 8%. The adjustment of the MC of seeds was based on the FW of the seeds, according to the formula:

$$\mathrm{FW2} = \frac{\mathrm{FW1}(100-\mathrm{MC_1})}{100-\mathrm{MC2}} \quad (2)$$

where FW_2 is the desired weight and MC_2 is the desired moisture content. The moisture content of the seeds was achieved specified by drying in the oven at 103 ± 2 °C for 17 h (4 replicates of 10 seeds). The exact seed MC levels achieved are shown in Table 2.

2.3 Seed storage

Seeds of each species were dry-stored at three levels of approximate MC (5, 8, and 11%; Table 2), in plastic air-sealed bags at temperatures of − 3 °C, − 18 °C, or cryopreserved at − 196 °C for 12, 24, and 36 months. As a control, we used nonstored seeds at approximately 11% MC. Cryopreserved seeds in sealed polypropylene bags were directly immersed in liquid nitrogen (LN). After storage, seeds were thawed in a water bath at 40 °C for 10 min. Subsequently, seeds were placed in a stratification substrate and underwent stratification.

2.4 Stratification and germination

After each storage interval and prior germination and seedling emergence tests, seeds of all the examined species underwent

Table 1 Location and date of the seeds from the six tested species

Species	Seeds collection date	Place	Coordinates
Malus sylvestris	29 Sep 2012	Kolumna	N51°36′51.1; E19°11′41.6
Pyrus communis	7 Sep 2012	Łopuchówko	N52°35′31.6; E17°05′11.5
Sorbus aucuparia	6 Sep 2012	Mosina	N52°14′40.0; E16°51′00.0
Prunus avium	17 May 2012	Śnieciska	N52°11′41.0; E17°10′53.0
Prunus padus	26 Jun 2012	Koniko	N52°18′38.0; E17°00′54.0
Cornus sanguinea	29 Aug 2012	Błażejewko	N52°12′51.0; E17°06′07.0

a species-specific stratification process, as presented in Table 3. The stratification substrate was composed of a moist mixture (v/v, 1:1) of quartz sand (fraction > 1 mm) with sieved peat of pH 3.5–4.5. Seeds were mixed with the substrate at a ratio of 1:3 and placed in loosely sealed plastic containers. The condition of the seeds and the substrate was checked every 2 weeks. Stratification continued until the appearance of the first germinating seeds (≤ 5%).

Directly after stratification, germination and seedling emergence tests were conducted in a laboratory under controlled conditions. Tests were performed in a growth chamber at cyclically alternating temperatures of 3°/20 °C with an interval of 16/8 h. Seeds with a radicle of ≥ 3 mm were counted as germinated. Germinated seeds were examined weekly, counted, and eliminated until the last seed germinated. After the test, non-germinated seeds were cut and evaluated as healthy and dormant or decayed. During the germination test, the total germination percentage (GP), as well as the mean germination time (MGT or $\overline{T}$), was calculated according to the formula given by Ranal and Santana (2006) based on Labouriau (1983). Germination time was given in weeks and counted from the beginning of cold stratification.

$$\overline{T} = \frac{\sum_{i=1}^{k} N_i T_i}{\sum_{i=1}^{k} N_i} \quad (3)$$

where T_i is the time from the start of the experiment to the ith observation and N_i is the number of seeds germinated in the ith time (the number of germinated seeds corresponding to the ith observation); k is the last observation.

Seedling emergence (SE) tests were conducted in a similar way using a mixture of sand and peat. Stratified seeds were sown in plastic boxes and covered with a 1 cm substrate layer. Seedling emergence tests were conducted at the same temperatures as germination tests. The seed was regarded as emerged when cotyledons and epicotyl were observed above ground. After seedlings reached 2–3 cm in height, boxes were moved into the chamber with light and a temperature of 20 °C. A sprouting index (SI) was calculated using the same formula as for calculating MGT, counted from the end of stratification. For germination and seedling emergence tests, 3 replicates of 30 seeds or 4 replicates of 50 seeds (in the case of mountain-ash and common dogwood) were used. For each test, separate seed samples were used.

2.5 Seedling emergence and further seedling growth in a greenhouse

In addition to the laboratory tests, a field emergence test was conducted. Stratified seeds were sown into plastic containers (FlexiFrame, BCC Sweden), with each seed in a separate cell, and placed in random blocks in the greenhouse. Seeds were placed into a mixture of peat, perlite (v/v, 2:1), and long-lasting mineral fertilizer (Osmocote Extract 5–6 M 15–912 + 2MgO + TE, 2–4.5 mm). The test was performed on 3 replicates of 30 seeds or 4 replicates of 50 seeds (in the case of mountain-ash and common dogwood). One month after

Table 2 Moisture content of each tested species

Species	Moisture content (%)		
Malus sylvestris	4.9	8.5	10.7
Pyrus communis	5.3	7.1	11.9
Sorbus aucuparia	5.5	8.4	11.1
Prunus avium	5.5	8.0	11.2
Prunus padus	5.9	8.3	11.4
Cornus sanguinea	5.5	8.0	11.0

Table 3 Stratification method used to induce germination in the six species

Species	Stratification	
	Temperature (°C)	Time (weeks)
Malus sylvestris	3	11
Pyrus communis	3	15
Sorbus aucuparia	25/3	2/20
Prunus avium	20/3/20/3	2/2/2/13
Prunus padus	25/3/25/3	2/2/2/22
Cornus sanguinea	15~20/3	Daily change 6/24

sowing, seedlings were counted. In autumn, after the vegetation period, all 1-year-old seedlings were measured for their height (H) and root collar diameter (RCD). These tests were repeated each year during the 3-year seed storage period. The temperature of the air and ground in the greenhouse was recorded during the experiment (Table 7).

2.6 Statistical analysis

The fixed effects of storage conditions on germination and seedling emergence were evaluated separately using a generalized linear model (GZLM) with a binomial distribution with log-link function (Ranal and Santana 2006). For the germination rate and sprouting index comparison, a linear regression model was used. Germination means used in statistical analyses were counted from 3 to 4 replicates (for each 30–50 seeds), each obtained from separate germination tests. A three-way analysis of variance (ANOVA) with interaction between main effects (storage time, moisture content, and storage temperature) was used in the linear regression model (Table 9). For GZLM, special analysis was based on deviance (Carvalho et al. 2018). For multiple comparisons between treatments, we adjusted p values using Bonferroni-Holm method. For the linear regression models, we tested normality and equal variance assumptions of residuals by examining the plots of residuals. For growth analysis (height and root collar diameter measurements), a nonparametric Kruskal-Wallis test was performed ($p < 0.05$; from *agricolae* package). For all statistical analyses of the data, R statistical software (R Core Team 2020) was used.

3 Results

3.1 Effects of storage conditions on seed germination

After the third year of storage, seeds' average germination capacity of all species was above 80%, regardless of the storage conditions used. The main effects (storage time, seed MC, and storage temperature) had visible impact on seed germination and seedling emergence process. The interaction effects on germination were recorded between storage time and seed MC in bird cherry and between storage time and temperature in common pear as well as wild cherry (Table 8). The MC of stored seeds mainly affected germination of crab apple and mountain-ash. Different storage temperatures had major effect on wild cherry seedling emergence. Although common dogwood seeds germinated and emerged at high percentage regardless of the storage conditions used, nonstored seeds germinated significantly better than stored seeds. The mean time to germinate and seedling emergence index in most cases were alike. The seedling emergence tests were more sensitive on influence of storage conditions and therefore better indicate seed quality after the storage in comparison with germination test.

3.1.1 European crab apple

The best results were for European crab apple seeds stored at − 18 °C at 8.5% seed MC, where germination and seedling emergence both in the laboratory and in the greenhouse were high, at approximately 93%, 89%, and 83% and similar for each tested year of storage, respectively (Fig. 2). Germination after storage at − 3 °C showed a slight but significant decrease. The germination percentage was significantly lower after 1 and 3 years of storage than that of nonstored and seeds that were stored for 2 years (Table 4). The mean germination time was the highest after 3 years of storage: 12.2 weeks after the start of stratification. Seedling emergence both in the laboratory and in the greenhouse significantly decreased in each tested year. The nonstored seed emergence was 90.7% in the laboratory and 91.5% in the greenhouse, in comparison with 76% and 78% emerged after the third year of storage, respectively. The mean time of seedling emergence varied after each year of storage, with the lowest after the first year (2.1 weeks) and the highest after the second year (2.6 weeks) of seed storage. The temperature used for storage did not alter the germination percentage, which was between 85 and 88% (Table 5). Seed desiccation to 4.9% significantly lowered germination, from 92% in the control to 77% (Table 6). However, the mean germination time was a week faster (11.4 weeks) in strongly desiccated seeds than in the control (12.3 weeks). A similar effect was recorded for seedling emergence, which was significantly lower in seeds desiccated to 4.9% both in the laboratory and in the greenhouse. Desiccated seeds of European crab apple (5.5%) had a significant decrease in seedling emergence after storage at each tested temperature. Seedling emergence of *M. sylvestris* seeds stored at − 196 °C decreased after the first year of storage, regardless of seed MC.

3.1.2 European pear

In general, nonstored seeds of European pear (*P. communis*) germinated at a slightly but significantly higher percentage (99%) than stored seeds (96–97%; Table 4). After the first year of storage of seeds desiccated to 7.1% MC and stored at − 196 °C, we recorded some decrease in all values of the conducted tests, but after the third year of storage, seeds maintained their germination ability at the same level as the control seeds (Fig. 3). The mean germination time was 16 weeks after the start of desiccation in each tested year. Seedling emergence was the highest in the nonstored control and after the third year of storage (95–97%). However, the mean time of seedling emergence differed significantly: after 1 year of storage, seedlings emerged almost 1 week faster (1.3 weeks) than after the other tested storage times (approximately 2 weeks). Seedling emergence in the greenhouse was significantly higher after the third year of storage (93%) than for nonstored seeds (83%). Seedling emergence after exposure in seeds

Table 4 Mean germination percentage (GP), mean germination time (MGT), and seedling emergence (SE) in laboratory and greenhouse, and sprouting index (SI) for 6 tested species after 3 years of storage. Statistical tests were run for each species separately

Species	Index	Storage time, years								
		Nonstored	1	2	3		Nonstored	1	2	3
Malus sylvestris	GP, %	90.7 ± 1.34a	85.8 ± 2.21b	90.4 ± 1.48a	80.0 ± 1.89c	SE in laboratory, %	90.7 ± 1.3a	81.2 ± 1.65b	82.5 ± 2.7b	76.3 ± 2.09c
	MGT, weeks	-	12.0 ± 0.08a	12.0 ± 0.09a	12.2 ± 0.1b	SI, weeks	2.4 ± 0.08b	2.1 ± 0.03a	2.6 ± 0.03c	2.4 ± 0.03b
						SE in greenhouse, %	91.5 ± 0.82a	83.6 ± 1.9b	77.9 ± 1.8c	78.8 ± 1.41c
Pyrus communis	GP, %	98.9 ± 0.31a	96 ± 1.32a	96.3 ± 0.8a	97.7 ± 0.66a	SE in laboratory, %	96.7 ± 0.5a	91.9 ± 2.06a	92.2 ± 1.27a	94.8 ± 1.01a
	MGT, weeks	-	16.0 ± 0.01a	16.0 ± 0.01a	16.1 ± 0.02b	SI, weeks	2.1 ± 0.02c	1.3 ± 0.02a	2.0 ± 0.05b	2.3 ± 0.03d
						SE in greenhouse, %	83.3 ± 1.98b	84.6 ± 2.84ab	91.9 ± 1.07a	92.8 ± 1.73a
Sorbus aucuparia	GP, %	84.7 ± 0.81a	72.3 ± 1.38c	75.1 ± 1.2c	79.5 ± 0.94b	SE in laboratory, %	77.0 ± 1.23a	68.1 ± 1.23b	67.5 ± 1.17b	67.7 ± 1.38b
	MGT, weeks	-	20.5 ± 0.03b	20.3 ± 0.04a	20.4 ± 0.04ab	SI, weeks	1.7 ± 0.03b	1.9 ± 0.02c	1.9 ± 0.03c	1.6 ± 0.02a
						SE in greenhouse, %	85.5 ± 1.11a	73.3 ± 1.9b	65.5 ± 1.57c	85.9 ± 1.16a
Prunus avium	GP, %	87.0 ± 1.00b	90.0 ± 1.63a	86.0 ± 1.3b	89.9 ± 1.77a	SE in laboratory, %	89.3 ± 1.14a	75.8 ± 3.16c	73.7 ± 3.38c	81.2 ± 3.03b
	MGT, weeks	-	15.8 ± 0.12b	15.9 ± 0.13b	15.6 ± 0.1a	SI, weeks	2.7 ± 0.02a	4.0 ± 0.04b	4.0 ± 0.05b	4.3 ± 0.05c
						SE in greenhouse, %	85.9 ± 1.15b	77.2 ± 3.32c	78.6 ± 2.8bc	88.8 ± 1.94a
Prunus padus	GP, %	89.3 ± 0.86	88.3 ± 1.35	89.6 ± 1.27	90.9 ± 1.33	SE in laboratory, %	83.7 ± 1.09a	68.8 ± 2.59b	79.9 ± 1.64a	79.6 ± 1.25a
	MGT, weeks	24.1 ± 0.06a	24.6 ± 0.07b	23.9 ± 0.05a	24.7 ± 0.06b	SI, weeks	2.7 ± 0.04b	2.7 ± 0.04b	2.4 ± 0.04a	2.8 ± 0.02b
						SE in greenhouse, %	86.3 ± 2.06ab	89.8 ± 1.07a	84 ± 1.45b	88.8 ± 1.26ab
Cornus sanguinea	GP, %	96.8 ± 0.56a	93.6 ± 0.73b	92.7 ± 0.7b	92.7 ± 0.87b	SE in laboratory, %	97.3 ± 0.52a	91.7 ± 0.87b	87.2 ± 0.92d	90.1 ± 0.78c
	MGT, weeks	27.0 ± 0.06b	26.4 ± 0.06a	27.2 ± 0.06b	27.6 ± 0.05c	SI, weeks	5.4 ± 0.02d	4.6 ± 0.03b	4.4 ± 0.03a	5.3 ± 0.03c
						SE in greenhouse, %	65.2 ± 1.69ab	64.7 ± 1.56ab	61.3 ± 1.32b	66.7 ± 1.19a

Index means in the same row marked with the same letter are not significantly different at $p < 0.05$. Means without letter marks are not significantly different at $p > 0.05$

stored in LN was significantly lower (90%) than the emergence for seeds stored at − 18 (97%) and − 3 °C (96%; Table 5). Seedling emergence in the greenhouse was 83% after storage in LN, 92% after storage at − 18 °C, and 90% after storage at − 3 °C. Seed moisture content had little or no effect on seed germination and emergence (Table 6). The highest germination percentage (99%) was recorded at 7.1% MC. Seedling emergence in the laboratory was significantly lower for seeds desiccated to 5.3% (91%) than for those in the control treatment (95%). There were no significant differences between seedling emergence percentages in the greenhouse; at all tested MC levels, the percentage was 87–89%. Seeds of *P. communis* desiccated to ca. 5% were more vulnerable to storage at − 196 °C. Higher MC during storage in LN resulted in better germination percentage and seedling emergence in greenhouse.

3.1.3 Mountain-ash

The seeds of mountain-ash (*S. aucuparia*) had significantly lower germination and seedling emergence in the laboratory after storage in all tested conditions in comparison with nonstored seeds. The germination percentage after the first and second year was 10% lower than in the control seeds

Table 5 Mean germination percentage (GP), mean germination time (MGT), and seedling emergence (SE) in laboratory and greenhouse, and sprouting index (SI) for 6 tested species at different storage temperatures. Statistical tests were run for each species separately

Species	Index	Storage temperature, °C						
		− 3	− 18	− 196		− 3	− 18	− 196
Malus sylvestris	GP, %	85.3 ± 1.92	88.2 ± 1.51	86.7 ± 1.56	SE in laboratory, %	83.2 ± 1.57a	84.5 ± 1.72a	80.3 ± 2.36a
	MGT, weeks	12.0 ± 0.1	12.0 ± 0.1	12.1 ± 0.09	SI, weeks	2.4 ± 0.05b	2.3 ± 0.05a	2.4 ± 0.05ab
					SE in greenhouse, %	83.5 ± 1.41	84.4 ± 1.54	80.8 ± 1.85
Pyrus communis	GP, %	97.8 ± 0.55a	98.1 ± 0.57a	95.8 ± 1.01a	SE in laboratory, %	95.5 ± 0.81a	96.6 ± 0.52a	89.6 ± 1.61b
	MGT, weeks	16.0 ± 0.02a	16.0 ± 0.02a	16.1 ± 0.02b	SI, weeks	2.0 ± 0.07a	1.9 ± 0.07a	1.9 ± 0.07a
					SE in greenhouse, %	89.5 ± 1.43ab	91.7 ± 1.4a	83.2 ± 2.32b
Sorbus aucuparia	GP, %	80.1 ± 0.99a	76.7 ± 1.15b	77 ± 1.29b	SE in laboratory, %	72.2 ± 1.18a	68.8 ± 1.1b	69.2 ± 1.34ab
	MGT, weeks	20.4 ± 0.04	20.4 ± 0.04	20.4 ± 0.03	SI, weeks	1.8 ± 0.03	1.7 ± 0.03	1.8 ± 0.03
					SE in greenhouse, %	79.5 ± 1.55	77.5 ± 1.82	75.6 ± 1.92
Prunus avium	GP, %	92.0 ± 0.94a	90.6 ± 0.93a	82.1 ± 1.25b	SE in laboratory, %	89.2 ± 1.11a	85.4 ± 1.26b	65.5 ± 2.96c
	MGT, weeks	15.4 ± 0.03a	15.4 ± 0.04a	16.6 ± 0.09b	SI, weeks	3.6 ± 0.1a	3.7 ± 0.1a	3.9 ± 0.12b
					SE in greenhouse, %	90.4 ± 1.16a	87.5 ± 1.03b	70 ± 2.46c
Prunus padus	GP, %	88.9 ± 1.03	90.9 ± 0.95	88.7 ± 1.15	SE in laboratory, %	77.8 ± 1.74	78.2 ± 2.2	78.0 ± 1.24
	MGT, weeks	24.4 ± 0.08	24.3 ± 0.08	24.3 ± 0.07	SI, weeks	2.7 ± 0.04	2.7 ± 0.04	2.7 ± 0.04
					SE in greenhouse, %	86.8 ± 1.27	87.3 ± 1.42	87.5 ± 1.36
Cornus sanguinea	GP, %	93.5 ± 0.71	94.6 ± 0.62	93.8 ± 0.68	SE in laboratory, %	92.3 ± 0.73	91.7 ± 0.87	90.8 ± 0.97
	MGT, weeks	27.1 ± 0.07	27.1 ± 0.08	27 ± 0.09	SI, weeks	4.9 ± 0.07	4.9 ± 0.07	4.9 ± 0.08
					SE in greenhouse, %	63.3 ± 1.2	64.3 ± 1.44	65.8 ± 1.17

Index means in the same row marked with the same letter are not significantly different at $p < 0.05$. Means without letter marks are not significantly different at $p > 0.05$

(85%; Table 4). The mean germination time was the lowest after the second year of storage (20.3 weeks). Seedling emergence in the laboratory also significantly decreased after storage. Seedling emergence in a greenhouse was significantly lower after the first and second years of storage. The highest germination percentage was recorded in seeds stored at − 3 °C (80%) in comparison with 77% at both lower temperatures (Table 5). Similar results were recorded for seedling emergence in the laboratory, where emergence after storage at − 3 °C was the highest (72%). The temperature had no effect on either the mean germination time (20 weeks) or the sprouting index (1.8 weeks). Desiccated seeds of mountain-ash (5.5% MC) had a significantly higher germination percentage and seedling emergence in the greenhouse compared with the control (11.1% MC; Table 6). Seedling emergence in the laboratory was recorded at 67% for the control and 74% for desiccated seeds. In seeds of mountain-ash, all tested temperatures and MCs after the third year of storage resulted in high viability of seeds in comparison with the nonstored control. Only desiccated seeds (8.4 and 5.5% MC) stored in LN had a significantly lower germination percentage after the third year. The most consistent results were recorded after 3 years of storage for seeds with 8.4% MC at − 3 °C (Fig. 4).

3.1.4 Wild cherry

After 3 years of storage of wild cherry seeds, its germination rate remained high (89%) at temperatures of − 18 °C and − 3 °C. Storage in LN significantly decreased seedling emergence both in the laboratory and in the greenhouse; however, germination after storage in LN remained high. We obtained the best results at a storing temperature of − 18 °C in seeds with 11.2% MC (Fig. 5). The seeds germinated at a similar level in the control and after all tested storage years (Table 4). Seedling emergence in the laboratory in all the tested conditions was significantly lower after storage (73–81%) than in the control (89%). The mean time of seedling emergence was 1 week faster in fresh nonstored seeds, 2.7 weeks in comparison with 4.3 weeks after the third year of storage. After the third year of storage, seedling emergence in the greenhouse was 89% in comparison with the control result, 86% of germinated seeds. The storage temperature significantly affected the percentage and the rate of germination and seedling emergence. After storage in LN, the germination percentage decreased from 92% at − 3 °C and 91% at − 18 °C to 82%, and the mean germination time was significantly longer, increasing from 15.4 to 16.6 weeks (Table 5). Seedling

Table 6 Mean germination percentage (GP), mean germination time (MGT), and seedling emergence (SE) in laboratory and greenhouse, and sprouting index (SI) for 6 tested species at different seed moisture contents. Statistical tests were run for each species separately

Species	Index	Moisture content, %						
		11	8	5		11	8	5
Malus sylvestris	GP, %	92.0 ± 1.1a	91.1 ± 0.84a	77.0 ± 1.61b	SE in laboratory, %	85.2 ± 1.11b	88.6 ± 1.08a	74.3 ± 2.38c
	MGT, weeks	12.3 ± 0.03b	12.4 ± 0.04b	11.4 ± 0.04a	SI, weeks	2.3 ± 0.04b	2.5 ± 0.05c	2.2 ± 0.05a
					SE in greenhouse, %	86.5 ± 1.39a	84.9 ± 1.51a	77.4 ± 1.56b
Pyrus communis	GP, %	97.4 ± 0.48a	98.5 ± 0.57a	95.7 ± 1.03a	SE in laboratory, %	95.3 ± 0.71a	95.0 ± 0.77a	91.4 ± 1.72a
	MGT, weeks	16.0 ± 0.02	16.1 ± 0.03	16.0 ± 0.02	SI, weeks	1.95 ± 0.06b	1.9 ± 0.07ab	1.9 ± 0.07a
					SE in greenhouse, %	88.5 ± 2	88.7 ± 1.41	87.2 ± 2.12
Sorbus aucuparia	GP, %	76.5 ± 1.07b	77.1 ± 1.3b	80 ± 1.06a	SE in laboratory, %	66.8 ± 0.97c	70.0 ± 1.05b	73.5 ± 1.42a
	MGT, weeks	20.4 ± 0.03b	20.5 ± 0.04b	20.3 ± 0.03a	SI, weeks	1.8 ± 0.02b	1.7 ± 0.03a	1.7 ± 0.03a
					SE in greenhouse, %	78.1 ± 1.38	77.1 ± 1.71	77.4 ± 2.18
Prunus avium	GP, %	90.2 ± 0.94a	88.6 ± 1.33a	85.9 ± 1.43a	SE in laboratory, %	82.0 ± 2.2a	81.3 ± 2.58a	76.7 ± 2.98b
	MGT, weeks	15.7 ± 0.1a	15.8 ± 0.14ab	15.9 ± 0.12b	SI, weeks	3.7 ± 0.11a	3.7 ± 0.1a	3.8 ± 0.11a
					SE in greenhouse, %	82.6 ± 2.26	82.9 ± 2.18	82.4 ± 2.34
Prunus padus	GP, %	89.0 ± 0.91	89.8 ± 1.02	89.7 ± 1.23	SE in laboratory, %	78.5 ± 1.42	79.9 ± 1.57	75.6 ± 2.15
	MGT, weeks	24.4 ± 0.08	24.2 ± 0.08	24.4 ± 0.07	SI, weeks	2.6 ± 0.04a	2.7 ± 0.04b	2.6 ± 0.05ab
					SE in greenhouse, %	86.8 ± 1.34	88.1 ± 1.46	86.8 ± 1.23
Cornus sanguinea	GP, %	93.7 ± 0.67	93.4 ± 0.74	94.8 ± 0.57	SE in laboratory, %	92 ± 0.91	91.6 ± 0.93	91.1 ± 0.75
	MGT, weeks	27.0 ± 0.07a	27.1 ± 0.09b	27.1 ± 0.08ab	SI, weeks	4.9 ± 0.07ab	5.0 ± 0.07b	4.9 ± 0.08a
					SE in greenhouse, %	63.9 ± 1.28	64.9 ± 1.31	64.7 ± 1.26

Index means in the same row marked with the same letter are not significantly different at $p < 0.05$. Means without letter marks are not significantly different at $p > 0.05$

emergence in the laboratory after storage at traditional temperatures was 89% at − 3 °C and 85% at − 18 °C, while seedling emergence for seeds stored in LN was 66%. Seedling emergence in the greenhouse after LN storage was almost 20% lower than that of seeds stored at the other tested temperatures. The mean seedling emergence time was higher after LN storage. Desiccation of *P. avium* seeds to 5.5% slightly decreased germination from 90% at 11.2% MC to 86% (Table 6). Seeds stored at higher MC levels germinated faster, from 15.9 weeks at 5.5% MC to 15.7 weeks at 11.1% MC. Seedling emergence in the laboratory was the lowest for desiccated seeds, at 77%. In the greenhouse, seedlings emerged at 82% for all tested MC levels.

3.1.5 Bird cherry

Storage conditions had little or no effect on bird cherry (*P. padus*) seed germination and seedling emergence (Fig. 6). Germination was similar among nonstored seeds and all storage times, varying from 88 to 91% (Table 4). The mean germination time varied at each year from 23.9 weeks after 2 years of storage to 24.7 weeks after the third year. Seedling emergence in the greenhouse showed no significant differences among all storage years except for the second year, where it was slightly lower (84%) in comparison with the first and third years of storage (89–88%). Seeds germinated in all the tested temperatures with a mean germination time of approximately 24 weeks after the start of the cold stratification phase. Desiccation of seeds did not result in changes in the germination percentage (89–90%; Table 6) or the mean germination time (24 weeks).

3.1.6 Common dogwood

Seeds of common dogwood (*C. sanguinea*) were characterized by similar durability, in which the only factor that slightly affected germination and seedling emergence was storage time. Nonstored seeds of common dogwood germinated at 97% in comparison with 93–94% for stored seeds (Table 4). The mean germination time varied from 26.4 weeks after the first year of storage to 27.6 weeks after the third year. Seedling emergence in the laboratory was significantly higher in nonstored seeds (97%) and decreased in storage seeds (87–92%). Additionally, the mean time of emergence varied each year, resulting in the fastest, 4.4 weeks in the seeds stored for 2 years, to the slowest, 5.4 weeks in nonstored seeds. The results of seedling emergence in the greenhouse were much lower than those in the laboratory, resulting in emergence between

61 and 67%. The temperature of storage had no effect on germination percentage or seedling emergence both in the laboratory and in the greenhouse (Table 5). Seeds germinated at a high level, 94–95%, at all the tested temperatures, and the mean germination time was 27 weeks from the beginning of stratification. Seedling emergence in the laboratory was between 91 and 92%, much higher than in the greenhouse, where it was 63–66% (Fig. 7). Similar results were obtained for different seed MC levels. At all MC levels, between 94 and 95% of seeds germinated with a mean time of 27 weeks (Table 6).

3.2 Height and root collar diameter of seedlings

The height of 1-year-old seedlings from seeds stored in controlled conditions was comparable in all tested conditions. All tested species' seedling height and diameter were the lowest in nonstored seeds and the highest after the third year of storage (Fig. 1a, d). In the case of seedlings of European crab apple, seeds stored at controlled temperatures did not differ among the tested temperatures, and seedling height varied from 34 to 36 cm (Fig. 1b, e). The root collar diameter in seedlings from seeds stored at − 3 °C was 3.92 mm and was significantly lower than that from seeds stored at other storage temperatures. The shortest seedlings (33 cm) were obtained from seeds stored at 8.5% MC, while seedlings grown from seeds with 5 and 10.7% MC were statistically significantly taller (Fig. 1c, f). A similar result was obtained for European pear, in which the shortest seedlings grew from seeds stored at 7.1% MC (34 cm) in comparison with 35 and 36 cm for the heights of seedlings grown from seeds stored at 5.3 and 11.9% MC, respectively (Fig. 1c). The thickest root collar diameter was 4.28 mm in seedlings grown from seeds at 7.1% MC. The tallest seedlings were obtained from mountain-ash seeds stored at − 3 °C, at 53 cm, and they were 2 cm higher than seedlings grown from seeds stored at lower temperatures (Fig. 1b). The root collar diameter in mountain-ash was similar at all the tested temperatures and varied from 5.29 to 5.34 mm. Seedlings grown from desiccated seeds (5.5%) were 53 cm in height in comparison with 51 and 52 cm heights of seedlings grown from 8.4 and 11.1% of MC, respectively. Seedlings of wild cherry grown from cryostored seeds grew 2 cm less (34 cm) than seedlings stored at higher temperatures (Fig. 1b). The thickest root collar diameter was obtained in seedlings from seeds stored at − 18 °C. The height and root collar diameter were similar for all tested MC levels. The moisture content of the stored bird cherry seeds had no effect on the height of seedlings, which was 29 cm for all tested MC levels of seeds. The root collar diameter (4.36 mm) was slightly larger in seedlings grown from seeds with 8.3% MC. In common dogwood, seedlings grown from cryostored seeds had the smallest root collar diameter of 3.56 mm, in comparison with 3.66 and 3.64 mm in seedlings from seeds stored at − 3 or − 18 °C (Fig. 1e). The shortest seedling, at 49 cm, was grown from seeds stored at 11% MC, 4 cm shorter than seedlings grown from seeds with greater MC. The root collar diameter for seedlings grown from seeds stored at 8% MC was 3.51 mm, in comparison with 3.7 and 3.65 mm for seedlings grown from seeds stored at 5.5% and 11% MCs, respectively.

4 Discussion

Seeds of the investigated species responded variously to storage conditions. Although they belong to the same storage behavior category (*orthodox* or uncertain in case of *C. sanguinea* according to the SID database) and originate from the same ecological niche, their MC and temperature during storage had different effects on germination and seedling emergence. Probert et al. (2009) showed that seed longevity is affected primarily by the environment and endosperm presence. Non-endospermic seeds from dry, hot environments tend to have a longer life span in storage. Oil content and seed mass, according to those authors, have no effect on seed longevity. However, the oligo-/disaccharide ratio in seeds plays an important role in seed longevity (Lin and Huang 1994). In our research, seeds were rather small, with 1000-seed weight of 7–32 g in *Malus sylvestris*, *Pyrus communis*, and *Sorbus aucuparia*, 52–84 g in *Cornus sanguinea* and *Prunus padus*, and 184 g in *P. avium*. The large size of *P. avium* seeds could have a negative effect during its freezing in LN. Also differences in seed coat thickness can influence seed longevity, as seeds with less permeable seed coats deteriorate slower (Debeaujon et al. 2000). Besides, Nguyen et al. (2012) show that dormancy in *Arabidopsis* mutants is negatively correlated with seed longevity. In our study, we observed that species with more complex dormancy (*C. sanguinea*, *P. padus*, and *S. aucuparia*; Table 6) tended to be more tolerant to both desiccation and storage temperature.

Storage of *Malus sylvestris* seeds at 5.5% of MC in our experiments resulted in a decrease in all performed seed viability tests. Storage of seeds below 5% of MC was considered to increase seed longevity by other authors (Perez-Garcia et al. 2009; Ellis 1995; Ellis et al. 1996). However, an experiment by Hong et al. (2005) shows that ultra-dry storage can be beneficial in ambient conditions but not always at subzero temperatures. This was also confirmed by experiments of Haiying et al. (2002) on tomato seeds (*Solanum lycopersicum*) stored at 2.3% of MC for 13 years at 20 °C and of Ellis et al. (1986), in which seed desiccation of *Sesamum indicum* from 5 to 2% of MC increased storage time 40-fold. Walters (2007) indicated that, below a critical moisture content, longevity does not increase with further drying. A similar conclusion was reported by Michalak et al. (2015b), as storage of European crab apple desiccated to 4% of MC also resulted in decreased germination and

Table 9 Analysis of variance results for mean germination time and seedling index at $p < 0.005$. Significant differences are marked in italics

	Factor	*df*	Sum Sq	Pr(>*F*)	Sum Sq	Pr(>*F*)		Sum Sq	Pr(>*F*)	Sum Sq	Pr(>*F*)		Sum Sq	Pr(>*F*)	Sum Sq	Pr(>*F*)
M. sylvestris			MGT		SI		*P. communis*	MGT		SI		*S. aucuparia*	MGT		SI	
	Storage (ST)	3	44.761	*< 0.000*	2.63817	*< 0.000*		13.7971	*< 0.000*	15.0703	*< 0.000*		27.7903	*< 0.000*	2.69383	*< 0.000*
	Moisture content (MC)	2	18.488	*< 0.000*	1.92211	*< 0.000*		0.1294	*< 0.000*	0.0610	*0.039*		0.6978	*< 0.000*	0.47777	*< 0.000*
	Temperature (T)	2	0.042	0.4481	0.13322	*0.017*		0.0216	0.058	0.1763	*< 0.000*		0.0489	0.459	0.06672	0.055
	ST × MC	6	2.490	*< 0.000*	3.12627	*< 0.000*		0.0544	*0.030*	0.4958	*< 0.000*		0.3238	0.120	0.26256	*0.001*
	ST × T	6	0.101	0.6882	0.19036	0.069		0.0444	0.073	0.4090	*< 0.000*		0.3249	0.119	0.21690	*0.006*
	MC × T	4	0.095	0.4562	0.13263	0.083		0.0038	0.901	0.2727	*< 0.000*		0.1667	0.261	0.13822	*0.019*
	ST × MC × T	12	0.182	0.8436	0.36553	*0.039*		0.0841	*0.046*	1.0518	*< 0.000*		0.8528	*0.013*	0.46261	*< 0.000*
	Residuals	72	1.850		1.10955			0.2626		0.6467	*< 0.000*		3.3649		1.21193	
P. avium							*P. padus*					*C. sanguinea*				
	Storage (ST)	3	82.222	*< 0.000*	39.165	*< 0.000*		12.1940	*< 0.000*	2.53180	*< 0.000*		26.9808	*< 0.000*	31.1419	*< 0.000*
	Moisture content (MC)	2	0.661	*0.002*	0.048	0.409		0.3680	0.122	0.23408	*0.036*		0.7713	*0.036*	0.2190	*0.004*
	Temperature (T)	2	18.367	*< 0.000*	1.067	*< 0.000*		0.2251	0.272	0.01698	0.778		0.4744	0.126	0.0189	0.606
	ST × MC	6	0.663	*0.040*	0.507	*0.008*		2.3342	*0.001*	0.48720	*0.035*		0.9935	0.194	1.1705	*< 0.000*
	ST × T	6	6.591	*< 0.000*	0.707	*< 0.000*		0.1241	0.960	0.13092	0.692		1.5951	*0.035*	0.2726	*0.031*
	MC × T	4	0.304	0.18108	0.179	0.162		0.0146	0.996	0.10803	0.528		0.3742	0.507	0.1107	0.216
	ST × MC × T	12	0.682	0.29824	0.222	0.748		0.8802	0.585	0.38902	0.493		0.3913	0.990	0.6181	*0.003*
	Residuals	72	3.401		1.909			6.1079		2.42707			12.1251		2.0307	

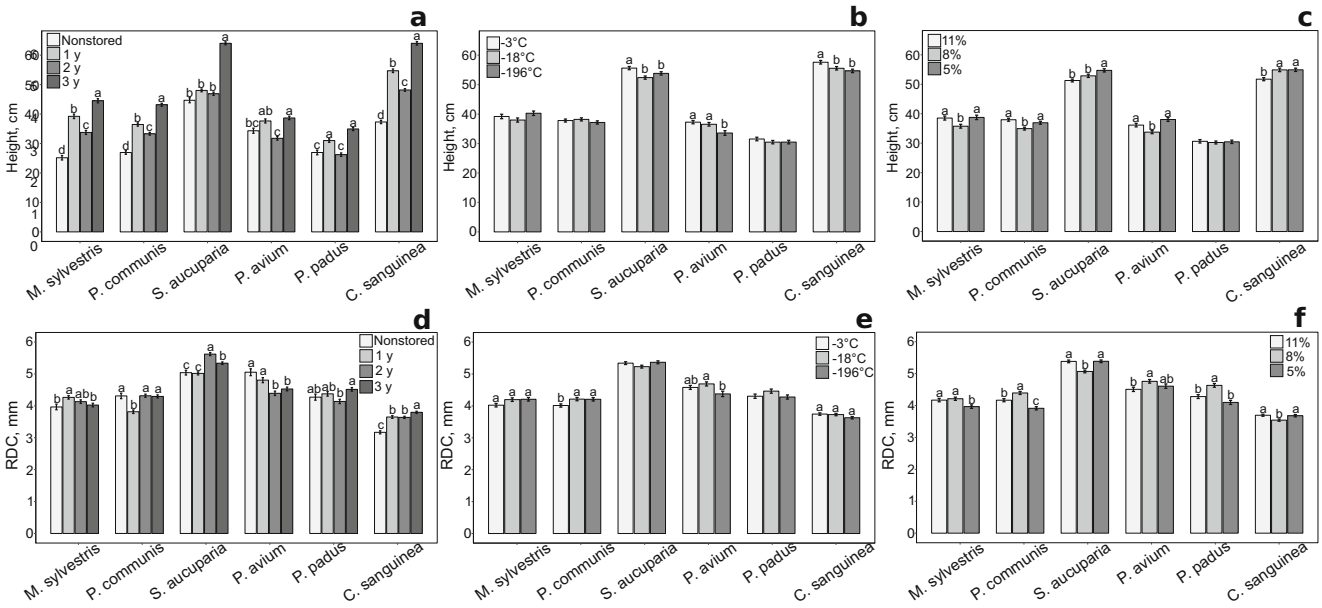

Fig. 1 Mean height (H) and root collar diameter (RCD) for seedlings grown from seeds stored in controlled conditions. **a**, **d** Storage time. **b**, **e** Temperature. **c**, **f** Moisture content. Statistical tests were run for each species separately. Means in the same row marked with the same letter are not significantly different at $p < 0.05$, by Kruskal-Wallis test. Means without letter marks are not significantly different at $p > 0.05$

seedling emergence. We noted that seed storage of *M. sylvestris* at MC 5.5% in LN resulted in a significant decrease in seedling emergence. Despite the decline in seedling emergence, desiccated seeds of European crab apple tend to release dormancy a week earlier than the control. In general, desiccation plays a crucial role in the after-ripening process, which prepares seeds to germinate. As noted by Angelovici et al. (2010), gene expression and metabolic processes during seed desiccation strongly resemble those observed during germination. This suggests that one of the functions of the desiccation process is preparation for germination. Seed storage conditions of *Pyrus communis* affected neither germination nor seedling emergence. However, despite being desiccation-tolerant (to MC 5.3%), seeds stored at low MC in LN germinated at a lower rate. There is little information about storing *Sorbus aucuparia* seeds in the literature. Gordon et al. (1992) recommend storage of *Sorbus* spp. at 6–8% of MC, at 1–4 °C for up to 8 years. As reported by Granstrom (1987), mountain-ash seeds survive and remain viable for up to 5 years in the soil bank. In our experiment, nonstored seeds germinated faster and better than seeds stored for 1–2 years. This might be due to the delay in seed sowing caused by a lack of space in the growth chambers, as the proportion of decayed seeds after

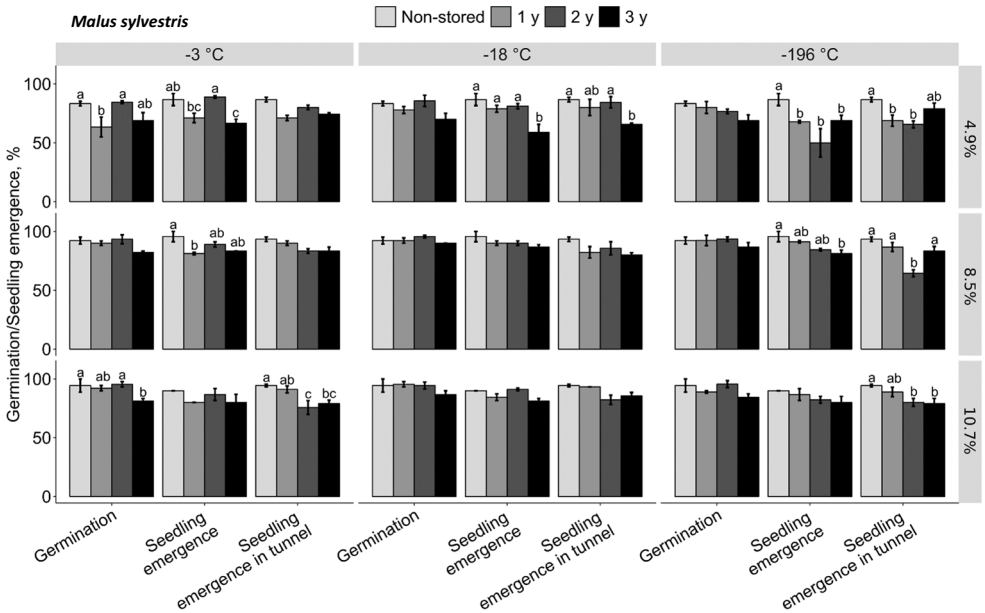

Fig. 2 Germination and seedling emergence in laboratory and greenhouse of seeds of *Malus sylvestris* after 1, 2, and 3 years of storage in controlled conditions. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

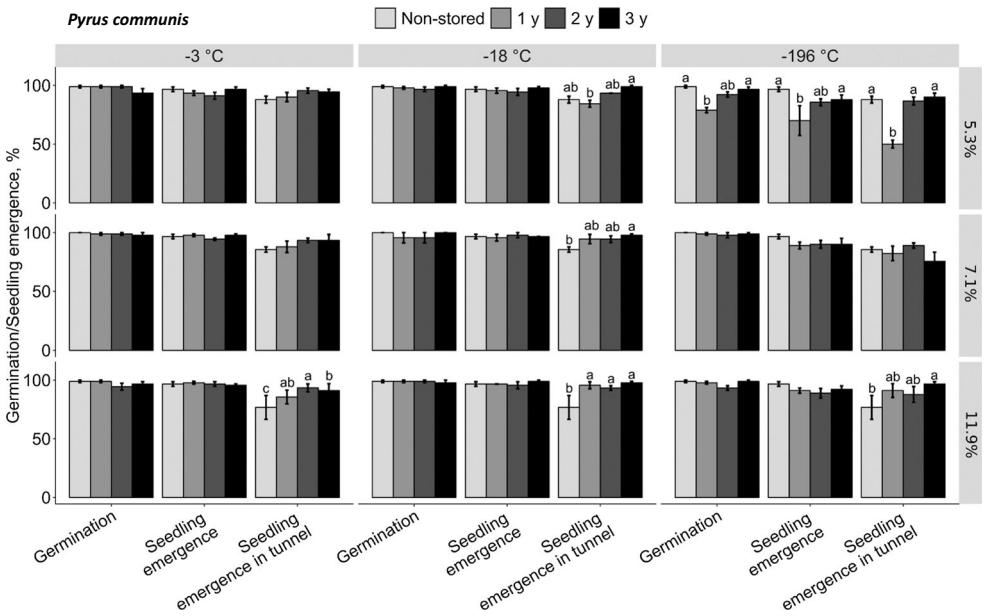

Fig. 6 Germination and seedling emergence in laboratory and greenhouse of seeds of *Prunus padus* after 1, 2, and 3 years of storage in controlled conditions. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

stratification never exceeded 4.5%. Therefore, to hinder germination, nonstored seeds were placed at − 1 °C for 2 weeks prior to sowing. According to Afroze and O'Reilly (2015), as well as our observations (Fig. 8), freezing can result in higher germination. For confirmation, additional seeds of mountain-ash were examined, with a 2-week treatment at − 1 °C after the end of stratification. Seeds subjected to freezing germinated approximately 10 times better than the control. The lower temperature may be beneficial in breaking seed-coat-induced dormancy (Raspé et al. 2000). Perez-Garcia et al. (2009) found that, in some species of the family Brassicaceae, long-term storage increased germination due to dormancy loss. However, after the third year of storage, both the rate and total germination showed no difference from nonstored seeds. Information on the storage behavior of *Cornus sanguinea* seeds is scarce in the literature. The Seed Information Database (Kew Royal Botanical Gardens 2019) states that its storage behavior is uncertain. Seed survival in the transient seed bank was less than a year, which may indicate that seeds are short-lived under ambient conditions (Thompson et al. 1997). In our study, seed desiccation to 5.5% of MC did not affect germination, which reached 95% (vs. 94% in the control). The seeds remained viable at all the tested storage temperatures for 3 years; therefore, seeds of this species should be classified as *orthodox*. Our study shows for the first time the feasibility of cryopreservation of common dogwood seeds in the safe range of 5.5–11.0% of MC. The seedling emergence test in the greenhouse showed much lower results than in its laboratory counterpart. This is most likely due to the late sowing date (second half of April), in which high soil temperatures could induce secondary dormancy (Benech-Arnold et al. 2000).

Seeds of *Prunus padus* germinate even after desiccation to 3.5% according to Popova et al. (2016). At the tested temperatures, the seeds remained viable; therefore, seeds of bird cherry can be successfully stored in conventional conditions defined by gene bank standards (FAO 2014). In the literature, for medium-term storage of this species, temperatures of 3–5 °C with MC below 12% were recommended (Gordon and Rowe 1982), whereas for long-term storage, a temperature of − 18 °C at ca. 5% of MC is recommended (Kew Royal Botanical Gardens 2019). Our results indicate that long-term storage of cryopreserved seeds of bird cherry is possible at all the tested MC levels (seedling emergence 76–79%). Popova et al. (2016) report that the safe MC for cryogenic storage of bird cherry seeds is 3.5–15.0%, which is a wider range than in other "oily" orthodox seeds. On the other hand, seeds of wild cherry in our study tolerated desiccation to MC 5.5% and germinated at 86%, compared with 90% in the control (MC 11%). In general, wild cherry seeds are desiccation-tolerant to 1.6% of MC (Chmielarz, 2009; Royal Botanic Gardens Kew, 2019; Jensen and Eriksen 2001). However, partial drying of freshly collected seeds can result in a significant decrease in germination after immediate stratification and sowing (Michalak et al. 2015c). Further storage of dry seeds in tightly closed vials restores germination capacity to higher values (Suszka 1964). In our research, storage of seeds at ultralow temperatures resulted in a significant decrease in seed viability, which was detected in the seedling emergence test (although in the germination test the results were comparable), so wild cherry seed can be classified as desiccation-tolerant and LN-sensitive. Cryopreservation considerably hampers seed emergence (66% in the laboratory and 70% in a greenhouse) but is feasible at a narrow range of seed MC (Chmielarz, 2009). The cited study suggests that low tolerance to subzero temperatures may result from the high content (45%) and composition of storage lipids in the seeds of wild cherry. Damages can occur during changes in the phase transitions of the membranes from liquid to

crystalline (Stanwood 1985). A similar composition of seed storage lipids is also observed in *Hippophaë rhamnoides*, which—like wild cherry—germinates but does not develop into viable seedlings after storage in LN (Wawrzyniak unpublished). Hor et al. (2005) report that there is a negative correlation between lipid content of seeds and unfrozen water, which affects their ability to regenerate after exposure to LN. Out of the investigated 6 species, *Prunus avium*, *Pyrus communis*, and *M. sylvestris* seeds do not fully tolerate storage in LN after severe desiccation to MC ca. 5%. However, in contrast to *Prunus avium* and *Pyrus communis*, whose seeds tolerate desiccation to MC 5%, *M. sylvestris* seeds are negatively affected by severe desiccation to MC ca. 5%. *S. aucuparia*, *Prunus padus*, and *C. sanguinea* seeds tolerate storage in LN at MC ca. 5%.

Although there are many publications describing the effect of seed storage on germination, few studies have addressed the further growth of seedlings. For example, seedlings of *Coffea arabica* grown from seeds stored for 9 months at 10–11% of MC and 10–15 °C were not suitable for nursery planting. Additionally, seedlings grown from seeds stored at 18% of MC and 20 °C were 50% shorter than from those stored at higher MC levels (da Rosa et al. 2011). Mucha et al. (2015) showed that seed storage temperature influences the root anatomy of black poplar (*Populus nigra*) and consequently may alter nutrient absorption. Good-quality seedlings with greater height and root collar diameter confer a higher chance of survival in natural conditions (Grossnickle 2012). Therefore, we conducted field research after the first year of the growing season in the greenhouse. In all the tested species, seedling emergence in the greenhouse was slightly lower than in laboratory conditions but still high, regardless of storage conditions. The height and the root collar diameter differ significantly between the tested years but probably due to differences in temperatures during the growing season (Table 7) rather than seed storage time. Differences in morphological attributes at different moisture levels and temperatures were small and did not affect seedling quality despite their statistical significance. However, this is not always the case. Arguedas et al. (2018) found that seedlings of corn (*Zea mays*) grown from cryostored seeds had delayed growth, although they did not differ phenotypically. The measurements in our study were made only at the end of the growing season, so we cannot conclude whether any differences were visible at the initial phase of growth. However, even if there were any differences initially, the seedlings from all the tested variants evened out by the end of the season.

Basing on our results, we can recommend storing seeds of *Malus sylvestris* at − 18 °C and ca. 8%. For seeds of *Prunus avium*, the safest temperature for storage is − 3 °C. Desiccation to ca. 8% before storage at low temperature can be recommended for seeds of *Sorbus aucuparia*. In the case of *Pyrus communis*, severe desiccation should be avoided if storage in LN is planned. *Prunus padus* and *Cornus sanguinea* can be safely stored at low seed MC and temperature, as they proved to be the most tolerant. All the tested seed storage methods can be successfully used in nursery production, as no or minimal morphological differences occurred in seedling growth.

5 Conclusion

The results of the study show the importance of species-oriented approaches, as even in related species (both taxonomically and ecologically), seeds responded differently to given moisture content levels and storage temperatures (5–11% and − 196°, − 18°, − 3 °C). Our research showed that, in terms of storage behavior, (i) seeds of *M. sylvestris* lose viability after desiccation below 6% MC despite being considered desiccation-tolerant; (ii) desiccation of *P. communis* seeds to ca. 5% MC decreases their storability in LN; (iii) *S. aucuparia* seeds are best stored at − 3 °C, below 11% MC; (iv) *P. avium* seeds, due to damage after cryopreservation and at − 18 °C storage, should be describe as desiccation-tolerant and sensitive to low temperatures (below − 18 °C); (v) seeds of *P. communis*, *P. padus*, and *C. sanguinea* can be stored long term at − 18 °C or in LN at 5–8% MC without losing viability; (vi) seeds of *P. padus* should be classified as *orthodox*; (vii) seeds of *C. sanguinea* are, for the first time, shown to be feasible for cryopreservation between 5.5 and 11% MC and should be considered an *orthodox* type; and (viii) seedlings grown from seeds stored for up to 3 years in the presented storage conditions show small or no morphological differences after the first growing season and can be utilized for nursery production.

In general, species with deeper physiological dormancy (*S. aucuparia*, *P. padus*, *C. sanguinea*) tended to be more tolerant to desiccation and low temperatures. However, our study confirms that severe desiccation can damage some seeds even if classified before as *orthodox* species. Therefore, more accurate experiments on the long-term storage of seeds of woody species should be conducted, especially for species subject to rapid environmental changes.

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Data availability The datasets generated and analyzed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.9080513.v2).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Appendix

Table 7 Monthly mean temperatures and minimum and maximum temperatures in greenhouse recorded during experiment in greenhouse and air and ground levels

Year	Month	Mean temperature, °C		Air temperature, °C		Ground temperature, °C	
		Air	Ground	Min	Max	Min	Max
2013	Apr	13.1	12.6	2.8	27.7	1.8	28.2
	May	16.7	15.8	4.5	34.4	3.0	35.7
	Jun	19.3	18.4	8.2	35.7	7.1	36.9
	Jul	20.3	19.5	11.0	33.4	9.8	36.6
	Aug	18.1	18.0	9.6	34.6	10.6	38.6
	Sep	12.0	12.4	2.2	19.4	5.2	20.9
	Oct	5.0	6.0	0.1	10.9	2.7	10.1
2014	Apr	12.6	13.0	-2.2	34.1	0.7	27.8
	May	15.3	14.7	-0.9	37.8	2.6	28.9
	Jun	17.4	16.4	5.8	35.5	8.7	28.3
	Jul	21.2	20.2	8.6	33.3	11.5	27.8
	Aug	16.9	16.6	4.8	34.7	8.7	25.2
	Sep	15.4	15.2	6.3	26.5	6.5	23.7
	Oct	11.8	11.5	4.7	18.5	4.2	18.1
2015	Apr	-	14.3	-	-	5.7	27.4
	May	-	15.9	-	-	4.9	28.2
	Jun	-	17.2	-	-	11.8	28.8
	Jul	-	19.2	-	-	12.7	26.9
	Aug	-	19.3	-	-	12.1	26.5
	Sep	-	14.0	-	-	7.8	23.6
	Oct	-	9.6	-	-	5.6	14.9
2016	Apr	12.5	11.9	-2.3	38.4	1.9	23.9
	May	18.8	17.2	2.5	44.5	6.7	30.4
	Jun	21.8	18.4	6.1	46.9	10.5	27.1
	Jul	21.9	18.6	9.4	44.0	12.9	24.9
	Aug	19.5	17.3	7.4	40.8	11.9	23.5
	Sep	16.7	14.9	2.5	36.5	7.4	21.6
	Oct	12.2	12.0	4.6	22.9	4.5	21.0

Table 8 Full model results for examined species. Analysis of deviance results for germination percentage and seedling emergence in controlled conditions and greenhouse at $p < 0.005$. Significant differences are marked in italics

Factor	*df*	Deviance	Resid. Df	Resid. Dev	Pr(>chi)	Deviance	Resid. Df	Resid. Dev	Pr(>chi)	Deviance	Resid. Df	Resid. Dev	Pr(>chi)
	Germination percentage					Seedling emergence				SE in greenhouse			
M. sylvestris NULL			107	291.93			107	295.501			107	291.93	
Storage (ST)	3	51.468	104	240.461	*< 0.000*	64.979	104	230.522	*< 0.000*	51.468	104	240.461	*< 0.000*
Moisture content (MC)	1	127.375	102	113.087	*< 0.000*	83.047	102	147.476	*< 0.000*	127.375	102	113.087	*< 0.000*
Temperature (T)	1	4.404	100	108.682	0.130	7.760	100	139.716	*0.021*	4.404	100	108.682	0.130
ST × MC	6	8.962	94	99.72	0.218	4.269	94	135.447	0.640	8.962	94	99.72	0.218
ST × T	6	4.959	88	94.761	0.598	28.576	88	106.871	*< 0.000*	4.959	88	94.761	0.598
MC × T	4	0.72	84	94.041	0.955	6.849	84	100.022	0.144	0.72	84	94.041	0.955
ST × MC × T	12	7.183	72	86.858	0.880	14.950	72	85.072	0.244	7.183	72	86.858	0.880
P. communis NULL			107	195.028			107	221.36			107	476.04	
Storage (ST)	3	17.317	104	177.711	*0.001*	21.076	104	200.29	*< 0.000*	35.364	104	440.67	*0.003*
Moisture content (MC)	1	15.856	102	161.854	*< 0.000*	15.370	102	184.92	*0.001*	4.308	102	436.36	0.423
Temperature (T)	1	11.251	100	150.603	*0.006*	51.162	100	133.75	*< 0.000*	36.280	100	400.09	*0.001*
ST × MC	6	16.077	94	134.527	*0.023*	7.732	94	126.02	0.348	54.197	94	345.89	*0.001*
ST × T	6	17.507	88	117.019	*0.014*	14.043	88	111.98	0.058	52.533	88	293.36	*0.002*
MC × T	4	12.630	84	104.389	*0.021*	1.430	84	110.55	0.871	33.482	84	259.87	*0.010*
ST × MC × T	12	11.437	72	92.953	0.576	6.225	72	104.33	0.943	43.818	72	216.06	0.131
S. aucuparia NULL			107	291.930			143	252.628			143	625.13	
Storage (ST)	3	51.468	104	240.461	< 0.000	56.859	140	195.768	< 0.000	305.665	140	319.46	< 0.000
Moisture content (MC)	1	127.375	102	113.087	< 0.000	25.410	138	170.358	< 0.000	0.781	138	318.68	0.821
Temperature (T)	1	4.404	100	108.682	0.1303	7.688	136	162.670	0.021	10.587	136	308.10	0.070
ST × MC	6	8.962	94	99.720	0.2175	31.505	130	131.165	< 0.000	39.353	130	268.74	0.003
ST × T	6	4.959	88	94.761	0.5975	8.199	124	122.966	0.22390	7.854	124	260.89	0.683
MC × T	4	0.720	84	94.041	0.9554	3.488	120	119.478	0.47976	17.896	120	242.99	0.061
ST × MC × T	12	7.183	72	86.858	0.8801	25.103	108	94.375	0.014	23.473	108	219.52	0.460
P. avium NULL			107	194.012			107	475.76			107	395.79	
Storage (ST)	3	9.372	104	184.640	*0.025*	78.191	104	397.57	*< 0.000*	54.332	104	341.46	*< 0.000*
Moisture content (MC)	1	9.601	102	175.038	*0.008*	11.509	102	386.06	*0.006*	0.083	102	341.38	0.960
Temperature (T)	1	57.200	100	117.838	*< 0.000*	216.815	100	169.25	*< 0.000*	178.036	100	163.34	*< 0.000*
ST × MC	6	2.290	94	115.548	0.891	20.861	94	148.39	*0.005*	4.766	94	158.57	0.592
ST × T	6	33.691	88	81.857	*< 0.000*	46.782	88	101.60	*< 0.000*	58.665	88	99.91	*< 0.000*
MC × T	4	2.474	84	79.383	0.649	7.074	84	94.53	0.178968	5.831	84	94.08	0.225
ST × MC × T	12	7.139	72	72.244	0.848	8.393	72	86.14	0.826162	17.744	72	76.33	0.140
P. padus NULL			107	149.908			107	174.590			107	186.38	
Storage (ST)	3	2.9922	104	146.916	0.464	49.258	104	125.332	*< 0.000*	14.6270	104	171.75	*0.039*
Moisture content (MC)	1	0.4769	102	146.439	0.815	4.471	102	120.862	0.155	1.0992	102	170.65	0.730
Temperature (T)	1	3.5758	100	142.863	0.216	0.493	100	120.369	0.814	0.2878	100	170.36	0.921
ST × MC	6	25.3883	94	117.475	0.001	7.444	94	112.925	0.400	16.8253	94	153.54	0.140
ST × T	6	9.0377	88	108.437	0.257	5.175	88	107.750	0.634	0.5558	88	152.98	0.999
MC × T	4	1.1001	84	107.337	0.918	9.074	84	98.675	0.109	3.4763	84	149.50	0.737
ST × MC × T	12	13.1876	72	94.149	0.503	10.898	72	87.778	0.695	17.4509	72	132.06	0.615
C. sanguinea NULL			143	285.87			143	401.12			143	247.42	
Storage (ST)	3	41.739	140	244.13	*< 0.000*	148.022	140	253.10	*< 0.000*	12.366	140	235.05	*0.060*
	1	4.444	138	239.69	0.272	0.520	138	252.58	0.840	0.584	138	234.47	0.831

Table 8 (continued)

Factor	*df*	Deviance	Resid. Df	Resid. Dev	Pr(>chi)	Deviance	Resid. Df	Resid. Dev	Pr(>chi)	Deviance	Resid. Df	Resid. Dev	Pr(>chi)
Moisture content (MC)													
Temperature (T)	1	3.176	136	236.51	0.395	3.018	136	249.56	0.365	3.227	136	231.24	0.361
ST × MC	6	8.430	130	228.08	0.552	26.235	130	223.33	*0.008*	12.646	130	218.59	0.239
ST × T	6	8.974	124	219.11	0.512	18.160	124	205.17	0.059	7.793	124	210.80	0.553
MC × T	4	4.166	120	214.94	0.656	5.745	120	199.42	0.428	8.834	120	201.97	0.232
ST × MC × T	12	9.950	108	204.99	0.925	11.397	108	188.03	0.815	31.910	108	170.06	0.064

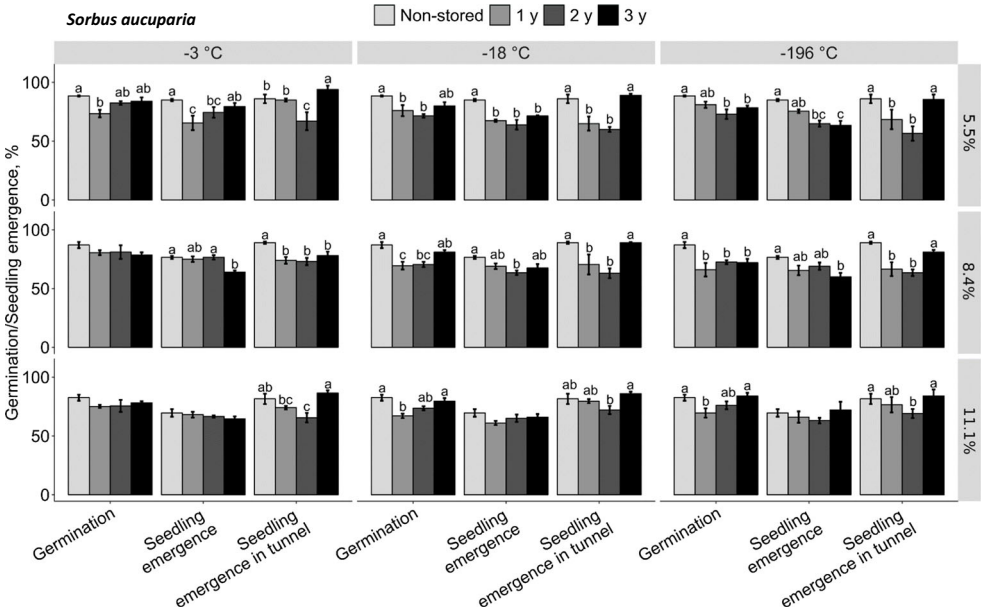

Fig. 3 Germination and seedling emergence in laboratory and greenhouse of seeds of *Pyrus communis* after 1, 2, and 3 years of storage in controlled conditions. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

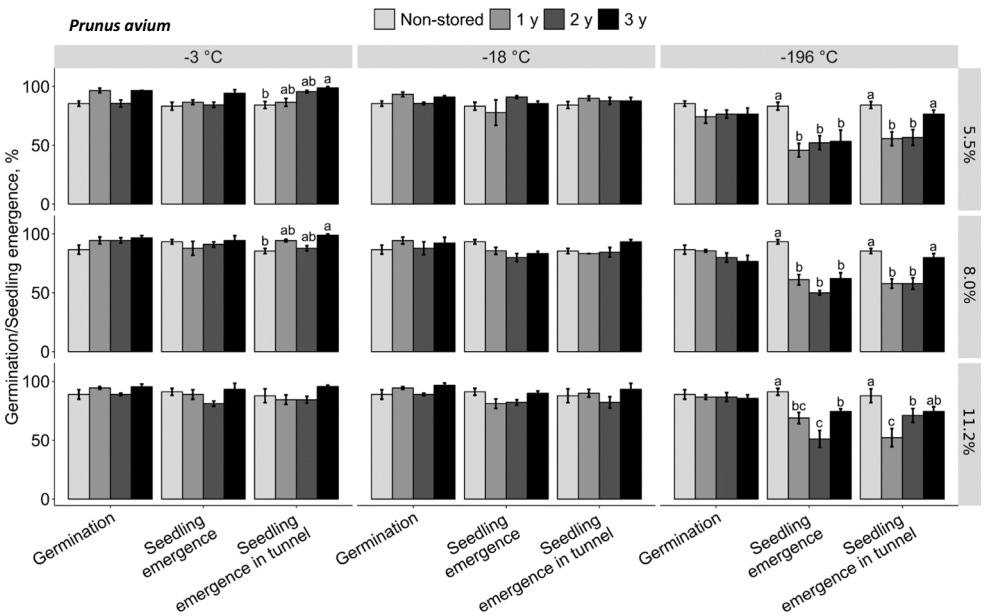

Fig. 4 Germination and seedling emergence in laboratory and greenhouse of seeds of *Sorbus aucuparia* after 1, 2, and 3 years of storage in controlled conditions. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

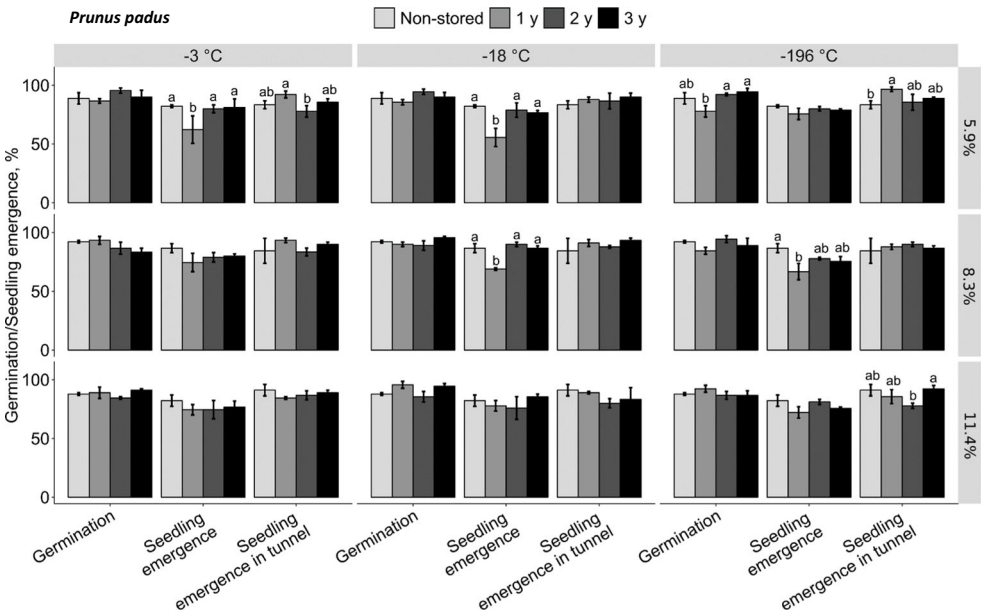

Fig. 5 Germination and seedling emergence in laboratory and greenhouse of seeds of *Prunus avium* after 1, 2, and 3 years of storage in controlled conditions. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

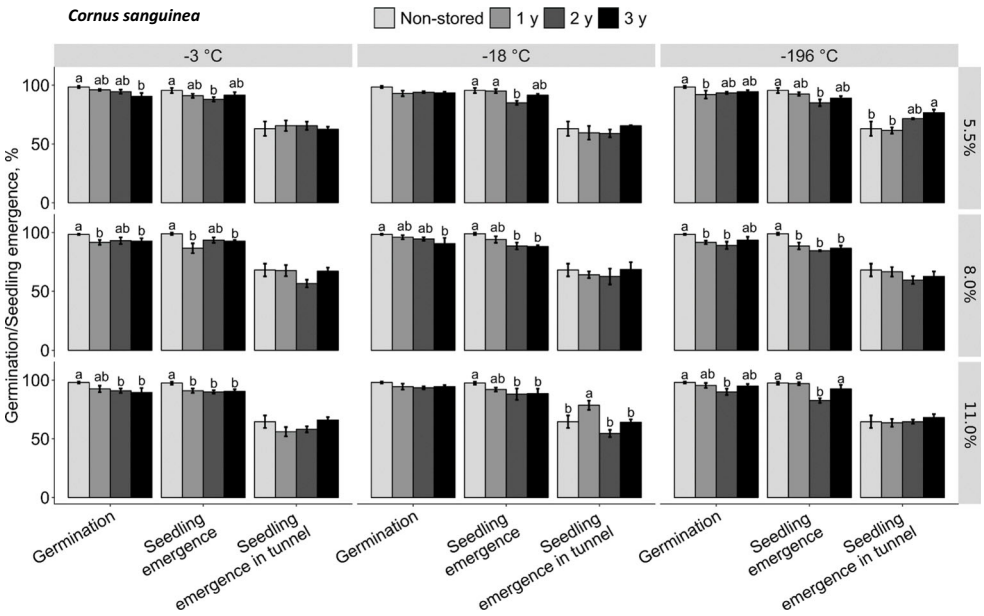

Fig. 7 Germination and seedling emergence in laboratory and greenhouse of seeds of *Cornus sanguinea* after 1, 2, and 3 years of storage in controlled conditions. $p < 0.005$, Tukey test. One-way ANOVA; columns marked with the same letter are not significantly different at $p < 0.05$, by Tukey's test. Means without letter marks are not significantly different at $p < 0.05$. Means ± SE

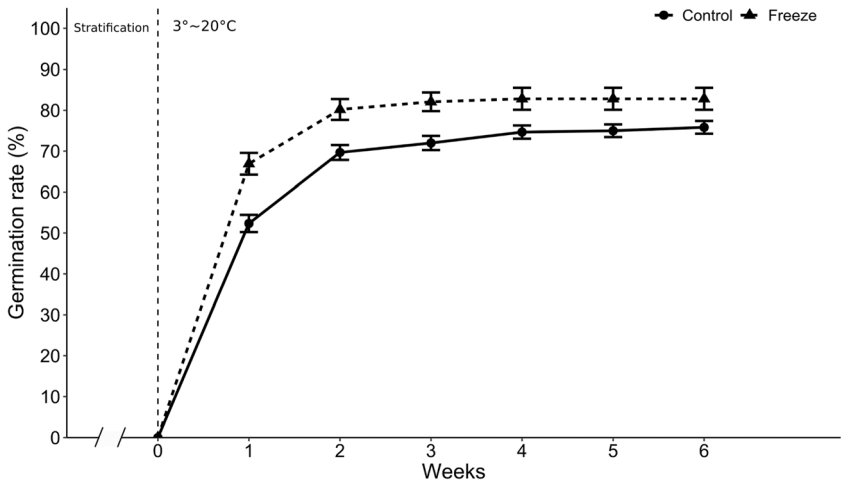

Fig. 8 Germination rate of seeds of *Sorbus aucuparia* after stratification in 3 °C and no additional storage (control) or with additional freezing in 2-week storage in − 3 °C (freeze). Error bars represent standard error for mean. Additional test was made for 3 replicates per 30 seeds, after 1 year storage in − 3 °C

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