

Effect of various lengths of cold stratification period on the germination of wild service tree (*Sorbus torminalis*) seed samples harvested in the Czech Republic

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Abstract: The paper deals with the assessment of the influence of the length of cold stratification period on the germination parameters of seeds of wild service tree [*Sorbus torminalis* (L.) Crantz]. The germination tests (4 × 100 seeds) were conducted in two runs in 2020 and 2021; they were performed for three seed lots and three cold stratification periods (two, three and four months) before the testing of germination in each run. The values of germination energy and germination capacity were assessed, and the germination rate was computed. The results showed that the two-month cold stratification period has not been sufficient to overcome the dormancy of tested seeds. Regardless of the year in which the tests were conducted, seeds stratified for two months showed almost zero germination. For seeds treated with three- and four-month cold stratification, the germination percentage varied from ca. 5% to 32% and from ca. 13% to 46%, respectively. Our results basically correspond with findings reported in the reviewed literature. In 2020, the seeds subjected to four-month stratification performed significantly better than the seeds subjected to three-month stratification. However, in 2021, the seeds stratified for three months reached slightly better results. Four-month stratification also led to an increased percentage of prematurely germinated seeds during stratification. To conclude, the two-month period of cold stratification proved to be insufficient and at least three months of cold stratification are needed to obtain the adequate germination of seeds in subsequent laboratory tests.

Keywords: germination capacity; germination energy; germination rate; moist chilling; premature germination; pretreatment

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Due to the expected changes in climate (Twardosz et al. 2021) and the occurrence of climatic stresses (King, Karoly 2017), much effort has recently been given to forest diversity and the need to alter species composition in forest ecosystems of Central Europe. European beech (*Fagus sylvatica* L.) and oaks [*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.] are considered important components in this transition. Nonetheless, the vulnerability to drought and pathogens may be limiting factors for beech (Martinez del Castillo et al. 2022; Jandl et al. 2023) and to droughts also for oaks (Doležal et al. 2010). Considering the drought tolerance and growth characteristics, some rare native tree species may be used as components for more diverse close-to-nature forests in Central Europe (Schmucker et al. 2023). Wild service tree [*Sorbus torminalis* (L.) Crantz] belongs among the promising trees in this regard (Paganová 2007; Pyttel et al. 2019). According to Paganová (2008), wild service tree represents a valuable admixture in oak forests and may play a significant role in urban forests (Prokopuk et al. 2022) as well as in landscape greening. Valuable timber with interesting properties (Paganová 2008; Bahmani et al. 2020) and newly developed technologies for the use of thinner timber assortments (Šedivka et al. 2023) could also contribute to greater utilisation of *Sorbus torminalis* in the foreseeable future.

Sorbus torminalis is mostly a small tree; in favourable conditions, it can reach a height of up to about 33 m (Thomas 2017). Seedlings are shade-tolerant, the light requirements rise with age. However, *Sorbus torminalis* can persist under the canopy of oaks (Pyttel et al. 2013), and it can benefit from traditional methods of forest management like coppicing and grazing (Barengo et al. 2001 in Rasmussen, Kollmann 2004b). *Sorbus torminalis* shows a broad amplitude in terms of edaphic conditions (Rasmussen, Kollmann 2004a). Although the overall competitive ability of this species is low (Thomas 2017), the wild service tree is a promising hardwood that can be used in forests of lower altitudes in Central Europe.

Sorbus torminalis is a typical forest admixed species with a scattered distribution (Bednorz 2007). In the Czech Republic, the reduced forest stand area of the species is only 86.42 ha, i.e. 0.003% (Maděra et al. 2013). In the last centuries, the proportion of wild service trees was probably higher than today. The decrease in its proportion is con-

nected with the cultivation of dark and dense high forests during the 20th century (Rasmussen, Kollmann 2004a; Angelone et al. 2007).

Small populations of *Sorbus torminalis* are often spatially and genetically isolated (Angelone et al. 2007). This phenomenon is partly compensated by the ability of the vegetative (clonal) propagation by root suckers and the efficient seed dispersal strategy by animal vectors (Hoebee et al. 2006), chiefly by birds and mammals (Demesure et al. 2000; Oddou-Muratorio et al. 2001).

In Southern (Oršanić et al. 2009) and Central Europe, *Sorbus torminalis* usually flowers in May and June and begins yielding at the age of 25 to 30 years. The species produces fleshy brown fruits (Rasmussen, Kollmann 2004a), usually collected in September for artificial regeneration (Hoffmann et al. 2007).

Similarly like in other *Sorbus* species, seeds of *Sorbus torminalis* are deeply dormant, which delays germination (Dinçer 2023). The cause of dormancy probably consists in the physiologically dormant embryo and chemical inhibition of the seed coat (Thomas 2017). Therefore, overcoming the seed dormancy is essential to ensure rapid, uniform and sufficiently effective germination (Pipinis et al. 2015). Different literature sources describe various approaches to the stratification of *Sorbus* seeds. International rules for seed testing (ISTA 2015) for *Sorbus* recommend prechilling (cold stratification) lasting four months to break dormancy. Pipinis et al. (2015) proposed 3 months of cold stratification. The length and methods of seed pretreatment differ also depending on whether the seeds were stored or not. For seeds stored over a longer time, Hoffmann et al. (2007) suggested modification based on a warm (20 °C) moist incubation period lasting 14 days and subsequent (cold) stratification lasting 110 days. However, under nursery (operational) conditions, the warm moist incubation at room temperature often led to the mould growth on the surface of pretreated seeds. Still, other authors reported various modifications of the pretreatment in the representatives of the genus *Sorbus* (Drvodelić et al. 2018), sometimes in combination with the application of growth stimulators (Pipinis et al. 2015). For operational use, the optimum pretreatment represents a compromise between maximising germination parameters, seed utilisation rate (reducing the number of seeds that start to germinate during stratification) and simplicity of the process.

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The paper aims to evaluate the effect of the length of cold stratification on the germination of seeds of wild service tree [*Sorbus torminalis* (L.) Crantz] harvested in the Czech Republic and to find out the optimum length for cold stratification under operational use in forest nurseries.

MATERIAL AND METHODS

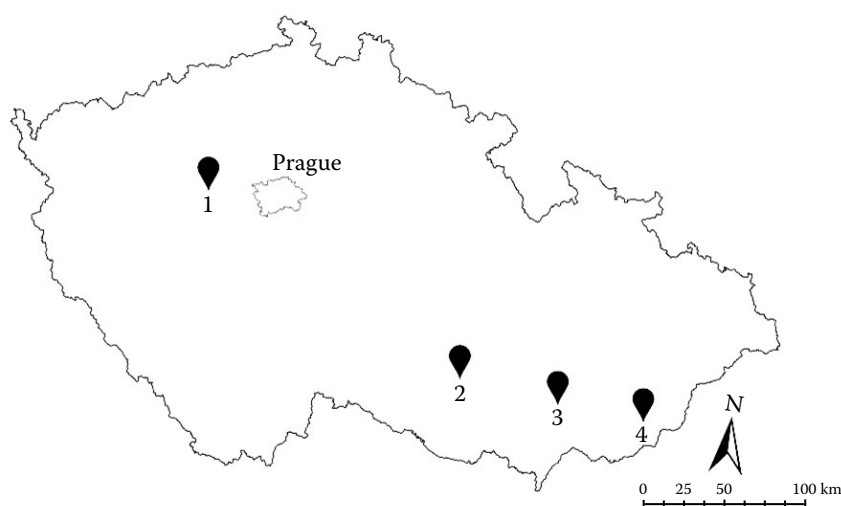
Seed sources and parameters. The fruits of *Sorbus torminalis* originated from four different regions across the Czech Republic where this tree species typically occurs (Figure 1).

The processed seed lots were purchased from the Týniště nad Orlicí Seed Production Plant (Forests of the Czech Republic, state enterprise) where the seed lots were stored in the standard conditions, i.e. dried (water content ~10%) and at a temperature of +3 °C. The study to assess germination was conducted in two separate runs. In 2020 (1st run), the seed lots CZ-3-3-BRK-00168-36-2-Z (SMK – Central Moravian Carpathians); CZ-3-3-BRK-00126-08-3-S (KCK – Křivoklát Region and the Bohemian Karst) and CZ-3-3-BRK-00001-33-2-B (PCV – Foothills of the Bohemian Moravian Highlands) were tested. In 2021 (2nd run), the seed lots CZ-3-3-BRK-00126-08-3-S (KCK); CZ-3-3-BRK-00001-33-2-B (PCV) and CZ-1-2C-BRK-00008-38-2-B (BKV – White Carpathians and Vizovice Hills) were tested. The tested

seed lots were not identical in the two runs, because one seed lot tested in 2020 was no longer available in 2021. The regions and environmental conditions on the locations of the seed sources are summarised in Table 1. The general parameters of the seed lots are summarised in Table 2. The structure of registration numbers of the seed source is specified in Decree No. 29/2004 Coll., Annex No. 20. Except for the BKV seed lot originating from a seed orchard, the remaining seed lots originated from forest stands.

Processing of the fruits and storage of the seeds before experiments. In the seed processing plant, the fresh fruits were pressed and mashed. The seeds were separated from the pulp mass with a stream of water on a sieve and then dried. After final cleaning, the seeds were stored in cold storage at a temperature of +3 °C.

Pre-sowing treatment. Before stratification, clean seeds were sterilised in sodium hypochlorite (0.5% solution) for 30 minutes. Then the seeds were thoroughly rinsed and soaked in clean water for two hours. A mixture of peat and perlite (1:1 by volume) was sterilised at 100 °C for two hours and used as a stratification medium. Seeds from each seed lot were divided into three parts and mixed with the stratification medium at a 1:1 volume ratio and placed in a cooling chamber for stratification. The first part of each seed lot was stratified for 60 days (2 months), the second part for 90 days (3 months)



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Figure 1. Approximate location of the sources of seed lots in the area of the Czech Republic

1 – Křivoklát Region and the Bohemian Karst (Křivoklátsko a Český Kras – KCK); 2 – Foothills of the Bohemian Moravian Highlands (Předhoří Českomoravské vrchoviny – PCV); 3 – Central Moravian Carpathians (Středomoravské Karpaty – SMK); 4 – White Carpathians and Vizovice Hills (Bílé Karpaty a Vizovické vrchy – BKV)

Map source: SALSC 2024

Table 1. Characteristics of *Sorbus torminalis* seed sources tested in the study

| Registration number of the seed source (source of a seed lot) | Region of the origin (Natural Forest Area) | Forest vegetation zone* | Elevation range (m a.s.l.) | Mean annual precipitation (mm) | Mean annual temperature (°C) |
|---|--|-------------------------|----------------------------|--------------------------------|------------------------------|
| CZ-3-3-BRK-00168-36-2-Z | Central Moravian Carpathians | beech-oak | 351–400 | up to 600 | 7.5–8.0 |
| CZ-3-3-BRK-00126-08-3-S | Křivoklát Region and the Bohemian Karst | oak-beech | 401–550 | 600–700 | 7.5 |
| CZ-3-3-BRK-00001-33-2-B | Foothills of the Bohemian Moravian Highlands | beech-oak | 351–400 | up to 600 | 7.5–8.0 |
| CZ-1-2-BRK-00008-38-2-B | White Carpathians and Vizovice Hills | beech-oak | 351–400 | up to 600 | 7.5–8.0 |

*Forest vegetation zones represent climate in the forest classification system of the Czech Republic, see e.g. Viewegh et al. (2003) for further details

and the third part for 120 days (4 months). The stratification temperature was 2.5 °C.

Germination tests. Assessment of seed pre-treatment methods to promote germination was conducted in two runs: from February to May 2020 (1st run) and from April to July 2021 (2nd run). Different timing was due to the COVID-19 situation. After the cold stratification was completed (2, 3 or 4 months), the seeds were rinsed thoroughly with clean water to remove the stratification medium.

Only the seeds with intact seed coats (no signs of initiated germination) were selected for germination tests. Prematurely germinated seeds were excluded from germination tests; the reasons for this approach are explained in the Discussion chapter. Within both runs, altogether 400 seeds (dis-

tributed in four transparent boxes with 100 seeds) were included in the germination tests for each combination of a seed lot (6 lots) and length of cold stratification (60, 90, 120 days). In the boxes, the seeds were placed on filter paper (50 g·m⁻²) soaked in demineralised water. The boxes were covered with a transparent plastic lid to keep the moisture inside.

The germination was conducted in two QCell 200 climatic chambers (POL-LAB, Poland) that were equipped with fluorescent lights. Each germination test lasted 28 days with alternating temperatures of 20/30 °C (dark/light) and eight hours of illumination (at 30 °C) per day. Illumination at an intensity of 10 000–12 000 lx was provided using warm-white light fluorescent tubes. In accordance with the international rules for seed

Table 2. The seed quality parameters of the *Sorbus torminalis* seed lots used in the study

| Run (year) | Registration number | Abbreviation | Year of seed maturation | Purity (%) | Weight of 1 000 seeds (g) | Number of viable seeds per 1 kg | Viability (%) |
|------------------------|--------------------------------------|--------------|-------------------------|------------|---------------------------|---------------------------------|---------------|
| 1 st (2020) | CZ-3-3-BRK-00168-36-2-Z | SMK | 2016 | 99.6 | 24.806 | 38 947 | 97 |
| | CZ-3-3-BRK-00126-08-3-S ^a | KCK | 2016 | 95.2 | 19.362 | 47 202 | 96 |
| | CZ-3-3-BRK-00001-33-2-B ^a | PCV | 2018 | 96.9 | 22.637 | 42 378 | 99 |
| 2 nd (2021) | CZ-3-3-BRK-00126-08-3-S ^b | KCK | 2016 | 95.2 | 19.983 | 44 306 | 93 |
| | CZ-3-3-BRK-00001-33-2-B ^b | PCV | 2018 | 96.9 | 23.632 | 39 774 | 97 |
| | CZ-1-2-BRK-00008-38-2-B | BKV | 2018 | 98.8 | 25.396 | 36 959 | 95 |

^aResults of repeated quality testing on the same seed lot in 2018; ^bresults of repeated quality testing on the same seed lot in 2020; SMK – Central Moravian Carpathians (Středomoravské Karpaty); KCK – Křivoklát Region and the Bohemian Karst (Křivoklátsko a Český Kras); PCV – Foothills of the Bohemian Moravian Highlands (Předhoří Českomoravské vrchoviny); BKV – White Carpathians and Vizovice Hills (Bílé Karpaty a Vizovické vrchy)

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testing (ISTA 2015), the seeds were classified as completely germinated if their primary root and hypocotyl together exceeded four times the size (length) of the seed. Completely germinated seeds were counted at weekly intervals (days 7, 14, 21 and 28) and removed from the germination boxes. The rotten seeds were also counted and removed to prevent the spreading of potential infection. A cutting test was carried out at the end of the germination test (day 28) to allow complete determination of the final number of dead, empty and alive but ungerminated seeds.

Within the germination test, we investigated germination energy and germination capacity. Germination energy was defined as the percentage of completely germinated seeds on day 7 of the germination test, in accordance with the Czech Technical Standard ČSN 48 1211 (ČNI 2006). The germination capacity was viewed as the cumulative percentage of completely germinated seeds on day 28. The germination rate was computed according to Equation (1) below.

Data processing and statistical analyses. The data from both runs conducted in 2020 and 2021 were processed separately. For the evaluation of seed germination parameters, the data of the tested seed lots were pooled within each run. Multiple comparisons for binomially distributed data (Agresti et al. 2008) were used for the analyses of germination capacity and germination energy.

Within particular runs, the seed germination rate (*GR*) was calculated for each length of cold stratification as the sum of values obtained by dividing the weekly percentages of completely germinated filled seeds by the number of days the seeds had been germinated in the growth chamber, using the following Equation (1) adapted from Maguire (1962):

$$GR = \frac{GP_1}{\Delta t_1} + \frac{GP_2}{\Delta t_2} + \dots + \frac{GP_n}{\Delta t_n} \quad (1)$$

where:

- n – the week of counting;
- GP_n – current (not cumulative) percentage of completely germinated seeds in the n -th week;
- Δt_n – number of days elapsed from the beginning of the germination test (7, 14, 21 and 28 days).

The Kruskal-Wallis test with multiple comparisons was used for germination rates since the

character of the data did not meet the assumptions for parametric two-way analysis of variance (ANOVA). Multiple post-hoc comparisons of mean ranks of all pairs of groups according to Siegel and Castellan (1988) were used. The chosen significance level α for all the conducted analyses was 0.05.

RESULTS

As for the comparison of the seeds of various geographical origins included in the study (Table 3), the highest values of germination capacity were recorded for the SMK seed lot in 2020 after a 4-month cold stratification and for the BKV seed lot in 2021 after a 3-month stratification. Low values of germination capacity, which did not exceed 16.5%, were recorded for the KCK seed lot. Germination parameters varied between seed lots (seed origins). Nonetheless, general trends of responses to the length of stratification were similar.

The development of the cumulative germination percentage of seeds during the 1st run of germination tests (in 2020) is depicted in Figure 2. On all counting dates (on days 7, 14, 21 and 28, respectively), the percentage of germinated seeds differed significantly between the compared stratification lengths. The seeds that were subjected to four-month cold stratification showed the highest cumulative percentages of germinated seeds. The seeds that were pretreated with cold stratification for two months did not almost germinate.

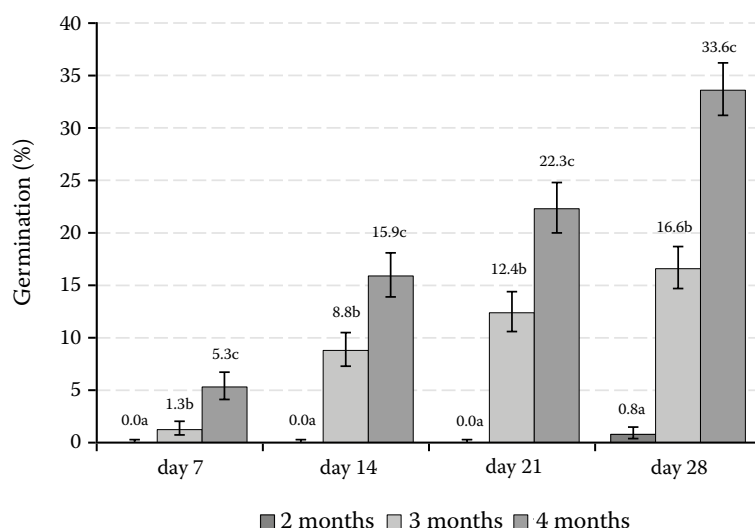
Also, in the 2nd run conducted in 2021 (Figure 3), the differences in cumulative germination percentages between the compared lengths of cold stratification were significant on all counting days. However, the differences between two-month and three-month cold stratification were considerably higher than in the run conducted in 2020. In contrast to the 1st run, the values of cumulative germination energy were slightly in favour of the three-month pretreatment. Like in the case of the 1st run, seeds stratified for only two months did not almost germinate practically in the 2nd run.

The values of the germination rate (Figure 4) confirm almost zero efficiency of the 2-month stratification. In the 1st run (2020), the germination rate indicated a markedly lower response of the seeds after a 3-month cold stratification in comparison with a 4-month cold stratification. On the other hand, the seeds included in the 2nd run (2021) re-

Table 3. Mean values of cumulative germination percentages (%) of seeds after various lengths of cold stratification (moist chilling)

| Seed lot | Run (year) | Stratification length (months) | Day of count | | | | | | | |
|----------|---------------------------|--------------------------------------|--------------|------|------|------|------|------|------|------|
| | | | 7 | | 14 | | 21 | | 28 | |
| | | | mean | SD | mean | SD | mean | SD | mean | SD |
| PCV | 1 st (2020) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.5 | 0.58 |
| | | 3 | 1.0 | 1.41 | 10.3 | 4.03 | 14.5 | 5.92 | 16.3 | 6.18 |
| | | 4 | 6.5 | 3.51 | 11.5 | 3.11 | 14.5 | 2.89 | 15.5 | 3.11 |
| KCK | 1 st (2020) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.8 | 0.50 |
| | | 3 | 0.8 | 0.96 | 3.5 | 2.65 | 4.8 | 2.63 | 5.3 | 2.06 |
| | | 4 | 1.8 | 1.71 | 7.8 | 1.71 | 11.8 | 2.22 | 13.3 | 2.22 |
| SMK | 1 st (2020) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 1.0 | 0.82 |
| | | 3 | 2.0 | 1.83 | 12.8 | 2.87 | 18.0 | 5.10 | 21.3 | 5.38 |
| | | 4 | 7.8 | 4.27 | 28.5 | 5.92 | 40.0 | 8.83 | 46.3 | 9.43 |
| PCV | 2 nd (2021) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.8 | 0.50 |
| | | 3 | 10.5 | 2.08 | 12.8 | 2.50 | 14.3 | 3.40 | 16.0 | 4.32 |
| | | 4 | 13.0 | 1.83 | 16.3 | 2.22 | 17.0 | 2.71 | 17.5 | 3.11 |
| KCK | 2 nd (2021) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.5 | 0.58 |
| | | 3 | 9.3 | 1.89 | 12.3 | 2.63 | 13.5 | 2.65 | 15.3 | 3.30 |
| | | 4 | 1.0 | 0.82 | 11.0 | 3.37 | 14.8 | 4.03 | 16.5 | 4.80 |
| BKV | 2 nd (2021) | 2 | 0.0 | 0.00 | 0.0 | 0.00 | 0.0 | 0.00 | 0.80 | 0.50 |
| | | 3 | 12.5 | 5.80 | 28.3 | 9.36 | 30.3 | 9.88 | 32.3 | 9.74 |
| | | 4 | 14.5 | 3.87 | 22.3 | 0.50 | 24.8 | 1.71 | 25.8 | 2.99 |

Mean – mean values referring to days 7, 14, 21 and 28 since the beginning of the germination tests; SD – standard deviation; PCV – Foothills of the Bohemian Moravian Highlands (Předhoří Českomoravské vrchoviny); KCK – Křivoklátsko Region and the Bohemian Karst (Křivoklátsko a Český Kras); SMK – Central Moravian Carpathians (Středomoravské Karpaty); BKV – White Carpathians and Vizovice Hills (Bílé Karpaty a Vizovické vrchy)

Figure 2. Cumulative germination percentages of seeds of wild service tree (*Sorbus torminalis*) on days 7, 14, 21, and 28 in the 1st run conducted in 2020

a–c – significant differences ($P < 0.05$) among the lengths of cold stratification within each counting day; error bars – 95% confidence interval

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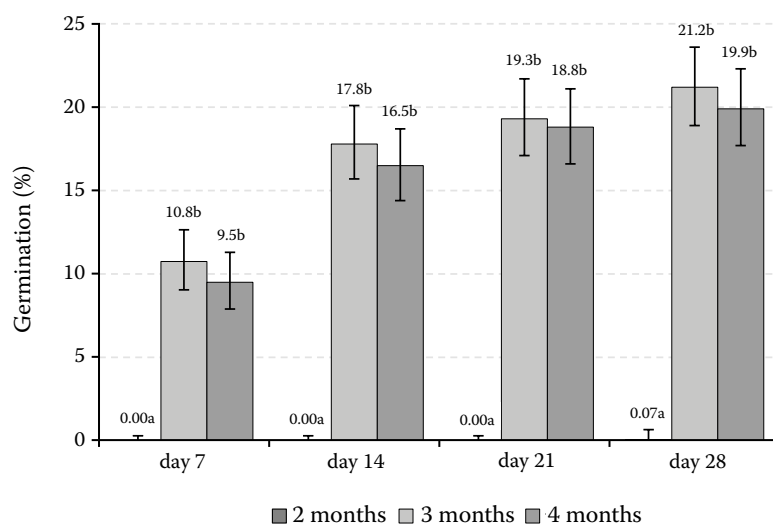


Figure 3. Cumulative germination percentage of seeds of wild service tree (*Sorbus torminalis*) on days 7, 14, 21, and 28 in the 2nd run conducted in 2021

a–c – significant differences ($P < 0.05$) among the compared lengths of cold stratification on the respective counting days; error bars – 95% confidence interval

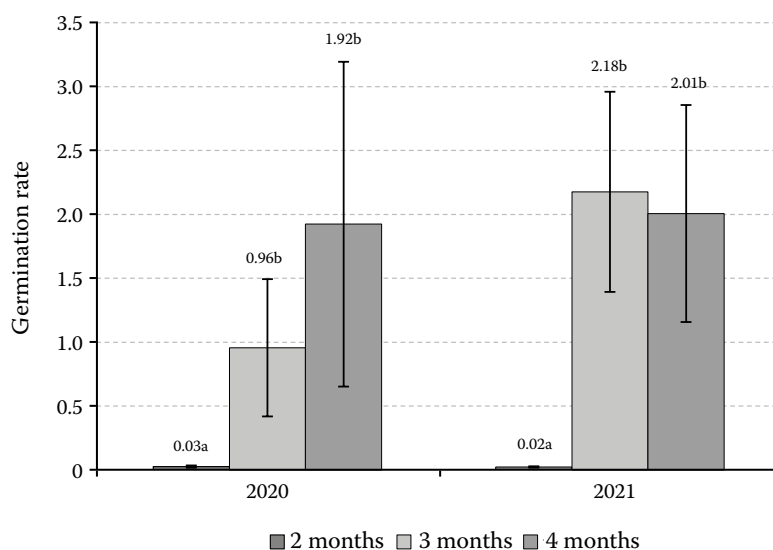


Figure 4. Comparison of the germination rate (mean \pm standard deviation) of seeds of wild service tree (*Sorbus torminalis*) a, b – statistically significant differences in germination rates for different lengths of cold stratification at $\alpha = 0.05$ for each run (2020 and 2021)

sponded to a 3-month cold stratification similarly as they did to a 4-month stratification. Also, the onset of more intense germination occurred earlier in the 2nd run from the start of the germination test than in the 1st run. However, the values of germination capacity in the 2nd run were markedly lower for both the 3-month and 4-month stratification than the germination capacity of the 4-month stratification in the 1st run.

DISCUSSION

The study aimed to evaluate the germination parameters depending on the duration of cold stratification and to find out the optimum length for cold stratification of *Sorbus torminalis* seeds in conditions of the Czech Republic.

Our results show that *Sorbus torminalis* seeds need a period of at least 3 months to break the dor-

mancy. This outcome basically corresponds with the findings reported in the literature. According to the review of Var et al. (2010), the *Sorbus torminalis* seeds did not germinate without stratification. Also, Pipinis et al. (2015) reported that non-stratified and 1-month stratified seeds of *Sorbus torminalis* exhibited very low or zero germination. According to Var et al. (2010), the maximum germination of *Sorbus torminalis* occurred after 4 months of cold stratification (2 °C) in a mixture of peat and perlite. This is in accordance with the results of our study and the ISTA (2015) rules. Similarly, Hoffmann (2007) recommended a 4-month seed pretreatment, although in the form of a combination of room-temperature incubation (~20 °C) and subsequent moist chilling.

Our results show interannual and regional variability in germination percentage. Generally, the lower germination percentage in the 2nd run could be related to the senescence of the seed lots. However, *Sorbus torminalis* seeds are classified as an orthodox seed type (Forest Research 2025). Therefore, we consider this aspect as negligible. It is difficult to assess different timing of the runs within a year. This is because the seed was stored in a cold store for a long time and the stratification as well as germination tests were conducted under controlled indoor conditions that were not directly influenced by the outdoor environment. We cannot completely exclude different conditions to which the seed may have been subjected during transportation from the supplier or simply undetected variability in the conditions of the individual runs (2020 and 2021) of the experiment, even though these runs followed an identical protocol. The variability of the seed quality among the provenances usually depends on stand conditions, i.e. soil, climate, local weather conditions, etc. (Var et al. 2010) and genetics.

However, rather than on comparing provenances, our study focused on germination response as a common function of stratification length. Different seed lots were included in the experiment as a source of variability despite which such a trend must be evident.

The overall values of germination capacity (derived from pooled outcomes for all seed lots within particular runs) did not exceed 35% in our experiment, although there were some seed lots with a significantly better value of this parameter. Also, Nicolescu et al. (2009) stated that the germination capacity of *Sorbus torminalis* seeds is low.

On the other hand, it should be reminded at this point that only the seeds which had not begun to germinate at the time of the start of the germination tests were included in the germination tests. In the operation conditions of forest nurseries or seed production facilities, premature germination (of species with small seeds in particular) is often a problem. In common forest nurseries, seeds should not be sown at an advanced stage of germination. The energy resources in the prematurely germinated seeds are at least partly depleted or the deformities in seedlings often occur. The prematurely germinated seeds are thus often practically lost for common production. Therefore, following the nursery practice, we did not include any prematurely germinated seeds in germination tests in our study.

For the above-mentioned reasons, the optimum stratification length under the given stratification conditions should also be considered concerning the need to reduce the number of seeds that prematurely germinate during the stratification period before the sowing date. During the cold stratification in our study, seeds spontaneously germinated within the 4th month of stratification despite the low cooling temperature. It was difficult to select the seeds that had not yet initiated germination for the germination test after the 4th month of stratification. Therefore, to set the appropriate length of stratification, it is needed to take into account not only the number of seeds germinated in the germination test but also the percentage of prematurely germinated seeds during the stratification period. For evaluation of the suitability of various lengths of stratification for forest nurseries, future studies should also include recording the proportion of prematurely germinated seeds.

The results by Pipinis et al. (2015) suggested that the seeds of *Sorbus torminalis* and *Sorbus domestica* respond to the length of cold stratification (moist chilling) similarly or analogously. According to these authors, the 3-month period of stratification at 3–5 °C is essential and probably sufficient for breaking dormancy in the seeds of both species. The germination tests by Pipinis et al. (2015) followed a different protocol from our study. From the methodological part, it was not clear whether the seeds that began to germinate during stratification were included in the tests or counted as germinated or not. Also, Prknová (2015) concluded for *Sorbus domestica* that dormancy was completely broken

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after stratification at temperatures of about 5 °C for 3 months (13 weeks). Takos and Efthimiou (2002) showed that the stratification process of *Sorbus torminalis* seeds can also take place in outdoor conditions, typically after being sown in autumn in the nursery. The last-mentioned method is applicable to bare-rooted nurseries; however, it cannot be applied easily to seeding into containers in nurseries with clearly timed production schedules.

To reduce the percentage of seeds that germinate prematurely during cold stratification (moist chilling), a shorter stratification period of about 3 months could be considered with subsequent sowing of seeds early enough in spring in a moist substrate where stratification could be completed. However, further research is needed.

The issue of dormancy and the necessary pretreatment of *Sorbus torminalis* seeds seem complex and more factors may play a role. For example, the physiological attributes of *Sorbus torminalis* seeds may differ between different trunk diameter classes of trees. The best germination rate was recorded in trees with a trunk diameter of around 30 cm and the seeds collected from the middle-aged trees needed a shorter stratification period than those from younger or older age classes (Espahbodi et al. 2007).

Seeds of some species of the genus *Sorbus* show deeper physiological dormancy than *Sorbus torminalis*. In contrast to findings by Pipinis et al. (2015), the typical European-Asian species *S. aucuparia* requires warm-cold or cold stratification up to 180 days according to the review by Raspé et al. (2000). Seeds of *Sorbus alnifolia* (originated from East Asia) need at least 150 days of cold stratification to overcome dormancy and enhance germination that, however, needs to be conducted at lower temperatures (5/15 °C) than in *Sorbus torminalis* (Tang et al. 2019). Prknová (2015), Tang et al. (2019), and Dinçer (2023) further reported that the removal or scarifying of the seed coat is quite an efficient way to break the seed dormancy of *Sorbus domestica*, *S. alnifolia* and *S. aucuparia*.

In the light of the obtained results and information in the literature, the pre-sowing treatment of *Sorbus torminalis* seeds is complicated by a portion of seeds which start to germinate during the stratification period. It may be considered to use the method of optical separation of prematurely germinating seeds. The separated seeds could be sown immediately after separation,

or theoretically, they could be frozen and stored for future sowing, if the freezing of germinated seeds shows to be possible for the wild service tree. This method has been successfully applied in the seed pretreatment of European beech (*Fagus sylvatica* L.), when germinated seeds with emerging radicles are separated using the optical sorter and then stored at a temperature slightly below 0 °C until the sowing time (Zeman 2019). However, according to our knowledge, the applicability of this approach to *Sorbus* has not been tested yet.

CONCLUSION

Two months of cold stratification (moist chilling) are insufficient to break the dormancy of *Sorbus torminalis* seeds. Seeds stratified for two months showed almost zero germination. At least three months of the pretreatment were needed for the successful initiation of germination of *Sorbus torminalis* seeds. Three-month and four-month stratification periods were generally comparable with each other, nonetheless, substantial differences were recorded within the individual seed lots and years of the experiment. Based on our observations, for future research to evaluate the suitability of a particular length of cold stratification period for *Sorbus torminalis*, the percentage of seeds that begin to germinate prematurely in the stratification medium before the germination tests should also be separately determined and taken into account.

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