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How to accelerate the germination of Scots pine and Norway spruce seeds?

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Abstract: The aim of the study was to confirm and compare efficiency of methods enhancing the vitality of Scots pine and Norway spruce seeds: soaking in water, cold stratification, additional moistening and incubation according to IDS method. The examined parameters included water content in the seeds, germination energy, mean germination time and germination capacity before the seed treatment, after its treatment by the tested methods and after drying. Results show that all the tested methods accelerate germination of seeds; cold stratification is the most efficient and recommended method for Scots pine and soaking of seeds in water is the most efficient and recommended for Norway spruce. The best results in spruce were also obtained with cold stratification (comparable with soaking in water) but the method is complicated, longer-lasting and more costly than soaking in water.

Keywords: vitality; water content; germination capacity; germination energy; mean germination time

Seeds of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* /L./ H. Karst.) are not dormant and hence they do not need pre-sowing treatment. The ongoing climatic change adversely affects the seed crop from the stands, both the incidence of seed years, crop amount and the quality of collected seeds (Martinec et al. 2019). Due to more frequent drought periods, vitality of seeds is lower in particular as it is affected among other factors also by weather at the time of maturation (Bezděčková, Matějka 2018). In the production of containerized planting stock the share of which has been continually increasing in the Czech Republic (Foltánek et al. 2018), fast achievement of homogeneous seedlings in forest nurseries provides more time for repeated sowing in plastic greenhouses and hence higher seed yields. These are reasons for including a pre-sowing treatment also in the system of Scots pine and Norway spruce seed management in order to speed up seed germination.

Vincent (1965) and Dušek et al. (1970) informed about many procedures that were tested for stimulating seed germination such as exposure of seeds to diverse forms of energy (heat, light, radiation, ultrasound) and treatment with chemicals (inorganic and organic compounds, growth stimulators). Although some positive results were reported in laboratory tests of seed germination capacity, evidences about faster and more uniform emergence of stimulated seeds are few. Moreover, the

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procedures were specific to individual tree species and provenances, and usually required preliminary testing of concentrations and treatment times on seed samples. In addition to their positive influence on germination rate (Sanberg 1988), growth stimulators improve morphological parameters of seedlings emerged from treated seeds (Khurshkaynen et al. 2019). Brassinosteroids accelerate germination and increase germination capacity of Scots pine and Norway spruce during drought stress (Kuneš et al. 2017). A positive influence on the germination rate of pine seeds was demonstrated after surface sterilization of seeds in the solution of sodium hypochlorite (Wenny, Dumroease 1987). The authors claimed this method to be particularly good for the seeds of Scots pine whose robust seed coat can resist oxidation effects of bleach but they did not advise it for Norway spruce. A positive effect on the germination of seeds of some plant species was also shown in nanomaterials, namely those containing Zn and Cu. However, Ryabinina et al. (2019) demonstrated a negative effect of some of them at certain concentrations exactly in Scots pine (reduction of the root system development and biomass) and warned against potential risks of their use.

Physiological treatment seems to be primarily a promising technique for increasing seed vitality. Kantor (1952), ČSN 48 2121 (1956), Sarvaš et al. (2010) and other authors described the soaking of both pine and spruce seeds in water prior to sowing, which accelerates water uptake by seeds and hence shortens the interval from sowing to emergence. Hrabí (1990) confirmed increased germination energy of seeds soaked in water after long-term storage up to a double of the initial value. Even better results were achieved by Barnett and McLemore (1967) after soaking loblolly pine seeds in oxidized water. Procházková (2004) and Hoffmann et al. (2005) mentioned cold stratification (so-called stimulation by prechilling) as a technique suitable for increasing the vitality of Scots pine and Norway spruce. Cold stratification favourably affects both germination energy and yield of seedlings in common coniferous tree species in spite of the fact that they are not dormant (Gordon 1976 in Gordon 1992). Procházková (2004) recommended the soaking of seeds in water at 3–5 °C for 24 h, their surface drying and return to a temperature of 3–5 °C for 3–6 weeks. Simak et al. (1984) found out that faster and more seeds of Scots pine germinated also following the osmotic stimulation by soaking in polyethylene glycol (PEG) solution. According to the authors, the positive effect of this treatment would be manifested especially in suboptimal conditions of germination (low temperature, deep sowing, diverse material for seed covering, worse climatic conditions). Huang and Zou (1989) warned however that drying after osmotic treatments may damage the seed membrane if the drying period is more than 24 hours. The positive effect of PEG on seed quality was also observed in Norway spruce (Procházková 2004). Bergsten (1987) described the IDS method by which the unproductive dead seeds are removed from a seed lot in three steps – incubation (water supply), drying (dead seeds lose water more quickly) and separation (floating in water). The first step of the IDS method – incubation of seeds with a water content of 30% at 15 °C for 8–12 days – has a comparable positive influence on the vitality of seeds with the PEG osmotic (Bergsten 1989). Moreover, the author claimed that it should have a greater potential in operational practice than the soaking of seeds in the PEG solution with regard to higher costs for the osmotic treatment. Downie et al. (1993) even confirmed that the germination of seeds after incubation applied within the IDS method was faster than after the osmotic stimulation.

The aim of this study was therefore to confirm and compare the efficiency of some methods in increasing the vitality of Scots pine and Norway spruce seeds. The selected methods were simple, easy to do and included soaking of seeds in water and cold stratification, and incubation of seeds, which is the first phase of the IDS method. The IDS method appears promising in terms of its use for precise sowing in the production of containerized planting stock. Equipment for its implementation was tested during the research.

MATERIAL AND METHODS

Germination parameters were evaluated in two Scots pine and two Norway spruce seed lots coming from the Czech Republic in 2019 (Table 1). In results, they are marked with the serial number of reproductive material from the number of accredited unit.

Six samples were collected from each seed lot. Sample 1 was always control with no pre-sowing treatment. Sample 2 was subjected to pre-sowing treatment by soaking in water for 48 h at 5 °C, with

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Table 1. The provenance of the Scots pine and Norway spruce seed lots analysed

Tree species	Labelling of seed lots	Region of provenance	National identification of seed origin	Year of collection	Weight of 1 000 seeds (g)
Scots pine	1443	Natural forest region (PLO) 16	CZ-2-2B-BO-1443-16-5-J	2007	6 653
	3154		CZ-2-2B-BO-3154-16-4-J		6 182
Norway spruce	unknown	unknown	unknown	unknown	8 066
	1361	Natural forest region (PLO) 27	CZ-2-2A-SM-1361-27-8-E	2005	5 840

water being replaced with a new portion after 24 h of soaking. Sample 3 was subjected to cold stratification; it was soaked in water at 5 °C for 48 h, the water was replaced with a new portion after 24 h, the seed surface was dried and the seed was placed into a polyethylene bag for stratification at 5 °C and occasional aeration for 21 days. The size of the first three samples was 15 g (2 × 5 g for water content determination and 1 × 5 g for germination capacity test). Sample 4 was subjected to the first phase of the IDS method. It was additionally moistened by adding water to the water content of 30% (Scots pine) and 35% (Norway spruce) at 15 °C, 100% relative humidity (RH) in the incubator in cylinders sealed with Gore-Tex membrane and after moistening, sample 5 was incubated for 3 days at ca. 10 °C with rinsing of seeds with water (i.e. further water content increase). Air temperature and RH in the incubators were monitored with a Minikin THi sensor (Environmental Measuring Systems s.r.o., Czech Republic). After the treatment, incubated samples 6 of Scots pine were dried on the surface and additionally dried in the laboratory dryer above silica gel back to water content suitable for storage, i.e. 6% (unlike the soaking and stratification, the IDS method need not be applied immediately before sowing). Samples 6 of Norway spruce were dried in the fluid dryer to water content of about 20% suitable for the separation of dead seeds from the seed lots. The size of samples for incubation within the IDS method was 500 g.

The control samples as well as the samples after the pre-sowing treatment were examined for water content and subjected to the test of germination capacity complying with prescribed standards (ČSN 48 1211:2006; ISTA 2011). Parameters evaluated within the germination test involved:

Germination capacity (GC) is defined to be equal to the number of normally germinated seeds at the end of the experiment (after 21 days) out of the total number of seeds:

GC (%) = total number of germinated seeds /total number of sown seeds × 100.

Germination energy (GE) is defined to be equal to the number of normally germinated seeds determined on Day 7 after setting up the germination test and specified in the percentage of germinating seeds:

GE (%) = total number of germinated seeds determined on Day 7/total number of sown seeds × 100.

Mean germination time (MGT) is a measure of the rate and *time*-spread of *germination,* it was calculated using the formula:

$$\text{MGT (days)} = \Sigma(T1 \times n1 + T2 \times n2 + \ldots + Tk \times nk)/\Sigma(n1 + n2 + \ldots + nk)$$

where:

n – the number of newly germinated seeds on the first, second and subsequent days;

T – the weight given to the number of germinated seeds on the first, second and subsequent days.

The data obtained for the germination characteristics were transformed into a normal distribution using the arc-sin transformation. Comparison of the individual variants was carried out by multi-factorial ANOVA with a significance level (α) of 0.05 using the Statistica programme (Ver. 12, 2013). The vertical columns in Figures represent 2× standard deviations. Multiple comparisons, i.e. the determination of differences or zero differences between the variants, were made using the LSD test.

RESULTS

The initial water content in the tested seeds was 5–7% (Table 2) and it increased to 27–36% by soaking in water and 21–27% by cold stratification. Higher initial water content in the seeds (spruce seed lot 1361) indicated higher water content after soaking and stratification. Additional moistening within the IDS method helped to reach water contents of ca.

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Table 2. Water content in the tested samples of Scots pine and Norway spruce seeds in variants of different pre-sowing treatments

Tree species	Labelling of seed lots	Water content (%)					
		control	soaking in water	stratification	IDS moistening	IDS moistening and incubation	IDS moistening, incubation and drying
Scots pine	1443	5.4	31.5	23.6	27.6	54.4	6.0
	3154	5.2	28.3	23.4	26.5	55.2	6.3
Norway spruce	Unknown	5.1	26.9	20.8	35.2	50.3	20.6
	1361	6.7	35.6	26.6	34.1	51.1	20.3

27% in pine seeds and ca. 35% in spruce seeds. After incubation by rinsing, the water content in all seeds increased to 50–55%. The drying for further storage reduced the water content in the incubated pine seeds to ca. 6%. The implemented following phase of the IDS method – drying – reduced the water content in the incubated spruce seeds to ca. 20%.

The germination capacity of most tested seed lots without the pre-sowing treatment was low (up to 50%), only the unknown seed lot of Norway spruce exhibited a higher value (85%) (Figure 1). Germination capacity was not much affected by any of the pre-sowing treatment variants, only in pine seed lot 3154 and spruce seed lot 1361, statistically significant differences were recorded between the control with no pre-sowing treatment and the variant of additional moistening and incubation with the IDS method ($P < 0.05$), where the germination capacity exhibited a decrease by ca. 10%. In contrast, the germination capacity markedly increased (by 10%) af-

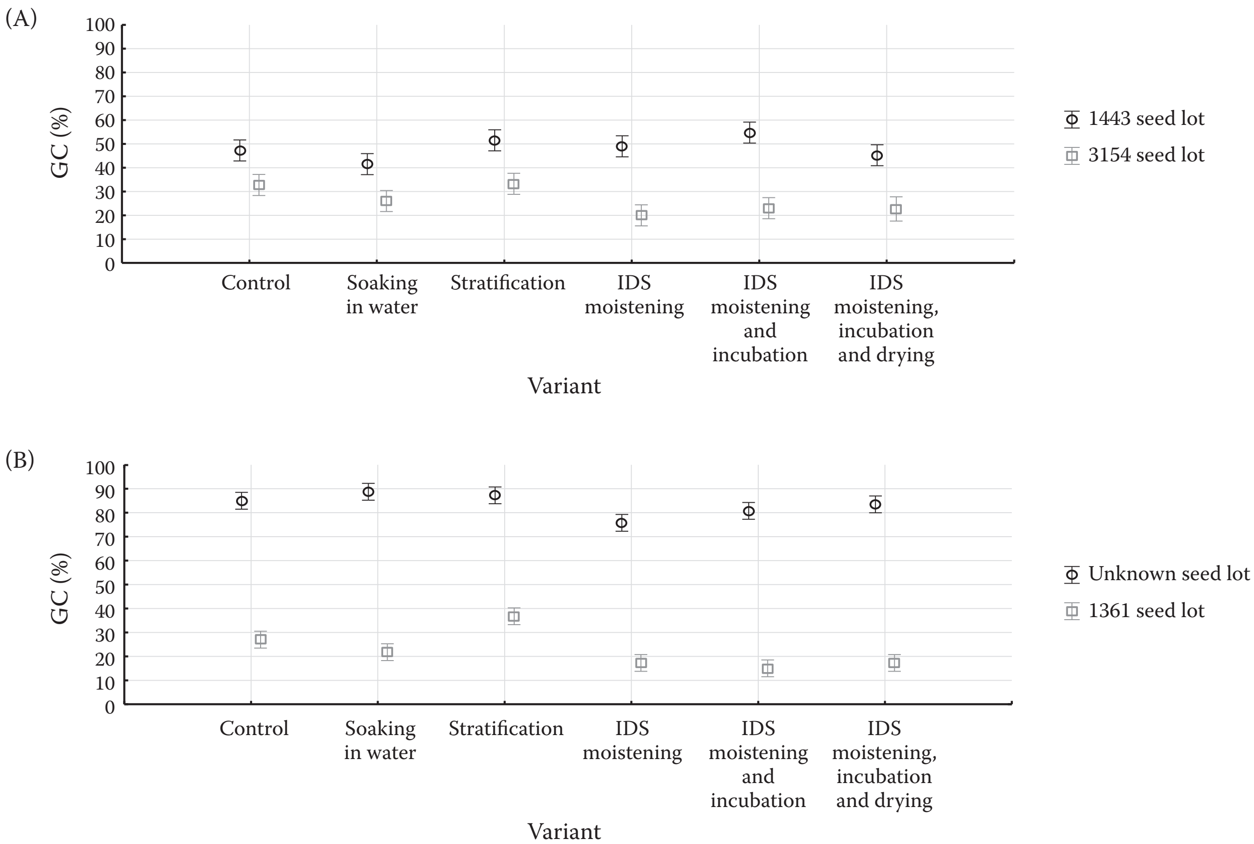

Figure 1. Germination capacity (%) of Scots pine (A) and Norway spruce (B) seeds in the variants of different pre-sowing treatments

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ter stratification in seed lot 1361 as compared with the control seeds without any pre-sowing treatment (P = 0.002).

All the tested techniques of pre-sowing treatment significantly ($P < 0.05$) increased the germination energy of the seeds (Figure 2). The highest values of germination energy, which approached the germination capacity of seeds, i.e. maximum values, were recorded in seeds after cold stratification. In the seeds of spruce, germination energy was high also after soaking the seeds in water, in the unknown seed lot even comparable with the seed after stratification (P = 0.69). On the other hand, soaking of pine seeds in water increased the germination energy at least of all pre-sowing treatment variants. Additional moistening of seeds within the IDS method statistically significantly increased the germination energy only in the seeds of higher quality (with the initial germination energy above 10%, i.e. pine seed lot 1443 and the unknown seed lot of spruce), and the subsequent incubation of seeds with the IDS method further considerably increased the energy of germination. Drying of incubated pine seeds to water content suitable for storage (6%) significantly reduced the germination energy ($P < 0.05$), but in the seed lot with the higher initial germination energy (above 10%) it was still higher than in the control untreated seeds. Drying of incubated spruce seeds to water content suitable for the separation of dead seeds by the IDS method (20%) did not change the germination energy increased by incubation (P = 0.31 for the unknown seed lot, P = 0.37 for seed lot 1361).

The longest mean germination time was exhibited by the control seed without pre-sowing treatment – both in the Scots pine and in the Norway spruce (Figure 3). The Scots pine did not show any statistically significant differences in mean germination time (at all times $P > 0.05$) in the variants of pre-sowing treatment; in the Norway spruce, the slowest emerging seeds were those after additional moistening within the IDS method and the most rapidly emerging seeds were those after soaking and stratification. No statistically significant difference was observed in the mean germination time between spruce seed soaking and stratification (P = 0.93 for

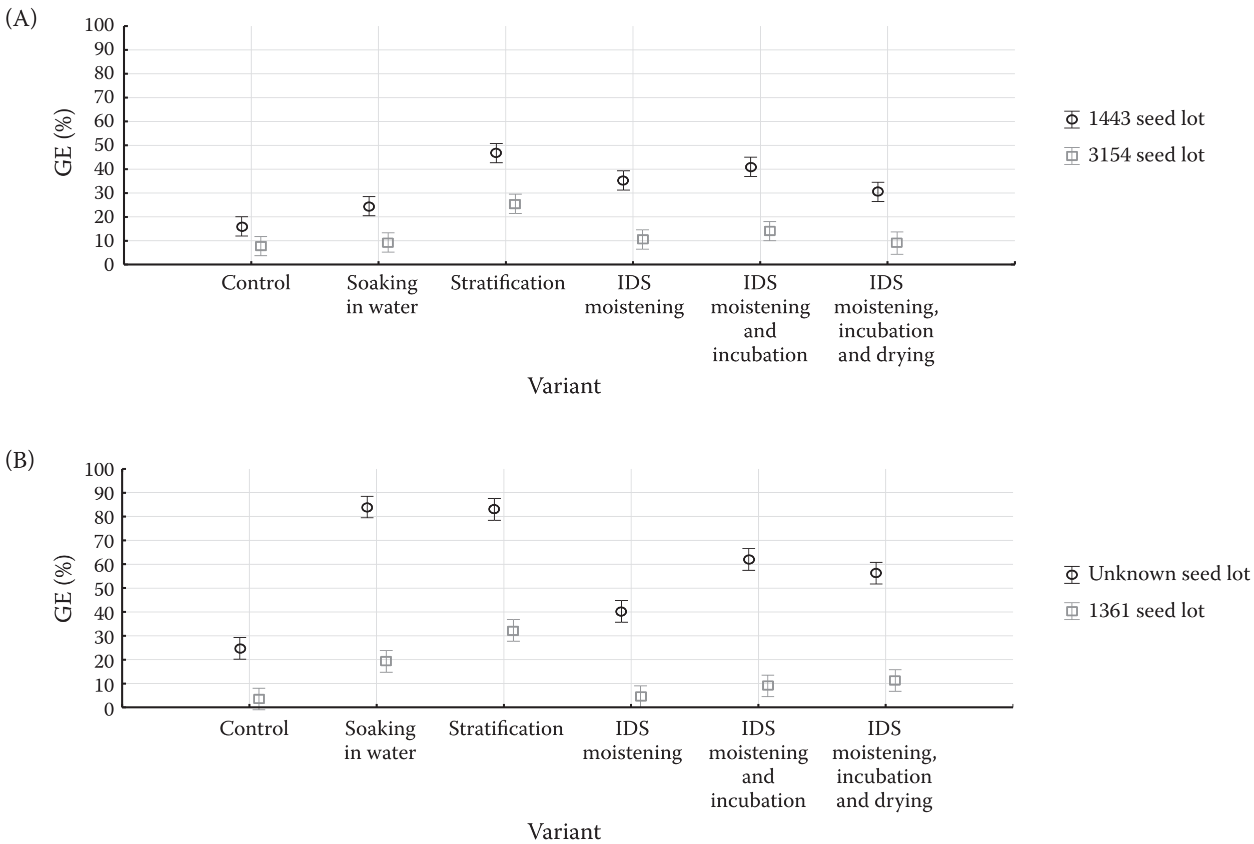

Figure 2. Germination energy (%) of Scots pine (A) and Norway spruce (B) seeds in the variants of different pre-sowing treatments

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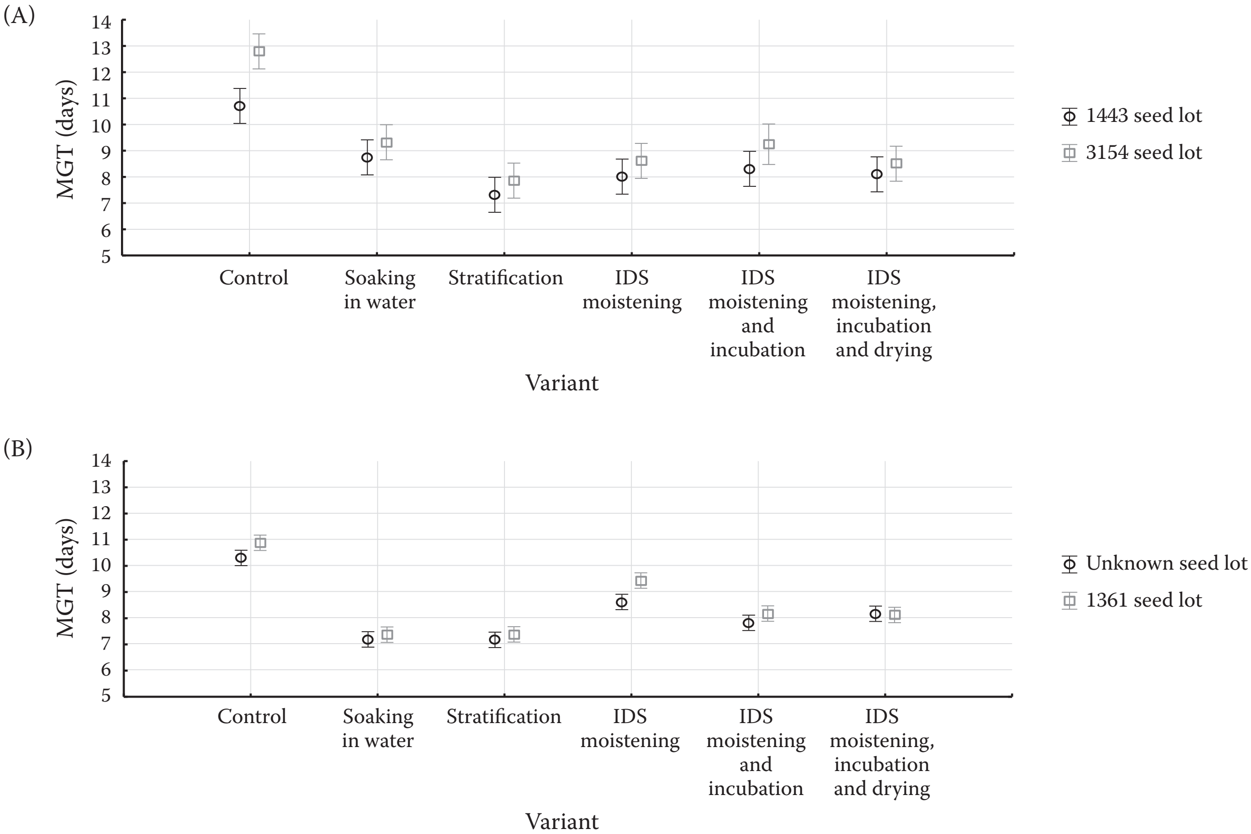

Figure 3. Mean germination time (days) of Scots pine (A) and Norway spruce (B) in the variants of different pre-sowing treatments

the unknown seed lot, $P = 0.95$ for seed lot 1361). Incubated spruce seeds (within the IDS method) germinated more slowly than after soaking and stratification but faster than after mere additional moistening or seeds without any pre-sowing treatment. Drying of incubated seeds had no influence on the germination rate expressed by the parameter of mean germination time; no statistically significant differences between the seeds before and after drying were found (at all times $P > 0.05$).

DISCUSSION

Recently, the rate of germination or seed vitality has been affected by frequent drought spells occurring in the vegetation season. The growing of containerized planting stock in particular needs the sown seeds to emerge as fast as possible and to be homogeneous, which is a prerequisite for the high yield of seedlings. According to Hrabí (1990), Procházková (2004), Hoffmann et al. (2005), the vitality of non-dormant seeds of Scots pine and Norway spruce can be enhanced by a pre-sowing treatment.

The seeds used in this study were of low quality as they had been stored for a long time (12–14 years). That is why mainly their vitality was not good but the effect of the pre-sowing treatment should be evident in such seeds. Seeds of Scots pine germinate the fastest after cold stratification. Mean germination time reached the lowest values after stratification albeit no statistically significant differences were demonstrated compared with the other variants of pre-sowing treatment. Nevertheless, seed germination energy was clearly the highest in both tested seed lots. This method of pre-sowing treatment was recommended for Scots pine also by e.g. Procházková (2004); Hoffmann et al. (2005) and Gordon (1976 in Gordon 1992). Chang et al. (1991) found out furthermore that one or two dehydration-rehydration cycles after cold stratification improved both the percentage and the rate of pine seed germination. Nonetheless, the majority of germinable seeds tested by us germinated within 7 days from the beginning of germination test; thus, the germination energy approached the germination capacity (difference ca. 10%). A further

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treatment of stratified seeds would have had only a low effect. The 21-day stratification therefore appears as an optimal pre-sowing treatment for Scots pine although Hofmann et al. (2005) recommended its duration to be 30–60 days. It also seems obvious that more different parameters should be monitored when assessing the seed vitality (here MGT and GE) in order to obtain precise information about the rate of seed germination.

In the Norway spruce, the efficiency of 21-day cold stratification for increasing the seed vitality was even higher than in the Scots pine. After this pre-sowing treatment, seeds were germinating considerably faster than the control, which was demonstrated by both monitored parameters of germination rate (MGT and GE). Germination energy was comparable with seed germination capacity in both seed lots, i.e. more or less all live seeds germinated within 7 days from the beginning of germination. Cold stratification was recommended for Norway spruce also by a number of authors (e.g. Procházková 2004; Gordon 1976 in Gordon 1992). Hofmann et al. (2005) set its duration at 28 days. Soaking the seeds in water, which notably increased germination energy and reduced mean germination time, had the same effect as cold stratification in Norway spruce. Germination energy was comparable with seed germination capacity once again. In the seeds of poorer quality (seed lot 1361), germination energy increased by ca. 15%, which is in line with results published by Hrabí (1990). In the seeds of better quality (unknown seed lot), germination energy increased even by 65%, which apparently has to do with the generally slower germination of spruce seeds compared to pine seeds, as described by Bezděčková and Matějka (2018). Most seeds of higher vitality are capable of germinating without any pre-sowing treatment within about 10 days from establishing the test of germination capacity but not within 7 days when germination energy is evaluated. The efficiency of pre-sowing treatment can be affected also by the age of seeds. The older the seed, the lower the GE and the more expressive the stimulating effect could be. Hrabí (1990) and Hofmann et al. (2005) claimed the optimal soaking time to be 24 h, which corresponds to water content in the seeds after surface drying (20–30%). According to Hrabí (1990), cytological analyses showed that seeds treated in this way exhibit the already started embryonic growth processes in them, and although the mitotic activity in the seeds is lower than for example in larch, extended soaking would not increase it further with respect to oxygen deficit in tissues. However, the author tested soaking in water for 24 h at a temperature of 17–20 °C (room temperature). Results published by Schmidt (1930 in Vincent 1965) show a frequent failure with soaking spruce seeds for 20 h at room temperature, and the author recommended a soaking time of 10 h. Slower uptake of water by the seeds at lower temperatures is considered friendlier and thus, the soaking of spruce seeds for 48 h at 5 °C tested by us appears an optimal pre-sowing treatment for spruce seeds. Although the cold stratification has the same effect, it takes more time and is therefore economically less favourable.

The soaking of pine seeds in water positively affected vitality only in better-quality seeds; unlike in spruce, the positive effect was however the lowest of all tested methods of pre-sowing treatment. Germination energy increased from ca. 15% to 25%, whereas Hrabí (1990) confirmed that in long-term stored seeds, germination energy of seeds can be increased at all times to a double by soaking in water. Similarly, Kantor (1952) confirmed the yield of pine seedlings after sowing seeds soaked in water into mineral soil to be twice as high and sowing them into humus to be even seven times higher. After sowing the soaked seeds, he also obtained seedlings with better morphological parameters, which was probably related to their faster emergence. Dušek et al. (1970) also recommended to increase water content in the seeds of conifers (pine too) before sowing by moistening on piles or by soaking in water for 24 hours while according to them longer soaking gave unfavourable results. Contrariwise, Schmidt (1930 in Vincent 1965) did not recommend soaking the pine seeds as the emergence rate of seeds after soaking was lower in many a case than in the control seeds with no pre-sowing treatment. Bogdanov (in Vincent 1965) mentioned the lower emergence rate of pine seeds after soaking in water, which was corroborated also by the results of our tests.

The additional moistening and namely the combination of moistening and incubation of seeds within the IDS method had a positive influence on the germination rate of both pine and spruce seeds, which is in line with the results published by Bergsten (1987, 1988, 1989). Creasey (2001) pointed out that a process of mitigating dormancy or accelerating germination within IDS incubation can be induced only in seeds with high water content (28–35%), and

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this is why the seeds that were just moistened did not exhibit so low MGT and so high GE values as the seeds after the subsequent incubation during which they received more water. In fractions of Scots pine seeds with the highest germination capacity (ca. 95%), Bergsten (1988) recorded mean germination time of 6 days. His slower germinating seeds had the mean germination time longer than 9 days, which corresponds to our results with the lower-quality seeds (GC 30–50%, MGT 8–9 days). The beneficial influence of moistening and incubation within the IDS method was not as great as the influence of cold stratification in either of the tree species or soaking in water of spruce seeds, perhaps due to the lower germinating capacity of tested seeds. To support vitality, Simak et al. (1984) recommended additional moistening of seeds in PEG solution, i.e. a combination of IDS method with osmotic stimulation. Zhu et al. (2005) stated that faster germination and higher germinating capacity of Scots pine in conditions of drought stress were advantages of the treatment in PEG solution. On the other hand, Downie et al. (1993) claimed that seeds of conifers additionally moistened within the IDS method germinated faster than after cold stratification or osmotic priming with PEG in spite of the fact that other spruce and pine species were subjected to the tests (white spruce, black spruce and jack pine). The advantage and at the same time the main asset of pre-sowing treatment by means of the IDS method is however the final separation of dead seeds from the seed lots as well as a possibility to dry back the seeds and store them until the nearest spring without any loss of invigorating and separating effects (Bergsten 1987, 1988). Thus, the IDS method can be scheduled for application in operationally less intensive periods whereas the seed soaking and stratification have to be implemented closely before sowing.

CONCLUSION

All the tested pre-sowing treatment techniques for the seeds of Scots pine and Norway spruce, i.e. soaking in water, cold stratification and additional moistening and incubation of seeds within the IDS method, had a positive influence on the rate of seed germination. The highest values of germination energy and the lowest values of mean germination time were recorded in the seeds of Scots pine after cold stratification; this pre-sowing treatment is therefore recommended for increasing the seed vitality. In Norway spruce, the best results in the parameter of germination rate were achieved both after soaking the seeds in water and after cold stratification. With respect to time duration, labour intensity and costs, we recommend the soaking of spruce seeds in water for enhancing their vitality. The combination of additional moistening and incubation within the IDS method essentially increased the germination energy and reduced the mean germination time of both pine and spruce seeds, especially those with higher germination capacity. This pre-sowing treatment can be used particularly in combination with subsequent drying, separation of dead seeds (the other IDS method phases) and storage until the spring sowing.

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