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The effect of prolonged stratification and shading on the emergence of *Pseudotsuga menziesii* (Mirb.) Franco seedlings

Abstract: The Douglas fir has been cultivated in Europe since the mid-19th century, but its artificial regeneration leads to a low yield of seedlings. It could be improved by prolonged pre-sowing treatment (cold stratification of seeds) or the early shading of sowings, so that their effect on five Douglas fir seed lots and their emergence and growth of seedlings were analysed. One lot came from the USA; four lots came from the Czech Republic. The germination capacity and germination rate were analysed for seed without pre-sowing treatment and seed after 21-day and 49-day stratification. The emergence rate was tested for variants of the 21-day and the 49-day stratification (shaded and unshaded treatment) and for autumn sowing (unshaded treatment). The results show that the maximal germination capacity of full seeds is achieved after the 21-day stratification. The prolonged 49-day stratification increases the germination rate, especially in seed lots with lower vitality. The biggest seedlings, in terms of length and biomass of the above-ground part and the biomass of the root system, emerged from seeds sown in autumn. The combination of the 49-day stratification of seeds and the 3-week shading after spring sowing can accelerate seedling emergence and increase the emergence rate of seedlings; seedlings achieve more biomass and length of the above-ground part, the root system compared to unshaded seedlings or seedlings emerged from 21-day stratified seeds. Comparable or better results were achieved from autumn sowing.

Key words: seed, germination, provenance, Douglas fir, yield of seedlings

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Introduction

Douglas fir (*Pseudotsuga menziesii* /Mirb./ Franco) is as a non-native conifer species with a high potential for cultivation in the region of Central Europe (Spiecker et al., 2019). One serious problem connected with artificial regeneration of this species is low yield of seedlings. Although there are more critical steps in nursery practice, in the case of Douglas fir, proper pre-sowing treatment, and also early microclimatic conditions are considered as the prevailing factors affecting the yield of seedlings (Heit, 1968; Martiník & Palátová, 2012).

Douglas fir seed is known as a species with "non-deep" relative dormancy (Gosling et al., 2003). Non-stratified dormant seeds of this species manifest a decline in germination when sowed in spring. While these seeds germinate only within a narrow range of optimum temperatures (i.e. 20–25 °C), a sub-optimal temperature (i.e. below 15 °C) will greatly reduce the germination capacity (Gosling, 1988; Bewley & Black, 1982). The extent of this dormancy depends on the provenance of Douglas fir seeds (Gosling & Peace, 1990; Dirr & Heuser, 2006; Martiník & Palátová, 2012).

Dormancy is the reason why seeds do not germinate, despite being exposed to favourable germination conditions (Black et al., 2006). These obstacles to germination may be removed using a selection of dormancy-breaking treatment, such as pre-chilling (cold stratification). According to Gosling (1988) and Martiník et al. (2014), cold stratification of Douglas fir seeds has a favourable effect on germination of seeds, which show an increase in total germination capacity, germination rate and extended range of temperatures at which the seeds germinate. In practice, 3 weeks of cold stratification is frequently used, when the seeds of Douglas fir are soaked in cold water (usually 1–5 °C) then stratification in temperatures between 3–5 °C follows (Gosling & Peace, 1990). Some authors recommend that the process of stratification be prolonged to 5-to-12 weeks (Edwards & El-Kassaby, 1995; Gosling et al., 2003; Seifert, 2005; Houšková & Martiník, 2015), which may help enhance the germination rate. Cold stratification is not only a technique for the enhancement of germination, it is also a means of reaching a higher uniformity of emergence and a positive effect on the seedling yield of Douglas fir seed (Houšková & Martiník, 2015).

Emerged seedlings are susceptible to fluctuation of temperatures, especially in terms of seed germination and cotyledons development, and are most susceptible to heat attack (Löffer, 1970). Dušek & Kotyza (1970) point out that the need for shading occurs in the early growth stages, i.e. from emergence to proper rooting (roots 6–10 cm long). According to Löffer (1970) seedlings usually emerge in half-shade in the forest in spaces where they are protected from adverse wind. Due to the superheating of a narrow layer of air above the ground in unshaded places, thin root collars of seedlings overburn easily and seedlings die back (Burda, 2009).

Shading is recommended for small seeds and late seeding although, due to a high demand for time and management, it is often replaced by watering (Burda, 2009). Duryea & Landis (1984) describe that irrigation water is applied to cool and shade young (1+0) and tender seedlings, but the light intensity can also be reduced with materials of light colour. Sharma et al. (1986) recommend shading for spruce (*Picea smithiana*), Sharma et al. (1987) for silver fir (*Abies pindrow* Spach), Solberg (1978) for *Pinus caribea* and *Pinus oocarpa*, Aliev (1975) for *Pinus nigra* var. *Caramanica*. Shading of seedlings is suitable for all conifers except pines (Dušek & Kotyza, 1970). Stoeckeler & Jones (1957) recommend shading even for Douglas fir. Duryea & Landis (1984) note that Douglas fir and western hemlock are less tolerant to heat damage than most pines.

In general, the sheltering of sowings facilitates the maintaining of optimal moisture conditions in the substrate, which quickly dries out. According to Dušek & Kotyza (1970), the sheltering protects sowings against damage by birds, extreme rains and late frosts, reduces flooding and accelerates germination. Stratified seed is susceptible to negative influence of air and solar radiation, particularly in terms of germination – the germs may dry out (Löffer 1970).

The working hypothesis of this study is that prolonged cold stratification (i.e. 49 days instead of the normal 21 days) of Douglas fir seed with early-sowing shading (i.e. 3 weeks after the beginning of the emergence of the seedlings) can lead to an increase in the emergence, growth and yield of seedlings. The specific aims of this study were: 1) to analyse the response of prolonged cold stratification and shading for different seed lots of Douglas fir; 2) to detect the effect of shading on the Douglas fir seedling morphology.

Material and methods

Material

For this experiment, 5 seed lots of Douglas fir were used. One lot was of North American origin; four were collected from stands growing in the Czech Republic (Table 1, Fig. 1). The origins of these stands are unknown.

Table 1. The provenance of the Douglas fir seed lots analysed

Seed lot	Country – Region of provenance	National identification of seed origin
I	USA Washington – Seed zone 422	SIA 473307, OECD 08137
II	Czech Republic – Natural forest area 16	CZ-2-2B-DG-3102-16-6-J
III	Czech Republic – Natural forest area 06	CZ-2-2A-DG-1740-06-3-P
IV	Czech Republic – Natural forest area 36	CZ-2-2A-DG-3151-36-3-Z
V	Czech Republic – Natural forest area 23	CZ-2-2A-DG-1005-23-5-L

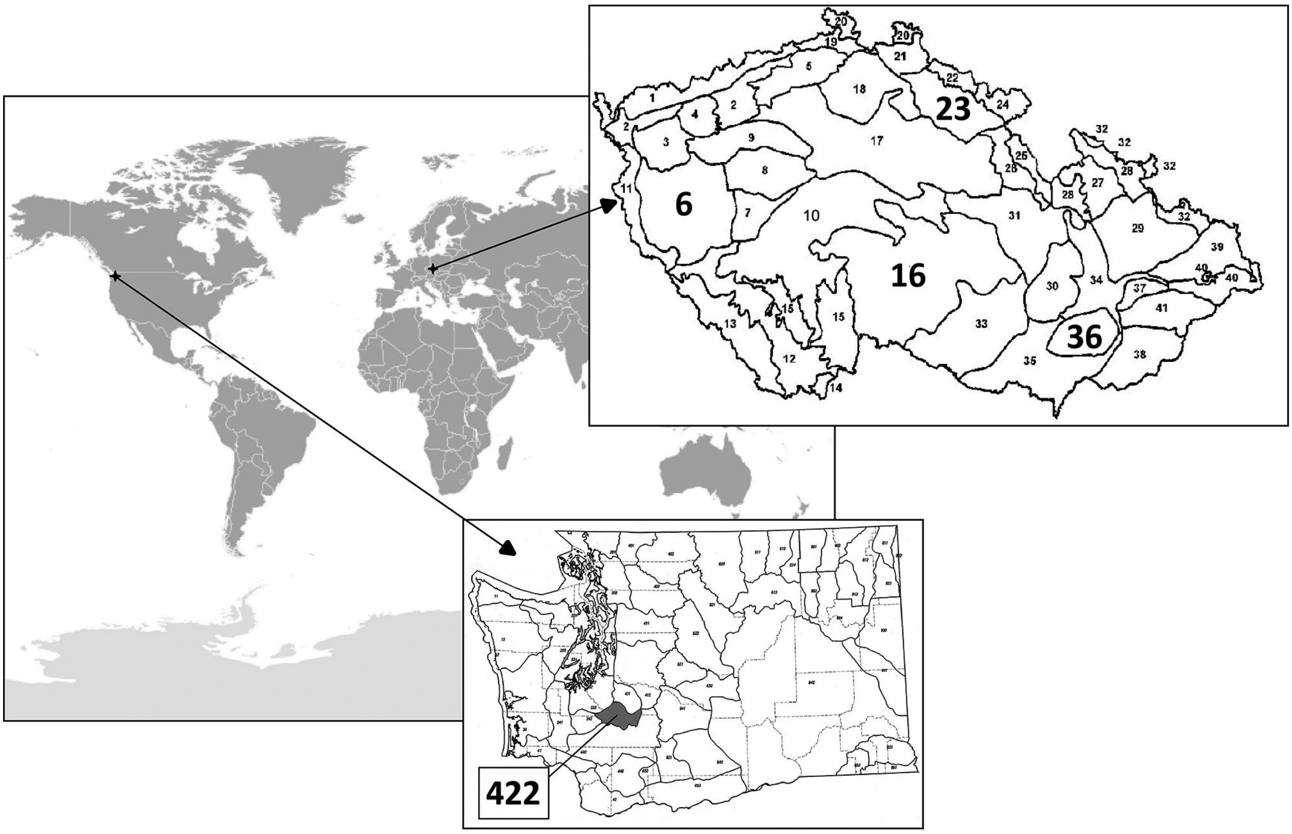

Fig. 1. The regions of provenance of the Douglas fir seed lots analysed

Methods

Experiment establishment

The germination capacity of the seed was determined in the laboratory at temperatures of 30/20 °C (8/16 h), according to ISTA (2014), for these treatments:

– "C" – Control – no pre-sowing treatment
– "21" – 21-day cold stratification (classical)
– "49" – 49-day cold stratification (prolonged)

The germination test was not realized for 21-day and 49-day treatments for seed lot III, due to a low number of seeds. Calculation of germination capacity was based on 8 replications and 50 seeds per replication for all treatments and seed lots. The pre-sowing treatment consisted of soaking the seeds in water at 5 °C for 48h, surface drying of the seeds by spreading them on filter paper at laboratory temperature for 2h, and pre-chilling (cold stratification) in plastic bags at a constant temperature of 5 °C.

The emergence (%) was conducted for the 21-day and 49-day treatments after spring (April) sowing and for autumn sowing "A" (non-stratified seed). The same seed lots, replication and seed number (as in the case of the testing of germination capacity) were used for evaluation of the emergence. Only the treatment of autumn sowing was not realized for seed lot I due to a later delivery of seed from the USA. The sowing substrate consisted of a mixture of peat and siliceous sand (4:1 ratio by volume). After sowing, seeds and emerging seedlings were irrigated every day in case it did not rain. To evaluate the effect of shading, the nursery-bed experiment was divided into non-shading ("A", "21", "49") and shading ("21s", "49s") treatments (i.e. the seedlings from autumn sowing were not shaded). The shade consisted of textile placed 10 cm above the bed. Shading was ended 3 weeks after the beginning of emergence. This field experiment was conducted in the forest nursery in Řečkovice (49°15'N, 16°35'E), which belongs to the Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University in Brno, Czech Republic.

Data collection and evaluation

The germination capacity and germination rate were determined at regular 2–3 day intervals from the beginning of germination when germinating seeds with a radicle longer than the length of the seed were removed. Germination capacity of full seeds "GC_{fs}" was determined as the number of germinated seeds at the end of the experiment (after 21 days) out of the total number of full seeds. The number of full (empty) seeds was determined at the end of the germination test by a cut test. The germination rate was measured using the following parameters:

- "GE_{fs}" – the germination energy of the full seeds, as the number of normally germinated seeds, determined 7 days after the beginning of the germination test, expressed as a % of the total number of full seeds;
- "PV" – the peak value, as the maximum quotient derived by dividing daily the accumulated number of germinants by the corresponding number of days, which is the mean daily germination of the most vigorous components of the seed lot (Czabator, 1962);
- "R_{50}" – the number of days required for 50 % of the full seeds to germinate (Edwards & El-Kassaby, 1995).

The germination synchrony (SYN) was initially proposed to evaluate the degree of overlap of flowering individuals in a population (Primack, 1985). When synchrony is equal to 1, seed germination occurs at the same time, whereas synchrony nearing 0 denotes that at least two seeds complete the germination process at different times (Lozano-Isla et al. 2019). As presented in Ranal & Santana (2016) and Lozano-Isla et al. (2019), germination synchrony is described as:

$$SYN = \frac{\sum C_{n_1,2}}{2}$$

where $C_{n_1,2} = \frac{n_i(n_i - 1)}{2}$ and $N = \frac{\sum n_i(\sum n_i - 1)}{2}$.

$C_{n_1,2}$ is a combination of the seeds germinated in time i, and n_i is the number of seeds germinated in time i.

The evaluation of the seedling emergence was carried out at 5–7 day intervals from the beginning of emergence (from 7th May for autumn sowing, from 15th May for spring sowing). Emerged seedlings were counted and considered as emerged when they dropped the seed coat. Monitoring of emergence was finished 3 weeks after the beginning of emergence, when the total emergence (%) was determined, i.e. the total percentage of seedlings emerged by this date, which was found to be the most appropriate in previous tests (Houšková et al. 2014, Martiník et al. 2014, Houšková & Martiník 2015), as seedlings that emerge later usually do not grow to the required morphological parameters and are usually cull stock. Their economic importance is low and they are not used further.

At the end of the vegetation period (when the high increment stopped growing), all emerged seedlings in all treatments were lifted and counted, and the lengths of their above-ground part (cm) and the root system (cm) were measured. Afterwards, the above-ground part was separated from the root system by cutting at the root collar; both parts of all plants, separately for particular treatments, were placed in an oven and dried at 105 °C until they reached a constant weight (24 hours). Then the total dry weight (g) of all above-ground parts and root systems of seedlings in particular treatments of stratification time, shading and origin (seed lots) were determined. Finally, this total weight was recalculated to the dry weight of one seedling.

Data analysis

Germination capacity (GC_{fs}), germination energy (GE_{fs}) and synchronization indexes (SYN) were calculated using GerminaQuant software; the principle was described by Lozano-Isla et al. (2019). The principle features of the application make it possible to calculate the analysis of variance and summary statistics. The post-hoc test (Tukey's HSD) was used to compare the differences in germination parameters (factors: seed lots and pre-sowing treatment).

Other data were statistically tested in R (R Core Team, 2017). Two-way ANOVA was applied to determine differences in the emergence, the length of the above-ground part, the root system and in the biomass (factors: treatment and shading). The differences between the treatments (time of sowing, time of stratification and performed shading) and shading were determined using Tukey's HSD test. Statistical differences among data were always recorded with a significance level of 0.05. The boxplots in the graphs of the results are a standardized way of displaying the distribution of data, based on a five number summary (minimum, first quartile, median, third quartile, and maximum).

Results

The quality of the tested seed lots varied. The proportion of empty seeds ranged from 9 to 60%, the proportion of dead seeds was minimal in all seed lots (Table 2). Two-way ANOVA found differences in all tested parameters of germination between seed lots, treatments (stratification time) and their interactions (Appendix, Table A1). Germination capacity of full seeds was statistically significantly increased by seed stratification. In most seed lots, the maximum (99%) germination capacity of full seeds was reached after only 21 days of stratification. Only in seed lot I, the germination capacity of full seeds was slightly lower after 21 days of stratification – 92%; when the stratification was prolonged to 49 days, it increased to 96% but the difference was not statistically significant. Seed stratification also had a positive effect on the germination rate of all seed lots. There was

Table 2. Germination parameters for tested seed lots of Douglas fir with different pre-sowing treatments

Seed lots	GC_{fs} (%)			Empty seeds (%)	GE_{fs} (%)			Peak value			R_{50} (days)			SYN		
	C	21	49		C	21	49	C	21	49	C	21	49	C	21	49
I	74^{d}	92^{b}	96^{ab}	9	2^{a}	20^{b}	43^{c}	*	3.3	4.2	14	8	8	0.11^{d}	0.19^{d}	0.33^{c}
II	94^{ab}	99^{ab}	99^{ab}	22	5^{a}	83^{d}	85^{d}	1.9	4.8	4.8	12	7	7	0.16^{d}	0.50^{b}	0.61^{a}
III	67^{e}	–	–	12	1^{a}	–	–	*	–	–	16	–	–	0.12^{d}	–	–
IV	84^{c}	99^{a}	99^{ab}	40	4^{a}	48^{c}	75^{d}	*	2.5	2.5	16	8	8	0.12^{d}	0.18^{d}	0.32^{c}
V	85^{c}	99^{a}	99^{a}	59	0^{a}	22^{b}	51^{c}	0.9	1.6	1.8	14	8	7	0.19^{d}	0.18^{d}	0.34^{c}

a, b, c – Indexes denoting statistically significant differences in germination capacity, germination energy of full seeds and germination synchrony

* – The peak value was not reached during the germination test.

GC_{fs} – Germination capacity of full seeds, the number of germinated seeds at the end of the experiment (after 21 days) from the total number of full seeds.

GE_{fs} – Germination energy of full seeds, the number of normally germinated seeds, determined 7 days after the beginning of the germination test, expressed as a % of the number of full seeds.

Peak value – Maximum quotient derived by dividing daily the accumulated number of germinants by the corresponding number of days, which is the mean daily germination of the most vigorous components of the seed lot (Czabator 1962).

R_{50} – The number of days required for 50 % of the full seeds to germinate (Edwards & El-Kassaby 1988).

SYN – Germination synchrony, the indice that evaluates synchronization or heterogeneity in germination (Lozano-Isla et al. 2019).

a significant increase in germination energy, peak value and decrease in the R_{50} value; in stratified treatments, the seeds germinated faster. There was no difference in the seed germination rate after the prolonged 49-day and the classical 21-day stratification in any seed lot in the value of R_{50}, and it was the peak value which was slightly smaller in favour of the 49-day stratification. However, from the differences in germination energy, it can be stated that prolonged stratification had a positive effect on the germination rate in the seed lots with lower vitality (i.e. I and V); for seed lots with greater germination energy (i.e. II and IV), there was no significant difference in the germination rate between seeds with different stratification time. Prolonged 49-day stratification caused synchronization of Douglas-fir germination; germination synchrony indices were always the highest. Classical 21-day stratification did not have such an effect; germination synchrony indices were not always higher than those of the control seeds without stratification, and they were always smaller than after 49-day stratification.

In the time of seed germination and seedling emergence, i.e. until the end of May, daily minimum temperatures were around 5 °C, the maximum around 20 °C and the mean usually 10–15 °C (Fig. 2). In the first half of June, temperatures rose by more than 5 °C but were still lower than in the germination test.

Differences in seedling emergence between treatments (sowing and stratification time) were found in each seed lot; shading caused differences in this parameter in 3 out of 5 tested seed lots; interaction between treatment and shading was not statistically

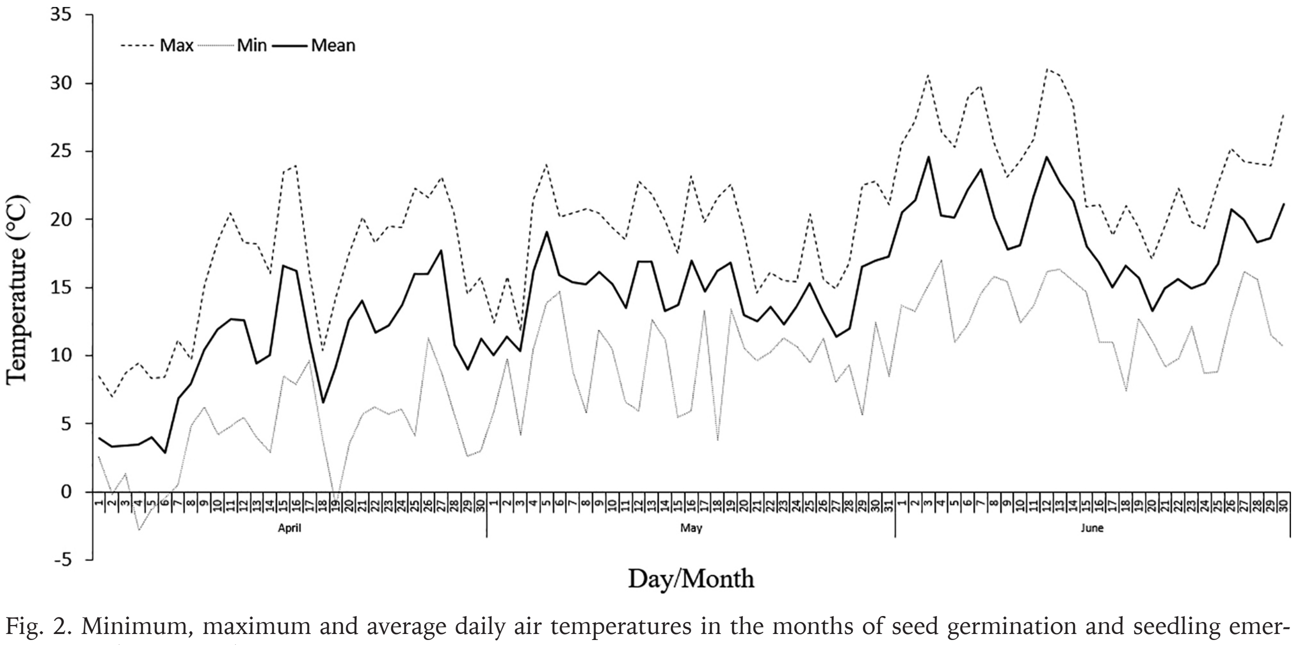

Fig. 2. Minimum, maximum and average daily air temperatures in the months of seed germination and seedling emergence (April–June) in the field experiment

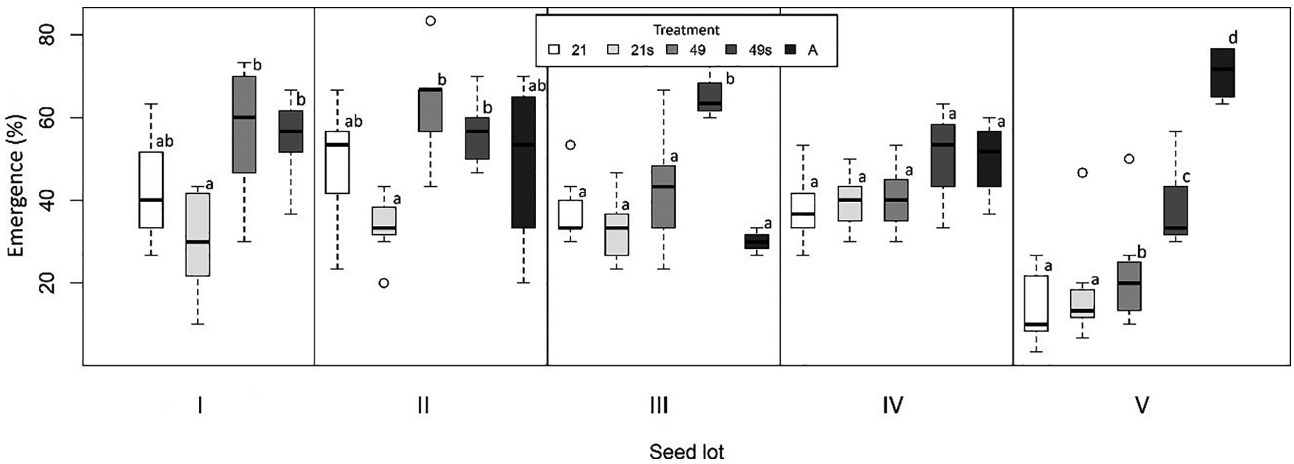

Fig. 3. Emergence (%) for shaded (marked "s") and unshaded seed lots of Douglas fir with different times of spring pre-sowing treatments ("21" and "49" days) and for autumn sowing ("A") 3 weeks after the beginning of emergence

significant (Appendix, Table A2). The average emergence of Douglas fir seedlings after prolonged 49-day stratification was higher for all seed lots, compared to the classical 21-day stratification, 3 weeks after sowing (Fig. 3), but the differences were not statistically significant (with the exception of seed lot V). The 49-day stratification significantly increased the seedling emergence only in seed lot III and V, and in the cases where the plants were shaded after sowing. For most treatments, there were mostly no statistically significant differences found between the emergence of shaded and unshaded seedlings; only in seed lot III and V, the shaded seedlings showed a higher emergence than the unshaded seedlings after 49 days of stratification. The emergence of the seedlings from autumn sowing was comparable to that from spring sowing after 21-day and 49-day stratification in the shaded and unshaded treatments. Only in seed lot III, the seedling emergence from the autumn sowing was statistically significantly lower than in the spring sowing after the prolonged 49-day stratification with shading. On the other hand, in seed lot V, the seedling emergence from autumn sowing was the highest of all treatments.

Douglas fir seedlings from autumn sowing reached the greatest amount of biomass at the end of the 1st growing season, in both the above-ground part and the root system (Table 3). There was about twice as much biomass from the seedlings gained from the spring sowing. For the seedlings from the spring sowing, the prolonged 49-day stratification or shading of the emerging seedlings did not always have a positive effect on biomass, but for most seed lots the seedlings that were shaded, while they emerged from seed after the 49-day stratification, had the greatest amount of biomass (of both the above-ground part and the root system). However, on average, the difference between the shaded treatment with 49-day seed stratification and the other treatments was not always detectable.

After the first year of cultivation, statistically significant differences were found in the length of the above-ground part of Douglas fir seedlings from different seed lots in the tested treatments of sowing time, stratification time and shading, and mostly in their interaction (Fig. 4, Appendix Table A2). The greatest length (approx. 10 cm) was reached by the seedlings from the autumn sowing; in spring sowing, the shaded seedlings were significantly taller (approx. 8 cm), compared to the unshaded seedlings (approx. 5–6 cm). There was a statistically significant difference between the treatments of stratification time, mainly in the shaded Douglas firs, in favour of the prolonged 49-day stratification.

Table 3. Dry matter of the above-ground part and the root system of the seedlings after different stratification times ("21" and "49" days), sowing times (spring or autumn "A"), with (marked "s") and without shading after sowing

Seed lots	Dry matter (g.plant^{-1})									
	Above-ground part					Root system				
	A	21	21s	49	49s	A	21	21s	49	49s
I	–	0.07	0.11	0.08	0.17	–	0.08	0.08	0.09	0.15
II	0.30	0.06	0.14	0.09	0.15	0.21	0.06	0.09	0.08	0.12
III	0.30	0.16	0.11	0.12	0.14	0.19	0.09	0.07	0.08	0.10
IV	0.33	0.14	0.10	0.13	0.17	0.24	0.12	0.07	0.08	0.13
V	0.38	0.14	0.09	0.06	0.20	0.29	0.14	0.07	0.06	0.13
Mean	0.33^{a}	0.12^{bc}	0.11^{bc}	0.10^{b}	0.17^{c}	0.23^{a}	0.10^{bc}	0.08^{b}	0.08^{b}	0.13^{c}

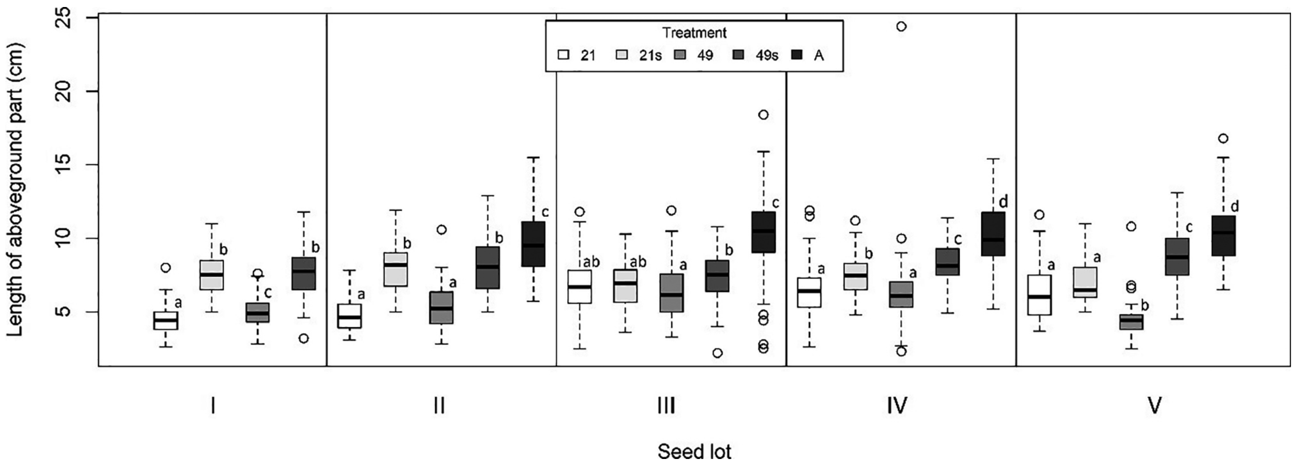

Fig. 4. The length of the above-ground part of the one-year-old Douglas fir grown from different seed lots and in different treatments of sowing time (spring or autumn "A" sowing), time of stratification ("21" or "49" days) and shading during emergence (shaded treatments marked "s")

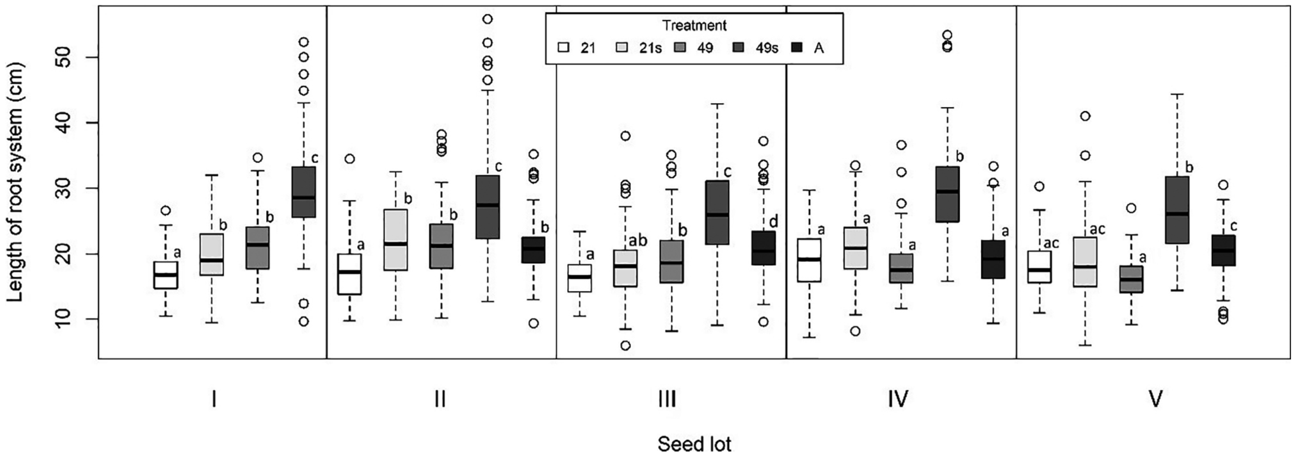

Fig. 5. The length of the root system of annual Douglas fir seedlings grown from different seed lots and in different treatments of sowing time (spring or autumn "A" sowing), time of stratification ("21" or "49" days) and shading during emergence (shaded treatments marked "s")

There were also statistically significant differences in the length of the root system of shaded and unshaded Douglas fir seedlings and the tested treatments; with the exception of seed lot II, interaction between shading and treatment was significant as well (Fig. 5, Appendix Table A2). The greatest length of the root system was recorded in shaded Douglas firs from spring sowing after the prolonged 49-day seed stratification (approx. 28 cm). For the other treatments, the root system was significantly shorter (approx. 20 cm) and, although statistical analysis also showed significant differences, the seed lots responded differently. Therefore, it is not possible to unambiguously determine which root system – of the autumn sowing, the spring unshaded sowing and the spring shaded sowing after the 21-day stratification – is longer or shorter.

Discussion

The optimal pre-sowing treatment, especially of dormant seeds, and the taking care of the sowing, are the basics of successful production of planting stock in forest nurseries. The low yield of Douglas fir seedlings in the Czech Republic, and also in other European countries, led to the initiation of the research that analysed the possibilities of pre-sowing treatment changes (i.e. the time of stratification) and the possibilities for the improvement of the conditions after sowing (i.e. shading). Our results confirmed that cold stratification before sowing influences Douglas fir seed positively, it improves both the germination capacity and the germination rate. Owston & Stein (1974), and also Martiník & Palátová (2012), came to the same conclusions, however, not only Gosling & Peace (1990) but also Müller et al. (1999) noticed that seeds of certain origins do not show any dormancy, and cold stratification is therefore unnecessary, and that is why both ISTA (2014) and ČSN 48 1211 (2006) recommend a double germination test – without pre-sowing treatment and also after 3-week stratification at 5 °C. A comparison of germination tests can show if pre-sowing treatment stimulates or inhibits the germination capacity, or if the germination capacity does not change. We can therefore say that some seeds of most tested seed lots were dormant because the cold stratification increased their germination capacity.

Classical cold stratification of Douglas fir seed carried out in nurseries takes 21 days. However, many authors point out the demand for the prolongation of the cold stratification from three to as many as several tens of weeks (Sorensen, 1991; Edwards & El-Kassaby, 1995; Gosling et al., 2003; Seifert, 2005). A series of studies (Houšková et al., 2014; Martiník et al., 2014; Houšková & Martiník, 2015) led to the conclusion that the optimal time of cold stratification is 49 days. When stratification was prolonged from 21 to 49 days in our research, the seed germination rate even improved and germination became synchronized. The prolonged 49-day stratification should also have a positive effect on the emergence of Douglas fir seedlings (Houšková et al., 2014; Martiník et al., 2014; Houšková & Martiník, 2015). A large number of fast emerging seedlings creates a homogenous plant culture with a high yield of seedlings, while the growth of slowly emerging seedlings is negatively influenced by the competition of the plants that had already emerged. These slowly emerging seedlings usually do not grow to the required size and they become cull stock. Our results confirmed a faster emergence of seedlings after the 49-day seed stratification only for two out of five tested seed lots, especially in combination with shading after sowing. Hofman (1964) also points out that not only careful stratification is a prerequisite of successful Douglas fir sowing, but recommends soaking of the seeds in water, in combination only with proper shading after sowing.

The emergence of seedlings in the individual treatments of sowing time, stratification time and shading was usually comparable but only seed lot V, sown in autumn, reached twice the emergence of that of the best treatment of the spring sowing. Seedlings from the autumn sowing were always tallest, and there was the most biomass of both the above-ground part and the root system. However, the root system of the seedlings from the autumn sowing was not longer than that after the spring sowing. Hofman (1964), in accordance with Duryea & Landis (1984), also points out that autumn sowing brings about higher seed yield and taller seedlings with a larger root system. He also presumes that the spring sowing of Douglas fir is a "European custom" from the time when it was possible to obtain seeds only in the countries of origin (i.e. America) and they arrived in Europe after harvest, later in the winter or in the spring. Papp (1961 in Hofman, 1964) warns that the seeds start to germinate after autumn sowing too soon and the seedlings then suffer from late frosts, to which Douglas fir is sensitive. There is also an increased risk of damage by birds and rodents from the moment the seeds are completely ripe until they begin to germinate (Duryea & Landis, 1984), although Hofman (1964) admits the problem of rodents only in the case of their overpopulation, which can also be solved by suitable protective means, and the damage by birds in autumn is comparable to that after spring sowing.

The time of spring sowing is important for Douglas fir. Lavender (1958) and Hofman (1964) indicate that earlier spring sowing is more successful than autumn sowing, and seedlings have a longer time to develop, they are bigger and have a larger root system. According to these authors, the emergence is also higher. Sorensen (1996) also recommends earlier spring sowing and long pre-sowing treatment by cold stratification, thus reducing the seedling mortality after sowing and during the first winter. Our results show that the prolonged 49-day seed stratification with shading after sowing in the spring is the most successful. The emergence of seedlings is usually not significantly higher and their emergence is not always faster, but the seedlings have a greater amount of biomass and length of both the above-ground part and the root system. Just the prolongation of stratification of the seed or mere shading of seedlings did not have such an effect. Hofman (1964) points out the importance of soaking the seeds in water before sowing and that the duration of cold stratification does not make a difference. However, Allen (1962) affirms that shorter stratification accelerates seed germination at 25 °C (which is optimal), whereas at lower temperatures, such as 15 °C or 10 °C, the stratification time must be longer (up to 120 days or more). Therefore, when choosing a suitable stratification time, it is necessary to take into consideration the temperature conditions after sowing. Considering the lower temperatures after sowing in our research, compared to the temperatures during the germination test, it is also possible that, as a result of this, the seedling emergence is much lower than the germination capacity of the seed.

Shading after sowing should mitigate the effects of direct solar radiation and strong air flow and protect seedlings from extreme fluctuations in humidity and temperature in the soil (rhizosphere) and in the ground layer (phylosphere) (Dušek & Kotyza, 1970). Toumey & Neethling (1923) recorded the best emergence of selected conifer seed, including Douglas fir, in a shaded bed. Although this positive effect of shading was not confirmed in all tested seed lots, Toumey & Neethling (1923) found the losses in the open bed to be about four times, and in the mulched bed more than five times, higher than those in the bed under half-shade. However, the authors warn that shading can reduce the size of seedlings, regardless of the tree species. They explain that, if there is insufficient irrigation in open beds and low soil moisture, the positive effect of shading on maintaining soil moisture may outweigh its negative effect on plant growth. However, not only was there no reduction in the growth observed in the tested seed lots after shading, the shaded and irrigated Douglas fir seedlings grew to an even greater length of the above-ground part than the unshaded ones.

Results of this experiment confirmed and clarified previous findings (Gosling et al., 2003; Seifert, 2005; Houšková & Martiník, 2015); i.e., that in the case of spring sowing, prolonged 49-day seed stratification and shading of beds during the emergence of seedlings leads to a higher number of larger plants of Douglas fir. Autumn sowing, without pre-sowing treatment of Douglas fir seed and with careful protection from biotic factors (e.g. rodents, birds) and abiotic factors (e.g. frost, sunlight), seems to be suitable in forest nurseries.

Conclusion

The results of this experiment with five seed lots of Douglas fir show that the maximal germination capacity of full seeds is achieved after 21-day stratification. Prolonged 49-day stratification increases the germination rate, especially in seed lots with lower vitality. Three-week shading after spring sowing of the 49-day stratified seeds accelerates emergence and increases the emergence of seedlings only in some of the seed lots. Comparable or better results were achieved in some seed lots after autumn sowing. The biggest seedlings in terms of length and biomass of the above-ground part, and the biomass of the root system, emerged from seeds sown in autumn; seedlings from spring sowing after 49-day seed stratification with 3-week shading after sowing achieved more biomass and a greater length of the above-ground part and root system, compared to the seedlings that came out of seed after classical 21-day stratification. These seedlings also had the longest root system.

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