Germination and emergence response of specific Douglas fir seed lot to different temperatures and prechilling duration

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ABSTRACT: One of the critical points of Douglas fir seedling production is to optimize the time of sowing and length of pre-sowing treatment. Germination and emergence of Douglas fir seed lot of the Czech origin (CZ-2-2A-DG-1740-6-3-P) were observed in simulated warm and cold conditions in two phytotrons for 3 months. Before this procedure the seeds were exposed to different prechilling duration. The temperature in control conditions of phytotrons was stable for 28 days and then it was increased: 11, 13, 17°C in cold phytotron and 13, 17, 20°C in the warm phytotron. Seeds without stratification and stratified for 3, 5, 7, 9 and 16 weeks were tested. The laboratory germination capacity of non-stratified seeds was only 58% and it was significantly ($P < 0.05$, Tukey's test) lower than in stratification treatments when it varied from 92% to 96%. Non-stratified seeds did not germinate in cold phytotron and they germinated very slowly (germination rate at the end of experiment was 7%) in warm phytotron. The germination capacity of seeds both in the phytotron and in the laboratory was observed in treatments with prechilling of seeds longer than 7 weeks in warm conditions and only for seeds after 16 weeks of prechilling in cold conditions. The highest emergence rate of seeds (84%) was found in the warm phytotron after 16 weeks of stratification. Comparable results were reached only for 9 weeks of stratification in the same phytotron. The results suggest that the prolongation of the standard 3-week prechilling period helps to increase germination capacity and emergence rate of seeds. Future research should focus on optimal length with regard to more seed lots and also on seedling quality parameters.

Keywords: stratification; germination capacity; emergence rate

From the aspect of forestry, Douglas fir is considered a promising tree species for the region of Central Europe (Kenk, Ehring 1995; Kantor et al. 2001; Tauchman et al. 2010). In the region of the Czech Republic it is recommended to increase its share from 0.2 to 2–4% of the total stand area (Beran, Šindelář 1996; Kantor et al. 2010). On sites without seed-producing trees and in areas where Douglas fir has not been present so far, artificial regeneration is necessary. One of the critical points in artificial regeneration of Douglas-fir is a small yield of seedlings in nursery practice of the Czech Republic. From many aspects which influence the yield of Douglas fir seedlings the pre-sowing treatment seems to be important because the seeds exhibit dormancy (Heit 1968; Gosling, Peace 1990; Müller et al. 1999).

Cold stratification of seeds has a favourable effect on subsequent germination and shows an increase in total germination capacity, increased germination rate and extended range of temperatures at which the seeds germinate (Gosling 1988). The main prerequisite for successful stratification is its sufficient duration. According to Jones, Gosling (1994), short stratification (2–6 weeks) of seeds with relative dormancy improves the germination capacity as well as the germination rate. However, in order to extend the range of temperatures at which the seeds germinate, a necessary time of stratification is 6–24 weeks, according to Gosling et al. (2003), nearly all live seeds

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of Douglas fir germinated after 48 weeks of stratification with no regard of temperature at which they germinated. The authors do not recommend a treatment longer than 64 weeks.

Such a long pre-sowing treatment is not usual in the nursery practice. A survey published by SEIFERT (2005) indicates that the pre-sowing treatment normally lasts between 2 and 12 weeks. The norm ISTA (International rules for seed testing) 2003 recommends 3 weeks of stratification prior to a germination test, and the same stratification time is used as a standard before sowing e.g. in Canada (LEADEM 1996), Denmark (POULSEN 1996) or in the Czech Republic (CSN 48 1211). EDWARDS and EL-KASSABY (1995) reported that germination capacity was maximal after three weeks of stratification in a laboratory experiment on germinators and did not change when the stratification was extended by 2 or 4 weeks. Nevertheless, the germination rate characterized by parameters such as MDG, PV, R50 (CSABATOR 1962) was increasing with the length of stratification. EDWARDS, EL-KASSABY (1995) pointed out the genetic heterogeneity of seed lots and recommend extending the stratification up to 5 weeks. Although the total germination capacity may not be increased, longer stratification reduces the time required for the emergence of seedlings, which shows in synchronized emergence and lower variability of seedlings. The increased tolerance to low temperatures after stratification makes it possible to implement earlier seeding in nurseries and hence a longer growing period. Based on the experience with the seed of Douglas fir from seed orchards in Oregon, SORENSEN (1991) also suggested the extension of the stratification time.

The above facts indicate that a suitable time of cold stratification depends on conditions to which the stratified seeds will be exposed after sowing. Maximum germination capacity of a seed lot can be ascertained by the germination test conducted at an optimum temperature of 30/20°C after three weeks of cold stratification. At a sub-optimum germination temperature, the outcome will depend on the length of the stratification period.

The experiment was aimed to analyse germination and emergence differences in an indigenous seed lot of Douglas fir under simulated conditions of early and late sowing for various time lengths of cold stratification (prechilling duration). The study is part of an extensive experiment aimed at Douglas fir. The partial task is optimizing the sowing date and prechilling duration of this species for the Czech Republic nursery practice.

MATERIAL AND METHODS

For this experiment, a seed lot of Douglas fir (*Pseudotsuga menziesii*, var. *menziensii*) from a certified unit CZ-2-2A-DG-1740-6-3-P was used. The origin of the stand is unknown.

Two growth chambers (phytotrons) with controlled conditions were used to simulate the effect of different temperatures on germination capacity and emergence rate of seeds. Phytotron I simulated conditions of early sowing and phytotron II simulated conditions of late sowing. Differences in the settings of phytotrons (temperature and illumination length) were established according to average temperatures and solar radiation time for conditions of the southeast region of the Czech Republic (TOLASZ et al. 2005). These temperature conditions can be related to a wider territorial complex of Central Europe.

For phytotrons I and II the sowing conditions were simulated for April and May, respectively. The temperature in the two growth chambers was changed after four weeks to correspond to average temperatures of the following month (Table 1).

Prechilling duration treatment tested for the selected seed lot was as follows: no pre-sowing treat-

Table 1. Simulated light and temperature conditions in phytotrons (e.g.) for analyzing 6 relations among stratification, environmental conditions and germination and emergence of 7 Douglas fir seeds

Number of days from sowing	Light conditions	Phytotron I		Phytotron II	
		time specification	temperature (°C)	time specification	temperature (°C)
0–27	day (full light)	6^{00}–20^{00}	11	6^{00}–21^{00}	15
	night (darkness)	2^{00}– 6^{00}	8	21^{00}– 6^{00}	11
28–55	day (full light)	6^{00}–21^{00}	15	5^{00}–21^{00}	17
	night (darkness)	21^{00}– 6^{00}	11	21^{00}– 5^{00}	13
56–83	day (full light)	5^{00}–21^{00}	17	5^{00}–21^{00}	20
	night (darkness)	21^{00}– 5^{00}	13	21^{00}– 5^{00}	16

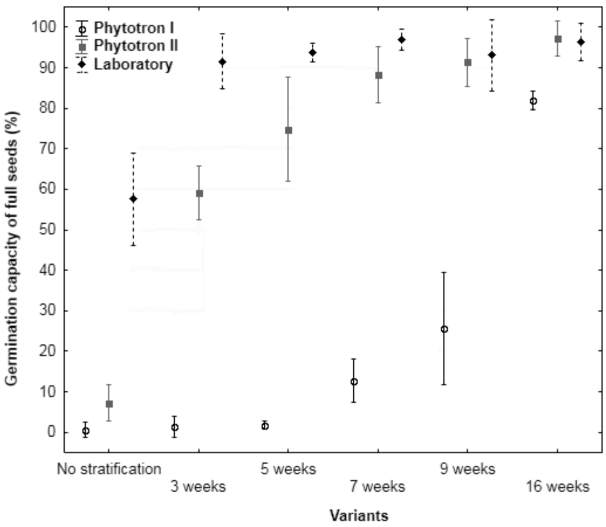

Fig. 1. Germination capacity of Douglas fir full seeds of analyzed variants with different prechilling duration in laboratory and phytotron conditions

ment (no stratification), 3-, 5-, 7-, 9- and 16-week stratification. The pre-sowing treatment consisted of soaking the seeds in water at 5°C for 48 h, surface drying of the seeds by spreading them on filter paper at laboratory temperature for 2 h, and prechilling (cold stratification) in plastic bags at a constant temperature of 5°C.

In the whole treatment, germination capacity of the seeds was determined in the laboratory at temperatures of 30/20°C (8/16 h) according to CSN 48 1211 (2006); germination capacity and emergence rate of the seeds were also ascertained in the conditions of growth chambers (Table 1). Germination tests were performed on germinators. For the assessment of emergence rate, boxes, into which the seeds of individual variants were sown, were filled with substrate (mixture of peat and silica sand at 4:1). These boxes were placed into the phytotrons. Watering boxes were controlled by ocular evaluation of the substrate in the phytotrons.

Germination capacity was evaluated three weeks after the establishment of the experiment; emergence rate was monitored for 12 weeks. The rate of germination/emergence was determined according to germination energy and peak values (PV), i.e. maximum quotient derived by dividing the daily accumulated number of germinants/young seedlings by the corresponding number of days, which is the average daily germination/emergence of the most vigorous components of the seed lot (Czabator 1962). To determine the peak value, the monitoring in the phytotrons was extended and evaluated for 28 days from the start of the experiment.

Each variant of the pre-sowing treatment was established in four repetitions with 100 seeds. Germination capacity and emergence rate of the individual variants at the end of observation were mutually compared by using multi-factorial ANOVA (temperature conditions represented by the place of analysis – laboratory, phytotron I and II – were the first factor; time of prechilling was the second factor) with a significance level of $P < 0.05$ in STATISTICA 10 (Tulsa, USA). Vertical columns in statistical diagrams represent 95% confidence intervals. Multiple comparisons, i.e. determination of differences between the variants, were made using Tukey's HSD test.

RESULTS

The test of germination capacity in the laboratory showed that in optimal laboratory conditions the tested seed lot reached a high germination capacity of full seeds (Fig. 1, Table 2) – over 90% in all strati-

Table 2. Germination and emergence parameters of the tested Douglas-fir seed lot and statistical differences between 6 types of prechilling duration treatment

Treatment	Laboratory					Phytotron I		Phytotron II	
No stratification	2.8^{a}	58^{g}	2.5	33.0	10	0.5^{e}	2^{k}	7.0^{e}	24^{lm}
3-week stratification	17.2^{b}	92^{j}	5.9	5.0	10	1.5^{e}	17^{kl}	59.0^{gh}	40^{mn}
5-week stratification	29.1^{c}	94^{j}	6.7	4.0	8	1.5^{e}	34^{lm}	75.0^{hi}	39^{mn}
7-week stratification	64.3^{d}	97^{j}	9.2	1.5	8	12.5^{ef}	29^{lm}	88.5^{ij}	52no
9-week stratification	56.3^{d}	93^{j}	8.7	1.0	9	25.5^{f}	53no	91.5^{ij}	67^{op}
16-week stratification	89.5^{d}	96^{j}	11.6	0.0	9	82.0^{ij}	66^{o}	97.0^{j}	84^{p}

means within the same parameter together for laboratory and phytotron test followed by the same letter are not significantly different ($P < 0.05$); $^{a–d}$ for GE, $^{e–j}$ for GC, $^{k–p}$ for ER; GE – germination energy of full seeds, the percentage of seeds that germinated within one week out of the total amount of full seeds, GC – germination capacity of full seeds, the percentage of seeds that germinated at the end of the test (within three weeks) out of the total amount of full seeds, ER – emergence rate, the percentage of seeds that emerged within 83 days out of the total amount of seeds

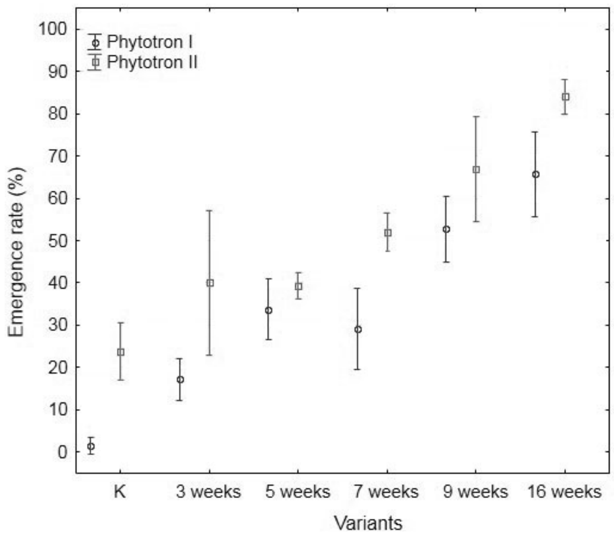

Fig. 2. Emergence rate of Douglas fir seeds with different prechilling duration in phytotron conditions

fication variants. A significantly lower germination capacity of full seeds (58%) was found only in non-stratified seeds. Although the prechilling duration did not affect the germination capacity in these conditions because the differences between the respective variants were non-significant, the germination energy of full seeds was increasing with the increase of prechilling duration (Table 2). Seeds stratified longer than 5 weeks showed a comparable germination rate.

In considerably colder conditions of the two growth chambers in which the initial temperature did not exceed 15°C seeds with no stratification germinated only minimally (Fig. 1). With the exception of the longest 16-week stratification, the germination capacity of stratified seeds was significantly higher in all types of treatment in warmer conditions of phytotron II in comparison with phytotron I (Table 2). At the same time there was a trend of germination capacity growing with the increasing length of stratification (Fig. 1, Table 2). The lower the temperature at germination, the longer the stratification that was necessary to achieve germination capacity comparable with that determined in optimal laboratory conditions. In conditions of phytotron II variants receiving the pre-sowing treatment longer than 5 weeks showed germination capacity that was statistically comparable with that found in the laboratory. In colder conditions of phytotron I this germination capacity was reached only in the variant with the longest 16-week stratification.

Higher temperature also had a positive influence on the emergence rate of the tested seeds (Fig. 2). With the exception of 5 and 9-week variants, emergence rate values in warmer conditions of phytotron II were statistically significantly higher for the analysed variants than in phytotron I (Table 2). Similarly like in the case of germination capacity, a beneficial effect of pre-sowing treatment length on the overall emergence rate was found as well. A statistically significant difference was observed between emergence rates of stratified and non-stratified seeds. In colder conditions of phytotron I the emergence rate of control seeds as well as seeds after 3-week stratification was minimal. In stratified seeds, the emergence rate was growing in both phytotrons with increasing stratification time. The highest values were found in phytotron II with seeds stratified for 9 and 16 weeks. The variants of seeds in phytotron II stratified for 7 weeks and seeds in phytotron I stratified for 9 and 16 weeks reached comparable results as well.

Different time of the pre-sowing treatment and different temperature also influenced the course of seed emergence (Fig. 3a,b). In warmer conditions simulating late sowing (phytotron II) stratified seeds started to emerge between day 20 and 25 after sowing; in colder conditions simulating early sowing (phytotron I) stratified seeds started to emerge as late as after 30 days from sowing. The beginning of control variant emergence without pre-sowing treatment was always substantially delayed. In phytotron I the seeds

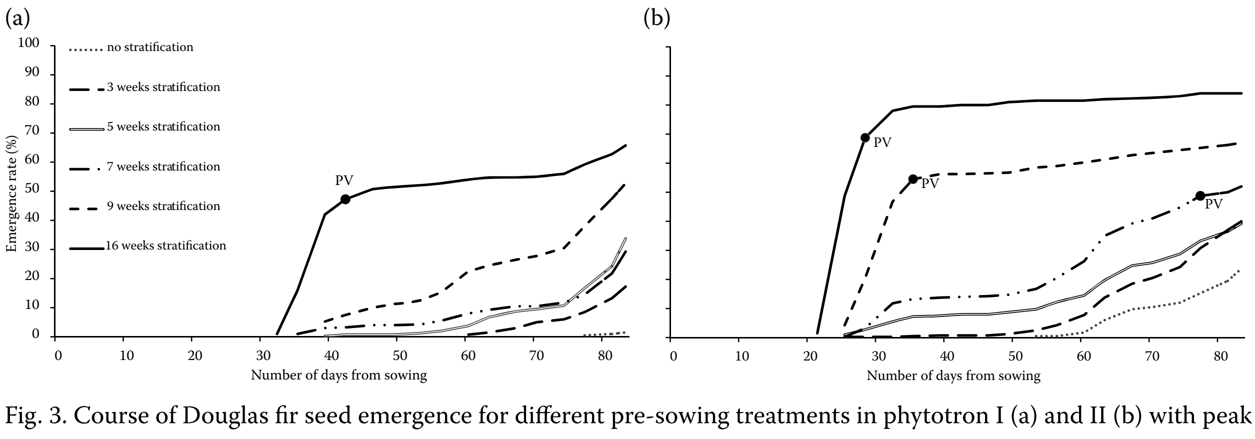

Fig. 3. Course of Douglas fir seed emergence for different pre-sowing treatments in phytotron I (a) and II (b) with peak value (PV) marking (maximum emergence speed)

Table 3. Peak value (PV) for germination capacity and emergence rate of non-stratified and stratified Douglas fir seed lot expressed by cumulative germination capacity (emergence rate) and number of days to arrive at the level of peak value (%/days)

Treatments	Cumulative				
	germination capacity/ number of days to arrive at the peak value			emergence rate/ number of days to arrive at the peak value	
No stratification	*	*	*	*	*
3-week stratification	*	54/21	65/11	*	*
5-week stratification	*	61/18	74/11	*	*
7-week stratification	*	70/14	73/8	*	49/77
9-week stratification	*	64/14	69/8	*	54/35
16-week stratification	75/21	70/11	81/7	47/42	69/28

*peak value was not reached in the monitored time

with the shortest 3-week stratification also began to emerge much later. Fig. 3 shows different emergence dynamics in colder conditions of phytotron I in the variant with the longest pre-sowing treatment as compared with other variants. In phytotron II a similar course of emergence was observed in the 9-week variant. A greater part of seeds in these variants emerged within 10 days from the beginning of emergence. According to the peak value, these seeds reached the maximum emergence rate relatively fast. In variants with shorter pre-sowing treatment the seeds emerged slowly, gradually during the entire experimental period, usually not reaching the maximal emergence rate within the time.

The beneficial influence of higher temperature and pre-sowing treatment time on the course of germination and emergence is also apparent from Table 3. The seeds without stratification germinated long and slowly, not reaching the peak value within the experimental period. The stratified seeds reached the maximum germination rate in laboratory conditions within 11 days. At significantly lower temperatures in the growth chambers, a comparable germination rate as in the laboratory was reached by the variant with 16 weeks of stratification in warmer conditions of phytotron II. The time of reaching maximum germination and emergence rates was increasing with the decreasing time of stratification and phytotron temperature. Also emergence rate at the time of peak value was the highest in the treatments with the longest stratification.

DISCUSSION

Besides the seed quality and soil moisture content, temperature can be considered as the most important factor in the process of germination or emergence. In the case of sowing dormant seeds in spring, an adequate pre-sowing treatment is also a vital condition of success (Sorensen 1991). In general, there are large differences in dormancy with respect to the origin of Douglas fir seed lots (Müller et al. 1999; Martiník Palátová 2012). According to Heit (1968) stratification is necessary only for Douglas fir var. *caesia* and *viridis (menziesii)*. As mentioned by Stein, Owston (Bonner et al. 2008), seed lots of green Douglas fir (*Pseudotsuga menziesii/Pseudotsuga menziesii* var. *menziesii*) from the original natural range even need such stratification. Results from the laboratory test of germination show that the analysed seeds of the indigenous Douglas fir provenance (*Pseudotsuga menziesii/Pseudotsuga menziesii* var. *menziesii*) of the Czech Republic origin were dormant. Germination capacity of these seeds without stratification was considerably limited. The manifestation of dormancy – reduced germination of non-stratified seeds at sub-optimum temperatures (Bewley, Black 1994; Müller et al. 1999; Gosling et al. 2003) – is also apparent from the germination outcome of non-stratified seeds in the conditions of both phytotrons. At temperatures of 11 and 15°C, the non-stratified seeds germinated either not at all or only minimally. This is in agreement with the conclusions of Gosling et al. (2003) that the temperature of about 15°C is limiting for germination of non-stratified Douglas fir seeds. Germination capacity determined in the laboratory test expresses the maximal production potential of the seed lot. Emergence rate more or less differs from the laboratory test. While the germination test lasts for 21 days, the counting of emerging seeds usually lasts much longer (Koloteo et al. 2001) – in our case 83 days from sowing. However, a major cardinal difference lies in the fact that while the germination test takes place in optimal conditions, emergence usually occurs under less favourable conditions. Thus, the closer the sowing conditions to optimal conditions, the lesser the difference between laboratory germination and field germination (Ferguson-Spears 1995). At the end of our experiment, the highest emergence rate – more than 84 % was found in seeds stratified in phytotron II (warmer conditions) for 16 weeks. Emergence rate over 50% was found in colder conditions of phytotron I in variants with pre-sowing treatment longer than 9 weeks, and 7 weeks in phytotron II. At the same time, the tested seed lot – when sown in a common nursery bed from March to May – reached emergence rate higher than 70% after 3–7 weeks of stratification in a comparable assessment period (Houšková et al. 2014). Conditions in the growth chambers were set to simulate average daily and night temperatures in the months of April – June (July), whilst in real conditions of emergence in forest nurseries, the temperature increases/decreases gradually during the day/night, reaching above/below the average. The daily maximum in April commonly exceeds 20°C in the conditions of the Czech Republic, which Gosling et al. (2003) considered as temperatures close to the optimum for the germination of Douglas fir seeds (25°C). These relatively short periods of higher temperatures apparently initiate the germination of seeds and their emergence. By contrast, low temperatures at early sowings may extend the process of cold stratification (Martiník et al. 2013; Houšková et al. 2014). This suggests that rather than by average temperature, the emergence rate of the tested seed lot is affected by the daily course of temperatures including maximal and minimal values.

From the practical point of view, however, the emergence rate is considerably important because the plants that emerged two or three weeks later than the majority of other seeds will not reach the required parameters and are usually eliminated from further cultivation of the nursery stock. Germination rate of forest tree seeds is assessed by using the R_{50} index, which is the number of days needed for the germination of 50% of seeds (Ching 1959), or by germination power or peak value (Czabator 1962). Since the sufficiently long pre-sowing treatment not only increases tolerance to unfavourable conditions of germination but also should show in accelerated germination and emergence, we used the peak value parameter in our experiment to objectivise the effect of the stratification time on emergence rate. In colder conditions of phytotron I, the peak value for emergence was reached only in the variant with 16 weeks of stratification. By contrast, the emergence rate in phytotron II culminated even after 7 weeks of stratification. In the variants with shorter stratification, the seeds were emerging slowly even in warmer conditions of phytotron II with the peak value not being reached until day 83 when the evaluation of emergence rate was ended. These results corroborate conclusions of Sorensen (1991) and Müller et al. (1999) that the course of the emergence of stratified seeds is influenced by the duration of pre-sowing treatment and by temperature to which the seeds are exposed after stratification.

The results of our experiment show that extended stratification apparently facilitated seed germination even in sub-optimal conditions, accelerating the beginning of emergence as well as its course, which is considerably important from the practical point of view. In conditions of the Czech Republic, the commonly used 3-week stratification can be expected to yield neither rapid and uniform emergence nor high emergence rate of Douglas fir seeds. Taking into account the planned sowing date, the stratification of seeds needs to be longer.

CONCLUSIONS

The research results show increased tolerance of dormant seeds of Douglas fir originating from the Czech Republic (*Pseudotsuga menziesii/Pseudotsuga menziesii* var. *menziesii*) to temperature conditions of germination and emergence with stratification extended beyond the standard duration of 3 weeks. The extended stratification resulted in increased rates of seed germination and emergence. At the same time, these results suggest that an optimal time of Douglas fir seed stratification could range at a level of the maximal stratification length tested, i.e. ca. 9–16 weeks. In order to define the time of stratification, it will be necessary to assess different seed lots, sowing times, conditions of emergence and growth of plants as well as the quality of cultivated seedlings.

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