doi: 10.17221/7/2015-JFS

Does prolonged stratification of Douglas fir influence the yield of seedlings?

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ABSTRACT: The paper analyses the influence of an extended period of cold stratification on the germination capacity, emergence rate and yield of seedlings in the Douglas fir as well as on the morphological parameters of one-year-old seedlings. A total of ten seed lots were used for evaluating germination capacity, originating from the Czech Republic, Slovakia, France and the USA. Germination capacity, emergence rate and yield of seedlings were analysed for two seed lots of Czech provenance on seed beds in the settings of a forest tree nursery. Tested variants involved (a) no pre-sowing treatment, (b) seed stratified for 21 days, and (c) seed stratified for 49 days. The germination capacity of the stratified seed was higher than that of the control variant for all of the seed lots. The effect of the stratification period length was zero for both germination capacity and emergence rate. Differences were identified between the stratification variants as regards germination energy and emergence rate. For the control variant, seedling yield reached only 1% and 2%. For the stratified seed, yield of seedlings of two selected Czech provenances was 18% and 16% (variant 21) and 26% and 36% (variant 49). Differences in the morphological parameters of seedlings were not straightforward.

Keywords: provenance; germination; emergence

The Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) has been cultivated in Europe since the mid-19th century (Nožička 1963; Hofman 1964). Despite initial aesthetic grounds, the cultivation in Europe is now associated with the production of timber (Kenk, Ehring 1995; Kantor et al. 2001; Kantor 2008; Podrázský et al. 2013b). The role of this species for forest stability and soil is discussed (Kupka et al. 2013; Ulbrichová et al. 2014). Regeneration in this woody species involves a high proportion of artificial methods, particularly in countries with low representation of Douglas fir. These are linked to the production of planting stock of the required quality. This applies e.g. to the Czech Republic, where the current representation of 0.22% is recommended to increase to 2% to 4% (Beran, Šindelář 1996; Podrázský et al. 2013a). However, the cultivation of Douglas fir planting stock is complicated by a low yield of seedlings, where proper pre-sowing treatment is one of the limiting factors (Heit 1968; Martiník, Palátová 2012).

Douglas fir seeds exhibit "shallow" relative dormancy (Gosling et al. 2003), which is manifested in the reduced germination of non-stratified seeds in spring sowing. The extent of dormancy is influenced by the provenance of Douglas fir seeds (Gosling, Peace 1990; Dirr, Heuser 2006; Martiník, Palátová 2012). While non-stratified dormant seeds germinate only within a narrow range of optimum temperatures, i.e. 20–25°C, sub-optimal temperature, i.e. below 15°C, will greatly reduce the germination capacity (Gosling 1988; Bewley, Black 1994). The variability in the extent of dormancy has led to dual germination tests being introduced: no pre-sowing treatment testing and testing after three weeks of cold stratification (ISTA 2014). While spring sowing with no pre-sowing treatment is recommended for non-dormant seeds, dormant seeds require a relevant pre-sowing treatment if applying the spring sowing method (Dirr, Heuser 2006; Martiník, Palátová 2012). However, a number of authors point to the necessity of extending cold

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QI112A172.

stratification from three to five or seven or even several dozen weeks (Edwards, El-Kassaby 1995; Gosling et al. 2003, Seifert 2005). While this will not cause the seed germination capacity to increase, it helps enhance the germination rate (Edwards, El-Kassaby 1995; Houšková et al. 2014; Martiník et al. 2014b), which is particularly of practical importance. In addition to the length of the pre-sowing treatment period, factors influencing the seed germination capacity/emergence rate include the humidity at which the seeds are stratified, as well as the conditions under which the germination/emergence process is underway (Gosling 1988; Huth et al. 2011).

Since the majority of studies focused on the effect of the length of stratification on seed germination in Douglas fir evaluated seed lots originating from the native range of the species (Sorensen 1991; Edwards, El-Kassaby 1995), while surveys evaluating the effect of extended stratification on germination and yield of Douglas fir seedlings of European provenance are lacking, save a few exceptions (Martiník et al. 2014a, b). Thus the report aims are to assess (a) whether Douglas fir seed lots of European provenance respond to cold stratification extended from 21 to 49 days by increased germination capacity as well as parameters to describe the germination rate; (b) on two seed lots of domestic (i.e. Czech) provenance, how the extended pre-sowing treatment is reflected in emergence rates, yield of seedlings and parameters of year-old seedlings.

MATERIAL AND METHODS

Material. Germination capacity was evaluated in nine Douglas fir seed lots of European provenance – of which five seed lots came from the Czech Republic, two from Slovakia and two from France, and one seed lot originated from North America (Table 1). Seed emergence and yield were tested for two domestic seed lots – Czech provenance (B and C).

Methods. ***Setting up the experiment.*** Seed germination capacity was tested under laboratory conditions using seed germinators complying with prescribed standards (ČSN 48 1211:2006; ISTA 2014). For all of the lots, seeds were analysed within 8 replications, 50 seeds per replication, with no pre-sowing treatment (C – Control variant), after 21 days of cold stratification (21), and after 49 days of stratification (49). Seed stratification was also realized according to applicable standards (ČSN 48 1211:2006; ISTA 2014). It consisted in soaking the seeds in water at 5°C for 48 h, surface drying of the seeds by spreading them on filter paper at a laboratory temperature for 2 h, and cold stratification in plastic bags at a constant temperature of 5°C.

Likewise, the emergence rate for variants C, 21 and 49 was tested in 8 runs, 50 seeds per replication. Sowing operations were underway in spring 2013 on the premises of the forest nursery in Řečkovice, which is a part of the Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University in Brno, Czech Republic; the location details: 49°15'N, 16°35'E. The sowing substrate consisted of a mixture of peat and siliceous sand (4:1 ratio by volume).

To evaluate the yield of seedlings, sowings were established simultaneously for lot B and lot C at the same nursery and on the same date and amounted to 0.25 g per 1 m^2 of seed bed.

Collecting and evaluation data. Germination capacity was assessed at regular intervals of 2 to 3 days from the start of the germination process, when any germinating seeds with radicles longer than the seed length were removed. Germination capacity was defined to equal to the number of germinated seeds at the end of the experiment (after 21 days) out of the total number of seeds; for filled seeds, germination capacity was defined to be a number of germinated seeds out of all filled seeds. The number of full/empty seeds was determined at the end of the germination test for each of the variants. Parameters evaluated in addition to the germination capacity involved: GE – Germination energy; a number of normally ger-

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

Seed lot	Country – region of provenance	National identification of seed origin
A	CR – PLO 6	CZ-2-2A-DG-1740-6-3-P
B	CR – PLO 36	CZ-2-2A-DG-3151-36-3-Z
C	CR – PLO 23	CZ-2-2A-DG-1005-23-5-L
D	CR – PLO 16	CZ-2-2B-DG-3102-16-6-J
E	CR – PLO 12	CZ-2-2B-DG-3883-12-4-P
F	SK	Okr/12
G	SK	PME-213-TO-017 (200-400)
H	FR	PME-VG-05 Washington 2VG
I	FR	PME-VG-02 Luzette -VG
J	Washington, USA – seed zone 422	SIA 473307, OECD 08137

Table 2. Germination parameters for tested seed lots of Douglas fir and pre-sowing treatment variants

Seed lots	Germination capacity of full seeds (%)			Empty seeds (%)	GE			R_{50}			PV		
	C	21	49		C	21	49	C	21	49	C	21	49
A	88.6	97.0	97.6	12.2	1.7	38.9	56.0	15	8	7	*	3.65	4.38
B	89.0	99.4	98.4	33.4	1.3	37.6	46.0	14	7	7	1.44	3.03	3.36
C	87.8	99.0	99.2	48.8	0.4	23.8	34.4	13	8	7	1.16	2.24	2.72
D	94.8	99.7	99.8	21.0	9.8	77.7	76.7	12	6	6	1.96	5.43	5.65
E	90.8	97.9	91.5	52.4	8.6	46.7	35.3	12	6	6	1.10	3.21	3.11
F	81.6	94.0	89.9	46.6	2.4	49.2	43.1	16	4	4	*	3.54	3.65
G	59.6	88.2	90.3	17.2	0.0	40.1	59.3	20	7	7	*	3.38	4.25
H	64.1	94.9	96.3	4.6	0.4	48.7	58.4	19	7	7	*	3.92	4.63
I	82.9	95.7	95.2	0.8	2.7	57.1	51.7	14	7	7	1.94	4.40	4.60
J	74.4	91.9	96.2	7.8	11.1	54.2	75.7	16	8	7	*	3.39	4.73

C – non-stratified seeds, 21, 49 – days of stratification, GE – germination energy, R_{50} – germination rate, PV – not reached in the time of germination test

minated seeds determined on day 7 after setting up the germination test and specified in the percentage of germinating seeds; PV – Germination value; a maximum quotient derived by dividing the number of germinants accumulated per day 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} – Germination rate; the number of days required for 50% of filled seeds to germinate (Edwards, El-Kassaby 1988).

For the emergence rate parameter, the assessment took place as part of 3–4 day periods from the beginning of germination. The seedlings that threw off the seed coat were considered as “emerged”. In addition to the total emergence rate over the reporting period, i.e. on 31 August, the emergence rate after three weeks from the start of germination was also assessed.

Yield of seedlings (%) was rated in the spring of the following year as the total amount of year-old seedlings obtained from the seeds sown per square metre. Analysed in addition to the quantity was the length of the aboveground part and the root system of all the emerged seedlings for each of the stratified variants/seed lots.

The data obtained for the characteristics of germination and emergence were transformed into a normal distribution using the arc-sin transformation. Comparison of the individual variants was carried out by multifactorial ANOVA with a level of significance (α) at 0.05 using the STATISTICA 12. The vertical columns in Fig. 1 represent 2× standard deviation. Multiple comparisons, i.e. the determination of differences or no differences between the variants, were made using the LSD test. Since the data sets characterising the length of aboveground part and root system met the basic assumptions about the data, it was possible to use the parametric t-test without editing data to compare both of the two variants of stratification (21 and 49).

RESULTS

Germination capacity

Germination capacity of filled seeds (Table 2) was different for each of the seed lots (P = 0.00). Differences between the control variant and the seeds stratified for both 21 and 49 days were also statistically significant (P = 0.00), all the analysed seed lots thus exhibited signs of dormancy. On the contrary, no differences in germination capacity were observed (P = 0.22) between stratification variants (21 and 49).

All the seed lots exhibit the high (typically over 90%) germination capacity of filled seeds after stratification (Table 2). The proportion of empty seeds was highly variable; for some seed lots, the proportion was as much as 50% (C, E, F), while for the seed from the seed orchards of French provenance or that coming from the native range of the Douglas fir, i.e. the USA., it was minimal (10%).

The ANOVA results showed that in addition to provenance (P = 0.00), the pre-sowing treatment (P = 0.00) had a significant effect on germination energy. The germination rate of non-stratified seeds was significantly lower at all times than that of stratified seed (Table 2) when expressed as germination energy (P = 0.00) and using parameter R_{50}, as well as the peak value coefficient. The parameter R_{50} did not detect a difference in the length of stratification with 50% of filled seeds germinating virtually at the same time after both 21 and 49 days of stratification. A significant difference between stratification variants 21 and 49 was found in germination energy (P = 0.00). Likewise, the germination rate expressed as the peak value was significantly higher in the extended (49 days) stratification variant compared with the conventional variant (21 days), except seed lot E where the peak value was slightly higher for variant 21 compared with variant 49.

Table 3. Comparison of emergence rates and yields for two selected seed lots of Douglas fir seed of Czech provenance

Seed lots	Treatment	Emergence rate (%)				Seedling yield
		at the end of observation		3 weeks after emergence beginning		
		all seeds	full seeds	all seeds	full seeds	
B	C	55	83	10	15	2
	21	58	83	39	56	18
	49	59	94	58	91	26
C	C	38	72	4	8	1
	21	49	97	25	50	16
	49	47	92	44	86	35

Table 3 illustrates the relatively high overall emergence rate of two seed lots of Czech provenance (B and C), with over 80% of filled seeds emerging from the stratified seed. The non-stratified seed exhibited a statistically significantly lower emergence rate (P = 0.00) compared with the stratified seed, with no differences observed between stratification variants 21 and 49 (P = 0.84). Seeds with no stratification also emerge very slowly; only a minimum amount of seedlings (up to 10%) emerged three weeks from the start of emergence. Stratified seeds emerged faster (P = 0.00). The length of stratification was also a key factor, when almost a maximum of the seedlings was able to emerge within 3 weeks from the start of emergence only after 49 days of stratification. After 21 days of stratification, the emergence rate was significantly lower (P = 0.00). The yield of seedlings from the seed with no stratification was minimal; it reached 20% after 21 days of stratification and was the highest after 49 days of stratification (26% for seed lot B and 35% for seed lot C).

Statistically significant differences between variants 21 and 49 in terms of parameters of one-year seedlings were found only for the length of aboveground parts in the case of seed lot B. Differences in the length of the root system were not statistically significant (Fig. 1).

DISCUSSION

The low yield of seedlings is a limiting factor for the production of Douglas fir planting stock. Out of many factors influencing the quantity of seedlings, we focused on the quality of seed, pre-sowing treatment or sowing time, as applicable. The seed lots analysed showed the high germination capacity of filled seeds. However, the proportion of empty seeds varied to a considerable extent while it is a key indicator of seed quality, important for determining the sowing rate.

Pre-sowing treatment is an essential part of nursery practice for tree species with dormancy (Kantor 1975). Although dormancy need not be the rule for Douglas fir (Müller et al. 1999; Dirr, Heuser 2006), seed lots that European nursery operations usually manage show certain signs of dormancy (Martiník, Palátová 2012). From the available references and the current practice (e.g. Seifert 2005; Martiník, Palátová 2012; ISTA 2014), there is an obvious need for cold stratification in spring sowing for this species.

Previous analyses (Martiník et al. 2013, 2014a, b) suggested that the option of 21 days of cold stratification of seeds (ISTA 2014, ČSN 48 1211:2006) recommended and applied in the field does not make full use of the seed potential. Although most of the

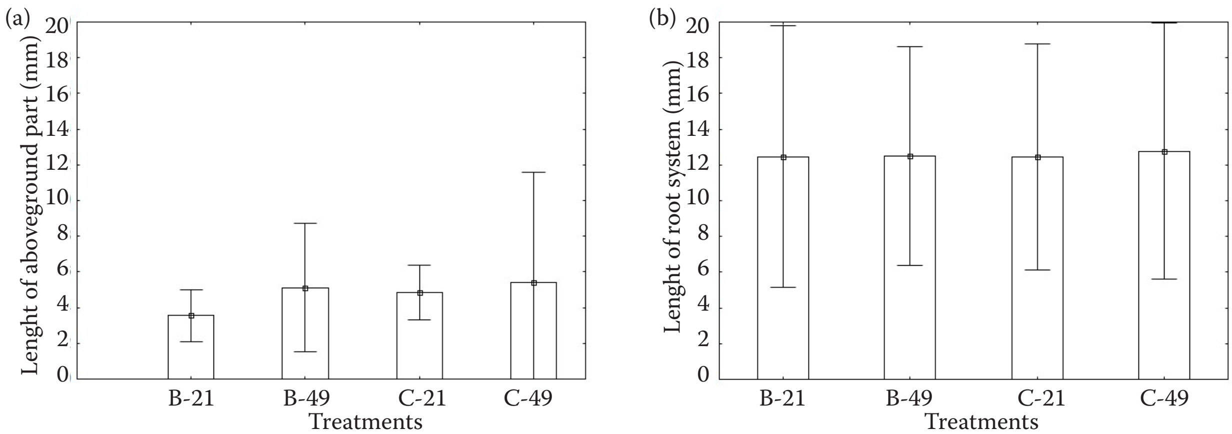

Fig. 1. Morphological parameters of one-year-old seedlings of Douglas fir provenance B and C after 21 and 49 days of stratification: aboveground part (a) and root system (b)

filled seeds emerge during the growing season, the emergence rate is lower than that of seeds with extended stratification period. With the objective of sowings at the forest nursery being to achieve the maximum quantity of seedlings over a relatively short period, i.e. about three weeks after the start of emergence, since it is a homogeneous and easier to treat sowing, giving a good premise for a maximum yield, the germination/emergence rate plays an important role in the production of planting stock, not only in the case of Douglas fir. Sorensen (1991) and Edward, El-Kassaby (1995) recommended extending the stratification period to make 35 days, while Seifert (2005) and Gosling et al. (2003) suggested that the success of seed germination is achieved for stratification periods exceeding even 70 days. Houšková et al. (2014) recommended to extend the Douglas fir seed stratification period to 49 days in the settings of the Czech Republic. The recommendation has been confirmed by the results of this study. The seed originating from four countries of Europe and America germinated faster with a 49-day stratification period than with the standard period of 21 days.

Compared with the germination of seeds under optimal laboratory conditions the rate of seed emergence in tree nurseries is influenced by moisture and temperature conditions in seed beds (Ferguson-Spears 1995). In addition to ecology the quantity of produced seedlings is then influenced by the growing practice. The yield testing made preferentially use of domestic seed lots which can be presumably sown under similar ecological and climatic conditions. A positive effect of extended stratification on the rate of emergence was also shown on the seed beds. Within three weeks from the start of emergence the emergence rate of seeds after 49 days of stratification reached values comparable with germination, meaning that the maximum quantity of seedlings emerged (91% and 86% of the quantity of filled seeds) unlike with the seed after 21 days of stratification, which produced about 30–40% less seedlings.

Even though the total rate of emergence of both seed lots tested did not differ between variants 21 and 49, the total number of seedlings that survived the first growing season was 8/19% higher for variant 49 compared with variant 21; it was thus the higher rate of emergence for seeds after 49 days of stratification which influenced the yield of seedlings.

With the results of observations also suggesting a high mortality of seedlings during the first year of cultivation of the Douglas fir planting stock, more limiting factors can be expected to influence the yield of seedlings in forest tree nurseries in this period (Thompson 1984). However, the extended 49-day stratification period is essential for maximising the potential of the seed.

Since the differences in the maturity of seedlings, more specifically in the length of the aboveground part and root, were not clear, the plant maturity may be influenced by the sowing time rather than the pre-sowing treatment period (Sorensen 1991). Subsequently, the spring timing of sowing commonly used and recommended for Douglas fir in the Czech Republic, i.e. April to May (Martiník et al. 2014c), should provide a prerequisite for the production of seedlings with required parameters.

CONCLUSIONS

The present results showed a positive effect of extending the stratification period from 21 to 49 days on the seedling yield of Douglas fir seed after the first year of cultivation. The differences identified may represent the yield of seedlings to be higher by 10% to 15%, thus ultimately 15 to 20 thousand more seedlings grown per 1 kg of seed. The results also indicated the future direction of research targeted at increasing the yield of seedlings in Douglas fir. This should involve optimising the technology of planting stock cultivation in the first year of life.

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Received for publication January 20, 2015
Accepted after corrections May 15, 2015

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