ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LXI 18 Number 1, 2013

http://dx.doi.org/10.11118/actaun201361010147

IMPACT OF PRE-SOWING TREATMENT AND SOWING SEASON ON DOUGLAS FIR EMERGENCE RATE IN A SPECIFIC SEED LOT

Antonín Martiník, Eva Palátová, Kateřina Houšková

Received: October 22, 2012

Abstract

MARTINÍK ANTONÍN, PALÁTOVÁ EVA, HOUŠKOVÁ KATEŘINA: *Impact of pre-sowing treatment and sowing season on Douglas fir emergence rate in a specific seed lot.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 1, pp. 147–155

Not only in the Czech Republic there is a problem with low yield of Douglas fir seedlings in forest tree nurseries. It can be caused mainly by two factors: the type of pre-sowing treatment and the temperature at the time of sowing. The aim of this study is to find out their influence on the emergence rate of Douglas fir. We have tested one specific seed lot originated from the Czech Republic subject to the following variants of pre-sowing treatment: soaking for 48 hours, stratification without a medium for 21 days and for 30 days after 48 hours of soaking and stratification for 30 days with a medium. The treated seeds were sown in a phytotron at temperatures of 13/8 °C – 10/14 hours (day/night) (simulation of early sowing season in February or March) and at temperatures of 17/13 °C – 14/10 hours (simulation of late sowing season in May). In case of the early sowing season, approximately half of the germinable seeds emerged in all treatment variants including the control variant (seeds without stratification). The late sowing season resulted in different emergence rate of the seeds that were subject to different variants of pre-sowing treatment (24–51 %). Then, 21day and 30day stratification were not sufficient for the tested conditions of the early and late sowing season. None of the combinations of pre-sowing treatment and sowing time resulted in full use of the seed potential of the tested seed lot.

Douglas fir, germination capacity, yield of seedlings

Douglas fir as a tree species introduced from western areas of the North American subcontinent has been grown in Europe including the Czech Republic for more than 120 years (Šindelář and Beran, 2004). Today, it covers 4400 ha which represents 0.17% of forest land in the Czech Republic (Kantor *et al.*, 2010). Annual regeneration, or rather reforestation task, is approximately 300 ha. From the aspect of forestry, Douglas fir is considered a perspective tree species and according to long-term conceptions of species composition, it is recommended to cover 2% of the total stand area. In 1994, the Forest Management Institute (ÚHÚL) even recommended its share to represent 4% of the stand area (in Beran and Šindelář, 1996). In suitable habitats (poorer types) and in areas with seed-producing stands, Douglas fir regenerates naturally (Šika, 1985; Kinský and Šika, 1987; Bušina, 2006; Kantor *et al.*, 2010). On fertile sites, on sites without seed-producing trees and in areas where Douglas fir is not present so far, artificial regeneration is necessary.

Effective pre-sowing treatment is essential for successful cultivation of Douglas fir planting stock for artificial regeneration as Douglas fir seeds exhibit dormancy, although, according to some authors, seed lots that are not dormant can also be found (Heit, 1968; Gosling and Peace, 1990; Müller *et al.*, 1999). The need of pre-sowing treatment can be eliminated by sowing in the autumn; however, it is connected with higher risk of damage to the young seedlings by the spring frosts (Papp, 1961). Hofman (1964) recommended preparing the seeds for the spring sowing, for example by soaking or by germinating in sand or in sawdust (Hofman and Heger, 1962). At present, pre-sowing treatment involves almost exclusively prechilling at 3–5 °C, usually after 24–48 hour soaking (Owsten and Stein, 1974; Gosling and Peace, 1990; Seifert, 2005, Czech National Standard, 2006; International Rules for Seed Testing, 1993 etc.). Duration of prechilling varies greatly according to authors from 2 to 12 weeks, or even longer using redrying. Edwards and El-Kassaby (1995) and Anonymous (2000) recommend prolonging of the prechilling period up to 5 weeks due to great genetic heterogeneity of the seed. Although it may not lead to increased germination capacity, it improves the germination rate of the heterogeneous seed lots, according to the authors.

Determination of the most suitable length of stratification is difficult because Douglas fir seeds show so-called conditional dormancy (Gosling, 1988) and their germination is influenced by temperature. Seeds without stratification germinate very slowly and in a very narrow range of temperatures. Prechilling at 3–5 °C has a positive effect involving higher germination capacity, faster germination rate and wider range of the temperatures allowing seed germination (Gosling, 1988; Gosling *et al.*, 2003). Conditional dormancy influences the need of stratification: stratification may not be necessary for germination at temperatures above 20 °C whereas at lower temperatures it is essential (Seifert, 2005). Sorensen (1991) also found out that germination of seeds is influenced not only by the length of stratification but also by the temperature of germination. The seeds that germinated at 15 °C required longer stratification to reach their maximum germination capacity while 21-day prechilling induced similar germination results at 25 °C. However, longer stratification period led to much faster and more uniform germination compared to shorter prechilling.

If temperature influences germination of the stratified seeds, it can also affect the emergence rate (field germination). In the Czech Republic, Douglas fir seeds are usually sown in spring after 21 days of prechilling (Czech National Standard, 2006). The aim of our research was to find out how the time of sowing in combination with the variant of stratification used influences the emergence and whether unfavourable temperature conditions in the seedbed after sowing cannot contribute to low seedling yields.

MATERIALS AND METHODS

For this experiment, a seed lot of Douglas fir (*Pseudotsuga menziesii*, var. *menziensii*) from a certified unit CZ-1-2C-DG-374-10-4-C was used. Origin of the stand is unknown.

We established an experiment under controlled conditions in a phytotron to find out how the post-sowing temperature influences the emergence of differently stratified Douglas fir seeds. Early and late sowing seasons were simulated by different temperature conditions as shown in Tab. I. The early sowing season simulated the conditions of February and March whilst the late sowing season simulated the conditions that the seeds may encounter in May. The selected conditions were based on the average temperatures and length of daylight according to sunrise and sunset in the respective months as presented in The Climate Atlas of the Czech Republic (2005). Four variants of pre-sowing treatment were tested within both early and late sowing season simulation (Tab. II). Seeds without stratification were used as a control sample. After the pre-sowing treatment, the seeds were sown into crates filled with a substrate of mixed peat and siliceous sand in a proportion of 4:1 and covered with a thin layer of siliceous sand. Each variant of pre-sowing treatment was set up in 4 repetitions by 100 seeds. Simultaneously, standard tests of germination capacity were established for each variant of pre-sowing treatment and the sowing period in germinators. The test of germination capacity was carried out according to the Czech National Standard (2006) at the temperatures of 30/20 °C for 8/16 hours. After 33 days, the crates

I: *Conditions simulating early and late sowing season*

Sowing season	Phytotron conditions		Period of germination in phytotron
	Day	Night	
Early sowing	13 °C, 10 hours	8 °C, 14 hours	11. 3.–13. 4. 2011
Late sowing	17 °C, 14 hours	13 °C, 10 hours	14. 4.–17. 5. 2011

II: *Variants of the pre-sowing treatment of Douglas fir seed*

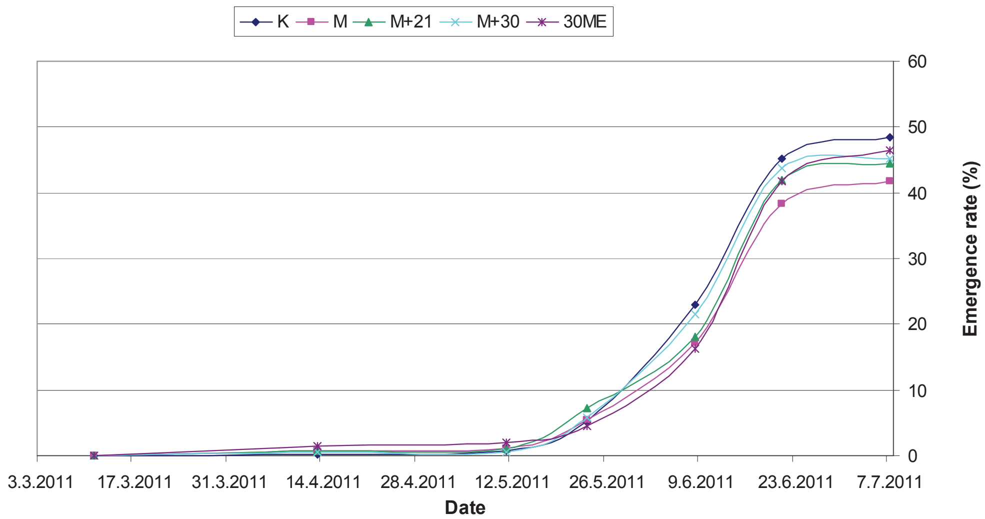
Variants	Pre-sowing treatment specification
K	Control – no pre-sowing treatment
M	Soaking in H_2O for 48 hours at 2 °C
M + 21	Soaking in H_2O for 48 hours at 2 °C and subsequent stratification without medium at 2 °C for 21 days
M + 30	Soaking in H_2O for 48 hours at 2 °C and subsequent stratification without medium at 2 °C for 30 days
30ME	Stratification with medium (1:4 mixture of sand and peat) at 2 °C for 30 days

were transferred from the phytotron to a nursery in Řečkovice, 49°15'N, 16°35'E (nursery of the Department of Silviculture, Faculty of Forestry and Wood Technology, Mendel University in Brno). The early sowing season seeds were moved on 13.04.2011 and late sowing season seeds 17.05.2011. Both in the phytotron and in natural conditions, the emergence rate was monitored and recorded for each variant of the treatment at regular intervals of 7–10 days as the number of the emerged young seedlings. The experiment was finished on 07.07.2011, i.e. after 118 days from the early sowing and 84 days from the late sowing.

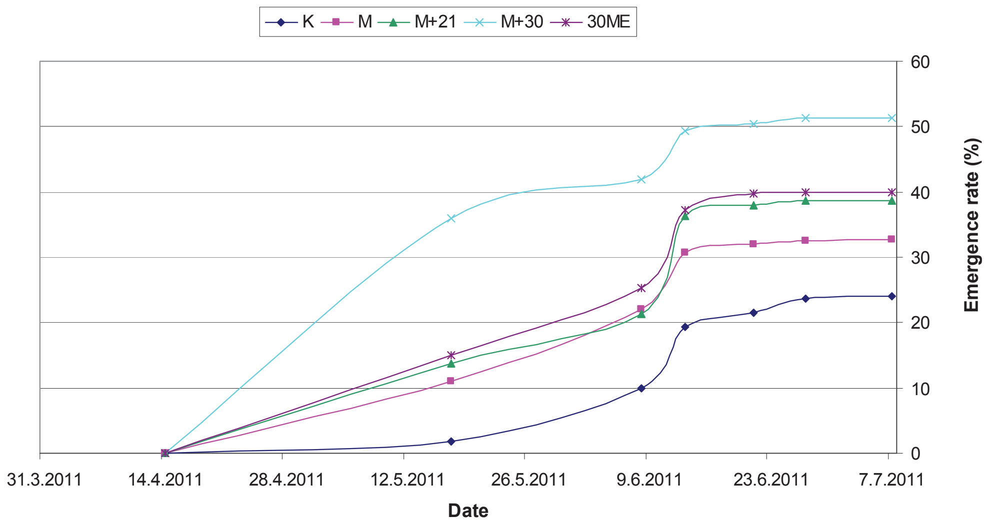
Differences in the overall emergence rate of individual variants of pre-sowing treatment for both dates of sowing were analysed by a Kruskal-Wallis test. Differences in the emergence rate between the particular dates of sowing and for the specific variants of pre-sowing treatment were evaluated by a t-test (α 0.05). Differences in the germination capacity of full seeds for each variant of pre-sowing treatment in early and late sowing season were also analysed by a t-test (α 0.05). Statistica and Excel software were used for data processing.

RESULTS

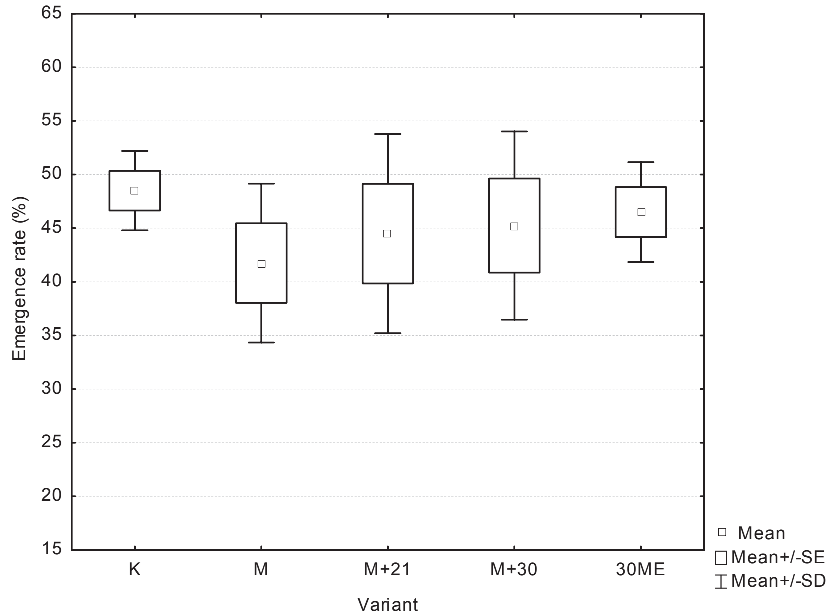
The experiment revealed that temperature after sowing influences the course of emergence as well as the total emergence rate of each pre-sowing treatment variant. The different courses of emergence in each variant are shown in Fig. 1 and 2. At low temperatures (early sowing), the seeds in a phytotron did not emerge in any variant of pre-sowing treatment. The seeds of all variants emerged at the end of May and at the beginning of June in a relatively short time after the crates had been transferred to natural conditions; the maximum (total) emergence rate achieved in the individual variants did not differ significantly. At the last counting (118 days after sowing), the highest



1: *Emergence of Douglas fir seeds subject to different variants of pre-sowing treatment – early sowing season simulation*



2: *Emergence of Douglas fir seeds subject to different variants of pre-sowing treatment – late sowing season simulation*



3: *Emergence rate of Douglas fir seeds in individual variants of pre-sowing treatment at the end of the experiment (07. 07. 2011) – early sowing*

III: *Kruskal-Wallis test results for early sowing*

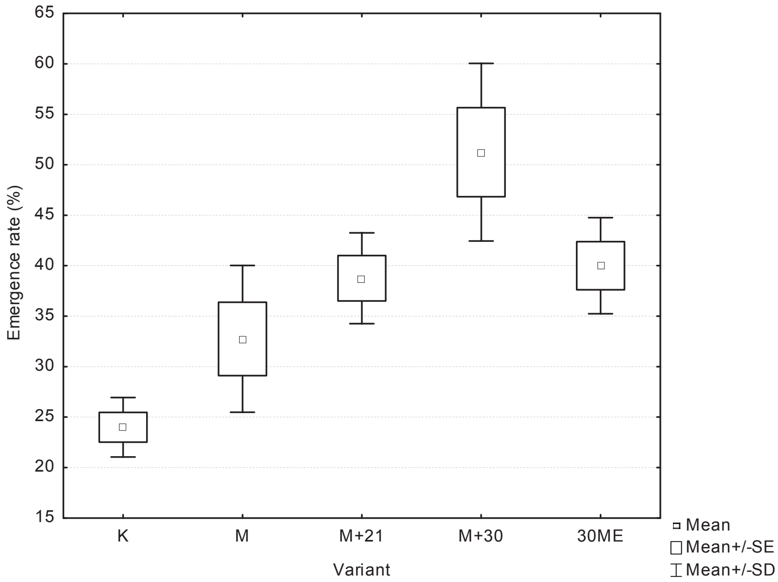
Variants	K R:13.125	M R:7.3750	M+21 R:10.000	M+30 R:10.750	30ME R:11.250
K		1.374513	0.747018	0.567734	0.448211
M	1.374513		0.627495	0.806779	0.926302
M+21	0.747018	0.627495		0.179284	0.298807
M+30	0.567734	0.806779	0.179284		0.119523
30ME	0.448211	0.926302	0.298807	0.119523	

IV: *Kruskal-Wallis test results for late sowing*

Variants	K R:2.7500	M R:8.1250	M+21 R:11.500	M+30 R:17.250	30ME R:12.875
K		1.284871	2.091650	3.466163	2.420338
M	1.284871		0.806779	2.181292	1.135467
M + 21	2.091650	0.806779		1.374513	0.328688
M + 30	3.466163	2.181292	1.374513		1.045825
30ME	2.420338	1.135467	0.328688	1.045825	

average emergence rate of 49% was found in the control variant of seeds without stratification (Fig. 3). The values of emergence rate obtained in other variants were lower (42–47%) but statistically not significantly (Tab. III). In slightly warmer conditions (simulation of late sowing), the control (non-stratified) seeds did not emerge at first whereas the stratified seeds started to emerge in the phytotron shortly after sowing. In contrast to early sowing, marked differences were observed between the emergence rates of the individual pre-sowing treatment variants. The seeds stratified for 30 days without a medium (M+30) were the fastest to emerge and this variant also showed the highest average emergence rate (51 %). No significant difference was found between the emergences of seeds stratified for 21 days and 30 days without a medium after 48hour soaking. The lowest statistically significant emergence rate was observed in the control variant (Tab. IV, Fig. 4).

Comparison of the differences in the emergence rate of each variant after early and late sowing (Tab. III, Tab. IV) revealed that only in case of M+30 variant after late sowing, the emergence rate was statistically significantly higher, and only when compared to the control variant. Besides, the control variant was the only one that exhibited statistically significant differences between the emergence rates after early and late sowing (Tab. V). Differences in the germination capacity of full seeds in each



4: *Emergence rate of Douglas fir seeds in individual variants of pre-sowing treatment at the end of the experiment (07. 07. 2011) – late sowing*

V: *T-test results comparing the emergence rate for individual variants of pre-sowing treatment between early and late sowing*

Variants	Average emergence rate – early sowing (%)	Average emergence rate – late sowing (%)	p – value (t-test)	Statistical significance
K	48.50	24.00	0.000047	sig.
M	41.75	32.75	0.133727	insig.
M + 21	44.50	38.75	0.307937	insig.
M + 30	45.25	51.25	0.371617	insig.
30ME	46.50	40.00	0.098724	insig.

VI: *Results of the t-test – comparison of germination capacity between early and late sowing for individual variants of pre-sowing treatment*

Variants	Early sowing				Late sowing				p – value (t-test)	Statistical significance
	G (%)	GE (%)	Coef. (%)	F (%)	G (%)	GE (%)	Coef. (%)	F (%)		
K	74	6	66	13	79	6	30	11	0.162068	insig.
M	80	12	52	11	79	10	42	9	0.755612	insig.
M + 21	89	26	50	1	89	20	44	3	0.984542	insig.
M + 30	95	48	48	1	87	19	59	4	0.002138	sig.
30ME	94	37	50	0	89	27	45	1	0.002725	sig.

G – germination capacity of full seeds, GE – germinative energy of full seeds, Coef. – percentage of emerged seeds from germinable seeds, F – percentage of fresh seeds

variant of pre-sowing treatment, as determined by standard germination capacity tests in germinators, were not statistically significant at the beginning of early and late sowing. Only in the M+30 and 30 ME variants, the germination capacity of full seeds was statistically significantly higher in the early sowing season compared to the late sowing (Tab. VI). Comparison of the germination capacity and the emergence rate allows us to assess to what extent the seeds have used their potential to produce viable seedlings. Despite the relatively high germination capacity of full seeds observed in individual variants of pre-sowing treatment, only about a half of the germinable seeds emerged (48–52%) in the early sowing. In the control variant, 66% of the seeds emerged. In the late sowing, the M+30 variant showed the best performance, with the emergence rate reaching 59% of the germination capacity of full seeds. Statistical significance of the differences is shown in Tab. V and VI.

DISCUSSION

One of the main problems of growing Douglas fir is the low seedling yield (Hofman and Heger, 1959; Gosling and Aldhous, 1994; Seifert, 2005; Cafourek, 2011 oral communication). This could be due to insufficient pre-sowing treatment that does not allow full use of the potential of the available seeds.

The yield of seedlings is defined as an amount of seedlings fully grown from the mass of seeds. It includes growth and possible loss within the first year of growing but is mainly affected by the emergence rate. Emergence rate depends on the quality of the seeds used, mainly on their germination capacity. Germination capacity, as determined by laboratory testing, expresses the maximum potential of a seed lot to provide viable seedlings. It is common to have a difference between emergence rate in natural conditions and results of laboratory examination. This can be caused by various factors, for example: in germination capacity tests under laboratory conditions, a seed is considered as germinated when the radicle is four times longer than the seed, whereas after sowing, a seed is considered as emerged when the primary leaves emerge from the seed coat (testa). At that moment, the radicle is approximately ten times longer than the seed. Besides, there are differences in the length of the testing periods. Germination capacity test takes 21 days, whereas counting of the emerged seeds usually requires much longer time (Coloteo *et al.* 2001). Emergence rate also depends on seed vigour. Seed lots with the same germination capacity can behave differently though the seeds were sown in identical but stressed conditions. The seeds with higher vigour are able to germinate in a wide range of conditions (Ferguson Spears, 1995; Coloteo *et al.*, 2001) and their emergence rate is therefore higher. The main difference is that the germination capacity tests in a laboratory are run under optimal conditions whereas emergence in soil is usually affected by less favourable conditions. The closer are the laboratory and natural conditions, the difference between the germination capacity and emergence rate is smaller (Ferguson Spears, 1995).

In our experiment, the seeds were kept for 33 days in simulated conditions in a phytotron. The early sowing season conditions simulated the outside conditions during February and March and in the simulated late sowing season, the temperature was similar to May. Germination capacity tests were carried out following the Czech National Standard (2006) at the temperatures of 30/20 °C (8/16 hours). These temperatures were significantly different compared to those chosen for emergence in our experiment. The differences between the laboratory and emergence rate can be partially ascribed to different temperatures to which the seeds were exposed. According to Gosling (1988), Douglas fir seeds are characterized by conditional dormancy. This means that the seeds germinate in a narrow range of conditions and preceding cold stratification at 3–5 °C has a positive effect on their germination. According to Sorensen (1991), the length of pre-sowing treatment required varies in relation to the temperature of germination. The author found out that to achieve maximum germination capacity of seeds, longer stratification was necessary when the seeds germinated at 15 °C, whereas at 25 °C, 21 days of cold stratification resulted in a similar germination capacity. According to Gosling *et al.* (2003), non-stratified Douglas fir seeds do not germinate at temperatures of 10, 15 or 35 °C; at temperatures of 20 and 30 °C they germinate at a lower extent. The author reports 25 °C as the optimal temperature for Douglas fir seeds germination. Extended time of cold stratification leads to higher germination capacity in a wide range of temperatures and after 48 weeks of stratification at 4 °C, all vital seeds germinated, regardless the temperature. Although the results are based on laboratory germination, we can apply them to a certain extent to emergence rate. The patterns described could explain the results detected in our study. For the 33 days of the early sowing season simulation (13/8 °C, 10/14 hours), signs of emergence were not observed in any variant of the pre-sowing treatment. In Sorensen`s (1991) results, three weeks (M+21) and thirty days (M+30) stratifications were not sufficient to allow emergence at such low temperatures. In simulation of the late sowing season (17/13 °C, 14/10 hours), seeds started to emerge in the phytotron already. The highest emergence rate was found in the variant with the longest 30-day (M+30) stratification whereas the seeds only soaked (M), stratified for 21 days (M+21) or non-stratified (K) emerged less (Fig. 2). With the same length of stratification (30 days), the variant 30ME reached a lower emergence rate than the variant M+30, but similar to the variant M+21. The detected difference could have been caused by slower seed hydration after their placing in stratification. In this variant, the seeds were not soaked for 48 hours but only mixed with a wet medium. Slower water intake could lead to shorter stratification period as stratification is effective only in seeds with water content above 20% (Gosling *et al.*, 2003).

After 33 days of emergence in a phytotron, the crates containing seeds were transferred to natural conditions in a nursery. The early sowing season seeds were moved on the mid-April and late sowing season seeds mid-May. Comparison of the courses of emergence and the total emergence rates is therefore subject to error resulting from exposure to different climate conditions from the dates stated above.

In the case of the early sowing season simulation, the subsequent process of emergence and the total emergence rate were not significantly different in the individual variants of pre-sowing treatment (Fig. 1). The fact that the control (non-stratified) seeds followed the same course of emergence and reached the same maximum value of emergence rate indicates that the low temperatures used in the early sowing simulation could have substituted the conditions of cold stratification. Germination of full seeds as detected in the individual variants of pre-sowing treatment following the conditions set by the Czech National Standard (2006) was relatively high, ranging from 74 to 94% in the early sowing simulation. However, the emergence rate reached only half of the germination capacity of full seeds. The best results were detected in the early-sown control variant (non-stratified seeds) where 66 % of the germinable full seeds emerged (this value probably included the seeds that did not germinate during the germination capacity test but were still germinable). Provided that the climate conditions are favourable and lower emergence rate can be accepted, very early sowing can replace autumn sowing that gives better results (Hofman and Heger, 1959; Hofman, 1964). According to Pappa (1961 in Hofman, 1964) and Sullivana and Sullivana (1984), autumn sowing eliminates the need of pre-sowing treatment but the seeds can suffer damage from birds, rodents, eventually from a spring frost. Very early spring sowing can have a positive effect on seedlings maturity (Carmichael, 1957; Sorensen, 1978).

Simulation of the late sowing season also did not have a significant effect on the emergence rate (Fig. 2 and 4). In contrast to the early sowing, the control (non-stratified) seeds were slowly emerging, but reached only 24% emergence rate which was statistically significantly lower than in the other variants of pre-sowing treatment. It means that the chosen temperatures did not have a positive effect on the non-stratified seeds and therefore, in this case, cold stratification is necessary. The differences in the total (maximum) emergence rate between the two sowing periods in each variant of pre-sowing treatment were not statistically significant with the exception of the control variant. Late sowing did not lead to full use of the seed potential either.

Our experiment confirmed that non-stratified Douglas fir seeds emerge slowly and only under certain conditions and that cold stratification prior to sowing has a positive effect on subsequent germination. An advantage of cold stratification is widening of the range of temperatures allowing the seeds to germinate (Gosling *et al.*, 2003). According to Jones and Gosling (1994), cold stratification for 6–24 weeks is necessary to induce seed tolerance towards the germination temperature. The periods of stratification tested in our study were significantly shorter (21 and 30 days) and therefore, the seeds could not react positively to suboptimal germination temperature. This could explain why the emergence rates observed in the two simulated sowing periods were not significantly different.

CONCLUSIONS

The aim of this experiment was to determine the effect of individual variants of pre-sowing treatment in a specific lot of Douglas fir seed on the emergence rate in simulated early and late sowing. The following findings have been made:

In simulated early sowing season in a phytotron (temperature 13/8 °C) for 33 days, seeds did not emerge in any of the variants of pre-sowing treatment. After being transferred to natural conditions, their emergence rate reached approximately 50 % of the germination capacity of full seeds in all variants, including the control variant (seeds without stratification).

In simulated late sowing season in a phytotron (temperature 17/13 °C), seed emergence rate after 33 days in individual variants of pre-sowing treatment varied, but only in the variant with 30day stratification without a medium it was significantly higher compared to the control variant. After the transfer to natural conditions, the emergence rate was between 24% (seeds without stratification) and 51% (30day stratification without medium).

Full seed germination capacity (as assessed by a laboratory test) in individual variants of pre-sowing treatment ranged from 79 to 94% in the early sowing simulation and from 79 to 89% in the late sowing. The seed potential was not sufficiently exploited in any combination of pre-sowing treatment and time of sowing.

Both 21day and 30day stratification were insufficient for the conditions chosen for the early and late sowing season simulation.

Further research will focus on verification of the obtained results in other Douglas fir seed lots and on determination of the optimal pre-sowing treatment length.

SUMMARY

One of the problems associated with artificial regeneration of Douglas fir (*Pseudotsuga menziesii* /MIRB./ FRANCO) is the low yield of seedlings. The main causes may be insufficient pre-sowing treatment of the seed or low temperature at the time of sowing. The aim of this study was to investigate the influences of different pre-sowing treatments and time of sowing on Douglas fir emergence rate. One specific seed lot originated from the Czech Republic was chosen. The following variants of pre-sowing treatment have been tested: soaking for 48 hours without stratification, soaking for 48 hours with stratification without a medium for 21 and for 30 days and stratification for 30 days with a medium without previous soaking. The control variant was left without any pre-sowing treatment. The early sowing (February, March) was simulated in a phytotron for 33 days with temperatures of 13/8 °C (day/night – 10/14 hours), the late sowing (May) with temperatures of 17/13 °C (14/10 hours); after that period, the experiment was transferred to seed beds. For all variants, the investigation included a laboratory test of germination capacity of full seeds; it ranged from 79 to 95 %. The results of emergence rate have shown that during simulation of early sowing in the phytotron, seeds did not emerge in any of the pre-

sowing treatment variants. After their transfer to natural conditions, the emergence rate reached about 50 % of the germination capacity of full seeds in all of the variants. During simulation of late sowing, seeds began to emerge in the phytotron already and at the time of the experiment termination, the emergence rate of the seeds subject to individual variants of pre-sowing treatment differed; however, only in the variant involving 30-day stratification without medium it was significantly higher (51%) compared to the other variants (24–40 %). Therefore, neither of the two dates of sowing and neither of the pre-sowing treatment variants has lead to full use of the seed potential. The results confirm that non-stratified seeds of Douglas fir germinate slowly and only in certain conditions and that cold stratification prior to seeding positively affects germination. An advantage of cold stratification is that it broadens the range of temperatures in which the seeds are able to germinate. Literature sources suggest that the length of cold stratification period set by both Czech and international standards (21 days) is not sufficient to fully promote the tolerance of seed to germination temperature. This could be the reason why the tested variants of pre-sowing treatment in the chosen seed lot did not provide satisfactory results.

Acknowledgement

This work was funded from the NAZV Grant No. QI112A172 – financial support by the Czech Republic Ministry of Agriculture.

REFERENCES

ANONYMOUS, 2000: Differences in Seed and Seedling Attributes Between Select (orchard produced) and Standard (wild stand) Seedlots. *Proceedings for the 2000 Forest Nursery Association of BC Meeting*. Prince George: Cone and Seed Improvement Program BCMoF Tree Seed Centre, 13.

BUŠINA, F., 2006: Natural regeneration of Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) in forest stands of Hůrky training Forest District, Higher Forestry School and Secondary Forestry School in Písek. *Journal of Forest Science*, 53, 1: 20–34.

CARMICHAEL, R. L., 1957: Relation of seeding date to germination of Douglas-fir seed. *Northwest Science*, 31, 4: 177–182.

COLOTEO, D., STEENIS, E. V., PETERSON, M., BENNETT, R., TROTTER, D., DENNIS, J., 2001: *Seed handling guidebook*. Ministry of Forests, British Columbia, 106.

Czech National Standard (ČSN 48 1211), 2006: Forest seed management – Collection, quality and test of seed stock quality of forest trees. ČNI, 60.

EDWARDS, D. G. W., EL-KASSABY, Y. A., 1995: Douglas-fir genotypic response to seed stratification. *Seed Science & Technologies*, 23, 771–778.

FERGUSON SPEARS, J., 1995: An introduction to seed vigour testing. In: VENTER, A. A. V. (ed.) *Seed vigour testing seminar*. ISTA, Zürich: 1–9.

GOSLING, P. G., 1988: The effect of moist chilling on the subsequent germination of some conifer trees over a range of temperatures. *Journal of Seed technology*, 12, 90–98.

GOSLING, P. G., ALDHOUS, J. R., 1994: Seed. Chapter 5. In: ALDHOUS, J. R., MASON, W. L. (eds.) Forest nursery practice. *Forestry commission bulletin 111*, London HMSO: 66–83.

GOSLING, P. G., PEACE, A. J., 1990: The analysis and interpretation of ISTA „double“ germination tests. *Seed Science & Technologies*, 18, 791–803.

GOSLING, P., SAMUEL, Y., PEACE, A., 2003: The effect of moisture content and prechill duration on dormancy breakage of Douglas fir seeds (Pseudotsuga menziesii var. menziesii [Mirb.] Franco). *Seed Science Research*, 13, 239–246.

HEIT, C. E., 1968: Propagation from seed. Part 17: Testing and growing Douglas fir seeds from different sources. *America Nurseryman*, 10, 12–60.

HOFMAN, J., 1964: *Silviculture of Douglas fir*. Státní zemědělské nakladatelství Praha, 253. ISBN 07-002-64.

HOFMAN, J., HEGER, B., 1959: Results of sowing operation of *Pseudotsuga menziesii* in 1956. *Work of VÚLHM*, 16, 85–100.

International Rules for Seed Testing (ISTA), 1993: International Seed Testing Association, Zürich: 288.

JONES, S. K., GOSLING, P., 1994: Target moisture content prechill overcomes the dormancy of temperate conifer seeds. *New Forests*, 8: 309–321.

KANTOR, P., BUŠINA, F., KNOTT, R., 2010: The position of Douglas fir (Pseudotsuga menziesii [Mirb.] Franco) and its natural regeneration at training forest district Hůrky of the secondary forestry schools Písek. *Zprávy lesnického výzkumu*, 55, 4: 251–262.

KINSKÝ, V., ŠIKA, A., 1987: Possibilities of natural regeneration of Douglas fir. *Lesnická práce*, 66, 9: 393–399.

MÜLLER, C., FALLERI, E., LAROPPE, E., BONNET-MASIMBERT, M., 1999: Drying and storage of prechilled Douglas fir, Pseudotsuga menziesii, seeds. *Canadian Journal of Forest Research*, 29, 2: 172–177.

OWSTEN, P. W., STEIN, W. I., 1974: Pseudotsuga Carr. Douglas-fir. In: SCHOPMEYER, C.S. (ed.) *Seeds of woody plants in the United States*. Forest Service, U.S. Department of Agriculture, Washington, D.C.: 374–683.

SEIFERT, S., 2005: *Treatment of seeds of conifer tree species*. Thesis. Faculty of Agricultural Sciences and Landscape Architecture, University of Applied Sciences Osnabrück, 154.

SORENSEN, F. C., 1978: Date of sowing and nursery growth of provenance of Pseudotsuga menziesii given two fertilizer regimes. *Journal of Applied Ecology*, 15, 1: 273–279.

SORENSEN, F. C., 1991: *Stratification period and germination of Douglas-Fir seed from Oregon seed orchard: Two case studies*. Research Note. Pacific Northwest Research Station, United States Department of Agriculture, Forest Service, PNW-RN-499, 23.

SULLIVAN, T. O., SULLIVAN, D. S., 1984: *Operational direct seeding of Douglas-fir and lodgepole pine with alternative foods in British Columbia*. Research note, Province of British Columbia, Ministry of Forest, 97, 16. ISSN 0226-9368.

ŠIKA, A., 1985: Reproduction possibilities of Douglas fir in ČSR from indigenous stands. *Work of VÚLHM*, 67, 41–62.

ŠINDELÁŘ, J., BERAN, F., 2004: To some current problems of Douglas fir cultivation (benchmarking study). *Lesnický průvodce*, VÚLHM Jíloviště-Strnady, 3, 34. ISBN 80-86461-38-6.

The Climate Atlas of the Czech Republic, 2007: Palacký University Olomouc, 255.

Address

Ing. Antonín Martiník, Ph.D., doc. RNDr. Ing. Eva Palátová, Ph.D., Ing. Kateřina Houšková, Ph.D., Department of Silviculture, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic, e-mail: martinik@mendelu.cz, eva.palatova@mendelu.cz, katerina.houskova@mendelu.cz