Generative and vegetative reproduction of *Pseudotsuga menziesii* (Mirb.) Franco in the Central Chernozem Region

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Abstract. The demand for wood products grows every year. There is an urgent need of fast-growing and economically valuable tree species. One of such species undoubtedly is *Pseudotsuga menziesii* (Mirb.) Franco, the fastest growing coniferous species with valuable wood. It was introduced in Russia a century ago but there is still virtually no research proving its efficiency and suitability for the Central Chernozem Region of Russia. Moreover, mass reproduction of *Pseudotsuga menziesii* in Russia has been held back by lack of parent trees and effective scientifically-based cultivation techniques. In this study we analyze the effectiveness of both generative and vegetative reproduction of the species in the studied region. Our findings show that the germination capacity of *Pseudotsuga menziesii* seeds ranges from 63 to 95% depending on the age of parent trees and growing conditions. Cold stratification is proved to be the optimal method of pre-sowing seed treatment for the species. Grafting can be used to introduce rare and decorative subvarieties of the genus. And on the whole, *Pseudotsuga menziesii* can be considered promising for forestry practice and green building of the region.

1. Introduction

Conifer species occupy approximately one-third of the Earth's forest area and play a crucial role in the world's ecosystems [1]. Currently, in light of the expected climate changes, forest sector desperately needs species with high adaptive potential and adaptive growth response to climate anomalies, especially to droughts, that are becoming increasingly common [2, 3]. Thus the choice of tree species is one of the most important tools for managing forest ecosystems, as it can help to increase forest productivity and industrial efficiency [4].

There are a number of factors to consider when selecting species for a sustainable forest. One of them undoubtedly is wood quality and value [3, 5]. *Pseudotsuga* is one of the most valuable conifer genuses. It is native to the west coast of the United States and Canada. The genus includes several varieties, which have been ranked as species by some botanists. Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) is one of the tallest tree species of the genus [6, 7]. It can grow up to 70 m (100 m under favorable conditions) attaining the diameter of 2 m, which exceeds that of coniferous species native to the Central Chernozem Region. The Central Chernozem Region (henceforth – the studied region) is a Russian economic region famous for its high quality soil, called Chernozem (Black Earth). It covers a total area of 167,700 km² and includes Voronezh, Belgorod, Kursk, Lipetsk and Tambov.
regions. The capital of the region is the city of Voronezh, located at latitude $51^\circ40'18"$N and longitude $39^\circ12'38"$W.

The wood of *Pseudotsuga* is second only to the genus *Larix* by its physical and mechanical characteristics and can be used for the same purposes [8]. It has a distinctive wood grain pattern and texture, which makes *Pseudotsuga* wood exceptionally suitable for furniture making. It can also be used as timber, plywood, lumber and for a whole range of materials from house construction to decorative woodwork. Since *Pseudotsuga menziesii* is one of the most fast-growing coniferous species, it far outstrips other species, native to the studied region. Also *Pseudotsuga menziesii* has high winter resistance, adaptability to nutrient-deficient soils, tolerance to air pollution and resistance to insect pests and diseases. These ecological characteristics and its exceptional resistance to drought and soil water deficit make the species suitable for city environment and allow its use for urban planting and landscaping [9-11]. By and large, a *Pseudotsuga* forest plot approaching maturity has 2 to 3 times higher standing crop than that of native conifer plantations [12]. *Pseudotsuga menziesii* is therefore an ideal species for forestry. It is also worth noting that *Pseudotsuga* is one of the most beautiful of the conifers. It has received national recognition in its home countries and deserves the widest possible application in the areas of its introduction.

The most decorative variety of the genus probably is 'glauca' (*Pseudotsuga menziesii* var. *glauca*), also known as Rocky Mountain Douglas-fir, ranked by some dendrologists as a distinct species. This variety is comparable to *Picea pungens* var. *glauca* due to the colour of its needles. *Pseudotsuga menziesii* var. *glauca* is inferior to *Pseudotsuga menziesii* in size, but has the same valuable ecological and biological characteristics. Despite the species' great economic value and its suitability for forestry there are still few scientific researches that sufficiently prove its fitness for Russia. The two types of *Pseudotsuga* have been thoroughly studied in the United States and Canada [13], but in our country there have been scarcely any attempt to introduce *Pseudotsuga menziesii* and *Pseudotsuga menziesii* var. *glauca* into new areas. Some contribution to the issue has been made on the territory of the Crimea and the Caucasus [14] but virtually no research has yet been conducted in the northern regions, and in particular in the studied region. The species therefore remains relatively unknown and neglected here by forestry specialists up to the present day.

It is noteworthy that generally the most productive and stable specimens prove to be those originated from the regions where climate conditions are similar to the region of introduction. Soil and climate conditions of the studied region are very similar to those of the west of North America and are therefore optimal for cultivation of *Pseudotsuga menziesii*. The species is of undeniable practical interest both for forestry and for green building and has great potential for its introduction into forestry practice of the region.

Since the XIX century *Pseudotsuga* seeds have been widely used for creation of forest plantations outside the natural habitat. In Europe, some plantations are registered as seed sources for forest plantations because the species is seen as a potential tool for adaptation to climate change [15].

In this article we analyze the effectiveness of generative and vegetative reproduction of *Pseudotsuga* species in the studied region. The aim of our research is to evaluate seed characteristics of *Pseudotsuga*, to reveal the dependence between germination capacity of *Pseudotsuga* seeds and conditions under which parent trees grow. Our long-range goal is to introduce the species into the forestry of the studied region.

We believe that the success of the species’ adaptation to new conditions depends primarily on reproduction quality and offspring vigor. Thus, determination of optimal conditions for better germination characteristics of seeds can contribute to development of methods of species’ reproduction and its further introduction in the studied region.

### 2. Materials and methods

The study was based on measurements and observations of *Pseudotsuga menziesii* seeds and seedlings. We used seeds collected in a breeding nursery of Voronezh State University of Forestry and Technologies named after G.F. Morozov (henceforth – the University), in the amenity forest of the
All-Russian Research Institute of Forest Genetics, Breeding and Biotechnology (henceforth – the Institute) and in the Semiluki forest breeding nursery in Voronezh region, created by the Institute. The choice of these three plots was not random. In 1972-1975 the Institute employee sowed *Pseudotsuga menziesii* seeds exported from Canada on a trial plot in the amenity forest of the Institute. In 1973 in the Semiluki forest breeding nursery scientists of the Institute planted 150 *Pseudotsuga menziesii* seedlings at the age of 8 years, grown in Forest Steppe Experimental Plant Breeding Station. Also in 1973 in a breeding nursery of the University a group of plant breeders planted 6-year-old *Pseudotsuga menziesii* seedlings grown in the Botanical Garden of Voronezh State University from seeds exported from the Baltic region. In our study we summarize the experience of previous generations of Voronezh breeders summing up the results of 45 years' work on *Pseudotsuga menziesii* introduction.

We evaluated seed productivity using the methodology devised by Vainagy [16].

Conifer seeds need to be pre-treated prior to planting. Some seeds have hard shells and the sleeping embryo inside it needs to be “woken up”. In 2013 we started experimental study of various pre-sowing seed treatment methods. The most common way of pre-sowing seed treatment is cold stratification, which is usually done by subjecting seeds to moist conditions at certain low temperature. Another way of pre-sowing seed treatment is storage in snow, when seeds are put under natural snow cover at temperatures near 0°C. Before sowing itself seeds are usually soaked in water for about 24 hours, which causes the seed coat to swell, beginning the initial stage of germination. We tried all these three ways of pre-sowing seed treatment as well as autumn sowing without any pre-treatment. The best results under laboratory conditions was achieved by soaking the seeds in water.

In order to evaluate seed characteristics of *Pseudotsuga* we selected four samples of 100 seeds each. We soaked them for 24 hours and then put them in glass Petri dishes with filter paper at the temperature of 22-25°C. After 7, 10, 15, 20, 25 and 30 days we checked the paper to see how many seeds had germinated. Some seeds sprouted already on the 15th day, but the germinating power (the number of seeds that germinated successfully, i.e. their primary root length reached the size of a seed itself) was measured on the 20th day. Cumulative germination percentage (the total number of seeds in the sample that germinated) was measured on the 30th day.

Experiments on green cuttings of *Pseudotsuga menziesii* were conducted in a cold greenhouse. We used various substrates: sand, peat, vermiculite, peat-sand mixture (1-1 ratio), vermiculite-peat mixture (1-1 ratio), and peat-soil-sand-vermiculite mixture (1-1-1 ratio). We selected 10 cuttings of 6-12 cm long, with 2 replications each, and treated them with ‘Kornevin’ – a stimulator of root formation, containing 5 g/kg of indole butiric acid. Another 10 cuttings, with 2 replications each, were taken without treatment.

Experiment on grafting was conducted on three-year-old seedlings of *Pseudotsuga menziesii* and *Abies alba* P. Mill. The branches for grafting were collected in March 2014 from 35-year-old trees in the amenity forest of the Institute. We used only shoots of one year old. The branches were kept under natural snow cover until the moment of grafting.

Grafting involved placing the cambium of an excised branch (the scion) on the duramen of the rootstock. This method was developed and first described by E.P. Prokazin [17]. First of all, we removed the needles from the rootstock, leaving a few of them around the apical bud. Then we made a slanting cut of 4 to 7 centimeter long at a narrow angle, which ran gradually across the stem passing through the duramen to the opposite side. Similarly we made a slicing cut of the same length on the rootstock, in this case, the section passed across the cambial layer, i.e, only a thing strip of bark was removed. After that we placed the scion on the rootstock so that the cambial regions matched as closely as possible. We used elastic grafting tape to wrap the graft in order to keep cuts tight and to prevent drying.

This method is recommended for grafting of thin conifers seedlings, as it usually results in highest survival rates. Experiment on grafting was carried out at the beginning of circulation of sap in plants (in the middle of April), and we used three samples of 100 seedlings each.
3. Results and discussion

According to the data obtained, the common cold stratification resulted in the highest seed germination capacity, that is to say it was almost equal to that figure under laboratory conditions (80-90%), as cold treatment usually results in higher germination percentages and better capacity for survival of the seedlings, as well as in their higher drought and cold tolerance. Table 1 presents the parameters of seeds of different Pseudotsuga menziesii trees according to our measurements.

Table 1. Germination of Pseudotsuga menziesii seeds harvested in 2011 under laboratory conditions.

| A reference number assigned to each Pseudotsuga menziesii tree | Weight of 1000 seeds, g | Germinatin, g power % | Cumulative germination, % |
|---------------------------------------------------------------|-------------------------|-----------------------|--------------------------|
| 7 (breeding nursery of the University)                        | 8.4±0.0025              | 9.8                   | 19.0±0.001               |
| 1-2 (breeding nursery of the University)                      | 10.5±0.0033             | 3.5                   | 36.5±0.005               |
| 2-4 (Semiluki forest breeding nursery)                        | 14.7±0.0035             | 6.3                   | 90.0±0.007               |
| 1-16 (amenity forest of the Institute)                        | 10.85±0.0029            | 7.5                   | 80.5±0.008               |

As shown in table 1, in the breeding nursery of the University cumulative germination percentage ranged from 19 to 36% for different trees at the age of 44 years old. In the amenity forest of the Institute this percentage was around 80% (at the age of 35-38 years old) and in the Semiluki forest breeding nursery – around 90%. The seeds from the Semiluki breeding nursery had the highest germination capacity (see table 1), we attribute it to more favorable conditions for cross-pollination there, to a larger number of bearing-age tree (46 years old) and to higher canopy density.

Plants growing in groups produce fewer empty seeds, because the pollination process is easier in that case. We used cones from an isolated tree (with reference number 7) as reference sample. What is more, we collected cones from different parts of the crown beginning with the lower part of the crown where cones were present (at the height of 5 meter from the ground) and then every 5 meters up to the summit of the tree (at the height of 10, 15, 20 and 25 m). Table 2 presents the results of the measurements.

Table 2. Germination of Pseudotsuga menziesii seeds harvested in 2011 under laboratory conditions.

| Height of Pseudotsuga menziesii tree at which cones were collected, m | Weight of 1000 seeds, g | Germinatin, g power % | Cumulative germination percentage, % |
|---------------------------------------------------------------------|-------------------------|-----------------------|---------------------------------------|
| 5                                                                   | 7.2±0.0025              | 11.5                  | 12.5±0.002                             |
| 10                                                                  | 8.4±0.0031              | 9.8                   | 19.0±0.001                             |
| 15                                                                  | 6.9±0.0018              | 23.8                  | 27.3±0.002                             |
| 20                                                                  | 7.6±0.0022              | 3.8                   | 21.5±0.002                             |
| 25                                                                  | 5.7±0.0027              | 1.3                   | 12.5±0.002                             |

Field germination was less than laboratory germination, because laboratory we maintained the optimal temperature and relative humidity, which helps the seed germination process. Introduction is considered to be successful if introduced plants demonstrate the ability to successfully reproduce by seeds in a new habitat. In that case natural regeneration from seed was abundant (40 plants per m²) only in the Semiluki forest breeding nursery (figure 1).

In winter 2014 we tested Pseudotsuga menziesii seeds collected in 2011 and 2012 from trees growing in the breeding nursery of the University. Table 3 presents results of measurements of seeds of different Pseudotsuga menziesii trees (weight of 1000 seeds, germinating power and cumulative germination percentage) in 2012, 2013 and 2014.
Figure 1. Natural regeneration of *Pseudotsuga menziesii* in the Semiluki breeding nursery.

Table 3. Germination capacity of *Pseudotsuga menziesii* seeds harvested in 2012, 2013 and 2014.

| Reference number assigned to each tree and a harvest year | Weight of 1000 seeds, g | Germinating power, % | Cumulative germination percentage, |
|----------------------------------------------------------|------------------------|----------------------|-----------------------------------|
|                                                          |                        | 2012  | 2013  | 2014  | 2012  | 2013  | 2014  |
| 9, collected in 2011                                      | 8.6±0.02               | 9.0   | -     | 7.3   | 12.0  | -     | 9.3   |
| 16, collected in 2011                                     | 6.3±0.02               | 7.0   | -     | 10.3  | 10.0  | -     | 10.8  |
| 9, collected in 2012                                      | 8.4±0.03               | -     | 9.8   | 11.2  | -     | 19.0  | 12.0  |

As shown in the table 3, the seeds collected from tree 9 in 2011 after two years of storage lost their laboratory germination capacity by 3%. The seeds collected from tree 9 in 2012 after one year of storage lost their germination capacity by 7%. Whereas the seeds, collected from tree 16 after two years of storage, retained the initial germination capacity, those seeds had been kept in a closed vessel in a fridge at 5°C.

In the spring of 2013, we launched an experiment on green cuttings of *Pseudotsuga menziesii*. The cuttings were planted in different substrates on 6 and 10 April 2013. The observations and calculations were made on 20 September 2013. We registered only callus formation, and though the percentage of it was quite high (71-100 %) the green cuttings had not formed roots.

In the spring of 2014, we repeated the experiment on green cuttings of *Pseudotsuga menziesii*. The cuttings were planted in different substrates on 22 May 2014. The observations and calculations were made on 1 November 2014. Shoots in question still hadn't formed roots, although callus formation was registered in 80-95 % of green cuttings.

We repeated the experiment in the spring of 2014. This time the green cuttings of *Pseudotsuga menziesii* were planted in substrates on 22 May 2014. The observations and calculations were made on 1 November 2014. Shoots in question still hadn't formed roots, although callus formation was registered in 80-95 % of green cuttings.

Also in 2014 we conducted a grafting experiment with *Pseudotsuga menziesii* var. *glauca*. The experiment was carried out from 10 to 15 April 2014. Survival rate for grafts ranged from 70 to 90%, depending on the type of rootstock. Table 4 shows the survival rate of *Pseudotsuga menziesii* var. *glauca* grafts.
Table 4. The survival rate of *Pseudotsuga menziesii* var. *glauc*a grafts.

| Type of rootstock | Number of grafts | Length of cuts, cm | Number of survived grafts | Survival rate, % |
|-------------------|------------------|---------------------|----------------------------|-----------------|
| *Pseudotsuga menziesii* | 100              | 5-7                 | 85±0.0025                  | 85±0.0014       |
| *Pseudotsuga menziesii* | 100              | 4-5                 | 89±0.0031                  | 89±0.0016       |
| *Pseudotsuga menziesii* | 100              | 5-7                 | 91±0.0027                  | 91±0.0022       |
| *Abies alba*        | 100              | 4-5                 | 72±0.0015                  | 72±0.0035       |
| *Abies alba*        | 100              | 5-7                 | 68±0.0017                  | 68±0.0029       |
| *Abies alba*        | 100              | 4-5                 | 74±0.0028                  | 74±0.0039       |

As shown in table 4, the survival rate was the highest for the most biologically close species (*Pseudotsuga menziesii*). The experiment did not reveal any statistically significant impact of cut length on survival rate. At the moment, the literature data indicate that the germination capacity of *Pseudotsuga menziesii* seeds ranges from 55 to 75% depending on the variety [3]. The optimal method of pre-sowing seed treatment for *Pseudotsuga menziesii* is the common 30-day cold stratification without treatment with stimulators. Our study confirms this observation, in our case this method of pre-sowing seed treatment resulted in cumulative germination percentage of 80-90%.

4. Conclusion

Our study focused on generative and vegetative reproduction of *Pseudotsuga* species. Based on the results obtained, we conclude that the seeds from one of our three experimental plots, namely, in the Semiluki forest breeding nursery, had the highest germination capacity. We attribute this to the fact that the parent trees of *Pseudotsuga mensiesi* grew in more favorable conditions there.

Among the pre-sowing seed treatment methods, cold stratification without treatment with stimulat ors has proved to be the most effective and cost-effective, both of which are essential for mass introduction of *Pseudotsuga menziesii* into forestry practice and green building of the studied region.

Comparing the findings of our research into some aspects of vegetative reproduction of *Pseudotsuga mensiesi* with literary sources we have noticed that existing theories assume that only cuttings from very young high-quality plants (no more than 5-10 years old) root relatively well [3]. As we were in possession of only 35-year-old trees in the amenity forest of the Institute, that might explain the fact that the shoots used for the cutting experiment described above did not deliver a favorable effect.

The experiments on green cuttings we conducted on various substrates showed a high percentage of callus formation (71%, sometimes even up to 100%). However, it was not followed up by root formation. Thus, the method requires further elaboration and clarification. Right now we can only assume that the process of rooting for *Pseudotsuga mensiesi* takes a longer period of time, perhaps such experiments should be conducted in warm greenhouses. The experiment on grafting of *Pseudotsuga menziesii* var. *glauc*a resulted in a high survival rate only for the most biologically close species (*Pseudotsuga menziesii*). Therefore, vegetative reproduction using grafting techniques can be recommended for introducing the rare and valuable *Pseudotsuga menziesii* var. *glauc*a into the forestry practice in the Central Chernozem Region of Russia.

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