Features of growth and development of *Abies alba* Mill. seedlings in the conditions of Voronezh (Russia)

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Abstract. Phenological observations are one of the methods for studying the reaction of introduced species to new conditions and subsequent introduction into regional phytocenoses. This research wants to assess growth and development of *Abies alba* Mill., currently exotic in the Central Black Earth region of Russia. Seeds, obtained from individuals growing in the natural range (the Carpathians) in 1987 were sown in the university nursery. Then five-year-old seedlings were planted in the University arboretum. At the age of 22 the arboretum habitat was inferior to natural analogues. Phenological observations of bud opening and formation of annual growth coincide with the beginning of the growing season in natural plantings. High rates of seed germination (80%), high annual growth rates of seedlings (and transplanted plant) determined final success of silver fir in forest-steppe conditions. Precipitation has a dominant influence on growth and development, as shown by the correlation analysis between climate and growth, despite the fact that the species is drought-resistant in natural habitats. The study emphasizes the factors that control growth and development of silver fir. The use of this method of sowing is ecologically sound, which can further improve the success of species adaptation to the individual conditions of regional phytocenoses.

1. Introduction

Genus *Abies* Hill. is the second largest genus of the *Pinaceae* Lindl family with the number of species ranging between 40 to 50, depending on the specific classification scheme [1]. About eight of these species are common in the Mediterranean Basin and represent a good model for studying the effects of range fragmentation.

Silver fir (*Abies alba* Mill.) grows in the mountain forests of Western, Southern, South-Eastern and Central Europe, where it is an ecologically and economically important species. It is usually associated with beech (*Fagus sylvatica* L.) at low and medium altitudes, and with spruce (*Picea abies* L. Karst.) at high altitudes. In subalpine zones, the species grows in pure stands [2]. On the territory of the Russian Federation, it can be found only in the northern part of the Transcaucasus, growing separately or together with beech species.

Genus *Abies* Hill. (despite their wide distribution and great economic importance) remain poorly studied from the point of view of practical experience in afforestation, which greatly narrows the possibility of rational use of its versatile properties.

In addition to its environmental importance (including its ease of natural regeneration), fir is prized for its high quality timber used for furniture and musical instruments. Fir seeds have also been a
valuable raw material for the production of essential oils, preventing them from being used in forestry and reducing the seed bank in the soil.

The impact of global climatic changes is causing profound transformations in natural environments and landscape structure, threatening biodiversity and habitats. The effects of climate change are widely studied on a variety of scales, ranging from the physiological response to the regional distribution of plants, using a variety of methods. “Among various methods of silvicultural adaptation, planting of various species, including alternatives, has been proposed as an effective management option,” George J et al. [2].

Abies alba (like other plants) is adapting to climate change. First, its growth rates are obviously changing. We all know that tree height and stem diameter can be influenced by a wide range of complex interacting factors – climate, soil conditions and competition for resources. Understanding the changes in tree growth is important for addressing environmental issues and for sustainable forestry. “The interaction between tree growth, climate and soil conditions shows that the growth and productivity of stands of the genus Abies Hill. positively correlated with the amount of precipitation, negatively with temperature, and poorly correlated with the nutritional quality of the site”, Kobal M et al. [3].

The dynamics of growth, as a response to climate, can be investigated by standard analysis: measuring the width of annual rings or obtaining data on stable isotopes of carbon and oxygen [4-6].

In Central Europe, there has been intense and controversial debate about the reasons for the reduction in area-coverage and seed production of Abies alba Mill. Hypotheses have been put forward suggesting the impact of phyto-diseases, insect damage, inappropriate forestry practices, etc. Currently, most of these hypotheses have been rejected in favor of two alternative ones.

The essence of the first hypothesis is reduction of Abies alba Mill. growth, primarily associated with the impact of climatic factors. The second hypothesis (1970-1990) indicates the suppression of Abies alba Mill. growth due to the impact of SO2, but in a complex interaction with climatic and biotic factors [7].

The confirmation of the first hypothesis is in publications, the meaning of which boils down to identifying the climate-induced differentiations of growth response of Abies alba Mill. Researchers believe that these cumulative effects can be a powerful tool for understanding forest dynamics in the face of uncertain climate change scenarios [8].

Changes in the response of growth to temporal climate variability can lead to a change in the equilibrium of the forest ecosystem due to changes in the phenological characteristics of plants, the species’ ecological amplitude and dynamics of the communities’ composition. Thus, there are serious concerns about the impact of climate change on the growth and distribution of species along altitude gradients, especially in Mediterranean forests at their extreme southern border of distribution, where these communities experience increased sensitivity to stress in the form of summer drought.

The most common method for obtaining information on the influence of climate on a tree species is the analysis of tree rings mentioned above. The result of the influence of environmental factors is an immediate reaction to the width of the growth rings. The effect on growth of tree rings is tested by comparing growth before, during and after known abiotic, biotic or anthropogenic stress. Particularly interesting data are data on intra-annual fluctuations in the density of early or transitional wood. They indicate past drought in spring or early summer, followed by wet conditions, but their formation and functional roles are not well understood [9].

We know that thanks to its deep root system, adults of Abies Hill. are drought tolerant. However, (in comparison with other conifers) Abies Hill. is sensitive to sudden frost and winter temperatures below -26 to -27 °C. Frost leads to the loss of needles and sharp reduction in the growth of wood in the next growing season.

In support of the second hypothesis, we find information in publications of a biological nature (for the period around the 1980s). It has been experimentally confirmed that the effects of SO2 have a comprehensive effect on the vital functions of Abies alba Mill.
Considering the two alternative hypotheses described above, we must understand that the characteristics of growth and development of any species ultimately affect vertical spatial structure of forest. Forest dynamics is the product of three partially opposite processes: tree growth, which leads to the accumulation of stand volume, tree death, which leads to a decrease in volume, and regeneration, which causes the exchange of tree generations [10].

Among these processes, the most deterministic one is tree growth, which is highly autocorrelated due to the slow rate of change in the tree architecture and strong dependence on a number of relatively stable plot variables.

*Abies alba* Mill. is a durable tree, as we have indicated earlier, has a multipurpose use, and can be recommended for the creation of forest plantations in the Central Black Earth region of the Russian Federation. The biology of the species is such that the fir is a slow-growing species even in its natural range. We assume that the introduction of *Abies alba* Mill. in the plantations of the Voronezh region and the Central Black Earth region as a whole will increase biodiversity. At this point in time, the studied species is an introduced species that has successfully passed acclimatization, but in terms of numbers in the territory of the Voronezh region, it still remains to be an exotic one. This species is recorded in botanical gardens, arboreta, in large city parks of the Central Black Earth Region of the Russian Federation.

Successful introduction of *Abies alba* Mill. in the Voronezh region is closely associated with the adaptation of functions and structures of the species to the new environmental conditions. Adaptive reactions of introduced species provide not only their survival in changed conditions, but also contribute to a higher biological and economic productivity, an increase in decorative qualities.

The purpose of this study is comprehensive research of the growth and development of *Abies alba* Mill. seedlings grown from the seeds of their natural range (the Carpathians) by direct sowing in the natural and climatic conditions of the Voronezh region.

Research objectives were to study the ecological and biological characteristics of *Abies alba* Mill. seedlings based on observations of the passage of phenophases; apply the method of direct sowing of seeds; to record the growth of seedlings grown from the seeds of the species natural range in the conditions of the Voronezh region, taking into account the influence of a complex of natural and anthropogenic factors; to determine the possibility of introducing the studied species to increase biodiversity in the conditions of the Voronezh region and the Central Black Earth Region as a whole.

2. Materials and methods

The Central Black Earth Region (hereinafter – the Central Black Earth Region) has a total area of 167.7 thousand km². It is an economic region located in Russia and including the Belgorod, Voronezh, Kursk, Lipetsk and Tambov regions. The capital of the region is Voronezh, located 51°40' 18" N, 39° 12' 38" E.

The method of sowing seeds and analysis of growth dynamics (specifically, tree height as a whole and the growth of shoots in one growing season) are the key factors determining the state of tree and shrub species and phytocenoses in general.

In pure stands of the same age, the plot index (height of the dominant trees at the control age) is almost independent of competition. However, some experimental research provides evidence of density dependence of height growth, even for dominant trees in unmixed stands. The observed variations in growth depending on distance are explained by competition for light [11].

In order to neutralize some of the abiotic factors in our region, we have undertaken the direct seeding method as a generally accepted alternative means of artificial regeneration. Seedlings obtained by direct sowing tend to demonstrate a high degree of adaptation to environmental conditions and have a well-developed root system [12].

However, direct sowing of introduced species on the territory of the Central Black Earth Region has always been of secondary importance due to the problems with low quality and quantity of seeds, wrong choice of a place for sowing and, as a consequence, poor seed germination. The main methods are planting crops (1-3 year old seedlings or seedlings).
In our research, we focused on the following tasks: to adapt the seedlings to the regional climatic conditions as much as possible and to fully satisfy the need for planting material by proper selection.

Research objectives are to study the ecological and biological characteristics of seedlings *Abies alba* Mill. based on observations of the passage of phenophases; apply the direct sowing method; record the growth of seedlings; to determine the possibility of introducing the studied breed to increase biodiversity in the conditions of the Voronezh region and the Central Black Earth Region in general.

Seeds of *Abies alba* Mill. were obtained by us from individuals from a natural range (the Carpathian mountains) in 1987 in order to study the introduction of the species in the Voronezh region. The seeds were sown in the nursery of our university and then introduced into the arboretum in 1992 with five-year-old seedlings, respectively. Since that time, we have carried out phenological observations of the species acclimatization. *Abies alba* Mill. refers to monoecious fir. In the arboretum of Voronezh State University of Forestry and Technologies, the number of individuals is 3 pcs. They are close-set, so pollination of the trees is straightforward. The ontogenetic development of the species has always been satisfactory. At the age of 22, the habit of three specimens is on average: tree height is 5.5 m; trunk diameter is 75 cm, crown diameter – 2 m, which is slightly inferior to natural analogs. At the age 22, *Abies alba* Mill. the first seed production was noted (in natural ecosystems in free standing from 30 years old, under the forest canopy much later from 60-70 years old).

Further, we set the goal of introducing the studied species into the forestry of the region. In autumn (end of September), 2009, we collected cones with seeds in the arboretum for subsequent sowing next spring in the nursery of All-Russian Research Institute of Forest Genetics, Selection and Biotechnology (hereinafter referred to as the nursery). This nursery is located in the city, and experiences the entire range of anthropogenic factors. Distance from the nursery to the arboretum is only 2 km.

Storing fir seeds for several months or years requires special equipment to slowly reduce the water content and then cool the seeds without damage [12]. We have obtained a short-term storage of fir seeds at the recommended seed moisture content from 9 to 12% and temperatures from 0 °C to -5 °C.

The factors determining the success or failure of an individual direct planting of trees can be divided into two categories: unpredictable, directly influenced by the environment, and the possibility of modification using forestry techniques and technical equipment.

The unpredictable natural factors for the Voronezh region are: sharp and sudden frosts in late spring; severe drought in summer; thaws in December and January. All this can lead to a complete loss of seedlings after their successful germination. When conducting field experiments, we also need to take into account that the time of bud opening may coincide with late spring frosts, otherwise this may lead to a partial loss of the vegetative season [13].

We took into account the sowing experience of some researchers. Some of them noted that the germination rate can vary from 15% -30%, others recorded the germination rate of less than 50%, and still others recorded high germination rate variability from 5 to 90%. Such a range in the percentage of seed germination can be explained by the fact that this indicator decreases with an increase in the terrain above sea level [14].

A direct seeding experiment was started in the nursery on stationary beds at the end of April 2010. A total of 5000 seeds were sown. We noted information on the use of various stimulants for pre-sowing preparation of seeds of coniferous plants, which makes it possible to increase seed germination, improve seedling resistance to unfavorable environmental factors, increase survival rate, enhance plant immunity, and also activate seedling growth [15]. We thought that the presence of resins and essential oils in the seeds of fir, contained in the seed coat, would protect the seeds from both biotic (for example, birds) and abiotic (for example, temperature) effects. Therefore, we did not carry out special pre-sowing seed treatment.

The soil in the nursery is from grey forest. This type of soil is the most common in the forest ecosystems of the region (in Europe, silver fir grows on loam). The relief is smooth, and soil preparation included only removal of weeds. Competition from terrestrial vegetation can be another factor damaging shoots and seedlings.
We have also eliminated the threat posed by competition from tree and shrub species by sowing silver fir in a free area. We consider this fact to be the key one in the method of direct sowing, since subsequently rooted plants have more access to nutrient reserves and at some stage will outstrip tree growth in ecosystems, or will be more stable in phytocenoses of the same age. We did not shade the young seedlings, despite the fact that the species is shade-tolerant one, but a significant linear increase in the growth of young individuals of *Abies alba* Mill. after a slight increase in illumination level has been recorded [12]. Despite the fact that the seedlings were grown in high light, the trees had conical crowns, and did not become umbrella-shaped.

There are different opinions about the amount of seeds needed for sowing in specific areas. We made the calculation as follows. Accepted sowing rate *Abies alba* Mill. in the Russian Federation is 5 grams of seeds per linear meter. We sowed 5000 seeds per 100 linear meters. The average weight of 1000 fir seeds is 65 grams. Thus, 3.3 grams of seeds were sown per linear meter. By reducing the plant population, we thereby increased the feeding area of each future seedling.

Subsequently, we did not transplant the seedlings into pits, which, according to some researchers, provide additional shelter, and future seedlings grew in furrows perfectly.

For further phenological observations, we randomly selected 30 seedlings.

3. Results and discussion

We know from the literature that reaction rates of forest-forming species of the *Abies* Hill. genus show that their limit is wider than the ecological conditions of the natural range of species introduction in terms of amount of warm during the growing season by 10-15%. The limit is also wider in terms of resistance to low winter temperatures by 5-45%, and in terms of moisture content of the growing season – by 45% [9]. Thus, we conclude that the significant plasticity of exotic species, which determines their ability to adapt to new growing conditions, is the main guarantee of successful development of *Abies alba* Mill. outside their natural distribution area.

Phenological observation is one of the methods for studying the reaction of introduced species to the new growing conditions. Research on the phenology of trees is becoming more and more relevant due to the ongoing climate change in the Central Black Earth Region.

The analysis of phenological observations of *Abies alba* Mill. showed that swelling of the buds in different years occurs at approximately the same time – in 3-4 weeks of April. Breaking of buds was seen from 8 to 10 of May at an average daily air temperature of +4°C-+11°C. On average, the duration of this phenophase was 20-26 days.

The beginning of shoot growth was observed from June 21, the end - from July 17 of the current year. According to our observations, the duration of shoot growth is 63-71 days. In terms of precipitation, the Voronezh region can be considered a well-to-do region, with an average of 682 mm and most of 655 mm during the growing season.

Direct seeding method was used to obtain seedlings of *Abies alba* Mill. It is a cost effective alternative to planting because of the lower cost of seeds and the method itself. As we have already discussed in the section materials and methods, we sowed 5000 seeds. Seedling germination rate was 80%. In the end, we received 4 thousand seedlings, which are still developing well on the territory of the nursery.

The most general regularity of the duration and energy of tree growth depending on the weather conditions of the year is as follows: in the first half of the growing season, when there is still sufficient moisture in the soil, the amount of growth depends on the sum of positive temperatures, and in the second half of the growing season, when there is little water in the soil, it depends on precipitation.

The first shoots appeared in 10-14 days in our region and approximately 2-4 weeks after the emergence of seedlings. Necrotic spots were found on the seedling cotyledons in cool and damp weather. The researchers associated this with pathogens belonging to the genera *Fusarium, Neonectria (Ascomycota), Pythium, Phytophthora (Oomycota), Rhizoctonia (Basidiomycota)* [16]. We did not record the presence of pathogens or the appearance of necrotic spots in our region (comparing to the mountainous regions of Central Europe).
The average height of a one-year seedling by the end of the growing season was from 4 cm to 10 cm (figure 1). From 2010 to 2014, the fir grew very slowly and we did not consider it necessary to make accurate measurements of the growth of each seedling. Then we resumed measuring the annual growth from 2015 to 2020 (figure 1). We recorded that sharp annual increases were noted in 2016-2017 (maximum growth was up to 17 cm) with an amount of precipitation of 789-644 mm, and with a sufficiently high average annual temperature of +8 °C (+8.3 °C). In 2018, precipitation fell slightly less – 612 mm of precipitation (temperature +7.6°C), but the positive dynamics of an increase in the length of shoots remained, on average, from 13.5 cm to 15.0 cm. It is possible that moisture from the previous year remained in the soil and in 2018 the soil was covered with a high snow cover for a long time.

The growing season of 2020 turned out to be extremely dry, precipitation was only in the form of light snow in February 2020, as a result of a continuous snow cover. Autumn 2020 was also not typical for the region (absence of the rainy season). As the result, average annual growth was 5-7 cm.

At the end of the 2020 growing season, we measured the total tree height and crown diameter. Average tree height in 30 specimens of *Abies alba* Mill. it varies from 84 cm (with a crown diameter of 60 cm) to 117 cm (80 cm) by the age of 10. Kapper O.G. gave the information that the species in natural populations reaches 70 cm in height by the age of 9 years. We see that the height of the seedlings grown by us and individuals of the natural population at the same age is practically the same.

Analyzing the growth of *Abies alba* Mill. in height in different years, we observe a clear increase in growth in 2018 for all the specimens (figure 1). The increment was at the same level in 2015 and 2019. The studied species shows the minimum values of increment in 2010, which is associated with an increase in air temperature (dry season).

According to our data, the average value of fir growth is 11.2 cm; statistical coefficients vary within different limits (table 1). The sensitivity coefficient is 0.31 (an increase in *Abies alba* Mill. responsive to changes in external factors). The inter-serial correlation coefficient is 0.66. The obtained values of the sensitivity and inter-serial coefficient are quite high; therefore, the values obtained by us (in terms of growth) are suitable for further analysis with climatic factors (figure 2).

**Figure 1.** Growth of *Abies alba* Mill. by years.

Mean increment of fir in 2018-2019 was significantly higher than in previous and subsequent years (figure 1). The inter-serial correlation coefficient between years is approximately the same. The magnitude of the 1st order autocorrelation indicates that the increment of the current year by 40-45% is associated with the increment of the previous year.
According to our data, fir growth has an average sensitivity (0.31). The relationship between the increment with the annual air temperature and the amount of precipitation in dynamics are shown in the graphs (figure 2).

Calculation of the correlation coefficients of increment with climatic variables for the general period showed that precipitation mainly affects the annual variability of the increment. The relationship with precipitation is significantly positive, in contrast to the relationship with air temperature. The correlation coefficient for precipitation in 2016 reaches its maximum value ($r = 0.47$). The correlation coefficient for temperature shows a slight negative relationship, reaching -0.32 in 2015.

**Table 1.** Statistical parameters for the increment of silver fir.

| Species      | Mean increment rate, cm | Coefficient of inter-serial differences | Coefficient of variation | Response factor | Percentage of variance explained by principal component |
|--------------|-------------------------|----------------------------------------|--------------------------|-----------------|-------------------------------------------------------|
| *Abies alba* Mill. | 11.2                    | 0.66                                   | 0.41                     | 0.31            | 333                                                   |

Analysis of temporal changes in the relationship between climate and growth highlighted the increasing impact of precipitation levels on species biology.

This can be interpreted as flexible morphological and physiological adaptation of seedlings to ecological conditions, which increases the probability of survival for many years.

Finally, the results highlight that changes in precipitation patterns have significant and beneficial effects on the growth of even drought tolerant species.

**Figure 2.** Correlation coefficients of *Abies alba* Mill. increment with climatic data: 1 – with precipitation; 2 – with temperature.

Thus, our task is solved: a thorough study of the features and timing of phenophases, high percentage of seed germination, positive growth dynamics enable to recommend silver fir for introduction into the landscapes of Voronezh and Central Chernozem region.
4. Conclusion
Each figure should have a brief caption describing it and, if necessary, a key to interpret the various lines and symbols on the figure. Captions should be below the figure and separated from it by a distance of 6 points, although to save space it is acceptable to put the caption next to the figure.

The introduction of exotic plants into regional phytocenoses presupposes a preliminary study of their ecological and biological characteristics. Studying the growth and development of the species is important.

Phenological traits are important aspects of plant adaptation determining the seasonal timing of biological events, ensuring optimal growth and minimizing the risks of damage causing large changes in response to climate fluctuations.

Phenological observations of *Abies alba* Mill. showed that bud opening and the formation of annual increment coincides in time with the beginning of the growing season in natural plantings. Apparently, the genetic feature of the plant organism enables it to adapt to a certain extent to the seasonal structure of regional meteorological rhythms. The influence of the extreme values of winter factors in the Voronezh region is relatively the same for the species of different geographic zones.

Based on phenological observations, we can talk about the correspondence of the seasonal rhythms of development of silver fir as an introduced species to their local ecological-phytocenotic analogs. This indicates their enhanced adaptation to the conditions of the Central Black Earth Region.

Before planting introduced plants in natural ecosystems, it is necessary to trace ontogenetic development starting from the moment of sowing seeds into the soil.

Although direct seeding has received a lot of attention over the past two decades, especially with regard to species of the genus *Abies* Hill., However, the theoretical and practical knowledge of this method among foresters is often limited. The risk of regeneration failure is considered to be high due to its strong dependence on environmental conditions during germination.

The results of our study (4000 seedlings grew out of 5 thousand seeds) emphasize that sowing from seeds obtained from trees of the natural population, an increase in the feeding area, a uniformity of the relief, the absence of competition from other vegetation – all these were the factors that control and enhance the growth and development of *Abies alba* Mill. When conducting an experiment on sowing seeds, we purposefully changed the seeding rate downwards, thereby increasing an additional inflow of water as a key factor for the growth and development of any tree.

Direct sowing from seeds using individuals of a natural region can be considered an ecologically sound approach that can further improve the success of the species adaptation to the individual conditions of regional phytocenoses. Further, the use of controlled silvicultural measures for the introduction of *Abies alba* Mill. into local flora can enhance the transformation and enrichment of forest plantations.

High rates of seed germination (80%), annual growth of seedlings (and then transplanted plants) with positive dynamics determine the final success of silver fir spread under forest-steppe conditions.

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