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MYCORRHIZA FORMATION IN *PINUS SYLVESTRIS* AND *PICEA OBOVATA* SEEDLINGS IN FOREST NURSERIES IN KAZAKHSTAN

D. Sarsekova[1]*, B. Osserkhan[2], T. Abzhanov[3] and A. Nurlabi[4]

[1]*Department of Forest Resources and Forestry, Faculty of Forestry, Wildlife and Environment, S. Seifullin Kazakh Agro Technical University, Apt. 18, 14V, Kerey and Zhanibek Khans Str., Nur-Sultan, 010000, Republic of Kazakhstan; E-mail: sarsekova.katu@bk.ru; corresponding author*
[2]*Department of Forest Resources and Forestry, Faculty of Forestry, Wildlife and Environment of Agronomy, S. Seifullin Kazakh Agro Technical University, Apt. 25, 14B, Kerey and Zhanibek Khans Str., Nur-Sultan, 010000, Republic of Kazakhstan; E-mail: osserkhan.katu@ro.ru*
[3]*Department of Forest Resources and Forestry, Faculty of Forestry, Wildlife and Environment of Agronomy, S. Seifullin Kazakh Agro Technical University, Apt. 27, 14A, Kerey and Zhanibek Khans Str., Nur-Sultan, 010000, Republic of Kazakhstan; E-mail: abzhanov.katu@ro.ru*
[4]*Department of Forest Resources and Forestry, Faculty of Forestry, Wildlife and Environment of Agronomy, S. Seifullin Kazakh Agro Technical University, Apt. 105, 29G, A. Moldagulova Str., Nur-Sultan, Nur-Sultan, 010000, Republic of Kazakhstan; E-mail: nurlabi.katu@ro.ru*

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This work presents research materials, the purpose of which is to grow seedlings of coniferous trees in the open field using artificial substrates of mycorrhizal macromycetes in forest nurseries of Central, North and Northeast Kazakhstan. The success of mycorrhiza formation in seedlings *Pinus sylvestris* and *Picea obovata* from forest nurseries of Akmola and Karaganda regions, and the survival rate of seedlings from forest nurseries of Akmola, Karaganda and Pavlodar regions of Kazakhstan are analysed. In the future, we want to expand the range of studied species and compare artificially mycorrhized seedlings with naturally mycorrhized species from natural forests. If we compare the survival rate of seedlings in nurseries, the highest in *P. sylvestris* seedlings in Shaldai is about 78%, low in Novodolenskoe, about 27%, and for *P. obovata*, on average, 66%. In the future, we want to expand the range of studied species and compare artificially mycorrhized seedlings with naturally mycorrhized species from natural forests.

Key words: ectomycorrhizae, forest nurseries, mycorrhiza, *Picea*, *Pinus*.

INTRODUCTION

About 8 thousand species of higher plants and 7–10 thousand species of fungi of the planet form mycorrhiza, which participates in the cycle of biogenic elements, activation of mineral nutrition, optimisation of plant metabolism, resistance to drought, pathogens, salinity, heavy metals. In most woody plants, ectomycorrhiza is formed, which is structurally the root of a higher plant that covers the mushroom sheath, and the fungal hyphae penetrate into

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its crustal parenchyma through the intercellular spaces, forming the Gartig net (Agerer 1987).

Ectomycorrhizae (EcM) play a key role in the cycle of substances and energy in boreal forest ecosystems. The mutualistic relationship between fungi and plants that form the EcM provides mutual benefits for both partners. The ecology, physiology, and anatomy of ectomycorrhiza have been studied in many species of woody plants growing in Europe, North America and Australia (Chen *et al.* 2016, Herrera-Martínez *et al.* 2014, Long *et al.* 2016, Sizonenko *et al.* 2017, Toju and Sato 2018).

The Republic of Kazakhstan, as well as other states of Central Asia, is one of the extremely forest-deficient countries, for which the preservation of existing forests and the development of green construction are the most urgent state task. The particular importance of these problems, first of all, is due to the fact that forests and plantations are irreplaceable enhancers of living conditions of the population and carriers of a favourable ecological environment.

The forest cover of the territory of Kazakhstan according to the intra-republican accounting and assessment system is 4.6% (Sarsekova *et al.* 2019), and according to international standards – 1.1%. This is due to the fact that saxaul thickets, which form 49% of the forest area, but do not reach an average of five metres, are not included in the forest category according to the UN FAO rules (Baizakov 2015).

The total area of the state forest fund of the Republic of Kazakhstan is 28.8 million hectares. Of these, forest-covered lands are 12.5 million hectares (43.6%), and forest lands – 19.0 million hectares (66%) (Mussayeva *et al.* 2019). Forestry is gradually gaining momentum and is on the rails of sustainable development. Now the reforestation work has been brought to 60.000 hectares per year. Despite the high reforestation work, the survival rate of the main forest-forming populations in forest cultures is low. This is due to the fact that the region has a very continental changeable climate and saline soil. Therefore, it is very important to increase the survival rate of forest cultures in these conditions.

For conifers and shrubs, mycosymbiotrophism is mandatory, and the intensity of mycorrhizal formation provides seedlings with a high survival rate when they are planted on a silvicultural area. It is also noted that the adsorbing surface of mycorrhizal ends is one and a half times higher than that of mycorrhizal roots (Eropkin 1973). Shubin (1973) carried out a long study of the process of mycorrhization from the formation of mycorrhizal endings and their further development in the seedlings of *Picea obovata* Ledeb. and *Pinus sylvestris* L.

Kenzin (1985) studied mycorrhiza on the roots of annual *P. sylvestris* seedlings. The author revealed that the widest range of mycorrhizal forms is characteristic of the root systems of *P. sylvestris* seedlings. It has clavate, bead-shaped, forked, coral-shaped, and single tuberous forms of mycorrhiza. The functional significance of mycorrhiza formation in annual seedlings *P. sylvestris* and *P. obovata* in forest nurseries was studied by Veselkin (2006).

Studying the role of mycorrhiza, many authors argue that mycorrhization increases the resistance of trees in various extreme forest conditions. Seedlings with weak mycorrhization were more susceptible to diseases compared to healthy well-mycorrhized seedlings.

In Kazakhstan, practical work concerning the isolation of mycorrhizal fungi into pure cultures and the study of their cultural characteristics was carried out by Meshkov (2010). The author expressed the opinion that the use of the obtained mycorrhized compost in forestry production will significantly improve the quality of cultivated planting material in forest nurseries, improve the survival rate and sustainability of forest crops, and also contribute to the natural regeneration of forests and the improvement of the ecological situation in forest biogeocenoses.

The purpose of our work in this research work is the cultivation of seedlings of coniferous trees on substrates of mycorrhizal macromycetes in forest nurseries of Central, northern and northeastern Kazakhstan in order to reproduce an artificial symbiosis of coniferous roots with mushrooms to increase survival, stimulate growth, endurance, and improve the decorative qualities of conifers. The growth and development of the aboveground organs of the seedling is influenced by the underground parts of the root systems. In particular, we determined and calculated several parameters of the underground and aboveground parts of *P. sylvestris* and *P. obovata* seedlings.

METHODS

The object of the study was the seedlings of Scots pine (*Pinus sylvestris*) and Siberian spruce (*Picea obovata*) collected in 3 forest nurseries in Kazakhstan: 1) the forest nursery of the "Akkol" State Forestry Institution, located in Akkol district, Akmola region; 2) the Novodolinsk Nursery of the "Karaganda Forestry and Wildlife Protection Farm" State Communal Institution; and 3) the nursery in the Shaldai branch of the "Ertis Ormany State Forest Nature Reserve" Republican State Institution in the Pavlodar region. In the forestry nursery of the "Akkol" in 2018, two-year-old seedlings of Scotch pine and three-year-old seedlings of Siberian spruce were planted in May, and in two other nurseries, seedlings were planted in May 2019. This text contains data and analyses of the 2018–2019 study of the Akkol forest nursery, as well as the materials of the Novodolinsk and Shaldai forest nurseries in 2019. In the nurseries, seedlings were grown in open ground.

During planting in 2018 in the Akkol forest nursery and in 2019 in the Novodolinsky and Shaldai nurseries for *P. sylvestris* and *P. obovata*, the mycorrhiza-forming drug was introduced into the root system simultaneously with planting. In order to study mycorrhiza formation in nurseries where the soil contains little of various types of mycorrhizal macromycetes due to remoteness from forest zones, from the environment and local climate. The drug was applied in the spring to non-frozen warm soil. When planting seedlings, mycorrhizal-forming preparations were introduced into the planting pit or near the selected plant. Since we have seedlings less than 0.5 m in height, the dose is 10–50 ml per plant.

We used a drug widely distributed in the CIS called "Mycorrhizal coniferous growth activator". "Mycorrhizal activator of growth of conifers" was developed as a result of the design of technological characteristics of active strains of living rhizosphere microorganisms and is grown on the basis of natural material taken from the roots of a specific culture of conifers. The drug is prepared for certain botanical genera of woody plants (*P. sylvestris* and *P. obovata*) by pre-order 2-3 months before the date of application to the soil in early May or in September – a period of active root growth. The preparation contains: mycelium of fungi of the genera *Suillus*, *Boletus*, *Paxillus*, and *Cortinarius*.

Samples of seedlings of the studied objects from forest nurseries were dug manually with a shovel from each variant, 10 samples. For each object of the study, 30 pieces were selected from 3 variations and 10 pieces from 1 control, for a total of about 240 pieces of seedlings. The aboveground and underground parts of *P. sylvestris* and *P. obovata* seedlings were measured. The aboveground parts of the seedlings were measured during the growing season, and the underground parts were dug out in late August and early September and stored in a refrigerator in plastic bags at a temperature of + 4 °C and analysed for no more than 10 days (Qian *et al.* 1998). Sampling points were confined to a row and were taken from the beginning, from the middle and from the end. The roots were cleaned and washed in water. The division of mycorrhizal endings into morphotypes was carried out using a binocular microscope based on the nature of branching, colour and shape of the endings.

From the parameters of the development of aboveground organs, the total height of the aboveground part and the length of the above-cotyledonous part of the stem or shoot were determined. The taken seedling samples were lightly shaken off and carefully cleaned of adhering soil particles. Each batch of seedlings was labelled (selection date, seedling age, breed, etc.). Under laboratory conditions, the root system of seedlings was washed in water and vegetative parameters were determined: the weight of the whole plant, the height of the aboveground part, the diameter of the stem at the root collar, the length of the root system, the width of the root system was measured (its area was calculated), the total (total) length of all roots was calculated seedlings, etc.

To calculate the increase in the total biomass and its component parts of seedlings, the weight of the assimilation apparatus (needles), stem and roots was determined separately.

The study of mycorrhiza formation on the roots of seedlings of coniferous trees was carried out according to the generally accepted methods of Selivanov (1981), Veselkin (2006), and Eropkin (1973).

The methods of studying the dynamics of the growth of root systems of seedlings were: in determining the branching order of the roots of the root system (roots of the I, II and III orders); in measuring the thickness of the roots: up to 1 mm – suction, from 1 to 3 mm – the bulk of the roots (conductive), more than 3 mm – skeletal roots; in measuring the length of the roots: suction, conductive, skeletal.

The identification of the types of mycorrhizal and the determination of the intensity of mycorrhizal infection was done by microscopic examination of the roots. Therefore, the roots of the seedlings were separated from the plant and immediately fixed. They made a description of the roots, a sketch of the roots and mycorrhiza. To study mycorrhizal formation on the roots of seedlings, it was determined by counting: the number of mycorrhizal roots (conductive), pcs. for 1 plant; number of non-mycorrhizal roots (suction), pcs. for 1 plant; number of mycorrhiza on the whole plant, pcs. for 1 plant; the degree of infection of the roots by the fungus, %; mycorrhizal density (the number of mycorrhiza per 100 mm of the length of the conducting roots, pcs. per plant. The intensity of mycorrhizal infection is determined according to Selivanov (1981) during microscopic studies by making long-term storage preparations of seedling roots. The dug roots are immediately fixed.

Mycorrhized roots were distinguished from non-mycorrhized ones according to the following criteria: in *P. obovata*, the modification of the shape of absorbing roots and the presence of superficial hyphal formations were taken into account; in *P. sylvestris*, dichotomously branching roots were considered mycorrhizal.

To determine the degree of mycorrhization of seedlings in forestry, Shubin (1973) developed a scale based on the ratio of mycorrhizal endings to the number of sucking roots: 0 = no mycorrhiza; 1 = mycorrhiza make up less than a quarter of the root ends; 2 = mycorrhiza make up from a quarter to half of all root ends; 3 = mycorrhiza more than half of all root endings.

Some authors believe that it is possible to assess the mycorrhization of coniferous seedlings using three parameters: 1) the number of sucking lateral ends on the root system of the seedling (which indicates the potential for mycorrhizal formation); 2) the ratio of the number of formed mycorrhiza to the total number of sucking ends (which shows the success of the process of mycorrhiza formation); 3) the density of mycorrhiza (which makes it possible to assess the intensity of mycorrhiza formation).

Statistical processing of the data was carried out using the Microsoft Excel 2003, SNEDECOR 10 software package. The work (tables and figures) shows the arithmetic mean values and their standard deviations. To view the mycorrhizal roots, we used a USB microscope (separation capacity 30 Mpix, magnification up to 500×). Also, in the text, the names of woody plants (IPNI 2019) and ectomyces (Index Fungorum 2019) used correspond to the world index.

RESULTS

Average morphological parameters and intensity of mycorrhization of *P. sylvestris* and *P. obovata* seedlings in forest nurseries are given in Tables 1 and 2. The weight of the whole plant in *P. sylvestris* in two-year seedlings between experiment and control, the difference in the Akkol forest nursery is 1.1±3.3 g; In the Novodolinsky forest nursery, 1.7±0.71 g, and it can be argued that in the last nursery, the weight of the entire plant exceeds about 0.6 g. In three-year-old *P. sylvestris* seedlings in the Akkol forest nursery, experimental variants where mycorrhizal-forming preparations were used, the weight of the whole plant exceeds 1.5 times general average control options (Table 1). The height of the aboveground part on average in the control variants of two-year-old *P. sylvestris* seedlings in the Akkol forest nursery in 2018 was calculated to be 6±1.02 cm, and in 2019 the average height of three-year-old *P. sylvestris* seedlings in the control variants was 22.9±2.41 cm. In the experimental variants (with the introduction of the drug) in the same years, the following indicators were recorded: 8±0.79 cm (2018) and 27.8±2.28 cm (2019). If we compare the height of the aboveground part of the experimental and control variants in years , then we will get an increase in the experimental ones by 25% higher than the control ones in 2018, and in 2019 the experimental ones exceeded by 18% in the Akkol forest nursery. In the Novodolinsk forest nursery, the difference between the experimental and control variants was 21%. We can say that in general, according to the experience, an average increase of 21% is higher than in the control variants. The third parameter that we measured and compared control with experience is the trunk diameter at the root collar. In this parameter, we can see from the table that two-year-old *P. sylvestris* seedlings in both nurseries control is 1.2 times higher than the experiment, but at three years of age, on the contrary, the experience exceeds the control by 1.5 times.

The growth and development of the aboveground organs of seedlings depends on the underground parts of the root systems. In this work, we died out, determined and calculated several parameters of the underground part of *P. sylvestris* and *P. obovata* seedlings.

In *P. sylvestris*, by the end of the second year of life, 26.2–43.7% (in control) and 40.9–57% (in experiment) of absorbing roots are converted into my-

Table 1

Morphological parameters and intensity of *Pinus sylvestris* mycorrhization in forest nurseries. Abbreviations: exp = experimental, con = control.

Parameters	M±m (nursery, year of selection, age of seedlings, experience (with application of the preparation), control (without the preparation) and one-factor accuracy of the difference									Reliability of difference (t ≥ 3) between nurseries		
	Akkol						Novodolinsk					
	2018 (two-year seedlings)			2019 (two-year seedlings)			2019 (three-year seedlings)					
	exp	con	t*	exp	con	t*	exp	con	t*	exp	con	t**
The mass of the whole plant, g	5.6±0.69	4.5±0.36	1.41	49.1±3.92	33.3±4.03	2.81	7.4±1.50	5.7±0.79	1.00	1.09	1.38	1.24
Aboveground height, cm	8±0.79	6±1.02	1.55	27.8±2.28	22.9±2.41	1.48	23.6±2.09	18.7±1.59	1.87	6.98	6.72	6.85
Stem diameter at root neck, mm	2.6±0.28	3±0.44	0.77	8.8±0.66	5.8±0.93	2.63	3.2±0.30	3.4±0.32	0.46	1.46	0.74	1.10
Main root length, mm.	65±4.69	56.1±4.00	1.44	213.3±13.3	132.9±16.9	3.74	125±7.29	78±7.82	4.39	7.04	2.49	4.77
Number of side conductive roots, pcs	7.2±0.80	4.5±0.36	3.08	9.3±0.76	7.9±0.71	1.35	8.3±0.56	6.2±0.83	2.09	1.13	1.88	1.51
Length of side conductive roots, mm	61.9±4.63	45.9±4.95	2.36	166.7±6.93	140.8±6.23	2.78	84.5±8.24	45.5±4.09	4.24	2.39	0.06	1.23
Length of all conductive roots, mm	337.6±16.0	203.4±35.7	3.43	836.3±46.7	737.0±49.5	1.46	455.8±47.8	266.4±42.5	2.96	2.34	1.14	1.74
Number of absorbing roots, pcs	112±19.1	83.9±12.9	3.08	148.5±8.90	115.8±13.1	2.06	122±24.4	103±21.9	0.58	0.32	0.75	0.54
Mycorrhizational intensity, %	57±4.29	43.7±4.55	2.13	61.6±6.14	51.4±6.27	1.16	40.9±10.8	26.2±8.12	1.09	1.39	1.88	1.64
Number of mycorrhizae, pcs	63.5±9.52	38.7±6.73	2.12	89.9±9.85	59.5±11.4	2.02	50.7±13.4	27.2±6.56	1.58	0.78	1.22	1.00
Number of mycorrhizal endings, pcs	204.8±32.8	92.5±16.2	3.07	167.7±15.7	87.2±11.3	4.16	132.3±32.2	48.1±9.03	2.52	1.56	2.39	1.98
Density of absorbing roots, pcs/100 mm roots	7.6±1.20	5.7±0.81	1.31	7.1±0.94	5.7±0.62	1.24	3.4±1.00	2.5±0.26	0.87	2.69	3.76	3.23
Mycorrhizal density, pcs/100 mm roots	5.9±0.87	4.3±0.64	1.48	20.1±3.76	7.7±1.19	3.14	6.6±1.86	2.6±0.45	2.09	0.34	2.17	1.26
Density of mycorrhizal endings, pcs/100 mm roots	17.5±1.38	10.5±1.92	2.96	62.8±15.4	26.3±5.40	2.24	23.4±2.20	12.9±2.37	3.25	2.27	0.79	1.53

Note: * = univariate significance of differences (when making the preparation) (when processing with the preparation); ** = two-factor significance of differences (when processing with the preparation and nursery)

Table 2

Morphological parameters and intensity of mycorrhization of *Picea obovata* in forest nurseries. Abbreviations: exp = experimental, con = control.

Parameters	M±m (nursery, year of selection, age of seedlings, experience (with application of the preparation), control (without the preparation) and one-factor accuracy of the difference									Reliability of difference (t ≥ 3) between nurseries		
	Akkol						Novodolinsk					
	2018 (two-year seedlings)			2019 (two-year seedlings)			2019 (three-year seedlings)					
	exp	con	t*	exp	con	t*	exp	con	t*	exp	con	t**
The mass of the whole plant, g	6.7±0.86	5.5±0.84	0.99	45.2±2.26	36.1±2.85	2.50	17.7±5.62	16.2±4.46	0.21	1.93	2.36	2.15
Aboveground height, cm	27.5±2.13	10.5±0.99	7.24	39.9±2.21	30.6±0.84	3.93	38.2±3.58	37.3±2.19	0.21	2.56	11.2	6.88
Stem diameter at root neck, mm	5.3±0.46	3±0.20	4.59	6.9±0.51	4.4±0.35	4.04	5.3±1.87	4.5±0.48	0.41	0	2.88	1.44
Main root length, mm	96.1±4.01	87.1±7.13	1.10	175±14.3	197.5±8.19	1.33	128±10.7	118±5.79	0.82	2.79	3.36	3.08
Number of side conductive roots, pcs	6.3±0.69	5.7±0.50	0.70	12.8±1.12	7.9±1.22	2.95	10±1.26	15.6±2.42	2.05	2.57	4.01	3.29
Length of side conductive roots, mm	69.5±10.5	81.2±6.79	0.93	146.3±12.4	119.8±12.4	1.51	90.5±9.34	75.6±8.43	1.18	1.51	0.52	1.02
Length of all conductive roots, mm	175.5±22.8	241.8±28.8	1.80	909.2±65.2	610.2±91.4	2.66	418.4±40.1	509.6±76.9	1.04	5.26	3.26	4.26
Number of absorbing roots, pcs	24.9±1.49	36.3±4.74	2.29	158.5±17.8	113.9±10.7	2.14	84.2±14.7	94.6±13.6	0.52	4.01	4.04	4.03
Mycorrhizational intensity, %	45.3±6.34	22±6.08	2.65	53.0±5.88	38.1±7.69	1.53	45± 7.25	31.9±3.99	1.58	0.03	1.36	0.69
Number of mycorrhizae, pcs	11.3±1.44	8.0±0.83	1.98	84.1±7.65	43.4±9.48	3.34	38±7.31	30.7±5.95	0.77	3.58	3.78	3.68
Number of mycorrhizal endings, pcs	65.4±10.9	23.1±2.57	3.77	169.0±16.7	70.0±8.27	5.31	213±52.8	141.2±43.2	1.05	2.73	2.73	2.73
Density of absorbing roots, pcs/100 mm roots	6.1±0.93	5.6±0.98	0.37	5.3±1.10	9.3±1.57	2.08	4±0.58	4.3±0.92	0.28	1.92	0.98	1.45
Mycorrhizal density, pcs/100 mm roots	3.0±0.40	2.7±0.36	0.55	18.5±2.76	9.4±1.96	2.68	13±2.23	6.8±1.09	2.49	4.41	3.72	4.07
Density of mycorrhizal endings, pcs/100 mm roots	12.9±2.55	4.5±0.66	3.18	44.6±4.02	16.2±2.89	5.73	36±3.25	19.6±3.89	3.24	5.59	3.83	4.71

Note: * = univariate significance of differences (when making the preparation) (when processing with the preparation); ** = two-factor significance of differences (when processing with the preparation and nursery) corrhiza (Table 1). By the end of the third year of life, the indicator of the intensity of mycorrhization reaches 51.4% (in control) and 61.6% (in the experiment). The distribution of mycorrhization intensity values observed in two-year and three-year-old *P. sylvestris* seedlings in the experimental and control variants are different. In three-year-old seedlings from the Akkol nursery, in comparison with two-year-old seedlings from two nurseries, the fashion shifts towards higher values (Fig. 1). The intensity of mycorrhization depends on the number of mycorrhizal roots, in our work we can see that in the Akkol forest nursery the number of mycorrhiza in two-year-old Scots pine seedlings is higher than in the Novodolinsk forest nursery.

We took the significance of the difference between the variants of the experiment and control as one-factor, between nurseries and variants of two-year-old *P. sylvestris* seedlings as a two-factor difference was determined by Student's t test. The significance of the difference in nurseries in different years was calculated using fourteen parameters (Table 1). In 2018, in the Akkol nursery, the maximum difference was 3.43, and the minimum was 0.77, on average 2.14. The significance of the difference in three-year-old seedlings in the Akkol nursery is a maximum of 4.16, a minimum of 1.16, and an average of 2.31. In Novodolinsky forest nursery between the experiment and the control, the difference was maximum of 4.39, minimum of 0.46 and on average of 2.07.

In *P. obovata*, in three- and four-year-old seedlings, 45.3–53% of the absorbing roots were transformed into ectomycorrhizae on average in the experiment and 22–38.1% of the absorbing roots in the control (Table 2), at the end of the third year the intensity of mycorrhization of the root systems of this

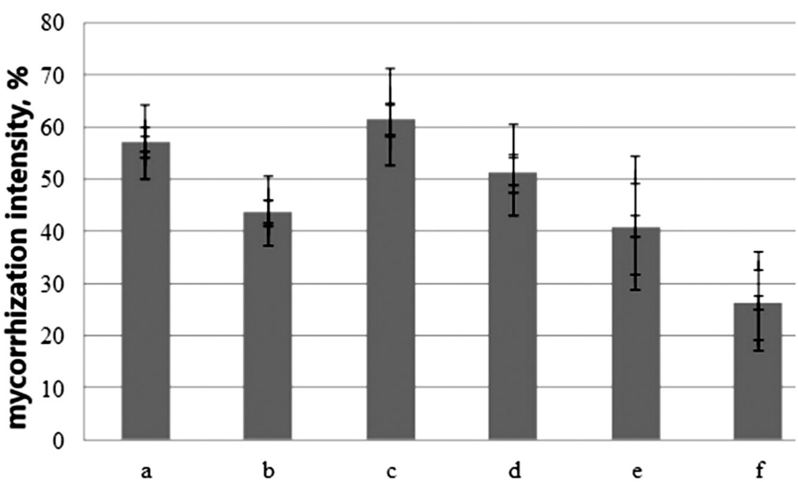

Fig. 1. Mean values (M±m) of *Pinus sylvestris* mycorrhization intensity in the nurseries: a = Akkul 2018 (experimental), b = Akkul 2018 (control), c = Akkul 2019 (experimental), d = Akkul 2019 (control), e = Novodolinsk 2019 (experimental), f = Novodolinsk 2019 (control)

species in experience increased by 7.7%, and control by 16.1%. The intensity of *P. obovata* mycorrhization in three-year-old seedlings in the Novodolinsk nursery experiment (with the introduction of a mycorrhizal drug into the root system) is higher by 13.1±3.26% than the control variants. The morphological data of the root system of the studied species are different in terms of experience and control, as well as in tree nurseries. In the first version, the experiment with control in the Akkol tree nursery in 2018, almost all the parameters of the study show that the experience exceeds the control, only in one the number of absorbing roots is higher by 46%, therefore the intensity of mycorrhization is less. If we compare the intensity of mycorrhiza by the ratio of the absorbing and mycorrhizal roots of *P. obovata* in nurseries, we get the following result that the greater the number of mycorrhiza or mycorrhizal roots, the higher the intensity of mycorrhiza (Fig. 2). The length of the main root of *P. obovata* seedlings according to the experiment and control in three-year-olds in Novodolinskoe according to the experiment is 7.8% higher and in four-year-olds in Akkolsky according to the control it is higher by 11.2%. In terms of the number of lateral conducting roots, the maximum indicator was recorded in the control variant, 15.6±2.42 pieces. The length of all conducting roots of two-year-old seedlings in Akkol exceeded the control by 27.4%, and in Novodolinsky it also exceeded the control by 17.8%. The following criteria were used to calculate the density of absorbing roots, mycorrhizal and mycorrhizal endings on the conducting roots of a 100 mm section. In the process of measuring and calculating averages and their deviations, we obtained the

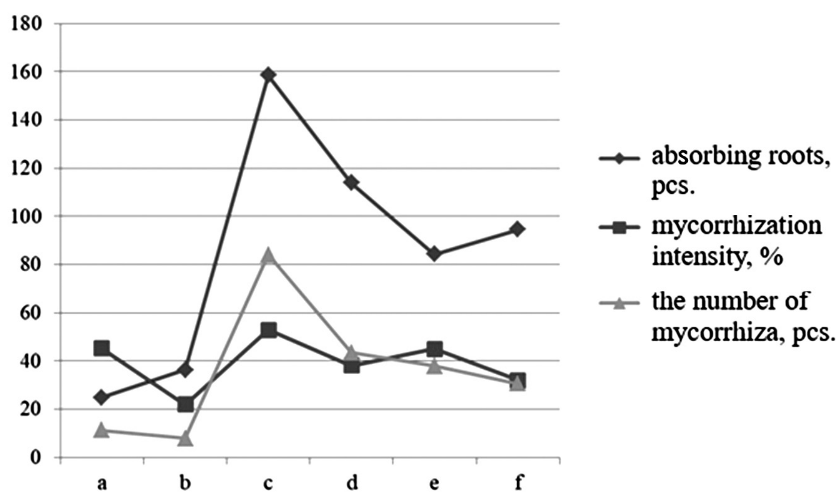

Fig. 2. Changes in the mycorrhization intensity to the ratio of the number of absorptive roots and *Picea obovata* mycorrhiza by nursery: a = Akkol 2018 (experimental), b = Akkol 2018 (control), c = Akkol 2019 (experimental), d = Akkol 2019 (control), e = Novodolinsk 2019 (experimental), f = Novodolinsk 2019 (control)

following indicators: according to the density of absorbing roots at the age of two, the fashion displacement for growth was 6.1 pieces and it refers to the experience of the first year of the Akkol forest nursery. In terms of mycorrhizal density, the maximum in the Novodolinsky forest nursery, the experience of two-year-old *P. obovata* seedlings exceeds the control variants by 47.6%. The density of mycorrhizal endings in three-year-old *P. obovata* seedlings in Akkol exceeded the control variants by 8.4±1.89 pieces, in four-year-old ones by 28.4±1.13 pieces. In the Novodolinsky forest nursery, the density parameters of mycorrhizal terminations in the experimental variants are 16.4±0.64 pieces higher than in the control variants.

The significance of the difference between *P. obovata* seedlings between the experimental and control variants was calculated in the same way as for the given species (Table 2). In three-year-old *P. obovata* seedlings in the Akkol forest nursery, the maximum difference in reliability was the height of the aboveground part of 7.24, and the minimum density of absorbing roots was 0.37, on average for all measurement parameters, 2.29. The significance of the difference in four-year-old *P. obovata* seedlings in the Akkol forest nursery, the maximum significance of the difference is 5.73, the minimum is 1.33, and the average is 2.98. Novodolinsky forest nursery between the experimental and control variants of *P. obovata* in all parameters, the reliability of the difference was a maximum of 3.24, a minimum of 0.21 and an average of 1.13. Two-factor significance is the difference, the first factor is comparison of experience (with the introduction of the drug) and control (without the drug), the second factor is forest nurseries. In this difference, the maximum height of the aboveground part was 6.88, the minimum intensity of mycorrhization was 0.69, and the average was 3.10.

The presented data allow us to assume the existence of a species specificity of the success of the formation of ectomycorrhizae in two species of conifers when they grow in nurseries. In this work, the intensity of mycorrhization of *P. sylvestris* and *P. obovata* in nurseries was compared. In spruce, the intensity of mycorrhization is lower than that of *P. sylvestris*. This is due to the fact that macromycetes forming mycorrhizal roots choose a host, there are species that can cohabit symbiotically with several species of trees, but there are also only one species. Spruce belongs to the latter, as well as in the control variants the mycorrhizal content is lower. *P. obovata* in its natural form does not grow in the studied regions where nurseries are located; for this reason, the soil of nurseries contains little of those macromycete species that form mycorrhiza. According to the dispersion analysis (Fig. 3) of the experimental data on the intensity of mycorrhization, the following indicators were obtained: Analysis of averages by the smallest significant difference – SSD (5%), F-criterion = 0.6307, degrees of freedom = 1, 4, Q = 0.4716, degree of influence according to

SNEDECOR = 0.0000, standard error = 4.8081 (9.53% of the total mean), SSD (1%) = 31.307, SSD (5%) = 18.879, and SSD (10%) = 14.496.

The average mycorrhizogenesis in the nurseries for two types of conifers was about 50%. As they age, the mycorrhization increased in the seedlings of the studied species; this can be seen in the example of the Akkol forest nursery in Tables 1 and 2, which shows the intensity of the mycorrhization of the root systems from 2018 to 2019. From two-year-old to three-year-old seedlings, the total height of plants, the value of vertical stem growth, and the length of the main root naturally increased.

The first goal of our work was to determine the mycorrhizal formation of common pine and Siberian spruce seedlings in forest nurseries. The second goal was the influence of mycorrhizal formation on growth and development as well as on the survival rate of seedlings. We have described mycorrhizogenesis and its influence on the growth and development of above- and underground organs of seedlings in detail in the text. Next, the influence of mycorrhizal formation on seedlings' survival rate in the open ground was analysed using the method of recalculating for the surviving specimens of the studied species for both the experimental and control variants at the end of each year. In the dispersion analysis of *P. sylvestris* survival rates, we obtained different parameters (Table 3). The analysis of the difference in factor averages for pine seedlings can be seen in Table 4. The difference in control and experience was 5.9 units or less, which is insignificant. The data for the dispersion analysis of the *P. obovata* survival rate are given in Table 5. According to the analysis of the difference between the factor averages, the experimental variants exceed

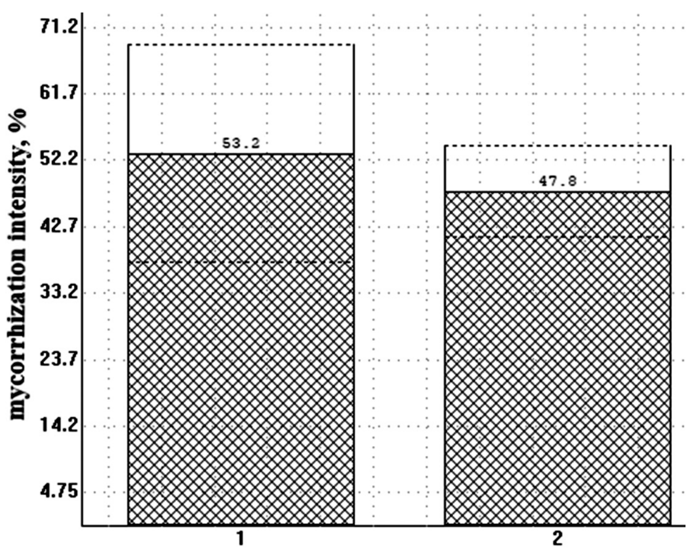

Fig. 3. Mycorrhizogenesis by species: 1 = *Pinus sylvestris*, 2 = *Picea obovata*

Table 3

Dispersion analysis of *P. sylvestris* survival in forest nurseries.

Dispersion	Sum of square	Proportion of variation	Degrees of freedom	Average square	F- criterion
General	1762.660	1.0000	7	251.809	0.247
Factor	69.620	0.0395	1	69.620	
Sl. Factors	1693.040	0.9605	6	282.173	

Total randomisation: analysis of SSD (smallest significant difference) (5%), F-criteria = 0.247, degrees of freedom = 1, 6, variation Q = 0.6371, degree of influence according to SNEDECOR = 0.0000, standard error = 8.3990 (13.8% of the total mean), SSD (1%) = 44.036, SSD (5%) = 29.064, SSD (10%) = 23.082

Table 4

Analysis of the difference between the factor averages of *P. sylvestris.*

Variants	Akkol		Novodolinsk	Shaldai	Middle	Variation	Meaningful?
	2018	2019	2019	2019			
	1	2	3	4			
1. *P. sylvestris*	64.00	75.00	53.60	62.40	63.75	Control	
2. *P. sylvestris* (on application of the preparation)	60.00	66.60	26.70	78.10	57.85	–5.900	Not
Middle	62.00	70.80	40.15	70.25	60.800	–2.950	Not

Randomisation in blocks (by nurseries): F-criterion = 0.4553, degrees of freedom = 1, 3, variation Q = 0.5482, degree of influence according to SNEDECOR = 0.0000, standard error = 6.1827 (10.2% of the total average), SSD (1%) = 51.070 SSD (5%) = 27.825, SSD (10%) = 20.578

Table 5

Dispersion analysis of *P. obovata* survival rate in forest nurseries

Dispersion	Sum of square	Proportion of variation	Degrees of freedom	Average square	F-criterion
General	491.673	1.0000	5	98.335	0.056
Factor	6.827	0.0139	1	6.827	
Sl. Factors	484.847	0.9861	4	121.212	

Table 6

Analysis of the difference between the factor averages of *P. obovata*

Variants	Akkol		Novodolinsk	Middle	Variation	Meaningful?
	2018	2019	2019			
	1	2	3			
1. *P. obovata*	58.00	72.40	67.20	65.87	Control	
2. *P. obovata* (on application of the preparation)	54.00	81.50	68.50	68.00	2.133	Not
Middle	56.00	76.95	67.85	66.933	1.067	Not

Total randomisation: analysis of SSD (5%), F-criteria = 0.0563, degrees of freedom = 1, 4, variation Q = 0.8241, degree of influence according to SNEDECOR = 0.0000, standard error = 6.3564 (9.50% of the total average), SSD (1%) = 41.388, SSD (5%) = 24.959, HC SSD (10%) = 19.164.

Randomisation in blocks (by nurseries): F-criterion = 0.3144, degrees of freedom = 1, 2, variation Q = 0.6314, degree of influence according to SNEDECOR = 0.0000, standard error = 2.6902 (4.02% of the total average), SSD (1%) = 37.759, SSD (5%) = 16.370, SSD (10%) = 11.109

the control variants in the average index of the Akkol and Novodolinsk nurseries (Table 6). The dispersion analysis of *P. sylvestris* and *P. obovata* survival rates showed that in both cases, the difference is not significant between the experimental and control variants.

DISCUSSION

A fairly high diversity of ectomycorrhizal morphotypes has been described in conifers (Agerer 1987, Courty *et al.* 2010, De Roman *et al.* 2005). Preliminary identification of ectomycorrhizae by morphological characters can significantly simplify identification. However, determining the morphotypes of ectomycorrhizae is not always an easy task, because often due to age, environmental conditions, insufficient preservation of ectomycorrhizae, their colour, shape and structure can change, and as a result, the identification of mycobiont is difficult (Burke *et al.* 2005, Horton and Bruns 2001, Sakakibara *et al.* 2002, Wurzburger *et al.* 2001).

In the studied nurseries, ectomycorrhizal associations are formed in two- and three-year-old *P. sylvestris* seedlings and three- and four-year-old *P. obovata* siberian seedlings, but under these conditions, in comparison with natural mycorrhiza formations, they are characterised by some peculiarities.

In theoretical terms, a significant conclusion arising from the results provided is the conclusion that mycorrhization of the root systems of *P. sylvestris* and *P. obovata* seedlings by certain species of ectomycorrhizal fungi of the genus *Suillus*, *Boletus*, *Paxillus*, *Cortinarius* increases the quality of the planting material. The main goal of mycorrhization measures is to increase the survival rate and safety of seedlings after planting them on a forest cultivated and landscaped area. At the same time, already in the second or third year after planting, an increase in growth is observed. Mycorrhization is more effective in poor and dry soils. The more unfavourable the conditions, the more mycorrhization can affect the sustainability of forest crops. Thin absorbing roots (at least in relation to conifers, it is more correct to use the term "ectomycorrhizas" rather than "roots" as usually 85–90% of thin roots are mycorrhized) are the most active part of the underground organs of trees (Veselkin 2006).

Artificial mycorrhization is an expensive undertaking, and the slightest violation of technology can have a negative effect. Therefore, its use is justified, first of all, when creating forest crops in extreme conditions or in plantation cultivation. In order to increase the efficiency of reforestation, it is possible to carry out measures to promote the natural mycorrhization of planting material and the selection of seedlings mycorrhized by species of fungi that correspond to the growing conditions of forest crops. The optimal methods of artificial mycorrhization in industrial cultivation and use of local fungal strains as a source of mycorrhiza is the introduction of spores during the growing season or the addition of mycelium to the nutrient substrate before sowing.

In Sweden, a group of scientists dealt with the problems of mycorrhization, both artificial and natural. In particular, an assessment of the efficiency of infection of *P. sylvestris* and *P. obovata* with ectomycorrhizal fungi was carried out (Menkis *et al.* 2007), as a result it was found that already at the end of the first year of cultivation in the process of natural mycorrhization, inoculated fungi were replaced by other species. The same researchers surveyed nurseries for growing seedlings with open and closed root systems in order to determine the degree of natural mycorrhization of the seedlings' roots. It was found that for *P. sylvestris* the highest degree of mycorrhization (48%) was observed on seedlings with an open root system, and for *P. obovata* (71%) on seedlings with a closed root system. A total of 27 species of ectomycorrhizal fungi were found in nurseries on the roots of *P. sylvestris* and *P. obovata* seedlings.

For *P. obovata,* Katenin (1972) distinguishes clavate, fern-like, aciniform forms of mycorrhizal endings. Mycorrhizal ends of conifers Eropkin (1973) divides it into simple, transitional, complex and believes that the forms of mycorrhizal endings are determined by the age of the mycorrhiza, the systematic affiliation of the host plant and ecological conditions. In this regard, the young mycorrhizal ends of conifers have a clavate shape; with age, they are transformed into coral-like mycorrhizal ends in *Pinus*, and in *Picea* and *Abies*, into loosely crest-like and fern-like ends. For *Picea*, according to (Veselkin 2006), characterised by monopodial and feathery branching. According to the classification of Selivanov (1981), *P. obovata* has eumycetic chalmophage ectomycorrhizae formed mainly by basidiomycetes.

In practical terms, the materials presented indicate that economic measures aimed at obtaining positive results at the level of species development (in our case, the introduction of a mycorrhizal-forming drug) can negatively affect the success of mycorrhizal formation if it is not properly dosed. Taking into account the variety of mechanisms of mycorrhization effect on plants, one of which, for example, an increase in resistance to pathogens, such a weakened mycorrhiza formation can have negative consequences and lead to a decrease in the effectiveness of silvicultural activities at the stage of creating forest crops.

Thus, studies of the mycorrhiza formation of *P. sylvestris* and *P. obovata* showed insignificant differences in the morphological structure and seasonal dynamics of the growth of root systems and growth in nursery conditions.

CONCLUSIONS

As a result of the conducted studies of mycorrhiza formation in seedlings of *Pinus sylvestris* and *Picea obovata* in forest nurseries, the following conclusions can be drawn: in Scots pine, mycorrhization is higher than in Siberian spruce. This is primarily due to the fact that the studied species, in particular, the spruce is not adapted to the bottom region. In order to analyse mycorrhizogenesis formation in seedlings, we carried out a morphological analysis of the above- and underground parts. The analysis revealed that the experimental options exceeded the control. The obtained results could be influenced by several factors, these are mycorrhizal-forming substrate, forest nursery (location or region), climate and other factors not taken into account by the authors.

In the future, to expand the foreign experience of artificial mycorrhization of seedlings of forest tree species with an open and closed root system of the material and the selection of seedlings, mycorrhizated by species of fungi, corresponding to the growing conditions of forest crops. Introduce the already researched methods into practice when creating forest cultures. The main goal of mycorrhization measures is to increase the survival rate and safety of seedlings after planting them on the forestry area. At the same time, already in the second or third year after planting, an increase in growth is observed. Mycorrhization is more effective in poor and dry soils. The more unfavourable the conditions, the more mycorrhization can affect the sustainability of forest crops.

In the future, we plan to continue research in this direction, for this it is necessary to expand the range of studied nurseries for a reasonable judgment about the presence of a connection between the characteristics of the soil and the technologies used for growing planting material with the success of mycorrhization, and also compare artificial mycorrhization with natural.

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