Influence of Nursery Substrata on Mycorrhizas of Seedlings in Different Scots Pine (*Pinus sylvestris* L.) Populations

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Mycorrhiza formation on the roots of Scots pine seedlings in nursery applying various substrata (pine and oak stand litter, decomposed peat) in different populations (Telšiai, Labanoras, Veisiejai) is analysed. In this paper the different positive impact of various substrata on the growth the development of Scots pine seedlings from separate populations, the peculiarities of spreading of various mycorrhiza morphotypes, their relation and impact on overground parameters of biennial seedlings were observed.

The total amount of mycorrhiza in the control ground without litter is by 9% higher than in oak stand litter, 15% – in pine stand litter, 16% – in decomposed peat. The variability of the most common (ectendomycorrhiza and ectomycorrhiza) mycorrhizae were evaluated in the examined nursery substrata. While ectendomycorrhiza dominates (60%) in decomposed peat, the occurrence of ectomycorrhiza decreases (12%). In oak stand litter the ectomycorrhiza increased (28%) in respect to ectendomycorrhiza and dead mycorrhiza. This was due to the abundance of mycorrhiza fungi and greater activity of microorganizms (bacteria). Concerning population level, the outspread of ectomycorrhiza in respect to other morphotypes of Labanoras population (19%) surpassed the representatives from Veisiejai (14%) and Telšiai (12%) populations. The reaction of mycorrhiza morphotypes highlighted higher sensitiveness of Telšiai population, which influenced weaker growth of seedlings. The representatives from Labanoras population reacted to new soil conditions most sensitively, though according to growth parameters they were more preeminent than other populations. The diversity of mycorrhiza fungi presupposes selection possibilities of seedlings according to its types and ensures more rapid growth and greater resistance of seedlings to various environmental factors.

Key words: Scots pine seedlings, populations, nursery substrata, morphotypes of mycorrhiza, the optimums of growth parameters.

Introduction

In our nurseries, due to highly intensive fertilization, application of pesticides and cultivation for many years in turn, soil mycobiota has undergone great changes, which led to the lack of necessary for mycorrhiza fungi. Therefore, the growth of seedlings has slowed down and their resistance to various environmental factors has decreased. Chemical protection measures have suspended not only the activities of pathogenic fungi, but have also reduced mycorrhiza formation and self-regulation possibilities of soil microorganisms (Rudawska 2000). Thus, new, ecologically sound and environmentally acceptable agrobiological measures to ensure growing of quality planting stock in nurseries have been sought. In Lithuania mycorrhiza studies were initiated only recently and at the present time have become very important both from scientific and practical point of view.

Seeking to be integrated into the EU, the country has to review essentially the strategy and tactics of further development, to afforest vast (200 – 600 thous. ha) abandoned and infertile land areas, to increase the woodedness of the country from 30.9% (Lithuanian state forest statistics 2001) to 38.4% (Riepšas 2002). Thus, it is necessary to reconsider the development strategy of protected areas, optimizing protected forest areas of various destinations, develop multiple, sustainable forestry, ensuring conservation of biodiversity and formation of stable ecosystems (Kairiūkštis 1996, Riepšas 2000).

To reduce transfer stress of seedlings from different populations and to sustain their genetic diversity, the soil should contain not only sufficient amount of nutrients, but also mycorrhiza fungi. Continuous soil biological and chemical processes substantially reduce the amount of organic compounds and the possibility of seedlings to assimilate them. To increase soil biological activity and productivity, one of the most widespread and efficient measures is applied – transfer of forest litter mixed with forest soil genetic horizons into the nursery (Маттис 1976, Gorzelak *et al.* 1989, Hilszczanska 2000). Mycorrhiza fungi, owing to their higher absorption capacity, facilitate seedlings in assimilating nitrogen and other nutrients from hardly decomposable organic compounds, alongside taking from plants carbohydrates (Šleinys 1986). Therefore, the advantage should be taken of this. Mycorrhiza increases surface capacity of the roots of seedlings and their resistance to biotic and abiotic factors during transplanting (Rudawska 1997). Better establishment is observed on seedlings having greater weight of fine roots (Смирнов 1981, Baltrušaitienė 1994). For Scots pine seedlings the litter of spruce stands mixed with peat is more suitable (Gorzelak *et al.* 1989). If there is a lack of mycorrhiza fungi, Scots pine grows slower, becomes dwarf or dies (Meyer 1973).

Ectomycorrhiza is the most suitable form of Scots pine symbiosis. Ectomycorrhiza, spread on the roots of Scots pine, shows soil conditions in the nursery and the efficiency of applied agrobiological measures (Kowalski 1997, Sierota *et al.* 2004).

Ectendomycorrhiza is mostly wide-spread in forest nurseries and young Scots pine stands, especially on soils having high nitrogen concentration (Rudawska 1993). An urgent environmental pollution problem due to an increased amount of nitrogen fertilizers in the soil in western and central Europe is said to be one of the possible causes of forest decline (Nihlgard 1985). A sequential, chain-like dependance has been ascertained: environmental pollution by nitrogen fertilizers → disappearance of mycorrhiza → forest decline (Rudawska 2000). Ectendomycorrhizae, appearing due to an increased amount of nitrogen in the soil, by the way of their prevalence reduce the possibility of appearance of other mycorrhizae and may be the main cause of the death of seedlings, transplanting them from nursery into agricultural land, characterized by a different microflora than forest soils (Dominik 1963). The roots of seedlings, failing to find a suitable symbiont (fungus) in the transfered soil, suffer from mass attack by plant disease agents and are more susceptible to unfavourable abiotic factors (Münzenberger *et al.* 2000). Other researchers (Stenström *et al.* 1986) pointed out, that a low concentration of nitrogen easily stimulates the appearance of mycorrhiza in poor and infertile soils, ensures successful establishment of seedlings and more rapid growth of them during the first year after transplantation.

The aim of studies is to study the influence of separate nursery substrata on mycorrhiza development and to evaluate the variability of mycorrhiza morphotypes and growth parameters in the progenies of different populations.

Material and methods

The experiment was carried out in 2000-2001 in Dubrava nursery.

To ascertain the peculiarities of mycorrhiza spreading on seedlings and to assess the sensitivity of Scots pine offsprings from different regions, the seeds from three populations (Telšiai, Labanoras and Veisiejai) were chosen and sown using a stencil (5×5 cm) with 4 replications in prepared substrata in Dubrava nursery. The maintenance and fertilization of seedlings were performed according to methodics worked out by V. Juška *et al.* (Юшка *et al.* 1982), L. Kubertavičienė (1998), K.Armolaitis (1999), J.Paičius (2001).

To ascertain the influence of substrata on mycorrhiza development, pine and oak stand litter as well as decomposed peat were prepared according to A. Gorzelak (1989). The control variant was represented by nursery soil. Mycorrhized soil comprised 40 kg/m² of a longitudinal strip. Totally, 1920 kg of mycorrhized soil were used for 48 m² of the longitudinal strip.

In Forest Research Institute (Warsaw) asessment analysis was done for 48 randomly selected seedlings according to M. Rudawska *et al.* (1994). 48 prepared samples in test-tubes were identified and classified according to the most frequently occurring in nurseries Scots pine mycorrrhiza morphotypes. Changeability analysis of mycorrhiza morphotypes was done with the help of two-factor dispersion analysis according to this model:

$$Y_{jlk} = m + A_j + K_l + A \times K_{jl} + E_{jlk},$$

where Y_{jlk} – trait value of k-seedling from l-population in j-substratum, m – average of the whole experiment, A_j – effect of j-substratum, K_l – effect of l-population, $A \times K_{jl}$ – interaction effect of substratum and population, E_{jlk} – random error.

To assess phenotypic (ecological) plasticity of different populations (Telšiai, Labanoras, Veisiejai) according to mycorrhiza morphotypes, parameters of regression analysis were applied (Федин *et al.* 1973): regression coefficient and standard deviation according to this model:

$$X_{ij} = m + r_i I_j + d_{ij},$$

where X_{ij} – trait value of i-population in j-substratum, m – mean of the whole experiment, r_i – regression coefficient of trait variance of i-population, I_j – index of environmental (ecological) conditions in j-substratum, d_{ij} – deviation of i-population from regression line in j-substratum.

The occurrence of mycorrhiza morphotypes of each seedling was described by an alternative (qualitative) trait, recalculating it into quantitative one and pointing out its spreading percentage (frequency). Variation coefficient of traits was ascertained by the usual for qualitative traits formula:

$$V = (\delta/M)\times 100\%,$$

where V – trait variance coefficient, δ – mean standard deviation, M – arithmetic mean of the trait.

Distribution probability of individual mycorrhizae of seedlings of different Scots pine populations was evaluated by the method of indicator species (Mccune *et al.* 1997). Their indication value was reliably estimated within 0-100% range. Dispersion of mycorrhiza optimums was defined by linear canon ordination method (RDA) of direct gradient analysis (Ter Braak 1987, Lepš *et al.* 1999). Statistical reliability of the correlation between preconditioning factors (different populations, individual substrata) and parameters (growth of seedlings, indices of biological productivity, mycorrhizae) in the first and second hypothetical axes was estimated after a Monte Carlo test (Ter Braak *et al.* 1998) that is independent of distribution laws. The dependance of indices among mycorrhizae and between mycorrhizae and growth as well as productivity indices were studied by direct regression analysis.

Statistical analysis was conducted using MEAN (MS EXCEL), GLM (STATISTICA 5.5) and other programs (CANOCO 4.0; PC-ORD 3.2).

Results and discussion

Autochtonous populations of forest trees are best adapted to the conditions of their habitats, forming a corresponding complex of genetic traits. Changes in environmental conditions growing nursery seedlings may influence the genotypic structure of future plantations, because some genotypes may be more sensitive to a certain factor (nursery substrata) of growth conditions and react (positively or negatively) in a different way. Essential changes in growth conditions may reveal the degree of population reaction to the factor.

To preserve naturally formed genotypic structure of populations, agrobiological measures applied in nurseries should be close to the natural ones. Thus, it is very important to ascertain favourable for the adaptation of populations agrobiological and especially mycotrophic factors under new environmental conditions at initial stages of their formation, i.e. in nurseries.

Having studied the results of mycorrhiza spreading in separate substrata, a positive influence of the transfer of pine and oak litters as well as decomposed peat into the nursery was ascertained. A sufficient amount of organic matter and soil pH_{KCl} 5.0÷6.0 provide favourable conditions for the development of mycorrhiza (Rudawska 1998). The most characteristic morphotypes of mycorrhiza – ectendomycorrhiza and ectomycorrhiza of type I - spread in the studied nursery substrata and different populations unevenly. Dead mycorrhiza, used to define the total amount of mycorrhizae, failed to comprise a separate type.

The applied method of indicator species enables to ascertain relative abundance of mycorrhizae and their occurrence frequency in different nursery substrata and various populations. Having generalized these indices, the probability of mycorrhiza ocurrence in the control (dead mycorrhiza) and oak stand litter (types I and IV of ectomycorrhiza) according to 0-100% scale is significant within the limits of allowable reliability (Table 1).

Table 1. Assessment of indicator values of mycorrhiza morphotypes in different populations and nursery substrata

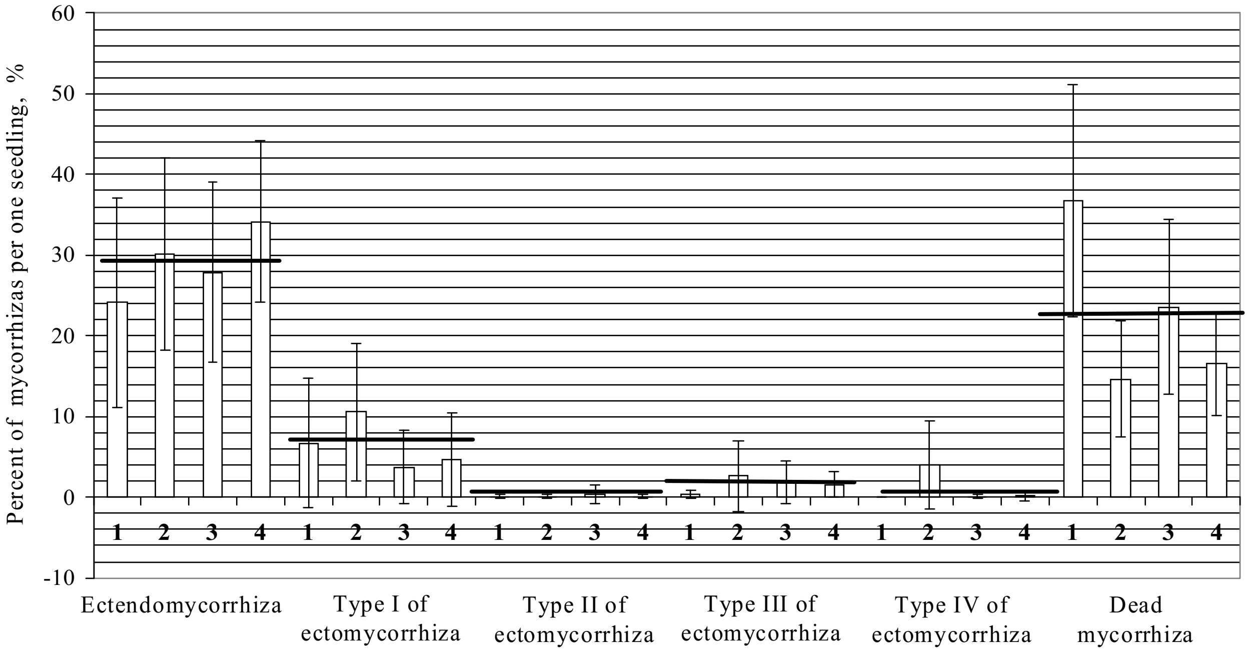
Morphotypes of mycorrhiza	Indicator values, %								
	Population			Reliability p	Nursery substratum				Reliability p
	Telšiai	Labanoras	Veisiejai		Control	Oak stand litter	Pine stand litter	Decomposed peat	
Ectendomycorrhiza	28	41	31	0,011	21	26	24	29	0,201
Type I of ectomycorrhiza	16	48	20	0,025	22	41	10	14	0,033
Type II of ectomycorrhiza	3	1	8	0,825	1	1	10	1	0,561
Type III of ectomycorrhiza	21	18	14	0,908	3	20	17	16	0,746
Type IV of ectomycorrhiza	0	15	12	0,508	0	63	0	0	0,001
Dead mycorrhiza	36	36	28	0,663	40	16	26	18	0,001

On the level of populations occurrence the probability of mycorrhiza morphotypes (ectendomycorrhiza, type I of ectomycorrhiza) only of the seedlings of Labanoras population was significant.

By the general percent of mycorrhiza falling per seedling, the control variant has by 9.5% higher index than oak litter, by 18% - than pine and by 19% than decomposed peat, however, the composition of mycorrhizae spread in separate nursery substrata is different. As compared to the control, the greatest ascertained difference in type I ectomycorrhiza percentage in oak stand litter comprises 59%. The percentage of dead mycorrhiza in the control is greater than that of pine and oak litters and decomposed peat respectively by 56%, 151%, 123% (Figure 1).

mycorrhizae (24%) is preconditioned by a high amount of humus of the mentioned substratum (3.0%).

Due to a relatively high amount (21.8 mg/100g of soil) of hydrolizable nitrogen in decomposed peat the highest percentage (60%) of ectendomycorrhiza has been ascertained. In the control variant (15.4 mg/100 g of soil) its percentage decreases down to 36%. The most wide-spread mycorrhizae in different populations are displayed in different ways: ectendomycorrhiza is



Nursery substrata: **1** – control, **2** – oak stand litter, **3** – Scots pine stand litter, **4** – decomposed peat.

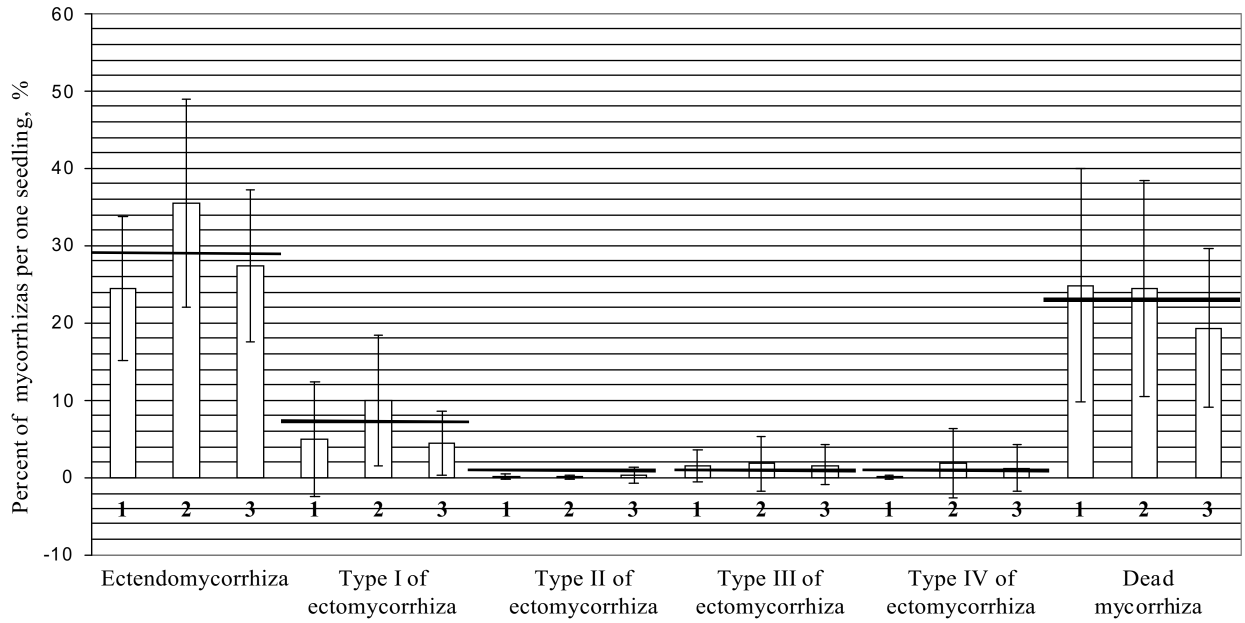
Figure 1. Variation of mycorrhizae morphotypes in nursery substrata. Bars show average of mycorrhizae morphotypes in the various nursery substratum, vertical lines – standart deviation. Horizontal lines show average of mycorrhizae morphotypes in different nursery substrata.

On population level the greatest total percentage (74%) of mycorrhiza was ascertained in Labanoras population, the variable of which is higher than those of Telšiai (32%) and Veisiejai (35%) populations. On population level the greatest difference of ectendomycorrhiza percentage between Labanoras and Telšiai populations reaches 45%. The greatest difference in type I ectomycorrhiza percentage between Labanoras and Veisiejai populations comprises 127% (Figure 2).

Due to an intensive application of mineral fertilizers for a long time, the most abundant dead mycorrhiza (54%) was ascertained in the control, in which the lack of minimal humus amount (1.9%) is described as a limiting factor for the development of mycorrhiza. In the litter of oak stand the least percentage of dead

most abundant in Labanoras population, while dead mycorrhiza – in Telšiai population.

The results of our studies have shown, that ectendomycorrhiza prevailing in decomposed peat and pine litter decrease the percentage of ectomycorrhiza by 12% and 11% respectively. The prevalence of ectendomycorrhiza decreases the possibility of appearance of other mycorrhiza types and may be the main cause of seedling decline (Dominik 1963). Without a suitable symbiont (fungus), the roots of seedlings are actively attacked by disease agents of plants and become more susceptible to unfavourable abiotic factors. The observed increase (28%) in ectomycorrhizae (type I, type III, type IV) in the litter of oak stands shows the diversity of mycorrhiza fungi, their toler-



Population: **1** – Telšiai, **2** – Labanoras, **3** – Veisiejai.

Figure 2. Variation of mycorrhizae morphotypes at the level of populations. The bar shows average of mycorrhizae morphotypes in various populations, vertical lines – standart deviation. Horizontal lines show average of mycorrhizae morphotypes in different populations.

ance (coehistance) due to the metabolism of substances. We have found a greater distribution (19%) of ectomycorrhizae in Labanoras than in Veisiejai (14%) or Telšiai (12%) populations. Similar study results were presented by M.Rudawska, pointing out, that ectomycorrhiza may be comprised of about 5000 species of fungi with 2000 species of woody plants (Rudawska 2000). By the manner of prevalence ectomycorrhizae reduce the possibility of seedlings mortality after their transfer from the nursery to agricultural land, which is characterized by different microorganisms than forest soil (Dominik 1963). Successful establishment of seedlings to a large extent depends on the qualitative and quantitative composition of mycorrhizae, revealed at the juvenile stages (Rudawska 2000). The longer arrows denote significant optimums of mycorrhizae: in the control (dead mycorrhiza), in the litter of oak stand (I and IV types of ectomycorrhiza), in decomposed peat (ectendomycorrhiza) (Fig. 3). In RDA diagram the first and the second axes of hypothetical ecological gradients together explain 49% of the variation of influenced parameters (mycorrhizae and growth, indices of biological productivity), while variation of influencing parameters (populations and substrata) – 84%. The obtained relationship (coefficient of canonic correlation) between influenced parameters and influencing factors in the first axis of ordinates comprise 0.85, in the second – 0.56 ($p<0.05$, Monte Carlo test).

Represented by a short arrow in the diagram the optimum of morphotype II of ectomycorrhiza is insignificant due to its insufficient spreading in all substra-

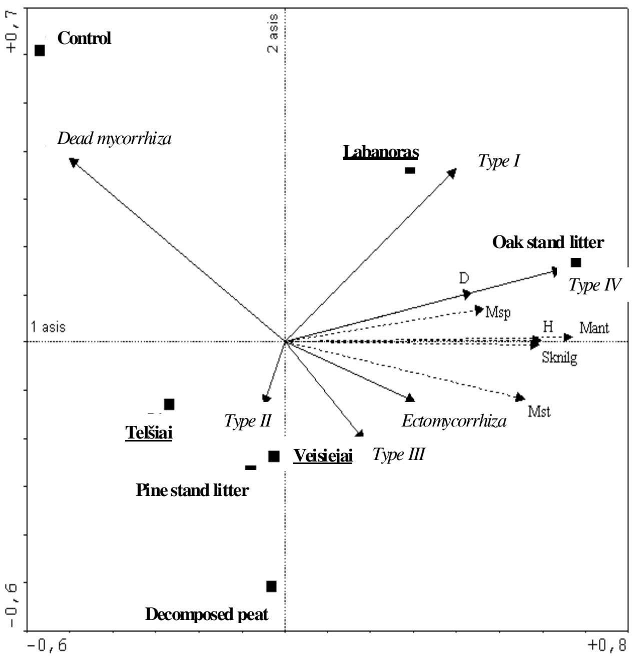


Figure 3. Ordination diagram of the influencing parameters (populations, substrata) and influenced parameters (mycorrhizas, growth, indices of biological productivity) of redundancy analysis (RDA).

ta. In oak litter alongside with ectendomycorrhiza obvious is the prevalence of ectomycorrhizae (type I and IV) optimums. In the first hypothetical axis (axis 1) a reliable difference between the dead mycorrhiza of the control and type IV ectomycorrhiza in oak stand litter has been ascertained. On population level the closeness of Labanoras population to optimal limits of ectomycorrhiza spreading (type I, type IV) is admitted. Meanwhile, ectendomycorrhiza is closer to Veisiejai population.

An obvious increase in ectomycorrhizae in the litter of oak stands predetermines the growth rate of seedlings: as compared to the control, they are by 23% higher and have thicker root collar, by 16% longer roots and by 45% greater aboveground as well as by 51% greater underground weight. The qualitative and quantitative composition of mycorrhizae in oak stand litter provide optimal growth conditions for the seedlings of Labanoras population. The least percentage of ectomycorrhizae in the control variant (11%) preconditions the least growth indices of seedlings. Assessing the mentioned indices on population level, it has been found, that Labanoras seedlings surpass all the other seedlings. Rapid growth of the seedlings of this population may be explained by hereditary genetic properties (Раманаускас 1978, Danusevičius 2000).

Studies of the reaction of population seedlings to new soil conditions have shown, that the greatest reaction by aboveground and underground portions was exercised by Labanoras population.

Variables and correlations of mycorrhizae of the latter show, that morphotypes studied in the roots to one or another extent precondition the development of the aboveground portion of seedlings. A close and reliable dependence is revealed by linear regression equations: Weight of needles = 4.9716 + 0.52003 x Type IV ($r=0.73$; $p<0.001$), Aboveground weight = 6.9826 + 0.53166 x Type IV ($r=0.69$; $p<0.001$).

Conclusions

1. The diversity of mycorrhiza morphotypes in pine and oak stand litter as well as decomposed peat is suitable for applying these substrata in nurseries and unsuitable for agriculture areas, where natural soil structure and properties have been damaged due to intensive cultivation and lack of mycorrhiza fungi.

2. Mycorrhization of seedlings increases genotypic diversity at the initial stage (nursery) of population development.

3. The influence of forest litter (pine, oak stand) and decomposed fen peat substrata on the growth of seedlings from different populations differs and it preconditions the distribution ratio of various mycorrhiza morphotypes. The most wide-spread are ectendomycorrhiza and ectomycorrhiza. In the decomposed fen peat ectendomycorrhiza (60%) prevails. The latter reduces the possibility for ectomycorrhiza formation. In the litter of oak stand a higher than in other substrata distribution of ectomycorrhiza has been ascertained. It shows a greater diversity of symbionts, preconditions a more rapid growth of seedlings: as compared to the control, they are by 23% higher and have thicker root collar, by 16% longer roots and by 45% greater aboveground as well as by 51% greater underground weight.

4. A different distribution of ectomycorrhizae was ascertained in the roots of seedlings of different populations (the most wide-spread in the population of continental (Labanoras), the least – in the populations (Telšiai) of maritime conditions). Ectomycorrhiza influences a more rapid growth of aboveground portion of seedlings. This leads to better growth and biological productivity of the progenies of the most continental population. Growth rate of the seedlings of Labanoras population is explained by inherited genetic properties.

5. The diversity of mycorrhiza fungi presupposes selection possibilities of seedlings according to its types and ensures more rapid growth and greater resistance of seedlings to various environmental factors.

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ВЛИЯНИЕ ПИТАТЕЛЬНОЙ СРЕДЫ В ПИТОМНИКАХ НА МИКОРИЗУ СЕЯНЦЕВ В РАЗЛИЧНЫХ ПОПУЛЯЦИЯХ СОСНЫ ОБЫКНОВЕННОЙ (*PINUS SYLVESTRIS* L.)

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Резюме

Применив в питомнике отдельные субстраты (подстилки соснового и дубового древостоев, разложившийся торф) и различные популяции (Тельшяй, Лабанорас, Вейсеяй), было исследовано формирование микоризы на корнях сеянцев сосны обыкновенной (*Pinus sylvestris* L.). Установлено различное позитивное влияние применения отдельных субстратов на рост и развитие сеянцев сосны обыкновенной изученных популяций, определены особенности распостранения разных морфотипов микоризы, их взаимосвязь и воздействие на параметры надземной части сосновых сеянцев.

Общий процент микоризы одного сеянца в контроли на 9% и 15% выше, чем в соответствующих подстилках дубового и соснового древостоев, а также на 16% выше, чем в разложившимся торфе. В исследованных субстратах было установлено наличие найболее распространённой микоризы - эктендомикоризы и эктомикоризы. Доминированное положение эктендомикоризы (60%) в разложившимся торфе снизило появление эктомикоризы (12%) в том самом субстрате. В подстилке дубового древостоя явное увеличение эктомикоризы (28%) за счёт уменьшения эктендомикоризы и мёртвой микоризы, указало на разнородность грибов микоризы и активную деятельность микроорганизмов. Распостранение эктомикоризы среди сеянцев Лабанорской популяции было выше (19%), чем у сеянцев Вейсеяйской (14%) и Тельшяйской (12%) популяций.

Реакция на распространение морфотипов микоризы показало большую чувствительность Тельшяйской популяции, что повлияло на слабый рост её сеянцев. Сеянцы Лабанорской популяции своими параметрами надземной части более чувствительно реагировали на изменение условий почвы из-за внесения субстратов, хотя показателями роста превосходили остальные популяции. Разнородность грибов микоризы, обусловленная эффективностью используемых средств, определила возможность выбора морфотипов микоризы для сеянцев различных популяций и обеспечила их активный рост, а также устойчивость перед разными неблагоприятными условиями окружающей среды.

Ключевые слова: сеянцы сосны обыкновенной, популяции, субстраты, морфотипы микоризы, показатели роста