Mycorrhizal formation of light-coniferous species of urbanized ecosystems

G A Zaitsev1, G M Mukhametova2* and L M Safiullina2

1 Ufa Institute of biology, Ufa Federal Research Centre of the Russian Academy of Sciences, 69 October avenue, Ufa, Russia
2 Bashkir State Pedagogical University named after M. Akmulla, 3A October Revolution street, Ufa, Russia

*E-mail: g_mukhametova@mail.ru

Abstract. Mycorrhiza formation plays a key role in the adaptation of light-coniferous species of urbanized territories, since the fungal symbiont acts as a barrier against technogenic impact. The article presents the study of mycorrhiza formation of Pinus sylvestris and Larix sukaczewii in urbanized ecosystems of the Strelitamak industrial center, Republic of Bashkortostan. The material was sampled in 2009 and 2016 at 8 sample plots. Anatomical and morphological structures of trees roots absorbing parts were analysed, micropreparations of cross sections were made. Mycorrhiza formation under the influence of technogenic pollution is not suppressed, the development of the fungal component of mycorrhiza increases. Pine mycorrhizal associations were found to be more sensitive to industrial pollution than larch mycorrhiza. The variety of fungus sheath of light coniferous species changes according to a single scheme. The plant makes for the unfavorable ecological conditions by a higher level of mycorrhiza formation development.

Mycorrhiza formation is the most widespread form of symbiotic relationships between plants and heterotrophs. The research results indicate that the symbiosis of plants with fungi is widespread in all main natural zones, urbanized areas are no exception. Mycorrhiza formation is an important and essential process for the sustainable functioning of ecosystem and adaptation of woody plants to unfavorable factors. Mycorrhiza are actively involved and, in turn, transformed when trees adapt to various environmental factors and play a leading role in stabilizing urbanized ecosystems of sanitary protection zones in industrial city centers.

In Russia, mycorrhiza was found in all gymnosperms and in 77.5% of angiosperms. It was found that mycorrhizal plants number tend to increase from polar regions to Variable zone, which is associated with better provision of heat and moisture. Plant-fungi symbiosis is widespread in mesophytic plants. More than 100 species of symbiont fungi are known for Scots pine. Symbiont mushrooms receive carbohydrates and vitamins (thiamine) from plants. The fungus provides the plant with elements of mineral nutrition (nitrogen, phosphorus). It does not increase the content of phosphorus and nitrogen in the soil, but ensures their more efficient use. Fungus supply plants with some trace elements, vitamins, nicotinic and pantothenic acid. They can absorb water from almost dry soil. Mycorrhizal plants have been noted on soils with a pH of 2.9 to 8.0 [1].

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For trees of boreal and temperate climates growing on the territory of the former USSR, many species are characterized by ectomycorrhiza. Ectomycorrhiza forms on young, non-corked plant roots. A sheath of fungal hyphae covers the root surface. Hyphae penetrate into the intercellular spaces, branching into them and forming a so-called Gartig network. In the soil, the hyphae forms are mycelium on which the fungal fruit can grow. The mycelium sheath is an effective organ that absorbs water and mineral salts. Sometimes its mass can reach up to 40% of the root mass. Significant and often irreversible physiological, anatomical and morphological changes in organs and tissues occur in woody plants under the influence of toxic emissions from industrial enterprises and transport. In this regard, works aimed at assessing the impact of industrial pollution and other types of technogenic impacts on mycorrhizal associations are of particular relevance.

The aim of the work is to investigate the features of mycorrhiza formation in Pinus sylvestris and Larix sukaczewii in urbanized ecosystems. The aim of the study is to compare the ecological and anatomical variability of the plant and ectomycorrhiza fungal symbionts of two light coniferous tree species - Pinus sylvestris and Larix sukaczewii of urbanized ecosystems of the Strelitamak Industrial Center (SIC), the Republic of Bashkortostan. Mycorrhiza formation in Larix has been studied less than mycorrhiza from Pinus and Picea [2]. The material was collected on the territory of a large industrial city of Sterlitamak (53°37'N, 55°57'E), Republic of Bashkortostan, forest-steppe zone of the Cis-Urals in 2009 and 2016. The northern part of the city is subject to powerful industrial impact - "pollution zone", the southern part of the city is the territory of forest nursery. For the Republic of Bashkortostan it is important to study the main trends in the natural environmental transformations, depending on the influence of technogenic factors [3].

Sampling was carried applying standard methods. Samples of the absorbing part of pine and larch roots were taken from each sample plot from the A1 soil horizon. The anatomical structure of ectomycorrhizae was analyzed on cross sections 8-10 μm thick prepared on a freezing microtome, diameter of the root ends and thickness of the mycorrhizal sheath in glycerin without staining were examined and measured under AxioImager A2 microscope (CarlZeiss, Germany); to show significance at least 100 cross sections from each trial plot were prepared. For the qualitative and quantitative analysis of the morphological and anatomical structure of ectomycorrhiza the technique of DV Veselkin [4] is used when describing the type of fungus sheath; for its structure - classification of I A Selivanov [5].

Typical ectomycorrhiza was found in Larixsukaczewii and Pinussylvestris on the investigated plots. It was revealed that the intensity of mycorrhization under contamination conditions increases on all test plots to 78-92%, while in the control test it ranges from 61 to 90%. At the same time, the intensity of micorosis formation in larch is higher than in pine. As it was shown by the results of the Shannon index in pine, the negative impact influenced the structural diversity of the fungus sheath; the Shannon index in the test samples shows higher contamination than in both years of research. On the other hand, with larch, no unambiguous reactions were found in structural diversity of mycorrhizal sheath type (table 1).

**Table 1. Mycorrhization intensity and variety of fungus sheaths Pinussylvestris and Larixsukaczewii growing under conditions of SIC.**

| Feature          | Larixsukaczewii | Pinussylvestris |
|------------------|-----------------|-----------------|
|                  | 2009 control    | 2016 contamina| 2009 control | 2016 contamination |
| Mycorrhization intensity, % | 77 82 90 92       | 61 78 75 87         |
Shannon Index
1.62 1.54 2.13 2.24 1.78 1.62 1.97 1.75
Subtypes number, pcs
9 10 12 13 9 10 11 11
Abundance of
plectenchymatic sheaths, %
21 5 35 25 34 27 51 25
Abundance of pseudo-
plectenchymatic and
double sheaths, %
13 26 31 54 32 26 28 28
Abundance of structurless
sheaths, %
44 46 27 14 34 47 21 47
Abundance of HN type
(absence of sheath), %
22 23 7 5 - - - -

The impact of pollution on a variety of fungi sheath is also expressed in the ratio change of mycorrhiza abundance with sheaths of different types. It is believed that the most physiologically active are light-colored, well-structured pseudo-parenchymal and double sheaths [6]. Analysis of the sampled material showed that in contaminated pine, the abundance of fungi sheaths of a structureless type increases 1.5-2 times on average. While in larch trees, on the other hand, under pollution the abundance of structureless subtypes of fungal sheath is half as much, since larch trees have a HN subtype, which was not detected during pine mycorrhiza formation. A characteristic feature of this subtype is the absence of external fungi sheath and, at the same time, the presence of a well-developed Gartig network between cells of the root cortex. The ratio of plectenchymatic, pseudoparenchymal and double subtypes abundance of fungi sheath in pine decreases, which confirms the negative effect of pollution on the structural diversity of pine mycorrhiza at a qualitative level. At the same time, pollution had a stimulating effect on the structural diversity of larch mycorrhiza.

Analysis of anatomical and morphological parameters of pine and larch variability under conditions of the Sterlitamak industrial center expansion in 2009 showed that the total diameter of the root end increases under the influence of the polymetallic pollution type. Total diameter of pine root end under control varied from 77 to 667 μm; in the zone of industrial pollution, the minimum diameter of the root end was fixed at 230 μm and increased to 836 μm. For larch general size characteristics varied from 120-130 μm to 494-678 μm. At the same time, for pine plant symbiont increases the dimensional characteristics under industrial action (root radius, number tannin cells layers, cells of cortex parenchyma), while in the fungal symbiont no significant differences were found, i.e. the thickness of the fungi sheath varies from 6-7 μm to 61-87 μm (table 2).

Table 2. Variability of the general size and quality parameters of ectomycorrhizal roots under conditions of the SIC, 2009.
Average number of tannin cells layers, pcs  
1.33±0.06  1.91±0.11***  1.52±0.06  1.67±0.07

The number of layers of root cortex "living" cells, pcs  
2.27±0.10  1.93±0.09*  3.16±1.10  3.60±0.13*

The number of Gartig net layers, pcs  
1.82±0.11  1.61±0.08  1.93±0.10  1.83±0.10

The occurrence of tannin cells, %  
83  86  87  65

The occurrence of endings with lost turgor (star-shaped on cut), %  
35  37  59  37

Significance of differences ***p<0.0001, **p<0.001, * p<0.01

In larch, both plant and fungal symbiont (total diameter, root radius and fungi sheath thickness) increase, which is caused by industrial pollution. The average thickness of fungi sheath during contamination significantly increases by 60%.

On the test plots in the pollution zone, pine is characterized by a greater number of tannin cells layers, dying root cortex cells, which in most cases flatten in the tangential direction. An increase in the occurrence of such cells corresponds to a decrease in the number of layers root cortex "living" cells, which is observed in pine. In larch, on the contrary, there is a significant increase in the number of layers of parenchyma cells and varies in the range from 1 layer to 6-9 layers, in contrast to pine in which this indicator varies from 1 to 4-5 layers.

On the urbanized territory of the Sterlitamak industrial center, in the zone of pollution near larch, an increase in the average thickness of fungi sheaths is observed; under the influence of heavy metals the volume of the fungal component of ectomycorrhiza increases [7].

Analysis of the data obtained in 2016 confirms that larch also has a plant and fungal symbiont (total diameter, root radius and thickness of the fungi sheath) with an increase in industrial pollution, which again confirms the stimulating effect of the overall effect on mycorrhiza formation (table 3).

**Table 3.** Variability of the general size and quality parameters of ectomycorrhizal roots under conditions of the SPC, 2016.

| Parameter                        | Pinussylvestris         | Larixsukaczewii         |
|----------------------------------|-------------------------|-------------------------|
|                                  | control | contamination | control | contamination |
| The total diameter of the root end, μm | 320.9±8.7 | 284.7±9.0** | 303.7±8.1 | 374.2±10.9*** |
| Root radius, μm                  | 128.0±4.1 | 147.0±4.05** | 137.1±3.5 | 167.6±4.8*** |
| Fungi sheath thickness, μm       | 14.5±0.9  | 18.8±2.4      | 17.63±1.3 | 22.45±1.3*    |
| Share of sheath size, %          | 17.0     | 22.6**        | 20.19    | 21.34         |
| Average number of layers tannin cells, pcs | 1.38±0.07 | 1.51±0.07 | 2.05±0.10 | 1.70±0.09    |
The number of layers of root cortex "living" cells, pcs

|                  | Value 1 | Value 2  |
|------------------|---------|---------|
|                 | 2.46±0.08 | 2.15±0.10*** |
| The number of Gartig net layers, pcs | 1.97±0.07 | 1.52±0.08*** |
|                 | 2.93±0.10 | 2.34±0.10 |
| The occurrence of tannin cells, % | 35 | 36 |
| 35 | 36 | 78 | 41 |
| The occurrence of endings with lost turgor (star-shaped on cut), % | 32 | 48 |
| 53 | 38 |

Significance of differences ***p<0.0001, **p<0.001, * p<0.01

Under unfavorable ecological conditions plants make for a higher level of mycorrhiza development. These changes are considered as adaptive responses aimed at ensuring sustainability of growth and development of Pinussylvestris and Larixsukaczewii urbanized ecosystems.

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