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# FUNGI COLONIZING CONIFEROUS SEEDLINGS IN FOREST NURSERIES OF MIDDLE SIBERIA

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***Abstract:*** *The study was carried out in four forest nurseries of Middle Siberia. Affected seedlings of Scots pine (Pinus sylvestris L.), Scots Siberian stone pine (Pinus sibirica Du Tour), and Siberian spruce (Picea obovata Ledeb.) were collected for molecular phytopathological examination. In total, 14 fungal taxa were identified in the needles and roots of the plants. The most dominant among them were Sydowia polyspora (Bref. & Tavel) E., Didymella glomerata (Corda) Qian Chen & L. Cai, Cladosporium herbarum (Pers.) Link, Lophodermium seditiosum Minter, Staley & Millar, Phialocephala fortinii C. J. K. Wang & H. E. Wilcox and Cadophora finlandica (C. J. K. Wang & H. E. Wilcox) T. C. Harr. & McNew. The richness of the fungal taxa was higher in the needles than in the roots of all the tree species studied. The obtained results could be used for implementing more effective phytosanitary measures in the studied nurseries.*

***Key words:*** *phytopathological monitoring; forest nurseries; phytopathogens; dark septate endophytes (DSE); DNA-analysis.*

## 1. Introduction

The state of future forests, their productivity, and biological stability depend on the quality of the planting material grown in forest nurseries. However, juvenile plants are very susceptible to various diseases which lead to their attrition and, therefore, to significant reduction in the yield of planting material.

Consequently, phytopathological monitoring of forest nurseries is one of the main aspects of the Russian forest sector. Annual losses of coniferous seedlings from diseases are at least 10-15

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and often 30-45%, but in some cases, they can reach up to 40-80% [30]. Most diseases causing problems for forest nursery grown plants are fungal. The threat to seedlings is caused mainly by parasitic fungi which trigger metabolic disorders in plants, leading to growth retardation and even mortality [29]. Intensive cultivation practices (e.g., monoculturing, high sowing density, intensive fertilization, excess of irrigation, chemical weed and pest control) are among the main reasons for the rapid spread of fungal infections in forest nurseries [21], [25]. Apart from that, the dominance of micromycetes in the microflora of diseased seedlings indicates the functional significance of fungi in the pathological process, which makes it necessary to study their species composition [30]. At the present time, one of the perspective methods of early diagnostics and taxonomical identification of pathogens in plant tissues is molecular-genetic analysis [5]. Direct sequencing of fungal DNA from plant tissues is a sensitive method for the detection of plant-inhabiting phytopathogens [18], [20]. Currently, this approach is extensively used within the state program for monitoring of forest genetic resourses in Russia. It allows performing more efficient phytopathological monitoring in forest nurseries and afforestation activities [30]. The goal of this study was to identify the fungi colonizing the needles and roots of diseased coniferous seedlings in forest nurseries of Middle Siberia.

## 2. Material and Methods

One-, two-, three-, and four-year old diseased seedlings of Scots pine (*Pinus sylvestris* L.), Siberian stone pine (*Pinus sibirica* Du Tour), and Siberian spruce (*Picea obovata* Ledeb.) were sampled during the 3rd decade of April 2020 in four bare root nurseries (Table 1 and Figure 1).

In total, 240 seedlings were collected using the methodology described in the state coordination program for the development of biotechnology in the Russian Federation [16]. The diseased seedlings were carefully excavated then packed into kraft paper bags, transported to the laboratory, and kept at 4°C. All samples were rinsed with distilled water before the analyses. DNA from each sample was isolated using the CTAB method [10]. For fungi identification, the internal transcribed spacer of the fungal ribosomal DNA (ITS rDNA) was sequenced using primers ITS1F and ITS4 [35]. PCR was performed using the GenPak® PCR Core Kit (Laboratory Isogen Ltd., Russia) following the manufacturer's instructions. The amplification cycle consisted of an initial heat denaturation step at 94˚C for 1 min, followed by 35 cycles of 94˚C for 15 sec, 60˚C for 20 sec, and 72˚C for 26 sec, and a final extension at 72˚C for 10 min. Electrophoresis was performed in a 2% agarose gel. Sequencing was performed on the ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Raw sequence data were analyzed using the Sequencing Analysis Software v.6.0 (Life Technologies Corporation, Carlsbad, USA). The UNITE database was used to determine the identity of the sequences [26]. The criteria used for identification were: sequence coverage >80%, identity to species level 98-100%, and identity to genus level 94-97%.

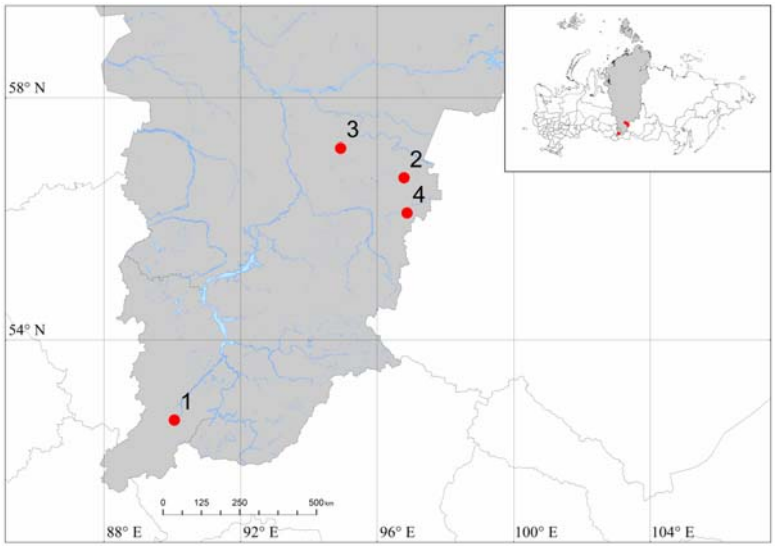


Fig. 1. *Map of Russia showing the locations of the investigated forest nurseries (numbered 1-4 as in Table 1) from which the seedlings were collected. The red dots indicate the forest nurseries*

Table 1

*Four forest nurseries in Middle Siberia where diseased seedlings of Pinus sylvestris L., Pinus sibirica Du Tour, and Picea obovata Ledeb. were studied using the direct sequencing method*

| | Forest nursery | Host tree species | Number of samples | Fungal taxa |
|---|---|---|---|---|
| 1 | Abazinskiy | *Pinus sylvestri* | 30 | *Didymella glomerata*, *Cadophora finlandica*, *Lophodermium conigenum*, *Sydowia polyspora* |
| | | *Pinus sibirica* | 60 | *Didymella glomerata, Sydowia polyspora, Phialocephala fortinii, Cadophora finlandica* |
| 2 | Dolgomostovskiy | *Pinus sylvestris* | 60 | *Cladosporium herbarum*, *Chalara* sp., *Didymella pomorum*, *Leptodontidium* sp., *Cenangium acuum*, *Sydowia polyspora* |
| 3 | Taseevskiy | *Picea obovata* | *30* | *Phialocephala fortinii*, *Paraphoma radicina*, *Pezicula radicicola*, *Cladosporium herbarum* |
| | | *Pinus sibirica* | *30* | *Lophodermium seditiosum* |
| 4 | Tinskiy | *Picea obovata* | 30 | *Phialocephala fortinii*, *Herpotrichia juniper*, *Lophodermium seditiosum*, *Didymella glomerata*, *Cenangium acuum*, *Lophodermium seditiosum* |

## 3. Results and Discussion

A total of 228 sequences were obtained by direct sequencing from 240 seedlings representing three tree species. The derived sequences resulted in the identification of 14 different fungal taxa (Table 2).

Table 2

*Frequency of fungi isolated from the needles and root tips of Pinus sylvestris L., Pinus sibirica Du Tour, Picea obovata Ledeb. seedlings bare-root cultivated in forest nurseries*

| **Fungal taxa** | Colonization [%] | | Host tree species | | |
|---|---|---|---|---|---|
| | Needles | Root tips | Scots pine | Siberian stone pine | Siberian spruce |
| *Cadophora finlandica* (C. J. K. Wang & H. E. Wilcox) T. C. Harr. & McNew | - | 28.3 | + | + | - |
| *Cenangium acuum* Cooke & Peck | 0.8 | - | + | - | - |
| *Chalara* (Corda) Rabenh. | - | 9.4 | + | - | - |
| *Cladosporium herbarum* (Pers.) Link | 15.6 | - | + | - | + |
| *Didymella glomerata* (Corda) Qian Chen & L. Cai | 21.3 | - | + | + | + |
| *Didymella pomorum* (Thüm.) Qian Chen & L. Cai | 11.5 | 5.7 | + | - | - |
| *Herpotrichia juniperi* (Sacc.) Petr. | 4.9 | - | + | - | - |
| *Leptodontidium* de Hoog | - | 0.9 | + | - | - |
| *Lophodermium seditiosum* Minter, Staley & Millar | 14.8 | - | + | + | - |
| *Lophodermium conigenum* (Brunaud) Hilitzer | 4.9 | - | + | - | - |
| *Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White | - | 4.7 | - | - | + |
| *Pezicula radicicola* (Kowalski & Bartnik) P. R. Johnst. | - | 2.8 | - | - | + |
| *Phialocephala fortinii* C. J. K. Wang & H. E. Wilcox | - | 48.2 | + | + | + |
| *Sydowia polyspora* (Bref. & Tavel) E. | 26.2 | - | + | + | - |

The richness of fungal taxa was higher in the needles than in the roots of all the studied tree species. The microflora of the diseased Scots pine seedlings harboured the highest number of fungal taxa (12 out of 14) (Figure 2).

The most dominant among them were *S. polyspora*, *D. glomerata*, *C. herbarum*, *L. seditiosum*, *P. fortinii,* and *C. finlandica*. Five fungal taxa were detected on the damaged seedlings of Siberian stone pine – *D. glomerata*, *L. seditiosum*, *S. polyspora*, *P. fortinii*, *C. finlandica*. The microflora of the Siberian spruce seedlings was mainly represented by *D. glomerata*, *C. herbarum,* and *P. fortinii*. Two micromecetes were found on the diseased seedlings of all the studied tree species - *D. glomerata* and *P. fortinii.*

The analysis showed that the most commonly identified fungus in the damaged needles was *Sydowia polyspora*

(anamorph *Sclerophoma pithyophila*) (26.2%) (Table 2). It was found in the needles of Scots pine and in the Siberian stone pine seedlings. *S. polyspora* is considered as a common needle endophyte of the *Pinaceae* species, but under certain conditions the fungus can act as a weak pathogen causing small foliar lesions [27]. It is also encountered as a saprophyte in litter layers [7]. In this study, the needles of one- and two-year old seedlings were commonly colonized by *Sydowia polyspora* (31.0%) (Figure 2). The stems of some of the plants infected by *S. polyspora* had S-shaped bending, which is one of the symptoms of the disease. The obtained results are in accordance with another study [30], which showed that *S. polyspora* was widely spread in forest nurseries located in taiga, forest-steppe, and Southern-Siberian mountain zones of Middle Siberia.

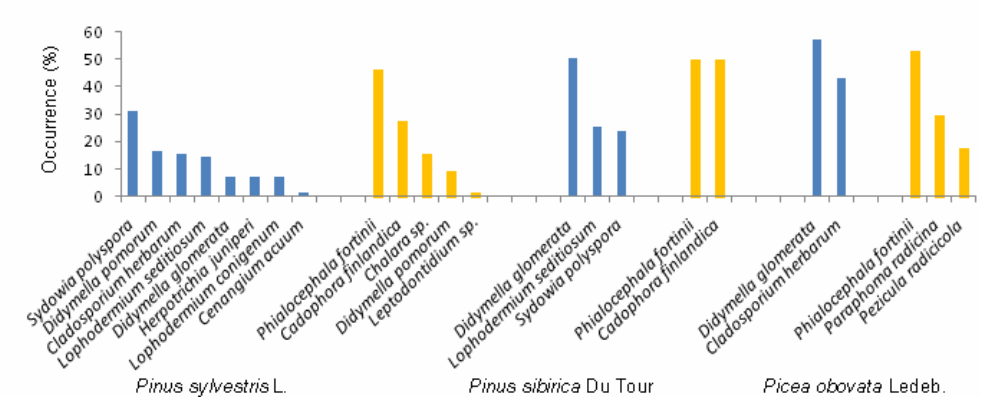


Fig. 2. *The occurrence of the main micromycetes in the needles and roots of Pinus sylvestris L., Picea obovata Ledeb., Pinus sibirica Du Tour seedlings. Blue bars – needles, orange bars – roots*

Fungi of the genera *Didymella* were also among the frequently identified pathogens in the needles. *D. glomerata* (21.3%) was found in the needles of all the studied tree species, while *D. pomorum* (11.5%) was only detected on Scots pine seedlings. Both species previously belonged to the genus *Phoma* (*P. glomerata* (Corda) Wollenw. & Hochapfel, *P. pomorum* Thüm., respectively) which is considered to be one of the largest fungal genera [3, 4], [30]. *D. glomerata* and *D. pomorum* are distributed throughout a broad range of environments and can cause Phoma blight disease of seedlings [23]. The disease causes chlorotic and necrotic foliage, tip dieback, and defoliation of seedlings [17]. In forest nurseries of Middle Siberia it is one of the most common but poorly studied infections of coniferous seedlings [30].

*Cladosporium herbarum* (15.6%) was detected on the Scots pine and Siberian spruce seedlings. It is considered as a facultative parasite often associated with weakened plants. It was reported that *C. herbarum* had also been detected in the epiphytic microflora of healthy-looking coniferous seedlings [30]. In forest nurseries it usually causes post emergence damping-off but in the present study it was also found on two- and four-year old seedlings (Figures 3 and 4).

The causal agent of Lophodermium

needle cast disease - *Lophodermium seditiosum* (14.8%) was found in the needles of Scots pine and in Siberian stone pine seedlings. *L. seditiosum* is widely prevalent on pine seedlings in forest nurseries of Siberia, especially in taiga and forest-steppe zones [12], [30]. In this study three- and four-year old seedlings were mainly infected by this fungus (31.6%).

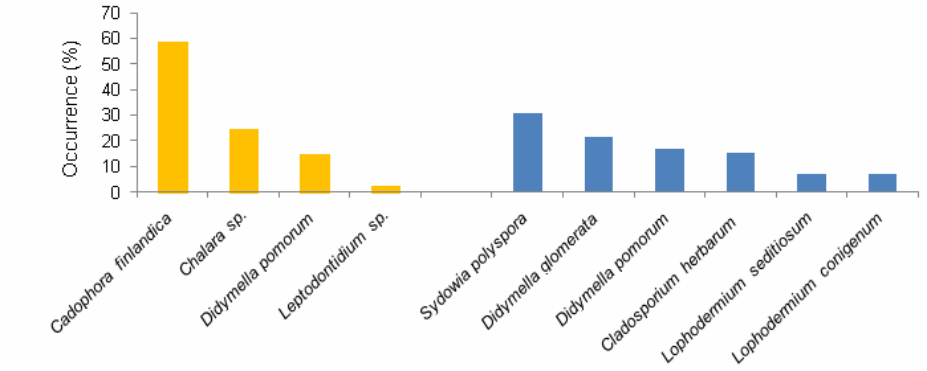


Fig. 3. *The occurrence of the main micromycetes in the roots and needles of one- and two-year old seedlings. Orange bars – roots, blue bars – needles*

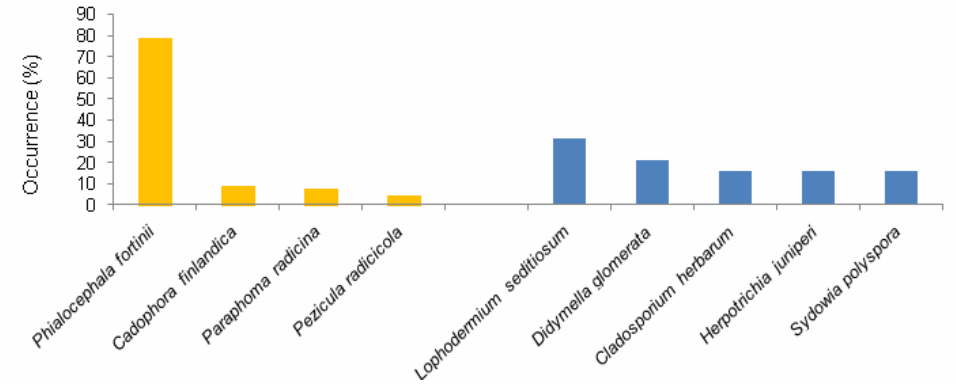


Fig. 4. *The occurrence of the main micromycetes in the roots and needles of three- and four-year old seedlings. Orange bars – roots, blue bars – needles*

Among rarely detected fungi in the damaged needles of the seedlings were *Herpotrichia juniperi* (4.9%), *Lophodermium conigenum* (4.9%), and *Cenangium acuum* (0.8%). *H. juniperi* is a causal agent of snow blight disease of coniferous seedlings which is quite easily diagnosed by the microscopic method, and there are certain guidelines for its control [19], [30]. *L. conigenum* is reported to be an endophyte of primary and secondary needles, and of cones of *Pinus* species [22]. It occurs in Europe, Asia, North America, and Australia [31]. *C. acuum* colonizes pine and spruce needles and causes needle necrosis and cast [6]. In this study, *H. juniper* and *L. conigenum* were detected only on the needles of Scots pine, while *C. acuum* was found on the needles of Scots pine and Siberian spruce seedlings.

The roots of diseased *Pinus sylvestris* L.,

*Pinus sibirica* Du Tour, and *Picea obovata* Ledeb. seedlings were commonly colonized by *Phialocephala fortinii* (48.2%) and *Cadophora finlandica* (28.3%). *P. fortinii* is the most frequent dark septate endophyte (DSE) in the natural forest ecosystems in the Northern hemisphere [14]. There is strong evidence for the assumption that the roots of each Norway spruce (*Picea abies*) tree in the forest habitats of Europe are colonized by *P. fortinii* [1], [13]. The fungus may also behave as a weak pathogen, a wood decomposer or a mutualist. Quite possibly the role of *P. fortinii* in trees may shift along with host and/or environmental conditions [13], [20]. It was also shown that *P. fortinii* may contribute to plant growth and stress tolerance in stressful ecosystems [32], [34]. Besides, it may act as an opportunistic pathogen [36]. However, taking into account its widespread distribution, it is very unlikely that *P. fortinii* is a primary pathogen [28]. Thus, more detailed studies of the interactions between *P. fortinii* and host plants are necessary to clarify the functional basis of this symbiosis. In the present study, *P. fortinii* was found in the roots of all the studied tree species, but only on three- and four- year old affected plants (78.5%) (Figure 4). *Cadophora finlandica* was the second most frequently detected fungi in the roots of coniferous seedlings. Most *Cadophora* species are predominantly isolated from soil and plants, interacting as plant pathogens, root colonizers or saprobes [2], [33]. The fungus *C. finlandica* can also influence metal uptake by plants as it seems to be commonly associated with the roots in metal contaminated sites [9]. Interestingly, the mycobiota in the roots of one- and two-year old Scots pine and Siberian stone pine seedlings was commonly represented by *C. finlandica* (58.5%) (Figure 3). Several other micromycetes were also found in the roots of diseased seedlings: *Chalara* sp. (Acc No. AY805549.1) (9.4%), *Didymella pomorum* (5.7%), *Paraphoma radicina* (4.7%), *Pezicula radicicola* (2.8%), and *Leptodontidium* sp. (Acc No. MH861023.1) (0.9%). These fungi are mainly known as facultative pathogens but their behavior may change depending on the environmental conditions and/or the health state of the plants [8], [11], [15], [24]. Given the low frequency of occurrence of these phytopathogens, they do not pose a significant threat to the seedlings in the studied forest nurseries.

Therefore, the main fungi colonizing Scots pine, Siberian stone pine, and Siberian spruce seedlings were determined using direct sequencing. The microflora of each studied forest nursery was represented by 4-6 fungal taxa. The most frequently detected fungi on one-four year old coniferous seedlings were *S. polyspora, D. glomerata*, *D. pomorum*, *C. herbarum, L. seditiosum*, *P. fortinii,* and *C. finlandica*.

## 4. Conclusions

In this study the fungal community in the diseased coniferous seedlings grown in bare root forest nurseries in Middle Siberia was assessed. *S. polyspora*, *D. glomerata*, *C. herbarum,* and *L. seditiosum* were the most commonly identified fungi in the damaged needles of *Pinus sylvestris* L., *Pinus sibirica* Du Tour, and *Picea obovata* Ledeb. seedlings. The roots of the infested seedlings were commonly colonized by *P. fortinii* and *C. finlandica*, and they were followed in a

decreasing frequency by *Chalara* sp., *D. pomorum*, *P. radicina*, *P. radicicola,* and *Leptodontidium* sp. Two micromecetes, *D. glomerata* and *P. fortinii*, were detected on the diseased seedlings of all the studies tree species. The fungus *P. fortinii* was observed in all the surveyed forest nurseries.

Summing up, molecular genetic diagnostics is essential for the precise detection of fungal communities on seedlings in forest nurseries. The obtained results can be taken into account in implementing the right phytosanitary measures in the affected nurseries.

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