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EXPERIMENTAL ARTICLES

Effect of *Trichoderma* Fungi on Soil Micromycetes That Cause Infectious Conifer Seedling Lodging in Siberian Tree Nurseries

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Abstract—Soils in the tree nurseries studied were characterized by a lower species diversity of fungi than adjacent virgin soils. In particular, the relative abundances of representatives of the genera *Mucor, Chaetomium*, and *Trichoderma* in the nursery soil were two times lower than in adjacent virgin soils. On the other hand, the nursery soil exhibited greater abundances of fungi of the genus *Fusarium*, which are causative agents of many diseases of conifer seedlings. To appreciate the efficiency of biocontrol of the infectious diseases of conifer seedlings, we introduced several indigenous *Trichoderma* strains into the nursery soil and found that this affected the species composition of soil microflora considerably. Changes in the species composition of mycobiota beneficially influenced the phytosanitary state of soils and reduced the infectious lodging of conifer seedlings.

Key words: nursery soils, virgin soil, phytopathogens, micromycetes, conifer seedlings, microbial antagonism, *Trichoderma*, biological control

Treatment of soil, seeds, and seedlings with fungicides is traditionally used to control the causative agents of conifer seedling lodging in Siberia. However, fungicides do not possess selective action and kill useful epiphytic and saprotrophic microbiota and mycorrhizal fungi [1]. One possible way to control phytopathogens and not affect beneficial soil microorganisms is to use biological methods of control [2, 3], such as the introduction of antiphytopathogenic microorganisms into the soil. The active antiphytopathogens are fungi of the genus *Trichoderma.*

The aim of the present work was to study the composition of micromycete complexes in the soils of two tree nurseries in the Krasnoyarsk krai and to appreciate changes in the soil microbiocenoses caused by the introduction of *Trichoderma viride* spore suspensions.

MATERIALS AND METHODS

Investigations were carried out in the conifer tree nurseries situated in the Bolshe-Murtinskii (southern taiga) and Ermakovskii (forest steppe) terranes of Krasnoyarsk krai. The first nursery lies on dark gray weakly podzolized soil, and the second nursery lies on podzolized chernozem.

Soil samples, which were collected four times a year (from May to September) in 1996–1998, were analyzed by plating on potato agar, wort agar, and Czapek agar medium [4, 5]. Analysis of these plates determined (1) the total number of fungal cells per g soil (N); (2) the number of cells of particular species per gram of soil (n); (3) the number of encountered fungal species; (4) the spatial abundance of species, which was expressed as the percentage of soil samples in which a given species was detected; (5) the temporal abundance of species, which was expressed as the ratio of the number of time periods during which a given species was detected in soil to the total number of time periods during which soil samples were collected; and (6) the Shannon–Weaver diversity index, which was calculated using the formula $H = -Ep_i \log_2 p_i$, where H is the data-processing index of diversity of microcommunities and p_i is the probability of species abundance to fall within a certain range of its values (from 1 to 10, from 11 to 20, etc.) [6, 7].

Phytopathogens from infected plants were isolated by the wet chamber method and by plating the slurries of homogenized infected plant parts on to wort and potato agars. Plants were superficially sterilized by immersing them in a 2% solution of $KMnO_4$ for 2–3 min and then repeatedly washing them with sterile water [5].

The fungi isolated were classified using the determinative tables of Mil'ko [8], Bilai [9], Kirilenko [10], Egorova [11], and Bilai and Koval' [12]. To reduce the time necessary for their identification, fungi were cultivated in microquantities. This method allows the observation of the whole life cycle of fungi within 24–72 h [13].

Indigenous *Trichoderma* strains antagonistic to phytopathogenic microorganisms were isolated by the method of dense populations and enrichment on Czapek and wort agars [4, 5].

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The correlational dependences of the effect of temperature and soil moisture on plant infection and the species diversity of micromycetes (Shannon–Weaver index) were obtained using routine statistical methods. Significance levels were estimated by an *F*-test using Statistica, v. 5 for Windows 95 [7, 14]. Confidence limits were determined for the 95% significance level.

RESULTS AND DISCUSSION

Species Diversity of Soil Micromycetes

The diversity of micromycetes in the nursery soil was sufficiently high (Fig. 1) to isolate 59 fungal species belonging to 21 genera from the dark gray weakly podzolized soil and 63 species belonging to 18 genera from the podzolized chernozem.

The dark gray weakly podzolized soil was dominated by fungi of the genus *Penicillium* (Fig. 1a). The relative abundance of these fungi in adjacent virgin soil was 1.4 times that of the nursery's soil. The species diversity of fungi of the genus *Trichoderma* in the virgin soil was 3.5 times as high as in the nursery's soil.

The podzolized chernozem was dominated by fungal species of the genera *Penicillium* and *Cladosporium*, which constituted 50% of all the fungal species isolated (Fig. 1b). The abundance of *Trichoderma* in the adjacent virgin soil was 2.5 times higher than in the nursery's soil. Species of the genus *Aspergillus* ranged from 5.1 to 9.6% of the total number of fungal isolates. The relative abundance of *Aspergillus* species in the nursery's soil was almost two times greater than in the adjacent podzolized chernozem. The dominant species were *A. fumigatus, A. niger,* and *A. ustus.* The abundance of other micromycetes (*Alternaria, Cladosporium, Trichoderma, Mucor,* and *Chaetomium*) in the virgin soil was higher than in the nursery's soil.

Both soil types were dominated by imperfect fungi, among which species of the genus *Penicillium* were most numerous (31.8 to 48.2% of the total number of fungal isolates). The number and diversity of the isolates of this genus in the nursery soil were lower than in the adjacent virgin soils.

Dark-colored hyphomycetes of the genera *Alternaria, Cladosporium,* and *Aureobasidium* were encountered in all the mycocenoses studied, and were more abundant in the podzolized chernozem than in the dark gray weakly podzolized soil. *Trichoderma* species (*T. koningii, T. viride,* and *T. polysporum*) were two times more frequent in virgin soils than in the nursery soil.

These data indicate that the mycocenoses of the nursery's and the adjacent virgin soils are qualitatively the same. However, the fungal diversity in the nursery soil is lower than in the adjacent soils. For the dark gray weakly podzolized soil, the Shannon-Weaver diversity index was 2.24 (nursery's soil) and 2.65 (virgin soil); and for the podzolized chernozem, these indices were 2.51 and 2.87, respectively. The total number and the number of dominant fungal species were greater in the nursery soil, while the number of minor species was greater in the adjacent virgin soils. The low abundance of fungi of the genera *Trichoderma, Mucor,* and *Chaetomium* in the nursery's soils is noteworthy.

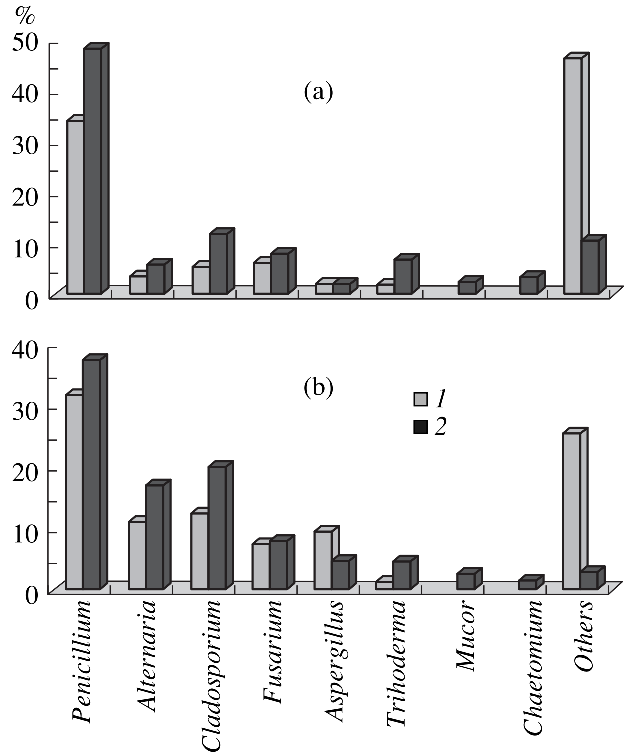

Fig. 1. Relative abundances of soil fungi in (a) dark gray weakly podzolized soil and (b) podzolized chernozem: (*1*) nursery's soil and (*2*) adjacent virgin soil.

Due to the high density of conifer seedlings in nurseries, they become susceptible to soil-born phytopathogenic microorganisms, whose population in nursery's soils is, as a rule, denser than in adjacent virgin soils. For instance, the populations of *Fusarium solani* var. *eumartii, F. culmorum, F. sporotrichiella* var. *sporotrichoides*, and *F. oxysporum* var. *orthoreceras* in the nursery's soils were 3.0–3.9, 2.1–3.3, 1.3–3.2, and 1.5–1.8 times denser than in the adjacent virgin soils. Two *Verticillium* species, *V. albo-atrum* and *V. tenerum*, were not detected in the virgin soils at all, although at the Ermakovskii-nursery, they were isolated from the soil, and germinated seeds and dead conifer seedlings.

Infectious conifer seedling lodging takes place when the population of phytopathogens at the early stages of seedling ontogenesis is high. The microbiological investigation of conifer seedlings in the nurseries showed that they had all the known signs of phytopathogenic affection, such as seed, germ, and root rotting, withering of seedling apices, and seedling lodging.

Analysis showed that the seedling roots and stems were mainly infected by fungi of the genus *Fusarium.*

Effect of the presowing treatment of Scotch pine seeds with *Trichoderma* species on seed germination and the morphometric parameters of seedlings at the end of the first month of vegetative growth

Species	Number of isolates used for treatment	Percentage of germinated seeds	Hypocotyl length, cm	Seedling biomass, mg dry wt
T. hamatum	8	61.3 ± 1.9	2.2 ± 0.2	43.23 ± 0.16
T. koningii	10	69.3 ± 3.9	3.1 ± 0.1	43.96 ± 0.14
T. viride	10	67.0 ± 2.5	2.9 ± 0.2	44.08 ± 0.10
Control (treatment with H_2O)	–	51.3 ± 1.7	2.1 ± 0.1	42.11 ± 0.08

The phytopathogenic species that prevailed in the dark gray weakly podzolized soil were *F. sporotrichiella* var. *sporotrichoides*, *F. heterosporum*, and *F. moniliforme*, while *Fusarium solani* var. *eumartii*, *F. oxysporum* var. *orthoreceras*, and *F. avenaceum* var. *herbarum* dominated the podzolized chernozem. Seedling leaves were mainly infected by fungi of the genera *Alternaria* (*A. alternata*, *A. geophila*, and *A. solani*) and *Cladosporium* (*C. cladosporioides* and *C. herbarum*).

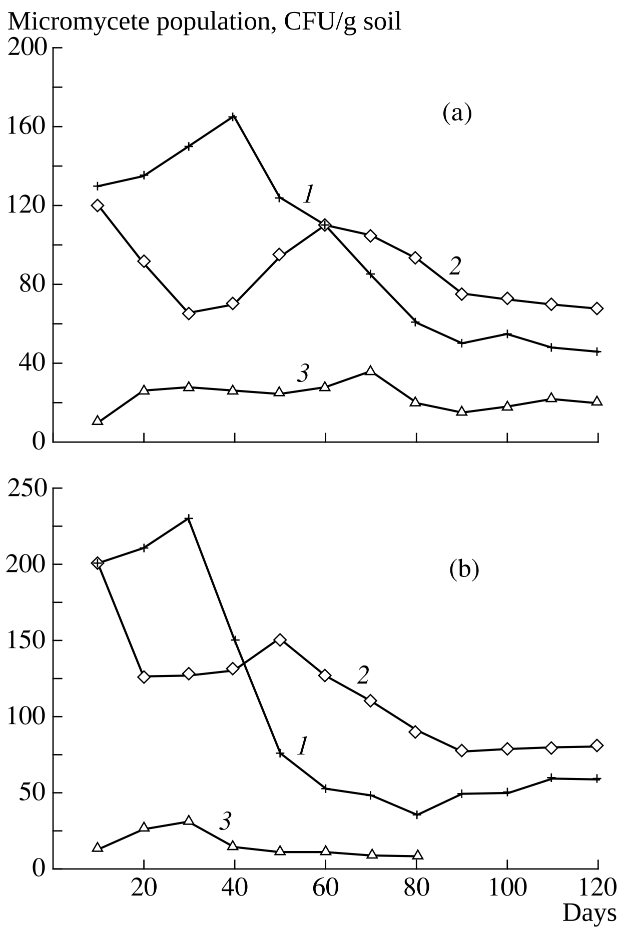

Fig. 2. Effect of the presowing treatment of soils with *T. viride* on the dynamics of soil micromycetes in (a) dark gray weakly podzolized soil and (b) podzolized chernozem: (*1*) dynamics of micromycetes in the control (untreated) soil; (*2*) dynamics of micromycetes in the treated soil; and (*3*) dynamics of *T. viride* in the treated soil.

Within two weeks of seedling growth, the high soil moisture content raised the degree of seedling infection by 78% (podzolized chernozem) and 55% (dark gray weakly podzolized soil).

The effect of *Trichoderma* fungi on micromycete population in the nursery's soils According to data available in the literature, microbial diversity in the soils of tree nurseries is low because of: (1) an insufficient amount of herbaceous plants, (2) frequent chemical treatments, which kill a great number of susceptible microbial species, and (3) monocultivation, due to which many of the indigenous microorganisms are excluded by the species that are more adapted to the conditions of artificial phytocenoses [1, 15]. This results in an increase in the population of phytopathogenic micromycetes and a decrease in the population of antiphytopathogenic species [16–18].

Our investigations showed that the relative abundance of microorganisms antagonistic to phytopathogenic fungi in the soils of tree nurseries is low. The antagonists isolated from the nursery's soils were mainly represented by bacteria of the genera *Pseudomonas* and *Bacillus* (53% and 32%, respectively, of the total number of antagonists). The dominant antiphytopathogens of virgin forest and peat soils were *Trichoderma viride* and *Penicillium chrysogenum.*

The control of phytopathogenic micromycetes in the soils of tree nurseries necessitates their efficient ecological management. It is known that *T. viride* is a soil saprophyte dominating the phylloplane of many plants and is antagonistic to many phytopathogenic micromycetes. This mycoparasite inhibits the growth of phytopathogens through the secretion of mycotoxins and some hydrolytic enzymes capable of degrading the cell walls of host fungi [1, 19, 20]. In light of this, many *Trichoderma* species are considered to be promising antiphytopathogenic agents.

In the present study, we attempted to use the isolates of indigenous *Trichoderma* species to suppress the activity of phytopathogenic micromycetes. The results of laboratory and plot investigations confirmed the high antagonistic activity of *Trichoderma* isolates, whose aqueous suspensions augmented the degree of germination of Scotch pine seeds by 6.7–18% and promoted the growth of seedlings. For instance, the treatment of pine seedlings with the suspensions of *T. koningii* and *T. viride* isolates increased the hypocotyl length and seedling biomass by 3–4 and 14–15%, respectively (see table). The role of hyphal interactions between different fungi in the biological control was studied by Inbar *et al.* [19].

Further studies were devoted to the effect of introduced *Trichoderma* species on the composition of micromycete complexes in the nursery's soils. The introduction of *T. viride* spores to the soils in the amount of 10^8 CFU/m^2 led to a decrease in the total population of micromycetes in 20 days by 3–4 times (dark gray weakly podzolized soil) and 1.5 times (podzolized chernozem) as compared to the control plots (Fig. 2). The degree and duration of depression in the development of soil micromycetes were higher in the dark gray weakly podzolized soil: the normal levels of micromycete population in this soil and the podzolized chernozem soil were restored in August and at the beginning of July, respectively. Later, fungal population in either type of soil became denser than in the control plots.

The introduction of *T. viride* into the soils considerably influenced the species composition of soil mycobiota, especially during the period of the maximum growth of the introduced species (in June), when its population density reached a value of 4.5×10^4 CFU/g air-dried soil as compared to 2.8×10^2 CFU/g air-dried control soil. The relative abundance of *T. viride* in the artificially contaminated soil was 59.6%. The decrease in the total population of micromycetes in the soils contaminated with *T. viride* was mainly due to the decrease in the populations of fungi of the genera *Cladosporium* (by a factor of 23.4), *Alternaria* (by a factor of 7.5), and *Fusarium* (by a factor of 2.3). At the same time, the populations of the *Penicillium* species *P. islandicum* Wehm, *P. verticulatum* Dang, and *P. spinulosum* Thom slightly increased. These data suggest that *T. viride* can suppress the growth of some mycotoxin-producing fungi.

Dennis and Webster emphasized that the relationship between indigenous *Trichoderma* and other soil micromycetes lies in the competition for nutrients [20]. In our opinion, when specially introduced to the soils, *Trichoderma* not only competes with other soil microorganisms for nutrients but may also use soil microbiota (including phytopathogens) for nutrition.

To conclude, experiments with introduced *Trichoderma* species showed that they beneficially influenced the phytosanitary state of the nursery's soils and decreased the infectious conifer seedling lodging in the Siberian tree nurseries.

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