Microbial Ecology

Fungal Diversity, Dominance, and Community Structure in the Rhizosphere of Clonal *Picea mariana* Plants Throughout Nursery Production Chronosequences

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Abstract

Fungal diversity in the rhizosphere of healthy and diseased clonal black spruce (*Picea mariana*) plants was analyzed with regard to nursery production chronosequences. The four key production stages were sampled: mother plants (MP), 8-week-old cuttings (B + 0), second-year cuttings (B + 1), and third-year cuttings (B + 2). A total of 45 fungal taxa were isolated and identified based on cultural, phenotypic, and molecular characters. Members of phylum Ascomycota dominated, followed by Basidiomycota and Zygomycota. Diagnosis characters and distance analysis of the internal transcribed spacer rDNA sequences allowed the identification of 39 ascomycetous taxa. Many belong to the order Hypocreales, families Hypocreaceae and Nectriaceae, which contain many clusters of potentially pathogenic taxa (*Cylindrocladium*, *Fusarium*, and *Neonectria*) and are also ecologically associated with antagonistic taxa (*Chaetomium*, *Hypocrea*, *Microsphaeropsis*, *Penicillium*, *Paecilomyces*, *Verticillium*, *Trichoderma*, and *Sporothrix*). This is also the first report of a *Cylindrocladium canadense* association with disease symptoms and relation with *Pestalotiopsis*, *Fusarium*, *Exserochilum*, *Rhizoctonia*, and *Xenochalara* fungal consortia. Both production chronosequence and plant health considerably influenced fungal taxa assemblages. Unweighted pair-group arithmetic average clustering showed that isolates from MP, B + 0, and B + 1 plant rhizospheres clustered together within healthy or diseased health classes, whereas isolates from healthy and diseased B + 2 plants clustered together. Canonical correspondence analysis revealed substantial alteration in community assemblages with regard to plant health and yielded a principal axis direction that regrouped taxa associated with diseased plant rhizosphere soil, whereas the opposite axis direction was associated with healthy plants. Two diversity indices were defined and applied to assess the fungal taxa contribution (Tc) and persistence (Pi) throughout the production.

Introduction

The boreal forests, which are the second most extensive terrestrial biome on Earth after tropical forests, occupy an area of 18.8 million km^2. *Picea mariana* forest ecosystems in Canada are a dominant part of this biome located north of 50°N latitude. Close to one million hectares of forest were harvested in Canada in 1990 [35], and the harvested area has increased by more than 3% per year ever since [7]. Because of the huge economical pressure on black spruce forests and the impact of climate change, fire, insects, and diseases, an extensive reforestation program was established in Canada. The efficiency of this program relies, in good part, on the safe production of high-quality pathogen-free plants. Although beneficial soil inhabitants and the undetected pathogenic fungi that can occur in the rhizosphere of healthy (asymptomatic) plants may be important factors influencing their survival, our knowledge of these microbial communities is very limited. Despite large replant efforts in the last decade, most Canadian provinces have a regeneration gap between the area harvested and the area regenerated, either naturally or

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through planting programs. Canada produces approximately one billion tree seedlings per year to reforest over 400,000 hectares [7]. The province of Quebec, in eastern Canada, contributes approximately 15% to this effort, mostly as black spruce [*P. mariana* (Mill.) B.S.P.]. The St-Modeste Forest Nursery, owned by the Government of Quebec, has developed a black spruce production approach based on controlled hybridization, propagation by cuttings, and growth in containers [51]. This technology provides genetically selected and more uniform plants, thus ensuring a more successful reforestation that depends strongly on a continuous supply of high-quality seedlings [10]. Unfortunately, as in other production approaches, the plants may suffer from diverse fungal pathogenic infections causing root-rot and damping-off outbreaks [25, 27, 32]. Indeed, the young conifers are threatened by these pathogens in the early stages of regeneration, significantly affecting the success of forest regeneration programs throughout North America [17, 28, 42].

In the 1990s, more than four million nursery seedlings showing root-rot or damping-off were destroyed in the province of Québec [25]. Despite large losses, little is known about the diversity of the fungal pathogens involved. Identification of the causal agents is often contradictory, even for the main pathogenic fungal species that belong to the Ascomycota genus *Cylindrocladium*. Previously, *Cylindrocladium floridanum* Sobers and Seymour has been considered to be a prevalent pathogenic species of black spruce in eastern Canada [15], although some genetic differences were found between Canadian and American isolates [16, 27]. However, based on cultural, phenotypic, and rDNA internal transcribed spacer (ITS) sequence analyses, Vujanovic and St-Arnaud [53] have recently shown that *Cylindrocladium* isolates from the St-Modeste nursery belong to *Cylindrocladium canadense* Kang, Crous, and Schoch [29] rather than *C. floridanum*. If *C. canadense* is prevalent in at least some eastern Canadian nurseries, the potential differences with *C. floridanum* ecology and a lack of knowledge about the ecological preferences of *C. canadense* may reduce the effectiveness of management strategies to control root diseases.

With the increasing concern about the environmental consequences of fungicide applications, microbial diversity associated with various coniferous species has recently received attention to explore the potential for alternative strategies of disease management [18, 31]. Among the various microbial ecological niches associated with plants, the rhizosphere is the most challenging. The rhizosphere is the soil zone directly influenced by the plant roots [58]; it is inhabited by complex microbial communities, which depend on the nutrients released by the roots [5]. Fungal mycelia constitute a significant part of the microbial biomass in close proximity to roots and some taxa may be important competitors of pathogens, acting as biocontrol agents [8, 14, 15, 30]. A shift in the fungal community structure related to plant development during the various nursery production stages may consequently be an important factor affecting disease outbreaks. Change in the plant health status and photosynthetic activity may also influence root exudation and modify microbial community structure or activity and, therefore, rhizosphere suppression of root pathogens [44]. Many fungal communities or functional groups are poorly known, particularly those classified as Ascomycota, which could be important competitors of fungal pathogens. The available data concerning spruce rhizosphere microbiota are very scarce, with the exception of mycorrhizal root symbionts [14, 31, 36].

In a previous work [19], profiling the rhizosphere microbial communities of fully grown (3-year-old) black spruce seedlings, we found 84 bacterial and 31 fungal sequences that belonged to a wide range of microorganisms. Based on cloned 18S rDNA gene sequence analysis, rhizosphere-associated fungal communities varied considerably between healthy and diseased plants. While accurate species identification was not possible in most cases, the results raised the possibility that some fungal taxa frequently or exclusively found in healthy rhizospheres may have competed with the pathogen and reduced its ability to colonize the rhizosphere and invade roots. Still, we lack knowledge of the fungal communities that characterize the *P. mariana* plants at the species level, and also of the population dynamics and dominant taxa throughout the production chronosequences. Moreover, propagation of genetically improved *P. mariana* plants by cloning of selected lines yield more uniform plants with superior phenotypes, but may also reduce the diversity of rhizosphere microbial communities and potentially lower the overall substrate antagonism toward root pathogens.

Recently, Mazzola [34] stated that effective implementation of strategies to manage root-rot and damping-off pathogens requires (1) initial identification of the biological components involved in the disease's etiology and (2) monitoring of the environmental impact on the abundance of the target microbial population's growth stages. Furthermore, additional data on the fungal diversity associated with economically important forest trees is urgently required to prevent and manage the involuntary introduction of genetically unique foreign pathogens associated with world trade [12]. The aim of this study was to compare the fungal community bioprofile and to identify dominant species and assemblages in the rhizosphere of healthy and diseased containerized *P. mariana* plants, in relation to the production chronosequences of a forest nursery. Fungal assessment and identification at the species level were based on a combination of phenotypic, cultural, and ITS rRNA gene sequence analyses. Special attention was given to the

Ascomycota fungal communities, as they are known sources of both black spruce pathogens and potential biocontrol species.

Materials and Methods

Experimental Design and Sampling. Black spruce cuttings and rhizosphere soil samples were collected in September 2001 and 2002 from a governmental tree nursery located in the eastern region of Canada (St-Modeste, Québec: 47°49′55″N, 69°24′11″W) where a high incidence of root rot diseases were previously reported (Innes, pers. comm.). In this nursery, black spruce propagation is routinely conducted by cuttings from 2–3-year-old mother plants (MP) produced from black spruce seeds harvested from controlled pollination orchards [10]. Plants are rooted in compartmentalized production trays in a mixture of peat, vermiculite, and perlite (2:1:1) and grown for 1 year under greenhouse conditions, followed by 2 years of growth outdoors before being shipped for reforestation [51]. Sampling was conducted over the regular *P. mariana* production chronosequences and included the following four key production stages: (1) MP, (2) 8-week-old cuttings rooted in compartmentalized production trays in a greenhouse (B + 0), (3) second-year cuttings where the production trays were arranged on outdoor plots for an additional 6-month growth period after 6 months in a greenhouse (B + 1), and (4) third-year cuttings having grown for a second year in outdoor plots (B + 2). Each year, five healthy-looking plants and five plants showing symptoms associated with root rot were randomly chosen within each of the four growth stages. Plants were carefully examined under a dissecting microscope to confirm the absence or presence of root necrosis. All plants were maintained in compartmentalized containers. For each plant, a 100-g soil sample was collected from the rhizosphere by vigorously shaking the *P. mariana* plant and collecting the soil portion in close contact with the root system. Roots and soil samples were placed in individual bags and transported back to the laboratory, where they were rapidly stored at 4°C before the isolation of fungi and determination of root biomass.

Data Collection and Identification of Fungal Taxa. Roots from asymptomatic and symptomatic MP, B + 0, B + 1, and B + 2 plants from 2001 and 2002 samplings were separately washed. Fresh and dry weights were determined after drying each sample for 24 h at 70°C. A 3-g subsample was taken from each 100-g soil sample after thorough mixing in a sterile bag. Dilution series were prepared in sterile water and inoculated onto plates of 2% potato dextrose agar (PDA, Difco) supplemented with antibiotics (100 mg l^{-1} streptomycin sulfate and 12 mg l^{-1} neomycin sulfate, Sigma, St. Louis, MO, USA). The dilution series were repeated five times for each plant. Assay plates were incubated at 22°C in the dark and observations were made after 7 and 14 days. All fungi were isolated generally from the 10^{-3} dilution, separated in morphotypes and quantified as colony-forming units (CFUs). Pure cultures were established using standard procedures. Fungi were morphologically identified at least to the genus level using standard diagnostic characteristics in fungal taxonomy. Representative isolates of Ascomycota occurring at a frequency of >1% in all samples, as well as ambiguous rare isolates, were further analyzed by polymerase chain reaction (PCR) amplification and sequencing of the ITS rRNA gene region. Most isolates belonging to Basidiomycota and Zygomycota were identified by morphology only. Reference DNA samples were deposited in the Institut de recherche en biologie végétale Micro fungus collection (MTF) under numbers MTF A01-F05 (see Table 2).

DNA Extraction, PCR Amplification and ITS Sequencing. Fungal isolates were grown for 14 days on 2% PDA plates, and mycelial mats were collected using a sterile scalpel blade. Mycelia were freeze-dried and ground with a mortar and pestle in liquid nitrogen [57]. Genomic DNA was extracted using the DNAeasy plant mini kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Internal transcribed spacer region 1, the 5.8S rRNA gene, and ITS region 2 were amplified using the primer pair ITS1F and ITS4 [21, 56]. PCR amplicons were purified on agarose gels using the QIAquick gel extraction kit and sequenced at the Montreal Genomics Centre (Montreal, Canada). Sequences were identified by similarity searches in GenBank using the BLASTn search algorithm. These identifications were confirmed in comparison with phenotypical characterization. All fungal ITS sequences generated in this study were deposited in GenBank under accession numbers DQ132810–DQ132848.

Data Analyses. Sequences were aligned using Clustal X software (version 1,82) [50]. Distance trees were produced with the PAUP 4.0b10 software [48] using a neighbor-joining approach, and support for groups in the tree was assessed using a bootstrap analysis with 1000 repetitions. A fungal distance tree was prepared with sequences that matched at 91% and higher.

Fungal diversity indices were computed for healthy and diseased health classes using the Shannon–Wiener index (H') calculated from $H' = -\sum p_i \ln p_i$ [40]. A Taxon contribution index was defined as $\mathrm{Tc} = (-p_i \ln p_i)/H'$ and is derived from the Shannon–Wiener index. Tc is a fraction and describes the contribution of each taxon to the normalized value of diversity. Furthermore, the persistence of a species through the production stages (MP, B + 0, B + 1, and B + 2) was analyzed using a persis-

Table 1. **Diversity, abundance and persistence of fungal taxa associated with the rhizosphere soil of healthy and diseased *P. mariana* plants throughout forest nursery production chronosequences (MP, B + 0, B + 1 and B + 2)**

		Species richness[b]													
		Healthy							*Diseased*						
		CFU × $10^1 \cdot g^{-1}$ soil							*CFU × $10^1 \cdot g^{-1}$ soil*						
Fungal taxon[a]	*Isolate #*	*MP*	*B + 0*	*B + 1*	*B + 2*	∑	*Tc*[c]	*Pi*[d]	*MP*	*B + 0*	*B + 1*	*B + 2*	∑	*Tc*[c]	*Pi*[d]
Phylum Ascomycota															
Dothideales, Dothioraceae															
A. pullulans (de Bary) Arn.	06	5	.	.	.	5	0.090	0	.	.	12	.	12	0.139	0
Dothideales, incertae sedis															
Septonema chaetospira (*Grove*) Hughes	40	.	.	.	.				.	.	.	4	4	0.063	0
Eurotiales, Trichocomaceae															
A. niger Tiegh	05	.	.	.	.				.	.	.	1	1	0.021	0
Paecilomyces sp.	28	1	2	7	2	12	0.166	0.807	.	.	2	3	5	0.050	0.485
P. brevicompactum Dierckx	31	.	.	.	1	1	0.026	0	.	.	.	.			
P. commune Thom	32	.	.	4	5	9	0.137	0.496	.	.	.	.			
P. daleae K.M. Zalessky	33	.	.	.	.				.	.	.	6	6	0.085	0
P. thomii Maire	34	3	12	7	9	31	0.285	0.929	.	2	.	3	5	0.074	0.485
Hypocreales, Hypocreaceae															
Gliocladium sp.	17	.	.	.	2	2	0.045	0	.	.	.	.			
Hypocrea sp.	19	3	11	6	3	23	0.245	0.699	.	1	3	4	8	0.105	0.703
T. viride Pers : Fr.	43	.	.	.	.				5	.	.	.	5	0.074	0
Hypocreales, Nectriaceae															
C. canadense Kang, Crous and Schoch	11	7	1	8	4	20	0.226	0.870	7	7	9	14	37	0.273	0.968
F. avenaceum (Fr.) Sacc.	13	.	.	1	.	1	0.026	0	.	.	3	.	3	0.050	0
F. oxysporum Schltdl	14	.	3	2	.	5	0.090	0.485	28	.	7	.	35	0.265	0.361
F. sambucinum Fuckel	15	.	2	1	.	3	0.062	0.459	10	8	4	.	22	0.205	0.747
F. tricinctum (Corda) Sacc	16	.	1	.	.	1	0.026	0	6	5	4	.	15	0.161	0.783
N. mauritiicola (Henn.) Seifert and Samuels	25	1	.	.	.	1	0.026	0	.	.	.	.			
Hypocreales, incertae sedis															
A. strictum W. Gams	01	.	.	.	8	8	0.126	0	.	.	.	2	2	0.036	0
V. bulbillosum W. Gams and Malla	44	3	.	.	.	3	0.062	0	.	.	.	.			
Microascales, Microascaceae															
Scopulariopsis sp.	39	.	1	.	.	1	0.026	0	.	.	.	.			
Mycosphaerellales, Mycosphaerellaceae															
C. cladosporioides (Fres.) de Vries	09	2	4	.	3	9	0.137	0.765	.	1	.	.	1	0.021	0
Ramularia sp.	37	.	.	.	.				.	.	.	6	6	0.085	0
Ophiostomatales, Ophiostomataceae															
O. nigrocarpum (R.W. Davidson) de Hoog	27	.	.	.	.				.	.	.	2	2	0.036	0
S. schenkii Hektoen and Perkins.	41	.	.	.	10	10	0.147	0	.	.	.	.			
Pleosporales, Leptosphaeriaceae															
Coniothyrium sp.	10	.	.	.	.				.	.	5	5	10	0.122	0.500
Microphoma sp.	21	2	.	.	.	2	0.045	0	.	.	.	.			
Pleosporales, Montagnulaceae															
Microsphaeropsis sp.	22	.	.	.	3	3	0.062	0	.	.	3	.	3	0.050	0
Paraphaeosphaeria sp.	30	.	.	.	.			0	.	.	2	.	2	0.036	0

Table 1. (continued)

Fungal taxon[a]	Isolate #	Species richness[b]														
		Healthy							*Diseased*							
		CFU × 10^1·g^{-1} soil							*CFU × 10^1·g^{-1} soil*							
		MP	*B + 0*	*B + 1*	*B + 2*	∑	*Tc*[c]	*Pi*[d]	*MP*	*B + 0*	*B + 1*	*B + 2*	∑	*Tc*[c]	*Pi*[d]
Pleosporales, Pleosporaceae															
A. alternata (Fr.) Keissler	04	8	5	2	.	15	0.191	0.700	9	.	6	2	17	0.175	0.690
Exserohilum novae-zelandie (Hughes) Upadhyay and Mankau	12	.	.	.	.				.	.	.	2	2	0.036	0
Sordariales, Chaetomiaceae															
C. globosum Kunze	08	.	.	3	2	5	0.090	0.485	.	.	.	2	2	0.036	0
Xylariales, Amphisphaeriaceae															
Pestalotiopsis spp.	35	10	3	10	.	23	0.245	0.714	.	.	.	13	13	0.146	0
Xylariales, Xylariaceae															
Geniculosporium sp.	18	.	.	.	.				.	.	.	5	5	0.074	0
Incertae sedis, Myxotrichaceae															
O. tenuissimum (Peck) Hughes	26	3	.	.	.	3	0.062	0	.	.	.	.			
Incertae sedis, incertae sedis															
M. radicis atrovirens sp. 1	23	.	.	.	.				.	.	.	8	8	0.105	0
M. radicis atrovirens sp. 2	24	.	.	.	.				.	.	.	2	2	0.036	0
Papulaspora sp.	29	.	.	.	.			0	.	2	2	.	4	0.063	0.500
Phialocephala sp.	36	.	1	.	.	1	0.026	0	.	2	.	.	2	0.036	0
X. juniperi M.J. Wingf. and Crous	45	.	.	.	.				.	.	.	9	9	0.114	0
Phylum Basidiomycota															
Agaricales, Agaricaceae															
Agaricus sp.	02	.	.	.	.				.	.	.	1	1	0.021	0
Agaricales, Tricholomataceae															
L. bicolor (Maire) P.D. Orton	20	.	.	.	5	5	0.090	0	.	.	.	1	1	0.021	0
Ceratobasidiales, Ceratobasidiaceae															
Rhizoctonia sp.	38	.	.	.	.				.	.	.	7	7	0.095	0
Incertae sedis, incertae sedis															
Unidentified anamorphic Basidiomycete	07	2	.	.	.	2	0.045	0	.	3	2	8	13	0.146	0.667
Thelephorales, Thelephoraceae															
T. terrestris (= T. americana) Ehrenb	42	.	.	.	2	2	0.045	0	.	.	.	.			
Phylum Zygomycota															
Mucorales, incertae sedis															
Actinomucor/Umbelopsis sp.	03	.	.	.	.				.	.	.	1	1	0.021	0
Total (CFU×10^1 g^{-1} soil)		50	45	51	61	207			65	31	64	110	270		
Number of taxa		13	12	11	15	28			6	9	14	23	35		
H′[e]							2.846						3.077		

[a]Identified both morphologically and by sequencing the fungal ITS rRNA gene region for all ascomycetous fungi occurring at a frequency >1%, as well as for ambiguous rare isolates. A dot mean that the taxon was not detected in a specific category. Taxa are presented by phylum and are ordered by order and family.

[b]Values are mean of five dilution series per plant, with five healthy and five diseased plants from each of the four key production stages in 2001 and 2002. Production stages were MP, B + 0, B + 1, and B + 2.

[c]Taxon contribution index, $\mathrm{Tc} = (-p_i \ln p_i)/H'$

[d]Persistance index, $\mathrm{Pi} = -[\sum q_i/Q \ln (q_i/Q)]/[2 \ln 2]$

[e]Shannon–Wiener index, $H' = -\sum p_i \ln p_i$

tence index defined as $\mathrm{Pi} = -[\sum q_i/Q \ln(q_i/Q)]/[2 \ln 2]$, where q_i is the CFU value for one production stage, Q is the sum of all the CFU values of a particular taxon, and 2 ln 2 is a normalizing constant. A value of Pi equal to 1 means that the fungal CFU values were constant through all production stages, whereas a value of Pi equal to 0 means that the fungal taxon was isolated in only one stage.

The isolation frequency of a single fungal taxon was calculated ($N_i/N_t \times 100$; where N_i is the CFU value of the isolated fungus and N_t the total CFU values for all combinations of health class and production stage). Data were then submitted to canonical and noncanonical correspondence analyses (CCA and CA) in CANOCO (Windows version 4.5) [49] using χ^2 ($P < 0.05$) distance to test the similarity of the isolated fungal assemblages between health class and production stages combinations. Outliers were eliminated by following the empirical method described by Borcard (unpublished; available at http://biol10.biol.umontreal.ca/BIO6077/outliers.html). Clustering analyses were carried out with the R package [9] using asymmetrical quantitative coefficient to relate production stage with shift in associated fungal assemblages in the rhizosphere and Fager's coefficient for fungal taxa ordination based on detected isolate variations through all chronosequences. The similarity matrix was then exported and the final figure was redrawn in Statistica 6.0 (StatSoft, Tulsa, OK, USA) using the unweighted pair-group arithmetic average (UPGMA) clustering method [43].

Results

Fungal Taxa Prevalence and Phylogenetic Affiliation. Most isolated fungal taxa belonged to phylum Ascomycota, whereas relatively few Basidiomycota and Zygomycota were recovered from the rhizosphere of either healthy or diseased black spruces (Table 1). Dominant taxa obtained from the rhizosphere of healthy plants were all classified as Ascomycota species of the genera *Cylindrocladium*, *Hypocrea*, *Penicillium*, and *Pestalotiopsis* (Tc > 0.2), followed by *Acremonium*, *Alternaria*, *Cladosporium*, *Paecilomyces*, *Penicillium*, and *Sporothrix* taxa (Tc = 0.1–0.2). Subdominating taxa were *Aureobasidium*, *Chaetomium*, *Fusarium*, *Microsphaeropsis*, *Oidiodendron* and *Verticillium* species (Tc = 0.05–0.1). The main rhizosphere inhabitants of diseased plants were *Cylindrocladium* and *Fusarium* species (Tc > 0.2), followed by *Alternaria*, *Aureobasidium*, an unidentified anamorphic Basidiomycete, *Coniothyrium*, *Fusarium*, *Hypocrea*, *Mycelium radicis atrovirens* I, *Pestalotiopsis*, and *Xenochalara* (Tc = 0.1–0.2), and then by *Geniculosporium*, *Microsphaeropsis*, *Paecilomyces*, *Papulaspora*, *Penicillium*, *Ramularia*, *Rhizoctonia*, *Septonema*, and *Trichoderma* (Tc = 0.05–0.1).

Among the Basidiomycota members, *Agaricus* was exclusively isolated from diseased plant rhizospheres (Tc = 0.021) and *Thelephora* from healthy plant rhizospheres (Tc = 0.045), whereas *Laccaria* was more prevalent in healthy (Tc = 0.090) than diseased (Tc = 0.021) plant rhizospheres. One unidentified anamorphic Basidiomycete dominated in diseased plant rhizospheres (Tc = 0.146), occurring rarely in association with healthy plants (Tc = 0.045). Other minor Ascomycete isolates and a single isolate of *Actinomucor*/*Umbelopsis* sp. (Zygomycota) from a diseased B + 2 plant (Tc = 0.021) are listed in Table 1.

Ascomycota taxa were the most diversified and abundant fungal isolates in the *P. mariana* rhizosphere, with 39 taxa isolated (Table 1). Among those, 37 taxa had ITS rRNA sequence similarities ≥91% with sequences from GenBank (Table 2). An unrooted neighbor-joining tree based on ITS rDNA sequences shows the phylogenetic affiliation of the dominant Ascomycota isolates into seven well-defined families and three incertae sedis (Fig. 1), whereas seven other families are represented with at least one taxon (Table 1). Fungal community structure was similar between 2001 and 2002, so results are presented as the mean of the two sampling years. Most rhizosphere isolates belonged to order Hypocreales, families Hypocreaceae or Nectriaceae, or incertae sedis. Many Hypocreales isolates belong to species having strains known to be pathogenic to plants such as *Acremonium strictum*, *C. canadense* (strains F01, F02, F04, F05, and B05), *Fusarium* species complex (*Fusarium avenaceum*, *Fusarium oxysporum* B09, *Fusarium sambucinum* C12, and *Fusarium tricinctum* D03), *Neonectria radicicola* F03 [anamorph: *Cylindrocarpon destructans* (Zinssm.) Scholten] and *Nectria mauritiicola* [anamorph: *Rhizostilbella hibisci* (Pat.) Seifert]. On the other hand, other isolates of Hypocreales are from fungal genera containing biocontrol strains, such as *Gliocladium* sp., *Hypocrea* sp. C06, anamorphic *Trichoderma* isolates (*Trichoderma atroviridae* C08, *Trichoderma viride* A10, and *Trichoderma* sp. D05), and one *Verticillium* sp. A01 isolate. The second largest clade was formed by members of the family Trichocomaceae and included *Aspergillus niger*, *Geniculosporium* sp., *Paecilomyces* (A02), and eight *Penicillium* taxa (*Penicillium brevicompactum* A09, *Penicillium daleae* C10, *Penicillium commune* A05 and E02, *Penicillium griseoroseum* D04, *Penicillium spinulosum* C02, *Penicillium thomii* A06 and B11, *Penicillium* sp.1 A08, and *Penicillium* sp.2 D06). Eleven less represented families were Amphisphaeriaceae (two taxa), Chaetomiaceae (one taxon), Dothioraceae (one taxon), Leptosphaeriaceae (two taxa), Microascaceae (one taxon), Montagnulaceae (two taxa), Mycosphaerellaceae (two taxa), Myxotrichaceae (one taxon), Ophiostomataceae (two taxa), Pleosporaceae (two taxa), Xylariaceae (one taxon). Six other taxa were of uncertain position.

Table 2. ITS gene sequence analysis of Ascomycota associated with the rhizosphere soil of *P. mariana* plants grown in a nursery

DNA sample #	*Isolate #*	*Most closely related taxon*[a]	*Accession number*[b]	*Similarity (%)*
C09	01	*A. strictum*	U57671	99
A07	10	*Coniothyrium* sp.	AY157492	98
F04	11-A	*Cylindrocladium* sp. (=*C. canadense* strain 1)	AF348256	97
F02	11-B	*C. canadense* strain 2	AF348256	98
F05	11-C	*C. canadense* strain 3	AF348256	98
F01	11-D	*C. canadense* strain 4	AF348256	98
B05	11-E	*C. canadense* strain 5	AF348256	98
B09	14	*F. oxysporum*	AY354397	97
C12	15	*F. sambucinum*	X65480	99
D03	16	*F. tricinctum*	AY188923	99
C06	19	*Hypocrea* sp.	AJ230662	96
D10	21	*P. herbarum*	AY337712	99
D09	22	*Microsphaeropsis* sp.[c]	–	
D07	25	*N. mauritiicola*	AJ557830	91
A03	27	*O. nigrocarpum*	AF484457	93
A02	28	*Paecilomyces* sp.[c]	–	
D12	30	*Paraphaeosphaeria* sp.	AB096264	91
A09	31	*P. brevicompactum*	AY373898	99
D06	32-A	*P. commune* strain 1	AF455436	98
A05	32-B	*P. commune* strain 2	AF455418	99
E02	32-C	*P. commune* strain 3	AF455418	100
C10	33	*P. daleae*	AF033442	97
A06	34-A	*P. thomii* strain 1	AY373934	100
B11	34-B	*P. thomii* strain 2	AY373934	100
A11	35-A	*P. uvicola*	AF409974	95
B06	35-B	*P. aquatica*	AF409956	97
B10	41	*S. schenkii*	AF117945	95
A10	43-A	*T. viride* strain1	AF486010	99
D05	43-B	*T. viride* strain 2	AF486010	96
A01	44	*V. bulbillosum*	AJ292410	99
C01	45	*X. juniperi*	AF184889	99
A12	*	*Cadophora* sp.	AY371512	95
D02	*	Leaf litter ascomycete strain 029	AF502621	99
F03	*	*N. radicicola*	AY295319	99
A08	*	*Penicillium* sp.	AY391833	94
D04	*	*P. griseoroseum*	AY425983	91
C02	*	*P. spinulosum*	AY373933	100
A04	*	*Phialophora* sp.	AF083200	96
C08	*	*Trichoderma atroviride*	AY154952	98

Asterisks mean not previously separated based on cultural and morphological features. Identification based only on ITS sequence and BLAST search in GenBank.

[a]Closest match as determined by sequencing and BLASTn search of the ITS gene sequence in GenBank.

[b]Accession number of the closest match in GenBank.

[c]Identification based only on cultural and morphological features. No significant match.

Relation Between Fungal Diversity, Production Stages and Plant Health. Both production chronosequence and plant health influenced fungal taxa assemblages considerably, as shown by an unweighted pair-group analysis using an asymmetrical quantitative coefficient (Fig. 2). Isolates from MP, B + 0, and B + 1 plant rhizospheres clustered together within healthy (linkage distance = 0.546) or diseased (linkage distance = 0.661) health classes. Isolates from healthy and diseased B + 2 plants clustered together but with considerably less linkage distance (linkage distance = 0.695). Root biomass was significantly higher ($P < 0.05$) in healthy plants compared to diseased plants for all production stages (Fig. 3). The highest proportion of fungal diversity was observed in diseased plant rhizospheres. From the 45 identified taxa (Table 1), 35 (78%) were associated with diseased ($H' = 3.077$) and 28 (62%) with healthy ($H' = 2.846$) plants. Fungal abundance was also generally higher in the rhizosphere of diseased plants. The range in the average number of viable fungal particles isolated from all four production stages was 160 CFU g^{-1} dry soil in the rhizosphere of healthy plants (450–610 CFU g^{-1} dry soil) and was considerably lower than the 790 CFU g^{-1} dry soil range measured from the diseased plants (310–1100 CFU g^{-1} dry soil). The total CFU number for all stages was also higher in samples from diseased (2700 CFU g^{-1} dry soil) than from healthy (2070 CFU g^{-1} dry soil) plant rhizospheres.

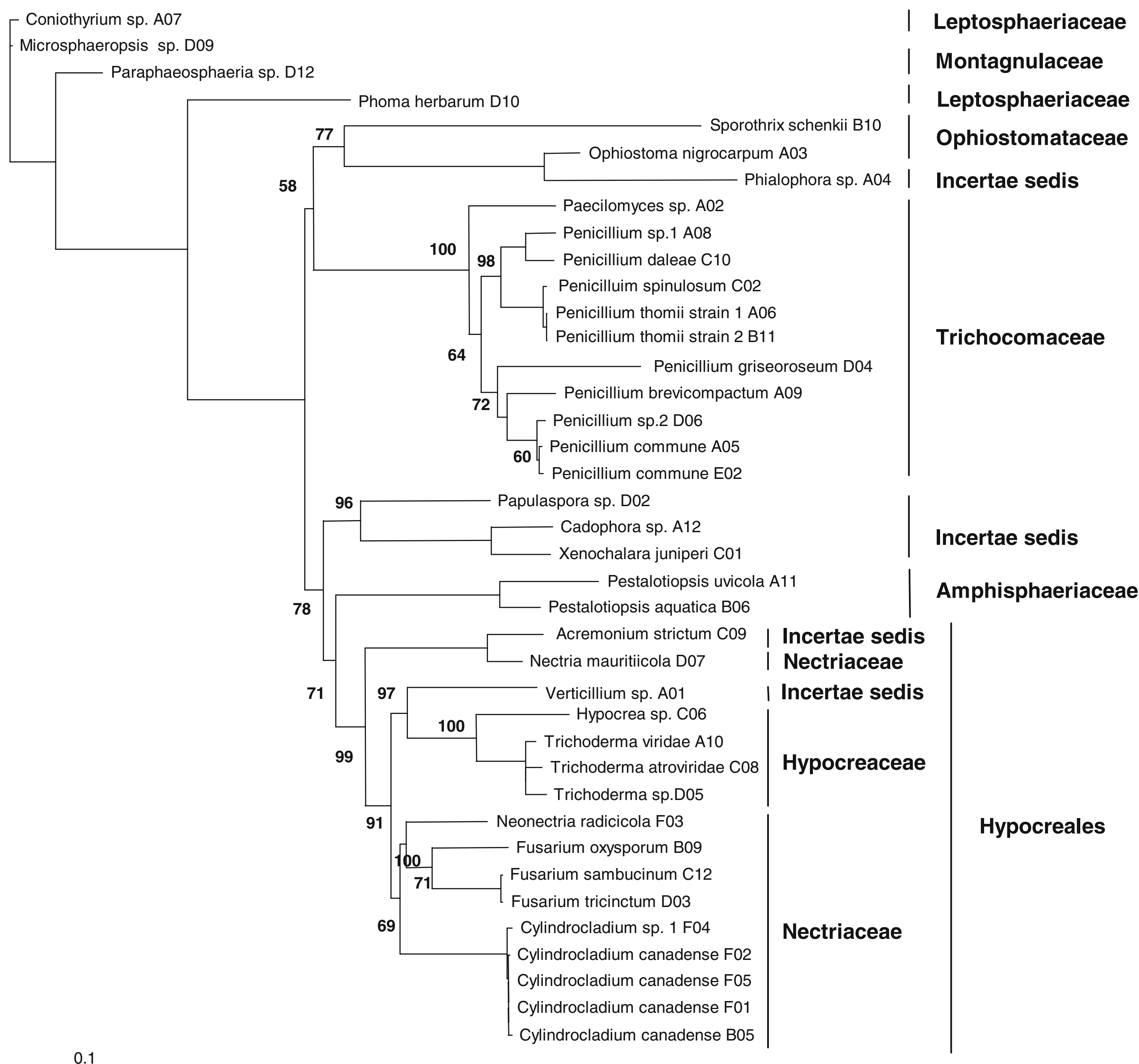

Figure 1. Unrooted neighbor-joining tree showing similarities between ITS gene sequences from 39 Ascomycota isolates from rhizosphere soil of *P. mariana* plants. Bootstrapping values greater than 60% calculated from 1000 replicates are given above the *branches*. *Scale bar* indicates the number of substitutions per site. Identification numbers of DNA samples are given after species names (see Table 2).

Relation Between Fungal Communities. CCA revealed substantial alteration ($P<0.05$) in fungal community assemblages with regard to plant health and yielded a principal axis direction that regrouped fungal taxa associated with diseased plant rhizosphere soil, whereas the opposite axis direction was associated with rhizospheres of healthy plants. The corresponding CA ordination is presented in Fig. 4. Four main fungal communities can be recognized in relation to: (1) healthy MP, B + 0, and B + 1 plant rhizospheres, (2) diseased MP, B + 0 and B + 1 plant rhizospheres, (3) healthy B + 2 plant rhizospheres, and (4) diseased B + 2 plant rhizospheres. The MP, B + 0, and B + 1 healthy plant rhizosphere fungal communities were dominated by isolates of *Cladosporium cladosporoides*, *Chaetomium globosum*, *Hypocrea* sp., *Microphoma* sp., *Microsphaeropsis* sp., *N. mauritiicola*, *Oidiodendron tenuissimum*, *Paecilomyces* sp., *P. thomii*, *Scopulariopsis* sp., and *Verticillium bulbillosum*. The MP, B + 0, and B + 1 diseased plant rhizospheres were associated with *Alternaria alternata*, *Aureobasidium pullulans*, *Coniothyrium* sp., *Fusarium* spp. (*F. oxysporum*, *F. avenaceum*, *F. sambucinum*, *F. tricinctum*), *Papulospora*, *Paraphaeos-*

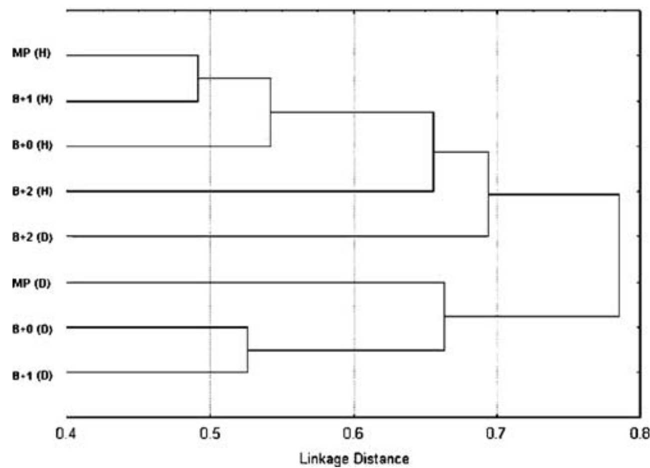

Figure 2. Unweighted arithmetic average clustering (UPGMA) between black spruce plant production stage and associated fungi isolated in the rhizosphere. The distance used is the asymmetrical quantitative coefficient. *H* healthy seedling, *D* diseased seedling.

phaeria sp., *Phialocephala* sp. and *T. viride*. The healthy B + 2 rhizosphere soil was associated with *A. strictum*, *Actinomucor*/*Umbeliopsis* sp., *Gliocladium* sp., *Laccaria bicolor*, *Penicillium* spp. (*P. brevicompactum* and *P. commune*), *Sporothrix schenkii*, *Thelephora terrestris*, and *T. viride*. Finally, the diseased B + 2 plant rhizospheres were mainly associated with *Agaricus* sp., *A. niger*, an unidentified anamorphic Basidiomycete, *C. canadense*, *Exserohilum novae-zelandiae*, *Geniculosporium* sp., *M. radicis atrovirens* (MRA-I and MRA-2), *Ophiostoma nigrocarpum*, *P. daleae*, *Pestalotiopsis* spp. (*Pestalotiopsis uvicola* and *Pestalotiopsis aquatica*), *Ramularia* sp., *Rhizoctonia* sp., *Septonema* sp., and *Xenochalara juniperi*.

The persistence of fungal taxa forming these communities varied widely. Indeed, some of the fungal taxa were isolated from a single production stage, whereas others were found in all production stages. In healthy plant rhizospheres, *A. alternata*, *Cladosporium cladosporioides*, *C. canadense*, *Paecilomyces* sp., *P. thomii*, and *Hypocrea* sp.

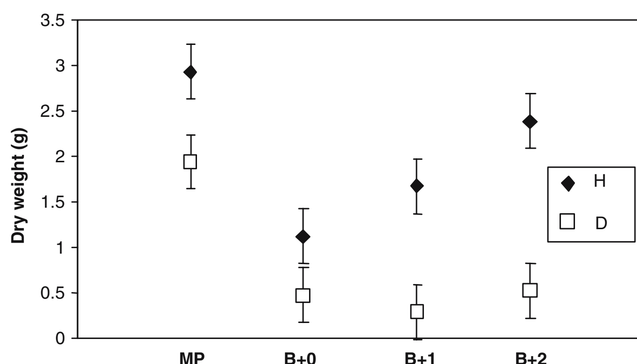

Figure 3. Relationship between root dry weight of healthy (*H*) and diseased (*D*) *P. mariana* containerized plants. *Bars* are the standard error of the mean.

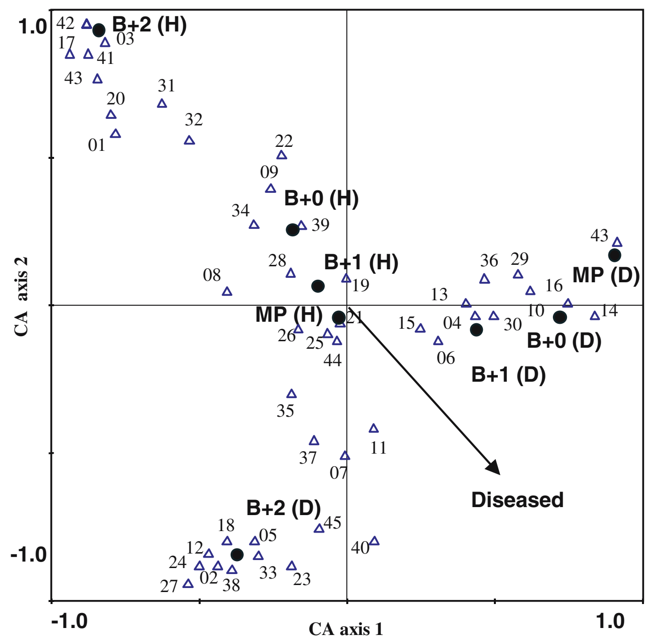

Figure 4. Correspondance analysis of the relationship between black spruce production chronosequences, health status, and fungal communities in rhizosphere soil. *H* healthy, *D* diseased plants. *Circles* nursery production stage, *triangles* fungal taxon. Fungal taxa numbers refer to identifications given in Table 1 and to ITS sequences closest matches in Table 2.

taxa showed the highest persistence index (Pi > 0.7) (Table 1), whereas in diseased plant rhizospheres, the most persistent taxon was *C. canadense* (Pi > 0.9), followed by *F. sambucinum*, *Fusarium trincinctum*, and *Hypocrea* sp. (Pi > 0.7).

UPGMA clustering demonstrated relationships in the occurrence of the 45 fungal taxa isolated from the rhizosphere soil (Fig. 5). *Cylindrocladium canadense* clustered with *Agaricus* sp. and MRA-I (linkage distance = 0.546), whereas *F. oxysporum* clustered with *A. strictum*, *E. novae-zelandiae*, *Penicillium* spp. (*P. brevicompactum*, *P. commune*, and *P. daleae*) and *S. schenkii* (linkage distance = 0.578). *Pestalotiopsis* species formed a cluster with *Geniculosporium* sp., *Gliocladium* sp., *C. globosum*, *F. tricinctum*, and *Parasphaeosphaeria* sp. and with an unidentified anamorphic Basidiomycete (linkage distance = 0.816). Others, mainly numerous rare isolates, were distributed into three separate clusters having linkage distances >0.9.

Discussion

This study provides the first comprehensive analysis of temporal changes in fungal communities in the rhizosphere of *P. mariana* plants over all nursery production stages. It was found that both plant health and production stages had a strong influence on fungal community structure. This may suggest that some fungal isolates found strongly associated with healthy plants

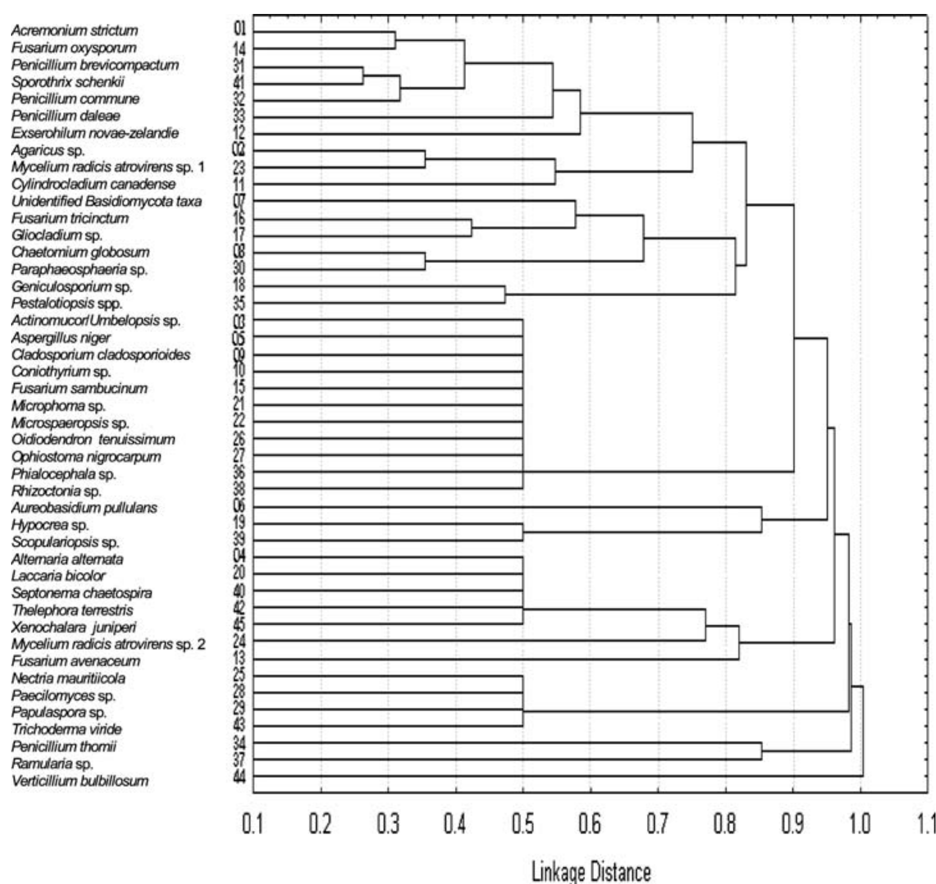

Figure 5. Unweighted arithmetic average clustering (UPGMA) between individual fungal taxa detected in rhizosphere soil of *P. mariana* plants. The distance used is Fager's community coefficient. *Numbers* after fungal species correspond to isolate numbers in Table 1, and fungal taxa closest matches are given in Table 2.

have the potential to compete with root-rot pathogens in the spruce rhizosphere and are potential biocontrol candidates. However, it is also possible that these organisms were simply less competitive in diseased plant rhizospheres.

One important finding shown by our data is the dominance of the root pathogen *C. canadense* in the rhizosphere of diseased *P. mariana* plants. *Cylindrocladium canadense* is a mitosporic fungus belonging to the Nectriaceae family (Fig. 1) with a possible teleomorphic stage in *Calonectria* [13, 29]. To the best of our knowledge, the present work is the first ecological report of *C. canadense* and its occurrence throughout nursery production chronosequences. Differences in biology are found when compared to *C. floridanum*, *Cylindrocladium scoparium*, and *Cylindrocladium crotalariae* data from conifer nurseries in the USA [11, 22]. Although *C. canadense* occurred predominantly in the rhizosphere of diseased B + 2 plants (the last production stage before outplanting), the fungus was found in all production stages and in both healthy and diseased (H/D ratio = 1:1.85) plants (Table 1). Indeed, it is obvious from our results that this fungus could jeopardize a safe nursery production. This raises the question of the possible impact of symptom-free plant infections on reforestation success, considering that pre-infected, asymptomatic black spruce seedlings can develop root-rot symptoms after transplantation, contributing to an increase in mortality of up to 25% [23].

In addition to *C. canadense*, several important sister phytopathogenic Hypocreales fungi were also frequently isolated in association with diseased plants. These are *N. radicicola* [anamorph: *C. destructans* (Zinssm.) Scholten], *N. mauritiicola* [anamorph: *R. hibisci* (Pat.) Seifert], and *Giberella* spp. (anamorph: *Fusarium* spp.) (Fig. 1, Table 1). Our results also showed the occurrence of a complex *Fusarium* clade represented by *F. avenaceum*, *F. oxysporum*, *F. sambucinum* and *F. tricinctum*. The *Fusarium* consortium as a whole was more abundant than *C. canadense* in the rhizosphere of diseased MP, as well as in the young B + 0 and B + 1 plants (Fig. 4). This suggests that the rhizosphere of younger plants may represent an excellent niche for colonization by fast-growing *Fusarium* species. Therefore, *Fusarium* taxa could very likely be a significant factor promoting the development of root-rot and damping-off symptoms preceding *Cylindrocladium* infection in these growth stages. *Fusarium*-associated root-rot has been previously documented in conifer seedlings grown in forest nurseries [2, 4, 6, 26] but has never been related to a specific *P. mariana* production stage in containers, nor to an associated fungal community. The third largest group of potentially deleterious species in our study belongs to the genus *Pestalotiopsis* (*P. aquatica* and *P. uvicola*) (Fig. 1).

This group changes in abundance from common in young healthy MP, B + 0, and B + 1 plants to exclusively present in diseased B + 2 plants (Fig. 4). In this study, the ITS sequence similarity searches in *Pestalotiopsis* spp. (*P. aquatica* and *P. uvicola*, 95 and 97%, respectively) did not allow identification to the species level without any doubt. Previously, *Pestalotiopsis funerea* has been reported as a seed-born species frequently found on various pine hosts in eastern Canada [54]. Further taxonomical and ecological studies will therefore be needed to interpret the significance of this result.

In this study, the main fungal inhabitants of the rhizosphere of diseased plants (Table 1) were *Cylindrocladium* and *Fusarium* (Taxon contribution index, Tc > 0.2), followed by *Alternaria*, *Aureobasidium*, an unidentified Basidiomycota taxa, *Coniothyrium*, *Hypocrea*, MRA I, *Pestalotiopsis*, and *Xenochalara* (Tc = 0.1 to 0.2). Several of the fungal species isolated here are known to cause losses of conifer seedlings during germination, early emergence, and growth [35]. In our study, the diseased MP, B + 0, B + 1, and B + 2 plants had 27, 32, 40, and 80% lower root biomass, respectively, compared with the equivalent healthy plants (Fig. 3). The results indicated that a decreased root biomass in diseased plants was associated with an increased fungal taxa richness and abundance in several treatments, as shown in Table 1. This relation may be linked to increased root decomposition, leading to higher carbon availability in the soil to support fungal growth [1, 21]. Our results also support findings that the major pathogenic community (*C. canadense*, *Fusarium* spp., and *Pestalotiopsis* spp.), as well as the less abundant community (*A. alternata*, *Paraphaeosphaeria* sp., and *Phoma herbarum*), may have a seed-born origin. In fact, many of these taxa were isolated from the rhizosphere of black spruce MPs produced from seed under greenhouse conditions, and still persisted in later production growth stages. The postgermination spread of the pathogens throughout the successive production stages may occur from disease loci originating from a seed-born inoculum [46], as reported on Norway spruce in Europe [33].

Overall, ascomycetous fungi showed the highest biodiversity with 39 different isolated taxa (Table 1). In those fungi, CA ordination revealed that many taxa that were mainly associated with healthy *P. mariana* plants were also related to fungal species known to have biocontrol properties (Fig. 4). The most important species known for their biocontrol properties belong to genera such as *Chaetomium*, *Gliocladium*, *Penicillium*, *Paecilomyces*, *Trichoderma* (*Hypocrea*), and *Sporothrix* [3, 38, 39]. Our results showed a clear ecological relationship between pathogenic species and potential biocontrol taxa (Fig. 5): *Cylindrocladium canadense* and *F. oxysporum* were closely associated with *Penicillium* spp. (*P. brevicompactum*, *P. commune*, and *P. daleae*), whereas *Pestalotiopsis* and *Paraphaeospheria* (pathogens) formed a cluster with *Gliocladium* sp and *C. globosum*. Moreover, we observed that other taxa, such as *Hypocrea* sp., anamorphic *Trichoderma* isolates (*T. atroviridae* and *T. viride*), *Verticillium* sp., *Paecilomyces* sp., and *Penicillium* (*P. daleae*, *P. griseoroseum*, and *P. spinulosum*), formed the largest clade among known biocontrol generalists [15, 30, 55]. However, it should be noted that, under favorable environmental conditions, some of these taxa could also act as weak, secondary seed-borne pathogens [47].

As previously observed [19], members of the Basidiomycota were abundant colonizers of the mature B + 2 plants grown outdoors. Indeed, *L. bicolor* and *T. terrestris* dominated in B + 2, although the latter was also found in B + 1. The two aforementioned species are known to form ectomycorrhizal associations and were mostly associated with healthy seedlings grown outdoors. This symbiotic association has been shown to protect host roots against root pathogens, including containerized *Picea glauca* and *Picea engelmanii* seedlings in western Canada [31]. Some isolates of *M. radicis atrovirens* are commonly found as inhabitants of the rhizosphere of *P. mariana* and were shown to be associated with the ectomycorrhizal mantle [45]. Here, *M. radicis atrovirens* isolates were exclusively found in diseased plants. Previously, they were shown to occur in up to 70% of nonmycorrhizal roots and were reported to have necrotrophic abilities in the presence of *C. destructans* in the rhizosphere of *Picea abies* [24].

This study showed the occurrence of marked contrast between specific fungal taxa assemblages associated with both production chronosequences and plant health that could affect (a) the effectiveness of the *P. mariana* clonal production system and, plausibly, (b) the survival of black spruce transplants *in situ*. Distance analyses based on ITS gene sequences showed that the ascomycetous taxa belong to seven large phylogenetic groups (Fig. 1). In addition, Basidiomycota were represented with four distinct groups and Zygomycota with one distinct group. Each of these groups showed differences in ecological preference to the health-production stage combination and might be seen as "ecological units" containing communities of species having similar interrelationships and mutually influencing each other. For instance, a recently described *C. canadense* and *Fusarium* spp. showed, among pathogenic associates, the highest persistence (Pi > 0.7) and a considerable environmental range of adaptability colonizing all production stages. Results also suggest that the best biocontrol candidates in this environment would be *Penicillium* taxa due to their uniform level of persistence, similar to the level measured for the main pathogens. In addition to being known antibiotic producers, *Penicillium* spp. occupied the same microniches as pathogens, showing competitiveness as a persistent population. Toxic metabolite production is involved in fungal competitive relationships in the soil biocenosis [52]. *Penicillium damascenum* was

shown to protect *Picea glehnii* seeds and reduce infections caused by *Pythium vexans* [30]. Antagonism against *Rhizoctonia solani* by various *Penicillium* spp., including *P. brevicompactum*, was also reported [37]. Biocontrol of fusarium wilt of tomatoes by *Penicillium oxalicum* was recently described [41]. We suggest that a high persistence index of potential antagonistic taxa, such as the *Penicillium* group, may indicate a beneficial mycobiota effect on plant health. Plant and soil types and soil management regime were previously shown to affect the soil microbial diversity and soil's disease suppressiveness [20]. Our results also support the hypothesis that taxon fluctuations may be driven by fungal interactions that may also affect the plant's health and susceptibility to disease. Further research is, however, needed to fully evaluate the potential of such fungal associations to control root rot pathogens under conditions prevailing in modern conifer nursery production.

This study provides new data on fungal species associated with nursery trees in current reforestation efforts in boreal forests. It also supports the importance of monitoring, an important asset against exotic pathogens within an international trade of coniferous plants.

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References

1. Anderson, IC, Campbell, CD, Prosser, JI (2003) Diversity of fungi in organic soils under a moorland—Scots pine (*Pinus sylvestris* L.) gradient. Environ Microbiol 5: 1121–1132
2. Asigbu, FO, Kacprzak, M, Daniel, G, Johansson, M, Stenlid, J, Manka, M (1999) Biochemical interactions of conifer seedling roots with *Fusarium* spp. Can J Microbiol 45: 923–935
3. Avis, TJ, Hamelin, RC, Bélanger, RR (2001) Approaches to molecular characterization of fungal biocontrol agents: some case studies. Can J Plant Pathol 23: 8–12
4. Axelrood, PE, Neumann, M, Trotter, D, Radley, R, Shrimpton, G, Dennis, J (1995) Seedborne *Fusarium* on Douglas-fir: pathogenicity and seed stratification method to decrease *Fusarium* contamination. New For 9: 35–51
5. Bais, HP, Park, SW, Weir, TL, Callaway, RM, Vivanco, MJ (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9: 26–32
6. Bloomberg, WJ (1981) Disease caused by Fusarium in forest nurseries. In: Nelson, PE, Toussoun, TA, Cook, RJ (Eds.) Fusarium: Disease, Biology and Taxonomy, Pennsylvania State University Press, University Park, pp 178–187
7. Canadian Council of Forest Ministers (2005) Compendium of Canadian Forestry Statistics, http://www.nfdp.ccfm.org
8. Capieau, K (2004) Biological control of grey mould in Swedish forest nurseries. Ph.D. Thesis, SLU, Acta Universitatis agriculturae Sueciae. Silvestria Vol. 325
9. Casgrain, P, Legendre, P (2001) The R package for multivariate and spatial analysis. Département de sciences biologiques, Université de Montréal, Québec
10. Colas, F, Tousignant, D, Mercier S (2003) Recent research in tree reproduction in Quebec. XII World Forestry Congress, Quebec-Canada. MRNFP-Research Note, http://www.mrnf.gouv.qc.ca/english/publications/forest/publications/Colas-A.pdf
11. Cordell, CE, Anderson, RL, Hoffard, WH, Landis, TD, Smith, RS Jr, Toko, HV (1989) *Cylindrocladium* USDA Forest Service, Diseases: Forest Nursery Pests No. 680: Agriculture Handbook. United States Government Printing, Washington DC
12. Crous, PW (2005) Impact of molecular phylogenetics on the taxonomy and diagnostics of fungi. EPPO Bull 35: 47–51
13. Crous, P, Wingfield, MJ (1994) A monograph of *Cylindrocladium*, including anamorph of *Calonectria*. Mycotaxon 51: 341–435
14. Duchesne, LC, Peterson, RL, Ellis, BE (1987) Interaction between the ectomycorrhizal fungus *Paxillus involutus* and *Pinus resinosa* induces resistance of *Fusarium oxysporum*. Can J Bot 66: 558–562
15. Dumas, MT, Strunz, GM, Boyonoski, NW, Finlay, HJ (1996) *In vitro* interactions between *Cylindrocladium floridanum* and species of *Trichoderma*. Can J Plant Pathol 18: 325–329
16. Dumas, MT, Greifenhagen, S, Halicki-Hayden, G, Meyer, TR (1998) Effect of seedbed steaming on *Cylindrocladium floridanum* soil microbes and the development of white pine seedlings. Phytoprotection 79: 35–43
17. Dumroese, RK, James, RL, Wemmy, D (2000) An assessment of *Cylindrocarpon* on container western white pine seedlings after outplanting. West J Appl For 15: 5–7
18. Enebek, SA, Wei, G, Kloepper, JW (1998) Effects of plant growth-promoting *Rhizobacteria* on loblolly and slach pine seedlings. For Sci 44: 139–144
19. Filion, M, Hamelin, RC, Bernier, L, St-Arnaud, M (2004) Molecular profiling of rhizosphere microbial communities associated with healthy and diseased black spruce (*Picea mariana*) seedlings grown in a nursery. Appl Environ Microbiol 70: 3541–3551
20. Garbeva, P, van Veen, JA, van Elsas, JD (2004) Microbial diversity in soil: selection of microbial populations by plant soil type and implications for disease suppressiveness. Ann Rev Phytopathol 42: 243–270
21. Gardes, M, Bruns, TD (1993) ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhiza and rusts. Mol Ecol 2: 113–118
22. Hadi, S (1974) Epidemiology and control of Cylindrocladium stem canker and root rot of conifers in nurseries. Ph.D. Thesis, University of Wisconsin
23. Hamelin, RC, Bérubé, P, Gignac, M, Bourassa, M (1996) Identification of root rot fungi in nursery seedlings by nested multiplex PCR. Appl Environ Microbiol 62: 4026–4031
24. Holdenrieder, O, Sieber, TN (1992) Fungal associations of serially washed healthy non-mycorrhizal roots of *Picea abies*. Mycol Res 96: 151–156
25. Innes, L (1993) Moyen de lutte contre la pourriture racinaire dans les pépinières forestières. MRN/RNC, Insectes et Maladies des arbres, St-Foy, Québec
26. James, RL, Dumroese, RK, Wenny, DL (1995) *Fusarium proliferatum* is common, aggressive pathogen of container-grow conifer seedlings. Phytopathology 85: 1129
27. Jeng, RS, Dumas, M, Liu, FH, Hubbes, M (1997) DNA analysis of *Cylindrocladium floridanum* isolates from selected forest nurseries. Mycol Res 101: 285–291

28. Juzwick, J, Honhart, C, Choung, N (1988) *Cylindrocladium* root rot in Ontario bare-root nurseries: estimate of spruce seedling losses. Can J For Res 18: 1493–1496
29. Kang, JC, Crous, PW, Schoch, CL (2001) Species concepts in the *Cylindrocladium floridanum* and *Cy. spathiphylli* complexes (Hypocreaceae) based on multi-allelic sequence data, sexual compatibility and morphology. Syst Appl Microbiol 24: 206–217
30. Keiko, Y, Yukiharu, F, Yasuyuki, H, Tadashi, Y, Satoshi, T (2001) *Penicillium* fungi from *Picea glehnii* seeds protect the seedlings from damping-off. New Phytol 152: 521–531
31. Kernaghan, G, Sigler, L, Khasa, D (2003) Mycorrhizal and root edophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA sequence analysis. Microb Ecol 45: 128–136
32. Kope, HH, Alexrood, PE, Sutherland, J, Reddy, MS (1996) Prevalence and incidence of the root-inhabiting fungi *Fusarium*, *Cylindrocarpon*, and *Pythium*, on container grown douglas-fir and spruce seedlings in British Columbia. New For 12: 55–67
33. Lilja, A, Poteri, M, Vuorinen, M, Kurkela, T, Hantula, J (2005) Cultural and PCR-based identification of the two most common fungi from canker container-grown Norway spruce seedlings. Can J For Res 35: 432–439
34. Mazzola, M (2004) Assessment and management of soil microbial community structure for disease suppression. Ann Rev Phytopathol 42: 35–59
35. Meyer, TR, Irvine, M, Harvey, EM, McDonough, T (1993) Integrated Pest Management in Canadian Forest Nurseries—Current Perspectives and Future Opportunities. USDA Forest Service, Rocky Mountain Forest Range Experimental Station, General Report, RM-243. USDA Forest Service, Washington DC
36. Morin, C, Samson, J, Dessureault, M (1999) Protection of black spruce seedlings against *Cylindrocladium* root rot with ectomycorrhizal fungi. Can J Bot 77: 169–174
37. Nicoletti, R, Stefano, MD, Stefano, SD, Trincone, A, Marziano, F (2004) Antagonism against *Rhizoctonia solani* and fungitoxic metabolite production by some *Penicillium* isolates. Mycopathologia 158: 465–474
38. Paulitz, TC, Bélanger, RR (2001) Biological control in greenhouse systems. Ann Rev Phytopathol 39: 103–133
39. Punja, ZK, Utkhede, RS (2003) Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol 21: 400–407
40. Rosenzweig, ML (1995) Species Diversity in Space and Time. Cambridge University Press,
41. Sabuquillo, P, Cal, AD, Melgarejo, P (2006) Biocontrol of tomato wilt by *Penicillium oxalicum* formulations in different crop conditions. Biol Control 37: 256–265
42. Saunders, JE, Juzwik, J, Hutchinson, R (1992) Outplanting survival of *Cylindrocladium* root rot affected black spruce seedlings. Can J For Res 2: 1204–1207
43. Sneath, PH, Sokal, RR (1973) Numerical Taxonomy: The Principles and Practice of Numerical Classification. WH Freeman, San Francisco
44. St-Arnaud, M, Vujanovic, V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. In: Hamel, C, Plenchette, C (Eds.) Mycorrhizae in Crop Production: Applying Knowledge, Haworth Press, Binghampton. In press
45. Summerbell, RC (1989) Microfungi associated with mycorrhizal mantle and adjacent microhabitats within the rhizosphere of black spruce. Can J Bot 67: 1085–1095
46. Sutherland, JR, Lock, W, Farris, SH (1981) Sirococcus blight: a seed born disease of container grown seedlings in coastal British Columbia forest nurseries. Can J Bot 59: 559–562
47. Sutherland, JR, Shrimpton, GM, Sturrock, RN (1989) Diseases and Insects in British Columbia Forest Seedling Nurseries. FRDA Report, ISSN 0835-0752. FRDA, Victoria
48. Swofford, DL (2000) PAUP*. Phylogenetic Analysis Using Parsimony (and other methods), Version 4,0b10. Sinauer Associates, Sunderland
49. ter Braak, CJF, Šmilauer, P (2002) CANOCO. Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination, (version 4.5). Microcomputer Power, Ithaca
50. Thompson, JD, Gibson, TJ, Plewniak, F, Jeanmougin, F, Higgins, DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24: 4876–4882
51. Tousignant, D, Périnet, P, Rioux, M (1996) Black spruce cutting propagation at the Pépinière de Saint-Modeste. Gouvernement du Québec, Ministère des Ressources naturelles, Quebec
52. Vey, A, Hoagland, R, Butt, T (2001) Toxic metabolites of fungal biocontrol agents. In: Butt, T, Jackson, C, Magan, N (Eds.) Fungi as Biocontrol Agents: Progress, Problems and Potential, CAB International, Wallingford, pp 311–346
53. Vujanovic, V, St-Arnaud, M (2005) Root rot of black spruce caused by *Cylindrocladium canadense* in eastern North America. Plant Dis 89: 204
54. Vujanovic, V, St-Arnaud, M, Neumann, P (2000) Susceptibility of cones and seeds to fungal infection in a pine (*Pinus* spp.) collection. For Pathol 30: 305–320
55. Whipps, JM (2001) Microbial interactions and biocontrol in rhizosphere. J Exp Bot 52: 487–511
56. White, TJ, Bruns, T, Lee, S, Taylor, JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, MA, Gelfand, DH, Sninsky, JJ, White, TJ (Eds.) PCR Protocols: Guide to Methods and Applications, Academic, New York, pp 315–322
57. Yergeau, E, Filion, M, Vujanovic, V, St-Arnaud, M (2005) A PCR-denaturing gradient gel electrophoresis (DGGE) approach to assess *Fusarium* diversity in asparagus. J Microbiol Methods 60: 143–154
58. Yung, C-H, Crowley, DE (2000) Rhizosphere microbial community structures in relation to root location and plant iron nutritional status. Appl Environ Microbiol 66: 345–351