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Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry

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Abstract Recent improvements in the management of oil sand tailings used by the Canadian oil sand industry have resulted in the production of composite tailing sands (CT): a new challenging material for reclamation work. Jack pine (*Pinus banksiana* Lamb.), hybrid poplar (*Populus deltoides* Bartr. ex Marsh. ×*Populus nigra* L.) and red clover (*Trifolium pratense* L.) plants were used in an 8-week greenhouse bioassay to evaluate the mycorrhizal inoculum potential of CT. This inoculum potential was compared with that of three other reclamation materials [common tailing sands (TS), deep overburden (OB) and muskeg peat (MK)], and with three sites reclaimed in 1982 (R82), 1988 (R88) and 1999 (R99). CT was devoid of active mycorrhizal propagules while all other materials showed some level of inoculum potential. Arbuscular mycorrhizal fungi were observed on roots of clover or poplar grown in TS, OB, and all substrates containing peat (MK, R82, R88 and R99). Pine roots were also colonized by vesicle-forming hyphae of an unidentified fine endophyte and by dark septate fungi. Ectomycorrhizas (ECM) were observed on pine and poplar grown in OB, MK, and in soils from the two older reclaimed sites (R82 and R88). Using morpho- and molecular typing, six ECM fungi were identified to the genus or species level: *Laccaria* sp., *Thelephora americana*, *Wilcoxina* sp. (E-strain), *Tuber* sp. (I-type), a Sebacinoid, and a Pezizales species. *Laccaria* sp. and *Wilcoxina* sp. were the most frequently observed ECM species.

Keywords Inoculum potential · Oil sands · Tailing sands · Revegetation · ITS-RFLP

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Introduction

In northeastern Alberta (Canada), surface mining of oil sands creates large areas of disturbed land that require reclamation (Fung and Macyk 2000). In 1998, Syncrude Canada alone produced 75 million m^3 tailings (Li and Fung 1998), including common tailing sands (TS) and composite tailings (CT). Opencast extraction of oil sands implies removing the organic layer [muskeg peat (MK)] and the deep geological overburden (OB). These spoils are retained and used to amend stabilized tailings in order to reconstruct the soil before revegetation (Danielson et al. 1983a; Danielson and Visser 1989; Li and Fung 1998; Fung and Macyk 2000).

According to soil remediation standards, a reclaimed growing medium should support a healthy plant community that will evolve toward an ecosystem comparable to that of neighboring natural areas. Revegetation of TS is now routinely conducted at an operational scale (Fung and Macyk 2000), whereas the reclamation of CT is still in the research phase (Khasa et al. 2002). Common TS are deposits of tailing slurry containing significant quantity of water, residual bitumen, and coarse-grained and fine-grained materials. CT are composed of the finest material fraction of these slurries, which barely consolidate and to which $CaSO_4$ is added to enhance compaction and stabilization rates. CT are challenging materials to reclaim because of their inherently low nutrient levels, and high alkalinity [pH(H_2O) >8] and salinity (Fung and Macyk 2000). Furthermore, CT may lack certain components of the soil microbial community essential for plant growth and survival. One such component, mycorrhizal fungi, plays a critical role in ecosystem dynamics and productivity (van der Heijden et al. 1998; Baxter and Dighton 2001; Dahlberg 2001; Jonsson et al. 2001; Leake 2001). In return for photosynthates (Smith and Read 1997), plants, as individuals or as a community, benefit from symbiotic mycorrhizal fungi through enhanced mineral nutrition and water status, and resistance toward pathogens (Kropp and Langlois 1990). Moreover, mycorrhizal fungi buffer host plants against environmental stresses and alter interplant competition (Malajczuk et al. 1994). Therefore,

symbiotic fungi should be considered as an essential component of soil reclamation programs.

The level of occurrence of arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi in highly disturbed mine spoils is low (Danielson et al. 1983b; Abbott and Robson 1991; Bellgard 1993; Malajczuck et al. 1994; Pfleger et al. 1994; Gould et al. 1996). Several authors have reported the presence of mycorrhizal fungi in different materials (TS, MK, OB and sewage sludge) used to reconstruct soil from oil sand tailings (Zak and Parkinson 1982, 1983; Zak et al. 1982; Danielson et al. 1983b, 1984; Danielson and Visser 1989). Thus, amending CT with OB and MK could provide a source of indigenous mycorrhizal fungal propagules. However, the inoculum potential of these materials is reduced during storage and by subsequent dilution with tailing sands (Danielson et al. 1983b; Malajczuck et al. 1994; Pfleger et al. 1994).

Most studies on the diversity and inoculum potential of AM and ECM fungi occurring in tailings from the oil sand industry were carried out in the 1980s (Zak and Parkinson 1982, 1983; Zak et al. 1982; Danielson et al. 1983b, 1984; Danielson and Visser 1989). However, reclamation practices have evolved, producing new types of tailing sands (e.g., CT) and new technologies are available for the identification of mycorrhizal fungi (e.g., molecular tools). Identification of any ECM fungus using morphology alone, is hampered by several drawbacks (Danielson 1984; Gardes et al. 1991; Sakakibara et al. 2002), but the use of molecular techniques provides greater precision for ecological studies. Restriction fragment length polymorphism (RFLP) is one of the simplest techniques, which usually allows identification to the species level using the internal transcribed spacer (ITS) region of rDNA (White et al. 1990; Egger 1995; Egger et al. 1996; Horton and Bruns 2001).

In this context, the present study used a modified bait plant system (Brundrett et al. 1996c) to: (1) evaluate the AM and ECM inoculum potential of CT compared to that of TS, OB and MK and soil from reclaimed sites of different ages; and (2) identify the ECM species present using molecular typing (ITS-RFLP and sequencing).

Materials and methods

Substrates

The substrates used were CT, TS, MK, OB, and undisturbed soil cores taken from sites reclaimed in 1982 (R82), 1988 (R88), and 1999 (R99). The samples were collected in May 2001 on the Syncrude Canada mining area, 30 km north of Fort McMurray (56°39′N/111°130′W, altitude: 369 m). Tailings (CT and TS) were collected from landfills. Soil cores of OB were randomly collected from a pile of geologic material freshly excavated from a depth of 30–40 m, and cores of MK were collected from freshly spread material. Reclaimed sites comprised TS amended with a layer of OB covered with a layer of MK. Fifteen soil cores were collected per site. Soil samples were collected at 10 m intervals along three parallel 40 m transects; transects were 20 m apart. Bulk soil samples were collected at both ends of each transect (six samples per site). Because fungal propagules are concentrated in the top 20 cm of the soil profile (Brundrett et al. 1996b), cores were taken using PVC tubes of 8×20 cm (diameter×height). Soil cores were then stored at 4°C prior to processing. To describe site conditions, plants occurring on reclaimed sites were listed (Table 1) and soil chemical and physical properties were analyzed from bulked samples (Table 2).

Inoculum potential bioassay

To evaluate the AM and ECM inoculum potential of each substrate, soil cores were planted with red clover (*Trifolium pratense* L., provided by BIOVISION Seed Labs Research, Edmonton, Alberta, Canada), hybrid poplar (*Populus deltoides* Bartr. ex Marsh. ×*Populus nigra* L., supplied by CRBF, Université Laval) and jack pine (*Pinus banksiana* Lamb., Syncrude source provided by Alberta tree improvement and seed center, Smoky Lake, Alberta, Canada). Red clover and jack pine seeds were surface sterilized for 2 min in 1% bleach, rinsed six times with distilled water, and sown on a sand:Turface MVP (Profile, Buffalo Grove, Ill.):loamy subsoil mix (1:1:1, v:v:v). The substrate was sterilized (121°C, 45 min) twice with a 24 h interval between each treatment. Seedlings were grown for 3 weeks before transplanting to the cores. The same sterile substrate was used for control treatments to check for contamination by greenhouse mycorrhizal fungi. Hybrid poplar cuttings were inserted directly into the soil cores.

The first bioassay experiment consisted of 64 soil cores with eight replicates per substrate type (CT, TS, OB, MK, R82, R88, R99 and control); each core was planted with one jack pine and one red clover seedling. The second bioassay consisted of 56 soil cores with seven replicates per substrate type; each core was planted with one hybrid poplar cutting and one clover seedling. All plants were watered as required. Seedlings were grown for 8 weeks under a 16 h photoperiod at 27/22°C (day/night) and 32% relative humidity. Plants growing on CT and TS showed nutrient deficiencies and were given four 20 mg applications of ammonium nitrate ($NH_4^+NO_3^-$) during the experiment.

At the end of the experiment, plants were harvested and the root systems washed. Fresh roots were weighed and large root systems sub-sampled (Brundrett et al. 1996c). Root length measurements were obtained by image analysis using WinRhizo 5.0A (Regent Instruments, Québec, Canada). Measurements performed on sub-samples were extrapolated to the whole sample using the weight ratio. Each root system was stored in 50% ethanol at 4°C (Brundrett et al. 1996c) prior to further analyses. Fresh and dry (65°C, 48 h) shoot weights were recorded.

Determination of inoculum potential

Substrate inoculum potentials were defined as the percentage colonization by AM and ECM fungi of each bioassay

Table 1 Planted ([a]) and naturally occurring plant species on sites reclaimed in 1982 (R82), 1988 (R88) and 1999 (R99)

	R82	R88	R99
Trees	*Populus tremuloides* Michx. *Pinus banksiana* Lamb.[a] *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roemer[a]	*Populus tremuloides* Michx. *Populus balsamifera* L. *Pinus banksiana* Lamb.[a]	*Populus tremuloides* Michx.[a] *Picea glauca* (Moench) Voss.[a]
Shrubs	*Cornus stolonifera* Michx. *Fragaria virginia* Duchesne *Rubus idaeus* L. *Shepherdia canadensis* (L.) Nutt.	*Cornus stolonifera* Michx.[a] *Fragaria virginia* Duchesne *Rosa acicularis* Lindl. *Salix* sp.	*Fragaria vesca* L. *Salix* sp.
Forbs and grasses	*Achillea millefolium* L.	*Arnica cordifolia* Hook.	*Agropyron trachycaulum* (Link) Malte ex H.F. Lewis
	Calamagrostis canadensis (Michx.) Beauv.	*Bromus inermis* Leyss.	*Artemisia absinthium* L.
	Corydalis aurea Willd.	*Dactylis glomerata* L.	*Cirsium arvense* (L.) Scop.
	Epilobium angustifolium L.	*Melilotus alba* Medikus	*Corydalis aurea* Willd.
	Medicago sativa L.	*Taraxacum officinale* G.H. Weber ex Wiggers	*Draba nemorosa* L.
	Taraxacum officinale G.H. Weber ex Wiggers	*Vicia americana* Muhl. ex Willd.	*Epilobium angustifolium* L. *Equisetum arvense* L. *Hordeum jubatum* L. *Medicago sativa* L. *Melilotus alba* Medikus *Phleum pratense* L. *Senecio eremophilus* Richards. *Sonchus arvensis* L. *Taraxacum officinale* G.H. Weber ex Wiggers *Trifolium repens* L. *Vicia americana* Muhl. ex Willd.

plant (Brundrett and Abbott 1994) and represented development of active propagules in a specific substrate under greenhouse conditions. Arbuscular mycorrhizal fungi (AMF) within red clover and poplar roots were stained using a slightly modified version of the method described by Vierheilig et al. (1998). Briefly, red clover and poplar roots were cleared for 5 and 20 min, respectively, using 10% KOH at 100°C, rinsed three times with distilled water before staining in a 5% solution of black ink (Sheaffer Skrip Ink) in white vinegar (5% acetic acid). Clover roots were stained for 4 min and poplar roots for 20 min. Root endophytes in jack pine roots were stained using a modified version of the method described by Koske and Gemma (1989). Briefly, roots were cleared for 75 min in 10% KOH

Table 2 Chemical and physical properties of bulked samples of composite tailings (CT), tailing sands (TS), deep overburden (OB), muskeg peat (MK) and reclaimed soils R82, R88 and R99. Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test. *EC* Electrical conductivity

Soil types	S	P	Ca	Mg	Na	K	OrganicC	N	pH_{H2O}[e]	EC[e]	Clay	Silt	Sand
	$cmol^+ kg^{-1}$						%	%		$\mu S\ cm^{-1}$	%	%	%
CT	0.9[b]	0.6[c]	3.6[d]	2.9[d]	0.8[c]	0.3[d]	0.3[d]	0.01[c]	7.8[a]	30[c]	3.8[c]	4.7[b]	91.5[a]
TS	0.7[b]	0.5[c]	4.2[d]	1.5[d]	0.04[c]	0.4[cd]	0.3[d]	0.01[c]	6.9[c]	29[c]	3.3[c]	3[b]	93.7[a]
OB	168.9[a]	0.5[c]	136.3[c]	28.8[bc]	19.6[a]	1.5[b]	1.1[d]	0.04[c]	7.7[a]	876[a]	32.2[a]	24.3[a]	43.5[c]
MK	64.6[b]	5.3[a]	276.7[ab]	133.6[a]	11.7[b]	2.8[a]	28[a]	0.7[a]	5.1[d]	407[b]			
R99	166.7[a]	1.8[b]	332.6[a]	46.9[b]	2.4[c]	1.3[b]	16[b]	0.6[a]	7.3[bc]	758[a]	12[b]	21.2[a]	66.8[b]
R88	3.8[b]	1.3[bc]	107.7[c]	19.7[cd]	0.4[c]	2.4[a]	1.6[d]	0.1[c]	7.4[ab]	98[c]	13[b]	20.5[a]	66.5[b]
R82	22.7[b]	1.4[bc]	241[b]	39.7[bc]	1.4[c]	1.2[bc]	6.7[c]	0.3[b]	7.1[bc]	188[bc]	12[b]	19.5[a]	68.5[b]

[e]EC and pH_{H2O} were recorded from 1:2 (substrate:dH_2O, v:v) slurries for all except MK, where they were recorded from 1:10 slurries (Kalra and Maynard 1992)

at 100°C and rinsed three times with distilled water prior to immersion in hydrogen peroxide (H_2O_2 10%) for 30 min. Roots were acidified in a 1% HCl solution for 10 min and then stained with 0.05% trypan blue. Stained roots were examined under a dissecting microscope, and the percentage colonization was scored using the gridline intersect method (Giovannetti and Mosse 1980; Brundrett et al. 1996c). The presence of ECM fungi was assessed on whole root systems of jack pine and hybrid poplar. Colonization rates of jack pine seedlings were defined as the proportion of mycorrhizal roots relative to the total number of short roots. Because of the limited number of ECM tips and their patchy distribution over the large root systems of hybrid poplar, colonization rates were expressed as the total number of ECM per meter of root.

Morphological description and ITS-RFLP identification of ECM fungi

Both morphological and molecular methods were used to identify ECM root tips. Morphotyping was performed to group mycorrhizas within each soil core using branching patterns, tip shape, color, texture, lustre, emanating hyphae, outer mantle anatomy and cystidia (Goodman et al. 1996; Agerer 1999). For each morphotype in a given soil core, molecular typing was performed on duplicate samples of 3–4 root tips. Total DNA was extracted following the method of Kårén et al. (1999). ITS amplification used primers ITS1 (White et al. 1990) and either the ascomycetes-specific NL6Amum or basidiomycetes-specific NL6Bmum primer (Egger 1995). Amplifications were carried out as follows: 0.1–0 ng DNA were added to a mix containing 20 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.1 mg/ml BSA (Roche, Basel, Switzerland), 0.2 μM each primer (Qiagen-Operon, Alameda, Calif.) and 0.025 U/μl Platinium *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif.). The PCR was performed using a PTS-225 thermocycler (MJ Research, Waltham, Mass.) and the following cycles: initial denaturation at 95°C for 2.5 min followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 55 s, and extension at 72°C for either 45 s (cycles 1–13), 2 min (cycles 14–26), or 3 min (cycles 27–30), and a final extension at 72°C for 10 min. PCR products were examined on a 1% agarose gel with 0.5% Synergel (Diversified Biotech, Boston, Mass.). A second amplification, using 1 μl of the first PCR product, was carried out when the first PCR yielded weak signals. An 8 μl sample of PCR products was digested at 37°C for 60 min using 3 U *Hinf*I, *Alu*I, and *Rsa*I (Roche, Basel, Switzerland). Restriction product lengths were evaluated on a 1% agarose gel with 0.5% Synergel (Diversified Biotech). The primers ITS1 and ITS4 (Operon-Qiagen) were used both to amplify and to sequence the ITS of DNA extracts for which the PCR-RFLP results did not match with any referenced patterns. Sequencing was performed using the BigDye Terminator Sequencing Kit (Applied Biosystems, Foster City, Calif.) and an ABI genotype 3100 automated sequencer (Applied Biosystems).

Statistical analysis

The two bioassays were set up as completely random designs (Steel et al. 1997) according to the linear model $Y_{ij}=\mu+S_i+\varepsilon_{ij}$, where Y_{ij} was the jth observation of the ith soil type treatment, μ the overall mean, S_i the effect of the ith soil type treatment, and ε_{ij} the experimental error. Data were analyzed with a one-way ANOVA, and a Duncan's multiple range test was performed to evaluate pairwise comparisons between the means (Steel et al. 1997). In addition, Pearson correlation coefficients (Steel et al. 1997) were calculated to detect linear relationship between soil properties, mycorrhizal status and plant growth parameters.

Results

Plant growth

Basic plant growth parameters (Table 3) and soil properties were measured to provide complementary information concerning evaluation of site inoculum potentials (Figs. 1, 2, 3). Bearing this in mind, N amendment of CT and TS was considered to have had a negligible effect on the results obtained, as these substrates exhibited the lowest N content

Table 3 Plant growth response on control substrate (CTRL), CT, TS, OB, MK, and reclaimed soils R82, R88 and R99. Means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test

Soil Type	Jack pine			Hybrid poplar			Red clover		
	Shoot: root	Total fresh biomass (g/plant)	Root length (cm)	Shoot: root	Total fresh biomass (g/plant)	Root length (m)	Shoot: root	Total fresh biomass (g/plant)	Root length (m)
CTRL	0.6^{b}	0.5^{a}	138^{a}	1.6	6.4^{b}	67^{a}	0.5^{cd}	13.7^{a}	160^{a}
CT	1^{ab}	0.2^{b}	40^{c}	1.8	10.2^{a}	52^{ab}	0.6^{cd}	0.4^{d}	5^{c}
TS	0.7^{b}	0.3^{b}	91^{b}	1.7	10.7^{a}	65^{a}	0.6^{cd}	0.6^{d}	6^{c}
OB	0.7^{b}	0.2^{b}	46^{c}	1.9	5.2^{b}	32^{b}	0.3^{d}	0.6^{d}	8^{c}
MK	0.8^{ab}	0.5^{a}	130^{a}	2.2	6.2^{b}	38^{ab}	0.6^{c}	2.5^{c}	20^{c}
R99	1.2^{a}	0.1^{b}	31^{c}	1.9	6.2^{b}	39^{ab}	1^{b}	7^{b}	64^{b}
R88	0.9^{ab}	0.2^{b}	55^{c}	1.9	6.2^{b}	43^{ab}	1.3^{a}	9.6^{ab}	63^{b}
R82	1.1^{a}	0.2^{b}	51^{c}	2	7^{b}	39^{ab}	1.1^{b}	6.5^{b}	54^{b}

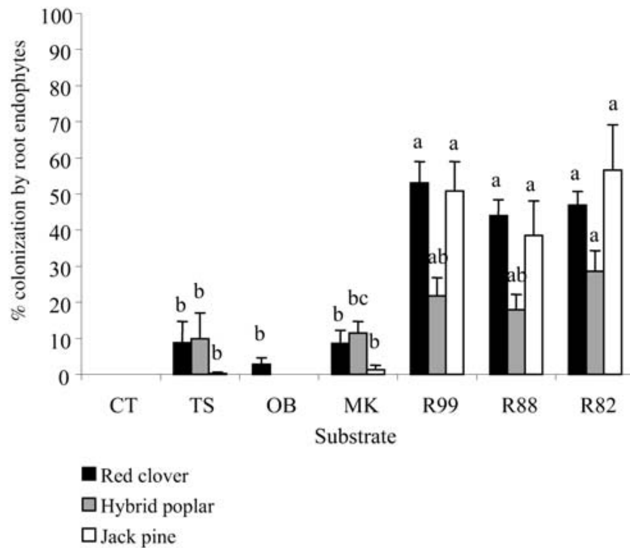

Fig. 1 Percentage colonization by root endophytes observed on plants grown on composite tailings (*CT*), tailing sands (*TS*), deep overburden (*OB*), muskeg peat (*MK*) and soil from sites reclaimed in 1982 (*R82*), 1988 (*R88*) and 1999 (*R99*). Root endophytes of red clover and hybrid poplar are arbuscular mycorrhizal (AMF). Jack pine colonization includes dark septate (DS) fungi and a fine root endophyte. Means within a plant species with the same letter are not significantly different at the 5% level according to Duncan's multiple range test

(0.01%). Clover growth in terms of total fresh biomass, root length and shoot:root ratio was ranked low for plants grown in CT, TS and OB. Clover growth was slightly improved on MK and highest on soil from the reclaimed sites. Biomass, root length and shoot:root ratio of clover seedlings were strongly positively correlated with percentage AMF colonization (Table 4). In contrast, there was no significant correlation with substrate texture, mineral content, EC or pH. Jack pine grown in MK had the highest total fresh biomass and root length. Jack pine shoot dry mass was strongly positively correlated with ECM fungal colonization (data not shown). Total fresh biomass and root length of jack pine were positively correlated with substrate C/N and

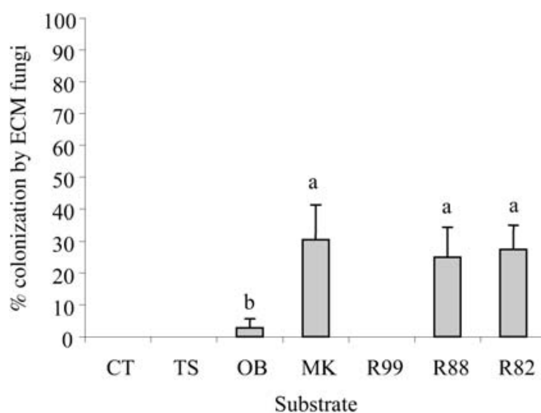

Fig. 2 Percentage colonization by ectomycorrhizal (ECM) fungi on jack pine seedlings grown on CT, TS, OB, MK and reclaimed soils R82, R88, and R99. Means with the same letter are not significantly different at the 5% level according to Duncan's multiple range test

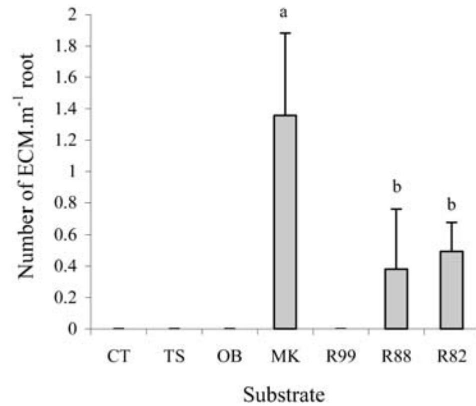

Fig. 3 ECM colonization expressed as the number of mycorrhiza per meter of root on hybrid poplar bioassays grown on CT, TS, OB, MK, and reclaimed soils R82, R88, and R99. Means with the same letter are not significantly different at the 5% level according to Duncan's multiple range test

phosphorus content (Table 4). Poplar plants grown in CT and TS had the highest total fresh biomass, which, together with root length, was positively correlated with sand content and negatively correlated with calcium, potassium, clay and silt content. The shoot:root ratio of poplar showed no significant difference between soil types. Nevertheless, this parameter was positively correlated with ECM fungal colonization, phosphorus, calcium, magnesium and potassium content, and negatively correlated with silt and sand content.

Mycorrhizal status and typing

All control seedlings were non-mycorrhizal and none of the bioassay species formed AM or ECM structures when grown on CT. Typical *Arum*-type (Gallaud 1905; Smith and Read 1997) colonization was observed on roots infected by AMF. Arbuscules were frequently observed in red clover root; however, fewer arbuscules and higher numbers of vesicles were observed on poplar roots (data not shown). Cortical cells of jack pine and poplar roots grown in R82, R88 and R99 were colonized by an unidentified fine endophyte with non-septate hyphae, from which developed small vesicles (20 µm diameter) but no arbuscules. Of those substrates where AM were observed, OB, TS and MK had AMF colonization levels lower than 10% (Fig. 1). Reclaimed sites had the highest AM inoculum potentials of all substrates tested. In addition, dark septate (DS) fungi (based on the presence of microsclerotia) were recorded in jack pine roots grown in R82 and R99.

With regards to ECM fungi, one should consider that molecular typing using PCR-RFLP and sequencing analyses of the ITS region is not an absolute identification and some degrees of uncertainty remain. Overall, six ribotypes were distinguished using ITS-RFLP (Table 5) and sequencing. By comparison with RFLP patterns from the in-house and BCERN database (http://www.pfc.forestry.ca/biodiversity/bcern/), three of the six fungi were identified at least to the genus level. These species were *Thelephora americana*, *Laccaria* sp. and *Tuber* sp. For the

Table 4 Correlation analysis of soil properties, mycorrhizal status and plant growth parameters across all substrate types (n=7). *ECM* Ectomycorrhizal fungi, *AMF* arbuscular mycorrhizal fungi

Soil properties and mycorrhizal status	Jack pine					Hybrid poplar					Red clover			
	Shoot: root	Total fresh biomass[c]	Root length (cm)	ECM (% root tips colonized)	Root endophytes[d]	Shoot: root	Total fresh biomass[c]	Root length (m)	ECM (number of ECM m^{-1} root)	AMF[d]	Shoot: root	Total fresh biomass[c]	Root length (m)	AMF[d]
S[a]	ns[e]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P[a]	ns	0.799**	0.723*	ns	ns	0.887***	ns	ns	0.927***	ns	ns	ns	ns	ns
Ca[a]	ns	ns	ns	ns	ns	0.679*	−0.719*	−0.714*	ns	ns	ns	ns	ns	ns
Mg[a]	ns	0.731*	ns	ns	ns	0.942***	ns	ns	0.892**	ns	ns	ns	ns	ns
Na[a]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
K[a]	ns	ns	ns	0.752*	ns	0.809*	−0.764**	ns	0.763**	ns	ns	ns	ns	ns
C/N	ns	0.808**	0.850**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
EC (μS.cm^{-1})	ns	ns	ns	ns	ns	ns	−0.725*	−0.730*	ns	ns	ns	ns	ns	ns
Clay[b]	ns	ns	ns	ns	ns	ns	−0.818**	−0.805*	ns	ns	ns	ns	ns	ns
Silt[b]	ns	ns	ns	ns	ns	−0.860**	−0.996***	−0.940***	ns	ns	ns	ns	ns	ns
Sand[b]	ns	ns	ns	ns	ns	−0.768*	0.953***	0.918***	ns	ns	ns	ns	ns	ns
ECM	ns	ns	ns	1.000	na[f]	0.916***	ns	ns	1.000	na	na	na	na	na
AM	na	na	na	na	na	ns	ns	ns	na	1.000	0.887***	0.932***	0.980***	1.000
Root endophytes	0.820**	ns	ns	na	1.000	na	na	na	na	na	na	na	na	na

*P<0.01; **P<0.005; ***P<0.001

[a] $cmol^{+}$ kg^{-1}

[b] Percent soil mass

[c] Grams/plant

[d] Percent root length colonized

[e] Not significant

[f] Not applicable

Table 5 Internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) patterns of ECM fungi found in OB, MK and reclaimed soils R82, R88 and R99. Molecular weights in parenthesis correspond to low intensity bands. NL6Bmun appeared able to amplify ascomycetes DNA even though it worked preferentially on basidiomycete DNA. The same RFLP patterns were recovered with NL6Amun or NL6Bmun. These primer pairs specifically amplified fungal DNA as the same RFLP patterns were obtained from pine or poplar mycorrhizas

Fungal species	Host	Soil type	Primer pair	Enzyme	Molecular weight (bp)					
Sebacinoid sp.	Pine	OB, R88	ITS 1/NL6Bmun	*Hinf*I	325	160	105			
				*Alu*I	675	175	110			
				*Rsa*I	470	(340)	260	225		
Thelephora americana	Pine	MK	ITS 1/NL6Bmun	*Hinf*I	(545)	325	265	175	160	(110)
				*Alu*I	(715)	555	190	155	120	
				*Rsa*I	780	(445)	(330)	210		
Laccaria sp.	Pine	MK	ITS 1/NL6Bmun	*Hinf*I	(440)	(360)	290	215	155	135
	Poplar	MK, R82		*Alu*I	435	240	190	(115)		
				*Rsa*I	980	185				
Pezizales sp.	Pine	R82	ITS 1/NL6Bmun	*Hinf*I	365	280	175	110		
	Poplar	R82		*Alu*I	405	195	155	115		
				*Rsa*I	460	215	170	105		
Wilcoxina sp. (E-strain)	Pine	R82, R88	ITS 1/NL6Bmun	*Hinf*I	500	185	170	(135)		
	Poplar	R82	ITS 1/NL6Amun	*Alu*I	400	260	180	(115)		
				RsaI	685	185				
Tuber sp. (I-type)	Pine	R82, R88	ITS 1/NL6Bmun	*Hinf*I	300	270	180	135		
	Poplar	R88	ITS 1/NL6Amun	*Alu*I	450	185	145	125		
				*Rsa*I	330	300	260			

other three, an E-strain fungus (*Wilcoxina* sp.) was identified using the patterns presented by Sakakibara et al. (2002) and the last two species were identified to the family level by sequencing. Of the latter, one was a Sebacinoid species (97% homology with the 18S-28S subunit; accession number: AF440656) and the other a Pezizales species (92% identity with the 18S-28S subunit and 100% homology in the ITS1 region; accession number: AF266709). While molecular identification of *Laccaria* sp., *Tuber* sp., Pezizales and Sebacinoid species agreed with morphotype grouping, mycorrhizas of *T. americana* and *Wilcoxina* sp. were variable in appearance, which lead to occasional inconsistencies between the analyses. CT, TS and R99 had no ECM inoculum potential (Figs. 2, 3). Among soil with ECM fungi, OB had the lowest ECM inoculum potential with the Sebacinoid species lightly colonizing roots (not higher than 14%) of jack pines. MK and reclaimed sites had the highest ECM inoculum potentials of all substrates tested. The ECM status of jack pine was positively correlated with potassium and the ECM status of poplar plants was positively correlated with phosphorus, magnesium and potassium content.

Discussion

Inoculum potential of pure reclamation materials

Symbiotic fungi are not expected to occur in tailing sands, which originate from deep old geologic materials that are further sterilized by the oil extraction processes. This is supported by our results, which showed that CT lacked mycorrhizal propagules. By contrast, TS cores that came from an older landfill site than the CT, showed a low inoculum potential. Zak and Parkinson (1982, 1983) also reported recolonization of TS. In the present study, TS cores were collected 20 m from the edge of R99, and the vicinity to this site with a high AM inoculum potential probably accounted for the colonization of TS by AM propagules. OB materials also originate from deep below the soil surface, and showed a low AM and ECM inoculum potential. The Sebacinoid species found in OB might have originated from topsoil residues remaining in the vehicles used to transport OB to the dump site. The low biomass of clover grown in OB was probably induced by the low AM inoculum potential as these two variables were strongly correlated. Furthermore, plant survival together with the negative correlation between poplar biomass and clay and silt content and EC, indicates that OB was the most unfavorable substrate for plant growth. One should consider that CT and TS were slightly enriched with mineral N that could have enhanced their fertility compared to OB. MK peat, the only pure material collected from the top of the geologic profile, had the highest ECM inoculum potential. This peat originated from mature stands dominated by ectomycotrophic plant species, which probably explains the high ECM and the low AM fungal inoculum potentials observed. Peat is a slow decaying organic substrate and probably benefits ECM fungi rather than AM fungi (Marschner and Dell 1994). The former are able to access organic nitrogen, which is the dominant nitrogen form in peat (Abuzinadah and Read 1989; Vogt et al. 1991; Finlay et al. 1992; Marschner and Dell 1994; Northup et al. 1995).

Inoculum potentials of reclaimed sites

The AM status of clover plants was highly correlated with plant biomass, which was higher on reclaimed sites with high AM inoculum potentials than on MK with a low AM inoculum potential. Reclaimed sites were colonized by pioneer herbaceous species capable of maintaining a high AM inoculum potential. Time since reclamation did not affect clover biomass or AM inoculum potentials. Although

percentage colonization was equivalent between the reclaimed sites, effect of site age could have an impact on the diversity of AMF encountered on these sites. The youngest reclaimed site, R99, was characterized by the highest richness of invading forbs and grasses, while older sites, R82 and R88, had a lower number of forb and grass species. According to van der Heijden et al. (1998), increasing plant biodiversity is concomitant with increasing AMF species richness; therefore, R99 had the potential to support a higher diversity of AMF.

The two older reclaimed sites, R82 and R88, had high inoculum potentials of both ECM and AM fungi, whereas MK and R99 had high inoculum potentials of only one of these fungal types. The site reclaimed in 1999 was the only one revegetated with *Picea glauca* and *Populus tremuloides* and not with *Pinus banksiana*, which may have reduced the number of ECM species compatible with the jack pine bait plants. Nevertheless, these three host species share a number of mycorrhizal species (e.g., *T. Americana*, *Wilcoxina* sp.) that we encountered on the older reclaimed sites. Therefore, difference in host species used for revegetation could not alone account for the difference between sites. In contrast with AM inoculum potentials, ECM inoculum potentials do seem to be dependent on the age of reclaimed sites and the plant community successional stage. The 2-year-old reclaimed site (R99) was colonized by early succession plant species such as *Draba nemorosa* L. (nonmycorrhizal), while the 14- and 20-year-old sites (R88 and R82) were dominated by ectomycotrophic species (Table 1). Therefore, the vegetation succession present on R88 and R82 (age of trees and plant community composition) probably enhanced the presence of ECM fungi, as suggested in a review by Malajczuk et al. (1994). Once ECM host species are able to successfully establish on a site, they produce a “stable” inoculum source through the maintenance and support of a dynamic hyphal network. This network is thought to represent the principal mechanism by which individual mycorrhizal fungi expand in undisturbed natural systems (Brundrett et al. 1996b). In addition to plant community succession, differences between reclaimed sites may also have been generated by three uncontrollable factors: (1) differences in the origin of tailings and their management due to technological developments; (2) variability in application rates (or dilution rates) of the amending materials, OB and MK; and (3) the new revegetation practice used for the R99 site, which comprised planting barley following the amendment to stabilize the substrate before tree planting. The latter practice could favor AM and limit ECM inoculum potential.

Jack pine is one of the main species used for revegetation of reclaimed mine sites in Alberta. However R99, which was reclaimed to support tree revegetation, generated pine seedlings with the lowest biomass production. Three reasons may explain poor growth of jack pine on R99. Firstly, the absence of ECM fungal propagules in this soil would be expected to result in a lower biomass production (Jonsson et al. 2001) than those of trees growing on reclaimed sites having a higher ECM inoculum potential. Furthermore, as no arbuscules were observed on the “fine endophyte” colonizing pine roots, there was no evidence of a mutualistic association between the host and the fungus, which could simply occupy the root without benefiting the plant (Johnson et al. 1997). Even if the association was symbiotic, this endophyte species might not be able to access nutrients from complex organic substrates. Moreover, it could be parasitic during early stages of the association (Johnson et al. 1997). Secondly, Jumpponen and Trappe (1998) showed that DS fungi may be pathogenic. Therefore, DS fungi colonizing jack pine seedlings on R99 could have a neutral or negative effect on pine growth. Thirdly, a soil parameter, such as the significantly higher level of sulfate salt (Table 2), could limit jack pine growth in R99 (Apostol et al. 2002).

ECM fungal identification

Because of imprecision in the ITS-RFLP and sequencing techniques used (Glen et al. 2001), fungal identification was limited to the family or genus level rather than to the species level. In spite of this limitation, of the six fungi ribotyped, four were of taxa commonly found on disturbed sites (e.g., Visser 1995; Danielson 1991; Danielson and Visser 1989; Danielson et al. 1983b, 1994). In the present study, *Laccaria* sp. and *Wilcoxina* sp. were the most frequently recorded. Danielson (1984) reported that *Laccaria proxima* was one of the most common symbionts encountered on disturbed sites in northern Alberta, and this species possibly corresponds to the *Laccaria* sp. observed in this study. *Thelephora americana* is an early-stage fungus on disturbed sites in the boreal forest with a wide host range; however, in the present study it was observed on pine roots but not on poplar.

The Pezizales species found in our study corresponds to a *Pyronemataceae* sp. recorded on plants grown in soil of ancient forests of *Pinus longaeva* located at an elevation of 2,800–3,500 m in the White Mountains of California (Bidartondo et al. 2001). The authors found this fungus to be dominant on substrates of low inoculum potential from harsh environments. These ancient forest soils were alkaline, which suggests that this ECM species is tolerant to high pH. The Sebacinoid species was recovered from OB, the second most alkaline substrate used. Sebacinoid species belong to the *Rhizoctonia* group of the heterobasiomycetes and were traditionally considered as mycoparasitic and/or saprophytic fungi. Selosse et al. (2002) reported two species able to form symbiotic associations with orchids and ECM host plants. One of these species corresponds to the species found on jack pine roots in the present study. To the best of our knowledge this species has never been described as an early-stage disturbance-tolerant mycorrhizal species. Furthermore, the Sebacinoid ecotype recorded in the present study has possibly undergone ecological adaptation (Molina et al. 1992), allowing tolerance to salinity and is therefore of interest for future CT revegetation programs.

The ectendomycorrhizal E-strain fungus and the I-Type fungus [the *Tuber* sp. found in the present study may correspond to the I-type fungi described by Danielson and

Visser (1989)] have already been recorded in this area (Danielson 1982; Danielson et al. 1983b; Danielson and Visser 1989; Visser 1995). Danielson et al. (1983b) found that mixing sand or OB with MK enhanced colonization by E-strain and that the I-Type fungus was the main colonizer on pure MK peat. In the present study, both fungi occurred on plants grown in reclaimed soil from R82 and R88, but were absent in R99.

In conclusion, CT and TS were devoid of active ECM propagules. However, native mycorrhizal fungi occurring in MK enhanced ECM inoculum potentials of TS reclaimed in 1982 and 1988. Nevertheless, all substrates were characterized by low levels of diversity and percentage colonization (<50%) by ECM. This greenhouse bioassay gives a minimum value for inoculum potential of the substrates assessed (Brundrett et al. 1996a). Such an experiment was inherently limited to the pool of fungal species that would colonize seedlings in greenhouse conditions following soil sampling disturbance on the mycelial network. Hence, colonization rates and species diversity from in situ bioassay studies would be expected to be higher. This is supported by preliminary results from an ongoing greenhouse bioassay experiment, using soil from other reclaimed sites in the same area, which indicates the presence of *Cenococcum geophilum*, *Suillus tomentosus* and *Suillus luteus*. Although jack pine is commonly used in the revegetation of reclaimed TS, it requires colonization by ECM fungi in order to survive and grow. On hostile substrates such as saline alkaline CT, controlled inoculation of seedlings in the nursery with selected strains of mycorrhizal fungi could compensate for the low natural inoculum potential and improve survival and growth of tree seedlings after outplanting.

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