NATIVE MYCORRHIZAL FUNGI WITH ASPEN ON SMELTER-IMPACTED SITES IN THE NORTHERN ROCKY MOUNTAINS: OCCURRENCE AND POTENTIAL USE IN RECLAMATION[1]

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Abstract: Aspen (*Populus tremuloides*) is an early successional tree that has naturally colonized large tracts of land near functioning and defunct smelter sites in the northern Rocky Mountains. This is evidenced by extensive aspen stands on the East Ridge of Butte (MT), behind the smelter stack at Anaconda (MT), near the (removed) smelter in Kellogg (ID), and downwind of the Trail, B. C. smelter (Canada). Aspen is able to colonize these areas due to mutualistic relationships with mycorrhizal fungi which increase phosphorus uptake, and ameliorate soil conditions such as low pH, high heavy metals, low fertility, and drought. Mycorrhizal fungi have been used to establish various trees on coal spoils and mine sites in eastern U.S., Ohio, and Utah, but use of aspen has not been examined. Typically a commercial fungal inoculum is added to trees, but inherent problems are: spread of exotic fungi, and use of expensive generic fungi which are not site/host specific. One solution is use of native fungi adapted to a particular tree species, soil type, and climatic region. This research is initiating investigation of the use of native mycorrhizal fungi to enhance aspen establishment on smelter-impacted sites. Thirty species of native fungi are reported on these sites, half of which grew under laboratory conditions. A few grew well enough to warrant further interest as inoculum, including: *Laccaria proxima, Tricholoma flavovirens, Tricholoma populinum, Scleroderma cepa,* and *Paxillus vernalis.* Inoculum is presently being developed for use in greenhouse and field studies with aspen. Potential for heavy metal immobilization and uptake are discussed.

Key words: ectomycorrhizal fungi, *Populus tremuloides*, smelters, heavy metals

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Introduction

Aspen (*Populus tremuloides*) is an early successional tree that has naturally colonized large tracts of land near functioning and defunct smelter sites in the Northern Rocky Mountains (Cripps 1996, 2001). This is evidenced by extensive aspen stands on the East Ridge of Butte, MT (inactive copper smelter), adjacent to the smelter stack at Anaconda, MT (inactive copper smelter), at the (removed) lead smelter at Kellogg, ID, and along the Columbia River downwind of the operating smelter in Trail, B.C., Canada (Cripps, 2001) (Fig. 1). In addition, aspen has been reported to occur naturally at the Sudbury smelter in Ontario (Freedman and Hutchinson, 1980), the copper tailings at Copper cliff, Ontario (Crowder et al., 1982), a phosphate mine dump in near Soda Springs, ID (Williams and Johnston, 1984), and on nickel tailings (Harris and Jurgensen, 1977). All of the smelter areas were once devoid of vegetation due to smelter emissions or tailings dumping (Quinn, 1989); aspen is apparently sustainable in these areas where soils are high in heavy metals. The clonal nature (reproduction by root suckering) of aspen is advantageous, and, once established, it can expand over large areas (Sheppard et al., 2001), as evidenced by the significant aspen stands regenerating south of the Anaconda Superfund site.

Aspen is able to colonize these areas due to mutualistic relationships with particular mycorrhizal fungi which increase phosphorus uptake, and ameliorate soil conditions such as low pH, high heavy metal content, low fertility, and drought. Aspen can associate with over 60 species of fungi, but only a subset occurs on acidic soils (Cripps, 2001; Cripps and Miller, 1993). Mycorrhizal fungi have been shown to be of importance in natural revegetation of anthracite wastes in the eastern U.S. and have been successfully used to establish conifers in these areas (Cordell et al., 2000; Marx 1975, 1980; Marx and Artman, 1979). Aspen is also an early colonizer on coal spoils, and is supported by a select group of mycorrhizal fungi (Schramm, 1966; Shuffstall and Medve, 1979). Some of the same fungal species that occur with aspen on coal spoils also occur with aspen on smelter-impacted sites in the western U.S. (Cripps, 2001).

In management situations, a commercial inoculum, often *Pisolithus tinctorius*, is added to seedlings propagated in greenhouses to enhance establishment and sustainability in the field when outplanted (Cordell et al., 2000; Marx and Artmen, 1979). This has primarily been accomplished with conifers (Malajczak et al., 1994; Perry et al., 1987), however, mycorrhizal fungi have been shown to stimulate the growth of aspen *in vitro* (Cripps 2001; Cripps and

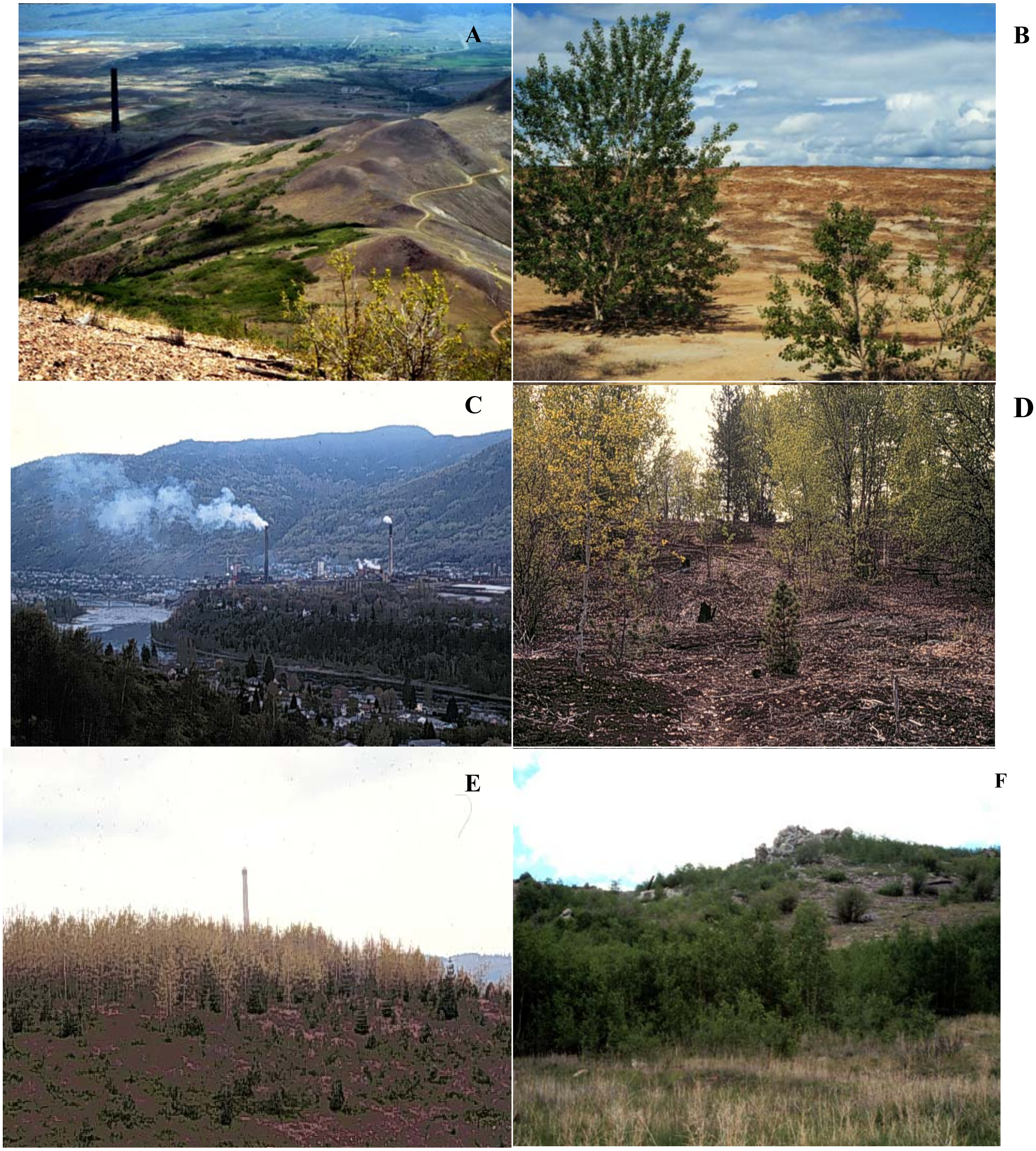

Fig. 1. Smelter-impacted sites where aspen (*Populus tremuloides*) has regenerated naturally in the Northern Rocky Mountains. A. Anaconda copper smelter, MT. B. Opportunity ponds, tailings impoundment, Anaconda, MT. C. Trail, B.C. (Canada) lead smelter. D. *Populus* spp. at Trail, B.C. E. Kellogg, ID lead

Miller, 1995; Lee et al., 1985; Navratil and Rochon, 1981), and have potential to enhance establishment of aspen on disturbed sites. In one study, the biomass of aspen seedlings in a closed sterile system was increased by various native mycorrhizal fungi, including *Amanita muscaria* (400% increase), *Amanita pantherina* (250%), *Paxillus vernalis* (300%), *Tricholoma scalpturatum* (430%), and non native fungi *Cenococcum geophilum* (275%), and *Pisolithus tinctorius* (350%) (Cripps 2001). It is not known if , or how, these results translates to enhanced aspen establishment or sustainability in the field.

Problems inherent in using commercial inocula with aspen in the western U.S. are: use of exotic fungi which prohibit colonization by native fungi, spread of exotic fungi, and use of expensive generic fungi which are not site/host specific. One solution is to use native fungi adapted to a particular tree species, soil type, and climatic region. Various species/strains of fungi need to be tested, since each affects trees differently. Problems with aspen in nursery settings include fast growth and need for fertilization, both of which preclude mycorrhization.

This research is initiating an investigation of use of native mycorrhizal fungi to enhance aspen establishment on smelter-impacted sites. The first goal is to catalogue ectomycorrhizal fungi which occur with aspen on smelter-impacted sites in the Northern Rockies, particularly in the Butte and Anaconda copper smelting area, in part, a superfund site. This paper reports on results of the survey, and the ability of these species to grow in culture, a necessary first step for development of mycorrhizal inoculum (Brundrett et al., 1996). The next step is to select mycorrhizal fungi which might have potential for use in revegetation of aspen in heavy metal soils such as those on smelter-impacted sites using greenhouse studies and field trials. This process is currently underway.

Methods

Survey and collection of fungi

Sporocarps (fruiting bodies) of ectomycorrhizal fungi were collected under naturally occurring aspen on smelter-impacted sites over several years. Fruiting bodies of putatively ectomycorrhizal fungi were described, identified, dried as vouchers, and deposited in the Montana State University Herbarium (MONT). Microscopic work was done in 3% KOH when necessary to confirm identifications. Fresh sporocarps were tissue cultured on modified Melin-Norkrans (MMN) to determine if it was possible to grow fungi *in vitro*, as the necessary first step in producing a soil or liquid inoculum (Schenck, 1982; Brundrett et al., 1996). Each culture was rated for no growth, slow growth, or fast growth. Fast growing species are currently being tested in greenhouse trials, but results are not available at this time.

Site descriptions

The primary collecting site was the Butte-Anaconda area in southwest Montana, a site of previous copper mining and smelting activity. The area was heavily impacted by sulfur oxide fumes in the late 1800's and early 1900's which resulted in soils with a high heavy metal content and areas devoid of vegetation, many of which persist today. Part of the area is within the Anaconda Superfund site, and includes the stack for the copper smelter (Smelter Hill), and vast landscapes of open tailings called Opportunity Ponds (Reclamation Research Unit, 1993). The smelter ceased to function in the 1960's. Aspen have since established on smelter hill and open slopes; large well-developed natural stands are visible just south of the smelter stack. At Opportunity Ponds aspen occur in an open moonscape, devoid of other vegetation. A soil analysis of the Butte area showed high levels of copper, nickel, aluminum and iron (Cripps, 2001), and high levels of arsenic, copper, lead, cadmium, and zinc have been recorded in the Anaconda area (Taskey, 1972). Both areas have a sandy, infertile soil with pH ranging from 1.83 (Anaconda) to 6.5 (Butte). Mycorrhizal fungi were also collected at Kellogg, Idaho near the silver refining site and lead smelter stack which was removed in the 1990's, and at Trail, B.C. where the lead smelter is still in operation and fumes move along the heavily impacted banks of the Columbia river. In all areas, aspen have established naturally.

Results

Over 30 species of ectomycorrhizal fungi were recorded on smelter-impacted sites, primarily from the Butte Anaconda areas which were the most intensively studied (Table 1). A majority of the fungi are from larger aspen stands where organic matter has built up and trees are older (up to 70 years). Many also occur on other soil types, including gravelly loam and deep volcanic loess with a higher pH (Cripps 2001). While all of the species in Table 1 were recorded from smelter sites, those primarily restricted to acidic soils are marked with a star*. *Laccaria proxima* (Fig. 2) and *Inocybe lacera* v. *lacera* were common in open areas with little or no organic matter, and near the youngest roots of the aspen stands at the perimeter.

Table 1. Ectomycorrhizal fungi associated with aspen (*Populus tremuloides*) on smelter-impacted sites in the Northern Rocky Mountains. Starred (*) species are more restricted to acidic soils. Growth indicates ability of a fungal isolate to grow *in vitro* on nutrient media.

species	locations	smelter-type	growth
AMANITACEAE			
Amanita muscaria v formosa (Pers per Fr.) Bert.	Butte	Cu	+
Amanita pantherina (DC per Fr.) Krombh.	Butte	Cu	+
RUSSULACEAE			
Lactarius controversus (Fr.) Fr.	Butte, Anaconda	Cu	-
Russula aeruginea Lindbl.:Fr.	Butte	Cu	-
Russula claroflava Grove *	Butte	Cu	-
Russula foetenula Peck *	Butte	Cu	-
Russula cf velenovskyi Miz-Zv.	Butte	Cu	-
TRICHOLOMATACEAE			
Laccaria proxima (Boud.) Pat. *	Butte, Anaconda, Kellogg, Trail	Cu, Pb	++
Tricholoma flavovirens (Pers. Ex Fr.) Lun & Nan*	Butte, Anaconda	Cu	++
Tricholoma populinum Lge. *	Butte	Cu	++
Tricholoma scalpturatum (Fr.) Quel.	Butte, Anaconda, Trail	Cu, Pb	++
CORTINARIACEAE			
Cortinarius subbalaustinus R. Hry.	Butte	Cu	-
Cortinarius cf talus Fr. *	Anaconda	Cu	+
Cortinarius trivialis Lge.	Anaconda	Cu	-
Cortinarius cf malachius Fr. *	Anaconda	Cu	NT
Hebeloma mesophaeum (Fr.) Quel.	Butte, Anaconda	Cu	++
Hebeloma populinum Romangn.	Butte	Cu	++
Inocybe flavella v flavella P. Karst	Butte	Cu	-
Inocybe geophylla (Fr.:Fr.) Kumm.	Butte	Cu	-
Inocybe lacera (Fr:Fr) Kummer v. *lacera* *	Butte, Anaconda, Kellogg, Trail	Cu, Pb	+
Inocybe cf longispora Lge *	Butte	Cu	-
Inocybe mixtilis (Britz.) Sacc.	Butte	Cu	-
Inocybe nitidiuscula (Britz.)	Butte	Cu	-
Inocybe phaeocomis (Pers.) Kuyper var *major*	Butte	Cu	-
Inocybe rimosa (Bull:Fr.) Kummer *	Butte, Anaconda	Cu	+
Inocybe sindonia (Fr.) P. Karst.	Butte	Cu	-
Inocybe squamata Lge. *	Anaconda	Cu	NT
BOLETACEAE			
Paxillus vernalis Watling *	Butte, Anaconda	Cu	++
Phylloporus rhodoxanthus (Schw.) Bres. *	Butte	Cu	NT
Leccinum aurantiacum (Bull:St.Amans) SF Gray	Butte	Cu	++
Scleroderma cepa (Vaill.) Pers. *	Butte	Cu	+
Xerocomus spadiceus Fr. *	Butte	Cu	+
THELEPHORACEAE			
Thelephora terrestris Fr. *	Kellogg, Trail	Pb	NT
ASCOMYCOTA *Cenococcum geophilum* Fr.	Butte, Anaconda	Cu	NT

(-) indicates no growth, (+) poor growth, (++) vigorous growth *in vitro,* and NT = not tested.

Fig. 2. Mycorrhizal fungus *Laccari a proxima* sporocarps from a smelter-impacted area near Butte, MT.

Fig. 3. Mycorrhizae comprised of fungus *Cenococcum geophilum* and aspen roots from the Anaconda Superfund site.

Other fungi associated with young roots, include *Scleroderma cepa, Thelephora terrestris,* and *Cenococcum geophilum* (Fig. 3) which produces no fruiting bodies.

Inocybe was the most diverse genus in this study, with ten species recorded, and the most prolific on smelter-impacted areas is *Inocybe lacera* v. *lacera*. It was recorded at Butte, Anaconda, Trail, B.C., and Kellogg, ID smelter sites with aspen in soil containing high levels of Pb, Cu, Cd, and As. Most of the *Inocybe* species did not grow in culture, or grew very slowly. Similarly, *Laccaria proxima* was recorded at the Butte, Anaconda, Trail, and Kellogg smelter sites in soils of high heavy metals including Pb, Cu, Cd, and As. It was also recorded on the McLaren tailings near Cooke City, MT with conifer and willow in soils high in iron. *Laccaria proxima*, grew fast in culture, and is currently being tested in green house trials.

Paxillus vernalis was common with aspen in the Butte-Anaconda area in sandy soils of low fertility and high heavy metal content. It grew well in culture in this study, and appears to have potential value for use in reclamation, and is currently being tested. *Scleroderma cepa* was only collected once under aspen at Butte, but *Scleroderma* species are not readily discovered since they are primarily hypogeous (underground) fungi. This species grew well in culture, and is also being tested in greenhouse trials. *Thelephora terrestris* was collected at the lead smelters at Trail, B.C. and Kellogg, ID, but is apparently absent from the intensively studied copper mining areas of Butte and Anaconda. While it was not tested for growth in this study, it was one of the few species collected at all smelter sites, and we report it so it will not be overlooked as a potential reclamation species. *Cenococcum geophilum* was commonly noted on aspen roots at Butte, and was particularly interesting for its prolific occurrence on Smelter Hill near the Anaconda smelter stack, and at Opportunity ponds, areas of high pollution. A viable isolate was not obtained from surface sterilized aspen roots for testing.

Other fungi of interest are various species of *Tricholoma* and *Hebeloma*. *Tricholoma scalpturatum* and *Tricholoma flavovirens* both occur at Butte and Anaconda with aspen. *Hebeloma mesophaeum* and *Hebeloma populinum* are found in some of the same areas. All species in these genera grew well in culture, and some are apparently specific for aspen. Some are currently being tested in the greenhouse as inoculum for aspen seedlings. *Leccinum aurantiacum* is common in the larger aspen forests at Butte and Anaconda. *Leccinum* was typically found with older trees and in forests with a developed understory. It grew well in culture and is specific for aspen. *Amanita* species such as *A. muscaria* and *A. pantherina* occur with aspen at both Butte and Anaconda. They both grew in culture, but rather slowly, precluding further testing.

Discussion

In the Northern Rocky Mountains, aspen naturally colonize areas laid bare by airborne smelter pollution and mine tailings. This includes active and inactive smelter sites, phosphate mine dumps, uranium tailings, coal spoils, and soils high in heavy metals such as copper, lead, cadmium, and arsenic. Aspen is rarely reported to successfully establish from seedlings in the Western U.S. (Sheppard et al., 2001), but it does seed on mine sites (Williams and Johnston, 1984). The few solitary aspen trees existing on tailings dumps over 2 meters deep at Anaconda can only be explained by seeding. While it is unclear if aspen is sustainable on mine tailings, large aspen forests near the Anaconda Smelter site and at Butte, have existed for over 70 years and provide a habitat for a diverse array of birds, mammals, and understory plants. We also know that aspen is precluded from seed establishment by the presence of uninterrupted grass (Williams and Johnston, 1984). This study found naturally seeded aspen in open areas between grass strips, and none within seeded grass strips on a phosphate mine dump, in a 10 plot study. The establishment of aspen on mine sites could conceivably reduce reclamation costs, since it is clonal i.e. self-propagates by root suckering, and can spread once established. The natural establishment of aspen relies on edaphic factors, including a suitable moisture regime. However, a lack of suitable mycorrhizal fungi (to ameliorate harsh conditions) could also be critical to aspen survival. Harris and Jurgensen (1977) found mycorrhizae to be necessary in the establishment of aspen in soils with high heavy metal content.

The community of mycorrhizal fungi which supports aspen on smelter sites in the Rocky Mountains is quite diverse, and only partially recorded at this point in time. Many of these fungal species are exclusive to aspen and are absent or uncommon in conifer forests. Some are known from other disturbed and mined areas in North Temperate regions. This includes the important genera *Laccaria, Inocybe, Paxillus*, and *Scleroderma*, along with groups whose roles on mine sites are less well known. Certain fungi in these genera are known to tolerate heavy metals and can ameliorate effects on plants (reviewed by Colpaert and Van Assche, 1987; Godbold et al., 1998; Hartley et al., 1997; Jentscke and Godbold, 2000; Tam, 1995).

The mycorrhizal fungi recorded on smelter-impacted sites in the Northern Rocky Mountains appear to tolerate stressed conditions, and particular strains could be valuable for use in reclamation. Information on their occurrence at mined sites in other regions, can help elucidate the range of conditions in which they can exist.

Many *Inocybe* species occur on disturbed and early successional sites, and in areas with open sandy soil (Cripps, 1997). Malloch (1982) described a new species (*I. immigrans* Malloch) from uranium and gold mine wastes in Ontario, and refers to *Inocybe* species as xerophytic (dry loving), which may be a key point to their existence on these sites. *Inocybe lacera,* the most prolific species on smelter-impacted sites in the Northern Rocky Mountains, is also known to occur with aspen on eastern coal spoils where it is important to tree establishment in hot soils (Schramm, 1966), and it has been recorded on uranium tailings with willow (Kalin and Stokes, 1981). While most *Inocybe* species do not grow well in culture, *Inocybe lacera* was previously shown to be capable of forming mycorrhizae with aspen in tubes under sterile conditions (Cripps and Miller, 1995). However, while it appears to benefit aspen in nature, in one study it was detrimental to seedlings in high nutrient soils, perhaps becoming pathogenic (Cripps, 2001). This counter-intuitive result suggests there is much to learn about each unique plant-fungus relationship, and that laboratory studies do not necessarily translate to environmental situations.

The genus *Laccaria* is also well known on disturbed sites with young trees. Both *Inocybe* and *Laccaria* are considered "weedy" early colonizers capable of forming mycorrhizae with a variety of young tree species, which are supplanted later by more competitive fungi as the trees age. *Laccaria laccata* has previously been recorded with aspen and other *Populus* species on uranium tailings (Kalin and Stokes, 1981), and on arsenic mine wastes in England (Benson et al., 1980; Pyatt, 1973). *Laccaria* species grow well in culture, and are considered to have high potential for reclamation with many tree species, and have been shown to be effective in aggregation of soil to prevent slope erosion (Graf, 1997). However, *Laccaria laccata* was not advantageous to spruce (*Picea abies*) on lead polluted soil in Slovania, which could reflect the poor nature of spruce as a reclamation tree (Vodnik et al., 1995). Reports of *Laccaria laccata* on mine spoils could actually refer to *Laccaria proxima*, a similar species.

Paxillus vernalis is specific to aspen and birch, while the closely related *Paxillus involutus* has a broad host range. The latter species has been recorded on toxic arsenic wastes (Benson et al., 1980) and on uranium tailings in Ontario with aspen (Kalin and Stokes, 1981). In a previous study of aspen inoculated under sterile conditions, the biomass of seedlings inoculated with *Paxillus vernalis* increased 300% after only 3 months in closed tubes (Cripps, 2001). Its ability to develop mycorrhizae on young aspen seedlings under greenhouse conditions is currently being tested.

Scleroderma species are well-known early colonizers, particularly with conifers. Species are often used in commercial inoculum, since sporocarps contain huge numbers of spores, and can be used as a spore slurry as well as a mycelial inoculum. *Scleroderma aurantium* has been recorded on coal spoils in Pennsylvania (Schramm, 1966), and with nine species of naturally invading trees on bituminous wastes in the Allegany mountains (Riley and Brown, 1978). *Scleroderma citrinum* is reported on arsenic mine waste (Benson et al., 1980). Recently, *Scleroderma cepa* (from eastern coal spoils) was used in reclamation of a copper mine in Utah, although specific results are not yet available (Cordell et al, 2000).

Thelephora terrestris is known to colonize young seedlings of many trees in nature and particularly in nurseries. It is reported to grow well in culture, and has been found on arsenic soils (Benson et al., 1980), on uranium tailings with aspen (Kalin and Stokes, 1981), and on coal spoils with aspen (Schramm, 1966). While not tested in this study for growth (difficult to isolate), it was one of the few species collected at all smelter sites, and we report it so it will not be overlooked as a potential reclamation species. It is, however, known to compete with more desirable fungi under some conditions.

Cenococcum geophilum is a ubiquitous mycorrhizal fungus found in low quantities with most trees in many areas, and is considered to play a role in drought tolerance. It differs from all other fungi discussed (basidiomycetes) in that it is an ascomycete and does not produce fruiting bodies. Since this fungus must be directly isolated from surface sterilized roots, a somewhat difficult procedure, it is not typically used as a reclamation fungus, but it does have potential for drought prone areas. It is of particular interest here for its prolific occurrence on Smelter Hill near the Anaconda smelter stack, and at Opportunity ponds, areas of high pollution, but has not, as yet, been successfully isolated from aspen.

Other genera of interest are *Tricholoma* and *Hebeloma*, since they grow well in culture, and are found with young trees. However, they are not generally considered fungi of disturbed habitats, and occur more often in high nutrient soils (particularly with high nitrogen) with a more basic pH. In a previous study, *Tricholoma scalpturatum* increased the biomass of aspen seedlings over 400% *in vitro* and doubled stem diameters (Cripps, 2001). An increase in stem diameter is considered beneficial for outplanted trees on disturbed sites in reforestation. *Hebeloma* is common in bare root nurseries and several species were collected at Butte and Anaconda. Many of these occur primarily with aspen, and are not well known for reclamation purposes.

Many *Leccinum* species are specific for aspen, and are capable of producing an extensive underground rhizomorphic system which aggregates soil. *Leccinum scrabrum* is recorded on arsenic wastes (Benson et al., 1980). *Leccinum aurantiacum*, common at Butte and Anaconda, is primarily found in older forests with a developed understory, and may not tolerate xeric conditions. The *Amanita muscaria* and *Amanita pantherina* occur with aspen, and are also found in conifer forests, and may be important cross-over species as conifers invade aspen in successional processes. In a previous study, each increased the growth of aspen in vitro, 400% and 250%, respectively (Cripps, 2001).

Pisolithus tinctorius, a fungus commonly used in reclamation, is currently being tested in greenhouse trials. Although it is not known to associate naturally with aspen, a local strain from conifers is being used. It is also recorded from Ontario (Malloch and Duja, 1979). *Pisolithus tinctorius* grows well in culture, and has the advantage of producing massive numbers of spores which can be used in spore slurries. In a previous study, it formed mycorrhizae faster than other fungi tested with aspen and increased seedling biomass 350% in a closed system (Cripps, 2001).

While *Pisolithus tinctorius* has been successfully used to help revegetate coal spoils and mine wastes (Cordell et al., 2000; Marx and Artman, 1979), it failed with conifers in southwestern Oregon (Castillano and Trappe, 1991). There is currently interest in using trees and shubs inoculated with mycorrhizal fungi such as *Pisolithus tinctorius* in mine land reclamation programs because cost savings can be enormous (Cordell et al., 2000). Aspen has a further advantage, since it continues proliferation by root suckering. In one project, over 5 million *Pisolithus*-inoculated oak and pine seedlings were planted on over 5,000 acres of Abandoned Mined Lands (AML) in Ohio (Cordell et al., 2000). The inoculated trees had an 85% survival rate compared to a 25-50% survival rate for un-inoculated trees. The same company is using similar methodology at a copper mine in Utah where *Pisolithus tinctorius* and *Scleroderma cepa* (from eastern coal spoils) are being used with native trees (Cordell et al., 2000). Results are apparently positive and compliant with regulatory agency requirements.

Conclusions

The use of exotic fungi, like the use of exotic trees and shrubs, must be considered in reclamation processes. Fungi can be considered exotic or native, depending on their source, and at present, we do not know if exotic fungi are capable of replacing native fungi and/or stalling out development of the diverse mycorrhizal community so necessary to forest health. The benefits of using non-native fungi to quickly cover areas devoid of vegetation must be weighed against effects on natural successional processes. The ability of transgenic aspen to associate with mycorrhizal fungi is a new concern and results are conflicting (Kaldorf et al. 2002).

One way to circumvent these concerns is to develop native inoculum for use on native trees for specific soil types and regions. At present, we have discovered over thirty species of ectomycorrhizal fungi with aspen on smelter sites, and the number is likely to grow. About half of the fungi grow in culture, but only a select few grow vigorously enough to be of interest as mycelial inoculum. At this point, native fungi of interest for their fast growth, include *Laccaria proxima, Tricholoma flavovirens, Tricholoma populinum, Scleroderma cepa, Paxillus vernalis.* and a local strain of *Pisolithus tinctorius*. Techniques and biosystems are presently being developed for nursery and field situations with aspen on smelter sites. There is concern about the decline of aspen in the western United States, about 40% over the last decade (Sheppard et al.

2001) and it should be noted that current aspen stands are the primary reservoirs of mycorrhizal fungi for future aspen forests, both on and off smelter-impacted sites.

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