Mycorrhizal Fungi of Aspen Forests: Natural Occurrence and Potential Applications

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Abstract—*Native mycorrhizal fungi associated with aspen were surveyed on three soil types in the north-central Rocky Mountains. Selected isolates were tested for the ability to enhance aspen seedling growth in vitro. Over 50 species of ectomycorrhizal fungi occur with* Populus tremuloides *in this region, primarily basidiomycete fungi in the Agaricales. Almost one-third (30%) were ubiquitous with aspen and were found on all three soil types. Over one-third (37%) were restricted to the acidic, sandy soil of the smelter-impacted Butte-Anaconda area, revealing a subset of fungi that tolerate these conditions. Mycorrhizal fungi were screened for their ability to enhance aspen growth and establishment. Of nine selected isolates, all but one increased the biomass of aspen seedlings 2–4 times. Stem diameter, height, and number of root tips increased with inoculation of some fungi. The native species* Paxillus vernalis, Tricholoma scalpturatum, Hebeloma mesophaem, Thelephora terrestris, *and* Laccaria s*pp. were most promising for further study.* Pisolithus tinctorius *(available as commercial inoculum) formed prolific mycorrhizae and stimulated plant growth but does not occur with aspen in the Rocky Mountains.*

Introduction

Over 80% of plant families are mycorrhizal, and this mutualistic association between plant roots and fungi are the rule in nature, not the exception (Malloch et al. 1980). Most terrestrial ecosystems depend on mycorrhizae, which promote the establishment, growth, and health of plants. Mycorrhizal fungi are particularly crucial in forest systems where they benefit trees by augmenting inorganic nutrient uptake and providing protection from heavy metals, drought, pathogens, grazers, and other organisms (Fogel 1980). Seven mycorrhizal "types" have been defined by the morphology of the root structures formed and the organisms involved (see Smith and Read 1997 for a review). The Pinaceae (pine family), Betulaceae (birch family), Salicaceae (willow and aspen family), Fagaceae (oak family), and Myrtaceae (eucalyptus family) are primarily *ecto*mycorrhizal and associate mostly with basidiomycete fungi, which produce mushrooms as reproductive structures (Malloch et al. 1980). Mushrooms produced by mycorrhizal fungi can be observed near host trees at certain times of the year and are evidence of mycorrhizae in the soil. It should be kept in mind that not all mushroom-producing fungi are mycorrhizal, and forests also host a diverse array of large, fleshy fungi that are saprophytic, parasitic, or mutualistic in other ways (Pilz and Molina 1996).

Aspen (*Populus tremuloides* Michx.) is predominantly ectomycorrhizal (Cripps and Miller 1993; Fontana 1963; Vozzo and Hackskaylo 1974). The prefix "ecto" refers to the intercellular nature of the fungal hyphae that remain external to the plant root cells. Hyphae form a layer over individual roots tips (mantle) and surround individual cortex cells (Hartig net) where nutrient exchange takes

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place, but they do not invade the root cells. The fungal mycelium proliferates into the soil, essentially extending the root system and enhancing the uptake of inorganic nutrients, primarily of phosphorus and nitrogen, which is considered a main benefit to the plant. In return, fungi subsist on carbohydrates from the plant, which are converted to fungal sugars. There are reports of *Populus tremuloides* associating with arbuscular mycorrhizal (AM) fungi that invade the root cells forming a type of endomycorrhizae, but we have found this to be rare. Other species of *Populus* are more likely to associate with AM fungi, and some are also ectomycorrhizal (Vozzo and Hackskaylo 1974).

There were two main objectives in the present study. The first was to survey the mycorrhizal fungi associated with aspen on three different soil types in the north-central Rocky Mountains and identify species with a narrow or broad range. The second objective was to evaluate the effectiveness of several mycorrhizal species in enhancing the growth of aspen seedlings. The second goal has potential application in mined-land reclamation.

In nature, an individual tree typically supports numerous species of mycorrhizal fungi simultaneously, and this mycoflora is dynamic, changing over the life of the tree. The potential number of fungal associates varies with the plant species. For example, Douglas-fir (*Pseudotsuga menziesii*) is capable of forming mycorrhizae with over 2,000 species of fungi (Trappe 1977), while alder (*Alnus* spp.) is limited to only a few (Brunner et al. 1990). In Montana and Idaho, we previously reported over 50 species of mycorrhizal basidiomycete fungi in aspen stands (Cripps and Miller 1993; Cripps 1997). That list has now been extended and refined with additional species determinations, particularly in the Cortinariaceae, a dominant family with aspen in terms of species richness. Ectomycorrhizal fungi exhibit various levels of specificity in plant-fungus interactions. Some fungi are restricted to one or a few hosts, while others have a preference for conifers or broad-leaf hosts. Some are known to occur with a wide range of trees. For example, *Suillus* occurs primarily with pine, occurs to a lesser extent with larch and Douglas-fir, and is rare with other trees. The genus *Gomphidius* appears restricted to pines, and *Lactarius controversus* to aspen and birch. The role that soil and other factors play in determining the mycobionts of a particular forest is only beginning to be understood. In Europe it is common to refer to a particular mycorrhizal fungus's preference for mull or mor soil, while in North America we know so little about the distribution of mycorrhizal fungi that patterns are yet to be completely discerned. Aspen occur in large, pure stands on many soil types and offer an opportunity to examine the role soil factors play in the distribution of mycorrhizal fungi on one host. Mycorrhizal fungi with aspen were surveyed on three very different soil types in Montana and adjacent areas of Idaho to determine if mycofloras varied or if the same subset of mycorrhizal fungi occurred in all stands. Some results have been reported in Cripps and Miller (1993). The updated list is given here along with the specifics of soil characteristics.

Potential Application of Mycorrhizal Fungi

The smelter-impacted area of Butte-Anaconda was of particular interest, because soils are acidic and high in concentrations of copper, iron, and zinc. Heavy metals such as cadmium have been found in aspen leaves on this Superfund site (Bissell 1982). The role of aspen as a pioneering tree in the recovery of smelter-impacted sites in this region is generally unrecognized, and reclamation efforts are typically focused on imported plants. The backside of the Anaconda smelter hill supports a vibrant aspen stand, and young aspen are

found within a few hundred feet of the smelter stack itself (Cripps 1996). Dotting the extensive moonscape of tailings waste, small isolated aspen appear to be healthy and thriving. Although their longevity is in question, the survival of even one is significant given aspen's clonal nature and potential to proliferate. At the smelter site in Kellogg, Idaho, a thriving natural aspen stand rises above the planted yellowing conifers. In Trail, British Columbia, where the smelter is still in operation, numerous *Populus* spp. line the banks of the Columbia River downwind of the smelter stack.

Whether aspen has seeded in or regenerated from protected pockets of aspen is an open question. It is difficult to account for the occurrence of isolated aspen trees located on tailings 10 m deep and surrounded by hectares of dumped tailings except by seeding, although this has not been verified. All of the roots examined at these sites were mycorrhizal and several species of fungi have been identified. Ectomycorrhizal fungi are crucial to aspen's survival in these areas, and their potential to expedite aspen colonization on these sites has not been examined. With this in mind, the effects of mycorrhizal fungi on early seedling growth of aspen were studied as an initial screening for native and nonnative fungi, which might be useful in aspen establishment and health and for mined-land reclamation. The parameters examined were the ability of the mycorrhizal fungi to (1) grow in vitro, (2) form mycorrhizae with aspen in vitro, and (3) affect biomass, height, stem diameter, and general health of aspen.

Methods

Sites

The three study areas are located in the north-central Rocky Mountains of southwestern Montana and southeastern Idaho at latitude 45° N, longitude 110–112° W, and elevations of 1,800 to 2,000 m (figure 1). The Butte-Anaconda (B) area lies near the towns of those names in Montana at an elevation near 1,800 m. Fumes from previously operating copper smelters killed much of the vegetation in the late 1800s and early 1900s, and aspen have colonized large areas within the last 70 years. The soil is a nutrient-poor sand (over 70%), with pH's from 4.3 to 5.7, and contains high concentrations of metals, particularly copper. The Cinnabar site (C) just north of Yellowstone National Park supports an older aspen stand with trees up to 114 years old that is gradually being invaded by conifers. The moist area is in a mountain basin, and the soil is a gravelly glacial till, high in P, Ca, and Mg. The large aspen stands of the Teton (T) area in SW Idaho lie on rolling uplands of well-drained soils formed from deep loess and composed of 75% silt. The soil is relatively fertile as attested to by adjacent agricultural land, and aspen tend to persist in this area without conifer replacement. Soil characteristics for the sites are shown in tables 1 and 2.

Mycorrhizal Synthesis and Growth Studies

Native and nonnative fungi that grew well in culture were selected for in vitro growth studies to examine the effects of individual fungal species on early aspen growth. Sterile technique was used to ensure that effects were due to the mycorrhizal fungi and not to extraneous organisms. Pot and field studies will be necessary to further examine effects of mycorrhizal inoculation under greenhouse conditions and for outplantings. Our purpose was to restrict each experimental unit to one mycobiont and one plant host (aspen). Sporocarp

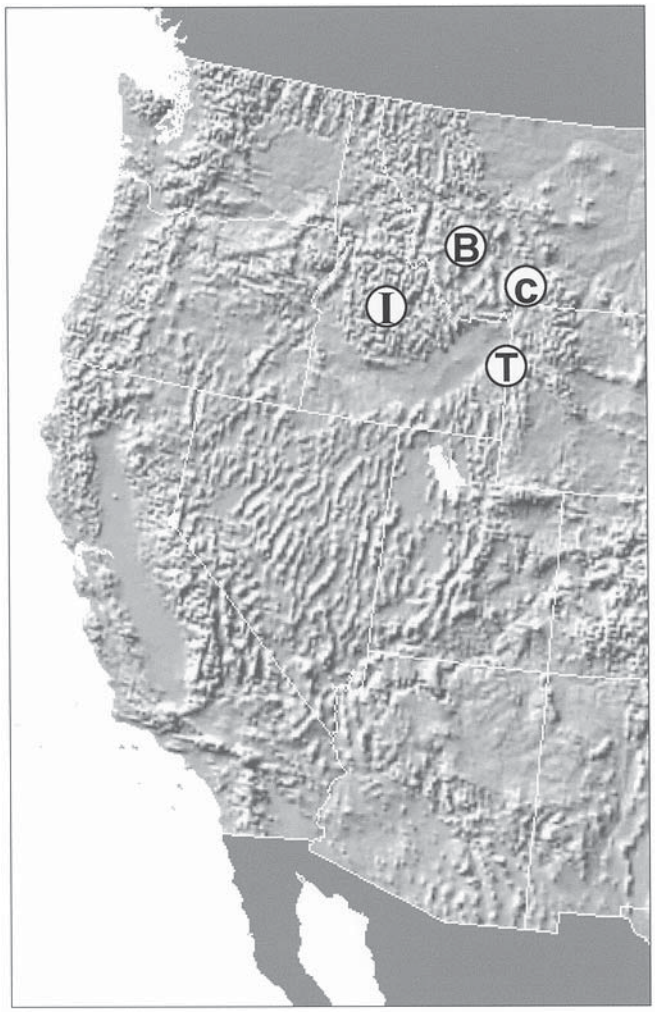

Figure 1—Location of aspen forests in study. B = Butte-Anaconda smelter-impacted area. C = Cinnabar Basin north of Yellowstone Park. T = Teton foothills. I = the town of McCall, ID. Map courtesy of Ray Steiner, John Hopkins University.

Table 1—Soil characteristics of aspen sites in relation to depth. OM = organic matter, CEC = cation exchange capacity.

Soil depth	Sand	Silt	Clay	pH	Exchangeable Ca in ppm	OM	Base saturation	CEC
cm	- - - - - - - - - *Percent* - - - - - - - - -					- - - - *Percent* - - - -		
Butte								
8	82	15	3	5.3	1.46	1.9	45	0.46
15	81	13	5	4.3	1.04	3.2	15	0.15
20	70	20	9	4.5	1.53	4.8	11	0.11
58	74	17	8	4.9	3.03	2.7	28	0.28
97	84	6	10	5.6	5.92	2.1	77	0.77
Cinnabar								
8	65	27	7	5.5	12.60	7.2	63	0.63
15	63	28	9	5.7	11.80	5.7	66	0.66
20	58	31	11	5.8	13.70	5.5	65	0.65
58	70	24	6	6.4	7.40	1.1	86	0.86
94	67	24	9	6.5	6.89	1.1	93	0.88
Tetons								
15	15	77	8	5.5	8.00	2.4	84	0.84
36	12	79	9	6.0	6.37	1.7	73	0.73
56	12	79	9	6.1	5.92	1.2	71	0.71
84	11	67	21	6.3	10.2	1.0	82	0.83
122	42	44	14	6.3	7.90	0.7	79	0.79

Table 2—Exchangeable macro- and micronutrients (ppm) of soil on aspen forest sites.

Soil depth	P	Ca	Mg	Zn	Fe	Al	Cu
cm							
Butte							
8	62	404	53	10.7	31.6	155	43
15	54	252	34	7.7	72.4	264	116
20	94	386	37	19.7	35.2	588	142
58	63	803	61	72.9	9.9	388	3.5
97	40	1,377	68	1.7	3.2	71	0.3
Cinnabar							
8	78	2,048	303	4.7	12.3	135	0.2
15	67	2,128	312	3.6	10.2	138	0.2
20	64	2,260	332	3.5	9.3	138	0.2
58	130	1,487	312	0.6	22.3	131	0.4
94	119	1,360	295	0.6	25.2	122	0.6
Tetons							
15	62	1,502	111	3.3	15.8	128	0.5
36	56	1,190	105	2.6	14.2	117	0.7
56	62	1,032	97	1.9	19.1	111	1.3
84	113	1,523	164	1.3	28.1	157	3.8
122	32	977	124	0.8	20.3	133	1.9

tissue of native fungi and mycelium of nonnative fungi was cultured according to the sterile technique described by Molina and Palmer (1982) and grown on Hagem's medium modified by Van Cotter (1987, unpublished): 4 g malt extract, 1 g yeast extract, 5 g d-glucose, 0.5 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 ml $FeCl_3$ (1% aqueous), 100 ml biotin (0.5 mg biotin/ml aqueous), and 100 ml thiamine-HCl (1 mg thiamine/ml aqueous) added to 1,000 ml of distilled H_2O. Eleven grams of agar/L were added to solidify the medium, which was autoclaved for 20 minutes. Cultures were incubated in the dark at 20 °C for a minimum of 1 month and used to inoculate 250 ml flasks containing 75 ml of liquid Cotter's modified Hagem's without agar.

Fresh aspen seeds were stored in a dry place at 0 to 5 °C. Seeds stored too long lose viability and the risk of contamination increases. Seeds were gently agitated in a 15% Clorox™ solution for 15 minutes and rinsed three times (10 minutes each) in double-distilled H_2O (Cripps and Miller 1995). Two drops of the detergent Tween™ were added to the first two solutions to reduce surface tension. Seeds were placed in petri dishes containing Cotter's modified Hagem's made with 11 g/L of agar and placed in a growth chamber under incandescent and fluorescent lights for 16 hours a day followed by 8 hours of dark. Seedlings that showed no signs of contamination were planted in synthesis tubes 23 days later.

Molina and Palmer's tube method of synthesis (1979) was followed using 10 ml peat, 90 ml of vermiculite, and 70 ml of Cotter's modified Hagem's (without agar) for each 200 ml synthesis tube. Five ml of mycelial slurry was added to each tube, which was autoclaved, and the lower part encased in aluminum foil. Ten replicates were used for each fungus and the uninoculated controls. After mycelium colonized the peat-vermiculite medium for 2 weeks, sterile seedlings were introduced and tubes were placed in a growth chamber. Tubes were periodically checked for mycorrhization and seedlings harvested after 3 months. At that time, the general condition of the aspen seedlings was noted, particularly leaf color. The stem diameter and height were measured. Roots were carefully washed and percent mycorrhization determined by counting the number of mycorrhizae per total number of root tips.

Seedlings were dried at 65 °C for 48 hours and weighed. The nonparametric Kruskal-Wallis *t*-test was used to compare responses in control seedlings versus those inoculated with individual fungi.

Results

Mycorrhizal Associates of Aspen

Over 54 species of ectomycorrhizal fungi occurred with aspen on the three study sites and additional aspen stands sampled in Montana and Idaho (table 3). The fungi are all Basidiomycota, primarily Agaricales (gilled mushrooms and boletes), and one Aphyllophorales (*Thelephora terrestris* Fr.). The fungi are distributed in seven families: Amanitaceae, Russulaceae, Tricholomataceae, Cortinariaceae, Paxillaceae, Boletaceae, and Thelephoraceae (figure 2). The dark-spored Cortinariaceae is the most diverse family, with 25 species of *Cortinarius, Inocybe*, and *Hebeloma* occurring in aspen stands. In casual observation, *Leccinum* species often dominate in terms of sheer biomass, with sporocarps occurring in large numbers. *Leccinum insigne* (orange-capped bolete) is considered one of the most characteristic species of aspen stands.

Two major categories of ectomycorrhizal fungi became apparent in the study. Nearly one-third (30%) of the ectomycorrhizal species occurred on all three soil types with *Populus tremuloides*. This percentage increased each year of the study as species fruited on additional sites, as is typical in fungal studies. In the second group, over 37% of the mycorrhizal species occurred only on the smelter-impacted, sandy, acidic soil of the Butte-Anaconda area, suggesting that there is a subset of mycorrhizal fungi more restricted to, or more tolerant of, these abiotic conditions.

Effect of Mycorrhizal Fungi on Aspen Seedling Growth

General condition of aspen

About half of the mycorrhizal fungi isolated grew in culture, and fewer grew well enough to be tested. Of the six native and three nonnative fungi selected for testing, all formed mycorrhizae with aspen, except *Chalciporus (Boletus) piperatus* (Bull.:Fr.) Singer. *Piloderma* formed a mantle, but no Hartig net. The general condition of plants at the end of the experiment is shown in table 4. None of the uninoculated control seedlings died, and leaves remained completely green throughout the experiment. This was also true for inoculation with four native fungi, *Amanita muscaria* v. *formosa* (Pers. Per Fr.) Bert., *Amanita pantherina* (DC Per Fr.) Krombh., *Paxillus vernalis* Watling, and *Tricholoma scalpturatum* (Fr.) Quel. In contrast, all plants inoculated with *Inocybe lacera* (Fr:Fr) Kummer became necrotic after forming a few mycorrhizae; the black leaves abscissed and dropped off, and the plants died. *Chalciporus piperatus* inoculated plants did not form mycorrhizae, but leaf color was affected. Leaves of seedlings inoculated with *Paxillus vernalis* developed red, yellow, and black coloration, but plants remained in good condition. With the exception of *Inocybe lacera,* which produced 100% mortality in aspen seedlings, and one plant with *B. piperatus*, all plants inoculated with native mycorrhizal fungi were alive at the end of the experiment. However, mycorrhizae were slow to form under these conditions with native fungi.

Seedlings inoculated with nonnative fungi had a higher mortality rate (10–20%), and extreme leaf tips turned black. While *Cenoccocum* and *Piloderma*-inoculated plants turned a pale yellow-green color, those with

Table 3—Ectomycorrhizal fungi occurring with *Populus tremuloides* in the north-central Rocky Mountains, U.S.A. B = Butte, C = Cinnabar, and T = Teton site.

Ectomycorrhizal fungi with *Populus tremuloides* in the north-central Rocky Mountains	State	Acidic, sandy infertile soil (Butte-Anaconda)	Calcareous, sandy loam (Cinnabar)	Calcareous silty soil (Tetons)	Soil types	Growth in vitro[a]
AMANITACEAE						
Amanita alba Gill.	MT, ID		+		C	–
Amanita fulva (Schaeff.) per Pers.	ID			+	T	–
Amanita muscaria v. *alba* Peck	ID				?	+
Amanita muscaria v. *formosa* (Pers per Fr.) Bert.		+	+	+	BCT	+
Amanita pantherina (DC. Per Fr.) Krombh.	MT, ID	+	+	+	BCT	+
Amanita vaginata (Bull. Per Fr.) Krombh.	MT, ID		+	+	CT	–
RUSSULACEAE						
Lactarius controversus (Fr.) Fr.	ID, MT	+	+	+	BCT	+
Lactarius cf. *zonarius* Fr.	MT, ID		+	+	CT	?
Russula aeruginea Lindbl.:Fr.	MT, ID	+	+	+	BCT	–
Russula claroflava Grove	MT	+			B	–
Russula cf. *krombholtzii* Kromb.	MT	+		+	BT	–
Russula foetenula Peck	MT	+	+		BC	–
Russula cf. *velenovskyi* Mlz-Zv.	MT	+	+	+	BCT	–
Russula xerampelina (Schaef.:Secr.) Fr.	MT		+		C	–
TRICHOLOMATACEAE						
Laccaria laccata v. *pallidifolia* (Peck) Peck	MT	+			B	+
Laccaria proxima (Boud.) Pat	MT	+			B	+
Laccaria tortilis (Bolt.) Cooke	MT	+	+		BC	?
Tricholoma flavovirens (Pers. Ex Fr.) Lun & Nan	MT	+			B	?
Tricholoma populinum Lge.	MT	+			B	+
Tricholoma scalpturatum (Fr.) Quel.	MT, ID	+	+	+	BCT	+
CORTINARIACEAE						
Cortinarius alboviolaceous (Pers.:Fr.) Fr.	MT		+		C	–
Cortinarius hedyaromaticus Cripps & Miller	MT		+		C	+
Cortinarius ochrophyllus Fr.	MT	+			B	–
Cortinarius subbalaustinus R. Hry.	MT, ID	+	+	+	BCT	–
Cortinarius talus Fr.		+			B	+
Cortinarius trivialis Lge.	MT, ID	+	+	+	BCT	–
Cortinarius cf *stuntzii* Rehner and Ammirati	MT	+			B	?
Cortinarius cf *sertipes*	MT		+		B	?
Hebeloma insigne Smith, Evenson & Mitchell	MT	+	+	+	BCT	+
Hebeloma mesophaeum (Fr.) Quel.	ID, MT	+	+		BC	+
Hebeloma populinum Romagn.	MT	+	+	+	BCT	+
Hebeloma spp. 1, 2, 3	MT				?	+
Inocybe dulcamara (Alb. & Schw:Pers) Kummer	MT	+	+		BC	+
Inocybe flavella v. *flavella* P. Karst	MT, ID	+	+	+	BCT	?
Inocybe flocculosa (Berk) Sacc. v. *flocculosa*	MT, ID	+	+	+	BCT	–
Inocybe geophylla (Fr.:Fr.) *Kumm.* v. *geophylla*	MT	+			B	–
Inocybe griseolilacina Lge.	MT		+		C	–
Inocybe lacera (Fr:Fr) Kummer v. *lacera*	MT, ID	+			B	+
Inocybe longispora Lge.	MT	+			B	–
Inocybe mixtilis (Britz.) Sacc.	MT	+	+		BC	–
Inocybe nitidiuscula (Britz.) Sacc.	MT	+	+		BC	–
Inocybe phaeocomis (Pers.) Kuyper v. *major*	MT	+			B	–
Inocybe rimosa (Bull:Fr.) Kummer	MT, ID	+			B	+
Inocybe squamata Lge	MT	+			B	?
Inocybe sindonia (Fr.) P. Karst	MT	+			B	–
Inocybe whitei (B & Br) Sacc. v. *whitei*	MT	+	+	+	BCT	–
PAXILLACEAE						
Paxillus vernalis Watling	MT, ID	+	+	+	BCT	+
BOLETACEAE						
Chalciporus piperatus (Bull.:Fr.) Singer	MT, ID		+	+	CT	+
Leccinum aurantiacum (Bull:St.Amans) SF Gray	MT, ID	+	+	+	BCT	+
Leccinum holopus *(Rostk.) Watl.*	*ID*				*?*	*?*
Leccinum insigne Smith, Thiers & Watling	MT, ID	+	+	+	BCT	+
Phylloporus rhodoxanthus (Schw.) Bres.	MT	+			B	?
Xerocomus spadiceus Fr.	MT	+			B	+
THELEPHORACEAE						
Thelephora terrestris Fr.	MT, ID	+			B	+

[a](+) fungus grew on MMN, (–) fungus showed no growth on MMN, (?) fungus was not tested on MMN.
MMN is Melin-Norkrans media (Molina and Palmer 1982).

Figure 2—Ectomycorrhizal fungi associated with aspen. Row 1: *Amanita muscaria, Amanita pantherina, Laccaria proxima*. Row 2: *Lactarius controversus, Russula aeruginea, Cortinarius trivialis*. Row 3: *Cortinarius subbaulaustinus, Inocybe squamata, Inocybe lacera*. Row 4: *Leccinum insigne, Boletus piperatus, Paxillus vernalis*.

Table 4—General condition of aspen seedlings inoculated with mycorrhizal fungi after 3 months. Native fungi were isolated from aspen stands in Idaho and Montana. Nonnative fungi are from VPI culture collection and origins are unknown.

	Seedling mortality	Leaf color general condition	Mycorrhizal	Average biomass as % of control
	Percent		*Percent*	*Percent*
Control	0	Green	0	100
Native fungi				
Amanita muscaria	0	Green	15	400
Amanita pantherina	0	Green	11	250
Boletus piperatus	10	Green with black tips	0	430
Inocybe lacera	100	Most black	1	100
Paxillus vernalis	0	Red/yellow/green/black	12	300
Tricholoma scalpturatum	0	Green	1	430
Nonnative fungi				
Cenococcum graniforme	20	Yellow-green, black tips	5	275
Piloderma croceum	10	Yellow-green, black tips	1	300
Pisolithus tinctorius	20	Dark green, black tips	86	350

Pisolithus tinctorius were a healthy looking dark green. Mycorrhizae were slow to form with the first two, but the root systems of aspen inoculated with *P. tinctorius* (PT) were heavily colonized by the fungus in a short period of time (table 4).

Aspen biomass, stem diameter, height, number of root tips

All of the inoculated aspen seedlings (except those with *I. lacera*) showed a significant increase in total plant biomass over uninoculated controls (figure 3a). In most cases, the increase in average biomass was substantial, and as a percent of the controls the biomass was 430% for *Tricholoma scalpturatum* and *Boletus piperatus*, 400% for *Amanita muscaria*, 350% for *Pisolithus tinctorius*, 300% for *Paxillus vernalis* and *Piloderma croceum*, 275% for *Cenococcum graniforme*, and 250% for *Amanita pantherina*. The biomass of aspen inoculated with *Inocybe lacera* was not significantly different from the control, and plants were in poor condition.

Stem diameter in aspen seedlings increased significantly with the addition of all the mycorrhizal fungi, except *I. lacera* (figure 3d). While the average height of aspen seedlings was increased by inoculation with some fungi, this was only marginally significant for others (figure 3b). Inoculation also affected leaf shape, size, and number differentially, with a general increase of surface area, but the details are not reported here. The average number of root tips doubled with inoculation for most fungi, even those with *Boletus piperatus* that did not form mycorrhizae (figure 3c). *Inocybe lacera* eventually killed the seedlings. The average number of root tips after inoculation with *Pisolithus tinctorius*, *Cenococcum graniforme*, and *Tricholoma scalpturatum* was generally four times that of the control (figure 3c).

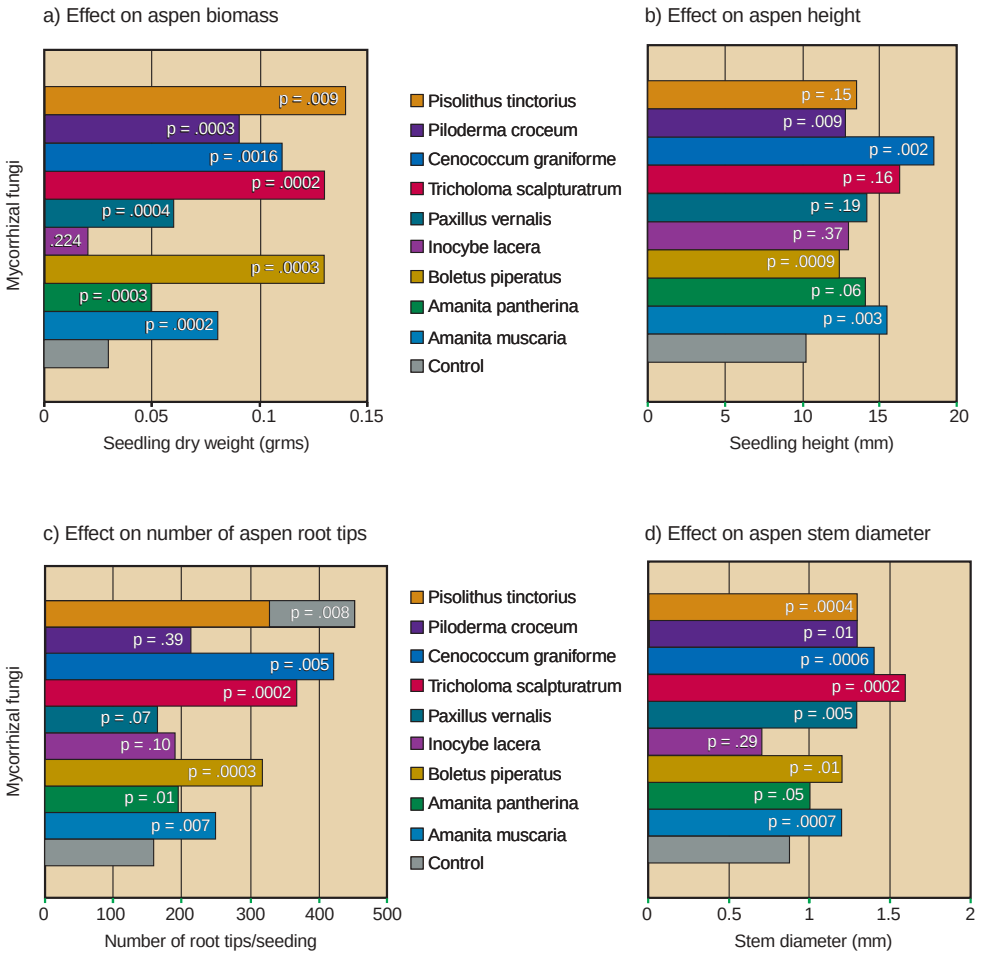

Figure 3—Effect of ectomycorrhizal fungi on early seedling growth of aspen. First three fungi are nonnatives, followed by six native species occurring with aspen in Montana and Idaho. A p-value of less than 0.05 is considered significantly different from the control.

Discussion

Mycorrhizal Associates of Aspen

Quaking aspen is now known to be associated with over 54 species of ectomycorrhizal fungi in the north-central Rocky Mountains and more species of fungi are yet to be identified, particularly in the *Russulaceae*. Aspen's ability to form a mutualistic association with such a diverse array of fungi could help account for its wide geographic range and its ability to proliferate in many different habitats (Cripps and Miller 1993). All of the mycorrhizal fungi are Basidiomycetes and members of the Agaricales (gilled mushrooms), except *Thelephora terrestris* (Aphyllophorales). Many of the same species have been reported with aspen in Canada and their mycorrhizae synthesized in the lab (Godbout and Fortin 1985). In that study, aspen formed mycorrhizae with several species of *Scleroderma,* which is of interest because of its use as a commercial inoculum (but not recorded for our study). Aspen did not form mycorrhizae with *Rhizopogon,* also used as a commercial inoculum, which occurs naturally with conifers. Sister species *Populus tremula* L. in Europe is mycorrhizal with the same fungal genera, and often with the same or related species (Anselmi et al. 1990; Heslin and Douglas 1986; Pirazzi et al. 1989). Many groups of mycorrhizal fungi found in conifer forests are absent from the aspen stands of Montana and Idaho. Mycorrhizal members of whole families such as the Sclerodermataceae, Hydnaceae, Clavariaceae, Cantharellaceae, Hygrophoraceae, Corticiaceae, and hypogeous (subterranean) Ascomycota and Basidiomycota, i.e., the truffle-like fungi, were not recorded in Rocky Mountain aspen forests. Conversely, many of the mycorrhizal fungi found in aspen forests are infrequent or absent from conifer forests.

The Cortinariaceae are a dominant group in terms of species richness, but many of the dark-spored species do not grow well in culture and are not useful for growth studies. *Inocybe* species are particularly diverse with aspen (Cripps 1997), as are *Cortinarius* species. Perhaps the most characteristic fungi of aspen stands are the *Leccinum* species (rough-stemmed boletes), which often fruit in abundance in mid-summer after significant amounts of rain.

Almost one-third (30%) of the mycorrhizal fungi occurred on all three diverse soil types examined, including (1) a nutrient-poor sandy soil, (2) a fertile gravelly loam/glacial till, and (3) a deep silty loess. Interestingly, these fungi appear to be those most closely allied with aspen. For example, the following fungi are almost strictly with aspen (or birch) and are extremely rare in conifer forests and possibly absent altogether: *Lactarius controversus* (Fr.)Fr.; *Russula aeruginea* Lindbl.:Fr.; *Cortinarius trivialis* Lge.; *Cortinarius subbalaustinus* R. Hry.; *Hebeloma insigne* Smith, Evenson, and Mitchell; *Hebeloma populinum* Romagn.; *Paxillus vernalis* Watling; *Leccinum aurantiacum* (Bull:St.Amans) SF Gray; and *Leccinum insigne* Smith, Thiers & Watling. This close alliance, regardless of soil type, appears more characteristic of older aspen stands with sufficient organic matter and nutrient availability. *Amanita* species are the exception, occurring also in conifer woods, and could possibly be "crossover" species in successional processes.

Over one-third (37%) of the mycorrhizal fungi were found only on the acidic, sandy, nutrient-poor soil of the Butte-Anaconda site, where smelters have impacted the area, and high concentrations of copper, lead, and zinc are present. This subset of aspen's mycorrhizal flora prefers or tolerates these conditions. Many of these species are rather nonspecific in regard to host plant, and are considered "early colonizers" occurring with many species of young trees.

Thelephora terrestris Fr., *Laccaria laccata* Peck, *Laccaria proxima* (Boud.) Pat, *Hebeloma mesophaeum* (Fr.) Quel., and *Inocybe lacera* (Fr.:Fr) Kummer have all been observed on smelter sites, in coal spoils, and with many species of young trees in open habitats. These same fungi have been observed with aspen on smelter sites in Kellogg, Idaho, and Trail British, British Columbia (Cripps 1996). Since aspen is often the pioneering species in smelter-impacted areas of Montana and Idaho, these fungi have a potential value for use in reclamation. In contrast, many species of ectomycorrhizal fungi are inhibited by low pH and high metal content in soils (Harris and Jurgensen 1977; Hung and Trappe 1983; McCreight and Schroeder 1982). Other mycorrhizal species are believed to ameliorate effects of heavy metals in plants (Hartley et al. 1997).

For birch, the succession of mycorrhizal fungi on a tree is predictable, with early stage fungi colonizing young seedlings, followed by the prevalence of late stage fungi with older trees (Last et al. 1987). The succession of mycorrhizal fungi on aspen in the study area appears to start with the early colonizers listed above, which are eventually replaced by fungi more restricted to aspen. Given aspen's clonal nature, microhabitat could play more of a role in species distribution. Early colonizers often occurred in young aspen stands or with young roots on the edge of older clones. Late colonizing fungi preferred the interior of aspen stands with a relatively well-developed soil and understory. It should be kept in mind, however, that fungal sporocarps are not necessarily indicative of the predominance of a fungus in the soil and on the plant roots.

Screening native mycorrhizal fungi as inoculum for aspen

Only a limited number of mycorrhizal fungi were examined for their effect on aspen seedlings because many of the species do not grow or grow well in culture. Others with a high potential for use as mycorrhizal inoculum such as *Hebeloma*, *Laccaria*, *Thelephora*, and some *Tricholomas* are yet to be tested. Although mycorrhizal fungi enhanced the growth of young aspen, sometimes remarkably so, with a two- to four-fold increase in biomass, this is not necessarily indicative of enhanced establishment and survival of aspen seedlings under natural conditions. Field and pot experiments need to follow this in vitro study to evaluate inoculated aspen as outplantings and in greenhouse conditions. Anselmi et al. (1990) did report a significant increase in aspen volume with fungal inoculation of most species in pot cultures. In our study, stem diameter and height increased with inoculation of about half of the fungal species. How growth parameters translate into increased fitness of aspen is another question.

What may not be obvious from our results is that each mycobiont affected the morphology of aspen in a recognizable manner for the given conditions. For example, inoculation with *Cenococcum* produced tall, pale seedlings with long, narrow leaves and long petioles. Aspen inoculated with *Tricholoma* had leaves that were two times as wide and long as the control, and plants in general were a deep rich green. Whether morphological changes produced by mycorrhizal fungi translate into form differences in older trees is not known but is an intriguing idea. The fact that various mycorrhizal fungi differ in their effect on aspen suggests that the physiology of each union is unique and that each fungus plays a particular role in the ecology of a host plant. For example, *Cenococcum* is known to tolerate drought conditions that inhibit other mycorrhizal fungi, and this fungus could be a crucial survival link in conditions of water stress. One could speculate that the diversity of fungi belowground in aspen stands enhances aspen's ability to survive a variety of conditions.

The percentage of mycorrhizal roots was not directly correlated with increases in aspen biomass, stem diameter, and height. The biomass of aspen increased substantially with addition of some fungi, but in most cases only a low percentage of roots were colonized in the given time period. This could be a result of high efficiency nutrient transfer through a small number of individual mycorrhizae or due to pre-mycorrhizal effects such as release of IAA. *Boletus piperatus* doubled the number of roots and increased the biomass of aspen seedlings without forming mycorrhizae, again suggesting a pre-mycorrhizal event such as hormone production by the fungus. *Pisolithus tinctorius* (PT) formed mycorrhizae quickly and extensively, covering the roots system in a few weeks and producing dark green healthy plants. PT is sold as a commercial inoculant, but is not native in Montana and Idaho and has failed in field trials in Oregon (Castellano and Trappe 1991). Inoculation with *Inocybe lacera* killed all the aspen seedlings, which could be due to an associated yeast or the high nutrient conditions that might increase its pathogenicity. *Inocybe lacera* typically occurs in sandy, nutrient-poor soil. The morphology of each type of mycorrhiza is unique and recognizable for each fungal species (Cripps and Miller 1995; Cripps 1997).

Nursery conditions can preclude or slow fungal colonization, since fertilizers are usually antagonistic to mycorrhizal formation. Mycorrhizae were slow to form in our study, and methods to speed up the process are necessary for commercial production. Some of the mycorrhizal inoculum tested produced aspen with discolored leaves, spotted black, red, and yellow. This is not a desirable quality for commercial plants, unless outplanting success can be proven to outweigh undesirable cosmetic problems. Another possibility is selecting a proper soil inoculum that could circumvent these problems (Helm and Carling 1990).

Native ectomycorrhizal fungi that are likely candidates for use in reforestation and reclamation with aspen are: *Paxillus vernalis, Tricholoma scalpturatum, Cenococcum graniforme* Fr.*,* and some yet to be tested (*Laccaria* spp., *Hebeloma mesophaem, H. populinum,* and *Thelephora terrestris)*. Care must also be taken in the nursery because *Pisolithus tinctorius* (Pers.) Coker & Couch and *Thelephora terrestris* have been known to adversely affect young plants, and proper timing for inoculation may be essential. It is also advantageous to know the soil type for outplantings. *Hebeloma* species are more likely to associate with young aspen under high fertility conditions such as lawns. Other fungi such as *Thelephora, Paxillus,* and *Cenococcum* may be more useful in heavy metal soils of low fertility.

Summary

Each aspen stand hosts a diverse community of mycorrhizal fungi as determined by soil type, age of the aspen stand, geographic region, and other edaphic and historical factors. Young aspen in pioneering situations, such as post-fire and smelter sites and previously unforested land, depend on "early stage" mycorrhizal fungi such as *Inocybe, Laccaria, Hebeloma, Thelephora,* and *Paxillus* for establishment and health. Their occurrence on the Butte-Anaconda smelter site also suggests a tolerance for heavy metals in some strains. Many of these "weedy" species of fungi also occur with young conifers. These are the fungi most likely to be of use in mined-land reclamation, and our results suggest they increase aspen biomass, height, and stem diameter in vitro. Further tests of outplantings are necessary to determine whether these mycorrhizal fungi

enhance establishment of aspen on actual mine sites. In older aspen stands, "late stage" mycorrhizal fungi make up a large part of the mycoflora, and these are species more closely allied with aspen than other tree species.

Soil type and other factors can affect the "succession" of mycorrhizal fungi. The impacts of various management strategies such as clear-cutting and fire on the mycorrhizal communities of aspen are not known. However, this should be given consideration, since management practices could apply selective pressures that promote certain species of mycorrhizal fungi, possibly to the exclusion of others, with long-term unintended consequences.

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