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There are three reports in this Volume:

GREENHOUSE POT STUDIES DEALING WITH AMENDATION OF OIL SAND TAILINGS: EFFECTS OF PEAT, SEWAGE SLUDGE AND FERTILIZER ON PLANT GROWTH, MYCORRHIZAE AND MICROBIAL ACTIVITY

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ABSTRACT

Carex and feather moss peats from northern Alberta were used as growth media for jack pine and slender wheatgrass and their microbiological properties assessed. Differences between the two peats were minimal. Following this assessment, mixtures of the two peats were used to amend oil sand tailings (55% peat, V/V) and either mineral fertilizers or sewage sludge were added at different rates. Slender wheatgrass and jack pine were grown in the greenhouse in the various growth media, and plant growth, microbial activity and mycorrhizal development were measured. Growth of both species was poor without the addition of either fertilizer or sewage. Fertilization up to the equivalent of 112:49:72 kg N:P:K ha^{-1} substantially increased shoot and root growth of slender wheatgrass. Shoot growth of jack pine reached a maximum at the 28:12:18 kg ha^{-1} rate, and pine roots weights were depressed when more than 56:24:36 kg ha^{-1} were applied. Maximum shoot growth of slender wheatgrass and jack pine occurred with the equivalent of 46 and 23 mT ha^{-1} sewage respectively. The addition of 92 mT ha^{-1} strongly depressed the growth of jack pine while slender wheatgrass was unaffected. In the absence of peat, slender wheatgrass was much more sensitive to sewage. VA mycorrhizal inoculum in the peat was sparse and high levels of fertilizer or the lowest level of sewage completely inhibited VA mycorrhizal infection. Ectomycorrhizal inoculum was abundant but infections were nil when more than 56:24:36 kg N:P:K ha^{-1} was applied and very strongly reduced when 23 mT ha^{-1} or more of sewage was applied. In the presence of slender wheatgrass, fertilizer increased microbial biomass but had no effect when no plants were present, i.e. fertilizer did not affect the decomposition of peat. Microbial activity (CO_2 efflux) was unaffected by the addition of fertilizer but decomposition of grass litter was reduced by high rates of fertilizer. When sewage was added to the growing medium, microbial activity and microbial biomass were increased and the decomposition potential was decreased. The presence of the fibrous rooted slender wheatgrass consistently inhibited the decay of grass litter as compared to unplanted systems.

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1. GENERAL INTRODUCTION

It is clear that in order to establish vegetation and soil microbial processes in mine tailings, it is necessary to add ameliorating materials. The particular types and amounts of amendments used will be dependent on the tailings characteristics, the availability of amendments, economic considerations and the chemical and biological properties of the amendments. In a previous field study, amendments were added in single, individual applications and plant growth (Visser et al., 1984a), microbial relations (Visser et al., 1984b) and mycorrhizal development (Danielson et al., 1984; Zak et al., 1984) were monitored for three to four growing seasons. From this study and from information garnered from operational procedures it was apparent that certain factors relating to high nutrient additions and to rates and combinations of amendments were worthy of careful study under more controlled conditions.

In the studies cited above, sewage sludge proved to be a highly effective amendment in promoting plant growth over a moderately long period of time. However, sewage sludge had adverse effects on some mycorrhizal relationships and on symbiotic N_2 fixation while increasing the rate of cellulose decomposition. How different application rates of sewage sludge or combinations of sludge with a recalcitrant source of organic matter affects these processes and plant growth is unknown.

Mineral fertilizers are easily applied to spoils but unless slow release types are used, repeated applications may be required to maintain satisfactory plant growth. A single application of fertilizer in the field study stimulated plant growth but a considerable portion of the nutrients were lost through leaching during the first growing season. High concentrations of fertilizers may be applied to partially counteract the leaching losses but such levels may inhibit formation of mycorrhizae and waste expensive fertilizers. It seems more reasonable to use low, noninhibitory fertilizer levels and aim at minimizing leaching losses by incorporating adsorptive amendments and maximizing root and mycorrhizal development.

In the field study, peat was a generally satisfactory organic amendment especially for woody plants. The primary deficiency of peat was the low level of available P which apparently limited the growth of some fibrous rooted species. It would appear that the combination of peat with a source of available P would result in a superior growth medium. However, rates of mineral P additions should not be so high as to inhibit mycorrhizal development or the growth of plants adapted to low nutrient regimes.

In view of the possible interactions among amendment combinations and possibilities of producing a planting medium with chemical and biological properties superior to single-amendment media a series of greenhouse pot studies were conducted. The intention was to supplement the data collected during the field study as well as to provide specific information applicable to future field studies on oil sand reclamation.

1.1. LITERATURE CITED

Danielson, R.M., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Ectomycorrhizae in amended oil sands and subalpine coal mine spoil and in undisturbed jack pine and spruce stands. Final Report to Research Management Division, Alberta Environment.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984a. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final Report to Research Management Division, Alberta Environment.

Visser, S., C. Griffiths and D. Parkinson. 1984b. Reinstatement of biological activity in severely disturbed soils: Effects of amendation and planting on the microbial development in three minespoils. Final Report to Research Management Division, Alberta Environment.

Zak, J.C., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Vesicular-arbuscular mycorrhizal development of slender wheatgrass on amended minespoils. Final Report to Research Management Division, Alberta Environment.

2. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH IN **CAREX** AND FEATHER MOSS PEATS FROM NORTHERN ALBERTA

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2.1. ABSTRACT

Slender wheatgrass and jack pine were grown in Carex and feather moss peats and a 1:1 mixture of the two peats in pots in the greenhouse. The three peat types were added to oil sand tailings at rates of 30, 55 and 80% (v/v) and all treatments were fertilized at a rate of 112:49:72 kg NPK ha^{-1}. Plant growth was only affected to a minor degree by either the type or quantity of peat. VA mycorrhizal infections were not detected and ectomycorrhizae were infrequent. Respiration was the same for all types but decomposition potential and microbial biomass were least in the Carex peat. The presence of slender wheatgrass increased respiration and microbial biomass and decreased the decomposition of roots in litter bags. It is recommended that, for future experiments, a mixture of peats be applied at a rate of 55% (v/v).

2.2. INTRODUCTION

Two basic types of peat deposits occur in northern Alberta, moss peat and Carex peat (Turchenek and Lindsay, 1978). Moss peats develop from sphagnum or feather mosses in bogs which are usually treed with black spruce and tamarack as well as having a ground cover of ericaceous shrubs. Carex peats originate in fens dominated by Carex species in open peat lands with few woody plants (Turchenek and Lindsay, 1978).

Differences in chemical and biological properties of these two peat types may affect their usefulness as amendments on extracted oil sand tailings. Vegetation on the undisturbed peat, as well as depth of the deposits, will affect the density and quality of mycorrhizal inoculum available for both VA and ectomycorrhizal hosts which may be planted on the extracted oil sands and overburden stockpiles. Microbial biomass, decomposition potential and nutrient levels in peat amendments may all affect nutrient release and retention and thus the economies of establishing and maintaining the necessary vegetation cover on areas in need of reclamation. This present study was conducted to determine if peat type should be considered in both experimental and operational reclamation situations.

The objectives of the study were: (1) to assess the effects of peat type on the growth of slender wheatgrass and jack pine in pots under greenhouse conditions, (2) to determine the effects of adding different amounts of peat to sand-silt-clay mixtures on plant growth and microbial activity, and (3) to determine how the presence or absence of plants in the peat-mineral mixtures affects microbial processes. Data obtained in this study will be used in planning additional greenhouse pot studies and will provide information for field studies.

2.3. MATERIALS AND METHODS

A sample of feather moss peat was obtained from a drained black spruce-tamarack bog on the Syncrude lease near Fort McMurray, Alberta. The sample of Carex peat was obtained from the Suncor site in the same vicinity. These peats plus a silt-clay mineral fraction from a Syncrude overburden deposit were mixed with extracted oil sand tailings from the Syncrude dyke to produce planting mixtures. The nine planting mixtures were composed of Carex or feather moss peat or a 1:1 (v/v) mixture of the two peats. These three peat types were then mixed into the mineral fractions to give final peat contents of 30, 55 and 80% (v/v). The mineral mixtures used were composed of tailing sand and silt-clay overburden at a ratio of 19:1 for the 30 and 55% peat applications and 4:1 for the 80% peat application. These mixtures of peat and mineral fractions are currently being recommended for the reclamation of oil sands tailings (P. Sims, pers. comm.).

The same amount of fertilizer was added to all nine treatments. The equivalent of 493 kg/ha of 23:23:0 and 146 kg/ha of 0:0:62 was used as these applications have been used in other reclamation studies (Visser et al., 1984). This amounted to 0.44 g of 23:23:0 and 0.13 g 0:0:62 per pot. The fertilizer was mixed into the planting mixtures and placed in 12.5 cm diameter pots. Three slender wheatgrass seeds or three pregerminated jack pine seeds were planted in two-thirds of the pots. The remaining pots were left unplanted but otherwise treated like the planted pots. Five replicate pots of each treatment (9 planting mixtures, 3 planting treatments) were prepared. In addition, 15 pots were prepared with autoclaved 80% mixtures and planted with jack pine to detect airborne ectomycorrhizal inoculum. The greenhouse temperatures varied from 18 to 25°C and supplemental light was used to give a 15 h day length with a minimum of 3.5 klx.

Prior to planting, each of the four components of the planting mixtures and each of the 9 planting mixtures were analyzed for key chemical properties. Organic matter content was estimated by determining loss on ignition at 400°C for 24 h, and pH was determined

in water. Total N, NO_3-N and extractable P was determined according to McKeague (1976).

The slender wheatgrass was harvested after 10 wk and the jack pine after 13 wk. The number of tillers of each slender wheatgrass plant were counted, the grain removed and grain and shoots dried at 80°C and weighed. Roots were washed free of planting mixture, subsampled for VA mycorrhizal evaluations, dried and weighed. Jack pine shoots were dried and weighed and the roots reserved for ectomycorrhizal evaluations prior to drying.

For VA mycorrhizal evaluations, slender wheatgrass roots were cleared and stained with .01% trypan blue (Phillips and Hayman, 1970). Roots were scanned for infections but not quantitatively evaluated. All the short roots of jack pine were rated for mycorrhizal infection in the 30 and 80% rate treatments. The number of short roots ranged from 41 to 611 per pot. Roots were examined at 12X and to confirm infections, whole mounts were examined with a 40X brightfield objective.

Following harvesting of the plants, basal respiration (CO_2 efflux) in each planting mixture was determined on samples placed in tubes connected to an "Ultragas 3" CO_2 analyzer (Wosthoff Company, Bochum, Germany). Microbial biomass C was determined on the same samples using the method of Anderson and Domsch (1978).

Decomposition potential of each planting mixture was estimated by determining the weight loss of slender wheatgrass roots in litter bags. One preweighed nylon litter bag (1.0 mm mesh) containing about 0.25 g of roots was buried in each pot prior to planting the plant species. When the plants were harvested, the litter bags were removed, cleaned, washed for 15 sec on each side, dried at 35°C and weighed.

2.4. RESULTS

The two peats were similar in pH, total N content and extractable P (Table 1). However, the Carex peat contained much higher levels of NO_3-N than the feather moss peat. The two mineral fractions were both low in organic matter and N. The pH of the planting mixtures were all similar and in a favorable range (Table 2).

Neither peat type nor rate of application affected the shoot growth of slender wheatgrass (Table 3). Root growth was uniformly superior with the 55% application. The number of tillers produced was least with plants that were grown in pots with 80% feather moss peat or the mixture (Table 4). Seed production was significantly less in the 30% Carex and feather moss peat treatments than in any of the other treatments.

Both shoot and root growth of jack pine seedlings were greater in planting mixtures containing Carex peat than with feather moss peat or a mixture of the two peats (Table 5). The rate of peat application also affected shoot and root growth with the 55% rate, allowing superior plant growth. About 10% of the pine seedlings in the feather moss and mixture treatments died of a damping off disease and attempts to replant the seedlings failed as new seedlings also died.

Basal respiration did not differ significantly among the three peats either in the absence or presence of plants (Table 6). In pots containing 30% peat, respiration was not affected by the presence of plants, whereas when 80% peat was used CO_2 efflux was significantly greater in the planting mixture with slender wheatgrass than with jack pine or in the unplanted mixture. Respiration was about 3 to 5-fold greater with the 80% treatment than with the 30% treatment.

Increasing the amount of peat from 30 to 80%, significantly increased the microbial biomass C 4 to 6 times (Table 7). When 80% peat was used, there was significantly less microbial biomass C in the Carex peat than in the feather moss peat or the mixture. At

Table 1. Characteristics of two peats, extracted oil sands and mineral amendment used to prepare planting mixtures.

	pH	Loss on ignition (%)	Total N(%)	NO_3-N $\mu g/g^{-1}$	Extractable P $\mu g/g^{-1}$
Feather moss peat	5.6	81	2.2	40	4.8
Carex peat	5.2	89	2.3	278	2.6
Silt-clay	7.4	5	.1	.5	2.6
Extracted oil sands	7.3	1	.01	1	1.6

Table 2. Amounts of peat added to pots and characteristics of the final mixtures prior to planting.

Peat type	% peat (v/v)	Field equivalent of dry peat (mT ha^{-1})	Final Mixture pH	Final Mixture % H_2O at 1/3 bar (wet wt.)	Final Mixture Loss on ignition (%)
Carex	30	25	6.0	12	3
Feather moss	30	21	5.9	12	4
Mixture	30	25	6.1	11	3
Carex	55	48	5.9	23	6
Feather moss	55	38	5.8	28	8
Mixture	55	45	6.0	21	10
Carex	80	69	6.1	49	21
Feather moss	80	54	5.8	56	30
Mixture	80	66	5.5	55	22

Table 3. Effect of peat type and rate of application on shoot and root growth of slender wheatgrass.

Rate of peat application (v/v)	Type of peat: Carex	Feather moss	Mixture	Row means
	Shoot weight (g)			
30%	3.6[a]	4.2[a]	4.3[a]	--
55%	4.3[a]	4.1[a]	4.7[a]	--
80%	4.2[a]	4.0[a]	3.8[a]	--
	Root weight (g)			
30%	1.0	1.4	1.6	1.4[a]
55%	1.9	1.7	1.7	1.8[b]
80%	1.2	1.1	1.2	1.2[a]
Column means	1.4[a]	1.4[a]	1.5[a]	

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values for shoot weights followed by the same letter not significantly different ($p = .05$). Values within root mean set or peat mean set followed by same letter not significantly different ($p = .05$).

Table 4. Effect of peat type and rate of application on tillering and seed production of slender wheatgrass.

Rate of peat application (v/v)	Type of Peat		
	Carex	Feather moss	Mixture
	Number of tillers per plant		
30%	10^{bc}	10^{bc}	12^{c}
55%	10^{bc}	9^{ab}	9^{ab}
80%	8^{ab}	7^{a}	7^{a}
	Seed weight per plant (mg)		
30%	22^{a}	35^{ab}	54^{abcd}
55%	73^{bcd}	76^{bcd}	43^{abc}
80%	88^{d}	80^{cd}	74^{bcd}

Data analyzed by two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values followed by the same letter are not significantly different ($p = .05$).

Table 5. Effect of peat type and rate of application on shoot and root growth of jack pine.

	Type of peat			
	Carex	Feather moss	Mixture	Row means
	Shoot weight (mg)			
30%	90	50	70	70[a]
55%	170	100	70	110[b]
80%	110	70	60	80[a]
Column means	120[b]	70[a]	70[a]	
	Root weight (mg)			
30%	19	13	14	15[a]
55%	31	24	21	25[b]
80%	21	12	11	15[a]
Column means	24[b]	16[a]	16[a]	

Data analyzed by two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values within each rate mean set or peat mean set followed by the same letter are not significantly different (P = .05).

Table 6. Effect of peat type and rate of application on basal respiration ($CO_2\uparrow$) of planting mixtures in pots planted with slender wheatgrass or jack pine or left unplanted.

Peat type	Application rate (%)	No plant	Slender Wheatgrass	Jack pine
		Respiration ($\mu l\ CO_2\uparrow\ 100\ g^{-1}$ of planting mixture h^{-1})		
Carex	30	46^{ab}	54^{ab}	53^{ab}
Feather moss	30	40^{ab}	82^{bcd}	57^{abc}
Mixture	30	34^{b}	67^{abc}	53^{ab}
Carex	80	117^{cde}	343^{fg}	199^{ef}
Feather moss	80	184^{ef}	356^{fg}	146^{de}
Mixture	80	244^{efg}	423^{g}	182^{ef}

Data could not be analyzed by using a three-way ANOVA due to significant interactions. Data in each planting treatment analyzed with a separate two-way ANOVA and differences among means tested by Scheffé pairwise comparisons. Values in each column followed by the same letter do not differ significantly ($p = .05$). Data was ln Y transformed prior to analysis.

Table 7. Microbial biomass C (mg C 100 g^{-1} mixture) in two types of peat and a mixture of the two peats and either planted with slender wheatgrass or jack pine or left unplanted. Data presented as overall treatment means[1].

Treatment	Rate of peat application 30%	80%
Carex peat	5.4[a]	21.9[a]
Feather moss peat	5.6[a]	32.8[c]
Mixture of peats	5.9[a]	37.0[c]

	Rate of peat application 30%	80%
No plants	4.0[a]	24.0[c]
Slender wheatgrass	10.0[b]	55.7[d]
Jack pine	4.4[a]	19.9[c]

	No plant	Slender Wheatgrass	Jack pine
Carex peat	7.0[a]	20.9[c]	8.8[ab]
Feather moss peat	11.5[b]	22.3[c]	9.7[ab]
Mixture of peats	11.8[b]	28.3[c]	9.5[ab]

[1] Data analyzed by 3-way ANOVA and no significant 3-way interactions were present. Each pair of parameters analysed by 2-way ANOVA and differences among overall treatment means compared with Scheffé pairwise comparisons. Values within each of the three sets not significantly different ($p = .05$) if followed by the same letter. Data ln Y transformed for analysis, values geometric means.

both the low and high rates of application of all peat types, the greatest amount of microbial biomass C was found in the planting mixtures in which slender wheatgrass had been grown. The presence of jack pine did not affect the amount of microbial biomass C. In the absence of plants the microbial biomass C was least in the Carex peat treatment but when plants were present, peat treatment had no effect.

The presence of slender wheatgrass roots significantly inhibited root decomposition in all three peat treatments (Table 8). The type of peat used in the planting mixtures affected the rate of decomposition, with the roots in the litter bags buried in the Carex treatment decomposing slower than those in the feather moss treatments. However all differences were relatively small.

Observations on the VA mycorrhizal status of slender wheatgrass roots indicated that there were no infections in any treatment. Mycorrhizal development of jack pine was very low and sporadic. No infection was detected in the 30% application rate in any of the three peat treatments nor in the autoclaved treatments. In the 80% Carex peat treatment, one pot contained heavily infected roots but the symbiont was Thelephora terrestris Ehrhard ex Fr. and it is assumed that the infection was due to airborne contamination. One other pot in the 80% carex treatment had infected roots but only 2% of total short roots were mycorrhizal.

In the 80% feather moss and mixture treatments 2% of the short roots were mycorrhizal and 8 of the 10 trees had some infection. All of the symbionts appeared to be Basidiomycetes except for one occurrence of the I-type Ascomycete.

Table 8. Decomposition of slender wheatgrass roots in litter bags buried in three types of peat planted with slender wheatgrass or jack pine or left unplanted.

	Peat type			
	Carex	Feather moss	Mixture	Row
Plant treatment	Percent weight remaining			means
No plants	61	49	52	54[a]
Slender wheatgrass	68	64	67	66[b]
Jack pine	57	46	47	50[a]
Column means	62[b]	53[a]	55[ab]	

Data analyzed with a two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within planting mean set or peat mean set followed by the same letter not significantly different ($p = .05$).

2.5. DISCUSSION

Although the two peats were of quite different origins, in the short term greenhouse study conducted here, differences in plant growth responses were minor. Differences may have been minimized by the application of fertilizer to all treatments although the growth of jack pine was 70% greater with the Carex peat than with the feather moss peat. Regardless, the jack pine seedlings were very small in all treatments considering the amount of fertilizer applied (Part 3) and differences should not be overemphasized. The feather moss peat contained damping off organisms which may have contributed to the poor growth of jack pine in this treatment. VA mycorrhizae were absent and ectomycorrhizae were poorly developed in both peat types. It appeared that ectomycorrhial inoculum was more abundant in the feather moss peat than in the sedge peat, but infections were too sporadic to make definite conclusions. The low level of ectomycorrhizal infection and the lack of VA infection in this experiment was due to suppression by the fertilizer applied (Part 3).

Basal respiration did not differ among the peat types but decomposition rates and microbial biomass were both least in the Carex planting mixture. The lack of differences in CO_2 efflux would suggest that both peats were of similar stability and would persist for equal lengths of time if used to amend tailings sand.

Growing slender wheatgrass in the mixtures enhanced respiration and increased the microbial biomass present. However, decomposition of introduced root material was inhibited by the presence of slender wheatgrass. Jack pine did not affect these factors, probably because of the much smaller root systems.

In general, no peat type or rate of application was clearly superior to the others. For greenhouse studies the medium (55%) rate of application is a reasonable choice. Although the mycorrhizal factor could not be evaluated, the use of a mixture of the two peat types would offer the best opportunity for including a variety of inoculum. Thus it is recommended that a 55% mixture of the carex and feather moss peats be used in future experiments.

2.6. LITERATURE CITED

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10: 215-221.

McKeague, J.A. 1976. Manual on Soil Sampling and Methods of Analysis. Prepared by the Canadian Society of Soil Science, Soil Research Institute, Research Branch, Agriculture Canada, Ottawa.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.

Turchenek, L.W. and J.D. Lindsay. 1978. Interim report on a soils inventory in the Athabasca Oil Sands area. Prepared for the Alberta Oil Sands Environmental Research Program by Alberta Research Council, Soils Div. AOSERP Rept. 28.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final report to Research Management Division, Alberta Environment.

3. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH RESPONSES TO FERTILIZATION OF PEAT AMENDED OIL SAND TAILINGS

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3.1. ABSTRACT

Extracted oil sands were amended with 55% (v/v) peat and fertilized with a fertilizer equivalent to 112:49:72 kg NPK ha^{-1} and 1/2, 1/4, 1/8 and 0 fractions of that rate. Slender wheatgrass and jack pine were grown in the greenhouse for 10 and 15 wk respectively in each of the five fertilizer regimes. Root and shoot weight of slender wheatgrass increased with each incremental addition of fertilizer whereas growth of jack pine reached a maximum with 1/4 of the highest rate. Further additions reduced root growth of jack pine while shoot growth remained constant. Mycorrhizal formation by jack pine also decreased substantially above the 1/4 level. Less than 2% of the roots of slender wheatgrass were infected in the control and 1/8 treatment and none with the high fertilizer. Respiration and microbial biomass were unaffected by fertilizer additions in the absence of plants but decomposition potential was reduced with the highest level of fertilizer. In the presence of slender wheatgrass, moderate levels of fertilizer significantly increased microbial biomass while the high fertilizer application resulted in a level of microbial biomass similar to the control. Decomposition potential was less when slender wheatgrass was present than in unplanted pots.

3.2. INTRODUCTION

Fertilizer is the most common amendment used in operational reclamation procedures as it is felt that the addition of nutrients is a prerequisite for the establishment of plants. However, fertilizer may have less favorable effects on other components of the system, in particular, below ground processes. Preliminary observations suggested that normal rates of fertilization can depress microbial activity (Visser et al., 1984) which may affect decomposition of litter and nutrient cycling.

The sensitivity of mycorrhizal symbioses to P is well known and fertilization may substantially inhibit mycorrhiza formation (Gerdemann, 1975). As pointed out by Rhodes (1980), the use of a P fertilizer may cause a suppression of VA mycorrhizae and, although P is abundant, micronutrient deficiencies may develop as the soil is inefficiently exploited by nonmycorrhizal root systems. On the other hand, when P levels are very low, mycorrhizal formation may also be inhibited (Mosse et al., 1981). Thus fertilization may either positively or negatively affect mycorrhizae and the specific effect is difficult to predict as both host and symbiont are determining factors (Mosse et al., 1981). Presumably, symbionts are adapted to nutritional conditions of the soil in which they exist (or more precisely, the nutritional status including carbohydrates and P levels of the host roots), but once an area has been severely disturbed, the nutritional status of the substrate is changed. Also, if nonindigenous hosts are also introduced, the symbionts indigenous to the undisturbed habitat may be less well adapted than species that could be introduced. It is of interest then, to determine how indigenous symbionts react to reclamation practices such as fertilization.

As concern has been expressed over the loss of C from peat amendments and the possible deterioration of reclaimed spoils (see discussion in Ziemkiewicz et al., 1980) it is of interest to determine the effects of nutrient enrichment on the rate of peat decomposition. By manipulation of fertilizer levels, it may be possible to use this valuable resource more economically and efficiently.

The objectives of this greenhouse pot experiment using peat amended oil sand tailings were to determine (1) the effects of different levels of application of NPK fertilizer on primary production, (2) the effects of fertilizer on the rates of decomposition of a standard introduced substrate (decomposition potential), (3) the effect of fertilizer and plants on the CO_2 efflux from the planting mixture, (4) the effect of fertilizer and plants on microbial biomass and (5) the effect of different amounts of fertilizer on VA and ectomycorrhizal development.

3.3. MATERIALS AND METHODS

The experimental materials used were similar to those used in the previous experiment (Part 2). One substrate mixture was used for all the treatments, a 1:1 mixture of Carex peat and feather moss peat plus 45% (v/v) mineral additive. The mineral portion was a 19:1 (v/v) mixture of extracted tailings sand and silt-clay overburden. Fertilizer was added at a top rate of 493 kg/ha of 23:23:0 and 146 kg/ha of 0:0:62 (field equivalent) and three fractions thereof plus a nonfertilized treatment. The amount of fertilizer added per pot and the treatments were: 1x = .44 g 23:23:0 + .13 g 0:0:62 and 1/2, 1/4 and 1/8 of these amounts. Each of these fertilizer combinations and a control (zero fertilizer) were mixed with the planting mixture and placed in 15 12.5 cm diameter pots per fertilizer level. Five pots of each fertilizer level were planted with three slender wheatgrass seeds per pot, five pots with three pregerminated jack pine seeds per pot and the remaining five pots were left unplanted. Thus the final treatments included five fertilizer rates and three planting treatments. In addition, five pots were filled with unfertilized planting mixture, autoclaved and planted with jack pine seeds to detect air-borne ectomycorrhizal inoculum.

The slender wheatgrass was harvested after 10 wk and the jack pine after 15 wk. Tillers of slender wheatgrass were counted, the seed removed and leaves and seed dried at 80°C and weighed. Roots were washed, subsampled for VA mycorrhizal evaluations, and dried and weighed. Jack pine shoots were dried and weighed and the roots washed and evaluated for mycorrhizal infection prior to determining dry weights.

Slender wheatgrass roots in the control, 1/8x and 1x treatments were cleared and stained with .01% trypan blue (Phillips and Hayman, 1970) and evaluated according to Zak and Parkinson (1983). Jack pine roots in all treatments were cut into 2-3 cm segments and the segments randomly sampled until 300 short roots had been rated for infection (Part 2).

Following harvesting of plants, the basal respiration of the spoil mixtures was determined with an "Ultragas 3" CO_2 analyzer

(Wosthoff Company, Bochum, Germany). Microbial biomass C was determined on the same samples using the glucose stimulated respiration method of Anderson and Domsch (1978).

Decomposition potential was estimated by placing approximately .25 g of slender wheatgrass leaves in 1.0 mm mesh nylon litter bags and burying one preweighed bag per pot prior to planting the test plants. When the plants were harvested, the litter bags were retrieved, cleaned, dried at 35°C and weighed.

3.4. RESULTS

Slender wheatgrass shoots and roots showed a clear and consistent response to increasing levels of NPK mineral fertilizer (Table 1). Seed production as well as the number of tillers per plant also responded to the fertilizer applications. Roots and shoots responded relatively uniformly as indicated by the shoot/root ratios. In the absence of any fertilizer, the peat-tailing sand mixture supported little growth of the grass and no reproductive capability as no flowers were formed. In all the other treatments, flowers were formed 7 wk after planting.

Jack pine shoot and root growth reached maximum weights with the 1/8x to 1/4x application rates (Table 2). Shoot growth did not change when more than 1/4x fertilizer was added. However, root growth was depressed by rates exceeding 1/4x such that shoot/root ratios increased dramatically. The reduction in root weights at high fertilizer levels also resulted in a decrease in the number of short (feeder) roots. Autoclaving the planting mixture did not significantly affect the growth of jack pine.

The amount of VA infection was very low in the control (zero fertilizer treatment), occurring only in one pot (Table 3). In the 1/8x treatment, infection occurred in four of the five pots but only small portions of the root systems were mycorrhizal. All infections were caused by a coarse endophyte. Total root lengths were markedly stimulated by fertilization although the rate of application did not result in different lengths.

Rates of ectomycorrhizal infections of jack pine were not significantly affected by additions of fertilizer except with the 1x treatment (Table 4). The major symbionts were unknown Basidiomycetes. It was not possible to determine if this group included one or more taxa. It appeared that the second most common group, termed white dichotomous, was sensitive to fertilizer additions as it was not encountered in the fertilizer treatments. The only two types that could be recognized were the Rhizopogon-like symbiont and the I-type Ascomycete, both of which were rare. The

Table 1. Growth of slender wheatgrass in peat amended with different amounts of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

	Fertilizer level				
Parameter	0	1/8x	1/4x	1/2x	1x
Shoot weight (g)[1]	.34[a]	1.78[b]	2.36[c]	3.54[d]	4.26[e]
Root weight (g)[1]	.27[a]	1.28[b]	1.56[c]	2.02[d]	2.27[e]
Seed weight (g)[2]	0	.09[a]	.39[b]	.77[c]	.74[c]
Shoot/root ratio	1.3	1.4	1.5	1.8	1.9
Tillers $plant^{-1}$	2.6[a]	4.9[b]	6.2[c]	7.6[d]	9.7[e]

[1] ln Y transformation required.

[2] ln (Y + 1) transformation required.

Data analyzed by one-way ANOVA and differences tested with Scheffé pairwise comparisons. Values in each row followed by the same letter not significantly different (p = .05).

Table 2. Growth of jack pine in peat amended with different amounts of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

Parameter	Fertilizer level 0	1/8x	1/4x	1/2x	1x	Auto-claved
Shoot weight (mg)	113^{a}	271^{bc}	384^{c}	398^{c}	378^{c}	185^{ab}
Root weight (mg)	74^{ab}	153^{c}	187^{c}	113^{abc}	69^{a}	130^{b}
Shoot/root ratio	1.5^{ab}	1.8^{ab}	2.1^{b}	3.5^{c}	5.4^{d}	1.4^{a}
Short roots $plant^{-1}$	340^{ab}	415^{abc}	661^{c}	354^{abc}	231^{a}	603^{bc}

Data ln Y transformed and analyzed by one-way ANOVA and differences tested with Scheffé pairwise comparisons. Values in each row followed by the same letter not significantly different ($p = .05$).

Table 3. VA mycorrhizal infection and root lengths of slender wheatgrass fertilized at two rates and unfertilized (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Control	1/8x Fertilizer	1x Fertilizer
Total root length (m l^{-1})	92[a]	489[b]	443[b]
Mycorrhizal root length (m l^{-1})			
Total	1.9	7.9	0
With hyphae	1.7	6.3	0
With vesicles	.03	.06	0
With arbuscules	.2	1.6	0
Percent infection ($\bar{x} \pm$ SD)	1.6±3.6	1.8±2.0	0

Root length data ln Y transformed prior to analysis by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter not significantly different (p = .05).

Table 4. Percent mycorrhizal infection of jack pine seedlings growing in peat with different levels of fertilizer (1x equivalent to 112:49:72 kg N:P:K ha^{-1}).

Infection	Fertilizer level									
	0		1/8x		1/4x		1/2x		1x	Autoclaved
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD		
Total	49[a]	29	47[a]	23	42[a]	29	16[a]	16	0	0
Unknown types	28	17	46	23	29	26	12	17	0	0
Hyaline basidiomycete	21	26	0	0	0	0	0	0	0	0
Rhizopogon-like	.3	.5	.4	.9	.3	.8	0	0	0	0
I-type ascomycete	0	0	1.1	2.4	12	27	4	8	0	0

Data analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly ($p = .05$).

Rhizopogon-type infected only 1-2% of the short roots of seedlings it colonized and the I-type infected 5 to 61% of the short roots. Roots in the 1x and autoclaved treatments were uninfected.

Basal respiration of mixtures planted with slender wheatgrass or jack pine did not differ from unplanted mixtures (Table 5). Fertilization had no effect on respiration of peat planted with slender wheatgrass. In peat planted with jack pine, respiration was significantly greater with the 1/8x fertilizer treatment than with the high application rate.

Ten and 15 wk after initiation of the experiment, microbial biomass C did not differ significantly among the unplanted treatments (Table 6). However, in peat planted with slender wheatgrass microbial biomass C was significantly higher in the 1/8x, 1/4x and 1/2x fertilizer treatments than in the control and the 1x treatment. The latter two treatments did not differ significantly from the unplanted peat. Fertilization did not affect amounts of microbial biomass C in peat planted with jack pine. Fertilization of slender wheatgrass resulted in increased amounts of microbial biomass C in all fertilized peats as compared with peats planted with jack pine although the jack pines were 5 wk older than the slender wheatgrass.

The presence of slender wheatgrass plants resulted in less decay of buried slender wheatgrass leaves than in unplanted peat (Table 7). Fertilization at the 1x level resulted in a decrease of decomposition potential in the peat planted with slender wheatgrass. Decomposition had apparently slowed considerably by 10 wk as there were no differences in weight remaining between 10 and 15 wk except in the 1x fertilizer treatment.

Table 5. Basal respiration of peat amended with different amounts of fertilizer and planted with slender wheatgrass, jack pine or left unplanted. Values are $\mu l\ CO_2 \uparrow 100\ g\ h^{-1}$ growing medium (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Fertilizer level					Row
Treatment	0	1/8x	1/4x	1/2x	1x	means
Control (10 wk)	117	96	96	106	96	102[a]
Slender wheatgrass	115	124	112	121	121	119[b]
Column means	116[a]	110[a]	104[a]	114[a]	109[a]	
Control (15 wk)	96	112	128	112	96	109[a]
Jack pine	102	140	115	96	64	103[a]
Plant mean	99[ab]	126[b]	122[ab]	104[ab]	80[a]	
Slender	115[ab]	124[ab]	112[ab]	121[ab]	121[ab]	
Jack pine	102[ab]	140[b]	115[ab]	96[ab]	64[a]	

Data analyzed by two-way ANOVA and differences tested using Scheffé pairwise comparisons. Values within each plant mean set or fertilized mean set followed by the same letter do not differ significantly ($p = .05$). For jack pine, slender wheatgrass comparisons, values followed by the same letter do not differ significantly ($p = .05$).

Table 6. Microbial biomass C in peat amended with different amounts of fertilizer and planted with slender wheatgrass or jack pine or left unplanted. Values are mg C 100 g h^{-1} growing medium (1x equivalent to 112:49:72 kg NPK ha^{-1}).

	Fertilizer level				
Treatment	0	1/8x	1/4x	1/2x	1x
Control (10 wk)	10.1^{a}	12.0^{a}	12.3^{a}	11.0^{a}	8.4^{a}
Slender wheatgrass	10.6^{a}	18.9^{b}	17.7^{b}	17.5^{b}	16.2^{a}
Control (15 wk)	7.4^{ab}	13.1^{ab}	13.8^{b}	10.6^{ab}	7.4^{ab}
Jack pine	9.3^{ab}	10.6^{ab}	9.3^{ab}	10.1^{ab}	7.8^{a}
Slender wheatgrass	10.6^{a}	18.9^{b}	17.7^{b}	17.5^{b}	16.2^{b}
Jack pine	9.3^{a}	10.6^{a}	9.3^{a}	10.1^{a}	7.8^{a}

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each set followed by the same letter do not differ significantly ($p = .05$).

Table 7. Decomposition of slender wheatgrass leaves buried in peat amended with different amounts of fertilizer. Values are percent weight remaining (1x equivalent to 112:49:72 kg NPK ha^{-1}).

Treatment	Fertilizer level 0	1/8x	1/4x	1/2x	1x	Row means
No plants (10 wk)	17	16	14	17	27	18[a]
Slender wheatgrass	16	20	25	24	34	24[b]
Column mean	16[a]	18[a]	20[a]	21[a]	31[b]	
No plants (15 wk)	15	18	14	14	15	15[a]
Jack pine	17	14	14	11	15	14[a]
Plant mean	16[a]	15[a]	12[a]	14[a]	16[a]	
No plant (10 wk)	17	16	14	17	27	
No plant (15 wk)	15[a]	18[a]	14[a]	14[a]	15[a]	

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each plant mean or fertilizer mean followed by the same letter not significantly different ($p = .05$). For the time comparison, values followed by the same letter do not differ significantly ($p = .05$).

3.5. DISCUSSION

Fertilization with a mineral fertilizer resulted in quite different growth response patterns with the two plant species. Slender wheatgrass positively responded to all levels applied with a maximum 12-fold increase in shoot growth over growth in the unfertilized control. In contrast, jack pine increased a maximum of 3.5-fold and shoot growth did not increase above a rate equivalent to 28:12:18 kg NPK ha^{-1} (1/4x). It is unknown whether similar results would occur with a top dressing of fertilizer in contrast to the incorporation technique used here. Clearly, planting slender wheatgrass rather than jack pine on oil sand would result in a more rapid accumulation of nutrients in plant biomass and litter. In a sandy spoil this would result in a conservation of fertilizer nutrients that are subject to leaching losses. As decomposition (of buried leaves) was not affected by nutrient levels, it might appear that increased fertilization would lead to increased litter accumulation. However, this might not be true as substrate quality will change with soil nutrient levels and affect decomposition rates.

Of considerable interest was the strong depression of root growth of jack pine by high nutrient levels, a feature not shown in shoot growth. This depression of root growth could result in reduced ability to survive under stress conditions on amended oil sand tailings. High levels of fertilizer resulted in small root systems and plants with high shoot/root ratios. During drought conditions, large shoot/root ratios may result in water deficits due to large transpirational areas and small absorptive surfaces. This would be further accentuated as high fertilizer levels resulted in a complete suppression of mycorrhizal development. The absence of mycorrhizae has been shown to restrict water transport to roots due to the lack of rhizomorphic "pipelines" (Duddridge *et al.*, 1980). VA mycorrhizal inoculum was apparently quite low and even when plants became infected the infections were not extensive. This suggests that spread through the root system was slow as was observed for some

ectomycorrhizal symbionts, especially the Rhizopogon-like symbiont. In order to obtain high levels of infection of VA hosts growing in materials such as used here, inoculum must be increased substantially. However, this would be futile if large amounts of fertilizer are used which would suppress infection.

Increasing the level of N, P and K generally did not affect basal respiration of the peat. This would infer that fertilization will not result in increased loss of peat-C and accelerate organic matter losses. A large portion of the peat is apparently composed of recalcitrant C compounds which decay very slowly even when N, P and moisture are abundant. However the presence of a fibrous rooted plant such as slender wheatgrass appeared to accelerate the loss of peat C. The increased CO_2 efflux from peat planted with slender wheatgrass as compared with unplanted peat could be due to a priming effect of the root exudates or it might be an artifact resulting from the inability to remove very fine root fragments. As the existence of a priming effect appears to be doubtful, the cause of increased CO_2 output remains to be determined.

In unplanted spoil, amounts of microbial biomass C was not affected by fertilization just as basal respiration was unaffected. However, when planted with slender wheatgrass, there was an increase in microbial biomass when moderate amounts of fertilizer were applied. It appears likely that the increased biomass was the result of utilization of root exudates by the rhizosphere and soil microflora (Visser et al., 1984). The amount of roots was very small with the slender wheatgrass control and all the jack pine treatments, resulting in small amounts of exudates available in the rhizosphere for microbial growth. The reduced amounts of microbial biomass at the higher fertilizer level was probably due to a fertilizer induced inhibition of microbial growth (Kowalenko et al., 1978). In all three pot systems, grass, pine and unplanted, there was a trend toward less microbial biomass as fertilizer levels increased above the 28:12:18 kg NPK ha^{-1} (1/4x) application rate. It might be expected that this would also result in a decrease in the amount of

decomposition as was determined in the slender wheatgrass treatments. The apparent lack of effects with jack pine was probably due to the long burial period. By 10 wk, most of the leaves had been decomposed to the extent that only the most resistant and stable portions remained. Clearly organic matter (leaf litter) decomposition was inhibited both by the presence of a fibrous rooted plant and by high levels of mineral nutrients.

3.6. LITERATURE CITED

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215-221.

Duddridge, J.A., A. Malibari and D.J. Read. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287:834-836.

Gerdemann, J.W. 1975. Vesicular-arbuscular Mycorrhizae. The Development and Function of Roots, eds. Torrey and Clarkson. Academic Press, London.

Kowalenko, C.G., K.C. Ivarson and D.R. Cameron. 1978. Effect of moisture content, temperature and nitrogen fertilization on carbon dioxide evolution from field soils. Soil Biology and Biochemistry 10:417-423.

Mosse, B., D.P. Stribley, and F. LeTacon. 1981. Ecology of mycorrhizae and mycorrhizal fungi. Advances in Microbial Ecology, ed. M. Alexander. Plenum Publishing Corporation, New York, p. 137-210.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-161.

Rhodes, L.H. 1980. The use of mycorrhizae in crop production systems. Outlook on Agriculture 10:275-281.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final report to Research Management Division, Alberta Environment.

Zak, J. and D. Parkinson. 1982. Initial VA mycorrhizal development of slender wheatgrass on two amended mine spoils. Canadian Journal of Botany 60: 2241-2248.

4. EFFECTS OF PHOSPHORUS ADDED TO PEAT ON THE GROWTH OF ALSIKE CLOVER AND ON MICROBIAL PARAMETERS

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4.1. ABSTRACT

A P deficient peat was amended with 272 μg P g^{-1} peat, 544 μg P g^{-1} peat or left unamended. Pots were planted with alsike clover and grown for either 6 or 12 wk in the greenhouse. Added P increased the growth of shoots, roots and increased flower production. No differences in growth were apparent between the two levels of P. Decomposition of filter paper buried in the peat was enhanced with added P with the most rapid rate occurring in the high P peat. With increasing levels of P, respiration (CO_2 efflux) and microbial biomass C both decreased significantly. Phosphorus levels did not affect the number of nodules on the roots or their ability to fix N_2 (C_2H_2).

4.2. INTRODUCTION

Peat obtained from muskeg sites is a standard amendment used to improve the physical and chemical properties of extracted oil sands following the surface mining of bitumen bearing deposits in northern Alberta. Peat provides a long term source of nutrients for plant growth and improves the water holding properties of spoils, thus enhancing the development of maintenance-free soils that are required by provincial guidelines.

In a trial of a variety of amendments to determine their value in reestablishing biological activity in three contrasting types of minespoils (Visser et al., 1984a, b; Danielson et al., 1984; Zak et al., 1984) a peat type obtained from a forested site was used to ameliorate the stressful conditions. The obvious characteristics of this peat were an unusually high content of NO_3-N, a moderate pH and a very low level of available P (Visser et al., 1982a). As P appears to be a regulating factor in plant growth and microbial processes, and that P is often available in only small quantities in peat (Brady, 1974) it appeared to be worthwhile to investigate the effects of P availability in detail.

Over a three year period it was observed that the growth of alsike clover did not differ on a subalpine minespoil and on the same spoil heavily amended with the feather moss peat described above (Visser et al., 1984a). A chemical analysis of the peat showed that the peat was very deficient in extractable P with less than 0.5 g extractable P per gram dry peat. It was therefore postulated that the lack of a growth response of alsike clover was due to the low level of available P. The objectives of this study were to determine (1) the effects of added P on the growth of alsike clover, and (2) the effect of added P on microbial biomass, basal respiration of the peat, decomposition potential and N_2 fixation potential of the clover.

4.3. MATERIAL AND METHODS

The peat was collected from unplanted pathways in the experimental tanks established 3 years previously (Visser et al., 1984a). The peat had been removed from a white spruce site 3 years previously and spread on a subalpine mine spoil. The peat was divided into 30 716g portions and moistened with water containing calcium phosphate, monobasic or super phosphate. Ten samples received no P, 10 received 272 μg P g^{-1} peat, and 10 received 544 μg P g^{-1} peat. The 272 μg P level was the same level that was applied in the field tank study (Visser et al., 1984a). After adding the P, the peat samples were left to equilibrate for 3 days in plastic bags. Then each bag of peat was emptied into a 12.5 cm plastic pot and a litter bag containing filter paper was placed in a vertical position in the middle of the pot. Each pot was planted with five 4 day old alsike clover seedlings and the pots were placed in the greenhouse in a complete random block design. The pots were kept at a constant moisture level by watering daily with distilled water to a specific weight.

After 6 and 12 wk, five pots of each P treatment were harvested and shoots, roots and flowers dried at 80°C and weighed. Filter papers were removed, washed, dried at 35°C and weighed to determine decomposition potential. Basal respiration (CO_2 efflux) and microbial biomass C of the root-free peat was determined using a Wosthoff CO_2 analyzer and the Anderson and Domsch (1978) technique. The number of root nodules was estimated by taking a subsample of the total root weight in each pot, spreading the roots in lactophenol on a slide, covering them with a coverslip and then counting the nodules under a dissecting microscope at 20x magnification. Prior to removing the plants from the peat, the peat plus plants minus the pot were placed in plastic containers, sealed and assessed for N_2 (C_2H_2) fixation capacity using the technique outlined by Visser et al. (1984b). Prior to injecting acetylene into the containers holding the plants, the plants were tested for natural ethylene production. Also unplanted peat was tested for asymbiotic N_2

(C_2H_2) fixation. No measureable ethylene was produced by the plants after one hour incubation. The low level of asymbiotic N_2 (C_2H_2) which was measured was subtracted from the N_2 (C_2H_2) fixation measured for each pot.

4.4. RESULTS AND DISCUSSION

Data on the effects of added P on alsike clover growth over the two sampling periods are presented in Table 1. These results show that there was an interactive effect of time and P level on shoot and root growth i.e., plant growth was not constant over the two sampling periods. When the treatment means were analyzed separately, it was observed that shoot, root and total plant weight were significantly greater in the P treated peat than in the control peat indicating that P was indeed limiting. However, there was no difference in plant weight produced at the low P level compared with that produced at the high P level demonstrating that the lower level of P would have been adequate over the short term. In all three treatments, there was a significant increase in shoot, root and total plant weight between 6 and 12 wk.

Results in Table 2 demonstrate that flower production was enhanced by the addition of P to the peat, but as for the roots and shoots, there was no difference in the weight of flowers produced at the two levels of P. Also the weight of flowers produced did not increase significantly from the first to the second sampling time. It is interesting to note that no flowers were produced by plants in the control pots, indicating the significance of adequate P availability to flowering and seed formation. The lack of seed production due to low P levels, would undoubtedly affect reseeding of alsike clover if the growing season was short (i.e. 3-4 months).

The decomposition of filter paper was enhanced by the addition of P to the peat (Table 3). This result indicates that the microorganisms involved in cellulose decomposition are, like the plants, limited in their activities by low P levels. As a result, low P levels in an amendment such as peat could markedly affect decomposition rates of incoming litter such as dead roots and shoots if these substrates were also low in available P.

The increased decomposition of filter paper in P-amended peat is interesting when the data in Table 4 are studied. These results show that with increasing levels of P, microbial biomass C

Table 1. Shoot weight, root weight and total plant weight of alsike clover grown in pots containing peat with and without added phosphorus.

	Time (wk)	Phosphorus added ($\mu g\ g^{-1}$ peat) 0	272	544
		Dry weight (g pot^{-1})		
Shoot wt	6	0.14[a]	1.16[b]	.85[b]
	12	1.02[b]	3.33[c]	3.46[c]
Root wt	6	0.04[a]	0.26[b]	0.23[b]
	12	0.42[b]	0.56[c]	0.51[c]
Total plant wt	6	0.18[a]	1.42[b]	1.08[b]
	12	1.44[b]	3.89[c]	3.97[c]

Values in each data set were analyzed with a two-way ANOVA after ln transformation. Scheffé confidence intervals were computed for treatment means. Values within each data set followed by the same letter are not significantly different ($p = 0.05$).

Table 2. Flower production by alsike clover grown in pots containing peat with and without added phosphorus.

Time (wk)	Phosphorus added ($\mu g\ g^{-1}$ dry peat) 0	272	544	Row means
	Dry weight ($g\ pot^{-1}$)			
6	0	.089	.036	.063[a]
12	0	.121	.094	.108[a]
Column means	0[b]	.105[a]	.065[a]	

By applying the Chi-square goodness of fit test for presence or absence of number of flowers produced pot^{-1}, the no phosphorus treatment plants produced a significantly lower number of flowers (no flowers) over each time period than the phosphorus treated plants. Hence the no phosphorus data was deleted from the two-way ANOVA.

Data for the phosphorus treatments were analyzed with a two-way ANOVA. Values in the amendment mean column or time mean row followed by the same letter do not differ significantly ($p = 0.05$).

Table 3. Decomposition of cellulosic filter paper in pots containing alsike clover grown in peat with and without added phosphorus.

	Sampling Time (wk)	Phosphorus level 0	272	544
% dry wt remaining of filters	6	79.2[a]	45.5[b]	21.8[c]
% dry wt remaining of filters	12	3.7[a]	1.3[b]	0.7[b]

Data at each sampling time were analyzed using a one-way ANOVA and Scheffé confidence intervals. Values in each row followed by the same letter do not differ significantly ($p = 0.05$).

Table 4. Basal respiration and microbial biomass C in peat with and without added phosphate after removal of alsike clover roots.

	Time (wk)	Phosphorus level ($\mu g\ g^{-1}$ peat) 0	272	544	Row means
Basal respiration ($\mu l\ CO_2 \uparrow 100\ g^{-1}$ dry peat hr^{-1})	6	98	54	35	62[a]
	12	111	57	35	68[a]
Column means		104[a]	55[b]	35[c]	
Microbial biomass C (mg C 100 g^{-1} dry peat)	6	303	216	155	225[a]
	12	302	228	168	232[a]
Column means		302[a]	222[b]	161[c]	

Data analyzed with a two-way ANOVA. Scheffé confidence intervals were computed for row and column means. Values within the time mean column or treatment mean row followed by the same letter do not differ significantly ($p = 0.05$).

and basal respiration decrease, an observation which is in direct opposition to the decomposition data.

Data in Table 5 demonstrates that there were no significant effects of P on the number of nodules which developed on the roots or on the N_2 fixing capacity of these nodules. Although many nodules were present on 6 wk old plants, they were not active as no acetylene was reduced. However, N_2 fixation did increase over the 6 to 12 wk sampling time indicating that the 6 wk nodules were either too immature to fix N_2 or their capacity to fix N_2 was retarded due to high levels of NO_3-N in the peat.

In conclusion, it appears that the low levels of extractable P in the peat used to amend the spoil material (Visser et al., 1984a) markedly inhibited plant growth and flower production by alsike clover. Since peat is generally low in P (Brady, 1974), its usefulness as an amendment for the revegetation of disturbed soils might be limited unless additional P is applied prior to planting plant species such as alsike clover. If additional P were not applied, the plants would presumably be heavily dependent on mycorrhizal infection and development for supplying P necessary for growth and reproduction.

Table 5. Nodule numbers and N_2 (C_2H_2) fixation of alsike clover grown in pots containing peat with and without added phosphate.

	Time (wk)	Phosphorus level (μg/g dry peat) 0	272	544	Row means
Nodule number	6	342	560	885	596[a]
g^{-1} dry root	12	521	548	867	645[a]
Column means		432[a]	554[a]	867[a]	
N_2 fixation	6	0	0	0	
capacity (nmoles N_2 (C_2H_2) fixed g^{-1} dry root hr^{-1})	12	60.4[a]	34.8[a]	19.5[a]	

Values in the nodule number data set were analyzed with a two-way ANOVA. Scheffé confidence intervals were computed for time and treatment means. Values within the time mean row or the treatment mean followed by the same letter do not differ significantly ($p = 0.05$). The 12 wk data analyzed with a one-way ANOVA. Values in the 12 week row followed by the same letter do not differ significantly ($p = 0.05$).

4.5. LITERATURE CITED

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215-221.

Brady, N.C. 1974. The Nature and Properties of Soils. Macmillan Publ. Co., New York.

Danielson, R.M., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Ectomycorrhizae in amended oil sands and subalpine coal mine spoil and in undisturbed jack pine and spruce stands. Final Report to Research Management Division, Alberta Environment.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984a. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final Report to Research Management Division, Alberta Environment.

Visser, S., C. Griffiths and D. Parkinson. 1984b. Reinstatement of biological activity in severely disturbed soils: Effects of amendation and planting on the microbial development in three minespoils. Final Report to Research Management Division, Alberta Environment.

Zak, J.C. and D. Parkinson. 1982. Initial VA mycorrhizal development of slender wheagrass on two amended mine spoils. Canadian Journal of Botany 60:2241-2248.

Zak, J.C., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Vesicular-arbuscular mycorrhizal development of slender wheatgrass on amended mine spoils. Final Report to Research Management Division, Alberta Environment.

5. MICROBIAL ACTIVITY, MYCORRHIZAL INFECTION AND PLANT GROWTH RESPONSES TO SEWAGE SLUDGE AMENDATION OF PEAT-TAILING SAND MIXTURES

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5.1. ABSTRACT

Extracted oil sands were mixed with 55% (v/v) peat and amended with dry sewage sludge at rates equivalent to 0, 23, 46 and 92 mT ha^{-1}. Slender wheatgrass and jack pine were planted in the amended oil sands and the plants were grown in the greenhouse for 10 and 15 wk respectively. Growth of both plant species was greatly enhanced by the sewage sludge with maximum growth of jack pine occurring with 23 mT ha^{-1} and maximum growth of slender wheatgrass occurring with 46 mT ha^{-1}. Additional sewage sludge did not affect the growth of slender wheatgrass and a rate of 92 mT ha^{-1} strongly decreased root and shoot growth of jack pine. VA mycorrhizal formation was completely inhibited by all sewage sludge applications while ectomycorrhizal development was severely reduced by sewage. Sewage sludge additions increased basal respiration and microbial biomass in the planting media and decreased the rate of decomposition of slender wheatgrass leaves. The presence of slender wheatgrass increased microbial biomass in the planting media and reduced rates of decomposition. Jack pine had little influence on respiration, microbial biomass or decomposition.

5.2. INTRODUCTION

Sewage sludge can be a highly beneficial amendment on severely disturbed soils or on mine spoils (Berry and Marx, 1977; Visser et al., 1984). It has the advantage over mineral fertilizers in that nutrients are slowly released over a period of years thus eliminating the need for successive fertilizer applications. In a previous study, sewage sludge and peat were applied separately to oil sand tailings and both benefited plant growth (Visser et al., 1984). In that peat is routinely applied to oil sand tailings but the peat is deficient in P, it was thought that combining peat with its recalcitrant C and sewage sludge with slowly available nutrients might result in a highly desirable growth medium. However, high soil nutrient levels can inhibit mycorrhizal formation and decomposition (Part 3) and sewage sludge does not always have favourable effects on plant establishment (Visser et al., 1984). Thus it was of interest to determine the effects of adding different levels of sewage to soil on plant growth and microbial factors.

The objectives of this study were to determine the effects of (1) adding different amounts of dried sewage sludge on plant growth, (2) sewage sludge on decomposition potential, respiration and microbial biomass of a peat-tailing sand mixture, (3) sewage sludge on VA and ectomycorrhizae formation and (4) plants on decomposition potential and microbial biomass.

5.3. MATERIALS AND METHODS

The general procedures used were the same as for the fertilizer experiment (Part 3). A 1:1 (v/v) mixture of *Carex* and feather moss peats (Part 2) were mixed with a second mixture of tailings sand and silt-clay overburden, with the peat composing 55% (v/v) of the final growing medium. Dry sewage sludge was obtained from the City of Calgary Sewage Treatment plant, broken up and sieved, with the fraction between 2 and 4 mm being retained for use. The sewage sludge was added to the growing medium at a rate equivalent to 46 mT ha^{-1}, the rate used in the tank study (Visser *et al.*, 1984) and at zero, one-half and twice this rate. The sewage sludge was thoroughly mixed with the planting medium and placed in 12.5 cm diameter pots and moistened. The actual amount added in the 46 mT ha^{-1} treatment was 40.5 g per pot. Five replicate pots of each sewage level were planted with three slender wheatgrass seeds per pot, five replicate pots were each planted with three pregerminated jack pine seeds and five replicates were left unplanted. In that it was suspected that the peat from Ft. McMurray contained low levels of ectomycorrhizal inoculum, peat from the Canmore site in the Rocky Mountain foothills (Danielson *et al.*, 1984) was utilized to duplicate the jack pine plant growth and mycorrhizal study. This peat,formed under white spruce, was known to contain high levels of mycorrhizal inoculum. In order to detect airborne inoculum, five replicate, untreated pots of each peat type were autoclaved and planted with jack pine. Pots were watered three times weekly.

When the slender wheatgrass was 10 wk old and the jack pine 15 wk old, the plants were harvested. Tillers of slender wheatgrass were counted and seeds and shoots dried at 80°C and weighed. Roots were subsampled for VA mycorrhizal evaluations and dried and weighed. Jack pine shoots were dried and the roots reserved for mycorrhizal counts.

Slender wheatgrass roots were cleared and stained in 0.01% trypan blue according to Phillips and Hayman (1970) and the degree of infection determined using a line intersect method (Zak and

Parkinson, 1983). Jack pine roots were cut into 2-3 cm segments and 300 short roots per sample were rated for infection (Part 3). To further characterize the mycorrhizae, attempts were made to culture the symbionts.

Basal respiration of the amended planting media was determined by measuring CO_2 efflux under standard conditions of temperature and moisture. An "Ultragas 3" CO_2 analyzer (Wosthoff Company, Bochum, Germany) was used to determine respiration rates. The same samples were used to determine the quantity of microbial biomass C present using the technique of Anderson and Domsch (1978). Decomposition potential was estimated by placing about .25 g of slender wheatgrass leaves in 1 mm mesh litter bags and burying one bag in each pot. When the plants were harvested, the bags were removed, washed and dried to constant weight at 35°C.

5.4. RESULTS

The growth of slender wheatgrass was strongly stimulated by the addition of sewage sludge (Table 1). Growth was very poor in the unamended peat and plants failed to flower. The addition of more than 46 mT ha^{-1} of sewage sludge did not result in any further increases in growth. Shoot/root ratios were very large when grown in the 46 and 92 mT ha^{-1} treatments.

The growth of jack pine was also greatly enhanced when either 23 or 46 mT ha^{-1} rates of application were used (Table 2). The addition of more sewage sludge reduced both shoot and root growth and resulted in very low numbers of short roots. All three levels of application resulted in a small number of short roots per unit root weight. Subordinate mother roots (Wilcox, 1968) were small in number and did not constitute a significant portion of the root systems.

The high level of sewage resulted in a planting medium that was lethal to about 10% of the recently germinated jack pine seedlings. All seedlings survived in pots without sewage. Needles of all the sewage treated seedlings were dark green with no apparent symptoms of nutrient imbalances.

VA mycorrhizal development on slender wheatgrass plants was of a limited extent, with only 9% of the root system being infected after 10 wk (Table 3). Sewage sludge completely inhibited infection by symbionts indigenous to the peat. Root length was substantially reduced by the addition of more than 23 mT ha^{-1} of sewage sludge.

Ectomycorrhizal inoculum compatible with jack pine was present in both peat mixtures resulting in root systems with a large portion of the short roots mycorrhizal (Table 4). However the addition of sewage sludge almost completely prevented the development of mycorrhizae with only 3% of the short roots mycorrhizal in the 23 and 46 mT ha^{-1} treatments. All of the symbionts appeared to be Basidiomycetes except for the I-type Ascomycete. Mycelial strands were formed by the Rhizopogon-type and Amphinema byssoides (Fr.) J. Erikss.

Table 1. Growth of slender wheatgrass in a tailings sand-peat mixture amended with three levels of sewage sludge.

Parameter	Sewage sludge (mT ha^{-1})			
	0	23	46	92
Shoot weight (g)	.56[a]	5.8[b]	7.6[c]	7.9[c]
Root weight (g)	.45[a]	2.4[b]	1.8[c]	1.8[c]
Seed weight (g)	0	.27[a]	.66[a]	1.6[b]
Shoot/root ratio	1.3	2.4	4.2	4.3
Tillers $plant^{-1}$	2.9[a]	13.2[b]	17.8[c]	19.7[c]

All data except seed weight ln Y transformed prior to performing one-way ANOVA, difference tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly (p = .05).

Table 2. Growth of jack pine on peat-tailings sand mixtures amended with three rates of sewage sludge.

	Peat source	Sewage sludge (mT ha^{-1}) 0	23	46	92	Autoclaved
Shoot weight (mg)[1]	Ft. McMurray	121^{a}	811^{c}	1235^{c}	312^{b}	148^{a}
" " "	Canmore	188^{ab}	894^{c}	842^{c}	267^{b}	95^{a}
Root weight (mg)[1]	Ft. McMurray	97^{a}	336^{bc}	583^{c}	163^{ab}	101^{a}
" " "	Canmore	120^{a}	366^{b}	350^{b}	113^{a}	51^{a}
Shoot/Root ratio	Ft. McMurray	1.3	2.4	2.1	2.2	1.5
" " "	Canmore	1.6	2.5	2.5	2.4	1.9
Branches/plant	Ft. McMurray	$.5^{a}$	2.3^{bc}	2.7^{c}	1.2^{ab}	0
	Canmore	1.2^{a}	2.1^{b}	1.5^{ab}	1.1^{a}	0
Short roots/plant[2]	Ft. McMurray	405^{b}	504^{c}	342^{abc}	104^{a}	477^{c}
	Canmore	378^{abc}	324^{abc}	164^{ab}	125^{ab}	165^{ab}
Short roots/mg root[1]	Ft. McMurray	12.3	3.5	1.6	.6	13.6
	Canmore	9.3	2.5	1.2	.7	9.4
Subordinate mother roots/plant	Ft. McMurray	2.1	12.4	11.9	3.4	2.3
	Canmore	1.5	6.9	11.5	7.4	2.3

[1]Data analyzed by one-way ANOVA after ln Y transformation and differences tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p = .05$).

[2]Data analyzed by two-way ANOVA and significant differences detected by Scheffé confidence intervals. Values followed by same letter(s) are not significantly different ($p \leq 0.05$).

Table 3. VA mycorrhizal infection and root lengths of slender wheatgrass grown peat amended oil sands with three levels of sewage sludge.

	Sewage sludge (mT ha^{-1})			
	0	23	46	92
Total root length (m l^{-1})	130	840	349	240
Mycorrhizal root length (m l^{-1})				
Total	12.8	0	0	0
With hyphae	7.9	0	0	0
With vesicles	1.5	0	0	0
With arbuscules	3.4	0	0	0
Percent infection ($\bar{x} \pm$ SD)	9.2 $\pm$ 9.8	0	0	0

Root length data ln Y transformed prior to analysis by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly ($p = .05$).

Table 4. Percent mycorrhizal infection of jack pine seedlings growing peat-tailing sand mixtures amended with three rates of sewage sludge.

	Peat Source	Sewage sludge (mT ha^{-1}) 0	23	46	92	Auto-claved
Total infection	Ft. McMurray	31	0	0	0	0
	Canmore	57	3	3	0	0
I-type Ascomycete	Ft. McMurray	5	0	0	0	0
	Canmore	15	0	3	0	0
Rhizopogon-like	Ft. McMurray	12	0	0	0	0
	Canmore	0	0	0	0	0
Tomentella sp.	Ft. McMurray	0	0	0	0	0
	Canmore	23	3	0	0	0
Amphinema byssoides	Ft. McMurray	0	0	0	0	0
	Canmore	0	0	0	0	0
Hyaline Basidiomycete	Ft. McMurray	0	0	0	0	0
	Canmore	17	0	0	0	0
Unknowns	Ft. McMurray	13	0	0	0	0
Unknowns	Canmore	0	0	.2	0	0

The addition of sewage sludge resulted in increased basal respiration of the planting medium after both 10 and 15 wk (Table 5). Amendation with a rate of 92 mT/ha of sewage sludge resulted in significant increases in CO_2 evolution as compared with a rate of 23 mT ha^{-1} in unplanted pots. The presence of slender wheatgrass did not result in a significant change in respiration at any level of application, but at an application equivalent to 92 mT ha^{-1} respiration was significantly higher in pots planted with jack pine than in unplanted pots. Root weights of jack pine in the 92 mT ha^{-1} treatment were low (Table 2) indicating that factors other than root mass were involved in the stimulation of respiration. The microbial biomass C was increased by sewage amendation and was similar at 10 and 15 wk (Table 6). The amount of microbial biomass in the planting media was greater when planted with slender wheatgrass than in unplanted pots at all three levels of sewage sludge application, but only at the highest level when jack pine was planted. In pots planted with either slender wheatgrass or jack pine, the microbial biomass increased steadily as the rate of sewage sludge application increased although root and shoot biomass did not increase (slender wheatgrass, Table 1) or decrease (jack pine, Table 2).

The decomposition rates of slender wheatgrass leaves were significantly reduced when sewage sludge was added to the growing medium (Table 7). In pots planted with slender wheatgrass, decomposition was strongly reduced and the amount of sewage sludge applied had no significant effects. The presence of jack pine did not affect decomposition rates as much as did slender wheatgrass but with the highest rate of sewage sludge application the weight of leaves remaining after 15 wk was significantly greater in both peat types when jack pine was present than in unplanted pots. The type of peat used to amend the tailings sand had only small effects on the rates of decomposition (Table 8). Autoclaving the peat appeared to decrease the rate of decomposition but not to a substantial extent.

Table 5. Basal respiration of a peat-tailings sand mixture amended with three levels of sewage sludge and planted with slender wheatgrass, jack pine or left unplanted. Values are µl $CO_2 \uparrow$ 100 g^{-1} h^{-1} growing medium.

Treatment	Sewage sludge (mT ha^{-1})			
	0	23	46	92
Control (10 wk)	125[a]	244[b]	355[bc]	502[cd]
Slender wheatgrass	113[a]	422[bcd]	568[cd]	786[d]
Control (15 wk)	175[ab]	207[b]	271[bc]	398[c]
Jack pine	121[a]	191[ab]	261[b]	541[d]

Slender wheatgrass data ln Y transformed. All data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each set followed by the same letter do not differ significantly (p = .05).

Table 6. Microbial biomass C in a peat-tailings sand mixture amended with three levels of sewage sludge and planted with slender wheatgrass or jack pine or left unplanted. Values are mgC 100 g^{-1} planting medium.

	Sewage sludge (mT ha^{-1})			
Treatment	0	23	46	92
Control (10 wk)	12[a]	25[b]	33[bc]	57[d]
Slender wheatgrass	12[a]	46[cd]	65[d]	103[e]
Control (15 wk)	13[ab]	21[bc]	28[cd]	55[e]
Jack pine	11[a]	22[c]	33[d]	65[f]

Slender wheatgrass data ln Y transformed. All data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each set followed by the same letter do not differ significantly (p = .05).

Table 7. Decomposition of slender wheatgrass leaves buried in a peat-oil sands mixture and amended with three levels of sewage sludge.

	Peat	Sewage sludge (mT ha^{-1})			
Treatment	Source	0	23	46	92
Control (10 wk)	Ft. McMurray	7^{a}	14^{ab}	29^{bc}	29^{bc}
Slender wheatgrass	Ft. McMurray	7^{a}	46^{cd}	51^{cd}	41^{c}
Control (15 wk)	Ft. McMurray	0^{a}	13^{bc}	24^{c}	21^{d}
Jack pine	Ft. McMurray	8^{ab}	15^{bcde}	23^{cdef}	31^{f}
Jack pine	Canmore	14^{bcd}	25^{f}	24^{cdef}	27^{f}

Data analyzed by two-way ANOVA and differences tested by Scheffé pairwise comparisons. Values within each set followed by the same letter do not differ significantly ($p = .05$).

Table 8. Decomposition of slender wheatgrass leaves buried in peat-oil sands mixture, planted with jack pine and amended with three levels of sewage sludge. Values are percent weight remaining.

Peat source	Autoclaved	Sewage sludge (mT ha^{-1})			
		0	23	46	92
Ft. McMurray	22^{bc}	8^{a}	15^{ab}	23^{bc}	31^{c}
Canmore	21^{ab}	14^{a}	25^{b}	24^{b}	27^{b}

Data analyzed by one-way ANOVA and differences tested by Scheffé pairwise comparisons. Values in each row followed by the same letter do not differ significantly ($p = .05$).

5.5. DISCUSSION

Sewage sludge served as an excellent source of plant nutrients for both slender wheatgrass and jack pine in this greenhouse pot study. However heavy applications resulted in either no benefit for growth or, in the case of jack pine, severely decreased primary production and survival of young seedlings. In addition, even the lowest level of application used resulted in a nearly complete suppression of mycorrhizal formation. The absence of mycorrhizae and their extensive network of extramatrical mycelium in conjunction with the reduction of root growth with high sewage sludge levels could result in severe consequences for plant establishment on minespoils where a variety of stresses, in addition to macronutrient deficiencies, may occur. In this study neither shoot nor root growth of slender wheatgrass gave any clear indication that the amount of sewage sludge might have any impact on tolerance to non-nutrient stresses. However a measure of root length, as well as mycorrhizal development, provides evidence that grasses subjected to high nutritional levels are probably less able to exploit soil or mine spoils for either nutrients of low mobility or water. Unlike an uncontrolled reclamation situation, these stresses are unlikely and probably underestimated within pots in greenhouse conditions.

Much heavier field applications of sewage sludge have been used without the negative effects described here. Berry and Marx (1976) used up to 275 mT ha^{-1} and did not find any adverse effects on either plant growth or mycorrhizal infection of two species of southern pines. These authors recommended that application rates on disturbed sites should be as high as environmental and economic considerations permit. In a later study, Berry and Marx (1977) found that growth of loblolly pine and mycorrhiza formation were depressed by adding 275 mT ha^{-1} of sewage sludge. However growth was excellent with rates similar to those used in this study. However, rates applied to pots and to field plots are not necessarily comparable. The sewage sludge used in the pots was in the form of small pellets, thoroughly mixed into the planting mixture and was kept constantly wet by frequent watering.

In contrast, field applied sludge may be in large chunks and some portion is on the surface where it is dry for much of the growing period. Regardless, the present pot study illustrated that high application rates can have highly deleterious effects on root growth and mycorrhizae. When evaluating the effects of sewage sludge, root length rather than weight, should be measured as well as root associated symbioses.

As expected, sewage sludge additions increased respiration and microbial biomass. It is presumed that the increase in respiration and microbial biomass was due to decomposition of material in the sewage sludge rather than a stimulation of peat decomposition. The addition of mineral nutrients to peat has been shown to have no effect on these parameters (Part 2). The presence of slender wheatgrass doubled the amount of microbial biomass in the sewage treated planting medium. This indicates that a large portion of the microbial biomass was more active in the decay of easily degraded substances (sloughed roots, root exudates) rather than in the decay of peat or recalcitrant materials in the sewage sludge. It is possible that, as was indicated by the inhibition of litter decomposition, sewage sludge may even inhibit decay of recalcitrant materials.

5.6. LITERATURE CITED

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215-221.

Berry, C.R. and D.H. Marx. 1976. Sewage sludge and Pisolithus tinctorius ectomycorrhizae: their effect on growth of pine seedlings. Forest Science 22:351-358.

Berry, C.R. and D.H. Marx. 1977. Growth of loblolly pine seedlings in strip-mined kaolin spoil as influenced by sewage sludge. Journal of Environmental Quality 6:379-381.

Danielson, R.M., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Ectomycorrhizae in amended oil sands and subalpine coal mine spoil and in undisturbed jack pine and spruce stands. Final Report to Research Management Division, Alberta Environment.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-161.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final Report to Research Management Division, Alberta Environment.

Wilcox, H.E. 1968. Morphological studies of the roots of red pine, Pinus resinosa. II. Fungal colonization of roots and the development of mycorrhizae. American Journal of Botany 55: 688-700.

Zak, J.C. and D. Parkinson. 1982. Initial VA mycorrhizal development of slender wheatgrass on two amended mine spoils. Canadian Journal of Botany 60:2241-2248.

6. PLANT GROWTH, MICROBIAL ACTIVITY AND CELLULOSE DECAY IN TAILING SAND TREATED WITH THREE LEVELS OF SEWAGE SLUDGE

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6.1. ABSTRACT

In a greenhouse pot experiment, extracted oil sand was left unamended or amended with dried sewage sludge at levels equivalent to 23, 46 and 92 mT ha^{-1} and subsequently planted with slender wheatgrass or left unplanted. Pots were sampled at 2, 6 and 12 wk to determine effects of sewage sludge on plant growth, microbial activity, mycorrhizal infection and cellulose decay potential. Shoot and root production were most enhanced by the 23 and 46 mT ha^{-1} sludge rates and were significantly reduced by the 92 mT ha^{-1} application. Over the first 6 wk the 46 mT treatment also had adverse effects on plant growth, but this inhibition was overcome during the following 6 wk. No vesicular-arbuscular (VA) mycorrhizal infection was detected in any of the treatments. Microbial respiration and biomass C increased with increasing sludge levels and were not substantially affected by the presence of plants. The addition of sewage sludge stimulated cellulose decay potential which was observed to be negligible in the untreated sand.

6.2. INTRODUCTION

Sewage sludge has been shown to be highly effective in ameliorating soil chemical/physical properties and promoting plant establishment and growth in nutrient-impoverished minespoils (Sopper, 1970; Berry and Marx, 1977; Stout et al., 1978; Bradshaw et al., 1978; Visser et al., 1984a). Application of sewage sludge to oil sand tailings, which are characterized by an absence of organic matter, essential plant nutrients and a microbial component, will improve both the nutrient and microbial status of the sand (Visser et al., 1984b). Also, because of its slow decay rate, sewage sludge behaves as a slow release fertilizer, making it a highly desirable amendment for reclaimed minespoils requiring a minimum of fertilizer maintenance. However, high levels of sewage may have adverse effects on plant establishment and growth by inhibiting seed germination and root elongation (Wong et al., 1981; Sabey and Hart, 1975; Wollen et al., 1978; McCormick and Borden, 1973). VA mycorrhizal infection rates also appear to be adversely affected when plants are grown in the presence of sewage sludge (Spitko and Manning, 1981; Zak and Parkinson, 1982), while high soil fertility levels can reduce soil microbial activity (Kowalenko et al., 1978). It was, therefore, believed necessary that additional information be obtained on the effects of different sewage sludge levels on plant response and microbiological parameters in tailings sand. The main objectives of this study were to determine (1) the effects of different levels of sewage sludge applied to tailing sand on primary production of slender wheatgrass, (2) the effect of sewage sludge on microbial respiration and biomass C, (3) the effect of sewage sludge on the decay of cellulose filter paper, (4) the effects of plant growth on soil microbial activity and cellulose decomposition, and (5) the VA mycorrhizal infection potential of sludge treated sand.

6.3. MATERIALS AND METHODS

Anaerobically digested sewage sludge from the City of Calgary sewage lagoons was air dried and sieved to produce pellets 2 to 4 mm in diameter. The dried sludge had a pH of 8.6 and contained approximately 30% organic C, 3% N (of which .32% was NH_4-N), 15,325 $\mu g\ g^{-1}$ P (of which 170 $\mu g\ g^{-1}$ were extractable), 11,950 $\mu g\ g^{-1}$ Fe, 815 $\mu g\ g^{-1}$ Cu, 1525 $\mu g\ g^{-1}$ Zn and 788 $\mu g\ g^{-1}$ Pb.

The sewage sludge pellets were thoroughly mixed with tailings sand (pH 7.5; .15% organic C, < .01% N, 1.1 $\mu g\ g^{-1}$ extractable P, 2.9 $\mu g\ g^{-1}$ Fe, .05 $\mu g\ g^{-1}$ Cu, 0.3 $\mu g\ g^{-1}$ Zn, and .5 $\mu g\ g^{-1}$ Pb) to obtain treatments equivalent to the following application rates: 0 (no sewage sludge); 23 mT ha^{-1}; 46 mT ha^{-1}; 92 mT ha^{-1}. The 46 mT ha^{-1} rate was equivalent to the rate applied to the tailing sand in the tank study (see Visser et al., 1982a). To avoid variation due to subsampling, each treatment was set up on a per pot basis so that the 23 mT treatment contained 2.66% sludge/pot (w/w); the 46 mT treatment contained 5.32% sludge/pot and 92 mT treatment contained 10.64% sludge/pot. In addition to improving the nutrient quality of the sand, the sludge also raised the moisture holding capacity of the sand from 20% in the untreated sand to 22%, 24% and 26% in the 23, 46 and 92 mT treatments respectively. The mixtures for each pot were remoistened with distilled water to 75% of field capacity. Nine replicate pots of each sewage sludge treatment were planted with five, 1 wk old slender wheatgrass seedlings and six replicates $treatment^{-1}$ were left unplanted. Plant growth parameters were measured at 2, 6 and 12 wk after planting and microbial respiration, microbial biomass C, and cellulose decay potential were assessed at 6 and 12 wk after planting. Three planted and then unplanted pots $treatment^{-1}$ were destructively sampled at each sampling time with the exception of the 2 wk samples when only planted pots were processed. Pots were incubated in the greenhouse and maintained at 75% field capacity with distilled H_2O during the experiment.

At each sampling time, the number of tillers produced by each grass plant was determined and shoots were dried at 80°C and weighed. Roots were washed free of planting mixture, dried and weighed. On the last sampling date, roots were subsampled, cleared, and stained in 0.01% trypan blue (Phillips and Hayman, 1970) for vesicular-arbuscular mycorrhizal evaluations.

Sand from the planted and unplanted pots, harvested 6 and 12 wk after planting, was analyzed for microbial activity and microbial biomass C. Microbial activity (CO_2 efflux) was determined under standard temperature and moisture conditions using an "Ultragas 3" CO_2 analyzer (Wosthoff Co., Bochum, Germany). The same samples were used to determine the quantity of microbial biomass C using the technique described by Anderson and Domsch (1978). Details for determining soil respiration and microbial biomass C have been outlined by Visser et al. (1982b). The cellulose decay potential of each planted and unplanted sand/sludge mixture was estimated at 6 and 12 wk after planting by bagging a pre-weighed 4.5 cm diameter filter paper in 1 mm nylon mesh and embedding one filter in each pot. At harvest, the bags were removed, washed, dried at 35°C and percent dry weight remaining for each filter calculated.

6.4. RESULTS

The poor nutrient status of the tailings sand resulted in very little shoot and root production by slender wheatgrass over the term of the study (Table 1). At 2 wk, plants in the untreated tailing sand appeared healthy with no obvious nutrient deficiencies but as growth progressed seedlings exhibited stunting and possible N and P deficiencies.

Plant production was most stimulated by the application of sewage sludge at the 23 and 46 mT ha^{-1} rates and significantly depressed by the 92 mT ha^{-1} application (Table 1). Six weeks after planting, shoot and root weights demonstrated a decreasing trend with increasing sludge levels, but at 12 wk this trend was not as pronounced. Although slender wheatgrass exhibited very little growth in the 92 mT treatment over the first 6 wk, growth was significantly stimulated between 6 and 12 wk. Six week-old plants in the high sludge treatments were stunted with yellowish foliage, but at 12 wk plants appeared healthier and exhibited stimulated growth. Shoot/root ratios measured at all three sampling times demonstrated an increasing trend with increasing sludge levels. Tiller production occurred between 6 and 12 wk after planting and was most enhanced by the 23 and 46 mT ha^{-1} application rates. Seedling mortality was greatest in the two high sludge treatments where 8% of the total number of seedlings died over the term of this study (2% were lost in the control treatment and none in the 23 mT ha^{-1} treatment).

Vesicular-arbuscular mycorrhizal infection was not detected in 12 wk old roots from any of the treatments.

Microbial respiratory activity demonstrated an increasing trend with increasing sludge application rates, while microbial biomass C increased significantly with each successive sludge level (Tables 2 and 3). The presence of slender wheatgrass at either 6 or 12 wk after planting had no significant influence on soil respiration, but resulted in a small but significant increase in microbial biomass C when results from both sampling times were

Table 1. Growth of slender wheatgrass in tailings sand amended with three levels of sewage sludge.

Plant parameter	Plant age (weeks)	Sludge application (mT ha^{-1}) 0	23	46	92
Shoot wt (mg $plant^{-1}$)	2	3.3^{ab}	3.2^{ab}	2.3^{ab}	1.5^{a}
	6	6.4^{bc}	52.2^{ef}	20.5^{de}	4.1^{abc}
	12	9.6^{cd}	240.1^{gh}	357.2^{h}	82.3^{fg}
Root wt (mg $plant^{-1}$)	2	3.4^{bc}	0.9^{ab}	0.4^{a}	0.2^{a}
	6	8.1^{cd}	17.8^{de}	6.6^{cd}	0.8^{ab}
	12	5.7^{cd}	72.5^{ef}	82.8^{f}	14.2^{cd}
Shoot/root ratio	2	1.0	3.6	5.8	7.5
	6	0.8	2.9	3.1	5.1
	12	1.7	3.3	4.3	5.8
Tillers $plant^{-1}$	2	1^{a}	3.2^{ab}	2.0^{ab}	1^{a}
	6	1^{a}	8.5^{c}	10.3^{c}	4.3^{b}

Data in each of the shoot wt., root wt. and tiller categories were analyzed by a two-way ANOVA and Scheffé multiple contrasts for pairwise comparisons. Values followed by the same letter(s) do not differ significantly ($p < 0.001$). Shoot and root wt. data required ln Y transformation.

Table 2. Microbial respiration (µl CO_2 ↑ 100 g^{-1} dry soil h^{-1}) of tailing sand treated with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level (mT ha^{-1})	Sampling time			
	6 weeks		12 weeks	
	unplanted	planted	unplanted	planted
0	49 ^{a}	57 ^{ab}	57 ^{ab}	57 ^{ab}
23	180 ^{cde}	172 ^{cde}	139 ^{abc}	155 ^{bcd}
46	270 ^{ef}	351 ^{fg}	270 ^{ef}	245 ^{de}
92	351 ^{fg}	425 ^{g}	400 ^{g}	425 ^{g}

Data analyzed by three way ANOVA (MSE = 500). Differences detected by Scheffé multiple contrasts for pairwise comparisons. Values followed by same letter(s) do not differ significantly ($p \leq 0.05$).

Table 3. Microbial biomass C (mg 100 g^{-1} dry soil) in tailing sand treated with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level ($mT\ ha^{-1}$)	Sampling Time					
	6 weeks			12 weeks		
	Unplanted	Planted	$\bar{x}$	Unplanted	Planted	$\bar{x}$
0	5.3	4.3	4.8[a]	4.3	4.6	4.5[a]
23	15.8	21.6	18.7[c]	11.8	13.8	12.8[b]
46	24.3	28.8	26.6[d]	20.0	22.6	21.3[c]
92	32.8	32.4	32.6[e]	30.8	35.0	32.9[e]

	Combined 6 and 12 week unplanted $\bar{x}$	Combined 6 and 12 week planted $\bar{x}$
All treatments	18.1[a]	20.4[b]

Data analyzed by three way ANOVA (MSE = 5.2). Planted and unplanted means at each sampling time were combined to determine sludge level and time effects, while all means in the unplanted or planted treatments were combined to test plant effects. Differences were detected using Scheffé multiple contrasts for pairwise comparisons. Values in the mean columns in each data set, followed by same letter do not differ significantly ($p \leq 0.05$).

combined. With the exception of the 46 mT ha^{-1}, planted treatment where CO_2 efflux decreased substantially between the 6 and 12 wk sampling times, no changes in microbial respiration were detected over the course of the study. However, microbial biomass C in the 23 and 46 mT ha^{-1} planted and unplanted treatments decreased between 6 and 12 wk after the initiation of the study. Microbial biomass C in the control and 92 mT ha^{-1} sludge treatments were not significantly altered over time.

No decomposition of cellulose filter paper occurred in the untreated tailings sand over the 12 wk study period (Table 4). In general, sewage sludge application stimulated the decay of the filters. The presence of plants did not influence the cellulose decay rate at any of the sludge levels, and amount of filter paper remaining after 6 wk decay was not significantly different from that remaining after 12 wk decomposition. The decomposition data were highly variable - a factor which may have been due to the heterogeneous distribution of sludge pellets within the sand matrix.

Table 4. Decay of cellulose filter paper in tailings sand amended with three levels of sewage sludge and left unplanted or planted with slender wheatgrass.

Sludge level ($mT\ ha^{-1}$)	% dry weight filter paper remaining			
	6 weeks		12 weeks	
	Unplanted	Planted	Unplanted	Planted
0	100^{c}	100^{c}	100^{c}	100^{c}
23	50^{abc}	18^{ab}	38^{ab}	3^{a}
46	16^{ab}	30^{ab}	38^{ab}	20^{ab}
92	37^{ab}	56^{bc}	63^{bc}	39^{ab}

Data analyzed by three-way ANOVA (MSE = 140). Differences detected by Scheffé multiple contrasts for pairwise comparisons. Values followed by same letter(s) do not differ significantly ($p \leq 0.05$).

6.5. DISCUSSION

The infertility of the tailing sand which, according to Turchenek (1976) is mainly due to a lack of organic matter and inorganic colloids, resulted in a very poor performance by slender wheatgrass. Similar results were obtained when growing slender wheatgrass in spent tailing sand held in large wooden tanks in the field (Visser et al., 1984a). The addition of sewage sludge to the extracted sand at rates equivalent to 23, 46 and 92 mT ha^{-1} increased the organic matter (as determined by loss on ignition) level of the sand to 1.2, 1.5 and 2.1%, respectively. The increase in organic matter levels would be instrumental in improving the nutrient status and water retention capability of the sand. Amendation of the spent sand with the nutrient rich sewage sludge was highly effective in stimulating plant growth, but only at the 23 and 46 mT ha^{-1} application rates. The addition of 92 mT ha^{-1} sewage sludge severely reduced the growth of the slender wheatgrass, and there was some indication that over the first 6 wk of the study the 46 mT application may also have inhibited plant growth. Toxic effects of sewage sludge on plant growth have been reported in other studies (Wong et al., 1981; Sabey and Hart, 1975; Wollen et al., 1978 and McCormick and Borden, 1973), and have been attributed to the presence of toxic concentrations of ammonia after sludge application (McCormick and Borden, 1973). The improvement in plant growth between 6 and 12 wk in the 92 mT ha^{-1} sludge treatment may be explained by the increased microbial oxidation of the ammonia to nitrate, thereby reducing plant inhibitory levels of ammonia. Interestingly, when the same levels of sewage sludge used in this study, were applied to a 55% (v/v) peat/sand mix and planted with slender wheatgrass, plant growth was not significantly reduced by the 92 mT ha^{-1} sludge treatment (see Part 5, this report). The high adsorptive capacity, chelating properties, and nitrification capability of peat may have lessened the inhibitory effects of the higher sludge levels on grass growth in the peat/sand mix. The increased supply of N provided by the sewage sludge was evidenced in the

of the size of the grass root systems and increase in shoot/root ratios, as sludge levels increased (Russell, 1977). As suggested in Part 5 the very small root systems produced by grasses subjected to high fertility regimes, may not be advantageous in a minespoil such as the tailing sand, where a well-developed fibrous root system would provide both better erosion control and retention of key nutrients within the soil/plant system.

The lack of VA mycorrhizal infection of the grass roots was to be expected since Zak and Parkinson (1982) have observed that oil sands tailings are essentially devoid of VA mycorrhizal inoculum. It is difficult to determine if the sewage sludge contained any mycorrhizal inoculum, since there is some evidence that sludge may be highly toxic to the VA mycorrhizal fungi (Spitko and Manning, 1981; Zak and Parkinson, 1982).

Microbial respiration and biomass C were low in the spent sand mainly because the spent sand lacks the organic matter required by the microorganisms for their growth and maintenance. The addition of sewage sludge not only improves the organic C and nutrient status of the sand, but also introduces large quantities of bacteria and fungal hyphae (Visser et al., 1984b). Hence it is not surprising that in this study, microbial respiration and biomass C rose considerably with the application of the sludge and increased as sludge levels increased. Macgregor and Naylor (1982) and Eiland (1981) have also noted that microbial respiration and biomass were higher in soil receiving sewage sludge applications. The presence of slender wheatgrass in the sludge-treated sand resulted in a stimulation of microbial biomass, but not to the same extent as that observed in the sludge-treated peat/sand mixture (Part 5). It has been postulated that much of the stimulation of microbial activity and biomass often observed in planted versus unplanted systems can be attributed to the input of easily degradable resources in the form of sloughed roots and root exudates (Visser et al., 1984b). Slender wheatgrass grown in the sludge amended peat/sand mixture produced over four times as much root material as slender wheatgrass grown in the sludge-treated tailing sand. The greater root mass produced in

the peat/sand presumably resulted in a larger input of root exudates which would account for the greater stimulation of soil microbial biomass in this treatment.

Untreated tailings sand exhibited no cellulose decay potential presumably because both the nutrients and microorganisms required for the degradation of pure cellulose were absent. The addition of sewage sludge with its high inoculum level greatly accelerated cellulose decay. Cellulolytic bacteria have been isolated from sewage sludge (Gude, 1980; Ramasamy et al., 1981), hence their presence in the sludge used in this study may explain the faster decomposition of cellulose in the sludge treated than untreated sand.

6.6. LITERATURE CITED

Anderson, J.P.E. and K.H. Domsch. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10:215-221.

Berry, C.R. and D.H. Marx. 1977. Growth of loblolly pine seedlings in strip-mined kaolin spoil as influenced by sewage sludge. Journal of Environmental Quality 6:379-381.

Bradshaw, A.D., R.N. Humphries, M.J. Johnson and R.D. Roberts. 1978. The restoration of vegetation on derelict land produced by industrial activity. The Breakdown and Restoration of Ecosystems, eds. M.W. Holdgate and M.J. Woodman. Plenum Publishing Corp., New York. pp. 249-274.

Eiland, F. 1981. The effects of application of sewage sludge on microorganisms in soil. Danish Journal of Plant and Soil Science. Report No. 1534, pp. 39-46.

Gude, H. 1980. Occurrence of cytophagas in sewage sludge. Applied and Environmental Microbiology 39:756-763.

Kowalenko, C.G., K.C. Ivarson and D.R. Cameron. 1978. Effect of moisture content, temperature and nitrogen fertilization on carbon dioxide evolution from field soils. Soil Biology and Biochemistry 10:417-423.

Macgregor, A.N. and L.M. Naylor. 1982. Effect of municipal sludge on the respiratory activity of a cropland soil. Plant and Soil 65:149-152.

McCormick, L.H. and F.Y. Borden. 1973. Percolate from spoils tested with sewage effluent and sludge. Ecology and Reclamation of Devastated Land. Vol. 1, eds. R.J. Hutnik and G. Davis. Gordon and Breach, Science Publishers, Inc. pp. 239-250.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-161.

Ramasamy, K., M. Meyers, J. Bevers and H. Verachtert. 1981. Isolation and characterization of cellulolytic bacteria from activated sludge. Journal of Applied Bacteriology 51: 475-481.

Russell, R. Scott. 1977. Plant Root Systems: Their Function and Interaction with the Soil. McGraw-Hill Book Co. (UK) Ltd. Maidenhead, Berkshire, England. 298 pp.

Sabey, B.R. and W.E. Hart. 1975. Land application of sewage sludge: 1. Effect on growth and chemical composition of plants. Soil Sci. Soc. Am. J. 42:252-256.

Sopper, W.E. 1970. Revegetation of strip mine spoil banks through irrigations with municipal sewage effluent and sludge. Compost Science 11:6-11.

Spitko, R.A. and W.J. Manning. 1981 Irradiated, digested sewage sludge: effects on plant-symbiont associations in the field. Environmental Pollution, Series A, 25:1-8.

Stout, W.L., O.L. Bennett and E.L. Mathias. 1978. Effect of sewage sludge, garbage mulch and lime on some chemical and physical properties of a strip mine spoil. Ecology and Coal Resource Development. Vol. 2, ed. M.K. Wali. Pergamon Press. pp. 920-925.

Turchenek, L.W. 1976. Soils research related to revegetation of the oil sands area. Proceedings of the First Annual Workshop of the Vegetation Technical Research Committee - AOSERP. pp. 42-48.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984a. Reinstatement of Biological Activity in Severely Disturbed Soils: Effects of Different Amendments to Three Different Minespoils on Selected Soil Physical and Chemical Properties and on Plant Growth. Final report submitted to Research Management Division, Alberta Environment.

Visser, S., C. Griffiths and D. Parkinson. 1984b. Reinstatement of Biological Activity in Severely Disturbed Soils: Effects of Mining of the Microbiology of Three Minespoils and the Microbial Development in the Minespoils after Amendation and Planting. Final Report submitted to the Research Management Division, Alberta Environment.

Wollan, E., R.D. Davis and S. Jenner. 1978. Effects of sewage sludge on seed germination. Environmental Pollution 17: 195-205.

Wong, M.H., W.M. Lau and S.W. Yip. 1981. Effects of sludge extracts on seed germination and root elongation of crops. Environmental Pollution, Series A, 25:87-98.

Zak, J.C. and D. Parkinson. 1982. Initial vesicular-arbuscular mycorrhizal development of slender wheatgrass on two amended minespoils. Canadian Journal of Botany 60: 2241-2248.

7. SUMMARY

From the data collected in the greenhouse pot studies, it is possible to make the following conclusions.

7.1. PLANT GROWTH AND MYCORRHIZAL STATUS

Although differences in plant growth in the feather moss peat, Carex peat and mixture of these two peats were minimal, there was some suggestion that slender wheatgrass root production and jack pine root and shoot production were superior in 55% (v/v) peat/mineral mixtures. The 55% peat/sand mixture has a field equivalent of 38 mT feather moss peat ha^{-1}, 48 mT Carex peat ha^{-1} or 45 mT peat mixture ha^{-1}. The application of mineral fertilizer at the initiation of the study may have obscured any growth differences caused by the different peat types.

Fertilization of the peat, up to a total of 112:49:72 kg NPK ha^{-1}, substantially increased both shoot and root growth by slender wheatgrass. Shoot growth by jack pine reached a maximum at 28:12:18 kg NPK ha^{-1} and was not further stimulated by additional fertilization. Both jack pine root growth and ectomycorrhizal infection of these roots were reduced at fertilizer levels above the 28:12:18 kg NPK ha^{-1} rate. There were negligible quantities of VA inoculum in the peat/sand mixture as evidenced by the lack of mycorrhizal infection of the slender wheatgrass roots in the unfertilized and low fertilizer treatments.

Successful establishment, growth, and seed production by alsike clover in the feather moss peat used in the field study (Visser et al., 1984) required the application of additional P. The low extractable P levels in this peat (2 $\mu g\ g^{-1}$) did not appear to be adequate to ensure the establishment of this species during the early stages of plant growth.

Growth of slender wheatgrass and jack pine in a 55% (v/v) peat/tailing sand mixture was greatly stimulated by the addition of dried sewage sludge with maximum shoot production occurring at 46 and 23 mT ha^{-1} sludge levels for grass and pine respectively. The

growth of jack pine was strongly inhibited at 92 mT sludge ha^{-1}, while root production by slender wheatgrass decreased sharply at sludge application rates exceeding 23 mT ha^{-1}. When slender wheatgrass was grown in pure tailings sand amended with dried sewage sludge, maximum growth occurred with the 23 mT ha^{-1} sludge rate and was significantly reduced with a sludge application of 92 mT ha^{-1}. These data suggest that the degree of inhibition by sewage sludge on plant growth is dependent not only on the particular plant species but also on the chemical/physical properties of the soil to which the sludge is applied. The adsorptive and chelating properties of peat appeared to decrease the inhibitory properties of sewage sludge. The presence of sewage sludge severely reduced ectomycorrhizal infection of pine roots and completely inhibited VA mycorrhizal infection of the grass roots. It is suggested that the inhibitory effects of sewage sludge may be a result of the generally high nutrient levels characteristic of sludge, the presence of heavy metals in the sludge, or high ammonium N levels which may be particularly problematical immediately after sludge application. Additional research is required to more accurately assess which characteristics of the sludge are most detrimental to plant growth and mycorrhizal formation.

7.2. MICROBIAL ACTIVITY AND DECOMPOSITION

Soil microbial activity was not significantly different amongst the feather moss, Carex and mixed peats, suggesting that the two peat types were very similar in terms of their stability. However, microbial biomass C and decay of grass roots were lowest in the Carex peat. Microbial respiration and biomass were significantly stimulated by the growth of slender wheatgrass, while decomposition potential was reduced in the presence of the grass. These data suggest that mineralization of dead plant parts would be slowest in Carex peat planted with slender wheatgrass.

Amendation of a 55% (v/v) peat/sand mixture with mineral fertilizer up to a total of 112:49:72 kg NPK ha^{-1} did not

accelerate the loss of stable C from the peat. Decomposition of slender wheatgrass leaves was reduced by high levels of fertilizer. Microbial biomass was significantly stimulated by the growth of slender wheatgrass in peat with all but the highest fertilizer application (i.e. 112:49:72 kg NPK ha^{-1}). Again, the presence of slender wheatgrass reduced the decay of slender wheatgrass litter.

When P alone was added (rates = 50 and 100 kg P ha^{-1}) to a feather moss peat used in a previous field study (Visser et al., 1984), respiratory activity and microbial biomass were significantly reduced, while decay of pure cellulose was enhanced.

Application of sewage sludge to pure tailings sand or a 55% (v/v) peat/sand mixture at rates ranging from 23 to 92 mT ha^{-1} was highly effective in increasing both microbial respiration and biomass C. This increase is believed to be due mainly to the introduction of high levels of microbial material with the sludge. The growth of slender wheatgrass in the sludge treated peat stimulated the microbial biomass to an even greater degree, but both high levels of sewage sludge and the presence of slender wheatgrass reduced the decomposition rate of slender wheatgrass leaves. It is interesting to note that in each of three separate experiments, the growth of slender wheatgrass inhibited the decay of slender wheatgrass litter - a factor which may be important in newly-revegetated minespoils where a high rate of nutrient turnover may be required to maintain the established vegetative cover. Also, very high nutrient concentrations in either inorganic or organic form may have adverse effects on nutrient release from decaying litter.

It is difficult to apply results obtained under controlled laboratory conditions to a field situation, but the data obtained in the present studies suggest that:

1. with a high initial application of fertilizer the type of peat or amount of peat has very little influence on tree or grass growth over the short term.

2. heavy application of mineral or organic fertilizers may have adverse effects on plant growth, with grasses being less

sensitive to high nutrient regimes than pine. In general, root growth was reduced when high fertilizer rates were used. This may be a disadvantage during the initial stages of revegetation when poor root development could lead to a greater degree of erosion on unstable slopes. Also, plants established in disturbed sites where chemical/physical stresses are high may require extensive root systems in order to survive such adverse conditions as high temperatures, low moisture or low nutrient availability.

3. it appears that many of the peats found in the Fort McMurray area are severly lacking in VA mycorrhizal inoculum. Both VA- and ectomycorrhizal infection of plant roots are inhibited by high nutrient regimes.

4. the decomposition rates of dead plant material may also be reduced by high soil nutrient levels.

5. although growth by slender wheatgrass stimulated microbial respiration and biomass C, decomposition rates were inhibited by the presence of slender wheatgrass. This suggests that during the early stages of reclamation nutrient release from decaying plant material may be more rapid in areas vegetated with trees than in those planted with grass.

7.3. LITERATURE CITED

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths and D. Parkinson. 1984a. Reinstatement of Biological Activity in Severely Disturbed Soils: Effects of Different Amendments to Three Different Minespoils on Selected Soil Physical and Chemical Properties and on Plant Growth. Final Report submitted to Research Management Division, Alberta Environment.

REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED SOILS: VESICULAR-ARBUSCULAR MYCORRHIZAL DEVELOPMENT OF SLENDER WHEATGRASS ON AMENDED OIL SAND TAILINGS AND SUBALPINE COAL MINE SPOIL

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ABSTRACT

The vesicular-arbuscular mycorrhizal (VAM) development of slender wheatgrass on extracted oil sands tailings and a subalpine coal mine spoil amended with either fertilizer, peat, or sewage sludge was examined over a 4 yr period. During the first growing season on the oil sands spoil mycorrhizae were limited to plants on the peat-amended spoil. While VAM infection was not detected in plants on the fertilized plots until the end of the second growing season, plants on the sewage- amended plots were not mycorrhizal until after the 4th yr. VAM infection in plants on the subalpine mine spoil was detected at 2 wk only in the peat-amended spoil. Although sewage initially suppressed the rate of mycorrhizal development, plants did develop VA mycorrhizae by the 10 wk sampling time. The mycorrhizal status of plants on the amended subalpine mine spoil did not change significantly between the 2nd and 4th year.

Glomus aggregatum and Glomus mosseae were the most common VA fungi in the amended spoils. In the oil sands tailings, VA fungal spores were detected only in the control and peat-amended plots. While there was no amendment effect on spore densities of G. mosseae in the subalpine coal spoil, spore numbers of G. aggregatum were significantly reduced in the sewage-treated spoil. The successful reestablishment of VA mycorrhizal in mine spoils will depend in part on the effects of soil amendments on VA fungal species occurrence and inoculum production.

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1. INTRODUCTION

The restoration of biological activity in soils disturbed through mining activity may involve the addition of nutrients, some form of organic matter, and the reintroduction of specific groups of decomposer and symbiotic microorganisms (Parkinson 1979). The type of amendment applied to a spoil and its rate of application may influence the development of the saprophytic microflora and the success in reestablishing the necessary symbiotic component. Eiland (1981) showed that the addition of slurry and farmyard manure to an agricultural soil every 2 yr resulted in higher fungal numbers than when applied every year. Within the context of mine spoil reclamation, very little information is available concerning the effects of nutrient addition or organic matter input on the soil microflora of these disturbed habitats.

It is now well recognized that the symbiotic associations of a specific group of phycomycetous fungi with higher plants, termed vesicular-arbuscular mycorrhizae (VAM), are ubiquitous in nature (Mosse 1973a; Crush 1975; Read *et al.* 1976; Davidson and Christenson 1977; Molina *et al.* 1979; Sparling and Tinker 1978a; Rabatin 1979) and that this symbiosis is required by many plants for absorption of phosphorus and other nutrients from soils of low nutrient status (Mosse 1973b; Crush 1976; Powell 1977a; Sparling and Tinker 1978b; Nicolson and Johnston 1979). Recent work by Allen *et al.* (1981) with blue gramagrass has shown that VA mycorrhizal plants may also be more drought tolerant; an important characteristic to consider in the reclamation of semi-arid environments. The occurrence of VAM associations with plants grown on mine spoils has been documented in studies by Daft and Nicolson (1974), Daft and Hacskaylo (1976), Khan (1978), Miller (1979), Reeves *et al.* (1979) and Allen and Allen (1980). Growth increases of mycorrhizal plants as compared with nonmycorrhizal ones on mine spoils have been demonstrated for many plant species (e.g. Aldon 1975; Daft and Hacskaylo 1977; Lindsey *et al.* 1977; Lambert and Cole 1980; Khan 1981).

Given that VA mycorrhizae are an integral component of most terrestrial ecosystems, if reclamation of disturbed habitats is to be effective, attention should be given to the rates at which plants become infected with VA fungi in these habitats and the means of enhancing the mycorrhizal status of plants used for the revegetation of mine spoils. The reestablishment of mycorrhizal relationships has come to be recognized as an important component of an overall reclamation strategy (e.g. Marx 1975; Aldon 1978; Reeves et al. 1979; Trappe 1981). The successful development of mycorrhizae in disturbed soils may be influenced by the type of inorganic and organic amendment applied and the frequency of application. Studies on the addition of N or P fertilizer on VA mycorrhizal development in agricultural systems (e.g. Hayman et al. 1975; Kruckelman 1975) have shown a decrease in VA infection following application. However, Sparling and Tinker (1978) found that the addition of a N fertilizer to a grassland soil (125 kg · ha) did not alter the amount of mycorrhizal infection. The effects of fertilizer application to mine spoils on VA mycorrhizae have received no attention, but it is presumed that the effects of fertilizer application on the development of VA mycorrhizae depend largely on nutrient levels in the spoil prior to fertilization (Hayman 1978). While numerous studies have examined the effects of sewage sludge on plant growth (e.g. Gaynor and Halstead 1976), there is only meager information concerning mycorrhizal development on sewage amended soils. Berry and Marx (1976) found that sludge application rates as high as 275t dry wt · ha did not reduce the ectomycorrhizal development of Pinus echinata Mill. and P. taeda L. on severly eroded soils by Pisolithus tinctorius (Pers.) Coker and Couch. However, Spitko and Manning (1981) reported that a single application of sewage sludge (4.7 and 9.4t dry wt · ha) on agricultural soils inhibited VA mycorrhizal development of onion.

The potential use of various inorganic and organic surface amendments in mine spoil reclamation warrants a critical examination of their effects, over the long term, on the rates of mycorrhizal development and the status of the infection of plants used for this

reclamation. Time-course studies of VA mycorrhizal development have dealt mainly with agronomic plant species growing under either controlled environmental conditions (Beveage and Bowen 1975; Sanders et al. 1977) or in agricultural habitats (Sutton 1973; Rich and Bird 1974; Saif 1977; Black and Tinker 1979). Apart from the investigation by Ponder (1979) which examined the rate of mycorrhizal development of three plant species grown in recently graded mine spoil under greenhouse conditions, there has only been the study by Allen and Allen (1980) which has followed the mycorrhizal development of herbaceous plants grown on mine spoils under field conditions. They found that infection levels in plants grown on topsoil amended mine spoil in Wyoming increased gradually over a 3 yr period following reclamation to within 50% of the infection levels in plants from undisturbed sites.

The rates at which plants develop VA mycorrhizae in habitats disturbed through mining activity will depend in part on the inoculum density and species composition of the VAM fungi in the mine spoil following reclamation. The VAM inoculum density and species composition of these disturbed habitats will be governed by reclamation practices (Allen and Allen 1980; Rives et al. 1980), plant species composition of the reclaimed site (Miller 1979; Reeves et al. 1979), chemical and physical characteristics of the spoil, and the rate of recolonization by mycorrhizal fungi from undisturbed areas around the mine.

The objectives of this study were:

1. To examine the vesicular-arbuscular mycorrhizal development of slender wheatgrass grown on extracted oil sands tailings and a subalpine coal mine spoil over a 4 yr period.
2. To determine the effects of amendation of these mine spoils on the rates of mycorrhizal development and the status of the infection over time.
3. To examine the effects of amendation on the occurrence VAM fungal species and inoculum densities.

This investigation was one aspect of a larger project which examined the effects of amendation, from an organismic and process perspective, on the reinstatement of biological activity in soils disturbed through mining activity (see Danielson *et al.* in prep. and Visser *et al.* in prep. for information pertaining to other aspects of this project).

2. MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN OF SPOIL TANK STUDY

The mine spoils chosen for this investigation were the extracted oil sands from the Great Canadian Oil Sands Ltd. processing plant located near Fort McMurray, Alberta and from a pit coal mine located in a subalpine spruce-fir habitat at Luscar, Alberta. Large quantities of each spoil were transported to a location near the University of Calgary in early 1977 and placed in wooden tanks which were subdivided in 16 subunits (5x7xlm) by wooden partitions (Figure 1). Only 12 subunits were used in this investigation. The soil tanks were built into the ground and had an open bottom in the gravel subsoil beneath the original topsoil layer at the site. Approximately 60 cm of spoil was placed in each subunit. After leveling, each subunit was randomly amended only once with either: 1) fertilizer (a mixture of 23-23-0 and 0-0-62) applied at a rate of 113 kg N, 113 kg P_2O_5 and 91 kg K_2O · ha); 2) a feather moss peat from a mountain site near Canmore, Alberta currently dominated by white spruce (applied to a uniform depth of 14 cm). The peat was removed from the site by a front-end loader, after the surface layer was stripped off to avoid introducing weeds, and transported to Calgary by truck. The material was stockpiled for one wk and then applied to the appropriate subunits by hand; 3) sewage sludge (anaerobically digested, applied at a level equivalent to 46 t (dry) · ha, or 4) left unamended. Three subunits were established per treatment. These amendments and application rates were chosen based on either literature information or current reclamation procedures at the mine sites. All amendments were rototilled into the spoils to a depth ot 15 cm prior to planting and sampling for chemical analysis.

Immediately following application of the amendments in June 1977 each subunit was divided into four plots (2.75 x 1.75 m), with 0.5 m walkways between them and the walls of the tank, and planted with four plant species:

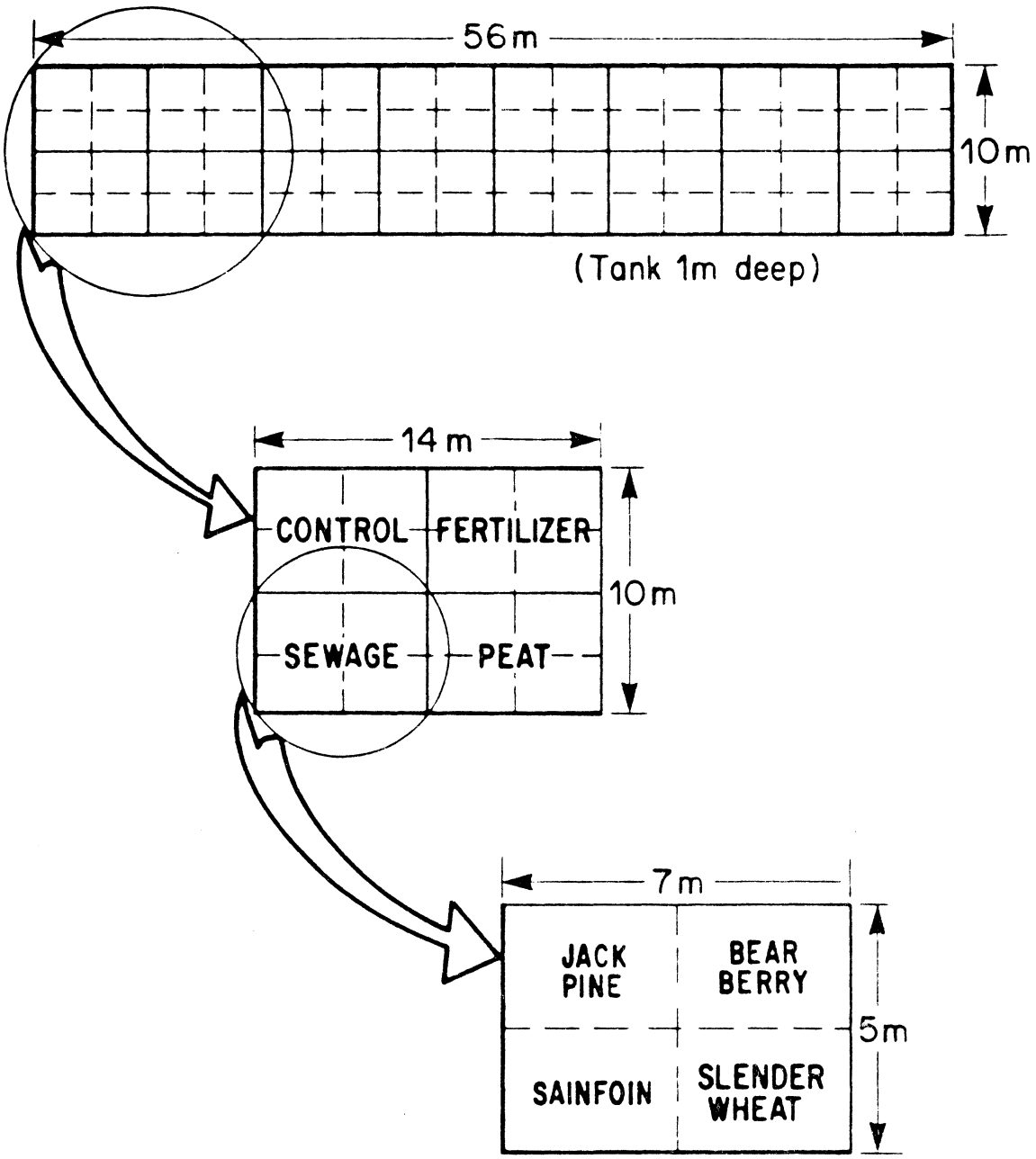

Figure 1. Plan of spoil tank.

Spoil Type	Plant Species
Oil Sands	Arctostophylos uva-ursi (L.) Spreng. (bearberry)
	Pinus banksiana Lamb (jack pine)
	Agropyron trachycaulum (Link) Malte. (slender wheatgrass)
	Onobrychis corniculatus L. (sainfoin)
Subalpine	Picea glauca (Moench.) Vass (white spruce)
	Salix sp. (willow)
	Agropyron trachycaulum
	Trifolium hybridum L. (alsike clover)

The plant species chosen for each spoil type represented species that were either being used in revegetation programs at the mine sites and at other locations in the province or were species that occurred in the undisturbed areas around the mines. Slender wheatgrass was chosen for this investigation since it was grown on both mine spoils.

Bearberry, jack pine, white spruce, and willow were planted out as seedlings. Known weights of seed of alsike clover, sainfoin, and slender wheatgrass were hand sown in each plot (Table 1). Plots were weeded and pathways kept clear of vegetation during each growing season.

2.2 SAMPLING PROGRAM AND QUANTIFICATION OF VESICULAR-ARBUSCULAR MYCORRHIZAE

To assess the initial rate of VAM development in roots of slender wheatgrass, five intact plants were dug from each plot (three plots per treatment) at 2, 6, and 10 wk after plant emergence. Samples not immediately processed were moistened and stored at 5°C. In the laboratory, roots were washed free of soil on a 2 mm sieve and shoots removed. Prior to assessing mycorrhizal infection, the root systems of the five plants from within each plot were subsequently

Table 1. Seeding rates of herbaceous plants used in the spoil tank study.

Plant Species	Amount of Seed Sown ($g \cdot m^2$)	No. of Seeds · g ($\bar{X}$ + S.E.)
Slender Wheatgrass	2.3	263 ± 1
Alsike Clover	0.4	1333 ± 2
Sainfoin	16.4	37 ± 1

pooled and cut into 1 to 2 cm segments. The entire amount of root obtained per plot was used to determine the amount and status of the VAM infection.

Plants were sampled the second and fourth year toward the end of the growing season (i.e. late August). Since it was impractical to excavate whole intact root systems after the first year, two random soil samples (14 cm deep x 6 cm diam.) were taken using a hand trowel from each plot (six replicates per treatment). Roots were obtained from each sample by washing the spoil through 2 mm and 500 µm sieves. Roots present on the 2 mm sieve were removed, blotted, weighed, and kept for quantification of VAM infection. To obtain the fresh weight of root in the 500 µm fraction, the organic material was first separated from the heavier inorganic component through successive decantings. This material was subsequently dispersed in water and roots removed, blotted, and weighed. If there was too much organic debris in the 500 µm fraction for easy separation of the root material, a random subsample (10%) was obtained and the weight of root in the subsample was used to estimate the weight of root in the fraction. This value was added to the weight of root from the 2 mm sieve to obtain the total wet weight of root in the soil sample. A random (10%) of the wet weight of root was used to estimate the amount and status of the VAM infection.

A modification of the procedure described by Phillips and Hayman (1970) was used to observe the mycorrhizal infection. Roots were cleared in simmering 10% KOH for 6 min, rinsed in distilled water for 5 min, acidified in 0.1 N HCl for 5 min, and stained in simmering 0.01% trypan blue. These changes were necessary in order to reduce fragmentation of the roots and to decrease background staining.

VAM infection was quantified using a modification of the line intersect technique (Newman 1966). An occular grid (11 x 11 lines, 60 µm between lines) was used to estimate root length (cm) rather than using a single line. Giovannetti and Mosse (1980) have shown that a grid system provides for a more accurate measurement. Prior to quantification, roots were placed within a 2.4 x 5.0 cm area

on a microscope slide. Care was taken to place as much root as possible in this area without having roots overlaying each other. This procedure was repeated until all the roots from a given sample were placed on microscope slides. Twenty five fields were examined per slide under a compound microscope at 160X magnification. Prior testing using filaments of known length showed that 25 fields per 2.4 x 5.0 cm area gave accurate, consistent estimates of filament length. For each slide, five kinds of measurements were made: length of root, length of mycorrhizal root, and length of mycorrhizal root containing either arbuscules, vesicles, or only hyphae. Although more than one type of mycorrhizal structure may be intersected by a grid line in any given field, only one structure was enumerated. The order of importance was arbuscules, vesicles and then hyphae.

Root length data from the first year samples were expressed on a per plant basis after dividing the total number of observations per sample by the number of plants sampled (five). The total lengths of root in each sample from the 2nd and 4th growing seasons were calculated from the total fresh wt of root and expressed as length of root (cm) per 10 cm^3 of spoil. Percent infection was determined by dividing total mycorrhizal root length by total root length. The necessity for expressing mycorrhizal infection levels on a root length basis rather than as a percentage of root length has been addressed by several investigators (e.g. Gerdemann 1968; Ambler and Young 1977).

2.3 QUANTIFICATION OF VAM FUNGAL SPORES AND SPOROCARPS

Spoil samples were taken from each slender wheatgrass plot on the amended oil sands tailing and subalpine coal mine spoil at the end of the third growing season (i.e. August 29, 1979) to estimate spore and sporocarp numbers of VA fungi. Three random soil samples to a depth of 10 cm were taken per plot, nine replicates per treatment. A modification (Smith and Skipper 1979) of the wet sieving and decanting technique described by Gerdemann and Nicolson (1963) was used to remove spores and sporocarps from the amended mine spoils. A 25 g (wet wt) subsample of spoil was placed in 200 ml of

water and stirred for 5 min before pouring the material through a series of sieves (500, 250, 125, and 53 μm). The subalpine mine spoil samples were first passed through a 4 mm mesh sieve to remove large rock fragments and other debris. Material remaining on each sieve after washing with tap water for 3 min was transferred into separate 150 ml beakers to which 100 ml of water was added and vigorously stirred. Heavy material was briefly allowed to settle (30 s) and spores collected by pouring the water onto a 6 cm diam. filter paper held in a Millipore filter holder which was under suction. The material remaining in each beaker was then extracted again.

A modification (Smith and Skipper 1979) of the sucrose centrifugation method (Allen et al. 1979) was used to collect VAM fungal spores and sporocarps in the peat-amended spoils due to interference from organic matter. Ten grams (wet wt) of peat-amended spoil were wet sieved and the material remaining on each sieve was washed into separate 50 ml centrifuge tubes and made up to 35 ml with distilled water. Following centrifugation (2000 rpm, 10 min) the supernatant was filtered as above. The pellet remaining after the first centrifugation was resuspended in 2 M sucrose solution to bring the volume up to 35 ml., vigorously stirred, and centrifuged at 2000 rpm for 10 min; the supernatant was filtered. More than one filter paper had to be used per centrifuge tube to avoid masking of the spores by suspended fine particulate matter. Spores and sporocarps on the filter papers were counted at a constant magnification (25 or 50X) under a dissecting microscope. Spores that were cracked or heavily pitted were not included in the count. Numbers of spores and sporocarps per VA fungal species were expressed per 10 g-dry-wt of spoil. A representative number of each spore and sporocarp type were mounted in lactophenol on slides for identification. Taxonomy of the VAM fungi followed Gerdemann and Trappe (1974) and Hall and Fish (1979).

2.4 THE OCCURRENCE OF THE VA FUNGI IN THE PEAT AMENDMENT

Samples were collected in June 1980 from the peat deposit near Canmore, Alberta, which provided the material used in the tank study, to determine if VA mycorrhizal inoculum was present in this amendment. The peat deposit was forested with mature white spruce and had a dense undergrowth of shrubs and herbs. Portions of the deposit had been mined previously and used as an amendment on nearby coal mine spoils. Three profiles were sampled around the perimeter of the excavated site. The face of each profile was cleaned and samples were taken at the 20 to 30, 50 to 60, and 80 to 90 cm depths from each of the profiles. All peat samples were stored at 5°C until they could be broken up by hand and thoroughly mixed. Extraction and quantification of VAM fungal spores were as previously described for the peat-amended mine spoils.

To determine if viable inoculum was present in the peat, three pregerminated slender wheatgrass seeds were planted in 13 cm pots containing peat from each depth and profile and grown in the greenhouse for 10 wk. Plants were watered once at 3 wk with a 20-20-20 fertilizer solution (0.5 g · 4 L) to alleviate phosphorus deficiencies. At 10 wk, roots were washed free of the peat on a 10 mm sieve, blotted dry, and their fresh weight determined. A random 10% of the total fresh weight of roots in each pot was subsequently cleared, stained, and assessed for VA mycorrhizal as previously described.

2.5 PRIMARY PRODUCTION

Five slender wheatgrass plants from each plot per treatment were obtained at 10 wk after plant emergence to determine shoot weight per plant. Shoots were separated from roots and dried at 80°C to constant weight. Shoot production values (g dry wt · m^2) for the second and third growing season were estimated at the time of sampling for assessment of VA infection and VA fungal spore and sporocarp numbers by clipping three random, 25 x 25 cm quadrats per plot (i.e. nine replicates per treatment). The soil samples obtained for mycorrhizal evaluation the second year were taken from the center

of the first two quadrats. Shoot material was dried at 80°C to constant weight.

2.6 CHEMICAL ANALYSIS

In June, 1977 (prior to planting) and after two growing seasons (September, 1978), soil samples were taken from the 0 to 5 cm depth in all amended and control plots for chemical analysis. Two random samples were taken per plot (three plots per treatment) and pooled. Allsamples were air dried and sieved through a 2 mm sieve. The following parameters were measured: NO^3-N, extractable P, DTPA extractable Pb and Zn and soil pH. Levels of NO^3-N were estimated by a phenol-disulphonic acid colorimetric procedure with a solution of copper and silver sulfate as the extractant. Extractable P was determined by a colorimetric measurement with molybdenum blue using ascorbic acid as the reductant following extraction with a sulfuric acid and ammonium fluoride solution. Amounts of Pb and Zn were measured by atomic absorption spectroscopy. The pH was determined after saturating the spoil with water. Chemical analyses and pH determinations were conducted as described by McKeague (1976). Additional information on the physical and chemical characteristics of the mine spoils and amendments can be found in Visser et al. (in prep.)

2.7 STATISTICAL ANALYSIS

Mycorrhizal and nonmycorrhizal root lengths, percent infection, spore and sporocarp numbers, shoot production, and nutrient analysis data, were statistically analyzed using one-way analyses of variance (Sokal and Rohlf 1969). Data sets underwent either ln, ln (x + 1), square root, or arcsine $\sqrt{p}$ transformation to render the variances homogenous as determined by the Bartlett's test. Several data sets were not tranformable and were analyzed using non-parametric tests. Details of these statistical tests are presented as footnotes to the appropriate tables.

3. RESULTS

3.1 VESICULAR-ARBUSCULAR MYCORRHIZAL DEVELOPMENT

3.1.1 Oil Sands

Vesicular-arbuscular mycorrhizal development during the first growing season was limited mainly to plants grown on the peat-amended plots (Table 2). There was some mycorrhizal infection in plants grown on the control plots at 10 wk after plant emergence, but the infection was very light and sporadic. VAM infection was first detected in plants grown on the peat-amended spoil at 2 wk (Table 2). Infection at this time consisted solely of hyphae (Table 3).

Infection levels at the end of the second growing season were higher ($p = 0.05$) in plants grown on the peat-amended plots as compared with plants on the other treatments (Table 4). VA mycorrhizae were observed for the first time in roots from the fertilized plots at the end of the second growing season, though at very low levels and sporadic in occurrence. VAM infection was not detected in roots from the sewage-amended plots.

After four growing seasons, infection levels were still highest ($p = 0.05$) in plants on the peat-amended spoil (Table 5). A low level of infection was detected for the first time in plants grown on the sewage-amended plots.

There were significant changes in the mycorrhizal status of slender wheatgrass plants between the second and fourth growing seasons (Table 6). The length of mycorrhizal root per 10 cm^3 of peat-amended spoil decreased ($p = 0.05$) during this period of time. Mycorrhizal root lengths and percent infection increased ($p = 0.05$) in the fertilized plots over the 2 yr period. The majority of plants in the control plots did not survive after 2 yr. However percent infection in the surviving plants increased from 4 to 36% ($p = 0.05$).

3.1.2 Subalpine Coal Mine

Vesicular-arbuscular mycorrhizal infection was detected at 2 wk after plant emergence only in plants grown on the peat-amended

Table 2. Initial VA-mycorrhizal development of slender wheatgrass grown on the amended oil sands spoil.[x]

	Time from Plant Emergence (weeks)	Treatment			
		Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length per Plant (cm)[y]	2	0	0.7	0	0
	6	0	6	0	0
	10	0.5[a]	37[b]	0	0
% Infection[z]	2	0	0.6	0	0
	6	0	4	0	0
	10	0.9[a]	23[b]	0	0

[x]Within a row, all non-zero means superscripted differently differ at $p = 0.05$, as indicated by Scheffé confidence intervals (Neter and Wasserman 1974).

[y]Data underwent ln $(x + 1)$ transformation.

[z]Data underwent arcsine $\sqrt{p}$ transformation.

Table 3. Status of the mycorrhizal infection (length of root which contained either arbuscules, hyphae, or vesicles) during the initial growth of slender wheatgrass on the amended oil sands spoil.[x]

Infection Status	Time from Plant Emergence (weeks)	Length of Root (cm) After the Following Treatments			
		Control	Peat	Fertilize	Sewage
Arbuscules	2	0	0	0	0
	6	0	3	0	0
	10	0	5	0	0
Hyphae	2	0	0.7	0	0
	6	0	4	0	0
	10	0.5^{a}	30^{b}	0	0
Vesicles	2	0	0	0	0
	6	0	< 0.1	0	0
	10	0	2	0	0

[x]Means superscripted differently, differ at $p = 0.05$ as indicated by Scheffé confidence interval. Data underwent $\ln(x + 1)$ transformation.

Table 4. VA-mycorhizal status of slender wheatgrass grown on the oil sands spoil, 2 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length ($cm \cdot 10cm^3$)[y]				
Total	0.6[a]	130[b]	1.2[a]	0
Arbuscules	0.1[a]	26[b]	0.5[a]	0
Hyphae	0.4[a]	98[b]	0.7[a]	0
Vesicles	0.1[a]	6[b]	0.0	0
% Infection[z]	4.0[a]	46[b]	1.0[a]	0

[x]Within a row all non-zero means superscripted differently differ at $p = 0.05$ as indicated by Scheffe confidence intervals.

[y]Data underwent ln $(x + 1)$ transformation.

[z]Data underwent arcsine $\sqrt{p}$ transformation.

Table 5. VA-mycorrhizal status of slender wheatgrass grown on the oil sands spoil, 4 years after application of surface amendments.[w,x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length ($cm \cdot 10cm^3$)[y]				
Total	ND	51^a	10^b	1^c
Arbuscules	ND	9^a	2^b	0
Hyphae	ND	41^a	7^b	1^c
Vesicles	ND	1	1	0
% Infection[z]	36^a	62^b	43^c	0.3^d

[w]Within a row all non-zero means superscripted differently differ at $p = 0.05$ as indicated by Scheffe confidence intervals.

[x]There were too few plants in the control to estimate root lengths. Two plants were dug from each plot to determine percent infection.

[y]Data underwent ln (x + 1) transformation.

[z]Data was statistically analyzed using the non-parametric Friedman test.

Table 6. Comparison of the mycorrhizal status of slender wheatgrass after 2 and 4 years on the amended oil sands spoil.[x,y,z]

Treatment		Year	
		Second	Fourth
Control	% Infection	4	36*
Peat	Mycorrhizal Root Length (cm · $10cm^3$)	130	51*
	% Infection	46	62
Fertilizer	Mycorrhizal Root Length (cm · $10cm^3$)	1	10*
	% Infection	0.6	43*
Sewage	Mycorrhizal Root Length (cm · $10cm^3$)	0	1
	% Infection	0	0.3

[x]Within a row, * indicates significant differences at $p = 0.05$ as indicated by a t-test.

[y]Root length data underwent square root transformation.

[z]% infection data underwent arcsine $\sqrt{p}$ transformation.

spoil (Table 7). Mycorrhizae were detected in plants grown on the control and fertilizer-amended plots at 6 wk after plant emergence. Infection was not detected in plants grown on the sewage-amended spoil until 10 wk. At 10 wk, there were no significant differences in total length of mycorrhizal root per plant among the amendments, although plants on the amended plots had more mycorrhizal roots than plants on the control ($p = 0.05$). The infection in plants grown on the peat-amended plots consisted solely of hyphae at the 2 wk sampling time (Table 8). While arbuscules were observed in roots of plants grown on the control and peat-amended plots at 6 wk, they were not observed in the roots of plants grown on the fertilizer- and sewage-treated plots until 10 wk after plant emergence.

Mycorrhizal root lengths after 2 years were highest ($p = 0.05$) in the peat-amended spoil as compared with the control and sewage-amended plots (Table 9). Mycorrhizal root lengths in the fertilized plots were not significantly different from the control, peat- or sewage-amended spoil. The status of the infection was also affected by the type of amendment applied. Lengths of root containing arbuscules were highest ($p = 0.05$) in the peat-amended spoil. Plants on the sewage-amended spoil had the lowest ($p = 0.05$) arbuscular root length. There were no significant differences in lengths of root with hyphae or vesicles among any of the treatments. Percent infection was suppressed ($p = 0.05$) in the sewage-treated plots compared with the percentages of infected roots from the control and peat-amended spoil.

Infection levels were still highest ($p = 0.05$) in the peat-amended spoil after four growing seasons (Table 10), than in the control or sewage-amended plots. Mycorrhizal roots lengths in the fertilized spoil were not significantly different than those in the control and peat-amended plots. The effects of the amendments on the status of the infection was similar to that observed after the 2nd year. Arbuscular root length was lowest ($p = 0.05$) in the sewage-treated spoil. However, the lengths of root which contained arbuscules were not significantly different among the other

Table 7. Initial VA-mycorrhizal development of slender wheatgrass grown on the amended subalpine coal mine spoil.[x]

	Time from Plant Emergence (weeks)	Treatment			
		Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length per Plant (cm)[y]	2	0	2	0	0
	6	3[a]	13[a]	1[a]	0
	10	19[a]	97[b]	160[b]	99[b]
% Infection[z]	2	0	5	0	0
	6	4[a]	10[a]	0.5[a]	0
	10	23[a]	58[b]	44[b]	16[b]

[x]Within a row, all non-zero means superscripted differently differ at $p = 0.05$, as indicated by Scheffé confidence intervals.

[y]Data underwent ln $(x + 1)$ transformation.

[z]Data underwent arcsine$\sqrt{p}$ transformation.

Table 8. Status of the mycorrhizal infection (length of root which contained either arbuscules, hyphae, or vesicles) during the initial growth of slender wheatgrass on the subalpine coal mine spoil.[x]

Infection Status	Time from Plant Emergence (weeks)	Length of Root (cm) After the Following Treatments			
		Control	Peat	Fertilizer	Sewage
Arbuscules	2	0	0	0	0
	6	0.3[a]	5[b]	0	0
	10	8[a]	40[b]	66[b]	31[ab]
Hyphae	2	0	2	0	0
	6	3[a]	8[a]	1[a]	0
	10	11[a]	57[b]	86[b]	68[b]
Vesicles	2	0	0	0	0
	6	0	0.2	0	0
	10	0	0.7[a]	7[a]	0.7[a]

[x]Within a row, all non-zero means superscripted differently, differ at $p = 0.05$ as indicated by Scheffé confidence intervals. Data underwent ln $(x + 1)$ transformation.

Table 9. VA-mycorrhizal status of slender wheatgrass grown on the subalpine coal mine spoil, 2 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length ($cm \cdot 10cm^3$)[y]				
Total	17^a	90^b	32^{ab}	24^a
Arbuscules	12^a	54^b	11^a	1^c
Hyphae	5^a	35^a	20^a	20^a
Vesicles	0.2^a	1^a	1^a	3^a
% Infection[z]	42^a	41^a	29^{ab}	9^b

[x]Within a row values superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y]Data underwent ln $(x + 1)$ transformation.

[z]Data underwent arcsine $\sqrt{p}$ transformation.

Table 10. VA-mycorrhizal status of slender wheatgrass grown on the subalpine coal mine spoil, 4 years after application of surface amendments.[x]

	Treatment			
	Control	Peat	Fertilizer	Sewage
Mycorrhizal Root Length ($cm \cdot 10cm^3$)[y]				
Total	19^{ab}	64^{c}	45^{bc}	12^{a}
Arbuscules	14^{a}	12^{a}	28^{a}	5^{b}
Hyphae	4^{a}	48^{b}	15^{c}	7^{ac}
Vesicles	0.5^{a}	4^{b}	2^{ab}	0.2^{a}
% Infection[z]	70^{a}	51^{b}	70^{a}	32^{c}

[x]Within a row values superscripted differently differ at $p = 0.05$ as indicated by Scheffé confidence intervals.

[y]Data underwent ln $(x + 1)$ transformation.

[z]Data underwent arcsine$\sqrt{p}$ transformation.

treatments. Percent infection was highest ($p = 0.05$) in the control and fertilizer-amended plots and lowest in the sewage-treated spoil.

There were no significant changes in mycorrhizal root lengths from any treatment between the second and fourth growing seasons (Table 11). However, percent infection did increase ($p = 0.05$) in the control, fertilizer- and sewage-amended plots over the two years.

3.2 ROOT LENGTHS

3.2.1 Oil Sands

Root length of slender wheatgrass over the first growing season, was affected by treatment ($p \leq 0.05$) (Table 12). Root lengths per plant were greater ($p = 0.05$) for those grown on the fertilizer- and sewage-amended spoil than for plants grown on the control plots. Root lengths of plants grown on the peat-amended spoil were not significantly different from root lengths of plants grown on the other treatments. Across all treatments root lengths were highest ($p = 0.05$) at 10 wk after plant emergence.

Root lengths at the end of the second growing season (Table 13) were greater ($p = 0.05$) in the amended plots than in the control. However, there were no significant differences in root lengths among the amendments. After four growing seasons, (Table 13) root lengths were greater in the sewage-amended plots than in the fertilized ones ($p = 0.05$). Root lengths in the peat-amended plots were not significantly different from lengths in the fertilizer- or sewage-amended spoil. Between 2 and 4 years, root lengths decreased ($p = 0.05$) in the peat- and fertilizer-amended spoil. There were no significant changes in the sewage-amended plots.

3.2.2 Subalpine Coal Mine

There was a significant ($p \leq 0.01$) interaction between time and treatment on root lengths (cm) of slender wheatgrass during the first growing season on the subalpine coal mine spoil (Table 12). Root lengths in the control plots were not significantly different

Table 11. Comparison of the mycorrhizal status of slender wheatgrass after 2 and 4 years on the subalpine coal mine spoil.[x,y,z]

		Year	
		Second	Fourth
Control	Mycorrhizal Root Length (cm · $10cm^3$)	17	19
	% Infection	42	70*
Peat	Mycorrhizal Root Length (cm · $10cm^3$)	90	64
	% Infection	41	51
Fertilizer	Mycorrhizal Root Length (cm · $10cm^3$)	32	45
	% Infection	29	70*
Sewage	Mycorrhizal Root Length (cm · $10cm^3$)	24	12
	% Infection	9	32*

[x]Within a row, * indicates significant differences at p = 0.05 as indicated by a t-test.

[y]Root length data underwent ln transformation.

[z]Percent infection data underwent arcsine$\sqrt{p}$ transformation.

Table 12. Total root lengths (cm • plant) of slender wheatgrass during the first growing season on the amended mine spoils.

Spoil Type	Time from Plant Emergence (weeks)	Treatment				Significance Level[x]		
		Control	Peat	Fertilizer	Sewage	Time	Treatment	Interaction
Oil Sands[y]	2	88	108	99	99	*	***	NS
	6	58	145	180	302			
	10	63	164	543	353			
Subalpine[z]	2	32	47	28	35	***	**	**
	6	113	123	192	203			
	10	98	166	349	586			

[x]Significance Level: * $0.05 \leq p > 0.01$; ** $0.01 \leq p > 0.001$; *** $p \leq 0.001$; NS nonsignificant.

[y]Data were found to be nontransformable and were statistically analyzed using a non-parametric test of interactions (Marascuilo & McSweeny 1977). Effects of time and treatments were determined using the Friedman's test at $p = 0.05$.

[z]Data was analyzed using a two-way ANOVA after ln transformation.

Table 13. Total root lengths of slender wheatgrass on the amended mine spoils 2 and 4 years after application of surface amendments.[x,y]

Spoil Type	Years Following Amendation	Root Length (cm · 10cm^3)			
		Control	Peat	Fertilizer	Sewage
Oil Sands[z]	2	15^a	304^b	159^b	258^b
	4	ND	86^a	35^b	124^a
Subalpine	2	49^a	200^b	104^b	222^b
	4	26^a	125^b	65^{ab}	47^a

[x]Within a row, means superscripted differently differ at p = 0.05 as indicated by Scheffé confidence intervals.

[y]Data underwent ln transformation.

[z]There were too few plants in the control to estimate root lengths.

among the sampling times. Also, at the 2 and 6 wk sampling times, root lengths in the control plots were not significantly different from lengths in the amended plots. At 10 wk, root lengths in the amended plots were greater than in the control plots, but there were no significant differences among the amendments.

Root lengths of slender wheatgrass at the end of the second growing season (Table 13) were greater ($p = 0.05$) in the amended plots compared with the control. However, there were no significant differences in total root lengths among the amendments. After four growing seasons, root lengths were higher ($p = 0.05$) in the peat-amended spoil as compared with root lengths in the control and sewage-amended plots (Table 13). Root lengths in the fertilized spoil were not being significantly different from those in the other treatments. Root lengths did not change significantly between 2 and 4 years on the control, peat- or fertilizer-amended subalpine mine spoil. There was a decrease ($p = 0.05$) in the sewage-amended plots though during this time.

3.3 SHOOT PRODUCTION

3.3.1 Oil Sands

All amendments increased ($p = 0.05$) shoot production above the control during the first growing season (Table 14). Shoot weights were highest in the sewage-amended spoil.

Shoot production for the second growing season was again highest ($p = 0.05$) in the sewage-treated plots (Table 15). Peat and fertilizer also increased plant growth compared with the control. By the third year (Table 15), shoot production had decreased ($p = 0.05$) in all but the peat-amended plots compared with estimates made during the second growing season.

3.3.2 Subalpine Coal Mine

Shoot growth was highest ($p = 0.05$) during the first growing season in the fertilizer- and sewage-amended spoil

Table 14. Shoot weights of slender wheatgrass plants at the end of the first growing season on the amended mine spoils (10 wk after plant emergence).[x,y]

	mg Dry Wt · Plant			
Spoil Type	Control	Peat	Fertilizer	Sewage
Oil Sands	5[a]	23[b]	145[c]	272[d]
Subalpine	15[a]	23[a]	147[b]	252[b]

[x]Within a row, means superscripted differently differ at p = 0.05 as indicated by Scheffé confidence intervals.

[y]Data underwent ln transformation.

Table 15. Shoot production estimates of slender wheatgrass for the 2nd and 3rd growing seasons on the amended mine spoils.[x]

Spoil Type	Growing Season	g Dry Wt · m^2			
		Control	Peat	Fertilizer	Sewage
Oil Sands[y]	2nd	2^{a}	110^{b}	167^{b}	766^{c}
Oil Sands[y]	3rd	0.02	80^{a}	18^{b}	50^{ab}
Subalpine[y]	2nd	43^{a}	111^{b}	210^{bc}	325^{c}
Subalpine[z]	3rd	27^{a}	102^{b}	45^{ab}	71^{ab}

[x]Within a row, means superscripted differently differ at $p = 0.05$ as determined by Scheffé confidence intervals.

[y]Data underwent ln transformation.

[z]Data underwent square root transformation.

(Table 14). Peat did not significantly increase plant growth the first year compared with the control.

Shoot production after two growing seasons (Table 15) was greater ($p = 0.05$) in all amendments than in the control. The highest production estimates were observed in the sewage-treated plots. Shoot production was significantly ($p = 0.05$) lower in the fertilizer and sewage-amended spoil after three years (Table 15) when compared with production estimates from the second growing seasons.

3.4 CHEMICAL ANALYSES OF THE MINE SPOILS

3.4.1 Oil Sands

Levels of extractable phosphorus in the amended oil sands spoil (0 to 5 cm) prior to planting were highest ($p = 0.05$) in the fertilized and sewage-amended plots (Table 16). Amounts of extractable P decreased ($p = 0.05$) over the first two years in the fertilizer- and peat-amended plots, and increased ($p = 0.05$) in the sewage-amended spoil (Table 16). There were no significant changes in P levels in the control.

All three amendments initially increased ($p = 0.05$) NO_3-N levels (0 to 5 cm) in the spoil, with the peat-amended plots having the highest ($p = 0.05$) levels (Table 16). Levels of NO_3-N in the fertilizer- and peat-amended spoil decreased ($p = 0.05$) over the two growing seasons. There were no significant changes in amounts of NO_3-N in the control and sewage-amended plots between the first and second growing seasons.

Initial levels of DTPA extractable Pb were not changed by amendation (Table 16). Zinc levels in the spoil were significantly increased ($p = 0.05$) by peat- or sewage-application compared with levels in the control and fertilized plots.

The pH of the spoil was very alkaline prior to the application of the surface amendments (Table 16). The addition of fertilizer and peat lowered the pH to neutrality. The addition of sewage also decreased pH of the spoil as compared with the control,

Table 16. Chemical analysis of the amended oil sands spoil (0 to 5 cm depth).[x]

Sampling Date	Treatment	µg · g Dry Wt of Spoil				
		Extractable[x] P	NO_3-N[x]	Pb[y]	Zn[y]	pH[z]
Prior to Planting	Control	1[1a]	0.1[1a]	0.6[a]	0.3[a]	8.7
(June, 1977)	Peat	3[2b]	251[2b]	3[a]	11[b]	7.3
	Fertilizer	53[4c]	40[4c]	1[a]	0.6[a]	7.0
	Sewage	26[6c]	16[6c]	3[a]	3[b]	7.8
September, 1978	Control	2[1d]	0.8[1d]			
	Peat	0.8[3d]	62[3e]			
	Fertilizer	14[5e]	3[5d]			
	Sewage	169[7f]	7[7d]			

[x]Data was statistically analyzed using 2-way ANOVAS following ln transformation. Letters compare treatments within one time, numbers compare time within one treatment at p = 0.05.

[y]Values not followed by the same letter differ at p = 0.05 as determined by a Scheffé confidence interval.

[z]Data was not statistically analyzed because of no variation amongst the replicates.

but the decrease was less than that observed for the other two amendments.

3.4.2 Subalpine Coal Mine

Levels of extractable P in the spoil (0-5 cm), prior to planting, (Table 17) were increased ($p = 0.05$) by the application of fertilizer or sewage. After two growing seasons, P levels were highest ($p = 0.05$) in the fertilizer- or sewage-amended spoil (Table 17). Amounts of extractable P in the peat-amended plots were lower ($p = 0.05$) than levels in the control plots. The levels of extractable P, within each treatment, did not change significantly over the first two growing seasons.

Prior to planting, NO_3-N levels were highest ($p = 0.05$) in the fertilizer- or peat-amended spoil (Table 17). After two growing seasons NO_3-N levels were still highest ($p = 0.05$) in the peat-amended spoil. There was a decrease ($p = 0.05$) in NO_3-N levels in the fertilized plots between the first and second growing season. Amounts of NO_3-N in the control, peat- or sewage-amended plots did not change significantly over the first two growing seasons.

Sewage amendation increased ($p = 0.05$) the amount of DTPA extractable Pb and Zn in the subalpine mine spoil (Table 17) prior to planting. Extractable Zn levels were also higher ($p = 0.05$) in the peat-amended plots than in the control.

The incorporation of peat into the spoil only resulted in slight changes in the pH of the material (Table 17). Fertilizer had no substantial effect on soil pH. However, sewage increased pH of the mine spoil.

3.5 VA FUNGAL SPECIES

Glomus aggregatum and Glomus mosseae were the most commonly encountered species of VA mycorrhizal fungi, occurring in all but the fertilizer- and sewage-amended oil sands plots (Table 18). The absence of VA fungi in these plots was consistent with the results obtained on mycorrhizal infection levels at the end of the second growing season. Glomus aggregatum has not been previously reported

Table 17. Chemical analysis of the amended subalpine coal mine spoil (0 to 5 cm depth).

Sampling Date	Treatment	µg · g Dry Wt of Spoil				
		Extractable[x] P	NO_3-N[x]	Pb[y]	Zn[y]	pH[z]
Prior to Planting	Control	3[a1]	4[a1]	0.5[a]	0.9[a]	7.5
(June, 1977)	Peat	2[a2]	449[b2]	0.9[a]	10[b]	7.4
	Fertilizer	51[b3]	150[b3]	0.8	1[a]	7.1
	Sewage	42[b4]	25[c5]	6[b]	8[b]	7.8
September, 1978	Control	5[c1]	3[d1]			
	Peat	0.3[d2]	97[e2]			
	Fertilizer	26[e3]	4[d4]			
	Sewage	89[e4]	25[f5]			

[x]Data was statistically analyzed using 2-way ANOVAS following ln transformation. Letters compare treatments within one time, numbers compare time within one treatment at p = 0.05.

[y]Values not followed by the same letter differ at p = 0.05 as determined by a Scheffé confidence interval.

[z]Data was not statistically analyzed because of no variation amongst the replicates.

Table 18. VA-mycorrhizal fungal species and numbers of spores and sporocarps present in the slender wheatgrass plots on the oil sands and subalpine amended mine spoils after three growing seasons.

Fugal Species	Spoil Type	Spore Numbers : 10 g Dry Wt of Spoil Treatment			
		Control	Peat	Fertilizer	Sewage
Entrophospora infrequens (Hall) Ames & Schneider	subalpine	< 1	0	0	0
Glomus aggregatum Schenck & Smith	oil sands	3	182	0	0
	subalpine[x]	52^{a}	180^{b}	19^{ac}	4
Glomus mosseae (Nicol & Gerd.)	oil sands	0.2	6	0	0
	subalpine[y]	14^{a}	8^{a}	20^{a}	12^{a}
G. mosseae (sporocarps)	subalpine[x]	21^{a}	0	18^{ab}	2^{b}

[x]Data underwent ln transformation. Values having a mean of zero were not included in the ANOVA. Means superscripted differently differ at $p = 0.05$ as indicated by Scheffe confidence intervals.

[y]Means are not significantly different at $p \leq 0.05$ as determined by post-hoc comparisons based on the nonparametric Krieskal-Wallis test.

from reclaimed mine spoils; having been found only under citrus (Schenck and Smith 1982). The spores of this species were also common in soil from a short-grass prairie southeast of Calgary and in the pasture lands and walkways around the spoil tanks. Glomus mosseae, the other commonly encountered endophyte has been reported from Western Canada (Molina et al. 1978) but not specifically from Alberta. Khan (1978) found G. mosseae in association with plants growing on coal tips in the Illawara region of New South Wales. A fifth species, Glomus tenuis (Green) Hall, with spores too small to be readily isolated from soil, was detected in the roots of slender wheatgrass grown on the peat-amended spoils during quantification of VAM infection levels by its characteristic hyphae and morphology of infection.

3.6 VA FUNGAL SPORE AND SPOROCARP NUMBERS

Spore numbers of Glomus aggregatum and G. mosseae in the oils sands spoil were highest ($p = 0.05$) in the peat-amended plots with low spore numbers in the control and no spores detected in the fertilizer- or sewage-amended spoil (Table 18).

Spore number of G. aggregatum in the subalpine spoil were highest ($p = 0.05$) in the peat-amended spoil followed by the control, with numbers the lowest in the sewage-amended plots. Numbers of spores in the fertilized plots were not significantly different from the values for the control or sewage-amended plots. There were no significant effects of amendation of the subalpine mine spoil on number of G. mosseae spores. Total spore numbers in the control and amended subalpine mine spoil were not significantly correlated with shoot production for the third growing season.

Sewage suppressed ($p = 0.05$) sporocarp production by Glomus mosseae in the subalpine mine spoil (Table 18). Fertilizer, however, had no effect on sporocarp numbers.

3.7 EFFECTS OF THE AMENDMENTS ON THE SIZE OF GLOMUS MOSSEAE SPORES AND SPOROCARPS

The numbers of spores and sporocarps of Glomus mosseae that were obtained on each sieve, after wet sieving the subalpine mine spoil, were used to examine the effects of the amendments on the size of these fungal propagules. G. mosseae was chosen since the range of spore and sporocarp sizes for this species (Gerdemann and Trappe, 1974) extended over the range of pore sizes used in the wet sieving procedure. There were an insufficient number of these propagules in the oilsands spoil to conduct a similar examination.

There were no substantial effects of fertilizer application to the subalpine mine spoil on the size class distribution of G. mosseae sporocarps compared with the control (Figure 2). Insufficient sporocarps were obtained from the peat- and sewage-amended plots to determine if these treatments had any effect on sporocarp size.

Single spores of G. mosseae obtained from the control and fertilized subalpine mine spoil occurred mainly within the 53 to 125 and 125 to 250 μm size classes (Figure 2). There were no significant differences in the numbers of spores occurring within these two size classes. A greater ($p = 0.05$) percentage of the total number of G. mosseae spores from the sewage-amended plots, however, occurred within the smallest size class. There were insufficient number of G. mosseae spores collected from the peat-amended spoil to examine the effects of this amendment on size class distribution.

3.8 THE OCCURRENCE OF VA FUNGI IN THE PEAT AMENDMENT

VA fungal spores were detected in samples from each of the sites in the peat deposit but their occurrence and numbers did not exhibit a consistent pattern with depth. While the majority of spores from site one and three were detected in the 50 to 60 cm depth, spores in site two occurred predominantly in the 20 to 30 cm layer.

Although VA fungal spores were detected from all the sites, they appeared to be nonviable. Spore walls were generally heavily

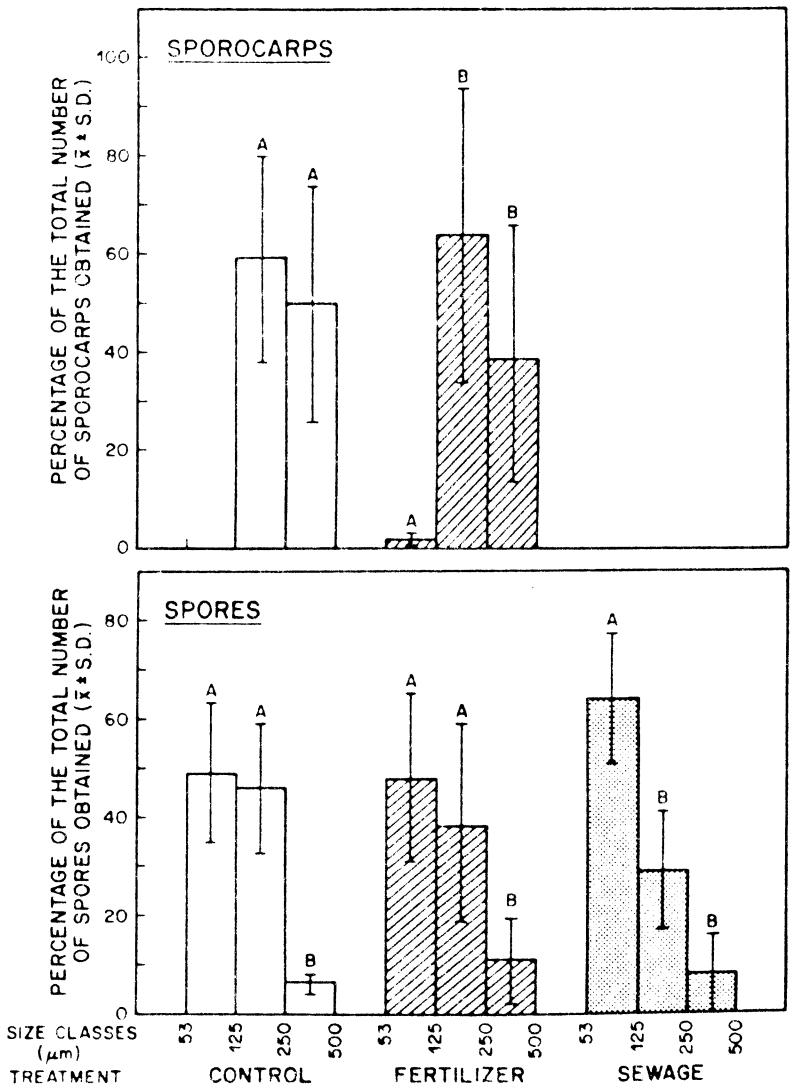

Figure 2. Size class distribution of Glomus mosseae sporocarps and spores obtained from the slender wheatgrass plots on the amended subalpine coal mine spoil at the end of the third growing season. Data underwent arcsine$\sqrt{p}$ transformation to obtain homogeneous variances. For each treatment, the same letter above a bar indicates nonsignificant differences at $p = 0.05$ as indicated by Scheffé confidence intervals.

thickened and pitted and spores lacked content. The characteristics of these old spores suggested affinity with Glomus fasciculatum. However, chlamydospores found associated with the roots of slender wheatgrass from the bioassay study were identified as Glomus aggregatum.

The distribution of VA mycorrhizal inoculum within the peat deposit was patchy (Table 19) There was no apparent relationship between the presence of VA fungal spores and the subsequent mycorrhizal development of slender wheatgrass in the baiting study. The amount of VA-fungal infection, expressed either as mycorrhizal root length or as a percent of the total root length was variable and did not follow a trend with depth (Table 19).

Table 19. The distribution of viable vesicular-arbuscular mycorrhizal inoculum within the peat deposit using Agropyron trachycaulum as the host.[x]

	Profile 1			Profile 2			Profile 3		
Depth (cm)	20-30	50-60	80-90	20-30	50-60	80-90	20-30	50-60	80-90
Total Root Length (m)	81	127	32	45	52	30	22	175	7
Mycorrhizal Root Length (m)	0	0	0	12	20	23	0	123	0.3
% Infection	0	0	0	27	38	78	0	71	4

[x]Root length values represent the total amount of root produced by three plants grown in a 13 cm diam. pot under greenhouse conditions for 10 weeks.

4. DISCUSSION

4.1 INITIAL MYCORRHIZAL DEVELOPMENT

Initial rates of vesicular-arbuscular mycorrhizal development in slender wheatgrass grown on the oil sands and subalpine mine spoils were significantly affected by amendation. The type of amendment applied to a mine spoil and the rate of application can control the initial mycorrhizal development of plants used in revegetation programs by:

1. increasing VA fungal inoculum densities in the spoil,
2. altering soil nutrient levels and/or other chemical and physical properties.

The appearance of infection at 2 wk only in the peat-amended spoils and the presence of VA mycorrhizae mainly in plants on the peat-amended oil sands spoil indicated that the peat amendment had added VA fungal inoculum. This was confirmed in a later study. The rapid infection rates in the peat-amended soils presumably were indicative of infections being established from hyphae or infected root fragments (Hall 1976; Johnson 1977; Powell 1977; Hayman & Stovold 1979). The oil sands tailings were found, by wet sieving and decanting and by establishing slender wheatgrass plants on the spoil in the greenhouse, to be initially devoid of VA fungi.

The initial rates of VA mycorrhizal development in plants grown on the peat-amended spoils were similar to those reported by Ponder (1979) for ryegrass and sudangrass on a recently graded strip-mine spoil from Illinois and for plants grown on agricultural and undisturbed habitats (Sutton 1973; Read *et al.* 1976; Powell and Sithamparanathan 1977). The longer time until infection in plants from the control and fertilizer-amended subalpine mine spoil suggested that inoculum levels were considerably lower and/or that the inoculum was in a different form as compared with the peat-amended spoil.

The delay in infection in plants from the sewage-amended subalpine mine spoil may be due to factors other than inoculum levels. Spitko and Manning (1981) found that application of sewage

sludge (4.7 and 9.4 t dry weight · per ha) to field plots initially inhibited the development of VA mycorrhizae. They suggested that the inhibition may have been the result of high levels of extractable P(222 to 269 ppm). In that initial extractable P levels were not significantly different between the fertilizer- and sewage-amended subalpine mine spoil, toxic substances(s), such as heavy metals, may have been responsible for the delay in infection in plants on the sewage-treated plots. Hepper and Smith (1976) showed that the addition of 0.7 ppm of Zn to culture media significantly reduced the germination of Glomus mosseae spores. While it is difficult to transpose laboratory results to field situation, the higher levels of heavy metals in the sewage-treated subalpine mine spoil than in the other treatments suggests that sensitivity of the VA fungal inoculum to heavy metals may have been one factor which contributed to the initial delay in infection. Zinc levels in the sewage-amended plots were not significantly different from levels in the peat-amended spoil, suggesting the Pb rather than Zn may be responsible for the suppression. However, the degree of sensitivity of the endophyte(s) in the peat- or sewage-amended plots to Zn levels could have been an ecotypic response. Since infection was initiated in plants grown on the sewage-amended plots between 6 and 10 wk after plant emergence, Pb and Zn levels may have been reduced over time to ineffective levels through absorption by the clay fraction, chelation with organic matter (Gaynor and Halstead 1976), and (or) immobilization in decomposer biomass.

Arbuscules have been shown to be the sites of P transfer between endophyte and host (Schoknecht and Hattingh 1976). The length of time until their appearance in roots of plants grown on spoils of low nutrient status may have significant effects on seedling survival. The length of root or more importantly the volume of root containing arbuscules that is necessary before the plant benefits from the symbiosis is unknown. Stribley et al. (1980) indicated that infection levels of less than 20% in onions did not appear to have an effect on shoot yield, but they gave no data on the proportion of the infection that was arbuscular. While slender

wheatgrass on the peat-amended spoils contained arbuscules at 6 wk, it is unknown whether the infection was functional at this time.

4.2 VA FUNGAL INOCULUM

The ability of a habitat to recover to predisturbance conditions following mining activity and subsequent reclamation, will depend in part on the successful reestablishment of mycorrhizal relationships. The success and rate of reestablishment will be determined by the effects of reclamation practices on fungal species present and inoculum densities in the reclaimed site. Spores of mycorrhizal fungi may constitute an important source of infection in reclaimed mine spoils where plant densities over the short term are usually low. Total spore numbers from the control and amended subalpine coal mine spoil [spore numbers per 10 g-dry-wt of spoil $\bar{X} \pm$ S.D., control (66 ± 62), peat (188 ± 115), fertilizer (39 ± 36) and sewage (16 ± 29)] were higher than spore numbers reported from coal mine wastes (Khan 1978) or from an agricultural field (Hayman *et al.* 1975) but were lower, except in the peat amended spoil, in comparison with numbers found by Sutton and Barron (1972) from agricultural fields in Ontario.

The application of sewage sludge to a mine spoil, by suppressing spore production, could significantly affect the success of reclamation programs by slowing the rate of VA fungal inoculum buildup in these sites. Spore production of *Glomus aggregatum* was significantly reduced in the sewage-amended subalpine coal spoil, 3 yr after the original application, compared with the other treatments. In mine spoils where VA inoculum levels are initially low or where mycorrhizal development is suppressed, nonmycorrhizal plant species, usually members of the Chenopodiaceae (Miller 1979) that colonize the site, could have a competitive advantage over mycorrhizal dependent species, especially if nutrient become limiting (Janos 1980). Over time, the composition of the site would change from mycorrhizal dependent to nonmycorrhizal species. Under these circumstances successional blocks may be created in the system, since nonmycorrhizal species do not provide for the buildup of VA fungal

inoculum and subsequent species which require VA mycorrhizae for nutrient uptake and growth are excluded from the habitat (Reeves et al. 1979; Janos 1980). Allen and Allen (1980) reported that Salsola kali, a nonmycorrhizal plant species, predominated on strip-mine soils with less than 1 spore per g for 10 yr.

Sewage sludge application to the subalpine coal mine spoil not only resulted in decreased numbers of G. aggregatum chlamydospores but also affected the size of G. mosseae spores. Spore size, as a function of the amount of nutrient reserves, may be a major factor factor determining VA fungal spore longevity, and the ability of these propagules to germinate and establish successful infection. Garrett (1973) has commented that the average size of the reproductive propagule of a plant pathogenic fungus is determined by the nutritional requirements for establishment of a new individual of the species in its typical habitat. Garrett's statement suggests that spores of VA fungi which occur at the small end of the spore size range for the species would have a lower probability of causing infection than spores larger in size. Ross (1980) reported that germination of small (< 75 um) Glomus macrocarpum Tul. & Tul. var geosporus (Nicol. & Gerd.) Gerdemann & Trappe chlamydospores from soybean fields was less than that of larger (> 105 um) ones.

4.3 VA MYCORRHIZAL DEVELOPMENT: 2ND AND 4TH YEAR

The low levels and sporadic nature of the VAM infection in the control and fertilized oil sands tailings during the second year suggested that the oil sands spoil, originally devoid of mycorrhizal inoculum, was being colonized by VA fungi, though at very low rates. There is some question as to the means by which VA fungal inoculum is dispersed and the distance over which effective dispersal can occur. Movement of inoculum either in the form of spores or root fragments can only be accomplished by the physical movement of soil particles (Gerdemann and Trappe 1974). McIlveen and Cole (1976) showed that worms, ants, and birds could be agents of dispersal for VA fungal spores by their movement of soil particles. It is doubtful whether this type of dispersal is a major contributor to long distance

transport and would likely be more important to within habitat movement of inoculum. The distance from the mine site to potential VA fungal inoculum pools and the rates of dispersal and subsequent recolonization would likely be important factors that need to be considered in the planning of reclamation programs. Success or failure of a revegetation strategy may be determined in part by the immigration rates of VA fungi into these disturbed habitats. This would be especially true if the spoil lacked mycorrhizal inoculum prior to reclamation, such as the oil sands tailings, and inoculum was not introduced either via an amendment or by planting mycorrhizal seedlings.

The decision to use a specific amendment or one application rate versus another could have significant consequences on the outcome of a revegetation program. The peat amendment consistently resulted in higher levels of infection in both spoils compared with sewage application, which suppressed mycorrhizal development, although more severly in the oil sands tailings than on the subalpine coal mine spoil. Because of the nutrient poor condition of most mine spoils, the addition of some form of nutrients will be necessary to achieve maximum plant growth even if mycorrhizae are present. Hughes et al. (1978) and Menge et al. (1978) showed that the addition of nutrients to mycorrhizal plants increased growth above levels obtained for mycorrhizae alone. Slender wheatgrass plants on the controls exhibited less total root lengths than plants on the amended spoils even though mycorrhizal root lengths may not have been significantly different. Consideration should be given to choosing an amendment application regime that will not only ensure optimum plant growth and survival but which will not retard or suppress mycorrhizal development. The initial absence of mycorrhizal inoculum coupled with high soil P levels could have been the primary factor responsible for the suppression of infection in plants on the sewage-amended oil sands spoil. The reduced infection levels of plants on the sewage-amended subalpine coal spoil may have resulted solely from high levels of soil P.

The effects of various reclamation practices on VA mycorrhizal development of plants used in a revegetation program may depend on the VA fungal inoculum potential on the site prior to revegetation, the species of endophytes present, and the abiotic characteristics (e.g. pH, nutrient content, moisture retention) of the spoil. While slender wheatgrass on the fertilizer- and sewage-amended subalpine coal mine spoil were infected by the end of the first growing season, plants on the fertilized- and sewage-treated oil sands tailings did not become mycorrhizal until the end of the second and fourth growing seasons respectively. These differences in mycorrhizal response to fertilizer- or sewage, between the two spoil types may have been due to the lack of inoculum in the oil sands spoil.

The lack of significant changes in the mycorrhizal status of slender wheatgrass on the subalpine coal spoil, over the four years, indicates that initial reclamation procedures will have long term effects on VA mycorrhizal development. Allen and Allen (1980) also found that plants on topsoil amended mine spoils in Wyoming were only 50% of infection levels in plants from undisturbed habitats after 3 yr. By influencing mycorrhizal development, the type of amendment applied to mine spoil could determine the rate of recovery and the success of a reclamation program.

4.4 PLANT GROWTH

Since mycorrhizal and nonmycorrhizal plants did not occur on the same treatment, it is difficult to ascertain whether VA infection was necessary for the growth of slender wheatgrass on the controls and amended spoils. Over the short term, the absence of infection in plants grown of the sewage-amended oil sands spoil and the low infection levels in the sewage-amended subalpine coal mine spoil had no apparent effect on plant growth. It is interesting however, that shoot production peaked in the fertilizer- and sewage-amended plots on both mine soils during the second growing season and was significantly lower the 3rd year, but not in the peat-amended plots where plants were heavily mycorrhizal. These results suggest

that while high nutrient applications may result in greater short term primary productivity, the subsequent suppression of mycorrhizal development, as a result of the high nutrient levels, may lead to wide fluctuations in plant growth. Mycorrhizal relationships may be important in maintaining stability in primary production from year to year.

5. CONCLUSIONS

1. The type of amendments applied to the mine spoils controlled the initial rates of VAM development in slender wheatgrass by increasing fungal inoculum densities in the spoils and by altering soil nutrient levels and/or other chemical and physical properties.

2. The peat used in this study was found to contain VA fungal inoculum. The rapid infection rates in the peat-amended spoils presumably were indicative of infections being established from hyphae or infected root fragments.

3. The initial application of the amendments had long term effects on the development of VA mycorrhizal relationships in these disturbed soils. Slender wheatgrass plants on the fertilizer- and sewage-amended oil sands tailing took 2 and 4 yr respectively to develop VA mycorrhizae. There was no significant change in the mycorrhizal status of plants on the subalpine coal spoil over the 4 yr period. In general, changes in the mycorrhizal status of plants on mine spoils may occur only slowly with time.

4. The rate of dispersal of VAM inoculum appears to be very low. The distance from the mine site to potential VA fungal inoculum pools and the rates of inoculum dispersal and subsequent recolonization are important factors that need to be considered in the planning of a reclamation program.

5. Glomus aggregatum and Glomus mosseae were the dominant endophytes in the two mine spoils. Spore numbers and occurrence of these species were influenced by the amendment and the type of mine spoil.

6. Sewage significantly decreased spore production of G. aggregatum and the size of G. mosseae spores in the subalpine coal spoil. By suppressing Va fungal spore production and by affecting the size of spores produced by an endophyte, the application of sewage to a mine spoil may significantly alter the outcome of the revegetation program by slowing the rate of inoculum buildup within the spoil.

6. RECOMMENDATIONS

1. If the rapid reestablishment of VAM infection in plants grown on mine spoils is to be a major concern of a reclamation strategy, attention will have to be given to determining: a) initial Va fungal inoculum densities in the mine spoils, b) application rates of amendments which do not suppress mycorrhizal development but allow for optimum plant growth and survival. The effects of specific amendments on mycorrhizal development will depend on the nutrient status of the mine spoil and the VA fungal species and spore densities in the mine soil prior to reclamation.

2. The determination of nutrient response curves for mycorrhizal plants of species to be used in a revegetation program would be important in determining the optimum levels of fertilizer application.

3. Where disturbance is severe enough to either eliminate or significantly reduce VA fungal inoculum levels in a mine spoil, such as the oil sands, inoculum must be added to the site. The rate of natural recolonization by VAM fungi appears to be too slow to allow for their rapid reestablishment in these sites. The addition of inoculum can be accomplished by the addition of an amendment which contains VA fungi, such as the peat used in this study, or through the preinoculation of transplanted seedlings.

4. The use of commercially produced inoculum for large scale field inoculations is not feasible at this time. Research is needed not only to develop techniques for the introduction of inoculum prior to seeding but for ensuring adequate infection from the introduced inoculum.

5. Further research should be directed toward obtaining information of VA fungal species occurrences in mine spoils, their ecological tolerance limits, the effects of nutrient addition, and VA fungal species efficiency in promoting plant growth and survival.

6. Further information is needed on the effects of sewage sludge on VAM development before recommendations can be made concerning its use in a reclamation program. Future research should examine: a) the effects of toxic compounds in sewage on VA spore

germination and mycorrhizal development, b) application of different levels of sewage on mycorrhizal development.

7. REFERENCES CITED

Aldon, E.F. 1975. Endomycorrhizae enhance survival and growth of four-wing saltbush on coal mine spoils. USDA Forest Service Research Note R.M.-294.

Aldon, E.F. 1978. Endomycorrhizae enhance shrub growth and survival on mine spoils. The reclamation of disturbed arid lands, ed. R.A. Wright. Albuquerque: University of New Mexico Press. 196 pp.

Allen, E.B. and M.F. Allen. 1980. Natural re-establishment of vesicular-arbuscular mycorrhizae following strip-mine reclamation in Wyoming. Journal of Applied Ecology. 17: 139-147.

Allen, M.F., W.K. Smith, T.S. Moore Jr., and M. Christensen. 1981. Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal Bouteloua gracilis H.B.K. Lag ex Steud. New Phytologist 88:683-693.

Ambler, J.R. and J.L. Young. 1977. Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Science Society of America Journal 41: 551-556.

Berry, C.R. and D.H. Marx. 1976. Sewage sludge and Pisolithus tincotrius ectomycorrhizae:their effect on growth of pine seedlings. Forest Science 22: 351-358.

Bevege, D.I. and G.D. Bowen. 1975. Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. Endomycorrhizas, ed. F.E. Saunders, B. Mosse and P.B. Tinker. London: Academic Press. 626 pp.

Black, R. and P.B. Tinker. 1979. The development of endomycorrhizal root systems. II. The effect of agronomic factors and soil conditions on the development of vesicular-arbuscular mycorrhizal infection in barley and on the endophyte spore density. New Phytologist. 83: 401-413.

Crush, J.R. 1975. Occurrence of endomycorrhizas in soils of the MacKenzie Basin, Canterbury, New Zealand. New Zealand Journal of Agricultural Research. 18: 361-364.

Crush, J.R. 1976. Endomycorrhizas and legume growth in some soils of the MacKenzie Basin, Canterbury, New Zealand. New Zealand Journal of Agricultural Research. 19: 473-476.

Daft, M.J. and E. Hacskaylo. 1976. Arbuscular mycorrhizas in the antracite and bituminous coal wastes of Pennsylvania. Journal of Applied Ecology. 13: 523-531.

Daft, M.J. and E. Hacskaylo. 1977. Growth of endomycorrhizal and nonmycorrhizal red maple seedlings in sand and anthracite. Forest Science. 23: 207-216.

Daft, M.J. and T.H. Nicolson. 1974. Arbuscular mycorrhizas in plants colonizing coal wastes in Scotland. New Phytologist. 73: 1129-1138.

Danielson, R., C. Griffiths, and D. Parkinson. In preparation. Reinstatement of biological activity in severely disturbed soils: Ectomycorrhizae in amended oil sands and subalpine coal spoil and in undisturbed jack pine and spruce stands. Prepared for Research Management Division, Albert Environment.

Davidson, D.E. and M. Christensen. 1977. Root-microfungal and mycorrhizal associations in a short-grass prairie. The belowground ecosystem: A synthesis of plant associated processes, ed. J.K. Marshall. Range Science Department Science Series No. 26. Fort Collins: Colorado State University. 351 pp.

Eiland, F. 1981. The effects of high doses of slurry and farmyard manure on microorganisms in soil. Danish Journal of Plant and Soil Science. 85: 145-152.

Garrett, S.D. 1973. Deployment of reproductive resources by plant-pathogenic fungi: an application of E.J. Salisbury's generalization for flowering plants. Acta Botanica Indica. 1: 1-9.

Gaynor, J.D. and R.L. Halstead. 1976. Chemical and plant extractability of metals and plant growth on soils amended with sludge. Canadian Journal of Soil Science. 56: 1-8.

Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhizae and plant growth. Annual Review of Phytopathology. 6: 397-418.

Gerdemann, J.W. 1971. Fungi that form vesicular-arbuscular type of endomycorhizae. Mycorrhizae, ed. E. Hacskaylo. Misc. Publ. 1189, USDA Forest Service. pp. 9-18.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society. 46: 235-244.

Gerdemann, J.W. and J.M. Trappe. 1974. The Endogonaceae in the Pacific northwest. Mycologia Memoir No. 5. New York: The New York Botanical Garden, New York. 76 pp.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist. 84: 489-500.

Griffin, D.M. 1972. Ecology of soil fungi. Syracuse: Syracuse University Press. 193 pp.

Hall, I.R. 1976. Response of Coprosma robusta to different forms of endomycorrhizal inoculum. Transactions of the British Mycological Society. 67: 409-411.

Hall, I.R. and B.J. Fish. 1979. A key to the Endogonaceae. Transactions of the British Mycological Society. 73: 261-270.

Hayman, D.S. 1978. Endomycorrhizae. Interactions between non-pathogenic soil microorganisms and plants, ed. Y.R. Dommergues and S.V. Krupa. Amsterdam: Scientific Publishing Company, Amsterdam. 626 pp.

Hayman, D.S., A.M. Johnson, and I. Ruddlesdin. 1975. The influence of phosphate and crop species on Endogone spores and vesicular-arbuscular mycorrhiza under field conditions. Plant and Soil. 43: 489-495.

Hayman, D.S. and G.E. Stovold. 1979. Spore populations and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales. Australian Journal of Botany. 27: 227-233.

Hepper, C.M. and G.A. Smith. 1976. Observations on the germination of Endogone spores. Transactions British Mycological Society. 66: 189-194.

Hughes, M., L.W. Martin, and P.J. Breen. 1978. Mycorrhizal influence on the nutrition of strawberries. Journal of the American Society of Horticultural Science. 103: 179-181.

Janas, D.P. 1980. Mycorrhizal influence tropical succession. Biotropica. 12: 179-181.

Johnson, P.N. 1977. Mycorrhizal Endogonaceae in a New Zealand Forest. New Phytologist. 78: 161-170.

Khan, A.G. 1978. Vesicular-arbuscular mycorrhizas in plants colonizing black wastes from bituminous coal mining in the Illawara region of New South Wales. New Phytologist. 81: 53-63.

Khan, A.G. 1981. Growth response of endomycorrhizal onions in unsterilized coal wastes. New Phytologist. 87: 363-370.

Kruckelman, H.W. 1975. Effects of fertilizers, soils, soil tillage, and plant species on the frequency of Endogone chlamydospores and mycorrhizal infection in arable soils. Endomycorrhizas, ed. F.E. Sanders, B. Mosse, and P.B. Tinker. London: Academic Press. 626 pp.

Lambert, D.H. and H. Cole, Jr. 1980. Effects of mycorrhizae on establishment and performance of forage species in mine spoil. Agronomy Journal. 72: 257-260.

Lindsey, D.L., W.A. Cress, and E.F. Aldon. 1977. The effects of endomycorrhizae on the growth of rabbit brush, four-wing saltbush, and corn in mine spoil material. USDA Forest Service Research Note RM-343.

Marascuilo, L.A. and M. McSweeney. 1977. Nonparametric and Distribution Free Methods for the Social Sciences. Monterey: Brooks/Cole Publishing Company. 596 pp.

Marx, D.H. 1975. Mycorrhizae and the establishment of trees on strip mined land. Ohio Journal of Science. 75: 288-297.

McIlveen, W.D. and H. Cole, Jr. 1976. Spore dispersal of Endogonaceae by worms, ants, wasps, and birds. Can. J. Bot. 54: 1486-1489.

McKeague, J. 1976. Manual on soil sampling and methods of analysis. Ottowa: Soil Resource Institute. 212 pp.

Menge, J.A., E.L.V. Johnson, and R.G. Platt. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytologist. 81: 553-560.

Miller, R.M. 1979. Some occurrences of vesicular-arbuscular mycorrhiza in natural and disturbed ecosystems of the Red Desert. Canadian Journal of Botany. 57: 619-523.

Molina, R.J., J.M. Trappe, and Gerald S. Strickler. 1978. Mycorrhizal fungi associated with Festuca in the western United States and Canada. Canadian Journal of Botany. 56: 1691-1695.

Mosse, B. 1973a. Advances in the study of vesicular-arbuscular mycorrhiza. Annual Review of Phytopathology. 11: 171-196.

Mosse, B. 1973b. Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soil given additional phosphate. Phytologist. 72: 127-136.

Neter, J. and W. Wasserman. 1974. Applied linear statistical models. Chicago: Richard D. Irwin Inc. 842 pp.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology. 3: 139-145.

Nicolson, T.H. and C. Johnston. 1979. Mycorrhiza in the Gramineae III. Glomus fasciculatus as the endophyte of pioneer grasses in a maritime sand dune. Transactions of the British Mycological Society. 72: 261-268.

Parkinson, D. 1979. Microbes, mycorrhizal and mine spoils. Ecology and coal resource development. Vol. 2, ed. M.K. Wali. New York: Pergamon Press. 1091 pp.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society. 55: 158-161.

Ponder, F. Jr. 1979. Presence of endomycorrhizal fungi in recently graded coal mine spoil. Journal of Soil & Water Conservation. 34: 186-187.

Powell, C.L. 1977a. Mycorrhizas in hill country soils. III. Effect of inoculation on clover growth in unsterile soils. New Zealand Journal of Agricultural Resources. 20: 53-57.

Powell, C.L. and J. Sithamparanthan. 1977. Mycorrhizas in hill country soils. IV. Infection rate in grass and legume species by indigenous mycorrhizal fungi under field conditions. New Zealand Journal of Agricultural Resources. 20: 489-494.

Rabatin, S.C. 1979. Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by Glomus tenuis. New Phytologist. 83: 95-102.

Read, D.J., H. Koucheki and J. Hodgson. 1976. Vesicular-arabuscular mycorrhiza in natural vegetation systems. 1. The occurrence of infection. New Phytologist. 76: 641-653.

Reeves, F.B., D. Wagner, T. Moorman and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural environments. American Journal of Botany. 66: 6-13.

Rich, J.R. and G.W. Bird. 1974. Association of early season vesicular-arbuscular mycorrhizae with increased growth and development of cotton. Phytopathology. 64: 1421-1425.

Rives, C.S., M.I. Bajwa, A.E. Liberta and R.M. Miller. 1980. Effects of topsoil storage during surface mining on the viability of VA mycorrhiza. Soil Science 129: 253-257.

Ross, J.P. 1980. Effect of nontreated field soil on sporulation of vesicular-arbuscular mycorrhizal fungi associated with sogbean. Phytopathology. 70: 1200-1205.

Saif, S.R. 1977. The influence of stage of host development on vesicular-arbuscular mycorrhizae and Endogonaceous spore population in field-grown vegetable crops. I. Summer-grown crops. New Phytologist. 79: 341-348.

Sanders, F.E., P.B. Tinker, R.L.B. Black and S.M. Palmerley. 1977. The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arabuscular endophytes. New Phytologist. 78: 247-268.

Schenck, N.C. and G.S. Smith. 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. Mycologia. 74: 77-92.

Schoknecht, J.D. and M.J. Hattingh. 1976. X-ray microanalysis of elements in cells of VA mycorrhizal and nonmycorrhizal onions. Mycologia. 68: 296-303.

Smith, G.W. and H.D. Skipper. 1979. Comparison of methods to extract spores of vesicular-arbuscular mycorrhizal fungi. Soil Science Society of America Journal. 43: 722-725.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. San Francisco: W.H. Freeman. 776 pp.

Spitko, R.A. and W.J. Manning. 1981. Irradiated digested sewage sludge: effects on plant-symbiont associations in the field. Environmental Pollution. 25: 1-8.

Sparling, G.P. and P.B. Tinker. 1978. Mycorrhizal infection in Pennine Grassland I. Levels of infection in the field. Journal of Applied Ecology. 15: 943-950.

Stribley, D., P.B. Tinker and R.C. Snellgrove. 1980. Effect of vesicular-arbuscular mycorrhizal fungi on the relations of plant growth, internal phosphorus concentration, and soil phosphate analysis. Journal of Soil Science. 31: 655-672.

Sutton, J.C. 1973. Development of vesicular-arbuscular mycorrhizae in crop plants. Canadian Journal of Botany. 51: 2487-2493.

Sutton, J.C. and G.L. Barron. 1972. Population dynamics of Endogone spores in soil. Canadian Journal of Botany. 50: 1909-1914.

Trappe, J.M. 1981. Mycorrhizae and productivity of arid and semiarid rangelands. Advances in food producing systems for arid and semi-arid lands. Part A, ed. J. Manassah and E.J. Briskey. New York: Academic Press. 676 pp.

Visser, S., J.C. Zak, R.M. Danielson, C. Griffiths, and D. Parkinson. In preparation. Reinstatement of biological activity in severly disturbed soils: Effects of different amendments to three different mine spoils on selected soil physical and chemical properties and on plant growth. Prepared for Research Management Division, Alberta Environment.

REINSTATEMENT OF BIOLOGICAL ACTIVITY IN SEVERELY DISTURBED SOILS: ECTOMYCORRHIZAE IN AMENDED OIL SAND TAILINGS AND SUBALPINE COAL MINE SPOIL AND IN UNDISTURBED JACK PINE AND SPRUCE STANDS

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Abstract

The mycorrhizal status of jack pine and bearberry grown in oil sand tailings treated with various amendments (peat, mineral fertilizer, liquid sewage sludge) and of white spruce and willow grown in subalpine coal mine spoil using the same amendments was monitored for 4 years. In addition, ectomycorrhizae associated with jack pine and white spruce on undisturbed and disturbed field sites were studied and compared to those found in the amendment study.

Application of sewage sludge increased the rate of ectomycorrhizal development of jack pine, slowed the mycorrhizal development of white spruce and bearberry, and apparently did not affect the mycorrhizae of willow. Peat introduced inoculum of several species of ectomycorrhizal symbionts and when ectomycorrhizal inoculum in the spoil was low, its application resulted in more rapid mycorrhizal development. Mineral fertilizer had little effect on mycorrhizal development. The most common ectomycorrhizal fungus was the E-strain which dominated white spruce in all treatments and jack pine in the peat. At the end of 4 years the E-strain fungi were being replaced on white spruce by Amphinema byssoides and was apparently replacing Thelephora terrestris on jack pine in all treatments except the peat amendment. Agarics constituted a minor portion of the ectomycorrhizal fungi whereas at least four Ascomycetes and three Aphyllophoreales were present. Once established, the specific symbioses were stable for at least several years.

More than 50 species of ectomycorrhizal fungi were identified from fruit bodies on a mature jack pine field site. On this site Cenococcum geophilum, Tricholoma flavovirens and Lactarius spp. formed a large proportion of the ectomycorrhizae. Species fruiting in an adjacent cutline were largely different from those occurring in the undisturbed mature stand. Amphinema byssoides was a dominant symbiont of both mature spruce trees and of spruce seedlings regenerating on roadcuts.

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1. INTRODUCTION

Mycorrhizal associations are present in the vast majority of vascular plants under natural conditions. Although many of these normally mycorrhizal plants can survive and even thrive without fungal symbionts under artificial conditions, it is generally accepted that mycorrhizae are essential for long term success in the field. Host plants may benefit from mycorrhizal associations in several ways. Foremost among these is enhanced nutrient uptake, especially P with endomycorrhizae but other nutrients as well with ectomycorrhizae. It has been proposed that ectomycorrhizae enhance water uptake and increase drought tolerance (Trappe, 1977) but definitive experimental evidence is still lacking to support this view. Mycorrhizal symbioses may also increase the tolerance of higher plants to other adverse conditions. Certain ectomycorrhizae can increase the tolerance of plants to high soil temperatures (Marx et al., 1970) and extremes of soil pH (Clement et al., 1977), offer protection from root pathogens (Marx, 1973) and perhaps increase plant tolerance to toxic metals (Benson et al., 1980).

Mine spoils and tailings can be generally characterized as adverse environments for plant growth. They are usually infertile, low in organic matter, possess small nutrient reserves, have low moisture-holding capacities, are subject to excessive leaching, erosion and high soil temperatures and, depending on the particular spoil, they may have unfavorable chemical characteristics (Jurgensen, 1979). Thus vegetation on spoils may be subjected to a variety of stresses that might be at least partially alleviated by mycorrhizal infection. Of primary importance is an increase in efficiency of water and nutrient uptake and the conservation of the nutrient pool. Mycorrhizae may function in conserving nutrients indirectly by stimulation of plant growth and incorporation of nutrients into biomass or directly by more efficient exploitation of the soil and interception of nutrients by extramatrical mycelium that might be leached away in coarse textured spoils.

For these reasons it was felt that mycorrhizal symbioses were a critical component in the establishment of biological activity in mine spoils and tailings and worthy of detailed studies. The objectives of the ectomycorrhizal studies were to (1) monitor the development of ectomycorrhizae of woody plant species in a subalpine coal mine spoil and extracted oil sand tailings, (2) determine how potentially operational amendation procedures would affect rates of ectomycorrhizal development and the fungal symbionts involved, (3) determine the stability of ectomycorrhizal associations of a 4 year period (observations on fruit body production for 5 years), and (4) determine the ectomycorrhizal fungi occurring in natural stands of jack pine and white spruce. It should be emphasized that it was not the intention of these studies to determine the effects of ectomycorrhizae on plant growth, nor to determine the relative merits of particular fungal-host combinations.

In order to facilitate the monitoring of ectomycorrhizal development, spoil from a subalpine coal mine and tailings from an oil sand extraction plant were transported to Calgary, placed in large wooden tanks and planted with plant species that were likely candidates for use in the reclamation of each mine waste. This allowed frequent observations and maintenance of the experimental plots.

2. TANK STUDIES

2.1 MATERIALS AND METHODS

2.1.1 Tank Set-up

The subalpine coal mine spoil was from a site near Luscar, Alberta forested with *Picea glauca* (Moench) Voss, *Picea engelmannii* Parry, *Picea* hybrids and *Abies lasiocarpa* (Hook.) Nutt. prior to mining. The spoil was transported to Calgary, deposited in large wood-enclosed tanks (Figure 1), amended, and the amendments

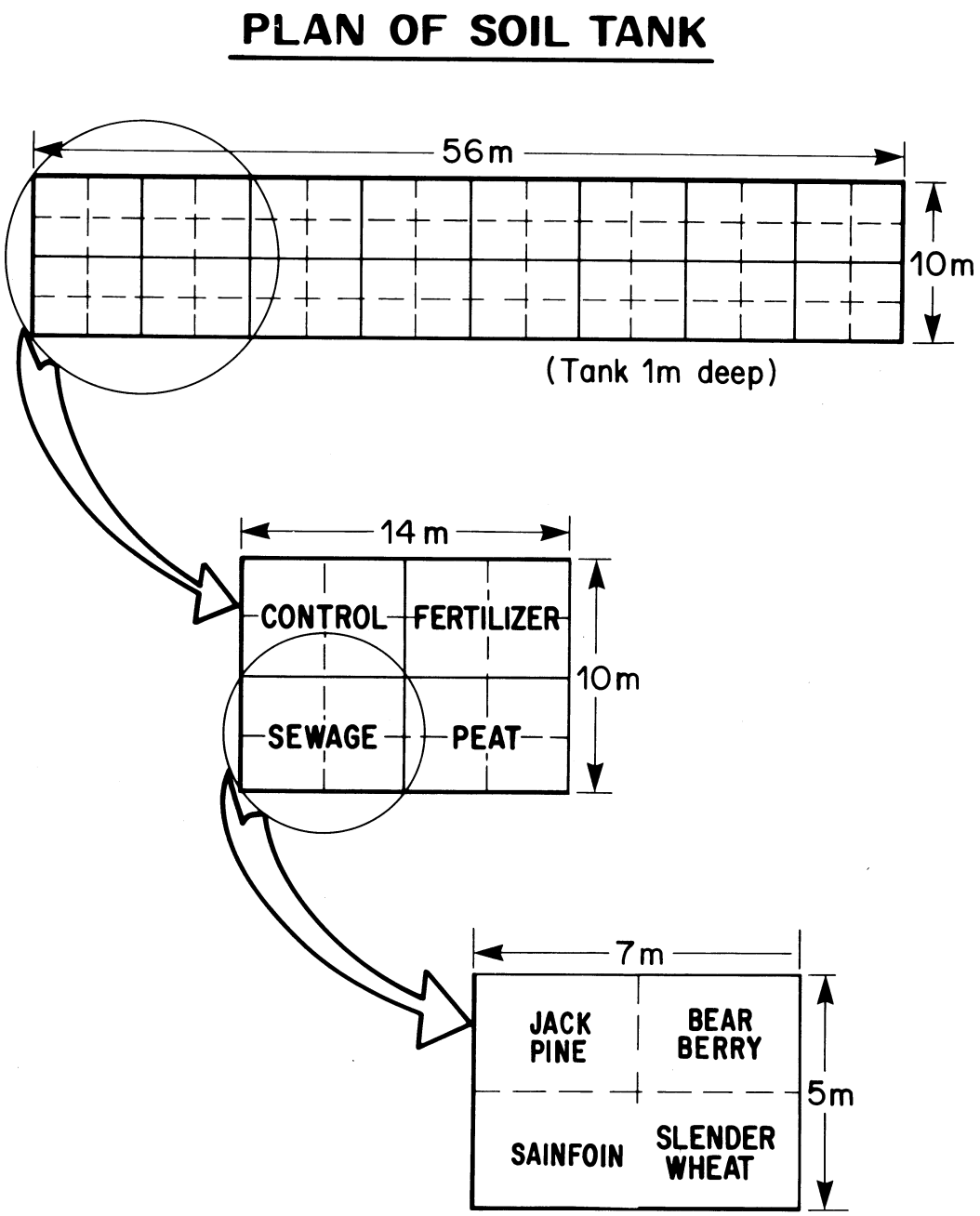

Figure 1. Plan of soil tanks

rototilled into the spoil. The amendments consisted of (1) none, (2) a 14 cm deep-layer of feather moss peat collected from a mature white spruce forest near Canmore, Alberta, (3) mineral fertilizer equivalent to 113 kg N, 113 kg P_2O_5 and 91 kg K_2O ha^{-1}, and (4) anaerobically digested liquid sewage sludge applied at a rate of 46 mT dry weight ha^{-1}. Each treatment was replicated three times.

The oil sand tailings were obtained from the Great Canadian Oil Sands plant near Fort McMurray, Alberta. The tailings consisted almost entirely of fine-grained sand, was extremely low in organic matter and virtually sterile. The tailings were transported to Calgary and amended in the same manner as was the subalpine spoil. The characteristics of the coal mine spoil and the oil sand tailings are given in Table 1. Additional details of the amendments are reported by Visser et al. (1984).

2.1.2 Planting and Harvesting for Primary Production Estimates

White spruce seedlings were grown under greenhouse conditions in 192 cm^3 Spencer-Lamaire containers (Spencer-Lamaire Industries Ltd., Edmonton, Alberta) containing commercial, unsterilized peat moss. The seedlings were planted in the subalpine spoil at the age of 20 weeks using 20 cm spacing. Laurl leaf willow (*Salix glauca* L.) was commercially grown from cuttings in 95 cm^3 plastic containers filled with peat moss. The willow was planted in the subalpine spoil using 25 cm spacing. Survival was determined each spring by counting all individual plants and accounting for plants destructively sampled. Winter injury and subsequent dieback of the willow following the third winter was estimated in June by randomly selecting five individuals from each of the three replicate plots and measuring total height and total live height.

Jack pine (*Pinus banksiana* Lamb.) seedlings were grown in the greenhouse in 47 cm^3 Spencer-Lamaire book-containers by the staff of the Northern Forest Research Centre, Edmonton, Alberta. The seedlings were planted in the oil sand tailings when 20 weeks old with 20 cm spacing. Common bearberry (*Arctostaphylos uva-ursi*

Table 1. Chemical characteristics of a subalpine mine spoil and oil sand tailings following amendation with either peat, sewage sludge or fertilizer, or left unamended.

Spoil Type	Treatment	Total N (%)	NO_3-N ($\mu g.g^{-1}$)	Available P ($\mu g/g^{-1}$)	pH
Subalpine	Control	0.1	4	3	7.1
	Peat	1.6	449	1	7.0
	Fertilizer	0.1	150	51	6.7
	Sewage	0.1	25	42	6.9
Oil Sands	Control	0	0	1	7.3
	Peat	0.8	251	3	7.1
	Fertilizer	0.1	40	53	6.4
	Sewage	0.1	16	26	6.8

(L.) Spreng.) were purchased from a commercial nursery and were grown from cuttings in 100 cm^3 plastic pots filled with a peat-sand mixture. The bearberry cuttings were planted in the tailings with 20 cm spacing. Survival was determined in the same manner as for the subalpine woody plants. Winter injury of bearberry was estimated by randomly selecting five individuals from each replicate plot and visually estimating the amount of each plant that was killed.

Plants were harvested in September and early October in successive years to determine dry matter production. Five randomly selected individuals from each plot were used except in the fourth year when only three spruce were removed from each plot. Willow was not sampled after the third year due to browsing by deer, winter injury, and the assumption that the root systems extended below the spoil material into the subsoil. All samples were dried at 80°C to a constant weight. Leaf and needle samples were ground in a Wiley mill and analyzed for nutrient content using methods described by McKeague (1976).

2.1.3 Mycorrhizal Assessments

2.1.3.1 Sampling and mycorrhizal counts. Mycorrhizae of all plant species were sampled in September and October by digging up three randomly selected plants from each plot. A flat spade was used to remove all the soil midway between adjacent plants to a depth of 15 cm. Loose soil was shaken off the roots in the field and the three root systems from each plot were pooled and stored in plastic bags at 5°C until they could be processed. Only roots extending beyond the planting plug were analyzed for mycorrhizae.

All active short roots in each subsample were counted and evaluated. Short roots were considered to be active and alive if (1) the meristem, if visible, was pale and turgid, (2) the short root was turgid but not necessarily terete, and (3) if dark coloured and the meristem not apparent, the cortex was torn open with fine

forceps and if brown inside, it was considered to be dead. All live short roots in which the length/diameter ratio exceeded 1.5 were considered to be susceptible to ectomycorrhizal infection and were rated. Short roots were rated as positive if any Hartig net was present, regardless of mantle development. Short roots were frequently checked using whole mounts in which very early stages of infection could be detected. The whole mounts were examined using a 40x brightfield, oil immersion objective. In order to detect intracellular infections, hand-cut sections were made, mounted in 0.05% trypan blue, heated until steaming occurred and examined with phase-contrast and brightfield optics. Additional details of the mycorrhizal assessments are given in Appendix 1.

At the end of the first growing season, all of the short roots of the spruce seedlings were rated for infection. In the evaluations after the second and fourth growing seasons, 300 randomly selected short roots per plant (i.e. 900/plot, 2700/treatment) were rated as to ectomycorrhizal status. All distinctive ectomycorrhizal types were counted separately.

The degree of infection of willow at the end of the first year was so low and morphological differentiation so slight that a staining technique was used to detect infection. Willow roots from each plot were cut into 2 to 3 cm lengths and 20 randomly selected segments were cleared in hot 10% KOH for 30 min, rinsed in water for 5 min, rinsed again in 0.1 N HCl for 5 min, strained in hot .01% trypan blue for 5 min and stored in lactophenol (Phillips and Hayman, 1970). At the end of the second growing season, the spoil was very hard and dry and a majority of the feeder roots were broken during sampling and thus unsuitable for ectomycorrhizal determinations. The actual number of feeder roots rated ranged from 40 to 300 per plot rather than the 900 desired. The roots were not cleared and stained at this sampling time.

Bearberry roots that extended beyond the planting plug were rated for mycorrhizal infection, dried at 80^{o}C and weighed. At the end of the first growing season, three plants in each of three

replicate plots were randomly selected, the root systems combined and the total number of short roots counted and the mycorrhizal status rated. After the second growing season, each of the nine plants per treatment were evaluated separately by rating 300 short roots per plant.

At the end of the first growing season, three jack pine seedlings from each plot were randomly selected, the roots extending beyond the planting plug cut off and pooled, and the total number of short roots counted and evaluated. At the end of the second and fourth growing seasons, the roots from each of nine individual seedlings per treatment were evaluated separately. Three-hundred short roots from each seedling were counted. In addition, each sample was recounted to determine the total number of short root tips and the number of short roots that had been broken off. All final counts were corrected for breakage with the assumption that the broken roots did not differ from intact roots. Root lengths were determined by direct measurements of all lateral roots (those roots exceeding 1 cm in length) that occurred outside the planting plug and by measuring 50 short roots per seedling.

2.1.3.2 Characteristics of specific ectomycorrhizae. The E-strain symbionts were identified using the criteria of Danielson (1982), the most important of which was the large size (4 to 8 μm) of the cells of the mantle. It could be distinguished from Thelephora terrestris (Ehrh.) Fr. and other Basidiomycetes by the nearly glabrous mantle in contrast to the presence of thin, hyaline hyphae on T. terrestris + jack pine ectomycorrhizae. It was not possible to recognize T. terrestris ectomycorrhizae with certainty without using cultural techniques.

Ectomycorrhizae were considered to be formed by Cenococcum geophilum Fr. if (1) the ectomycorrhizae were black, (2) stiff pigmented hyphae radiated out from the surface, (3) the hyphae were 4 to 6 m in diameter with simple septa, and (4) the mantle consisted of the hyphal arrangement illustrated by Trappe (1971).

Roots infected with Mycelium radicis atrovirens Melin (MRA) were not counted but could possibly be mistaken for C. geophilum ectomycorrhizal infections, as the short roots may become black with extensive development of MRA. However, MRA differs from C. geophilum by the more olivaceous colour of the extramatrical mycelium of MRA, and most importantly, hyphal diameters which do not exceed 4 µm.

I-type ectomycorrhizae were characterized by the presence of thin setoid crystidia on the mantle, thus fitting into the I-subtype of Dominik (1962). Cystidia may be absent on some ectomycorrhizae of the I-type, but even if absent, these ectomycorrhizae can still be identified by the textura epidermoidea (Eckblad, 1968) hyphal structure and similarity to adjacent cystidial forms. The septa of the cystidia and young mantle cells possessed Woronin bodies.

Amphinema byssoides ectomycorrhizae were characterized by (1) the abundance of cream-coloured extramatrical hyphae, (2) pale yellowish mycelial strands, (3) hyphae 2 to 3 µm in diameter with large keyhole type clamps, and (4) mycelium which became dark-yellow in KOH. Culturing was required to confirm the identity of A. byssoides.

What is referred to as Suillus ectomycorrhizae was characterized by (1) dichotomous to subtuberculate form, (2) abundant extramatrical mycelium, (3) conspicuous mycelial strands, (4) colours varying from white to vinaceous, (5) mycelium covered with crystalline material and/or resinous deposits and simple septa, (6) a colour change to vinaceous or purple-brown of some of the excreted material on the hyphae when placed in 3% KOH. These characters would also apply to ectomycorrhizae formed with certain Rhizopogon species but for convenience, such ectomycorrhizae were assumed to be formed by Suillus species.

2.1.3.3 Direct isolation techniques. Direct isolation techniques were used to quantify and identify symbionts that did not produce

distinctive ectomycorrhizae. For spruce, the entire root systems of three plants from each plot was cut into 2 to 3 cm segments and 12 segments from each plot were randomly selected and given 15 2-min wash cycles in an automatic root washing machine (Bissett and Widden, 1972). At the end of the second year, 10 washed segments from each plot were randomly selected and the first three mycorrhizae from one end were plated directly on benomyl-MMN agar (Appendix 1). At the end of the fourth growing season, the roots were sampled and washed in the same manner, but 12 root segments were selected and the first five mycorrhizae were plated on benomyl-MMN agar resulting in a total of 60 mycorrhizae plated per plot (180 per treatment). In addition, washed roots were surface sterilized and plated on MMN+ agar (Appendix 1) to isolate Ascomycete symbionts. Root segments were dipped in 95% ethanol, soaked in 30% H_2O_2 for 15 sec, rinsed in two changes of ice-cold sterile water for 1 h and plated, five/plate, on MMN+. Ice water was used for rinsing off the H_2O_2 in hopes of stopping the action of the H_2O_2 more quickly than with water at room temperature (Appendix Tables 2 and 3). Representative isolates were subcultured on MMN and PDA for identification and grouping.

Attempts to isolate the symbionts from the bearberry mycorrhizae were made in the second year by plating 30 mycorrhizae per seedling directly on benomyl-MMN and by plating 20 tips per seedling on the same medium after being given 15 2-min washes.

Isolation procedures for jack pine were similar to those used with white spruce. The combination of surface sterilization and benomyl-MMN agar resulted in the failure to recover any probable symbionts after the first growing season. Thus, after the second growing season, washed ectomycorrhizae were plated directly on benomyl-MMN and after the fourth year, surface sterilization was combined with plating on MMN+ agar to detect Ascomycetes. Isolations were made from jack pine ectomycorrhizae within the planting cores in the second year to obtain estimates on the symbionts that were introduced with the seedlings. Fungi were

identified by matching cultures with those obtained from fruit bodies. Specimens and cultures of all symbiont species are deposited at the Biosystematic Research Institute (DAOM), Agriculture Canada, Ottawa.

2.2 RESULTS FOR THE SUBALPINE COAL MINE SPOIL

2.2.1 Primary Production of White Spruce and Willow

The survival of both willow and spruce was high for the first two growing seasons and amendation had no effect (Table 2). Willow was subject to winter injury and dieback of stems which was particularly severe in the peat amended spoil after the third winter. Subsequent observations indicated that winter injury occurred in all amendments and that the total height of willow was limited by stem dieback.

The shoot growth of white spruce was not significantly affected by amendation until the fourth growing season (Table 3). Growth in the sewage and peat amended spoil was significantly greater than in the fertilizer amended spoil. Fourth year seedlings in the control treatment were notably paler green than in the sewage or peat treatments, indicating N deficiency. In contrast to white spruce, willow showed responses to the amendments every year it was sampled (Table 4). Sewage sludge resulted in an average 10-fold increase in shoot weight after three seasons as compared to the unamended spoil, whereas peat and fertilizer resulted in 3- to 4-fold increases. The growth of willow in the fertilizer treatment was highly variable.

2.2.2 Foliar Analysis

Amendation had no effect on concentrations of K, Ca, Mg, Mn or Zn in spruce needles after one growing season (Table 5). Phosphorus levels of needles were significantly higher in the control than in the peat and fertilizer treatments. Iron and Cu levels of needles were lower in the peat than in the other three treatments.

Table 2. Survival of white spruce and survival and height dieback of willow grown in subalpine coal mine spoil that was amended with either peat, sewage sludge or fertilizer, or left unamended[1].

Amendment	Spruce		Willow		Percent height dieback of willow
	Number of Growing Seasons				
	1	2	1	2	
	% Survival				
Control	93[a]	90[a]	95[a]	91[a]	7[a]
Peat	92[a]	88[a]	95[a]	92[a]	60[b]
Fertilizer	93[a]	89[a]	97[a]	94[a]	12[a]
Sewage	89[a]	84[a]	92[a]	91[a]	8[a]

[1] Data within each column analysed by 1-way ANOVA after data arcsin $\sqrt{p}$ transformed. Where F-test was significant differences in means was determined by Tukey pairwise comparisons. Numbers within each column superscripted by the same letter are not signifcantly different ($p = .05$).

Table 3. Shoot weight of white spruce grown in subalpine coal mine spoil that was amended either with peat, fertilizer or sewage sludge, or left unamended.[1]

Amendment	Number of Growing Seasons			
	1	2	3	4
	Shoot dry weight (g)			
Control	0.3[a]	1.5[a]	6.1[a]	13.8[ab]
Peat	0.3[a]	1.3[a]	6.9[a]	21.4[b]
Fertilizer	0.4[a]	1.7[a]	5.6[a]	10.8[a]
Sewage	0.4[a]	2.3[a]	9.4[a]	21.2[b]

[1] Data within each column analysed by 1-way ANOVA. Numbers superscripted with the same letter within each column do not differ significantly at $p = .05$, as determined by Scheffé multiple contrasts where F-test significant.

Table 4. Shoot weight of willow grown in subalpine coal mine spoil that was amended either with peat, fertilizer or sewage sludge, or left unamended.[1]

Amendment	Number of Growing Seasons		
	1	2	3
	Shoot dry weight (g)		
Control	0.6[a]	4.5[a]	14.4[a]
Peat	0.9[a]	13.7[b]	41.1[b]
Fertilizer	2.3[c]	11.1[b]	62.0[ab]
Sewage	3.0[c]	38.8[c]	151.8[c]

[1] Data within each column analysed by 1-way ANOVA. Numbers superscripted with the same letter within each column do not differ significantly at $p = .05$, as determined by Scheffé multiple contrasts where F-test significant.

Table 5. Elemental concentrations of white spruce needles after the plants were grown for one growing season in subalpine coal mine spoil either amended with peat, fertilizer or sewage, or left unamended.

Amendment	P	K	Ca	Mg	Mn	Fe	Zn	Cu
				$\mu g.g^{-1}$				
Control	2778^{b}	5627^{a}	12295^{a}	2175^{a}	2015^{a}	423^{b}	48^{a}	9^{b}
Peat	2085^{a}	4765^{a}	13314^{a}	2374^{a}	1541^{a}	116^{a}	41^{a}	2^{a}
Fertilizer	2207^{a}	5130^{a}	13208^{a}	2462^{a}	1600^{a}	546^{b}	40^{a}	7^{b}
Sewage	2562^{ab}	5102^{a}	12575^{a}	2549^{a}	1966^{a}	574^{b}	50^{a}	8^{b}

[1] Data within each column analyzed by one-way ANOVA. Scheffé multiple contrasts ($p = .05$) used to locate significant differences. Values in each column superscripted by the same letter to not differ significantly.

Nitrogen concentrations were not measured in spruce needles due to the large sample size required but foliar N of the willow after 2 years was significantly higher in the peat amended spoil than in the control (unamended) spoil.

2.2.3 White Spruce Ectomycorrhizae

Initially sewage sludge had a suppressive effect on ectomycorrhizae formation of white spruce (Table 6). However, by the end of the second growing season there were no differences in percent short roots that were ectomycorrhizal of plants from the variously amended spoil. Overall ectomycorrhizal infection rates increased significantly ($p = .001$) between 1 and 2, and 2 and 4 years. After 4 years, nonmycorrhizal short roots were rare. On all occasions, a majority of the ectomycorrhizae were considered to be active. As determined by direct counts, the E-strain was the dominant ectomycorrhizal symbiont at the end of the fourth growing season (Table 6). The E-strain fungi were not evaluated in previous years as criteria for identification had not been developed. *Cenococcum geophilum* was the only other ectomycorrhizal fungus that could be recognized by direct observation. It was not detected in the first year, occurred at low levels in all treatments except the peat at the end of the second year, and had almost disappeared by the fourth year.

On benomyl-MMN agar, which is selective for Basidiomycetes, *Amphinema byssoides* was isolated from 15 $\pm$ 21% ($\bar{x} \pm SD$) of the ectomycorrhizae in all four treatments in the fall of the fourth year (Table 7). Variation was such that no significant differences could be detected among treatments (i.e., types of amendment). There was also no significant differences ($p = .05$) in isolation frequency of *A. byssoides* between 2 and 4 years. However, in that *A. byssoides* occurred in 8 of the 12 samples (all treatments considered) in the fourth year and was not detected in the second year, it is certain that it was increasing in abundance. It was also readily detected by direct observation in the fourth year,

Table 6. Ectomycorrhizal development of white spruce seedlings planted in subalpine coal mine spoil which was either amended with peat, fertilizer or sewage sludge, or left unamended.

	All Fungi[1]			E-strain[2]	Cenococcum geophilum[2]		
	Number of growing seasons[3]						
Amendment	1	2	4	4	1	2	4
Control	74[a]	93[a]	100[a]	80[a]	0	1.5[ab]	0
Peat	51[a]	88[a]	99[a]	60[a]	0	0[a]	0
Fertilizer	66[a]	96[a]	99[a]	94[a]	0	1.3[ab]	0
Sewage	1[b]	91[a]	100[a]	48[a]	0	3.6[b]	0.3

1 Data analysed by 2-way ANOVA after 2 arcsin $\sqrt{p}$ transformation. Numbers within the total infection group superscripted by the same letter are not significantly different ($p = .05$) as determined by Scheffé multiple contrasts.

2 Data analysed by 1-way ANOVA. Numbers within the same column superscripted by the same letter are not significantly different ($p = .05$) as determined by Scheffé multiple contrasts where F-test significant.

3 At the end of first growing season, all short roots on the sample plants were evaluated for ectomycorrhizal status, the actual number ranging from 91 to 471 per seedling, with a mean of 261. After the second and forth growing seasons, 300 short roots per seedling were evaluated.

Table 7. Percent isolation frequency of two symbionts of white spruce (*Amphinema byssoides* and *Tomentella* sp.) and nonmycorrhizal fungi from ectomycorrhizae grown in amended and nonamended subalpine coal mine spoil for 2 or 4 years.[1]

	Amphinema byssoides[2]		*Tomentella* sp.		Nonmycorrhizal fungi[3]		No growth of any fungi[3]	
Amendment	2 yr	4 yr	2 yr	4 yr	2 yr	4 yr	2 yr	4 yr
			% isolation frequency					
Control	0	5[a]	0	0	28[b]	7[a]	72[a]	88[a]
Peat	0	15[a]	8	4	27[b]	1[a]	66[a]	81[a]
Fertilizer	0	7[a]	0	0	19[b]	5[a]	81[a]	88[a]
Sewage	0	35[a]	0	0	40[b]	5[a]	60[a]	60[a]

1 For the second year, 30 mycorrhizae were plated/plot; for the fourth year, 60 mycorrhizae/plot were plated. All nonsurface sterilized and plated on benomyl-MMN agar.

2 Data within 4 year column analysed by 1-way ANOVA. Values within column superscripted with the same letter do not differ significantly at $p = .05$ as determined by Scheffé multiple contrasts where F-test significant.

3 Data analysed by 2-way ANOVA. Values within a group (nonmycorrhizal or no growth) superscripted by the same letter do not differ significantly ($p = .05$) as determined by Scheffé multiple contrasts where F-test significant.

whereas all the ectomycorrhizae observed in the second year appeared to be the E-strain type. Yellow mycelial strands and floccose mycelium typical of A. byssoides were common in the fourth year but were never observed previously. After 4 years, some ectomycorrhizae were observed to be infected with the E-strain at the base and with a clamped Basidiomycete (presumably A. byssoides) at the tip. It thus appeared that A. byssoides was able to replace the E-strain after the E-strain was established on short roots.

The only other ectomycorrhizal fungus isolated on benomyl-MMN agar was a Basidiomycete, Tomentella sp., which occurred only in the peat amended spoil at low levels. This species may produce ectomycorrhizae with cystidia (R.M. Danielson, unpublished data) but none were seen on the white spruce mycorrhizae. However, cultures from white spruce matched those obtained from cystidoid jack pine ectomycorrhizae. There were no differences in the ectomycorrhizae yielding no growth of any fungai either among amendments or between years. It is obvious that the major symbiont(s) was not capable of growth on the benomyl-MMN medium.

An average of 9 $\pm$ 14% ($\bar{x} \pm$ SD) of the ectomycorrhizae from all four treatments plated on MMN+ after surface sterilization yielded Amphinema byssoides (Table 8). The major symbiont isolated was the E-strain which grew from 44 $\pm$ 21% ($\bar{x} \pm$ SD) of the mycorrhizae plated from all treatments. The low percentage of E-strain isolated from ectomycorrhizae from the peat treatment may have been due to the inability of the E-strain fungi indigenous to the peat to grow on MMN agar. The growth of nonmycorrhizal fungi from the root tips reduced the chance of detecting slow growing symbionts. Cylindrocarpon destructans (Zins.) Scholten, Myxotrichum sp., Mycelium radicis atrovirens (MRA) and a sterile dark form were the major nonmycorrhizal fungi that grew from surface sterilized mycorrhizae (Table 9).

Table 8. Frequency of isolation of three symbionts of white spruce (E-strain, Amphinema byssoides and Tomentella sp.) and nonmycorrhizal fungi from surface sterilized ectomycorrhizae grown in amended and nonamended subalpine coal mine spoil for 4 years.[1,2]

Amendment	E-strain	Amphinema byssoides	Tomentella sp.	Non-mycorrhizal fungi	Mycorrhizae yielding no growth of any fungi
		% isolation frequency			
Control	64[a]	2[a]	0	43[ab]	6[ab]
Peat	22[a]	8[a]	3	28[a]	41[b]
Fertilizer	46[a]	3[a]	0	54[b]	13[ab]
Sewage	44[a]	25[a]	0	54[b]	1[a]

1 Sixty ectomycorrhizae were plated per plot on MMN+ agar.

2 Data within each column analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at $p = .05$ as determined by Scheffé multiple contrasts where F-test significant.

Table 9. Percent frequency of isolation of nonmycorrhizal fungi from surface sterilized white spruce mycorrhizae plated on MMN+ at the end of the fourth growing season. Seedlings planted in a subalpine coal mine spoil that was amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Taxa	Amendment			
	Control	Peat	Fertilizer	Sewage
Cylindrocarpon destructans	3[a]	11[a]	6[a]	2[a]
Myxotrichum sp.	7[a]	7[a]	19[a]	11[a]
Mycelium radicis atrovirens	0[a]	1[a]	7[a]	7[a]
Symbiont 2118	0[a]	<1[a]	0[a]	0[a]
Sterile dark 2013	13[a]	<1[a]	1[a]	10[a]
Ascomycete 1977	1[a]	0[a]	4[a]	1[a]

[1] Data within each row analysed by 1-way ANOVA. Values in each row superscripted with the same letter do not differ significantly at $p = .05$ as determined by Scheffé multiple contrasts where F-test significant.

2.2.4 Willow Mycorrhizae

At the end of the first growing season, willow roots were ectomycorrhizal only in the peat treatment and then at a low rate (Table 10). The absence of ectomycorrhizae in three treatments in the first year suggest that all inoculum originated in the peat. In the stained roots from the first year, no VA mycorrhizae were detected but resting spores of Olpidium sp. were present in several samples. After 2 years growth, infection rates were uniform among treatments with a small percentage of the feeder roots infected with Cenococcum geophilum except in the peat amended spoil. Although symbionts other than C. geophilum were not quantified due to lack of distinctive features, Tomentella sp. and the E-strain fungi were observed to be present. In the fifth growing season, Hebeloma sp. fruited prolifically in all three of the willow plots amended with sewage and in one of the fertilizer amended plots. Tomentella sp. produced large diffuse, resupinate fruit bodies on the surface of the peat under the willow canopy. Hebeloma sp. could not be cultured but Tomentella sp. was successfully brought into pure culture.

2.3 DISCUSSION OF AMENDING SUBALPINE COAL MINE SPOIL

The relative values of peat, sewage sludge and fertilizer for enhancing primary production on the subalpine spoil depended upon the test plant. White spruce, a slow growing conifer, was a poor short-term indicator of changes in soil nutrient levels. Its slow growth rate would do little to prevent leaching losses of soluble nutrients from the spoil. In contrast, willow responded quickly to amendation and incorporated large quantities of nutrients in stems and leaf litter. Sewage sludge was clearly the best spoil amendment for primary production of willow, but peat was equally good for spruce.

Amendation had two major effects on ectomycorrhizal development of white spruce; a short-term inhibition of ectomycorrhizal formation by sewage and the introduction of some symbionts (Tomentella sp., A. byssoides, and possibly E-strain) in the

Table 10. Ectomycorrhizal development of willow planted in subalpine coal mine spoil which was either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

	All fungi		Cenococcum geophilum	
	Number of growing seasons			
Amendment	1	2	1	2
Control	0	51[a]	0	2[b]
Peat	12	67[a]	0	0[a]
Fertilizer	0	65[a]	0	1[ab]
Sewage	0	63[a]	0	1[ab]

[1] Data for second growing season analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at $p = .05$ as determined by Scheffe multiple contrasts where F-test significant.

peat. It would not seem that the temporary delay in ectomycorrhizal development had no significant effect on growth and survival of spruce seedlings.

The peat contained a non-chlamydospore producing taxon of the E-strain, which was characterized in culture by a slow growth rate, dark pigmentation and abundant stiff, ornamented hyphae. The major form of the E-strain indigenous to the spoil produced chlamydospores and was lighter in colour than the E-strain fungi from peat. The term E-strain refers to a species or complex of similar species that have Ascomycete affinities (Danielson, 1982). Information on geographic distribution and ecological preferences are limited in that it cannot be linked to a known teleomorph, but observations on ectomycorrhizae indicate that the E-strain is a common and widespread symbiont. With pine hosts the E-strain is very common under nursery conditions in both Europe and North America (Laiho, 1965; Mikola, 1965). Under forest conditions, E-strain associations with pine are considered to be rare, especially in mature forests (Laiho, 1965). However, Mikola (1965) did find E-strain ectomycorrhizae associated with seedlings growing in burned clearcut areas, situations in some ways like those of mine spoils. Pines infected with E-strain and transplanted to forest soils appear to experience a change of symbionts soon after outplanting (Mikola, 1965); this is unlike the relatively stable condition found in the subalpine spoil.

The success of the E-strain in the subalpine spoil may be due in part to low levels of inoculum of other symbionts, or it may be more competitive than Laiho (1965) suggests. The occurrence in undisturbed forests has been based largely on the intracellular infection of pine ectomycorrhizae and little is known about its association with other tree species in which the infection is restricted to intercellular positions (e.g. spruce). Ectomycorrhizal data from this study strongly suggests that large amounts of inoculum were present in both the subalpine spoil and the peat amendment. Both of these materials were from mature forests

dominated by spruce and indicate that the E-strain was an active symbiont under forest conditions. It may be that the E-strain is a common symbiont of mature spruce or other conifers but not of pines except in highly disturbed systems.

The fungus that appeared to be replacing the E-strain was Amphinema byssoides. Recently, ectomycorrhizal isolates were matched with cultures obtained from fruit bodies, thus permitting identification (R.M. Danielson, unpublished data). Amphinema byssoides was only detected once after 2 years, but was the second most common symbiont after 4 years. It appears likely that A. byssoides will continue to replace the E-strain and eventually become dominant in all spoil treatments.

Amphinema byssoides appears to occur wherever spruce and pine occur, and very probably is one of the most common ectomycorrhizal fungi. Despite this, there is just one report of A. byssoides as an ectomycorrhizal fungus. Fassi and De Vecchi (1962) describe it as being common in pine nurseries in Italy. Ectomycorrhizae formed by A. byssoides are surrounded by extremely large amounts of extramatrical mycelium, and Fassi and De Vecchi (1962) suggest that loss of nutrients by leaching is reduced by direct uptake from decomposing litter. Whether A. byssoides is directly involved in the decomposition of litter remains to be determined but the presence of such large amounts of hyphae may indeed reduce leaching losses as compared to fungi which produce little external mycelium (e.g. E-strain).

Tomentella is another genus of resupinate fungi that has been considered to be entirely saprophytic (Larsen, 1968). At least some Tomentella species are closely related to members of the genus Thelephora and it is not unreasonable that the genus Tomentella may include ectomycorrhizal species. The Tomentella species associated with white spruce in this study was reaily cultured although Larsen (1968) states that species of Tomentella do not grow in culture. Tomentella sp. was observed fruiting on the surface of the peat beneath willow in the fifth growing season. Cultures were

established from fruit bodies and matched with cultures from spruce ectomycorrhizae. Tomentella was introduced with the peat and it is of interest that a species indigenous to cool, wet organic soils has persisted in the mine spoil for over 4 years.

2.4 RESULTS FOR THE OIL SAND TAILINGS

2.4.1 Primary Production of Jack Pine and Bearberry

Sewage sludge was clearly the best amendment for the growth of jack pine seedlings (Table 11). Mineral fertilizer had a minimal and short-term effect on growth whereas the beneficial effect of peat was more long lasting. Browsing by deer during the fourth winter introduced additional variation and limited any further primary production measurements. Survival of jack pine was good in all treatments except the control (Table 12). Survival of bearberry was lower than jack pine and after the first winter was lowest in the high nutrient treatments. Extensive dieback of bearberry occurred during the second winter but it could not be related to the amendment treatments due to variation caused by differences in exposure to wind.

2.4.2 Development of Bearberry Mycorrhizae

Sewage suppressed mycorrhizal infection of bearberry for at least 2 years (Table 13). It is presumed that most of the inoculum was introduced with the rooted cuttings. Thelephora terrestris was observed fruiting on some containers but the actual abundance of mycorrhizae when planted is unknown. The suppression of mycorrhizal development may have been due to the inhibition of specific fungi as one species was frequently isolated from plants grown on all types of amended tailings except the sewage amendment (Table 14). The mycorrhizae were all monopodial in the sewage amended spoil, whereas most mycorrhizae in the other three amendments were cruciate or coralloid. All sections examined showed the anatomy of the mycorrhizae to be of the arbutoid type (Harley, 1969) with the infection limited to the first layer of cortical cells.

Table 11. Snoot weight of jack pine seedlings grown in oil sand tailings amended either with peat, fertilizer or sewage, or left unamended.[1]

Amendment	Number of growing seasons			
	1	2	1	2
	Shoot Dry Weight (g)			
Control	.5[a]	.6[a]	.6[a]	1.2[a]
Peat	1.1[b]	2.6[b]	7.7[c]	5.8[a]
Fertilizer	1.0[ab]	2.6[b]	2.4[b]	3.1[a]
Sewage	1.0[b]	10.4[c]	24.5[d]	54.6[b]

[1] Data for second growing season analysed by 1-way ANOVA. Values in each column superscripted with the same letter do not differ significantly at p = .05 as determined by Scheffé multiple contrasts where F-test significant.

Table 12. Survival of jack pine and bearberry and winter dieback of bearberry of plants grown on oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.

Amendment	Percent Survival				Percent dieback of bearberry
	Jack Pine		Bearberry		
	1 yr	2 yr	1 yr	2 yr	
Control	86[a]	54[a]	62[a]	40[ab]	20[a]
Peat	92[ab]	88[ab]	74[a]	58[b]	17[a]
Fertilizer	86[ab]	80[ab]	31[b]	21 [a]	19[a]
Sewage	98[b]	97[b]	38[b]	30[ab]	1[a]

[1] Data within each column analysed by 1-way ANOVA. Values in each column superscripted by same letter not significantly different ($p = .05$) as determined by Tukey pairwise comparisons where F-test significant. All but percent dieback data were 2 arcsin $\sqrt{p}$ transformed prior to analysis.

Table 13. Mycorrhizal development of bearberry plants grown for 1 and 2 years in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

Number of growing seasons	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent of short roots mycorrhizal			
1	52[a]	81[a]	68[a]	0.1[b]
2	74[a]	70[ab]	79[a]	24[b]

[1] Each year's data analysed with the same letter by 1-way ANOVA. Means superscripted in each row not significantly different ($p = .05$) as determined by Scheffé multiple contrasts where F-test significant.

Table 14. Percent frequency of isolation on benomyl-MMN of bearberry symbiont R-1444 after 2 years from oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.

Root Treatment	Amendment			
	Control	Peat	Fertilizer	Sewage
None	4	5	2	0
Washed	12	20	38	0

2.4.3 Development of Jack Pine Ectomycorrhizae

2.4.3.1 Direct counts and overall ectomycorrhizal infections. During the first year, ectomycorrhizal infection of jack pine short roots was very low with all amendments except the peat (Table 15). After the second growing season, infection levels increased in all treatments with levels in the peat being significantly ($p = .05$) greater than with the other three amendments. A large amount of variation existed among rates of ectomycorrhizal infection. The low rates were associated with small quantities of mycorrhizae (low inoculum potential) within the planting cores. When seedlings with low inoculum in the plugs were omitted from the data analysis, peat and sewage amendments did not differ from one another and they had significantly greater infection rates than plants in the control and fertilizer amended tailings. Cenococcum geophilum formed ectomycorrhizae with 10% of the short roots of one seedling.

Between the second and fourth years, the rates of ectomycorrhizal infection increased significantly ($p = .05$) in all types of amended tailings except the control. Rates of ectomycorrhizal infection differed significantly among all amendments in the fourth year. The increases of mean ectomycorrhizal infection with time were probably largely due to the decrease in variability by virtue of seedlings with low levels of inoculum in the planting core gradually becoming heavily ectomycorrhizal (Figure 2). Variability in the mycorrhizal status of the short roots decreased significantly ($p = .005$) between the second and fourth years in all amendment treatments except the fertilizer.

The largest root systems, in terms of weight, length, and number of short roots were found in the sewage amended spoil (Table 16). The lateral roots were 1.5 times as long as the total length of short roots with no significant differences among treatments. There appeared to be differences in the frequency of short root initiation and lateral root diameters after the second year

Table 15. Ectomycorrhizal development of jack pine seedlings grown in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended.[1]

Number of growing seasons	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent of short roots ectomycorrhizal (SD)			
1	7[a] (5)	25[a](6)	5[a] (2)	5[a] (2)
2	33[ab](27)	72[a](26)	24[b](17)	49[ab](38)
4	29[a] (19)	91[d] (6)	60[b](11)	83[c] (17)

[1]Data analysed by 1-way ANOVA. Differences among means detected by Scheffé pairwise comparisons. Values in each row followed by the same letter to not differ significantly (p = .05).

Table 16. Characteristics of the root systems of jack pine seedlings after two seasons growth in oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Root Characteristics[2]	Amendment			
	Control	Peat	Fertilizer	Sewage
Short roots mycorrhizal (%)	33.5^{ab}	72.2^{b}	24.5^{b}	49.4^{ab}
No. of short roots/seedling	1200^{a}	2236^{ab}	3103^{b}	7609^{c}
No. of tips/seedling	1436^{a}	2772^{ab}	3368^{b}	8746^{c}
Mean short root length (mm)	1.94^{ab}	2.08^{a}	1.55^{a}	2.34^{a}
Short root length/seedling (m)	2.18^{ab}	4.77^{a}	4.88^{b}	17.44^{c}
Lateral root length/seedling (m)	2.69^{a}	5.90^{b}	7.15^{b}	23.92^{c}
Total root length/seedling (m)	4.87^{a}	10.68^{b}	12.03^{b}	41.36^{c}
Root weight in core/seedling (g)	.32^{a}	.79^{b}	.53^{c}	1.61^{d}
Root weight in soil/seedling (g)	.23^{a}	.69^{b}	.75^{b}	2.82^{c}
Total root weight/seedling (g)	.51^{a}	1.48^{b}	1.27^{b}	4.43^{c}
No. short roots/cm lateral root	4.6^{a}	3.8^{ac}	4.4^{a}	3.2^{bc}
No. of tips/short root	1.3^{ac}	1.4^{b}	1.1^{a}	1.3^{bc}
Length of laterals/length short roots	1.34^{a}	1.54^{a}	1.76^{a}	1.39^{a}
Root length (cm)/root weight (mg)	2.22^{a}	1.67^{b}	1.62^{b}	1.48^{b}
Root length in soil (cm)/shoot weight (g)	747^{a}	364^{b}	612^{ac}	469^{bc}

[1] Data within each row analysed by 1-way ANOVA. Numbers within a row superscripted by the same letter not significantly different ($p = .05$) as determined by Scheffé multiple contrasts.

[2] Except where noted, data is for only roots that extended out from the planting core.

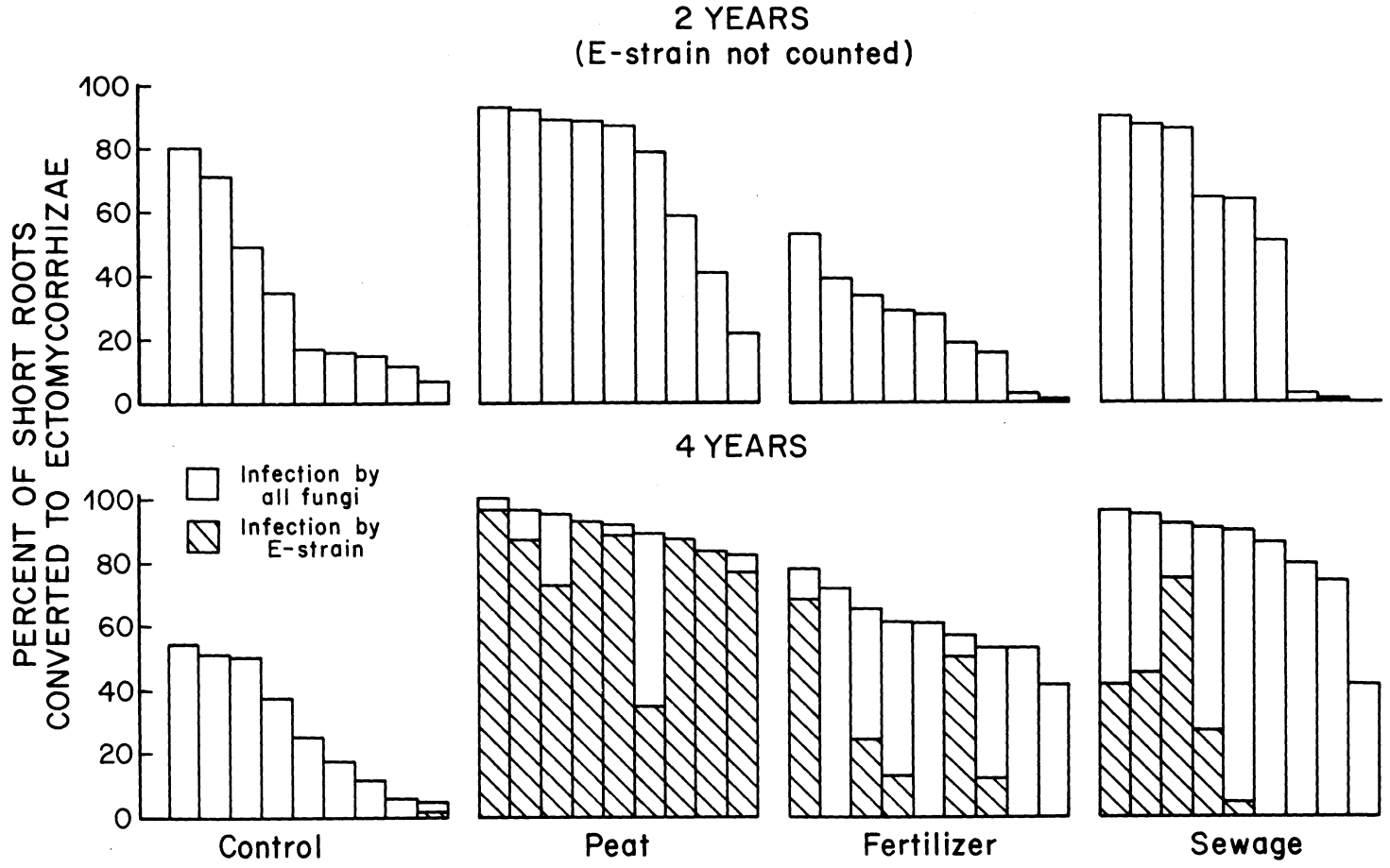

Figure 2. Percent ectomycorrhizal infection of individual jack pine seedlings 2 and 4 years after planting in tailings sand amended with either peat, fertilizer or sewage or left unamended. Indicated is infection by all ectomycorrhizal fungi and E-strain fungi. Each bar represents an individual seedling (nine replicates per treatment).

but the differences were nonsignificant ($p = 0.05$). Few changes appeared to occur in the long lateral root portions of the root systems between the second and fourth year (compare with data in Table 17). The soil volume sampled each year was approximately equal but it did not include the entire root system as the sample included only the top 20 cm of spoil. Within this volume the amount of roots remained relatively unchanged although there was a considerable increase in the shoot weights (Table 11) except in the control. However, the number of root tips per seedling in the sewage amendment did not appear to increase. This, coupled with an increase in ectomycorrhizal infection (Table 15), suggests that there may have been a considerable increase in the amount of extramatrical mycelium which would effectively increase the absorbing surface of the root system without increases in lateral root length.

Amendment and temporal effects of nonmycorrhizal root parameters were minimal. The amount of short root initiation was slightly greater in the improverished control than in the other three treatments. An increase in lateral root diameter between 2 and 4 years is suggested by the decrease in the length/weight ratio (Tables 16 and 17).

2.4.3.2 Ectomycorrhizal fungi in the planting plug. Preplanting observations on jack pine indicated that most seedlings were heavily infected with ectomycorrhizal fungi. Thelephora terrestris was fruiting on many containers and a single specimen of Inocybe sp. was observed. Thelephora terrestris was cultured from a fruit body and this culture was used for comparison with ectomycorrhiza isolates. Inocybe sp. failed to grow in culture. At the end of the second growing season, T. terrestris was the most common symbiont isolated from roots in the planting plug (Table 18). Suillus was present in plugs from all amendments except the sewage.

Table 17. Characteristics of the root systems of jack pine after four growing seasons. Plants grown in oil sand tailings amended with either peat, fertilizer or sewage sludge, or left unamended.[1]

Root Characteristic[2]	Amendment			
	Control	Peat	Fertilizer	Sewage
No. short roots/ seedling	1002[a]	2248[ab]	2477[b]	9678[c]
No. root tips/ seedling	1347[a]	2772[ab]	3611[b]	14810[c]
Lateral root length/ seedling	1.8[a]	5.5[b]	5.8[b]	24.6[c]
Root weight in soil/ seedling (g)	.27[a]	.96[b]	.78[b]	3.58[c]
No. short roots/cm lateral root	5.5[b]	4.1[a]	4.3[a]	3.9[a]
Root length (cm)/ root weight (mg)	.70[a]	.59[a]	.74[a]	.70[a]
No. tips/short root	1.37[a]	1.24[a]	1.46[a]	1.56[a]

[1] Data within each row analysed by 1-way ANOVA. Numbers within a row superscripted by the same letter not significantly different ($p = .05$) as determined by Scheffe multiple contrasts.

[2] Data is for only roots that extended out from the planting core.

Table 18. Isolation frequency of ectomycorrhizal fungi from jack pine ectomycorrhizae on benomyl-MMN agar. Roots sampled after the second growing season from within the planting plug.

Fungi	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent isolation frequency			
Thelephora terrestris	30	24	41	27
Suillus sp.	7	9	16	0
Basidiomycete	4	3	0	0
No growth of any fungi	0	30	6	1

2.4.3.3 Ectomycorrhizal fungi in the amended tailings sand. On roots growing out from the plugs, T. terrestris was the dominant symbiont isolated from the control, fertilizer and sewage amendments (Table 19). Other Basidiomycete symbionts were rarely isolated. The large percentage of ectomycorrhizae from the peat-amended tailings sand that remained sterile suggested that the symbiont was not T. terrestris and was unable to grow on the benomyl-MMN medium. Procedures were modified in the fourth year so that Ascomycetes could be isolated. Results after the fourth year using benomyl-MMN were similar to the second year results (Table 20) with T. terrestris being isolated at similar rates in each of the two years. Two additional Basidiomycetes were isolated from ectomycorrhizae in the peat treatment, Amphinema byssoides and Tomentella sp., both of which were also isolated from white spruce (see Section 2.2.3). The large percentage of ectomycorrhizae from all treatments which remained sterile on the benomyl-MMN suggests that the species intolerant to benomyl had become more abundant between the second and fourth years. Plating of ectomycorrhizae on MMN+ demonstrated that the benomyl-sensitive species was the E-strain (Table 20). The mantle of ectomycorrhizae formed by T. terrestris was usually very thin or absent and H_2O_2 treatment was lethal to this species. The dominant nonmycorrhizal fungi that were isolated from surface sterilized mycorrhizae were Cylindrocarpon destructans from the peat treatment and Mycelium radicis atrovirens (MRA) from the other three treatments. A single isolate of the ectomycorrhizal discomycete, Sphaerosporella brunnea (Alb. & Schw. ex Fr.) Svrcek & Kub., was obtained from an ectomycorrhiza in the control treatment.

Direct counts substantiated the culturing results (Table 21). The E-strain was dominant in the peat and frequent in the fertilizer and sewage amended tailings sand. The low estimate of the E-strain in the peat using culturing techniques was due to the small portion of the E-strain population indigenous to the peat that was capable of growth on the MMN medium. Evidence strongly suggests that

Table 19. Isolation frequency of ectomycorrhizal fungi from jack pine ectomycorrhizae using benomyl-MMN agar. Roots extending out from the planting plug sampled after the second growing season.

Fungi	Amendment			
	Control	Peat	Fertilizer	Sewage
	Percent isolation frequency			
Thelephora terrestris	73	5	76	44
Suillus sp.	3	1	2	0
Basidiomycete	3	1	1	.5
No growth of any fungi	2	52	4	2

Table 20. Isolation frequency of fungi from jack pine ectomycorrhizae after four growing seasons using either a medium selective for Basidiomycetes (Benomyl-MMN) or a nonselective medium (MMN+).

	Isolation[1] medium	Amendment			
		Control	Peat	Fertilizer	Sewage
		% isolation frequency			
E-strain	Benomyl-MMN	0	0	0	0
Thelephora terrestris[2]	Benomyl-MMN	62[b]	.6[a]	60[b]	51[b]
Suillus sp.	Benomyl-MMN	2	0	.5	8
Amphinema byssoides	Benomyl-MMN	0	.5	0	0
Tomentella sp.	Benomyl-MMN	0	3	0	0
No growth of any fungi[3]	Benomyl-MMN	24[a]	37[a]	33[a]	12[a]
E-strain[3,4]	MMN+	2(.4)[a]	18(11)[b]	23(6)[ab]	23(5)[ab]
Thelephora terrestris	MMN+	2	0	2	6
Suillus sp.	MMN+	0	1	0	0
Cylindrocarpon sp.	MMN+	.5	44	0	.5
Mycelium radicis atrovirens[3,5]	MMN+	41[a]	0	49[a]	29[a]
No growth of any fungi	MMN+	34(24)[b]	10(5)[ab]	9(5)[ab]	12(3)[a]

1 Ectomycorrhizal and other fungi isolated from roots extending from the planting core either after washing and plating on benomyl-MMN or after washing, surface sterilization and plating on MMN+.

2 Data analysed by Krusal-Wallis test. Numbers superscripted by the same letter not significantly different ($p = .05$) as determined by post hoc, multiple comparisons.

3 Data analysed by 1-way ANOVA. Numbers superscripted by the same letter not significantly different ($p = .05$) as determined by Scheffé multiple contrasts.

4 Data ln ($x + 1$) transformed before analysis. Arithmetic means are followed by geometric means in parenthesis.

5 Peat not included in analysis as there was no variation in replicates.

Table 21. Abundance of four ectomycorrhizal fungi on jack pine planted in oil sand tailings either amended with peat, fertilizer or sewage sludge, or left unamended. Determinations made after four growing seasons using direct counts of short roots and ectomycorrhizae.

	Amendment							
	Control	Peat	Fertilizer	Sewage	Control	Peat	Fertilizer	Sewage
	% of short roots ectomycorrhizal with:				Number of samples each type occurred in of 9 possible			
E-strain[2]	0.2(0.6)	80(19)	18(25)	21(27)	2	9	5	5
Suillus sp.	1.7	0.9	1.8	6	4	1	5	2
I-type ascomycete	0	1.8	0	0	0	1	0	0
Amphinema byssoides	0	0.5	0	0	0	1	0	0

[1] Data not analysed due to the large number of samples with zero frequencies.

[2] Mean and standard deviation.

E-strain inoculum was introduced with the peat but the source of the inoculum in the other treatments is unknown.

When the second year assays were made, the E-strain ectomycorrhizae could not be recognized and it thus is not known for certain if the frequency of E-strain mycorrhizae changed between 2 and 4 years. However, the possibility of there being more than one symbiont forming the rather nondescript ectomycorrhizae was investigated after the second growing season. Forty samples of ectomycorrhizae were selected to cover the range of morphology, colour and mantle characteristics; photographed, and either plated directly on benomyl-MMN or surface sterilized prior to plating. Of the 27 types from the control, sewage and fertilizer treatments, 25 yielded cultures of Thelephora terrestris and two yielded no symbionts. It is possible that these two types were E-strain ectomycorrhizae. Of the 13 types from the peat, a subtuberculate type yielded cultures identical to Suillus tomentosus and the other 12 yielded no symbionts while 86% of the surface sterilized tips remained sterile. It appears that if the E-strain was present in treatments other than the peat at the end of the second year, it was rare.

Based on the 25 ectomycorrhizal samples that yielded cultures of I. terrestris, ectomycorrhizae formed by this fungus were monopodial to coralloid with up to 16 tips per short root, glabrous, lacking mycelial strands or rhizomorphs, elements short and robust to long and slender, buff to pale snuff-brown or occasionally snuff-brown. The Hartig net was well developed but the mantle was usually very thin or lacking and there was no intracellular penetration.

2.5 DISCUSSION OF AMENDING OIL SAND TAILINGS

Plants in the amended oil sand tailings became mycorrhizal either by virtue of inoculum present in the planting core, inoculum introduced with the peat and, possibly, by air-borne sources. It is apparent that once a mycorrhizal fungus became established in spoil material, changes in mycorrhizal associations were slow to occur.

Plants that lacked inoculum in planting plugs only slowly became mycorrhizal, presumably by mycelium from neighboring plants. Some jack pine seedlings had very low mycorrhizal infection rates even after 2 years. The consequence of having some plants with very low infection rates after 2 years in the field was a significant decrease in variability with time. Thus a portion of the jack pine seedlings were unable to benefit from mycorrhizal associations for several years when container-grown seedlings were used in inoculum poor spoil.

Amendation affected the rates of colonization by mycorrhizal fungi, in the case of the peat, by increasing the inoculum density and thus decreasing the variability. Fertilizer had no effect on rates of ectomycorrhizal development for the first 2 years but then caused an increase in ectomycorrhizal infection rate compared to the control due to better seedling growth. Sewage both greatly enhanced plant growth and ectomycorrhizal development of jack pine while depressing ectomycorrhizal formation and having a smaller effect on growth of bearberry.

Thelephora terrestris + jack pine ectomycorrhizae were very difficult to identify even when using high magnification techniques. It was necessary to use culturing techniques and comparison with known fruit body isolates to identify and quantify T. terrestris ectomycorrhizae. Frequency of recovery of T. terrestris from surface sterilized ectomycorrhizae was very low and this was probably due at least in part to the very thin mantle. The formation of a thin mantle or its complete absence on jack pine has been previously noted by Marx and Bryan (1970) and Hacskaylo (1965). Thus it appears that mantle development is normally limited in jack pine + T. terrestris ectomycorrhizae. Ectomycorrhizae formed by T. terrestris have been described as having hyphal strands (Hacskaylo, 1965; Marx et al., 1970; Thomas and Jackson, 1979) but no strands were associated with jack pine + T. terrestris mycorrhizae in this study. This suggests a taxonomic difference between Alberta and southern U.S. Thelephora's. Thelephora terrestris is also noted for its ability to fruit on and around young seedlings in nurseries (Corner,

1968). Thelephora terrestris was not observed to fruit in the amended sands until the fourth year and then only in the sewage treatment. In southern pine nurseries and on plots amended with sewage, T. terrestris may fruit prolifically during the first growing season (Berry and Marx, 1976; Marx et al., 1970). It appeared that the T. terrestris strain introduced with the seedlings required large plants prior to fruting, perhaps due to plant induced environmental modifications as it fruited on the containers prior to outplanting.

Although the data from the second year were not detailed with respect to specific symbionts, it appeared that the E-strain was stable within the peat and increased in abundance in the other treatments. The E-strain fungus is considered to be a species of low competitive abilities and thus is rapidly replaced by other fungi (Mikola, 1965). In particular, Thomas and Jackson (1979) stated that in spruce nurseries, the E-strain was replaced by Thelephora terrestris. In oil sand tailings, the opposite appeared to take place with jack pine. In addition, T. terrestris was largely able to colonize roots in peat amended tailings sand in the presence of the E-strain. From the data gathered, it appeared that the E-strain persisted longer in amended sands than T. terrestris. Although symbionts typical of mature forests (Suillus sp. and Cenococcum geophilum) were present, they did not appear to increase in abundance and remained minor components of the ectomycorrhizal populations.

3. CHLAMYDOSPORE POPULATIONS OF THE E-STRAIN SYMBIONT IN AMENDED COAL MINE SPOIL.

3.1 INTRODUCTION

The E-strain fungus, which was the dominant symbiont associated with white spruce (see Section 2.2.3), is unusual in that it produces large soil-borne chlamydospores (Danielson, 1982). It is likely that these chlamydospores serve as resistant resting propagules and are important sources of inoculum. They may function to

infect roots of newly introduced plants or to re-infect roots of established plants following periods of severe stress that are lethal to mycelium. It was thus of interest to know if the type of amendment affected chlamydospore populations and whether or not the E-strain symbiont is more or less beneficial than other symbionts that occur in the spoils. Chlamydospore populations in the sub-alpine coal mine spoil were quantified in the spring of 1980:

1) to determine the effects of peat, sewage sludge and fertilizer on the production of chlamydospores by the E-strain;
2) to determine if any chlamydospores of VA mycorrhizal fungi were present in the spoil with a nonhost species; and
3) to compare amendment effects on chlamydospore populations of two dissimilar mycorrhizal systems, i.e. E-strain + spruce and VA + slender wheatgrass (see Zak et al., 1984).

3.2 MATERIALS AND METHODS

Three randomly chosen soil samples per plot (three replicate plots for each type of spoil amendment) were taken prior to any new plant growth in spring 1980. The samples were taken to a depth of 10 cm and located 3 cm north of each randomly chosen seedling. The soil fraction larger than 4 mm was discarded, the roots were removed for separate examination and the remaining soil was stored at $5^{o}C$ until it could be processed.

Twenty-five grams of mineral soil (wet weight) or 10 g of peat of each sample were added to about 350 mL water and mixed with a magnetic stirrer for 3 min. The soil suspension was wet sieved through a series of 500 m, 250 m, 125 m and 53 m sieves. The material on the 53 m and 125 m was washed into centrifuge tubes and brought up to volume with water. The tubes were centrifuged at 2000 RPM for 10 min and after standing for 15 min, the liquid decanted onto coarse filter paper in a Millipore filter holder under a vacuum. Additional filters were used if a large amount of suspended material was present. The material in the tubes was then resuspended in a 2M sucrose solution and centrifuged again at 2000 RPM

for 10 min and filtered as above. The filters were scanned at 50X and all E-strain chlamydospores and all VA-type spores picked off the filters. The VA-type spores were mounted on slides in lactophenol for examination and the E-strain spores were mounted in Shear's mounting fluid or lactophenol. This method was tested by adding a known quantity of spores to peat and successively extracting more than 50% of them (Appendix Tables 4 and 5).

In order to determine if the E-strain fungus was present in each sample, the mycelium and mycorrhizae were examined. Each filter from the 125 μm-sucrose extraction was examined for E-strain hyphae. The criteria used to identify the E-strain hyphae were (1) the size range of 4-6 μm diameter, (2) the presence of ornaments in the form of small blisters, (3) simple septa with associated Woronin bodies and plugs, (4) brown pigmentation evident at 30X magnification, and (5) infrequent branching. The mycorrhizae were closely examined for diagnostic characteristics in whole mounts using a 40X oil immersion objective. The roots found in each sample were dried and weighed to determine if the number of chlamydospores was related to the amount of roots present.

3.3 RESULTS

E-strain ectomycorrhizae and hyphae were present in all treatments (Table 22). The quantity of roots in all treatments was similar and thus did not preclude the production of chlamydospores if conditions were otherwise favorable. There was no apparent correlation between the amount of roots and the number of chlamydospores.

Very few chlamydospores were produced in the peat and sewage amended spoil while substantial numbers were found in the control and fertilizer amended spoils. These differences were not due to differences in seedling sizes as these were similar in all treatmets (see Table 3). The greatest number of spores in any one replicate was 739/100 g dry soil in one control sample.

Table 22. Numbers of E-strain chlamydospores in amended coal mine spoil, presence of E-strain hyphae and mycorrhizae and VA chlamydospores after 3 years growth of white spruce.

Amendment	Amount of root in sample (mg)	No. of samples (of 9 possible) with E-strain:		E-strain spores per 100 g dry soil	VA-type chlamydospores per 100 g dry soil[2]
		Mycorrhizae	Hyphae		
Control	20	5	9	174[a]	28[a]
Peat	21	8	8	0	1118[b]
Fertilizer	32	9	6	177[a]	28[a]
Sewage	52	8	9	3	36[a]

[1] A Chi-square goodness-of-fit test was used to determine if amendment affected the number of samples with spores. Control and fertilizer had significantly greater numbers of samples with spores. They were then tested by a t-test to determine if the actual numbers of spores produced were different.

[2] Data analysed by Kruskal-Wallis test. The difference in means was determined by post-hoc multiple comparisons. Numbers within the same column superscripted by the same letter not significantly different ($p = .05$).

VA-type chlamydospores were rare in all treatments except the peat. Very few of these spores appeared to be viable and were probably introduced with the peat.

Water was ineffective in floating out the chlamydospores of the E-strain (Appendix Tables 4 and 5). A much higher percentage of VA-type spores were extracted with water, however, almost all of these were nonviable.

3.4 DISCUSSION

Amendation had a strong influence on the production of E-strain chlamydospores. The suppression of spore production in the sewage amended spoil was apparently not due to plant size, which was similar in all treatments, nor to the absence of E-strain mycorrhizae. At the end of the fourth year, the E-strain infected 47% of the short roots in the sewage amended spoil and 80 and 94% of the roots in the control and fertilizer amended spoil, respectively. A similar suppression of Glomus aggregatus spore production in the sewage treatment occurred in the slender wheatgrass plots (Zak et al., 1984). The lack of spore production and the gradual replacement of the E-strain on the roots by Amphinema byssoides (see p. 26) may indicate that conditions in sewage treated spoil was somewhat unfavorable for the E-strain symbiont.

The lack of E-strain chlamydospores in the peat was not an artifact of the extraction procedure (see Appendix Tables 4 and 5) but is most likely due to the presence of another taxon of the E-strain. E-strain isolates for the peat differed significantly from those in the other treatments and they did not produce chlamydospores in culture (Danielson, 1982). The absence of chlamydospores in the peat suggests that the E-strain inoculum from the spoil has been unsuccessful in competing with the taxon that was introduced with the peat.

The procedure used here for extracting chlamydospores should be modified when the substrate is peat or forest floor material. The water extractant and the 125 μm sieve washings can be eliminated. It is recommended that settling times of 15 and about

75 min be used and then a 15 min settling time after resuspension and centrifugation.

4. ECTOMYCCORHIZAL INOCULATION IN THE CANMORE PEAT USED AS AN AMENDMENT.

4.1 INTRODUCTION

Data for mycorrhizal infection of jack pine strongly indicated that the peat was a source of the inoculum of the E-strain (see Section 2.4.3.3). However, no initial evaluation of the peat was made for ectomycorrhizal inoculum. In the subalpine spoil, the E-strain was present in all treatments and could have been introduced with the spoil or in the root plugs as well as with the peat. However, no chlamydospores were recovered by wet sieving the peat around the spruce seedlings (see Section 3) or the spoil in the absence of spruce (see Zak et al., 1984). Thus the source of the E-strain inoculum was still questionable. Information on the source of the inoculum will increase our knowledge of the ecology of this symbiont and may permit predictions on mycorrhizal associations in other revegetation studies. It was felt that it would be possible to determine the potential inoculum source of the E-strain by sampling the site at Canmore in June 1980 where the peat was obtained in 1977. The objectives for resampling the Canmore peat were:

1) to determine if the E-strain symbiont was present in the peat deposit;
2) to determine if chlamydospores of the E-strain were present in the peat; and
3) to determine what other symbionts compatible with jack pine were indigenous to the peat.

4.2 MATERIALS AND METHODS

Peat was collected from the Canmore site in June, 1980. The area was forested with mature white spruce with a shrub undergrowth. Three profiles (soil pits) were sampled around the perimeter of the excavated peat site. The face of each profile was

cleaned and samples taken at the 20-30, 50-60, and 80-90 cm depths from each of three profiles. When the peat was originally harvested, the surface layer was stripped off and discarded. The top sample (20-30 cm) was within the rooting zone of the surrounding white spruce and the peat was fairly soft and fibrous. The middle depth (50-60 cm) contained few roots and the peat was much more amorphous. The lowest depth (80-90 cm) was at the bottom of the profile just above the clay bottom of the old bog. The material at the bottom was hard and was removed in layers. A few roots reached the bottom of each profile. An area of approximately 50 x 15 cm was removed from each depth. The peat was stored at $5^{o}C$ until it could be broekn up by hand and thoroughly mixed.

For detection of E-strain chlamdospores, 10 g samples were wet-sieved as described in the E-strain chlamydospore section (Section 3.2). To determine if viable propagules of the E-strain and other symbionts were present, a baiting technique was used. Jack pine was used rather than white spruce due to the more rapid growth of jack pine and the more distinctive E-strain mycorrhizae formed with jack pine than with spruce.

Peat from each sample was put into two 15.5 cm diameter pots and the surface covered with a layer of granite grit. One sample of the 20-30 cm depth from each pit was autoclaved and pots prepared in the same manner to serve as indicators of air-borne contamination. All pots were planted with three surface sterilized (30% H_2O_2 for 30 min), pregerminated jack pine seeds and one-half of the pots were watered twice weekly with a solution of 75 $mg.L^{-1}$ of 20:20:20 fertilizer containing trace elements and iron (Plant Prod). To the other half (i.e. one pot of each sample), water only was added. The plants were harvested when they were 18 weeks old, the tops dried and the roots cleaned and evaluated for mycorrhizal infection.

The degree of infection was estimated by examining the short roots with a dissecting microscope and checking for infection by observing wholemounts at 500X magnification. Each morphological type was estimated separately and isolation procedures were used to

confirm differences among types. For isolations, the mycorrhizae were washed under cold, running water for 1 to 2 h, either plated directly or surface sterilized with 30% H_2O_2 for 15 sec and plated on MMN+ and benomyl-MMN agar. Chemical analysis of the peat was performed according to McKeague (1976).

4.3 RESULTS

There was no sighnificant effect of depth or profile location on soil pH or levels of NO_3-N (Table 23). Ammonium-N did not vary with depth but was significantly higher in profile 3 than in the other two sites. Due to depth-site interactions, the P data could not be analysed but it is obvious that P concentrations were very low in all samples except the one from the bottom of profile 3. This sample was unusual in virtually all factors examined and contributed a major portion of the variation. Autoclaving resulted in a large release of NH_4-N, no change in NO_3-N and a nonsignificant increase in the level of extractable P.

The jack pine seedlings grew very poorly in all the unfertilized samples except in the surface sample of profile 3 (Table 24). All seedlings in the unfertilized series, except those in the 20-30 cm depth of profile 3, showed P deficiency symptoms. Fertilization strongly stimulated growth of jack pine in all samples except those from the 80-90 cm depth of profile 3. This sample had a visible slimy growth of bacteria at the time of sampling and the pot had an anaerobic odor when sampled. The amount of stimulation by fertilization was quite variable and was not consistent with depth. Seedlings in three pots of the fertilized peat showed apparent Fe deficiency despite the fact that the fertilizer contained Fe. The apparent Fe deficiencies could not be related to the ectomycorrhizal status of the roots. Autoclaving the peat also stimulated seedling growth by releasing nutrients. Addition of fertilizer to the autoclaved peat had no apparent effect on growth.

No ectomycorrhizae were formed in the autoclaved peat, indicating that most or all infections in the nonsterilized pots were the result of indigenous inoculum. Ectomycorrhizal inoculum

Table 23. Chemical characteristics of the Canmore peat.[1]

	Depth (cm)	pH	NO_3^--N ($\mu g.g^{-1}$)	NH_4^+-N ($\mu g.g^{-1}$)	P ($\mu g.g^{-1}$)
Profile 1	20-30	5.8	242	14.5	2.5
	50-60	5.8	630	15.5	1.7
	80-90	6.3	714	12.5	1.2
Profile 2	20-30	6.4	104	11.5	1.5
	50-60	6.0	270	7.0	1.5
	80-90	6.2	396	10.5	2.5
Profile 3	20-30	5.8	468	20.0	3.0
	50-60	5.7	374	38.0	1.2
	80-90	6.0	44	26.0	35.0
Autoclaved					
Profile 1	20-30	-	248	512	7.5
Profile 2	20-30	-	105	407	2.5
Profile 3	20-30	-	396	462	12.5

[1] Data for nonautoclaved sampled analyses by 2-way ANOVA, without interactions after testing for interactions using Tukey test for additivity. Effect of autoclaving tested by paired t-test, differences tested at $p = .05$.

Table 24. Growth of jack pine on Canmore peat from three pits and three depths with and without fertilization.[1]

Depth (cm)	Pit 1		Pit 2		Pit 3	
	Fert	Non Fert	Fert	Non Fert	Fert	Non Fert
	Shoot weight (mg)					
20-30	107^{bcd}	50^{abc}	117^{cd}	40^{ab}	893^{g}	160^{de}
50-60	206^{def}	29^{a}	344^{efg}	41^{ab}	677^{fg}	53^{abc}
	Shoot weight (mg)					
80-90	227^{b}	43^{a}	394^{b}	38^{a}	36^{a}	32^{a}
	Root weight (mg)					
20-30	24^{a}	29^{a}	27^{a}	24^{a}	209^{b}	60^{ab}
50-60	48^{ab}	20^{a}	97^{ab}	30^{a}	171^{b}	23^{a}
	Root weight (mg)					
80-90	58^{a}	24^{a}	116^{c}	22^{a}	20^{a}	22^{a}
	Autoclaved - Shoot weight (mg)[2]					
20-30	160	160	100	78	660	417
	Autoclaved - Root weight (mg)					
20-30	30	76	41	41	141	194

[1] Data were separated into 2 groups based on depth. The shoot and root weight data for 20-30 cm and 50-60 cm group was analysed by 3-way ANOVA. The 80-90 cm group was analysed by 2-way ANOVA. Numbers within a group superscripted by the same letters not significantly different ($p = .05$) as determined by Scheffe multiple contrasts where F-test significant.

[2] Autoclaved data not analysed.

was present throughout the peat profile (Table 25). The only sample in which there was no infection was the one from the 80-90 cm depth of profile 3. It was estimated that at least 13 species of symbionts infected jack pine. There did not appear to be qualitative differences with depth except possibly in pit 3 where the E-strain, a form designated 'glabrous-dark buff' and Amphinema byssoides were restricted to the 20-30 cm depth. Amphinema byssoides occurred in the 20-30 cm depth in all three profiles but did not occur at the 80-90 cm depth in any of the three profiles.

Ectomycorrhizal development was strongly stimulated by application of mineral fertilizer. Considering all depths, infection was 48 and 87% in nonfertilized and fertilized pots, respectively. The most abundant symbiont was an Ascomycete designated as the I-type. This symbiont (or perhaps two or more similar species) formed greater than 78% of the ectomycorrhizae in seven of the nine fertilized profile-depth combinations. This symbiont formed ectomycorrhizae with jack pine which were simple or dichotomous, pale fulvous, inflated, glabrous or with hyaline cystidia which arose from the outermost cells of the mantle. The cystidia were of a setoid type, up to 100 µm long, thin-walled, septate with acute tips. Woronin bodies were associated with the septa of the setae. The mantle was a textura epidermoidea, perfectly smooth except for the setae with no extramatrical mycelium or mycelial strands. This form conforms to the I subtype of Dominik (1962).

A second cystidial type was less frequently found and was assigned to the genus Tomentella. The ectomycorrhizae were simple to dichotomous, snuff-brown to umber and glabrous except for the presence of light-brown setae. The setae were 2.5 µm at the base, clamped where attached to the mantle, walls slightly thickened, pale-brown, about 200 µm long with acute tips. The mantle was a textura epidermodea with cells up to 6 µm diameter. The culture was moderately fast growing, pale-brown to hyaline on MMN, dark-brown with a black reverse on PDA. The hyphae were clamped, 4 µm diameter and verruculose. ON PDA brown, spinulose crystals 6-8 µm in diameter were produced in the agar or brown pigments occurred within

Table 25. Fungi forming ectomycorrhizae with jack pine planted in peat from under a white spruce stand under greenhouse conditions.

	External features*	Hyphal pigments**	Sampling Depth (cm) 20-30	50-60	80-90	Number of seedlings colonized (of 18)	Percent Infection of colonized seedlings
			Percent Infection+				
Ascomycetes							
I-type	Cystidia	-	47	47	33	9	84
E-strain	EMM	+	16	0	0	1	99
Cenococcum geophilum	EMM	+	<1	0	0	1	<1
Basidiomycetes							
Tomentella sp.	Cystidia	+	<1	11	<1	7	10
Amphinema byssoides	EMM+R	-	3	1	0	5	2
Rhizopogon-like sp.1	EMM+C+R	-	0	0	1	1	<1
Rhizopogon-like sp.2	EMM+C+R	-	0	0	1	1	1
Unknown 1	EMM	+	16	11	0	2	82
Unknown 2	EMM	+	1	0	0	1	9
Unknown 3	Glabrous	-	13	0	0	1	76
Unknown 4	EMM	-	0	1	0	1	5
Unknown affinity		-	<1	<1	<1	4	1

* EMM = extramatrical mycelium, C = crystals on the mycelium, and R= rhizomorphs or mycelial strands.

** Individual hyphae with brown pigments.

\+ Represents the mean of six plants per sampling depth.

the hyphae. These brown materials turned green with the application of 3% KOH or 10% NaOH and dissolved.

Two other species of Basidiomycetes with brown pigmented hyphae were observed and isolated. One was very slow-growing and the other was similar to Tomentella sp. but lacked the KOH-reactive pigments. It formed ectomycorrhizae only with nonfertilized seedlings. Fertilized seedlings in the same peat samples were dominated by I-type ectomycorrhizae.

The E-strain symbiont formed ectomycorrhizae in only one of the nine samples. The infection was typical for this species, having a thin mantle and intracellular hyphae. Although mycorrhizal tips plated on MMN produced the typical straight, ornamented hyphae, only one isolate grew enough to be subcultured. This isolate was identical to isolates from the peat amended spoils and did not produce chlamydospores in culture. No E-strain chlamydospores were recovered by wet sieving of the nine samples. Other symbionts in the Canmore peat included Cenococcum geophilum, which was very rare, Amphinema byssoides, which occurred at low densities, several unknowns and two isolates which were similar in appearance to Rhizopogon species.

The use of isolation procedures was useful in symbiont identification as 10 of the 13 fungi were successfully isolated. Root washing plus direct plating of mycorrhizae on MMN+ or benomyl-MMN was the most successful isolation technique with 64% recovery from 250 mycorrhizae. When the mycorrhizae were also surface sterilized, the recovery was only 19% from 165 tips.

4.4 DISCUSSION

E-strain ectendomycorrhizae were formed in one sample of the Canmore peat. Isolation attempts and wet sieving for chlamydospores indicate that the taxon in the Canmore peat is distinct from that in the subalpine spoil and that it does not produce chlamydospores either in culture or in the peat substrate. However, it does appear that the E-strain taxon from the peat produces resistant propagules. A sample of the original peat amendment which had

been stored outside in a small pile for 3 years was planted with jack pine. After 13 weeks growth, all the seedlings were infected with the E-strain. No chlamdospores could be recovered by wet sieving from this stored peat. It appears that resistant spores are produced by E-strain fungi in addition to large chlamydospores.

Although no pines were on the sampling site there was a large number of nonhost-specific symbionts. It can be assumed that most or all the symbionts recorded here also formed mycorrhizae with white spruce. It would appear that the use of peat formed under spruce stands would provide a wide spectrum of symbionts compatible with pines. In the experimental soil tanks the only symbionts apparently introduced successfully with the Canmore peat were the E-strain and Tomentella.

The identity of the I-type is unknown but its Ascomycete affinity is clear. It is unusual that nearly all of the mycorrhizae formed on the fertilized jack pine had Ascomycete symbionts. The frequency of Ascomycete symbionts of the native white spruce in the field was not determined. Future studies on ectomycorrhizae in organic spoils should examine the relative importance of Ascomycetes in more detail.

The ectomycorrhizae formed with jack pine grown in the Canmore peat had two distinct morphological characteristics that were uncommon or absent in native jack pine mycorrhizae in sandy, well-drained soils (i.e. Mildred Lake) (see Section 5.2). Firstly, four tentative taxa of Basidiomycetes plus the E-strain found on jack pine grown in Canmore peat all produced pigmented hyphae, whereas in the jack pine from the field site, symbionts with brown pigmented hyphae were very rare. It is likely that all four of the Basidiomycetes from the Canmore peat are in the Aphyllophorales and it would appear that the nonhost--specific symbionts in the peat are not agarics. Fruit bodies of Gasteromycetes, Corticium-like fungi and Ascomycetes should be collected and cultured to try to identify these symbionts.

The second morphological characteristic of interest is that mycelial strands, rhizomorphs and extramatrical hyphae were rarely

associated with the ectomycorrhizae of plants grown in the Canmore peat. The only types which had mycelial strands were the Rhizopogon-like and Amphinema byssoides mycorrhizae, all of which were uncommon. Species with a loose network of extramatrical mycelium included the E-strain, Brown Basidio, Cenococcum geophilium and Triangular branch types. The low incidence of ectomycorrhizae with mycelial strands suggests that these structures are of less importance in wet organic soils than those subject to periodic water stress. In jack pine growing at Mildred Lake, glabrous ectomycorrhizae were uncommon and well-developed rhizomorphs were common (See Section 5.2). In the peat, where water conductance and ion diffusion rates would be expected to be high, the glabrous or cystidial type may be equally effective in water and nutrient uptake as rhizomorphic species. Nonglabrous species would also have to expend carbon and energy on the production of rhizomorphs or extramatrical mycelium.

The cystidial morphological type would appear to be intermediate between rhizomorphic and glabrous types. The cystidia would effectively increase the surface area and amount of soil exploited but the amount of extension would be genetically limited unlike the unlimited potential growth of mycelial strands. However, the actual production of cystidia is apparently environmentally controlled as in the Canmore peat the I-type mycorrhizae exhibited a great range in the quantity of cystidia produced, i.e. glabrous to bristling with cystidia. Thus during dry periods, the fungus may expend energy to produce cystidia with a resultant increase in the capacity for water uptake and be able to conserve energy during wet periods by not producing cystidia. It therefore follows that the symbionts native to peat may not be as effective on reclaimed land surfaces as would be rhizomorphic species.

To obtain the maximum benefit from ectomycorrhizal infection in reclamation efforts, it still may be desirable to establish symbionts which possess morphological adaptations for extracting water and nutrients in dry, low conductance soils. For example, the mycelium of Cenococcum geophilium, a species reputed to

be effective in droughty situations (Trappe, 1977), can extend over 2 m from the roots (Fogel, 1980) thus vastly increasing the volume of soil exploited. Duddridge et al. (1980) have shown that water can be transported through rhizomorphs of Suillus bovinus for distances more than 0.5 m and be utilized by the host. The use of peat as a source of inoculum may introduce nonagaric, nonrhizomorphic symbionts ill-adapted to the variable moisture conditions and high soil temperatures that are likely to be found on reclaimed mine spoils.

5. FIELD STUDIES

5.1 ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH SPRUCE AT THE SUBALPINE COAL MINE SITE.

5.1.1 Introduction

The spoil that was used in the tank study was from a coal mine at Luscar, Alberta. It was thus of interest to obtain some information in the ectomycorrhizal associations that occurred in both undisturbed and disturbed sites for comparison with the associations that developed in the tank study. The objective of this brief study was to determine the identity of ectomycorrhizal fungi occurring in the subalpine site.

5.1.2 Materials and Methods

The spruce-fir site has been described briefly in Section 2.1.1 and in more detail by Visser et al. (1984). White spruce naturally regenerating in adjacent areas, an abandoned roadbed and a roadcut, were sampled to determine the symbionts occurring in disturbed areas. Five samples of the forest floor in the mature stand, 15 x 15 x 5 cm, were taken in June 1978. Five spruce seedlings were dug up from the roadbed in June and five more removed from a roadcut in another location in September. The roots were washed free of litter and soil and examined. All active ectomycorrhizae (Appendix 1) in the forest samples were counted and

all ectomycorrhizae formed by Cenococcum geophilium were counted in the June samples. After direct examinations, the roots were cut into 2-3 cm segments and 10 segments from each sample were randomly selected and the first five ectomycorrhizae on one end surface sterilized in H_2O_2 and plated on benomyl-MMN. Twenty ectomycorrhizae from each sample were also plated without being surface sterilized but Mortierella sp. was so abundant that no symbionts could be isolated.

At the time the isolation sampling was done, criteria for the identification of the E-strain were not developed and it was not known if this fungus was indigenous to the subalpine site. In that the E-strain produces large soil-borne chlamydospores that can be recovered by wet sieving, the area was resampled in June, 1981 and the soils sieved. The undisturbed area was sampled by removing 15 cm square cores every 4 m along a transect. The forest floor (LFH layers), which was about 5 cm deep, was sampled separately from the clayey mineral soil which was sampled to a depth of 5-10 cm. Five volunteer white spruce seedlings growing on a roadcut were dug up as well as five seedlings that had been transplanted from a roadcut 2 years previously and planted on reclaimed spoil. These 20 samples were wet sieved as described previously (see Section 3) and examined for E-strain chlamydospores.

5.1.3 Results and Discussion

Ectomycorrhizae formed by Cenococcum geophilum were the only ones that were distinctive enough to be quantified by direct counts. Cenococcum geophilum was not present on any of the seedlings growing in the abandoned roadway (mean dry root weight: 238 $\pm$ 120 mg, x $\pm$ SD/sample). In the mature stand, 14.5 $\pm$ 9.5% of the ectomycorrhizae were formed by C. geophilum. The samples, about 1L in volume, contained 746 $\pm$ 505 ectomycorrhizae on 458 $\pm$ 190 mg of roots. All ectomycorrhizae in the abandoned road samples were indistinguishable from those formed by Amphinema byssoides. The most common symbiont recovered from the surface sterilized ectomycorrhizae was A. byssoides (Table 26). It formed slow growing, cream coloured

Table 26. Fungi isolated on benomyl-MMN from surface sterilized white spruce ectomycorrhizae from a mature stand and from seedlings in cutlines at the subalpine coal mine site.

Taxa	Roadcut (June)	Mature Stand (June)	Roadcut (Sept.)
		% isolation frequency[1]	
Amphinema byssoides	41	18	16
Lactarius-like	3	0	17
Basidiomycete	5	0	.4
Sterile hyaline	12	15	34
Mortierella sp.	5	5	1
Mucor sp.	1	.4	0
Tolypocladium sp.	1	0	0
Unknown	1	4	0
Yeast	0	0	2
No growth of any fungi	31	60	30

[1] Fifty ectomycorrhizae plated for each sample site.

colonies that turned pale to bright yellow when a drop of 2% KOH, 10% KOH or concentrated NH_4OH was applied. The colonies also reacted with phenol aniline (Singer, 1975) to turn livid vinaceous within 2 min. Colonies did not react to H_2SO_4, formalin, phenol, $FeCl_3$ or sulfoformal. The hyphae were 2-3 m diameter, clamped with keyhole- type clamps and smooth or lightly encrusted.

Lactarius-like fungi were the only other symbionts isolated. They produced slow-growing colonies lacking aerial hyphae except for erect fascicles in the center. The hyphae were hyaline, smooth and with simple septa. Piloderma croceum Erikss. & Hjortst. was also present in one sample from the mature forest as evidenced by the golden yellow mycelium (Mikola, 1961) in the forest floor but it was not isolated.

Chlamydospores were found in only one sample, a seedling sample from the cutline. Thirty chlamydospores were recovered from 25 g of soil. This spore density was similar to that found in the tanks (see Table 22). This observation demonstrates that E-strain inoculum is present in disturbed subalpine systems and suggests that the E-strain in the spoil in the tanks may have originated with the spoil itself.

5.2 ECTOMYCORRHIZAL FUNGI OCCURRING IN A JACK PINE-LICHEN WOODLAND LOCATED IN NORTHEASTERN ALBERTA.

5.2.1 Introduction

The ectomycorrhizal inoculum for jack pine in the tank study originated from either the peat, air-borne spores or was introduced with the planting stock. It is thus unlikely that the ectomycorrhizal associations that developed in the tank study would be similar to those developing under natural conditions. In order to determine the degree of similarity, ectomycorrhizal associations were studied in a native jack pine-lichen woodland near Fort McMurray, Alberta where oil sands are currently being mined. The data obtained from this study should serve to determine the species diversity of ectomycorrhizal fungi, the abundance of morphologically

distinctive ectomycorrhizae and allow comparisons between disturbed and undisturbed jack pine stands. In addition, the collection of fruit bodies will provide valuable cultures which can be used in future inoculation programs and for comparison with unknown ectomycorrhizal isolates.

5.2.2 Materials and Methods

The study site was located adjacent to the Alberta Environment Mildred Lake Research Facility about 40 km north of Fort McMurray, Alberta ($57^{o}5'N$, $111^{o}45'W$). The jack pine stand was about 40 years old with little ground vegetation other than lichens (primarily Cladina mitis). Adjacent to this stand was an area cleared several years previously of vegetation and regenerating to jack pine and bearberry. This area (cutline) was used as an example of a disturbed site for comparison with the jack pine-lichen woodland.

The identify of ectomycorrhizal fungi was determined by two methods:

1) Fruit bodies of all suspected symbionts in the vicinity of the plot were collected on 22 September 1977, 29 September 1978, 20 June 1979, 20 September 1979, 2 July 1980 and 16 September 1980. Collections were made from the mature stand and along the adjacent cutline. These collections provided qualitative information on a portion of the ectomycorrhizal symbionts but no information on the abundance of any symbiont-host combination.

2) Fifteen cores, 10 cm deep and 5.7 cm in diameter, were taken at 1.5 m intervals on a transect across the stand on 20 June and 20 September 1979. The September cores were taken 15 cm from the June samples. Ten cores, 10 cm diameter and 15 cm deep, were also taken adjacent to jack pine seedlings in the cutline. The roots in all the cores were washed free of soil and all active ectomycorrhizal tips (not short roots) were counted.

Specific ectomycorrhizae were identified on the basis of similarity to ectomycorrhizae previously synthesized in pure culture (R.M. Danielson, unpublished data). Briefly, jack pine + Tricholoma

flavovirens ectomycorrhizae were characterized by (1) elements monpodial or irregularly branched 1 to 3 times, loosely arranged, (2) colour pale livid vinaceous to livid vinaceous, hyaline if hyphae sparce, (3) hyphal strands abundant, concolourous with the ectomycorrhizae, branching into fine strands.

Jack pine + Lactarius paradoxus ectomycorrhizae were (1) simple to coralloid and robust, (2) mantle very smooth, appearing as a translucent halo in profile, (3) rhizomorphs fused to lateral roots, (4) ectomycorrhizae and rhizomorphs cream to brown and tinted grey-green at least in part, (5) in plan view, mantle a textura epidermoidea. Other members of the subgenus Lactarius probably form very similar ectomycorrhizae with green pigmentation.

Unknown Lactarius + jack pine ectomycorrhizae were those with (1) smooth mantle with none or very little extramatrical mycelium, and (2) mantle cells forming a textura epidermoidea. This concept may include fungi other than those in the genus Lactarius but at least L. rufus + sitka spruce has the same hyphal arrangement (Alexander, 1981).

As a further aid in identification, distinctive ectomycorrhizal types were washed, surface sterilized in 30% H_2O_2 for 10 sec and 20 tips of each type plated on benomyl-MMN agar. Cultures resulting from these isolations were compared to stock cultures obtained from fruit bodies.

Specimens and cultures of a majority of the symbionts are on deposit with the Biosystematics Research Institute (DAOM), Agriculture Canada, Ottawa.

5.2.3 Results

About 50 species of confirmed or suspected ectomycorrhizal fungal symbionts of jack pine were collected at the Mildred Lake site (Table 27). Although there were a large number of ectomycorrhizal fungi fruiting on the site, specimens were sparce and widely scattered for all but a few species. In the cutline the most common species were Thelephora terrestris, Laccaria proxima, Hebeloma sp., and Scleroderma macrorhizon. None of these were observed in the

Table 27. Presumed ectomycorrhizal symbionts of jack pine collected in a mature jack pine-lichen stand and an adjacent cutline at Mildred Lake in northeastern Alberta (4 years observations).

Fungal Species	Number of years observed in: Mature Stand	Cutline	Growth in Culture	Mycorrhizal Status confirmed in pure culture by:[3]
Armillaria ponderosa (Pk.) Sacc.	1	0	+	NT
Astraeus hygrometricus (Pers.) Morgan	1	4	NA[2]	RMD
Chroogomphus rutilus (Schaeff.: Fr.) O.K. Miller	1	0	NA	NT
Coltrichia perennis (Fr.) Murrill	0	1	+	RMD
Cortinarius semiganguineus Fr.	3	0	-	NT
Cortinarius spp. (about 10 species)	4	3	-	NT
Entoloma sp. 2585[1]	0	1	NA	NT
Entoloma sp. 3012	1	0	+	NT
Hebeloma sp. 2657	0	3	+	RMD
Hydnellum zonatum (Fr.) Karst.	3	0	+	RMD
Hydnum sp. 3009	1	0	+	RMD
Hygrophorus eburneus (Fr.) Fr.	3	0	-	NT
Hygrophorus sp.	4	0	-	NT
Inocybe sp.	0	3	-	NT
Laccaria proxima Boudier	0	3	+	RMD
Lactarius affinus Pk. var. affinus	2	0	+	NT
L. paradoxus Beardslee & Burlingham	0	2	+	RMD
L. deliciosus (Fr.) S.F. Gray	1	0	+	JWK
L. resimus (Fr.) Fr.	1	0	+	NT
L. rufus (Scop.: Fr.) Fr.	2	0	+	IJA
L. villosus Clements	0	1	+	NT
Leccinum aurantiacum (Bull.) S.F. Gray	2	0	+	NT
Leptonia sp. 3021	1	0	+	NT
Neolecta vitellina (Bres.) Korf & Rogers	2	0	-	NT
Ramaria sp. 2954	1	0	NA	NT

Table 27. Presumed ectomycorrhizal symbionts of jack pine collected in a mature jack pine-lichen stand and an adjacent cutline at Mildred Lake in northeastern Alberta (4 years observations). (continued)

Fungal Species	Number of years observed in: Mature Stand	Cutline	Growth in Culture	Mycorrhizal Status confirmed in pure culture by:[3]
Rhizopogon rubescens Tul. var. *rubescens*	0	1	+	RMD
Russula spp. (about four species)	4	4	-	NT
Scleroderma macrorhizaon Chev.	0	3	+	RMD
Sistotrema confluens Fr.	2	0	+	RMD
Suillus albidipes (Pk.) Sing.	3	1	+	RMD
S. brevipes (Pk.) Kuntze	2	0	+	LFG
S. granulatus (Fr.) Kuntze	1	0	+	JWR
S. tomentosus (Kauff.) Sing. Snell & Dick	3	0	+	RMD
Thelephora terrestris (Ehrh.) Fr.	1	3	+	RMD
Tricholoma flavovirens (Pers. ex Fr.) Lundell	4	1	+	RMD
T. pessundatum var. *montanum* (Fr.) Gillet	2	0	+	RMD
T. saponoceum (Fr.) Staude	2	0	-	BN
T. zelleri (Smith & Stuntz) Ovrebo	1	0	+	RMD
T. spp. (about 5 species)	4	2	NA	NT

[1] Numbers refer to voucher specimen numbers.
[2] NA = Culturing not attempted.
[3] NT = Not Tested; RMD = R.M. Danielson; LFG = L.F. Grand (1968); JWR = J.W. Riffle (1973); IJA = I.J. Alexander (1981); BN = B. Norkrans (1949).

adjacent mature stand except for one record of T. terrestris. The most common species in the mature stand were Hygrophorus subalpinus, Lactarius resimus and Tricholoma flavovirens. At a generic level, the most common symbionts fruiting in the jack pine stand were Cortinarius, Hygrophorus, Lactarius, Suillus and Tricholoma. The only hypogeous species found was Rhizopogon rubescens and all the suspected symbionts were Basidiomycetes except Neolecta vitellina, an inoperaculate Ascomycete.

Fruit bodies of fungi presumed to be saprophytes were also collected and cultured. These included two species of Clitocybe, three species of Collybia, Gymnopilus sp., Hygrophoropsis aurantiacus (Fr.) Schroeter, Hygrophorus conicus (Fr.) Fr. Lyophyllum sp., Melanoleuca sp., Pholiota sp., Cystoderma granulosum (Batsch ex Fr.) Fayod, Lycoperdon marginatum Vitt. and L. pusillum Pers.

Ectomycorrhizae recovered from cores taken in the cutline were not quantified in that most of the roots were shriveled and inactive. A small percentage of the root tips were infected with Cenococcum geophilum but no Tricholoma flavovirens or Astraeus hygrometricus types were observed. The most common type present was a coralloid form which resumbled ectomycorrhizae formed by Lactarius species. Ornamented hyphae resumbling those of the E-strain were also observed but no ectendomycorrhizae were found.

At both sampling dates, a majority of the ectomycorrhizae in the mature stand were active and were quantified. There was a large amount of variation in the total tips per liter soil and matched T-tests did not detect significant differences between the June and September samples (Table 28). Cenococcum geophilum exhibited the least variation and occurred in 27 of the 30 samples. It occurred at fairly low levels throughout the stand, exceeding 20% of the total ectomycorrhizae in only four samples. In contrast, Tricholoma flavovirens which occurred in 16 samples, exceeded 20% in 9 samples. Overall, about 40% of the ectomycorrhizae were formed by Lactarius spp. and T. flavovirens. Suillus spp., which fruited fairly commonly in the area appeared to form an insignificant portion of the ectomycorrhizae.

Table 28. Total number of active ectomycorrhizal root tips and frequency of occurrence of specific ectomycorrhizal fungi in the top 10 cm of mineral soil in an undisturbed jack pine-lichen woodland at Mildred Lake in northeastern Alberta.

Sampling Date	Total tips/L soil	% of ectomycorrhizal tips infected with:				
		Tricholoma Flavovirens	*Cenococcum geophilum*	*Lactarius paradoxus*	*Lactarius* spp.	*Suillus* spp.
June Mean ± SD[1]	4020 ± 2324	30.7 ± 33.7	8.5 ± 11.5	12.9 ± 20.4	19.5 ± 34.0	.4 ± .9
June Range	1513 - 10455	0 - 86.2	.2 - 36.8	0 - 62.6	0 - 90.8	0 - 2.1
Sept Mean ± SD	3204 ± 1689	11.2 ± 18.3	10.0 ± 13.4	3.0 ± 4.7	26.5 ± 38	1.8 ± 1.2
Sept Range	835 - 5790	0 - 53.4	0 - 44.9	0 - 14.5	0 - 96.5	0 - 3.6

[1] No significant differences due to time in any category as determined by matched pair t-tests ($p = .05$).

With the exception of Cenococcum geophilum and a darkly pigmented Basidiomycete, Tomentella sp. (R-1851), which formed 25 of the 27,600 ectomycorrhizae examined, all of the ectomycorrhizae were formed by species with hyaline or very lightly pigmented hyphae. The Tomentella isolate was identified when it fruited on MMN agar after 3 months incubation. The Basidiospores were ornamented like species of Thelephora (Corner, 1968) and the tomentelloid fungi (Larsen, 1968) and since the fruit bodies were strictly resupinate, it is referred to the genus Tomentella.

5.4 DISCUSSION

In contrast to most other studies of ectomycorrhizae in natural forests, a large portion of the ectomycorrhizal fungi could be associated with fungi fruiting on the site. Past studies have indicated that few ectomycorrhizae were formed by the agarics and boletes that fruit commonly in forests (Lamb, 1979; Lamb and Richards, 1970; Riffle, 1973; Zak and Bryan, 1963). The current study as well as that of Chu-Chow (1979) in which a large percentage of the ectomycorrhizae of Pinus radiata D. Don were shown to be formed by Rhizopogon spp., suggests that in some forests, agarics and their allies are important ectomycorrhizal symbionts. The failure to identify additional ectomycorrhizae may be due to restricted spacial distribution, i.e. widely spaced clumps of many species which would necessitate extremely intensive sampling for detection. In addition, many species are extremely variable in culture, thus making match-ups between ectomycorrhizal isolates and fruit body isolates very difficult unless the full range of variability has been accounted for. It is clear that large numbers of isolations must be made from fruit bodies and monoxenic syntheses performed in order to identify the important symbionts.

Although the soil at the Mildred Lake site was subject to drought, there was no significant change in the quantity of ectomycorrhizae between early summer and fall. In drought-prone conifer forests in Montana, very substantial seasonal decreases occur in the numbers of active ectomycorrhizae (Harvey et al., 1978). The

density of ectomycorrhizae in the Mildred Lake soil was similar to that found by Mikola and Laiho (1962), less than found in a loblolly pine plantation (Menge et al., 1977) and considerably greater than found by Harvey et al. (1978). Harvey et al (1979) suggested that the number of ectomycorrhizae increased with increasing site productivity.

Two instances of specialized fruiting were noted in the mature stand. Cortinarius semisanguineus fruited most frequently on jack pine logs which were at the brown cubical rot stage of decay. It is very likely that decayed wood served as a substrate for jack pine + C. semisanguineus ectomycorrhizae. In forest soils in Montana, decayed wood has been shown to be an important site of ectomycorrhizae formation as well as a source of moisture for plant growth during dry periods (Harvey et al., 1979). Thus, C. semisanguineus may be an important symbiont in mature jack pine stands which contain substantial amounts of decaying wood.

The second instance of specialized fruiting involved the apparent stimulation of fruiting of certain symbionts by disturbance. Sistotrema confluens fruited exclusively on the sides of soil core holes both 3 and 15 months after the cores had been removed. Also observed fruiting in the core holes were Hydnellum zonatum, Entoloma sp., Hygrophorus sp. and an unknown agaric. Intentional disturbance (e.g. trenching) might be used to induce fruiting of other species.

The inclusion of Neolecta vitellina as a potential symbiont is provisional and based on the field observations of Ogawa (1977) who considered it to be ectomycorrhizal with Pinus densiflora. However, Redhead (1979) observed it to be parasitic on ectomycorrhizal roots. Attempts to culture it from ascospores and pieces of the fruit bodies during this study failed. It is worthy of additional study in that it and N. irregularis (Pk.) Korf & Rogers are widespread in Canadian coniferous forests (Redhead, 1977).

Hydnums were only found in mature stands and have not been reported by others to occur in disturbed areas (e.g. Hintikka and Naykki, 1967). This probably is due to their very slow rates of

growth (Hintikka and Naykki, 1967) and subsequent high stability as indicated by species fruiting in the same spot for decades (Harrison, 1971). It is likely that all the hydnums that fruit on soil are ectomycorrhizal even though they have never been considered in mycorrhizal research programs. Although species such as Hydnellum zonatum are very slow growing in culture, they might be useful in inoculation programs due to their apparent stability and long lived nature. Trials are required to test some hydnums against some of the pioneer (and perhaps more ephemeral) species that are currently being used in nursery inoculation research.

The most likely traditional candidates for inoculation trials are those found in the cutline. Species of Laccaria and Hebeloma, very similar to those found at Mildred Lake, are promising candidates for seedling inoculations (J. Trappe, pers. comm.). Observations reported here indicate that species in these genera are successful symbionts in northern Alberta.

Pisolithus tinctorius is not known to occur in Alberta and is rare in other parts of Canada (Malloch and Kuja, 1979). This indicates that it is ill-adapted to Canadian conditions and despite its success in the southern U.S., other fungi should be considered for inoculation in Canada. In addition to Hebeloma sp. and Laccaria proxima, Astraeus hygrometricus should be considered. The fruit body development of A. hydrometricus shows a clear adaptation to droughty, sandy soils and it is likely that the ectomycorrhizae formed by A. hygrometricus would show similar adaptations. Although more slow growing than P. tinctorius, it has several similarities with P. tinctorius which should justify its inclusion in any inoculation program with jack pine. Both Astraeus hygrometricus and Pisolithus tinctorius are Gasteromycetes and have pigmented hyphae and form mycelial strands. They, along with species of Scleroderma, especially S. macrorhizon, occur in sand blows in Wisconsin (Phelps, 1973), mine wastes in Pennsylvania (Schramm, 1966) and in the mountains of Italy (Pacioni, 1978). This Astraeus-Scleroderma-Pisolithus association suggests that either Scleroderma or Astraeus might be

equally as useful a symbiont as P. tinctorius. Scleroderma macrorhizon forms ectomycorrhizae with jack pine but is slow-growing and difficult to maintain in culture (R. Danielson, unpublished data). Thus, of the Gasteromycete species found in droughty areas, A. hygrometricus appears to be the best choice for inoculum testing. It has been used successfully in a preliminary greenhouse inoculation trial (R. Danielson, unpublished data). In that Pisolithus tinctorius has been shown to possess considerable variation in growth rates and effectiveness in forming mycorrhize (Molina, 1979), additional isolates of Astraeus hygrometricus should be sought and cultured.

6. GENERAL CONCLUSIONS

1) The use of complimentary techniques (direct counts and isolations) allowed nearly all the fungal components of the ectomycorrhizae in the tank study to be tentatively identified and quantified. Mycological methodology is required to monitor ectomycorrhizal populations in disturbed areas.

2) There were only a small number of species of mycorrhizal fungi in the amended spoils and these were almost entirely Aphyllophorales and Ascomycetes. Hebeloma sp., Inocybe sp., and Suillus spp. were the only Agaricales detected over the course of 5 years. The most abundant species all had brown pigmented hyphae unlike the species found in undisturbed jack pine or white spruce stands.

3) The initial mycorrhizal associations formed were relatively stable, persisting for at least several years. Thus, mycorrhizal management must be fully considered prior to spoil treatments in order to establish symbioses and to ensure that effective symbionts are introduced.

4) The spread of ectomycorrhizal fungi from plant to plant was relatively slow. Thus if the entire plant population is to benefit from the symbiosis during the period of plant establishment, the inoculum must be abundant and dispersed over the entire spoil area or present on each individual when planted.

5) The effects of amending spoils and tailings on ectomycorrhizal associations cannot be predicted unless specific host-symbiont combinations have been studied previously. For example, sewage sludge additions inhibited the formation of some symbioses whilst stimulating the formation of others.

6) The peat amendment was an important source of inoculum, introducing ectomycorrhizal fungi that persisted on the root systems for long periods.

7) Most ectomycorrhizae in the peat differed morphologically from those found in undisturbed sites. Notably, ectomycorrhizal fungi from peat lacked mycelial strands and large amounts of external mycelium, features which may be important in water conduction and uptake as well as nutrient conservation in coarse textured, droughty spoils.

8) A large portion of the ectomycorrhizae in the native jack pine stand were apparently formed by agarics, especially Tricholoma spp. and Lactarius spp. Hypogeous fungi and Ascomycetes (except Cenococcum geophilum) were rare.

9) Most ectomycorrhizal symbionts fruiting in the cutline adjacent to the jack pine stand were much less frequent in the mature stand. This suggests that most species present in mature forests are ill-adapted to conditions existing in barren disturbed soils.

10) The corticoid fungus, Amphinema byssoides, was found to be a widespread symbiont of pine and spruce, and displayed a very wide ecological amplitude.

11) A species of Tomentella, a genus previously thought to contain only wood decay fungi, was found in the peat and formed ectomycorrhizae with spruce, pine, bearberry and willow.

7. RECOMMENDATIONS FOR FUTURE RESEARCH

1) The jack pine, white spruce, and willow should be sampled in the sixth growing season to determine the persistence of Thelephora terrestris, the E-strain, Amphinema byssoides and Tomentella sp. in the two spoils.

2) Detailed studies should be conducted on species that hold promise for use in greenhouse ectomycorrhizal inoculation trials as well as those species that are likely to be encountered in reclamation efforts. These would include Amphinema byssoides, Thelephora terrestris, the E-strain, Laccaria proxima, Astraeus hygrometricus, Cenococcum geophilum, Tricholoma species and Sphaerosporella brunnea. Host specificity, efficiency, ectotypic variability and inoculation difficulties should be determined.

3) The persistence of the species named in (2) should be determined in field situations on amended spoil or tailings materials.

4) The field testing of a complete range of ectomycorrhizal symbionts is currently impossible due to the inability to establish many mycorrhizal combinations using current techniques for rearing container-grown seedlings. Intensive investigations should be made of the inoculation process of container-grown seedlings with regard to fertilizer regimes, planting mixture composition, and the planting mixture microflora.

5) As drought would be expected to be a prime stress factor on oil sand tailings, morphological, cultural and taxonomic factors should be evaluated in order to permit the selection of fungi that are adapted to low moisture conditions.

6) A survey should be conducted of nursery stock in Alberta in order to determine the ectomycorrhizal status of the roots and the potential for incorporating inoculation procedurers into the production of seedlings.

7) Additional stands of white spruce and jack pine should be studied with regard to mycorrhizal associations. Information is required on the general abundance of specific fungi for each forest type.

8. LITERATURE CITED

Alexander, I.J. 1981. The Picea sitchensis + Lactarius rufus mycorrhizal association and its effects on seedling growth and development. Trans. Br. Mycol. Soc. 76: 417-423.

Benson, L.M., R.L. Evans and P.J. Peterson. 1980. Occurrence of basidiomycetes on arsenic-toxic mine waste. Trans. Br. Mycol. Soc. 74: 199-201.

Berry, C.R. and D.H. Marx. 1976. Sewage sludge and Pisolithus tinctorius ectomycorrhizae: Their effect on growth of pine seedlings. For. Sci. 22: 351-358.

Berry, C.R. and D.H. Marx. 1978. Effects of Pisolithus tinctorius ectomycorrhizae on growth of loblolly and Virginia pines in the Tennessee Copper Basin. U.S. For. Serv. Res. Note SE-264.

Bisset, J. and P. Widden. 1972. An automatic, multichamber soil washing apparatus for removing fungal spores from soil. Can. J. Microbiol. 18: 1399-1404.

Chu-Chow, M. 1979. Mycorrhizal fungi of Pinus radiata in New Zealand. Soil Biol. Biochem. 11: 557-562.

Clement, A., J. Garbaye and F. LeTacon. 1977. Importance des ectomycorhizes dans la resistance au calcaire du Pin noir (Pinus nigra Arn. spp. nigricans Host). Oecol. Plant. 12: 111-131.

Conners, I.L. 1967. An annotated index of plant diseases in Canada. Publ. 1251. Canada Dept. of Agric., Ottawa. 381 pp.

Corner, E.J.H. 1968. A monograph of Thelephora (Basidiomycetes). Beih., Nova Hedwigia 27: 1-110.

Danielson, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines. Can. J. Bot. 60: 7-18.

Dominik, T. 1962. Tentative proposal for a new classification scheme of ectotrophic mycorrhizae established on morphological and anatomical characteristics. Roczn. Nauk Les. 14: 223-245 [English transl., U.S. Dept. of Commerce OTS 60-21383],

Duddridge, J.A., A. Malibari and D.J. Read. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature 287: 834-836.

Eckblad, F.-E. 1968. The genera of the operculate discomycetes - A re-evaluation of their taxonomy, phylogeny and nomenclature. Norw. J. Bot. 15: 1-191.

Eriksson, J. and L. Ryvarden. 1973. The Corticiaceae of North Europe Vol. 2: Aleurodiscus-Confertobasidium. Fungiflora, Oslo. 261 pp.

Fassi, B. and E. De Vecchi. 1962. Ricerche sulle micorrize ectotrofiche del pino strobo in vivaio I. Descrizione di alcune forme pias diffuse in Piemonte. (Researches in ectotrophic mycorrhizae of Pinus strobus in nurseries I. Description of some of the most common forms in Piedmont.) Allionia 8: 133-152.

Fogel, R. 1980. Mycorrhizae and nutrient cycling in natural forest ecosystems. New Phytol. 86: 199-212.

Gilbertson, R.L. 1974. Fungi that Decay Ponderosa Pine. Univ. Arizona Pr., Tucson. 197 pp.

Grand, L.F. 1968. Conifer associates and mycorrhizal syntheses of some Pacific Northwest Suillus species. For. Sci. 14: 304-312.

Hacskaylo, E. 1965. Thelephora terrestris and mycorrhizae of Virginia pine. For. Sci. 11: 401-404.

Harley, J.L. 1969. The Biology of Mycorrhiza. Interscience Publ., New York. 334 pp.

Harrison, K.A. 1971. The evolutionary lines in the fungi with spines supporting the hymenium. In: Evolution in the Higher Basiodiomycetes, R.H. Petersen (ed.), Univ. Tennessee Press, KNoxville, pp. 375-338.

Harvey, A.E., M.F. Jurgensen and M.J. Larsen. 1978. Seasonal distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in Western Montana. For. Sci. 24: 203-208.

Harvey, A.E., M.J. Larsen and M.F. Jurgensen. 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. For. Sci. 25: 350-258.

Hintikka, V. and O. Naykki. 1967. Notes on the effects of the fungus Hydnellum ferrungineum (Fr.) Karst. on forest soil and vegetation. Comm. Inst. Forest. Fenn. 62(2): 1-22.

Jurgensen, M.F. 1970. Microorganisms and the reclamation of mine wastes. In: Forest Soils and Land Use, C.T. Youngberg (ed.), pp. 251-286.

Kalin, M. and P.M. Stokes. 1981. Macrofungi on uranium mill tailings - associations and metal content. Sci. Total. Environ. 19: 83-94.

Laiho, O. 1965. Further studies on the ectendotrophic mycorrhiza. Acta Forest. Fenn. 79(3): 1-35.

Lamb, R.J. 1079. Factors responsible for the distribution of mycorrhizal fungi of Pinus in eastern Australia. Aust. For. Res. 9: 25-34.

Lamb, R.J. and B.N. Richards. 1970. Some mycorrhizal fungi of Pinus radiata and P. elliottii var. elliottii in Australia. Trans. Br. Mycol. Soc. 54: 371-378.

Larsen, M.J. 1968. Tomentelloid fungi of North America. Tech. Publ. No. 93, State Univ. Coll. Forest. Syracuse Univ., Syracuse, N.Y. 157 pp.

Liberta, A.E. 1966. Resupinate hymenomycetes from Gaspe and adjacent countries (Canada) I. Mycologia 58: 927-933.

Malloch, D. and A.L. Kuja. 1979. Occurrence of the ectomycorrhizal fungus Pisolithus tinctorius in Ontario. Can. J. Bot. 57: 1848-1849.

Martin, K.J. and R.L. Gilbertson. 1977. Synopsis of wood-rotting fungi on spruce in North America. Mycotaxon 6: 43-77.

Marx, D.H. 1969. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59: 153-163.

Marx, D.H. 1973. Mycorrhizae and feeder root diseases. In: Ectomycorrhizae - Their Ecology and Physiology. C.G. Marks and T.T. Kozlowski (eds.), Academic Press, N.Y., pp. 351-382.

Marx, D.H. and J.D. Artman. 1979. Pisolithus tinctorius ectomycorrhizae improve survival and growth of pine seedlings on acid coal spoils in Kentucky and Virginia. Reclam. Rev. 2: 23-31.

Marx, D.H. and W.C. Bryan. 1970. Pure culture synthesis of ectomycorrhizae by Thelephora terrestris and Pisolithus tinctorius on different conifer hosts. Can. J. Bot. 48: 639-643.

Marx, D.H., W.C. Bryan and L.F. Grand. 1970. Colonization, isolation and cultural descriptions of Thelephora terrestris and ectomycorrhizal fungi of shortleaf pine seedlings grown in fumigated soils. Can. J. Bot. 48: 207-211.

McKeague, J.A. 1976. Manual on Soil Sampling and Methods of Analysis. Prepared by the Canadian Society of Soil Science, Soil Research Institute, Research Branch, Agriculture Canada, Ottawa.

Menge, J.A., L.F. Grand and L.W. Haines. 1977. The effect of fertilization on growth and mycorrhizae numbers in 11-year-old loblolly pine plantations. For. Sci. 23: 37-44.

Mikola, P. 1961. The bright yellow mycorrhiza of raw humus Separatum - Inter. Union of Forest Res. Organizations: 24-4.

Mikola, P. 1965. Studies on the ectendotrophic mycorrhiza of pine. Acta Forest. Fen. 79(2): 1-56.

Mikola, P. and O. Laiho. 1962. Mycorrhizal relations in the raw humus layer of northern spruce forersts. Comm. Inst. Forest. Fenn. 55: 1-13.

Molina, R. 1979. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of Pisolithus tinctorius. For. Sci. 25: 585-590.

Norkrans, B. 1949. Some mycorrhiza-forming Tricholoma species. Svensk. Bot. Tidskr. 43: 485-490.

Ogawa, M. 1977. Microbial ecology of mycorrhizal fungus - Tricholoma matsutake (Ito et Imai) Sing. in pine forest IV. The Shiro of T. matsutake in the fungal community. Bull. Gov. For. Expt. Sta. No. 297: 59-104.

Pacioni, G. 1978. Notes of mycological flora of Circeo National Park: I. Scleroderma meridionale; II. Gyrophragmium dunallii. Micol. Ital. 7(2): 39-45.

Phelps, J.W. 1973. Microfungi in two Wisconsin sand blows. Trans. Br. Mycol. Soc. 61: 386-390.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158-161.

Redhead, S.A. 1977. The genus Neolecta (Neolectaceae fam. nov., Lecanorales, Ascomycetes) in Canada. Can. J. Bot. 55: 301-306.

Redhead, S.A. 1979. Mycological observations: 1, on Cristulariella; 2, on Valdensinia; 3, on Neolecta. Mycologia 71: 1248-1253.

Riffle, J.W. 1973. Pure culture synthesis of ectomycorrhizae on Pinus ponderosa with species of Amanita, Suillus and Lactarius. For. Sci. 19: 242-250.

Ryvarden, L. 1976. Studies in the Aphyllophorales of the Canary Islands. 3. Some species from the western islands. Quad. Bot. Canar. 26/27: 29-40.

Schramm, J.R. 1966. Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans. Amer. Phil. Soc. 56: 1-194.

Shaw, G.H. 1973. Host fungus index for the Pacific Northwest. II. Fungi. Bull. 766 Washington Agric. Expt. Sta., Coll. Agric., Washington St. Univ., Pullman, 162 pp.

Singer, R. 1975. The Agaricales in Modern Taxonomy. Cramer, Vaduz. 912 pp.

Thomas, G.W. and R.M. Jackson. 1979. Sheathing mycorrhizas of nursery grown Picea sitchensis. Trans. Br. Mycol. Soc. 73: 117-195.

Trappe, J.M. 1971. Mycorrhiza-forming ascomycetes, pp. 19-37, In: Mycorrhizae, E. Hacskaylo (ed.), U.S. Dept. Agric. Misc. Publ. 1189, Washington.

Trappe, J.M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. Ann. Rev. Phytopathol. 15: 203-222.

Visser, S., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Effects of different amendments to three different minespoils on selected soil physical and chemical properties and on plant growth. Final report submitted to Research Management Division, Alberta Environment, 128 pp.

Zak, B. and W.C. Bryan. 1963. Isolation of fungal symbionts from pine mycorrhizae. For. Sci. 9: 270-278.

Zak, J.C., C. Griffiths and D. Parkinson. 1984. Reinstatement of biological activity in severely disturbed soils: Vesicular-arbuscular mycorrhizal development of slender wheatgrass on amended mine spoils. Final report submitted to Research Management Division, Alberta Environment.

9. APPENDIX 1: EVALUATIONS OF ECTOMYCORRHIZAL ROOT SYSTEMS WITH EMPHASIS ON JACK PINE.

9.1 INTRODUCTION

The purpose of this Appendix section is to present the routine techniques used in evaluating the ectomycorrhizal status of root systems. The techniques have been used primarily with white spruce and jack pine but illustrate the background information required and methods that can be applied in studies of these as well as other host species. It is felt that quantification of total ectomycorrhizal infection alone is insufficient in field studies of ectomycorrhizae and more effort must be placed on identifying the symbionts, i.e. symbiont + host relationships. This is a difficult task, but without it the effects of environmental changes, e.g. land clearance, mining, silvicultural practices and toxic chemical inputs, cannot be properly evaluated. General aspects of ectomycorrhizal quantifications have been given by Grand and Harvey (1982).

9.2 GENERAL OBJECTIVES OF ECTOMYCORRHIZAL ASSESSMENTS

1. To determine the intensity of ectomycorrhizal infection as indicated by the percentage of short roots converted to ectomycorrhizae. Short roots of a single plant may become infected by soil-borne propagules, external hyphal growth from an ectomycorrhiza to adjacent noninfected short roots or by internal spread of the fungal symbiont within the cortex of long lateral roots. Thus, percent ectromycorrhizal infection in itself cannot be considered to be a measure of soil infectivity or inoculum potential (sensu Garrett, 1970) of the fungal symbionts. Once infection has occurred the inoculum potential of the fungus will be increased dramtically and soil infectivity over-estimated by short root and ectomycorrhizae counts.

2. Determination of the species of fungi forming ectomycorrhizae. This is far more difficult than simply determining the

level of total ectomycorrhizal infection and usually requires additional techniques. Few fungi can presently be identified from observations of the ectomycorrhizae although repeated reports on the occurrence of Cenococcum geophilum suggests otherwise. However, C. geophilum is one of the few exceptions and few mycorrhizal associations have been studied in sufficient detail to allow identification of the fungal symbiont. Standard mycological techniques, especially for obtaining fungi in pure culture, are required to identify and quantify specific fungal symbionts.

9.3 SHORT ROOTS VERSUS LONG LATERALS (SENSU SUTTON, 1980).

In order to quantify ectomycorrhizal infections it is first necessary to recognize the basic unit of quantifying ectomycorrhizal infection, the short root. Short roots, or feeder roots as they are referred to by some authors, are all considered to be susceptible to infection by ectomycorrhizal fungi whereas long laterals are not susceptible or the hyphae are limited to intercellular spaces of the cortex. If young lateral roots are included in counts, the degree of infection may be underestimated and the total number of roots involved in nutrient and water uptake overestimated. These two functionally different types of roots of pine are distinguished by the following features.

Short Roots	Long Lateral Roots
1. Root apcies rounded with a small, sharply delimited meristermatic zone which is lens-shaped.	1. Root apices acute, strips of cortical cells are frequently seen sloughing off from the tip. The meristem is paraboloid, longer than in short roots.
2. Branching, if it occurs, is bifurcate or dichotomous at an acute angle.	2. Branching which produces either short roots or a higher degree of long laterals, is racemose at right angles to the mother root.

3. Length is usually less than 5 mm (rarely 10 mm), i.e. of limited extent.	3. Length is indeterminate.
4. Root diameter is thin (less than 0.5 of the mother root diameter) unless a thick fungal mantle is present.	4. Usually much thicker than short roots with diameter equal to or greater than 0.5 the diameter of the mother root.
5. Apices may be covered with a fungal mantle.	5. Apices not covered with a mantle of hyphae.
6. Root hairs may be present on uninfected short roots but are rarely present on infected short roots.	6. Root hairs common even on old laterals.
7. The root diameter does not vary greatly with changes in nutrient levels.	7. Under high nutrient levels the apical 2-3 cm may be enlarged 2-3 times the diameter of the older root.

A third type of root is found on pine species and termed subordinate mother roots by Wilcox (1968). These roots appear intermediate between short roots and long laterals and can either be grouped with short roots due to their susceptibility to infection by ectomycorrhizal fungi and limited potential for growth or counted separately. They constitute a minor part of the root system of pines but can cause confusion during ectomycorrhizal evaluations. These roots are characterized by the following features:

1. Apices rounded similar to short root tips but capable of obtaining lengths of 2-3 cm.
2. Branching is racemose and at right angles but infrequent.

3. Size is between long laterals and short roots.

4. The fungal mantle may cover the apical meristem unlike long laterals.

9.4 PREPARATION FOR COUNTING AND FUNGAL ISOLATIONS

1. Samples of roots are collected, which may be in soil cores of known volume from the field or entire root systems of small seedlings from greenhouse evaluations.

2. Wash roots to remove soil or planting mixture or soil under running water using a 2 mm sieve to prevent losses of broken roots. Further cleaning with an artist's brush or fine forceps (Number 5 jeweler's forceps) and needles may be necessary to remove soil or roots of nontarget plants so the short roots can be observed. It is necessary to use a dissecting microscope during the fine cleaning so as few ectomycorrhizae as possible are broken off.

3. Once clean, place the root system in petri dishes, cover with water to suppress the growth of molds and store at $5^{o}C$. If possible, the roots should be evaluated within several days following removal from the soil.

4. If a random sampling of the ectomycorrhizae is desired, the entire root sample should be cut into 2-3 cm long segments, each of which will have 10-20 short roots.

5. A preliminary examination of the samples is useful in determining if any distinctive types are present or if special procedures will be required.

9.5 GENERAL RULES FOR COUNTING SHORT ROOTS

The counting and determination of the ectomycorrhizal status is often complicated by the complexity of morphology, root activity and artifacts due to the extraction and cleaning processes. In order to make the evaluations objective, it is necessary to follow some rules in the counting process.

1. Only "active" short roots are counted and rated. This determination is subjective as dead or inactive roots are often very

difficult to distinguish from live, active roots. Simple criteria have been reported by Harvey et al. (1976). Active short roots are those that are turgid, with sound colours, with a hyaline meristem, and with a pale-coloured cortex and stele.

2. Broken roots are not counted or rated with regard to ectomycorrhizal infection status as only the missing tip may have been infected. This is especially important with species such as Populus, Salix, and other fine-rooted species in which only the final millimeter of a 10 mm short root is often ectomycorrhizal. If the total number of short roots is required, the scars and broken roots must be counted separately and added to the number of intact roots. In such cases, it can only be assumed that breakage occurs equally to ectomycorrhizal and nonmycorrhizal roots (an invalid assumption in many cases).

3. The minimum size of short root that can be evaluated for infection is one in which the short root meristem extends beyond the cortex of the mother root. For convenience and to make decisions objective, all short roots are counted and rated in which the ratio of length of short root to diameter of short root exceeds 1.5.

4. Regardless of the amount of branching, each short root is considered a single ectomycorrhizal infection point or unit as branching may be induced by the fungal symbiont. In addition, all root tips can be counted to obtain data on the degree of branching and total ectomycorrhizal development (excluding extramatrical hyphal development and long lateral root colonization).

5. If a dichotomous root has one tip infected and one uninfected it is rated as one infected short root with one additional uninfected tip.

6. If the stem or base is infected with the dichotomous portion uninfected it is rated as one infected short root plus one additional infected tip as it is assumed that the branching is symbiont induced.

7. If two fungi are infecting the same short root, only the species on the tip (the youngest part) is recorded. Additional notes may be made on the apparent succession of fungal symbionts in progress.

9.6 COUNTING PROCEDURES AND CONFIRMATION OF ECTOMYCORRHIZAL INFECTION.

Many of the techniques used in quantifying the ectomycorrhizal status of root systems are based on experiences with the genus Pinus which have some of the most morphologically distinct ectomycorrhizae. However, ectomycorrhizae formed with many other genera of plants are much less easily recognized and require more critical evaluations (Wilcox, 1982). Even with pines, it is often not possible to confirm infection by ectomycorrhizal fungi using low magnifications. Evaluations must balance the factors of adequate sample size (number of short roots counted) and time required for the evaluations using the low magnification, high magnification and sectioning techniques.

1. All intact, active short roots are examined with the aid of a dissecting microscope at 10-50X (12X is the most efficient magnification) with incident light and rated as ectomycorrhizal or uninfected. The root sample is placed in a rectangular dish and covered with water. A clear plastic dish (a flat plastic lid works well) is preferable as the background colour can be changed from white to black readily. In case of inconspicuous ectomycorrhizae, a black background is best as individual hyaline hyphae can be seen radiating from the root surface, a feature not discernible with a white background. The sample size (number of short roots) will depend on a variety of factors but 300 short roots per sample is adequate in most cases. This sample is drawn randomly from the roots that were previously cut into 2 to 3 cm segments. The criteria used to determine if short roots are ectomycorrhizal using low magnification techniques are as follows.

Ectomycorrhizal	Nonmycorrhizal
1. The most obvious indication of infection is when short roots are converted into coralloid structures by infection with genera such as Suillus and Rhizopogon. More common is simple dichotomous branching in species of Pinus.	1. Usually monpodial with occasional dichotomous branching. However container-grown pine seedlings may exhibit extensive dichotomous branching in the absence of ectomycorrhizal fungi.
2. The presence of extramatrical hyphae or mycelial strands on short roots often indicate infection.	2. Large amounts of hyphae are usually absent.
3. The surface may be very smooth and polished.	3. The surface is coarse and flaky due to the large size of the exposed cortical cells.
4. The cortical region is opaque due to Hartig net development.	4. The cortical region is translucent and appears to "sparkle" internally as no hyphae are present to obstruct the view.
5. Root hairs are almost always absent from infected portions but they may be present on the base of a short root while the tip is infected.	5. If root hairs are present along the entire length of a short root, it is uninfected. Rarely, short roots may become infected after root hairs have developed.

6. Infection results in an increased short root diameter, often appearing clavate with swollen tips.	6. Uninfected short roots are slender, often constricted 2 to 3 times and taper gradually to the tip.
7. Colour is useful only when bright, white or pure black.	7. Colours are nondistinct and bland.

2. To verify infections in doubtful cases, which may be frequent, the most efficient and sensitive technique is to examine whole mounts of short roots using high magnification. Short roots are mounted in water (do not squash) and the upper surface is examined with a 40X brightfield objective. The mantle can then be observed in plan view and if the mantle is thin or absent, the Hartig net can be easily seen. The presence of either a mantle or Hartig net is the critical and confirming criterion of the ectomycorrhizal state. Very early stages of infection can be detected as well as the hyphal arrangement of the mantle using whole mounts.

3. Final verifications of infections can be made with hand-cut sections (using Gillette double-edge stainless steel razor blades and elder pith) mounted in 0.05% trypan blue in lactophenol. The stain will intensify in colour if heated briefly with an alcohol lamp. These sections should be sealed the following day with clear fingernail polish. The trypan blue will increase in intensity for about a week during storage after which the hyphal elements can be easily seen with brightfield optics. The disadvantage of sectioning is the time required to cut and mount sections and the possibility that light infections may be overlooked. Whole mounts circumvent both of these disadvantages.

9.7 ISOLATION OF FUNGAL SYMBIONTS FROM SPECIFIC TYPES OF MYCORRHIZAE.

This supplements ectomycorrhizal counts and permits the identification of many symbionts that cannot be recognized by ectomycorrhiza morphology. The isolation technique is limited in that (1) many ectomycorrhizal fungi will not grow in pure culture on agar, (2) it is necessary to match ectomycorrhizal isolates with cultures from fruit bodies, and (3) it is necessary to observe a large number of cultures to account for interspecific variability.

Two procedures are very useful in isolating fungal symbionts from ectomycorrhizae; surface sterilization and the use of selective media. The addition of the common fungicide benomyl to media largely eliminates hyphomycetes while allowing Basidiomycetes and Phycomycetes to grow. A medium containing benomyl is the medium of choice if the fungus is known to be a Basidiomycete but it cannot be used alone if Ascomycetous symbionts are present. A simple but very effective method of surface sterilizing mycorrhizae is to briefly dip the mycorrhizae in 95% ethanol, immerse in 30% H_2O_2 for 15 sec and rinse in ice cold sterile water for 30 min. A general procedure for isolating ectomycorrhizal fungal symbionts is as follows:

1. Select 2 to 3 cm long segments of lateral roots with the desired ectomycorrhizal type. Wash thoroughly for 30 to 60 min with cold tap water in a 50 mL beaker with 1 mm mesh mylon netting secured over the top of the beaker.

2. For fungal symbionts bearing clamps on the hyphae, either plate directly on benomyl-MMN (Appendix Table 1) if the mantle is thin or surface sterilize prior to plating if the mantle is thick. For Ascomycete symbionts (identified by the presence of Woronin bodies associated with the septa), surface sterilize and plate on MMN+ (Appendix Table 1). For symbionts of unknown affinities surface sterilize and plate on both benomyl-MMN and MMN+. Ten tips, 1 to 2 mm long, are the minimum number plated for each type, five per plate.

Appendix Table 1. Media used for the isolation of mycorrhizal symbionts (MMN modified from Marx, 1969).

A. Modified Melin-Norkrans (MMN) plus antibiotics (MMN+)

$(NH_4)_2HPO_4$	0.25 g
$CaCl_2$	0.05 g
NaCl	0.025 g
KH_2PO_4	0.50 g
$MgSO_4{\cdot}H_2O$	0.15 g
Sequestrene iron	0.048 g (10 mL stock solution[1])
Thiamine HCl	100 g (1 mL stock solution[2])
Malt extract	3 g
Dextrose	10 g
Agar	15 g
Water	1000 mL
Streptomycin	100 mg[3]
Chlorotetracycline	50 mg[3]

1 Stock solution of 480 mg Sequestrene in 100 mL water; Green Cross brand, 10% Fe

2 Stock solution 10 mg Thiamine HCl in 100 mL water

3 Add the two antibiotics directly to 10 mL sterile water, dissolve and add to molten media just prior to pouring plates.

B. Benomyl-MMN Agar

Add 5 mL of stock solution of Benomyl to MMN+ just prior to pouring plates. Stock solution contains 0.2 g Benomyl in 100 mL acetone. Benomyl contains 50% active ingredients (Later Chemicals Ltd.).

3. Parafilm plates and check daily for at least one week for the growth of nonmycorrhizal fungi. If fast growing fungi such as Mucor grow from some of the mycorrhizal transfer the other tips to a fresh plate containing the same medium. The plates should be observed for 2 months before discarding as some ectomycorrhizal fungi are very slow growing.

4. Record recovery of probable symbionts, nontarget fungi and tips that remain free of fungal growth.

5. Attempt to match cultures with those obtained from fruit bodies of known ectomycorrhizal fungi.

9.8 PRESERVATION OF SPECIMENS OF MYCORRHIZAE.

Root and ectoectomycorrhizae can be preserved indefinitely in a weak solution of FAA if care is taken to prevent evaporation of the preservative. Weak FAA consists of 50% ethanol (90 mL), acetic acid (5 mL) and formaldehyde (5 mL) (Johansen, 1940).

9.9 KEY MORPHOLOGICAL CHARACTERISTICS OF "WILD" ECTOMYCORRHIZAE.

In a majority of instances, the taxonomic identity of the fungal symbionts occurring in natural populations cannot be determined. Regardless, attempts should be made to characterize the ectomycorrhizae. Dominik (1962) developed a wholly artificial scheme which utilized extramatrical hyphal and colour characteristics as major descriptors and other authors have simply used colour to subdivide ectomycorrhizae into groups. The latter approach is inadequate as, with a few exceptions, colour varies with age and environmental factors. A discussion of the classification of ectomycorrhizae and evaluations of the factors used in classifying them have been given by Zak (1973).

The characteristics selected here are stable and are indicators of taxonomic affinities or likely to be of importance in defining ecological preferences. Collection of such information over a wide range of soil types may result in defining the importance of certain morphological features under specific environmental

conditions. The features to be noted are given in decreasing order of importance.

1. Ascomycete or Basidiomycete affinities. This can often be determined by observing whether clamps (Basidiomycetes) or Woronin bodies (Ascomycetes) are present at the septa. Dolipore septa cannot be seen with the light microscope except in very rare cases. An Ascomycete affinity is often suggested if the hyphae exceed 5 m in diameter and Woronin bodies should be searched for at young septa. For fungi that have simple septa and in which Woronin bodies cannot be seen, the growth on media containing benomyl can be used to demonstrate Basidiomycete affinities (Danielson, 1982).

2. Hyphal arrangement of the mantle. The arrangement of the hyphae of the mantle is one of the most stable and useful characteristics in identifying different types of mycorrhizae. The plan view (the view perpendicular to the surface) of the mantle is easily seen using whole mounts and 400-500X magnification with a high quality brightfield objective lens. The organization of the mantle hyphae can be categorized according to the textura types as illustrated by Korf (1973) and Eckblad (1968). Other terms have been used to describe hyphal or tissue arrangements but the textura type is the preferred system. Synenchyma, a term used by Chilvers (1968), and pseudoparenchyma refers to compact tissue with no interhyphal spaces in which the hyphal basis is difficult or impossible to discern. In the textura system, this would include textura prismatica, textura globulosa and textura angularis. Prosenchyma (Chilvers, 1968) and plectenchyma describe moderately compact tissue in which the hyphal elements are clearly distinguishable. This type is divided into four textura types including textura intricata with distinct interhyphal spaces and textura epidermoidea which lacks interhyphal spaces.

3. Presence of cystidia. Cystidia are sterile terminal cells whose morphology distinguishes them from nondifferentiated terminal hyphal cells. Ectomycorrhizae must be very carefully examined at 12-25X with a dissecting microscope to detect cystidia

as they are often less than 100 μm long. The determinant growth, which results in a fringe of cells projecting to a uniform length, distinguishes cystidia from young hyphae. The shape, size, wall thickness and septation are determined using whole mounts at high magnification. Care must be exercised when quantifying cystidial types as the cystidia are inducible by unknown factors and thus may be absent on some ectomycorrhizae formed by cystidial fungal species.

4. Presence of mycelial strands or rhizomorphs. If present, mycelial strands are usually obvious, although they may be lost during the root cleaning process. The degree of cell organization is an important factor in recognizing mycelial strands produced by different species.

5. Amount of extramatrical mycelium. This varies from apparently none to so copious that the roots cannot be seen. The actual amounts may be underestimated on cleaned root systems, thus this determination is somewhat subjective.

6. Hyphal pigmentation. The only pigments recorded here are brown wall pigments present in quantities sufficient to colour hyphae when examined at 500X. Other pigments, e.g. yellow or green, cannot be seen except in mass, i.e. on intact ectomycorrhizae.

7. Presence of materials on hyphal walls (excluding ornaments). Materials excreted and deposited or formed de nova on the outside of hyphal walls can be in the form of crystals, resinous exudates or encrustations. They may be so abundant that the hyphae are completely covered or occur as occasional patches. The presence of such materials appears to be consistent within a species and is particularly useful in recognizing Rhizopogon-Suillus ectomycorrhizae.

9.10 LITERATURE CITED

Chilvers, G.A. 1968. Some distinctive types of eucalypt mycorrhiza. Aust. J. Bot. 16: 49-70.

Danielson, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines. Can. J. Bot. 60: 7-18.

Eckblad, F.-E. 1968. The genera of the operculate discomycetes. A re-evaluation of the taxonomy, phylogeny and nomenclature. Norw. J. Bot. 15: 1-191.

Garrett, S.D. 1970. Pathogenic root-infecting fungi. Cambridge Univ. Pr., Cambridge.

Grand, L.F. and A.E. Harvey. 1982. Quantitative measurements of ectomycorrhizae on plant roots. In: Methods and Principles of Mycorrhizal Research, N.C. Schenck (ed.). Amer. Phytopath. Soc., St. Paul, pp. 157-164.

Harvey, A.E., M.J. Larsen and M.F. Jurgensen. 1976. Distribution of ecomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. 22: 393-398.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book Co., N.Y.

Korf, R.P. 1973. Discomycetes and tuberales. In: The Fungi, an Advanced Treatise, Vol. IVA, G.C. Ainsworth et. al. (eds.), Academic Pr., N.Y., pp. 249-319.

Marx, D.H. 1969. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59: 159-163.

Sutton, R.F. 1980. Root system morphogenesis. N.Z. J. For. Sci. 10: 264-292.

Wilcox, H.E. 1968. Morphological studies of the roots of red pine, Pinus resinosa II. Fungal colonization of roots and the development of mycorrhizae. Am. J. Bot. 55: 688-700.

Wilcox, H.E. 1982. Morphology and development of ecto- and ectendomycorrhizae. In: Methods and Principles of Mycorrhizal Research, N.C. Schenck (ed.). Amer. Phytopath. Soc., St. Paul. pp. 103-113.

Appendix Table 2. Effect of time of exposure to H_2O_2 and temperature of rinse water on recovery of the E-strain from jack pine mycorrhizae formed in 3 year old peat used to amend the subalpine spoil and oil sand tailings.

	Time of exposure to 30% H_2O_2 (Sec)			
	15	5	15	5
	Ice Water		Room Temp.	
Taxa	Percent Recovery			
E-strain[1]	88[a]	66[ab]	64[ab]	52[b]
Nonmycorrhizal	4[a]	14[ab]	18[ab]	36[b]

[1] Data within each row analysed by 2-way ANOVA. Numbers superscripted with the same letter within each row not significantly different at p = .05 as determined by Scheffe multiple contrasts where F-test significant.

Appendix Table 3. Recovery of symbionts from mycorrhizae with and without surface sterilization and with rinsing in water at room temperature and in ice water.

	Not surface sterilized[1]		H_2O_2-RT		H_2O_2-Ice	
	Pine	Spruce	Pine	Spruce	Pine	Spruce
	Percent Recovery					
Thelephora	38	-	1	-	9	-
Unknown symbiont	2	-	1	-	0	-
Nonmycorrhizal	57	61	19	10	15	12
No Growth	3	0	74	0	76	4
E-strain - pure	-	11	-	39	-	56
E-strain - overgrown	-	28	-	51	-	28
E-strain total	-	39	-	90	-	84

[1] Jack pine mycorrhizae from sewage treated spoil, plated on benomyl-MMN. Spruce from subalpine control, plated on MMN+. Both washed for 1 h prior to treatment.

Appendix Table 4. Efficiency of water and sucrose in the extraction of large chlamydospores from amended coal mine spoil.[1]

Sieve size and extractant	Percent of total chlamydospores extracted	
	E-strain	VA-type
53 µm water	4.3	54.6
125 µm water	1.0	11.5
53 µm sucrose	83.5	30.4
125 µm sucrose	11.2	3.5

[1] In order to determine if E-strain chlamydospores could be extracted from the peat by the method used, chlamydospores collected from a synthesis jar were added to a sample of the Canmore peat. Using the standard settling time of 15 min, only 14% of the chlamydospores were extracted (Table 5). Extending the settling time to 75 min permitted extraction of an additional 18% of the total chlamydospores added. Resuspension and centrifugation of the sample resulted in the recovery of a similar number of chlamydospores. Overall, 64% of the added chalmydospores were recovered and 96% of these were on the 53 m sieve.

Appendix Table 5. Efficiency of recovery of 640 E-strain chlamydospores added to a single 10 g sample of the Canmore peat.

Sieve size	Extractant	Time elapsed after centrifugation (min)			Resuspended and Centrifuged		
		0	15	75	150	15	75
		Number of chlamydospores extracted					
53 μm	water	14	NT[1]	NT	NT	NT	NT
53 μm	sucrose	14	90	116	15	114	21
125 μm	sucrose	0	8	7	NT	3	6

[1] NT - Not Tested.