Report No. RRTAC 88-5

ECTOMYCORRHIZAE OF JACK PINE AND GREEN ALDER: ASSESSMENT OF THE NEED FOR INOCULATION, DEVELOPMENT OF INOCULATION TECHNIQUES AND OUTPLANTING TRIALS ON OIL SAND TAILINGS

by

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Prepared for
The Oil Sands Reclamation Research Program
ALBERTA LAND CONSERVATION AND RECLAMATION COUNCIL
(Reclamation Research Technical Advisory Committee)

1988

STATEMENT OF OBJECTIVE

The recommendations and conclusions in this report are those of the authors and not those of the Alberta Government or its representatives.

This report is intended to provide government and industry staff with up-to-date technical information to assist in the development of guidelines and operating procedures. The report is also available to the public so that interested individuals similarly have access to the best available information on land reclamation topics.

ALBERTA'S RECLAMATION RESEARCH PROGRAM

The regulation of surface disturbances in Alberta is the responsibility of the Land Conservation and Reclamation Council. The Council executive consists of a Chairman from the Department of Forestry, Lands and Wildlife. Among other functions, the Council oversees programs for reclamation of abandoned disturbances and reclamation research. The Reclamation Research Program was established to provide answers to the many practical questions which arise in reclamation. Funds for implementing both the operational and research programs are drawn from Alberta's Heritage Savings Trust Fund.

To assist in technical matters related to the development and administration of the Research Program, the Council appointed the Reclamation Research Advisory Committee (RRTAC). The Committee first met in March 1978 and consists of eight members representing the Alberta Departments of Agriculture, Energy, Forestry, Lands and Wildlife, Environment and the Alberta Research Council. The Committee meets regularly to update research priorities, review solicited and unsolicited research proposals, arrange workshops and otherwise act as a referral and coordinating body for Reclamation Research.

Additional information on the Reclamation Research Program may be obtained by contacting:

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Additional copies may be obtained from:

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RECLAMATION RESEARCH REPORTS

** 1. RRTAC 80-3: The Role of Organic Compounds in Salinization of Plains Coal Mining Sites. N.S.C. Cameron et al. 46 pp.

DESCRIPTION: This is a literature review of the chemistry of sodic mine spoil and the changes expected to occur in groundwater.

** 2. RRTAC 80-4: Proceedings: Workshop on Reconstruction of Forest Soils in Reclamation. P.F. Ziemkiewicz, S.K. Takyi, and H.F. Regier. 160 pp.

DESCRIPTION: Experts in the field of forestry and forest soils report on research relevant to forest soil reconstruction and discuss the most effective means of restoring forestry capability of mined lands.

N/A 3. RRTAC 80-5: Manual of Plant Species Suitability for Reclamation in Alberta. L.E. Watson, R.W. Parker, and P.F. Polster. 2 vols, 541 pp.

DESCRIPTION: Forty-three grass, fourteen forb, and thirty-four shrub and tree species are assessed in terms of their fitness for use in Reclamation. Range maps, growth habit, propagation, tolerance, and availability information are provided.

N/A 4. RRTAC 81-2: 1980 Survey of Reclamation Activities in Alberta. D.G. Walker and R.L. Rothwell. 76 pp.

DESCRIPTION: This survey is an update of a report prepared in 1976 on reclamation activities in Alberta, and includes research and operational reclamation, locations, personnel, etc.

N/A 5. RRTAC 81-3: Proceedings: Workshop on Coal Ash and Reclamation. P.F. Ziemkiewicz, R. Stien, R. Leitch, and G. Lutwick. 253 pp.

DESCRIPTION: Presents nine technical papers on the chemical, physical and engineering properties of Alberta fly and bottom ashes, revegetation of ash disposal sites and use of ash as a soil amendment. Workshop discussions and summaries are also included.

N/A 6. RRTAC 82-1: Land Surface Reclamation: An International Bibliography. H.P. Sims and C.B. Powter. 2 vols, 292 pp.

DESCRIPTION: Literature to 1980 pertinent to reclamation in Alberta is listed in Vol. 1 and is also on the University of Alberta computing system. Vol. 2 comprises the keyword index and computer access manual.

N/A 7. RRTAC 82-2: A Bibliography of Baseline Studies in Alberta: Soils, Geology, Hydrology and Groundwater. C.B. Powter and H.P. Sims. 97 pp.

DESCRIPTION: This bibliography provides baseline information for persons involved in reclamation research or in the preparation of environmental impact assessments. Materials, up to date as of December 1981, are available from the Alberta Environment Library.

N/A 8. RRTAC 83-1: Soil Reconstruction Design for Reclamation of Oil Sand Tailings. Monenco Consultants Ltd. 185 pp.

DESCRIPTION: Volumes of peat and clay required to amend oil sand tailings were estimated based on existing literature. Separate soil prescriptions were made for spruce, jack pine, and herbaceous cover types. The estimates form the basis of field trials.

N/A 9. RRTAC 83-3: Evaluation of Pipeline Reclamation Practices on Agricultural Lands in Alberta. Hardy Associates (1978) Ltd. 205 pp.

DESCRIPTION: Available information on pipeline reclamation practices was reviewed. A field survey was then conducted to determine the effects of pipe size, age, soil type, construction method, etc. on resulting crop production.

N/A 10. RRTAC 83-4: Proceedings: Effects of Coal Mining on Eastern Slopes Hydrology. P.F. Ziemkiewicz. 123 pp.

DESCRIPTION: Technical papers are presented dealing with the impacts of mining on mountain watersheds, their flow characteristics and resulting water quality. Mitigative measures and priorities were also discussed.

N/A 11. RRTAC 83-5: Woody Plant Establishment and Management for Oil Sands Mine Reclamation. Techman Engineering Ltd. 124 pp.

DESCRIPTION: This is a review and analysis of information on planting stock quality, rearing site preparation, planting and procedures necessary to ensure survival of trees and shrubs in oil sand reclamation.

*** 12. RRTAC 84-1: Land Surface Reclamation: A Review of International Literature. H.P. Sims, C.B. Powter, and J.A. Campbell. 2 vols, 1549 pp.

DESCRIPTION: Nearly all topics of interest to reclamation including mining methods, soil amendments, revegetation, propagation and toxic materials are reviewed in light of the international literature.

** 13. RRTAC 84-2: Propagation Study: Use of Trees and Shrubs for Oil Sand Reclamation. Techman Engineering Ltd. 58 pp.

DESCRIPTION: This report evaluates and summarizes all available published and unpublished information on large-scale propagation methods for shrubs and trees to be used in oil sand reclamation.

* 14. RRTAC 84-3: Reclamation Research Annual Report - 1983. P.F. Ziemkiewicz. 42 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 15. RRTAC 84-4: Soil Microbiology in Land Reclamation. D. Parkinson, R.M. Danielson, C. Griffiths, S. Visser, and J.C. Zak. 2 vols, 676 pp.

DESCRIPTION: This is a collection of five reports dealing with re-establishment of fungal decomposers and mycorrhizal symboints in various amended spoil types.

** 16. RRTAC 85-1: Proceedings: Revegetation Methods for Alberta's Mountains and Foothills. P.F. Ziemkiewicz. 416 pp.

DESCRIPTION: Results of long-term experiments and field experience on species selection, fertilization, reforestation, topsoiling, shrub propagation and establishment are presented.

* 17. RRTAC 85-2: Reclamation Research Annual Report - 1984. P.F. Ziemkiewicz. 29 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 18. RRTAC 86-1: A Critical Analysis of Settling Pond Design and Alternative Technologies. A. Somani. 372 pp.

DESCRIPTION: The report examines the critical issue of settling pond design and sizing and alternative technologies.

** 19. RRTAC 86-2: Characterization and Variability of Soil Reconstructed after Surface Mining in Central Alberta. T.M. Macyk. 146 pp.

DESCRIPTION: Reconstructed soils representing different materials handling and replacement techniques were characterized and variability in chemical and physical properties was assessed. The data obtained indicate that reconstructed soil properties are determined largely by parent material characteristics and further tempered by materials handling procedures. Mining tends to create a relatively homogeneous soil landscape in contrast to the mixture of diverse soils found before mining.

* 20. RRTAC 86-3: Generalized Procedures for Assessing Post-Mining Groundwater Supply Potential in the Plains of Alberta - Plains Hydrology and Reclamation Project. M.R. Trudell and S.R. Moran. 30 pp.

DESCRIPTION: In the Plains region of Alberta, the surface mining of coal generally occurs in rural, agricultural areas in which domestic water supply requirements are met almost entirely by groundwater. Consequently, an important aspect of the capability of reclaimed lands to satisfy the needs of a residential component is the post-mining availability of groundwater. This report proposes a sequence of steps or procedures to identify and characterize potential post-mining aquifers.

** 21. RRTAC 86-4: Geology of the Battle River Site: Plains Hydrology and Reclamation Project. A Maslowski-Schutze, R. Li, M. Fenton and S.R. Moran. 86 pp.

DESCRIPTION: This report summarzies the geological setting of the Battle River study site. It is designed to provide a general understanding of geological conditions adequate to establish a framework for hydrogeological and general reclamation studies. The report is not intended to be a detailed synthesis such as would be required for mine planning purposes.

** 22. RRTAC 86-5: Chemical and Mineralogical Properties of Overburden: Plains Hydrology and Reclamation Program. A. Maslowski-Schutze. 71 pp.

DESCRIPTION: This report describes the physical and mineralogical properties of overburden materials in an effort to identify individual beds within the bedrock overburden that might be significantly different in terms of reclamation potential.

* 23. RRTAC 86-6: Post-Mining Groundwater Supply at the Battle River Site: Plains Hydrology and Reclamation Project. M.R. Trudell, G.J. Sterenberg and S.R. Moran. 49 pp.

DESCRIPTION: The report deals with the availability of water supply in or beneath cast overburden at the Battle River Mining area in east-central Alberta to support post-mining land use. Both groundwater quantity and quality are evaluated.

* 24. RRTAC 86-7: Post-Mining Groundwater Supply at the Highvale Site: Plains Hydrology and Reclamation Project. M.R. Trudell. 25 pp.

DESCRIPTION: This report evaluates the availability of water supply in or beneath cast overburden to support post-mining land use, including both quantity and quality considerations. The study area is the Highvale mining area in west-central Alberta.

* 25. RRTAC 86-8: Reclamation Research Annual Report - 1985. P.F. Ziemkiewicz. 54 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

** 26. RRTAC 86-9: Wildlife Habitat Requirements and Reclamation Techniques for the Mountains and Foothills of Alberta. J.E. Green, R.E. Salter and D.G. Walker. 285 pp.

DESCRIPTION: This report presents a review of relevant North American literature on wildlife habitats in mountain and foothills biomes, reclamation techniques, potential problems in wildlife habitat reclamation, and potential habitat assessment methodologies. Four biomes (Alpine, Subalpine, Montane, and Boreal Uplands) and 10 key wildlife species (snowshoe hare, beaver, muskrat, elk, moose, caribou, mountain goat, bighorn sheep, spruce grouse, and white-tailed ptarmigan) are discussed.

** 27. RRTAC 87-1: Disposal of Drilling Wastes. L.A. Leskiw, E. Reinl-Dwyer, T.L. Dabrowski, B.J. Rutherford and H. Hamilton. 210 pp.

DESCRIPTION: Current drilling waste disposal practices are reviewed and criteria in Alberta guidelines are assessed. The report also identifies research needs and indicates mitigation measures. A manual included provides a decision-making flowchart to assist in selecting methods of environmentally safe waste disposal.

** 28. RRTAC 87-2: Minesoil and Landscape Reclamation of the Coal Mines in Alberta's Mountains and Foothills. A.W. Fedkenheuer, L.J. Knapik, and D.G. Walker. 174 pp.

DESCRIPTION: This report reviews current reclamation practices with regard to site and soil reconstruction and re-establishment of biological productivity. It also identifies research needs in the Mountain-Foothills area.

** 29. RRTAC 87-3: Gel and Saline Drilling Wastes in Alberta: Workshop Proceedings. D.A. Lloyd (compiler). 218 pp.

DESCRIPTION: Technical papers were presented which describe: the mud systems used and their purpose; industrial constraints; government regulations, procedures and concerns; environmental considerations in waste disposal; and toxic constituents of drilling wastes. Answers to a questionnaire distributed to participants are included in an appendix.

* 30. RRTAC 87-4: Reclamation Research Annual Report - 1986. 50 pp.

DESCRIPTION: This report details the Reclamation Research Program indicating priorities, descriptions of each research project, researchers, results and expenditures.

* 31. RRTAC 87-5: Review of the Scientific Basis of Water Quality Criteria for the East Slope Foothills of Alberta. Beak Associates Consulting Ltd. 46 pp.

DESCRIPTION: The report reviews existing Alberta guidelines to assess the quality of water drained from coal mine sites in the East Slope Foothills of Alberta. World literature was reviewed within the context of the east slopes environment and current mining operations. The ability of coal mine operators to meet the various guidelines is discussed.

** 32. RRTAC 87-6: Assessing Design Flows and Sediment Discharge on the Eastern Slopes. Hydrocon Engineering (Continental) Ltd. and Monenco Consultants Ltd. 97 pp.

DESCRIPTION: The report provides an evaluation of current methodologies used to determine sediment yields due to rainfall events in well-defined areas. Models are available in Alberta to evaluate water and sediment discharge in a post-mining situation. SEDIMOT II (Sedimentology Disturbed Modelling Techniques) is a single storm model that was developed specifically for the design of sediment control structures in watersheds disturbed by surface mining and is well suited to Alberta conditions.

* 33. RRTAC 87-7: The Use of Bottom Ash as an Amendment to Sodic Spoil. S. Fullerton. 83 pp.

DESCRIPTION: The report details the use of bottom ash as an amendment to sodic coal mine spoil. Several rates and methods of application of bottom ash to sodic spoil were tested to determine which was the best at reducing the effects of excess sodium and promoting crop growth. Field trials

were set up near the Vesta mine in East Central Alberta using ash readily available from nearby coal-fired thermal generating station. The research indicated that bottom ash incorporated to a depth of 30 cm using a subsoiler provided the best results.

* 34. RRTAC 87-8: Waste Dump Design for Erosion Control. R.G. Chopiuk and S.E. Thornton. 45 pp.

DESCRIPTION: This report describes a study to evaluate the influence of erosion from reclaimed waste dumps on downslope environments such as streams and rivers. Sites were selected from coal mines in Alberta's mountains and foothills, and included resloped dumps of different configurations and ages, and having different vegetation covers. The study concluded that the average annual amount of surface erosion is minimal. As expected, erosion was greatest on slopes which were newly regraded. Slopes with dense grass cover showed no signs of erosion. Generally, the amount of erosion decreased with time, as a result of initial loss of fine particles, the formation of a weathered surface, and increased vegetative cover.

** 35. RRTAC 87-9: Hydrogeology and Groundwater Chemistry of the Battle River Mining Area. M.R. Trudell, R.L. Faught and S.R. Moran. 97 pp.

DESCRIPTION: This report describes the premining geologic conditions in the Battle River coal mining area including the geology as well as the groundwater flow patterns, and the groundwater quality of a sequence of several water-bearing formations extending from the surface to a depth of about 100 metres.

** 36. RRTAC 87-10: Soil Survey of the Plains Hydrology and Reclamation Project - Battle River Project Area. T.M. Macyk and A.H. MacLean. 62 pp. plus maps.

DESCRIPTION: The report evaluates the capability of post-mining landscapes and assesses the changes in capability as a result of mining, in the Battle River mining area. Detailed soils information is provided in the report for lands

adjacent to areas already mined as well as for lands that are destined to be mined. Characterization of the reconstructed soils in the reclaimed areas is also provided. Data were collected from 1979 to 1985. A series of maps supplement the report.

** 37. RRTAC 87-11: Geology of the Highvale Study Site: Plains Hydrology and Reclamation Project. A. Maslowski-Schutze. 78 pp.

DESCRIPTION: The report is one of a series that describes the geology, soils and groundwater conditions at the Highvale Coal Mine study site. The purpose of the study was to establish a summary of site geology to a level of detail necessary to provide a framework for studies of hydrogeology and reclamation.

** 38. RRTAC 87-12: Premining Groundwater Conditions at the Highvale Site. M.R. Trudell and R. Faught. 83 pp.

DESCRIPTION: This report presents a detailed discussion of the premining flow patterns, hydraulic properties, and isotopic and hydrochemical characteristics of five layers within the Paskapoo Geological Formation, the underlying sandstone beds of the Upper Horseshoe Canyon Formation, and the surficial glacial drift.

* 39. RRTAC 87-13: An Agricultural Capability Rating System for Reconstructed Soils. T.M. Macyk. 27 pp.

DESCRIPTION: This report provides the rationale and a system for assessing the agricultural capability of reconstructed soils. Data on the properties of the soils used in this report are provided in RRTAC 86-2.

** 40. RRTAC 88-1: Eccles, T.R., R.E. Salter and J.E. Green. A Proposed Evaluation System for Wildlife Habitat Reclamation in the Mountains and Foothills Biomes of Alberta: Proposed Methodology and Assessment Handbook. 101 pp. plus appendix.

DESCRIPTION: The report focuses on the development of guidelines and procedures for the assessment of reclaimed wildlife habitat in the Mountains and Foothills regions of Alberta. The technical section provides background documentation including a discussion of reclamation planning, a listing of reclamation habitats and associated key wildlife species, conditions required for development, recommended revegetation species, suitable reclamation techniques, a description of the recommended assessment techniques and a glossary of basic terminology. The assessment handbook section contains basic information necessary for evaluating wildlife habitat reclamation, including assessment scoresheets for 15 different reclamation habitats, standard methodologies for measuring habitat variables used as assessment criteria, and minimum requirements for certification. This handbook is intended as a field manual that could potentially be used by site operators and reclamation officers.

** 41. RRTAC 88-2: Plains Hydrology and Reclamation Project: Spoil Groundwater Chemistry and its Impacts on Surface Water. M.R. Trudell (Compiler). Alberta Land Conservation and Reclamation Council Report #RRTAC 88-2. 135 pp.

DESCRIPTION: Two reports comprise this volume. The first "Chemistry of Groundwater in Mine Spoil, Central Alberta," describes the chemical make-up of spoil groundwater at four mines in the Plains of Alberta. It explains the nature and magnitude of changes in groundwater chemistry following mining and reclamation.
The second report, "Impacts of Surface Mining on Chemical Quality of Streams in the Battle River Mining Area," describes the chemical quality of water in streams in the Battle River mining area, and the potential impact of groundwater discharge from surface mines on these streams.

** 42. RRTAC 88-3: Revegetation of Oil Sands Tailings: Growth Improvement of Silver-berry and Buffalo-berry by Inoculation with Mycorrhizal Fungi and N_2-Fixing Bacteria. S. Visser and R.M. Danielson. 98 pp.

DESCRIPTION: The report provides results of a study: (1) To determine the mycorrhizal affinities of various actinorrhizal shrubs in the Fort McMurray, Alberta region; (2) To establish a basis for justifying symbiont inoculation of buffalo-berry and silver-berry; (3) To develop a growing regime for the greenhouse production of mycorrhizal, nodulated silver-berry and buffalo-berry; and, (4) To conduct a field trial on reconstructed soil on the Syncrude oil sands site to critically evaluate the growth performance of inoculated silver-berry and buffalo-berry as compared with their uninoculated counterparts.

** 43. RRTAC 88-4: Plains Hydrology and Reclamation Project: Investigation of the Settlement Behaviour of Mine Backfill. D.R. Pauls (compiler). 135 pp.

DESCRIPTION: This three part volume covers the laboratory assessment of the potential for subsidence in reclaimed landscapes. The first report in this volume, "Simulation of Mine Spoil Subsidence by Consolidation Tests," covers laboratory simulations of the subsidence process particularly as it is influenced by resaturation of mine spoil. The second report, "Water Sensitivity of Smectitic Overburden: Plains Region of Alberta" describes a series of laboratory tests that have been used to determine the behaviour of overburden materials when brought into contact with water. The report entitled "Classification System for Transitional Materials: Plains Region of Alberta" describes a lithological classification system developed to address the characteristics of the smectite rich, clayey transition materials that make up the overburden in the Plains of Alberta.

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* A $5.00 fee is charged for handling and postage.
** A $10.00 fee is charged for handling and postage.
*** A $20.00 fee is charged for handling and postage.
N/A Not available for purchase but available for review at the Alberta Environment Library, 14th Floor, 9820-106 Street, Edmonton, Alberta T5K 2J6.

EXECUTIVE SUMMARY

The overall objectives of these studies were to characterize the mycorrhizal status of jack pine and green alder which are prime candidates as reclamation species for oil sand tailings and to determine the potential benefits of mycorrhizae on plant performance. This entailed determining the symbiont status of container-grown nursery stock and the quantity and quality of inoculum in reconstructed soils, developing inoculation techniques and finally, performance testing in an actual reclamation setting. Much emphasis has been placed on gaining information on the fungi involved in the symbioses as little is known about the ecology of mycorrhizal fungi and this information is essential for successfully applying the technology.

Seedlings from seven nurseries in Alberta and British Columbia were examined for mycorrhizae. The conversion of short root to mycorrhizae varied from 0 to 100% depending upon the particular nursery, fertilizer regimes, and residence time in the nursery. It is without doubt that many sites are being planted with nonmycorrhizal jack pines, lodgepole pine, and white spruce. The most common fungi in the infested nurseries were E-strain, Thelephora terrestris and Mycelium radicis atrovirens. Amphinema byssoides occurred exclusively on spruce and succeeded E-strain and Thelephora in the nursery. Coltricia perennis, a polypore, was found on nursery stock for the first time. In one nursery, E-strain appeared to prevent the development of a seedling disease caused by a species of Cylindrocarpon. Alder seedlings from three nurseries were all nonmycorrhizal but most seedlings were nodulated.

The second major source of natural inoculum is the reconstructed soils and the inoculum source within the soils is the muskeg peat. Inoculum in undisturbed and stockpiled peat was assayed by using a greenhouse baiting technique and jack pine seedlings. The most common fungi were E-strain, I-type (Tuber sp.), and a hyaline Basidiomycete. Stockpiling the peat reduced the infectivity of the peat and plants

were still mycorrhizal deficient after 4 months in the greenhouse. Additional experiments showed that the greenhouse baiting technique accurately reflected the species that develop in the field but overestimates the rates of mycorrhization. The reconstructed soil contained adequate quantities of Frankia inoculum to nodulate alder but mycorrhizal inoculum compatible with alder was present only in very small quantities. From the nursery and indigenous inoculum studies it could be concluded that both jack pine and green alder planted on oil sand tailings amended with muskeg peat would potentially be deficient in mycorrhizae and this deficiency could adversely affect plant performance.

In order to determine if mycorrhizae would benefit plant performance in the field, two outplanting trials were conducted; the first with jack pine and the second with jack pine and green alder. The second trial was limited in scope due to heavy winter mortality soon after planting.

In the first trial, container-grown jack pine seedlings were inoculated with 12 fungi and mycorrhizae were formed by 9 of the 12 species. The most aggressive fungi (those producing the highest levels of short root infections) were Thelephora terrestris, Laccaria proxima, Hebeloma sp. and E-strain, all of which are known as "weedy" nursery species. A lower degree of infection was achieved with Cenococcum geophilum, Pisolithus tinctorius, Astraeus hygrometricus, Lactarius paradoxus, and Sphaerosporella brunnea. Amphinema byssoides, Hydnum imbricatum, and Tricholoma flavovirens failed to form any mycorrhizae. All the above inoculation treatments plus two uninoculated controls, one grown with the inoculated seedlings and the other from the Syncrude greenhouse, were outplanted in the spring on the Syncrude dyke. The reconstructed soil consisted of extracted oil sands, muskeg peat, and clayey overburden.

After one season in the field, T. terrestris, L. proxima, Hebeloma sp., and E-strain had all readily infected the new roots that extended into the reconstructed soil. The other fungi were poor colonizers of jack pine roots in the field. Competition from indigenous fungi was not a factor in the degree of success as only 4% of the short roots were infected by indigenous species. Growth of jack pine

was not significantly affected by the presence of mycorrhizae during the first growing season.

Laccaria proxima completely disappeared after 1 year and between the second and third year, Hebeloma sp. and Thelephora terrestris almost completely disappeared. Of the introduced fungi, only E-strain was present in substantial quantities after 3 years and it appeared also to be disappearing. It appeared that the major replacement process was noninteractive, i.e., the resident fungi died, and the roots were subsequently reinfected by another fungus. The colonization by indigenous fungi increased each year, rising to 33% in the second year and to 72% by the end of the third year. The most common indigenous fungi were E-strain, I-type (Tuber sp.), Mycelium radicis atrovirens, a Rhizopogon-like fungus, and a hyaline Basidiomycete. The latter four species increased with time, whereas E-strain appeared to be decreasing in abundance after 3 years. Shoot weights of seedlings inoculated with E-strain and Thelephora terrestris were 2- to 3-fold larger than the controls after 2 years growth but the differences in size decreased in the third year.

In the second outplanting study, inoculation could not be assessed due to heavy mortality but it was observed that three E-strain fungi and an I-type isolate all readily colonized new roots in the reconstructed soils. Alder lacked both nodules and mycorrhizae when planted but all plants became nodulated in the first year. However, mycorrhizal development was still often poor even after 2 years in the field. The major fungus associated with alder was Alpova diplophoeus which was also the dominant fungus on naturally regenerating plants.

In the jack pine outplanting study fertilizer was used conservatively in the greenhouse phase so as to ensure maximum mycorrhizal development. Consequently, the seedlings were small and thus some losses were encountered in the field due to flooding of low areas of the experimental plot. Subsequent fertilizer trials have demonstrated that seedling size need not be sacrificed for successful mycorrhizal development when certain, aggressive fungi are used. The most fertilizer tolerant fungus tested was an E-strain isolate. All short roots were ectomycorrhizal when fertilizer containing 60 mg N L^{-1} was

applied three times weekly. This rate approaches that used in commercial operations. Even when the rate was doubled, E-strain still infected a substantial portion of the short roots. Hebeloma sp. and Lactarius paradoxus were more sensitive to fertilizer levels infecting 20-30% of the short roots at the 60 mg N L^{-1} level. Astraeus hygrometricus and Amphinema byssoides did not form ectomycorrhizae at this level, however this may have been due to an overall low inoculum potential of these two species. It is proposed that host resistance is elevated by high fertilizer levels and that it may be possible to overcome this resistance by increasing inoculum potential and thus, to expand the taxonomic spectrum of fungi that can be successfully introduced onto container-grown seedlings. A simple change from using an organic growing medium to an inorganic medium did not result in any ectomycorrhizal formation by two fungi which continue to resist artificial inoculation attempts, Amphinema byssoides and Suillus tomentosus. As these fungi appear to persist through several succession stages (multi-stage fungi) they may be more desirable than species currently being tested such as E-strain, Laccaria proxima and Thelephora terrestris which are early-stage (pioneer) fungi.

One group of ectomycorrhizal fungi not included in any inoculation program are those species occurring in the late successional stages of a forest stand (late-stage fungi). At this point mycorrhizal infections cannot be initiated from either spores or mycelium. However, at least some of these fungi can be very aggressive when spreading from established ectomycorrhizae to nonmycorrhizal seedlings. Nothing is known of the relative benefits on plant performance of these late-stage fungi versus the early-stage fungi whose use is currently in vogue.

It appears that multi- and late-stage fungi are more sensitive to certain biological soil factors than early-stage fungi. Preliminary attempts to reduce fungal antagonism by treating planting mixtures with the fungicide benomyl was unsuccessful. However, fly maggots which were observed consuming inoculum, were eliminated by drenching the planting mixtures with Diazinon. By using intact cultures of fungi it was possible to increase inoculum potential and to observe consumption and colonization of the inoculum. Evidence from a series of trials

indicate that if the antagonistic mold, Trichoderma harzianum and fungal feeding fly larvae can be controlled, it may be possible to inoculate many multi- and late-stage fungi and test efficiencies in the field.

Attempts to inoculate green alder with pure cultures of Frankia and Alpova diplophloeus failed, however, a soil inoculum was used successively to promote both nodulation and mycorrhization. It was necessary to reduce fertilizer levels to concentrations well below those used commercially to obtain mycorrhizal infections. Field-collected nodules also served as a good inoculum source for Frankia. Although antagonistic fungi and maggots could be controlled with pesticides, bacteria rapidly colonized inoculum of Alpova and killed the fungus. Further work is necessary to improve inoculation procedures so a wider range of fungi can be introduced onto ectomycorrhizal hosts and be subsequently tested for efficacy on reclamation sites.

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ACKNOWLEDGMENTS

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1. GENERAL INTRODUCTION: ECTOMYCORRHIZAL TECHNOLOGY IN ALBERTA

Mycorrhizae have been studied for 100 years and been shown to be the normal status for the roots of nearly all vascular plants. Early phases of study were concerned with descriptive morphology and determining that these particular fungus-plant associations were mutualistic rather than pathogenic. In the 1920's and 1930's, studies concentrated on the nature of the symbiosis, the dependence of one partner on the other, and the exchange of materials between partners. It became established during this period that for the host plant, "any mycorrhizal fungus was better than none."

It was not until the 1950's that this concept was refined and narrowed into one that suggested practical, technological applications of the mycorrhizal symbiosis. As most soils already contained mycorrhizal inoculum, little application of the science could be seen except where trees were introduced into treeless areas (e.g., prairies) or where conifers were planted in tropical or southern hemisphere locations where conifers and their mycorrhizal fungi had never existed.

The refinement that changed the approach of research to that which continues to this date was the recognition that "some mycorrhizal fungi were better than others." Thus, species and strain selection became essential and promised practical results in terms of enhancing plant performance. Detailed studies on stressed environments such as coal mine spoils showed that few fungi were adapted to survive in these habitats and that it was these areas, where plants were potentially exposed to chemical, nutrient, moisture and temperature stresses, that mycorrhizae were most essential.

The potential application of the science became a small-scale technological reality when techniques were developed for propagation of mycorrhizal inoculum and procedures for inoculating large numbers of plants matured. In the 1980's it is possible to propagate and inoculate certain mutualistic combinations with rewarding results. However, only a few of the potential applications of mycorrhizae have been examined and tested. One of the most promising areas of application is on extracted oil sand tailings which present a variety of problems for

the establishment of vegetation. A wealth of raw material for inoculations in the form of fungal species exists in Alberta; nearly all awaiting critical evaluations and/or the development of techniques which will allow such evaluations.

No less promising are the possibilities and difficulties involved with the use of N_2-fixing shrub species on reclamation sites. These may enrich the young soils with surplus nitrogen, thereby speeding reclamation. However, these plants may also depend heavily upon mycorrhizal associations to perform reliably. Even the most basic information is lacking on these shrubs with regard to mycorrhizae--a situation which must be corrected before practial applications can be considered.

The research program reported here attempted to fill some of the numerous voids in information concerning ectomycorrhizae in northern forests and in particular, in regard to reclamation of oil sand tailings with ectomycorrhizal plants. A majority of the work was done with jack pine, the most common woody plant naturally occurring on sandy soils in Northern Alberta and one adapted to regeneration following severe natural disturbances (fire). Lesser attention was paid to the ectomycorrhizal N_2-fixing shrub, green alder. Other N_2-fixing shrubs that have potential for reclamation planting are reported upon in the companion report by S. Visser.

The research was designed to answer a number of questions concerning ectomycorrhizae and their manipulation under Alberta conditions. It was not expected that this research would revolutionize reclamation practices but rather evaluate the desirability and feasibility of incorporating root symbioses into nursery and reclamation practices. The first question was whether seedlings that are being grown in operational nurseries become mycorrhizal during their nursery residence time and, if so, with what fungi. A second part to this question of primary inoculum sources, was whether reclamation planting sites, i.e., the reconstructed soils, contained mycorrhizal inoculum compatible with the species being planted. These questions were addressed by sampling nursery stock and examining stockpiled peat and reconstructed soils, and determining the mycorrhizal status of plants grown in the nurseries and in muskeg peat.

The second major question was how rearing conditions of container-grown seedlings might be modified to enhance mycorrhizal development by fungal species other than those commonly known to inhabit nurseries. Modifications attempted included reduced fertilizer regimes, altered planting mixtures, and the use of a selective fungicide to inhibit fungal antagonists and an insecticide to control fungal consumers.

The most important practical question was whether the use of inoculated mycorrhizal seedlings would outperform non-mycorrhizal or normal nursery seedlings under uncontrolled field conditions on a routine reclamation site. To answer this, two outplanting studies were conducted on reconstructed soils and the host response and role of both introduced and indigenous in mycorrhizal associations carefully monitored.

Finally, based on observations on these and other studies, the question of how to most efficiently and logically select fungi for artificial inoculation was addressed. Only a small fraction of the total fungi can ever be field tested and factors other than convenience must be used to select those that are tested. It is hoped that the results of the studies reported here will lead to a rational and productive approach to future studies on the applications of the mycorrhizal symbiosis.

2. MYCORRHIZAL INOCULUM: INDIGENOUS AND FROM NURSERIES

Are seedlings that are being planted on reclamation sites mycorrhizal when they leave the nursery, and is there compatible inoculum in reclaimed soils?

2.1 THE ECTOMYCORRHIZAL STATUS OF CONTAINER-GROWN JACK PINE, LODGEPOLE PINE, AND WHITE SPRUCE REARED IN COMMERCIAL AND PROVINCIAL NURSERIES

2.1.1 Abstract

The ectomycorrhizal status of container-grown jack pine, lodgepole pine, and white spruce grown in six Alberta and one B.C. nursery was determined and the fungal associates identified. Depending on the nursery, the quantity of short roots converted to ectomycorrhizae varied from 0 to 100% when the seedlings were ready for planting. The differences appeared to be due to the history of nursery (length of time growing conifers), fertilizer rates used, and the period of time that crops were retained in shadehouses. The fungi forming mycorrhizae varied among nurseries and between crops and species within particular nurseries. The two most common symbionts were Thelephora terrestris and E-strain (Complexipes sp.), both of which are well-known nursery fungi. Also present and common in some nurseries on young stock was Mycelium radicis atrovirens (MRA). Amphinema byssoides was found exclusively on white spruce and appeared to replace Thelephora and E-strain when seedlings were retained in the nurseries for periods exceeding one year. The polypore species, Coltricia perennis, was recorded from nurseries for the first time and was occasionally abundant. Other fungi were rarely encountered except in one nursery where the seedlings had apparently been exposed to forest inoculum for a prolonged period of time. In one provincial nursery, portions of a pine crop were chlorotic, infected with Cylindrocarpon, a root pathogen, and largely nonmycorrhizal. As healthy seedlings were consistently mycorrhizal, it is suggested that E-strain mycorrhizae had a protective role in pre- venting this nursery disease.

2.1.2 Introduction

Container-grown conifers are currently being used for revegetating oil sand tailings in northeastern Alberta. Some of the advantages container-grown seedlings have over bare-root stock are the short

rotations, the flexibility in the control of cultural practices, and the intact, undisturbed root systems of the container-grown seedlings. The container-grown system also offers an unexcelled opportunity to manipulate and manage the mycorrhizal status of the roots as the growing media is generally free of ectomycorrhizal fungi. This provides the opportunity to introduce beneficial ectomycorrhizal fungi if the proper time can be determined for the inoculation. In experimental conditions, the fungi can be introduced as the growing medium is being prepared, i.e., preplanting. However, the fertilizer regimes used in some operational greenhouses are so high that mycorrhiza formation may be prevented or severely reduced. With the preplant time eliminated, the most obvious inoculation "window" is the time just after seedlings have been moved to the shadehouse and fertilizer levels are reduced. One factor that will determine if this window is to be effective is the presence and competition offered from ectomycorrhizae fungi indigenous to the nursery. Indeed, if ectomycorrhizae formation by nursery fungi is heavy, inoculation may not be necessary. This will depend in turn on the species of fungi and their relative efficiency and persistence after outplanting.

As a first step in determining the role of nursery fungi in plant performance, this study undertook to determine the mycorrhizal status of conifers in nurseries that provide planting stock for reclamation purposes.

2.1.3 Materials and Methods

2.1.3.1 Smoky Lake and Syncrude Nurseries. Seedlings were sampled in the Alberta Forest Service Pine Ridge Nursery in Smoky Lake in May 1983 and in the Syncrude Canada Limited nursery located north of Fort McMurray in October 1983. The age of the stock and the species selected was dependent upon the material available at each nursery. Jack pine (Pinus banksiana Lamb.) was not available at either nursery.

Two crops of lodgepole pine (Pinus contorta Loud. var. latifolia Engelm.) and one crop of white spruce (Picea glauca (Moench)

Voss) were sampled at the Pine Ridge Nursery. Fertilizer rates varied with each crop but in general the seedlings were grown for 3 months in the greenhouse utilizing 125-60-159 mg L^{-1} of NPK in the irrigation water. The exact volume was varied by adjusting the volume of irrigation water but the volume was usually between 16 and 25 L per 1000 seedlings per week. Once the seedlings were in the shadehouse, fertilization rates were reduced to one-half to one-third of those used in the greenhouse. In the final weeks prior to hardening off, a formulation of 44-101-150 mg L^{-1} NPK was used. The first crop of lodgepole pine had been in the shadehouse from September 1982 to May 1983 and the second from June 1981 to May 1983. The white spruce had also been in the shadehouse from June 1982 to May 1983. Both species were grown in 47 cc Spencer-Lamaire book containers.

Twenty-five seedlings of each crop were randomly selected, placed in plastic bags and held at $2^{o}C$ until they could be examined. For mycorrhizal assessments, the growing media was washed off and each root system was examined with a dissecting microscope. Overall mycorrhizal development (conversion of short roots to ectomycorrhizae) was visually estimated and the presence of each distinctive mycorrhizal type recorded. The presence of poorly differentiated mycorrhizae were confirmed by observing whole mounts of short roots under high (500X) magnification. Identification of the fungi was aided by attempting to culture them from the ectomycorrhizae. Both surface sterilized and washed ectomycorrhizae were plated on benomyl-MMN and MMN^{+} agars (Danielson et al., 1984).

At the Syncrude nursery, three crops of white spruce were sampled in the same way as at the Pine Ridge Nursery. The crops had been moved to the shadehouse in August 1982, June 1983, and August 1983. All the seedlings had been reared in 160 cc Spencer-Lamaire book containers.

For the mycorrhizal assessments, the percent mycorrhizal development by each individual fungus was visually estimated and checked by observations of whole mounts and culturing techniques as outlined above. Shoots and roots from both nurseries were dried at $80^{o}C$ and weighed.

2.1.3.2 Other Alberta and B.C. Nurseries. Seedlings were obtained from five other commercial nurseries in August 1985. These included the Alberta Tree Nursery and Horticulture Research Centre in Edmonton (Oliver), Reid, Collins Nurseries LTD (Aldergrove, B.C.), Prairie Sun Vine-Ripened (Noval, Joffre), Native Fruit Nursery LTD (Laidlaw, Tofield), and Whitecourt Mountain Seedling Nursery. Few details were available on rearing conditions other than planting dates. All crops present were sampled by randomly selecting 25 seedlings except 10 for Oliver 1981 spruce and the Laidlaw jack pine. Seedlings from the Oliver, Noval, and Laidlaw nurseries were washed free of planting mixtures and the entire root system scanned (12X) and mycorrhizal development estimated. Intact plugs of seedlings from Reid Collins and Whitecourt nurseries were surface scanned for mycorrhizal estimates. The 1985 lodgepole pine crop from Oliver was divided into two sections as a portion of the crop was severely chlorotic and unsuitable for planting. Mycorrhizae of the chlorotic seedlings were compared to the normal green pine seedlings.

2.1.3.3 RRTAC Reconstruction Plants. In order to improve the reclamation of extracted oil sand tailings, methods of soil reconstruction and the growth of shrubs and trees on reconstructed soils are currently being studied. Plots utilizing various amounts of muskeg peat and surficial overburden were constructed and planted with four species of potentially ectomycorrhizal plants. On August 29, 1984, 25 plants each of jack pine (Pine Ridge Nursery, March 1984), white spruce (Pine Ridge Nursery, January 1984), and northwest poplar (Laidlaw, April 1984) were randomly selected to determine plant size and mycorrhizal status. Sand box willow (Reid, Collins) was sampled (10 plants) in June 1985, as it was planted later than the other species. Ectomycorrhizal estimates were made as above on washed root systems. Roots of five individuals each of poplar and willow were cleared and stained, and examined for the presence of VA mycorrhizae (Phillips and Hayman, 1970). All species were grown in 150 cc Spencer-Lamaire Hillson book containers.

2.1.4 Results

2.1.4.1 Visual estimates of the Pine Ridge nursery stock indicated that greater than 90% of the short roots of both crops of lodgepole pine had been converted to ectomycorrhizae, and ectomycorrhizal conversion was about 75% with the white spruce. Regardless of time or species, Thelephora terrestris Ehrh.:Fr. was the dominant ectomycorrhizal fungus (Table 1). Once the seedlings had been in the shadehouse a year, T. terrestris was fruiting on about 75% of the seedlings. Thelephora terrestris was readily isolated (about 90% success from 350 tips) when the roots were washed and plated directly on the selective medium, benomyl-MMN. However, when the ectomycorrhizae were surface sterilized first, the recovery rate was less than 10%.

E-strain was the second most common ectomycorrhizal fungus associated with the Pine Ridge stock. It appeared to increase in abundance with lodgepole pine as the residence time in the shadehouse increased. Thelephora terrestris and E-strain often occurred together in the same short roots and it appeared that the latter was replacing T. terrestris.

An unidentified Basidiomycete was almost as common as E-strain but apparently decreased in abundance with time. An Ascomycete, Sphaerosporella brunnea (Alb. & Schw.:Fr) Svrcek & Kubicka, two Basidiomycetes, Amphinema byssoides (Fr.) J. Erkiss. and Coltricia sp., and two species of unknown affinity formed mycorrhizae with a minor portion of the seedlings. Amphinema byssoides readily overgrew both mycorrhizae formed by T. terrestris and the E-strain fungus. Descriptions of the ectomycorrhizae are given in Appendix 7.1.

White spruce seedlings which had been in the Syncrude shadehouse for 2 months were predominantly nonmycorrhizal or only infected with Mycelium radicis atrovirens (Table 2). Once the seedlings had been in the shadehouse over one summer, a majority of the seedlings were infected with one or more ectomycorrhizal fungi. In the June 1983 crop, the dominant fungi were the E-strain and Coltricia. When E-strain and Coltricia sp. occurred together on the same roots, E-strain appeared to overgrow Coltricia.

Table 1. Fungal species forming ectomycorrhizae with lodgepole pine and white spruce that were in the shadehouse for various lengths of time in the Pine Ridge Nursery, Smoky Lake, Alberta.

	Lodgepole Pine		White Spruce
	Time in shadehouse		
	Sep. 82 - May 83	June 82 - May 83	June 82 - May 83
Fungi	% of seedlings ectomycorrhizal with each fungus (% fruiting)		
Thelephora terrestris	96(24)	84(68)	100(84)
E-strain	16	40	16
Amphinema byssoides	0	0	12
Sphaerosporella brunnea	0	0	4
Basidiomycete	44	16	2
Brown cystidial	0	4	0
Coltricia sp.	0	4(8)	0
Unknown affinity	0	0	4

[1] For each set, n = 25, all seedlings in 47 cc Spencer-Lamaire book containers. Pine and spruce 3 months old when moved to shadehouse.

Table 2. Fungal species forming ectomycorrhizae with white spruce that were held in a shadehouse various lengths of time after rearing in the Syncrude greenhouse, Fort McMurray, Alberta.

Fungi	Time in shadehouse: Aug. 83[2] - Oct. 83	June 83[3] - Oct. 83	Aug. 82[4] - Oct. 83
	% of seedlings ectomycorrhizal with each fungus[1]		
E-strain	0	52	76
Amphinema byssoides	0	0	52
Coltricia sp.	0	40	0
Mycelium radicis atrovirens	76	20	28
Unknowns	16	12	36
Nonmycorrhizal	24	16	0
Percent of short roots ectomycorrhizal (± SD)	21 ± 16	56 ± 42	89 ± 20

[1] For each set, n = 25. All seedlings grown in 160 cc Spencer-Lamaire book containers.

[2] Shoot dry weight (±SD) 170 ± 64 mg, root dry weight 105 ± 34 mg.

[3] Shoot dry weight 346 ± 143 mg, root dry weight 366 ± 105 mg.

[4] Shoot dry weight 946 ± 257 mg, root dry weight 633 ± 146 mg.

Seedlings which had spent a year in the shadehouse were all mycorrhizal with about 90% of the short roots converted to ectomycorrhizae. E-strain occurred on more seedlings than any other fungus and Amphinema byssoides occurred on half the seedlings. E-strain ectomycorrhizae were commonly found to be overgrown with A. byssoides. Unknown fungi included four species of Basidiomycetes and they occurred on 36% of the seedlings. In the two younger crops there was only one unknown species detected in each group of seedlings. These are described briefly in Appendix 7.2.

Shoot and root characteristics of the pine and spruce seedlings sampled in 1985 are given in Table 3. The seedlings sampled from the 1984 crops from the Whitecourt nursery were small as they were culls. The age of the Reid, Collins seedlings is unknown but is judged to be at least one year old and perhaps two.

All seedlings from the Whitecourt nursery completed lacked mycorrhizae (Table 4). The 1985 seedlings were still in the greenhouse and being fertilized so heavily that many roots were bluish-green. The 1984 seedlings had been outside and exposed to inoculum from the surrounding forest, yet remained nonmycorrhizal. Jack pine seedlings from the Laidlaw nursery were also predominantly nonmycorrhizal but a small amount of MRA mycorrhizae occurred on the very healthy seedlings.

The Noval nursery has only recently began growing conifers and is located in a prairie region of Alberta, and thus, inoculum may be present in only very small quantities. Eight of the 50 seedlings examined had some mycorrhizal infection. MRA reached as high as 50% and Amphinema infected 75% of short roots of the single plant it occurred on. This indicates that good mycorrhizal development is possible if inoculum is present.

In contrast to the Whitecourt, Laidlaw, and Noval nurseries, the long-established provincial nursery at Oliver produced heavily mycorrhizal seedlings (Table 5). The 1985 and 1981 white spruce was dominated by E-strain and Amphinema byssoides mycorrhizae with small amounts of MRA. Amphinema was most abundant on the oldest seedlings. The normal lodgepole pine seedlings were also heavily infected by

Table 3. Size of container-grown spruce and pine seedlings sampled from five nurseries.

Nursery	Species	Crop	Height (cm)	Root Collar Diameter (mm)	Shoot Weight (g)	Root Weight (g)	S/R Ratio
Whitecourt	Lodgepole pine	1984[1]	8.2 ± 2.4	2.7 ± 0.6	0.97 ± 0.45	0.62 ± 0.31	1.8 ± 0.5
Whitecourt	Lodgepole pine	1985	14.9 ± 0.3	2.3 ± 0.5	1.00 ± 0.40	0.55 ± 0.25	1.9 ± 0.3
Whitecourt	White spruce	1984[1]	7.2 ± 1.6	2.5 ± 0.5	0.66 ± 0.26	0.54 ± 0.21	1.2 ± 0.3
Whitecourt	White spruce	1985	11.2 ± 1.8	2.2 ± 0.5	0.50 ± 0.16	0.26 ± 0.09	2.0 ± 0.4
Laidlaw	Jack pine	1985	19.5 ± 2.1	2.9 ± 0.4	0.77 ± 0.27	0.32 ± 0.06	2.3 ± 0.6
Noval	Lodgepole pine	1985	14.0 ± 2.7	3.4 ± 0.6	2.01 ± 0.55	1.20 ± 0.49	1.8 ± 0.4
Noval	White spruce	1985	19.7 ± 3.4	3.3 ± 0.5	1.60 ± 0.43	0.64 ± 0.14	2.5 ± 0.6
Reid, Collins	Lodgepole pine	?	30-40	NM[3]	2.23 ± 0.45	0.82 ± 0.17	2.7 ± 0.3
Reid, Collins	Jack pine	?	~20	NM[3]	2.31 ± 0.92	2.00 ± 0.94	1.2 ± 0.3
Reid, Collins	White spruce	?	~25	NM[3]	2.86 ± 0.95	2.11 ± 0.68	1.4 ± 0.4
Oliver	Lodgepole pine	1985(C)[2]	10.7 ± 2.7	2.0 ± 0.4	0.52 ± 0.26	0.23 ± 0.15	2.6 ± 1.1
Oliver	Lodgepole pine	1985(G)	11.4 ± 5.3	2.2 ± 0.5	0.69 ± 0.46	0.33 ± 0.18	2.1 ± 0.6
Oliver	White spruce	1984	15.4 ± 3.1	2.0 ± 0.3	0.64 ± 0.31	0.38 ± 0.16	1.7 ± 0.6
Oliver	White spruce	1981	22.2 ± 4.7	4.6 ± 0.9	3.66 ± 1.80	1.95 ± 0.69	1.8 ± 0.4

[1] All substandard culls remaining after crop shipped.

[2] C = chlorotic seedlings, G = normal green seedlings.

[3] Not measured

Table 4. Percent mycorrhizal infection and frequency of occurrence of mycorrhizae on pine and spruce seedlings from three nurseries.

Nursery	Species	Crop	Total Infection $\bar{x} \pm$ SD	Total Infection Occ.[1]	MRA[2] $\bar{x} \pm$ SD	MRA[2] Occ.	*Amphinema byssoides* $\bar{x} \pm$ SD	*Amphinema byssoides* Occ.	Unknown $\bar{x} \pm$ SD	Unknown Occ.
Whitecourt	Lodgepole pine	1984	0	0	0	0	0	0	0	0
Whitecourt	Lodgepole pine	1985	0	0	0	0	0	0	0	0
Whitecourt	White spruce	1984	0	0	0	0	0	0	0	0
Whitecourt	White spruce	1985	0	0	0	0	0	0	0	0
Laidlaw	Jack pine	1985	<1	3/10	<1	2/10	0	0	<1	1/10
Noval	Lodgepole pine	1985	0	0	0	0	0	0	0	0
Noval	white spruce	1985	7 ± 18	8/10	4 ± 12	7/25	3	1/25	0	0

[1] Occurrence, number of seedlings with mycorrhizae over total number of seedlings.

[2] MRA = *Mycelium radicis atrovirens*

Table 5. Ectomycorrhizal infection of container-grown pine and spruce from the Alberta Tree Nursery and Horticultural Centre (Oliver).

Ectomycorrhizal Fungi	Chlorotic 1985 Lodgepole pine		Normal 1985 Lodgepole pine		1985 White spruce		1981 White spruce	
	Percent Mycorrhizal Infection							
	$\bar{x}$ ± SD	Occ.[1]	$\bar{x}$ ± SD	Occ.[1]	$\bar{x}$ ± SD	Occ.[1]	$\bar{x}$ ± SD	Occ.[1]
E-strain	29 ± 42	10/25	83 ± 29	25/25	77 ± 37	25/25	34 ± 33	8/10
Thelephora terrestris	2 ± 10	1/25	3 ± 11	4/25	0	0	0	0
MRA	5 ± 19	3/25	<1	7/25	<1	5/25	3 ± 6	4/10
Rhizopogon-like	0	0	<1	1/25	0	0	0	0
Amphinema byssoides	0	0	0	0	23 ± 37	13/25	55 ± 37	10/10
Unknown	0	0	1 ± 5	1/25	0	0	8 ± 25	1/10
None	64 ± 47	19/25	12 ± 28	11/25	0	0	0	0

[1] Occurrence, number of seedlings with mycorrhizae over total number of seedlings.

E-strain and a small amount of Thelephora. In contrast, only 36% of the short roots of the chlorotic pine seedlings were mycorrhizal and only 9 of 25 seedlings were moderately to heavily mycorrhizal with most of the remaining seedlings being nonmycorrhizal. Nearly all the chlorotic seedlings had a species of Cylindrocarpon sporulating on the upper portion of the root system or had Cylindrocarpon chlamydospores within root tissue. Nectria, the teleomorph of Cylindrocarpon, was also observed on one seedling.

Each of three conifer species from the Reid-Collins nursery differed in the species of fungi forming mycorrhizae. Lodgepole pine was almost exclusively associated with Thelephora terrestris which was fruiting on 60% of seedlings (Table 6). Thelephora was also common on jack pine seedlings but the dominant fungus was MRA. The Rhizopogon-like fungus produced dark, well formed rhizomorphs identical to those observed on seedlings grown in dyke soils (Part 4.1). Also present in small quantities were I-type and Lactarius mycorrhizae. In contrast to the pines, spruce lacked Thelephora and was dominated by E-strain. Amphinema formed one-third of the mycorrhizae and in all cases the Amphinema infections were initiated at the top of the plugs and were spreading downward, overgrowing E-strain.

Jack pine and white spruce reared in the Pine Ridge nursery for the RRTAC plots were all heavily infected with Thelephora terrestris (Table 7). Small amounts of E-strain, Amphinema, and MRA occurred on the spruce seedlings. Poplar from the Laidlaw nursery and willow from Reid, Collins lacked both ectomycorrhizae and VA mycorrhizae.

2.1.5 Discussion

It is evident that the mycorrhizal condition of container-grown conifers at the time of planting can be extremely variable depending on the nursery of origin. Seedlings produced at private nurseries were often totally nonmycorrhizal which appeared to be the result of the use of high fertilizer levels or the age and location of the nursery. In the older provincial nurseries the seedlings were

Table 6. Ectomycorrhizal infection of container-grown pine and spruce from the Reid, Collins Nursery.

	Jack pine		Lodgepole pine		White spruce	
			Percent Mycorrhizal Infection			
Fungi	$\bar{x}$ ± SD	Occ.[1]	$\bar{x}$ ± SD	Occ.	$\bar{x}$ ± SD	Occ.
E-strain	0	0	0	0	61 ± 38	24/25
Thelephora terrestris	31 ± 38	17/25	97 ± 11	25/25	0	0
MRA[2]	60 ± 40	23/25	0	0	1 ± 3	11/25
Amphinema byssoides	0	0	0	0	35 ± 38	19/25
I-type	<1	3/25	0	0	0	0
Rhizopogon-like	5 ± 20	4/25	0	0	0	0
Lactarius sp.	<1	2/25	0	0	0	0
Cenococcum geophilum	0	0	<1	4/25	0	0
Unknown	1 ± 4	2/25	1 ± 5	2/25	2 ± 12	1/25
None	0	0	2 ± 10	1/25	0	0

[1] Occurrence, number of seedlings with mycorrhizae over total number of seedlings.

[2] MRA = *Mycelium radicis atrovirens*

Table 7. Ectomycorrhizal status and plant weights (mean ± standard deviation) of potentially ectomycorrhizal woody plants outplanted on the RRTAC soil reconstruction project.

Plant species	Plant wt (g dwt per plant) Shoot	Root	Total	Percent of short roots converted to ectomycorrhizae
Jack pine	3.78 ± 1.33	1.85 ± 0.67	5.64 ± 1.91	100[1]
White spruce	2.37 ± .85	1.21 ± 0.49	3.58 ± 1.24	99[2]
Northwest poplar	2.02 ± 0.76	0.25 ± 0.13	2.27 ± 0.87	0[3]
Willow	ND[4]	ND	ND	0[3]

[1] All ectomycorrhizae formed by Thelephora terrestris and T. terrestris fruiting on all seedlings.

[2] Thelephora terrestris responsible for 95% of the ectomycorrhizae and fruiting on 76% of the seedlings. E-strain occurring on 5 seedlings, Amphinema byssoides on 8 seedlings, Mycelium radicis atrovirens on 2 seedlings and Olpidium on 3 seedlings; infections not exceeding 5% except for one seedling with 75% E-strain ectomycorrhizae.

[3] VA mycorrhizae absent.

[4] ND = Not determined.

usually heavily ectomycorrhizal but the dominant fungi varied between the two nurseries, Thelephora at Pine Ridge and E-strain at Oliver. Even within a single nursery, the fungal associates can be quite different as evidenced by the data from the Reid Collins nursery. The seedlings were probably among the oldest sampled as indicated by the large size and the presence of normally non-nursery fungi such as

Cenococcum, Lactarius, I-type (Tuber sp.), and Rhizopogon. Unfortunately, no details on the seedlings were made available by the grower.

The comparison of the roots of the chlorotic and normal lodgepole pine at the Oliver Nursery resulted in interesting observations. There is little doubt that the chlorosis was due to a root disease caused by Cylindrocarpon and that most of these plants were also weakly mycorrhizal. The size of the chlorotic seedlings were similar to the nonchlorotic ones but the shoot/root ratio of the chlorotic seedlings was larger (2.6 vs. 2.1) due to smaller root system. It would appear that the chlorotic seedlings grew normally for some time and then became infected with Cylindrocarpon which damaged the entire root system. It is likely that the pathogenic infection was facilitated by the nonmycorrhizal condition of the roots, i.e., E-strain afforded protection against the pathogen. Although it has been suggested by several researchers (e.g., Sinclair et al., 1982) that mycorrhizae aid in disease prevention, no previous examples are known from forest nurseries.

The data on crops of different ages indicate that mycorrhiza populations in nurseries are more dynamic than has been previously suggested, i.e., once Thelephora, always Thelephora. In fact, there appears to be a predictable succession pattern assuming inoculum is not limiting. Mycelium radicis atrovirens Melin (MRA), which has been considered as both a weak parasite (Richard et al., 1971) and as a true ectomycorrhizal fungus (Thomas and Jackson, 1979) is likely to be one of the earliest root colonizers. It is geographically widespread and very common in forest soils and nurseries (Richard and Fortin, 1974). Its exact effect on the host is still unclear but it formed mycorrhizae with good mantles and Hartig nets on some of the best nursery stock observed in this study (Noval). MRA can persist on the stock in limited quantities for as long as the seedlings are in the nursery but to a large measure it will be replaced by the more conventional ectomycorrhizal fungi, especially Thelephora terrestris and E-strain. It appears that E-strain fungi will replace Thelephora in nurseries if residence time is long enough in the same manner as has been observed on mine spoils (Danielson et al., 1984). In bare root nurseries,

replacement may also occur, as seedlings in recently fumigated soil were infected with a species with small hyphae (Thelephora?) whereas seedlings in soil that was not recently fumigated were infected with what almost certainly appears to be E-strain (Danielson and Davey, 1969). Thelephora terrestris is considered to be the most common naturally occurring fungus in southern U.S. bare root nurseries (Marx et al., 1984) but E-strain mycorrhizae may have been overlooked due to their superficial similarity to mycorrhizae formed by Thelephora (Danielson et al., 1984).

Coltricia perennis (Fr.) Murrill has not previously been reported from nurseries but does occur on jack pine in Alberta (Danielson, 1984). It occurred on one crop of white spruce in the Syncrude nursery and on lodgepole pine in the Pine Ridge nursery. Amphinema byssoides occurred only on spruce and appeared to replace E-strain and Thelephora as occurs on minespoils (Danielson et al., 1984). For Amphinema to become abundant, the seedlings need to remain for a year or more in the nursery. Amphinema occurs on pines in forests (Visser and Danielson, 1988) and the absence on pine in nurseries is probably due to the increased host resistance of the heavily fertilized seedlings.

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2.2 JACK PINE ECTOMYCORRHIZAL INOCULUM IN UNDISTURBED AND STOCKPILED MUSKEG PEAT

2.2.1 Abstract

A greenhouse baiting technique was used to determine the effects of stockpiling muskeg peat on the infectivity of peat with regard to ectomycorrhizal formation of jack pine. Seedling growth was poor due to nutrient deficiencies and the high pH of the stockpiled peat. Fertilization of the stockpiled peat was ineffective in stimulating plant growth unless the seedlings were mycorrhizal. Stockpiling resulted in reduced infection levels, fewer mycorrhizal seedlings and fewer symbionts as compared to undisturbed muskeg. The most important fungal symbionts in the stockpile were E-strain fungi, a cystidial Ascomycete (Tuber sp.), and a hyaline Basidiomycete. Ascomycetes accounted for 37% of the infected short roots and 32% of all the mycorrhizae had cystidia on the mantle. Rhizomorphs were rarely formed. The most aggressive fungi were those mentioned above and the least aggressive were a Rhizopogon-type and Cenococcum geophilum.

2.2.2 Introduction

During the course of surface mining of bitumen-bearing oil sands in northern Alberta, the muskeg peat deposits are routinely stockpiled for reclaiming the extracted tailings. Stockpiling mineral topsoil during mining operations in the U.S. and the resultant effects on VA mycorrhizal inoculum has received some attention (Rives et al., 1980; Gould and Liberta, 1981) but data on either the stockpiling of mineral soils containing ectomycorrhizal inoculum or peat soils is totally lacking. The stockpiling of topsoil generally reduces the VA infectivity with time but not necessarily to levels which will detrimentally affect plant growth or survival after application of the stored soil to spoils as compared to freshly spread soil.

Peat deposits differ substantially from mineral soils in their inherent mycorrhizal components and stockpiling peat may have more serious consequences on mycorrhizal associations than stockpiling mineral soil. The native vegetation of muskeg peat bogs is largely

coniferous and ericaceous and, therefore, VA mycorrhizal inoculum is probably rare. The drastic changes in soil moisture between undisturbed bogs and the reclamation sites where the peat is applied suggests that the indigenous fungi may be ill-adapted to the new conditions. Stockpiling may reduce species diversity and thus the ecological and physiological versatility of mycorrhizae. In addition, nutrient availability may be altered by stockpiling and creat nutrient conditions which may favour nonindigenous symbionts.

Whereas VA fungi are not host specific, ectomycorrhizal fungi are often generically host specific, although some species form mycorrhizae with a wide taxonomic range of higher plants. These fungi, e.g., Cenococcum and E-strain, are found over broad geographic ranges, encompassing many types of ecosystems. It is unlikely that a soil supporting any ectomycorrhizal hosts would completely lack inoculum for nonindigenous hosts unless the hosts exhibited strong ectomycorrhizal specificity, e.g., Alnus spp. However, the effectiveness of these broad-spectrum hosts is unknown. It has been suggested that host-specific ectomycorrhizal fungi may be more effective, i.e., benefit the host more, than broad spectrum fungi (Mikola, 1970). This suggestion has yet to be critically tested but it is worthy of consideration rather than simply recording overall infection rates and relating these to the success of reclamation treatments incorporating inoculum-bearing amendments. A first step in testing potential efficiencies is to determine the spectrum of compatible symbionts present in peat amendments and to attempt to characterize these taxonomically and with regard to adaptive morphological features.

It is thus of interest to determine the fate of ectomycorrhizal inoculum in stockpiled peat and to evaluate the characteristics of the mycorrhizae as they may relate to the efficiency of nutrient and water uptake in reclaimed tailings subjected to critical stresses. The objectives of this study were to determine:

1. the potential of undisturbed and stockpiled peat for initiating mycorrhizal associations of a prime reclamation candidate, jack pine;
2. the morphological characteristics of the ectomycorrhizae;

3. the identity of the symbionts indigenous to the peat; and
4. the inherent growth potential of the peat for jack pine seedlings.

2.2.3 Materials and Methods

The muskeg peat was located on the Syncrude Oil Sands lease ($57^{o}5'N$, $111^{o}45'W$) in northern Alberta. The peat was about 2 m deep and supported several ectomycorrhizal plants including eastern larch (Larix laricina (DuRoi) K. Koch) and swamp birch (Betula pumila L. var. glandulifera Regel). Black spruce (Picea mariana (Mill.) BSP) occurred at one end of the bog and small willows (Salix sp.) were widely scattered in the area. Ericaceous plants, especially Labrador tea (Ledum groenlandicum Oeder), were abundant as were feather mosses and hummocks of sphagnum mosses.

The peat was stripped from a portion of the bog and stored in the period between December 1980 and February 1981 to form a stockpile about 300 m wide and 3 m deep. When sampled in the second week of September 1981, the only ectomycorrhizal hosts present were widely scattered seedlings of aspen (Populus tremuloides Michx.) and willow. The major colonizer was lamb's quarters (Chenopodium album L.) which is nonmycorrhizal (Hirrel et al., 1978). Also present was fireweed (Epilobium angustifolium L.).

At the two sampling locations, bog and stockpile, both surface and subsurface samples were taken. On the stockpile, the surface was subject to drying and the 0-15 cm depth was sampled to represent this layer. Due to difficulty in inserting the Macauly peat sampler at some sample points on the stockpile, the subsurface samples varied from 50-100 cm deep but all samples were moist and well below the level subject to drying. In the undisturbed bog the surface samples were taken by hand to a depth of 15 cm below the bases of the feather or sphagnum mosses. The material sampled was moist, but due to the dry summer conditions, never saturated. The tops of all mosses on the hummocks were dry. The surface layer sampled was largely a mass of roots (many of which were ericaceous) and active ectomycorrhizae were frequently abundant. The subsurface samples were from the 50-100 cm

depth and were much moister and contained far fewer roots than the surface samples. About 200 cm^3 of peat was taken for each sample.

One 250 m long transect across the stockpile and one 250 m transect across the undisturbed bog were established. Every 10 m along each transect line, a surface sample was taken at a randomly selected point 1 to 5 m to the left of the line. The subsurface samples were taken at random points 1 to 5 m to the right of the transect to eliminate depth dependency. Thus, a total of 25 replicates were taken for each of the four depth-site combinations.

The samples were stored at 5^oC for about 5 weeks and then each sample was thoroughly mixed and one 65 cc Leach Cone-tainer filled and planted with a single pregerminated jack pine seedling (Experiment I). The seedlings were placed in a greenhouse with daylength supplemented with 3.5 klx lights to give a 15 h daylength for the first 8 weeks and 18 h daylength for the next 9 weeks. The seedlings were watered twice weekly for the first 10 weeks after which they were fertilized twice weekly with a solution containing 125 mg L^{-1} of 15:15:18 Plant Prod complete fertilizer. After a total of 17 weeks, the seedlings were placed at 5^oC, shoots harvested and dried, and the roots evaluated for mycorrhizal development and then dried at 80^oC. All short roots were rated for mycorrhizal infections, taxonomic affinity of the symbiont, and morphological characters of the mantle and extramatrical mycelium. Isolations on benomyl-MMN agar were used to further characterize the symbionts (Danielson et al., 1983).

In that seedlings in all treatments grew poorly, the entire experiment was repeated with samples that had been stored at 5^oC for 6 months (Experiment II). Daylength was extended to 20 h and 100 mg L^{-1} of Plant Prod 15:15:18 was added throughout the 15 week growth period. After 10 weeks, growth in the stockpiled peat was still poor, so additional Fe (56 mg L^{-1} Sesquestrene) and micronutrients (1 mL of micronutrient stock, Arnon, 1938) was added. Only mycorrhizal development of seedlings grown in stockpiled peat was evaluated in the second experiment. Five randomly chosen seedlings were used to determine seedling size on the undisturbed samples. The pH of the samples was determined with 10 g peat in 20 mL water. Initially, the values for materials were: undisturbed surface pH 6.5, undisturbed

subsurface pH 5.7, stockpile surface pH 7.4, and stockpile subsurface pH 7.7.

2.2.4 Results

Growth of the jack pine seedlings was poor in all of the peat sources (Table 1) and the application of fertilizer after 10 weeks of growth appeared to have no effect. Both root and shoot growth were significantly greater in peat from the undisturbed bog than in stockpiled peat. However, the total number of short or feeder roots was greater in the stockpiled peat than in the bog peat. The depth from which the peat was obtained had little effect on the weight of the seedlings. The poorest appearing seedlings were in the subsurface stockpile peat and, unlike seedlings in the other treatments, failed to form any needle fascicles. No height growth beyond the cotyledon stage occurred in any seedling.

The degree of mycorrhizal development was much greater in seedlings grown in the undisturbed peat than in stockpiled peat (Table 2). Depth of peat down to 0.5-1 m had no significant effect on infection rates although the data suggest that inoculum was less plentiful on the stockpile surface than in the body of the stockpile. The number of seedlings infected, total numbers of infections, and the coarse Ascomycete, I-type, and the hyaline Basidiomycete. Particularly inefficient in spreading was Rhizopogon (7) which occurred 16 times but only infected an average of 5% of the short roots. Cenococcum geophilum also occurred frequently but was very slow to extend from the original infection point. The golden floccose species (6) and Tomentella were intermediate in aggressiveness.

Fertilization throughout the growing period in Experiment II resulted in increased seedling growth but the seedlings were still small in comparison to seedlings grown in commercial peat (compare with 1x treatment in Part 3.1). Shoot weights (± standard deviations) of seedlings grown in the undisturbed peat were 76 ± 15 mg - control surface, 38 ± 8 mg - control subsurface, 166 ± 64 mg - fertilized surface, and 114 ± 26 mg - fertilized subsurface. In stockpiled peat, fertilization increased shoot weight 63% and root weight 76% and

Table 1. Characteristics of jack pine seedlings grown in peat from a muskeg bog and peat stockpiled for 8 months (Experiment I).

Peat Source	Depth 0-15	Depth 50-100	
	Shoot Weight (mg)		Row Means
Undisturbed	36	32	34[b]
Stockpile	21	20	21[a]
Column means	28[a]	26[a]	
	Root Weight (mg)		Row Means
Undisturbed	26	34	30[b]
Stockpile	20	22	21[a]
Column means	23[a]	28[b]	
	Short Roots/Seedling		Row Means
Undisturbed	353	276	265[a]
Stockpile	300	320	310[b]
Column means	277[a]	298[a]	

Shoot and root weight analyzed by two-way ANOVA, short roots/seedling analyzed by analysis of covariance with adjustment for total root weight. Differences between means tested by Scheffé pairwise comparison. Values (geometric means) within each set followed by the same letter in each row or column do not differ significantly ($p = .05$).

Table 2. Mycorrhizal infection of jack pine seedlings grown in undisturbed muskeg and stockpiled peat (Experiment I).

Peat Source	Depth (cm)	% Infection $\bar{x} \pm SD$	Total Seedlings	Number of Seedlings Infected	Total Number of Infections	Number of Species
Undisturbed	0-15	$78^{b} \pm 23$	24	24	89	25
Undisturbed	50-100	$53^{b} \pm 36$	25	24	58	15
Stockpile	0-15	$1^{a} \pm 4$	23	3	3	3
Stockpile	50-100	$15^{a} \pm 29$	20	7	8	6

[1] Data analyzed by Kruskal-Wallis test.

number of symbiont species were all much reduced by stockpiling the peat. The number of infections was estimated by assuming that all the internal colonization by each species on each seedling was due to a single infection event.

A total of 31 taxa of symbionts of jack pine were recorded from all the peat sources (Table 3, Appendix 7.3). Few of these taxa could be assigned to known genera or species and most were given descriptive trivial names which correspond to names in Parts 2.3 and 4.1. Two criteria were used to denote the importance of the various taxa, the percent of all the infected short roots infected by each taxon, and the percent of the seedlings infected by each taxon. In order to determine post-infection behaviour of the symbionts, i.e., aggressiveness, the degree (%) of infection of each seedling internally colonized by each taxon was determined.

Of the total roots infected, 37% were infected by Ascomycetes and Ascomycetes composed 25% of the symbiont species. Accordingly, Basidiomycetes were responsible for 61% of the short root infections. Eight species (25%) had pigmented hyphae and these eight formed 13% of

Table 3. Types of mycorrhizae developing in the greenhouse on jack pine seedlings grown in muskeg peat from an undisturbed bog and from a stockpile.

Taxa (No.)	External Features[1]	Hyphal Pigments[2]	% of Total Number of Mycorrhizae	% of Seedlings Infected		% Infection of Colonized Seedlings
				Bog	Stock	x ± SD
Ascomycetes						
I-type (1)	Cystidia	-	22.4	41	5	34 ± 31
E-strain (3)	EMM	+	5.8	6	2	63 ± 43
Cenococcum (5)	EMM	+	0.5	16	0	3 ± 3
T. angularis (9)	[EMM]	-	1.2	2	0	63 ± 0
Black asco (11)	EMM	+	0.3	2	0	14 ± 0
Coarse asco (13)	Glabrous	-	6.3	10	2	34 ± 27
No. 22	[EMM]	+	<.1	2	0	2 ± 0
No. 25	EMM	+	<.1	2	0	1 ± 0
Basidiomycetes						
Hyaline basid (12)	[EMM]	-	28.8	41	9	40 ± 36
Golden floccose (6)	EMM	-	8.5	35	0	14 ± 14
Tibiiform (10)	Cystidia	-	4.4	2	0	97 ± 0
Tomentella I (2)	[EMM]	+	4.3	35	0	10 ± 10
Tomentella II (21)	Cystidia	+	2.3	10	0	15 ± 15
Yellow cystidia (4)	Cystidia	-	3.1	24	0	11 ± 17
Rhizopogon-like (4)	EMM + C + R	-	1.8	31	2	5 ± 7
Rhizopogon-like (8)	EMM + C + R	-	0.6	4	0	12 ± 14
White floccose (30)	EMM	-	3.3	0	2	45 ± 0
White floccose (14)	EMM	-	0.4	2	0	24 ± 0
No. 16	[EMM]	-	1.0	2	0	58 ± 0
No. 18	EMM	-	<.1	2	0	2 ± 0

continued...

Table 3 concluded.

Taxa (No.)	External Features[1]	Hyphal Pigments[2]	% of Total Infected Short Roots[3]	% of Seedlings Infected		% Infection of Colonized Seedlings
				Bog	Stock	$\bar{x} \pm SD$
Basidiomycetes (cont.)						
Tom-like (20)	Glabrous	+	<.1	2	0	2 ± 0
No. 24	EMM	-	0.3	2	0	10 ± 0
No. 26	EMM	-	3.3	8	0	20 ± 25
No. 27	EMM	-	<.1	2	0	1 ± 0
No. 29	Glabrous	-	0.9	2	0	30 ± 0
Dense floccose (31)	EMM	-	0.5	0	2	14 ± 0
Unknown Affinity						
No. 15	?	-	0.1	4	0	3 ± 3
No. 17	Glabrous	-	1.1	2	0	30 ± 0
No. 19	EMM	-	0.8	4	0	16 ± 18
No. 23	[EMM]	-	0.2	2	0	6 ± 0
No. 28	?	?	<.1	2	0	2 ± 0

[1] Presence of cystidia on the mantle, EMM = extramatrical mycelium, C = crystals on the hyphae, R = rhizomorphs or mycelial strands, [EMM] = sparse EMM.

[2] Individual hyphae with definite brown pigments in the wall.

[3] A total of 10,091 mycorrhizae.

the mycorrhizae. With regard to external features, 8% of the mycorrhizae were glabrous, 32% had cystidia, and 26% formed extensive extramatrical hyphae. Rhizomorphs and crystalline deposits on the hyphae were found with only two species and these formed 2.4% of the mycorrhizae.

The species forming the most mycorrhizae were the I-type Ascomycete (I-subtype of Dominik, 1962) and a hyaline Basidiomycete. These two species accounted for 50% of the infections. Also of improtance were E-strain fungi and the coarse Ascomycete. It is likely that the coarse Ascomycete is related to the E-strain but it did not grow well enough in culture to confirm the relationship. These same four taxa were also responsible for a majority of the infections initiated in the stockpiled peat. In the undisturbed peat, the average number of species per seedling were 3.8 in the surface and 2.2 in the subsurface peat.

Post-infective aggressiveness could only be reliably estimated for species which occurred four or five times or more. Those species best able to internally colonize jack pine roots were E-strain, decreased number of short roots per seedlings 11% (Table 4). Seedlings that did not receive any fertilizer all appeared to be N deficient and/or P deficient. Those that were fertilized appeared to be deficient in N or were a healthy green colour, i.e., fertilization alone did not correct the nutrient deficiencies (see below). The application of additional micronutrients and Fe did not result in any apparent changes in the plants. It is very likely that the pH of the stockpiled peat was responsible for the poor growth. The pH (± standard deviation) of the fertilized peats at the end of the growth period were 7.4 ± 0.4 - undisturbed surface, 6.3 ± 0.4 - undisturbed subsurface, 7.8 ± 0.3 - stockpile surface, and 7.9 ± 0.2 - stockpile subsurface.

Mycorrhizal infection was clearly enhanced by fertilization of both depths of peat (Table 5). Once fertilizer was applied it became apparent that the surface peat was less infective than the subsurface peat. This was true for percent short roots infected, number of seedlings infected, and total number of infections. The number of species detected did not vary with depth.

Table 4. Characteristics of jack pine seedlings grown in stockpiled peat unfertilized or with fertilizer added twice weekly (Experiment II).

	Depth		
	0-15	50-100	
Fertilized	Shoot Weight (mg)		Row Means
-	25	27	26[a]
+	37	42	40[b]
Column means	31[a]	34[a]	
	Root weight (mg)		Row Means
-	23	26	24[a]
+	33	45	38[b]
Column means	27[a]	34[b]	
	Short roots/seedling		Row Means
-	368	377	372[b]
+	349	308	329[a]
Column means	358[a]	343[a]	

Shoot and root weight analyzed by two-way ANOVA, short roots/seedling analyzed by analysis of covariance with adjustment for total root weight. Differences between means tested by Scheffé pairwise comparison. Values (geometric means) within each set followed by the same letter in each row or column do not differ significantly ($p = .05$).

Table 5. Mycorrhizal infection of jack pine seedlings grown in fertilized and nonfertilized stockpiled peat (Experiment II).[1]

Peat Source Depth (cm)		% Infection $\bar{x} \pm SD$	Total Seedlings	Number of Seedlings Infected	Total Number of Infections	Number of Species
0-15	-	$3^a \pm 8$	23	3	3	3
0-15	+	$22^b \pm 33$	24	10	11	6
50-100	-	$5^a \pm 15$	22	4	5	3
50-100	+	$51^c \pm 42$	21	17	21	6

[1] Data analyzed by Kruskal-Wallis test.

The dominant symbiont was the hyaline Basidiomycete and the only other fungi which infected a large number of short roots were the I-type and E-strain fungi (Table 6). Fertilization did not result in a change in the species that infected jack pine but increased the number of seedlings infected. As in the previous experiment, E-strain, hyaline Basidiomycete and I-type were the most aggressive symbionts.

Of the 24 surviving fertilized seedlings in the surface peat it was noted that 14 exhibited N deficiency symptoms and 10 seedlings were healthy green. Eight of the 10 healthy seedlings were mycorrhizal, whereas only one N deficient seedling was infected (15% mycorrhizal). In the subsurface fertilized peat all 14 healthy green seedlings were mycorrhizal. A comparison of sizes of infected and noninfected (including those with less than 30% infection) seedlings was made using a t-test. In the surface, infected seedlings were significantly ($p \leq 0.05$) larger (54 mg) than noninfected seedlings (32 mg). The subsurface peat was almost identical, 34 mg versus 54 mg, with the infected seedlings being significantly larger.

Table 6. Types of ectomycorrhizae developing on jack pine seedlings planted in stockpiled peat which was either fertilized or had no fertilizer added.

Taxa (No.)	External Features[1]	Hyphal Pigments[2]	% of Total Number of Mycorrhizae[3]	% of Seedlings Infected		% Infection of Colonized Seedlings
				-Fert	+Fert	$\bar{x} \pm SD$
Ascomycetes						
I-type (1)	Cystidia	-	14.1	2	9	63 ± 30
E-strain (3)	EMM	+	15.1	0	7	57 ± 39
Coarse asco (13)	Glabrous	-	1.5	2	0	44 ± 0
Basidiomycetes						
Hyaline basid (12)	[EMM]	-	57.2	7	36	54 ± 35
Tomentella I (2)	[EMM]	+	2.0	2	4	19 ± 19
Rhizopogon-like I (7)	EMM + C + R	-	1.0	4	2	10 ± 10
Dense floccose (23)	EMM	-	3.8	0	7	20 ± 16
Unknown Affinity						
No. 24	EMM	-	5.1	0	2	35 ± 23
No. 32	?	-	<0.1	0	2	<1

[1] Presence of cystidia on the mantle, EMM = extramatrical mycelium, C = crystals on the hyphae, R = rhizomorphs or mycelial strands, [EMM] = sparse EMM.

[2] Individual hyphae with definite brown pigments in the wall.

[3] A total of 7,267 mycorrhizae.

2.2.5 Discussion

The stockpiled peat was a poor growth medium for jack pine seedlings whether fertilized or not. The difference in pH between the undisturbed muskeg and the stockpile may well account for the differences in growth between the two sources. At nearly pH 8, the availability of P and micronutrients would be reduced which may account for the small growth response to fertilizer. It may be of considerable importance that the mycorrhizal plants appeared to respond to fertilization while the nonmycorrhizal plants did not respond. However, it is possible that the larger plants became infected because they were more susceptible rather than mycorrhizae enhancing growth. It seems more likely however, that mycorrhizae enhanced nutrient uptake, probably N, which resulted in better growth. McVean (1963) also found pine seedlings to grow very poorly in peat unless the plants were mycorrhizal. Mycorrhizal seedlings took up more P and N than nonmycorrhizal seedlings and McVean (1963) concluded that the enhanced growth was a result of becoming infected rather than a cause of infection. He credited mycorrhizae as having a major influence on the field success of scots pine planted on open moorlands and bogs.

There was no clear indication that any one symbiont was superior to another. However, it was observed that the only seedling with healthy colour grown in the stockpile peat in the first experiment was heavily infected with E-strain. In the fertilized stockpile peat, the largest seedling produced (shoot weight 162 mg) was 100% mycorrhizal with the E-strain. This suggests that E-strain fungi may be particularly beneficial but further testing is required.

The spectrum of fungal symbionts was typical for peat soils. In other baiting experiments and greenhouse studies, the I-type Ascomycete (Tuber sp., see Part 4.1), E-strain, hyaline Basidiomycete, Rhizopogon-like and Tomentella are constant associates of jack pine seedlings (Danielson et al., 1984a,b; Danielson et al., 1983, see Part 4.1). These fungi also developed mycorrhizae in the field on extracted oil sands amended with peat which had been stockpiled for 5 years (Part 4.1). Although additional observations are necessary to determine the role of these fungi in field reclamation sites, there is little doubt that field performance evaluations are warranted.

It is unknown if the mycorrhizal status of jack pine grown in the greenhouse reflects the status of ectomycorrhizal plants in the native bog. It is tempting to extrapolate the data obtained in this study to muskeg bogs but it is impossible without field observations. It would be of considerable interest to determine the properties of the mycorrhizae in undisturbed muskeg to determine the taxonomic and morphological similarity between indigenous plants in the greenhouse and in the field.

Although the amount of mycorrhizal inoculum was less on the surface of the stockpile than in the stockpile interior, this probably has little practical significance. The portion of a pile that is subject to moisture and temperature extremes is a relatively small volume and would be mixed and diluted with the unexposed peat when applied to tailings sand. Stockpiling peat resulted in a less infective and less species diverse growth medium as compared to the surface meter of native bog. These reductions may be due to dilution with deeper layer or death of propagules following stockpiling. Regardless, it would be expected that sufficient inoculum is present to eventually infect plants in the field. However, the time required to form infections of sufficient magnitude to influence host performance might be considerable.

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2.3 MYCORRHIZAL DEVELOPMENT OF CONTAINER-GROWN JACK PINE AND LARCH SEEDLINGS OUTPLANTED ON AMENDED OIL SANDS TAILINGS OR REARED IN THE GREENHOUSE.

2.3.1 Abstract

Two experiments were performed to determine the degree of similarity of ectomycorrhizal development of container-grown seedlings either planted on the Syncrude containment dyke or in the same oil sand-muskeg peat reconstructed soil in the greenhouse. In the first experiment, after one growing season, jack pine shoots and egressed roots were substantially heavier in the greenhouse than in the field. Seedlings in the field remained nonmycorrhizal, whereas two-thirds of the short roots of the greenhouse seedlings became mycorrhizal with five symbionts. In the second experiment, both jack pine and larch were reared in the greenhouse and planted on the Syncrude dyke. After one season, the size of the plants did not differ among the planting sites. Total jack pine short roots becoming ectomycorrhizal in the dyke, greenhouse, and in a mature jack pine stand were 10, 41, and 54%, respectively. In the greenhouse, E-strain and a hyaline Basidiomycete were the dominant fungi but they were rare on seedlings grown on the dyke. Jack pines grown in the jack pine stand were infected largely by multi- and late-successional stage fungi in the genera *Suillus*, *Tomentella*, and *Lactarius*. Twelve percent of the short roots of larch became ectomycorrhizal in the field and 33% in the greenhouse. The most common fungus was one tentatively referred to the genus *Fuscobolitinus*. The greenhouse bioassay appears to reflect the species of fungi present in severely disturbed habitats but overestimates the rates of mycorrhizal development.

2.3.2 Introduction

In order to determine if mycorrhizal inoculum is present in a particular soil or minespoil, it is necessary to grow the appropriate species of plant in the material. This can be done either in the field or in the greenhouse. Growing the plants in the greenhouse has the advantage of convenience and ensuring that if different treatments are used, they are all subjected to the same conditions. However,

conditions in the field and the greenhouse may differ so strongly that rates of mycorrhizal development and the symbionts involved may be substantially different.

Schoenberger and Perry (1982) have used what they termed a greenhouse bioassay to determine the mycorrhizal potential of forest soils where clear-cutting and burning had taken place. They suggested that results of the bioassay may not be extrapolated to the field but that the technique was valid for comparing the effects of disturbance. The objective of this study was to determine the degree of similarity between mycorrhizae of seedlings grown under field and greenhouse conditions.

In a previous study using stockpiled peat in the greenhouse and planted with jack pine germinants, the plants grew poorly and the mycorrhizae were primarily formed by three species of fungi (Part 2.2). In this study, container-grown seedlings were used as the bait plants. It is likely that larger seedlings in peat:vermiculite plugs may fare differently than germinants in undiluted muskeg peat.

2.3.3 Materials and Methods

2.3.1.1 Experiment I. Container-grown jack pine were grown as described by Danielson et al. (1984b). The control treatment trees planted in 1982 on the Syncrude dyke (Part 4.1) were used for comparison with seedlings retained in the greenhouse. Extracted oil sand amended with muskeg peat and overburden clay was collected from five sites immediately adjacent to the outplanting plot (Part 4.1). The samples consisted primarily of peat which was packed into 12.5 cm pots and planted with uninfected seedlings identical to those planted on the dyke. The pots were placed in the greenhouse which had supplemental lighting to provide a 20 h daylength with a minimum of 3.5 klx. Temperature ranged from 18 to 25°C. Ten replicate seedlings were watered three times weekly with deionized water and ten replicates were watered once weekly and fertilized twice weekly with a solution containing 100 mg L^{-1} of Plant Prod 15:15:18 fertilizer in deionized water. Plants were removed from the field after 13 weeks and from the greenhouse after 9 weeks, and weights and mycorrhizal status determined.

2.3.3.2 Experiment II. A further comparison was conducted in 1983 to elucidate the differences in ectomycorrhizal development under field and greenhouse conditions using a species alien to muskeg (jack pine) and a species indigenous to muskeg (eastern larch, Larix laricina (Du Roi) K. Koch). The use of these two species would be expected to more fully access the ectomycorrhizal inoculum status of the reconstructed soil.

Container-grown seedlings were prepared in a manner similar to experiment I. The plants were grown in 150 cc Cone-tainers (Ray leach, Canby, OR) filled with a 1:1 (v/v) mixture of autoclaved peat moss and vermiculite. Each cell was planted with a single germinant and fertilized with solution containing 200 mg L^{-1} of 15:15:18 Plant Prod Soilless Feed three times weekly. Sequestrene 330 was used every 4 weeks at a rate of 56 mg L^{-1} to prevent Fe chlorosis. The jack pines were 12 weeks old when outplanted on June 8 and the larch was 11 weeks old.

Ten replicates of each species were planted in 10 randomly chosen sites on the Syncrude dyke plot. The sites were at positions where seedlings had died or were harvested at the end of the first growing season and were thus distinct from any live jack pine roots. All the seedlings were apparently nomycorrhizal at the time of planting as indicted by the examination of 5 other larch and 10 other jack pine seedlings. After 11 weeks (September 8), the seedlings were dug up and the mycorrhizal status determined.

At the time of outplanting, soil samples were collected from each of the 10 planting sites for the greenhouse phase of the study. The pH of the samples varied from 6.3 to 7.5. The soil was placed in 12.5 cm pots (volume approximately 800 mL) and 10 replicates prepared for each species. In that the Leach containers were deeper than the pots, it was necessary to remove 30-50% of the root system prior to planting. In addition, the peat-vermiculite growth medium was washed from the roots. As a result, the seedlings suffered from water stress after being placed in the greenhouse and several plants died. The plants were watered as needed (no fertilizer added) and harvested at the same time the seedlings in the field were harvested. The shoots and roots were dried at 80°C to determine plant growth and 300 short

roots that extended into the soil were rated as being mycorrhizal or nonmycorrhizal on each plant.

In addition to planting jack pine on the Syncrude dyke, 10 seedlings were also planted in a mature jack pine-lichen woodland at Mildred Lake. This was done to compare the mycorrhizal development in a soil with high levels of inoculum (Mildred Lake) versus a low inoculum site (Syncrude dyke).

2.3.4 Results and Discussion

2.3.4.1 Experiment I. Shoot weights of seedlings grown in the greenhouse were significantly larger than those grown in the field (Table 1). Fertilization did not significantly affect either shoot or root growth. Root weights within the planting plug apparently reached a maximum as weights in all three treatments were very similar. However, the amount of egressed roots was much greater under greenhouse conditions than in the field. In terms of plant nutrition and survival, the amount of roots extending into the soil is very likely much more important than roots in the plug. Extending roots will serve to anchor the seedlings and prevent frost-heaving, exploit a large volume of peat for nutrients and water, and greatly increase the chance of roots encountering mycorrhizal inoculum. Shoot/root ratios may be misleading when the root system exists in two distinct compartments; emphasis should rather be placed on the amount and mycorrhizal condition of egressed roots.

Differences between mycorrhizal development of greenhouse and field-grown seedlings were clear and dramatic (Table 2). Nearly two-thirds of the short roots of seedlings grown in the greenhouse were mycorrhizal, whereas no mycorrhizae were detected on seedlings grown on the Syncrude dyke. Fertilization had no effect on mycorrhizal formation. Five species of fungi were involved as symbionts, but one, referred to here as the hyaline Basidiomycete, was clearly dominant. It is fairly certain that all the inoculum of these species originated in the peat as other jack pine seedlings grown in the greenhouse simultaneously for other experiments remained nonmycorrhizal. In addition, the hyaline Basidiomycete, the I-type, and the Rhizopogon-like fungus

Table 1. Growth characteristics of container-grown jack pine seedlings either reared in the greenhouse or on the Syncrude dyke.

	Syncrude Dyke	Greenhouse Fertilized	Greenhouse Unfertilized
Shoot weight (mg)	646[a]	1823[b]	1363[b]
Root weight in plug (mg)	540[a]	640[a]	608[a]
Root weight, total (mg)	636[a]	1067[b]	1002[ab]
Shoot/root ratio	1.0	1.8	1.3

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 ($p = .05$).

Table 2. Mycorrhizal development of container-grown jack pine seedlings either reared in the greenhouse or on the Syncrude dyke.

	Percent Infection		
	Syncrude Dyke	Greenhouse Fertilized	Greenhouse Unfertilized
Symbiont	$\bar{x}$	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
All mycorrhizae	0	67 ± 28	60 ± 37
I-Type Ascomycete	0	1 ± 2	2 ± 5
Hyaline Basidiomycete	0	62 ± 26	52 ± 39
Rhizopogon-like	0	<1 ± <1	5 ± 7
White floccose	0	3 ± 7	<1 ± 2
Hyaline cystidial	0	0 0	<1 ± <1

were all detected on other seedlings in the outplanting trial (Part 4.1).

The success of the hyaline Basidiomycete appears to be due to two factors; inoculum density and the ability to rapidly spread through the root system once an initial infection occurs. Three of the 20 greenhouse seedlings were not infected by the hyaline Basidiomycete indicating that few propagules were present in each pot. Where infection was present, it exceeded 30% except on one seedling. In contrast, the Rhizopogon-like symbiont infected eight seedlings but only an average of 5.8% of the short roots were infected. Both the white floccose and the I-type were found on six seedlings each, infecting 6.7 and 7.7% of the short roots respectively. Thus, it appears these latter three fungi were less able to spread through the root system (i.e., less aggressive) than was the hyaline Basidiomycete. The pattern of producing small localized infections appears to be typical of Rhizopogon-like fungi found in peat (Part 2.2).

In the field where the hyaline Basidiomycete occurred, it did not spread extensively (Part 4.1). As this species is indigenous to muskeg sites, it may be that the frequent watering in the greenhouse favoured rapid spread. Conditions on the dyke were droughty throughout the summer and moisture probably limited plant growth and may have affected mycorrhizal development.

2.3.4.2 Experiment II. The size of the plants in the greenhouse and on the Syncrude dyke were all similar after 3 months (Table 3). In Experiment I, greenhouse-grown jack pines were substantially larger than their field counterparts. The lack of difference in the second experiment was probably due to the root pruning and the subsequent water stress of the greenhouse plants. No apparent differences were found between the growth of jack pine planted in the dyke and jack pine planted in the mature stand.

The overall ectomycorrhizal formation of both jack pine and larch was greater in the greenhouse than in the field (Table 4). In the first experiment, there was no ectomycorrhizae formed on the seedlings planted in the dyke, whereas about 10% of the short roots were ectomycorrhizal in the second experiment. The difference may be

Table 3. Growth characteristics of jack pine and larch prior to planting and after one season's growth on the Syncrude dyke, in dyke material in the greenhouse, and in a mature jack pine stand.

Species and Planting Sites	Shoot Dry Weight (mg ± SD)	Total Root Dry Weight (mg ± SD)	Dry Weight of Egressed Roots (mg ± SD)
Jack pine - preplanting	480 ± 42	273 ± 47	--
Jack pine - Syncrude	938 ± 189	564 ± 160	215 ± 98
Jack pine - greenhouse	1225 ± 351	643 ± 285	182 ± 97
Jack pine - Mildred Lake	803 ± 118	627 ± 79	282 ± 69
Larch - preplanting	243 ± 102	82 ± 37	--
Larch - Syncrude	720 ± 251	511 ± 305	187 ± 112
Larch - greenhouse	973 ± 182	792 ± 243	232 ± 71

related to the more extensive root development in the reconstructed soil in the second experiment which would have increased the chances of the roots encountering inoculum.

Eight species of fungi were found to form mycorrhizae with jack pine, seven in the greenhouse, and four in the field. The E-strain was the dominant fungus on the greenhouse plants and was much less common in the field. The second most common fungus was the hyaline Basidiomycete which was the dominant fungus on greenhouse grown plants in the first experiment. Astraeus hygrometricus, which was found on two seedlings, probably originated from seedlings introduced to the dyke (Part 4.1). It is curious that it was so unsuccessful when introduced but was able to spontaneously form mycorrhizae with the plants in this study.

Eight fungi also formed mycorrhizae with larch with five being in common with jack pine. Of the most interest was the very frequent occurrence of what is tentatively referred to here as Fuscoboletinus in the field-grown seedlings and its infrequent occurrence in the

Table 4. Amount of mycorrhizal development and number of trees with mycorrhizae of jack pine and larch either planted on the Syncrude dyke or in Syncrude dyke peat in the greenhouse after one growing season.

	Jack Pine Syncrude (n=10)		Jack Pine Greenhouse (n=9)		Larch Syncrude (n=10)		Larch Greenhouse (n=8)	
Taxa	%	No.	%	No.	%	No.	%	No.
Total	10	8	41	7	12	9	33	7
E-strain	<1	1	23	3	0	0	13	4
Suillus	3	4	<1	3	0	0	0	0
Hyaline basid	0	0	14	4	0	0	0	0
MRA	0	0	<1	1	<1	2	0	0
Ascomycete	0	0	1	2	0	0	8	1
Basidiomycete	0	0	<1	1	0	0	12	3
Unknown	2	2	1	1	<1	1	0	0
Astraeus	4	2	0	0	0	0	0	0
Fuscoboletinus	0	0	0	0	11	9	<1	2
Cenococcum	0	0	0	0	<1	2	0	0
I-type	0	0	0	0	1	1	0	0

greenhouse. In most other cases, e.g., E-strain, the frequency was higher in the greenhouse than in the field. Fuscoboletinus only forms mycorrhizae with species of larch which explains its absence on jack pine (Miller, 1977).

The short roots of jack pine planted at Mildred Lake in the mature jack pine stand were 54 ± 19% (x ± SD) ectomycorrhizal with 11 species of fungi. The dominant fungus was a species of Suillus which infected 23 ± 23% of the short roots and occurred on eight of the 10 seedlings. The second most common fungus was a species of Tomentella which formed cystidial mycorrhizae. It occurred on seven seedlings and infected 12 ± 18% of the short

roots. Also present were Cenococcum geophilum (2 ± 4%, five plants) and a species of Lactarius (6 ± 9%, seven plants). Green mycorrhizae as formed by Lactarius deliciousus (L.: Fr.) S.F. Gray (Molina and Trappe, 1982) and L. paradoxus Beardslee and Burlingham (Danielson, 1984) occurred on two seedlings.

Clearly ectomycorrhizal development occurred much more rapidly in the jack pine stand than in the reconstructed soil and the fungi responsible were largely different. Nonetheless, no differences were apparent in the growth of the host plants during the first growing season. Of considerable interest is that Suillus and Tomentella formed so many mycorrhizae on the seedlings in the pine stand, whereas in a previous study, they had been shown to be rare in the same jack pine stand (Danielson, 1984). In fact, the seedlings were planted within 15 cm of the sampling points used in the earlier study.

This over-representation of certain species has been found to occur in birch seedlings planted under an 11-year old birch tree (Fleming, 1983). Fleming attributed the success in colonization of the late successional stage fungus Lactarius to the presence of mycelial strands. This could explain the success of Suillus which is a multi-stage fungus (Danielson, 1984) and produces numerous mycelial strands. Tomentella occurs rarely in an ectomycorrhizal state on mature pine and it is curious how it manages to have enough inoculum potential to infect seedlings planted in a stand dominanted by Lactarius and Tricholoma (Danielson et al., 1984a).

From the two experiments, it can be concluded that the greenhouse bioassay technique overestimated the rate of ectomycorrhizal development and underestimated the number of species present. One species, Fuscobolitinus sp., was favoured by field conditions, whereas others, e.g., the E-strain, were favoured by greenhouse conditions. Nonetheless, the species found with the greenhouse bioassay accurately reflected those eventually developing on reconstructed soils (Part 4.1). However, if soils that contain large amounts of inoculum (e.g., clear-cuts) or those having living ectomycorrhizal hosts on them are used, the bioassay may not reflect the mycorrhizal potential in the field.

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3. INOCULATION: FERTILIZER AND REARING CONDITIONS

How can the rearing procedures of container-grown conifers be altered to enhance mycorrhization?

3.1 SHOOT, ROOT AND ECTOMYCORRHIZAL DEVELOPMENT OF CONTAINER-GROWN JACK PINE SEEDLINGS ARTIFICIALLY INOCULATED AND GROWN UNDER FOUR FERTILIZER REGIMES

3.1.1 Abstract

Jack pine seedlings were grown in 150 cc containers and fertilized with a soluble fertilizer (15:15:18 NPK) three times weekly using concentrations of 100, 200, 400 and 800 mg L^{-1}. The highest concentration exceeded operational levels currently being used in Alberta. Additional seedlings were also grown in 65 cc containers and fertilized with 200 mg L^{-1} of the same fertilizer. The seedlings were either left uninoculated or inoculated with a solid carrier inoculum of Astraeus hygrometricus, Amphinema byssoides, Hebeloma sp., Lactarius paradoxus or an E-strain fungus. The E-strain formed ectomycorrhizae at all fertilizer levels although the rate was reduced when the highest level of fertilizer was used. Hebeloma sp. and Lactarius paradoxus converted 48% or greater of the short roots to ectomycorrhizae when 100 or 200 mg of fertilizer was applied, 20 and 36% respectively when 400 mg was used, and none when 800 mg was used. Astraeus hygrometricus and Amphinema byssoides performed poorly but formed some ectomycorrhizae even though basidiospores and a homogenized fruitbody were used as inoculum in addition to the mycelium. Nonmycorrhizal seedlings reached maximum shoot weights with the 200 mg fertilizer rate but 400 mg was required by ectomycorrhizal plants to reach maximum weights. Increasing the container size resulted in increased plant weights with little effect on ectomycorrhizal development. Ectomycorrhizal seedlings had reduced lateral root lengths and increased numbers of short roots per unit length of lateral root.

3.1.2 Introduction

Increasing amounts of seedlings are being grown commercially in containers under greenhouse conditions where the potential exists for the introduction of mycorrhizal inoculum. One of the major factors possibly limiting inoculations is the restrictions imposed by the fertilizer levels used in greenhouse operations. Too much fertilizer and mycorrhizae formation may be suppressed and, too little fertilizer

and the seedlings may be below standard in size. It is also apparent that mycorrhizal fungi differ in their abilities to initiate mycorrhizal infections under moderate to high fertilizer regimes (Danielson et al., 1984a). Whether seedlings mycorrhizal with fungi able to tolerate high fertilizer levels or those only tolerant to low levels would ultimately be more beneficial when outplanted can only be determined once tolerance levels are established for a range of fungi. At present only a very few species have been successively tested and a majority of the information on fertilizer-inoculation interactions is based on a single fungus.

The objectives of this study were to (1) determine the effect of fertilizer regimes, ranging from low to operational, on the ectomycorrhizal development of container-grown jack pine (Pinus banksiana Lamb.) seedlings inoculated with five known ectomycorrhizal fungi; (2) determine the effects of ectomycorrhizae and fertilizer levels on shoot and root dry matter production of the jack pine seedlings, (3) determine the effects of fertilizer levels and ectomycorrhizae on lateral root lengths, total numbers of short roots and frequency of short root initiation, (4) to determine the nitrogen content of jack pine needles of ectomycorrhizal and nonmycorrhizal plants, and (5) to determine the effect of container size on seedling growth using one moderate level of fertilization.

3.1.3 Materials and Methods

3.1.3.1 Ectomycorrhizal Fungi. Five ectomycorrhizal fungi, all confirmed as associates of jack pine in monoxenic culture and potentially of use in inoculation programs, were used in this study. Astraeus hygrometricus (Pers.) Morgan RMD 2186, Hebeloma sp. RMD 2657, Lactarius paradoxus Beardslee & Burlingham RMD 2454, and an E-strain anamorph R 947, have been used in a previous study where details of their origin are described (Danielson et al., 1984b). Amphinema byssoides (Fr.) J. Erikss. RMD 3140 was isolated from a fruitbody on a black spruce log in a muskeg bog in the Fort McMurray area of northeastern Alberta, September 10, 1981. Voucher specimens and cultures of

the five fungi are on deposit at the Biosystematics Research Institute, Agriculture Canada, Ottawa, Canada.

3.1.3.2 Inoculum Preparation. Inoculum was prepared as described by Danielson et al. (1984b) in a peat-vermiculite mixture (1:15,v:v) in glass tubes containing 150 cc of substrate moistened with modified Melin-Norkrans (MMN) solution (Marx and Bryan, 1975). The tubes were inoculated with mycelial slurries, incubated for 3 months, the inoculum checked for contamination and viability on potato dextrose agar and the inoculum washed in cold tapwater. This inoculum was incorporated at a ratio of 1:9 (v/v) into the growing medium, a 1:1(v/v) mixture of peat and vermiculite.

In that Amphinema byssoides had failed to initiate mycorrhizal formation when peat-vermiculite inoculum was used previously (Danielson et al., 1984b), an attempt was made to double inoculate the A. byssoides cells. Fruitbodies of A. byssoides were collected in a mixed Picea-Populus stand (RMD 3192 and 3195), homogenized in water and an aliquot injected into each cell when the seedlings were 6 weeks old.

Problems were also encountered with Astraeus hygrometricus as no ectomycorrhizae were present as indicated by an inspection after 10 weeks growth. The same isolate had been previously used successfully (Danielson et al., 1984b) so a basidiospore suspension was made to try to salvage the A. hygrometricus phase of the study. Basidiospores were removed from fruitbodies which had been collected 6 years previously and stored at room temperature and were added to water (1.5 g in 500 mL) and wetted with Tween 20. Ten milliliters of suspension was injected into each 150 cc cell and 5 mL to each 65 cc cell when the seedlings were 12 weeks old.

3.1.3.3 Containers. The containers used for the fertilizer rate comparisons were 150 cc volume Leach Cone-tainers (Ray Leach, Canby, OR). For the container-size comparison, 65 cc Leach Cone-tainers were used at a single fertilizer level. The containers were packed with the appropriate growing media and each cell planted with a single jack pine germinant. The inoculation treatments then consisted of five fungi plus an uninoculated control for each container size.

3.1.3.4 Fertilization. In the first week a solution containing 80 mg L^{-1} of 10:42:10 fertilizer was applied uniformly to all the seedlings. In the period from 2 to 18 weeks the fertilizer treatments applied to 150 cc containers were 100, 200, 400 and 800 mg L^{-1} of soluble fertilizer (15:15:18 complete Plant Prod Soilless Feed with Fe EDTA and micro- nutrients) made up in deionized water. These rates can also be expressed as 15, 30, 60 and 120 mg N L^{-1}. Only the 200 mg L^{-1} level was used with the small containers.

All containers were fertilized thrice weekly until the solutions dripped freely from the drain holes. Once per week, Sequestrene 330 (56 mg L^{-1}) was added to all treatments to prevent Fe deficiencies. The overall design of the fertilizer rate experiment was six inoculation treatments (five fungi and one control) x four fertilizer levels. In addition, the six inoculation treatments were also used in small containers at one fertilizer rate. Ten replicate cells of each treatment were prepared.

3.1.3.5 Greenhouse Conditions. The seedlings were grown in a greenhouse in the period between September 6 and January 24 for a total of 20 weeks. The photoperiod was extended to 20 h with a minimum of 3 klx (65 μEm^{-2} sec^{-1}, 16 Wm^{-2}) provided by Gro-lux bulbs. Temperatures ranged from 18 to 25^{o}C. The seedlings were grouped according to treatments and moved once a week to prevent bias according to location in the greenhouse.

3.1.3.6 Shoot and Root Evaluations. When the seedlings were 10 and 12 weeks old, height measurements were made. The normal pattern of height growth of jack pine is for growth to start when the seedlings are about 5 to 6 weeks old and to halt when between 10 and 12 weeks old (Danielson, unpubl. data). The height measurements were made to confirm these observations.

When 20 weeks old, the 10 replicates were harvested and shoot height and number of branches per seedling determined. The shoots were removed dried at 80^{o}C and weighed. The root systems were washed free of growing medium and cut up into 2 to 4 cm long segments to permit sampling for ectomycorrhizae.

Segments were randomly selected until 300 short roots had been rated (the counted sample) as to their being nonmycorrhizal or converted to ectomycorrhizae. The roots were examined using 12x magnification of whole mounts to detect obscure mantle development and Hartig nets. The presence of either or both constituted an ectomycorrhizal structure, the basic unit of infection being the short root.

3.1.3.7 Root Characteristics. The total number of short roots per seedling for all treatments was calculated by using the ratio of dry weight between the counted sample with 300 short roots and the weight of the remainder of the root system. The possible effect of ectomycorrhizal status and fertilizer levels on the length of long lateral roots (sensu Sutton, 1980) was determined by measuring the laterals in the counted sample and converting to total seedling lengths by using the ratios of weights as above. The fertilizer effect was determined using the control and the E-strain treatments only (i.e. nonmycorrhizal versus heavily ectomycorrhizal). The possible effects of species of ectomycorrhizal fungi on lateral root length was determined using only the 200 mg L^{-1} fertilizer level.

The frequency of short root initiation (number of short roots per length of long lateral root) was determined by using the ratios of short roots per lateral root length in the counted samples. In a similar way, the number of short roots per unit weight of root was calculated. Both of these parameters may indicate fundamental, nonmycorrhizal changes in the root system of jack pine induced by either species of ectomycorrhizal fungi or fertilizer levels.

3.1.3.8 Foliar Nitrogen Determinations. Total nitrogen content of the needles of plants heavily ectomycorrhizal (E-strain treatment) or nonmycorrhizal (control treatment) were determined using the microkjeldahl technique (Allen, 1974).

3.1.4 Results

3.1.4.1 Ectomycorrhizal Development. The E-strain fungus was the most aggressive ectomycorrhizal fungus of the five species tested (Table 1).

Table 1. Effect of fertilizer levels and container size on ectomycorrhizal development of jack pine seedlings inoculated with five species of ectomycorrhizal fungi.[1]

Inoculation Treatment	Fertilizer level (mg L^{-1})				
	100	200[2]	200	400	800
	Percent of short roots ectomycorrhizal				
Control	0	0	0	6	0
Astraeus hygrometricus	6[b]	0	1[a]	0	0
Amphinema byssoides	19[a]	16[a]	8[a]	0	0
Hebeloma sp.	98[b]	87[b]	48[a]	20[a]	0
Lactarius paradoxus	84[b]	65[ab]	64[a]	36[a]	0
E-strain	100[b]	100[b]	100[b]	100[b]	36[a]

[1] Data analysed by Kruskal-Wallis test and post hoc comparisons. Values in each row not followed by the same letter do not differ significantly ($p = .05$).

[2] Grown in 65 cc containers, all others in 150 cc containers.

It infected all the short roots except when the highest level of fertilizer was used. Nonetheless, heavy fertilization decreased ectomycorrhizal development by E-strain as indicated by the amount of extramatrical hyphae, dichotomous branching of the ectomycorrhizae and mantle development. The number of tips per short root were 1.08, 1.26, 1.08 and 1.00 for the 100, 200, 400 and 800 mg L^{-1} treatments respectively. Extramatrical hyphae were common with the two low levels of fertilizer, sparse (not visible to the unaided eye) when 400 mg L^{-1} was used and nearly non-existent at the highest fertilizer level. Even when a dissecting microscope was used for examination of plants grown

with 800 mg L^{-1} it was nearly impossible to determine which short roots had been converted to ectomycorrhizae. Mantle hyphae were absent and many ectomycorrhizae had root hairs, situations not observed when less fertilizer was used. At the three lower levels all the individual seedlings were infected whereas with the highest level, only 6 of the 10 replicate seedlings had E-strain ectomycorrhizae.

Hebeloma sp. and Lactarius paradoxus both formed no mycorrhizae when 800 mg L^{-1} of fertilizer was applied but formed some when 400 mg L^{-1} was used. Although the mean number of short roots infected at this level were similar for the two species, the patterns of infection were different. Lactarius paradoxus formed ectomycorrhizae with 9 of 10 seedlings whereas Hebeloma sp. only infected 2 of 10 seedlings. Once Hebeloma sp. initiated infection, it appeared to be highly successful at spreading through the remainder of the root system.

The other two species of fungi tested were poor performers in all fertilizer treatments although there was a tendency to form less ectomycorrhizae with 200 mg L^{-1} fertilizer than with 100 mg L^{-1}. Peat-vermiculite inoculum of A. hygrometricus was ineffective and it is assumed that the ectomycorrhizae formed resulted from basidio- spores as previously uninoculated seedlings became mycorrhizal when basiodispores were added. The source, fruitbody slurry or peat- vermiculite inoculum, for A. byssoides cannot be determined with certainty.

During the mycorrhizal evaluations it was noted that some roots appeared to have been fed upon by insects. No animals were seen but sometimes extensive portions of the ectomycorrhizae and lateral roots had been decortinated. The damage appeared to be caused by chewing as discrete patches of the cortex was often missing. A small amount of damage was seen on seedlings with Hebeloma sp. (100 mg L^{-1}) and L. paradoxus (100 and 200 mg L^{-1}). However, in the E-strain treatment fertilized with 200 mg L^{-1}, most of the short roots as well as the laterals were decortinated. Among the other E-strain treatments, only the 100 mg L^{-1} treatment had a few damaged roots. No damage was seen in any other treatments.

The control treatments were free of ectomycorrhizae except for one seedling. The fungus responsible for the infections was determined to be Thelephora terrestris Ehrh.:Fr.

A single fruitbody of Hebeloma sp. was produced in a Hebeloma sp. inoculated tube in the 400 mg L^{-1} treatment in the 18th week.

3.1.4.2 Shoot and Root Dry Weight Production. In the large containers, there was a clear response of shoot production in all inoculation treatments to increased fertilizer rates (Table 2). In all cases there was a very large increase in shoot weight (2 to 3-fold) when the fertilizer concentration was increased from 100 mg L^{-1} to 200 mg L^{-1}. In nonmycorrhizal plants and those with small quantities of ectomycorrhizae (A. hygrometricus and A. byssoides), maximum shoot weights were obtained with 200 mg fertilizer per liter. However with the Hebeloma sp., Lactarius paradoxus and E-strain inoculation treatments about 400 mg L^{-1} of fertilizer was required to reach maximum shoot weights.

Root weights were much less affected by the amount of fertilizer than were shoot weights. In the large containers there were no significant differences due to fertilizer concentration except between the 100 and 800 mg L^{-1} treatment with L. paradoxus inoculation treatment and the 100 and 400 mg L^{-1} treatment with the E-strain. There were no significant effects on root weights when 200 to 800 mg L^{-1} fertilizer was applied except for A. hygrometricus.

Increasing the container size from 65 to 150 cc resulted in about a 50% increase in shoot weights of both nonmycorrhizal and mycorrhizal plants when 200 mg L^{-1} of fertilizer was applied. Container size did not significantly affect root weights except in the E-strain inoculation treatment where root weights in the small containers were unusually low.

Inoculation with Hebeloma sp., Lactarius paradoxus and E-strain resulted in significantly reduced shoot weights with the low fertilizer level in the large containers and in the small containers as compared to the nonmycorrhizal control (Table 3). The E-strain also significantly reduced shoot weight when 200 mg L^{-1} was applied but it did not affect growth above that level.

There were no effects on root growth in the large containers when 200 mg L^{-1} or more fertilizer was applied. In the small containers E-strain significantly reduced root weights as compared

Table 2. Effect of fertilizer level on shoot and root growth of mycorrhizal and nonmycorrhizal container-grown jack pine seedlings after 20 weeks in the greenhouse.[1]

Inoculation Treatment	Plant part	Fertilizer Level ($mg.L^{-1}$) 100	200[2]	200	400	800
		Dry weight (g)				
Control	Shoot	$.76^{a}$	$.93^{a}$	1.35^{b}	1.40^{b}	1.60^{b}
	Root	$.45^{a}$	$.45^{a}$	$.45^{a}$	$.42^{a}$	$.47^{a}$
Astraeus hygrometricus	Shoot	$.64^{a}$	$.87^{a}$	1.50^{b}	1.56^{b}	1.55^{b}
	Root	$.52^{ab}$	$.42^{ab}$	$.65^{b}$	$.51^{ab}$	$.41^{a}$
Amphinema byssoides	Shoot	$.57^{a}$	$.85^{b}$	1.50^{c}	1.59^{c}	1.51^{c}
	Root	$.59^{a}$	$.41^{a}$	$.54^{a}$	$.55^{a}$	$.40^{a}$
Hebeloma sp.	Shoot	$.48^{a}$	$.65^{a}$	1.36^{b}	1.81^{bc}	2.05^{c}
	Root	$.43^{ab}$	$.42^{a}$	$.59^{ab}$	$.62^{b}$	$.59^{ab}$
Lactarius paradoxus	Shoot	$.55^{a}$	$.70^{a}$	1.18^{b}	1.55^{bc}	2.00^{c}
	Root	$.39^{ab}$	$.37^{a}$	$.49^{ab}$	$.59^{ab}$	$.56^{b}$
E-strain	Shoot	$.36^{a}$	$.49^{a}$	1.03^{b}	1.78^{c}	1.65^{c}
	Root	$.34^{ab}$	$.22^{a}$	$.52^{bc}$	$.65^{c}$	$.43^{bc}$

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

[2] Grown in 65 cc containers, all others in 150 cc containers.

Table 3. Effect of mycorrhizal inoculation on shoot and root growth of container-grown jack pine seedlings using four fertilizer regimes after 20 weeks in the greenhouse.[1]

	Fertilizer level (mg L^{-1})									
	100		200[2]		200		400		800	
Inoculation treatment	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	Dry weight (g)									
Control	$.76^{c}$	$.45^{abc}$	$.93^{d}$	$.45^{b}$	1.35^{b}	$.45^{a}$	1.40^{a}	$.42^{a}$	1.60^{a}	$.47^{a}$
Astraeus hygrometricus	$.64^{bc}$	$.52^{bc}$	$.87^{cd}$	$.42^{b}$	1.50^{b}	$.65^{a}$	1.56^{a}	$.51^{a}$	1.55^{a}	$.41^{a}$
Amphinema byssoides	$.57^{bc}$	$.59^{c}$	$.85^{cd}$	$.41^{b}$	1.50^{b}	$.54^{a}$	1.59^{a}	$.55^{a}$	1.51^{a}	$.40^{a}$
Hebeloma sp.	$.48^{ab}$	$.43^{abc}$	$.65^{b}$	$.42^{b}$	1.36^{b}	$.59^{a}$	1.81^{a}	$.62^{a}$	2.05^{a}	$.59^{a}$
Lactarius paradoxus	$.55^{b}$	$.39^{ab}$	$.70^{bc}$	$.37^{b}$	1.18^{ab}	$.49^{a}$	1.55^{a}	$.59^{a}$	2.00^{a}	$.56^{a}$
E-strain	$.36^{a}$	$.35^{a}$	$.49^{a}$	$.22^{a}$	1.03^{a}	$.52^{a}$	1.78^{a}	$.65^{a}$	1.65^{a}	$.43^{a}$

[1] Data analysed by one way ANOVA. Differences among means detected by Scheffé pairwise comparisons. Values in each column followed by the same letter not significantly different ($p = .05$).

[2] Grown in 65 cc containers, all others in 150 cc container.

to the other treatments. At the lowest level of fertilizer used, root systems infected with E-strain weighed less than those lightly infected with Astraeus hygrometricus or Amphinema byssoides. Inocu- lation with Lactarius paradoxus also resulted in lighter root systems than those inoculated with A. byssoides.

The shoot:root ratios of all the inoculation treatments in the large containers combined together were 1.2, 2.5, 2.9 and 3.6 for the 100, 200, 400 and 800 mg L^{-1} treatments, respectively. Shoot heights (to terminal bud) for the same sequence of treatments were 5.9, 9.2, 10.1 and 10.0 cm. The mean number of branches per seedling for the same treatment sequence was 1.8, 3.3, 3.8 and 4.1

3.2.4.3 Short Root Production and Lateral Root Lengths. The total number of short roots produced per seedling varied from about 2000 to over 6000 (Table 4). In the control treatment there were less short roots produced at the two higher fertilizer levels than at the two lower levels. However, this trend was less evident in the inoculation treatments, there being a difference between 100 and 200 mg L^{-1} in the large containers only with Lactarius paradoxus. There were no significant differences in number of short roots between 200 and 400 mg L^{-1} although there was a decrease between 400 and 800 mg L^{-1} in the E-strain inoculation treatment. Between the 400 and 800 mg L^{-1} levels the degree of ectomycorrhizal development by E-strain also decreased sharply (see Table 1).

The lengths of lateral roots reflected the dry weight data with there being no difference in lengths in the control due to fertilization (Table 5). In the E-strain treatment in the large containers the laterals were significantly shorter at the two lower levels than at the two higher levels. There was a clear trend in both the controls and seedlings inoculated with E-strain to initiate short roots less frequently as the fertilizer levels increased (Table 5). However, fungi less agressive than E-strain, Lactarius paradoxus and Hebeloma sp., affected short root production to a somewhat lesser degree, at least at the 200 mg L^{-1} fertilization rate (Table 6).

Table 4. Number of short roots on jack pine seedlings grown in two container sizes, exposed to four fertilizer regimes and inoculated with five ectomycorrhizal fungi.[1]

Inoculation Treatment	Fertilizer level (mg L^{-1})				
	100	200[2]	200	400	800
	Number of short roots per seedling				
Control	4347[b]	2695[a]	4183[b]	2254[a]	2072[a]
Astraeus hygrometricus	6578[b]	4568[a]	6637[b]	ND[3]	ND
Amphinema byssoides	6649[b]	3864[a]	6126[b]	ND	ND
Hebeloma sp.	2715[ab]	1860[a]	4558[b]	3918[b]	ND
Lactarius paradoxus	1976[a]	2463[ab]	3449[b]	3439[b]	ND
E-strain	2638[abc]	1516[a]	3070[bc]	4037[c]	2095[ab]

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

[2] Grown in 65 cc containers, all others in 150 cc containers.

[3] ND = not determined

The lateral root lengths as affected by ectomycorrhizae did not follow the same pattern as was observed for root weights. In both container sizes, the length of the lateral roots in the E-strain treatment were significantly shorter than in any other inoculation treatments (Table 7). Inoculation with Hebeloma sp. and Lactarius paradoxus also reduced root length in comparison with the control and the lightly infected treatments. The only difference in root weights in the 200 mg L^{-1} treatments was that E-strain in small containers reduced root weight in relation to the other treatments (see Table 3).

Table 5. Effects of fertilization and ectomycorrhizal formation by an E-strain fungus on lateral root lengths and frequency of short initiation of container-grown jack pine seedlings

Inoculation Treatment	Fertilizer level (mg L^{-1}) 100	200[2]	200	400	800
	Length of lateral roots per seedlings (cm)				
Control	1204^{cde}	966^{bcd}	1491de	1430de	1361de
E-strain	557^{b}	316^{a}	744^{bc}	1366de	1772^{e}
	Number of short roots per centimeter lateral root				
Control	3.6^{ab}	2.8^{b}	2.8^{b}	1.6^{c}	1.5^{c}
E-strain	4.7^{a}	4.8^{a}	4.1^{ab}	3.0^{b}	1.3^{c}

[1] Data analysed by two-way ANOVA and differences among means determined by Scheffé pairwise comparisons. Values within each set followed by the same letter are not significantly different ($p = .05$)

When short root production of nonmycorrhizal plants was considered on the basis of root weight, the number of short roots per unit weight decreased with increasing fertilizer levels (Table 8). However, there were no significant differences in short roots among fertilizer treatments in the large containers of any of the inoculated seedlings.

3.1.4.4 Nitrogen Content of Needles. As expected, total foliar N increased as the amount of N applied increased (Table 9). Mycorrhizal infection appeared to have little influence on levels of N in the needles. Nitrogen levels continued to increase between the 400 and 800 mg L^{-1} fertilizer rates although shoot growth did not increase (see Table 2).

Table 6. Effect of different ectomycorrhizal fungi and container size on the frequency of short root initiation of container-grown jack pine seedlings fertilized three times weekly with a solution of 200 mg L^{-1} of 15:15:18 fertilizer.[1]

Inoculation Treatment	Container size (cm^3)	
	65	150
	Number of short roots per centimeter lateral	
Control	2.8^{c}	2.8^{c}
Astraeus hygrometricus	4.0^{ab}	2.9^{bc}
Amphinema byssoides	3.5^{abc}	2.6^{c}
Hebeloma sp.	3.1^{bc}	3.3^{abc}
Lactarius paradoxus	4.8^{a}	3.6^{abc}
E-strain	4.8^{a}	4.1^{ab}

[1] Data analysed by two-way ANOVA and differences among means determined by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly (p = .05).

3.1.5 Discussion

The highest level of fertilizer applied exceeded that used in some operational nurseries. The Syncrude industrial nursery fertilizes with a solution of 20:20:20 at a rate of 500 mg L^{-1} applied twice a week (B. Fessenden, Alberta Research Council, pers. comm.). In terms of N, this amounts to 100 mg L^{-1} as compared to 120 mg L^{-1} used in this study. In addition, the plants in this study were fertilized three times a week ensuring that the high rate was clearly in or exceeded operational ranges. Despite this, the E-strain fungus was able to infect and form ectomycorrhizae with jack pine at operational levels, albeit of reduced morphological distinctiveness and quantity. Few other fungi have formed mycorrhizae with conifers at operational levels, these being Laccaria laccata (Scop.:Fr.) Berk. ê Br. (Molina and Chamard, 1983), Cenococcum geophilum Fr.,

Table 7. Effect of different ectomycorrhizal fungi and container size on lateral root length of container-grown jack pine seedlings fertilized three times weekly with a solution of 200 mg L^{-1} of 15:15:18 fertilizer.[1]

Inoculation Treatment	Container size (cc) 65	150	Row means
	Length of lateral root per seedling (cm)		
Control	966	1491	1200[c]
Astraeus hygrometricus	1082	2161	1529[c]
Amphinema byssoides	1093	2318	1592[c]
Hebeloma sp.	588	1399	907[b]
Lactarius paradoxus	519	968	708[b]
E-strain	316	744	484[a]
Column means	692[a]	1400[b]	

[1] Data analysed by two-way ANOVA and differences among means determined by Scheffé pairwise comparisons. Values followed by the same letter do not differ significantly ($p = .05$).

Piloderma croceum (Bres.) Erikss. ê Hjortz. and Thelephora terrestris (Kropp, 1982).

Lactarius paradoxus and Hebeloma sp. were more sensitive to high fertilizer levels and it is likely that they would not form mycorrhizae under commercial conditions. The same probably applies to Pisolithus tinctorius (Pers.) Coker ê Couch but Laccaria proxima Boudier appears to be only slightly less sensitive to high nutrient levels than is the E-strain (Danielson et al., 1984b).

It is apparent that fungi are available to use under nursery conditions but whether these will prove to be the best performers in the field remains to be determined. In a field trial on the Syncrude dyke the E-strain was the most successful fungus introduced after three

Table 8. Number of short roots per unit root weight of jack pine seedlings grown in two container sizes, exposed to four fertilizer regimes and inoculated with five ectomycorrhizal fungi.[1]

Inoculation Treatment	Fertilizer level (mg L^{-1})				
	100	200[2]	200	400	800
	Number of short roots per mg root weight				
Control	9.7^{c}	6.1^{ab}	9.3^{bc}	5.3^{a}	4.4^{a}
Astraeus hygrometricus	12.1^{a}	10.2^{a}	9.7^{a}	ND[3]	ND
Amphinema byssoides	11.3^{a}	9.5^{a}	9.4^{a}	ND	ND
Hebeloma sp.	6.3^{ab}	4.4^{a}	7.7^{b}	6.5^{ab}	ND
Lactarius paradoxus	5.1^{a}	6.7^{a}	7.0^{a}	5.8^{a}	ND
E-strain	7.8^{a}	6.7^{a}	5.9^{a}	6.2^{a}	5.4^{a}

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

[2] Grown in 65 cc containers, all others in 150 cc containers.

[3] ND = not determined.

growing seasons (Part 4.1). Hebeloma sp. also persisted for 2 years whereas both Astraeus hygrometricus and Lactarius paradoxus did not persist in the reconstructed soil. It is apparent that performance in the greenhouse is not an indicator of field performance.

With regard to the ectomycorrhizae assessments of nursery grown stock the results with E-strain indicate that great care must be taken to detect all ectomycorrhizae. Increasing fertilizer levels resulted in strong reductions in extramatrical and mantle mycelium making evaluations difficult. This has also been shown to occur with Laccaria proxima (Danielson et al., 1984a) and probably also occurs with most or all other ectomycorrhizal fungi. Unless roots exposed to

Table 9. Nitrogen content of the needles of container-grown jack pine seedlings which were grown in two sizes of containers with four fertilizer regimes and ectomycorrizal with E-strain or nonmycorrhizal.[1]

	Fertilizer level (mg L^{-1})				
Inoculation Treatment	100	200[2]	200	400	800
	Total Nitrogen (% $\pm$ SD)				
Control	1.46 $\pm$ .31	1.40 $\pm$ .15	2.05 $\pm$ .32	2.16 $\pm$ .23	2.41 $\pm$ .24
E-strain	1.42 $\pm$ .21	2.17 $\pm$ .40	1.80 $\pm$ .22	1.91 $\pm$ .34	2.31 $\pm$ .40

[1] Grown in 65 cc containers, all others in 150 cc containers.

high fertilizer levels are examined using high magnifications, the effects of fertilization may be misinterpreted.

Seedlings grown in large containers with none or few ectomycorrhizae reached maximum size with thrice weekly applications of 200 mg L^{-1} 15:15:18 fertilizer (30 mg N L^{-1}). When a substantial portion of the short roots were ectomycorrhizal, double that amount of fertilizer was required for seedlings to reach maximum size. However, even with heavy infection by the E-strain, shoot weights exceeded 1 g, a size well above the 0.67 g ideal recommended by Carlson (1979). Under the lighting regime currently in use, 200 mg L^{-1} of fertilizer is a good level, both resulting in large seedlings and heavy ectomycorrhizal formation by the aggressive fungi.

The reason for the failure of the vegetative inoculum of Astraeus hygrometricus to be effective is unknown. The same isolate has been used successfully several times previously (e.g. Danielson et al., 1984b). The abrupt failure of this isolate illustrates one of the potential problems of using artificial inoculations on a large scale. The continued ineffectiveness of mycelial inoculum of Amphinema byssoides (Danielson et al., 1984b) indicates that much more information is required on inoculum potential, host resistance and microbial antagonism before inoculation procedures can become routine operations.

The most sensitive parameters of root development were lateral root length and frequency of short root initiation per unit length of lateral root. Neither total number of short roots or number of short roots per unit root weight were good indicators of treatment induced changes in the root system. The fact that mycorrhizal infection can change both the length of lateral roots (negative effect) and the frequency of short roots (positive effect) could affect the efficiency of nutrient uptake of low mobility ions. Whether these effects would be realized in soils remains to be determined but are worth considering and remain hidden if only root weights are measured.

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3.2 ECTOMYCORRHIZAL FORMATION OF CONTAINER-GROWN JACK PINE USING PEAT-VERMICULITE AND TERRAGREEN AS GROWING MEDIA.

3.2.1 Abstract

Jack pine seedlings were grown in containers with either peat-vermiculite or an inorganic (Terragreen) growing medium to determine the influence of medium on ectomycorrhizal development. The fungi used included two species which readily form ectomycorrhizae in containers, an E-strain fungus and Hebeloma sp., and two species that have failed to form ectomycorrhizae in open containers, Amphinema byssoides and Suillus tomentosus. The seedlings grew poorly in the Terragreen due to low water holding capacity. The E-strain and Hebeloma sp. formed ectomycorrhizae in both media with a higher percentage of the short roots being converted to ectomycorrhizae using E-strain with the peat-vermiculite and the opposite for Hebeloma sp. Suillus tomentosus and Amphinema byssoides did not form any ectomycorrhizae in either medium.

3.2.2 Introduction

The repeated failures to successfully initiate ectomycorrhizal infections of container-grown jack pine seedlings using certain fungi has prevented a full-scale assessment of the efficacy of ectomycorrhizal fungi. Species of Suillus and the related genera, Fuscoboletinus and Rhizopogon, have usually failed to infect roots of what should be susceptible hosts in containers (Molina, 1980; Riffle and Tinus, 1982; Ivory and Munga, 1983; Danielson, 1984). Tricholoma is another genus in which mycorrhizal formation with container-grown hosts is poor or nil (Shaw et al., 1982; Danielson et al., 1984a) as well as Hydnum imbricatum L.: Fr. and Amphinema byssoides (Fr.) J. Erikss. (Danielson et al., 1984a).

The cause of the failure of these fungi to initiate mycorrhizal formation is unknown but it does not appear to be due to the use of excessive nutrients as spore suspensions of Suillus tomentosus (Kauff.) Sing., Snell & Dick, and Amphinema byssoides can be used successively (Danielson, unpubl. data). Indeed with certain species and under some conditions, successful inoculations using mycelium have been performed. Rhizopogon luteolus Fr. was successful in steamed or

fumigated soil (Theodorou, 1967) or autoclaved soil (Theodorou and Bowen, 1970). Suillus granulatus (L.: Fr.) S.F. Gray was successfully introduced into autoclaved soil (Theodorou and Bowen, 1970) and Suillus (Boletus) plurons into nursery transplant beds (Mikola, 1970). A possible factor that is responsible for the failures is the presence of an antagonistic microflora and that some fungi are more susceptible to antagonism than others. This has been suggested by Theodorou (1967) but direct evi- dence is lacking. The strongest evidence for an antagonistic mechanism is that all the fungi mentioned above readily form ectomycorrhizae in monoxenic cultures.

If microbial antagonism is a factor affecting inoculations then it might be possible to induce shifts in the fungal and bacterial populations or generally reduce microbial activity and allow the desired fungi to initiate infections. As a first step in altering microbial activity the standard growing medium used in rearing container-grown seedlings was changed. Terragreen, a calcined montmorillonitic clay, was substituted for the peat-vermiculite mixture to eliminate all organic materials in the root zone except for root exudates. It was assumed that the use of an inorganic substrate would reduce the number of fungal and bacterial species and also reduce the activity of those species present.

3.2.3 Materials and Methods

The growing media were a 1:1 (V/V) mixture of autoclaved peat and vermiculite and Terragreen sieved to exclude particles less than 1 mm. The containers were 150 cc Leach Cone-tainers (Ray Leach, Canby, OR) with ten replicates of each inoculation treatment being prepared so that five replicates could be harvested just prior to inoculation and five at the end of the experiment. The fungi used were two species with which successful inoculations had been performed, E-strain R 947 and Hebeloma spp. RMD 2657 (Danielson et al., 1984a) and two fungi which had failed to form ectomycorrhizae, Amphinema byssoides RMD 3140, and Suillus tomentosus RMD 3138 (Danielson et al., 1984a, Danielson, 1984). Also included was an uninoculated control for a total of five inoculation treatments.

Each cell was planted with one jack pine (Pinus banksiana Lamb.) germinant and the plants were grown in a greenhouse (December 8 to March 9) with a 20 h extended photoperiod (minimum light 3 klx, 65 μEm^2sec^{-1} or 16 wm^{-2}) and temperatures ranging from 18-24°C. All cells were fertilized twice weekly to saturation with a fully soluble fertilizer, 15:15:18 Plant Prod Soilless Feed at a concentration of 200 mg L^{-1}. Iron, in the form of Sequestrene 330, was added at 4 and 8 weeks at a rate of 56 mg L^{-1}.

After the plants were 8 weeks old, five replicates were harvested from each inoculation treatment and plant size, number of short roots, and substrate pH determined. The other five replicates were inoculated with a mycelial slurry using liquid cultures rather than plate cultures as described by Danielson et. al. (1984b). Three 1 L flasks containing 150 mL MMN solution (Marx, 1969) were prepared for each fungus. The fungi were grown on MMN plates and colonies were cut out and added to 150 mL MMN in a Virtis homogenizer jar and the colonies fragmented for 15 to 30 sec. This suspension was added to the flasks and the cultures grown for 5 (E-strain and Hebeloma sp.) or 10 days (A. byssoides and S. tomentosus) on a reciprocal shaker. At the end of the incubation period the liquid cultures were centrifuged at 15,000 RPM for 10 min and the supernatent decanted off. The mycelium was washed by adding distilled water, shaking the suspension and leting it sit for 10 min before centrifuging again. After decanting, the volume was brought up to 100 mL and the mycelium mascerated in the Virtis homogenizer. Twenty milliliters of the slurries were injected into each cell except the control cells.

Five weeks after inoculation, the seedlings were harvested and plant growth assessed as before and the ectomycorrhizal development determined. Each root system was scanned and if any ectomycorrhizae were present, 300 randomly selected short roots were evaluated as to whether they were converted into ectomycorrhizae or not.

3.2.4. Results

At the time of inoculation the seedlings were growing rapidly in the peat-vermiculite medium and much slower in the Terragreen (Table 1). However, both sets of plants had a large number of short

roots (potential infection sites) with no difference between growing media. There was also no significant ($p = .05$, Wilcoxon t test) difference in media pH at 8 weeks with the peat-vermiculite at pH 5.7 and Terragreen at pH 5.4

After a total of 13 weeks, the shoot weights in all five inoculation treatments in peat-vermiculite were greater than those in Terragreen (Table 2). Inoculation had no significant effect in the peat-vermiculite but inoculation in Terragreen with both E-strain and Hebeloma sp. resulted in significantly larger shoots than in the control. Root weights were also much smaller in the Terragreen than in the peat-vermiculite and only Hebeloma sp. caused a significant increase in root weight (Table 3). The total number of short roots per seedling was significantly greater (3934 verses 764) in the peat-vermiculite than in the Terragreen ($p = .05$, ANOVA, Scheffé).

No ectomycorrhizae were formed in the control treatment or on seedlings inoculated with Amphinema byssoides or Suillus tomentosus using either growth medium (Table 4). The effect of growth medium on ectomycorrhizal formation was dependent on the fungus used as infection was higher with the peat-vermiculite than Terragreen when E-strain was used and the converse was true when Hebeloma sp. was used. E-strain was more aggressive than Hebeloma sp. in both growth media. Although ectomycorrhizal formation was high in the peat-vermiculite, the characteristic chlamydospores were relatively uncommon.

3.2.5 Discussion

Growth of jack pine was poor in the Terragreen growing medium although Plenchette et al. (1982) considered it a good medium for VA mycorrhizal studies. However, the moisture holding capacity of the material is poor and thus either large containers must be used or very frequent watering must be done to provide sufficient nutrients for reasonable plant growth. With coarse rooted species such as conifers, continuous or very frequent fertilization would be necessary during the early stages of growth while the root system is small. The positive response of jack pine to inoculation by E-strain and Hebeloma sp. in the Terragreen probably was due to enhanced nutrient uptake via the mycelium as inoculation of container-grown seedling almost always

Table 1. Size and condition of the root system of 8 week old jack pine seedlings prior to being inoculated.[1]

Parameter	Growth Media	
	Peat:Vermiculite	Terragreen
Height (cm)	4.1[b]	2.7[a]
Shoot dry weight (mg)	108[b]	38[a]
Root dry weight (mg)	44[b]	29[a]
No. of short roots/seedling	260[b]	221[b]

[1] Differences between means determined with the Wilcoxon t test.

Table 2. Shoot weight of 13 week old jack pine seedlings grown in two growing media after being inoculated 5 weeks previously.[1]

Parameter	Growing Media	
	Peat:Vermiculite	Terragreen
	Shoot dry weight (mg)	
Control	450[c]	70[a]
E-strain	375[c]	110[b]
Hebeloma sp.	426[c]	105[b]
Amphinema byssoides	476[c]	94[ab]
Suillus tomentosus	449[c]	82[ab]

[1] Data analysed by 2-way ANOVA after ln (Y+1) transformation and differences detected by Scheffé pairwise comparisons. Values (arithmetic means) followed by the same letter do not differ significantly (p = .05).

Table 3. Root weight of 13 week old jack pine seedlings grown in two growing media after being inoculated 5 weeks previously.[1]

Inoculation Treatment	Growing Medium: Peat:Vermiculite Root dry weight (mg)	Growing Medium: Terragreen Root dry weight (mg)	Row means
Control	240	57	117[a]
E-strain	218	83	135[ab]
Hebeloma sp.	251	115	170[b]
Amphinema byssoides	277	85	154[ab]
Suillus tomentosus	308	82	159[ab]
Column means	257[b]	82[a]	

[1] Data analysed by 2-way ANOVA after ln (Y+1) transformation and differences detected by Scheffé pairwise comparisons. Values (geometric means) in each row or column followed by the same letter do not differ significantly (p = .05).

Table 4. Development of jack pine ectomycorrhizae in two growth media after being inoculated at age 8 weeks and grown an additional 5 weeks.[1]

Inoculation Treatment	Growing Medium: Peat:Vermiculite Root dry weight (mg)	Growing Medium: Terragreen Root dry weight (mg)
Control	0	0
E-strain	88[d]	64[c]
Hebeloma sp.	16[a]	43[b]
Amphinema byssoides	0	0
Suillus tomentosus	0	0

[1] Data analysed by 2-way ANOVA after ln (Y+1) transformation and differences detected by Scheffé pairwise comparisons. Values (geometric means) in each row or column followed by the same letter do not differ significantly (p = .05).

results in no response or a negative growth response (Danielson et al., 1984).

Whether peat-vermiculite or Terragreen was used as a growing medium, no ectomycorrhizae were formed when the plants were inoculated with Amphinema byssoides or Suillus tomentosus. It is still possible that microbial activity in the rhizosphere was preventing the sensitive species from colonizing the roots. If so, measures must be taken other than using an inorganic growing medium to reduce the activity of the presumbed antagonistic microflora. Or conversely, the inoculum potential of the introduced species must be increased so they can survive long enough to infect the roots. This might be accomplished by increasing the size of the inoculum units, a factor which can affect the ability of plant pathogens to infect their hosts (Garrett, 1970).

3.2.6 Literature Cited

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3.3 EFFECTS OF PLANTING MIXTURES AND INOCULUM POTENTIAL ON ECTOMYCORRHIZAL DEVELOPMENT OF CONTAINER-GROWN JACK PINE SEEDLINGS

3.3.1 Abstract

Attempts were made to introduce a wide ecological range of ectomycorrhizal fungi onto container-grown jack pine seedlings. With the exception of some weedy nursery fungi, conventional methods of inoculation failed in the past. In order to offer a broader range of fungi for field studies, the root environment was altered to favor ectomycorrhizal fungi and suppress consumers and antagonists. The type of peat used in planting media had dramatic effects on mycorrhization by Tomentella sp. but had little effect with three other fungi. A single preinoculation drench with the fungicide benomyl appeared to slightly increase the amount of mycorrhizae formed by Suillus tomentosus. Inoculum potential was manipulated by using fragmented mycelium, non-fragmented mycelium and intact cultures, all of which were applied directly to root systems. The use of intact cultures only increased success of multi-stage fungi slightly and late-stage fungi not at all. However, the use of intact cultures permitted visual observations of the inoculum and revealed that fly maggots rapidly consumed the mycelium and that Trichoderma also colonized it. Drenching the seedlings with Diazinon did not result in increased mycorrhization. Nonetheless evidence suggests that both fly larvae and Trichoderma were having adverse effects on root colonization by many of the ectomycorrhizal fungi.

3.3.2 Introduction

Although some species of ectomycorrhizal fungi will readily induce mycorrhization of nursery-grown conifer seedlings, a majority of the symbiotic fungi fail to form mycorrhizae under such conditions. The successful fungi are those thought to be early-stage fungi (sensu Mason et al. 1984) and the unsuccessful ones are multistage or late-stage (Mason et al. 1984) fungi. Prior to embarking on a tree inoculation program, it would be highly desirable to test multi- and late-stage fungi in field situations. Nursery stock in Alberta becomes

spontaneously infected with certain early-stage fungi however these fungi may not persist long enough to colonize new roots in the field (Part 2.1). It is possible that the ecologically distinct and more host specialized multi- and late-stage fungi may be better adapted to field conditions and impart more benefit to the host than the ubiquitous nursery fungi.

Past attempts have been made to introduce multi- and late-stage fungi using the conventional vermiculite-peat carrier (Part 4.1), mycelial slurries (Danielson et al. 1984) and by utilizing an inorganic growth medium (Part 3.2). All of these procedures failed to result in plant mycorrhization. Based on the hypothesis that the interfering factor(s) was of biological nature involving antagonisms or mycelial consumption by insect larvae, additional inoculation trials were attempted using modified growth media, a fungicide and an insecticide. To counterbalance host resistance and biological antagonisms, inoculum potentials (sensu Garrett, 1970) were amplified by increasing the mass of intact mycelium being introduced.

3.3.3 Materials and Methods

3.3.3.1 Experiment I. The first experiment was designed to test the effect of planting medium and the selective fungicide, benomyl, on mycorrhization by two early stage fungi, E-strain (R-947) and *Tomentella* sp. (RMD 3093), and two multi-stage fungi, *Suillus tomentosus* (RMD 3138) and *Amphinema byssoides* (RMD 3140). Two peats were used that differed in pH in hopes the most acidic peat would suppress bacterial activity. The four growing media treatments were:

1. North Carolina peat, pH 5.0, autoclaved, mixed 1:1 (V/V) with vermiculite (<2 mm).
2. The same as in (1) but drenched with a solution containing 0.55 g L^{-1} of the fungicide, benomyl, the day prior to inoculations.
3. Beaver Brand peat, pH 3.8 not autoclaved, no vermiculite.

4. Beaver Brand peat, autoclaved and reinoculated with the washings from white spruce needles collected in February and mixed 1:1 (V/V) with vermiculite (pH 3.9). Needles washings were used to introduce a microflora excluding Trichoderma; however, the peat became heavily infested with Trichoderma regardless.

All seedlings were grown in 150 cc Leach Cone-tainers with five replications per treatment. Jack pine seeds were surface sterilized in H_2O_2, germinated on PDA and one germinant planted per cell December 8, 1983. All seedlings were fertilized twice weekly with a solution containing 200 mg L^{-1} of Plant Prod 15:15:18 fertilizer. When the seedlings were 8 weeks old they were inoculated with mycelial slurries. The fungi were grown in liquid culture, fragmented, washed twice with distilled water and injected into the cells (Danielson et al., 1984a). After an additional 8 weeks growth, the roots were washed free of planting media, examined at 12X magnification and the quantity of mycorrhization of the short roots estimated. Shoots and roots were dried at 80°C and weighed.

3.3.3.2. Experiment II

This experiment consisted of a series of inoculation trials in which the inoculum potential was manipulated by fragmentation of the mycelium or placing intact cultures directly on the roots. Greenhouse conditions were similar to those in Exerpiment I and inoculum type and plant ages are indicated in the tables.

Fungal cultures were grown on MMN agar and fragmented by blending in water for 5 - 15 sec with a Virtis Homogenizer. Non-fragmented liquid cultures were washed and injected directly.

Intact cultures were prepared on 47 mm 0.2 µm Nuclepore polycarbonate filters which were sterilized and placed in the surface of MMN agar. Each filter was inoculated with an agar plug and grown until colonies were about 2 cm diameter. The seedlings were inoculated by peeling the filter off the agar, slitting the Leach Cone-tainers, pressing the colony onto exposed roots, and taping the container closed. The fate of the inoculum and mycorrhization were determined periodi-

cally by opening the container and lifting up the filter. For one test, 5 mm diameter agar plugs cut from plates were placed directly on the roots. The insecticide Diazinon was applied as a drench at one-fourth the manufacturers recommended rate (1.25 mL L^{-1}).

3.3.4 Results

The acid peat was a poor growth medium for the jack pine seedlings (Table 1). When mixed with vermiculite, growth was improved although still inferior to the less acid peat. Benomyl did not influence growth although in a preliminary test needle dieback occurred when three applications at the same concentration were given within a 12 day period. Inoculation had little or no effect on shoot growth. Root growth followed the same pattern as shoot growth with inferior growth in the acid peat.

The type of growing medium influenced mycorrhizal development (Table 2). Tomentella sp. was highly aggressive in the North Carolina peat but only infected one of the 10 seedlings grown in the more acid peat. E-strain mycorrhizae were also less frequent in the pure acid peat than in treatments utilizing vermiculite. The development of mycorrhizae by Suillus and Amphinema was erratic and generally poor. The use of benomyl appeared to increase mycorrhization by multi-stage fungi, but had no effect with either Tomentella, a basidiomycete, or E-strain, an ascomycete.

When a second isolate of Tomentella was injected in a solution containing benomyl no mycorrhizae were formed (Table 3). Fragmenting the mycelium also greatly reduced the amount of mycorrhizae. The E-strain fungus was more infective than Tomentella following fragmentation.

Mycorrhiza formation was good with non-fragmented inoculum for Coltricia perennis, I-type and E-strain fungi (Table 4). However, the late-stage fungus Leccinum aurantiacum was non-infective as was MRA. The growing medium in the MRA treatment was nearly black with mycelium.

When intact colonies were placed directly on the root system small amounts of mycorrhizae were formed by Suillus tomentosus (Table 5). No mycorrhizae were formed when non-fragmented mycelium from liquid cultures were used but small quantities developed when the

Table 1. Shoot and root weights of jack pine seedlings grown in four growing media utilizing two peat types.

Inoculation Treatment	Growing Medium Treatment			
	N.C. Peat pH 5	N.C. Peat + benomyl	Pure Peat pH 3.9	Autoclaved peat pH 3.8
	Shoot weight (g ± SD)			
Suillus tomentosus	0.34 ± 0.03	0.36 ± 0.10	0.14 ± 0.10	0.17 ± 0.05
Amphinema byssoides	0.36 ± 0.10	0.36 ± 0.11	0.19 ± 0.08	0.20 ± 0.12
Tomentella sp.	0.31 ± 0.05	0.29 ± 0.07	0.16 ± 0.05	0.27 ± 0.11
E-strain	0.26 ± 0.09	0.30 ± 0.05	0.16 ± 0.10	0.23 ± 0.05
Control	0.34 ± 0.06	0.37 ± 0.10	0.15 ± 0.08	0.26 ± 0.02
	Root weight (g ± SD)			
Suillus tomentosus	0.24 ± 0.03	0.25 ± 0.07	0.10 ± 0.08	0.13 ± 0.04
Amphinema byssoides	0.17 ± 0.06	0.22 ± 0.06	0.09 ± 0.05	0.10 ± 0.06
Tomentella sp.	0.18 ± 0.04	0.17 ± 0.06	0.08 ± 0.03	0.13 ± 0.06
E-strain	0.19 ± 0.07	0.22 ± 0.05	0.08 ± 0.05	0.10 ± 0.02
Control	0.25 ± 0.03	0.25 ± 0.09	0.10 ± 0.03	0.15 ± 0.02

Table 2. Ectomycorrhizal development of jack pine by four fungi introduced into four growth media when seedlings 8 weeks old and grown for an additional 8 weeks.

Inoculation Treatment	Growing Medium Treatment							
	N.C. Peat pH 5		N.C. Peat + benomyl		Pure Peat pH 3.9		Autoclaved peat pH 3.8	
	Mycorrhizal Infection							
	No.[1]	%[2]	No.	%	No.	%	No.	%
Suillus tomentosus	1[3]	10	3[3]	23	1	80	0	0
Amphinema byssoides	0	0	1	10	0	0	0	0
Tomentella sp.	5	100	5	100	1	10	0	0
E-strain	5	100	5[3]	99	5[3,4]	52[3]	5	98
Control	0	0	0	0	0[3,4]	0	1	1

[1] Number of seedlings with ectomycorrhizae

[2] Percent infection of the colonized seedlings only

[3] Maggots present

[4] Collembola present

Table 3. Effects of fragmentation and benomyl on inoculation and ectomycorrhizal development of jack pine seedlings.

Inoculation Treatment	Type of inoculum[1]	Seedling Age (weeks preinoc+ inoc)	Number of Seedlings[2] Mycorrhizal	Percent Short Roots Mycorrhizal
Tomentella 2437	Nonfragmented	8 + 10	5/5	80
Tomentella 2437	Fragmented	8 + 10	2/5	10
Tomentella 2437	Fragmented and benomyl	8 + 10	0/5	0
E-strain 2410	Fragmented	8 + 10	5/5	95

[1] All inoculum grown in liquid shake culture for 12 days and washed twice in distilled water.

[2] Ectomycorrhizal status estimated visually on intact planting cores; final shoots weights about 0.6 g.

mycelium was fragmented. Mycelium from liquid cultures of Amphinema byssoides was non-infective and the use of intact colonies only resulted in a few mycorrhizae. Fly maggots were observed to consume nearly all the mycelium of the intact colonies.

When intact colonies were used the condition of the inoculum could be monitored visually. Late-and multi-stage fungi failed to infect regardless of the addition of the insecticide Diazinon (Table 6). However it did prevent the consumption of the mycelium by the fly larvae which completely consumed the mycelial colonies within a week in the untreated containers. In all but two instances Trichoderma harzianum was observed fruiting on the introduced fungi. Mycorrhizae formation by Tomentella and E-strain occurred even if the mycelial pads were eaten by larvae or if they were colonized by Trichoderma.

Table 4. Inoculation of container-grown jack pine seedlings with mycelial slurries of six mycorrhizal fungi.

Inoculation Treatment	Type of inoculum[1]	Seedling Age (weeks pre-inoc+ inoc)	Number of Seedlings[2] Mycorrhizal	Percent Short Roots Mycorrhizal	Comments
Coltricia perennis 3236	Liquid non-fragmented	8 + 10	5/5	80	
I-type 2420	Liquid non-fragmented	14 + 4	5/5	60	
Leccinum aurantiacum 3128	Liquid	17 + 16	0/5	0	E-strain contamination
E-strain 2407	Liquid	19 + 7	5/5	90	
E-strain 2410	Liquid	19 + 7	5/5	90	
MRA 2417	Liquid	19 + 7	0/5	0	maggots abundant

Table 5. Effects of inoculum type on ectomycorrhizal formation of container-grown jack pine seedlings.

Inoculation Treatment	Type of inoculum[1]	Seedling Age (weeks pre-inoc+ inoc)	Number of Seedlings[2] Mycorrhizal	Percent Short Roots Mycorrhizal	Comments
Suillus tomentosus 3138	Intact on filter	12 + 6	4/5	10	maggots ate some inoculum
S. tomentosus 3138	non-fragmented	Liquid, 12 + 6	0/5	0	
S. tomentosus 3138	Liquid, fragmented	12 + 6	4/5	4	
Amphinema byssoides 3140	Intact on filter	13 + 5	2/5	< 1	maggots ate all inoculum
A. byssoides 3140	Liquid, fragmented	12 + 6	0/5	0	maggots common
A. byssoides 3140	Liquid, non-fragmented	12 + 6	0/5	0	

Table 6. Effect of diazinon on mycorrhizal formation of jack pine seedlings when inoculated with intact fungal colonies.

Species	Diazinon	No.of Seedlings Infected		% Infection		Condition of Inoculum after 1-3 weeks
		1 week	10 weeks	1 week	10 weeks	
Leccinum aurantiacum	-	0/2	0/2	0	0[1]	Pads eaten, + Trichoderma
L. aurantiacum	+	0/3	0/3	0	0[1]	+ Trichoderma
Rhizopogon rubescens	-	0/2	0/2	0	0	Pads eaten
R. rubescens	+	0/3	1/3	0	1	+ Trichoderma
Amphinema byssoides	-	0/3	0/3	0	0[1]	Pads eaten, + Trichoderma
A. byssoides	+	2/2	0/3	1	0[1]	+ Trichoderma
Tomentella (grey)	-	2/2	2/2	1	50[1]	Pads eaten
T. (grey)	+	0/3	-	0	-	Trees died
Tomentella 2437	-	2/2	2/2	1	100	± Trichoderma
T. 2437	+	2/2	2/2	1	100	+ Trichoderma
E-strain 2410	-	2/2	2/2	1	100	± eaten, + Trichoderma
E-strain 2410	+	2/2	2/2	1	100	+ Trichoderma

[1] Contaminated with E-strain

The late-stage and slow growing fungi Hydnum and Tricholoma were noninfective on filters or plugs except for one instance of T. flavovirens which slowly spread through the root system forming the characteristic vinaceous mycorrhizae (Table 7). Trichoderma was present on all the inoculum except for some T. flavovirens. Trichoderma was not observed on the inoculum of Astraeus 2432 or Lactarius which were successfully introduced with agar plugs or filters. Out of a total of five Astraeus hygrometricus 2186 plugs, two were colonized by Trichoderma after one week resulting in no new growth of Astraeus, while those plugs which remained uninfected by Trichoderma did exhibit growth. The jack pine seedlings in contact with the Trichoderma infested plugs developed few or no Astraeus mycorrhizae, while the seedlings inoculated with plugs which remained uncolonized by Trichoderma all developed Astraeus mycorrhizae. No maggots were observed 10 days after inoculation. When examined 11 weeks after inoculation larvae were present and some plugs had been reduced to feces. Some agar plugs were also colonized by Penicillium.

In a preliminary test of green alder, 4 month old plants were inoculated with cultures of Alpova diplophloeus on Nuclepore filters. After 7 days the Alpova cultures which experienced colonization by Trichoderma (n=2) or grazing by maggots (n=3) exhibited no growth. On the seedlings lacking both maggots and Trichoderma, there was definite growth of Alpova onto the roots surfaces. After 5 weeks, most of the Alpova colonies were completely consumed resulting in no mycorrhizae. Although the remaining, intact colony was colonized by Trichoderma the seedlings did produce some few weakly developed mycorrhizae.

3.3.5 Discussion

The composition of the growing media not only influenced seedling growth but also had the potential to alter mycorrhizal associations. The isolate of Tomentella used here was especially sensitive to the growing medium, illustrating that simple changes may sometimes spell the difference between complete inoculation success and complete failure with certain fungi. Until the causes of such differences are discovered and the biology of mycorrhizal fungi more fully understood, results of growing media manipulations will remain unpredictable.

Table 7. Mycorrhiza formation of jack pine seedlings inoculated with colonies on filters or agar plugs and drenched with Diazinon to control maggots.

Inoculation Treatment	Type of Inoculum	Number of Seedlings Mycorrhizal[1]	Condition of Inoculum after 10 days
Hydnum sp. 3220	Filter	0/4	+ *Trichoderma*
Hydnum imbricatum	Agar plug	0/5	+ *Trichoderma*
Astraeus hygrometricus 2432	Filter	5/5	- *Trichoderma*
A. hygrometricus 2186	Agar plug	4/5	- *Trichoderma*
Tricholoma pessundatum	Agar plug	0/5	+ *Trichoderma*
Tricholoma flavovirens	Agar plug	1/5	+ *Trichoderma*
Lactarius paradoxus	Agar plug	5/5	- *Trichoderma*
Suillus umbonatus	Agar plug	3/5	± *Trichoderma*

[1] Examined 10 days and 11 weeks after inoculation.

Altering the intact mycelial mass by either fragmentation or "planting" intact cultures on the root systems indicated that inoculum potential may be a major factor limiting the introduction of fungi other than "weedy", early-stage fungi. The use of intact cultures grown on membrane filters resulted in increased success (although low) with multi-stage fungi (*Suillus* and *Amphinema*) but late stage fungi failed to initiate infection under any conditions except for one seedling with *Trichoderma flavovirens*. The results with the multi-stage fungi were still highly erratic even though large colonies were placed directly in contact with roots indicating that factors other than inoculum mass were affecting mycorrhization. The prime factor is probably inoculum potential maintenance, i.e. the prevention of rapid viability losses of the mycelium after inoculation.

The use of intact cultures allowed the fate of mycelium to be monitored; however, it cannot be concluded that fragmented mycelium or that from liquid cultures necessarily suffered the same fates. The intact mycelium was observed in many instances to be completely consumed by fly larvae within a week after placement in the containers. This would unquestionably reduce the inoculum potential and would most severely affect the survival of slow growing fungi. Elimination of the larvae should then favor mycorrhization of the roots; however, this did not occur after treatment with Diazinon.

The second factor possibly altering mycelial survival was then observed on the intact cultures - the fruiting of Trichoderma harzianum directly on the mycelium. Species of Trichoderma are well-known antagonists of other fungi and have been used extensively in the experimental biocontrol of plant pathogens (e.g. Wells et al., 1972). It is thus conceivable, even likely, that Trichoderma was responsible for reducing the inoculum potential of the introduced fungi, in particular those that escaped the rapid attack of the maggots. If so, it would appear that multi- and late-stage species are more susceptible to fungal antagonism as Trichoderma occurred on inoculum of all three successional types while only early stage fungi maintained sufficient inoculum potential to grow and overcome host resistance. A preliminary attempt to control Trichoderma with benomyl failed. This level was probably too low to control Trichoderma as there was no apparent inhibition of formation of mycorrhizae by E-strain, a species which is susceptible to benomyl (Danielson, 1982). Additional application rates of benomyl should be tested to exploit the differential toxicity of the fungicide to basidiomycetes and ascomycetes.

The studies on inoculation of fungi from different stages of the proposed successional sequence have not resolved the practical problems but have yielded clues as to the solution. All the fungi are capable of forming mycorrhizae with young seedlings under monoxenic conditions characterized by high inoculum potential and low to moderate host resistance. In open conditions in normal planting mixtures only the early stage fungi are infective from mycelium (Part 4.1) and early- and multi-stage fungi from spores. This suggests that failures are due, not to chemical factors, but to the activity of other organisms. From

direct observations on colonies of mycorrhizal fungi it has been seen that the mycelium can be rapidly consumed by fly larvae and/or colonized by Trichoderma. Action by the former is certain to reduce infectivity and action by the latter is highly likely to adversely affect infectivity. It is felt that these organisms act to rapidly reduce inoculum potential in the planting mixture. They would have little effect on spores. The means to chemically control these organisms is at hand and the use of intact cultures offers an easy and sensitive method to monitor growth of the introduced fungus, consumption by maggots, colonization by Trichoderma and finally, mycorrhizal development - or failure.

3.3.6 Literature Cited

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4. OUTPLANTING: HOST RESPONSE AND FUNGAL ECOLOGY

Will inoculation enhance the field performance of seedlings outplanted on tailing sands and what is the fate of the introduced fungi?

4.1 HOST RESPONSE TO INOCULATION AND BEHAVIOUR OF INTRODUCED AND INDIGENOUS ECTOMYCORRHIZAL FUNGI OF JACK PINE GROWN ON AMENDED OIL SAND TAILINGS

4.1.1 Abstract

Nine fungi were initially introduced on jack pine seedlings which were planted on the Syncrude containment dyke. E-strain, Hebeloma sp., Thelephora terrestris, and Laccaria proxima all formed mycorrhizae with greater than 40% of the new short roots after one growing season. Cenococcum geophilum, Pisolithus tinctorius, Astraeus hygrometricus, Lactarius paradoxus, and Sphaerosporella brunnea formed less than 6% mycorrhizae. Laccaria proxima completely disappeared after 1 year and, between the second and third year, Hebeloma sp. and Thelephora terrestris almost completely disappeared. Of the introduced fungi, only E-strain was present in substantial quantities after 3 years. It appears that the major replacement process was noninteractive, i.e., the resident fungus died, the ectomycorrhizae reverted to uninfected short roots, and infection by another fungus occurred. The quantity of the total short roots converted to ectomycorrhizae by indigenous fungi was 4% after one growing season, and increased to 33% after 2 years and to 72% after 3 years. This resulted in a relatively uniform rate of infection regardless of inoculation treatment. Substantial variability within seedling treatments was caused by excessive moisture in one area of the plot which made detection of differences in seedling growth difficult. No differences due to inoculation could be detected after one growing season but inoculation with E-strain and Thelephora terrestris resulted in a two- to three-fold increase in shoot weight after 2 years compared to uninoculated seedlings. After three years shoot weights of the E-strain and Thelephora seedlings were still substantially larger than the controls but overall mycorrhizal infection had become uniform among treatments and the stimulation by inoculation would be expected to disappear with time. Of the indigenous fungi, E-strain decreased, whereas the I-type Ascomycete (Tuber sp.), Mycelium radicis atrovirens, Rhizopogon-like fungi, and hyaline Basidiomycetes increased in abundance between 2 and 3 years.

4.1.2 Introduction

Soils reconstructed from oil sand tailings, muskeg peat, and overburden are inherently nutrient-poor and measures must be taken to enhance plant growth. This has been done in the past by the periodic application of mineral fertilizers. An alternative to the use of fertilizers is the use of effective strains of mycorrhizal fungi. It has been demonstrated numerous times in the past two decades that the appropriate mycorrhizal fungi can stimulate the growth of plants, particularly on adverse sites. Nonetheless, the commercial application of mycorrhizal technology has not occurred in Canada and considerable research efforts are needed for selecting fungi and testing their effectiveness in the field. At this time, nearly all the field trials in North America have been with Pisolithus tinctorius (Pers.) Coker & Couch, a fungus indigenous to mineral soils and adverse sites. Despite the success of P. tinctorius in the southeastern United States, it can be anticipated that other fungi will be much better adapted to the conditions of the boreal forest. If mycorrhizae are to be used to increase nutrient uptake efficiency of plants used on reclaimed oil sand tailings, extensive field trials are necessary to select the "best" fungi. Factors to consider include adaptation to the peat-overburden amendments, persistence on the root system, ability to colonize new roots rapidly, ability to withstand competition from indigenous mycorrhizal fungi, and effects on host performance.

As a first step in screening ectomycorrhizal fungi for use in an inoculation program, an outplanting study of container-grown jack pine seedlings was initiated on the Syncrude dyke in 1982. The objectives of the study were (1) to compare the growth and survival of experimental seedlings inoculated with one of nine ectomycorrhizal fungi with noninoculated seedlings and with seedlings grown under operational conditions, (2) to determine the persistence and agressiveness of the introduced fungi, and (3) to determine the degree of ectomycorrhizal development of the indigenous fungi and their effects on the growth of jack pine seedlings.

4.1.3 Materials and Methods

4.1.3.1 Inoculation and Seedling Production. Jack pine seedlings were grown in 65 cc containers, inoculated with peat:vermiculite permeated with mycelium and fertilized at suboptimal rates (for details, see Danielson et al., 1984b). Eleven native fungi and Pisolithus tinctorius representing an ecological (early, multi and late stage fungi), taxonomic (Ascomycetes, gasteromycetes, agarics, aphyllophorlles), and morphological (± mycelial strands, pigmentation) range of characters were chosen for the test fungi. Of the 12 fungi introduced, nine successfully formed ectomycorrhizae in the greenhouse. Although three inoculation treatments failed, seedlings from all 12 treatments plus uninoculated control seedlings were used in the outplanting phase of the study. In addition, seedlings were obtained from the Syncrude Canada Ltd. nursery. These were raised under a higher nutrient regime and were hardened off after 12 weeks and were 20 weeks old, as were the experimental seedlings when planted. The experimental seedlings were moved directly from the greenhouse to the field site. The Syncrude seedlings were significantly ($p = .05$) taller (9.1 cm) and heavier (shoots = 440 mg, roots = 251 mg) than the experimental seedlings. The Syncrude seedlings were nonmycorrhizal (based on evaluations of a total of 3000 short roots on 10 seedlings).

4.1.3.2. Outplanting Site and Sampling

The planting site was the Syncrude tailings pond dyke near Fort McMurray, Alberta ($57^{o}5N$, $111^{o}45'W$). The climate is cool continental with long cold winters and short cool sumemrs. The growing season lasts 60 to 80 days with a maximum daylength of 18 h.

The extracted oil sands, from which the contaiment dyke was constructed, was amended with 15 cm of stockpiled muskeg peat and 10 cm of clay mineral overburden. The amendments were coarsely mixed into the sand with a cultivator. At the time of planting, the area was devoid of vegetation. The peat was from a muskeg bog and was stockpiled in 1976 and planted with a mixture of grasses in 1977 to prevent erosion. The peat was spread in December 1981 and cultivated in May 1982. The area was not fertilized.

The experimental plot consisted of 27 rows planted down the face of the dyke. Seedlings were planted 0.5 m apart with 1 m spacing between rows. Seventy-five seedlings of each treatment (only 30 for Tricholoma) were planted in two randomly selected rows, i.e., each row contained 36 to 38 seedlings. The seedlings were planted June 16 and 17, 1982, each one marked with a stake and numbered consecutively. After one, two, and three growing seasons (September 1982, 1983, and 1984), 10 seedlings from each of the 14 treatments were randomly selected and the seedling dug up so that the soil volume sampled each time was the same, i.e., that within a 10 cm radius from each stem. It is crucial to note that roots extending further from the seedlings were never sampled. Survival was determined for the entire plant population. Total height, annual height increment, and shoot dry weight (80°C) were determined for each plant sampled.

4.1.3.3 Ectomycorrhizal Assessments

The peat-sand-overburden mixture was washed from the root systems and the roots that egressed from the planting plugs were cut off and used for ectomycorrhizal assessments. No examinations were made of the roots inside the planting plugs as new root colonization was of primary interest and not static survival. Roots in the plugs were cleaned, dried at 80°C, and weighed. The egressed roots from each seedling were cut into 2 to 3 cm segments and the segments were randomly selected, examined with a stereomicroscope at 12-25X, and each short root rated for mycorrhization until 300 live short roots had been evaluated for each seedling. Frequent checks on suspected infections were made with whole mounts at 500X. Each short root was rated as non-mycorrhizal or mycorrhizal, and whether infected with the introduced fungus or an indigenous fungus (or fungi). Attempts were made to identify or otherwise characterize each indigenous infection (Danielson et al., 1984a).

Identification of the fungal symbionts were based on previous studies (e.g., Danielson, 1982) and where precise identifications were not possible, the Ascomycete-Basidiomycete affinity was determined by observations on Woronin bodies, clamp connections, and growth on benomyl-MMN agar (Danielson, 1982). In addition, each mycorrhiza was

examined for morphology, colour, amount of extramatrical mycelium (EMM), presence of mycelial strands or rhizomorphs, presence of cystidia, hyphal ornamentation, crystals or other hyphal exudates, and hyphal wall pigmentation when examined at 500X with brightfield illumination.

4.1.3.4 Plant Tissue Analysis

Needles produced during the second growing season were ground in a Wiley mill and digested in concentrated H_2SO_4 and 50% H_2O_2. The needles were digested in a BD-40 Technicon block digester for 30 min at 360°C using 0.25 g needles, and 5 mL H_2SO_4. After cooling for 5 min, 0.5 mL 50% H_2O_2 was added, the sample heated for 10 min, cooled, and H_2O_2 added repeatedly until the samples cleared (3.5 mL H_2O_2/sample). The samples were diluted so they contained about 6.7% acid and filtered through Whatman #1 filter paper, and further diluted to 1% acid concentration. The digest was analysed for orthophosphate and total N using a Technicon Autoanalyser with orthophosphate and NH_3-N modules.

4.1.4 Results

Seedlings grown in the Syncrude greenhouse were larger and heavier than the experimental seedlings after 3 months in the field (Table 1). There was only one significant difference of height and weight parameters among the inoculation treatments. Only three seedlings died during the first growing season although many were in poor condition. Egressed root weight of seedlings inoculated with Hebeloma was significantly larger than seedlings inoculated with Astraeus, Pisolithus, and control seedlings. The roots were clean of debris and the differences were not due to errors of including non-root material. The total number of short roots was also least with the latter three treatments and greatest in the Syncrude Tricholoma and Cenococcum inoculated seedlings.

Excluding the Syncrude treatment, shoot weight of all experimental seedlings increased 3-fold during the first growing season. Total root weight increased 2-fold during the same period (excluding Syncrude and Hebeloma). Root mass within the planting plug increased substantially while in the field, and egressed roots accounted for only

Table 1. Growth characteristics of inoculated jack pine seedlings after one growing season on amended oil sands.[1]

Inoculation Treatment	Height[2] (cm)	Branches[3] per Seedling	Shoot[2] Weight (mg)	Root Weight (mg)		No. Short Roots Outside Plug	Short Roots per mg Root
				Total[2]	Outside Plug		
Thelephora terrestris	6.8[a]	2.1[ab]	1005[a]	797[ab]	213[ab]	1102	5.3
Laccaria proxima	5.6[a]	4.3[ab]	827[a]	611[a]	103[ab]	730	6.1
Hebeloma sp.	6.4[a]	1.6[a]	809[a]	762[ab]	226[b]	978	4.3
E-strain	6.0[a]	2.5[ab]	592[a]	563[a]	155[ab]	1017	6.1
Cenococcum geophilum	6.2[a]	2.3[ab]	836[a]	714[ab]	165[ab]	1644	9.0
Pisolithus tinctorius	5.7[a]	2.6[ab]	486[a]	409[a]	59[a]	480	7.7
Astraeus hygrometricus	6.1[a]	1.8[ab]	801[a]	516[a]	88[a]	703	8.3
Lactarius paradoxus	5.5[a]	1.5[a]	729[a]	628[a]	108[ab]	719	8.3
Sphaerosporella brunnea	5.6[a]	2.8[ab]	658[a]	665[a]	116[ab]	823	6.7
Amphinema byssoides	5.6[a]	3.3[ab]	858[a]	719[ab]	118[ab]	759	6.5

continued...

Table 1 Concluded.

Inoculation Treatment	Height[2] (cm)	Branches[3] per Seedling	Shoot[2] Weight (mg)	Root Weight (mg)		No. Short Roots Outside Plug	Short Roots per mg Root
				Total[2]	Outside Plug		
Hydnum imbricatum	5.8[a]	2.5[ab]	866[a]	721[ab]	130[ab]	823	7.4
Tricholoma flavovirens[4]	6.3	2.8	848	504	167	1438	8.6
Syncrude	14.1[b]	6.0[b]	2580[b]	1400[b]	315[b]	2297	7.3
Control	6.1[a]	1.8[ab]	646[a]	636[a]	95[a]	703	6.3

[1] Data anlyzed by one-way ANOVA and difference detected by Scheffé pairwise comparisons. Values in each column followed by the same letter not significantly different ($p = .05$).

[2] Data ln Y transformed.

[3] Data ln (Y + 1) transformed.

[4] Not included in analysis as only five replicates taken.

20% of the total root weight. The height of the experimental seedlings increased from 4.8 to 5.9 cm during the first growing season, while the Syncrude seedlings increased from 9.1 to 14.1 cm in height. Branches were formed while in the field as the experimental seedlings increased from 1.1 to 2.3 branches per seedling.

By the end of the second growing season clear differences in shoot growth existed among inoculation treatments (Table 2). The heaviest seedlings were those inoculated with either E-strain or Thelephora terrestris or the noninoculated Syncrude seedlings. The Syncrude seedlings were 2.5 to 4-fold heavier than the E-strain and T. terrestris seedlings at the end of the first growing season but there were no significant differences among them after two growing seasons. The seedlings inoculated with Laccaria proxima and Hebeloma sp. were also heavier than the uninoculated seedlings. Root development of the Syncrude, E-strain, and T. terrestris seedlings was also greater than in the other treatments. The height growth of the Syncrude seedlings and those inoculated with E-strain, T. terrestris, L. proxima, and Hebeloma sp. were significantly greater than the non-inoculated plants. Total height of the seedlings was a much less sensitive indicator of growth than was weight. The number of branches produced was affected by inoculation but the number of short roots (infection sites) was unaffected (Table 3). Survival was variable but, in general, appeared to be favoured by inoculation.. The survival data was complicated by mortality of many small seedlings caused by water accumulation in one corner of the plot.

The nutrient content of the jack pine needles varied from 1.13 to 1.57% N and 0.134 to 0.165% P (Table 3). There were no apparent effects of inoculation on N and P concentrations although distinct differences existed between the colour of the foliage. For example, seedlings inoculated with E-strain were darker green than those from Syncrude.

Growth was related to the degree of infection by the introduced fungi but not necessarily to the total infection (Table 2) or mycorrhizal development by indigenous fungi. This cannot be interpreted as saying that the indigenous fungi are less effective than some of those introduced, however. Although no clear relationship was found

Table 2. Plant responses and total (introduced + indigenous) ectomycorrhizal development after two growing seasons of jack pine seedlings inoculated with various fungi and planted on a reconstructed soil on the Syncrude dyke.[1]

Inoculation Treatment	Shoot Dry (g)	Root Dry Weight: Total (g)	Root Dry Weight: Outside Plug (g)	Height Increment (cm)	Short Roots Mycorrhizal with all Fungi (%)
None-Syncrude	4.4^{e}	1.62	0.81	15.3^{d}	58^{cdefg}
E-strain	3.9^{de}	1.25	0.51	11.7^{bc}	85^{g}
Thelephora terrestris	3.6^{cde}	1.37	0.49	14.3^{cd}	62^{efg}
Laccaria proxima	2.5^{bcd}	0.96	0.34	13.1^{cd}	46^{bcdef}
Hebeloma sp.	2.3^{bcd}	0.98	0.40	11.9^{bc}	68^{fg}
Astraeous hygrometricus	2.1^{abc}	0.99	0.37	9.6^{ab}	80^{g}
Cenococcum geophilum	1.9^{ab}	0.77	0.30	9.5^{ab}	56^{cdefg}
Tricholoma flavovirens	1.8^{ab}	1.12	0.30	8.0^{a}	28^{abc}
Control	1.6^{ab}	0.83	0.27	7.9^{a}	37^{abcdef}
Hydnum imbricatum	1.5^{ab}	0.89	0.37	8.0^{a}	32^{abcd}
Lactarius paradoxus	1.4^{ab}	0.86	0.32	9.6^{ab}	45^{bcdef}
Pisolithus tinctorius	1.3^{ab}	0.40	0.23	8.0^{a}	35^{abcdef}
Amphinema byssoides	1.2^{a}	0.87	0.14	9.2^{ab}	24^{ab}
Sphaerosporella brunnea	1.1^{a}	0.60	0.23	7.5^{a}	13^{a}

[1] Shoot weight, root weights, and ectomycorrhizal data based on the destructively sampled seedlings, n=10 except for *Thelephora terrestris* (n=9) and *Tricholoma flavovirens* (n=5). Height increments measured in the field on all seedlings with n between 45 and 65 except *T. flavovirens* (n=22). Data analysed by one-way ANOVA and differences detected by Scheffé pairwise comparisons. Values in each column followed by the same letter do not differ significantly (p=.05).

Table 3. Growth characteristics of the seedlings destructively sampled and N and P contents of the current year's needles of jack pine seedlings inoculated with various fungi and grown on reconstructed oil sand tailings soil for two growing seasons.[1]

Inoculation Treatment	Total Height (cm)	Height Increment (cm)	Number of Branches per Seedling	Number of Short Roots per Seedling (outside plug)	Survival[2] (%)	Needle Content N (%)	Needle Content P (%)
None-Syncrude	26.6^{b}	12.8^{c}	4.8^{ab}	2010^{a}	99^{è}	1.47	.161
E-strain	17.4^{ab}	12.7^{bc}	2.7^{ab}	1358^{a}	95^{è}	1.40	.165
Thelephora terrestris	16.7^{ab}	11.7^{bc}	5.0^{b}	1418^{a}	90	1.13	.144
Laccaria proxima	18.1^{ab}	12.8^{c}	2.1^{ab}	1425^{a}	91	1.46	.151
Hebeloma sp.	16.4^{ab}	10.9^{bc}	1.2^{ab}	1442^{a}	93	1.57	.159
Astraeus hygrometricus	15.6^{ab}	10.0^{abc}	1.1^{ab}	1302^{a}	87	1.29	.148
Cenococcum geophilum	16.6^{ab}	11.1^{bc}	1.5^{ab}	1659^{a}	88	1.49	.155
Tricholoma flavovirens	13.4^{a}	10.4^{abc}	1.4^{ab}	1273^{a}	66	1.48	.158
Control	12.7^{a}	7.8^{abc}	1.7^{ab}	1215^{a}	77	1.32	.152
Hydnum imbricatum	13.6^{a}	7.8^{abc}	0.9^{a}	1617^{a}	72^{è}	1.50	.158
Lactarius paradoxus	13.3^{a}	10.3^{abc}	1.2^{ab}	1370^{a}	69	1.38	.159
Pisolithus tinctorius	14.1^{a}	8.1^{abc}	1.1^{ab}	1260^{a}	80	1.55	.165
Amphinema byssoides	9.2^{a}	7.6^{ab}	1.3^{ab}	816^{a}	76^{è}	1.30	.140
Sphaerosporella brunnea	10.6^{a}	5.7^{a}	1.0^{ab}	1411^{a}	82	1.29	.134

[1] All data except survival based on destructively sampled seedlings where n=10 except for *Thelephora terrestris* (n=9) and *Tricholoma flavovirens* (n=5). Data analyzed by one-way ANOVA and differences detected with Scheffé pairwise comparisons. Values followed by the same letter in each column do not differ significantly (p=.05).

[2] Values followed by an asterisk differ significantly from the expected values as determined by a chi-squared test.

between shoot weight and percent infection by indigenous fungi, two factors are apparent. When infection was low (<40%), the plants were nearly all small (<2g). Secondly, nearly all the large plants (>2.5g) had more than 40% of their short roots converted to ectomycorrhizae. It thus appeared that the indigenous fungi did stimulate the growth of jack pine seedlings.

Shoot growth after 3 years within inoculation treatments exhibited considerable variation with the coefficient of variance of shoot weights ranging from about 50 to 100% (Table 4). The noninoculated trees from the Syncrude nursery were larger (both height and weight) than the experimental seedlings but differences among the inoculation treatments largely disappeared between the second and third years. Height increments were similar for all treatments and ranged from about 10 to 15 cm. The rate of survival after 3 years (unadjusted for destructive sampling) appeared to be better for the Syncrude seedlings and those inoculated with E-strain, Laccaria proxima and Hebeloma sp. than seedlings in the other inoculation treatments. However, this was probably largely due to excessive moisture which adversely affected noninoculated seedlings the most due to chance assignment of the rows of seedlings. This poor drainage was responsible for a major portion of the variability in seedling growth. Some seedlings in all treatments grown in the wet area exhibited chlorosis and had reddish needles.

Overall infection of short roots exceeded 70% after 3 years except in the Hebeloma sp. and Thelephora terrestris inoculation treatments (Table 4). In these two treatments the introduced fungi had largely died and had not been replaced by indigenous species. When individual plant size and ectomycorrhizae were examined, no relationship was apparent between size and the dominant indigenous fungi forming ectomycorrhizae. Growth in the third growing season appeared to be largely dependent upon preseason plant condition (branch development, condition of apical bud) rather than ectomycorrhizal status.

Roots growing into the dyke soil in the first growing season were successfully colonized by Thelephora terrestris, Laccaria proxima, Hebeloma sp., and E-strain (Table 5). Of the other five fungi which infected the roots in the greenhouse, all but Sphaerosporella brunnea,

Table 4. Shoot growth and survival of jack pine seedlings after three growing seasons (mean ± standard deviation) and total (introduced + indigenous) ectomycorrhizal infection.

Inoculation Treatment	Height Growth (cm)		Shoot Weight	Survival	Short Roots Mycorrhizal With All Fungi
	Total	1984	(g)	(%)	(% ± SD)
None-Syncrude	41.2 ± 14.3	14.4 ± 8.3	22.8 ± 16.8	83	92 ± 11
E-strain	28.8 ± 6.2	14.9 ± 4.8	11.3 ± 6.2	93	85 ± 16
Thelephora terrestris	31.9 ± 8.1	13.6 ± 6.5	13.3 ± 2.5	78	53 ± 29
Laccaria proxima	27.5 ± 5.6	10.9 ± 4.2	9.7 ± 6.0	85	84 ± 13
Hebeloma sp.	27.2 ± 6.8	12.4 ± 5.9	8.4 ± 5.6	86	44 ± 17
Astraeus hygrometricus	22.9 ± 9.7	11.2 ± 7.1	5.8 ± 4.6	79	83 ± 25
Cenococcum geophilum	24.3 ± 7.5	12.8 ± 4.3	7.6 ± 3.7	78	79 ± 17
Tricholoma flavovirens	18.0 ± 7.1	9.4 ± 3.8	2.6 ± 2.6	58	71 ± 17
Control	22.0 ± 8.6	10.2 ± 4.9	6.3 ± 6.1	65	77 ± 36
Hydnum imbricatum	17.6 ± 6.1	8.3 ± 6.8	4.0 ± 5.8	54	86 ± 15
Lactarius paradoxus	28.4 ± 9.5	13.3 ± 8.1	11.1 ± 10.3	60	90 ± 15
Pisolithus tinctorius	22.3 ± 5.6	11.2 ± 3.8	5.8 ± 5.1	75	74 ± 26
Amphinema byssoides	23.8 ± 8.5	10.7 ± 5.8	6.3 ± 4.7	66	76 ± 29
Sphaerosporella brunnea	25.4 ± 10.4	11.7 ± 5.2	7.9 ± 6.5	73	76 ± 26

Table 5. Ectomycorrhizal infection of jack pine seedling roots growing out from planting plugs into amended oil sands by introduced and indigenous fungi after one growing season.

Inoculation Treatment	Percent Infection By: All Fungi x ± SD	Introduced Species x ± SD
Thelephora terrestris	75 ± 18	75 ± 18
Laccaria proxima	43 ± 23	42 ± 23
Hebeloma sp.	48 ± 13	48 ± 12
E-strain	93 ± 11	93 ± 11
Cenococcum geophilum	3 ± 4	2 ± 3
Pisolithus tinctorius	14 ± 16	6 ± 4
Astraeus hygrometricus	24 ± 22	4 ± 6
Lactarius paradoxus	9 ± 19	1 ± 2
Sphaerosporella brunnea	<1 ± 1	0 ± 0
Amphinema byssoides	6 ± 12	NA
Hydnum imbricatum	1 ± 1	NA
Tricholoma flavovirens	3 ± 4	NA
Syncrude	5 ± 14	NA
Control	0 ± 0	NA

NA = not applicable

which did not form mycorrhizae on new roots, colonized between 1 and 6% of the short roots on egressed laterals.

Indigenous mycorrhizal fungi were present in the amended oil sand but did not result in large scale mycorrhizal development (Table 5). Overall, 3.9 ± 11.5% (x ± SD) of the short roots from all treatments were infected with indigenous symbionts. Indigenous fungi occurred on 31% of the total seedlings with a total of 55 indigenous occurrences. A maximum of three fungi were found on a single seedling. The most common indigenous species was the E-strain (Table 6). It was found on 16% of the outplanted seedlings and infections

Table 6. Ectomycorrhizal infection of jack pine seedlings after one growing season by mycorrhizal fungi indigenous to amended oil sands.

Indigenous Fungi	Number of Seedlings Infected (total = 135)	Percent of Total Seedlings Colonized	Percent Infection of Colonized Seedlings
E-strain	22	16.2	31.5
I-type Ascomycete	10	7.4	3.4
Hyaline Basidiomycete	9	6.6	7.9
Rhizopogon-like	8	5.9	1.8
Cenococcum geophilum	1	0.7	4.3
Unknown Basidiomycete	1	0.7	2.3
Unknown Ascomycete	1	0.7	45.3
Floccose Basidiomycete	1	0.7	2.9
Unknown affinity 1	1	0.7	2.1
Unknown affinity 2	1	0.7	0.3

resulted in a substantial portion of the root systems being colonized. The I-type, the hyaline Basidiomycete and the *Rhizopogon*-like species occurred on about half as many seedlings and were much less successful in colonizing the root systems than E-strain. Six other species were detected on only one seedling each and except for the unknown Ascomycete, they infected only a few roots. Half the indigenous species were Basidiomycetes and half were Ascomycetes. Only the *Rhizopogon*-like species produced mycelial strands and all had hyaline hyphae except for E-strain and *Cenococcum*.

By the end of the second growing season, only three of the nine fungi originally present on the jack pine seedlings formed a substantial quantity of ectomycorrhizae (Table 7). The E-strain was the most successful fungus introduced as it readily colonized new roots and

Table 7. Ectomycorrhizal infection of jack pine seedling roots by introduced species of fungi prior to planting and after one, two, and three growing seasons on the Syncrude dyke.

Inoculation Treatment	Number of Growing Seasons in the Field			
	0[1]	1[2]	2[2]	3[2]
	% ± SD Ectomycorrhizal Infection			
E-strain	91 ± 7	93 ± 11	82 ± 15	55 ± 28
Hebeloma sp.	99 ± 2	48 ± 12	44 ± 22	7 ± 5
Thelephora terrestris	100	75 ± 18	52 ± 20	3 ± 7
Laccaria proxima	99 ± 2	42 ± 23	0	0
Cenococcum geophilum	57 ± 14	2 ± 3	12 ± 15	<1
Pisolithus tinctorius	54 ± 11	6 ± 4	6 ± 4	0
Astraeus hygrometricus	48 ± 17	4 ± 6	7 ± 9	2 ± 4
Lactarius paradoxus	32 ± 11	1 ± 2	0	<1
Sphaerosporella brunnea	17 ± 27	0	0	0
Amphinema byssoides	0	0	0	0
Hydnum imbricatum	0	0	0	0
Tricholoma flavovirens	0	0	0	0
Control	0	0	0	0

[1] All roots in planting cores sampled in preplanting assessments (Danielson et al.,1984b).

[2] Only those roots extending into the reconstructed soil and within 10 cm radius of the stem sampled.

persisted on old portions of the root system. Hebeloma sp. also persisted but infected less than half of the short roots. The ectomycorrhizae formed by Hebeloma sp. never appeared very healthy as they were dark coloured, thin, constricted, and lightly floccose. In contrast, ectomycorrhizae formed by indigenous species on the same plants were turgid, inflated and with healthy colours. Ectomycorrhizae formed by Thelphora terrestris were also in poor condition with most of them appearing shriveled and with small meristems. On most seedlings, T. terrestris was confined to older portions of the root system with the young roots either nonmycorrhizal or mycorrhizal with indigenous fungi. Among all the inoculation treatments, the roots of plants inoculated with T. terrestris appeared the least healthy.

Although Laccaria proxima was a successful colonizer in the first growing season, it had completely disappeared by the end of the second growing season. No trace of L. proxima was present on any of the 10 seedlings sampled although 4 of the 10 had less than 20% of the short roots converted to ectomycorrhizae by indigenous fungi, i.e., competition was not responsible for the demise of L. proxima. Lactarius paradoxus also had disappeared and Astraeus hygrometricus, Cenococcum geophilum, and Pisolithus tinctorius formed only a small number of ectomycorrhizae. Pisolithus tinctorius was largely confined to older roots indicating that it was unable to spread and colonize new roots. Astraeus hygrometricus was also not observed on new roots and its occurrence was very patchy and did not dominate even small portions of the root systems. Ectomycorrhizae formed by Cenococcum geophilum were only found on older roots as well, and the ectomycorrhizae were thin and usually with uninfected apices.

E-strain was the only introduced fungus present in significant quantities after 3 years (Table 7). It had decreased from 82% occurrence to 55% in the third year and many of the remaining E-strain ectomycorrhizae were dark, thin, and constricted. The Thelephora terrestris ectomycorrhizae looked very similar at the end of the second year prior to largely disappearing in the third year. It would appear that the introduced E-strain would be much further reduced in abundance in future years.

Both Hebeloma sp. and Thelephora terrestris ectomycorrhizae faded to almost negligible quantities in the third year. It would appear that changes in the host physiology or soil conditions were responsible for the demise as uninfected roots were abundant (about 50% of the total) and competition from indigenous fungi was not strong. In the case of the I. terrestris treatment, short roots were common and had I. terrestris on the base of the roots but the hyphae and cortical region was collapsed and inactive. However, the roots were alive, based on the turgid condition of the meristem region, and it appeared they continued to grow and frequently became reinfected on the tips by other ectomycorrhizal fungi. It thus appeared that I. terrestris died while in the ectomycorrhizal state, the short roots reverted to being nonmycorrhizal briefly and were then reinfected by the process of noninteractive replacement by indigenous fungi. This also indicates that short roots may live longer than ectomycorrhizae. The same process appeared to have taken place with Hebeloma as the proximal end of many short roots had Hartig nets composed of dead hyphae while younger (distal) portions of the roots were nonmycorrhizal. Copious amounts of extramatrical hyphae of Hebeloma were also present around some roots but they lacked cellular contents. E-strain (both introduced and indigenous) also appeared to be dying but the process was less advanced than with I. terrestris and Hebeloma. In addition, interactive replacement of E-strain by Rhizopogon was also occurring.

Considering all treatments, infection of short roots by indigenous fungi increased from 4% in the first year to 33% in the second year, and increased to 72% by the third year. Indigenous E-strain decreased slightly in abundance in the third year, whereas I-type, Cenococcum geophilum, Mycelium radicis atrovirens, Rhizopogon-like type 2, and hyaline Basidiomycetes all increased in abundance (Table 8). Of particular interest was the abrupt increase in the occurrence of Rhizopogon-like type 2. It was deteced on only one seedling in the second year but on 83 seedlings in the third year. The very dark rhizomorphs associated with the ectomycorrhizae makes its detection easy and, thus, it is not likely that it was misidentified. Once estabished, this species appeared to be quite aggressive as it

Table 8. Ectomycorrhizal infection by indigenous fungi of jack pine seedlings outplanted on the Syncrude dyke after two and three growing seasons.

Mycorrhizal Fungi (Code Number)	Percent of Seedlings Infected		Ectomycorrhizal Infection, % ± SD			
			All Seedlings		Colonized Seedlings Only	
			Number of Growing Seasons			
	2	3	2	3	2	3
Ascomycetes						
E-strain (1)	45	39	12 ± 22	9 ± 18	26 ± 26	23 ± 23
I-type (2)	37	58	7 ± 14	15 ± 24	17 ± 19	28 ± 27
Cenococcum geophilum (10)	15	36	<1 ± 1	2 ± 4	2 ± 3	4 ± 6
Mycelium radicis atrovirens(15)	48	65	5 ± 11	10 ± 15	10 ± 14	15 ± 17
Nondescript (22)	2	0	<1	0	2 ± 2	0
Nondescript (23)	<1	2	<1	<1	6	9
Basidiomycetes						
Hyaline species (5, 12)	34	42	4 ± 12	12 ± 20	17 ± 19	25 ± 23
Rhizopogon-like 1 (6)	30	28	2 ± 6	3 ± 9	6 ± 9	12 ± 14
Rhizopogon-like 2 (14)	<1	61	<1	15 ± 20	13	25 ± 21
Rhizopogon-like 3 (17)	15	0	2 ± 8	0	13 ± 17	0
Tomentella sp. 1 (13)	2	8	<1	1 ± 4	1	8 ± 11
Tomentella sp. 2 (19)	<1	1	<1	1	<1	
Golden floccose (16)	5	3	1 ± 5	<1	11 ± 21	14 ± ?
White crystals (21)	<1	<1	<1	<1	1	1
Unknown Affinity						
Nondescript (8)	6	7	1		3 ± 2	

infected 25% of the short roots of the plants it occurred on, a value similar to that found for the indigenous E-strain.

Interactive replacement (where one fungus overgrows an established ectomycorrhiza formed by another fungus) of indigenous fungi was observed for Rhizopogon-like types 1 and 2 replacing E-strain and I-type, I-type replacing Astraeus hygrometricus, Pisolithus tinctorius, Hebeloma sp., and Cenococcum geophilum, and Mycelium radicis atrovirens replacing E-strain. The actual number of replacements observed was small compared to the total ectomycorrhizae observed.

4.1.5 Discussion

The introduced fungi varied widely in their ability to colonize new roots and to persist in the oil sand-muskeg environment. Although new-root colonization efforts of six fungi were feeble, four species initially colonized substantial portions of the short roots formed in the reconstructed soil. The degree of persistence on the root system, at least within 10 cm of the planting plug, varied from one to three growing seasons. Laccaria proxima disappeared after one growing season, Thelephora terrestris and Hebeloma sp. after two, and it is predicted that the E-strain will not endure the fourth year. There is no assurance, however, that these fungi were, or will be, completely eliminated. Roots extending more than 10 cm from the plant plug were not sampled and it is possible that these fungi moved outward in a series of temporal waves, colonizing roots of the same age and physiological condition, and undergoing interactive or noninteractive replacement as the roots aged. Such a progression in fruitbody production and ectomycorrhizae as plants age has been recorded in detail in birch trees (Mason et al., 1982; Fleming et al., 1984). The replacement process appears to be dependent upon plant age, lateral root age, and perhaps short root age. As fine roots and ectomycorrhizae may turnover several times in one year (Alexander and Fairley, 1983), new short roots are continually being produced both close to the stem and on distant young lateral roots. Thus, if short root age were the only factor in replacement, no spatial sequence would exist.

It is difficult to see how plant age per se functions as a mechanism in controlling replacement unless the allocation of photosynthate changes with age and distance from the stem. Nonetheless, the host appeared to largely control the fungal sequence on jack pine in this study as the replacement process appeared to be a noninteractive one. The key feature in the noninteractive replacement process is the death of the resident ectomycorrhizal fungus. This occurred with Thelephora terrestris and Hebeloma sp. and was occurring after 3 years with E-strain. This resulted in seedlings that were inoculated with Hebeloma sp. and Thelephora terrestris to have the lowest overall mycorrhizal development after 3 years (see Table 4). Both these fungi died out between 2 and 3 years, leaving uninfected short roots that had not yet been colonized and infected by a second wave of fungi, a group of fungi more finely tuned to the soil conditions, or to the physiology of the host. The reason for the death of the fungus is unknown but interruption of the carbon flow from the host is a likely cause. Marks and Foster (1967) found that an interruption of root growth was necessary for replacement to occur.

Without more information on short root longevity, neither successional patterns of ectomycorrhizal fungi nor whole plant strategies for stress tolerance or avoidance can be explained. As a majority of the net primary production of trees may go into fine root biomass (Fogel, 1983), factors which reduce this, such as an increase in the life of short roots, may result in greater allocation of carbon to aboveground parts (Alexander and Fairley, 1983). The persistence of jack pine short roots after the death of ectomycorrhizal fungi may constitute a mechanism for conserving nutrients in this species. The suggestion here is that the regulation of fungal succession and inherent tree nutrient conservation mechanisms are intimately linked and should not be considered separately. Nonquantitative observations on jack pine roots over nearly a decade suggest that the turnover of short roots is considerably less than that observed for other temperate conifers as dead short roots are not abundant. Dead short roots are slow to decompose in the peat-tailings sand mixture and thus, unlesss broken off during extraction, they should be obvious, permitting turn-

over measurements. Rigorously designed experiments are required to challenge these observations.

The substandard size of the outplants resulted in excessive mortality and variability which reduced the chance of detecting inoculation treatment differences. In addition, the poor drainage conditions on one portion of the plot further increased within treatment variability. As a result, treatment differences were not readily apparent although inoculation with aggressive fungi (E-strain, Thelephora terrestris) resulted in short-term stimulations of growth. Indigenous inoculum was sufficient to swamp the effects of inoculation by the third year, at least as indicated by infections of roots near the planting plug. The much larger initial size of the Syncrude seedlings gave them an advantage that was not overcome by the experimental seedlings regardless of inoculation treatment. Seedlings which are held in the Syncrude shadehouse and become mycorrhizal may actually perform better than the nonmycorrhizal seedlings used in this test. Manipulation of seedling size by varying fertilizer regimes and planting container size is a more practical solution to increasing the early growth of seedlings than mycorrhizal inoculation unless inoculum is severely deficient in the planting sites or highly efficient fungi are found and introduced. In areas where less peat is used to amend the sand, the quantity of inoculum might play a role in vegetation development although certain ectomycorrhizae are usually introduced with operational planting stock. However, it is characteristic of the nursery fungi so far tested to have limited persistence in the reconstructed soils and to die even in the absence of competing fungi. The introduction of fungi better adapted to the sites and with morphological characteristics more resembling fungi in native jack pine stands might result in improved plant performance.

The majority of the non-introduced ectomycorrhizal fungi present after 3 years were those originally present in the stockpiled muskeg pet (Part 2.2). This suggests that these species are either very adaptable with regard to moisture conditions, are active only near the soil surface in bogs, or exist in bogs as inactive propagules except following disturbance (e.g., fire). Introduction of new species from adjacent stands appears to be slow, although if the resultant

ectomycorrhizae are nondescript (e.g., hyaline Basidiomycete category) detection would be very difficult. It seems unlikely that the rapid increase in Rhizopogon-like type 2, which increased in occurrence from 1 to 83 seedlings in one year, was due to airborne Basidiospores. However, the alternative explanation is that inoculum was originally present in the peat and remained dormant for two or more years. Evidence supporting spread by Basidiospores has been given by Lamb (1979) who found that only species of Rhizopogon and Suillus were found on pine seedlings distant (>800 m) from pine plantations rich in ectomycorrhizal fungi. Suillus tomentosus fruitbodies were common but no Rhizopogon fruitbodies were observed in jack pine stands in 1984. There is no evidence to suggest that Basidiospores of Suillus ¨re better adapted for dispersal than other boletes or agarics. The long distance dispersal of Rhizopogon is curious as the fruitbodies are hypogeous and the basidiospores are presumed to be dispersed by small mammals (Fogel and Trappe, 1978).

The identities of the ectomycorrhizal fungi referred to here as Rhizopogon-like are uncertain and are based on the similarity to known associations and cultures. Of key importance was the presence of mycelial strands, resinous-like deposits, and crystalline material on the hyphae (Danielson et al., 1984b). These features have been reported for a large number of species of Suillus and Rhizopogon (Appendix Table 7.5). The only species of these genera that are known to occur in the oil sands region are Rhizopogon rubescens, Suillus tomentosus, S. neoalbidipes (=S. albidipes), S. granulatus, and S. brevipes (Danielson, 1984; Danielson et al., 1984a). Cultures of Rhizopogon type 3 closely matched those of R. rubescens obtained from fruitbodies. It is of particular interest to identify the Rhizopogon type 2 as it was replacing other fungi and was very common. Its most distinctive feature was the dark, very compact rhizomorphs. Such well-formed mycelial strands occur in both Suillus and Rhizopogon. Apparently similar rhizomorphs are found in S. brevipes but not R. rubescens, S. tomentosus, S. neoalbidipes, or S. granulatus. Additional syntheses are required to resolve the identity of the Rhizopogon-like fungi.

The I-type Ascomycete also forms distinctive ectomycorrhizae but the teleomorph remains unknown (Danielson et al., 1984c).

Nonetheless, it can be speculated what the most likely taxonomic position is, based upon descriptions of ectomycorrhizae. The most distinctive features are the slender cystidia, the irregular synenchyma arrangement of the mantle, and the very smooth appearing surface. In that the cystidia are inducible by unknown factors and they may be absent, emphasis is placed on mantle structure. Considering these features, the only published descriptions of ectomycorrhizae that fit the I-type are ones formed by the genus Tuber. Chu-Chou and Grace (1983a,b) illustrate and describe ectomycorrhizae formed by Tuber sp. with cystidia and general morphology almost identical to the I-type. The only difference is in the mantle elements which are irregular synenchyma (jig-saw puzzle-like) in the I-type and a regular synenchyma (isodiametric cells) with Tuber sp. Voiry (1981) describes the mycorrhizae formed by T. albidum and illustrates cystidia identical to those of the I-type and describes the mantle as pseudoparenchymatous in the form of a puzzle. Further, Giovanetti and Fontana (1982) briefly describe mycorrhizae formed by six species of Tuber in which the surface is either glabrous or bearing hyphae or setae and the mantle is pseudoparenchymatous (=synenchyma). The colour ranged from light to dark amber, while I-type mycorrhizae are hyaline to rusty tawny - a similar range of colours. The Tuber mycorrhizae illustrated by Miller (1985) also closely resemble I-type ectomycorrhizae. The taxonomic association between the I-type fungus and the genus Tuber remains tentative but provides a testable suggestion as to the identity of the I-type. Its common occurrence in peat does not suggest Tuber or other hypogeous fungi as they have rarely been collected in such habitats. However, it is also likely that collectors have ignored muskeg areas in favour of other forest types. Indeed, hypogeous fungi have been only superficially studied in Alberta and there are only two records of the genus Tuber, both T. sphaerosporum Gilkey from the province (Danielson, unpublished data). Collecting, culturing, and pure culture syntheses must be performed to confirm the identity of the I-type fungus, an important symbiont in peat soils.

4.1.6 Literature Cited

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4.2 INOCULATION OF JACK PINE AND GREEN ALDER AND OUTPLANTING ON RECONSTRUCTED SOILS

4.2.1 Abstract

Plots were established on two soils reconstructed from (1) oil sand tailings and muskeg peat and (2) oil sand tailings, muskeg peat and clayey surficial overburden. Alder and jack pine were reared in the greenhouse and planted on the soils. The pine was successfully inoculated with three E-strain fungi and an I-type fungus. Inoculation of green alder failed and seedlings lacked both mycorrhizae and nodules when planted. After 2 years all the introduced fungi colonized new roots of pine growing into the reconstructed soils. After 1 year all the alder plants were nodulated but only 2% or less of the short roots were mycorrhizal. After 2 years, mycorrhizal development on alders was still often poor. Only two indigenous fungi were associated with alder, Alpova diplophloeus and Thelephora sp., and one with pine, a Suillus-like fungus.

4.2.2 Introduction

A large scale test is currently being conducted on the effects of using various amounts of muskeg peat and surficial clay overburden and depth of incorporation into oil sand tailings on the long term performance of woody shrubs and trees. The resulting reconstructed soils are anticipated to be relatively low in available nutrients, especially N and P, as well as coarse textured which may result in excessive drainage and droughly conditions.

It is now considered desirable to establish woody plants on oil sand tailings as quickly as possible as these plants will constitute the major biological elements in permanent, maintenance-free forests. Grasses provide quick ground cover to physically stablilize the sands but offer severe competition for woody plants introduced at a later stage (Vogel, 1980), provide excellent habitat for destructive rodents and tend to accumulate on the soil surface after death (Klym, 1982), rendering critical nutrient elements unavailable for plant growth. Nonetheless, grasses and forbs, including weeds, have been readily established on the tailings dykes. However, woody perennial

plants may offer greater problems in establishment and survival than grasses (Dai et al. 1983). One key factor responsible for this may be the great differences in root system morphologies. Whereas grasses have root systems composed of very fine and very abundant thread-like roots, the roots of woody plants are coarser, less frequent and provide much less surface area per unit weight (Bowen, 1980) than grasses. Consequences of these differences are that the root systems may differ substantially in ability to exploit the soil for low mobility nutrients and root distribution may affect water uptake and drought avoidance. The prime method of compensating for low root densities is the mycorrhizal habit in which external hyphae and hyphal aggregations effectively increase root densities. Thus the mycorrhizal status of shrubs and trees would probably be more critical for plant performance than for grasses.

As stable forest stands and/or wildlife habitat are the desired end product of oil sand reclamation and as certain shrubs may provide a long term input of growth limiting N and as these plants may particularly benefit from symbiont management, a study was undertaken to determine the root-symbiont relations of selected woody plants growing in reconstructed soils. The objectives of the study were to determine, on two recon- structed soils, one amended with peat and one amended with peat and clay:

1. the effect of inoculating silver-berry and buffalo-berry with soil containing both VA mycorrhizal and Frankia inoculum on plant growth and symbiont development,
2. the effect of inoculating alder with the ectomycorrhizal fungus Alpova diplophloeus,
3. the effect of inoculating jack pine with the best ectomycorrhizal fungus used in a previous study and three fungi indigenous to muskeg peat,
4. the contribution of indigenous symbionts to mycorrhizal development and nodulation,
5. the temporal and spatial distributions of some symbionts on developing root systems and,
6 to provide planning information for future projects involving microbial symbionts and reclamation practices.

4.2.3. Materials and Methods

The two University of Calgary plots were located on the east side of the RRTAC soil reconstruction-woody plant experimental area on a specially prepared pad of oil sand tailings from the Syncrude Canada Ltd. extraction plant (Figure 1). The two soil treatments, mixed to a depth of 20 cm, were the application of (1) 11 cm (3% organic C) of muskeg peat (P-1) and (2) 11 cm of muskeg peat plus 2.9 cm (12% clay) of surficial overburden clay (P-2). The plots were 12 x 44 m with buffer strips on all sides leaving 10 x 40 m for planting. The plots were constructed by Hardy Associates in June 1984. The muskeg peat was from the Syncrude NT-2 stockpile and the clay from the J-pit located close to a mixed aspen woodland. The clay was from a depth of approximately 1 to 3 m and consisted of 39% clay, 29% silt and 32% sand (Hardy Associates, 1983). Both plots were fertilized with 0-45-0 rock phosphate at a rate of 112 kg ha^{-1} and all amendments thoroughly mixed into the top 20 cm of sand with a Madge Rotoclear machine. Further details on plot construction are given in reports by Hardy Associates (1983, 1984).

Each 10 x 40 m plot was staked to delineate 40 rows with 20 planting positions per row. This resulted in 1 m spacing between rows and 0.5 m spacing within rows. Provisions were made to accommodate a total of 10 treatments per plot, each treatment with four randomly assigned rows (i.e. 20 plants/row x 4 rows = 80 replicates).

Seeds of alder (Alnus crispa (Ait.) Pursh) were obtained from Syncrude and collected in the Mildred Lake area in September 1981. Jack pine seed from the Fort McMurray area, lot 88-8-4 collected in August, 1978, were provided by the Alberta Forest Service.

The jack pine were grown in a 1:1 (V/V) mixture of peat (North Carolina, pH 5, autoclaved) and vermiculite (>2 mm) in 150 cc Leach Cone-tainers (Ray Leach, Canby OR). Seeds were washed overnight in cold running water, surface sterilized in 30% H_2O_2 (30 min) and germinated on PDA. Each container was planted with one germinant April 24, 1984 and plants were placed in the greenhouse. After three weeks, seedlings were fertilized twice weekly with a 200 mg L^{-1} solution of Plant Prod Soilless Feed 15:15:18 until the solution dripped through. Sequestrene Fe (56 mg L^{-1}) was added June 4 and July 13. Day

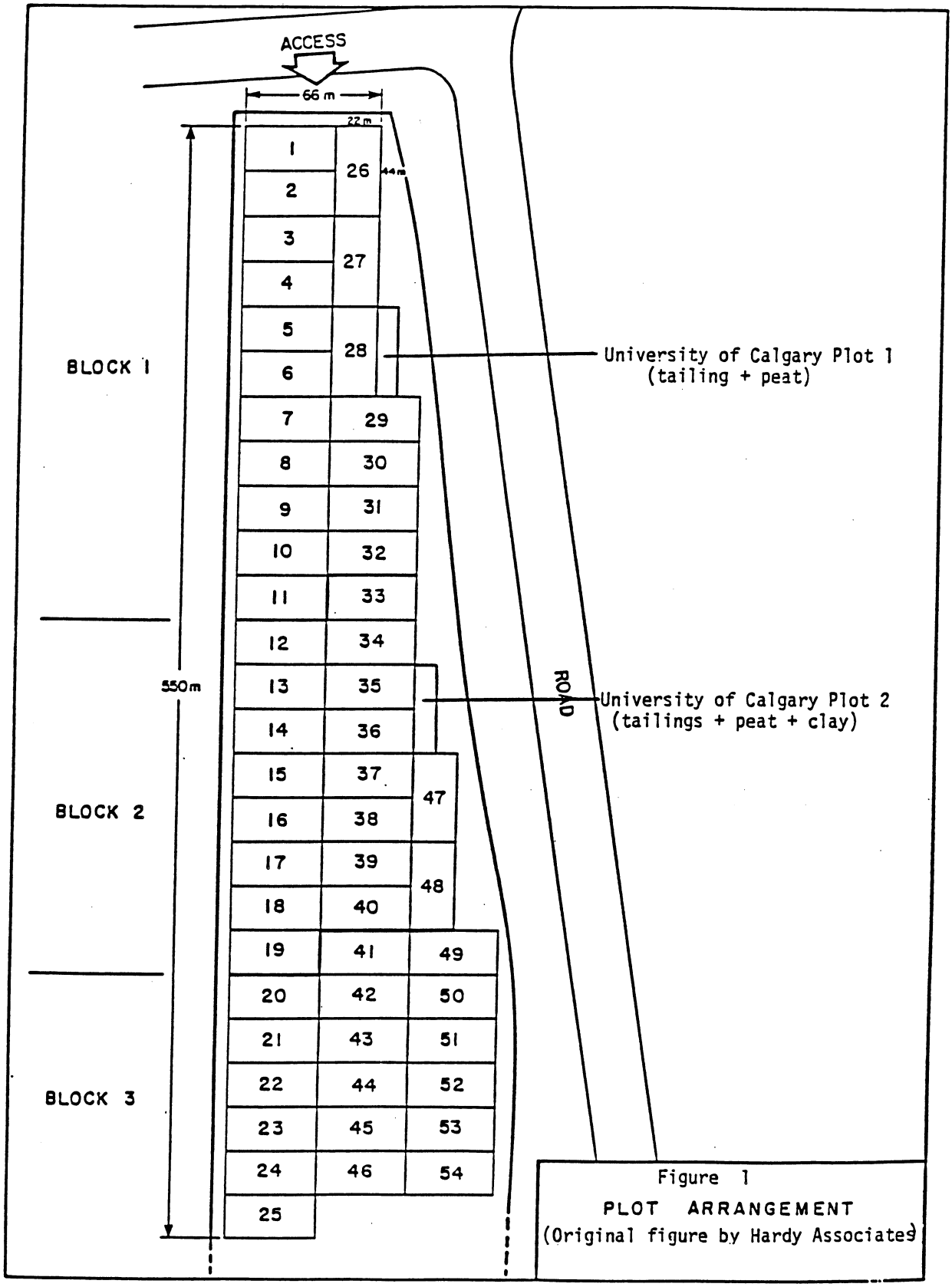

Figure 1
PLOT ARRANGEMENT
(Original figure by Hardy Associates)

length was extended to 20 h with a minimum of 3.2 klx (60 $\mu E\ m^{-2}\ sec^{-1}$) soil temperatures generally ranged from 18 to 30°C, with a few days reaching 40°C.

The jack pine were inoculated when 11 weeks old (July 11) with mycelial slurries of the most successful fungus used in Syncrude dyke study (E-strain 947) and three fungi indigenous to the muskeg peat (E-strain 2411, E-strain 2407 and I-type 2420). The latter three fungi were isolated from ectomycorrhizae of jack pine growing on the Syncrude dyke. Liquid shake cultures of each fungus were prepared in MMN solution, grown for 1 to 2 weeks, centrifuged, washed with distilled water, centrifuged, resuspended and 5 mL of the resulting suspension injected into each individual cell. A total of 120 seedlings of each of the four inoculation treatments were prepared. When the seedlings were 19 weeks old, fertilization was stopped and the seedlings were placed outside in partial shade for 2 weeks prior to planting.

The alder was grown under the same greenhouse conditions as was jack pine but differed somewhat in details of inoculation and substrate. Alder seeds were washed in cold running water for two days and germinated on moist filter paper. Germinants were planted in 160 65 cc Leach Cone-tainers filled with a 1:1 (V/V) mixture of autoclaved peat and vermiculite and placed in the greenhouse May 14. After 6, 8 and 13 weeks half the seedlings were injected with a mycelial slurry of Alpova diplophloeus (R-2402) prepared in the same manner as the jack pine fungi. The alder was fertilized twice weekly with a solution containing 100 mg L^{-1} Plant Prod 15:15:18 complete fertilizer for weeks 4 to 8 after which the concentration was increased to 200 mg L^{-1} until the plants were hardened off.

The preplanting mycorrhizal and nodulation assessments were made by randomly selecting 10 plants from each species-inoculation treatment, removing the shoots, washing the planting mixture from the roots systems and performing standard evaluations. Each jack pine root system was cut into 3-4 cm segments, the segments randomly selected and 300 short roots rated as to being nonmycorrhizal or ectomycorrhizal (Danielson et al. 1984b.). Entire alder root systems were examined at 12X magnification for nodules and obvious mycorrhizae and short roots checked at 500X for mycorrhization. All seedlings were planted on

September 18, 1984 in a randomized row pattern. The approximate number of seedlings planted per species-inoculation treatment was 60 for each jack pine and alder treatment.

Extremely cold conditions occurred about 1 week following planting which resulted in high mortality of both jack pine and alder. As a result, 10 alder were sampled after 1 year for growth and mycorrhizal development and all the remaining alders after 2 years. No pine were sampled after 1 year and after two years up to 10 seedlings were sampled with both soil treatments combined.

Entire plants were dug up and stored at $5^{o}C$ until they could be processed. Mycorrhizal assessments of pine were done by removing roots that had egressed from the planting plug and making visual estimates (12-25x) of the percentage of mycorrhizae formed by each species. For alder the entire root system was cleaned and in the first year the number of mycorrhizae and nodules were counted. In the second year, when mycorrhizae were more plentiful, the degree of mycorrhization was visually estimated on the whole root system and fresh weights of the nodules were obtained.

4.2.4 Results and Discussion

The shoot weight of jack pine (Table 1) for all treatments exceeded the ideal outplanting size as given by Carlson (1979) but was considerably less than the weight of the jack pine planted on the RRTAC plots (Part 2.1). Weights among the four treatments were similar although inoculation with E-strain 947 depressed growth as has been observed previously (Danielson *et al.*, 1984a).

The jack pine were all heavily ectomycorrhizal with all four fungi used and E-strain 947 was the most aggressive fungus (Table 2). Typical E-strain chlamydospores were formed in the containers by E-strains 947 and 2407. The I-type ectomycorrhizae were mostly cystidial and extramatrical hyphae were sparse. The use of a mycelial slurry of *Alpova diplophloeus* failed to result in mycorrhization of the alder even though it was applied three times. The alders also lacked *Frankia* nodules.

The poor survival permitted only one assessment of mycorrhizal development and growth of jack pine. The surviving pine seedlings were

Table 1. Pre-planting size of jack pine and green alder that were outplanted on The University of Calgary Soil Reconstruction plots.

Species	Inoculation Treatment	Shoot	Root	Total	Height
		g dry weight ± SD			cm
Jack pine	E-strain 947	0.80 ± 0.11	0.63 ± 0.08	1.43 ± 0.17	7.8 ± 1.0
Jack pine	E-strain 2411	1.02 ± 0.24	0.75 ± 0.10	1.77 ± 0.28	7.6 ± 1.7
Jack pine	E-strain 2407	1.09 ± 0.26	0.87 ± 0.13	1.50 ± 0.37	8.3 ± 1.3
Jack pine	I-type 2420	1.28 ± 0.27	0.88 ± 0.38	2.28 ± 0.46	11.0 ± 1.7
Green alder	Alpova	0.30 ± 0.09	0.16 ± 0.06	0.45 ± 0.15	11.0 ± 1.9
Green alder	None	0.26 ± 0.06	0.18 ± 0.05	0.44 ± 0.10	8.2 ± 1.4

Table 2. Pre-planting ectomycorrhizal status of jack pine and green alder that were outplanted on The University of Calgary Soil Reconstruction plots.

	Percent of short roots converted to ectomycorrhizae	Number of seedlings ectomycorrhizal
Jack pine + I-type 2420	80 ± 19	10
Jack pine + E-strain 947	99 ± 2	10
Jack pine + E-strain 2407	79 ± 13	10
Jack pine + E-strain 2411	92 ± 9	10
Alder + Alpova	0[1]	0
Alder + nothing	0[1]	0

[1] No Frankia nodules present.

healthy and grew substantially during the first two growing seasons (Table 3). There were no apparent differences in growth between plants inoculated with E-strain fungi and the I-type. All four introduced fungi aggressively colonized new roots and formed mycorrhizae. Only one indigenous fungus formed mycorrhizae, a Rhizopogon-like species that formed dark, well-formed rhizomorphs. This is apparently the same species that became common on pines planted in the Syncrude dyke and is referred to as Rhizopogon-like type 2 and is discussed in Part 4.1.

Table 3. Mycorrhizal status and shoot size of jack pine planted on peat-amended oil sands after 2 years.

Introduced Fungus	Percent Infection (± SD) Introduced	Suillus-like	Shoot Height (cm)	RCD[1] (mm)	Shoot Weight (g)
E-strain 947 (n=7)	99 ± 2	1	15 ± 3	4.5 ± 1.3	3.8 ± 3.2
E-strain 2407 (n=10)	99 ± 1	<1	28 ± 8	5.5 ± 1.8	9.8 ± 7.3
E-strain 2411 (n=10)	91 ± 16	9 ± 16	29 ± 8	5.1 ± 1.0	8.5 ± 3.1
I-type 2420 (n=10)	99 ± 2	1	21 ± 8	5.1 ± 1.0	5.7 ± 3.8

[1] RCD = root collar diameter

Alders grown in the peat only plot were larger than those grown in the peat and clay plot (Tables 4 and 5) but due to small samples no conclusions can be made as to the suitability of the two reconstructed soils for plant growth. After 1 year, all of the alders were nodulated and 70% were mycorrhizal (Table 4). However, the quantity of mycorrhizae were very low with only about 2% of the total short roots being infected. Susceptible short roots were abundant, about 3000 per plant, thus it appears that inoculum in the reconstructed soils was sparse. After two growing seasons all the 15 surviving alders were nodulated and mycorrhizal (Tables 5 and 6). Nonetheless, in the peat and clay plot only 25% of the short roots were mycorrhizal whereas 81% of the short roots in the peat plot were mycorrhizal.

Table 4. Size, numbers of nodules and ectomycorrhizae of green alder after 1 year's growth on the University of Calgary Soil Reconstruction Plots (x ± SD).

Plot	Height (cm)	Shoot Weight (g)	Root Weight (g)	Number of Plants Nodulated	Number of Nodules Per Plant	Number of Plants Mycorrhizal	Number of Mycorrhizae Per Plant[1]
Peat (n=10)	6.5 ± 2.8	0.84 ± 0.51	0.64 ± 0.34	10/10	36 ± 22	7/10	64 ± 109
Peat + Clay (n=10)	5.0 ± 2.1	0.47 ± 0.27	0.37 ± 0.15	10/10	13 ± 9	7/10	31 ± 66

[1] Total number of short roots on one average sized plant was 3,069.
in the peat and clay plot only 25% of the short roots were mycorrhizal whereas 81% of the short roots in the peat plot were mycorrhizal.

Table 5. Plant size and weight of nodules of green alder 2 years after being planted on oil sands amended with either peat or peat + clay overburden ($\bar{x} \pm SD$).

Plot	Height (cm)	RCD[1] (mm)	Shoot Weight (g)	Root Weight (g)	Nodule Weight (g)
Peat (n=9)	37 ± 13	8.5 ± 2.2	11.3 ± 7.4	13.0 ± 8.6	1.47 ± 0.95
Peat + clay(n=6)	23 ± 16	6.0 ± 2.9	4.6 ± 5.0	6.0 ± 5.7	0.49 ± 0.47

[1] RCD - root collar diameter

Table 6. Mycorrhizal infection of green alder roots after 2 years on oil sands amended with peat or peat + clay overburden.

	Alpova		Thelephora		None	
Plot	%	Occ.	%	Occ.	%	Occ
Peat (n=9)	54	6/9	27	6/9	19	0
Peat + clay (n=6)	25	6/6	0	0/6	75	0

Only two fungi formed ectomycorrhizae with alder, Alpova diplophleous and a species referred to as Thelephora (Table 6). The Thelephora + alder mycorrhizae bore hyphoid cystidia 60-200 µm long, clamped at the base, and had retraction septa and mycelial strands that were fawn to date brown and villose. A culture (R 2616) from the cystidial mycorrhizae produced black crystals which turned green after the application of KOH. These characters are close but not identical to mycorrhizae of T. terrestris. Species of Thelephora have not previously been identified as forming mycorrhizae with alder.

Although harsh winter conditions severely reduced the anticipated results from this field study, it did demonstrate several factors. Both E-strain fungi and I type readily colonized new roots and persisted for 2 years, and the dark rhizomorphic Suillus-like fungus is an important indigenous symbiont. Alder was strongly mycorrhizal deficient through the first growing season and in one soil deficient for at least 2 years. This indicates that alder could benefit substantially from inoculation with the appropriate fungi.

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5. ALDER: SYMBIONT STATUS AND GREENHOUSE REARING

Alder is often difficult to establish on reclamation sites. Could this be due to a deficiency in inoculum for mycorrhization and nodulation?

5.1 THE MYCORRHIZAL STATUS OF NATURAL AND GREENHOUSE-GROWN GREEN ALDER

5.1.1 Abstract

Brief surveys were made of the mycorrhizal and nodulation status of commcerical nursery stock of green alder, of naturally regenerated plants, and of seedlings grown in reconstructed soils. Nursery stock lacked any mycorrhizae and inoculum levels in soil from the Syncrude dyke were very low. The major symbiont of natural seedlings was the hypogeous Basidiomycete, Alpova diplophloeus, which is host specific for the genus Alnus. Alder became nodulated in the nursery and when grown on Syncrude dyke soil. VA mycorrhizae did not occur on the seedlings examined in this study. The results indicate that artificial inoculation of alder is necessary for rapid development of ectomycorrhizae.

5.1.2 Introduction

Nitrogen deficiency is one of the main factors limiting plant growth during the reclamation of mine spoils such as oil sand tailings (Bloomfield et al. 1982). Although this deficiency can be alleviated over the short term by applying fertilizers, long-term accumulation of N is more desirable to ensure the success of established plant species. Nitrogen fixing shrubs are one of the mechanisms by which a slow but long-term accumulation of N can be achieved and may be particularly valuable as a nurse crop for trees. As a result, interest has been expressed in establishing the N_2-fixing shrub green alder (Alnus crispa (Ait.) Pursh) to stimulate N accretion on oil sand tailings.

Unlike most other ectomycorrhizal plants, alder is associated with a restricted number of fungal species and these are often exclusively associated with alder. One of the main symbionts associated with alder is Alpova diplophoeus (Zeller & Dodge) Trappe & Smith (Molina, 1981). Species of Alpova are hypogeous Basidiomycetes (i.e., false truffles which develop completely underground) and are dependent on small mammals for dispersal. Thus it can be expected that unless specific inoculum is present in reconstructed soils or unless alder

seedlings are mycorrhizal at the time of planting, mycorrhizal development on tailings would be very slow and patchy.

The objectives of this study were to (1) make assessments of the mycorrhizal and nodulation status of green alder currently being grown in commercial nurseries, (2) to determine the symbionts associated with alder in the abandoned Vaartnou plots and with wildling shrubs, and (3) to assess the inoculum status of reconstructed soil on the Syncrude dyke by use of a greenhouse bioassay technique.

5.1.3 Materials and Methods

Green alder was available at only four of the nurseries surveyed for mycorrhizae (Part 2.1). These included the Hillson Nursery (Rochester, Alberta), Reid, Collins Nurseries Ltd. (Aldergrove, B.C.), Whitecourt Mountain Seedling Nursery (Whitecourt, Alberta), and the Syncrude Industrial Nursery (Fort McMurray). Ten or 25 seedlings were randomly sampled from the available stock and the roots examined for ectomycorrhizal development (Part 2.1) and nodulation. Roots of five plants each from Whitecourt and Syncrude were also examined for VA mycorrhizae (Phillips and Hayman, 1970). Shoot and roots were dried at 80°C and weighed.

Young green alders were collected from the Fort McMurray area and examined for ectomycorrhizal status. Roots of shrubs about 6 years old were sampled from the abandoned Vaartnou plots at Mildred Lake in June 1983. The area was formerly a jack pine-lichen woodland and the topsoil had been removed prior to planting. Other seedlings were collected from roadsides and other disturbed areas. Large green alders growing in a dense stand in a borrow pit near Suncor were also sampled as this was the most extensive stand of green alder observed in the area.

To determine the inoculum potential of the soils being reconstructed on the Syncrude site, a baiting (Part 2.3) or bioassay technique was used. Five samples of the reconstructed soil around the jack pine outplanting study plot (Part 4.1) were collected and placed in 150 cc Leach Cone-tainers. These were planted with green alder germinants, watered when required (no fertilizer added), and grown in

the greenhouse for 7 months, after which the roots were examined for ectomycorrhizal development.

5.1.4 Results

Container-grown alders from all four nurseries are completely nonmycorrhizal (Table 1). The seedlings represented stock that was ready for outplanting and thus, the nursery provided no inoculum of alder-specific ectomycorrhizae. Nodulated plants were present in all four nurseries but some non-nodulated plants occurred in all the crops sampled.

Twenty-eight samples of green alders were examined for naturally occurring mycorrhizae (Table 2). Quantitative estimates of the mycorrhizae were not possible due to the poor condition of some of the root samples but it was observed that Alpova diplophoeus was a common associate of alder. The soils were generally sandy but the two cutbanks sampled were composed of heavy, clayey subsoil, and Alpova occurred on both soil types. Other fungi were also mycorrhizal with alder, but with the exception of Cenococcum (occurring on <1% of the roots) could not be identified. Mycorrhizae were best developed in the borrow pit and all those observed were formed by Alpova.

As indicated by the greenhouse baiting technique, the reconstructed soil was very deficient in alder compatible inoculum (Table 3). No Alpova was observed although three other fungi caused the formation of a few ectomycorrhizae.

5.1.5 Discussion

Nursery stock of green alder was totally deficient in ectomycorrhizae unlike coniferous species grown in some of the same nurseries (Part 2.1). It is apparent that the common nursery fungi, Thelephora terrestris and E-strain are incompatible with alder and that if mycorrhizal seedlings are a desired nursery product, artificial inoculation will likely be necessary. The one area of the Syncrude dyke sampled for inoculum was also strongly deficient in mycorrhizal fungi compatible with alder. The weak infectivity of reconstructed

Table 1. Seedling size, nodulation and mycorrhizal status of green alder grown in three nurseries (x ± SD) and sampled in August 1985 (except Syncrude, part of RRTAC crop, sampled August 1984).

Nursery	Crop	Height (cm)	Shoot Weight (g)	Root Weight (g)	S/R Ratio	Percent Ectomycorrhizae	Percent VA-mycorrhizae	Number of Plants Nodulated
Hillson	1983	ND[1]	0.45 ± 0.12	0.49 ± 0.10	0.9	0	ND[1]	1/10
Reid, Collins	?	ND	0.56 ± 0.26	0.33 ± 0.16	2.0	0	ND	22/25
Whitecourt	1984	12.6 ± 2.8	0.89 ± 0.39	0.62 ± 0.23	1.4	0	0	7/10
Syncrude	1983	ND	1.55 ± 0.63	0.83 ± 0.36	1.9	0	0	20/25

[1] Not Determined

Table 2. Ectomyocrrhizal status and nodulation of green alder seedlings growing in the Fort McMurray area.

Source	Fungi Present
Road cutbank Muffaloose Trail (n=5)	Alpova 4/5; hyaline Basidiomycete 3/5; Cenococcum 1/5; reddish brown unknown 1/5 (3/5 nodulated)
Roadcut (n=2)	Alpova, hyaline Basidiomycete, Cenococcum. (all nodulated)
Below powerline (n=2)	Alpova 1/2, hyaline Basidiomycete 2/2. (all nodulated)
Vaartnou plot (n=5)	Most roots dead, Alpova present.
Wildlings near Vaartnou plot (n=5)	25% of roots infected, no Alpova present, four other fungi present
Borrow pit near Suncor (n=4)	50% of roots infected, all mycorrhizae formed by Alpova

Table 3. Ectomycorrhizal development of green alder grown in dyke peat in the greenhouse for 7 months. All plants were nodulated with Frankia sp.

Plant number	Plant weight (mg) Shoot	Root	Mycorrhizal Status % of short roots infected	Fungi involved in symbiosis
1	485	311	2	Unknown Basidiomycete
2	ND	142	0	
3	151	64	0	
4	175	152	1	Unknown Basidiomycete, Unknown affinity
5	75	26	0	

ND = Not determined

soils for alder was substantiated where alder was outplanted and ectomycorrhizal development was negligible after one growing season (Part 4.2).

The brief survey of wildling alders in the Fort McMurray region confirmed the laboratory suggestions that Alpova diplophoeus is a major symbiont of alder. The presence of few mycorrhizae associates has been noted from field observations by Mejstrik and Benecke (1969) although they did not identify any of the fungi. Molina (1981) determined that few fungi would form ectomycorrhizae with species of alder under monoxenic conditions and even fungi known as associates of a wide spectrum of hosts failed to infect alder. Similar results were found by Godbout and Fortin (1983) with green alder in which only Alpova diplophloeus of 46 fungi tested formed abundant ectomycorrhizae. Other fungi were capable of forming a few ectomycorrhizae but as was observed in the roots studied in this study, infections by these fungi were very limited in extent. There was no evidence that green alder could form VA mycorrhizae as was found by Malloch and Malloch (1982) and Rose (1980).

Unlike the ectomycorrhizal symbionts, Frankia, the N_2-fixing actinomycete, was widespread, occurring on nursery stock as well as being native to the reconstructed soils. It remains to be determined how efficient the native strains are and whether inoculation might increase the growth of alder as has been observed for other N_2-fixing plants on the reconstructed soils (Visser and Danielson, 1988).

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5.2 EFFECTS OF FERTILIZATION ON THE GROWTH, MYCORRHIZAL DEVELOPMENT, AND NODULATION OF CONTAINER-GROWN GREEN ALDER

5.2.1 Abstract

Green alder seedlings were grown in the greenhouse for 14 weeks and were inoculated with a mycelial slurry of Alpova diplophloeus after 12 weeks, inoculated with soil from an alder stand that was mixed into the growing medium, or left uninoculated. A commercial fertilizer (15:15:18) was applied to saturation twice weekly at concentrations of 0, 100, 200, or 400 mg L^{-1}. The plants grew very poorly without the addition of fertilizer, and satisfactory growth occurred when either 200 or 400 mg L^{-1} fertilizer was used. The mycelial slurry was non-infective at all fertilizer levels. The soil served as a moderately good source of ectomycorrhizal inoculum, although ectomycorrhizal development was strongly inhibited when 400 mg L^{-1} fertilizer was used. The principal ectomycorrhizal fungus was Alpova diplophloeus. All plants inoculated with soil became nodulated with Frankia, and optimum development occurred when 200 mg L^{-1} fertilizer or less was used. No VA mycorrhiza were present.

5.2.2 Introduction

Examination of green alder nursery stock from four nurseries revealed that although nodules formed by Frankia were common, all plants were nonmycorrhizal (Part 5.1). Further, under greenhouse conditions, alder developed very few ectomycorrhizae when grown in peat material from the Syncrude dyke (Part 4.2). It thus appears that alder planted on reclamation sites may be deficient in ectomycorrhizae, a condition which may be detrimental to establishment and growth of nursery stock. In that the "weedy" mycorrhizal fungi that normally occur in nurseries on pine and spruce are not compatible with alder, inoculation with specific fungi might have special benefits with alder. For inoculation to succeed, however, fertilizer levels must not be too high, such that fertilization supresses infection. It was the objective of this experiment to determine the effects of fertilization on

ectomycorrhizal development and growth of green alder and the effectiveness of two inoculum sources, a pure culture slurry and an infested soil.

5.2.3 Materials and Methods

Soil used for inoculation was collected from beneath a stand of green alder growing in a borrow pit close to the Fort McMurray-Fort MacKay highway. Previous examination of alder roots from this stand had shown them to be heavily infected with one fungus which is tentatively referred to as Alpova diplophloeus.

The pure culture inoculum originated from an ectomycorrhiza of alder growing in the "Vaartnou" plots near the Mildred Lake camp. It was isolated in July 1983 and is referred to as Alpova diplophloeus R-2402. Inoculum was prepared in liquid shake cultures in MMN medium inoculated with a homogenate from MMN agar. After about 2 weeks the mycelium was washed and used to inoculate alder seedlings (Danielson et al. 1984c).

The seeds were washed in cold running water overnight and germinated on moist filter paper. The germinants were planted in 65 cc Leach Cone-tainers March 8, 1984. The control (uninoculated) and the slurry inoculum cells contained a 1:1 (V/V) mixture of autoclaved peat moss (North Carolina, pH 5) and vermiculite (>2 mm fraction). The soil inoculum treatment contained a 1:4 (V/V) mixture of the alder soil and peat-vermiculite. Due to difficulties in culturing Alpova, the cells were not inoculated until May 28 (12 weeks old). The fertilizer used was 15:15:18 Plant Prod Soilless Feed at rates of 0, 100 mg L^{-1}, 200 mg L^{-1}, and 400 mg L^{-1}. Beginning the third week, the plants were fertilized twice per week until they were harvested when 14 weeks old. Ten replicates of each treatment were prepared for a total of 120 plants (4 fertilizer levels x 3 inoculum treatments x 10 replications).

After harvesting, the roots were washed free of planting mixture and the degree of ectomycorrhizal formation estimated visually at 12x, with infections being confirmed using whole mounts at 500x (Danielson et al., 1984b).

The specific characteristics of Alpova + alder ectomycorrhizae are as follows: FORM - Monopodial or occasionally branched, elements long and fragile, subglabrous; COLOUR - Whitish silvery due to trapped air, buff to fulvous or umber where compressed; MYCELIAL STRANDS - Abundant or sparse when young, well formed, fawn to purplish brown subglabrous; EXTRAMATRICAL HYPHAE - Clamped, 3-4 μm diameter, walls pale ochraceous; MANTLE - Texture intricata or more commonly cells very obscure, some cells containing intercellular rusty tawny pigments; DISTINCTIVE FEATURES - The dark colour (when mature), purplish tinted mycelial strands, clamped hyphae with some rusty tawny inclusions appear to be a distinctive combination for Alpova. Hyphae on young alder roots must be examined carefully for the presence of clamps and rusty tawny inclusions.

Each root system was also examined for nodules formed by Frankia and their presence or absence noted. Each root system in the soil inoculation treatment was also subsampled and cleaned and stained to determine if VA mycorrhizae were present (Phillips and Hayman, 1970). The roots and shoots were dried at 80°C and weighed.

5.2.4 Results

The seedlings grew very poorly without the addition of fertilizer (Table 1). The application of 200 mg fertilizer per liter twice a week resulted in satisfactory sized plants, and additional fertilizer only resulted in slightly larger shoots. The variability in growth within fertilizer treatments was considerable, probably as a result of the initial slow development. After 5 weeks, many seedlings still remained with only one true leaf while others were growing vigorously. Beyond a minimum application rate, fertilizer had little effect on the amount of roots produced.

The use of a mycelial slurry of Alpova was ineffective for initiating ectomycorrhizal formation (Table 2). A few apparently infected roots were noted; however, they were so poorly developed that the positive rating is questionable. In contrast, good ectomycorrhizae were formed when infested soil was added to the growth medium. Nonetheless, 8 of the 27 seedlings grown under the three lowest fertilizer

Table 1. Effects of four fertilizer levels on shoot and root weights of green alder reared in the greenhouse and either inoculated with soil or Alpova diplophloeus or not inoculated.

Fertilizer Level (mg L^{-1})	Inoculum Treatment		
	None	Alpova	Soil
	Shoot weight mg (± S.D.)		
0	15 ± 8	40 ± 25	82 ± 82
100	197 ± 24	239 ± 43	413 ± 122
200	403 ± 163	559 ± 86	363 ± 129
400	506 ± 159	640 ± 272	461 ± 163
	Root weights mg (± S.D.)		
0	25 ± 16	38 ± 25	45 ± 32
100	85 ± 26	100 ± 26	127 ± 55
200	128 ± 45	194 ± 50	132 ± 27
400	115 ± 54	137 ± 59	151 ± 52

regimes were nonmycorrhizal. This suggests that soil may not be a totally satisfactory source of inoculum.

The major symbiont provided by the soil was Alpova diplophloeus which colonized a major portion of root systems of the infected plants. The only other fungus present was a hyaline basidiomycete with clamped hyphae. It is possible that this fungus was also Alpova but not recognizable as such due to the young age. No VA mycorrhizae were detected.

Table 2. Ectomycorrhizal development and nodulation by Frankia of green alder grown in the greenhouse, treated with four fertilizer levels and either inoculated with Alpova or soil or left uninoculated.

Inoculation Treatment	Fertilizer Level mg L^{-1}	Number of Seedlings Nodulated	Mycorrhizal formation by: Alpova Number of Seedlings	Alpova % [2]	Other [1] Number of Seedlings	Other [1] % [2]
None	0	0/10	0/10	0	0/10	0
None	100	1/10	0/10	0	0/10	0
None	200	5/9	0/9	0	0/9	0
None	400	0/10	0/10	0	0/9	0
Alpova	0	1/9	1/9	<1	0/9	0
Alpova	100	0/10	1/10	<1	0/10	0
Alpova	200	3/8	1/8	<1	0/8	0
Alpova	400	2/8	1/8	<1	0/8	0
Soil	0	9/9	5/9	90	0/9	0
Soil	100	9/9	5/9	50	5/9	30
Soil	200	9/9	8/9	60	1/9	40
Soil	400	9/9	1/9	<1	0/9	0

1 No VA mycorrhizae detected in the soil inoculation treatment.

2 Percent of short roots of only the colonized plants that were ectomycorrhizal.

The use of soil also introduced Frankia and nodules were present on all plants in the soil inoculum treatment. Although not counted, it was apparent that fewer nodules were present when 400 mg of fertilizer was used than when 200 mg was used. Some plants in the non-soil inoculum treatments were also nodulated, probably as a result of the spread of Frankia by insects. In the soil-no fertilizer treatment, where all plants were nodulated, infection with Alpova appeared to result in increased growth. The shoot weights ($\bar{x} \pm$ standard deviations) of plants with Alpova ($n = 5$) were 136 ± 71 mg and those without Alpova ($n = 4$) were 19 ± 11 mg. The alternative explanation is that larger plants were more likely to become ectomycorrhizal.

5.2.5 Discussion

The mycelial slurry of Alpova was ineffective for inoculating container-grown alders. This failure could have been due to the late timing of the inoculum; however inoculations performed sooner have also completely failed (Part 5.3). It is more likely that Alpova is behaving in a manner similar to the multi-stage fungi in which mycelial slurries are noninfective (Part 6). Alpova is a segregate of Rhizopogon (Trappe, 1975) and it thus is likely that it would behave similarly to Rhizopogon and the epigeous relative, Suillus. To date, there are no reports of successful inoculation of container-grown alders with Alpova.

Inclusion of infested soil in the planting mixture resulted in both nodulation and mycorrhization of the alder seedlings. Even so, about 25% of the seedlings lacked mycorrhizae. As the soil used should have been high in mycorrhiza inoculum and it is unlikely that more than 20% soil could be used on a large scale, soil may not be a suitable inoculum source in a nursery operation. The use of soil also prevents evaluating the separate contributions of the mycorrhizae and Frankia to plant growth.

The optimum fertilizer level (15:15:18) for growth and symbiont development was 200 mg L^{-1} applied twice weekly. This allowed both good nodulation and development of Alpova ectomycorrhizae. This fertilizer regime is also suitable for the growth and mycorrhization of jack pine (Danielson et al., 1984a).

Once again it appears that Alpova diplophloeus is a major fungal symbiont of alder. This has previously been shown to be true on young alders in the Fort McMurray area (Part 5.1). As has been pointed out previously, Alpova is a hypogeous basidiomycete (false truffle) in which the spores are spread only by animal vectors, primarily small mammals. In addition, Alpova is very likely restricted to a single host genus, Alnus. Considering these factors, it is likely that most soils would be mycorrhizal deficient for alder, and special efforts may be necessary to establish Alpova mycorrhizae on reclaimed soils.

5.2.6 Literature Cited

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5.3 DEVELOPMENT OF NODULES AND MYCORRHIZAE ON GREEN ALDER AS INFLUENCED BY SOIL TEMPERATURE, PESTICIDES AND INOCULUM TYPE

5.3.1 Abstract

Soil from a green alder stand was used successfully to inoculate container-grown alder and produce nodulated, ectomycorrhizal plants. Inoculated plants were three times larger than uninoculated plants when grown at a soil temperature of 26°C. At 16°C no differences in size developed between inoculated and non-inoculated plants. Crushed nodules were also a good source of Frankia inoculum. Pure cultures of Alpova diplophloeus rapidly became colonized and killed by bacteria when they were put on the roots of alder. Some ectomycorrhizae were formed but Alpova did not spread far from the inoculum colony. Mycorrhization was slightly enhanced by growing the plants at 16° rather than 26°. In containers benomyl and Diazinon controlled Trichoderma and maggots, respectively.

5.3.2 Introduction

Green alder has been considered as a candidate for use in the reclamation of oil sand tailings but survival and growth on reconstructed soils has often been poor. Examination of nursery stock showed that potential outplants lacked mycorrhizae and were deficient in the N_2-fixing symbiont, Frankia (Part 5.1). Inoculum of Frankia was present in the reconstructed soils but alder only became mycorrhizal very slowly indicating a deficiency in the soil as well as the planting stock. Further, few fungi have been observed to form mycorrhizae with alder, the principal one being Alpova diplophoeus. As outplants may lack mycorrhizal inoculum, it may be desirable to artificially inoculate alder with Alpova. However, to date pure culture inoculations with Alpova have failed and techniques must be improved in order to determine the potential benefits of inoculating alders with mycorrhizal fungi. The use of alder as a reclamation species should not be rejected until a proper test of the whole plant - Alnus + Alpova + Frankia - is made.

In order to evaluate plant dependency on each of the symbiotic systems, the symbionts must be separated and inoculated independently.

Problems with inoculation of Alpova parallel those with jack pine and Suillus and the solution to one problem will probably resolve the second. Therefore, attempts were made to control potential inoculum grazers and antagonists with pesticides as were attempted with pine (Part 3.3). In addition, the effects of temperature on mycorrhizae formation were tested as an inoculum growth trial conducted in 1984 at relatively moderate temperatures (about 26^{o}C) stopped growth of Alpova in liquid cultures. Soil temperatures in this range are common under the greenhouse conditions used at The University of Calgary. Thirdly, different sources of Frankia inoculum were evaulated for effectiveness in producing nodules under greenhouse conditions.

5.3.3 Materials and Methods

5.3.3.1 Experiment 1. This experiment was designed to determine if either benomyl (a fungicide) or Diazinon (an insecticide) had obvious adverse affects on alder seedlings and if nonphytotoxic concentrations were effective on soil-borne insect larvae and fungi. Alder germinants were planted in 150 cc Cone-tainers containing a 1:1 mixture of autoclaved peat and vermiculite. They were placed in a greenhouse with extended day length (20 h, minimum 3.2 klx) and watered as needed until 6 weeks old. For the remainder of the experimental period all plants were fertilized twice weekly with a solution of 15:15:18 Plant Prod (200 mg L^{-1}). When the plants were 12 weeks old the pesticides were applied. Solutions of benomyl (100, 250 and 500 mg L^{-1}), Diazinon (1.25, 0.6 and 0.3 mL L^{-1}) and a combination of pesticides (benomyl 250 mg L^{-1} and diazinon 0.6 mL L^{-1}) were applied to three replicate tubes until the solution dripped out the bottom of the containers (30 mL per cell). Shoots were rinsed immediately with water. The day following, three 10 mm diameter plugs 1 mm thick of of Alpova diplophloeus (R-2402) inoculum from MMN agar plates were placed directly on fine roots exposed by cutting open the tubes. The tubes were closed with tape and after 5 days the inoculum was examined for growth and colonization by bacteria, fungi and insect larvae. Alpova was checked for growth in situ using a dissecting microscope and one plug from each tube examined for maggots (12-25x), sporulation of Trichoderma (12-25x)

and for bacteria and actinomycetes (500x, phase contrast). The general condition of alder leaves was also noted periodically to determine if the pesticides had phytotoxic effects. Thirteen days after the first pesticide application, the pesticides were reapplied, this time drenching each of the three replicate containers with a total of 500 mL of solution. The day following, a fresh agar plug of Alpova inoculum was placed in each tube. Thirteen days later the second inoculum plugs were examined for growth and colonization. Plants were observed for an additional 20 days.

5.3.3.2 Experiment 2. This experiment was done to determine the effect of two soil temperatures on mycorrhizal development. One alder germinant was planted in each of 84 150 cc Cone-tainers containing a 1:1 mixture of autoclaved peat and vermiculite. In addition, 28 containers were prepared with a soil inoculum collected a month earlier from a borrow pit in the Fort McMurray area which supported a dense stand of alder (Part 5.1). The soil was added at 20% (V/V) to the peat-vermiculite. One half of plants were placed in a growth chamber programmed to maintain a constant temperature of 26^{o}C in the root zone and the other half were placed in chamber programmed to maintain a 16^{o}C root zone temperature. The day length was 18 h and the air temperature was 24^{o}C day, 26^{o} night in the 26^{o} chamber and 11^{o}C day, 16^{o} night in the 16^{o} chamber. Light intensity 25 cm from the lights was 180 $\mu E\ m^{-2}sec^{-1}$ (170 w m^{-2}, 30 klx) in the 26^{o} chamber and 135 $\mu E\ m^{-2}$ sec (170 w m^{-2}, 21 klx) in the 16^{o} chamber. A solution containing 200 mg L^{-1} of 15:15:18 fertilizer was applied once per week.

Sufficient plants were grown to provide four treatments, (1) uninoculated, (2) soil inoculum (which would contain both Frankia and mycorrhizal fungi), (3) pure culture of Alpova diplophloeus (R-2402), and (4) Alpova plus benomyl (250 mg L^{-1}) and Diazinon (0.6 mL L^{-1}). Inoculum of Alpova was prepared on Nuclepore filters so intact colonies could be placed on the roots (Part 3.3). When the 26^{o} plants were 8 weeks old and the 16^{o} plants were 10 weeks old the pesticides were applied as a drench and 17 day old colonies were placed on the roots. The inoculum was visually checked for growth and

colonization by other organisms after 5 and 20 days. All plants were harvested when 17 weeks old, the percent of the short roots converted to mycorrhizae visually estimated (12x), number of nodules counted, and height, root collar diameter, and dry weight of shoots and roots determined. Growth medium pH was determined at the end of the experiment using 10g medium in 20 mL water.

5.3.3.3 Experiment 3. Alder was grown in 150 cc Cone-tainers in autoclaved peat: vermiculate (1:1) and fertilized twice weekly with a solution of 15:15:18 Plant Prod fertilizer at 200 mg L^{-1}. Plants were grown in the greenhouse under the same conditions as Experiment 1. When 12 weeks old, 10 replicate plants were inocululated with (1) a soil slurry, (2) nodule slurry, (3) a pure culture of Frankia or (4) left uninoculated.

Soil was collected from the borrow pit alder stand near the Suncor plant on June 13 and stored at 2°C until August 21. Nodules were sieved from the soil and 5.5 g (wet weight) was ground in mortar and pestle and 10 mL of suspension was injected into each container. For the soil slurry, 100 g of soil which had the roots and nodules sieved out, was placed in 500 mL water, mixed, and 10 mL was injected into each container. A pure culture of Frankia was obtained from M. Lalond and propagated in liquid culture. A 10 mL aliquot of the culture was injected into each container. The plants were grown for an additional 8 weeks and then harvested. The number of nodules on each plant were counted and the roots examined (12.5X, 500X) for mycorrhizae, and height, root collar diameter, and root and shoot dry weight (80°C) determined.

5.3.4 Results

5.3.4.1 Experiment 1. The application of 1.25 mL Diazinon per liter resulted in necrosis of the lower leaves and an obvious stunting of height growth. It was apparent that rinsing with water after applying the insecticide was insufficient to prevent damage to the plants. However all the rates of Diazinon applied were effective in controlling the maggots that otherwise colonized the Alpova inoculum (Table 1).

Table 1. Inoculation of alder with agar discs of *Alpova diplophloeus* and the development of maggots, *Trichoderma*, bacteria and actinomycetes on the inoculum as influenced by pesticides.

Treatments	Number of three replicates positive with respect to:									
	Alpova		Maggots		Trichoderma		Bacteria		Actinomycetes	
	Days from inoculation									
	5	13	5	13	5	13	5	13	5	13
Benomyl 100 mg L^{-1}	0	0	0	2	0	0	3	3	0	2
Benomyl 250 mg L^{-1}	0	0	1	2	0	0	3	3	0	3
Benomyl 500 mg L^{-1}	0	0	0	2	0	0	3	3	0	3
Diazinon 1.25 mL L^{-1}	0	0	0	0	0	3	3	3	0	3
Diazinon 0.6 mL L^{-1}	0	0	0	0	0	3	3	3	0	3
Diazinon 0.3 mL L^{-1}	0	0	0	0	0	3	3	3	0	2
Benomyl 250 mg L^{-1} and Diazinon 0.6 mL L^{-1}	0	0	0	0	0	0	3	3	0	2

Trichoderma sporulated on all the inoculum that had not been treated with benomyl but was absent when benomyl was applied. Examination of the inoculum with phase contrast microscopy indicated that few alien fungal hyphae were in the agar.

At both 5 and 13 days all inoculum was heavily colonized by bacteria. After 5 days most Alpova colonies were soft and gelatinized and showed no sign of life. By 13 days the colonies were mushy, actinomycetes were sporulating on the colonies and few intact hyphae of Alpova were observed. Actinomycete hyphae densely colonized the agar.

5.3.4.2 Experiment 2. Plants inoculated with soil and grown at 26°C soil temperature were three times taller and had three times the shoot mass as plants that were uninoculated (Table 2). The leaves of the soil inoculated plants were dark green and broad whereas leaves in all the other 26° treatments were narrow and light green. Inoculation at 16°C resulted in no growth response but leaves of plants grown in soil inoculum were broader and darker green than leaves in the other treatments. Inoculation with intact cultures of Alpova had no effect on plant growth at either temperature. Soil pH was very similar in all treatments.

Incorporation of infested soil into the planting medium was an effective means of introducing both Frankia and mycorrhizal fungi (Table 3). All plants at both temperatures became nodulated in the soil treatment and 5 of the 70 uninoculated plants became spontaneously nodulated.

Four mycorrhizal types developed at 26°C soil temperature and one type was found at 16° on plants inoculated with soil. Alpova occurred only at 26°. The dominant mycorrhizal type was a tomentose type with hyaline, clamped hyphae.

Intact colonies of Alpova initiated infection at 16° on all seedlings and on six seedlings at 26°. Alpova spread less than 20 mm from the inoculum at 16°. Five days after placing the Alpova colonies on the roots at 26°, all the colonies were heavily colonized by bacteria and no growth of Alpova could be detected. At 16° few bacteria were present on the inoculum and hyphae growth of Alpova had occurred. However, after 10 days bacteria were abundant on the 16° inoculum. At the end of the experiment all of the Alpova colonies had

Table 2. Effects of soil temperatures and inoculation with intact cultures of Alpova diplophloeus or soil and application of two pesticides, benomyl and diazinon, on alder grown in growth chambers for 17 weeks.

Inoculation Treatment	Soil Temp. °C	Soil pH	Height (cm)	Root Collar Diameter (mm)	Shoot Weight (g)	Root Weight (g)	S/R Ratio
Control	26	6.2	5.5 ± 0.8	2.7 ± 0.4	0.53 ± 0.16	0.47 ± 0.16	1.2 ± 0.3
Alpova Cultures	26	6.1	6.7 ± 1.6	2.5 ± 0.8	0.56 ± 0.29	0.50 ± 0.18	1.1 ± 0.3
Alpova + Pesticides	26	6.0	7.2 ± 1.6	2.8 ± 0.3	0.53 ± 0.10	0.40 ± 0.05	1.3 ± 0.2
Soil Inoculum	26	5.9	18.9 ± 2.2	3.8 ± 0.3	1.61 ± 0.29	0.76 ± 0.31	2.4 ± 0.9
Control	16	6.1	5.3 ± 1.0	2.7 ± 0.5	0.36 ± 0.08	0.41 ± 0.12	0.9 ± 0.2
Alpova Cultures	16	6.1	5.6 ± 0.1	2.8 ± 0.4	0.46 ± 0.88	0.65 ± 0.19	0.7 ± 0.2
Alpova + Pesticides	16	6.0	5.2 ± 1.3	2.6 ± 0.3	0.32 ± 0.06	0.41 ± 0.09	0.8 ± 0.1
Soil Inoculum	16	6.3	5.3 ± 0.4	2.3 ± 0.3	0.32 ± 0.10	0.32 ± 0.09	1.0 ± 0.3

Table 3. Effects of two soil temperatures and cultures of intact Alpova diplophloeus and soil inoculum on nodulation and mycorrhizal development of alder grown in growth chambers for 17 weeks.

Treatment	Soil Temp. °C	Number of Plants Nodulated	Nodules Per Plant x ± SD	Number of Seedlings Mycorrhizal	% Mycorrhizal Infection ($\bar{x}$ ± SD)			
					Total	Alpova	Basidio	Unknown
Control	26	1	<1	0	0	0	0	0
Alpova Cultures	26	2	<1	6	<1	<1	0	0
Alpova + Pesticides	26	2	<1	0	0	0	0	0
Soil Inoculum	26	10	20 ± 9	9	71 ± 35	11 ± 21	51 ± 39	1
Control	16	0	0	0	0	0	0	0
Alpova Cultures	16	0	0	10	1	1	0	0
Alpova + Pesticides	16	0	0	6	<1	<1	0	0
Soil Inoculum	16	10	10 ± 6	10	81 ± 7	0	81 ± 7	0

been completely destroyed. No Trichoderma or maggots were seen in either of the Alpova treatments. The addition of benomyl and diazinon inhibited mycorrhizal development by Alpova although it was poor at both temperatures. At 26^{o} only a very few mycorrhizae (<5) were formed and these were usually weakly developed. The same was true for the 16^{o} pesticide treatment, i.e. infection occurred but there was no further root colonization.

5.3.4.3 Experiment 3. Both the soil slurry and crushed nodules were effective sources of Frankia and inoculation with them resulted in nodulated plants (Table 4). Differences in growth due to nodulation were small although leaves of nodulated plants were larger and darker green than uninfected plants. No nodules were present on plants inoculated with the Frankia culture. No mycorrhizae were formed on any of the plants.

5.3.5 Discussion

Soil collected from under alder can be used as a source of both Frankia and ectomycorrhizal inoculum. However, it appears that a relatively large quantity of soil (20%) must be mixed into the growing medium to provide mycorrhizal inoculum. Nodules also proved to be a very good source of Frankia and can be easily added to the plants at any time in the growing cycle. However nodules would be more difficult to collect than soil. Nonetheless inoculation with simple symbiont sources offers several advantages especially for the small nursery grower. Fertilizer levels can be substantially reduced with no loss in seedling size. Outplant performance should be enhanced if one or both of the symbionts are established on the roots while in the nursery.

The use of pure cultures of Alpova appears to be limited by the rapid colonization of inoculum by bacteria. Infection from intact colonies occurred on about one-half the seedlings inoculated but in many cases only a very few mycorrhizae developed. This suggests that infection from the colony occurred very quickly, i.e. in a few days, before extensive bacterial colonization. Once the bacteria had proliferated further mycorrhizal development was inhibited. At low temperatures bacterial colonization was slow, allowing Alpova to infect a larger number of roots before the inoculum was killed.

Table 4. Effect of different inoculum types on growth of alder and nodulation and mycorrhization in the greenhouse. Plants 12 weeks old when inoculated and 20 weeks old when harvested (x±SD)

Inoculation	Height (cm)	Root Collar diameter (mm)	Shoot Weight (g)	Root Weight (g)	S/R Ratio	Number of seedlings nodulated	Number of Nodules per plant	Percent Mycorrhizae
Control	6.6 ± 1.8	3.3 ± 0.6	0.62 ± 0.24	0.37 ± 0.09	1.6 ± 0.4	0	0	0
Frankia culture	7.4 ± 1.0	3.0 ± 0.4	0.65 ± 0.18	0.37 ± 0.10	1.8 ± 0.6	0	0	0
Soil slurry	9.9 ± 1.9	2.9 ± 0.2	0.66 ± 0.10	0.30 ± 0.05	2.2 ± 0.4	10	12 ± 6	0
Nodule slurry	10.2 ± 1.4	3.2 ± 0.3	0.86 ± 0.13	0.34 ± 0.03	2.5 ± 0.4	10	158 ± 48	0

6. EPILOGUE: FUNGAL SUCCESSION AND INOCULATION

Can the knowledge of the ecology of mycorrhizal fungi be a guide for the rational selection of species for inoculation programs?

6.1 EPILOGUE: ASPECTS OF THE SUCCESSION OF ECTOMYCORRHIZAL FUNGI AND THEIR RELATIONSHIPS TO THE INOCULATION OF CONTAINER-GROWN SEEDLINGS

6.1.1 Abstract

Basidiospores and fragmented fruitbodies of ectomycorrhizal fungi were used in attempts to inoculate container-grown jack pine seedlings in the greenhouse. Hebeloma sp., which occurs with seedlings (early-stage fungus) and Suillus spp., which occurs with young and old plants (multi-stage fungi), all formed abundant ectomycorrhizae from both types of inoculum. Inoculation with fungi associated with mature stands of pine (late-stage fungi), Tricholoma sp., Chroogomphus vinicolor, Hygrophorus sp. and Russula sp., did not produce any ectomycorrhizae. It is suggested that the multi-stage fungi may be the best canidates for inoculating seedlings due to their relative ease in handling and persistence on field-grown plants. However, to achieve successful inoculations, it is necessary to modify procedures currently used for early-stage fungi. These changes would involve increasing the inoculum potential and/or decreasing host resistance.

6.1.2 Introduction

The existence of an orderly succession of ectomycorrhizal fungi as forest stands mature has been suggested in a casual context but only very recently have attempts been made to collect data that would confirm or deny the successional concept. If such a progressive replacement of fungi occurred, it could have implications on the selection of species of ectomycorrhizal fungi as the successional pattern could be used to predict persistence of introduced species. As well, it has been speculated that the more host specific symbionts are more efficient than "facultative" species (Mikola, 1970). In general, fungi associated with young seedlings are less host specific than those associated with mature trees. However, most of these observations are based not only on young versus old trees but also complicated by the fact that the young trees surveyed were usually in nurseries with soil and nutritional conditions much different than in the field.

In a series of investigations on conifers introduced to New Zealand and their similarly introduced fungal symbionts, Chu-Chou (1979) and Chu-Chou and Grace (1981, 1982) have shown that there is a progression of species producing fruitbodies in the sequence from the nursery to mature plantations. In nurseries, Rhizopogon spp., Laccaria laccata (Scop.: Fr.) Berk. & Br., Hebeloma crustuliniforme (Bull.: St. Am.) Quel., and Thelephora terrestris Ehrh.: Fr. were associated with radiata pine, Douglas-fir, or both. In eucalyptus nurseries, Laccaria laccata, Scleroderma spp., and Hydnangium carneum Wallr. (a close hypogeous relative of Laccaria) were the only fungi commonly fruiting. In plantations of eucalyptus up to 50 years-old, these species continued to fruit but additional species also became common such as Hymenogaster spp. (a secotioid genus related to Cortinarius), Cortinarius spp., and others (Chu-Chou and Grace, 1982). In Douglas-fir plantations, L. laccata and Rhizopogon vinicolor A.H. Smith persisted from the nursery and Amanita muscaria (L.: Fr.) Pers.: Hooker, Suillus lakei (Murr.) Sing., Russula sp., Scleroderma spp., and Tricholoma sp. also fruited. Suillus spp., Inocybe spp., A. muscaria and Scleroderma verrucosum Vaill.: Pers. were found only in stands over 5 years old.

From these observations there appears to be a limited number of species associated with young seedlings, some of which may persist for the life of the plantation, to which a larger number of species are added which never fruit in association with young seedlings. However, this does not show that the mycorrhizae differ with different aged trees, only that the spectrum of fungi fruiting differ. Isolation data by Chu-Chou and Grace (1981) of Douglas-fir mycorrhizae show that for some species, Rhizopogon vinicolor and Amanita muscaria, the fruiting pattern accurately reflected mycorrhizal development and persistence. However, the observations on fungal succession of totally introduced populations must be treated with caution as a full range of associates will not have been introduced. Missing elements in introduced populations may allow early successional species to persist after they would have been normally replaced in native forests.

Similar situations may exist in minespoils which are inoculum-poor, species-poor, and where competition is minimal. In coal mine spoil, qualitative estimates of the ectomycorrhizae of white spruce

seedlings showed that an early-stage fungus (sensu Mason et al., 1982), E-strain, could persist and dominate for over 4 years (Danielson et al., 1984). However, after 7 years over three-fourths of the ectomycorrhizae were formed by a multi-stage fungus, Amphinema byssoides (Fr.) J. Erikss. Multi-stage fungi are those that appear to occur throughout the life of the host. Amphinema byssoides has been observed on nursery seedlings (Part 2.1), wildling seedlings and in mature stands (Danielson et al., 1984).

In contrast to the situation on the coal mine spoil, Thelephora terrestris, an early-stage fungus that was introduced with jack pine planting stock, was dominant in oil sand tailings lacking peat for 4 years. In the peat-amended sand, E-strain fungi were dominant. However, between 4 and 7 years, T. terrestris was completely replaced by E-strain and Suillus spp., the latter probably also being a multi-stage fungi. In the peat, the resident E-strain was mostly replaced by Suillus spp. and Tomentella sp. Thus, in these spoils with a limited number of species, succession occurred in a dramatic fashion but the order was dependent upon the inoculum initially present and introduced.

The clearest demonstration that a regular, predictable succession of ectomycorrhizal fungi takes place is the long term study by Mason and associates (Mason et al., 1982). They have followed fruitbody formation around young birch trees in Britain for over 10 years and have found a definite sequential appearance of fruitbodies beginning with what they term early-stage fungi (species of Laccaria, Thelephora, Hebeloma), followed by late-stage fungi (Cortinarius, Leccinum, Lactarius, Russula). Most importantly, they have demonstrated fundamental differences between early and late-stage fungi, differences which may be relevant to inoculation success. Early-stage fungi can readily colonize roots in unsterile soil from indigenous inoculum or introduced mycelium (Deacon et al., 1983) or from basidiospores (Fox, 1983). In contrast, late-stage fungi cannot initiate mycorrhizal infections from basidiospores (Fox, 1983) or from mycelial inoculum unless it is attached to a parent tree (Fleming, 1983). This infers that the late-stage fungi are lower in inoculum potential (sensu Garrett, 1970) than early-stage fungi and/or are more

susceptible to microbial antagonism in the soil than early-stage fungi. It also suggests that late-stage fungi cannot be established on container-grown seedlings under conditions similar to those used for early-stage fungi. Nonetheless, it must be possible for late-stage fungi to initiate infections from detached mycelium or basidiospores, or these species could never spread beyond the area occupied by the parent tree.

As it would be desirable to determine the relative efficiencies of early and late-stage fungi as ectomycorrhizal symbionts and to further characterize differences between them, attempts were made to inoculate jack pine seedlings with basidiospores and fragmented fruitbodies. If this simple procedure fails, more elaborate measures will be necessary to inoculate trees with late-stage fungi.

6.1.3 Materials and Methods

Fruitbodies of ectomycorrhizal fungi were collected as they became available and were identified and voucher specimens preserved. Collections were made in lodgepole pine stands in the Calgary region and in jack pine stands in the Fort McMurray area. Collections 3185, 3186, 3187 and 3188 were from jack pine stands at Mildred Lake and the remainder were from the Calgary area. Spore prints were taken when possible and these were used as basidiospore inoculum as well as entire fruit-bodies which were fragmented in a blender. Suspensions of the two inoculum types were injected into Leach Cone-tainer cells containing 6 to 13-week old jack pine seedlings in a 1:1 (v/v) mixture of sterilized peat and vermiculite. The seedlings were fertilized twice per week with a solution containing 100 mg L^{-1} of 15:15:18 Plant Prod soluble fertilizer. After 2 to 4 months, the roots were examined for ectomycorrhizal development.

6.1.4 Results

Three species of Suillus and Hebeloma readily formed ectomycorrhizae with jack pine using either basidiospores or fragmented fruitbodies as inoculum (Table 1). In addition, the resupinate fungus Trechispora vaga (Fr.) Liberta formed ectomycorrhizae when fruitbodies were used as inoculum. This fungus has been

generally associated with white rots of pine and spruce (Gilbertson, 1974; Martin and Gilbertson, 1977). Inoculations with Tricholoma spp., Chroogomphus vinicolor, Hygrophorus sp. and Russula sp. all failed to cause ectomycorrhizal formation. All of the seedlings inoculated with Dentinum repandum resulted in Suillus-like ectomycorrhizae which probably resulted from basidiospore contamination.

The descriptions of the ectomycorrhizae are as follows:

Suillus tomentosus + jack pine. Mycorrhizae dichotomously branched 1 to 3 times, elements short and stout, pallid or very pale brown, extramatrical mycelium (EMM) abundant, mycelial strands fair to poorly formed, some small ones consisting of five or six strands fused together, EMM emmanating all along the length of mycelial strands. Hyphae 3-6 µm diameter, simple septate, smooth encrusted, some parts vinaceous in KOH. Cultures livid vinaceous in KOH, no crystals or mycelial strands.

Suillus umbonatus + jack pine. Mycorrhizae dichotomously branched 1 to 3 times, elements thick and stout, pure white in situ, more-or-less villose with curly hyphae of determinate length extending about 200 µm, EMM sparce to moderate in quantity. Rhizomorphs well formed, translucent in situ, distinct branching, few individual hyphae radiating out, main strands 60-100 µm diameter with all hyphae fused together, some crystalline material on the surface, occasionally slightly pink in KOH. Hyphae 2-3(4) µm diameter, simple septate, hyaline, smooth or when on mantle with a moderately dense covering of angular crystals 0.5-2 µm diameter, no reaction to KOH. Cultures with little aerial hyphae, brown, rhizomorphs well formed, very large amounts of crystalline material which turn livid vinaceous in KOH and culture almost black. The distinctive features are the pure white, hirsute mycorrhizae.

Table 1. Results of inoculation of container-grown jack pine seedlings with suspensions of basidiospores or fruitbody slurries of ectomycorrhizal fungi.

Species and voucher number		Spore or slurry inoculum	Ectomycorrhizal formation (positive/total tubes)	
			Test fungus	Other
Suillus tomentosus	3138	spores	10/10	0/10
Suillus tomentosus	3172	slurry	10/10	0/10
Suillus umbonatus	3124	spores	5/5	0/5
Suillus umbonatus	3167	spores	10/10	0/10
Suillus pseudobrevipes	3191	slurry	3/3	1/3
Hebeloma sp.	3178	slurry	5/5	0/5
Hebeloma sp.	3178	spores	5/5	0/5
Trechispora vaga	3184	slurry	5/5	0/5
Tricholoma flavovirens	3187	slurry	0/3	0/3
Tricholoma flavovirens	3187	spores	0/3	0/3
Tricholoma sp.	3186	slurry	0/3	1/3
Tricholoma sp.	3186	spores	0/3	1/3
Chroogomphus vinicolor	3148	spores	0/10	10/10
Chroogomphus vinicolor	3181	slurry	0/5	1/5
Chroogomphus vinicolor	3181	spores	0/5	0/5
Dentinum repandum	3182	slurry	0/5	5/5
Hygrophorus sp.	3185	slurry	0/3	3/3
Hygrophorus sp.	3185	spores	0/3	1/3
Russula sp.	3188	slurry	0/3	1/3
Russula sp.	3188	spores	0/3	0/3

Suillus pseudobrevipes + jack pine. Mycorrhizae coralloid, dichotomously branched up to four times, elements stout, light to moderately heavily floccose, elements easily seen, creamy yellow (8G-9H) or fulvous, EMM white and obviously encrusted at 12X. Mycelial strands hyaline, loosely to moderately well formed. Hyphae 3-4 µm diameter, simple septate, encrusted with resinous lens, some turning pink in KOH. The only distinctive feature was the yellow colour but it may disappear with age.

Trechispora vaga + jack pine. Mycorrhizae dichotomously branched one to two times, cream (6F) becoming fulvous, finely floccose, mantle thin, good Hartig net; mycelial strands pale yellow. Hyphae 2-3 µm diameter, simple septate, smooth, very faintly pigmented, no change in KOH. Cultures yellow. The yellow colours and simple septa may be distinctive.

6.1.5 Discussion

The fungi that were successful in infecting short roots and producing ectomycorrhizae were either early-stage (Hebeloma sp.) or what can be termed multi-stage fungi (Suillus spp.). Late-stage fungi (Tricholoma spp., Chroogomphus vinicolor, Russula sp.) all failed despite the introduction of large numbers of fresh basidiospores. It is not known whether the spores germinated or not or whether the germination requirements of early- and late-stage differ. Under aseptic conditions, germination of all mycorrhizal fungi is poor, with no apparent differences among early-stage and late-stage fungi (Fries, 1978).

In general, distinct differences exist between early- and late-stage fungi (Table 2) although some species transcend this classification and occur throughout the successional sequence. Prominant among these multi-stage species are probably many boletes (e.g., Suillus spp.), some Ascomycetes (Cenococcum geophilum Fr.) and Amphinema byssoides. It may be within this group that the most desirable fungi exist for artificial inoculations. These fungi can be cultured, basidiospores are infective, and their existence on both seedlings and mature trees indicates that they are competitive and

Table 2. Known and speculative characteristics of ectomycorrhizal fungi occurring at different successional stages

Feature	Successional Position	
	Early-stage	Late-stage
Capable of growth in culture	most or all	less than half
Growth rate in culture	moderate to rapid	slow
Fruiting in containers	few	none
Tolerance to fertilizers (R_H)	high	low
Number of host species	many	few or one genus
Infection from nonmycorrhizal hyphae	most	very few
Infection from spores	readily	difficult
Root to root infection spread	internal	external
Rhizomorphs	some	some
Hypogeous development	few	many
Salt tolerance	high	low
C-efficiency	high	low
Monokaryon infection	most	few
Soil type specificity	low	high
Sensitivity to antagonisms	low	high
Taxa	Asco or Basidio	few Asco

persistent. Their effects on host performance remains to be determined.

Of major importance in inoculation programs is the ability of the fungi to form mycorrhizae in the greenhouse when nutrient levels have been elevated by fertilization. There is no doubt that different fungi exhibit different fertilizer tolerances (Part 3.1) and it may be desirable to alter these tolerances, if possible, in order to produce satisfactorily sized seedlings. It is likely that a majority of the ectomycorrhizal fungi are "sensitive" to high fertilizer levels.

This sensitivity could be due to two causes, an external root sensitivity or internal root sensitivity (or both). In the first case, sensitivity could be due to the presence of fertilizer salts in the soil solution which have direct adverse effects on fungal hyphae or spores. This hypothesis has received very little consideration but recent experiments have indicated that direct effects may be important in mycorrhizal formation. Ingestad et al. (1986) grew Scots pine and inoculated it with Suillus bovinus in hydroponic growth chambers in which nutrients could be supplied continuously to the plants at low concentrations. In this way, the plants were given optimum nutrition using low conductivity solutions and despite this optimum nutrient status, the roots quickly became mycorrhizal and the fungus spread readily over the root system. Ingestad et al. (1986) concluded that previous reports of fertilizer inhibition were due to salt effects rather than to the nutrient status of the host.

This direct effect would also serve to explain a phenomenon encountered frequently in inoculation studies, that of limited infections, i.e., formation of a few good mycorrhizae near the inoculum source but the failure to spread and colonize adjacent, noninfected short roots. According to the inoculum potential theory (see below), once infection occurs, the inoculum potential of the fungus should increase due to the freely available carbon provided by the host, and subsequent infections should occur readily. However, this often does not occur (see Parts 3.3 and 5.2) and it could be suggested that either host resistance or the external environment is inhospitable to the spread of the fungus. Increased host resistance is a common feature of woody plants following infections or wounding by fungi,

insects or bacteria (Shigo, 1984). This process, referred to as compartmentalization, is the major defense reaction of trees and serves to localize and contain the invading organisms. Whether this also occurs on fine roots remains to be determined. In the absence of compartmentalization, the spread of mycorrhizal fungi could be limited by the external medium, for example, salt concentration.

The alternative theory to external adversity suggests that the fertilizer effect is not a true sensitivity to mineral nutrients per se but rather an inability to overcome host resistance which increases with improving mineral nutrition (Garrett, 1970). If this is true, then the sensitivity expressed should be dependent upon the inoculum potential of the fungi and by increasing inoculum potential, ectomycorrhizae formation should be possible at higher nutritional levels than with low inoculum potentials. This also infers that the various species of ectomycorrhizal fungi vary considerably in their inherent inoculum potentials which could account for the degrees of success expressed by the different fungi when exposed to different fertilizer levels (Part 3.1). It could be suggested that early-stage fungi possess high inoculum potentials and are thus capable of initiating infection from spores or small fragments of mycelium. At the other extreme, inherent inoculum potential is low and this must be compensated for by establishing a large mycelial base with high inoculum potential or preconditioning hosts to lower host resistance. Individual hosts may vary in resistance seasonally or with age as well, and thus vary in susceptibility to late-stage, low inoculum potential fungi. The failure of some multi-stage (e.g., Amphinema byssoides) and late-stage (Russula spp.) fungi to form mycorrhizae with seedlings may be due to a combination of low inoculum potential (inoculum as spores or small fragments of mycelium) and high host resistance (high light intensity, moderate to high nutrient levels).

An example of the dependency of ectomycorrhizal formation on the balance between inoculum potential and host resistance can be seen in synthesis experiments where the inoculum potential is very high due to the availability of a soluble, readily utilizable sugar which permits saprophytic growth. Molina and Trappe (1982) have reported numerous cases of fungi formally thought to be host specific based on

a wealth of field observations of fruitbody occurrence, forming mycorrhizae in monoxenic culture with "nonhosts." They interpreted these results as indicating that so-called host specific fungi really associated with other hosts in the field but did not produce fruitbodies with those hosts. However, their results could also be interpreted that in monoxenic culture inoculum potential was greatly elevated by the readily available carbon which permitted the fungus to overcome the resistance of species that were marginal in inherent resistance.

Host resistance in synthesis experiments may also be reduced by the environmental conditions. For example, Molina and Trappe (1982) used light levels of 10.5 klx, a level well below full sunlight. They also used an MMN nutrient solution that contains nine-fold greater concentrations of P than N, just the reverse of the nutrient ratios recommended for conifers (Brix and van den Driessche, 1974). That, and the fact that the plants do not transpire in culture tubes, could lead to nutrient imbalances and reduced host resistance.

Godbout and Fortin (1983) synthesized mycorrhizae with two species of Alnus, a genus considered to be highly specialized in regard to fungal associates (Molina, 1981). Using nonsterile growth pouches, and a low light intensity (10 klx), they found that seven species of ectomycorrhizal fungi that were not known to have fruitbody associations with alders, formed ectomycorrhizae in the pouches. Many of these formed only a few mycorrhizae in contrast to Alpova diplophloeus, a known associate of alders, which colonized most of the root system. Godbout and Fortin (1983) suggest that the positive results in the pouches apply to field conditions and that many alder associates simply do not produce fruitbodies in nature. It seems more likely that the synthesis results are due to the artifical conditions which favor infections by reducing host resistance and/or by introducing cultures of fungi with high inoculum potentials.

The prevalent thought is that when light intensity is reduced, mycorrhizal frequency is reduced as a result of lowered levels of carbohydrates in the roots (see Slankis, 1973), the opposite of that is suggested above. However, as Slankis (1973) points out, there are conflicting results concerning light intensity and ectomycorrhizal

infection. He cites several examples of where the ectomycorrhizal frequency was higher at low light intensities than at high light levels. Furlan and Fortin (1977) found that VA mycorrhizal infection was higher under light intensities of 5 or 10 klx than when plants were exposed to light intensities of 15 or 20 klx. However, others (e.g., Hayman, 1983) have reported that VA mycorrhizal infection was greater at high (25 klx) light intensities than at low (8 klx) light intensities. Differences among studies could be due to the use of different fungi (inoculum potential factor) or nutritional factors (host resistance factor). Giltrap (1983) has shown that mycorrhizal development in monoxenic culture with a birch-Cenococcum combination was higher with low light than high light when sugar levels were low (i.e., low host resistance and low inoculum potential) but with high sugar levels, infection was greatest at the highest light level (i.e., high host resistance overcome by high inoculum potential). However with Paxillus involutus, infection was always highest at the highest light level (a difference in inherent inoculum potential?). Further, with pine, both C. geophilum and P. involutus converted more short roots to mycorrhizae at high light levels (differences in host resistance?). It is well to note that the "high" light level used by Giltrap (1983) was really very low (18 W m^2). Nonetheless, it is apparent that low light intensities may under some circumstances favor mycorrhizal infections. In another study, photosynthetic stress was induced by partially defoliating soybean plants with the result that VA mycorrhizal root colonization increased with increasing defoliation (Boyne et al., 1984). This increased mycorrhization in spite of decreased carbon availability might have been due to changes in host resistance, as the results are contrary to the established theory on carbon availability and mycorrhizal infection.

Once host resistance has been initially overcome, a fungus may be quite a successful colonizer of the remainder of the root system as the host is providing energy for further expansion. This may explain the over-representation of specialized late-stage fungi (as indicated from mycorrhizal density) on seedlings planted in mature stands (Fleming, 1983; Part 2.3). This may also be indicated by the

all-or-nothing mycorrhizal formation by Hebeloma sp. on seedlings subjected to high fertilizer levels (high host resistance) (Part 3.1).

The attempt here has been to rationalize the artificial inoculation of seedlings by approaching the problem in terms successional patterns, inoculum potential and host resistance. Unfortunately, little is known about these factors for even the most common of the ectomycorrhizal fungi. However, this approach indicates that as succession progresses and the ectomycorrhizal fungi become more specialized, techniques for artificially inoculating these fungi must become increasingly refined with high regard given to the biology of the individual fungi. These refinements are basically concerned with elevating and maintaining inoculum potentials and reducing host resistance. The maintenance of high inoculum potentials will certainly be the most challenging aspect as it involves inoculum interactions with the soil microflora (antagonisms), the soil microfauna (grazing and feeding) and adaptations to soil abiotic factors. Until these factors are better understood, the question of what the effects of late-stage fungi are on host performance cannot be determined.

6.1.6 Literature Cited

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7. APPENDICES

7.1 Appendix. Ectomycorrhizae Occurring on Lodgepole Pine and White Spruce in the Smoky Lake Nursery.

1) Thelephora terrestris: Mycorrhizae whitish at tip to dark brown, smooth appearing. Mantle well developed, EMH common, 3-4 µm diameter, hyaline or lightly pigmented brown, clamped, dark brown mycelial strands very common. Readily isolated if not surface sterilized.

2) E-strain: Mycorrhizae dark brown, mantle discontinuous, EMH 6-10 µm diameter, Woronin bodies present, well ornamented. Intracellular hyphae present in some root cortical cells. No growth in culture, some stiff hyphae emerging from mycorrhizae but no growth on agar.

3) Unknown Basidiomycete No. 1 (All three crops): Mycorrhizae near glabrous to floccose, white, 2-3X dichotomous with pine. EMH 2-4 µm diameter, hyaline, smooth, clamped. Growth in culture with white frosty hyphae 2-3 µm diameter.

4) Unknown cystidial: Cystidia 20 x 80 µm, mantle a texture epidermoidea, EMH sparce, 4 µm diameter, septate, pigmented brown. No growth in culture.

5) Coltricia sp.: Mycorrhizae monopodial, inflated, brown, no EMH, hyphae 3-4 µm diameter, simple septa. No cystidia.

6) Sphaerosporella brunnea: Mycorrhizae subglabrous, tan to dark brown, mantle thin, a textura epidermoidea. EMH 4-10 µm diameter, smooth or finely ornamented, large Woronin bodies. Growth very rapid in culture (2 cm/day), hyaline on MMN, brown on PDA.

7) Amphinema byssoides: Mycorrhizae covered with dense whitish to pale yellow hyphae which filled the entire root plug. EMH 2-3 μm diameter, finely ornamented, clamped; yellow mycelial strands common. Addition of 3% KOH caused mycelium to turn dark yellow in colour. EMH easily stripped off leaving non-descript mycorrhizae. Growth slow in culture.

7.2 Appendix. White spruce ectomycorrhizaae occurring in the Syncrude nursery.

1) E-Strain: Mantle development sporadic, discontinuous, individual cells large. Extramatrical hyphae (EMH) sparse, septate, with Woronin bodies, 4-8 µm diameter, smooth or occasionally ornamented, hyaline or pale brown. No growth in culture.

2) Amphinema byssoides: EMH abundant, pale yellow, 2-3 µm diameter, clamped and usually finely ornamented, turns bright yellow with the application of 3% KOH.

3) Coltricia sp.: Mantle bristley with long, brown cystidia. Cystidia 200-400 µm long, 2-3 µm diameter, with 3 to 4 septa, occasionally branched, septa simple, terminal cells acute. Mantle in plan view a textura epidermoidea with cells 3-5 µm diameter. Growth good in culture, hyphae smooth with simple septa, a fertile, poroid hymenophore forming after several weeks. Cultures identical to a culture obtained from a fruitbody of Coltricia sp. obtained from the June 1983 sample. Fruitbody too weathered for a species identification.

4) Mycelium radicis atrovirens: Very sparse hyphal development, no mantle, EMH 2 µm diameter and pale brownish, Hartig net present.

5) Unknown Basidiomycete No. 1 (August 1983): Mycorrhizae inflated, shiny white to yellowish, sparse EMH 3-4 µm diameter, hyaline, clamped. Growth good in culture.

6) Unknown Basidiomycete No. 2 (June 1983): Poor to moderate mantle development, a textura intricata. EMH 3 µm diameter, clamped. Growth in culture.

7) Unknown Basidiomycete No. 3 (August 1982): Mycorrhizae with inflated tips, EMH abundant throughout the root plug, 3 µm diameter, hyaline, clamped, mantle well developed. Growth poor in culture.

8) Unknown Basidiomycete No. 4 (August 1982): Mycorrhizae smooth, swollen, good mantle development, EMH sparce, 3-4 µm diameter, hyaline, clamped, smooth. Growth good in culture.

9) Unknown Basidiomycete No. 5 (August 1982): Mycorrhizae covered with dense white EMH, mantle well developed, a textura intricata, EMH 3-4 µm, clamped. No growth in culture.

10) Unknown Basidiomycete No. 6 (August 1982): Mycorrhizae covered with dense rosy pink coloured EMH, hyphae 3-4 µm diameter, smooth, clamped, mantle a textura intricata.

7.3 Appendix - Distinguishing features of jack pine mycorrhizae developing in muskeg peat under greenhouse conditions. Numbers conform to those in Tables 3 and 5, in Part 2.2.

1. I-type Ascomycete. Dichotomously branched once or twice, cream becoming rusty-tawny with age, glabrous or bristling with hyaline cystidia. In plan view (500X) the mantle a textura "jig-saw" (jig-saw puzzle-like), septa with associated Woronin bodies, cells up to 20 µm diameter. Cystidia hyaline, acute, 3 x 110-150 µm, wall slightly thickened to 0.8 µm, septate, base inflated, Woronin bodies at the septa. Growth in culture moderately rapid but sparse and with no aerial hyphae.

2. Tomentella sp. I. Dichotomously branched up to three times, dark brown to almost black, glabrous or occasionally with cystidia or a few extramatrical hyphae, hyphae 3-4 µm diameter, ochraceous, clamped, pigmented and smooth. In plan view the mantle a textura angularis, individual cells (5) 10-14 (20) µm diameter, surface smooth or furfuraceous with pigmented flakes deposited between cells. Individual cells easily seen at 25X. Cystidia generally absent; hyphoid, undifferentiated, tip obtuse, wall thin and lightly pigmented, 15-20 X 2-3µm, septate with one simple septum. In culture colonies brown floccose, aerial hyphae 3-4 µm diameter, smooth or verruculose with encrustations, pale ochraceous, occasional cell contents turn green in KOH.

3. E-strain. Dichotomously branched once or twice, cream becoming sienna, rusty-tawny to baY, nearly glabrous; extramatrical hyphae pale fawn, blister-like ornaments, 5.5 µm diameter excluding ornaments, 8.0 µm with ornaments. Mantle a textura epidermoidea-textura intricata, hyphal walls smooth, Woronin bodies present. Heavy intracellular infection or hyphae apparently restircted to Hartig net. Cultures typical of E-strain with stiff,

frosted-appearing hyphae growing from the mycorrhizal root tips, initially hyaline, gradually becoming pale brown.

4. Yellow cystidia. Monopodial, greenish yellow (3B4) on tips, base brown over yellow (4A8) (like Pluteus lutescens), sometimes only the meristematic region yellow, no extramatrical hyphae, cystidia difficult to see. Mantle a textura angularis, cells 6-15 µm diameter. Cystidia hyphoid, walls thin or thickened, up to 2 µm thick at the base, septate and clamped, 4 x 30-75 (105) µm, occasionally branched at right angle, tip obtuse and slightly inflated and occasionally with yellow resinous exudate on the tip. In culture, colony bright yellow.

5. Cenococcum geophilum. Black with stiff radiating hyphae. Hyphae 4-5.5 µm, smooth, walls 0.8-1 µm thick, septa thickening with age, Woronin bodies present at young septa. Cells of mantle nested.

6. Golden floccose. Densely floccose with yellow or golden brown hyphae. Hyphae smooth, 4 µm diameter with large hemi-sphaerical clamps. Mantle a textura intricata. Despite repeated attempts, the symbiont could not be cultured.

7. Rhizopogon-like I. Stout coralloid, floccose to felty with loose mycelial strands, white becoming vinaceous purple or nearly black with age. Microscopically the hyphae covered with plate-like crystals and lens of resinous exudate, septa simple. Crystaline material and resinous material hyaline or pale vinaceous, the colour intensifying when mounted in KOH. Infection usually in localized areas of the root. Readily cultured.

8. Rhizopogon II. Coralloid, bay, small amount of extramatrical hyphae, hyphae clamped; rhizomorphs dark, well developed. Hyphae with abundant crystals and vinaceous resin-like deposits. Growth good in culture.

9. T. angularis. Monopodial or occasionally dichotomous, pale cream, sparce extramatrical hyphae, hyphae 4-5 µm diameter, simple septate, hyaline, smooth. Mantle composed of blocky elements forming a textura angularis, cells 8-14 µm diameter, Woronin bodies present. Growth in culture unknown.

10. Tibiiform cystidia. Cream, appearing finely hairy due to cystidia. Mantle a textura angularis, cells 10-14 µm diameter. Cystidia tibiiform, 80-110 µm long, arising from narrow hyphae on the surface, all thin walled, smooth, tip 6-10 µm diameter, base 3-4 µm diameter with a simple septum. Woronin bodies absent. Not obtained in culture.

11. Black asco. Jet black with abundant black hyphae radiating out as with Cenococcum; hyphae stiff, infrequently branched, densely ornamented with blister-like warts, 1.5 µm high, hyphae 4-4.7 µm diameter exclusive of ornaments, 6.3-7.1 µm with ornaments. Not obtained in cultured.

12. Hyaline basidio. Dichotomous, cream, unpigmented, extramatrical mycelium sparse or not apparent and the mycorrhizae appearing glabrous. Hyphae hyaline, smooth, 4 µm diameter, clamped, mantle a compact textura intricata, thin. The mycorrhizae are undistinguished and culturing was required to confirm identification. In culture on MMN, growth rapid, aerial hyphae lacking, colony hyaline, spreading, submerged hyphae 4-6 µm diameter, smooth, clamped, remaining separate or forming small aggregations giving the colony a spotty appearance. Hyphae in aggregations moniliform 10-15 (26) µm diameter, walls 1-1.5 µm thick. All colonies turn

pale yellow with the application of KOH or NaOH and this character separates this taxa from all the other hyaline, clamped species.

13. Coarse asco. Short roots appearing uninfected except for the opaque nature of the root tips or becoming dichotomous, no extramatrical mycelium lacking or very sparse, 4-6 μm diameter, walls slightly thickened, hyaline or tinted brown, nonornamented, Woronin bodies conspicuous at the septa. Microscopically the mantle thin as with E-strain, often discontinuous, cells 6-12 (22) μm diameter, suggestive of a textura globulosa or a textura intricata with inflated cells or textura intricata-textura epidermoidea. Intercellular infections absent. Slight growth from washed tips in pure culture but no growth after transfer.

14. White floccose. Monopodial to dichotomous, stout, floccose; hyphae 2.5 μm diameter, hyaline, thin-walled, clamped.

15. Unknown affinity. Material too scanty to characterize.

16. Unknown affinity. Dichotomously branched 1-2X, long elements, cream, consistently with sparse extramatrical hyphae; hyphae hyaline, 2.5 μm diameter, smooth, simple septate.

17. Unknown affinity. Dichotmously branched once or twice, elements stout, cream becoming pale brown with age, completely glabrous. Mantle dense and compact, a textura epidermoidea, cells 2.5-2.5 μm diameter; hyphae simple septate, no Woronin bodies seen. No growth on MMN^{+} from washed tips.

18. Basidiomycete. Unbranched, floccose, tinted yellow; hyphae 2.5 μm diameter, clamped, smooth or verruculose. Suggests Amphinema but culturing required to confirm.

19. Unknown affinity. Floccose, cream, robust, hyphae 2 µm diameter, smooth, simple septate, Woronin bodies lacking. No growth on benomyl-MMN from washed tips.

20. Tomentella-like. Dark brown, glabrous and very similar to Tomentella. Mantle a textura angularis, cells up to 10 µm diameter, with discontinuous bits of pigments between cells which project out and at 25X give a rough appearance, i.e., flake-like deposits. In culture (R-2350) growth slow with abundant long erect fascicles of hyphae, hyaline becoming dingy brown, rarely cells globose and 10 µm diameter. Hyphae 2-4 µm diameter, smooth, hyaline, clamped.

21. Tomentella-II. Dichotmously branched up to 3 times, snuff brown, bay becoming fuscous black, glabrous or with a dense proliferation of flexuous cystidia, occasional hyphae radiating out, 3 µm diameter, pale yellow brown, finely roughened. Mantle a textura epidermoidea, cells 3-6 (10) µm diameter, with or without flaky incrustations. Cystidia 2-2.5 x 200-250 (340) µm, acute, walls hyaline to ochraceous, clamped at the base, nearly occluded, aseptate or with one simple septum; arising from a low turf of much branched hyphae 2.5-5.5 µm diameter. In culture deep floccose, fawn, reverse dark brown, aerial hyphae 4 µm diameter, smooth or finely encrusted, hyaline to ochraceous, in 10% KOH a few encrusted areas flash through violet then turn green and slowly dissolve. ON PDA small areas of hyphal walls are green in KOH.

22. Ascomycete. Blackish, hyphae brown, simple septate with Woronin bodies, 6 µm diameter; mantle a textura intricata, cells 4-8 µm diameter.

23. Unknown affinity. Sparse mycelium.

24. Basidiomycete. Floccose, white, monopodial. Hyphae 3-4 μm diameter, smooth, clamped. No growth from surface sterilized tips.

25. Ascomycete. Black and Cenococcum-like but dark radiating hyphae not as stiff, hyphae 4 μm diameter, smooth, simple, obvious Woronin bodies; mantle cells up to 20 μm diameter.

26. White floccose. Pure white, floccose, white mycelial strands. Hyphae smooth, 4 μm diameter, large clamps present. Growth in pure culture.

27. Basidiomycete. Insufficient material to determine features.

28. Unknown affinity. Insufficient material to determine characteristics.

29. Basidiomycete. Elements slender, reddish brown, no external mycelium. Hyphae 2.5-5.4 μm diameter, clamped, mantle thin, discontinuous.

30. White floccose. Dense floccose, hyphae 4 μm diameter, hyaline, smooth, clamped.

31. Dense floccose. Densely floccose with hyaline mycelium, surface pale cream; hyphae 2.5-3.5 μm, smooth, clamped.

7.4 Appendix - Descriptions of Ectomycorrhizae Formed by Inidgenous Fungi of Jack Pine Planted on the Syncrude Dyke. Numbers Correspond to those in Table 8, Part 4.1.

1. E-strain fungi. The key characters are the (1) thin, discontinuous mantle composed of cells up to 10-12 μm diameter; (2) extramatrical hyphae 4-6 μm which is ornamented in part and lightly pigmented brown; (3) the presence of Woronin bodies at the septa; (4) hyphae usually, but not always, intracellular in the cortical cells; and (5) when ectomycorrhizae plated, the hyphae are stiff and frosted, white gradually turning brown. Most or all of the E-strain fungi indigenous to the dyke could be cultured. In general, growth was slow on MMN and PDA (1-2 cm diameter after 6 weeks) and aerial hyphae was sparce. One culture produced a few large chlamydospores. Cultures on MMN were often arachnoid and had moniliform submerged hyphae; on PDA the cultures were much darker and aerial hyphae more abundant (R2394, 2395, 2396, 2405, 2406, 2407, 2408, 2411, 2412).

2. I-type Ascomycete. The distinctive features are the hyaline cystidia, 100-150 μm long, and the textura arrangement of the mantle as seen in plan view. The cystidia are frequently absent but ectomycorrhizae can still be recognized by the extremely smooth mantle, the cell shape, and the presence of Woronin bodies. The fungus is readily cultured and produces hyaline colonies without aerial hyphae (R2420).

3. Unknown Basidiomycete (first season only). Subfloccose, hyphal 2-3 μm diameter, smooth, clamped.

4. Unknown Ascomycete (first season only). Mycorrhizae stout, dichotomous, branched once or twice, pale cream to pale fulvous, abundant EMH, dirty; mantle hyaline, a texture intricata with cells 6-8(14) µm diameter; EMH 2, 5-4(6) µm diameter, encrusted with fine hyaline angular granules, Woronin bodies obvious (R2393).

5. Hyaline Basidiomycete. Ectomycorrhizae subfloccose to floccose, mycelium hyaline, hyphae 3-4 µm, smooth, clamped. No growth in culture. A rather nondistinctive group which could include more than one taxon.

6. Rhizopogon-like (vinaceous). Ectomycorrhizae simple to large coralloid structures, floccose, white becoming tinted vinaceous; mycelial strands present; hyphae encrusted with small crystals and with droplets of resinous exudate, 3-4 µm diameter, septa simple; exudate or some crystalline matter turning livid vinaceous in KOH. Grows readily in culture (R2421, 3445, 2446, 2448).

7. Unknown Basidiomycete (first season only). Dichotomous, floccose, hyphae 3 µm diameter, smooth clamped.

8. Unknown affinity. Nondistinctive ectomycorrhizze lacking both clamps and Woronin bodies, and too few in number of attempt culturing.

9. Unknown floccose (first season only). Floccose, hyphae 2 µm diameter, simple septate, hyaline.

10. Cenococcum geophilum. Ectomycorrhizae black with stiff radiating hyphae that are 5-6 µm diameter, smooth, with Woronin bodies at young septa. Cells of mantle in nested, radiating patterns.

11. White floccose.

12. Basidiomycete (2μ). Ectomycorrhizae subfloccose, occasionally floccose, hyphae 2 μm diameter, clamped, smooth, hyaline. Growth in culture good, all submerged on MMN and appearing spotty due to hyphal aggregations. Colonies on PDA hyaline or pale brown, no aerial hyphae but some colonies becoming rough as hyphal aggregations develop on the surface of the agar. Colonies turn yellow with the application of KOH (R2426, 2429, 2431, 2435). The difference between this group and group 5 is not clear and requires further study on hyphal sizes, KOH reaction and ability to grow in culture.

13. Tomentella sp. 1(vin-rhubarb). Mycorrhizae fulvous, dichotomous, mantle cells 4-6 μm diameter, a textura epidermoidea dense with setoid cystidia; cystidia long and flexuous up to 2 x 300 μm, tip acute and often bent, clamped(?), and lightly pigmented at base. Growth in culture (R2419) on MMN fair, floccose, vinaceous buff, reverse pale brown, no odor. On PDA growth good, deep floccose, fawn, reverse cigar brown, no odor (6 weeks old).

14. Rhizopogon-black rhizomorph. Mycorrhizae white with black rhizomorphs.

15. Mycelium radicis atrovirens. Mantle absent or poorly developed, usually with a few or many loose olivaceous brown hyphae on and around the root surface; Hartig net well developed and composed of very small hyphal cells; hyphae 2-2.5 μm diameter, finely roughened hyaline if part of mantle or Hartig net, walls of EMH pigmented.

16. Golden floccose. Densely floccose with golden brown hyphae; hyphae smooth, 3-4 μm diameter, clamped, walls slightly thickened, ochraceous.

17. Rhizopogon-Large crystals. Ectomycorrhizae simple to coralloid, pure white; floccose mycelial strands, white or tinted pink. Hyphae totally encrusted, small crystals or typically with large crystals 10-20 µm diameter, no resinous exudates, no colour change in KOH. Growth in culture good, closely matches isolate from fruitbody of Rhizopogon rubescens (RMD 3228, R2442, 2443, 2444, 2447, 2449, 2450).

18. Golden brown. Floccose with golden brown hyphae, abundant cystidia up to 120 µm long, septate, clamped, 2 µm diameter, walls pale brown.

19. Tomentella sp. 2. Mantle a textura epidermoidea with cells 6-8 µm diameter, deposits of brown pigments on walls, cystidia up to 120 µm long, hyphae clamped. Colonies on MMN hyaline and very slow growing. On PDA growth moderately rapid, subfloccose,fawn, lumpy, sparse dark exudate drops, agar all nearly black (R2414).

20. Setae = Tomentella sp. 1 (13).

21. Snowball. White clusters of angular crystals up to 10 µm diameter, clusters of crystals up to diameter of short roots; hyphae 3 µm diameter, clamped,smooth, hyaline.

22. Ascomycete. Subglabrous, cream, hyphae 2-3 µm diameter, Woronin bodies present.

23. Ascomycete. Short roots look uninfected, mantle discontinuous, cells 10-20 µm diameter, Woronin bodies present.

Appendix 7.5, Table 1. Key features of ectomycorrhizae formed by *Suillus*, *Rhizopogon*, and closely related genera.

Ectomycorrhiza	Gross Features	Extramatrical Hyphae	Mycelial Strands	Reference
Pine + *S. luteus*	Yellowish pink, smooth	Encrusted, - clamps	Weakly formed	Palm and Stewart (1984)
Pine + *S. neoalbidipes*	White to brown, wet-like	Granules and globules, + clamps	Light brown	Palm and Stewart (1984)
Pine + *S. brevipes*	Brownish black to deep brown	Granules and globules, + clamps	Brown	Palm and Stewart (1984)
Pine + *S. americanus*	Light grey-brown	Granules and globules, + clamps	Compact ± synenchymatous	Palm and Stewart (1984)
Pine + *S. pictus*	moderate orange	Brown to orange granules and globules, - clamps	Moderate orange	Palm and Stewart (1984)
Pine + *S. brevipes*	Grey-brown	Dark brown granules & globules, - clamps	narrow wiry	Palm and Stewart (1984)
Pine + *S. granulatus*	Pale brown	Granules and globules, - clamps	Absent	Palm and Stewart (1984)
Pine + *S. punctipes*	Grey brown	Hyaline granules & globules, - clamps	±	Palm and Stewart (1984)
Pine + *S. granulatus*	Brown	Smooth, - clamps	+	Riffle (1973)
Douglas-fir + *S. subolivaceus*	Buff pink, tomentose	Incrusted, - clamps	Concolorous compact	Trappe (1967)
Pine + *S. granulatus*	?	Incrusted	?	Fassi and De Vecchi (1962)
Pine + *S. luteus*	?	Incrusted	?	Fassi and De Vecchi (1962)

Appendix 7.5, Table 1 Continued.

Ectomycorrhiza	Gross Features	Extramatrical Hyphae	Mycelial Strands	Reference
Pine + S. bovinus	?	Incrusted		Fassi and De Vecchi (1962)
Pine + S. luteus	Dark brown, downy	Brown, - clamps	Sepia, densely packed, + globules	Chu-Chou and Grace (1983)
Pine + S. placidus	Brown, tomentose	Hyaline, - clamps	?	Froidevaux and Amiet (1975a)
Pine + S. variegatus	Brown, tomentose	Hyaline, - clamps	Absent	Froidevaux and Amiet (1975c)
Pine + S. brevipes	White to brown, tomentose	?	+	Molina and Trappe (1982)
Larch + S. cavipes	White to brown, tomentose	?	?	Molina and Trappe (1982)
Larch + S. grevillei	White, tomentose	?	?	Molina and Trappe (1982)
Douglas-fir + S. lakei	Pale pink, tomentose	?	?	Molina and Trappe (1982)
Pine + S. tomentosus	White to brown, floccose	Encrusted, - clamps	White to brown, loose	Danielson (1984)
Pine + "Boletus"	Brown, wefts	Bead-like deposits, - clamps	Pale to black, compact	Schramm (1966)
Pine + S. umbonatus	White, villose	Angular crystals, - clamps	Translucent, compact	Danielson and Visser (1984)
Pine + S. pseudobrevipes	Yellow to brown, floccose	Resinous lens, - clamps	Hyaline, loose	Danielson and Visser (1984)
Douglas fir + R. colossus	White to brown tomentose	Smooth ?, - clamps	White to dark brown	Trappe (1967)

Appendix 7.5, Table 1 Concluded.

Ectomycorrhiza	Gross Features	Extramatrical Hyphae	Mycelial Strands	Reference
Pine + R. rubescens	White, pubescent	Smooth ?, - clamps	White	Chu-Chou and Grace (1983b)
Pine + R. luteolus	Cream to brown	Smooth ?, - clamps	Fawn, brown hyphae	Chu-Chou and Grace (1983b)
Douglas-fir + R. parksii	White to buff pubescent	- clamps	Dark isabelline	Chu-Chou and Grace (1983a)
Pine + R. rubescens	Brown, tomentose	Not incrusted	None	Froidevaux and Amiet (1975b)
Pine + R. cokeri	Yellow, tomentose	?	-	Molina and Trappe (1982)
Pine + R. fuscorubens	White to tan, tomentose	?	Pale brown	Molina and Trappe (1982)
Pine + R. occidentalis	White, tomentose	?	+	Molina and Trappe (1982)
Douglas-fir + R. vinicolor	White to brown, tuberculate	Encrusted, + clamps	White to black, thread-like	Zak (1971)
Pine + Melanogaster intermedius	Brown to dark brown	?	Dark brown	Molina and Trappe (1982)
Douglas-fir + Truncocolumella citrina	Brown, tomentose	?	Dark brown	Molina and Trappe (1982)
Larch + Fuscoboletinus aeruginascens	White to pale brown, tomentose	?	Pallid translucent	Molina and Trappe (1982)

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