

## The state of the art in mycorrhiza research in Alberta and Saskatchewan

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### Abstract

Earlier studies described indigenous mycorrhizae development on forest tree seedlings grown in prairie soils. The reclamation needs in Alberta in the 1970s stimulated intensive research of mycorrhizal associations in disturbed soils and undisturbed forest stands and supportive inoculation studies. Similarly, the increased reforestation targets of the 1980s generated an applied research program aimed at application of mycorrhiza technology in nursery production. The program, which consists of greenhouse inoculation, field outplantings, and collection of local isolates, has branched into several in-depth taxonomic, biochemical, and physiological studies of mycorrhizal symbiosis at universities. The current status and strategy of the program will be discussed.

### Introduction

My task to review the state of the art of mycorrhiza research in Saskatchewan and Alberta is made easier by having Dr. R. Danielson as the next speaker. He will describe more appropriately the work and important contributions to mycorrhiza research in Alberta by his research group at the University of Calgary. Since about the mid 1970s, his team, headed by Dr. D. Parkinson, has conducted in-depth, often very extensive, research on soil microbiology and the use of mycorrhizae in land reclamation. I will describe the development and current status of the applied mycorrhiza research program in Alberta designed to produce benefits in reforestation. I will also discuss and expand on the strategy and management of mycorrhiza research, where I believe we have not done enough collectively.

Though my present affiliation is with the Canadian Forestry Service at the Northern Forestry Centre in Edmonton, I wish to acknowledge that the work reported here was conducted while I was with the Alberta Forest Service (AFS).

The provinces of Alberta and Saskatchewan have well-established industries and corresponding reforestation programs. The planting stock production averages approximately 50-60 million seedlings; approximately half of that is in containerized seedlings.

Early mycorrhiza studies related to reforestation and afforestation were conducted in Saskatchewan

by R.D. Whitney of the Canadian Forestry Service. Whitney and his co-workers investigated the presence of mycorrhizal fungi in prairie soils, on burned-over forest sites and resolved the question of symbiosis and causality of *Polyporus tomentosus* with white and black spruce (Whitney 1965, 1969; Whitney et al. 1972).

The increased reforestation targets of the 1980s in Alberta coupled with the establishment of new nurseries designed for large scale production of containerized seedlings generated much interest in the use of mycorrhiza technology. At about the same time, forestry periodicals documented successes of the inoculation programs with *Pisolithus tinctorius* in the southern U.S.A. (Marx 1980; Marx et al. 1982, 1984) and the potential benefits of mycorrhizae in reforestation.

Many foresters interpreted the potential use of mycorrhizae as the end of reforestation problems and the lack of mycorrhizae on their planting stock, perceived or real, as an explanation of low survival and poor initial growth in plantations. In any event, the impetus, justification, and support for an applied mycorrhiza research and inoculation program was available.

Mycorrhiza research designed to derive benefits in reforestation should consist of several components (Fig. 1): collection of local isolates, inoculation trials that should include screening of isolates with local seed sources for compatibility, and field out-

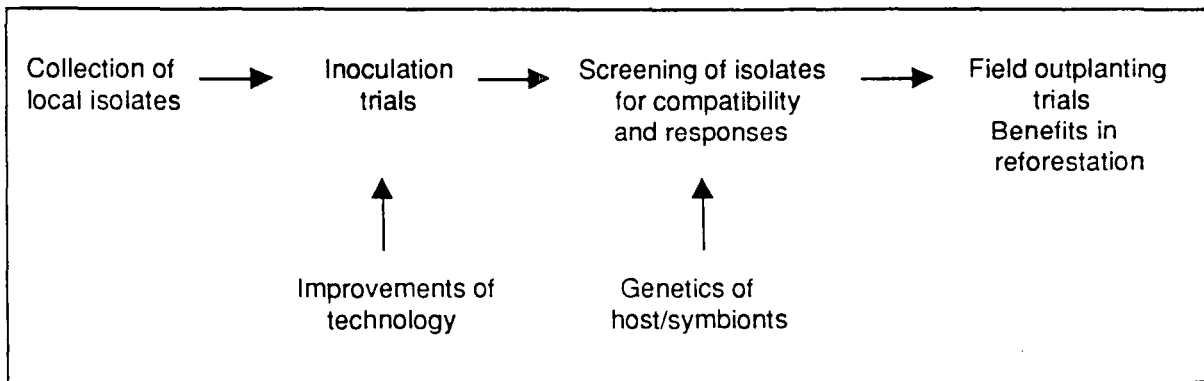


Figure 1. Mycorrhiza technology in reforestation

In many programs, as has been the case in our program, not all these steps were adhered to in the proper order. The intent to improve mycorrhization of nursery stock in the shortest time possible, was the major concern at times. For example, inoculation trials were initiated with imported isolates or commercial inocula without taking time to develop a well-integrated program involving collection of local, site adapted isolates.

#### *Applied research program on use of mycorrhizae in reforestation in Alberta*

The Forest Research Branch of AFS initiated an applied research program on the use of mycorrhizae in reforestation in 1983. Since its initiation, the program has branched into a number of integrated components in response to the progress, results, and logistics of the program (Fig. 2). Many supportive studies have been conducted in cooperation with the University of Alberta and University of Calgary. Inoculation and field outplanting trials were sup-

ported by the Alberta Forest Service and by local forest industry (Champion Forest Products Ltd.).

#### *Inoculation trials in containerized production*

Inoculations of white spruce and lodgepole pine under the routine growing regimes of containerized production used at the AFS' Smoky Lake Forest Nursery were successful in forming ectomycorrhizae with the "early stage" fungi such as *Hebeloma* and *Laccaria* but was less successful with other fungi.

Vermiculite-based, vegetative inocula of *Hebeloma crustuliniforme* and *Laccaria laccata* produced abundant mycorrhizae on seedlings in containers (Navratil 1985). Commercial inocula (Sylwan Spawn Laboratory) of these early stage fungi performed as well as laboratory inocula. The results also showed that the growing conditions and occurrence of propagules of indigenous fungi were conducive to a high degree of mycorrhizal development by *Thelephora terrestris* and E-strain. A brief summary of inoculation results is shown in Table 1.

Table 1. Summary of inoculations of containerized white spruce and lodgepole pine seedlings at the Pine Ridge Forest Nursery, Alberta Forest Service

Fungi	Mycorrhizal development
<i>Homa crustuliniforme</i>	total conversion of short roots
<i>Laccaria laccata</i>	total conversion of short roots
<i>H. crustuliniforme</i> + <i>L. laccata</i> combined	<i>Laccaria</i> dominated
<i>Pisolithus tinctorius</i>	successful, but rapidly replaced
<i>Cenococcum geophilum</i>	sporadic or failed
<i>Suillus granulatus</i>	sporadic or failed
<i>Thelephora terrestris</i>	indigenous, very aggressive
E-strain	indigenous, very aggressive

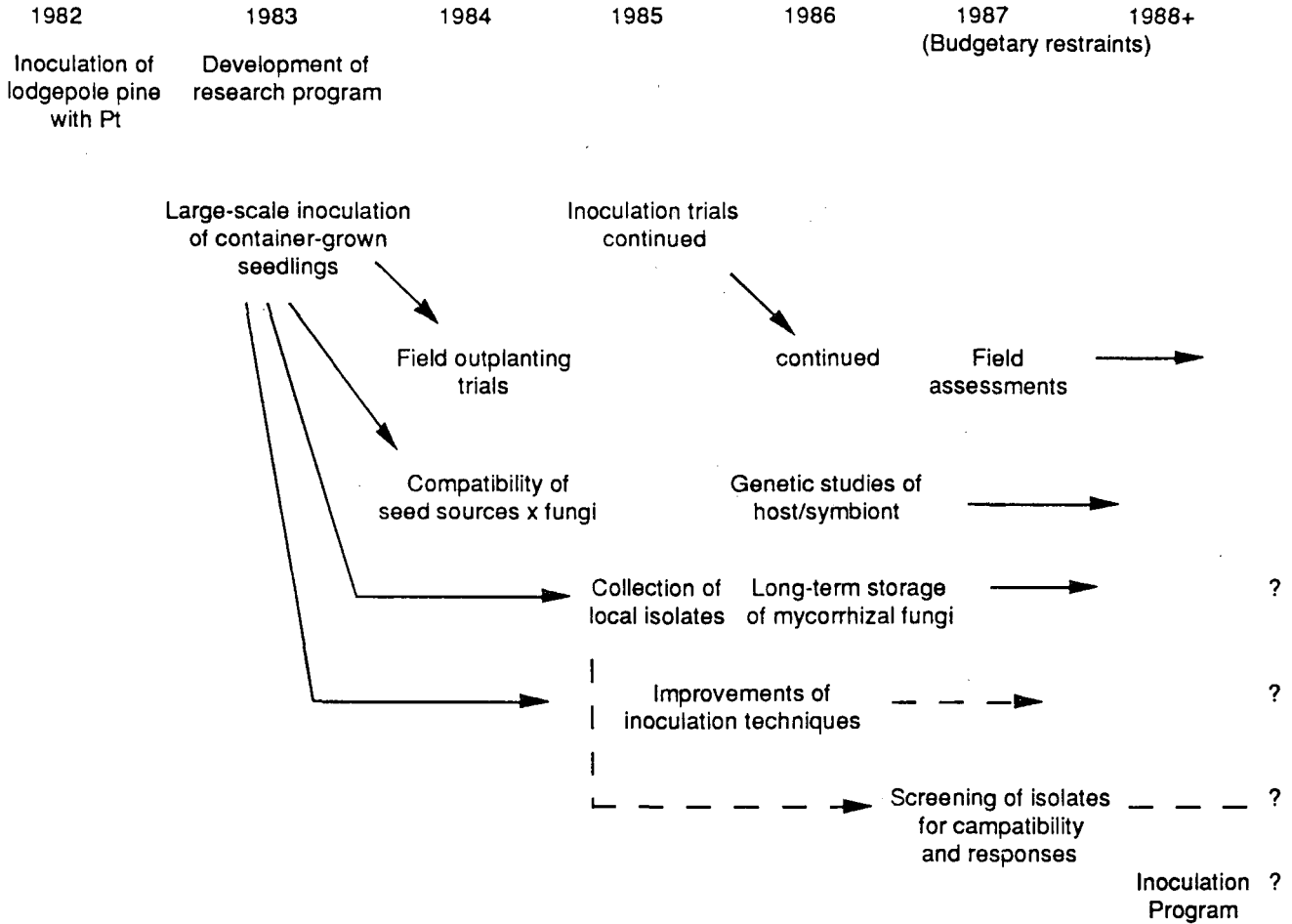


Figure 2. Research program on use of mycorrhizae in reforestation in Alberta.

- *Improvements of Inoculation Techniques*

Inoculation trials showed that shortcomings of the methods of inoculation may prevent testing of a broader ecological spectrum of fungi and that improvement of inoculation procedures may be necessary to introduce late- or multi-stage fungi. Dr. Danielson has conducted several studies to evaluate various approaches, some in conjunction with our programme, to achieve more effective inoculations.

The mycelial slurry technique has been successful (Danielson et al. 1984). The other modifications (fruitbody slurries, modification of planting mixtures, and inclusion of pesticides and antibodies) did not result in substantial improvements and only permitted inoculation by the same early stage fungi.

- *CO<sub>2</sub> Enrichment in Greenhouse*

Carbon dioxide enrichment is a feasible practice for shortening rearing schedules in tree nurseries. Significant growth responses and increased biomass

allocation to roots of white spruce and lodgepole pine when grown in CO<sub>2</sub>-enriched environments have been observed (Higginbotham 1983; Higginbotham et al. 1985).

In our study, we examined the effect of CO<sub>2</sub> enrichment (600 ppm as compared to ambient) on the growth, total nonstructural carbohydrates (TNC), and mycorrhizae formation with *Laccaria laccata* of white spruce and lodgepole pine seedlings. Enhanced CO<sub>2</sub> increased the root-shoot ratio of *Laccaria*-inoculated seedlings but not overall seedling size. Total nonstructural carbohydrates concentration in shoots was increased by CO<sub>2</sub> enrichment, but not in roots, indicating that nonstructural carbohydrates were rapidly used after translocation to root systems. Mycorrhizal development was very high (above 90% of short roots of both species in both) CO<sub>2</sub> environments.

The technique appears to have merit in alleviating carbohydrate drain and possibly size reduction of seedlings inoculated with high-energy demanding fungi.

### Fungal strain variation

It is well known that different isolates of mycorrhizal fungi can exhibit great variation in their ecological and physiological functions and in symbiotic effectiveness. In fact, the concept of tailoring seedlings with strains of fungi adapted to particular soil types, drought or temperature extremes may reflect and depend on the intraspecific variations. Thus, selection of the most beneficial isolate for large scale inoculations or specific stress sites may need to be preceded by a considerable screening process.

To gain more information on this subject we examined the extent of genetic variations in a natural population of *Suillus tomentosus* collected from several forest regions and sites in Alberta using the isozymes technique. Figure 3 shows the relative genetic distance existing among and within regions as separated by a principal component analysis scatterplot of 43 isolates.

Conclusions drawn from this recently published study (Zhu *et al.* 1988) were as follows: 1) intraspecific genetic variation exists among and within forest regions and sites, and 2) this variation is greater among forest regions than within forest regions. The research is being continued by H. Zhu, who is a PhD. candidate at the University of Alberta. He intends to determine the genetic variation in enzyme activity and biomass production of *Suillus* and *Hebeloma* and how this variation is related to mycorrhizal effectiveness of lodgepole pine seedlings. Other supportive research in this direction includes the study of interactions of phosphorus nutrient status, lodgepole pine provenances, and three ectomycorrhizal fungi conducted by B.N. Johnson, PhD. candidate, University of Alberta.

PCA scatterplot of 43 isolates of *Suillus tomentosus*

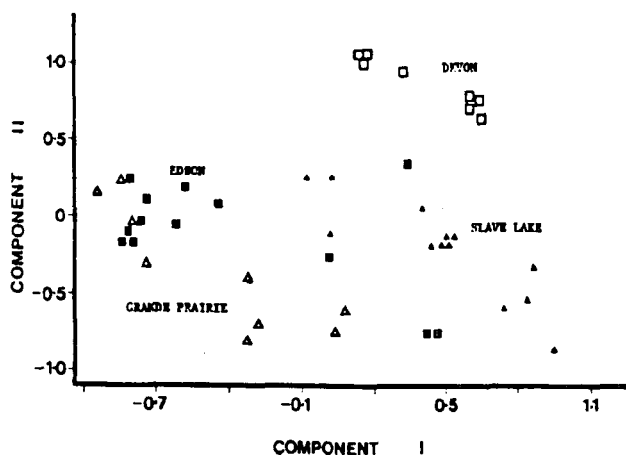


Figure 3. Principal component analysis scatterplot of 43 isolates of *Suillus tomentosus* from four forest regions in Alberta. (From Zhu *et al.* 1988).

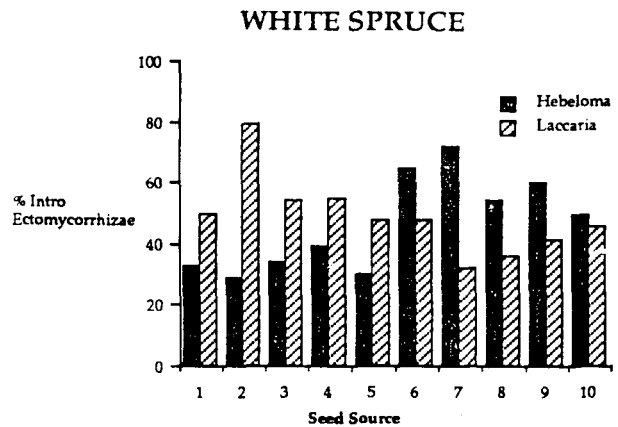


Figure 4. Seed source averages of % mycorrhizal infection with the fungi in *Hebeloma crustuliniforme* and *Laccaria laccata* inoculated white spruce seedlings.

### Seed source variation

The annual production of 30 million seedlings in Alberta consists of province-wide seed sources with wide differences in latitude, longitude, and elevation. In planning mycorrhization programs we felt that the question of host-fungus genotype interaction and its effects on mycorrhizal formation and seedling quality should be addressed. We examined in detail the compatibility of broad host-range fungi *Hebeloma*, *Laccaria*, *Cenococcum* with seed sources of white spruce and lodgepole pine (Navratil 1985, 1986).

The effects of seed sources were very pronounced for all mycorrhizal variables. Depending on the seed source, the percent mycorrhizal infection with the fungi introduced as inoculum varied from 32 to 77% and 28 to 63% for *Laccaria*- and *Hebeloma*-inoculated white spruce seedlings (Fig. 4); and from 31 to 79% and 38 to 84% for *Laccaria*- and *Hebeloma*-inoculated pine seedlings, respectively.

In *Cenococcum*-inoculated seedlings the differences among seed sources were also significant, but the infection rates were low (less than 5%).

Seed source + fungus interactions affected the frequency of mycorrhizal short roots per unit length of lateral roots. This was true for mycorrhizal short roots with both introduced and indigenous fungi. These differences were particularly pronounced when the total mycorrhizae (irrespective of the symbiont) were considered (Fig. 5).

We used principal component analysis to discriminate the groups of the most and least compatible seed sources with particular target fungi. The pattern of variation in white spruce compatibility with the introduced symbionts delineated the group of seed sources in the central region of the province (Fig. 6), which in turn coincides with the pattern of variation based on branching habits illustrated by Dunsworth and Dancik (1983).

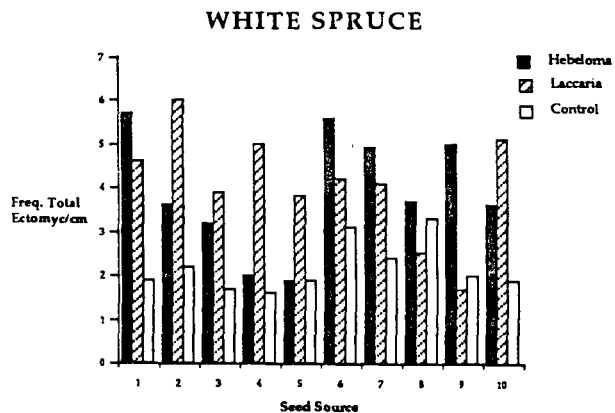


Figure 5. Seed source averages of the frequency of mycorrhizal (target and indigenous symbionts combined) short roots in control, *Hebeloma* and *Laccaria* treatments of white spruce seedlings.

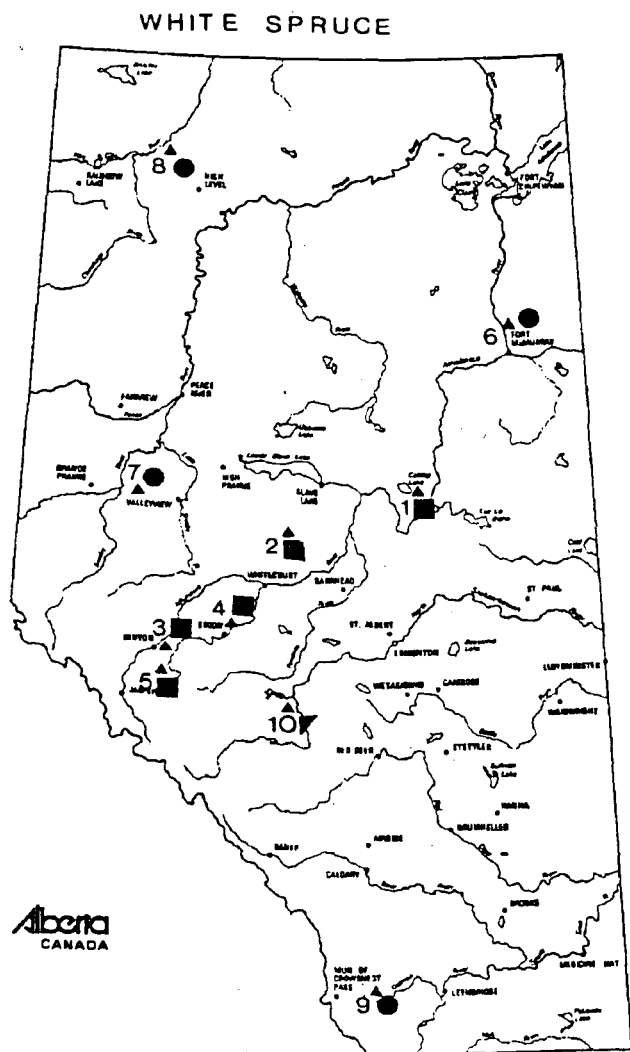


Figure 6. Distribution of white spruce seed sources and the geographic pattern of their compatibility with *Hebeloma crustuliniforme* and *Laccaria laccata*, compatible with *Hebeloma* compatible with *Laccaria* intermediate.

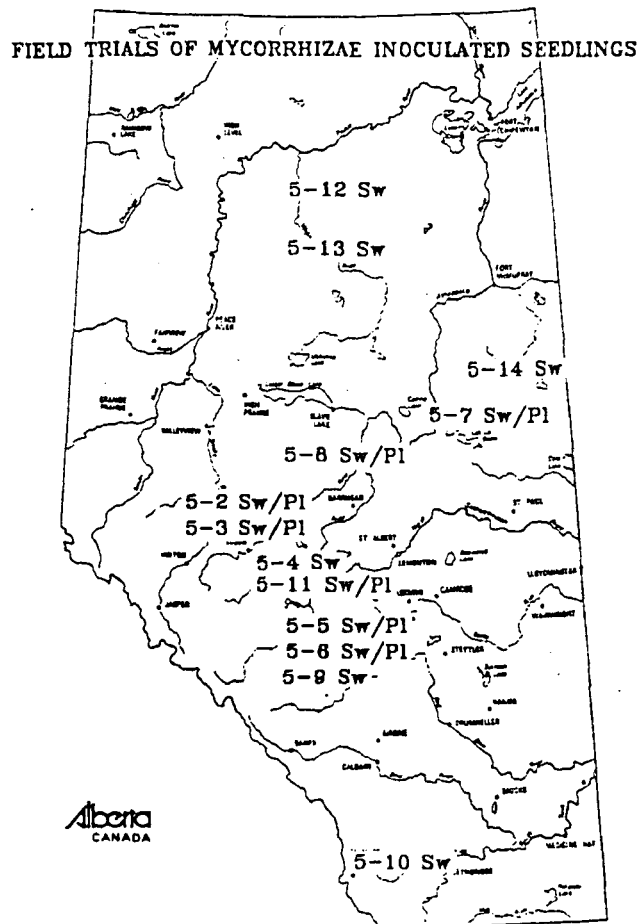


Figure 7. Location of field trials with mycorrhiza-inoculated seedlings in Alberta.

In another study (Zhu and Navratil 1987), we found that seed source had significant effects on mycorrhizae formation and growth of tamarack seedlings when inoculated with *Laccaria laccata*. We used four provenances and 17 open-pollinated families from northern Ontario. The difference in mycorrhizal formation among provenances was up to 20%, and it was up to 32% among families. Seed source also affected morphological attributes of seedlings, and significant provenances effects were found in shoot height and root variables. Effects of family-within-provenance were significant in shoot height, diameter, and total dry weight.

These results suggest that a wide matrix of seed sources and fungi may need to be screened in search of the best host-symbiont response for mycorrhizae formation, seedling quality, and ultimately for benefits after outplanting. These results also confirm the concept that the most promising approach may be in matching a local isolate with a local seed source.

### Field outplanting trials

Currently, 14 field trials with inoculated containerized seedlings of white spruce and lodgepole and jack pine are established on a variety of forest sites in Alberta (Fig. 7). The majority of sites are routine reforestation sites with bracke, disc trencher, or drag scarification. Three sites, an ameliorated abandoned gravel pit, abandoned landing, and ameliorated well site, had very disturbed soil conditions.

Mycorrhizae formation on inoculated seedlings at the time of planting was abundant with the target fungi *Hebeloma crustuliniforme* and *Laccaria laccata*, low (less than 5%) for *Cenococcum geophilum*, and very low to sporadic for *Pisolithus tinctorius*. Control seedlings had moderate-to-high mycorrhizae development with indigenous fungi, primarily *Thelephora* and E-strain.

One-year and three-year remeasurements of the trials showed that inoculation with selected mycorrhizal fungi provided no growth or survival benefits to seedlings after outplanting. Control seedlings performed equally well or in some cases slightly better than inoculated seedlings (Tables 2 and 3).

The ineffective performance of inoculated seedlings after planting has left unanswered questions: First, the selection and effectiveness of fungus/

host/site combinations; secondly, the need for mycorrhizae inoculation of seedlings planted on routine reforestation sites where sensitive management of resident microflora can ensure sufficient and rapid mycorrhizal development should be examined; and thirdly, the importance of using local fungal isolates has again been emphasized.

### Collection of local isolates of mycorrhizal fungi

Based on the concept advanced early by Trappe (1977) that adaptation of inoculated seedlings to the specific planting sites is enhanced by inoculating with fungi originating from the outplanting site or its ecological equivalent, we initiated a collection of local isolates from reforestation regions in Alberta. We were fortunate to engage a mycologist, Dr. R. Currah, and the Microfungus collection facility headed by Dr. L. Sigler at the University of Alberta to carry out this task with support from the Alberta Forest Service. The collection currently contains over 200 isolates of mycorrhizal fungi from forest sites of Alberta. In addition to identification, herbarium processing, and culturing of specimens, the project made it possible to access specialized equipment; tests are conducted on the viability of mycorrhizal fungi in ultra-low temperature storage.

Table 2. Survival and growth of control and inoculated white spruce seedlings outplanted in Edson and Lac La Biche Forests.

Site and Year planted	Total Height (cm)	1 year after planting		3 years after Planting	Annual Increment (cm)
		Annual Increment (cm)	% survival		
<b>Edson Forest 1984</b>					
Control	15.24 a	3.8 a	89.5	23.1 a	4.4 a
<i>Laccaria laccata</i>	14.19 a	3.5 a	91.0	20.4 a	4.0 a
<i>Hebeloma crustuliniforme</i>	14.24 b	2.6 b	87.0	20.0 a	3.5 a
<b>Hinton 84</b>					
Control	12.96 ac	3.70 a	93.2	21.5 a	6.2 a
<i>Laccaria laccata</i> (com. noc.)	11.88 bc	3.11 b	97.5	18.3 b	4.5 b
<i>Hebeloma crustuliniforme</i> (comb. inoc.)	11.16 b	2.53 c	99.0	15.1 c	3.7 b
<i>Laccaria laccata</i> + (comb. inoc.)	12.19 c	3.18 b	96.5	17.7 b	3.7 b
<i>Hebeloma crustuliniforme</i>					
<b>Lac La Biche Forest 1984</b>					
Control	15.95 a	3.3 a	96.0	25.4 a	7.6 a
<i>Laccaria laccata</i>	14.67 b	3.3 a	98.5	25.4a	8.6 a
<i>Hebeloma crustuliniforme</i>	14.88 b	2.5 b	98.5	23.9 a	7.6 a
<i>Laccaria laccata</i> + <i>Hebeloma crustuliniforme</i>	14.71 b	3.2 a	98.5	24.8 a	8.3 a

Table 3. Survival and growth of control and inoculated jack/lodgepole pine seedlings outplanted in Whitecourt, Edson and Lac La Biche Forests.

Site and year planted	1 year after planting			3 years after Planting		
	Total Height (cm)	Annual Increment (cm)	% survival	Total Height (cm)	Annual Increment (cm)	% survival
Whitecourt Forest 1984						
Control				45.3 a	17.7 a	94.4 a
<i>Laccaria laccata</i> (lab. inoc.)				35.0 a	12.7 a	94.4 a
<i>Laccaria laccata</i> (com. inoc.)				41.4 a	16.1 a	88.9 a
<i>Hebeloma crustuliniforme</i> (lab. inoc.)				36.2 a	11.9 a	100.0 a
<i>Hebeloma crustuliniforme</i> (com. inoc.)				40.6 a	17.2 a	94.5 a
Hinton 1984						
Control	19.95 a	2.87 ac	99.0	32.4 a	8.7 a	
<i>Laccaria laccata</i> (lab inoc.)	16.77 b	2.65 a	96.5	24.7 b	6.2 b	
<i>Laccaria laccata</i> (com. inoc.)	16.01 b	2.83 ac	96.3	25.4 b	7.1 b	
<i>Hebeloma crustuliniforme</i> (com. inoc.)	18.06 c	2.23 b	98.5	24.0 b	5.0 c	
<i>Cenococcum geophilum</i> + <i>Hebeloma crustuliniforme</i>	18.13 c	3.16 c	97.5	31.4 a	9.1 a	
Lac La Biche Forest 1984						
Control	29.9 a	8.4 a	99.5	69.1 a	24.9 a	
<i>Laccaria laccata</i> (lab. inoc.)	25.6 b	6.9 b	100.0	62.5 b	23.5 a	
<i>Laccaria laccata</i> (com. inoc.)	24.4 b	7.1 b	99.5	62.1 b	23.7 a	
<i>Hebeloma tuliniiforme</i> (com. inoc.)	26.1 b	6.9 b	99.5	62.2 b	23.3 a	
<i>Laccaria laccata</i> + <i>Hebeloma crustuliniforme</i> (comb. inoc.)	24.9 b	5.9 c	99.5	59.3 b	22.7 a	

My account of mycorrhiza research in Saskatchewan and in Alberta would not be complete without including the nursery inoculation trials at the Prairie Farm Rehabilitation Administration (PFRA)

Shelterbelt Centre in Indian Head, Saskatchewan (Table 4), and the recent studies investigating the effects of herbicides on mycorrhizal fungi conducted at the Northern Forestry Centre, Canadian Forestry Service (Chakravarty and Sidhu 1987a, b).

Table 4. Summary of nursery bed inoculations at the PFRA Shelterbelt Centre, Indian Head, Saskatchewan. (Compiled from Schroeder 1986; 1987.)

Fungi	Scots pine	Effects on host
<i>Hebeloma crustuliniforme</i> <sup>1</sup>		- improved seedling quality - increased mycorrhizae colonization - improved survival and growth after outplanting
	Colorado spruce	- no effect
<i>Laccaria laccaria</i>	Scots pine	- no effect
	Colorado spruce	- no effect

<sup>1</sup> Inocula from Sylvan Spawn Laboratories

### Strategy for management of mycorrhiza research

The Alberta program, though modest in scale, has progressed, I believe, in the right direction. It has, however, reached the stage when a serious decision concerning its future and strategy needs to be undertaken. The same may be said, and it has been said this morning, about mycorrhiza research in Canada in general. Scientific frontiers have been advanced, and many scientists and mycologists can be proud of their achievements. Collectively, however, we have to review the current status and reevaluate the future direction of mycorrhiza research in forestry. This workshop is a viable and important step forward, and it is appropriate to thank Dr. Fortin and other organizers for getting us together for this task.

Let me offer some observations using the outline in Figure 8.

#### MANAGEMENT OF MYCORRHIZA TECHNOLOGY

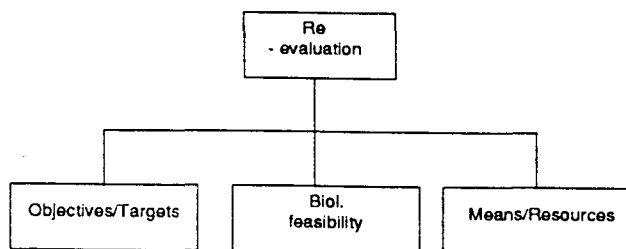


Figure 8. Concepts for reevaluation and management of mycorrhiza research.

The targets we want to achieve by mycorrhiza technology in reforestation need to be better defined. The research approaches should be different if the target is to increase survival and growth by 5-10% on all reforestation sites or if improved reforestation is primarily needed on localized stress- and drought-prone sites.

We should continually ask the question of biological feasibility of our expectations and adjust our goals accordingly.

Needless to say, the targets must be balanced with the needs, means, and resources. This seems obvious, but is not always accepted. We must keep in mind that mycorrhiza research is done for a purpose. We must decide who our client is. So far the working relationship has been between a scientist and perhaps a research forester. Ultimately, our client must be an operational forester.

### Benefits from Mycorrhiza Research in Forestry

The key concept underlying evaluation of research is that research produces new knowledge that, when put into use, affects production. In competition for research funds in applied, mission-oriented research programs, the enthusiasm of researchers may not be sufficient to convince managers and decision-makers. This is particularly true in times of budgetary restraints.

In a forest management setting, where the benefits are measured in increased growth and yield, the cost-benefit analysis of mycorrhiza research does not as yet rate very high.

Much has been learned about mycorrhizae and mycorrhiza technology over the past 10-15 years, but we have not yet been able to demonstrate benefits in practice, at least not in Canada. Benefits of mycorrhiza research must be extended to field environments.

In our strategy, two approaches could be pursued:

1. Emphasis should be shifted towards management of on-site microflora, and native populations of mycorrhizal fungi;
2. Mycorrhiza research must become an inherent component of reforestation research programs. It should be recognized that mycorrhizae is one of many quality attributes of seedlings and that soil microflora is one of many site variables that govern the success of plantations and thus the benefits from research investment.

Finally, I submit, that to gain extended support for mycorrhiza research we have to accept this challenge. Again, the Québec researchers and the Canadian Forestry Service should be applauded for arranging this workshop. It should serve as a platform for refocusing a long-term strategy of mycorrhiza research in forestry in Canada.

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