COMMERCIAL MYCORRHIZAL INOCULANTS: VALUE-ADDED CONIFERS FOR SITE REHABILITATION OR JUST ANOTHER WAY TO SPEND MONEY?

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WHAT IS MYCORRHIZA?

Mycorrhiza (literally "fungus root") is a mutually beneficial symbiotic association between fungi and plants. Globally, plants host many different kinds of mycorrhizae, but only ectomycorrhizae and VA mycorrhizae are directly relevant to the forest nursery business in British Columbia. For those producing other native plants, ericoid mycorrhizae are also important.

Ectomycorrhizae are distinguished by a structure called the Hartig net which consists of fungal hyphae surrounding cells of the short root cortex. Many ectomycorrhizae also have a mantle of fungal tissue that envelopes the short root and separates it from the soil or growth medium. Hyphae from the mantle surface connect the ectomycorrhizae with the soil. Some combinations of fungus and plant result in gross morphological changes to short roots that are fairly easy to spot with 10x magnification. All members of the Pinaceae form ectomycorrhizae, including Douglas-fir, hemlock, pine, spruce, larch, and true fir, as do birch, cottonwood, aspen, and alder. Certain basidiomycetes, ascomycetes and some zygomycetes are the fungi that form ectomycorrhizae. The ectomycorrhizal fungi of which we are most aware are those that form mushrooms or other conspicuous, above-ground fruiting bodies.

VA, or vesicular-arbuscular, mycorrhizae are distinguished by a finely-branched modified hypha called an arbuscule that develops inside of the cortical cells of fine roots. Between cortical cells, some VA mycorrhizae also form hyphal swellings called vesicles that serve as storage organs for the fungus. As with the ectomycorrhiza, hyphae connect the VA mycorrhizal fine root with a large volume of soil distant from the root, but, in contrast with the ectomycorrhiza, the surface of the VA mycorrhizal root is in direct contact with the soil or growing medium. Without clearing and staining of the roots, it is not possible to detect whether VA mycorrhizae are present since almost no morphological changes to the fine roots result from formation of VA mycorrhiza. Although VA mycorrhizae are formed by the vast majority of land plants, in the forest nurseries of BC, only western redcedar and Alaska yellow cedar form exclusively VA mycorrhizae, although poplars can form VA as well as ectomycorrhizae. Most of the native shrubs now being produced commercially would form VA mycorrhizae if the inoculum was present in the growing medium. Certain zygomycetes form VA mycorrhizae but the spores are smaller than the head of a pin and form belowground, so we are seldom aware of them.

Ericoid mycorrhizae are characterized by highly branched and coiled hyphal structures, variously called pelotons or hyphal complexes, within cortical cells of the fine roots. Fine roots of ericaceous plants are very fine indeed consisting of only one or two layers of cortical cells of which the ericoid mycorrhizal fungi occupy only the outermost layer. Examination under the microscope is necessary to determine if the roots of most ericaceous plants are colonized. Native plant producers who grow rhododendron, huckleberry, salal, and other Ericaceae need to be aware of this kind of mycorrhiza. A small number of ascomycetes and apparently some basidiomycetes form ericoid mycorrhizae.

WHAT DO THE FUNGUS AND PLANT DO FOR EACH OTHER?

The plant provides most or all of the carbon or energy used by the fungus. The fungus provides mineral nutrients to the plant from the relatively huge volume of soil that its hyphae can explore. Water is also supplied to the plant as well as some protection from root and other diseases. In the growing environment provided in container nurseries, the plant does not really need the fungus for water and nutrient uptake since the supplies are not limited, so the ectomycorrhizal fungi that dominate our commercial nurseries are not necessarily the best for the environment that seedlings face when outplanted. Rather, they are fungi that do well when the living is easy.

HOW CAN YOU TELL WHETHER PLANTS ARE MYCORRHIZAL?

Good colonization of commercial nursery stock by some ectomycorrhizal fungi can be detected by simply looking at the plugs at lifting because the hyphae on the surface of the growing medium are abundant and colourful. Ectomycorrhizal colonization by most fungi can be seen by direct observation under about 10 times magnification. However, colonization by other ectomycorrhizal fungi is undetectable if the roots are not cleared and stained. In a small preliminary trial, we took stock produced by Ministry of Forests, Extension Services and observed ectomycorrhizal colonization by these two different methods.

For hybrid spruce and lodgepole pine, clearing and staining detected more colonized roots than did direct observation (Table 1). This is what we would expect because clearing and staining makes visible ectomycorrhizal fungi that form little or no mantle while direct observation is quite dependent on the presence of a fungal mantle. In lodgepole pine, clearing and staining detected over 90% of short roots colonized while direct observation detected much lower levels in inoculated and non-inoculated stock. Part of the reason why the two methods produced such large differences for lodgepole pine may be that a fungus called "E-strain" occupied much of the pine roots and E-strain does not form a noticeable mantle.

Direct observation can also depend on the gross morphological response of short roots to mycorrhizal fungi. Ectomycorrhizal fungi can cause short roots to swell and branch. In pines, for instance, ectomycorrhizal development results in characteristic dichotomous branching of the short roots. Sometimes the swelling that results from ectomycorrhiza development can resemble swelling that arises for other reasons, and this may explain why direct observation of Douglas-fir detected more short roots that appeared mycorrhizal than we detected with clearing and staining.

Table 1. Percent colonization of inoculated and non-inoculated conifer seedlings as determined by two different methods. Clearing and staining was carried out by Guoping Xiao, UBC, and direct observation by Wayne Smith, Mikro-Tek, Timmins, ON.

	Interior Douglas-fir (Fdi)		Hybrid Spruce (Sx)		Interior lodgepole pine (Pli)	
	Not inoculated	Inoculated	Not inoculated	Inoculated	Not inoculated	Inoculated
Clearing and staining	<1	7	61	75	92	93
Direct observation	10	21	52	68	39	76

VA mycorrhizae can only be detected after clearing and staining. From the small amount of work that has been done in BC (Berch et al. 1992, 1993), it appears that, without intentional inoculation, western redcedar is essentially non-mycorrhizal in container nurseries. This would also be true for Alaska yellow cedar and most of the native plants being grown in containers. The mycorrhizal status of ericaceous native plants in nurseries is not known to me.

WHAT MYCORRHIZAL FUNGI ARE IN OUR NURSERIES?

The ectomycorrhizal fungi in container nurseries in BC include species of *Thelephora*, *Amphinema*, and *Laccaria* as well as E-strain (ectendomycorrhizae), MRA (Mycelium Radicis Atrovirens) and a probably a few others (*Inocybe*, *Suillus*, for example) (Hunt 1991).

WHAT IS THE CURRENT STATUS OF ECTOMYCORRHIZAL COLONIZATION OF COMMERCIAL NURSERY STOCK?

As part of a Forest Renewal British Columbia research project involving commercially-available ectomycorrhizal inoculum, we have been assessing mycorrhizal status of container nursery stock of Fdi, Pli and Sx. In 1997 and 1998, twenty commercial nurseries provided 20 seedlings each of these species. We cleared and stained the root systems and determined percent colonization. The results from the 1997 stock are presented in Table 2 and the results from 1998, though not yet complete, appear to be similar.

Interior lodgepole pine was generally the best colonized, hybrid spruce was intermediate, and Douglas-fir was poorly colonized. There was tremendous variation from

Table 2. Mycorrhizal colonization of conifers raised in container nurseries in British Columbia

Nursery Code	Sx	Pli	Fdi
1	4	84	17
2	12	35	0
3	46	93	9
4	75	66	9
5	42	69	30
6	67	66	-
7	22	35	-
8	36	33	0
9	68	64	5
10	75	83	0
11	30	61	6
12	56	71	1
13	47	72	11
14	40	79	3
15	7	12	0
16	83	49	8
17	40	43	12
18	-	-	28
19	-	44	-
20	-	-	4
Mean	45	59	9
Range	4 - 83	12 - 93	0 - 30

nursery to nursery and this can probably be accounted for by differences in seedlot and growing conditions, especially root zone aeration and fertilizer. Poor ectomycorrhizal colonization of container-grown Douglas-fir is a common occurrence (Castellano and Molina 1989, Hunt 1990) though the reasons for this have not been nailed down.

WHAT CAN BE DONE TO ENCOURAGE MYCORRHIZAL COLONIZATION?

Gary Hunt (1992) recommended that nursery managers limit fertilizers to soluble forms with an upper limit of 80 - 100 ppm N and 30-35 ppm P, and use peat with coarse:fine ratio greater than 0.5 and less than 5% fine fraction (less than 0.15 mm) to achieve adequate root zone aeration. By so doing, he found that spontaneous ectomycorrhizal colonization increased dramatically and the diversity of fungi increased as well.

Even so, ectomycorrhizal colonization in container nurseries can be quite variable even within the same nursery. This is because the fungi cannot grow from plug to plug or container to container directly and each plug must be colonized by spores. In nurseries where *Thelephora* fruits abundantly or the spore load from adjacent forests is high, this may not be much of an issue, but if the nursery manager wants to introduce a specific mycorrhizal fungus, the crop must be inoculated. A few commercial ectomycorrhizal inoculants are currently available and they come in two forms: mycelial slurry or spore suspension both of which are applied through the irrigation system.

VA mycorrhizal inoculum, in the form of spores, hyphal fragments or colonized root fragments, is also available from a small number of commercial sources. There is no commercially available inoculum of ericoid mycorrhizal fungi, but the development of mycelial slurry inoculum is certainly possible if there was sufficient demand.

IS IT IMPORTANT THAT STOCK BE MYCORRHIZAL?

Does mycorrhizal colonization add value to stock? There is a contested belief that, for nursery seedlings, "any mycorrhizal fungus is better than none". As the first step in working with mycorrhizae in the nursery, I believe that this is true because at least good mycorrhiza development in the nursery indicates good root development. However, different mycorrhizal fungi affect growth in the nursery in different ways and, in this sense, it is not true that "any fungus will do". In addition, responses in the nursery are not necessarily related to performance after outplanting because the two sets of growing conditions are so dramatically different. Of course, when even poorly colonized stock is planted promptly on adequate sites in years with adequate moisture and temperature conditions, roots that grow into the soil tend to become quickly colonized by indigenous mycorrhizal fungi. Well colonized, healthy stock is more likely to perform better in the field when conditions are not adequate. Because it is relatively easy to achieve reasonable colonization in the nursery by modifying growing conditions, mycorrhization is a good goal. Before a nursery manager should even consider inoculation of a crop, cultural conditions should be adjusted so that spontaneous mycorrhization is routine.

Does mycorrhizal colonization add enough value to justify the cost of inoculation? Given the variable results of out-planting trials and the limited variety of commercial inoculants available, I would not currently recommend routine inoculation of stock. If you are going to try mycorrhizal inoculum, use an adaptive management approach and incorporate research. From around the world, results vary tremendously; sometimes inoculation improves survival and growth in the field, sometimes it worsens growth, and sometimes there is no significant effect. The inconsistency of response to mycorrhizal inoculation comes from a number of sources including genetic differences within and between plant and fungus species, cultural differences in the nursery and seedling quality at time of planting, site conditions such as soil and climate, weather conditions during the year of planting, silvicultural differences from site prep to veg control, and duration of the field trial. All of this just means that the answer to the question is complex, it doesn't mean that we should stop trying.

Would inoculation add enough value to justify the cost in unusual circumstances? Maybe so. When seedlings are planted on highly disturbed or very poor sites, having the right ectomycorrhizal fungus on the roots could make a difference. Specifically in BC, we are studying the value of applying commercially-available ectomycorrhizal inoculants to seedlings intended for planting on rehabilitated landings and roads. Chuck Bulmer has found good evidence to support the value of planting landings and roads (Plotnikoff et al. in preparation) in that trees planted on rehabilitated roads and landings can approach stock performance on adjacent cutblocks. On landings and some roads, the planting medium is often compacted subsoil that lacks indigenous mycorrhizal fungi. Rehabilitation can be adequate to restore favourable growing conditions and mycorrhizal inoculation might provide adequate mycorrhizal fungi.

Inoculation with certain ectomycorrhizae will absolutely add value and warrant the cost, if by so doing one can provide not one crop but two. In Europe, New Zealand, and the United States, researchers have produced value-added seedlings that are colonized by choice edible ectomycorrhizal fungi such as chanterelles, boletes, and truffles. Truffle-inoculated plantations are already producing truffles in France, Italy, New Zealand, and the United States. Outplanting trials with chanterelles and boletes are just getting underway in Europe. Imagine how valuable seedlings colonized by pine mushroom would be in this province.

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