

Cost of Mycorrhizal Inocula

The sources of mycorrhizal inoculants are increasing. Several years ago, about the only source for inoculum was Abbot Laboratories. Now, several companies in North America are selling inoculum in one form or another. Below is an incomplete list of some of the different types of inoculum available. Costs can range from as low as 43 cents per thousand seedlings to as high as 10 cents per seedling. The cost and applications vary. The most expensive treatments are those applied to tree roots at time of transplanting.

A few attempts to inoculate seedlings at time of outplanting have failed. Alvarez and Trappe (1983) found that dusting roots of conifers with P.t. reduced seedling survival in some cases. High rates of spore application may have desiccated roots of the true firs and spore amounts applied need careful attention. Therefore, a later study tested a slurry of P.t. spores (Pilz and Znerold 1986). In this study, about 1 mg of spores were applied to the roots but this treatment also did not improve either survival or growth. The authors concluded that "the application of P.t. spores to a seedling's roots immediately preceding outplanting appears to be ineffective."

We fully agree with the following statement by Don Marx (1980). "The ultimate proof of the value of inoculation of bare-root or container grown nursery seedlings with specific fungi is their performance under diverse field conditions. Meaningful conclusions can only be obtained from properly designed, installed, and maintained field experiments which include periodic tree measurements and mycorrhizal assessments conducted over several years. Only limited field data of this type is available in the literature." In regards to mycorrhizal inoculation at outplanting, this statement is as true today as it was 16 years ago.

Table 1. Cost of various mycorrhizal inoculants

| INOCULUM TYPE | STOCK TYPE | APPLICATION TIME | USE RATE PER SEEDLING | COST PER THOUSAND SEEDLINGS |
|---------------------------------------|--------------------------|-------------------------|------------------------------|------------------------------------|
| double-sifted P.t. spores | bare-root | at sowing | 2.6 mg | \$0.43 |
| P.t. spores + humate | containers | in mix prior to sowing | 156 mg | \$1.52 |
| P.t. spore spray | containers | just after sowing | 1 mg | \$2.00 |
| P.t. spore pellets | bare-root | prior to sowing | 36 mg | \$2.75 |
| VA spores + clay | containers | in mix prior to sowing | 2.2 g | \$5.00 |
| vegetative P.t. mycelium | bare-root | at sowing | 0.75 ml | \$7.50 |
| vegetative P.t. mycelium | bare-root | at sowing | 1 ml | \$10 |
| VAM dry spore pellets | bare-root | at sowing | 1 ml | \$10 |
| P.t. spores + gel + other | bare-root | at transplanting | 425 mg | \$40 |
| P.t. spores + VA spores + gel + other | bare-root (conifer) | at transplanting | 425 mg | \$51 |
| P.t. spores + VA spores + gel + other | bare-root (hardwoods) | at transplanting | 850 mg | \$103 |

The following is an edited thread that appeared on the MICRONET listserv: Each color represents a different author.

I happened to stumble into the report at the INVAM site before it was >removed. Only confirmed what I already intuitively knew. There are many >mycorrhizal formulations that certainly don't provide any mycorrhizal >benefit. Have you ever looked at the endo spore count of the various >products. Most are not stated because the numbers are too low to be of any >significance. The obvious reason is to keep the price low. >The users don't understand the importance. The academic community doesn't >know or ignores.

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David South replied:

Regarding the claim that the academic community doesn't know or ignores the above....
(1) Who (if anybody) in the academic community has recommended adding mycorrhizal inoculum to seedling roots at the time the tree is placed in the planting hole?

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I don't understand the comment about who in the academic community has recommended putting inoculum in holes.. - lots of us do that (i.e recommend it and do it). Does the comment refer to recommending some commercial products? That's a different issue.

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David South replied:

Why do you do it? As far as I know, there is no scientific publication to support such a use (there are publications to support using mycorrhizal inoculum in other situations: especially when inoculating seed after soil fumigation)! Why does the academic community recommend putting inoculum in holes at the time of planting when no benefit has been demonstrated? Why add spores to the soil on sites that already have plenty in the soil? Why add spores to the soil (or roots) when the planted tree already has mycorrhiza on the roots? Why spend 4 to 10 cents extra per seedling on inoculating at time of transplanting when it may cost only 0.4 to 1 cent extra to inoculate in the nursery! Why would a scientist ever reject the null hypothesis when the data collected so far support the null hypothesis! Why would a scientist make such recommendations after reading the papers that show no benefit from such a treatment?

Several attempts to inoculate seedlings at time of outplanting have failed. Alvarez and Trappe (1983) found that dusting roots of conifers with P.t. reduced seedling survival in some cases. High rates of spore application may have desiccated roots of the true firs and spore amounts applied need careful attention. Therefore, a later study tested a slurry of P.t. spores (Pilz and Znerold 1986). In this study, about 1 mg of spores were applied to the roots but this treatment also did not improve either survival or growth. The authors concluded that "the application of P.t. spores to a seedling's roots immediately preceding outplanting appears to be ineffective."

South and Skinner (1998) reported no benefit from injecting freeze dried Rhizopogon spores into the soil after planting and fertilization with P.

I fully agree with the following statement by Don Marx (1980). "The ultimate proof of the value of inoculation of bare-root or container grown nursery seedlings with specific fungi is their performance under diverse field conditions. Meaningful conclusions can only be obtained from properly designed, installed, and maintained field experiments which include periodic tree measurements and mycorrhizal assessments conducted over several years. Only limited field data of this type is available in the literature." In regards to mycorrhizal inoculation at outplanting, this statement is as true today as it was 16 years ago.

Therefore, I am willing to send \$20 to the first person who can send me a refereed journal article (no sales brochures please) that shows a significant (0.05 level) increase in either survival or height growth by adding JUST spores to the planting hole at time of transplanting. Since I have references where a root-gel increased survival, there can be no confounding effects by adding the spores to a clay slurry, or a gel-slurry; no nutrients can be included in the mix. The paper must clearly demonstrate that just spores (no soil or anything else) produced the increase in performance of the seedling. Any takers? Oh, I will also give \$10 to the first person who sends me paper in the journal "Mycorrhiza" that shows NO benefit from adding spores to the planting hole at time of transplanting (since this will save me some time in the library).

>Does the comment refer to recommending some commercial
>products? That's a different issue.

No, the comment refers to adding spores ONLY to the planting hole. Some commercial products include other stuff than spores (such as root gels; yucca, etc.).

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>Perhaps we should start by deciding whether we are talking about AM or EM >inoculation- you and I were each talking about different kinds of fungi on >different kinds of plants in very different environments. We may want to save >the heated statements and challenges until everyone is talking about the same >thing.

David South replied:

I am talking about either AM or EM. I do not know of any refereed journal articles where ONLY EM spores were applied to holes at time of transplanting. Could this be because the trials showed no differences and the author did not write up and submit the results? Or did I just miss these journal articles?

>>I will not be collecting your reward, but I would like to say that in commercial >restoration projects in California almost every customer does an informal trial >of some sort. On newly graded ground, by far the most common situation for >planting, native endomycorrhizal inoculum is almost always lacking. We commonly >see growth response, greatly increased plant

diversity from a common seed mix, >and improved survival through our long dry summers. > >
>The best use of inoculum is probably not with transplants;

I fully agree. In my view, the cost of inoculation is increased greatly when applied in the transplanting hole. Use of inoculum in the bare-root or container nursery is more cost effective.

>I happen to think >that research on restoration is better focused on use of seeds, which give
>better root systems at a much lower cost than transplants.

I think inoculation of seed would work more often than inoculation of transplants. I have cited one reference where it worked using *Rhizophogon* on pine (Davis, Grace and Horrell 1996).

>That may not be true in southern forests, so don't offer me a reward to prove it for your plants
in >your conditions.

I know of references here in the South where inoculation of seed in a bareroot nursery in the South worked (so there is no need for me to offer a reward). However, as you indicate below, there may not be a need to use inoculum when direct seeding in wet climates in southern forests.

>In fact, I am not sure there is much point in inoculating at >all in wetter climates in North
America. It seems that AM inoculum moves in >very quickly in the eastern half to two thirds of
the continent.

We occasionally have problems with early inoculation of AM in the nursery with sweetgum, maple and dogwood. However, by the end of the year, the plantable bare-root seedlings usually have AM at time of lifting. Also, we usually outplant the sweetgum into soil that contains AM.

>In the arid >west, I have on several occasions seen container plants that had been in the
>ground for three years (always with artificial maintenance to keep them alive) >that were still
not mycorrhizal.

Maybe you should consider planting top-pruned bareroot seedlings with large roots that have mycorrhiza. Considering that it costs 4 to 10 cents per tree to put inoculum in the planting hole (for non-mycorrhizal container stock), I assume people would be willing to pay 4 to 10 cents more to plant better (seedlings with more roots, mycorrhizal roots, and a better root/weight ratio) seedlings.

>Are you arguing that they should not have been inoculated?

I am saying that I am not aware of data to back up the recommendations made by the "academic community" that spending an extra 4 to 10 cents per planting hole is cost effective. In my opinion, too often we make recommendations based on dogma instead of science or economics. [I would say that poor nursery management practices were used since the seedlings were non-mycorrhizal at time of transplanting.]

> No, you are not. You are talking about a very different situation >and very different fungi.

Are you so sure of what I am saying? The \$20 reward is for either AM or EM in either dry or wet climates.

>>Even in the arid west, ectomycorrhizal fungi do at least a fair job of taking >care of themselves. However, I know of one rather informal experiment with >Engelmann oak where the person used native duff to inoculate every other tree in >a line of plantings on an agricultural experiment station. They called me in >during year 2 or 3- at that time the inoculated trees were 8 to 10 feet tall and >the others either dead or only 1 to 3 feet tall. By that time, all were >mycorrhizal, so any effect had taken place early. Sadly, the experiment did not >answer the important questions, but is interesting enough that someone ought to >do it right.

My reward does not include any study using soil or duff (if it works, wouldn't adding duff cost less than 4 to 10 cents/hole? I am not sure. BTW, In my MS work, I reported data using duff inoculum for sweetgum in the nursery). Adding duff or soil can sometimes cause improvements in seedling performance (I have one reference where adding soil improved seedling performance). In fact, we recently wrote a report where we found that washing soil off the roots of bare-root pines can reduce seedling performance.

<http://www.ag.auburn.edu:80/aaes/information/highlights/fall98/roots.html>

But as you know, we should not assume the benefits from adding duff are solely mycorrhizal. It may or may not be that some other factor is involved in improving seedling performance. That is why I limited the reward to only using spores.

>>Perhaps the cost of the work could be partially offset by >collecting your reward ;).

Perhaps my "heated" statements will result encourage some researcher to test the following null hypothesis!

HO: adding AM or EM spores to a planting hole at time of transplanting a large container-grown seedling or large, top-pruned bare-root seedling does not affect seedling performance.

IMO, too often I see tree planting where the stock size used is woefully inappropriate for the site conditions.

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Dear micornetters, aloha kakou from the Big Island of Hawai'i The sore loser has been an interesting discussion that brought up old memories, triggered especially by Ted's comments about the complexity of the belowground system. In the late '70s a group of us at Oregon State began working on the problem of inability to reforest high elevation clearcuts in the west. Mike Amaranthus added forest soil to planting holes on three sites in Sw Oregon, and got highly significant survival and growth responses on one (all responses were published, positive and negative). In earlier work Sharon Rose had found relatively low MIP on that site, so we naturally assumed the problem was low EM inocula. But by that time in my career I had been humbled too many times by nature to trust any assumption, so Carlos Colinas (then a PhD student) took on the

task of figuring out what in the soil transfers was having a positive effect. Carlos did an elegant exp in which he used various approaches to knock out different functional groups in forest soils, which he then added to planting holes. To make a long story short, Carlos' results showed two things that surprised me. First, there was plenty of EM inocula in the clearcut, but either (a) something was inhibiting mycorrhiza formation (that something was very probably streptomyces, which has spread widely in the CC), or (b) some trigger for EM formation was missing (I believe the missing trigger was ethylene, which we measured at very low levels in the CC and high levels in the forest). Actually, both these mechanisms were probably operating. The second result that particularly got my attention was the importance of the belowground grazers--soils that had protozoa and microarthropds knocked out consistently lost their beneficial effect. I knew those critters were important in the nutrient cycle, but never guessed they could make the difference between dying and surviving. That the grazers were mediating a nutrient effect (nitrogen) was supported by Carlos' trtmnts adding fertilizer to planting holes. So I became a believer in the importance of the belowground ecosystem rather than any one part. As my old major Prof (Jack Rumely) told me 30 yrs ago, never be a one factor ecologist. Dave

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I don't know, but this debate has certainly featured an awful lot of trumpety moonshine* as well as some common sense and good science. For a while it seemed fatuous to get involved. It is pleasant to remember how sage Jim Trappe watched patiently as we squabbled over the pluralisation of "mycorrhiza" and then wearily, but masterfully waded in to finish it all off with a single, well-aimed blow. In the case of "Sore Losers?" might I make so bold as to offer something along the lines of: "Watch my lips: Mycorrhiza, including inoculant application, is an ecology issue" in order to inject some sanity. Andrew Smith asked if Alastair Fitter would be listening. I'm afraid not, but I stay plugged in to keep an eye on things, and will add my four penn'orth, hopefully reflecting with reasonable accuracy views held by us in York as well as by others around the world. A lot of the argument in this correspondence (and in the claims of those producing commercial inoculants) has ignored the complexity and unpredictability of mycorrhiza and the lamentably poor state of our understanding of how the association behaves in the field. It's a very difficult area of study so few have the courage to attempt it, but surely we have to acknowledge that mycorrhiza is not solely a glasshouse or trial plot phenomenon. Every mycorrhiza paper begins with extravagant claims for the ubiquity and importance of these symbioses the world's biomes, not flower pots. However, we have to do pot experiments in order to examine the component parts of mycorrhizal communities, but pot experiment results do not necessarily represent ecological reality (possibly a gross understatement). Unfortunately, there has been precious little research in natural ecosystems, so how can we know? Well, the little that there has been done, frequently contradicts laboratory results. Our understanding of mycorrhizal biology is mostly based on results from experiments with culturable fungi. It is becoming clear that ecologically significant AMF may not those culturable species. It is the culturable species which, of course, are found (if present) in commercial inocula. Offer *G. mosseae*, *G. fasciculatum*, *G. intraradices* (whatever that is), *P. tinctorius* etc., the commonly used, culturable fungi, to a plant in a pot and frequently the plant will become phosphorus rich and grow better (n.b. sometimes they don't). But are those fungi the ones that actually enhance plant performance beyond the glasshouse (which is where inoculants are made to be used)? Are *G. mosseae* etc., actually found other than in highly disturbed agricultural soils, potted culture collections and

inoculants? Do such fungi survive if added to a competitive natural mycorrhizal community? Even if culturable, the performance of ecologically significant mycorrhizas in pot experiments may be entirely different from their performance in the field. (see Fitter et al. to be published soon). We now have tools with which to examine field communities in great detail, taking into account the probably important unculturables (See Radajewski, Ineson, Parekh & Murrell (2000) Nature 403(10): 646-649). It is very unwise to extrapolate from the pot to the field and then make wild claims that a cocktail of easily cultured fungi (AM and/or EcM) will have predictable benefits to any plant, crop or plant community. especially 1. if not tested in proper experiments with the plants to be targeted under realistic field conditions, or 2. if applied accompanied by management practices or nutrients which may themselves result in measurable benefit, which is then inappropriately attributed to the fungi. Anecdotal evidence, no matter how convincing individual cases may appear to be, can never provide proof of claimed efficacy of added mycorrhizal fungi. We desperately (with good reason) aspire to employ these astonishing fungi to the improvement of our environment, but success will not happen overnight, especially if we ignore (and this is not scientist-elitist, but down to earth practical talk) carefully designed research - which, for the greater part, has yet to be conducted. If we succeed, it is unlikely to be attributable to the naive expedient of adding a biological remediation agent, which is presumed ought to work because it was reported to have done so in reputable scientific literature specially selected to fit the producer's argument, mixed with a large dash of optimism. This will be especially true if, as is likely, the claimed active ingredients of that mixture are ecologically irrelevant, ineffective, of sub-standard quality or absent. Indeed, we are unlikely to succeed simply by intelligently, scientifically adding mycorrhizal fungi. There are too many diverse interactive elements in soil, and you can't simply add something to make degraded soil good again - how often have ironic terms such as magic dust or golden bullet been used in discussion of commercial mycorrhizal inoculants? Land wrecked by decades of exploitative maltreatment requires informed manipulation not magic additives. We must apply intelligent management regimes (TLC) to agricultural or natural communities which need to be mycorrhizal (and other-fungal, and bacterial, and collembolal, and protozoal, and nematodal, and insectal, and botanical etc. etc. almost ad infinitum) in order to function properly. En route we must do our best to understand how natural communities work and feed that understanding back into restoration and management techniques to be employed in wild, agricultural and horticultural situations.

James Merryweather

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To just enter the latest debates, I would like to reiterate a point I made some time ago. I received some very negative comments about it, saying I should 'lighten up', but nevertheless I still hold the same view, i.e. We must get away from this ridiculous idea, prevalent implicitly, if not explicitly, in much mycorrhiza work, that a mycorrhiza is a mycorrhiza is a mycorrhiza ... This has been mentioned already in the recent debate, but needs to be reinforced. What we should be looking at is the interaction among plants, mycorrhizal fungi, other fungi and other organisms both in and above the ground. Of course, as has been pointed out, this is not really possible, but as a start, those of us who want to do good mycorrhiza research should make sure that we identify at least the phytobiont and the mycobiont or mycobionts as precisely as possible. One can get into debates about what level we use to define either -- species, cultivar, isolate, etc., but

at the very least we should be able to recognise the fungi we use and, through observation and non-inoculated controls, ensure we are not getting inadvertent contaminant mycorrhizal fungi. This is true for all types of mycorrhiza. So, when you see a paper entitled 'The effects of mycorrhiza on' or some such generalised phrase, the alarm bells should be ringing. Editors (and referees) in particular are responsible for allowing this sort of thing to continue. I do have a few single spore isolates available for research purposes, though I will have to charge a small handling fee, and you will have to obtain all necessary import permissions. I can carry out identifications and quality control of inoculum for a reasonable fee. I am also happy to attempt to isolate single spore cultures from mixed ones, but this is very time-consuming and therefore expensive. I would be happy to be part of grant proposals as a route to funding. A reminder also, that I am trying to organise a short-course in September or October on isolating and characterising AMF. At present, there are insufficient expressions of interest to make it worthwhile, but another 6 or 7 participants would be enough. Contact me personally if you are interested.

Chris Walker

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Post Script. As of Feb 26, 2000, no one has yet come forward to collect the \$20 or \$10 reward!