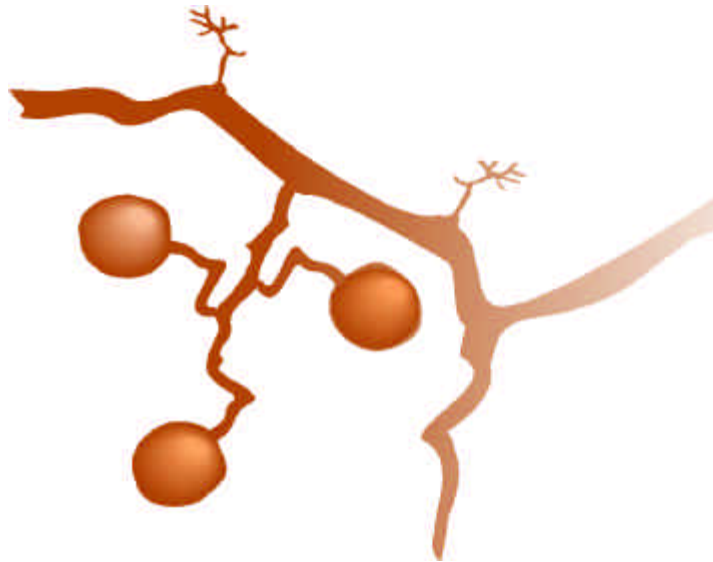


The Instant Expert Guide to

Mycorrhiza

The Connection for Functional Ecosystems



Ted St. John, Ph.D.

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Myco-What?

Most plant species form a symbiosis (mutually advantageous living arrangement) with beneficial fungi. The roots are colonized by the fungus, which also ramifies through the soil. The combination of root and fungus is called mycorrhiza. Mycorrhiza is considered such a fundamental part of the plant that most species could not survive in nature without it. The few plants that do not need mycorrhiza (mostly weeds) are considered to be departures from the normal state of the plant kingdom.

The mycorrhizal symbiosis appeared in the fossil record along with the earliest land plants, and may have made possible the transition from the aquatic to the terrestrial environment. Mycorrhizal fungi constitute the dominant microorganisms in most undisturbed soils—estimated at about 70% of microbial biomass. They make plant growth possible, link the roots of different species, control the mix of plant species on the site, and dominate the microflora, selecting a soil full of “good bugs” when the site might otherwise fill up with pathogens. Is it any wonder that Dr. R. M. Miller has called restoration without mycorrhiza “lipstick on a corpse?”

Mycorrhizas are fundamental to ecosystem function: the sum of energy flow and mineral cycling processes that characterize a natural community and allocate the resources that maintain it. It hardly states the case to say that mycorrhizas are *important* to ecosystem function. It is much more accurate to say that mycorrhizas *are* ecosystem function.

It is important to understand what mycorrhizal fungi are not. These are not the organisms that fix nitrogen (make atmospheric nitrogen available to plants) in association with legumes (those are bacteria of the genus *Rhizobium*) or with alders and *Ceanothus* (those are certain

The Terms: Lets Get It Right!

The first job is to learn the language of this discipline. Misuse of these terms is a giveaway that you are operating on the edges of your expertise.

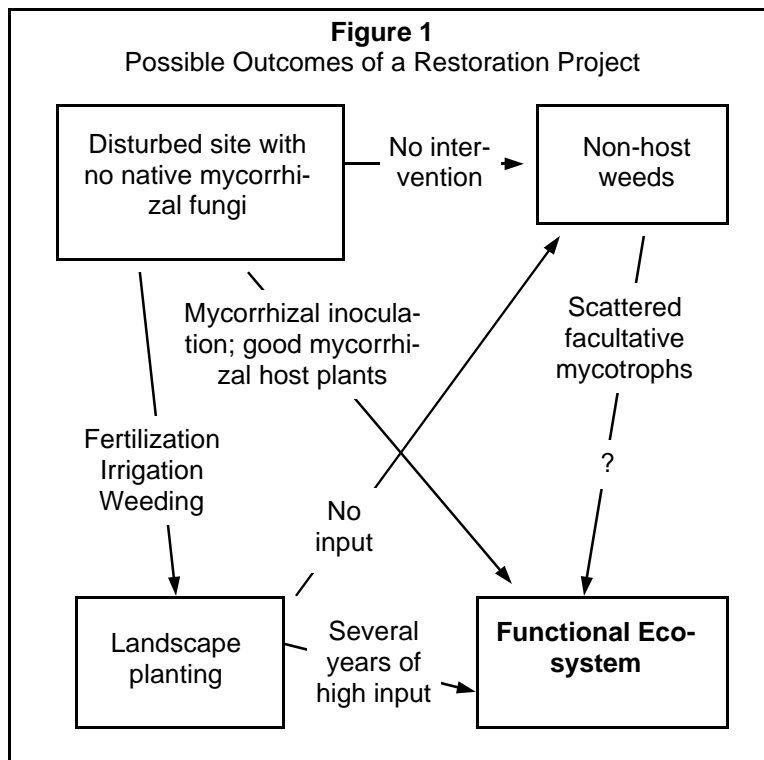
Noun: The word mycorrhiza comes from Greek origins: *myco* for fungus and *rhiza* for root. The extra “r” is free. The most common way to make this into a plural in North America has been the Latinized *mycorrhizae* (say my-co-RIZ-ee), a mixture of Latin and Greek in the same word. The British have no patience with this unsuitable mixture of languages and pluralize the word by simply adding an s, a mixture of Greek and English instead. The British practice is somewhat more defensible, since the earliest uses of the word was by a German in the late 1800s who pluralized the term in accordance with his own language.

Adjective: The adjectival form is *mycorrhizal*. Speak of a *mycorrhizal plant*, but please do not speak of a *mycorrhizae plant*.

The fungus: The term mycorrhiza refers to a combined structure. **The mycorrhiza is not the fungus - the mycorrhiza is the *symbiotic combination of plant and fungus*.** This subtlety is elusive enough that even specialists have lapses from time to time, and say mycorrhiza when they really mean the fungus. Even if you occasionally slip, be sure you grasp the central idea that a mycorrhiza is the combination, which includes the *mycorrhizal fungus* and the *mycorrhizal host plant*.

specialized actinomycetes). Mycorrhizal fungi do not fix nitrogen at all; in most cases what they do for the individual plant is aid in uptake of phosphorus.

Native mycorrhizal fungi are present in healthy ecosystems, but are often destroyed by disturbance. They are always missing from freshly graded sites, the most common situation for commercial restoration projects. **The lack of mycorrhizal fungi on disturbed sites is the**



basis for inoculation.

Numerous successful trials show that we now have the means to not just make individual plants mycorrhizal, but to quickly fill the soil with mycorrhizal roots and the network of mycelium (the mass of fungal filaments) that mediates ecosystem function. By putting the network in place on a restoration job, we can realize the same benefits that the network extends to natural ecosystems.

Why Become an Expert on Mycorrhiza?

The purpose of this booklet is to bring restoration and revegetation consultants up to speed on the symbiosis, its importance in ecosystem function, and its use in habitat restoration. The level of expertise we are hoping to achieve is an ability to understand the symbiosis and its role in a particular project, and an ability to make the procedures ap-

appropriate for each project.

The most important reason for you to know about mycorrhiza is that its use in restoration is here to stay. This is now an established technique for greatly improving the success of revegetation efforts, and a method that successful consultants need to know in order to stay current with the industry.

Learn the Terms

The sidebar indicates the most central, and most often misused terms. Here are a few more with which you should be comfortable:

Background

Host status: There is potential for confusion when speaking of the plant and its mycorrhizal status. In this booklet a plant that is incapable of becoming mycorrhizal is a *non-host*. A plant that can become mycorrhizal but just happens to be without symbiotic fungi is called a non-mycorrhizal plant.

Mycotrophy: Host plant species differ in the degree to which they depend on the symbiosis. That is, some (mostly weedy) species benefit little, even though they are capable of becoming mycorrhizal. Other species are *mycotrophic*: they depend upon the symbiosis and make little growth without it unless heavily fertilized.

Some particularly mycotrophic plants are trees and shrubs with roots that are sparsely branched and have few root hairs (cellular extensions that help roots take up nutrients). Perennial grasses are often strongly mycotrophic. Most weedy plants are either non-mycotrophic (i.e. annual grasses and weedy composites) or non-hosts (e.g. the families Amaranthaceae, Chenopodiaceae, Brassicaceae, Aizoaceae, Cyperaceae, and others).

Colonization: Until the 1970s, mycorrhizal plants were said to be *infected* by mycorrhizal fungi. Since *infection* sounded too pathological, we began saying *colonization* instead. Today, hard looks will befall those who speak of mycorrhizal *infection*.

Types: Mycorrhiza come in about seven types, which differ by kind

of fungus, kind of host plant, and morphology of the interface. The fungi of these several kinds of symbiosis may differ so completely that they cannot be related to each other. In other words, mycorrhiza was so successful that evolution produced it several times in several different versions.

These various types of mycorrhiza share certain characteristics. Mycorrhizal fungi plug into the *cortex* (a layer of cells found only on relatively young roots), and at the same time pass into the soil, forming a bridge to the plant. They all provide soil nutrients to the plant and draw energy compounds from the host.

Ectomycorrhizal fungi enter the roots, where the hyphae (fungal filaments) pass between root cells. They do not enter the root cells, as do endomycorrhizal fungi. There is often a mantle (covering) of interwoven fungal mycelium (mass of fungal filaments) on the surface of the finest roots, and an internal network, the Hartig net, that weaves between the cells in the root. The mantle is often visible to the unaided eye or by use of a hand lens. Ectomycorrhiza is found on many dominant forest trees and involves a “higher” (often mushroom-forming) fungus. The term is abbreviated ECM or EM.

Endomycorrhiza is not really a natural group; it simply refers to the fact that fungal hyphae enter the root cells. Under this name are the very dissimilar mycorrhizas of orchids, Ericaceae and relatives, and the largest group, the arbuscular (AM), or vesicular-arbuscular (VAM) type of mycorrhiza. This last group is so dominant in the plant kingdom that we might simplify the whole discussion by giving AM primary rights to the term endomycorrhiza. The less common types would then go by their own separate names. This book is almost entirely directed toward endomycorrhiza (AM).

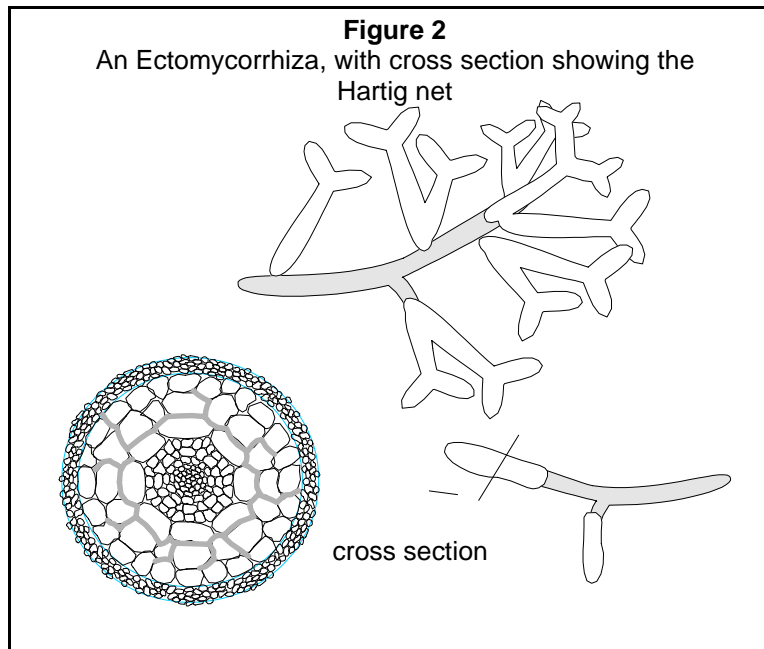
The arbuscules and vesicles, for which AM/VAM were named, are structures found in the roots of mycorrhizal plants. AM fungal species form arbuscules (branched structures inside the root cells) at some point in the colonization cycle, but not all form vesicles (oil storage organs in the roots). Thus, the current trend to drop the V part of VAM. Among mycorrhizal specialists, those who still say VAM are thought to be badly out of date, and there has been a stampede to the more current term. Say VAM at your peril.

The Questions that Clients Always Ask

Mycorrhiza is invisible to anyone but a specialist, and this leads newcomers to dismiss the symbiosis as non-existent, insignificant, or self-maintaining. The claims made for its effectiveness resemble the claims made for a large number of amendments, solutions, vitamins, and other "miracle" products. Mycorrhiza is distinguished from the other plant growth enhancers by a large body of scientific work, extending over more than a century. Nevertheless, clients and customers always have a certain sense of skepticism, and almost always ask one or more of the questions in the following list:

Background

- What beneficial effects can I expect on my project?
- Why is inoculation better than fertilization?
- Microbes are everywhere, especially in soil. Why do I need to add them?
- Can I get the same effects by replacing the topsoil?
- Do my plant species need to be mycorrhizal?
- Does it take a separate kind of mycorrhizal fungus for each species of plant?
- Is a mixture of fungi better than a single species of fungus?
- Are native fungi better than the generic fungi in commercial inoculum?
- Will the non-native fungi in commercial inoculum become microbial weeds at the project site?
- How much will inoculation cost?
- How do I know I do not already have mycorrhizal fungi?

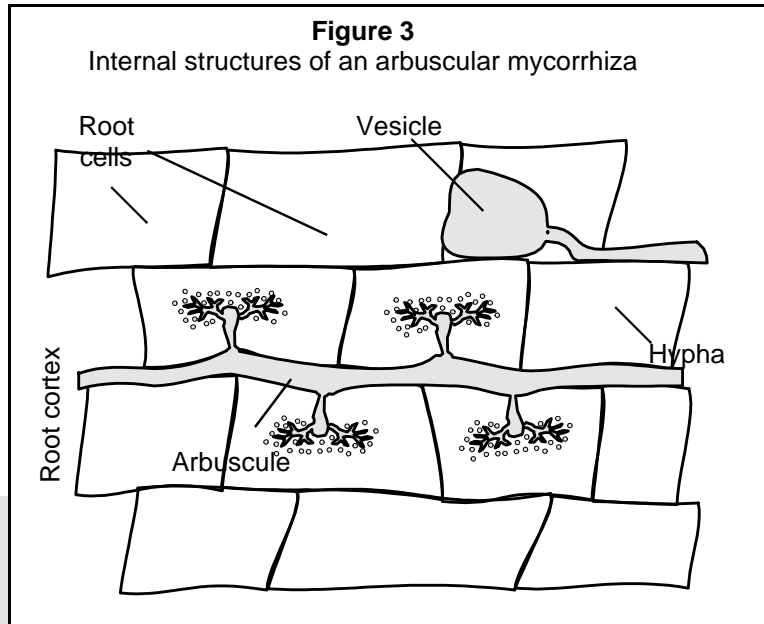


The remainder of this booklet addresses the questions and ways to explain the underlying principles to your clients.

Learn the Benefits of Mycorrhiza

Growth response: The best known mycorrhizal effect is that mycorrhizal plants take up more soil phosphorus and grow faster than corresponding non-mycorrhizal control plants. Hundreds of photographs of this growth response have been published. The pictures of big plants and little plants are largely responsible for the common perception that mycorrhizal inoculation ought to bring on a "Jack and the Beanstalk" response.

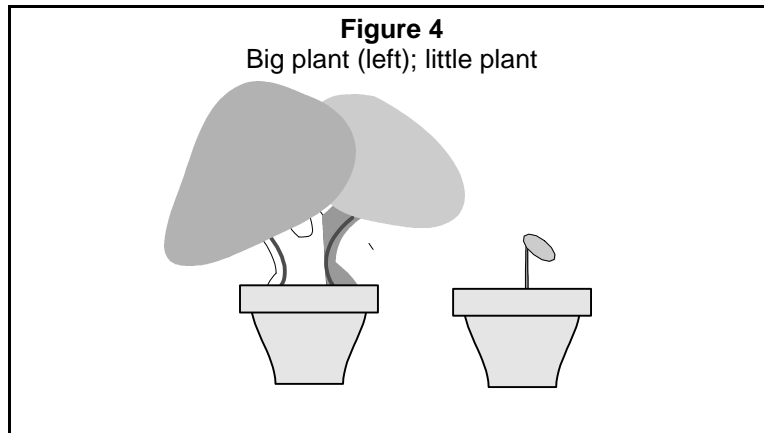
The growth response is probably the least important and indeed, the least likely of the effects of inoculating a restoration project. The growth response may be duplicated, and usually exceeded, by adding phosphorus fertilizer. The difference in growth rate is simply a meas-



ure of what one might have gained by spending pennies on fertilizer instead of dollars on inoculum. By dwelling on the relatively unimportant growth response instead of the enormously important ecosystem effects, you will set your client up for disappointment. You and your client may then miss out on a chance to make a real ecosystem instead of a fertilized garden.

Greenhouse trials: The growth response is common in greenhouse trials, and many hundreds of these experiments have been published. Specialists are rather tired of them, as the point was well established by the early 1970s. Nevertheless, people want to see it work with *their* plant, in *their* conditions, in *their* greenhouse. It is unlikely that these experiments will end any time soon.

In the field, growth responses are considerably less evident than in the greenhouse. This does not mean that mycorrhiza is less important; it is probably more so. Non-mycorrhizal plants in the field can extend their root systems to find new nutrient sources, one of the reasons the growth response may be much less than in containers. If you compare



only growth rates between inoculated and uninoculated plots, you may overlook the fact that many more plant species appeared from the same seed mix on the inoculated plot, or that many more individuals of some species survived on the inoculated plot.

If the plot was designed as an experiment to test the effects of inoculation, you may have used only the few plant species known to perform well on disturbed sites. If so, you have pre-selected species that will show little or no growth response. **The major reason that informal field trials are considered failures is that people look only for a growth response in plant species that have been pre-selected for non-mycotrophy.**

A more useful measure of success would be survival from a seed mix of many plant species. Many native plants fail if they are unable to become mycorrhizal soon after germination. This failure rate is differential: the most mycotrophic species are the least likely to survive. Many species are badly underrepresented on the uninoculated plot, and others do not appear at all (zero survival). Any difference in average plant size is accidental and largely beside the point. This important improvement in plant species diversity would be very unlikely from fertilization.

Seedlings become mycorrhizal very quickly if the soil is full of mycorrhizal hyphae, but more slowly if the soil contains only dormant

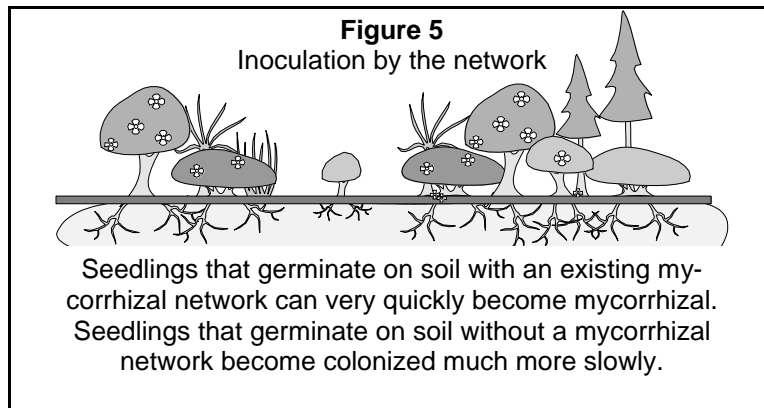
spores (fungal reproductive structures). The living mycelial network favors the diverse native species that must become mycorrhizal quickly. Soil with little inoculum selects against most natives and favors the plant species that do not need to become mycorrhizal early in life. **These plants are better known as weeds.**

Links: The effects of the fungus on the soil are even more significant than its effects on the plants. As more and more plants become mycorrhizal, the fungus links one root system to the next. This is possible because the fungus can colonize almost any plant species. Experiments have shown movement of soil nutrients and even photosynthate between plants of different species, as the mycorrhizal fungi pass materials back and forth. The early ecologists who spoke of the community as a “super-organism” were not entirely wrong. Below ground, the community is to some extent a super-organism with a single nutrient uptake system. The active hyphae that make up the network are by far the biggest component of the soil microbiota, and make the essential difference between living soil and inert “dirt.”

The structure of the network is much more complex than this simple picture. It includes not only a mixture of plant species, but a mixture of mycorrhizal fungal species. Different fungi are most active in different parts of the soil, they change seasonally, and to some extent associate with preferred plant species.

Structure: Fungal hyphae, and the bacteria they encourage, are the primary agents that bind soil particles into *soil aggregates*. These fungi control aggregates in the visible size range; ionic and other processes operate in the smallest aggregates. The hierarchy of soil aggregates, and the voids, pores, and cracks that form between aggregates constitute the *structure* of the soil. Soil structure allows water and air to infiltrate the soil and keeps soil particles from washing or blowing away. Root growth and animal movement generally follow the cracks between aggregates (the *macropore* space of the soil). In this way, the life of the soil and the structure of the soil are heavily interdependent. New research is showing that the fungi that best form soil structure are the ones that produce the most *Glomalin*, a biological glue that helps hold the soil together.

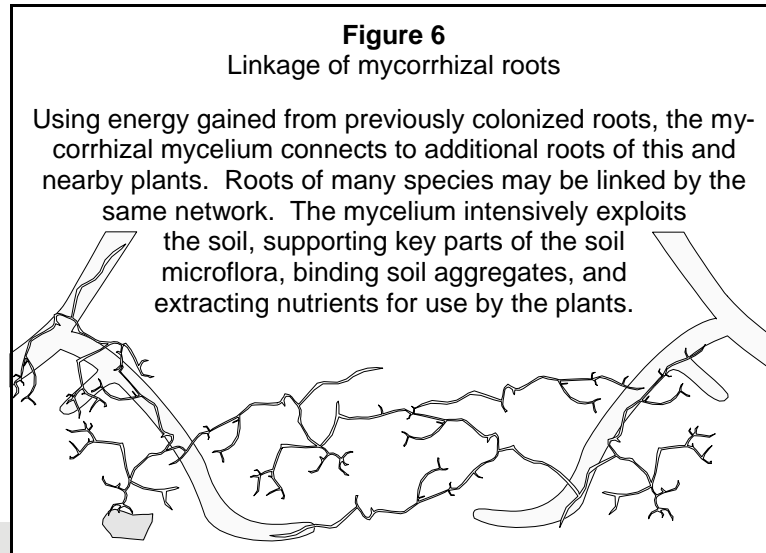
Soil structure is poorly developed or non-existent on badly disturbed



sites, and very well-developed in native ecosystems. Successful creation of soil structure should be a primary objective of habitat restoration, and is only possible after the mycorrhizal network is in place.

Soil microbiology: Because mycorrhizal fungi are so pervasive, they exert a large effect on other soil organisms. Research by the USDA has shown that beneficial soil bacteria are more abundant in soils permeated by mycorrhizal fungi, and pathogenic organisms less abundant. Protection by AM against pathogens probably depends primarily on independent *pathogen antagonists*. ECM appear to protect by other mechanisms, including physical shielding of the root by the fungal mantle, and perhaps by production of antibiotics.

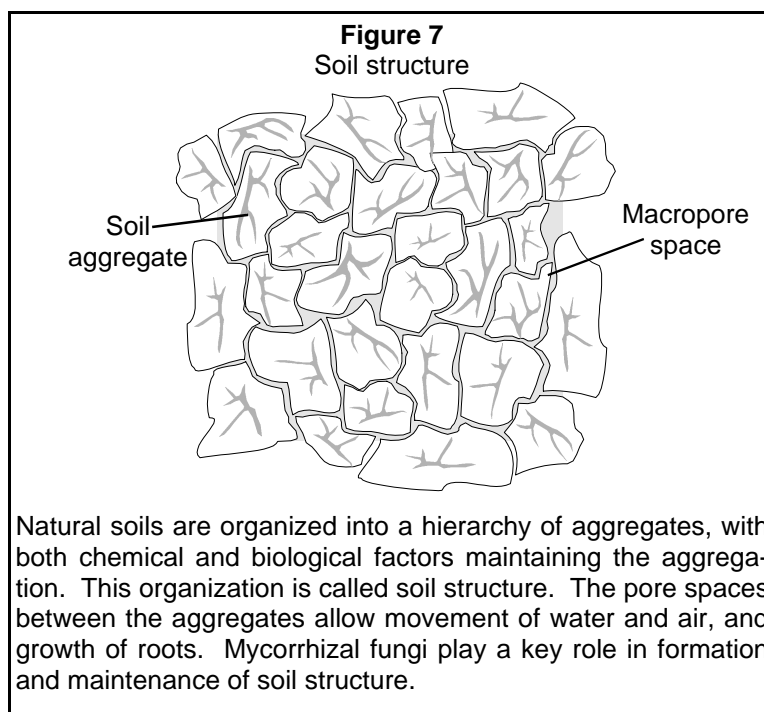
Function: As the first organ of nutrient uptake, the mycorrhizal network mediates nutrient cycling. As the instrument of rapid root colonization, it determines the plant species composition of the community. As the medium of soil structure, it determines the flow of water, nutrients, and air, directs the pathways of root growth, and opens channels for the movement of soil animals. As the moderator of the microbial community, it determines the metabolic processes of the soil. In other words, **the mycorrhizal network is practically synonymous with ecosystem function.**



Where is the Proof?

Mycorrhiza is probably the best studied of all plant-microbial relationships. There are thousands of publications in the technical literature that demonstrate the importance of mycorrhiza in plant growth and ecosystem function, but you will have to represent this base of knowledge to your client. At the end are lists of books and articles that can take you to the next step.

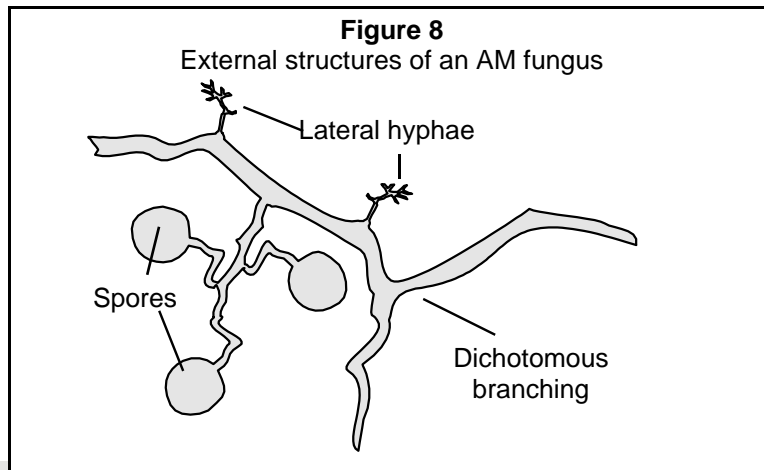
Since the scientific literature is difficult to read without a solid background in biology, you may want to show and interpret some examples for your client. You might try showing your client graphs and tables from scientific papers, documenting the growth response, seedling survival, species diversity, or effects on soil structure. Suitable papers might be the review by Brundrett, or that by Frances and Read in the bibliography. These papers are available in the technical libraries of universities that have good agricultural or biological programs. I have written for both scientific and semi-popular audiences, and have included some of those in the bibliography.



What Your Clients Can Reasonably Expect

If your clients have heard of mycorrhiza at all, they will expect dramatically larger plants on the inoculated plots. While there may be some growth response, plants in the field have a habit of expanding their root systems, taking over resources left behind by plants that did not survive, and otherwise compensating for the nutrient uptake that should be provided by symbionts. There are usually fewer plants on uninoculated sites, so each plant gets a larger share of soil resources. **The main reason for modest growth responses in the field is that the few species traditionally used on disturbed sites are those that do not need to be mycorrhizal.** Among those species, there will be little difference between inoculated and uninoculated plots.

Realistic benefits that your client can expect include survival, diversity, protection from disease, improved soil structure, and resistance to invasion by exotic plant species. Most of these benefits depend on



development of the mycorrhizal network in the soil. If the weeds win the race for control of the soil, none of the benefits will develop and the restoration effort will fail. That does not mean inoculation is not worthwhile; it means that timing, choice of host plants, and other details are critical and must be carried out with a firm commitment.

Why not Just Fertilize Instead of Inoculate?

Fertilization can produce large plants, but it often suppresses mycorrhiza formation. Fertilization lacks or even suppresses the other important benefits of mycorrhiza. Fertilization cannot increase plant species diversity; it tends to favor large individuals of the few most vigorous species. Fertilization cannot improve plant survival, but rather tends to favor a few large plants rather than many smaller ones. Fertilization does not make the site unfit for weeds, but instead gives them a nearly insurmountable competitive edge against native plants. Fertilization does nothing to decrease root disease, favor beneficial bacteria, or improve soil structure, perhaps the most important effects of mycorrhiza in natural systems. In a revegetation project, fertilization is often a serious mistake.

Learn Why Some Sites Require Inoculation

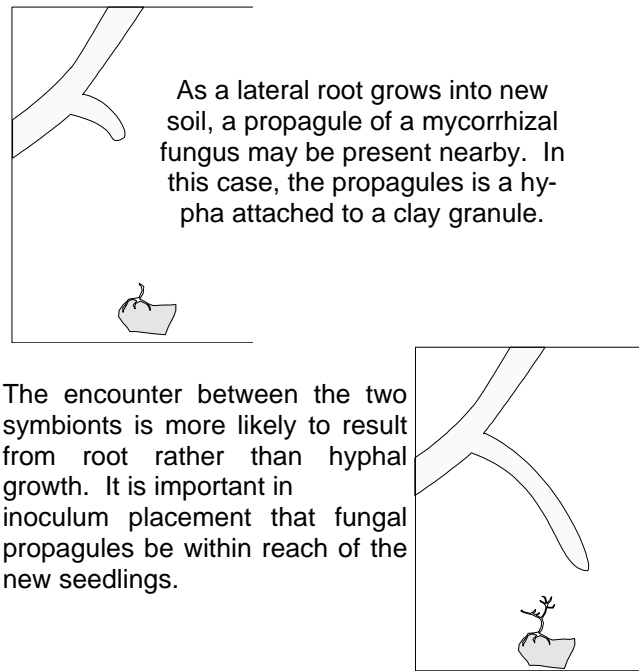
If mycorrhizal fungi are in all soils, why do we have to worry about them? Actually, the correct statement is that mycorrhizal fungi are in all **natural** soils. Any serious disturbance takes a heavy toll on the soil microbes, and such activities as grading, erosion, or overgrazing can destroy the fungi completely. The fungi do not disperse with the wind like mold fungi, but instead move by growing from root to root, or by moving with quantities of soil. Unless your site is within a few feet of healthy native vegetation, mycorrhizal fungi are very unlikely to show up fast enough to benefit your plants in the critical early stages. There are confirmed cases of native plants that sat three years (surviving only with artificial maintenance) before native mycorrhizal fungi moved to the site.

Topsoil as Inoculum For Restoration

For restoration of native vegetation, the ideal way to inoculate is to salvage and re-apply quality native topsoil. It is very important that the soil be as free of weeds as possible, and that it previously supported diverse, healthy native vegetation. Topsoil contains a range of valuable organisms and chemical properties, and often contains seeds of native plants.

Topsoil salvage is expensive and destroys the donor site; thus it should be considered only if the site to be graded can furnish the topsoil. Topsoil should be collected during a dormant season: the dry part of the year in warm climates, or the cold part of the year if there is no distinct dry season. The stockpile should be close to the restoration site; ideally, the soil is moved immediately from donor to receiving site. If it must be stockpiled, do not pile more than 30 cm deep for clay soils or two meters deep for sandy soils. While viable mycorrhizal propagules have been documented in stockpiles as much as twelve years of age, in general two to three years is the longest that stored soil should be considered reliable mycorrhizal inoculum. Salvaged topsoil may be spread over about twice the area from which it was collected.

Figure 9
Mycorrhizal colonization of roots

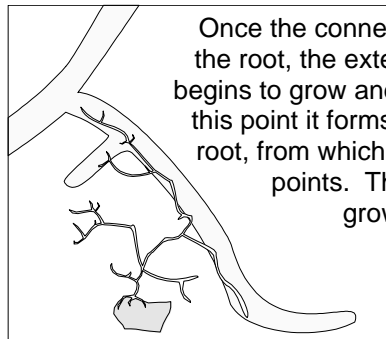
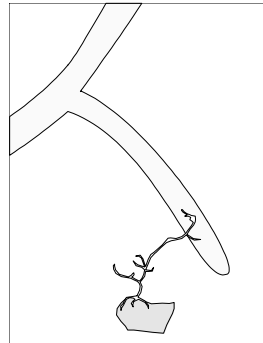


Determine Whether Your Plants Need to Be Mycorrhizal

Most plant species- probably 70 to 80%- are normally mycorrhizal in nature, and most of those are AM rather than some other kind. If in doubt, assume that your plants need to be AM. If your plant list contains few AM hosts, you should in most cases add some to the species list to be sure you gain the benefits of soil structure and favorable microbiology.

ECM hosts include members of the Pinaceae (most timber species), the oak and beech family, some tropical legume trees, and scattered members of other families from the arctic to the tropics. ECM-

At very close range, the hypha is attracted to the root surface. There it forms an appressorium and penetrates the epidermis between adjacent cells. Once inside the root, the hypha branches and forms arbuscules within the cells of the cortex.



Once the connections have formed inside the root, the external portion of the fungus begins to grow and branch more rapidly. At this point it forms runner hyphae along the root, from which it produces multiple entry points. The external mycelium also grows into surrounding soil to contact new roots and absorb nutrients.

Site Evaluation

dominated forests tend to be low in species diversity compared to AM forests, and may have a thick layer of organic debris on the forest floor.

Among ECM hosts are some species that can be simultaneously ECM and AM. These include willows, cottonwoods, alders, *Eucalyptus*, and some of the tropical legumes. There is a tendency for these doubly symbiotic plant species to be AM as seedlings and both AM and ECM as mature trees.

Ericads: The family Ericaceae, found worldwide on impoverished, acid soils, has two mycorrhiza types of its own. *Arbutoid* mycorrhizas, found in *Arbutus* and *Arctostaphylos*, look like and are related to ECM, except that the fungi penetrate the cells of the cortex. Ericoid mycorrhizas, found in most ericads, are quite different, and involve

fine, specialized roots. Some of the non-photosynthesizing plant species of families related to Ericaceae share the ectomycorrhizal fungus with a nearby ECM tree, and extract sugars from the tree by way of the mycorrhizal fungus. Their roots look much like arbutoid mycorrhizas.

AM hosts include almost everything else: grasses, shrubs, trees including redwoods and cedars, most domesticated plant species, and many members of the forest understory. You cannot distinguish AM roots from those without mycorrhiza except by a laboratory clearing and staining procedure.

Determine the Best Fungal Species

Site Evaluation

Specificity to hosts: AM fungi are very non-specific in their ability to associate with plants. That is, almost any AM fungus can form mycorrhiza with almost any AM host plant. However, there are preferences, in that host plant species may select different mycorrhizal partners from the mix of fungi available in the soil.

Specificity to soils: Mycorrhizal fungi are in general more specific to soil type than to host plant. Soil pH is the biggest selective factor, but soil texture and organic matter may also influence the suitability of the soil for particular fungi. The fungi commonly available as commercial inocula tend to have wide tolerance ranges. *Glomus intraradices*, the most widely available species, is suitable for soils from about pH 6 to 9. Another widely available fungus, *G. etunicatum*, is at its best in the acid range. There are fungi that tolerate cool spring temperatures and others that remain dormant until the soil warms up. Some do best in new plantings and others do not appear to take well in plantings, but may be abundant in mature native vegetation.

There is reason to believe that some fungal species are better than others at promoting soil structure. Some species appear to produce more “Glomalin,” the newly-discovered glycoprotein that acts as a glue for soil structure.

Consider a Mixture of Mycorrhizal Fungi

Several scientific studies have concluded that growth responses were improved with mixtures of fungi rather than single species. However, none of these studies has included a "wonder fungus" of the type sometimes isolated in large-scale screening projects. *G. intraradices* has turned up as a "wonder fungus" in several surveys, and field experience so far has shown it to be equal or superior to mixtures of other fungi. There is a concern that less effective fungi could dilute the propagules of the fungus that works best, perhaps decreasing its effectiveness. Even so, many researchers believe that mixtures of fungal species are preferable.

Plant diversity depends to some extent upon fungal species diversity. There may be a benefit to some rare plant species of having particular fungi that grow at the right time of year or produce some other specific effect. Until we know exactly how the effects are produced, the only way to include such fungi would be in quality topsoil from the native habitat of the rare plant species. What is very clear, from every study that has done the tests, is that inoculation is greatly superior to no inoculation, with differences between fungal species forming a secondary effect.

The pattern has been that high quality commercial inoculum allows a diversity of plant species to become established, and a diversity of fungi from nearby undisturbed land moves onto the site in subsequent months. Neither scientific nor commercial experience has sufficiently precise information to know which fungi provide the widest range of benefits, or may be required for particular rare plant species. If native topsoil is not available, the best strategy at this point is make sure a diversity of plant species succeed and are available to propagate more fungal species as they find their way onto the site.

Consider Local Native Fungi

Genetics of the organisms used in restoration is always a concern. Since mycorrhizal fungi are not strongly specific to hosts, there is usually no need to culture fungi from the same plant species. However, the fungi must be suitable for the soil and the climate of the res-

toration site.

In some cases introduced fungi meet these tests better than the natives. This is likely to be the case if the soil has been so modified by disturbance that its properties no longer resemble the original native topsoil. Subsoil, which usually differs in many ways from topsoil, is the material into which most restoration projects are planted. There is no assurance, or even likelihood, that the native fungi that came from the topsoil are better suited to the subsoil than exotic fungi.

Mixtures of local native fungi are available on contract from BioNet LLC. Their production requires appropriate material from the field site and takes several months. Because restoration projects often run on a compressed or unpredictable schedule, there will be times when neither topsoil nor native fungi are available and "generic" commercial inoculum will be the only realistic alternative to no inoculation at all.

Are There Fungal "Weeds"?

Another question is the possibility of introducing a "weedy" mycorrhizal fungus, which might displace native fungi. This appears unlikely, since attempts to do so in agriculture have consistently met with failure. In every documented case that I have found, natives replaced the introduced species between one and three years after introduction. As commercial inoculation increases, we must be alert for any evidence that there can be "weedy" mycorrhizal fungi.

Determine Whether Your Site Requires Inoculation

Laboratory tests can indicate whether the soil contains spores or whether the roots of existing plants are already mycorrhizal. However, in most cases you can make a very good guess without laboratory work, just by considering the condition of the site.

Mycorrhizal fungi are removed entirely by grading, and newly graded land always requires inoculation if the objective is a functional terres-

trial ecosystem. Eroded land is in nearly the same condition. Overgrazed land usually has greatly reduced amounts of native inoculum, and may have none at all. Agricultural sites that have been fumigated or disked several times a year may have a low concentration of native inoculum.

A good first look at your site should consider the existing vegetation. If there is none at all, or if it is occupied by weeds of the mustard, Chenopod, and Amaranthus families, you may assume that inoculation is mandatory. If it is occupied by weedy annual grasses and composites, or exotic perennials known to be highly invasive (brooms, *Arundo*, salt cedar), there may be some inoculum but the site would probably benefit from inoculation. If the site includes natives in a degraded state, say scattered early-successional native shrubs with weeds in between, inoculation is also likely to help, but the problem might be solved by overseeding or imprinting with aggressive natives that are good mycorrhizal hosts. These natives will propagate the fungi and if not overwhelmed by weeds, will build a continuous network that should favor improvement of the native community.

Sites that receive carefully stored topsoil, less than a year or two in age, should not require inoculation.

Some scientists believe that in humid climates native fungi invade so rapidly that inoculation is unnecessary. This requires more research, since the most mycotrophic species must become mycorrhizal very early in life, perhaps too early for even the fastest natural recolonization.

You can examine the soil directly for mycorrhizal spores during the season of plant dormancy. Combine ten or more soil samples from the upper soil and have a specialized laboratory look for spores, or find the methods for wet-sieving and spore identification to try it yourself. It is also possible to clear and stain field-collected roots or carry out a bioassay with the soil. There are very few laboratories prepared to provide these services. One good one is Soil Foodweb Incorporated of Corvallis, Oregon (541/752-5066).

Determine Whether Your Soil is Toxic to Mycorrhizal Fungi

Mine spoils are often toxic because of processing or because of the sometimes toxic nature of the ores that contain mineral resources. In most cases toxicity will have been determined as an early step in the remediation process, and detailed analyses will be available. A bioassay with radish seedlings (radish is a non-host) grown in representative soil samples will indicate whether the medium is suitable for plant growth. If it is suitable for plants, it is possible but less likely that there are factors that are toxic for spore germination or root colonization by mycorrhizal fungi.

The Inoculation Process

In examining a soil analysis, look for high concentrations of Na, Cl, B, Cd, Zn, and Mn. Any of these have been shown to interfere with colonization or may reasonably be suspected of doing so. Look also for extreme pH values. Soils in a more acid range may have toxic levels of aluminum ions.

If the soil is receiving organic amendments consider their possible effects on mycorrhizal colonization. Many forms of peat are tolerated only as a low proportion of the mix. Very raw compost materials can sometimes be inhibitory, although mature compost and most humic materials can be neutral or even stimulatory to fungal growth.

It is possible for artificial media to be so low in some nutrient ions that colonization is inhibited. I know of no example of this in a natural temperate zone soil, but it happens in subgrade or spoil material.

Use Mycorrhizal Inoculum Correctly

Root zone: One of the most important points is that endomycorrhizal inoculum must be placed in the soil, where new roots will grow through it. Colonization will succeed only if the fungi are properly placed and if the roots are healthy and growing. ECM spores are better able to penetrate the soil due to their small size.; even so surface application is not the best use even of ECM inoculum.

Propagules: In a soil with an established mycorrhizal network, the most active kind of propagule (structure that can produce new fungus) is fungal mycelium. Most new seedlings in healthy native vegetation are colonized in this way. Commercial inoculum contains several kinds of propagules, including spores, mycorrhizal root fragments, and living mycelium.

Most species of AM fungi form resting spores, either in the soil or in the root. Spores are more resistant to environmental stress than other propagules, but do not produce mycorrhiza as quickly as live mycelium or fragments of mycorrhizal roots. Spores can be separated from inoculum or the soil, and form the basis for classification for these fungi.

Fragments of roots may contain live fungi, and these fragments are often good propagules. In some cases the root fragments contain spores, and in other cases only fungal mycelium. Hyphae deteriorate once separated from the host plant, but continue for weeks or months as active propagules in whole inoculum.

As a living material, mycorrhizal inoculum is susceptible to environmental stress. It is important not to allow the inoculum to sit in the sun or expose it to freezing temperatures. Temperatures over about 50° C (122° F) may be lethal for many temperate zone isolates. The life span of mycorrhizal spores as given in the scientific literature is in the neighborhood of 6 months to a year. Certain kinds of carriers appear to provide protection, and in good storage conditions, with the original production vessel kept intact, inoculum in calcined clay has retained its viability for two or more years.

Choose the Best Way to Place Inoculum

The best options are mechanized and can be carried out at the same time as something else already being done on the project. For example, a compacted site may have to be ripped before planting. It works out well to rip, broadcast the inoculum, then finish with a process that incorporates the inoculum into the cracks and openings created by ripping. This might be another pass with the ripping equipment, disking, or dragging with a heavy timber. Some land imprinters are

equipped to drop inoculum from a fertilizer box, such as those sold under the brand names Clampco or Gandy. From the fertilizer box it falls through delivery tubes behind short ripping shanks. On agricultural land, where compaction and large rocks are not a problem, the inoculum can be delivered below ground with a fertilizer box and chisel type shanks, designed for placing fertilizer in the root zone.

Land imprinters and some other land preparation machinery moves the soil around during operation, and somewhat “accidentally” incorporate a significant portion of the inoculum. Imprinter operators are increasingly deciding that this somewhat inefficient means of incorporating inoculum is preferable to the problems caused by shanks, which tend to rake up weeds, bend during turns, and otherwise require continuous attention.

If the inoculum is laid down in lines, as by a fertilizer box, the lines should be about a foot apart. This is difficult on sites with vegetative debris, which becomes tangled in the shanks. Eighteen-inch spacing gives less trouble, and has given good colonization in most cases.

When growing from root to root, the fungi spread between $\frac{1}{2}$ and 1 meter per year. Soil animals may move it somewhat faster. Most often, the roots grow to the inoculum rather than the reverse, so the real requirement is to be sure that there is some inoculum close enough to each new seedling that its roots can find the fungi quickly. With wide spacing, there are probably a fair number of seedling deaths, but enough survive to give good representation of the seed mix.

Broadcast: It is possible to broadcast the inoculum (this has even been done by air), followed by disking or chaining. In one case a contractor with his own hydroseeding equipment blew it on, then disked to incorporate the inoculum.

Hydroseeding: a California consultant and landscaping company established some careful trials of EndoNet inoculation with hydraulic seeding equipment. There was a surprising degree of success, especially when seeds and inoculum were applied in the first pass, then mulch and other components in a second pass. The California Department of Transportation (Caltrans) has prepared some specifications

for mycorrhizal inoculation by hydroseeding.

Hand labor is less efficient but may be the only option on small inaccessible or rough sites. Mycorrhizal container plants or salvaged wild plants can be used to introduce inoculum. This is not generally a satisfactory way to introduce inoculum for plants that follow from seed, since the spacing of containers is generally too wide for access by any but the closest seedlings. Container plants may be inoculated at the time of planting, either by adding a small amount of bulk inoculum to the root zone, or by dropping in a biodegradable “teabag” package.

It is possible to put inoculum, rather than container plants, in the ground at intervals. A worker can make a slit in the soil with the blade of a shovel, drop a few inoculum granules into the slit, and press the hole shut. Seed can be placed by hand in the loose soil above the slit and pressed into the soil as the hole is firmed shut. These spot applications should be spaced as closely as possible to quickly fill the soil with mycorrhizal roots and mycelium. Again, one foot spacing is better, although much more labor-intensive, than eighteen inch spacing. At one foot spacing, there are 43,560 applications per acre. Even if it takes only 30 seconds to do each application, this process may cost thousands per acre in labor.

For very small jobs, you can collect topsoil from the root zone of a known mycorrhizal host. For AM inoculum, try a late-successional native shrub or tree, or a perennial grass. For ECM or arbutoid hosts, use the duff or upper soil from under the target plant species. In any of these cases, look for soil that is free of weeds, contains roots of the wild host plant, and has good structure. The disturbance caused by removing topsoil may negate any expediency of using wild-collected inoculum.

Machine application is generally much less expensive than hand application. There is need for some kind of hand-operated machinery that can reduce this high labor cost on inaccessible sites. Some kinds of antique corn planting tools might be modified to release inoculum from a backpack, and might reduce the time for each application to a few seconds. Other possibilities include a modified walk-behind tiller or lawn edger that would drop a line of inoculum into a narrow slit in the soil.

Mycorrhizal status of some California plant species

Facultative mycotrophs

Lotus scoparius California broom
Baccharis pilularis Coyote brush
Eriogonum fasciculatum California buckwheat
Nassella pulchra Purple needlegrass
Artemisia californica California sagebrush

May be facultative mycotrophs

Salvia mellifera Black sage
Salvia apiana White sage
Encelia californica Brittlebush

Net-builders

Iva hayesiana Poverty weed
Epilobium canum California fuchsia
Eriophyllum confertiflorum Golden yarrow
Ericameria spp. Goldenbush
Hazardia squarrosa Goldenbush
Isocoma menziesii Goldenbush
Bromus carinatus California brome
Hemizonia fasciculata Tarweed

Obligate mycotrophs

Most long-lived perennials, including
Nassella lepida Foothill needlegrass
Nassella cernua Nodding needlegrass
Rhus ovata Sugar bush
Rhus integrifolia Lemonade berry

May be non-hosts

Atriplex spp. Saltbush
Sambucus mexicana Mexican elderberry

The Cost of Mycorrhizal Inoculum

The direct components of this cost are materials and application. The cost of materials depends upon bulk cost, propagule count, and desired number of propagules per unit land area. The bulk inoculum itself varies in cost depending on supplier and quantity purchased. Commercial inoculum costs from near \$100 to thousands per acre at the time of this writing, with the trend in cost downward over time.

Since mycorrhizal inoculum must go into the root zone, there is a cost associated with placing the material below ground. If the site is already being imprinted, ripped, or tilled, inoculum incorporation adds little to the existing costs. If inoculum application must be a separate step, the cost depends on the method. See the section on getting inoculum into the root zone, and the model specifications for details on some of the available methods.

Form a Strategy for Building the Mycorrhizal Network

Distance: The shorter the distance the root must grow before encountering a propagule, the more quickly the plant will become mycorrhizal and the more quickly the mycelial network will form. It is important to minimize the average distance between propagules.

An important consideration is how much inoculum to use. Suppliers may make recommendations based on their own economic ambitions rather than the best interests of the customer. You may have to satisfy yourself that their recommendations are appropriate for your project.

All inocula registered in the state of California have to guarantee the number of propagules. You can determine this yourself with a bioassay, but for now accept the figure given by the supplier. Most suppliers determine their number of propagules with a bioassay method that takes into account all means of forming new colonization. If the supplier offers no propagule count, look for a better product.

Carrier: Commercial inocula usually come in the carrier that was used to grow the host plants. If so, dispersal is essentially a matter of distributing the carrier. EndoNet comes in a carrier that consists of porous clay granules. A granule of the carrier material with attached fungal structures may be considered a single propagule in practice, even if several fungal structures are attached to each granule.

Calculations: You can use some general guidelines to help decide how much inoculum to apply. In native soils with an active mycelial network, new roots need grow only a few millimeters before encountering propagules. In stored topsoil most surviving propagules are probably spores. With 10 to 1000 spores per liter of soil, a root needs to grow from one-fourth to four inches before encountering a spore.

The Caltrans recommendation of 3.6 million propagules per acre would be diluted in a six-inch layer of soil to give an average spacing of 2.2 inches between propagules. To calculate the distance for some other amount of inoculum, find the number of propagules to be applied to an acre, then interpolate from the table. The table is based on the simplifying assumption that propagules are distributed evenly through the top six inches of soil, and that they sit at the intersections of a cubic grid.

To give another example, a commercial inoculum supplied as a liquid suspension claims 2 propagules per ml (2000 per liter). They recommend 15 gallons (57 liters) per acre. Using the above calculation, this application would give 6.9 inches between propagules.

How close is close enough? This is a fair question, and one without a firm answer. Greater spacing means slower colonization, and a greater chance that the natives will lose the race to the weeds. The figure given above for top soil (one-fourth to four inches) would be about the right range. A guideline might be that the wider spacing should make use of fast growing native plants that are very active mycorrhizal hosts. Closer propagule spacing should be used when slower-growing natives are the dominant plant species. If host plants were in rows, as with some agricultural crops, a line of inoculum would give a predictable distance of propagules from seedling roots. Careful use of this principle might make possible very low rates of inoculum application, but would not result in a uniform mycorrhizal

Spacing of Mycorrhizal Propagules		
	Number of propagules per acre	Separation Distance, inches
Pure EndoNet	60,000,000,000	0.086
Best native topsoil	6,000,000,000	0.19
	3,000,000,000	0.23
Average topsoil	600,000,000	0.40
	300,000,000	0.50
	60,000,000	0.86
	30,000,000	1.1
	6,000,000	1.8
Subsoil with EndoNet	3,600,000	2.2
Poorest topsoil	600,000	4.0
	300,000	5.0
Subsoil with very low inoculation rate	60,000	8.6
	30,000	10.8

network between the rows.

Choice of host plants: it is important to choose host plants that will become mycorrhizal quickly, propagate the network aggressively, and grow quickly enough to help suppress weeds. Every local flora includes such plants. Many turn out to be short-live perennials from the grass and composite families. The decision of which host plants to use is a matter of seed availability, cost, and suitability of the site for the potential choices. Be sure to include several candidate plant species, not just one or two.

Detect the Scent of Sales Tactics

Unfortunately, the relatively immature inoculum industry has made use of a time-honored sales technique: BS. BS has been applied liberally by newcomers to the industry, many of whom are veterans of

BS: that in which we try not to step while crossing a pasture; that which is not so.

miracle plant growth formulas and potions of all kinds. These people give an unfortunate flavor to the whole concept, and have no doubt convinced many that mycorrhiza is just one more form of snake oil.

Mycorrhiza is a natural part of the soil and a part of plant nutrient uptake. The fungi are the dominant soil microorganisms, and soil biology depends heavily upon the presence, density, and types of mycorrhizal fungi. However, mycorrhizal fungi cannot make it rain, cannot decompact a fill slope, cannot compensate for planting out of season, and cannot make up for methods that are otherwise very poor. Here are some of the claims that should raise a red flag:

Evaluating Inoculum

Plants show dramatic growth increases within a few days: Mycorrhizal growth responses are slow to develop; a rapid response would have come from fertilizer in the inoculum.

Growth response in spinach, broccoli, or other non-host: plants known to be non-hosts are good tests of fertilizer or other non-mycorrhizal factor in the inoculum.

No guarantee of propagule count on label: California and several other states require guarantees of propagule or spore counts. Even so, a number of inoculum products are being sold with no indication of propagule density. On close examination, these have usually proven to have no detectable propagules, or at best a very low count. Others claim multiple fungal species but offer only a total propagule count.

Very low propagule counts: propagule and spore counts vary from as low as two to several hundred per cubic centimeter of inoculum. Be aware that the cost of the material should reflect the propagule density.

Inoculum offered in liquid or powder form: ECM fungal spores are fine enough to be incorporated in a powder form, but AM fungal spores are relatively large and are destroyed by grinding. The small-

est viable root fragments are about 1/10 mm in length, and the spores themselves are also in that size range.

Mycorrhizal propagules settle out quickly in water and must be continuously agitated to remain in suspension. Inoculum suspensions have been recommended for application through drip irrigation systems with hundreds of yards of pipe and tubing. AM inoculum is singularly unsuited for such use; see the earlier section on BS.

How Mycorrhizal Inoculum is Produced

ECM fungi are generally grown in laboratory media by methods of industrial microbiology. Some fungal species cannot be easily cultured in laboratory conditions, and those are either unavailable as commercial inocula, or are collected from the wild. Certain fungi that fruit belowground, including truffle-like species of the genus *Rhizopogon*, lend themselves to wild collection. The fruiting structures are essentially pouches of spores that may be applied at the user's convenience.

AM fungi must be cultured with a host plant; these fungi remain the last important holdouts among microorganisms that cannot be grown on defined culture media. Inoculum typically consists of roots, spores, fungal hyphae, and growth medium from the "open pot culture" method of inoculum production.

Carrier materials include soil, sand, calcined clay (the same material often used for cat litter), and organic mixes. The trade-offs involve suitability for plant and fungal use, convenience of storage and handling, and suitability for application by machinery.

An experimental method that may be finding its way into commercial inoculum production is the use of "transformed roots". These are roots that can grow on laboratory media without leaves, and can support mycorrhizal fungi. It is not yet clear whether this relatively slow and exacting method can produce inoculum that is economically competitive with more conventional methods. Also potentially useful are hydroponic and aeroponic methods, which depend upon a nutrient solution rather than a solid medium. With certain combinations of

plant and fungus these methods can produce very concentrated inoculum. Most such material has been short-lived compared to that produced in solid media, but there are some indications that the life span can be considerably extended with post-production treatment.

Conduct a Field Trial

There are thousands of experiments in scientific journals that have documented every aspect of the mycorrhizal symbiosis. These studies began to appear in the early part of the 20th century. Much of the scientific work has incorporated careful experimental design and made use of laboratory equipment, specialized knowledge, and university facilities that are not available to field practitioners. Even so, it has turned out that almost every purchaser of mycorrhizal inoculum ignores decades of good experiments and performs one bad experiment, his own, before becoming convinced of the efficacy of mycorrhizal inoculation.

Evaluating Inoculum

This attitude among users is not entirely unreasonable. Scientific work is usually designed to eliminate all variables but the one under study. Scientists are unfamiliar with commercial restoration practice, just as contractors are unfamiliar with scientific methods. Practical work must tolerate conditions that change over space and time, with details of methodology that are often determined on the job. Every inefficiency results in lost time and high costs. It is very difficult to imagine that a scientific project could be done by the methods that are actually used in practice; these complex, variable, and subjective factors are to a large extent beyond the reach of the scientific method. It therefore not surprising that practitioners want to do at least an informal trial in their own conditions, using their routine planting methods.

There can be no illusion that these trials are scientific, or that their results can be taken as scientific evidence. Every imaginable factor, from usage history of each spot to position on the slope, influences the experimental outcome. However, there is considerable value in seeing the results in practical conditions and gaining an understanding of the realistic possibilities. Over several such trials, we can begin to see whether the result is predictable, and to build a basis for confident decisions in the future. It is thus important to make these trials as ob-

jective and enlightening as they can be, given the considerable constraints of time, cost, and facilities.

The most common design is to divide the project area into two portions and apply inoculum to half. A serious weakness of this procedure is that the two halves are never equivalent; one side has some advantage regardless of inoculation. Watch for different amounts of sunlight, initial weed loads, amounts of native topsoil incorporated into the slope, proximity of native vegetation, water distribution, and activity by animals and human visitors.

When locating control and treatment plots, consider factors that may cross contaminate them. Inoculum may be introduced onto control plots by re-use of the same equipment for each treatment, slope position that lets soil and water move from treated to control, and movement of personnel and equipment. The plots should be at the same elevation, and controls should be treated before inoculated plots to avoid residual inoculum in the equipment. There should be no foot or wheel traffic after inoculation, especially from treated to control plots. If at all possible, there should be several plots of each treatment, spread over the entire available area. All plots must be carefully marked, photographed, and documented in written records. Many of these trials have proven useless because of poor documentation.

Plan at the beginning for the evaluation of the trial. Growth response is a less common result than improved plant diversity, greater seedling survival, improved root systems, greater vegetative cover by natives, and reduced weed growth. Use a diverse seed mix, including species that have traditionally been difficult to use in your conditions. For this purpose, do not include species with difficult germination requirements, since mycorrhizal fungi generally have no effect on the germination stage.

Judge the Success of an Inoculation Trial

The effects of inoculation on native ecosystems are so profound and such a natural part of the vegetation, that the only complete failure is a lack of colonization. This can be detected by laboratory examination of root samples, determination of the presence of mycorrhizal spores

during an appropriate season after planting, or the formation of soil structure as the vegetation grows. These tests require facilities and training, or the services of a specialized laboratory.

Plant growth response in itself is not likely to tell the story. If uninoculated plots have been kept healthy by fertilization, any mycorrhizal effects will have been masked. Uninoculated plots will likely have a different set of plant species, dominated by the least mycotrophic, and least responsive, species in the seed mix. Plant species diversity and species-by-species determinations of survival rate are more likely than growth response to clearly indicate successful inoculation.

Evaluating Inoculum

In order to see an effect with diversity and survival, the seed mix must contain mycotrophic plant species. That is, it must not consist only of species known to be “reliable” on disturbed sites. These species almost always turn out to be non-hosts or weakly mycotrophic species.

If improved diversity and greater dominance by natives are not apparent by visual examination, use transects or other quantitative measure to compare inoculated with uninoculated plots. Doing so has turned up as much as a two-fold difference in native cover on a site where the difference was not visually apparent.

Habitat restoration on graded land is the situation in which inoculation is virtually always required. Within other disciplines, there are particular circumstances in which inoculation is required, or would allow a departure from established methods that have been in effect compensating for a lack of the natural soil microflora.

Inoculate In a Production Nursery

At least one native plant nursery has been shipping mycorrhizal plants for over a decade. To make the production process reliable, it was necessary to re-examine almost every aspect of nursery operation. Difficulties were at various times traced to shade conditions, watering methods, fertilizer formulations and application rates, planting media, and water sources. Certain kinds of plants became vulnerable to root disease on a seasonal basis, and were unreliable hosts during those

periods. The smallest containers were found much more troublesome than larger containers. Containers directly on the ground or on weed control fabric were more likely to become mycorrhizal without intentional inoculation. Containers kept in full sunlight, or those that had been in production a year or more, were also more likely to become mycorrhizal on their own. The nursery was surrounded by native ectomycorrhizal hosts, and container-grown plants of those species often became mycorrhizal spontaneously.

A nursery program for mycorrhizal plant production is a major undertaking. The full range of considerations is beyond the scope of this booklet. The grower must be prepared to modify existing procedures, sometimes to the extent of a complete overhaul. The economic and public relations value of doing so will in some cases compensate for the costs, and in other cases will be irrelevant.

Inoculate for Erosion Control

Mycorrhizal fungi bind the soil in ways that the plants alone cannot do. In addition, mycorrhizal fungi promote the formation of soil structure, allowing movement of air and water through the rooting volume rather than across the surface. These have obvious implications for erosion control. In addition, erosion control plants introduced without inoculation may have a difficult time becoming self-reliant. In that case, non-host weeds may quickly claim the site, reducing it to an unsightly, easily eroded condition.

Use of inoculation in erosion control requires a suitable means of introducing both seed and inoculum, and incorporation of a diverse seed mix that includes several good mycorrhizal hosts. The use of fertilizer should be limited to the forms and amounts that approximate the actual needs of the vegetation. Excess fertilization, the rule in erosion control, will discourage formation of the mycorrhizal network and will encourage the growth of weeds.

Inoculate Agricultural Crops

This is a complex topic. The value of inoculation will vary from zero to great, depending on crop and history of the site, among other

things. The value of inoculating must be balanced against both cost and benefits of established or alternative procedures. A foremost consideration is cost; a crop that can only produce \$100 net income per acre can scarcely support an inoculation cost of \$100 per acre.

The clearest application is in the establishment of orchards or vineyards on newly fumigated ground. This is now standard practice in some areas and there have been good results in citrus, grape, stone fruits, and apples. The benefits may be less from improved nutrient uptake than from improved soil microbiology. Mycorrhizal roots encourage greater populations of native bacteria that fight root pathogens, and that otherwise promote plant growth. In addition, improved soil structure may be a worthwhile benefit of inoculation. Done at the planting stage, inoculation is a one-time cost that may be spread over the lifetime of the crop. The usual procedure is to drop some inoculum into the planting hole and backfill so that the roots encounter it as early as possible when they begin to grow.

There is not likely to be much benefit from inoculating an established vineyard or orchard. If mature plants are not mycorrhizal, look to inhibitory conditions to find the cause, not lack of inoculum. Possible inhibitory conditions include excessive fertilization, frequent disking, soil compaction, or the presence of root disease.

A second category of crop that may benefit from inoculation is nursery transplants, whether annual or perennial, especially those that go into fumigated soil. These tend to be high value crops that can support the added expense. Further, inoculation at the nursery stage makes very efficient use of inoculum. Possible candidate crops are strawberry, hops, and melons. Growth responses may be masked by heavy fertilization, especially with phosphorus. A program that involves inoculation should also reduce fertilizer and chemical input. It will require some research to understand the best balance between "natural" and chemical methods for each crop and each set of growing conditions.

Row crops, especially those of relatively low value, will be the lowest priority for inoculation. Unless the soil has been fumigated it is likely that native mycorrhizal fungi will meet the needs of the crop. Even with fumigation, the heavy fertilization typical of modern agriculture

will very likely mask the growth benefits of inoculation.

Any future for mycorrhiza in row crops will depend upon particular problems that can be solved only by a system that involves inoculation. Constraints may at some point be imposed upon use of phosphorus fertilizer, especially near bodies of water. Mycorrhizal inoculation may permit a shift to less mobile forms of phosphorus, including rock phosphate. Refinement of application techniques and inoculum placement may allow dramatic reductions in the amount of inoculum required for rapid root colonization. When those circumstances are combined, inoculation of carrots, onions, cotton, and certain other annual crops may become a reality.

Learn more about mycorrhiza

Further reading

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St. John, Ted, Bob Dixon, and Mick St. John. 1998. Habitat restoration at Discovery Park, Arizona. Land and Water March/April 1998:6-11.

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Resources

Web resources

INVAM: By far the best mycorrhizal web site for fungal identification, you can learn more about AM fungi and fungal taxonomy from this web site than from any available book: <http://Invam.caf.wvu.edu/>

Microbe Zoo: <http://commtechlab.msu.edu/ctlprojects/dlc-me/zoo/>

Mycorrhiza Information Exchange: <http://mycorrhiza.ag.utk.edu/>

MycoInfo: <http://www.mycoinfo.com/>

Practical use of mycorrhiza: www.mycorrhiza.org

Dr. David Sylvia: <http://www.ifas.ufl.edu/~dmsa/>

Examples of experimental results with mycorrhiza from the scientific literature

Growth Response and Other Benefits to Plants

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AB: These forest tree species responded dramatically to AM inoculation, especially *Thuja*.

2. Sylvia, D. M. 1990. Inoculation of native woody plants with vesicular-arbuscular mycorrhizal fungi for phosphate mine soil. Agriculture, Ecosystems, and Environment. 31:252-261.

AB: Height and stem growth of swamp dogwood was double controls in the nursery, but no effect after 18 months if planted into soil with native VAM.

3. Gemma, J. N., R. E. Koske, E. M. Roberts, N. Jackson, and K. De Antonis. 1997. Mycorrhizal fungi improve drought resistance in creeping bentgrass. Journal of Turfgrass Science 73: 15-29.

AB: Creeping bentgrass (*Agrostis palustris* cv. 'Penncross') inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* was able to tolerate drought conditions significantly longer than nonmycorrhizal turf. Protection against drought was conferred by *G. intraradices* when turf was grown under conditions of low phosphorus fertilization (11 mg l-l), but the benefits disappeared when the P concentration of the fertilizer was quadrupled to 44 mg l-l. Mycorrhizal turf maintained significantly higher chlorophyll concentrations (avg. 29% more) than did nonmycorrhizal turf during the 10-day-long drought in the field.

Need for inoculation

1. Bellgard, S. E. 1993. Soil disturbance and infection of *Trifolium repens* roots by vesicular-arbuscular mycorrhizal fungi. Mycorrhiza 3(1):25-29.

AB: Severe experimental disturbance disturbed the external hyphal network and root fragments (containing hyphae and vesicles), which in turn *temporarily reduced the infective potential of the fungus to zero*. An observed delay in the initiation of VAM in the most disturbed blocks can, therefore, be explained by the time required for hyphae to grow from other propagules in the soil which survived the disturbance event.

2. Powell, C. L. 1980. Mycorrhizal infectivity of eroded soils. Soil Biology and Biochemistry 12 (3):247-250.

AB: Mature pasture soils in North Island, New Zealand, had 6-19 mycorrhizal propagules per gram. Of 31 samples of eroded soils, 22 had less than 1 propagule per gram and 13 less than 0.2. In a pot trial mycorrhizal inoculation of white clover increased shoot growth in 7 eroded soils 1-12 fold.

3. Reeves, F. B., D. Wagner, T. Moorman, and J. Kiel. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. I. A comparison of incidence of mycorrhizae in severely disturbed US natural environments. *American Journal of Botany* 66 (1): 6-13.

AB: A comparison of a natural, undisturbed ecosystem, a mid-elevation sage community, with a severely disturbed old roadbed through this community, revealed that more than 99% of the plant cover in the natural community was mycorrhizal (vesicular-arbuscular), whereas less than 1% of the plant cover in the disturbed area (roadbed) was mycorrhizal. Examples of non-mycorrhizal plants as primary successional species in severely disturbed habitats are discussed. *The importance of maintaining or re-establishing the mycorrhizal fungal component in reclamation programs* designed to produce stable ecosystems is emphasized.

Effects on soil

1. Miller, R. M., and J. D. Jastrow. 1992. The role of mycorrhizal fungi in soil conservation. P. 29-43 in: G. J. Bethlenfalvay and R. G. Linderman (eds.). *Mycorrhizae in sustainable agriculture*. American Society of Agronomy Special Publication Number 54. American Society of Agronomy, Madison, WI.

2. Simmons, G. L., and P. E. Pope. 1987. Influence of soil compaction and vesicular-arbuscular mycorrhizae on root growth of yellow poplar and sweet gum seedlings. *Canadian Journal of Forest Research* 17(8):970-975.

AB: Weight, length and fibrosity of sweetgum seedling root systems decreased with increase in bulk density. Inoculated yellow poplar seedlings had greater root weight at each bulk density than uninoculated seedlings, but root length was not influenced by mycorrhizal treatments at higher bulk densities. Fibrosity of yellow poplar roots varied with mycorrhizal treatment at each bulk density. Results indicate that compaction effects may outweigh mycorrhizal benefits for yellow poplar at higher bulk densities. At each bulk density, sweetgum seedlings inoculated with *G. fasciculatum* showed the greatest root growth, suggesting that this fungus *may alleviate the effects of soil compaction* for this tree species.

3. Tisdall, J. M.; Oades, J. M. 1979. Stabilization of soil aggregates by the root systems of ryegrass. *Australian Journal of Soil Research* 17 (3):429-441.

AB: The root system of perennial ryegrass was more efficient than that of white clover in stabilizing aggregates of Lemos loam because the ryegrass supported a larger population of vesicular-arbuscular mycorrhizal hyphae in the soil. Electron micrographs showed that the hyphae were covered with a layer of amorphous material to which clay particles were attached.

4. Sustainable Agriculture: <http://www.ars-grin.gov/ars/PacWest/Corvallis/hcrl/rps003.htm>

Top soil storage and handling

1. Abdul-Kareem, A. W. and S. G. McRae. 1984. The effects on topsoil of long-term

storage in stockpiles. *Plant and Soil* 76 (1/3): 357-363.

AB: A study of 18 topsoil stockpiles of different size, age and soil type has revealed that biological, chemical and physical changes occur, mainly as a result of anaerobic conditions within the heaps, but also as a result of mechanized handling during the stripping and stockpiling. Visible changes occur within 0.3 m of the surface of stockpiles of clayey textured soils, but only below about 2m depth for sandy textures. These visible changes are accompanied by chemical changes, particularly in the forms of nitrogen, manganese and iron present but also in the content of available nutrients, pH and organic matter levels. Biological changes include reductions in potential for mycorrhizal infection, soil biomass and especially earthworm population. The soil atmosphere contains high levels of carbon dioxide, methane, ethane and ethylene. Physical changes include reduction in aggregate stability and resistance to compaction, increase in bulk density and changes in pore size distribution and micro-structure, as revealed by scanning electron microscopy. Limited evidence suggests that many of the adverse effects quickly disappear when the soil is re-spread.

2. Gould, A. B., and A. B. Liberta. 1981. Effects of topsoil storage during surface mining on the viability of vesicular-arbuscular mycorrhiza. *Mycologia* 73(5):914-922.
AB: Population levels of viable VA mycorrhizal inocula were lower in stored topsoil than in undisturbed soil, and decreased with increasing periods of storage. The populations of viable VA mycorrhizal inocula decreased after topsoil storage for 4 years compared with storage for 3 years.

Plant survival

1. St. John, T. V. 1996. Specially-modified land imprinter inoculates soil with mycorrhizal fungi (California). *Restoration and Management Notes* 14:84-85.
AB: The land imprinter was an effective and economical way to add mycorrhizal inoculum on a California restoration site. Mycorrhizal inoculation led to a five-fold increase in plant survival and doubling of plant species richness.
2. Sylvia, D. M. 1986. Effect of vesicular-arbuscular mycorrhizal fungi and phosphorus on the survival and growth of flowering dogwood (*Cornus florida*). *Canadian Journal of Botany* 64 (5): 950-954
AB: Mycorrhizal development and growth of *Cornus florida* seedlings were investigated in a field nursery and greenhouse. After 12 weeks., seedlings inoculated with *G. etunicatum* had greater survival, shoot dry mass and root fresh mass than seedlings inoculated with *G. intraradices* or the control. However, *G. etunicatum* did not affect the concentration or total uptake of P into shoots. This fungus can apparently enhance the survival and growth of dogwood seedlings without improving P nutrition.

Species diversity

1. Doerr, T. B., E. F. Redente, and F. B. Reeves. 1984. Effects of soil disturbance on plant succession and levels of mycorrhizal fungi in a sagebrush-grassland community. *Journal of Range Management* 37(2):135-139.

2. Gange, A. C., V. K. Brown, and L. M. Farmer. 1990. A test of mycorrhizal benefit in an early successional plant community. *New-Phytologist* 115(1):85-91.

AB: The fungicide Rovral (iprodione) was applied in granular form in an attempt to reduce VA mycorrhizal infection of plants during the early stages of secondary plant succession, namely the first year of colonization of bare ground. In 7 out of 11 plant species examined, infection levels were reduced by the fungicide. Four of these also showed reduced cover abundance as a result of fungicide application. Since 3 were annual forbs, which as a plant life-history grouping comprised 73% of the community, total cover of the vegetation (as measured by point quadrats) was significantly reduced by the fungicide. In 3 species, reduced infection levels did not appear to result in reduced vegetation cover. The reasons for this are discussed in relation to the dependency of plants on mycorrhizal infection. It was found that fewer plant species recruited into communities where iprodione was applied. The implications of these results, in terms of the role that mycorrhizas play in the structuring of early successional plant communities, are discussed.

3. Grime, J.P., J. L. M. Mackey, S. H. Hillier, and D. J. Read. 1987. Floristic diversity in a model system using experimental microcosms. *NATURE*; 328(6129):420-421.

AB: Most investigations of floristic diversity have involved studies of natural vegetation. Progress using these approaches has been limited because some potentially important factors are not amenable to precise field measurement or manipulation. Here the authors describe an alternative research strategy in which communities were allowed to develop in turf microcosms providing factorial combinations of soil heterogeneity, grazing and mycorrhizal infection, all of which are capable in theory of promoting diversity. The effect of grazing is shown to be due to the differential sensitivity of the canopy dominant to defoliation.

Resistance to weeds

1. Allen, M. F., E. B. Allen, and C. F. Friese. 1989. Responses of the non-mycotrophic plant *Salsola kali* to invasion by VA mycorrhizal fungi. *New Phytologist* 111:45-49.

AB: *S. kali* reacted to VAM fungi as to a pathogen.

2. Francis, R., and D. J. Read. 1994. The contributions of mycorrhizal fungi to the determination of plant community structure. P. 11-25 in: A. D. Robson, L. K. Abbott, and N. Malajczuk (eds.). *Management of mycorrhizas in agriculture, horticulture, and forestry*. Kluwer Academic Publishers, The Netherlands.

AB: Ruderal species that have been tested are suppressed in undefined ways by proximity of the hyphal network to their root systems (Francis and Read 1992). This suppression is very likely a major component of the resistance to invasion of functional ecosystems.

Pathogen resistance

1. Graham, J. H., and D. S. Egel. 1988. *Phytophthora* root rot development on mycorrhizal and phosphorus-fertilized nonmycorrhizal sweet orange seedlings. *Plant Dis-*

ease 72(7):611-614.

AB: Root dry weight and leaf P content of noninfested VAM sweet orange seedlings were greater than those of nonmycorrhizal plants, which were nearly deficient in phosphorus. *P. parasitica* reduced leaf P status of VAM and non-mycorrhizal seedlings alike but reduced dry weight of only VAM plants. There were significantly fewer rotted root tips on VAM seedlings. *P. parasitica* reduced VAM colonization as a result of the loss of root tips.

2. Linderman, R. G. 1994. Role of VAM fungi in biocontrol. P. 1-26 in: F. L. Pfleger and R. G. Linderman (eds.). Mycorrhizae and plant health. APS Press, St. Paul.

Specifications for inoculation by hydroseeding equipment

Endo (arbuscular) mycorrhizal inoculum shall consist of spores, mycelium, and mycorrhizal root fragments in a solid carrier suitable for handling by hydro-seeding equipment. The carrier shall be the material in which the inoculum was originally produced, and may include organic materials, vermiculite, perlite, calcined clay, or other approved materials consistent with mechanical application and with good plant growth.

Each endomycorrhizal inoculum shall carry a supplier's guarantee of number of propagules per unit weight or volume of bulk material. If more than one fungal species is claimed by the supplier, the label shall include a guarantee for each species of mycorrhizal fungus claimed. Endomycorrhizal fungal species shall be suitable for the pH of the soil at the planting site. If the inoculum consists of a mixture of species, no more than 20% of the claimed propagule count shall consist of fungal species known to be unsuitable for the pH of the soil at the planting site.

Endomycorrhizal inoculum shall be applied at the rate of 8,900,000 live propagules per hectare (3,600,000 live propagules per acre), based on the guarantee of the supplier or the analysis returned by an independent laboratory.

Endomycorrhizal inoculum shall be applied in the same application as the seeds. In no case shall endomycorrhizal inoculum be applied after the seeds. Inoculum must be applied within one hour of addition to the mixing tank. The inoculum shall be applied with hydro-seeding equipment within 60 minutes after the seed has been added to the mixture.

Endomycorrhizal inoculum is a live material. It shall be stored, transported and applied at temperatures of less than 32° C (90° F). If temperatures exceed 32° C (90° F), remaining erosion control applications must be applied within three hours of the application of the inoculum.

A sample of approximately 28 grams (one ounce) of inoculum will be taken from each inoculum container by the Engineer. The number of propagules will be determined by laboratory testing. Propagules shall include live spores, mycelial fragments, and viable mycorrhizal root fragments.

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