

Controlling Root Pathogens with Mycorrhizal Fungi and Beneficial Bacteria,

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Abstract.--Mycorrhizae help plants to acquire water and nutrients from soil, tolerate drought, and ward off root pathogens. These effects are due largely to mycorrhiza-induced physiological changes, including altered root exudation which changes the microbial composition in the mycorrhizosphere soil. Mycorrhizae and their associated rhizobacteria can antagonize root pathogens. Our goal is to find and manage these microbes to effect biological control of the widespread *Fusarium* root rot of conifers.

INTRODUCTION

Most terrestrial plant roots are colonized by soil fungi in a symbiotic association called mycorrhizae. The symbiosis causes physiological changes that profoundly affects the growth and survival of the host plant. For example, mycorrhizae induce changes in membrane permeability that affect root exudation and thus the microbial community in the mycorrhizosphere soil. In this paper we discuss strategies for isolating and characterizing mycorrhizal fungi and associated bacterial antagonists, and for developing the application technology to suppress root rot pathogens.

THE MYCORRHIZAL SYMBIOSIS

Different fungi form different types of mycorrhizae on different host plants. A comparison of anatomical differences between the two major types, ectomycorrhizae and endomycorrhizae, are shown in figure 1. Ectomycorrhizae are formed by higher Basidiomycete or Ascomycete fungi on relatively few families - such as the Pinaceae, Betulaceae, Fagaceae, and Myrtaceae - but those families represent many of the major forest trees. The ectomycorrhizal association is characterized by the formation of a mantle of fungal hyphae surrounding colonized short roots and a Hartig net of hyphae penetrating between root cortical cells. Many ectomycorrhizal fungi form extensive mycelium or rhizomorphs that extend out into the soil, and many of these fungal symbionts can be cultured and inoculum produced. Endomycorrhizal fungi form a symbiotic association with most of the remainder of the

of the plant kingdom forms vesicular-arbuscular mycorrhizae (VAN), characterized by the fungal structures - vesicles and arbuscules - formed in the cortical cells. As with ectomycorrhizae, endomycorrhizal fungi can form extensive extraradical hyphal networks that extend out into the surrounding soil, greatly expanding the nutrient and water absorbing capacity for the host plant.

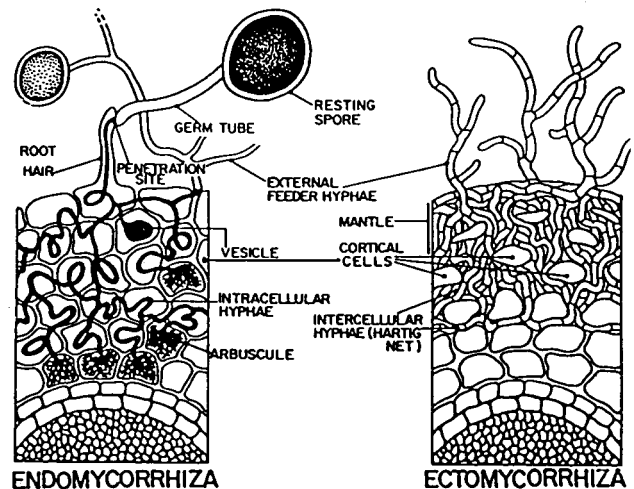


Figure 1.--Anatomical differences between the two major types of mycorrhizae - ectomycorrhizae and endomycorrhizae - shown in cross section.

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The major benefits to the plant of having mycorrhizae, regardless of the type, are (a) enhanced nutrient and water absorption, (b) increased drought tolerance, (c) improved transplantability, and (d) reduced susceptibility to root diseases. While the mechanisms for these effects are not clearly understood, they all appear to involve some morphological or physiological changes that occur when the mycorrhizal association is well established. The net effects of the formation of mycorrhizae may vary, depending on the fungus, host plant, soil, and climatic conditions. In natural ecosystems, plants may

have multiple mycorrhizal associations with different fungi, each of which may have different degrees of influence on the plant's growth and survival.

The physiological changes that occur when mycorrhizae form can be dramatic, and include increased rate of photosynthesis, altered nutritional state, altered phytohormone balance, altered chemical constituents, and altered membrane permeability. The latter change influences the quality and quantity of root exudation, which in turn affects the kinds and numbers of microorganisms that grow in the rhizosphere soil, now more appropriately termed the "mycorrhizosphere" (Linderman, 1988a). In addition, the exudates from extraradical hyphae or rhizomorphs that extend out into the soil from mycorrhizae can influence microbial populations.

Some microbes in the mycorrhizosphere soil can antagonize fungal root pathogens, some can alter the availability of mineral nutrient elements, and others can biologically fix atmospheric nitrogen. Several studies indicate that those microbial functions occur to a greater extent in the mycorrhizosphere soil than in soil from non-mycorrhizal plants (Linderman, 1988a, b). Therefore, in natural ecosystems, the community of microbes associated with mycorrhizae account for many fundamental plant growth and development processes. There are many of us that believe that without these microbial systems, plants probably would not survive because they could not effectively recycle nutrients (in the absence of external amendments and under nutrient deficient conditions), could not tolerate drought cycles, and could not withstand invasion by root pathogens.

NATURAL DISEASE SUPPRESSION

With few exceptions, plants in natural ecosystems have minimal levels of plant disease. There are several reports that document the capacity of undisturbed forest soils to exclude or suppress *Fusarium*, compared to nursery soils (Table 1 and figure 2). Even when plants with known pathogens in or on their tissues are transplanted into the wild, the pathogens disappear within a relatively short period. For example, Smith (1967) planted pine seedlings with high levels of pathogenic *Fusarium oxysporum* on or in their roots out into forest sites. Subsequent isolations from those seedlings documented the pathogen's disappearance within 1.5 years. The exclusion of *Fusarium* from the roots and from littercovered forest soils was documented by Toussoun et al. (1969) and Hammerschlag and Linderman (1975), and attributed to the presence of organic acids in needle leachates that stimulated germination of *Fusarium* chlamydo spores and blocked formation of replacement spores. Later, Schisler and Linderman (1984) demonstrated that *Fusarium* was abundant in forest nurseries, but was absent from adjacent undisturbed forest sites. Furthermore, they showed that the forest soil microbiota could reduce the survival of *Fusarium*, compared to nursery soil.

What microbes in the forest soil ecosystem can adversely affect the survival of *Fusarium*? The first and probably best possibility is mycorrhizal fungi (Sinclair et al., 1975). Zak (1964) and Marx (1972) discussed mechanisms whereby ectomycorrhizae may suppress root pathogens, including (a) production of a fungal mantle as a physical barrier to pathogen ingress, (b) production of antibiotics by the

Table 1.--Summary of reports indicating that coniferous forest soils suppress and exclude *Fusarium*, compared to nursery soils.

Observation	Source
1. <i>Fusarium</i> excluded from roots of infected pine seedlings outplanted into pine forest.	Smith (1967)
2. Needle extracts stimulate germination of <i>Fusarium</i> chlamydo spores without forming replacements.	Toussoun et al. (1969)
3. Decline of <i>Fusarium</i> in test soils correlated with needle suppression of annual plants (from Smith study).	Toussoun (1973)
4. Organic acids in needle leachates stimulate germination of <i>Fusarium</i> chlamydo spores.	Hammerschlag & Linderman (1975)
5. <i>Fusarium</i> absent from forest soils, present in nursery soils; microbes in forest soils reduce <i>Fusarium</i> populations in forest soils.	Schisler & Linderman (1984)

ectomycorrhizal fungus, (c) synthesis of fungistatic, antifungal substances by roots in response to mycorrhiza formation, (d) competition for nutrients needed by the pathogen, and (e) increased populations of antagonistic microflora in the mycorrhizosphere soil. Linderman (1993) described somewhat different mechanisms for VA mycorrhizae, including (a) enhanced nutrition, (b) competition for host photosynthate and infection site, (c) morphological changes in roots and root tissues, (d) changes in chemical constituents of plant tissues, (e) reduction of abiotic stresses, and (f) microbial changes in the mycorrhizosphere. In reality, disease suppression is probably some combination of one or more of these mechanisms. Least studied, but very likely to be involved, is increased populations of antagonists in the mycorrhizosphere soil, at the rhizoplane, or imbedded in the fungal mantle of ectomycorrhizae.

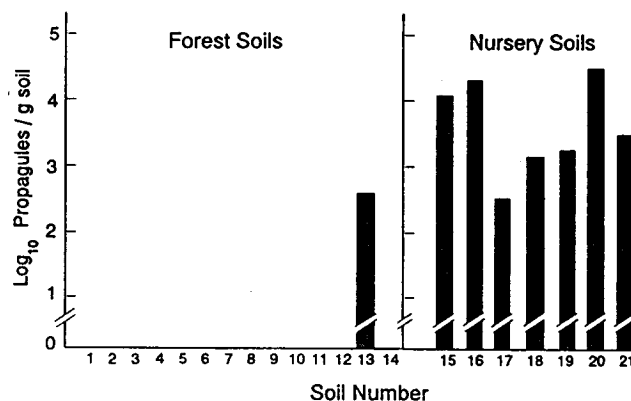


Figure 2.--Relative populations of *Fusarium* isolated from conifer forest soils vs conifer nursery soils (Data from Schisler and Linderman, 1984).

STRATEGIES FOR BIOCONTROL OF FUSARIUM ROOT ROT IN NURSERIES

We are convinced that microbes in natural ecosystems significantly contribute to the suppression of root diseases like *Fusarium* root rot of conifers (Linderman et al. 1983). Cultural practices in forest nurseries may reduce disease incidence and/or severity, but may also reduce the potential beneficial impact of microbes antagonistic to pathogens. For example, soil fumigation, practiced in most western forest nurseries, effectively controls weeds, insects, and root pathogens, but may also reduce populations of antagonists, including mycorrhizal fungi and rhizobacteria or fungi. Mycorrhizal fungi recolonize nursery soils from below the fumigation layer or they are reintroduced by wind or water from adjacent areas (Tanaka et al. 1989), but not in time, within a growing season, to influence the infection of seedlings by *Fusarium* (Bloomberg, 1981) (figure 3). Similarly, antagonistic bacteria or fungi may not reach effective population levels in time to suppress pathogen infections. Both antagonistic bacteria and mycorrhizal fungi may need to be introduced at time of seeding to effectively block *Fusarium* infection which occurs during the first weeks after conifer seed germination. The goal is to find and characterize candidate microbes to biologically suppress initial infections by *Fusarium*.

Finding and Characterizing Candidate Antagonists

We hypothesize that ectomycorrhizal fungi and bacteria antagonistic to fusaria are present in undisturbed forest soils adjacent to forest nurseries, based largely on studies documenting the absence of fusaria from forest soils contrasted to cultivated nursery soils (table 1). Therefore, we believe we can isolate and transfer these microbes from the forest into the nursery. Isolation of ectomycorrhizal fungi from seedlings growing in both the nursery and adjacent forest soils could yield good candidate fungi. Isolation of antagonistic bacteria from the same soils by dilution plating should yield more good candidates from the forest soils than from the nursery soils. Soil extracts are first heated at 50°C for 15 min to eliminate all but spore forming bacteria or actinomycetes which can withstand the rigors of inoculum production and handling by nursery personnel. Candidate bacteria can be identified *in vitro* by spraying



Figure 3.--Mortality from *Fusarium oxysporum* in conifer seedling nursery due to severe root rot. (photo courtesy Everett Hansen)

Fusarium spores over dilution plates from test soils (figure 4). Similar isolations can be made of bacteria cultured from the mantles of ectomycorrhizae from seedlings within the nursery or from forest sites. Bacterial colonies which inhibit the growth of *Fusarium*, as indicated by a zone of inhibition around the colony, can be isolated for further testing. Such tests will confirm our hypothesis that more antagonists occur in the undisturbed forest soils than the cultivated nursery soils. Candidate bacteria should also be tested on different media, including a soil extract medium from the nursery soil, and against other possible fungal pathogens like *Phytophthora*, *Pythium*, *Cylindrocladium*, and *Rhizoctonia*. The best candidates for further testing will be those bacteria that inhibit a wide range of pathogens on several media, especially the soil extract agar made from the nursery soil in test. An additional strategy is to test candidate bacteria for antagonism at low soil temperatures, since those are the conditions under which they must perform in the nursery.

The ectomycorrhizal fungi isolated from forest and nursery seedlings and the best candidate bacterial antagonists will be further tested in short term greenhouse tests. The best of those organisms will be advanced to actual nursery trials.

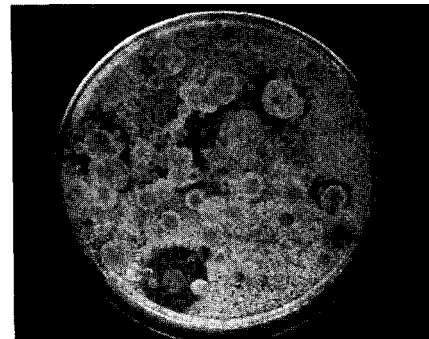


Figure 4.--Soil dilution plate showing bacterial colonies oversprayed with pathogen spores. Note zone of inhibition of pathogen around bacterial colony at lower left of plate. That bacterium can be isolated as a candidate antagonist.

Short Term Greenhouse Tests

Candidate ectomycorrhizal fungi and bacterial antagonists will be tested in greenhouse tests, the specifics of which are to be developed. Mycorrhizal fungal inoculum will be produced in vermiculite culture and introduced into the growth medium. We plan to use each separate forest nursery soil as the growth medium in these tests. Soil was collected from nursery sites where *Fusarium* root rot was evident. By that means, we will know that the soil is infested, without adding more inoculum, at levels high enough to cause disease naturally. We also know that the edaphic factors needed for disease are appropriate. Candidate bacterial antagonists initially will be introduced on the seed at time of sowing. Control seed will be dipped in water. Bacteria that effectively suppress pre-emergent damping off or post-emergent mortality in these tests will be advanced to actual nursery trials. The best ectomycorrhizal fungi, i.e. those that form mycorrhizae and also suppress *Fusarium* disease will also be advanced to nursery trials. Further greenhouse tests will also be run using combinations of the most effective

ectomycorrhizal fungi and bacterial antagonists.

MANAGING THE MYCORRHIZOSPHERE

The concepts presented here key on the facts known from previous studies and observations that root diseases like *Fusarium* root rot of conifer seedlings do not occur in natural forest ecosystems, in part due to the presence of microbes capable of suppressing disease. Our strategies are to isolate ectomycorrhizal fungi and stress-tolerant bacteria or actinomycetes from undisturbed sites adjacent to forest nurseries where soil fumigation and other cultural practices may have reduced populations of the best antagonists. In effect, we hope to transfer natural biological disease suppression systems into the nursery. Each nursery will be unique, so microbes that will be compatible and function in each nursery will be unique to that nursery and may not function in any other. Candidate microbes need to be introduced at the time of seeding in order for them to be well established prior to invasion by *Fusarium*. It is possible that reestablishment of antagonistic microbes can be enhanced by providing part of the natural forest environment, such as forest needle litter or humic substances (Schisler and Linderman, 1989; Linderman, 1989) which could encourage the functioning of antagonists. It would be interesting to evaluate the effects of needle or sawdust amendments, as discussed at this meeting by Bill Krelle from the Magalia Nursery in California, on levels of antagonists or the maintenance of introduced antagonists and ectomycorrhizae. Needle litter is known to be a source of mycorrhizal inoculum (Parke et al. 1983), but it has not been tested for antagonists.

Management of the mycorrhizosphere (Linderman, 1986) is a worthy but difficult endeavor. Nonetheless, with the loss of soil fumigants and effective chemicals to combat root diseases, alternative systems are needed. The key is to find effective biological control systems and introduce microbial agents at the right time and place to block infection by root pathogens. Maintenance of those microbial systems from season to season without fumigation could reconstruct populations of rhizosphere colonists that can biologically control *Fusarium* root rot of conifers without chemicals.

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