Biological interactions in the mycorrhizosphere

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Summary. Microbial communities in the mycorrhizosphere (i.e. in the small volume of soil immediately surrounding a mycorrhizal root) actively interact with the establishment and functioning of the mycorrhizal symbiosis. Examples of competitive and mutualistic interactions are given, and are discussed in the light of recent observations and experimental results. The significance of biological interactions in the mycorrhizosphere is considered from the standpoints of plant ecology and of practical application.

Key words. Rhizosphere; mycorrhizas; symbiosis; soil microbial ecology; biological interactions; bacteria; fungi; antagonism.

Introduction: The concept of the mycorrhizosphere

Soil biologists are familiar with the concept of the 'rhizosphere' [10], defined as the volume of soil in the immediate vicinity of the root which is predominantly affected by the activity of the plant (water and nutrient uptake, exudates, respiration, etc.). The rhizosphere differs from the surrounding soil with regard to most physico-chemical factors: lower pH (due to proton extrusion linked with cation uptake, and to production of organic acids); lower water potential (due to water uptake); lower partial pressure of oxygen and higher partial pressure of carbon dioxide (due to root respiration); and, most of all, higher concentrations of soluble carbohydrates (due to root exudation which can account for up to 30% of the carbon assimilated by the aerial part of the plant). As a consequence of this constant supply of carbohydrate, the number of microorganisms per gram of soil is larger by two or three orders of magnitude in the rhizosphere than in the surrounding soil, and the species found differ because of the different environmental conditions.

Since most actively absorbing rootlets are mycorrhizal under natural conditions, it is useful to extend this concept by defining the 'mycorrhizosphere', which is the rhizosphere of a root infected by a mycorrhizal fungus. As the fungus uses some of the root exudates and modifies root functions, microbial communities in the mycorrhizosphere differ from those in the rhizosphere and in the soil. The specificity of mycorrhizosphere microorganisms has been shown under many conditions [1,22,31,34-36,40,42,43,52].

The aim of the present paper is to analyze the consequences of these facts for the establishment and function of mycorrhizas, and for all the microorganisms more or less closely associated with roots. Firstly, the principal groups of mycorrhizosphere microorganisms will be presented. A second part will deal with some competitive interactions between mycorrhizal fungi and other mycorrhizosphere microorganisms. Then, the mutualistic interactions will be considered, and the mechanisms involved will be discussed. To conclude, the significance of these interactions will be examined from two different standpoints: evolution of plants and ecosystems, and practical applications in agriculture and forestry.

The living community in the mycorrhizosphere

A wide variety of organisms lives in the close vicinity of roots or mycorrhizas, taking advantage of the various organic compounds released by the plant. These organisms include members of most taxonomic groups of aerobic and anaerobic heterotrophic organisms, from bacteria through fungi and protozoa to animals.

To discuss this extremely varied community, it is useful to sort its components into trophic groups. Five main categories are to be considered:

1) *Purely saprophytic organisms.* These organisms are able to use a wide range of complex organic molecules (lignins, proteins, glycoproteins, cellulose and other polysaccharides). They do not differ very much from those living in the soil far away from the roots: they are able to live on dead organic material. However, they are more numerous in the rhizo/mycorrhizosphere, where large amounts of their substrates are produced: dead bodies of more specialized organisms, sloughed root cells, dead rootlets, decaying mycorrhizal fungi, polysaccharides and glycoproteins exudated or secreted by the root or the symbionts, etc. As a consequence of their ability to use complex substrates, these organisms do not depend on the root and do not compete for food to any significant extent with strictly rhizospheric or mycorrhizospheric organisms. However, some of them (like *Trichoderma* sp.) may produce antibiotics and/or quickly occupy the ecological niche of the mycorrhizosphere. This first group includes all the taxons previously cited.

2) *Specialized mycorrhizosphere organisms.* These organisms depend completely on the simple organic molecules (sugars, amino acids, organic acids) released by the plant and its fungal symbionts. They cannot multiply significantly in the non-rhizospheric soil because of the lack of these short-lived substances. As a consequence, they compete strongly with each other as well as with the symbionts and pathogens in their root surface-coloniz-

ing, pre-infection stage. This group includes some fungi, but the most common and the most studied microorganisms in this category are the so-called 'rhizobacteria', such as fluorescent Pseudomonads, Bacilli, *Azospirillum* sp., etc. Some of them are able to fix atmospheric nitrogen and significantly contribute to the maintenance of the fertility of ecosystems; some others are the extensively studied PGPB (Plant Growth Promoting Bacteria), which are used for improving crop yields.

3) *Root pathogens.* These are more or less strictly biotrophic, and penetrate the root tissues. Their relationship to the environment and to other rhizosphere-inhabiting microorganisms is particularly complex, because their life cycle occurs partly outside and partly inside the root. They cause damage to the root tissue, alter its absorbing function, and are detrimental to the plant. During their colonizing stage outside the root, they are in competition with other rhizo/mycorrhizosphere organisms for root exudates. This group includes some bacteria, but mostly fungi such as *Phytium* sp., *Fusarium* sp. and *Rhizoctonia* sp., which are major pathogens in agriculture and forestry.

4) *Root symbionts.* Like those belonging to the previous group, these microorganisms are biotrophic and root-dependent, and complete their life cycle both inside and outside the root. The only difference is that they are beneficial to the plant by providing either fixed atmospheric nitrogen (like the bacteria *Rhizobium* sp. and *Frankia* sp. which form nodules with the roots of some plant families), or by providing water, minerals and growth substances, as mycorrhizal fungi do. The last two trophic groups described (parasites and symbionts), as well as the specialized rhizospheric microorganisms, are dependent on small molecules provided by the plant. Therefore, when in their rhizospheric, pre-infection stage, they compete strongly with the latter.

5) *Predators.* Members of this last group of rhizosphere-inhabiting organisms (protozoa, nematodes, enchytreïds, mites, collembola and other insects) feed on the microorganisms previously cited and reduce their populations. For instance, it has been shown that some amoebae are active consumers of germinating spores of *Rhizopogon luteolus* in the rhizosphere of *Pinus radiata*, reducing mycorrhizal development.

Among the great diversity of interactions existing within and between these groups, we will focus on the well-documented cases where mycorrhizal symbiosis is involved: mycorrhizal fungi vs. rhizobacteria, vs. root pathogens and vs. themselves. Competitive and cooperative interactions will be discussed separately.

Competition in the mycorrhizosphere

Among the numerous competitive interactions in which mycorrhizal fungi are involved, three main types have been more extensively studied because of their practical significance: microorganisms inhibiting infection of roots by mycorrhizal fungi, interactions of mycorrhizal fungi with root pathogens, and competition between different mycorrhizal fungi.

Microorganisms inhibiting infection of roots by mycorrhizal fungi. Many free-living organisms in the rhizosphere are antagonists of mycorrhizal fungi during their growth on the surface of the root, just before the establishment of the symbiosis. It has been shown that these antagonists, when dominant in the rhizosphere community, can severely reduce mycorrhizal formation, and therefore indirectly reduce plant growth[9,23]. Among the organisms which are the most commonly involved are bacteria[4,8,16,17,20], fungi such as *Trichoderma* sp.[51], and even protozoa, nematodes, enchytreïds, mites and collembola feeding on mycelium. Antagonistic bacteria and fungi often produce toxic (antibiotic) substances[9], or are efficient competitors for root exudate, the substrate used by the mycorrhizal fungus before it penetrates the root and becomes biotrophic.

Interactions of mycorrhizal fungi with root pathogens. Root pathogens are a major limiting factor for plant production. Globally, the most widespread among the various crop plants and forest trees are fungi belonging to the genera *Phytophthora, Fusarium, Phytium* and *Rhizoctonia.* They kill the roots or reduce their ability to absorb water and nutrients by penetrating root tissues and producing toxins. They share the mode of nutrition (biotrophy) and the habitat with mycorrhizal fungi. Thus, the two categories of fungi strongly compete with each other.

When both organisms are free-living in the rhizosphere, before penetrating the root, pathogens often have a competitive advantage because of their saprophytic ability: many of them are less strictly biotrophic than mycorrhizal fungi[41]. On the other hand, most mycorrhizal fungi produce antibiotics, compensating for their poor saprophytic ability[28].

The situation is different when the mycorrhizal symbiosis is already established. The fungus does not have to compete for a substrate and is in a better position to use its antibiotic weapons. In the case of ectomycorrhizas, the fungal mantle even forms a physical barrier against penetration by pathogens. In this respect, it has been clearly demonstrated that dense, continuous mantles are more protective than loose and incomplete ones[28].

As a consequence, mycorrhizal symbiosis generally protects the plant against root pathogens, but this protection depends on the mycorrhizal fungus, on the pathogen species, and on the priority. For instance, young plantlets of *Chamaecyparis lawsoniana* are efficiently protected against *Phytophthora cinnamomi* only when VA endomycorrhizal formation takes place *before* inoculation with the pathogen[6]. In the same way, plants of citrus are protected against *P. cinnamomi* root rot when the soil is previously inoculated with the VAM fungus *Glomus fas-*

ciculatum[11]. With regard to ectomycorrhizas, *Pinus taeda* is protected against *P. cinnamomi* by a range of ectomycorrhizal fungi, but some of them (like *Pisolithus tinctorius*) only act as a barrier when the mycorrhizal mantle has been formed, while others (like *Laccaria laccata, Suillus luteus* and *Leucopaxillus cerealis*) display a preventive, long-range action possibly due to antibiotics. Indeed, in the case of *L. cerealis*, two antibiotic substances have been identified: diatretyne 3 and diatretyne nitrile[28].

Interestingly, it has been shown that Douglas fir roots are protected against *Fusarium oxysporum* by a free-living antagonist microflora which is stimulated by the presence of the ectomycorrhizal fungus *Laccaria laccata*, even before the formation of any mycorrhiza[47].

Another indirect protection mechanism has often been discussed as well: the enhancement of the natural resistance of the plant to the invading pathogen by mycorrhizal association. In the case of ectomycorrhizas, the establishment of the symbiosis dramatically increases the root content of polyphenols or terpenes[28] (in conifers). Most of these substances inhibit the growth of pathogens. With VA endomycorrhizas of legumes such as soybean, mycorrhizal development enhances the production of isoflavonoids, which play a major role in the natural defence of the plant[33].

In summary, mycorrhizal symbiosis appears to protect the roots against fungal diseases, by one or more of the various mechanisms previously described. In field experiments where mycorrhizal inoculation is involved, a large part of the recorded growth stimulation may be due to this protection, besides the improvement of mineral uptake or the hormonal effect[38,39]. The ability of a mycorrhiza-forming fungus to protect a crop against root pathogens should be a major criterion when selecting strains for controlled mycorrhization.

Competition between mycorrhizal fungi. In nature, a single plant is generally associated with several species of mycorrhizal fungi. The number of fungal partners is particularly high in the case of large, perennial plants such as trees, and more particularly in trees with ectomycorrhizas[53,54]. Many factors determine the structure of these mycorrhizal populations: micro-heterogeneity of the soil, age and activity of the roots, etc. However, a key factor is the differential ability of the different fungi to infect the roots under a given range of conditions and in the presence of other symbionts. New roots are continuously formed, and the different fungi compete with each other to infect them[20].

Most information about these competitive interactions is purely descriptive, and little is known about the mechanisms involved[12,20]. However, it is clear that the dispersal strategy of each fungal species plays a major role: for example, the size and survival of propagules[5], the growth rate in the rhizosphere, the ability to form far-reaching mycelial strands to infect new roots from a preexisting mycorrhiza, etc. Chemical interactions through antibiotic substances are also probably involved, as in the case of competition between symbionts and pathogens previously described. An interesting model for studying competitive interaction between mycorrhizal fungi is provided by trees which have both vesicular-arbuscular mycorrhizas and ectomycorrhizas, such as alders, poplars, and eucalypts. The temporal and spatial distribution of these two clearly distinct types of symbiotic associations can be monitored particularly easily[7].

Mutualistic interactions in the mycorrhizosphere

It is now well established that the rhizosphere microflora is often favorable to mycorrhizal development and stability. Beneficial effects of microorganisms have been found in a range of conditions with VAM[32,37] or ectomycorrhizal fungi[16-18,21], the 'helper' organisms being mostly bacteria[32,37] (including some actinomycetes), but also fungi (including some yeasts[16]). There are many possible mechanisms for this stimulation, and there is experimental support for most of them, as discussed below.

Direct trophic stimulation. The helper microorganisms may produce a substrate used by the mycorrhizal fungus. For instance, the growth of *Hebeloma crustuliniforme* in its free-living form is stimulated by citric acid produced in large quantities by some bacteria in the mycorrhizosphere[14]. It has also been reported that the germination of spores of VAM fungi is enhanced by volatile substances produced by actinomycetes[2,3]. Thus, this direct trophic effect may involve large amounts of a carbon source (such as organic acids) as well as low quantities of growth factors (such as the volatiles in the latter example). As rhizosphere microorganisms are known to produce large quantities of a wide range of vitamins and other growth factors[48-50], it is highly probable that some of these substances can be used by the mycorrhizal fungi.

Free-living, nitrogen-fixing bacteria in the rhizosphere may also favor mycorrhizal development, contributing ammonium and amino acids to the fungus[26].

Detoxification in the rhizosphere. Like most microorganisms, mycorrhizal fungi accumulate secondary metabolites in their growth medium (polyphenolic compounds and other substances), which are toxic to the fungus itself and limit its growth[14,24]. Some rhizospheric bacteria are able to break down these molecules, and are thus favorable to the development of the fungus[4]. For instance, in axenic in vitro culture, some fluorescent pseudomonads detoxify the dark polyphenolic compounds produced by *Paxillus involutus*, and stimulate its growth[14]. However, care should be taken when extrapolating such results to soil conditions where adsorbing colloids may completely change these interactions.

Modification of root exudates. A given microorganism could alter exudates by stimulating the root to produce more or different substances, or by metabolizing part of the exudate. This possible mechanism has not yet been documented.

Increase in the susceptibility of the root to penetration by the mycorrhizal fungus. It has been shown that infection of *Trifolium parviflorum* by a VA mycorrhizal fungus was stimulated by a strain of *Pseudomonas* sp. which released cellulolytic and pectinolytic enzymes, making it easier to separate the outer cells of the root cortex[4]. Phytohormones could also be involved; they are produced by a wide range of rhizosphere microorganisms, and control the morphology of rootlets[49, 50].

Interaction with the host fungus recognition mechanisms. The early events of recognition between a plant and a symbiotic fungus, leading eventually to mycorrhizal symbiosis, are being actively studied at present (Martin and Hilbert, this review). In analogy to plant pathogen interactions, the cell walls of the two organisms are believed to be the sites where signals are emitted and received, leading to changes in gene expression in both partners. Other rhizosphere organisms could obviously interact positively or negatively with these recognition mechanisms. To date, no results have been published in this field.

'Four-organism' relationship. In the examples above, we have only considered the plant, the mycorrhizal fungus, and a 'helper' microorganism. But it should not be forgotten that a very complex community lives around them. Therefore, contributions by a fourth partner may occur also. For instance, if microorganism A is an antagonist of the mycorrhizal fungus, and microorganism B an antagonist of A, B will act as a 'helper' of mycorrhizal development. This case is probably frequent, but out of reach of simple experiments.

A better understanding of all these mechanisms is necessary for a rational management of the soil microflora in agriculture. A more basic question is whether or not helper organisms obtain any benefit from stimulating mycorrhizal development. If so, helpers should be found mainly in the immediate vicinity of the fungus *in its functional symbiosis*, i.e. near mycorrhizas[15], and it should be possible to demonstrate a mutual benefit experimentally. To date, the small number of experiments undertaken in this direction have not led to clear conclusions[16, 40]. The matter remains open.

Ecological significance of biological interactions in the mycorrhizosphere

The previous examples have clearly shown that the ecology of the whole plant cannot be completely described if rhizosphere communities are not considered. The main fact is that most of the water and mineral uptake by the roots is controlled by fungal symbionts, but these mycorrhizal fungi themselves are constantly interacting with other microorganisms in the rhizosphere. In turn, those microbial communities are dependent on the plant through its symbionts.

From the standpoint of scientific investigation, this means that results obtained with plants growing in simple, axenic laboratory conditions, even if they are artificially associated with some mycorrhizal fungi, are of little relevance to the real situation in the field because of the lack of accompanying microbial communities. Experiments under such simple conditions are necessary for understanding basic physiological mechanisms, but their results should not be too hastily extrapolated to natural ecosystems. The challenge for modern plant biologists is to deal with this complexity and analyze the respective importance of the different components of the rhizosphere community.

For instance, a key factor determining whether a plant can survive and grow in a nutrient-deficient soil is its ability to mobilize some elements, such as phosphorus, from insoluble minerals or organic molecules. In this respect, mycorrhizal fungi are not necessarily more efficient than the root itself, and it has previously been shown that mycorrhizosphere bacteria play a major role in mobilizing elements for the benefit and the plant/fungus association[25]. In this regard, questions such as the following arise. Are these bacteria necessary for the plant to colonize poor sites? Did they evolve together with the plant? To what extent are they closely associated and specific? The answers could cast new light on plant distribution and succession, and more generally on the ecology of terrestrial ecosystems.

Another interesting aspect is the accumulating evidence, discussed above, that some rhizosphere microorganisms actually *help* symbiotic fungi to establish a symbiotic association with the root, even if the mechanisms involved have not been fully elucidated. Together with antagonist organisms, these 'helpers' contribute to the regulation of mycorrhizal development, and thus to the physiology and adaptability of the plant. One may wonder how this co-operation developed and ask the question whether the microbial community or the physicochemical environment of the roots have a more decisive influence on the distribution of plants.

These two examples show that the relationships between plants and their soil environment involve a living interface, the mycorrhizosphere, which should be considered as an integral part of the root, determining most of its functions.

Practical consequences of biological interactions in the mycorrhizosphere

Many research programs and much commercial development are presently aimed at controlling mycorrhizal symbioses in order to improve plant growth, both with ectomycorrhizas for forest plantation and with VA mycorrhizas for farming, horticulture and forestry. Controlled mycorrhization is a complement, or even an alternative, to traditional techniques such as soil tilling, mineral fertilization, pesticide use, etc. Its main advantage is that it does not involve any extra input of energy or raw material in agricultural systems. Controlled mycorrhization involves selection of highly-performing symbiotic fungi, aseptic culture to produce pure inocula, inoculation of the soil/plant system, and management of the environment in order to optimize mycorrhiza formation by the introduced fungus[29].

As we have seen previously, the microbial community of the rhizosphere is a key factor in the development, the stability and the efficiency of mycorrhizas. But biological interactions in the mycorrhizosphere have only recently been considered from a practical standpoint.

A common practice associated with controlled mycorrhization is disinfection of the soil prior to inoculation in order to get rid of native symbionts which could interfere with the introduced one[19,44,45]. Disinfection is obtained using heat (steam) or various fumigants such as methyl bromide. The side effects of these treatments are complex and rather unpredictable on a range of factors: soil type, native populations, contaminants (air-borne or water-borne microorganisms), climate, disinfection technique, etc. More particularly, disinfection treatments differentially kill different groups of microorganisms which may have different effects on mycorrhizal development. As the surviving ones and the contaminants quickly occupy the vacant niches, the result is a simpler and less balanced community[44]. If antagonist organisms, instead of helpers, take over, mycorrhizal inoculation fails. Comparative results show that total disinfection (i.e. by hot steam or methyl bromide) is not always favorable to mycorrhizal infection[19,44].

Therefore, alternative, milder and more specific treatments, selectively killing indigenous mycorrhizal fungi and root pathogens but conserving the accompanying bacterial populations, should be developed.

Another important factor regulating microbial communities in the mycorrhizosphere is the water potential in the soil. It has been shown that ectomycorrhizal development by the truffle fungus (*Tuber melanosporum*) is indirectly reduced by excessive irrigation: high moisture in the rhizosphere induces a rapid development of siderophore-producing rhizobacteria (flourescent pseudomonads) which strongly compete for iron with the fungus and reduce its activity[27,35].

These two examples show that artificially introducing a mycorrhizal fungus is just a part of controlled mycorrhization; managing the microflora of the mycorrhizosphere should be the next step.

However, the recent results on helper organisms suggest another way: introducing pure cultures of selected helpers at the same time as the fungus, taking further advantage of soil disinfection. To date, only a small number of field trials have been performed with this technique, but encouraging results have been obtained in a forest nursery with Douglas fir (*Pseudotsuga menziesii*) inoculated with *Laccaria laccata* and bacteria isolated from the mantle of *L. laccata* ectomycorrhizas. Some bacterial isolates provided a 50% increase of the mycorrhizal development[18]. It is probable that, in the near future, commercial mycorrhizal inoculum will contain associate helper bacteria, improving the efficiency of inoculation in a wider range of conditions and reducing the quantity of inoculum needed.

In addition, taking advantage of the protective effect of mycorrhizal symbiosis against root pathogens[41] is one of the most promising consequences of our recent knowledge on biological interactions in the mycorrhizosphere.

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