

Opportunities and Challenges for the use of Microbial Inoculants in Agricultural Practice

J. Zarb¹, C. Leifert² and A. Litterick²

¹*Specialist consultant in sustainable farming and plant/microbe interactions, 17 High Street, Helmsley, York, YO62 5AE*

²*Aberdeen University Centre for Organic Agriculture, MacRobert Building, King Street, Aberdeen, AB24 5UA*

Introduction

Microorganisms are ubiquitous in the soil where they play a vital role in nutrient cycling, and where they mediate various processes through their interactions with plants and other soil organisms. The aim of this paper is to provide an overview of some of these interactions, and to demonstrate ways in which they might be managed, agronomically or otherwise, for the benefit of farmers in a sustainable agriculture.

The importance of microorganisms (fungi, bacteria, actinomycetes, and yeasts) can hardly be overstated, yet their presence and activities are often disregarded in conventional agricultural systems, which rely heavily on non-sustainable inputs of energy, fertilizers, and pesticide. The legume-rhizobium symbiosis, the mycorrhizal symbiosis, and the numerous other relationships between plants and microorganisms, have evolved since plants first appeared on land. They are naturally occurring phenomena and, in the case of mycorrhizas, they are the rule rather than the exception. Clearly it makes sense to utilize these resources, particularly through direct agronomic management, which would be of the greatest benefit to farmers. This can only be fully realizable through improving our present understanding of agro ecosystems.

Mycorrhizal Fungi in Agriculture

Mycorrhizal fungi have received considerable interest from researchers, particularly for the past 40 years or so when their role as modulators of plant growth began to be universally recognized. It is only more recently that their interactions with other soil organisms have been studied in more detail. Difficulties in the commercial scale culture of some mycorrhizal fungi have limited their use in microbial inoculant products, yet they remain extremely promising candidates for this approach.

Vesicular arbuscular mycorrhizas (VAM) and ectomycorrhizas (EM) are associated with about 95% of plant species world-wide. In fact, VAM form the largest component of all fungal material in the soil. Mycorrhizal fungi form mutualistic symbioses with the roots of host plants, which are beneficial to the plant. The complex infection procedure culminates in the formation of the mycorrhiza: an entity with distinct structure and function. Mycorrhizal hyphae extending from the roots of the host plant enhance the ability of the root to take up water and minerals. This contributes to the frequent observation of improved plant nutrition and drought tolerance of mycorrhizal plants.

Most soil microbial activity occurs in the region directly adjacent to the plant root – the area termed the rhizosphere. Here root exudates provide a substrate for microbial life. Mycorrhizal hyphae are present in the plant roots and extend

through the rhizosphere and into the bulk soil. In each of these zones, mycorrhizal fungi interact with plant roots, other microorganisms, and soil constituents at molecular, chemical, and physical levels. These interactions have important consequences for plants; both through the direct effects of mycorrhizal fungi on plant nutrition, and through indirect effects via other soil microbes and plant pathogens. Mycorrhizal fungi, together with other organisms, also profoundly affect soil formation, aggregation and fertility.

Practical Benefits of Mycorrhizal Fungi in Agriculture

Mycorrhizal plants show increased growth (Thompson et al., 1994; Bloss & Pfieffer, 1984), and are generally more tolerant of adverse conditions such as drought (Parke et al., 1983), soil pathogens (Cooper & Grandison, 1986; Dehne, 1982; Duponnois & Ba, 1998), transplantation (Haas et al., 1986; Scagel, 1998), poor soil nutrient status (Smith, 1988), and soil pollution (Leyval et al., 1997), compared to non-mycorrhizal controls.

Improved plant growth and increased tolerance to adverse conditions can often be attributed to enhanced water and nutrient acquisition made possible by the extensive hyphal network which effectively increases the absorptive area of the root. However, the effectiveness of mycorrhizal fungi in increasing plant growth is not always directly related to the extent of root colonization or hyphal growth. In *Eucalyptus globulus*, plant dry weights were positively correlated with the length of mycorrhiza-colonised root for some EM species. In others, plant growth responses to EM inoculation could not be related to hyphal development (Thompson et al., 1994). In other cases, the benefits of EM inoculation are more clear-cut and this approach has been used to aid the establishment and growth of young transplants in horticulture and forestry e.g. *Eucalyptus tereticornis* (Reddy & Satyanarayana, 1998), *Acacia tortilis* (Munro et al., 1999), Pinus species (Scagel & Linderman, 1998).

Interestingly, many of these beneficial effects are associated with a range of other phenomena such as mycorrhizal IAA and ethylene production, and mycorrhiza-mediated plant disease suppression (Morin et al., 1999; Edwards et al., 1999). Black spruce (*Picea mariana*), for example, is susceptible to the root rot fungus *Cylindrocodium floridanum*. When tree seedlings were inoculated with the EM fungi *Paxillus involutus* and *Hebeloma cylindrosporum*, 50% of seedlings remained unaffected by root rot (Morin et al., 1999).

Mycorrhizal Fungi and Phosphorous Nutrition

A primary effect of mycorrhizal symbiosis is improved P nutrition made possible by the extensive hyphal network. This not only allows the plant to overcome the P depletion zone around the root but also allows it to reach immobile P that the fungus can solubilise. This phenomenon is most apparent in low P soils. P can substitute the effects of mycorrhizal infection on plant survival in non-mycorrhizal controls in many cases. However, with increasing soil P, the benefits of mycorrhizal infection decline and mycorrhizal infection is reduced. In general, the benefits of mycorrhizas are lost to plants that have other means of obtaining P from the soil. The use of fertilizers in conventional farming generally ignores the activity of mycorrhizal fungi. This could have important long-term consequences for crop production.

Mycorrhizal Fungi Can Activate Plant Defence Mechanisms

Studies of the interaction between mycorrhizal fungi and sedentary parasitic nematodes have provided evidence that resistance to soil pathogens can be related to factors other than improved plant nutrition. Investigations of the interactions between root knot nematode (*Meloidogyne hapla*) and VAM fungi on susceptible cultivars of tomato and white clover revealed that P nutrition was negatively correlated with nematode numbers in mycorrhizal roots (Cooper & Grandison, 1986). Furthermore, nematode numbers per gram of root were consistently less in mycorrhizal soils, and plants pre-infected with mycorrhizal fungi showed improved growth compared to uninoculated controls.

The Activation of Plant Defence Mechanisms Involves Range of Microorganisms

It is known that mycorrhizal infection can stimulate induced resistance against soil-borne pathogens at the site of infection. More recent work which examined interactions between *M. javanica* and a range of microorganisms including mycorrhizal fungi and the actinomycete *Pasteuria penetrans*, suggested that a number of soil organisms modulate the activity of soil pathogens, and together contribute to the phenomenon of the suppressive soil (Duponnois & Ba, 1998).

In this particular study, two similar soils from adjacent vegetable plots were examined. Whereas both were infested with *M. javanica*, one contained *P. penetrans*, an obligate parasite of the nematode. *P. penetrans* was also associated in the soil with fluorescent pseudomonads and nematophagous and mycorrhizal fungi whose presence stimulated the attachment of *Pasteuria* spores to juvenile nematodes.

Tomato and aubergine showed significantly improved growth in soils containing natural populations of *P. penetrans*. Populations of root knot nematodes were much reduced in these soils. Finally, non-autoclaved powdered root preparations containing *P. penetrans* suppressed *M. javanica* in autoclaved soil containing *P. penetrans*, but not in soils lacking the actinomycete. This suggests strongly that *P. penetrans*, and the microorganisms associated with it, act together in antagonism towards *M. javanica*.

Although mycorrhizal fungi and other soil microorganisms are known to confer resistance, tolerance, or other forms of bioprotection to host plants, the actual mechanisms involved are not clear. The work briefly reviewed above suggests that microbial products may be important, but many other mechanisms have also been postulated for the microbial control of plant pathogens. Condier et al. (1998) demonstrated that the VAM fungus *Glomus mosseae* stimulated localized and induced systemic resistance to *Phytophthora parasitica* in tomato using mycorrhizal and non-mycorrhizal roots in a split root experimental system. In this scheme, decreased pathogen development in mycorrhizal and non-mycorrhizal parts of mycorrhizal root systems was associated with the accumulation of phenolic compounds, and with typical plant cell defense responses. Mycorrhizal cortical cells were immune to *P. parasitica* and showed callus development at sites of parasite infection. The systemic component of resistance was characterized by host cell wall thickenings of pectins and proteins in non-mycorrhizal root parts, as well as callus deposition at infection sites. None of these observations were apparent in non-mycorrhizal pathogen-infected root systems.

A protein molecule associated with *Phytophthora* has been identified as a possible elicitor of mycorrhizal-induced activation of plant defence mechanisms since this

molecule is associated with the pathogen only in mycorrhizal tissues. These findings create a further avenue for the development of microbial inoculant technology in plant bioprotection.

Crude extracts of the growth medium of the AM fungus *Glomus intraradices* have been demonstrated to reduce the incidence and severity of the fungal pathogen *Fusarium oxysporum* in carrot whilst, at the same time, stimulating the growth of rhizosphere bacteria (*Pseudomonas chlororaphis* and *Clavibacter michiganensis*) (Filion et al., 1999). Observations of this type in which pathogens are suppressed whilst non-pathogens are stimulated in various experimental models have been reviewed in detail (Azon-Aguillar & Barea, 1996). Data such as these illustrate the complexity of the interactions that underlie the application of essential microorganisms in agriculture and emphasize the need to adopt a holistic, multidisciplinary, approach to the study of plant-soil microbiology.

Having established that actinomycetes, fluorescent pseudomonads, other bacteria, mycorrhizal fungi, and extracts of these, all contribute in various ways to disease control in agricultural ecosystems, their influence on other vital soil-plant processes shall now be examined.

Plant Growth Promoting Rhizobacteria

Among the various antagonists of fungal pathogens are the rhizobacteria, including *Pseudomonas* and *Bacillus* sp. Of these, a number are collectively known as plant growth promoting rhizobacteria (PGPR). They are able to stimulate plant growth in several ways, and they are promising species for use as soil and compost inoculants. Their role in biological control is largely through their ability to colonize the rhizosphere and suppress potential pathogens by competitive exclusion. PGPR are also associated with the production of antibiotics, hormones, siderophores, and HCN, as well as with molecular mechanisms, which can stimulate induced systemic resistance in plants. It is interesting to note that these mechanisms do not affect the mycorrhizal fungi with which PGPR are associated (e.g. Edwards et al., 1999).

As well as having effects on plant pathogens that lead to enhanced plant performance, PGPR can influence plants directly, or in association with other soil microorganisms. Some PGPR can directly promote root and shoot growth, and as nodulation promoting rhizobacteria (NPR) stimulate nodule formation in legumes by *Rhizobium*. Mycorrhiza helper bacteria (MHB) have also been identified (Fitter & Garbaye, 1994). These interact with mycorrhizal fungi to aid mycorrhizal infection. Indeed, these organisms appear to co-operate in several ways, which include aiding their mutual establishment in the rhizosphere; improved plant rooting, growth and nutrition; biological control of root pathogens and improved legume nodulation.

Soil Microbial Associations are Organized

Notwithstanding the difficulties involved in culturing microorganisms for commercial application in agriculture, a major obstacle to the production of effective microbial products is insufficient understanding of the mechanisms involved in bioprotection and plant stimulation. One important matter in this respect is the need to identify the most effective plant-mycorrhiza, and microbial associations. Microbial interactions in the soil do not occur at random but in a dynamic order, the nature of which may depend on compatible species being present (Andrade et al., 1998).

In examining this hypothesis, the soil bacteria *Alcaligenes eutrophus* and *Arthrobacter globiformis* were found to differ significantly in their preference for AM fungi associated with sorghum. *A. eutrophus* was shown to depend on the presence of *G. mosseae* for survival rather than on the plant root, whereas *A. globiformis* persisted equally well in both mycorrhizal and non-mycorrhizal soils. This example serves to show that understanding microbe-plant- microbe interactions in the soil will be fundamental for the management of sustainable agroecosystems involving intentional manipulation of the soil biota. Again, a holistic approach to research will be essential.

Application of Microorganisms in Soil Stability and Bioremediation The most fundamental and essential component of farming systems is the soil itself: that waterlogged, compacted, desiccated, salinised, wind- and rain-eroded, and generally abused habitat, which is in fact one of our most precious resources. It is not only home to the microorganisms with which we are presently concerned, but is constantly modified and maintained by their activities.

Soils consist of particles of sand, silt, and clay in varying proportions, held together into aggregates of various sizes by organic and inorganic materials. The structure of the soil profoundly affects the infiltration, drainage, and storage of water; the activity of the soil biota; crop production; and the stability of the soil to erosion. Root and microbial exudates, as well as various derivatives of organic matter decomposition, are essential in binding micro-aggregates to maintain a porous soil structure, although the extent to which individual species contribute to this process is not clear (Tisdal, 1994).

The activities of the soil organisms, in turn, depend much on the soil in which they occur, or to which they might be introduced, and, as already suggested, soil organisms influence one another in various ways. Studies of the possible interrelationships between VAM fungi, associated bacteria, actinomycetes, and fungi, and the stability of soil aggregates have suggested that mycorrhiza-mediated improvements in soil aggregation can lead to increased numbers of other microorganisms known to positively influence plant growth (Andrade et al., 1998). The fact that greater numbers of soil microorganisms were apparent in aggregated soils suggests that the creation of favorable growth conditions should be a prerequisite for introducing microorganisms to the soil.

Further evidence that VAM fungi contribute to the formation of favorable soil conditions comes from work on pot-grown soybean (*Glycine max*) in natural soil inoculated with *Bradyrhizobium japonicum*. The formation of water-stable soil aggregates was positively correlated with root and VAM mycelium development, irrespective of N source (nitrate or ammonia) (Bethlenfalvay et al., 1999). However, actinomycetes known to promote water stable aggregate formation, declined with increasing pH. Soil acidification is thus an important factor in soil aggregation and stabilization, and this in turn could be influenced by agronomic and industrial practices.

Potential for the Management of Microorganisms in Agriculture Agronomic practices have a profound effect on soil organisms. Intervention with essential microorganism inoculants should not be regarded as a single solution to the problems caused by damaging agricultural operations. Firstly, agricultural management practices should at least be designed to minimize undesirable impacts on the soil environment. At best they should be designed to work in harmony with biological processes in order to support sustainable agricultural systems.

Plant Breeding The indirect management of VAM, EM, and other microorganisms is possible through plant breeding. Rather than traditional selection for desirable agronomic traits, selection should aim for efficient mycorrhizal associates, which would contribute to improved agroecosystem stability.

Inoculation Reports on the successful inoculation of trees with EM fungi in temperate and tropical situations are numerous. The following have shown improved growth and resistance to pathogens following EM inoculation: Black spruce (*Picea mariana*)/*Paxillus involutus* (Morin et al., 1999); *Eucalyptus tereticornis*/*Pisolithus tinctorius* (Reddy & Satyanarayana, 1998); Douglas fir/*Laccaria bicolor*/*Pseudomonas fluorescens* (Frey-Klett et al., 1999).

VAM inoculants from natural soils have also been used to achieve improved plant establishment and growth: Indian leguminous trees / VAM / Rhizobium (Rao & Tarafda, 1998); guinea grass-coconut intercropping system / VAM / Azotobacter / Azospirillum (George et al., 1999); Oil palm/VAM (Moawad & Vlek, 1998).

Whereas EM fungi can be cultured axenically on simple media, VAM are obligate symbionts and are not amenable to culture in the same way. However, inoculants of VAM have been produced under experimental conditions from powdered mycorrhizal roots (e.g. Duponnois & Ba, 1998). The economic cost of such a procedure will preclude its rapid commercial application but there remain promising reports of progress towards achieving this aim. Munro et al. (1999) describe a successful low-cost method of inoculating *Acacia tortilis* with a range of native EM fungi and rhizobia, which significantly enhanced nodulation, and growth of tree seedlings. Techniques such as these could be further developed for the benefit of farmers by maintaining healthy agro ecosystems from which soil-based inoculants of native microorganisms could be derived. Developments in these areas will be significant in the rehabilitation of degraded soils and should benefit farmers, particularly in the Southern Hemisphere where tree establishment and growth are limited by nutrient-poor soils.

Microbial Inoculants in Composting And Bio fertilisers Now that we are becoming more aware of the need to recycle materials that were, until recently, regarded as waste products, techniques employing a range of microorganisms are being applied to enhance the breakdown of various products in order to produce composts. Fish processing waste, animal manures, and hops and sugar beet waste are all important candidates for composting. Sugar beet waste treated by *Aspergillus niger* fermentation was reported to improve the growth of Lucerne (alfalfa), with the greatest improvements (233%-343%) occurring when a VAM fungus was included in the treated medium (Rodriguez et al., 1999). With regard to biofertilisers, Azospirillum, Azotobacter, Azolla, P-solubilising microorganisms, and mycorrhizal fungi have successfully been used experimentally in tropical cropping systems in cereals (Hegde et al, 1999; and in guinea grass (George et al., 1999).

Conclusions Such promising reports of the use of a natural resource, namely soil microorganisms, in agriculture warrant further research into our understanding of how soil microorganisms function in agro ecosystems. Pilot and small-scale field projects which involve farmers directly, and which employ or create local resources, is more likely to bring practical benefits to farmers than global corporate operations.

What essential microorganisms should not do for farmers is to create a vicious circle of technological dependence.

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