

## USING ARBUSCULAR MYCORRHIZAL FUNGI TO IMPROVE INPUT USE EFFICIENCY

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### ABSTRACT

Biotechnology is expected to bring about a second Green Revolution in which more food is produced with fewer inputs and in a sustainable manner. Arbuscular mycorrhizal (AM) fungi possess important attributes to be major players in tomorrow's agriculture. They have evolved as sun-powered resource managers in successful ecosystems. Advances in mycorrhizal research are revealing AM fungi as a heterogeneous group of soil fungi with requirements of their own, a picture contrasting with our initial understanding of these organisms. Their use in agricultural production can be enhanced through plant breeding, soil fertility management, inoculation and use of signal molecules.

### INTRODUCTION

There are 6.52 billion people on Earth, a population that is growing at a rate of 6 million per month (US Census Bureau, 2006). Population growth increases food demand and pressure on agricultural lands. Farmers in many countries do not have the resources to practice high input agriculture and in countries where it is practiced, effluents are threatening environmental quality. After decades of fertilization, in rich countries, soil P levels have increased sometimes to reach threatening levels (Fixen, 2006). We need to produce more food with fewer inputs. The efficiency of nutrient utilization by crop plants can be enhanced using arbuscular mycorrhizal (AM) fungi.

Some 45 years have gone by since the beginning of experimental research on AM fungi, but applications derived from this research are still largely limited to the inoculation of plants, an approach that can be highly profitable for transplanted crops, but that has limitations in directly seeded fields. Here, I present three key features to consider in the development of AM applications: the nature of AM fungi, their dependence on the soil environment, and the functional diversity existing among this group. I will also present some recent results on the assessment of the mycorrhizal AM potential of soils.

### MATERIALS AND METHODS

The nature of the extraradical AM fungal mycelium was photographed. Fig. 1 was obtained after recovering, drying, and staining with chlorazol black E (Brundrett, 1984), an agar-coated slide that had been buried for three months under a pot-grown and *Glomus intraradices* inoculated alfalfa (*Medicago sativa* L.) plant. Fig. 2 presents *G. intraradices* grown on a carrot root culture in a 2-compartment Petri plate. Compartments allowed mycelium growth outside of the influence of non-volatile root exudates. Detailed methodology is given in St-Arnaud, 1996.

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Illustration of the dependence of AM fungi extraradical development on the soil environment comes from a field experiment. Eighty three asparagus plantations were sampled at harvest in four regions of the St. Lawrence Mixed Wood Plain, Québec. Soil microbial communities were characterized using phospholipid fatty acid profiling (Spedding, 2004), and asparagus mycorrhizal root colonization level was measured using the grid-line intersect method (Giovannetti, 1980). Soil environments were described according to soil texture, pH, content of organic matter and available NH<sub>4</sub>, NO<sub>3</sub> (Maynard, 1993), P, K, Na, Mg, K, Ca, Mn, Fe, Cu, Zn (Tran, 1993).

Data from a field experiment involving inoculated strawberry plants in Québec illustrates the functional diversity existing among the AM fungi. The experiment was set in a nutrient rich soil containing 498 mg P kg<sup>-1</sup>. *In vitro* propagated ‘Elite’ class plants of five strawberry (*Fragaria x ananassa* Duch.) cultivars (‘Chambly’, ‘Glooscap’, ‘Joliette’, ‘Kent’, ‘Sweet Charlie’) were pre-inoculated in the greenhouse with one of three AM fungal treatments (control, *Glomus intraradices* Schenck & Smith, or a mixture of *G. intraradices*, *G. mosseae* Gerdemann & Trappe, and *G. etunicatum* Becker & Gerdemann) and transplanted into the field in four blocks. Mother plants’ root colonization was determined prior to transplanting; that of daughter plants, 7 and 14 weeks after planting. Crown diameter, root and shoot mass and average number of daughter plants produced per mother plant was determined in fall. Details of the methods are given in Stewart (2005).

Measurements of the fatty acid 16:1 $\omega$ 5 in the phospholipid and neutral lipids extracted from 20 African and Canadian soils were compared to a standard method of soil AM potential determination, the Most Probable Number (MPN) method (Porter, 1979). The fatty acid was analyzed as per the method of Clapperton and Lacey (Dalpé, 2007).

## RESULTS AND DISCUSSION

### The nature of AM fungi

AM fungi are soil organisms. Most of their biomass consists of extraradical hyphae, which are importantly involved in plant growth stimulation. The extraradical phase of AM fungi takes up water and nutrient for most plants. It is also at the forefront of microbe-plant interactions and soil aggregate stabilization, a process which has much bearing on the aeration, water relations (Augé et al., 2001; Rillig and Steinberg, 2002), and quality of soils (Lupwayi et al., 2001).

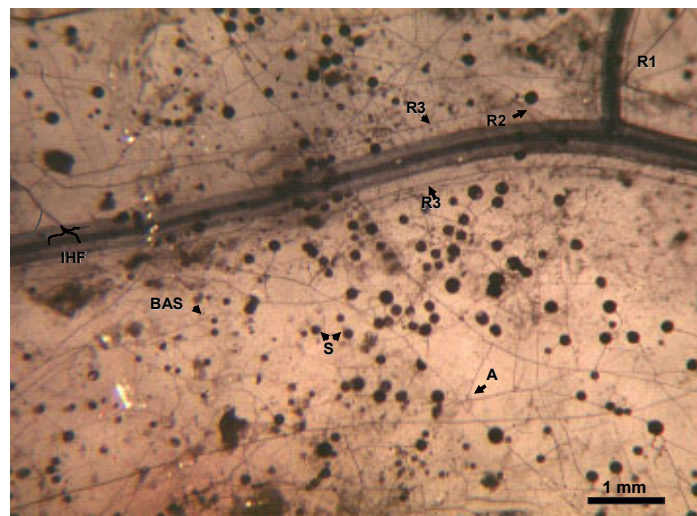


Fig. 1. Extraradical mycelium of *Glomus intraradices* associated with *Medicago sativa*, trapped in a buried agar-coated microscope slide. R1, 2, and 3, runner hyphae; A, anastomosis; S, spores; BAS, branched absorbing structure; IHF, infective hyphal fan and appressoria formation on the root surface

An extraradical AM mycelium appears as a simple structure made of coenocytic multinuclear hyphae growing 3-dimensionally through the soil while older hyphal segments are being emptied of their cytoplasm. AM networks, however, bear some level of complexity that can be manipulated by signal molecules. For example, isolated hyphal regions may reconnect to the network through of anastomosis formation (Fig. 1, A), which is a way to insure good resource distribution in the network. The observation of precontact tropism, protoplasm retraction from the tip and septum formation in the incoming hyphae, and in incompatible reactions between isolates of *G. mosseae* from different geographical areas (Giovannetti, 2003) suggest, interestingly, that specific recognition signals may be involved for anastomosis formation.

The occurrence of different morphologies and the observation of different hyphal behaviours within localized portions of the extraradical AM mycelium suggest both the specialization of some segments and probably the occurrence of differential plant-fungus exchanges of signal molecules at different locations along the roots. For example, three types of coarse running hyphae are recognized. Incoming running hyphae may branch just before contacting the root, as if they were responding to the root environment. Other coarse hyphae growing along the root send lateral hyphae toward the root surface (Fig. 1, R2). Other coarse hyphae protrude out of infection points heading straight off into the bulk of soil (Fig. 1, R1). Differential signalling within the mycorrhizae could explain the different growth patterns observed. Different signals could be exchanged at different times. Conversely, the same signal could trigger different responses depending on the recipient's physiological status. Signal localization in space or time, or different recipients' physiological status, could explain how attraction to root (Fig. 1, R2), parallel development (Fig. 1, R3), and repulsion from root may exist simultaneously (e.g. Fig. 1, R1).

The co-occurrence of such contrasting behaviours are necessary for effective mycorrhizal function. Attraction to root favours fungal spread between plants and roots. Parallel growth of hyphae and roots coordinates root growth and infection spread. Efficient foraging for water and nutrients by AM hyphae, on the other hand, takes place outside of the root depletion zone. Fig. 2 further indicates that some diffusible root-produced compounds are involved in AM hyphal growth control, triggering proliferation of mycelial growth away from the zone of nutrient depletion developing around plant roots (St-Arnaud, 1996). Plant produced flavonoids may be involved in recognition and development in AM symbioses (Lum, 2002).

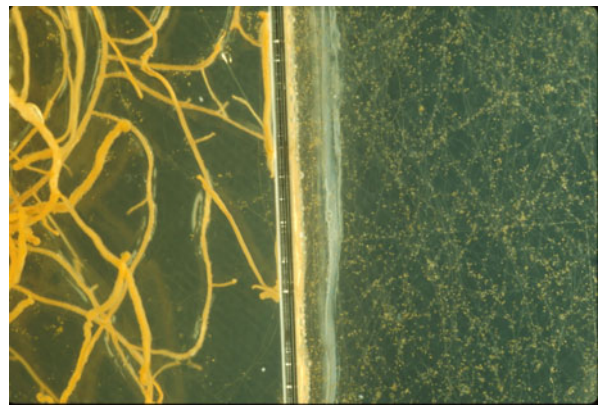


Fig. 2. Dense proliferation of *Glomus intraradices* hyphae in the compartment without carrot roots.

A chemotactic response of a *Gigaspora gigantea* germ-tube was triggered by maize root-produced volatile compounds (Suriyapperuma, 1995). The strength of the response produced differed with maize cultivars. This observation suggests that cultivars with improved symbiotic ability may be produced through plant breeding.

The careful observation of AM fungal growth and the examination of the scientific literature suggest that AM fungal development can be manipulated with appropriate signal molecules. Manipulation could be direct through application of manufactured active molecules to soil or to inoculants, or through plant breeding.

### Dependence on the soil environment

The development pattern of the AM hyphal network may be affected by the soil conditions affecting the diffusion of signal molecules. Soil temperature, distance and path tortuosity, and the functional groups on the soil solids may modulate the influence of diffusive signal molecules. Other soil organisms may also influence AM fungal development directly or indirectly. Thus, the nature of the soil and of the soil habitat influence extraradical AM fungal development. This influence may be selective, resulting in different AM fungal taxa developing better in different soil environments. This would concur with results showing that the symbiotic performance of effective AM strains depend on the soil type in which they are introduced through inoculation (Rivera, 2007).

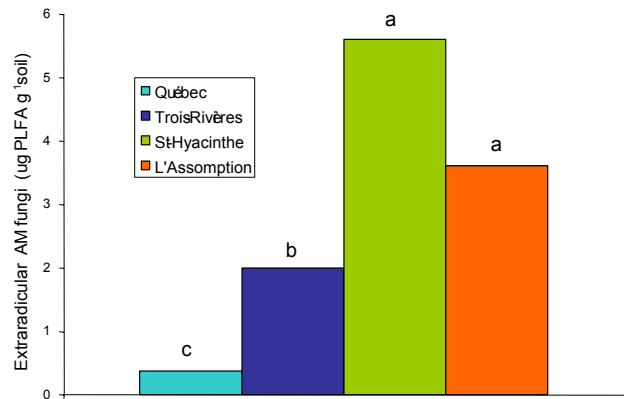


Fig. 3. Effect of region on the abundance of the AM fungi biomass PLFA indicator 16:1ω5. Means with the same letter are not significantly different ( $P = 0.05$ ) according to LSD.

In a survey of 83 asparagus plantations in four regions of the St-Lawrence valley, the abundance of AM extraradical mycelium varied with soil type. Fatty acid methyl ester profiling revealed that the structure of microbial communities in soils around Québec City (north) was different from that occurring in the soils of L'Assomption (south) (Canonical discriminant analysis,  $P < 0.0005$ ). Soil microbial communities in different regions differed most importantly in their levels of AM fungi (16:1ω5), gram- bacteria (18:1ω9), and fungal saprobes (18:2ω9) fatty acid indicators (data not shown). The regional variation in the biomass of extraradical AM fungi, as indicated by 16:1ω5 PLFA abundance was confirmed by ANOVA ( $P < 0.0001$ ). It was scarce in Québec soils, fair in soils of Trois-Rivières, the intermediate region, and similarly high in the two southern regions (Fig. 3). Soil analysis revealed that, like the structure of soil microbial communities, soils were similar within a region but different across regions ( $P < 0.0005$ ), although Trois-Rivières and St-Hyacinthe soils (at intermediate locations) were not different. Soils in different regions differed most importantly in their levels of available P, Ca, Fe, and K, and in the abundance of their silt size particles. Positive Pearson correlations were found between AM fungal biomass and soil available P and Zn ( $P = 0.01$ ;  $r = 0.42$  and  $r = 0.34$ , respectively). Thus, the development of AM fungi extraradical mycelium in asparagus fields seems influenced by soil characteristics and favoured in P- and Zn-rich soils. While the literature indicates that a plant's unfulfilled need for P is the main factor controlling AM root colonization, AM fungi appears to have requirements of their own for extraradical mycelium development. Reduced extraradical development in the northern soils may be associated with the presence of different AM fungal taxa. This hypothesis is being tested.

### Functional diversity among the ‘AM fungi’

The appellation ‘AM fungi’ refers to a range of different organisms with different properties and requirements. This is a reality that is sometimes overlooked for the sake of simplicity in the use of AM inoculants. Even strains selected as highly effective plant growth enhancers may not be effective in all conditions. This was demonstrated in a field experiment involving strawberry plant production where *Glomus intraradices* increased (protected LSD  $P = 0.03$ ) strawberry plant productivity by 46% in the cultivar Glooscap, but reduced it (protected LSD  $P = 0.008$ ) by about 30% in the cultivar Chambly (Fig. 4).

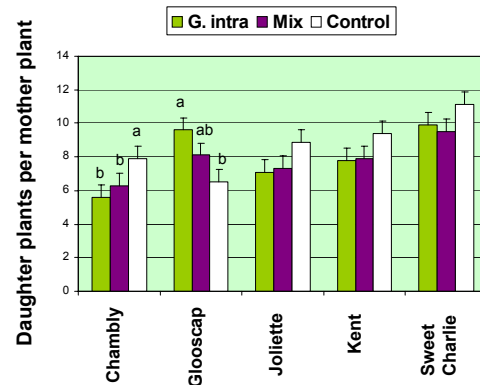


Fig. 4. AM isolates have different effect on the multiplication of strawberry plants.

The most important benefit brought by AM fungi to their host plant is probably improved phosphorus uptake. In P rich soil, AM fungi may provide little benefit to crops. It was concluded from this study that the carbon drain related to the maintenance of *G. intraradices* exceeded the benefits provided by the symbiosis in Chambly and that care should be taken to select the proper inoculum for responsive cultivars to improve rather than reduce the profitability of nurseries with high P fertility soils (Stewart, 2005). The infective capability of the indigenous AM fungal population of this soil was very low. Inoculated plants’ AM colonization level did not even reach 2% seven weeks after transplantation, suggesting that inoculation is a better approach than indigenous AM population management in soil with excessively available P level. In this experiment, soil available P was almost twice the threshold defining a soil as excessively rich for strawberry production in Québec.

P is very reactive and fertilizer P accumulates in soil over time (Beauchemin, 1999). Excessive P levels are encountered commonly in soils growing high value crops in Québec. The available P level in soils with a long history of intensive production, such as those in European countries for example, may largely exceed the level of our study soil. Water quality problems related to high P in soil have been identified. Furthermore, it is well known that too high soil P availability represses AM symbiosis formation, thus inhibiting a natural soil fertility mechanism. Excessive P fertilization is a threat for the environment and reduces soil quality while bringing no economical benefits to farmers.

### Assessment of the AM potential of soils

Recommendations for P fertilization of agricultural soils are not very accurate and larger amounts of fertilizer P than necessary are often applied as a yield insurance. While the capacity of a soil to supply P can be estimated by soil testing, there are currently no methods available to estimate the contribution of indigenous AM fungi to crop P nutrition. The methods used by researchers to determine soil mycorrhizal potential are labour intensive bioassays involving the cultivation of trap plants spanning over months. Obviously,

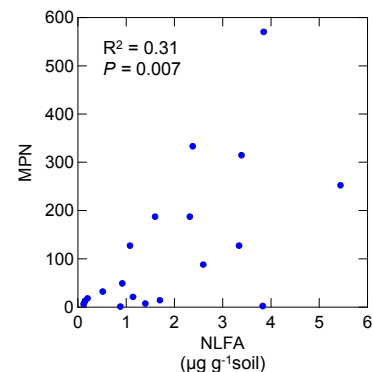


Fig. 5. Relationship between 16:1ω5 NLFA and MPN

they can not be used on a routine basis for soil testing.

The amount of the fatty acid 16:1 $\omega$ 5 in the phospholipid fraction of soil extracted lipids has recently been used as indicator of AM fungal biomass (Balsler, 2005), and that in the neutral lipid fraction (NLFA) is thought to represent the size of energy storage in AM fungi (Bååth, 2003). Recent results indicate that the measurement of fatty acid 16:1 $\omega$ 5 from the NLFA fraction of soil extracted lipids is correlated with results of the Most Probable Number Method (Fig. 5), the bioassay most widely used to determine soil AM potential in the research community.

The measurement of NLFA 16:1 $\omega$ 5 as an indicator of the soil mycorrhizal potential could possibly be calibrated and used on a routine basis to predict the likely contribution of AM fungi to plant P uptake and thus improve the accuracy of P fertilization recommendations.

## CONCLUSION

It is clear that AM fungi offer potential benefits to crop production. These fungi could become a useful tool for crop management. The development of chemicals molecules controlling AM fungi development appears as a promising way to manipulate the AM symbiosis of crops. The selection of AM strains for specific cultivars may be a good approach in some cases, such as the production of strawberry plants, which is a high value transplanted crop with only a few cultivars. Currently available inoculant may be very profitable for many plants grown in soil-less media or transplanted in the field. Highly mycorrhizae dependent transplanted crops may benefit from inoculation, even if they are grown in soil with excessively high P availability, as seen in the case of strawberry. Excessive soil P availability, however, reduces the contribution of AM fungi to plant growth and may prevent the use of AM biotechnologies in most productions. The development and use of a soil test taking into account the contribution of AM fungi indigenous to agricultural fields would improve the accuracy of P fertilization recommendations, reducing production costs and protecting soils against over fertilization and unhealthy P build-ups.

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