Inoculum of arbuscular mycorrhizal fungi for production systems: science meets business[1]

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Abstract: The development of an industrial activity producing microbial inocula is a complex procedure that involves for companies not only the development of the necessary biotechnological know-how, but also the ability to respond to the specifically related legal, ethical, educational, and commercial requirements. At present, commercial arbuscular mycorrhizal (AM) inocula are produced in nursery plots, containers with different substrates and plants, aeroponic systems, or, more recently, in vitro. Different formulated products are available on the market, which creates the need for the establishment of standards for widely accepted quality control. Progress should be made towards registration procedures that stimulate the development of the mycorrhizal industry. Biotechnology science linked to this industrial activity needs to be reinforced, particularly with regards to (*i*) the development of molecular probes for monitoring arbuscular mycorrhizal inocula in the field, (*ii*) increasing knowledge on the ecophysiology of AM fungi in anthropogenically disturbed ecosystems and on the interactions of AM fungi with other rhizosphere microbes, and (*iii*) selection of new plant varieties with enhanced mycorrhizal traits and of AM fungi with new symbiotic traits. However, one of the main tasks for both producers and researchers is to raise awareness in the public about potentials of mycorrhizal technology for sustainable plant production and soil conservation.

Key words: Glomeromycota, biotechnology, quality control, legal aspects, commercial aspects.

Résumé : Le développement d'activités industrielles pour la production d'inocula microbiens est un processus complexe impliquant, pour les compagnies, non seulement le développement du savoir-faire biotechnologique, mais aussi la capacité de satisfaire aux exigences législatives, éthiques, éducatives et commerciales spécifiquement concernées. Présentement, les inocula commerciaux mycorhizogènes à arbuscules (MA) sont produits en parcelles dans des pépinières ou en conteneurs, en utilisant des substrats et des plantes variées, ou encore en aéroponique et, plus récemment, en culture in vitro. On retrouve, sur le marché, des produits différemment formulés, et ceci crée le besoin d'établir des standards de contrôles de qualité largement acceptés. On devrait se diriger vers des processus d'homologation qui stimulent le développement de l'industrie des inocula mycorhizogènes. La science biotechnologique liée aux activités de cette industrie doit être soutenue, en particulier à ce qui a trait (*i*) au développement de sondes moléculaires pour suivre les inocula MA au champ, (*ii*) à l'augmentation des connaissances sur l'écophysiologie des champignons MA dans les écosystèmes affectés par l'homme, et sur les interactions des champignons MA avec les autres microorganismes de la rhizosphère, et (*iii*) à la sélection de nouvelles variétés de plantes ayant des caractéristiques mycorhiziennes accrues et de champignons MA ayant de nouveaux caractères symbiotiques. Cependant, une des principales tâches rencontrées par les producteurs aussi bien que les scientifiques consiste à élever le degré de connaissances du public sur les potentiels de la technologie mycorhizienne pour la production et la conservation des sols, de façon durable.

Mots clés : Glomeromycota, biotechnologie, contrôle de qualité, aspects légaux, aspects commerciaux.

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Introduction

The number of new small- to medium-sized companies producing arbuscular mycorrhizal (AM) fungal inocula around the world has been increasing, particularly in the last few years: in 2001, D. Sylvia (personal communication) listed 21 companies in North America, 8 in Europe, 2 in South America, and 2 in Asia, but there are certainly many more established companies aiming to produce and use AM fungal inocula in various sectors of plant production.

The reasons for the development of this agricultural biotechnology industry producing AM fungal inocula are multi-

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ple: (*i*) AM fungi are increasingly being considered as a natural plant health insurance (Gianinazzi and Gianinazzi-Pearson 1988), and examples of their positive impact on plant development and health, land reclamation, and phytoremediation are continually increasing (Leyval et al. 2002; Turnau and Haselwandter 2002); (*ii*) there is higher awareness of biodiversity issues, including those concerning soil microbial communities, and acceptance of these natural technologies as alternatives to agrochemicals (Barea 2000; Gryndler 2000); and (*iii*) society is demanding more sustainable means of production, with a consequent feedback to farmers and land conservationists.

Producing microbial inocula is a complex procedure that involves not only the development of the necessary biotechnological expertise, but also the ability to respond to the specifically related legal, ethical, educational, and commercial requirements. This is particularly true in the case of obligate endosymbiotic microorganisms such as AM fungi, because satisfying the aforementioned requirements is closely associated with the particular method of inoculum production.

Inoculum production systems with commercial applications

Production systems of AM fungi have evolved considerably during recent years, from relatively simple technologies to more complex ones, for example, in vitro methods (Jarstfer and Sylvia 1994). At present, inoculum is produced for commercial purposes in the following ways:

(*i*) nursery plots with soil (Sieverding 1991), in which inoculated plants are cultured in open field or nursery beds. Advantages: simple, adapted for local use, low costs; disadvantages: limited in application, easily contaminated, not well adapted for the development of an industrial activity.

(*ii*) containers (pots) with different substrates (Feldmann and Idczak 1994; Feldmann and Grotkass 2002). Advantages: low technology input, undesirable contaminations fairly easily eliminated, reasonable costs; disadvantages: not pure, limited in its industrial development.

(*iii*) aeroponic systems (Jarstfer and Sylvia 1994), where preinoculated plant roots are continuously misted with nutrient solution sprayed within cultivation boxes. Advantages: easier control of contaminants, carrier-free inoculum, adapted for microplants; disadvantages: relatively complicated technological setup.

(*iv*) in vitro on roots transformed with *Agrobacterium rhizogenes* (Becard and Fortin 1988; Declerck et al. 1996). Advantages: pure cultures, permits industrial development; disadvantages: high technological investment, high costs, not all AM fungi successfully culturable in this system, and suitability of inoculum produced in vitro, in particular its competitive ability toward other microbes in field soil, has yet to be tested.

Formulation of the inocula

Basically, the formulation procedure consists of placing fungal propagules (root fragments colonized with AM fungi, fragments of fungal mycelium, and spores) in a given carrier (perlite, peat, inorganic clay, zeolite, vermiculite, sand, etc.) for a given application. Biological inoculants belong to diverse taxonomic groups varying considerably in physiology and, as a consequence, in their nutritional and environmental requirements (Cost Action 8.30 2001). Therefore, the final configuration of the formulation will result from a more or less technologically complex procedure, determined by the microbe involved, the way of producing inoculum, and the target inoculum application (bare-root plants, containerized plants, cuttings, seeds, potting mixes, soils, etc.). The fungi should be selected to be compatible with the target environment (Estaún et al. 2002; Vosátka and Dodd 2002; Requena et al. 1996). Following mass production, fungal propagules must be formulated in such a way that they can be stored and distributed under a wide range of temperatures and without losing viability. Formulation should be simple and economical, and the formulated inocula should be easy to transport and apply. Some companies producing AM fungal inocula have adopted the approach of one type of formulation (i.e., single fungal species) for all markets, while others produce a range of products for their target buyers.

Quality control of mycorrhizal inocula

The industrial activity of inoculum producers has developed using different AM fungi, which are quite often not well characterized in terms of ecological requirements and stability. This, and the lack of quality control for several marketed inocula, are amongst the main reasons for the low acceptance of mycorrhizal technology in horticultural and agricultural practices. This situation has led to the need for this industry to develop, in its own interest, criteria that will satisfy minimum requirements of quality for the produced inoculum.

Whatever the mode of inoculum production chosen and the formulation procedure adopted by the companies, the marketed product has to meet the expected requirements of end-users (e.g., reduce phosphorus fertilizer inputs, increase plant tolerance to pollutants, improve flowering, favour ecological land restoration, and many others). Although these objectives may vary according to the companies, they should all aim at the use of AM fungi as a natural plant health insurance (Gianinazzi and Gianinazzi-Pearson 1988). In this context, the following criteria should be fulfilled by the companies: (*i*) plants to be inoculated must be able to form mycorrhizas; (*ii*) the AM fungal inoculum must be free of agents that could negatively affect normal plant growth and development; (*iii*) the shelf life of the inoculum should be sufficient to suit the end-user markets.

The introduction of such criteria by the inocula producers could contribute to the definition of conditions for the registration of products at national or international levels (von Alten et al. 2002). Furthermore, in the product description, inclusion of the following recommendations for quality standards may be considered.

I. Physical and chemical properties of the inoculum

Data on pH, nutrient carriers, and additives must be provided to end-users; amendments by additives can only be accepted if their primary aim is to support mycorrhizal development (e.g., additives should not be general fertilizers).

II. AM fungal propagule density

The relevant number of AM fungal propagules can be determined using various published techniques, such as the most probable number (Gianinazzi-Pearson et al. 1985; An et al. 1990) or the inoculum potential assay (Liu and Luo 1994). The most probable number is more often applied; however, results can vary according to the plant, substrate, and environmental conditions used (Feldmann and Idczak 1994; An et al. 1990). Therefore, there is need for an independent testing service that can be used by producers to check that batches of inocula meet baseline standards that have been established and agreed to by individual companies on the basis of a voluntary code of best practices.

III. Guaranteed effectiveness

The outcome of the arbuscular mycorrhiza symbiosis depends on environmental factors, AM fungal characteristics, and plant variables; our present knowledge makes it difficult to predict the effectiveness of inoculum. As an example, the procedure called "direct inoculum production process" could help to improve predictability of AM fungal inoculum effectiveness (Feldmann and Grotkass 2002). Quality control of commercial inoculum must deal with this aspect, and a reference system for information concerning AM fungal effectiveness based on the results of standard tests should be established for the buyers as well as a list of examples where the relevant inoculum had already been successfully used.

IV. Absence of microbial contaminants

With the exception of inoculum produced in vitro, all other inocula produced in nonsterile greenhouses or open-air systems will not be free from other associated microorganisms. AM fungi have also been shown to harbour bacteria inside their cytoplasm (possibly also symbiotic organisms within the symbiotic fungus), therefore even the in vitro cultivated inocula are not necessarily microbe free (Bonfante 2003). The use of good horticultural practice can prevent contamination and spread of unwanted (plant pathogens) microorganisms (plant protection products compatible with AM fungi can be used during inoculum production when the latter is not for the organic market). During inoculum production, root samples should be microscopically checked for the presence of potential pathogenic fungi. Additional tests based on trap plants susceptible to soilborne pathogens can also be used. Examples include the cress test for root rots (cress seeds are sown on the surface of the tested inocula, and inhibition of seed germination indicates presence of toxic elements or pathogens (von Alten et al. 2002). However, such methods are not really satisfactory, and there is an urgent need for molecular tests to detect microbial pathogens in AM fungal inocula.

V. Storage and use

How to store the inoculum together with recommended dates of usage and maximum dilution of the content should be clearly indicated, as it is well known that the infectivity of inocula can decline rapidly (Tommerup 1988).

VI. Absence of transgenic elements

This applies to inoculum produced on transformed roots, for which it may be necessary to provide such information, at least in Europe.

The mycorrhizal industry should take necessary measures to ensure that inoculum producers will respect the defined criteria of quality. For example in Europe, a Federation of European Mycorrhizal Fungi Producers (FEMFiP, http://www.femfip.com/) was founded in 2003, with the aims of achieving and maintaining high standards of inoculum quality. Its Memorandum states that "Methods for evaluating the quality of mycorrhizal fungi inocula will be standardized and a certification programme for producers will be introduced to promote end product efficacy". The procedures for compliance will be developed in collaboration with its members and administered through the offices of an independent laboratory", for example, IBG (http://www.kent.ac.uk/bio/beg/) and INVAM (http://invam.caf.wvu.edu/). The FEMFiP aspires in this way to promote the use of standardized criteria applicable to all inoculum producers in the European Union (EU).

Legal and ethical aspects of inoculum use

Suitable legislation based on quality control adapted to AM fungal inocula is essential for the development of mycorrhiza technology. At present, registration procedures for AM fungal inocula vary between countries, with some having very strict regulations (e.g., France and Canada) and others being less demanding or even without regulations. No regulation or slack regulation will favour the presence on the market of bad products, which could destroy the market. Overregulation could also destroy the market by preventing the development of small and medium enterprises, inoculum producers, and distributors of what is potentially one of the few biotechnologies applying natural microbes to plant production.

For example in France, beneficial microbes such as Rhizobium and AM fungi are considered biofertilizers, and their registration requires a complex and expensive procedure that implies detailed description of the biological properties of the relevant microbes (identification, dissemination, toxicity, etc.), demonstration of the beneficial effects of the microbe via several controlled field trials (three to five per year) during two production cycles, and demonstration via appropriate tests of the lack of toxicity or allergenicity of the formulated products for humans, animals, and plants.

At the EU level, there is no registration for biofertilizers. However, the directive 91/414/EEC regulates the use of microbial products for plant protection. The data requirements for approval of plant protection products focus on possible unacceptable impacts on plants or the environment, harmful effects on human or animal health, and contamination of groundwater. Therefore, to avoid registration under the directive 91/414/EEC, AM fungal inoculum should not be declared as a biocontrol agent. The cost of such a process would handicap attempts to introduce mycorrhizal technology into plant production systems.

Because of attempts to apply this directive to AM fungi, the European network on AM fungi, Cost Action 8.38 (2001), has initiated discussions within the EU on the need for a registration procedure for AM fungi. We consider that, because AM fungi do not produce toxins, they should be regarded as a natural part of the plant, the guidelines for the approval of microbial plant protection products should not be directly applicable to them, and the part concerning "risk assessment" criteria is particularly inappropriate. Cost Action 8.38 (2001) has elaborated a position paper (http://www.dijon.inra.fr/cost838/index.html) where data requirements are based on the above-mentioned directive, but focusing on questions considered important for approval of a commercial AM fungal product.

Link and feedback of inoculum production biotechnology to basic science

The development of AM fungal inoculum products requires further input of research, and there is a need to establish biotech science links to this industrial activity. This is in line with EU programmes to strengthen links between small and medium enterprises and research institutions. There have been several projects already funded by the EU associating academic institutions and inoculum producers (e.g., MYCHINTEC, GENOMYCA, MYCOREM, etc.). Several research priorities in AM fungal inoculum biotechnology can be identified and are outlined below.

Development of molecular probes of AM fungi

The classical, micoscopical procedures used to identify and evaluate AM fungi in roots and soil are not adequate for efficiently monitoring the inoculum. A key point for promoting mycorrhizal biotechnology is the development of simple, fast, and reliable molecular tests to identify AM fungi in roots and soils. This is important for inoculum producers, as it allows identification and protection of the product, and for the customers, as it provides a means of assuring quality of the marketed product. Rapid and accurate methods, such as polymerase chain reaction (PCR) techniques, need therefore to be adapted for quality control; not only AM fungal species have to be distinguished in the quality control, but even strains and substrains. Primers based on large subunit rDNA sequences, and recognizing a wide range of Glomeromycota (Schuβer et al. 2001) when used in nested PCR on soil and (or) root DNA, were recently identified by Gollotte et al. (2004) (Fig. 1). These generic probes are useful for assessing the mycorrhizal status of soil and roots. In a pioneer work, van Tuinen et al. (1998) developed a method for characterizing and quantifying colonization profiles of AM fungi present in roots using nested PCR, targeted to 25S rDNA, of roots stained with trypan blue. However, molecular probes defined at present are species specific, so that their practical use is limited to situations where a given inoculated AM fungal species is not present in the substrate or soil used.

In the context of an EU–China project, "Mycorrhiza technology for staple food crop production in small-scale sustainable agriculture in China" (ICA4-CT-2000–30014), a standard procedure applicable to field material has been developed for conserving roots for several months and subsequently extracting fungal DNA. These methodologies have been successfully applied to field samples of maize, sweet potato, and cassava roots (V. Gianinazzi-Pearson, unpublished data).

Although procedures for identifying AM fungi in roots and soil are established, progress is required to obtain "strain"-specific probes and the construction of kits better adapted to commercial activities.

Increased knowledge on the ecophysiology of AM fungi

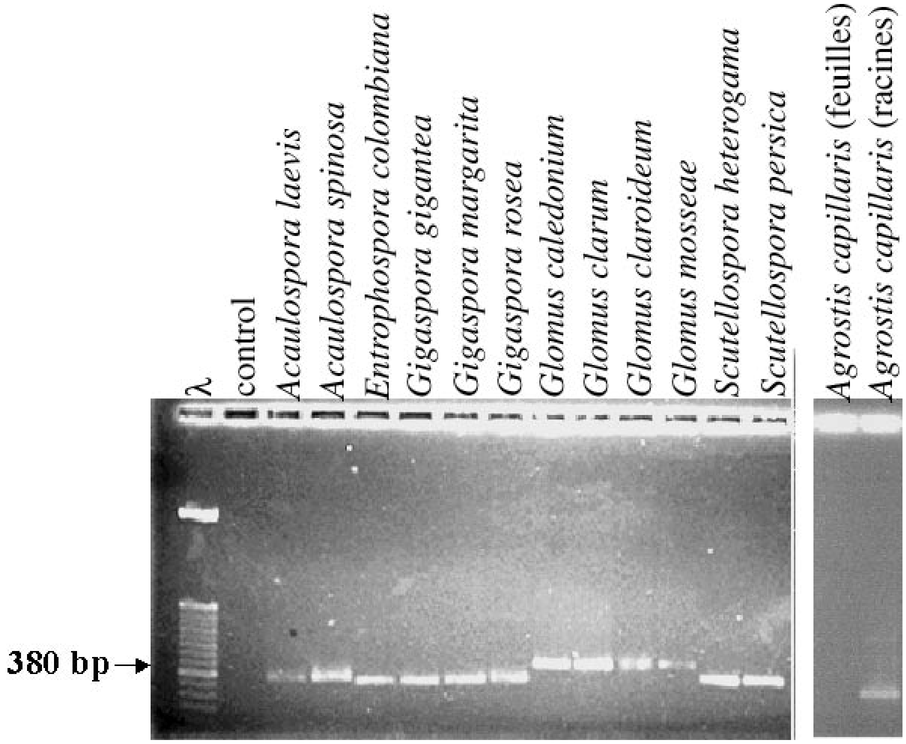
Definition of ecological attributes is essential for selecting adapted AM fungi for plant production systems. This should be an imperative of all inoculum producers, if the market for these products is to be maintained in the future. Strong collaboration between inoculum producers, plant growers, and researchers is necessary to understand more about AM fungal ecophysiology for their efficient manipulation. Urgent progress is needed in two different situations where AM fungal technology can be successfully applied in anthropogenically disturbed ecosystems (land reclamation, phytoremediation; Leyval et al. 2002) or for plant biotization (microbial ecology of the rhizosphere; Vestberg et al. 2002). One of the important tasks of fundamental research is to obtain more information on how AM fungi become tolerant to certain environmental stresses (drought, soil contamination, pH fluctuation, etc.) and to what extent this tolerance can be stable through subculturing under stress-free conditions. It has been repeatedly shown that fungi can lose some of their features, for example, tolerance to heavy metals through cultivation in media free of heavy metals (Malcová et al. 2003), and the environment can change the composition of the fungal population characteristics (Feldmann and Grotkass 2002).

There is always a risk linked to the selection of fungal strains under optimized production systems, where there can be a shift in features originally possessed by a particular strain. There is lack of knowledge about how changing conditions of subculturing can affect symbiotic efficacy or adaptation of AM fungal strains. This knowledge is particularly essential not only for the use of inoculum in stressed or polluted soils where resistance of inoculants to a particular type of stress is required, but also for determining appropriate conditions for inoculum production.

In nature, plant biodiversity and fitness rely on the development on roots of combinations of beneficial microbes with complementary functions in promoting plant growth and health. Plant inoculation with more than one beneficial microbe (plant biotization) is an emerging technology and a new challenge for the industry of microbial inocula, where AM fungal inocula producers in particular could easily acquire such technology (Vestberg et al. 2002). However, inoculation using different combinations of beneficial rhizosphere microbes has shown how difficult it is to predict the outcome in terms of plant growth and health. In fact, results vary according to the microbial combination used (Cordier et al. 2000; Gianinazzi et al. 2003).

Time of inoculation of the different inocula may be crucial, because, as pointed out by Barea (2000), AM fungi play a key role in root morphology and functioning and therefore in the establishment of a microbial community. Increase in knowledge about microbes and microbe–plant interactions in the rhizosphere is necessary to choose suitable beneficial microbial combinations for inoculants and for optimizing the effects of AM fungal inoculation (Puppi et al. 1994). This could open a new way to the mycorrhizal industry for promoting a promising technology based on multi-

Fig. 1. PCR amplification of a partial region of the large subunit of ribosomal DNA using the primers FLR3 and FLR4, which are general for AM fungi (Gollotte et al. 2004). PCR gives a positive signal with spore extracts from the AM fungi *Acaulospora laevis* AU211, *Acaulospora spinosa* NC105A, *Entrophospora colombiana* CL356, *Gigaspora gigantea* NC150, *Gigaspora margarita* BEG34, *Gigaspora rosea* BEG9, *Glomus caledonium* UK301, *Glomus clarum* BR143, *Glomus claroideum* DN987, *Glomus mosseae* BEG12, *Scutellospora heterogama* BR154, and *Scutellospora persica* VA102C. A band is also generated from root DNA extracts from the plant *Agrostis capillaris* sampled in a grassland. There is no amplification from leaf extracts. No DNA has been added in the negative control. λ, molecular weight marker (Gollotte et al. 2004).



microbial inoculation, probably better adapted to a more sustainable system of production.

AM fungi with new symbiotic traits?

Another interesting line of research is to obtain genetically modified AM fungi with new biological properties (Harrier et al. 2002), for example, for plant growth enhancement in polluted soils (bioremediation). This is one of the objectives of the EU Genomyca project "Genes and genetic engineering for arbuscular mycorrhiza technology and applications in sustainable agriculture" (QLK5-CT-2000-01319 NAS QLRT-CT-2001-02804).

Identification of "green" molecules

Because of its obligate biotrophy (Azcón-Aguilar and Barea 1995), inoculum of AM fungi has to be produced on living roots, which is usually considered a major disadvantage. The identification of plant molecules promoting root colonization by AM fungi and (or) fungal molecules stimulating root receptivity to the fungi is of particular importance for the mycorrhizal industry. These so-called green molecules could easily be introduced into the formulation of the inoculum to promote mycorrhizal formation by inoculated plants.

Selection of new plant varieties with enhanced mycorrhizal traits

A frequent problem of field inoculation is a high level of indigenous fungi, which makes introduction of inoculum of high quality and (or) for a precise aim unpredictable. Researchers have for a long time suggested the selection of varieties that enhance specificity towards some AM fungi (Cost Action 8.21 1995; Gianinazzi et al. 1995), but this has never been achieved in terms of research. The recent discovery of specific, mycorrhizal-activated plant genes (Brechenmacher et al. 2004), together with the tilling technology developed to speed up selection programmes of new varieties, open new possibilities to obtain plants with enhanced mycorrhizal traits.

Educational aspects of mycorrhizal technology promotion

There is still lack of public awareness concerning the potential of mycorrhiza for sustainable (agro)ecosystem management. Therefore, basic and higher education should emphasize the importance of beneficial soil microbes and their interaction at the soil–plant interface in the wider context of society demands. As agriculture and forestry are rather conventional and conservative markets, it is sometimes difficult to penetrate them with new biotechnologies. Moreover, mycorrhizal technology is relatively complex, as it encompasses several diverse aspects of plant production, that is, cultivation media, nutrient cycling, plant physiology, interactions with other microbes, and numerous environmental factors. Currently, each company needs to be not only producer and marketer but also, to a certain extent, a research and educational body, because of the very low public awareness of mycorrhizal products. However, this requires strong feedback between the commercial and the scientific world. For many years, mycorrhizal research has been funded by different national and international bodies, as there have been, and still are, relatively great expectations for the implementation of potential results in practice. Most of the scientific papers, reports, or project proposals in the mycorrhiza field begin with claims about the importance of mycorrhiza in plant communities and (or) their potential use in plant production systems. Future research funding could diminish substantially if the "promise" of application is not forthcoming or if there is no market demand for further fundamental research.

Certain markets still continue to focus on aboveground plant attributes when considering plant health and vigor. For example, in forestry, many growers still consider height, root collar diameter, or shoot branching as the main parameters of plant health, without taking into account that numbers of lateral roots and presence of mycorrhiza might be determining factors for transplanted plant survival. What is crucial is to raise awareness through the popular press of the multifunctional assets of mycorrhizal symbioses. Current markets should be focused on illustrating to end users all mycorrhiza-mediated potential solutions to problems encountered in the plant production system being used (e.g., high plant mortality due to drought, pathogens, excessive inputs of agrochemicals, high maintenance costs, etc.). A common language has to be employed, as there are numerous specific terms that nonscientists can easily understand, and for a scientist this "art" of explaining in simple words the complexity of mycorrhiza and their interactions with numerous environmental factors is not always obvious.

Commercial aspects of arbuscular mycorrhizal fungi inocula

Awareness of existing products and industry: science cooperation and feedback

As mentioned previously, awareness about mycorrhiza should be constantly increased through lectures to students at universities, special schools for forestry, horticulture, etc. Seminars for growers, landscapers, developers, nurseries, etc., and articles in technical or professional journals should be organized, as well as specific advertisements (product leaflets, advertisement, popularization programmes, etc.) launched by inoculum producers and distributors. There are fields of potential use of mycorrhiza that have not been fully exploited yet, such as using mycorrhiza in phytoremediation schemes (Vosátka 2001) or in biomass production for energy. Nevertheless, as fundamental knowledge about the function and mechanisms of plant–soil–fungi interactions increases, these areas for mycorrhiza application are probably going to grow, and potential users should be educated in this respect. The involvement of plant and inoculum producers in specific scientific projects (e.g., projects funded within EU Framework programmes) seems to be vital for achieving an essential threshold of practical knowledge for such specific, large-scale field applications. These joint projects involve appropriate-sized field trials with inoculum producers to investigate suitability of inoculation for certain sectors of plant production and therefore contribute to the development of market niches. On the other hand, feedback from "real-world" application to fundamental science can identify demands where fundamental knowledge will facilitate new areas of mycorrhizal technology use.

Testing of products

Declared effects of mycorrhiza can vary according to culture procedures and application targets, and each user wants usually to first test inoculum on a small scale. It is common practice that inoculum producers support research by supplying products for trials at low marginal costs and that they also help clients to evaluate the establishment of mycorrhiza. These trials are commonly cofunded by clients and producers, with the aim of increasing sales. Apart from testing inoculum efficacy, the mode of application has to be evaluated on a large scale. Most important is the economic feasibility of including inoculation into production technology. Any new market development concerns not only identifying suitable products and relevant promotion strategies, but also establishing appropriate and reasonable product pricing.

Tuning inocula and their application to plant producers' requirements

There is no universal mode of inoculum application, and some planting or plant production systems need specific procedures of application. The user should be aware that an important issue is to optimize the introduction of mycorrhiza as early as possible in plant growth, by layering inoculum below seeds or mixing inoculum into the growth substrate for containerized plants. Micropropagated plants can be inoculated post vitro at the transplantation stage, whilst bare-root plants can be dipped into gel formulations of mycorrhizal inocula before transplanting, or dry formulations of inocula can be spread into the planting site. Machinery is needed for larger-scale application, that is, mixing tanks for substrates or sowing machines for field inoculations. Numerous ecological factors have to be taken into account for the successful introduction of inocula into the field; these include soil properties, level of fertilizer input, or potential of existing fungal populations (Vosátka and Dodd 2002). One specific marketing branch of mycorrhizal products encompasses the production of biotized plants; this approach is being used, e.g., for endomycorrhizal trees for recultivation (Czech Republic), micropropagated plants (France), or medicinal plants (Germany). There is definitely a demand to tune inocula products to meet the ultimate needs of the end user.

Generic products or tuned products?

Plant species – fungal partner specificity in AM fungi is not very high, if any exist. On the other hand there are differences in ecophysiology and effectiveness of different fungal species and even their geographical isolates of AM fungi (Schweiger and Jakobsen 1999; del Val et al. 1999). For example, AM fungal isolates originating from stressed or contaminated soils of anthropogenic or degraded ecosystems can perform significantly better when inoculated back into the original soil, as compared with other symbionts that are not native to the degraded soils (Joner and Leyval 1997;

Batkhuugyin et al. 2000; Rydlová and Vosátka 2003). A remaining question is whether or not it is necessary to look for AM fungal "super strains" with a high degree of plasticity to adapt to wide ranges of environmental conditions, or rather focus on tailoring specific mixes of suitable symbionts for particular soils and ecosystems, or even isolate indigenous fungi for target ecosystems. Inoculation in extreme environments has shown that some nonindigenous AM fungi cannot adapt to environmental stresses, whereas others are able to cope with a wide range of soil conditions. At least for these specific environments, there is definitely a need to preselect inocula to ensure a successful outcome of the mycorrhization.

General or specific marketing of the products?

There is definitely an opportunity for introducing AM fungal products onto supermarket shelves, but this entails a certain risk of their misuse. Professional suppliers (e.g., cooperatives, landscaping companies, nurseries) can be selected to sell the products; however, this again requires an educational investment in the form of seminars, training, and site support. Alternatively, there can be a case-by-case service with development of the most suitable formulations and application modes contracted between end user and inoculum producer (with possible involvement of a research institute). This is presently occurring in many ways (EU projects, etc.) and seems to be an efficient way forward. Building a market in this way builds trust of end users in biological efficacy and economic feasibility of mycorrhizal products. There are still many examples of "conventional" farmers who prefer to empty an extra bag of NPK fertilizer into the soil rather than bother about some weird fungi that are never seen by the naked eye. It should be an ultimate aim of both scientists and their industrial partners to promote mycorrhizal inoculation as a third-millennium biotechnology, with potential implementations in most of the sectors of sustainable plant production.

Conclusions

There are a number of entrepreneurs developing inoculum production and marketing AM fungi or mycorrhiza. However, there are still technical hitches to the large-scale utilization of AM fungal inoculum as well as numerous legal, ethical, economical, etc., aspects of this technology to be resolved and that should not clash or be neglected. Important issues still remain for research (at best in cooperation with producers) to fill gaps in fundamental knowledge and to optimize appropriate maintenance and application schemes for AM fungi in plant production systems. The task for producers and distributors of inoculum is to convince end users that this ecologically sound technology is also economically feasible. Therefore, the customers will perceive good value for their money, and expectations from mycorrhizal inoculation will be fulfilled. More applied and near-market studies are needed and should be funded to aid the food and plant production, in particular in countries where climatic conditions favour the mycorrhizal symbiosis, for example, semiarid regions and the tropics, but also in countries where sustainable ways of agriculture or horticulture are developing. The main task for both producers and researchers is to raise awareness in the public about potentials of mycorrhizal technology for sustainable plant production and soil conservation.

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