

## **Trade with beneficial mycorrhizal fungi in Europe: suitable quality management avoids distribution of unwanted organisms in mycorrhizal inoculum**

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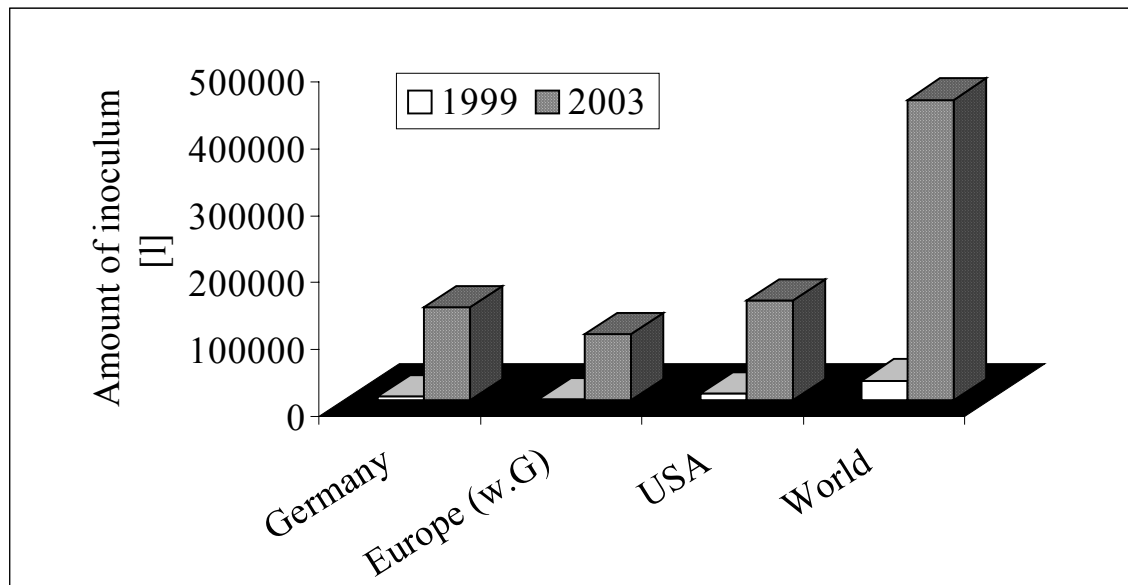
### **ABSTRACT**

Mycorrhiza is the most important mutual symbiosis between plant roots and soil borne fungi. An established symbiosis in the plant roots guarantees the adaptation even under unsuitable soil or climate conditions. The advantages have lead to an increasing demand for mycorrhiza products in the last years in Europe. As a consequence, large amounts of mycorrhizal inoculum are transported through the “borderless” Europe and are even imported from North and South America, and Asia. Because international regulations on the trade with beneficial microorganisms are failing, serious producers of mycorrhizal inoculum founded national and international organizations who are recently voluntarily working out quality control procedures to avoid the spread of unwanted organisms accompanying mycorrhizal inoculum.

### **INTRODUCTION**

Mycorrhiza is the most important mutual symbiosis between plant roots and fungi. More than 90 % of all plants live in a community with soil borne fungi. The advantages of a functioning symbiosis are manifold: the mycelia of the fungi enlarge the function of the root system and increase water and nutrient uptake of the plants. The symbiosis also results in a higher drought tolerance, the plants become more resistant to diseases, the soil structure is stabilized and the soil aeration, water permeability and aggregation is improved through the mycelia of the mycorrhiza fungi. The advantages of the symbiosis are evident in stress situations like bad soil or climate conditions or transplanting shock: the symbiosis guarantees the adaptation to the conditions and a faster growing of mycorrhizal plants.

Consequently, wherever potential hosts of mycorrhizal fungi are produced companies are interested to test the new mycorrhizal technology in practice. The German Federal Research Centre for Agriculture and Forestry surveyed in 2003 that the world wide production and trade of mycorrhiza products increased immensely during the last years (Figure 1). The USA and Germany are recently the largest producers and retailers of mycorrhizal inoculum, but a considerable amount of companies all over the world are preparing for the market and already started to spread the inoculum throughout Europe.



**Figure 1: Increasing production of mycorrhiza products show the necessity for a standardized quality management in Europe (w. G. = without Germany) source: Feldmann (2003)**

Plant species of all growth form types establish a symbiosis with arbuscular mycorrhiza. Others, like most of the needle trees need ectotrophic mycorrhiza for their growth. Ericaceae grow together with ericoid mycorrhiza. These major groups of mycorrhiza are produced with specific production methods defined by specific demands of the fungi produced: the arbuscular mycorrhizal fungi are obligatory biotrophic fungi, the others are facultative saprophytes. The first can only be produced on living plants; the second could be produced on sterile nutrient media. But, caused by resulting inefficiency, culturing media are recently recognized to be less and less suitable for the production of ectomycorrhizal fungi which leads to biotrophic production of these fungi as well. Overall it can be summarized, that mycorrhiza products differ in the fungi contained and their carrier material, but the majority is produced on living plants. The inoculum sold therefore is a mixture of substrate the multiplying plant already grew in, mycorrhizal hyphae and spores and accompanying organisms potentially including saprophytic, hyperparasitic and pathogenic fungi, phytophagous faunistic elements or weeds (Feldmann, 2003). While the risk of damages on useful plants, detrimental influences to environment and biodiversity caused by mycorrhizal fungi is recently estimated to be low (Feldmann, 2003), the risk to spread unwanted alien or even invasive accompanying organisms is known to be much higher. Suitable quality control procedures are therefore necessary to be applied.

## **VOLUNTARY COMMITMENT FOR QUALITY CONTROL IN EUROPE**

In Germany, trade of mycorrhiza products is regulated by the fertilizer act which classifies them as soil supplements. Supplements are defined as substances other than fertilizers, manufactured, sold or represented for use in the improvement of the physical condition of the soil or to aid plant growth or crop yields. Only some physical parameter have to be

declared, so that they do not have any relevance for the quality description of mycorrhizal inoculum. All serious German inoculum manufacturers unified in the German lobby "Application of mycorrhizal fungi in practice" (for more information see [info@mykorrhiza.de](mailto:info@mykorrhiza.de)), therefore, agreed on a standardized voluntary quality control in 1997 to increase the quality of products even without legal preconditions (Backhaus and Feldmann, 1997). Today, the main biological and physical parameters cited below are controlled in the different companies of the German lobby. Nevertheless, it became obvious that already slight differences between procedures and protocols applied could lead to significantly different results. The German lobby therefore induced the process to install voluntary quality control procedures on the European level (on the basis of COST Action 838) to discuss the problems on an international level and to include European companies as well. One important result was a joined publication of the largest European inoculum producers as a basis for a joined Europe wide voluntary quality control (von Alten et al., 2002). Another result was the foundation of the 'Federation of European Mycorrhizal Fungi Producers' which started to work out a methodology for a standardized quality control for the EU since May 2003 ([www.femfip.com](http://www.femfip.com)). The aim is to define reproducible, valuable parameters for later external quality control carried out by independent institutions. A seal for "good inoculum quality" will be developed which guarantees minimum requirements for certified products. The Federation also works on the transfer of scientific results to the practical application for the benefit of the customers. Already today high quality inoculum is on the market for a broad range of application.

## **SPECTRUM OF UNWANTED ACCOMPANYING ORGANISMS FOUND IN COMMERCIAL MYCORRHIZAL INOCULUM WITHOUT QUALITY CONTROL DECLARATION**

For the production of arbuscular mycorrhiza fungi living host plants are necessary because of their obligate biotrophy. The host plants (e. g. *Zea mays*) can be planted in pots or ground beds in the greenhouse. They are inoculated with starter inoculum in this unsterile production system. Peat, expanded clay, lava or vermiculite with different grain sizes are used as carrier materials depending on the later application methods. After the cultivation of at least three months the host plant is removed and the remaining carrier material now contains spores of the fungus and also mycelia and infected roots. The sterile production of arbuscular mycorrhiza fungi is also possible but products are not competitive on the market at the moment. The production of ectotrophic and ericoid mycorrhiza is possible sterile as well as unsterile. The fungal mycelia is propagated on nutrient media in the laboratory for a sterile product that is often mixed with a carrier material e.g. vermiculite. The mycelium overgrows the carrier material and can be mixed with substrates during planting or e. g. tree restorations. But investigations show that fungi can stop establishing a symbiosis with their natural hosts after some time in sterile culture. Therefore, inoculated trees grow in nurseries for the unsterile production. After some months the substrates can be used as inoculum. It is important to know that the inoculum normally contains variable mixtures of symbiotic fungal populations. The production in such an open system in the greenhouse can lead to contaminations of the inoculum with several organisms (Table 1).

Table 1: Unwanted accompanying organisms in commercial mycorrhizal inoculum with and without quality control declared on the packing

	<b>Inoculum No.</b>					
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
<b>Contaminants</b>	<b>with quality control</b>			<b>without quality control</b>		
<b>Fungi</b>	+	+	-	+	+	+
Potential phytopathogens	-	+	+	+	-	+
Hyperparasites	+	-	-	-	-	-
Saprophytes	+	+	-	+	+	+
<b>Phytophagus faunistic elements</b>						
Diptera (-larvae)	-	-	-	+	-	-
Coleoptera (-larvae)	-	-	-	-	+	-
Collembola	-	-	-	+	+	-
Acari	-	-	-	+	+	+
Nematoda	-	-	-	-	-	+
Gastropoda	-	-	-	-	-	-
<b>Plants/seeds</b>						
Algae	+	+	-	+	+	+
Weeds	-	-	-	-	+	+

## CONCURRENT QUALITY CONTROL DURING INOCULUM PRODUCTION

Most of the contaminations of mycorrhizal inoculum could be avoided by concurrent quality control procedures (Table 1). The protocol for the achievement of high quality inoculum without contaminations covered the following relevant aspects:

- Certification of substrate quality necessary for inoculum production: avoidance of pre-contaminations (of special importance in case of peat substrates)
- Suitable preparation of the substrate with special respect to the porosity: guarantee of good aeration for best root development
- Moderate irrigation to prevent the substrate from water logging and from the establishment of algae and moss
- Control of water quality (risk of contamination high if water from a well is used)
- Low, but frequent fertilization to optimize the forming of the symbiosis and to prevent from luxury growth and excessive root exudation of host plants
- Frequent investigation of host plants in order to detect disease and pests
- Adequate phytosanitary actions to eradicate disease, pests or weeds
- In case of disease or pest epidemic application of adequate plant protection actions
- Frequent measuring of propagule development of the target organism to cut the inoculum production time as short as possible
- Drought period at the end of the production without substrate disturbance followed by nearly complete disappearance of microfaunistic elements
- Removal of host plant parts and rapid drought of the substrate to reduce saprophytic activities

## POST-HARVEST QUALITY CONTROL

The post-harvest inoculum control investigates the outcome of the inoculum production after drying and mixing the substrate, but before packing it. The post-harvest quality control includes several physical properties of the inoculum, biological measures to describe quantity and effectivity of the mycorrhizal fungus multiplied (for details see von Alten et al., 2002), and a survey of accompanying organisms according to Table 1. Of principal importance is the focus on species and/or genus specific detection of numerous plant pathogens as well as beneficial fungi (Table 2). This test could be out-sourced because it is recently offered as service by an independent company ([www.DNA-SCAN.de](http://www.DNA-SCAN.de)).

Table 2: Spectrum of microorganisms checked in mycorrhizal inoculum after harvest

<i>Athelia (Sclerotium) rolfsii</i>	<i>Phytophthora citricola</i>	<i>Pythium spp.</i>
<i>Botrytis cinerea</i>	<i>Phytophthora cryptogea</i>	<i>Pythium sylvaticum</i>
<i>Colletotrichum acutatum</i>	<i>Phytophthora drechsleri</i>	<i>Pythium ultimum</i>
<i>Colletotrichum coccodes</i>	<i>Phytophthora fragariae</i>	<i>Ralstonia solanacearum</i>
<i>Colletotrichum fragariae</i>	<i>Phytophthora infestans</i>	<i>Rhizobium radiobacter</i>
<i>Colletotrichum gloeosporioides</i>	<i>Phytophthora nicotianae</i>	<i>Rhizoctonia solani</i>
<i>Colletotrichum spp.</i>	<i>Phytophthora ramorum</i>	<i>Sclerotinia minor</i>
<i>Cylindrocarpon destructans</i>	<i>Phytophthora spp.</i>	<i>Sclerotinia sclerotiorum</i>
<i>Cylindrocladium spp.</i>	<i>Plectosphaerella cucumerina</i>	<i>Sclerotinia spp.</i>
<i>Didymella spp.</i>	<i>Pseudomonas cichorii</i>	<i>Sclerotinia trifoliorum</i>
<i>Fusarium oxysporum</i>	<i>Pseudomonas marginalis</i>	<i>Thielaviopsis basicola</i>
<i>Fusarium solani</i>	<i>Pseudomonas syringae</i>	<i>Trichoderma asperellum</i>
<i>Fusarium spp.</i>	<i>Pseudomonas syringae pv. porri</i>	<i>Trichoderma hamatum</i>
<i>Gnomonia comari</i>	<i>Pseudomonas viridiflava</i>	<i>Trichoderma harzianum</i>
<i>Penicillium spp.</i>	<i>Pyrenochaeta lycopersici</i>	<i>Trichoderma spp.</i>
<i>Phoma destructiva</i>	<i>Pythium aphanidermatum</i>	<i>Verticillium albo-atrum</i>
<i>Phytophthora cactorum</i>	<i>Pythium dissotocum</i>	<i>Verticillium dahliae</i>
<i>Phytophthora capsici</i>	<i>Pythium irregulare</i>	<i>Verticillium spp.</i>
<i>Phytophthora cinnamomi</i>	<i>Pythium polymastum</i>	<i>Xanthomonas fragariae</i>

## IS THE CONTROL OF TRADE WITH MYCORRHIZAL INOCULUM ADVANTAGEOUS?

Trade with mycorrhizal inoculum is a reality all over Europe. Inoculum is transported between East and West and sometimes is crossing five borders without knowledge of the authorities. Very often, the carrier material of the inoculum itself is declared and not the micro-organisms bound to it. Would the duty to declare mycorrhizal inoculum handicap the trade of such products? We as producers of inoculum together with consultants of national authorities involved in questions of unwanted organisms demonstrated since years that we are prepared to carry responsibility for consequences resulting from our products. Therefore, we developed high standards of quality control which limit the potential risk of mycorrhizal inoculum already in the pre-competitive phase of the broad introduction of the mycorrhizal technology. Declaration at the borders would support our efforts to offer our customers high

quality goods with reliable characteristics instead of low quality goods without any certification.

The producers of mycorrhizal inoculum unified in the Federation of European Mycorrhizal Fungi Producers (FEMFiP) are supporting companies and other entities involved in the production and/or developing products based on mycorrhizal fungi across Europe. As already cited, particular aims are to achieve and maintain the highest standards of inoculum quality. This will be done by educational and training programmes aimed at promoting using of mycorrhizal fungi as a technology suitable for sustainable agriculture, forestry, horticulture, landscaping etc. Methods for evaluating the quality of inocula will be standardized and certification programme for producers will be introduced. The procedures for compliance will be developed in collaboration with its members and administered through the offices of an independent laboratory. FEMFiP aims to participate to the discussions for establishment of registration procedures of products based on beneficial rhizospheric micro-organism being prepared by European Committee. The quality control procedures outlined and the later certification of the products will support to close an important gap with view to the spread of unwanted organisms.

## LITERATURE

- Backhaus, G. F., Feldmann, F. (1997): Anwendung arbuskulärer Mykorrhizapilze im Pflanzenbau. Mitt. Biol. Bundesanst. für Land- und Forstwirtsch. 332, 85 pp.
- Feldmann, F., Hutter, I., Grotkass, C. (2003): Mycorrhizal fungi as factors of integrated plant protection in urban horticulture: the state of the art, Mitt. Biol. Bundesanst. für Land- und Forstwirtsch. 394, 205-210.
- Feldmann, F. (2003): Weltweiter Handel mit Inokulum arbuskulärer Mykorrhizapilze – Eine Risikoanalyse, Schriftenreihe BMVEL, Angewandte Wissenschaft, Heft 498, 165 – 175.
- von Alten, H., Blal, B. Dodd, J.C., Feldmann, F., Vosatka, M.(2002): Quality control of arbuscular mycorrhizal fungi inoculum in Europe. In: Gianinazi, S., Schuepp, H., Barea, J.M., Haselwandter, K.: Mycorrhizal Technology in Agriculture, 281-296.