

## Quality Control of Arbuscular Mycorrhizal Fungi Inoculum

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### Introduction

Most published papers by scientists working in the area of mycorrhizal fungi will mention in the first few lines of their introduction the potential importance of these fungi as a natural biotechnology and of potential applications of their data. The growing number of new small to medium sized companies (SMEs) around the world (Sylvia, 2001) producing inocula of mycorrhizal fungi indicates that many scientists have seen market opportunities for the commercial use of these fungi increase in the last decade. Many companies have therefore "spun-out" of the academic and research world into the business world. It is at this point that, in recent years at least, their products have come under increasing scrutiny by fellow scientists and the end-users alike. Many find that the promises made about their product and the results seen by the end-users are often world's apart. This has led to sweeping generalisations, positive and negative, about the efficacy of mycorrhiza products currently available. As natural biological agents and, for arbuscular mycorrhizal fungi at least, non-axenically culturable fungi, there are great problems in presenting

the product in the best state for the target markets. Some have taken the approach of single formulations for every market whilst others produce a range of products for their target buyers. Whatever the approach, it is increasingly likely that greater regulation and controls over the production and selling of such inocula will be introduced in the coming years. This is the reality of the business world but scientists and businesses alike need to begin to look at how a series of "Best Practices" can be adopted by these SMEs to allow the market to develop. At present regulation of these products varies between countries in Europe with some having very tight regulations e.g. France while others are less demanding. Over-regulation will prevent the development of SMEs and could destroy the market for what is potentially one of a few natural microbial biotechnologies available for natural plant production.

In 2002, a group of European SMEs have come together to compare notes and make suggestions for a way forward (Alten et al, 2002). This did not lead to a voluntary code of best practice, and even a Federation of Mycorrhizal Fungi Inoculum Producers in Europe could not progress in agreeing about principles of inoculum production. In fact, each company still has its specific quality procedure adapted to its specific demands and target markets. Our article here demonstrates the principal problems of the quality assurance, control and efficacy issues of mycorrhizal products like carried out in Germany since some years.

### The Issues in Quality Control

There are many issues associated with "hying" the potential benefits of using mycorrhizal fungi but these are no different from the marketing of other biological products. Trying to sell a product, which can (according to the literature):

1. increase plant P uptake and reduce demands for fertilisers
2. potentially increase plant growth and crop uniformity
3. reduce plant mortality
4. reduce root disease
5. increase plant tolerance of pollutants
6. allow earlier and better flowering
7. increase soil aggregation (soil structure)

8. increases tolerance of water stress
9. act as a mechanism for ecological land restoration

amongst other claims, means that it can have a broad range of possible benefits to the end-user. Many of these can justifiably be used to support the use of AMF as a natural "Plant Health Insurance". There are criteria that should be fulfilled by the producer of inoculum; one is that in recommending use of the product that the plants treated should form mycorrhizas as a result (given proper use of the product); secondly that the producer has taken every care in producing a product free of potential agents which could negatively affect normal plant growth and development; and last that while the production costs have to be kept to a minimum the shelf-life of the product should be sufficient to suit the end-user markets. This should mean that the producer also has a degree of responsibility to educate the end-user and supply back up support. We will look here at several aspects of Quality Control and Product Declaration, knowing that not all of them will be listed in a final agreement between SMEs on the European level.

## **1. Declaration of physical and chemical properties of AMF inoculum**

### Nutrient content of the inoculum

Customers who intend to introduce mycorrhizal fungal inoculum to target plants must be provided with some basic information concerning the chemical and physical characteristics of the inoculum. The nutrient content and the pH of the substrate solution can be of special importance if high doses of inoculum must be used under controlled fertilizer regimes. Besides this it must be possible to differentiate between the effects of additives like fertilizers or gels and the AMF themselves. Because of the processing a certain amount of nutrients will unavoidably remain in the substrate of conventionally produced AMF inoculum. Nevertheless the processing can reduce the phosphate (P) content of the substrate (Table 1). Conventional soil analytic examinations in independent official laboratories can provide inoculum producers with the necessary information about the macro-nutrient content of the inoculum at no great expense. Additionally the independent laboratory can provide a certificate,

which guarantees the customers the validity of the data. This enables AMF effects and available nutrient influences to be separated when using the inoculum.

**Table 1.** Fertilizer content of a commercial inoculum (IFP 01/99). The analysis was carried out in an independent laboratory (LUFÄ, Germany) with standard methods of soil analysis (data presented by IFP).

Nutrients	content [mg/l]
Salt content (KCl)	912
Nitrogen (N)	27
Phosphate (P <sub>2</sub> O <sub>5</sub> )	7
Potassium (K <sub>2</sub> O)	29
Magnesium (Mg)	87

Excessive amounts of added nutrients or organic compounds can inhibit seed germination even if they are useful for improving plant growth and mycorrhization in latter stages of plant development. Therefore if inocula are combined with biofertilizers or slow release fertilizers, careful tests should be undertaken to check the inhibition of seed germination. Amendment of inocula by additives should be primarily aimed to support mycorrhiza development and therefore the components of these additives should not be general fertilisers but they have to be specifically tuned to be compatible with AMF.

#### The pH of the inoculum substrate

Information about the substrate pH is of little importance for the target plant production system because of the relatively low amounts of inoculum (normally 10 % v/v) applied to a normally well-buffered soil system. Nevertheless, the range of adaptability of the AMF population within the inoculum to variable pH conditions can be estimated from the basic information provided by the inoculum producer. To our knowledge (IFP), the majority of AMF propagules of an AMF inoculum colonise best under the previous production conditions. Inoculation under differing pH regimes may often require a longer time to show the desired mycorrhizal effects.

## Carrier material

AMF can be bound to a wide range of carrier materials (see e.g. Backhaus. and Feldmann, 1996, Jarstfer and Sylvia, 1994). Information about the carrier material is important for the subsequent inoculation procedure. For instance, the inoculation of plants on "roof tops" requires a carrier, which effectively protects the AMF propagules during the "blowing-up" procedure of the substrate. In our experience (IFP), turf substrates or expanded clays did not survive this rough procedure but other materials like lava were resistant enough and guaranteed effective AMF colonization after a short time. Similarly attapulgitic clays, expanded clays or other inert carriers have been very successful carriers in Horticulture (companies Biorize, France, Mycotec, Germany) and Landscaping (Plantworks, UK). It, therefore, depends on the target use for the inoculum as to which carrier is best for mycorrhizal fungal propagules.

## **2. Testing propagule density**

### Quantification of mycorrhizal infection units ("MPN"-estimation)

The number of infection units in an inoculum of AMF depends on the number of spores, colonised root fragments and mycelial fragments, which can actually lead to root colonization under favourable conditions. The relevant number of propagules can be determined with various published techniques; the MPN standardized "most probable number" estimations (An et al., 1990; Daniels et al., 1981; Porter, 1979), IP inoculum potential assay (Liu & Luo, 1994) and spore counts. In practice, however, the MPN is not a constant and stable measure (Table 2). Within the first year after harvest the MPN in a commercial inoculum could vary significantly, probably due to inherent changes in spore maturity, spore dormancy phenomena and the activity of extraradical mycelium (ERM) or colonised root fragments as propagules for later root colonization. After four years of storage the MPN and the number of living spores was nearly identical indicating that the infectivity of the inoculum was mostly based on the spore content at this time. After eight years the remaining infectivity was still 17% of the maximum MPN and the content of living spores still reached

38% of the maximum count. These data maybe of special importance for inoculum producers and their customers calculating infectivity losses in the course of storage. Normally inoculum producers have as a policy of producing to order when possible and inoculum is recycled or discarded after 2-4 years.

**Table 2.** Most Probable Number (MPN) of propagules in a commercial inoculum (IFP 03/89)

Months after harvest	2	6	8	12	24	36	44	82	98
MPN [n]	73	54	94	116	91	68	28	24	23
	$\pm 9$	$\pm 5$	$\pm 7$	$\pm 12$	$\pm 4$	$\pm 3$	$\pm 6$	$\pm 4$	$\pm 1$
Spore numbers [n]	48	51	42	47	37	24	22	24	20
	$\pm 3$	$\pm 2$	$\pm 0$	$\pm 1$	$\pm 1$	$\pm 3$	$\pm 2$	$\pm 1$	$\pm 1$

The analysis was carried out according to Feldmann and Idczak (1994). The inoculum was stored at room temperature; the carrier material was standard soil (Einheitserde) Spores were counted after the method described by Daniels and Skipper (1984), the most probable number estimation followed the procedure of Porter (1979) and Feldmann and Idczak (1994). (Data provided by IFP)

The MPN estimation method, however, has its own limitations not the least the inference that one spore leads to one infection unit which underestimates the potential of spores of species of AMF, particularly those in the Gigasporaceae (Dodd et al., 2000). Comparisons between inoculum sources can only occur under single testing conditions due to specific host/AMF interactions, differences in substrates used and the prevailing environmental conditions. In a MPN estimation of a commercial inoculum six host varieties of four plant species were tested. The difference between the lowest and the highest MPN estimates in the same substrate was 477% (Table 3). Such a variation between data makes it impossible to compare estimates made by different laboratories using the MPN procedure, unless attempts at standardisation were made (identical abiotic and biotic factors).

**Table 3.** Most probable number estimation (MPN) of propagules in an inoculum of *Glomus etunicatum* with different test plant species

Test plant	Zea mays		Petroselinum crispum		Linum usi-tatissimum	Anagallis arvensis
Variety	Felix	Bad.LM	Blizzard	Mooskrause		
MPN	$13 \pm 7$	$20 \pm 8$	$28 \pm 6$	$32 \pm 4$	$37 \pm 12$	$62 \pm 9$

Before the test a spore content of 50/ml was counted in the substrate after wet sieving. The experiment was repeated three times (data provided by IFP).

This raises the possibility of creating an independent testing service, which could be used by producers in Europe to check that batches of inocula meet the baseline standards established and agreed by individual companies – a voluntary code of best practice. Such a code has been offered to US producers (Sylvia, 2001) but at a price of US\$925.

### Spore numbers

As mentioned earlier spore numbers present can be used as a measure that is more stable than the MPN when single species AMF (*Glomus* or *Gigaspora* spp.) products are used. Unfortunately the method of spore extraction from the substrate and the type of carrier influences the number of spores counted. Usually the mycorrhizal spores of an inoculum are extracted by wet sieving procedures (Pacioni, 1994, <http://www.bio.ukc.ac.uk/beg/Protocols/extraction.htm>). Variations in the procedure like preparation of substrate samples, centrifugation times, sieve mesh width and so on can, of course, influence the extraction results. But even a standardized extraction procedure leads to mis-estimations if inoculum carriers are tested which retain the spores and do not allow the extraction (Table 4).

**Table 4.** Spore yield from an AMF inoculum on different carrier materials after wet sieving (data from IFP)

Carrier type	spore number [n/g] <sup>a</sup>	extractable spores [n]	extractable spores [%]	living spores [%]
Expanded clay	115 ± 39	23 ± 19	23 ± 6	78 ± 5
Standard soil	76 ± 28	48 ± 23	59 ± 4	87 ± 4
Quartz sand	57 ± 36	49 ± 29	84 ± 7	88 ± 4

<sup>a</sup>The spore number was counted microscopically after diluting 1g of substrate in water. The same substrate sample was wet sieved afterwards and the extractable spores counted. The portion of living spores was counted according to Glenner (1977). The experiment was repeated three times.

It could be argued that inoculum producers should provide information about the number of extractable spores in their product but as many have shown the infectivity of colonised root fragments is greater than that of spores (Sieverding, 1991). It is also important to distinguish end use of inoculum in that certain inoculum formulations will have to function in the field situation (landscape use) not in greenhouse assays or production (horticulture) so the adaptability of spores of AMF

alone to alien soil conditions may mean that they do not germinate at such high rates (Dodd & Krikun 1984; Tommerup, 1983). It is clear, therefore, that only spore counts along with the MPN would serve as a basis for dilution recommendations.

There are other sources of inoculum e.g. aeroponically produced AMF (Sylvia & Jarstfer, 1992; Jarstfer & Sylvia, 1994) and *in vitro* colonised transformed roots (Declerck et al., 1996) are potential alternative inoculum sources but have been successful with a limited number of AMF, cannot be provided in commercial quantities as yet nor at commercially acceptable rates.

### **3. Guaranteed effectiveness of AMF inoculum**

This again finds considerable debate between companies involved in the commercial production of AMF. It has been noted that there are specific host/AMF strain relationships or perhaps "preferential selection" of AMF by plants (Dodd et al. 1990a,b) and the idea of functional compatibility has arisen as a consequence. This maybe particularly important where single species AMF inocula are produced on monocultures of plants. The same is true in case of qualitative estimates such as predicted effectiveness of the inoculation process. No prediction of the future mycorrhizal effectiveness (as "strength of desired effect") can be given by the producer if the plant the inoculum is produced on is not the target plant because the outcome of a symbiosis depends on environmental factors, AMF characteristics and plant variables (Table 5). Subsequent multiplication on the same host genotype could lead to a decrease of mycorrhizal efficacy. This is probably due to AMF population dynamical processes because it can be reversed by use of other host varieties of the same species (Feldmann, 1997).

Furthermore, the type of desired effect can be decisive for the selection of a fungal strain. Strains that enhance biomass in some plants may not increase the stress tolerance of their host and vice versa. In different end-user markets it may be more appropriate that the selection that the consortia of AMF used establish on trees for the long-term ecological benefits rather than biomass increases. Similarly, increased flowering by inoculated grasses maybe more beneficial than greater vegetative biomass (Dodd et al., 2001). Companies often use multiple host plants in the production of their mixed AMF consortia to increase the potential range of benefits

from inoculation. The directed inoculum production process (Feldmann and Grotkass, 2002) appears to cover most phenomena and helps to enhance the predictability of AMF inoculum effectiveness because of a technologically adaptation between fungal partner and target plant species with regard to desired effects and explicit environments.

**Table 5.** Effectiveness (MEI) of three AMF strains on the biomass accumulation of different host plants inoculated with subsequently produced inocula (data provided by IFP).

Test year	<i>Glomus etunicatum</i> HH6				<i>Glomus etunicatum</i> HH13				<i>Glomus intraradices</i> HH267			
	1.	2.	3.	4.	1.	2.	3.	4.	1.	2.	3.	4.
<i>Zea mays</i>	<b>31</b>	<b>20</b>	<b>15</b>	7	<b>43</b>	<b>37</b>	<b>14</b>	<b>14</b>	<b>27</b>	<b>24</b>	4	5
<i>Pelargonium zonale</i>	<b>26</b>	-	<b>49</b>	<b>30</b>	<b>28</b>	-	<b>56</b>	<b>25</b>	<b>20</b>	-	<b>23</b>	<b>28</b>
<i>Trifolium repens</i>	-	<b>12</b>	<b>20</b>	-	-	<b>30</b>	-1	-	-	<b>13</b>	-1	-
<i>Petroselinum crispum</i>	-	9	<b>13</b>	<b>21</b>	-	<b>11</b>	-8	-4	-	<b>17</b>	-5	<b>21</b>
<i>Baptisia tinctoria</i>	-	7	-	18	-	5	-	<b>20</b>	-	-2	-	5
<i>Helianthus annuus</i>	1	-3	-	-	-4	2	-	-	5	5	-	-
<i>Triticum aestivum</i>	1	4	-2	-	<b>-16</b>	-9	<b>-15</b>	-	9	<b>19</b>	-1	-

Values printed in bold are significantly different (t-test,  $p < 0,05$ ) from values for control plants. The mycorrhizal effectiveness index was calculated according to Plenchette et al. (1983). Positive values indicate an increase of fresh weight; negative values demonstrate lower fresh weight than in control plants. The number of plants was  $n=30$  per year; the maximum standard deviation was 9.5% of the cited average. The inoculum for the tests was produced each year in a subsequent process on *Zea mays* cv. Felix.

Nevertheless, a quality control of commercial inoculum must deal with the cited problems and find procedures which give the customer some basic information he needs for the decision to introduce effective mycorrhizal fungi. We A "reference system" of information concerning AMF effectiveness could include results from standard tests (Table 6) and furthermore a list of examples where the relevant inoculum had already successfully be used before (Feldmann, 1998). This could be presented in associated company literature or on the company's website.

**Table 6.** Mycorrhizal effectiveness index of two commercial inocula on three test plant varieties of different mycorrhizal dependency under standardised conditions (data from IFP).

Commercial inoculum	<i>Zea mays</i> Blizzard	<i>Tagetes erecta</i> Orange Prince	<i>Phaseolus vulgaris</i> Saxa
IFP	48 ± 12	27 ± 9	14 ± 2
Mycotec	44 ± 7	31 ± 7	7 ± 4

Note: the mycorrhizal dependency of a test plant is a variable of the chosen environmental conditions! The standard test was carried out in climate chambers with light ( $480\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) of e.g. SON-T AGRO 400 Philipps bulbs, 14h/d; minimal temperature 15°C, maximum temperature 25°C; 40-60% relative air humidity; irrigation below field capacity; substrate 30% loam, 50% quarzsand, 10% peat and 10% expanded clay; fertilization 2 times per week with a quantity calculated as 10% of the pot volume. The fertilizer used is Flory 9 (1%), pH 5.5; content: 15% N (10% Nitrate, 5% Ammonium), 7% P<sub>2</sub>O<sub>5</sub>, 22% K<sub>2</sub>O, 6% MgO, 0.03% B, 0.05% Mn, 0.01 Zn. Three test plant species were chosen which were known to be hosts but a) highly dependent on AMF to reach maximum growth under the relevant conditions, e.g. *Zea mays* cv. Blizzard, b) intermediately dependent, e.g. *Tagetes erecta* cv. Orange Prince, and c) of low dependence, e.g. *Phaseolus vulgaris* cv. Saxa

If these data are provided for customers they can be sure that the offered inoculum has a principle ability to act mutualistically with the target host. Nevertheless, they should not compare inocula on the basis of the tests. The real validation can only occur if the target plant was included in the efficacy tests.

#### 4. Detection of microbial contaminants

As long as the production of large (m<sup>3</sup> or tons) amounts of AMF inocula is connected to the necessity for open culturing of plants in non-sterile greenhouses or open-air systems, these inocula will not be free from other associated microorganisms. Bacteria and fungi are present on particles of the substrate used as carrier material for propagules of AMF. The composition of the microbes found varied with the host plant and in particular with the AMF isolate used. These microorganisms may include saprophytes that live in the rhizosphere of the host plants; however, there is the possibility that phytopathogenic organisms could be transferred via the inoculum from one host plant to the next albeit in very low propagule numbers. In contrast there are micro organisms that can accelerate the development of the symbiosis or improve plant health. They are called MHB (mycorrhiza helper bacteria) or PGPR (plant growth promoting rhizobacteria).

To avoid unwanted micro-organisms producers have in principle two possibilities, the selection of a host plant for inoculum production that is more or less resistant to root diseases or the control of root health during production as often as possible, and using all measures to keep away soil-borne pathogens from the host plants.

The selection of a suitable host plant is of primary importance. In general the host plant should never be the same as the plant to be inoculated later by the user of the inoculum during production. This is a basic rule of plant pathology and does not contradict with the idea of directing inoculum production. The efficacy of the mycorrhizal fungi is improved and transfers of specific pathogens e.g. the wilt pathogens within the genus *Fusarium* is avoided. It should be noted that the detection of the genus *Fusarium* does not infer pathogenic presence, as many species are saprophytic. Non-specific pathogens causing root-rots, like species of *Pythium*, *Rhizoctonia*, and *Thielaviopsis* are potentially much more dangerous. If present they can threaten many possible target host plants. It is best if these fungal pathogens can be excluded from the inoculum production totally.

Contamination and spread of plant pathogens can be avoided by Good Horticultural Practice (GHP, e.g. Feldmann et al., 1999). If there is any risk of infection suitable fungicides can be used to control pathogens of the host plant where the product is not used for the organic market. A removal of pathogens from contaminated inocula at present is not possible because of the recently changing legal situation of Plant Production Products. However, due to the fact that fungicides have to be chosen for the host plant/pathogen combination without consideration of AMF they can be severely influenced by the fungicide application itself. In older studies oomycetes having a special metabolism, could be controlled easily using fungicides like prothiocarb, which can selectively kill the pathogen but leave the AMF unaffected. Given the background of new legal regulations (directive 91/414/EEC) there is an urgent need to find and also to test registered plant protection products, which are useful in the inoculum production process. Preliminary studies are on the way and will be presented soon.

For the producer of AMF inoculum it is surely impossible to guarantee zero presence of pathogens for his material if the inoculum is produced in open pot culture, even if they took all precautions. In consequence a quality control of AMF inoculum must cover this aspect of unwanted presence of micro organisms. When

selling his material the producer needs to know whether they might have to face any economic risk arising from claims for indemnity. Some companies take out a liability insurance of some sort. However, the strict European product liability legislation has the consequence that insurance policies will not protect pathogen contamination. So producers cannot relax about undertaking appropriate checking procedures for potential pathogens.

During the inoculum production root samples are microscopically checked for the presence of potential pathogenic fungi. Of course it will not be possible to check each plant or each pot, however, root samples are taken in a representative way to provide a maximum of safety. These microscopic checks could become a standard operating procedure for companies and scientific institutions working with the commercial production of AMF and plant pathogens. For SME's, however, there can be an economic problem, not only a good (often expensive) microscope is needed but also personnel with the ability to recognise and identify pathogens as well as to judge their relative significance.

Additional tests can also be used to back-up microscopic examination, for example, a simple possibility is the use of trap plants for inoculation with the inoculum to be tested. The plants used must be highly susceptible to root pathogens. It might even be appropriate to use assortments of plant species covering susceptibilities to all possible pathogens but this may not be feasible. The ideal trap plant should germinate and grow fast be extremely susceptible and show clear symptoms easily recognisable even for unskilled personnel. One example for such a plant is cress because it is very susceptible to root rots caused e.g. by *Pythium* spp. Seeds of *Lepidium sativum* (Cress) can be sown in a Petri-dish containing a sterilized attapulgate clay control as well as pure inocula and dilutions (recommended application rates) of AMF. The percentage germination can be assessed after 8 days of culture in the dark.

Arbuscular mycorrhizal fungi can colonise an enormous variety of plants. So inoculum of these fungi can be used for the majority of plants cultured. A producer of commercial inoculum can offer its product for use in only one plant species or as a more non-specific promoter of many plants. Even when the inoculum is used only with one species different cultivars can show significant differences regarding susceptibility to root pathogens. In consequence the best way of testing for

contaminating pathogens is to use the same plant cultivar under more or less the same growing conditions as the buyer of the inoculum would do. This should be part of the service to be sold with the inoculum together with a small screening for the best AMF isolate for the customer's plants.

## **Conclusions**

The quality control of AMF inoculum recently is still an obligation of the inoculum producing companies and is not yet under control of independent institutions. The producer declares, e.g. on a control sheet which is accompanying the product, what he proved, which methods he used and what results he found. As an example the control sheet of the Committee of Mycorrhizal Application Germany (CMAG, 1997) is demonstrated in Table 11.

During consultation (or on the package) the producer should in addition give a recommendation of maximum and/or minimum dilution factors for the inoculation process but use of the optimum amount can be specified to ensure mycorrhization. The producer can also state any relevant adaptations of their fungi to target conditions, which will help the end-user choice. The aim of this is to ensure that the buyer, in paying a premium price for a mycorrhiza product, is receiving a product that should, if used properly, ensure mycorrhization of the plants treated not merely a support carrier or the action of other additives in the mix. The issues involved with ectomycorrhizal fungi and their infectivity characters and shelf-life have not been discussed here but it should be noted that some products do mix AMF and ECMF in their mixes for the tree markets.

The companies unified under the cover of this article agree that independent institutions should carry out the quality control of mycorrhizal products, possibly on the national level or even on the European level. It is not clear who could do that job, but first contacts to independent laboratories do exist in different countries. The criteria, which have to be proved, will be defined by the market for mycorrhizal fungi inoculum itself and will hopefully be standardized on the European level after discussion between the related companies. We think that the foundation of a specific

certificate for quality of mycorrhizal fungi inoculum would lead to the spread of the idea to sell only high quality products on the European market.

**Table 11.** Quality control parameters of AMF inoculum according to the agreement of the Committee of Mycorrhiza Application Germany (CMAG, 12/1997)

<b>Test Parameter</b>	<b>value</b>
<b>pH</b>	
<b>Content of fertilizer of the substrate [mg/l]</b>	
Salt content (KCl)	
Nitrogene (N)	
Phosphate (P <sub>2</sub> O <sub>5</sub> )	
Potassium (K <sub>2</sub> O)	
Magnesium (Mg)	
<b>AMF species/strain</b>	
<b>Most probable number of propagules</b> (on <i>host plant variety</i> ...[n/cm <sup>3</sup> ])	
<b>Effectiveness</b> (on <i>Zea mays</i> , <i>Tagetes erecta</i> , <i>Phaseolus vulgaris</i> ) [MEI]	
<b>Germination inhibition</b> (on <i>Lactuca sativa</i> , <i>Lolium perenne</i> , <i>Phaseolus vulgaris</i> , <i>Lepidium sativum</i> )	
<b>Fungal contaminants</b>	
Potential phytopathogens	
Hyperparasitic fungi	
other saprophytic fungi	
<b>Pathogenicity of contaminants</b> (on <i>Tagetes erecta</i> , <i>Zea mays</i> , <i>target plants</i> )	
<b>Potential phytophageous faunistic contaminants</b>	
Diptera	
Coleoptera, -larva	
Collembola	
Acari	
Nematoda	
Gastropoda	
<b>Botanical contaminants</b>	
Algae (Diatomeae, Cyanophyceae, Chlorophyceae)	
“Weeds”	

Each company, of course, defines the marketing strategy. But we would like to stress a point of special importance: no producer of mycorrhizal fungi inoculum

should declare, as an intended use of the product, the phytosanitary effect of the inoculum. If the marketing strategy points out any effect against phytopathogens the mycorrhizal fungi inoculum would have to be treated as biological plant protection product with the consequence of the necessity to reach authorised registration under the directive 91/414/EEC. The costs of that process would eliminate our attempts to introduce the mycorrhizal technology to plant production systems. This problem already was intensively discussed in the Cost Action 8.38 and formulated in a related position paper (Annual report of COST 8.38, 1999). That paper, in addition deals, with data requirements for a registration process that seems to be of low importance if no one is trying to register mycorrhizal fungi inoculum as a plant protection product. We, as inoculum producers, would appreciate support by the Cost Action 8.70 to find viable and low cost ways of quality control and proposals for the establishment of that quality control in independent institutions. Furthermore rapid and accurate methods, e.g. PCR-techniques have to be adapted to the demands of quality control. For example, not only AMF species have to be separated in the quality control, but even strains and sub-strains. That problem remains unsolved. Research activities of European partners of the Cost Action 8.70 are needed on another field of quality control: we urgently need rapid assessment protocols for the recognition of the degree of host dependency on AMF.

The aims of controlling unwanted microorganisms in inocula have been discussed above and approaches to limiting their impact made. The use of mycorrhizal fungi for natural plant production is still in its infancy and will require added value by companies to reassure end-users of its great potential. This means more information and guidance to growers and not a hard-sell approach. There are few natural biotechnologies available to aid sustainable plant production and the risks in not applying these are that little or no funds for applied research will be made available for scientists. It is therefore imperative that scientists and business collaborate more regularly to develop this market, as much further research will be needed to tune the products for the markets. We hope that this article is taken as a first attempt to bridge the mycorrhizal science-business gap.

## References

- An Z-Q, Hendrix JW, Hershman DE, Henson GT (1990) Evaluation of the "Most Probable Number" (MPN) and wet-sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. *Mycologia*, 82:576-581
- Annual report of COST 8.38, 1999:  
<http://www.dijon.inra.fr/cost/cost838/rapports/rapport1999.html>
- Backhaus GF, Feldmann F (1996) Mykorrhiza in gärtnerischen Substraten - endlich einsatzreif? *Taspo Gartenbaumagazin* 4:12-14
- Daniels BA, Skipper HD (1984) Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck NC (ed) *Methods and principles of mycorrhizal research*, The American Phytopathological Society, St. Paul, Minnesota, USA, 29-38
- Declerck S, Strullu DG, Plenchette C (1996) In vitro mass-production of the arbuscular mycorrhizal fungus, *Glomus versiforme*, associated with Ri T-DNA transformed carrot roots. *Mycol. Res.* 100:1237-1242
- Dehne H-W, Backhaus GF (1986) The use of vesicular-arbuscular mycorrhizal fungi in plant production. I. Inoculum production. *J. Plant Dis. a. Prot.* 93 (4): 415-424
- Dodd JC, Dougall TAG, Clapp JP, Jeffries P (2001) The role of arbuscular mycorrhizal fungi in plant community establishment at Samphire Hoe, Kent, UK –The reclamation platform created during the building of the Channel Tunnel. *Biodiversity and Conservation* (In Press)
- Dodd JC, Krikun J (1984) Observations on endogonaceous spores in the Negev desert (Israel). *Transactions of the British Mycological Society* 82:536-540
- Dodd JC, Boddington CL, Rodriguez A, Gonzalez-Chavez C, Mansur I (2000) Mycelium of Arbuscular Mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant and Soil* 226(2):131-151
- Dodd J C, Arias I, Koomen I, Hayman DS (1990a) The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem. I. The effect of pre-cropping and inoculation with VAM-fungi on plant growth and nutrition in the field. *Plant Soil* 122:229-240
- Dodd J C, Arias I, Koomen I & Hayman DS (1990b) The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem. II. The effect of pre-crops on the spore populations of native and introduced VAM fungi. *Plant Soil* 122:241-247
- Feldmann F (1997) Stabilität und Reproduzierbarkeit der Wirksamkeit von Inokulum arbuskulärer Mykorrhizapilze. *Mitt. Biol. Bundesanstalt* 332, 54-65
- Feldmann F (1998) *Symbiontententechnologie in der Praxis: Arbuskuläre Mykorrhiza im Gartenbau*. Thalacker-Medien, Braunschweig, Germany. ISBN 3-87815-109-8
- Feldmann F, Idczak E (1994) Inoculum production of VA-mycorrhizal fungi. In: Norris JR, Read DJ, Varma AK (eds) *Techniques for mycorrhizal research*. Academic Press, San Diego, 799-817
- Feldmann F, Hutter I, Niemann P, Weritz J, Grotkass C, Boyle C (1999) Einbindung der Mykorrhizatechnologie in die Heil- und Zierpflanzenproduktion sowie den Endverkauf. *Mitteilungen der Biologischen Bundesanstalt* 363:6-38
- Glenner GG (1977) Formazans and tetrazolium salts. In: Lillie RD (ed) *Biological stains*, Williams & Wilkins Co., Baltimore, 225-235

- Jarstfer AG, Sylvia DM (1994) Aeroponic culture of VAM fungi. In: Varma AK, Hock B (eds) *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology*. Springer-Verlag, Berlin, 427-441
- Liu R-J, Luo X-S (1994) A new method to quantify the inoculum potential of arbuscular mycorrhizal fungi. *New Phytol.* 128: 89-92
- Plenchette C, Fortin JA, Furlan V (1983) Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant Soil* 70:199-209
- Porter WM (1979) The most probable number method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research*, 17:515-519
- Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agro-systems. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Eschborn, Germany.
- Sylvia DM, Jarstfer AG (1992) Sheared-root inocula of vesicular-arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* 58:229-232
- Sylvia DM, Jarstfer AG (1994) Production of inoculum and inoculation with arbuscular mycorrhizal fungi. In: Robson AD, Abbott LK, Malajczuk N. (eds) *Management of Mycorrhizas in Agriculture, Horticulture and Forestry*. 231-238
- Sylvia DM (2001) <http://dmsylvia.ifas.ufl.edu/Commercial.htm>
- Tommerup IC (1983) Spore dormancy in vesicular-arbuscular mycorrhizal fungi. *Trans. Brit. Mycol. Soc.* 81:37-45