# Effects of Soil Management on Arbuscular Mycorrhizal Fungi in Autumn-Sown Crops in Mediterranean Climates

*Isabel Brito, Mário de Carvalho, Diederik van Tuinen and Michael J. Goss*

## Abstract

Soil tillage may reduce the rate of arbuscular mycorrhiza (AM) establishment markedly by breaking up living AM fungal mycelium. No till or reduced till management can allow earlier AM formation because less damage occurs to mycelium.

Work under field conditions in a Mediterranean climate clearly confirmed that wheat plants cultivated under no-till system had a 6 fold greater mycorrhizal colonization than those grown using a conventional tillage system.

Pot experiments were initiated to determine the benefit of the timing of colonization on plants. Soil disturbance induced by tillage practices was simulated by passing the soil through a 4 mm sieve at the start of each successive period of 3 weeks plant growth cycles. After 4 cycles of plant growth with winter wheat, significant effects in all colonization parameters were detected. Arbuscular, vesicular and hyphal colonization were clearly higher in undisturbed soil.

To gain a global overview of the diversity of Glomeromycota under the 2 cultivation systems in the experimental field, rDNA sequences from the fungi have been amplified successfully from DNA extracted directly from field soil. In total, 87 sequences were analysed, half from each kind of soil (undisturbed and disturbed). Based on differences observed in the frequency of the ribotypes present in soils under different tillage treatments, the results support the view that AMF are differently vulnerable to soil disturbance, not only in terms of diversity, but also in terms of the community structure.

**Keywords**: Arbuscular mycorrhiza, tillage, wheat, diversity.

## Introduction

Arbuscular mycorrhiza (AM), the most widespread plant-fungus symbiosis on earth, is formed by soil fungi of the Phylum Glomeromycota and roots of a majority of the terrestrial plants. More than 70% of all plant species in both agricultural and natural environments form mycorrhizal associations and they can be found in a wide range of habitats (Smith and Read, 1997), including deserts, lowland tropical rainforests, high latitudes and altitudes and aquatic ecosystems.

ISBN 3-923381-52-2, US ISBN 1-59326-246-9

The AM is a mutualistic symbiosis where the fungus gains all of its carbon from the plant, between 10% and 20% of the photosynthates they produce (Graham, 2000). The extensive extraradical mycelium produced constitutes a link between the plant roots and the soil environment and, in exchange, transports mineral nutrients from the soil to the plant. This is particularly important for minerals that are transported through the soil by slow diffusion processes and are easily immobilized, such as P. The extraradical mycelium increases the soil volume accessible for mineral nutrient uptake, beyond the phosphorus depletion zone (Clark and Zeto, 2000) that is established around actively absorbing roots. Besides this, many other benefits are recognised for AM plants: water stress alleviation, protection from root pathogens (nematodes and fungi), tolerance to toxic heavy metals, tolerance to adverse conditions such as very high or low temperature, high salinity, high or low pH, better performance during transplantation shock and aggregation of soil particles (Gupta et al., 2000).

Changes in the chemical, physical and biological variables in the soil, that are important for the formation and function of arbuscular mycorrhizas, may be caused by human activities such as agriculture. Soil tillage has been shown to reduce the rate of mycorrhiza establishment by breaking up the living AM fungal mycelium in the soil, which can colonise roots of plant seedlings more rapidly. If the extraradical mycelium is disrupted, new AM formation will depend mainly on the slow growth of infective hyphae from spores or colonised root fragments. In soils that are less disturbed, the increase in AM colonization is commonly, though not always (McGonigle et al., 1999) associated with a comparative advantage to plants in the early growth period.

Agricultural practices that maximise utilisation of naturally occurring symbiotic organisms such as arbuscular mycorrhizal fungi (AMF) are desirable, due not only because of the impracticality of inoculating on large scale in a relatively harsh environment but also to aid development of a greater understanding of the significance of AMF diversity in the Mediterranean region (Abbott et al., 1995).

Some of the most important challenges in AM research include the need to find good and practical methods to describe communities and the identification and evaluation of functional fungal biomass in relation to effectiveness in nutrient acquisition. The need for strategies involving cross-disciplinary research for solving these questions becomes increasingly evident, and results are now emerging which are based on integration of molecular, physiological and ecological approaches (Jakobsen et al., 2004).

Our research investigated if conventional tillage and no-tillage regimes produce any effect on the mycorrhizal colonization of winter cereals in Mediterranean climate under a particular rotation (wheat, triticale, sunflower). We used a combination of field trials, pot experiments and molecular biology studies to determine the effects of soil cultivation on plant performance, AM colonization and on the AMF diversity. The data from this work will help to inform on the best soil management practices to improve the natural occurring inoculum, thereby establishing a hopefully more productive and definitely more sustainable agriculture systems.

## Material and methods

### Experiment 1

In Alentejo region (southern Portugal, 38º28´N - 7º28´W) a field experiment was conducted to examine the effect of conventional tillage (CT) versus no-till (NT) on the evolution of AM colonization of wheat and triticale as winter crops of a particular rotation, during the vegetation period. The soil is a Luvisol (pH 5 to 6, P content 14 to 28 ppm in CT, 18 to 44 ppm in NT) and the experiment was design as a split plot design with tillage as main treatment, 6 plots replicated 3 fold. Four plants were sampled from each plot every two 2 weeks from March to June. AMF % root colonization was accessed (Giovanneti and Mosse, 1980) in 3 sub-samples.

### Experiment 2

Field and climatic variability limit the ability to isolate the influence of each factor contributing to the plant development. To understand if the differences in colonization observed under field conditions have any effect on plant performance, the use of pot experiments under controlled conditions was adopted. In pot experiments the objective was to promote indigenous mycorrhizal development within the soil and to establish a differential potential through contrasting soil disturbance by passing the soil through a 4 mm sieve (McGonigle and Miller, 2000).

The soil was collected on the field experiment site (pH 6,2, P - 25 ppm, total N% - 0,06, $NO_3$ - 14 ppm, OM % - 0.6 ), air dried and sieved (4mm). The experiment was conducted under greenhouse conditions with 6 l plastic pots. Four pre-germinated wheat seeds were sown into each of the 24 pots and 5 days later thinned to two plants per pot. Three weeks after emergence shoot high and dry weight were evaluated. In half of the pots the soil was removed as two 10 cm layers and passed separately through a 4 mm sieve. All root material separated on the sieve was cut into 2 cm long segments and mixed into the soil of the appropriate layer. Soil was repacked in the pots and arranged in the same two layers. In the other twelve pots the soil remains undisturbed. More pre-germinated seeds were then added to each of the 24 pots and a new cycle initiated. In each cycle, except the last, one week after transplant, ammonium nitrate (50 µg N/g dry soil) was applied (1.7 ml of 1M solution of $NH_4NO_3$ diluted on 100ml of distilled water). After the last cycle AMF % root colonization (McGonigle et al., 1990) was accessed in 3 sub-samples per pot.

### Experiment 3

Recently rDNA sequences from the Glomeromycota have been amplified successfully from DNA extracted directly from field soil (van Tuinen et al., 2004). It may be assumed that this novel approach gives a more global view of the diversity of fungi belonging to the Glomeromycota, as it avoids the biological bias introduced when trap cultures are used to isolate AMF (Jansa et

al., 2002). This approach was used in order to give a global overview of the Glomeromycota diversity in the experimental field.

Soil samples were taken from 2 adjacent field plots. The first one was disturbed (D) with a conventional tillage and the second undisturbed (U) with no tillage for the last 9 years. For each plot, ten soil cores of 200 ml (0-15 cm) were mixed together in order to have a composite sample. This sample was sieved and a subsample kept at 4ºC until use.

Three 200 mg sub-samples of each plot were used to isolate total DNA according to Martin-Laurent et al. (2001). Purified DNA was used to perform the first PCR amplification of the 5' end of the LSU rDNA with the eukaryotic specific primers LR1 (van Tuinen et al., 1998) and the fungal specific primer FLR2 (Trouvelot et al., 1999). The amplification product was diluted and used as template for the second PCR with the AMF specific primer FLR4 (Gollotte et al., 2004) in combination with LR1. PCR amplification products were cloned into the vector TOPO (TOPO TA Cloning Kit for Sequencing, Invitrogen) according to the manufacturer's instructions. Plasmid preparation was done using the Nucleo-plasmid kit (Macherey-Nagel) and sequencing performed by MWG Biotech-AG (D). All sequences were checked for the presence of chimeric sequences, and aligned with ClustalW. The alignment was optimized manually using the Se-Al v 2.0 software (University of Oxford). Phylogenetic analyses were performed using the neighbour joining (NJ) algorithm included in the ClustalW programme, using *Mortierella multidivaricata* as an outgroups. Positions with gaps were ignored and the reliability of the internal branches of the NJ tree was assessed using the bootstrap method with 1,000 replicates. Tree files were drawn using njplot (http://biom3.univ-lyon1.fr) and the sequences grouped together in ribotypes.

## Results

### Experiment 1

The results obtained indicate that plants cultivated on NT plots had a higher mycorrhizal colonization and triticale appeared to be more mycotrophic then wheat (Fig.1). In NT plots colonization rose gradually until late spring and dropped thereafter for both crops, following a typical colonization pattern.

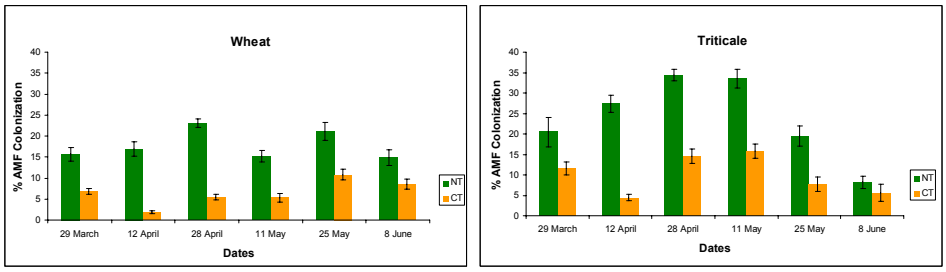


*Fig. 1: Wheat and triticale AMF % root colonization in field conditions. Error bars are standard error of mean.*

## Experiment 2

After 4 cycles there was an effect of differential soil disturbance on plant height and shoot dry weight (Fig.2). On undisturbed soil plants were significantly higher and shoot dry weight greater.

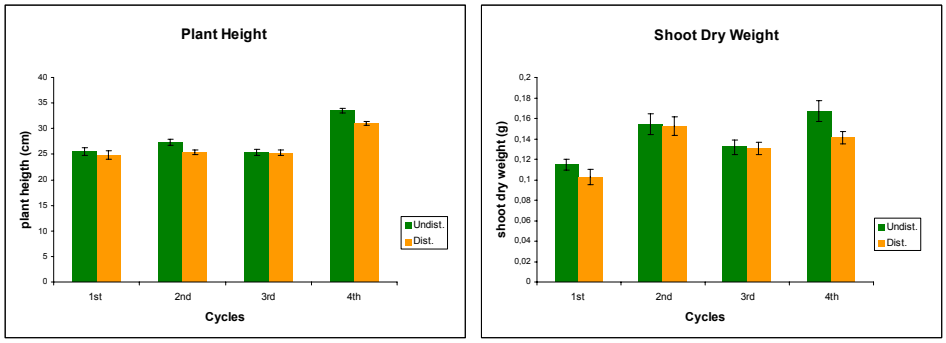


*Fig. 2: Plant height (cm) and shoot dry weight (g) over the 4 cycles. Error bars are standard error of mean.*

Differential soil disturbance significantly affected all colonization parameters (Fig. 3). Arbuscular (AC), vesicular (VC) and hyphal colonization (HC) were clearly higher in undisturbed soil. The differences observed on AC and VC on 3 weeks old plants indicate that in undisturbed soil, where the extraradical mycelium is not broken, the colonization takes place more efficiently / rapidly (vesicles are produced only when the colonization is well stabilised) and in greater extension.

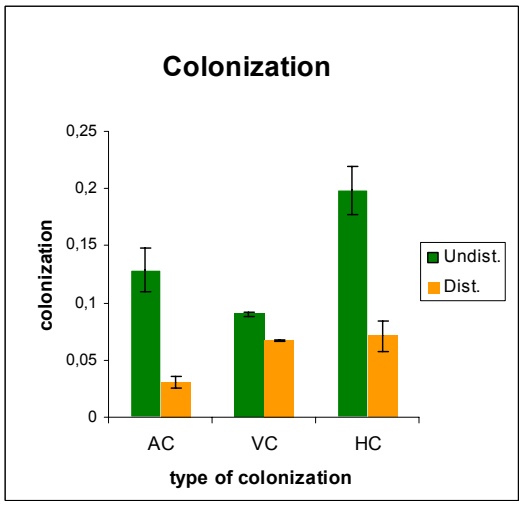


*Fig. 3: Colonization parameters. AC - Arbuscular Colonization, VC - Vesicular Colonization, HC - Hyphal Colonization. Error bars are standard error of mean.*

### Experiment 3

After the alignment of the 5' end of the large ribosomal sub-unit covering the D1 D2 region (616 bases) of the 87 sequences, 11 ribotypes, with different frequencies, were detected (Fig. 4). With the exception of one ribotype, identified as a *Scutellospora*, all the other ribotypes belonged to the Glomineae, and mainly to Glomaceae. Four ribotypes could be identified at the species level, namely *G. mosseae*, *G. intraradices*, *G. claroideum-etunicatum* and *G. occultum*. The ribotype corresponding to *Glomus intraradices* was the most abundant ribotype found in both experimental conditions. According to the data obtained only 3 of the ribotypes found (*G. mosseae*, *G. intraradices* and *Glomus II*) were present in both soil types, 6 could only be found in undisturbed soil and 2 only in disturbed soil.

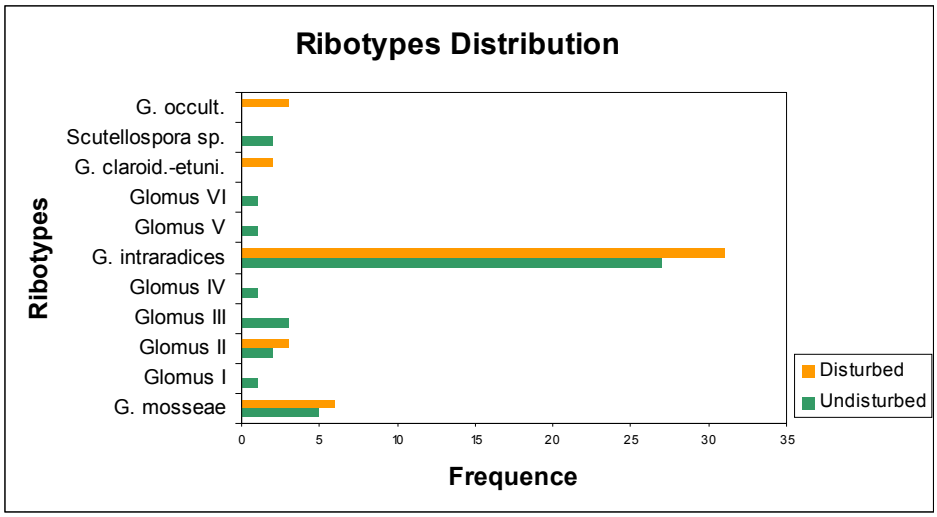


*Fig. 4: Total ribotypes found in each type of soil and its frequencies.*

## Discussion/conclusion

Several studies have confirmed that disrupting the living extraradical mycelium of AMF has an influence on the subsequent rate of root colonization on plants (Fairchild and Miller, 1990; Douds Jr. et al., 1995; Martins and Read, 1997; Goss and de Varennes, 2002). Most of these studies also state the need to verify this trend under field conditions, which the results obtained on experiment 1 seem to confirm.

The "mise au point" of the methodology of disturbance cycles overcame the edaphoclimatic variability of the field work. Another important advantage of this approach is to use the natural occurring AMF inoculum, enclosing all the diversity and forms.

Presently the methodology of disturbance cycles is being used to evaluate different aspects of the mycorrhization such as survival of inoculum over summer and its ability to start new colonization in the crop season.

The molecular data obtained so far seem to confirm that AMF are differently vulnerable to soil disturbance, not only in terms of diversity (Oehl et al., 2003), but also in terms of the community structure (Franke-Snyder et al., 2001), considering the differences observed in the frequency of the ribotypes present in both soils. The ribotype diversity found in the undisturbed system is clearly higher when compared to the disturbed system indicating that species richness may be reduced by soil disturbance. The data obtained will allow the generation of ribotype specific primers (Turnau et al., 2001), which will give the opportunity to follow in a more detailed study the distribution of selected taxa or ribotypes in the roots of the plants through out the growth season.

## References

Abbott, L.K., Robson, A.D. and Scheltema, M.A. (1995): Managing soils to enhance mycorrhizal benefits in mediterranean agriculture. Critical Reviews in Biotechnology **15**: 213-228.

Clark, R.B. and Zeto, S.K. (2000): Mineral acquisition by arbuscular mycorrhizal plants. Journal of Plant Nutrition **23**: 867-902.

Douds Jr., D.D., Galvez, L., Janke, R.R. and Wagoner, P. (1995): Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric., Ecosis. and Envir. **52**: 111-118.

Fairchild, G.L. and Miller, M.H. (1990): Vesicular-arbuscular mycorrhizas and the soil-disturbance-induced reduction if nutrient absorption in maize. New Phytol. 114: 641-650.

Franke-Snyder, M., Douds Jr, D.D., Galvez, L., Philips, J.G., Wagoner, P., Drinkwater, L. and Morton, J.B. (2001): Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA. Applied Soil Ecology **16**: 35-48.

Giovanneti, M. and Mosse, B. (1980): An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. **84**: 489-500.

Gollotte, A., van Tuinen, D. and Atkinson, D. (2004): Diversity of arbuscular mycorrhizal fungi colonizing roots of the grass species Agrostis capillaries and Lolium perenne in a fiels experiment. Mycorrhiza **14**: 111-117.

Goss, M.J. and de Varennes, A. (2002): Soil disturbance reduces the efficacy of mycorrhizal associations for early soybean growth and N2 fixation. Soil Biology and Biochemistry. **34**: 1167-1173.

Graham, J.H. (2000): Assessing costs of arbuscular mycorrhizal symbiosis agroecosystem fungi. In: G.K. Podila and D.D. Douds Jr. (eds.), Current Advances in Mycorrhizal Research. APS Press. St. Paul, pp 127-140.

Gupta, V., Satyanarayana, T.and Garg, S. (2000): General aspects of mycorrhiza. In: K.G. Mukerji, B.P. Chamola and J. Singh (eds.), Mycorrhizal Biology. Kluwer Academic/Plenum Publishers, New York, pp 27-44.

Jakobsen, I., Munkvold, L., Smith, A. and Smith, S. (2004): Functional diversity in nutrient acquisition by arbuscular mycorrhizal fungi. In: J. Baar and E. Josten, Programme and abstracts of the COST-Meeting Role of Mycorrhiza in sustainable land management. Applied Plant Research, WUR, Netherlands.

Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R. and Frossard, E. (2002): Diversity and structure of AMF communities as affected by tillage in a temperate soil. Mycorrhiza **12**: 225-234.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J.C., Soulas, G. and Catroux, G. (2001): DNA extraction from soils: old bias for new microbial diversity analysis methods. Appl Environ Microbiol **67**: 2354-2359.

Martins, M. and Read, D.J. (1997): Efeitos da distribuição do micélio externo de fungos micorrízicos arbusculares sobre o crescimento vegetal. Pesq. Agropec. Bras. **32(11)**: 1183-1189.

McGonigle, T.P. and Miller, M.H. (2000): The inconsistent effect of soil disturbance on colonization of roots by arbuscular mycorrhizal fungi: A test of the inoculum density Hypothesis. Appl. Soil Ecol. **14**: 147-155.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. and Swan, J. (1990): A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. **115**: 495-501.

McGonigle, T.P., Miller, M.H. and Young, D, (1999): Mycorrhizae crop growth and crop phosphorus nutrition in maize-soybean rotations given various tillage treatments. Plant and Soil 210: 33-42.

Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T. and Wiemken, A. (2003): Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Appl Environ Microbiol **69**: 2816–2824.

Smith, S. E. and Read, D.J. (1997): Mycorrhizal Symbiosis. Academic Press, London.

Trouvelot, S., van Tuinen, D., Hijri, M. and Gianinazzi-Pearson, V. (1999): Visualization of ribosomal DNA loci in spore interphasic nuclei of glomalean fungi by flurescence in situ hybridisation. Mycorrhiza **8**: 203-206.

Turnau, K., Ryszka, P., Gianinazzi-Pearson, V. and van Tuinen, D. (2001): Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. Mycorrhiza **10**: 169-174

van Tuinen, D., Charvolin, E. and Gianinazzi-Pearson, V. (2004): Ribossomal sequences as a tool to study and monitor arbuscular mycorrhizal fungi. In: J. Baar and E.Josten, Programme and abstracts of the COST-Meeting Role of Mycorrhiza in sustainable land management. Applied Plant Research, WUR, Netherlands

van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A. and Gianinazzi-Pearson, V. (1998): Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Molecular Ecology **7**: 879-887

Addresses of authors:
**Isabel Brito**
**Mário de Carvalho**
Universidade de Évora
ICAM
Apartado 94
7002 - 554 Évora, Portugal
ibrito@uevora.pt
**Diederik van Tuinen**
UMR INRA 1084, CNRS 5184
Université de Bourgogne
PME INRA CMSE
BP 86510
21065 Dijon Cedex, France
**Michael J. Goss**
Kemptville College
University of Guelph
Kemptville, Ontario K0G 1J0, Canada