DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

CSG 15

Research and Development

Final Project Report

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Executive summary (maximum 2 sides A4)

Arbuscular mycorrhizal fungi (AMF) are potential contributors to plant nutrition and pathogen suppression in low input agricultural systems, although individual species of AMF vary widely in their functional attributes. Recent studies at HRI and elsewhere have suggested that in some agricultural systems inoculum of AMF is substantially lower under conventional management relative to that under organic management. Further studies have suggested that conventional management selects AMF communities with limited benefits to their plant hosts relative to those in organic systems. There is a need to investigate the generality of these findings, and their implications for the productivity of organic systems, particularly during the period following conversion to organic management. This project had three objectives:

01 To deliver a literature review covering current understanding of the role of AMF in conventional and organic agricultural systems.

The review considered the ways in which management influences the structure and functioning of AMF communities, including their contributions under conventional and organic management, and recommendations for future research needs.

02 To establish the extent of differences in AMF inoculum between organic and conventional systems, covering a range of management practices.

Paired organic and conventional fields at 12 sites from across England were selected to investigate the relationships between management, AMF communities and soil chemistry. Organic and conventionally managed soils showed no significant difference in soil chemical properties (Organic C, total N, total P, extractable P, K, Mg). However, organically managed soils had greater AMF spore numbers and root colonisation potential, and therefore higher AMF inoculum potential, than conventionally managed soil. The relative difference in AMF spore numbers between organic and conventionally managed fields increased

with time since conversion. Differences in AMF inoculum potential between organic and conventionally managed fields, and between farm sites, could not be related to differences in soil chemistry.

03 To develop a method suitable for characterising AM fungus communities in soil libraries, based on 18S rRNA terminal restriction fragment length polymorphism.

T-RFLP was shown to provide a rapid semi-quantitative method for analysis of AMF community diversity. However it was clear that primers currently used to amplify AMF are selective and do not allow diversity of the whole AMF community to be determined. Additionally these primers amplify contaminant fungi which need to be removed from the T-RFLP profile prior to analysis. However, contaminant diversity was shown to be low.

The project has highlighted a number of key areas in which further research is needed in order to harness AMF to improve sustainability and productivity of organic and other agricultural systems. In particular, there is a need to determine the extent to which AMF diversity varies between organic and conventional management, the rate and mechanisms by which AMF diversity increases following conversion to organic production, the relationships between AMF diversity and crop nutrition/ pathogen control, and the soil factors controlling the effectiveness of added AMF inoculum.

Scientific report (maximum 20 sides A4)

1. INTRODUCTION

The UK demand for organic produce is increasing at rates of 15 % per annum. However the UK supply of some organic produce is insufficient to meet consumer demand, with the result that most produce (65 % in 2002), including 82 % of organic vegetables and 85 % of organic cereals is imported. The DEFRA Organic Action Plan for England has been implemented with the aim of ensuring the stable growth of organic farming in England and an increase in the domestic sourcing of organic produce. One of the key issues in the expansion of organic agriculture is production costs, which are often higher in the UK than other parts of Europe. The pressure on retailers to bring down the costs of organic produce has resulted in a preference for imported produce as well as downward pressure on the price paid to domestic farmers. This has had the consequence that many organic farmers barely cover their production costs. There is clearly a need to harness all available and suitable management technologies so that productivity can be increased in the organic sector.

Recent studies have indicated that one important contributor to plant productivity in low input systems, arbuscular mycorrhizal (AM) fungi, have very low inoculum in conventional management systems (Madder et al., 2000). Since these fungi are potential contributors to plant nutrition and disease control in the absence of fertilizers and pesticides, low inoculum potential in soil on conversion to organic agriculture could limit subsequent productivity in the short to medium term.

Arbuscular mycorrhizas (AM) are mutualistic associations between the roots of plants and fungi in the phylum Glomeromycotina (Schussler et al., 2001). Over 80 % of land plants are able to form mycorrhizas, and for these plants, mycorrhizas, not roots, represent the primary nutrient absorbing organs (Smith and Read, 1996). Fungal hyphae radiate into the soil from AM roots and improve the effectiveness of uptake of immobile nutrients such as P, Zn and Cu relative to non-mycorrhizal plants, although AM may also have a role in providing mineral forms of N, K and other nutrients to the host. Enhanced nutrient uptake by AM plants relative to non-mycorrhizal plants arises from a number of mechanisms; 1. Fungal hyphae extend beyond the area of nutrient depletion surrounding the root 2. Fungal hyphae greatly increase the surface area for absorption of nutrients relative to non-mycorrhizal roots 3. Hyphae are able to extend into some soil pores which are too small for roots to enter 4. Some mycorrhizal fungi can access forms of N and P which are unavailable to non-mycorrhizal plants, including organic forms of these nutrients. Additionally, mycorrhizal fungi are able to provide protection to the host plant against root and shoot pathogens, with a number of mechanisms proposed. These include antibiotic production, induced resistance and competition for root infection sites (Bending, 2003).

Over 150 species of AMF (AMF) have been described, and both niche differentiation and functional diversity have been recognised within the Glomeromycotina (Newsham et al., 1995). In natural environments the diversity of AMF is a key contributor to the diversity and productivity of plant communities (van der Heijden et al., 1998). A variety of agricultural practices are known to impact on AMF, with fertilizers, cultivation, crop monocultures and non-mycorrhizal crop plants known to reduce inoculum (Kurle and Pfleger, 1994). The diversity of AMF in conventional arable fields is known to be extremely low compared to adjacent semi-natural habitats (Helgasson et al.,1998; Daniell et al., 2001). Relative to conventional management, there is evidence that organic farming practices can enhance the amounts of AM inoculum (Bending et al. 2004; Mader et al., 2002). Further, there is evidence that the AMF communities selected by conventional practices are relatively less beneficial for crop yields than those from organic practices (Scullion et al., 1998; Eason et al., 1999). There is a need to determine the implications of these findings for the productivity of organic systems, particularly during the conversion phase, when low AMF inoculum and diversity, in conjunction with low P availability, could have an impact on productivity.

This project consisted of a literature review and a short experimental programme. The literature review was conducted to bring together existing knowledge of the contribution of AMF to productivity in organic systems, and management options available to optimise that contribution. The research compared amounts of AMF inoculum in conventional and organic systems of a range of agricultural practices, in order to investigate whether the evidence for reduced inoculum under conventional management has general applicability. The potential role of soil P availability in controlling the relative contribution of AMF in conventional and organic

systems was also determined. Current methods for assessing AMF diversity rely on plant bioassays in conjunction with expensive and time-consuming cloning/ sequencing of partial 18S rRNA genes. These methods are not suited to the analysis of multiple samples. We will investigate the potential for developing a rapid semi-quantitative method for assessing AMF diversity based on 18S rRNA terminal restriction fragment polymorphism using total soil DNA extracts or extracted spores.

2. OBJECTIVES

01 To conduct a literature review to assess state of the art knowledge about the role of arbuscular mycorrhizal fungi in organic agriculture and management options to enhance their contribution

02 To establish the extent of differences in arbuscular mycorrhizal fungus inoculum between organic and conventional systems, covering a range of management practices

03 To develop a method suitable for characterising arbuscular mycorrhizal fungus communities in soil libraries, based on 18S rRNA terminal restriction fragment length

3. MATERIALS AND METHODS

3.1 Objective 01

To conduct a literature review to assess state of the art knowledge about the role of arbuscular mycorrhizal fungi in organic agriculture and management options to enhance their contribution

A key component of this project was a literature review considering current understanding of the role of AMF in agricultural systems, including their contributions under conventional and organic management, and recommendations for future research needs. ADAS co-ordinated and delivered the review, with contributions made by ADAS, HDRA, HRI and the Department of Biology, York University.

3.2 Objective 02

To establish the extent of differences in arbuscular mycorrhizal fungus inoculum between organic and conventional systems, covering a range of management practices

3.2.1 Farm characteristics

Soil was collected from paired organic and conventional fields on twelve English farms (Table 1). The sites included reference farms monitored by HDRA as part of OF0316 (Conversion to organic vegetable production), OF0316 (The development of improved guidance on the use of fertility building crops in organic farming) and OF0164 (Understanding soil fertility in organically farmed systems), and included sites on grower holdings and research institutions. The sites covered a range of soil types and included stocked and stockless farms. All but one of the farms had arable/field vegetable rotations; the remaining farm was an upland grassland system. The organic fields had been under certified organic management for periods from 1 to 18 years.

Table 1 Farm locations and characteristics

Site	Soil type	Years since conversion	Enterprise
Ryton, Warks.	Sandy loam	15	Vegetable
Wellesbourne, Warks.	Sandy loam	4	Vegetable/Arable
Terrington, Norfolk	Silt clay loam	7	Vegetable/Arable
Kirton, S. Lincs.	Silty loam	4	Vegetable
Sutterton, S. Lincs.	Silty loam	3	Vegetable
Epworth, N. Lincs.	Sandy loam/peat	2	Vegetable
Duggleby, N. Yorks.	Clay loam over chalk	2	Arable
Great Coxwell, Oxon.	Sandy silt loam over limestone	10	Arable
Cirencester, Gloucs.	Silty loam over limestone	18	Arable
Tarleton, Lancs.	Peat	4	Vegetable
Ormskirk, Lancs.	Peat	1	Vegetable
Redesdale, Northumberland	Fine sandy loam	12	Grassland

3.2.2 Soil collection and preparation

At each site, soil was collected from an organically managed and a conventionally managed field. The organic and conventional fields were selected to have close proximity and cropping regimes at the time of sampling, as possible. Within each field five 10x10 m areas were marked out and from within each area 20 soil cores (0-30 cm depth) were collected with a 5 cm diameter soil auger and pooled to provide a c. 5 Kg sample. The samples were passed through a 6 mm sieve and mixed before use. The water holding capacity (WHC) of each soil sample was determined. 25 g fw soil was weighed into a Whatman No.1 filter paper in a funnel and 25 ml 0.1 M $CaCl_2$ added. The filter was covered and left to equilibrate overnight, before the moisture content of the soil was determined by drying in an 80^{o}C oven. Water content of the soil reflected 100 % WHC.

3.2.3 Soil chemical analysis

Each soil sample was analysed for pH, total C, N and P content, ~~extractable (~~extractable Olsen~~)~~ P, and extractable K by ADAS using standard ADAS methods.

3.2.4 Mycorrhizal inoculum potential

The inoculum potential of AMF was determined by extracting and counting numbers of AMF spores, and by a root colonization bioassay

a. Spore extraction and quantification

Spores were extracted from soil using a sucrose density gradient centrifugation method adapted from Daniels and Skipper (1982). 20 g fw soil was dispersed in 50 ml distilled H_2O and shaken for 30 mins. 20 ml of the suspension was removed and added to a 50 ml Falcon tube containing 20 ml saturated sucrose solution (299 g sucrose dissolved in 130 ml distilled H_2O). The tube was centrifuged at 1000 rpm for 3 min using an MSE Centaur centrifuge. The supernatant was poured onto a 37μm sieve and the sieve washed until the sucrose was completely removed. The material caught on the sieve was washed into a fresh Falcon tube with distilled H_2O and made up to 50 ml volume. Spore extracts were stored at 4^{o}C prior to counting. Between 10 and 50 ml of the spore suspension was filtered through a 45 μm grided milipore membrane using vacuum filtration. AMF spores caught on the membrane were counted using a dissecting microscope.

b. AMF colonization assay

Soil samples were adjusted to 40 % WHC and 200g fw soil placed into plastic plant pots. In the case of peaty Huntapac and Woodland soils, 8.5x8.5x10cm pots were used, while for the other soils 7x7x7cm pots were used). Three organically produced onion seeds (*Allium sepa* cv. Balstora) were sown 5mm deep into each pot. The pots were placed in a Sunbag (Sigma Chemical Co., Poole, Dorset), which possess a 24x24 mm window of 0.02 μm membrane, allowing gas exchange without the potential for cross-contamination. Tops of the bag were rolled and secured with two paper clips. The pots were placed into a glasshouse, and positioned in a randomised block design consisting of 5 blocks, each containing 1 soil from each site. The design was generated in Genstat. The glasshouse temperature was maintained under 20/15^{o}C day/night temperatures. Pots were weighed weekly, and water added to maintain soil H_2O content at 40 % WHC.

Seedlings were harvested after 14 weeks. Roots and shoots were separated and fresh weight determined. Roots were washed thoroughly in distilled H_2O to remove adhering soil, and blotted dry on a paper towel. The roots were cut to approximately 2 cm lengths and mixed thoroughly. 100- 500 μg roots were stored frozen at -20^{o}C. 200 μg roots were retained for analysis of mycorrhiza colonization. The remainder of the roots, and the shoots, were dried in an oven at 80^{o}C and the dry weight measured.

Root samples analysed for AMF colonization were stained according to the method of Grace and Stribley (1991). Roots were covered in 10 % w/v KOH and incubated at 90 ^{o}C for 30 minutes. The KOH was poured off and the roots washed with distilled H_2O. Roots were acidified in 2 % v/v HCL for 5 minutes at room temperature, following which the HCL was poured off and replaced with aniline blue (0.05 % w/v in 70 % glycerol). Roots were placed in an oven at 90 ^{o}C for 10 minutes. The aniline blue was poured off and the roots de-stained in 70 % glycerol. Root colonization was quantified using the gridline intersect method, in which the presence of mycorrhizal structures (arbuscules, vesicles, spores, runner hyphae) was determined at the point of intersect of plant roots on a 1.27 cm grid placed under a Petri dish, viewed under a dissecting microscope. Up to 120 intersects were assessed for each sample, and the % root length colonised by AMF determined.

3.2.5 Statistical analysis

Statistical analysis was conducted using Genstat. For analysis, organic vs conventional management was compared using analysis of variance by treating each farm site as a block. With the exception of pH, data sets were log transformed prior to analysis.

3.3. Objective 03
To develop a method suitable for characterising arbuscular mycorrhizal fungus communities in soil libraries, based on 18S rRNA terminal restriction fragment length

3.3.1 AMF cultures and DNA extraction

AMF pure cultures were obtained from the Prof. V. Gianinazzi-Pearson, INRA-Dijon, France. The cultures consisted of either root mats/ soil ~~or spore extracts~~ derived from pure cultures of individual fungi, or spore extracts. DNA was extracted from samples of the pure AMF cultures using an Ultraclean Soil DNA extraction kit (Cambio), and from 2 root samples from the experiment described above (High Mowthorpe organic treatment) using a Plant DNA Extraction kit (Cambio).

Table 2 Cultures of AMF used in the project

Culture ID~~ID~~	Species	Taxonomic group
BEG 8	*Acaulospora longula*	Diversisporales (Acaulosporaceae)
BEG 11	*Glomus geosporum*	Glomus group A
BEG 12	*Glomus mosseae*	Glomus group A
BEG 14	*Glomus claroideum*	Glomus group B

BEG 20	*Glomus caledonium*	Glomus group A
BEG 22	*Glomus coronatum*	Glomus group A
BEG 34	*Gigaspora margarita*	Diversisporales (Gigasporaceae)
BEG 35	*Scutellospora heterogama*	Diversisporales (Gigasporaceae)
BEG 47	*Glomus versiforme*	Diversisporales (Diversisporaceae)
BEG 144	*Glomus intraradices*	Glomus group B

3.3.2 PCR Primers and Amplification

Partial AMF small subunit (SSU) DNA fragments (c 550 bp) were amplified from cultures and soil samples using the primers AM1 (Helgason et al., 1998) and NS31 (Simon et al., 1992), and the PCR conditions outlined by Daniell et al. (2001). For Terminal restriction fragment length polymorphism (T-RFLP) AM1 and NS31 primers were labelled with the fluorescent dyes HEX and 6-FAM respectively.

3.3.3 Terminal restriction fragment length polymorphism

T-RFLP was conducted on amplified pure cultures and soil according to the method of Vandenkoornhuyse et al. (2003). This method was published after the current project had started. In this method ~~was published after this project had started. In this method,~~ the PCR products ~~amplified from the AMF pure cultures and root samples were~~are digested with the restriction endonucleases HinfI and Hsp92II. Digestion products amplified from the AMF pure cultures and root samples were purified and run on an Applied Biosystems 377 automated DNA sequencer.

3.3.4 Cloning reactions and sequence analysis

To test the specificity of the AMF primers and the measure of AMF diversity provided by T-RFLP, PCR products from the High Mowthorpe root samples were cloned using a Toppo TA cloning kit. A total of 40 positive clones were selected, and amplified with the sequencing primer SP6 and the AMF primer NS31, to check they contained DNA of the correct fragment size. Only 7 of the 40 clones contained a c. 550 bp fragment, with the remaining clones probably containing oligonucleotide contaminants from the PCR reactions. Sequencing reactions with the 7 clones containing the correct size fragment were conducted using a PRISM BigDye Terminator Cycle Sequence reaction kit (Applied Biosystems), with products analysed on an Applied Biosystems 377 automated DNA sequencer. Partial SSU DNA sequences were edited and assembled using the DNAstar II sequence analysis package (Lasergene Inc, Madison, USA). Sequences were compared to those on the EMBL DNA database using the program FASTA3.

4. RESULTS AND DISCUSSION

4.1 Objective 02

To establish the extent of differences in arbuscular mycorrhizal fungus inoculum between organic and conventional systems, covering a range of management practices

4.1.1 Soil chemical analysis

Table 3 pH, organic-C and total-N in organic and conventional fields at each farm location

	pH		Organic C		Total-N	
Site	**Conventional**	**Organic**	**Conventional**	**Organic**	**Conventional**	**Organic**
Collymore	8.00	7.98	3.66	3.79	0.42	0.44
High Mowthorpe	7.70	7.90	2.07	2.82	0.25	0.33
Huntapac	6.56	6.22	10.10	20.66	0.58	0.96
Kirton	8.04	7.94	1.37	1.38	0.19	0.19
Polybell	6.74	7.30	17.48	18.62	1.58	1.91
Redesdale	6.06	6.38	2.83	2.34	0.31	0.24
RAC	8.06	7.48	1.83	3.41	0.22	0.41
Ryton	6.18	6.10	1.59	2.01	0.17	0.21
Terrington	7.98	8.12	2.10	1.73	0.25	0.21
WCF	7.10	7.26	1.91	1.77	0.16	0.17
Wellesbourne	7.32	7.84	1.05	0.91	0.12	0.11
Woodland	8.36	8.02	1.36	2.13	0.16	0.26
Average	7.34	7.38	3.95	5.13	0.37	0.45

There were significant differences in pH between sites (P<0.001), with pH ranging from 6 (Redesdale) to over 8.3 (Woodland). There were significant differences in total organic-C between the sites, with values ranging from 1 (Wellesbourne) to over 20 % (Huntapac). There were significant differences (P<0.001) in total N between the sites, with values ranging between 0.11 (Wellesbourne) and 1.9 % (Polybell). However, there were no significant differences in pH, organic-C or N between organic and conventional management.

Table 4 Total soil P, extractable P and K in organic and conventional fields at farm locations

	Total-P (mg kg soil)		Extractable-P (mg kg soil)		Extractable K (mg kg soil)	
Site	Conventional	Organic	Conventional	Organic	Conventional	Organic
Collymore	1216	1764	29.8	55.8	277.2	850.6
High Mowthorpe	706	849	22.6	20.4	135.6	180.2
Huntapac	1389	1662	95.6	76.6	301.0	334.6
Kirton	737	798	24.0	36.6	163.0	81.2
Polybell	1354	1120	17.2	17.2	82.8	72.4
Redesdale	503	661	8.8	11.0	91.6	60.2
RAC	899	888	25.4	16.8	239.2	160.2
Ryton	650	460	45.6	8.2	98.2	65.0
Terrington	974	691	54.2	16.4	355.2	175.4
WCF	823	705	70.4	54.6	149.8	143.4

Wellesbourne	952	891	71.4	75.2	146.2	129.2
Woodland	695	997	49.8	68.2	189.6	425
Average	908.2	957.2	42.9	38.1	185.8	223.1

There were significant differences (P<0.002) in total P between the sites, with values ranging between 460 (Kirton) to over 1700 mg Kg soil (Collymore). There were significant differences (P<0.017) in extractable P between the sites, with values ranging between 8.2 (Ryton) and 95 mg Kg soil (Huntapac). There were significant differences (P<0.01) in extractable K between the sites, with values ranging between 60 (Redesdale) and 850 mg Kg (Collymore). However, there were no significant differences in total P, extractable P, or K between the organic and conventional treatments.

4.1.2 AMF inoculum potential

Table 5 AMF inoculum potential in organic and conventional fields at farm locations

	AMF spores (per g dw soil)		**% root length colonised**	
Site	**Conventional**	**Organic**	**Conventional**	**Organic**
Collymore	8.9	11.1	43.1	83.8
High Mowthorpe	11.8	8.2	12.0	64.9
Huntapac	6.0	9.3	16.3	34.5
Kirton	10.5	14.7	20.2	53.0
Polybell	7.6	10.2	51.7	54.9
Redesdale	6.7	16.5	50.5	61.7
RAC	3.9	27.1	20.1	71.6
Ryton	0.5	1.4	25.5	43.3
Terrington	3.3	10.2	3.8	51.9
WCF	2.2	8.9	33.4	36.0
Wellesbourne	1.8	1.6	34.4	38.9
Woodland	11.0	22.7	35.1	57.8
Average	6.2	11.8	28.8	54.4

There were significant differences (P<0.001) in the number of AMF spores between the sites, with amounts ranging from 0.5 (Ryton) to 27 per g soil (RAC). Numbers of AMF spores were significantly higher under organic relative to conventional management (P<0.004). There were no significant differences in AMF colonization between the sites. However, AMF root colonization was significantly higher in organically managed relative to conventionally managed fields (P<0.001).

Several studies have shown that AMF inoculum potential in soil is enhanced under organic management. Ryan et al. (1994, 2000) showed enhanced AMF colonization of a variety of crop plants in organic relative to conventional farms in Australia. Mader et al. (2000) showed that AMF root colonisation potential was 30-60 % higher in wheat, ryegrass and clover grown under organic compared to conventional management. Similarly, our study has shown an average 26 % increase in root colonization in organic relative to conventional fields. In the studies of Ryan et al. (1994, 2000) and Mader et al. (2000) differences in AMF colonization between organic and conventional systems were related to differences in P, which was found to be lower in organic relative to conventional systems. P fertilizer is known to have direct effects on AMF colonisation, which decreases with increasing available P. However, in our study, there were no differences between the organic and conventional systems with respect to any soil chemical property, including total P and extractable P.

A variety of management practices other than fertilizer use are known to directly influence AMF communities, including the diversity of crops included in rotations, fallow periods, the use of organic amendments, pesticide use and cultivation practices (Hodge et al., 2003). Since conversion, N, P and K fertilizer application was in the form of organic manures and composts in the organic fields and mineral fertilizers in the conventional fields. The conventionally managed fields received applications of growth promoters, fungicides, insecticides and herbicides, although the precise chemicals and doses varied between farms. No such chemicals had been applied to the organic farms since conversion. Organic and conventional fields had received various cultivation practices, including ploughing, subsoiling and disking although specific practices varied considerably between locations, with none specific to either the conventionally or organically managed fields.

4.1.3 Relationships between soil chemical properties, time since conversion to organic management and AMF inoculum potential

There were no significant correlations between any of the soil chemical properties and either AMF spore number or AMF colonization. There was a significant correlation ($r=0.53$, $P<0.02$, $df=9$) between AMF spore number and % AMF colonization. There was a significant correlation ($r=0.60$, $p<0.05$, $df=11$) between the ratio of spores in organic:conventional at each site and the time since conversion to organic production.

4.2. Objective 03
To develop a method suitable for characterising arbuscular mycorrhizal fungus communities in soil libraries, based on 18S rRNA terminal restriction fragment length

4.2.1 PCR amplification of AMF cultures and root samples

The primers AM1 and NS31 amplified SSU DNA from BEG cultures 11, 12, 20, 22, 34, 35 and 47 as well as the High Mowthorpe root samples. Schussler et al. (2001) separated AMF into 5 different phylogenetic groups. In our study the AM1 and NS31 primers were able to amplify DNA from AMF cultures within the Glomus group A within the Glomerales, but not the Glomus-group B cultures. Analysis of AMF SSU DNA sequences taken from the EMBL database suggested that AM1 and NS31 will additionally not amplify some members within the Diversisporales, or AMF in the Paraglomales and Archaeosporales. New primer sets are needed so that the diversity of all AMF fungi can be assessed in environmental samples, rather than just Glomus group A/ selected Glomus group B and Diversisporales, as is the case with primers AM1 and NS31.

4.2.2 T-RFLP analysis of pure cultures and root samples

The pure cultures gave single fragments, the size of which differed between the isolates. The High Mowthorpe root samples showed multiple peaks, with at least 14 different peaks, suggesting that diverse AMF species were present on the root sample. However, AM1 and NS31 clearly amplify other root inhabiting fungi in addition to AMF (see 4.3.3) and the T-RFLP profile will reflect both AMF and contaminant fungi. T-RFLP has now been used in several studies to compare AMF communities (e.g. Vandenkoornhuyse et al. (2003)). However, the potential contribution of contaminants to the profile has not been determined. Clearly T-RFLP should be conducted in conjunction with cloning so that contaminants can be identified and their fragments removed prior to analysis of the T-RFLP profile.

4.3.3 Identity of AMF inhabiting roots as determined by cloning

7 clones derived from High Mowthorpe root samples were sequenced. 4 of the 7 clones showed sequence homology to *Glomus* sp. within Glomus groups A and B (Table 5), and each clone was different, again

suggesting high diversity of AMF on the root sample. The remaining 3 samples all showed identical homology (97 %) to the ascomycete *Madurella mycetomatis* AF527811, indicating that the diversity of contaminant non-AMF sequences amplified by AM1/NS321 was low, and that the T-RFLP fragments reflect mostly AMF (c80 %).

Table 6 Characteristics of AMF clones obtained from High Mowthorpe root samples

Clone	EMBL partial 18S rRNA gene sequence similarity to next related sequence	Taxonomic group
MoR4	97.5 % to *Glomus* sp AF480153	Glomus group A
MoR7	99.8 % to *Glomus mosseae* GM0505616	Glomus group A
MoR17	99.8 % to *Glomus intraradices* GIN536822	Glomus group A
MoR341	97.8 % to *Glomus lamellosum* GLA276087	Glomus group B

5. CONCLUSIONS

- Paired organic and conventional fields at 12 sites from across England were selected to investigate the relationships between management, arbuscular mycorrhizal fungus (AMF) communities and soil chemistry
- Organic and conventionally managed soils showed no significant difference in soil chemical properties (Organic C, total N, total P, extractable P, K, Mg)
- Organically managed soils had greater AMF spore numbers and root colonisation potential, and therefore higher AMF inoculum potential, than conventionally managed soil
- The relative difference in AMF spore numbers between organic and conventionally managed fields increased with time since conversion
- Differences in AMF inoculum potential between organic and conventionally managed fields, and between farm sites, could not be related to differences in soil chemistry
- T-RFLP was shown to provide a rapid semi-quantitative method for analysis of AMF community diversity
- Initial results from T-RFLP and cloning analyses suggested that a diverse AMF community was present on the root samples from the High Mowthorpe site
- The small subunit rRNA gene primers currently used to amplify AMF from root and soil samples are selective and do not amplify members of the Paraglomales and Archaeosporales

6. FUTURE WORK

The project has highlighted a number of key areas in which further research is needed in order to harness AMF to improve sustainability and productivity of organic and other agricultural systems.

- We have shown that the size of AMF inoculum is different in organic and conventional soil. However, the extent to which AMF diversity contrasts between organic and conventional systems is unclear. Since AMF species show functional diversity and niche differentiation, diversity may be critical in determining the extent to which AMF can influence plant growth/nutrition/pathogen control of diverse crop plants. This and other studies have demonstrated that T-RFLP/cloning can provide an assessment of AMF diversity; techniques are now available to characterise AMF diversity under different management

systems. Such studies will identify the potential to manage AMF communities, particularly during the conversion of land from conventional to organic management, when AMF diversity is likely to be low.

- The impact of AMF diversity on crop nutrition and pathogen control is unclear. Most studies which have investigated the impacts of AMF on crop nutrition, and interactions between AMF and pathogens, have been conducted with single AMF isolates. In real field soil communities are complex; in samples from this study, onion roots grown in High Mowthorpe soil for only 14 weeks appear to have developed a community comprising of up to 13 species. The relationship between AMF diversity and crop nutrition/ pathogen control needs to be resolved in order that deficiencies within communities can be identified, and practices to optimise and manage the diversity of AMF communities can be developed.

- A variety of commercial AMF inocula are available to growers. However, in order to justify the expense for using these products, the yield/ quality improvements provided by inoculum use need to be established. However, the effectiveness of added AMF inoculum will depend on both the characteristics of existing AMF inoculum within the soil, and soil chemical properties, particularly P content. The thresholds in these properties required for added inoculum to be effective are unclear. Research needs to be conducted to establish the soil conditions under which AMF inoculum use could prove cost effective to growers.

- Our data has suggested that AMF communities in organically managed soils increase with time since conversion from conventional management. The mechanisms and time scales involved in recolonisation of organic land by AMF species following conversion need to be established, including the role of management practices (e.g. use of cover crops and manures, existing pest/weed/disease control measures, crop diversity within the rotation).

7. ACTION RESULTING FROM THE RESEARCH

(IP, Technology Transfer, Publications)

- A literature review outlining current understanding of the role of arbuscular fungi in agricultural systems, including the impact of management and organic practices, was delivered to DEFRA

Hodge, A.,Gosling P., Goodlass G., Bending G.D. (2003) Arbuscular Mycorrhizal Fungi (AMF) in Organic Farming. Literature review for DEFRA, UK. 70pp.

- A poster presentation of some key project findings was made at the Colloquium of Organic Researchers, and a paper submitted to the conference proceedings;

Ozaki, A, Rayns, F., Gosling, P, Bending, G.D., & Turner, M.K. (2004) Does organic farming favour arbuscular mycorrhizal fungi? Proceedings of the UK Organic Researchers Conference, Newport, Shropshire, 20-22 April 2004, p260-262.

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