Arbuscular Mycorrhizal Fungi (AMF) in Organic Farming (Defra Project OF0333)

Literature Review

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Executive Summary

Mycorrhizal associations between a fungus and a plant root are widespread in the natural environment. There are several different types of which the arbuscular mycorrhiza (AM) is the most common, it involves a relatively small number of fungi, but around two-thirds of all plant species. Mycorrhizal association provides many benefits to the plant. Of most importance from an agricultural point of view, is improved nutrition that enhances growth and fitness of the plant and improved resistance to soil-borne pests and diseases resulting from antagonistic processes associated with mycorrhiza. In dry climates improved drought resistance can also be an important benefit. For these reasons, mycorrhiza have been highlighted as plant growth promoters and possible biological control agents. However the transition from showing these effects in the laboratory or glasshouse to demonstrating them in the field has proved difficult. Conventional systems, particularly high input systems, probably have little to gain from encouraging AM colonisation, as the carbon drain on the crop by the fungi may be substantial, while the benefits which the plant gains, greater access to nutrients and reduced disease pressure, can be achieved at low cost through inputs of fertiliser and biocides. This situation may change in the future as a result of increasing pressure to reduce the use of inputs and develop more sustainable systems of food production. Organic and other low input systems potentially have more to gain from encouraging AM colonisation of crops, and changes to tillage and cropping could easily be made to encourage AM fungal establishment. However, though activity by arbuscular mycorrhizal fungi (AMF) has frequently been shown to be higher in organic systems, this has not always been shown to be beneficial, particularly where soil phosphorus concentrations are moderate to high.

In organic farming the functions of AMF most likely to bring enhanced productivity are the increased potential for nutrient capture and increased protection against pest and disease attack by AM colonised roots. To achieve these improvements a better understanding in field scale UK Organic Farming conditions is required of host/AMF specificities, infectivity and effectivity and interactions with normal organic farming practices such as growth of legumes and cover crops; use of livestock manures, composts and green manures and also existing pest/weed/disease control measures such as stale seedbeds. It is also necessary to identify whether inoculation or enhancement of natural AMF is the most successful pathway to achieve effective enhancement of AM colonisation.

1: Introduction

Mycorrhizal associations between a fungus and a plant root are widespread in the natural environment. The association can be categorised into one of seven types (arbuscular, ectomycorrhizal, ectendomycorrhizal, ericoid, arbutoid, orchid and monotropoid) based upon the fungus involved, and the resulting structures generated in the host root by the fungus-plant combination. Of these different types of association the most common is that of the arbuscular mycorrhiza (AM), which involves a relatively small number of fungi, but around two-thirds of all plant species. The arbuscular mycorrhizal fungi (AMF) cannot grow for any considerable time in the absence of a host plant and despite numerous attempts it is currently impossible to grow AMF in pure culture (unlike the fungi involved in the ectomycorrhizal association for example) thus, hampering the study of this association. Previously it was thought that the restricted growth of the AM fungus was because DNA replication and nuclear division could not occur in the absence of host plant colonisation (Burggraaf & Beringer, 1989). However, this has subsequently been demonstrated not to be the case (Bécard & Pfeffer, 1993; Bianciotto & Bonfante, 1993) and it is now believed the restricted fungal growth is due to blocking of stages in the fungal metabolic pathways (Bago *et al*., 1999, 2000). Through the production of spores however, AMF can persist in the soil until a host plant is present. The AM association is so called because of the formation of highly branched intracellular fungal structures or 'arbuscules'. In some cases, arbuscules may be absent (see ***section 1.3***). The AM association is the most ancient and probably aided the first plants to colonise land between 353-462 million years ago by scavenging for phosphate (Simon *et al*., 1993). Fossil evidence of arbuscule-like structures in *Aglaophyton* material from the Rhynie chert also confirm the existence of AM-like associations between 360-410 million years ago (Remy *et al*., 1994; Taylor *et al*., 1995). As the association is still very much in evidence today, it indicates that it must be beneficial to both plants and fungi.

The AM association consists of an internal phase inside the root and an external phase, or the extraradical mycelium (ERM) of the fungus, which is the phase in contact with the soil. The association may not be obviously mutualistic at all points in time, and it is possible under some conditions the AMF may 'cheat' their host plant into supplying carbon (C) with no apparent benefit to the plant. Proving that AMF are cheating, however, is difficult (see Fitter, 2001) not least because AMF have been demonstrated to confer a wide range of benefits to their host and it is likely more benefits are yet to be unearthed. AMF were principally believed to benefit their host by increasing uptake of the relatively immobile inorganic phosphate ion, due to the ability of the fungal ERM to grow beyond the phosphate depletion zone that quickly develops around the root surface (Sanders & Tinker, 1971; Koide, 1991; George *et al*., 1995). The identification of a inorganic P transporter expressed in the ERM of the AM fungus, *Glomus versiforme* (Harrison & van Buuren, 1995), and more recently, the identification of StPT3, a inorganic P transporter gene that is only expressed in AM colonised roots and which appears particularly associated with cells containing arbuscules (Rausch *et al*., 2001), has provided a molecular basis for the AM-plant inorganic P transfer mechanism, long assumed to occur but for which direct evidence was lacking. In addition, colonisation by AMF has also been demonstrated to increase resistance to soil pathogens (Newsham *et al*., 1995a; Lingua *et al*., 2002; Pozo *et al*., 2002) and foliar-feeding insects (Gange & West, 1994), alter drought resistance

(Augé *et al*., 1994), increase uptake of nitrogen (Hodge *et al*., 2001) and micronutrients including zinc (Faber *et al*., 1990; Kothari *et al*., 1991a) and copper (Gildon & Tinker, 1983; Li *et al*., 1991a) and improve soil aggregation stability (Tisdall & Oades, 1979; Tisdall, 1991; Degens *et al*., 1996). With such a range of roles for the AM association has posed a problem in producing a definition to best describe it. Currently, the most useful definition is perhaps that proposed by Fitter and Moyersoen (1996) of 'a sustainable non-pathogenic biotrophic interaction between a fungus and a root', although as pointed out by Hodge (2000), this does not emphasise the importance of both intra-, and particularly the extraradical, fungal mycelia in the association.

1.1. AM diversity

AM spores are large (up to 1 mm in diameter), thick walled and contain up to several thousand nuclei (Burggraaf & Beringer, 1989). Spores, which are formed either in the root cortex or in the soil, are assumed to be survival structures with some capacity for dispersal by wind, water, invertebrates, birds and mammals (Smith & Read, 1997). Approximately 150 species of AMF have been described based upon characterisation of their spores but there is little doubt that the true morphological diversity is much higher. Yet, as pointed out by Fitter (2001) there is somewhat of a paradox between the number of described species and the results of actual AM diversity surveys. Such surveys have been conducted at a range of scales (at the single habitat, system and regional level, see Table 1), and it would be expected as sampling scale increases so would the diversity of AMF found. However, the data in Table 1 demonstrate this is not necessarily the case (i.e. average diversity values for single habitat and at a system level are 17 and 13 respectively). Remarkably, even at the regional level, average diversity is not much higher than that found in a single habitat (i.e. 26 v 17). Furthermore, all the values in Table 1 are considerably lower than the total 150 described species. However, most of the studies shown in Table 1 also include a list of currently unidentified species, implying diversity is higher than the values in Table 1 suggest, and the International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, USA (http://www.invam.caf.wvu.edu.) currently holds *c*. 40 spore isolates which have yet to be characterised. In addition, Husband *et al*. (2002) using molecular techniques, identified 30 AM fungal types from sampling roots of two plant species, but more than half of these had not been previously recorded. Additionally the primers used in this study were selective for members of Glomus group-A within the Glomerales, and some members of the Diversisporalesand would not have amplified AMF in the orders Diversisporales, Archaeosporales and Paraglomales (see **section 2.3**). The application of molecular techniques has also demonstrated that while morphological differences may not always be apparent, different isolates can vary in their DNA sequence data (see ***section 1.3***; Schussler, 1999; Schussler *et al*., 2001), again implying a higher diversity than previously believed. Moreover, viable spores tend to be ephemeral, thus unless repeated sampling of the soil is carried out the full extent of the fungal diversity at any one location may be underestimated. The viability of spores is also reduced by dormancy (Tommerup, 1983) and pathogen attack (Boyetchko & Tewari, 1991), which also can vary between sampling times. Increased sampling effort tends to increase the diversity of spores found. Morton *et al*. (1995) demonstrated that with successive use of 'trap' cultures more species were identified. 'Trapping' AMF can occur in an assortment of forms (Bever *et al*., 1996). Usually soil from the field is removed and added to pots containing a host plant. The fungi

colonise the host and sporulates, thus increasing (or amplifying) the fungal spores to an identifiable amount. In other cases plants are removed directly from the field, washed and placed in sterile media. Again, the fungi in the roots sporulate and the spores can then be identified. The process of generating trap cultures generally takes 5-6 months each time and is not always successful, which is presumably why few studies conduct more than a single trapping event. It can however, show the presence of fungal species that would otherwise be missed by analysis of the soil alone. Using soil samples collected at different times and a variety of trap culture techniques (including manipulation of the environmental conditions under which the trap cultures where grown) Bever *et al*. (2001) have recently demonstrated that no single methodological technique results in all the AM species identified from their site being revealed. Moreover, the number of AMF identified from their study site, a 1-ha abandoned agricultural field, has increased from 11 species identified in 1992 to at least 37 in 2001, of which one-third have not been previously described. This level of AM diversity in a 1-ha field site is similar to the levels reported in Table 1 for diversity at the regional level. Thus, the more intense and varied the sampling strategy, the higher the diversity recovered. Had Bever *et al*. (2001) also used molecular techniques, then the diversity value may have been even higher. It has also been demonstrated that the species of AMF recovered can vary with the host plant present (Sanders & Fitter, 1992; Bever *et al*., 1997; Eom *et al*., 2000; Helgason *et al*., 2002; Vandenkoornhuyse *et al*., 2002) and that different AMF have varying effects on different plant species (van Heijden, 2002; van Heijden *et al*., 1998, 2003; O'Connor *et al*., 2002). These findings are particularly important as it has long been assumed, mainly due to the large number of plant species and the relatively small number of fungi involved, that AMF must lack any degree of specificity. In addition, the microcosm study of van der Heijden *et al*. (1998) demonstrated that increasing the diversity of AMF improved plant performance. Collectively, the results of these studies imply that plants may at least be able to select the AMF that benefit them the most and/or AMF can demonstrate a host preference. This, together with the increasing awareness that AMF are multifunctional (Newsham *et al*., 1995b), and their potential role in sustainable agriculture systems has renewed interest in the AM symbiosis.

Table 1 Examples of counts of numbers of AM fungal species based on either spore collections or trap cultures found in single habitats, ecosystems or entire regions. Studies marked with * used molecular techniques to investigate actual AM diversity *in planta*. Data updated from Fitter (2001)

SINGLE HABITAT			**ECOSYSTEM**			**REGION**		
Habitat	No.	Source	System	No.	Source	Region	No.	Source
Grassland, USA	27	Bever *et al*.,1996	Cacao, Venezuela	8	Cuenca & Meneses, 1996	Poland	21	Blaszkowski, 1989
Old Field, USA	24	Beaver unpublished+	Disturbed rainforest, Mexico	16	Guadarrama & Alvarez Sanchez, 1999	Nutrient-poor soils, Venezuela	24	Cuenca *et al*., 1998
Old Meadow, Canada	13	Hamel *et al*., 1994	Wheat, USA	13	Hettrick & Bloom, 1983	Atlantic coastal dunes, USA	23	Koske, 1987
Tallgrass prairie, USA	20	Hettrick & Bloom, 1983	Old field succession to forest, USA	25	Johnson *et al*., 1991	Apple orchards, USA	43	Miller *et al*., 1985
Sand dune, USA	17	Koske & Morton unpublished+	Sand dunes, USA	17	Koske & Gemma, 1997	Ando soils, Japan	16	Saito & Vargas, 1991
Desert, USA	11	Morton *et al*., 1995	Sand dunes, USA	6	Koske & Halvorson, 1981	Tropical forest, Panama*	30	Husband *et al*., 2002
Desert, USA	10	Morton *et al*., 1995	Lake dunes, USA	14	Koske & Tews, 1987			
Old Field, Canada	23	Van der Heijden *et al*., 1998	Turf grass, USA	19	Koske *et al*., 1997			
Poplar plantation, USA	10, 12	Walker *et al*., 1982	Wetlands, USA	9	Miller & Bever, 1999			
Woodland, UK*	11, 13	Helgason *et al*., 1998, 1999, 2002	Dunes, Brazil	12	Stürmer & Bellei, 1994			
Grassland, UK*	24	Vandenkoornhuyse *et al*., 2002	Mesquite scrub, USA	11	Stutz & Morton, 1996			
			Sand dunes, USA	9	Tews & Koske, 1986			
			Arable, UK*	8	Daniell *et al*., 2001			
AVERAGE =	**17**		**AVERAGE =**	**13**		**AVERAGE =**	**26**	

+ Cited in Morton *et al*. (1995)

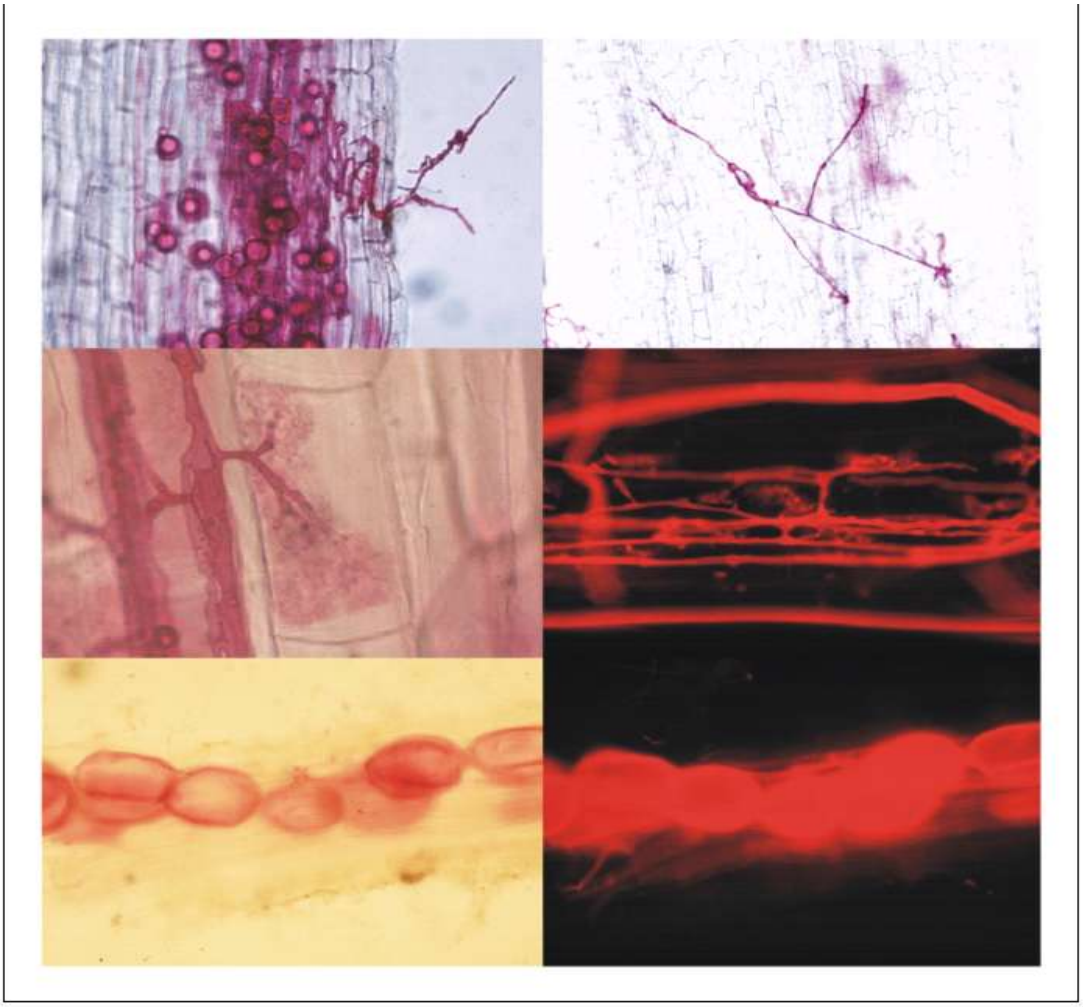

Fig. 1. Fungal structures which can form in roots colonised by mycorrhiza. A (top left): An entry point of an AM fungus at the start of the root colonisation process . B (top right): As A but showing three points of AM hyphal attachment. C (mid left): An arbuscule of a *Glomus* species in detail to show the highly branched intracellular nature of the structure. D (mid right): Arbuscular formation along a colonised root viewed under epifluorescence. E (bottom left): Vesicles of *Glomus mosseae* in *Plantago lanceolata* roots believed to be fungal storage structures although they do not always form in all AM associations. F (bottom right): Same as E except under epifluorescence (Photographs by J Merryweather (A-C) and A Hodge (D-F)).

1.2 AM propagules, fungal culture and the common mycelial network (CMN)

AM spores, colonised root fragments and hyphae, collectively termed 'propagules', are the three sources of inoculum by which living roots can become colonised. Although both spores and colonised root fragments have been shown to produce small amounts of mycelium on agar and in soil, unless colonisation of a growing root system occurs, hyphal growth will cease (Smith & Read, 1997). The exact mechanism by which roots stimulate AM hyphal growth from spores or root fragments has yet to be discovered although there is evidence that host plant root exudates, and in particular flavonoid compounds, play a role (Graham, 1982; Gianinazzi-Pearson *et al*., 1989; Giovannetti *et al*., 1993) in combination with elevated CO_2 concentrations (Bécard *et al*., 1992). Root fragments colonised by a particular AMF are commonly used as a source of inoculum in experimental systems. Although they are ideal for experimentation purposes, little is actually known about how long the AM hyphae can remain viable in such fragments. Tommerup & Abbott (1981) suggested hyphae may be able to survive for 6 months or more which may be longer than the life of the root fragment itself. If the AMF in the dying root is also attached to a living plant by the network of extraradical mycelium, then the nutrients from the dying roots may be transferred to that of the living root via the hyphal network (Ritz & Newman, 1985). However, given that the growth of the AMF is so restrictive in the absence of a potential host plant, it is currently impossible to grow the AMF in pure culture. In order to conduct experiments upon known types of AMF it is therefore necessary to first identify spores of the fungus required. Single spores are removed and grown in 'pot culture' with a host plant. The AMF colonises the root and sporulates, thus after a period of time, further spores of the fungus can be removed and the process repeated, allowing a 'pure' culture of a particular AMF to be maintained. Careful regular checking of the cultures is required to ensure spores of other AMF do not contaminate the pot.

Initially it was believed that AM spores were the only source of inoculum, probably as spores are the only AM structure that can be reliably identified taxonomically by experienced personnel. However, it is now widely recognised that rapid colonisation by new seedlings can occur after contact with the extraradical hyphae (Read *et al*., 1976) and this now seems the main mechanism by which colonisation occurs *in situ*. In undisturbed systems the AMF forms a permanent external mycelium network and plants are linked by a common mycelial network (CMN). Remarkably, this CMN can survive and retain its ability to act as a colonisation unit when the vegetation upon which it has developed is dormant or dead, enabling rapid colonisation when conditions are again favourable for plant growth (Smith & Read, 1997). The CMN can also withstand freezing conditions if pre-cooling occurs first (Addy *et al*., 1994, 1998), but soil disturbance, such as tillage, can disrupt the CMN and reduce AM inoculum potential (see ***section 3***; O'Halloran *et al*., 1986; Anderson *et al*., 1987; Evans & Miller, 1988), while high levels of P application can reduce total AM hyphal length (Abbott *et al*., 1984). Although AMF differ in the amount of external mycelium they produce (Jakobsen *et al*., 1992; Hodge, 2001) little is actually known about the ecology of this network in the field such as the distances it can extend, how many plants can be linked and the interaction between CMN's produced by different AMF (see Hodge, 2000). The reason why we know so little is due to the difficulties in studying the CMN *in situ*. Thus, most of the information comes from microcosm studies, which suggest the spread of hyphae differs among AM species, for example *Glomus fasciculatum* spread through unplanted soil at a rate of 1.66 mm day^{-1}

(Harinikumar & Bagyaraj, 1995) while *Glomus mosseae* spread to colonise a soybean plant at a rate of 3 mm day^{-1} (Camel *et al*., 1991). In their review, Smith and Read (1997) found rates of infection front hyphae spread of between 0.2-2.5 mm day^{-1} depending on the plant and AM species present.

There has been much speculation as to the quantity and distance of nutrient transfer along the CMN. In a field experiment, Chiariello *et al*. (1982) applied ^{32}P to the leaves of a 'donor' *Plantago erecta* plant. After 6-7 days high levels (> 40% above background counts per min) were measured in the shoots of neighbouring plants at a distance of *c*. 45 mm. Neither the size nor type of the neighbouring plants nor the distance between the donor and receiver plants were indicators of the amount of ^{32}P transferred, implying the AM fungus connected to the donor root system was not similarly connected to all its closest neighbours. To what extent internal colonisation of the root system was related to the amount of P transferred is unknown. Studying the CMN in the field is further complicated by the fact that different AMF, all with varying capacities for external mycelium production and hence CMN distributions, may colonise the one root system. Thus, determining the *total* root length colonised by AMF will not provide information upon the respective contribution to the total by each of these fungal types. Thus, before the CMN under natural conditions can be effectively studied, there is considerable scope for microcosm experiments with known identities of AMF to gauge the extent and interaction between nutrient transfer by different mycelial networks. The results of the study by Chiariello *et al*. (1982) suggest that while nutrient transfer between plants via the CMN can occur, the distances involved may be small. Newman (1988) upon reviewing the functional significance of CMNs reached a similar conclusion. In addition to nutrient movement, there has been much interest and debate upon the possibility of carbon (C) movement between plants via the CMN (Grime *et al*., 1987; Read, 1997; Simard *et al*., 1997; Fitter *et al*., 1999; Robinson & Fitter, 1999). If such a mechanism occurred, it would have considerable impact upon competitive interactions among plants, depending upon the extent to which they were linked into a CMN. Although initial studies did suggest C could move from one plant to another via an AM CMN (Grime *et al*., 1987) subsequent studies have demonstrated this 'moved' C remains in the roots (Watkins *et al*., 1996; Graves *et al*., 1997; Fitter *et al*., 1998) and probably is retained in the fungal structures rather than donated to the plant. This differs from the ectomycorrhizal association where C movement into *the shoots* was detected (Simard *et al*., 1997), thus, demonstrating true C movement from one plant to another. The form the C moved in however, is still unknown, and could simply have been due to amino acid transfer from the ectomycorrhizal fungal symbiont to the plant rather than C movement *per se* (see Robinson & Fitter, 1999).

In the field both AM spores and mycelia will be subject to grazing by other soil organisms including other fungi, actinomycetes, collembola, earthworms and mammals (Fitter & Sanders, 1992). Burrowing activities of some organisms may also result in severing the network of hyphae. Most work on AM mycelial grazing has focused upon the activities of collembola (or springtails) again using microcosm units. Although AM hyphae are not their preferred food source (Klironomos & Kendrick, 1996), collembola can graze upon both mycelia and spores of AMF as revealed by examination of their gut contents (Warnock *et al*., 1982; McGonigle & Fitter, 1988). Although this may aid in dispersal of AM spores (see ***section 1.1***) which can survive passage through the collembola's gut, it will have an adverse effect on the CMN.

When given a choice between AM hyphae or their preferred food source, conidial fungal hyphae, the collembola did not actually feed upon the AM hyphae but did bite and sever the AM hyphae until it was detached from the roots. Thus rendering it useless as a resource capture mechanism. At the highest population of collembola studied this severing of AM hyphae from the root was as high as 50% of the total produced (Klironomos & Ursic, 1998). As the internal AM structures will be unaffected by collembola activity, this could represent a considerable C drain on the plant, with little net benefit, until the external hyphae is re-established and functional again. However, this picture may be over pessimistic, as in most of the microcosm studies collembola populations are generally much higher than in the soil, generally only one species of collembola (i.e. *Folsomia candida*) has been used thus it may not be true for all collembola types, and the collembola are given few choices of substrate, thus the detrimental effects may not be as severe in the field where the collembola themselves will also be subject to attack by other organisms higher up the food chain.

1.3 Taxonomy and phylogeny of AMF

Originally AMF were classified in the family Endogonaceae (Zyomycota), but as this genus grew into an unruly assemblage of species with little in common the need for reclassification became apparent. Morton & Benny (1990) placed AMF into a new order, Glomerales (formerly Glomales) containing three families based upon their spore characteristics: Glomeraceae (formerly Glomaceae), Acaulosporaceae and Gigasporaceae. Although this reclassification of AMF did meet with considerable opposition (see Smith & Read, 1997), mainly due to its rather restrictive definition of *only* including those AMF known to produce arbuscules, it was significant owing to the fact it was the first attempt to classify AMF based upon a phylogenetic approach. More recently two new families, the Archaeosporaceae and the Paraglomeraceae, have been identified (Morton & Redecker, 2001).

AMF in common with some other fungi currently classified as Zycomycota, have not been demonstrated to produce zygosorangia (Benny, 1995), the key criteria of placing fungi in this phylum. Indeed their sexual stage, if they have one, is thus far unidentified. This has led to the proposal that AMF should be removed from the zygomycota completely and placed into a new phylum, the Glomeromycota, containing four orders based upon their small subunit (SSU) ribosomal gene sequences namely the Glomerales, Diversisporales, Archaeosporales and Paraglomerales (Schussler, 1999; Schussler *et al*., 2001) The Glomerales contain many of the 'original' *Glomus* spp, with the remainder forming a separate family (Diversisporaceae *fam. ined*.) within the order Diversisporales, along with the families Acaulosporaceae and Gigasporaceae. The order Archaeosporales as currently defined contains two families, the Geosiphonaceae and the Archaeosporaceae. The type species for the Geosiphonaceae is a non-mycorrhizal fungus, *Geosiphon pyriforme*, which forms an association with cyanobacteria (Gehrig *et al*., 1996) but which forms spores similar to those of AMF (Schussler *et al*., 1994). The order Paraglomerales contains a single family Paraglomeraceae. Although the classification proposed by Schussler *et al*. (2001) is not ideal due to a lack of morphological characteristics to link the proposed orders together, it does finally remove AMF from the Zyomycota. Clearly further investigations into AM fungal taxonomy and systematics are required before a final working classification of AMF is finally achieved, however the

classification proposed by Schussler *et al*. (2001) provides a useful foundation on which to build.

1.4 Anatomy of AM mycorrhizas

AM colonised roots form one of two general anatomical groups depending on the plant species. These are the *Arum*-type and the *Paris*-type. In the *Arum*-type, the intercellular hyphae spread rapidly in the root cortex. Short side branches of the fungal hyphae penetrate the cortical cells and the hyphae branches divide repeatedly to produce the characteristic arbuscule structure. These fine fungal branches invaginate the plant plasma membrane which is modified to form a periarbuscular membrane. Thus, the fungus is always located outside the plant cell cytoplasm with plant and fungal membranes separated by a specialised interfacial zone or 'apoplastic' region. Arbuscule structures can be relatively short-lived and their production and degeneration in the root is a dynamic process. The *Arum*-type is formed in many crop species and other cultivated herbaceous species. In the *Paris*-type the intercellular phase of the fungi is largely absent, but extensive intracellular hyphal coils develop which spread from cell to cell in the root cortex. Arbuscules can form on these coils but their occurrence is often low or absent altogether. The *Paris*-type tends to occur mainly in ferns, gymnosperms and some wild angiosperms (Smith & Read, 1997; Smith & Smith, 1997). As these plant species are less well studied, reports of the *Paris*-type are less frequently reported than that of the *Arum*-type. The arbuscule is the organ of phosphate exchange from fungus to plant (see ***Introduction***; Rausch *et al*., 2001). Carbon may also be transferred at this site in the other direction although carbon may also be transferred to the fungus via hyphae or intercellular hyphal coils (Smith & Read, 1997). Previously, the AM association was known as the vesicular-arbuscular mycorrhizal (VAM) association because the majority of fungal species involved also form vesicles within or between the cortical cells of the root. These vesicles are believed to be important storage organs for the fungus but as not all AMF form vesicles, their occurrence is no longer used as a diagnostic feature. However, because arbuscules are not always present in the *Paris*-type, this has lead to the suggestion that the AM association should instead be called the 'Glomalean' mycorrhizal association after the fungi that are involved, rather than the structures which may be present in the root. This also has drawbacks however, as colonisation by the fungus does not demonstrate the symbiosis is functional. Moreover, there is considerable debate concerning the phylogeny of AMF, thus the 'Glomalean' title may not be appropriate (see ***section 1.3***). As this review is mainly concerned with arbuscular mycorrhizal associations with crop species and as these form the *Arum*-type, arbuscules would be expected to be present thus the association will be referred to as 'AM'.

2: Measurement and identification of AMs

Identification of spores by expert personnel is still the main way in which AMF are identified, although molecular identification is becoming more common place. As AMF cannot be grown in pure culture, 'pot cultures' (see ***section 1.2***) are used instead to maintain a pure strain of a particular fungus. To identify AMF in the field, 'trap' cultures are also useful (see ***section 1.1***). The problem with trap cultures is, that they may select AMF which are comparatively 'easy' to culture, leading not only to an underestimation of the total population but potentially missing key functions

performed by untrapped individuals. Moreover, as there is increasing evidence that AMF are more selective in their choice of host (and vice-versa) than previously thought (see ***section 1.1***), a more careful selection of host plant-AM fungal combination is required. Certainly, in field studies, molecular techniques have aided the study of AMF *in planta*, more so than any technique that was previously available. In addition to molecular technologies (see ***section 2.3***), methodological advances have also been made in other areas, including AM biomass and hyphal length estimations (see ***section 2.2***) and the application of stable isotope techniques to follow the nutrient benefits of AM colonisation (Hodge, 2001; Hodge *et al*., 2001; Hodge, 2003). Recent development of new techniques, such as the stable-isotope probing method (Radajewski *et al*., 2000), fluorescent *in situ* hybridisation combined with microautoradiography (Lee *et al*., 1999) and tracking of labelled substrate uptake (Ouverney & Fuhrman, 1999) that have been used to follow active populations of bacteria (rather than just culturable organisms) in the soil may also have some potential for AM research. If not directly, then by following the bacterial population – AM fungal interaction *in situ*. The combination of these new techniques, along with the more traditional methods of estimating AM colonisation (see ***section 2.1***) enables much more detailed examination of both the diversity and function of AMF in the environment.

2.1. AM root colonisation assessment

Unlike the ectomycorrhizal association where the fungus forms a fungal sheath encasing the root which in most cases allows colonised root tips to be distinguished from uncolonised root tips by the naked eye, it is usually impossible to state with any certainty that a root is colonised by AMF without staining and microscopic examination. Root tissues are usually cleared first in potassium hydroxide (KOH), acidified in hydrochloric acid (HCl), washed and stained. A variety of stains have been found to be suitable for staining of the fungal structures inside the root including ink, trypan blue (although now less often in use as it is a registered carcinogen), aniline blue, chlorazole black and acid fuchsin. Acid fuchsin has the advantage of giving particularly clear results under epifluorescence (Merryweather & Fitter, 1991). The extent of AM colonisation is usually expressed as a percentage of the total root length colonised (RLC; the percentage of total intercepts where hyphae or other AM fungal structures are present) recorded for each intersection using a modified grid intersect method (see McGonigle *et al*., 1990a; Giovannetti & Mosse, 1980). Usually a minimum of 100 intersects are checked for each sample. In addition, percentage of arbuscules and vesicles (if present) are usually recorded at the same time. To determine the amount of fungal tissue that is actually active, rather than the total amount present, requires vital staining of the fresh root tissue. A number of vital stains that determine levels of fungal enzymatic activity, including succinate dehydrogenase and alkaline phosphatase, are used for this purpose usually in comparison to the total amount of root length colonised (Hamel *et al*., 1990; Schaffer & Peterson, 1993; Tisserant *et al*., 1993). However, in addition to being hazardous, vital stains often give variable results due to failure to penetrate the fungal tissues properly or uniformly and/or interference with background staining of the plant material.

2.2. AM external hyphal length assessment and problems

In addition to internal AM colonisation, it is also necessary to determine the length of AM hyphae produced externally, as it is this which is in contact with the soil. A

number of techniques have been used to extract the extraradical mycelium (ERM) of AMF including wire mesh (Vilariño *et al*., 1993), sucrose flotation centrifugation (Schubert *et al*., 1987) and membrane filtration (Jakobsen *et al*., 1992). Of these different methods the membrane filtration technique produces the most reliable results (Green *et al*., 1994). The AM hyphae collected on the membrane are stained with acid fuchsin or another appropriate AM stain (see ***section 2.1***). Assessment of hyphal length is carried out using the gridline intercept method (Miller & Jastrow, 1992) for a minimum 50 fields of view under microscopic examination. However, all these techniques tend to overestimate the length of AM hyphae when unsterilised soil samples are analysed due to the presence of other, non-AM, aseptate fungi. To try and reduce this error some workers have used an AM-minus control and subtracted hyphal lengths recorded in the absence of the AMF compared to when AMF are present (Abbott *et al*., 1984; Abbott & Robson, 1985; Jakobsen *et al*., 1992). However, this is not ideal, as the AMF themselves may influence the presence of other fungal species present through competitive interactions. As with the internal AM fungal structures a number of metabolic stains have been used to estimate the activity of the ERM, including fluorescein diacetate and tetrazolium salts (Schubert *et al*., 1987; Sylvia, 1988; Hamel *et al*., 1990). Sylvia (1988) found that most of the external mycorrhizal hyphae (EMH) still attached to root fragments was active, while that extracted from the soil ranged from 0-32% active. It is unknown to what extent the EMH extracted from the soil in the study by Sylvia (1988) was still part of the common mycelium network (CMN) or simply represented pieces which had broken free and were in the process of decomposition. Additional problems including loss of hyphal viability may occur during sampling and the sampling procedure itself never results in complete recovery of all the ERM.

It has been proposed that the longevity of the EMH produced by AMF may contribute to their efficiency, and should be considered when selecting AM inoculants for use in the field (Powell, 1982a). Yet, so little is known about the rate of EMH turnover in soils that this information is required first before such suggestions can be proven to be true. Certainly, roots produced by many plant species are remarkably short-lived, dying only a few days after they are produced (Fitter, 1999), thus the EMH of AMF may similarly be short-lived and have high turnover rates. Labelling of the hyphae by stable (^{13}C) or radioactive (^{14}C) isotopes of carbon would enable such turnover rates to be followed.

Techniques which have been used to estimate the biomass of the EMH of AMF include determination of chitin content (Bethlenfalvay & Ames, 1987; Frey *et al*., 1994), ergosterol content (Frey *et al*., 1994) and fatty acid profiles (Olsson *et al*., 1995). Determination of chitin content of AM EMH is problematic when applied to soil systems as it is produced naturally in large amounts, with estimates for its annual production and standing crop in the region of 10^{10}-10^{11} tons (Gooday, 1990), thus background levels are naturally high. Moreover, chitin also occurs in many insects and arthropods as well as other soil fungi. Ergosterol contents of AMF tend to be low (unlike those of ectomycorrhizal fungi) and again, applying this technique to soil, is confounded by interference from other species (Olsson *et al*., 1996, 1998). Some fatty acids have been found to have potential as specific markers for AMF. For example, the fatty acids 16:1ω5c, 18:1ω7c, 20:3, 20:4 and 20:5 have been detected in high amounts in AM spores and colonised roots (Graham *et al*., 1995; Jansa *et al*., 1999). Fatty acids are present in various types of lipids. The phospholipid fatty acids

(PLFAs) are located in membrane structures and neutral lipid fatty acids (NLFAs) are located in storage structures. Both PLFA and NLFA analysis have been used to estimate the ERM biomass of AMF (Olsson *et al*., 1995), study nutritional effects upon AM ERM (Olsson *et al*., 1997; Ravnskov *et al*., 1999) and follow interactions between AMF and other micro-organisms (Olsson *et al*., 1996, 1998; Larsen *et al*., 1998; Green *et al*., 1999). In addition, the ratio between the concentration of the NLFA 16:1ω5 and the PLFA 16:1ω5 has been suggested to be a good indicator of the carbon supply from the plant to the AM fungus (Olsson, 1999). For example, after the addition of phosphorus (P) to soil both the internal colonisation and ERM of a AM fungus declined as did the ratio of NLFA 16:1ω5/PLFA 16:1ω5 from *c*. 60 before P addition to *c*. 20 after P addition (Olsson *et al*., 1997). PLFA and NLFA analysis is not without its problems, because most of the markers used for AMF also occur in other soil micro-organisms or fauna, albeit in lower amounts (Jansa *et al*., 1999; Olsson, 1999). These difficulties can be overcome, or at least vastly reduced, by following changes in a combination of these fatty acid markers, following changes in both PLFAs and NLFAs, where possible including a non-mycorrhizal control, and also monitoring the bacterial community using other techniques, to ensure the changes observed are not simply due to alterations in this community. That said, the technique has been demonstrated to show considerable promise in monitoring AM ERM biomass changes under a range of conditions and for the identification of AMF and their spores (Madan *et al*., 2002).

2.3. Identification of AM fungi by molecular techniques

One of the most significant developments in AM research has been the application of the polymerase chain reaction (PCR) to identify AMF actually *in planta*. Prior to this development, the diversity of AMF in the field was established on the basis of AM spore counts. This approach was necessitated, because while the vegetative characteristics of the AMF show little variation (certainly not below the family level and even then differences are not always apparent), that of their spores do. However, molecular techniques demonstrated that spore diversity found in the vicinity of the root is not readily translated into diversity found in the actual colonised root (Clapp *et al*., 1995). Several PCR-based methods have been developed for use in AM research, with most targeting the ribosomal RNA (rRNA) genes (reviewed by Clapp *et al*., 2002). Generally, ribosomal genes are present in multiple copies arranged in tandem arrays. Each repeat unit comprises of genes encoding a small-(SSU or 18S) and a large-(LSU or 28S) subunit separated by an internal transcribed spacer (ITS), which includes the 5.8S gene. The SSU and 5.8S genes evolve relatively slowly, whereas the LSU and ITS regions evolve more rapidly. Thus, the SSU and 5.8S regions are useful for studies of distantly related organisms, while the LSU and ITS regions can be used to identify organisms at the species level. Generally, through the process of concerted evolution, the multiple copies of rDNA are kept identical. However, AMF differ in this respect. Early studies demonstrated that, within populations of morphologically identical *Glomus* spores, there was considerable ITS sequence variation (Sanders *et al*., 1995). Subsequent studies have also concluded that various AM species show considerable intrasporal variation in the ITS (Lloyd-MacGilp *et al*., 1996; Pringle *et al*., 2000; Jansa *et al*., 2002), the SSU (Clapp *et al*., 1999) and the LSU ribosomal genes (Clapp *et al*., 2001; Rodriguez *et al*., 2001). This complicates matters, not only for the molecular biologist, but for anyone working on AMF as it effectively means there are no genetically identifiable 'species' of AMF *per se*. Thus, trying to decode the actual number of AMF present in a colonised root from genetic codes which

themselves can vary, is obviously problematic. Although these drawbacks may seem insurmountable, they can be partially overcome by grouping the AMF together based upon sequence similarity, and making the assumption that the AMF within each grouping share at least some phenological characteristics. Moreover, although the SSU sequences in single spores do vary, this variation is relatively small compared to differences among what is currently classified as different AM 'species' (Clapp *et al*., 1999; Schussler *et al*., 2001), whereas, the ITS regions are more variable parts of the ribosomal genes in any case. More usually, a definitive set of ribosomal primers would be found, and used as a benchmark for all subsequent investigations upon the organisms in question. The fact that no single molecular marker is available for AMF, makes their study all the more challenging. It also means that, while some AM markers have been developed which may be excellent for detecting some AMF, others may be missed, thus the true diversity in the root may be underestimated. Such problems have not prevented the application of molecular techniques in ecological investigations upon AMF (see Table 1), and these techniques still represent the most effective measurement of AM diversity *in planta*.

To date, molecular techniques have not been widely used to characterise the structure of AMF communities. 18S rDNA profiling has largely been done by cloning / sequencing of PCR amplified partial 18S rRNA fragments, which is expensive and time consuming. Addititionally the primers used in these studies are specific for members of Glomus-group A within the Glomerales, and members of the Diversisporales, (see **section 1.3**), and will not amplify species in the Glomus-group B within the Glomerales, some members of the order Diversiporales, or AMF in the orders Archaeosporales and Paraglomerales (Husband *et al*., 2002). Although there is potential for rapid and inexpensive analysis of AMF community composition and diversity by adapting exisiting molecular profiling techniques such as terminal restriction fragment length polymorphism and denaturing gradient gel electrophoresis using partial 18S rDNA fragments (Kowalchuck *et al*., 2002) there has been little progress in development of these methods and protocols to characterise all AMF orders are not currently available. Development of such methods is essential if key questions relating to the role of AMF in agriculture are to be answered.

Future developments using such molecular applications could include determining relationships between species diversity and AMF functioning, and elucidating the relative role and contribution of different AMF colonising the roots and the identification and quantification of AM fungal ERM in soil.

3: AMF in agriculture

AMF are perhaps the most abundant fungi found in agricultural soils, making up between 5 and 50% of the total soil microbial biomass (Olsson *et al*., 1999), although their actual *diversity* is low (Helgason *et al*., 1998). Benefits to the crop include improved nutrition (Lambert *et al*., 1979; Thompson 1987; Graham 2000; Srivastava *et al*., 2002), enhanced resistance to pests and disease (Schonbeck, 1979; Paulitz & Linderman, 1991; Linderman, 1994; Borowicz, 2001; Calvet *et al*., 2001) and improved water relations (RuizLozano & Azcon, 1995; Smith & Read, 1997; Mohammad *et al*., 2003). Most agricultural crop species, with the exception of those of the Cruciferae, Polygonaceae and the Chenopodiaceae, are able to form AM fungal

associations and in most cases do so (Sylvia & Chellemi, 2001). However, the degree to which these benefits are manifest is dependant on many factors, both biotic and abiotic. These include fertilisation (Sanders, 1975; Jasper *et al*., 1979; Thomson *et al*., 1986; Braunberger *et al*., 1991), tillage (O'Halloran *et al*., 1986; Anderson *et al*., 1987; Evans & Miller, 1988; Douds *et al*., 1995), use of biocides (Sreenivasa & Bagyaraj, 1989; Kurle & Pfleger, 1994; Schreiner & Bethlenfalvay, 1997), use of organic amendments (Harinikumar & Bagyaraj, 1989; Douds *et al*., 1997; Kabir *et al*., 1998) and rotation design (Johnson *et al*., 1992; Miller, 2000; Menéndez *et al*., 2001). As a result, the precise role of AMF in improving yield and crop quality in the field is largely unquantified. Results are often contradictory and do not bear out the promise of laboratory and glasshouse based research. Much of this variability is down to the different species, strains and isolates of AMF that are likely to colonise the root in the field. Little is known about actual AM populations and diversity in arable situations, although it has been found to be much reduced compared to natural systems and dominated by *Glomus* species (Helgason *et al*., 1998; Daniell *et al*., 2001). Despite such problems, management practices that maintain and enhance naturally occurring mycorrhizal populations and the introduction of new inoculum has been shown to result in benefits to crop yield and quality in the field.

3.1 *Nutrition*

The most widely recognised role of AMF in the plant-fungus relationship is that of improving the uptake of nutrients by the host plant, particularly of phosphorus (P). Indeed P nutrition is generally regarded as the main controlling factor in the plant-fungal relationship (Thompson, 1987; Smith & Read, 1987; Graham, 2000). In soils with low phosphorus availability AMF can play a significant role in crop growth. However, it has become increasingly evident that AMF also have an important role in the uptake of a range of other nutrients particularly zinc (Zn), but also including copper (Cu), iron (Fe), nitrogen (N), potassium (K), calcium (Ca) and magnesium (Mg) (Smith & Read, 1997; Clark & Zeto, 2000). For example; Azaizeh *et al*. (1995) grew maize (*Zea mays*) with and without AMF, colonised plants had higher concentration of P, Zn and Cu. Koide *et al*. (2000) grew lettuce (*Lactuca sativa)* and velvetleaf (*Abutilon theophrasti*) in P deficient soil with and without AM inoculation. Inoculation increased growth, phosphorus content and phosphorus use efficiency compared with non-mycorrhizal plants. Li *et al*. (1991b) found better growth in mycorrhizal than non mycorrhizal white clover (*Trifolium repens*), with 70-80% of P uptake being contributed by AMF in the mycorrhizal plants. Wellings *et al*. (1991) demonstrated that AM colonisation increased dry weight, P and Zn uptake and yield in pigeon pea (*Cajanus cajan*). Ibijbijen *et al*. (1996) examined the effect of AMF on the growth and nutrient uptake of the common bean (*Faseolus vulgaris*) on a P deficient soil. Dry matter production was increased 8-23% and P uptake by 160-335%. K concentration was also increased in half of the bean varieties tested. Other examples include: Lambert *et al*. (1979); Kothari *et al*. (1990); Smith & Read (1997); Srivastava *et al*. (2002) and Mohammad *et al*. (2003). In legumes, as well as a direct increase in nutrient uptake there is an important synergistic effect of AM infection (Ibijbijen *et al*., 1996; Dar *et al*., 1997). The legume-rhizobium symbiosis is dependant on high concentrations of phosphorus and therefore enhanced P nutrition arising from AM colonisation, results in an increase in nodulation and N fixation (Ganry *et al*., 1985; Ibijbijen *et al*., 1996; Vazquez *et al*., 2002). This is especially relevant to organic and other low input systems that rely on fixation of atmospheric N by legumes to supply nitrogen to crops.

Much of the work on AMF and their role in enhancing the nutrition of agricultural crops has been done in the laboratory or glasshouse. These highly simplified systems can be criticised on the grounds that they do not replicate field conditions. However, despite difficulties in controlling the experimental conditions it is also possible to demonstrate a positive role for AMF in increasing nutrient uptake under field conditions. A good example comes from southern Queensland in Australia. Generally, phosphorus fertiliser has not been applied to agricultural soils in this region and they have a low index of phosphorus availability. Many crops exhibit P and Zn deficiency when grown after long bare fallows or after non-mycorrhizal rape (*Brassica napus*). The severity of this so called *long fallow disorder* was shown to be directly related to AM inoculum density and P deficiency (Thompson, 1987, 1991, 1994). Crops more dependant on AMF, such as maize, were more susceptible to *long fallow disorder* than those less reliant on AMF such as wheat (*Triticum aestivum*). Other examples of enhanced nutrient uptake in the field include, enhanced Zn uptake in wheat and pea (*Pisum sativum*) (Ryan & Angus, 2003), enhanced Zn, Cu and P uptake in bean (Hamilton *et al*., 1993), both cases associated with high levels of native AM colonisation, and inoculation of field plots with *Glomus fascicilatum* increasing growth and P uptake of garlic (*Allium sativum*) at low levels of P fertilisation (Al-Karaki, 2002).

In situations where available soil phosphorus concentration is high, either because of soil type or fertilisation, the reliance of the host plant on AMF is reduced. Thingstrup *et al*. (1998) demonstrated reduced shoot growth of oilseed flax in soils fumigated with dazomet (a fungicide) at low soil phosphorus concentrations, but not at soil phosphorus concentrations above 40 mg kg^{-1} (Olsens P). Bethlenfalvay and Barea (1994) measured a significant (57%) increase in yield of peas grown in the glasshouse in response to inoculation with *Glomus mosseae* in a clay loam low in P, but an insignificant (8%) increase in a silt loam high in P. While Hetrick *et al*. (1996) demonstrated reduced response of six wheat cultivars to AM inoculation at increasing levels of P fertilisation.

This reduced reliance on AMF at high soil P concentration is generally accompanied by reduced levels of AM root colonisation (Al-Karaki & Clark, 1999; Kahiluoto *et al*., 2001). There is also evidence that fertilisation actually selects AMF species that are inferior in terms of providing a benefit to the host. Johnson (1993) compared fungal diversity on plots fertilised with P for 8 years, with plots left unfertilised. Relative abundance of different species changed as a result of P fertilisation, with increasing dominance by *Glomus intraradix*. Glasshouse experiments showed that AMF from the fertilised plots were less able to support the growth of big bluestem grass (*Andropogon gerardii*), an effect that appeared to be the result of increased carbon drain on the host (a smaller number of arbuscles, but as many vesicles in fertilised plots, as with AMF from unfertilised plots).

Despite the apparently overwhelming influence of P nutrition, it has become increasingly evident that the availability of other nutrients also has a role in determining AM colonisation and activity. The influence of nutrients other than P on AM colonisation and host growth is illustrated by Hawkins & George (1999), who found that hyphal growth of *Glomus mosseae* in wheat was reduced in conditions of severe N deficiency. Liu *et al*. (2000) also demonstrated a controlling effect of soil N

on root colonisation and extra radical hyphae production, this time in maize, while Jamal *et al*. (2002) demonstrated a controlling effect of soil Zn and nickel (Ni) on AM colonisation of wheat on soils low in Zn. The controlling effect of other nutrients on the plant-fungal relationship also means that fertilisers other than P can reduce AM colonisation (Miller & Jackson, 1998).

However, the role of AMF in the uptake of other nutrients is less clear than in the case of P and in some cases results are contradictory, particularly in the case of micronutrients. Liu *et al*. (2000) examined the role of AMF in micronutrient uptake of maize. AM colonisation increased total shoot Fe at low soil micronutrient level but reduced it at high soil micronutrient level. Kothari *et al*. (1990) also examined the role of AMF in nutrient uptake of maize, though P, Zn and Cu uptake were enhanced by AMF, K concentration was reduced in root and shoot and Fe concentration in shoots. Manganese (Mn) concentration was also reduced in root and shoot, a common effect of AMF, even where other nutrient concentrations may have been increased, e.g. Azaizeh *et al*. (1995). An effect attributed to a decrease in Mn reduction in the rhizosphere (Kothari *et al*., 1991b).

This apparently contradictory role of AMF in nutrient uptake is not confined to micronutrients however. The relationship between soil nutrient concentrations and AMF mediated uptake is complex and there are many cases where AM colonisation has been shown have either a neutral effect on nutrient uptake and growth or even reduce it. Sainz *et al*. (1998) were unable to increase dry matter yield or nutrient content of red clover (*Trifolium pratense*) or cucumber (*Cucumis sativus*) on a soil of low nutrient status by inoculation with *Acaulospora* sp. Ryan *et al*. (2002) found no increase in the yield of wheat on soils with a range of P concentrations, in response to AM colonisation by native AMF in the field, with an increase in grain P and Zn concentration in only one of five experiments. Ryan and Angus (2003) obtained a similar result with wheat and peas, with colonisation by native AMF in the field having no correlation with crop growth, yield or nutrient uptake (with the exception of Zn). While Kahiluoto *et al*. (2001) showed that on a soil that had received 45 kg ha^{-1} yr^{-1} of P fertiliser for 20 years, AM colonisation actually reduced the growth of flax by 30% and barley (*Hordeum vulgare*) by 7%, while AM colonisation was retarded in white clover.

Why AMF have an apparently positive effect on nutrient uptake in some cases and a neutral or negative effect in others, even where soil phosphorus concentrations are similar, is unclear, but may be related to soil moisture, temperature, disease, soil micronutrient concentrations or the species or strains of AMF colonising the plant (Ryan & Graham, 2002).

3.2 *Control of disease and pests*

Although enhanced nutrient uptake is generally considered to be the principle benefit of AMF to crop growth, it has become increasingly apparent that they play a multifunctional role in the plant-fungal relationship (Newsham *et al*., 1995b). Of particular interest in an agricultural context, is their role in protecting the host plant. Indeed, some authors have proposed AMF as an alternative to the use of synthetic biocides for control of pest and disease.

The initial response to early laboratory research into the effect of mycorrhiza on pathogens was a surge in their use as a biological 'control agent' in the 1980s. Mycorrhizas were seen as a biological magic bullet to problems with disease. Though laboratory and glasshouse trials were encouraging, results in the field were often disappointing, due to a lack of understanding of the biology behind mycorrhiza and their use in a non-specific manner. However, as understanding has improved, and pressures to limit pesticide use have increased, along with an increasing number of promising field trials, commercial interest has once again increased.

3.2.1 Disease Control

There is now strong evidence that AMF play an important role in the suppression of soil borne disease, particularly fungal diseases (Schonbeck, 1979; Paulitz & Linderman, 1991; Linderman, 1994; Borowicz, 2001) which are particularly difficult to control, even with biocides (Dar *et al*., 1997). Examples include inoculation of onion with *Glomus* species delaying the development of white rot in onions by two weeks and providing significant protection compared with non AM plants for 11 weeks (Torres-Barragán *et al*., 1996). Inoculation of asparagus with *Gigaspora* and *Glomus* species, resulting in a reduction in the occurrence of Fusarium root rot (*Fusarium oxysporum*) symptoms from 90% in uninoculated plants to 20-50% in inoculated plants (Matsubara *et al*., 2001). There was also an increase in plant biomass, with a similar result being obtained by Matsubara *et al*. (2002). A reduction in the severity of *Fusarium* on roots of french bean by 34-77% (Dar *et al*., 1997), and a 24% increase in dry weight of verticillium wilt (*V. dahliae*) infected tomatoes (*Lycopersicon esculentum*) and a 10% increase in similarly infected aubergines (*Solanum melongena*) in response to AM inoculation (Karagiannidis *et al*., 2002). There are also reports of reductions in *Phytopthora* on citrus (Davis & Menge, 1980) and on cherry (*Prunus avium*) (Cordier *et al*., 1996). Reductions in the severity of disease occur despite the fact pathogen infection generally reduces AM colonisation (Dar *et al*., 1997; Karagiannidis *et al*., 2002). Though there is likely to be an indirect effect of AMF infection on plant disease, through enhanced nutrition and thus generally stronger growth of the host plant, this effect can be isolated (Trotta *et al*., 1996).

One of the principle mechanisms by which AMF reduce the impact of diseases on the host plant is through competition with the pathogenic organism for space and resources within the host root. Though the exact mechanism is unclear, there is generally a close inverse relationship between the number of host cells infected with AMF and the number infected with the pathogen (Matsubara *et al*., 1995; Matsubara *et al*., 2001). Infection of a cell by AMF effectively excludes the pathogen, as a result, colonisation by AMF must occur before attack by the pathogen if protection is to be effective (Matsubara *et al*., 2001; Sylvia & Chellemi, 2001).

Though competition between the pathogen and AMF within the host roots certainly plays a part in disease resistance, it seems likely that there are a number of mechanisms involved. AMF may change the environment of the rhizosphere, upsetting pathogen equilibrium (Filion *et al*., 1999). Dar *et al*. (1997) suggest that an observed reduction in *Fusarium* root rot in common bean colonisation by AMF, could be attributed to a general improvement in the nutrient status of the rhizosphere soil, increasing rhizosphere microbial biomass, and thus the level of competition to which the *Fusarium* was exposed. Other mechanisms may involve very subtle physical or

biochemical changes to the root caused by the AMF, which make infection by the pathogen more difficult. For example, AM colonisation is known to result in changes to the host root architecture (Yano *et al*., 1996) and this may reduce pathogen access. Vigo *et al*. (2000) looked at the mechanism by which *Glomus mossae* can control the root infecting fungi *Phytopthora parasitica*. The AMF appeared to cause physical changes to the root reducing the number of infection sites for the pathogen by 30-39%, resulting in decreased intensity of disease and a 30 % reduction in fruit necrosis.

There is also evidence that AMF cause changes to root exudates, which inhibits the pathogen. Norman & Hooker (2000) showed that root exudates from mycorrhizal strawberry plants caused a 64-89 % reduction in sporulation of *Phytopthora fragariae*, compared with spores treated with non-mycorrhizal root exudates. Filion *et al*. (1999) examined the effect of exudates of *Glomus intraradices* colonised carrot (*Dacus carota*) on four soil inhabiting micro-organisms. Exudates increased the growth of *Pseudomonas chlororaphis* (a beneficial bacteria) and the conidial germination of *Trichoderma harzianum* (a beneficial fungus) but reduced the conidial germination of *Fusarium oxysporum* spp. *chrysanthemi* (a root rot fungus), the growth of *Clavibacter michiganensis* subsp. *michiganensis* (which causes canker) was not affected. Such results indicate a complex interaction between AMF and other soil micro-organisms and also suggests that a viable method of disease control using AMF could be to intercrop mycorrhizal plants with non-mycorrhizal plants.

Even where disease control has apparently failed, there may be more subtle effects on the pathogenic organism. For instance Kjøller and Rosendahl (1996) measured similar infection levels of the pathogen *Aphanomyces euteiches* in both *Glomus intraradices* inoculated and non-inoculated pea plants. However, AMF inoculated plants were more tolerant to the pathogen. Closer examination of the relationship showed that pathogen enzyme activity was lower in the inoculated plants. Bødker *et al*. (2002) examined the effect of native AMF on the infection of pea by *Aphanomyces*. Though AM colonisation did not seem to reduce disease severity, it did inhibit the reproductive stage of the fungi, again indicating the subtle and complex nature of the relationship.

Though these results are encouraging, as with enhanced nutrition, the relationship between fungi, host and environment is complex and enhanced host resistance is not always manifest. Prados-Ligero *et al*. (2002) failed to delay the onset of white rot in garlic through inoculation of soil with *Glomus intraradices*, despite the fact that garlic was extensively colonised by mycorrhizal fungi, while Garcia-Romera *et al*. (1998) found no effect of *Glomus mosseae* on fusarium in soybeans (*Glycine max*). Often the degree of control achieved with AMF varies between AMF species (Matsubara *et al*., 2001), though whether this is the result of host or disease specificity is unclear.

Research into the effect of AMF on disease has mostly focused on soil borne diseases, however, there is evidence that AMF can also reduce the severity of above ground fungal disease. Feldman and Boyle (1998) showed a direct reduction in powdery mildew on begonias induced by AMF inoculation, though as with soil borne disease, AMF inoculation had to be established before exposure to the pathogen to offer significant protection. West (1995) demonstrated that AMF inoculation of *Senicio vulgaris* could mitigate the effects of the rust (*Puccinia lagenophorae*) on the host plant and that this resulted in increased growth of offspring of AM plants. However,

the interaction between AMF and above ground diseases is complex. Gerns *et al*. (2001) demonstrated a *greater* susceptibility to the mildew *Erysiphe graminis* in barley plants colonised with AMF than those not colonised. However, grain yield and quality were higher in the AM plants. AM colonisation somehow delayed leaf senescence caused by the mildew, despite higher pathogen infection rates.

3.2.2 Pest Control

As well as reducing damage to roots caused by soil borne fungal diseases there is some evidence that AMF may reduce damage to roots caused by pathogenic nematodes. For instance, Jaizme-Vega *et al*. (1997) were able to suppress root galling and nematode numbers in roots of banana (*Musa spp*.) by inoculation with *Glomus mossacea*. Vaast *et al*. (1998) were able to increase the growth of coffee (*Coffea arabica*) exposed to the nematode *Pratylenchus coffeae* by inoculation with *Acaulospora mellea* and *Glomus clarum* and Nagesh *et al*. (1999) were able to reduce numbers and egg production of the nematode *Meliodogyne incognita* in tomato grown in pots and nursery beds by inoculation with *Glomus fasciculatum*. Similar results have been obtained by Schonbeck (1979), Rao *et al*. (1995), Habte *et al*. (1999) and Mularwaman *et al*. (2002). However, the relationship is complex and is affected by environment. For example Waceke *et al*. (2002) found that the suppressive effects of AMF on a root-knot nematode was in most cases reduced by P fertilisers. A similar effect was demonstrated by Carling *et al*. (1996) who showed that tolerance of peanut (*Arachis hypogaea*) to the nematode *Meloidogyne arenaria*, was induced by AMF at two lower levels of P fertilisation but not at two higher levels. There are also interactions with other beneficial soil fauna as mycorrhizal spore number in the soil are reduced by Collembola (see ***section 1.2***). Bakonyi *et al*. (2002) found the optimum level to be 0.2 and 0.4 collembola g^{-1} soil density for maximum AM colonisation of maize and red fescue. As with protection against soil borne disease, there is evidence that for AMF inoculation to be effective in protecting against pathogenic nematodes, it must occur before exposure to the nematode. Talavera *et al*. (2001) was able to demonstrate tolerance of tomato to the nematode *Meloidogyne incognita*, with 24 % higher shoot weight compared with non-AMF plants, but inoculation with AMF had to occur 3 weeks in advance of inoculation with the nematode to be effective. Similarly Vaast *et al*. (1998) failed to protect coffee from the nematode *Pratylenchus coffeae* unless inoculation with the AMF occurred four months before expose to the nematode. Some authors have questioned the whole importance of AMF for nematode control in the field as there is so much contradictory evidence. For instance, in some cases AMF seen to increase nematode reproduction. Carling *et al*. (1996) showed that despite increased tolerance of AMF inoculated peanut to the nematode *Meloidogyne arenaria*, root galling and egg production per gram of root were increased. More contradictory evidence comes from Calvet *et al*. (2001) who were able to protect peach-almond hybrids by inoculation with 3 *Glomus* sp. but native soil AMF offered no protection. The evidence that suggests AMF can protect plants against pathogenic nematodes is undoubtedly weaker than for protection against soil fungal diseases and though Linderman (1994) concluded the balance of evidence suggested AMF could play a role in the control of root nematode damage, Borowicz (2001), using a meta analysis of work published between 1970 and 1998, concluded that AMF tend to exacerbate the harmful effects of nematodes. Though this conclusion was somewhat qualified, with AMF tending to protect against sedentary nematodes and encourage migratory nematodes.

As well as influencing resistance of the host plant to soil nematodes, there is some evidence that AMF can also protect the host plant from above ground herbivores. However, the evidence is contradictory and some suggests that AMF can increase damage. For example, Gange and West (1994) examined the effect of native AMF on the resistance of *Plantago lanceolata* to foliar feeding insects. Though AMF colonised plants suffered less from generalist chewing and leaf mining insects, both in the field and in laboratory studies, the leaf sucking insect *Myzus persicea* performed better on AMF colonised plants. Vicari *et al*. (2002) examined the effect of AMF on the feeding of a moth larvae on the grass *Lolium perenne*. Whether AMF increased or decreased damage was dependant on soil P concentration. Goverde *et al*. (2000) compared the performance of a lepidopteron caterpillar on AMF inoculated *Lotus corniculatus* with performance on non-inoculated plants. Growth and survival of caterpillars on AMF plants was generally significantly greater, depending on the species of AMF used. While Borowicz (1997) also measured a higher level of damage on AMF inoculated soybean, caused by Mexican bean beetle, than on non-inoculated plants.

Clearly AMF have an influence on the resistance of the host plant to pests and diseases. Though much of the published evidence suggests that AMF have a positive effect, the relationship is a complex one and both negative and neutral effects are also commonly reported. Much of the work done so far has been laboratory or glasshouse based and as such, the importance of AMF in protecting against pests and disease in the field is still in question. Negative or neutral effects of AMF demonstrated in the laboratory and glasshouse may be the result of ineffective host-AMF combinations and may not reflect the situation in the field where AMF species diversity may be higher. Much work is still required if AMF are to used effectively in the control of pests and disease.

3.3 *Water Relations*

As well as increasing nutrient uptake and reducing the effect of pests and diseases, there is evidence that AMF are able to increase the host plants tolerance to water stress (Smith & Read, 1997). The mechanisms involved are unclear but may include increased root hydraulic conductivity, improved stomatal regulation, hyphal water uptake and osmotic adjustment of the host; or be due to the ERM helping to maintain contact with the soil particles to extract water from smaller pores (RuizLozano & Azcon, 1995). Though the exact nature of the effect is unclear, often both water and nutrient uptake appear to be higher in drought stressed mycorrhizal plants than in non mycorrhizal plants. Davies *et al*. (1992, 2002) demonstrated increased drought resistance in pepper (*Capsicum annuum*) inoculated with AMF; Borkowska (2002) measured increased drought resistance in micropropagated strawberries inoculated with AMF; RuizLozano and Azcon (1995) demonstrated increased drought tolerance in AMF lettuce; von Reichenbach and Schonbeck (1995) demonstrated increased drought tolerance in AMF flax and Osonubi (1994) and Amerian (2001) demonstrated increased drought tolerance in AMF maize. Examples of enhanced nutrient uptake in water stressed conditions include Al-Karaki and Clark (1999) who measured higher nutrient uptake and drought tolerance in AM barley compared with non AM barley; Subramanian and Charest (1997, 1999) measured considerably more N, P, K, Mg, Mn and Zn in water stressed AM maize than in un-colonised plants and Srivastava *et al*. (2002) measured increased nutrient uptake as well as improved water relations in citrus inoculated with AMF. Perhaps paradoxically, evidence suggests that drought

resistant plant varieties are less reliant on AMF for their drought tolerance than drought sensitive varieties. Subramanian and Charest (1997) examined the effect of AMF on the drought tolerance of two maize cultivars. Water stress reduced yield in the sensitive cultivar more when it was non-mycorrhizal than it did the less sensitive cultivar. Al-Karaki and Al-Raddad (1997) similarly showed that a drought sensitive wheat genotype was much more dependant on AMF for dry matter production and nutrient uptake than a drought resistant genotype.

Despite results such as these, some authors argue that a substantial role for AMF in water uptake and water relations has not been demonstrated unequivocally (RuizLozano & Azcon, 1995; Smith & Read, 1997) and that inoculation with AMF does not always increase drought tolerance. Bryla and Duniway (1998) were unable to increase drought resistance in safflower (*Carthamus tinctorius*) or wheat by inoculation with *Glomus etunicatum*, though they were able to increase drought tolerance by acclimation. Ryan and Ash (1996) found that water stress reduced AM colonisation in wheat, (5-16% compared with 40-70% without water stress) in a field situation, and that this was accompanied by very poor growth. They suggested that AMF play little role in alleviating severe water stress in wheat. These apparently contradictory results may be the result of ineffective combinations of AMF and plant host. For instance Davies *et al*. (2002) failed to alleviate drought stress in Chile ancho pepper (*Capsicum annuum*) using an inoculum of *Glomus fasciculatum*, but a mixture of native Mexican AMF did alleviate drought stress. While drought stress reduced hyphal growth of *Glomus fasciculatum* it increased both hyphal growth and arbuscle formation with the mixed native AMF. Differences in the efficiency of AMF in alleviating drought stress may be subtle. RuizLozano and Azcon (1995) examined the effect of *Glomus deserticola* and *G. fasciculatum* on water uptake in lettuce. Though both AMF species improved water relations of lettuce, *G. fasciculatum* was much more sensitive to the level of water in the growing medium, becoming less effective as the level of water stress increased. When drought stress becomes severe, the evidence suggests that AMF fail to improve the growth of host plants (Ryan & Ash, 1996). Bryla and Duniway (1997) for instance, reported that mycorrhizal plants of safflower and wheat survived moderate drought stress better than non-AMF plants, but there was little difference between mycorrhizal and non-mycorrhizal plants when the drought conditions were severe.

As well as benefiting plants undergoing drought stress, AMF also seem to increase the host plant tolerance to water stress induced by saline conditions. Mohammad *et al*. (2003) measured higher dry weight and P, Fe and Zn uptake in barley plants grown on saline soils when they were inoculated with AMF. The exact mechanism involved is unclear, though Feng *et al*. (2002) found that improved tolerance of AM colonised maize plants to salt stress, was related to higher accumulation of soluble sugars in roots. Again there is also a variety effect. Al-Karaki *et al*. (2001) examined two varieties of tomato growing on saline soils in the glasshouse. The more salt tolerant variety had a significantly higher rate of AM colonisation, higher shoot dry matter and P, K, Zn, Cu and Fe, though colonisation increased shoot dry matter in both varieties. Dependence of both varieties on AMF was greater in saline conditions, with a greater enhancement of growth on the saline soil than on a non saline control soil. Different AM species also differ in their efficiency, which can in turn be modified by the degree of salinity. Pande and Tarafdar (2002) examined the salt tolerance on a number of AM species colonising neem (*Azadirachta indica* Linn.), *Glomus mosseae* being the most

salt resistant species, though *G. fasciculatum* produced the highest plant biomass at low to intermediate salt levels.

The available evidence suggests that AMF do play some role in improving water relations of the host plant, particularly in saline conditions. How significant this effect is remains unclear. There certainly seems to be a limit to the degree of water stress that AMF can mitigate against and once again there seems to be some degree of specificity in the host-fungal relationship, which may explain the failure of some experiments to show an effect.

3.4 *Soil Structure*

Interest in AMF has tended to focus on their role in directly influencing the growth of the host plant. However, it has become increasingly apparent that AMF play a wider role in ecosystems. In an agricultural context, the most important of these is in maintaining soil structure. All soil fungi promote soil aggregation through the enmeshing effects of fungal hyphae, binding soil microaggregates into larger macroaggregates and thus promoting aggregate stability. Fungi work in combination with bacteria to stabilise macroaggregates, bacteria dominating in the moist less aerobic centre of aggregates, producing polysaccharide gums, and fungi dominating the drier aerobic outer aggregate, enmeshing the aggregate (Tisdall, 1991). In addition, AMF hyphae are also covered in an extracelluar glycoprotien called glomalin, which sticks hyphae to soil. Glomalin is very resistant to microbial decay and accumulates in soils (Rillig *et al*., 2001). Some work suggests that glomalin exerts a strong influence on soil aggregate stability, an effect that can be manipulated by changing crop rotations (Wright & Upadhyaya, 1998; Wright & Anderson, 2000), though other work shows no correlation between the glomalin content of soil and aggregate stability (Borie *et al*., 2000; Franzluebbers *et al*., 2000). The role of glomalin aside, a host plant transfers as much as 20% of all fixed carbon to the fungal partner, which in itself forms an important flow of carbon to the soil microbial community, promoting aggregate stability (Jastrow *et al*., 1998). The overall effect of hyphae and carbon inputs can be significant. Thomas *et al*. (1986) measured a 72% increase in water stable aggregates >2mm, with AMF colonised onions (*Allium cepa*) grown in pots, compared with un-colonised onions. The effect tends to be greater with greater fungal hyphal length (Tisdall & Oades, 1979). Factors that reduce fungal colonisation, such as fertilisers, therefore reduce the effect. Bethlenfalvay and Barea (1994) measured a 400% increase in soil aggregation in a silt loam low in P as a result of the growth of AMF colonised peas but only a 50% increase in a clay loam high in P. Though Bethlenfalvay *et al*. (1998) found that nitrogen status had no effect on the interactions between AMF and roots in the formation of water stable soil aggregates.

Though not a directly measurable benefit of AMF to crops, improved aggregate stability is especially important in an agricultural context, where cultivations, trafficking and low levels of soil organic matter all tend to result in damaged soil structure. This can in turn result in reduced root growth, reduced drainage and reduced workability of the soil.

3.5 *Impact of agriculture on AMF*

Agricultural systems are very different from the natural systems in which the AMF-plant relationship has evolved. The continual cycle of crop planting, removal and

tillage disrupts the plant fungal relationship, meaning the AMF has to continually re-establish itself (O'Halloran *et al*., 1986; Anderson *et al*., 1987; Evans & Miller, 1988). (Though this situation is similar to many laboratory and glasshouse studies on AMF, where the AMF inoculum is added as colonised root fragments or spores, after which the fungus has to under go a period of development and establishment.) Bare fallow periods and non-mycorrhizal crops may also occur within an agricultural rotation, further disrupting the plant-fungus relationship (Black & Tinker, 1979; Thompson, 1987, 1990, 1994; Harinikumar & Bagyaraj, 1988; Ryan & Graham, 2002). Other agricultural practices, which may adversely impact on AMF include the use of fertilisers, particularly P (Sanders, 1975; Jasper *et al*., 1979; Thomson *et al*., 1986; Braunberger *et al*., 1991); the application of biocides (Sreenivasa & Bagyaraj, 1989; Kurle & Pfleger, 1994; Schreiner & Bethlenfalvay, 1997) and in certain cases the application or organic amendments (Harinikumar & Bagyaraj, 1989; Douds *et al*., 1997, Kabir *et al*., 1998). All of these factors interact, meaning that it is not always easy to predict the behaviour of the AMF-plant relationship in an agricultural situation.

3.5.1 Fertilisers

The use of P containing fertilisers has had a significant impact on agricultural productivity throughout the world (Wild, 1988), however, in areas such as the UK, P fertiliser use has been well in excess of crop requirements, leading to a build up of total and easily available P (Withers *et al*., 2001). High soil phosphorus availability generally results in reduced reliance of plants on AMF (Bethlenfalvay & Barea, 1994; Thingstrup *et al*., 1998) and a reduction in AM root colonisation due to the enhanced nutritional status of the plant (Sanders, 1975; Jasper *et al*., 1979; Thomson *et al*., 1986; Braunberger *et al*., 1991). Kahiluoto *et al*. (2001) demonstrated reduced AM colonisation of roots and reduced AMF spore density in soil with increasing P fertilisation levels for several crop types on two soils of low and intermediate phosphorus status. Jensen and Jakobsen (1980) examined AM colonisation of wheat and barley at five sites in Denmark with varying P fertility. AM colonisation was highest at the sites with lowest soil P. Further-more, high levels of P fertiliser reduced AM colonisation and spore numbers at all sites. Al-Karaki and Clark (1999) measured declining AM colonisation of durum wheat (*Triticum turgidum*) with increasing P fertilisation. Douds and Schenck (1990) inoculated Bahiagrass (*Paspalum notatum*) with three species of AMF on a soil low in P. Nutrient solutions high in P reduced percentage root colonisation by all three AMF species. Miller and Jackson (1998) examined AM colonisation in lettuce under intensive production, increasing use of P fertilisers reduced AM colonisation. Similar results were obtained by Jaizme-Vega *et al*. (1997) with banana.

Reduced root colonisation is usually accompanied by reduced sporulation. Martensson and Carlgren (1994) showed a strong relationship between AM spore numbers in soil and phosphorus fertilisation. Moderate amounts of phosphorus (45 kg ha^{-1} yr^{-1}) reduced spore numbers by as much as 50% over five years, while zero additions of phosphorus increased spore numbers by 100% in 5-14 years. Although this did not appear to have an impact on crop dry matter production, P uptake was reduced in the fertilised plots. However, there are exceptions, Khalil *et al*. (1992) found high levels (60-100%) of AM colonisation in soybean on 15 Iowa soils, despite very high soil P concentrations. Anderson *et al*. (1987) also found high levels of AM colonisation (up to 89%) in maize, despite high soil P. Davis *et al*. (1984) examined

colonisation of hops (*Humulus lupulus*) and peppermint (*Mentha piperita*) by native AMF at several sites. Extractable soil P was high, in the range of 21-244 mg kg^{-1} (Bray) and though colonisation of hops was low, colonisation of peppermint was moderate, 26-32%. Furthermore, on soils very low in P a small amount of P fertiliser will often increase AM colonisation (Jensen & Jakobsen, 1980). However, though high soil P may not always reduce AM colonisation, it may select less efficient species (Johnson, 1993).

It is not only fertilisation with P that can reduce AMF activity. Several authors have reported reduced AM colonisation with high levels of N fertilisation. Miller and Jackson (1998) examined AM colonisation in lettuce under intensive production at 18 sites, increasing use of N as well as P fertilisers reduced AM colonisation. Liu *et al*. (2000) showed that root colonisation of maize by AMF and production of extraradical hyphae were influenced by N fertilisation as well as P, high N depressing AMF. Burrows and Pfleger (2002) examined AMF spore production in plots containing 1-16 species, in semi natural ecosystems. Spore production of several species was negatively correlated with mid season soil nitrate concentrations, suggesting that nitrogen fertiliser is likely to select against some species and reduce overall AMF diversity. Treseder and Allen (2002) suggest that use of nitrogen fertiliser may select for *Glomus* species, which are indeed the most common in agricultural systems (Helgasson *et al*., 1998). There is also a complex interaction between fertiliser use and the concentration of other soil nutrients. Valentine *et al*. (2001) showed that AM colonisation in cucumber depended on both P supply and the supply of other nutrients. Plants grown at low P, with high concentrations of other nutrients, had the highest AM colonisation, and the highest biomass.

Generally speaking, fertilisers have a negative impact on AM colonisation levels and spore production, particularly soluble P fertilisers. There is also some evidence that fertilisers may select for less efficient AMF species. The main exception to this being very low fertility soils where AM colonisation may be inhibited by the low nutrient conditions. However, there are instances where AM colonisation remains high, despite high soil nutrient concentrations. It is unclear if this is because the AMF are providing some other benefit to the host, such as resistance to disease, or if the AMF are semi-parasitic P tolerant species.

3.5.2 Biocides

The role of biocides in the plant-fungal relationship is a complex one and is not easily predictable (Kurle & Pfleger, 1994). It seems reasonable however, to assume that fungicidal biocides will reduce AMF activity. Schreiner and Bethlenfalvay (1997) examined the effect the fungicides benomyl, pentachloronitrobenzene and captan on spore germination and colonisation of pea by 2 species of *Glomus* and one of *Gigaspora*. All fungicides were all capable of reducing spore germination, AM root colonisation or spore production, but the interactions were highly variable and depended on AMF species-fungicide combinations and environmental factors. Sreenivasa and Bagyaraj (1989) tested the effects of 9 fungicides on *Glomus fasciculatum* in pot trials. At the recommended application rates, all fungicides reduced root colonisation of Rhodes grass (*Chloris gayana*) and spore production. Though at half recommended rates, captan increased all mycorrhizal parameters and mancozeb, quintozene and ceresin increased some mycorrhizal parameters, though not significantly. Indeed paradoxically, some fungicides are regularly shown to have no

deleterious effect on AMF and in some cases increase AMF activity, especially at reduced application rates. Pattinson *et al*. (1997) found no long-term effect of the fungicides Terrazole or Terraclor on AM colonisation in cotton (*Gossypium hirsutum*), though there was an initial delay in the onset of mycorrhizal development, while Ryan *et al*. (1994) found no effect of fungicidal seed dressing. Udaiyan *et al*. (1999) looked at the effect of 6 fungicides on AMF activity in three types of millet (*Eleusine coracana, Panicum miliaceum* and *Paspalum scrobiculatum*) under field conditions. At recommended application rates, some reduced AM colonisation and sporolation, though others had no effect or increased AMF activity, depending on the species of millet involved.

As well as fungicides, other biocides in common use in agriculture can have an effect on AMF. The mechanisms involved are unclear, though biocides are known to alter root exudate type and quantity and this may be an important factor. Sreenivasa and Bagyaraj (1989) examined the effect of three nematicides on AMF, all three reduced AM colonisation and spore production at recommended application rates, though at half the recommended rate, all three increased spore production and had a neutral or positive effect on root colonisation. Pattinson *et al*. (1997) in contrast, found no long-term effect, either positive or negative, of the nematicide fenamiphos on AMF. Sreenivasa and Bagyaraj (1989) also examined the effect of 5 insecticides/acaricides, all caused a significant reduction in root colonisation and spore production of AMF at recommended application rates, while even at half the recommended rate dinocap caused a significant reduction in root colonisation and spore production. For herbicides Ryan *et al*. (1994) found no effect of Hoegrass and Jaguar on levels of AM colonisation of wheat.

From the available evidence, it seems likely that many biocides used in agriculture will have a deleterious effect on AMF when used at the recommended application rates. However, there is a complex interaction between AMF species, host plant species and biocide and some biocides seem to have a positive effect on AMF, particularly at reduced application rates. These positive effects are likely to be as a result of suppression of organisms which either compete with AMF or which attack AMF directly. The balance of positive and negative effects in the field is unclear.

3.5.3 Tillage

Tillage of the soil to incorporate crop residues and inputs, control weeds, and manage soil structure, forms an integral part of most agricultural systems. In some, such as horticulture, there may be intensive and repeated tillage operations throughout the year. In undisturbed conditions, AMF hyphae form a common mycelial network (CMN). Contact with this CMN is the main method by which seedlings become inoculated in the field (Read *et al*., 1976) and it is especially important in the early establishment of AM colonisation and early nutrient uptake. Soil tillage causes severe disruption to the CMN (see ***section 1.2***; O'Halloran *et al*., 1986; Anderson *et al*., 1987; Evans & Miller, 1988), resulting in delayed root colonisation and ultimately limits the volume of soil that is exploited by the AMF, which in turn translates into reduced nutrient uptake and yields in crop plants (Evans & Miller, 1988, 1990; Jasper *et al*., 1989a, 1989b, 1991; McGonigle *et al*., 1990b; McGonigle & Miller, 1993). Anderson *et al*. (1987) showed lower AM colonisation, P uptake and yield of maize under conventional tillage compared with zero tillage. A similar result was obtained by Evans and Miller (1988), O'Halloran *et al*. (1986) and Miller (2000), again with

maize. Galvez *et al*. (2001) compared mouldboard ploughed soils with chisel disced and 'no-till' systems. AMF spore numbers and colonisation of maize roots was highest in the 'no-till' system, however phosphorus use efficiency was highest under the mouldboard plough system. Mozafar *et al*. (2000) examined the effect of tillage on AM colonisation in wheat and maize in a long term field experiment. The 'zero-tillage' treatment increased AM colonisation in maize though it did not in wheat. Tillage also had an effect on nutrient content, increasing the concentration of some nutrients, and reducing others, but it did not have an effect on nutrient concentration in non-mycorrhizal rape, suggesting the nutrient differences were due to AMF, not tillage directly. Mulligan *et al*. (1985) demonstrated a reduction in AM colonisation and plant dry weight of young soybean plants as the amount of secondary cultivation increased. Kabir *et al*. (1998) studied AMF hyphal length in agricultural field plots, which had been managed under no-tillage, reduced tillage or conventional tillage for 11 years. AMF hyphal length densities were highest in the no-tillage plots, and lowest in the conventional tillage plots, while the reduced tillage plots contained intermediate AMF hyphal length densities. The highest maize P, Zn and Cu concentrations were also observed in the no-tillage and reduced tillage plots.

Glasshouse trials have confirmed the damaging effects of soil disturbance on AMF. Goss and de Varennes (2002) used sieved soil to represent tilled and un-sieved soil to represent untilled soil. Maize growth and P and N uptake were reduced in the sieved soil. Soybean plants grown after were 42% larger in the un-sieved soil, as was AM colonisation of roots and nodulation. McGonigle *et al*. (1990b) examined the effect of increasing soil disturbance by dividing soil cores collected in the field into increasingly smaller blocks. As the size of block decreased (degree of disturbance increased) dry weight and P and Zn uptake of maize declined, though Mn, Ca, K and N uptake increased with increasing disturbance. However, AM colonisation, assessed at harvest, was not influenced by disturbance. Suggesting a delay in AM colonisation, rather than an overall reduction. In the field, there was a similar difference between different tillage intensities. However, again there was no difference in AM colonisation and the authors question the importance of AMF in improved plant performance under reduced tillage conditions. It may be that tillage favours those AMF species which colonise principally from spores rather than hyphae, which may or may not influence AMF effectivity, depending on the efficiency of the AMF present in the soil which can colonise from spores.

Tillage certainly causes severe disruption to the AMF hyphal network which develops during crop growth. The more intensive the cultivation the greater the damage. This is likely to favour species which colonise mainly from spores. In certain cases this can result in a delay in root colonisation and this may translate into delayed growth and nutrient uptake, though this may not ultimately result in reduced yields.

3.5.4 Rotations

The diversity of AMF in soils used for agricultural production has been shown to be greatly reduced compared with natural ecosystems. Helgason *et al*. (1998) showed that arable soils at three sites in the UK were dominated by *Glomus mosseae*, which sporolates abundantly and can colonise plants easily from spores, which is likely to be important where the soil is regularly cultivated, disrupting the CMN. Daniell *et al*. (2001) similarly showed that arable soils from a number of sites were dominated by two *Glomus* species, while Oehl *et al*. (2003) found that increased land use intensity

was correlated with a decrease in AMF species number and the selection of species which sporolate rapidly. As agriculture has developed over the last 50 years, there has been an increasing move towards specialisation, resulting in a reduction in crop diversity and a geographical separation of animal husbandry and crop production. Arable cropping in the UK is frequently based upon one or two cereal crop species, with occasional non-graminaceous break crops. Reductions in host species number results in reduced AMF diversity (Burrows & Pfleger, 2002). This lack of diversity of arable crop species results in a further reduction in AMF diversity, beyond that caused by agriculture in general. Menéndez *et al*. (2001) measured diversity of AMF in soils under different crops and adjacent semi-natural grassland. The dominant species was different in the four crops, however, total species number was greater in the native grassland and in clover plots than in continuous wheat or barley. Replacing cereal monoculture with clover increased AM diversity and spore numbers over three years, emphasising the negative impact of cereal monoculture on AMF. Oehl *et al*. (2003) compared AMF species in low input grasslands with rotational arable and monocropped maize. AMF species number was greatest in the grassland and lowest in the maize, which was dominated by "generalist" species. Selection of generalist species by monoculture may be accompanied by a reduction in AMF effectivity. Johnson *et al*. (1992) found that maize yielded higher and had higher nutrient uptake on soils which had grown continuous soybean for five years than on soil which had grown continuous maize for five years. Conversely, soybean performed better after five years maize than five years soybean. The most abundant AMF species in continuous maize soil was negatively correlated with maize yield but increased soybean yield; there was a similar effect with soybean soil. Johnson *et al*. (1992) hypothesised that continuous cropping selects species which grow and sporolate most rapidly, and that these offer the least benefit to the plant because they divert more resources to their own growth and reproduction. Meaning that monoculture will result in increasingly smaller benefits of AM colonisation to the host plant.

Though increasing crop diversity is generally beneficial to AMF, adding a weakly mycorrhizal or non-AMF host crop, a brassica for instance, can have a severe negative impact on AMF and on the production of subsequent AMF crops (Black & Tinker 1979; Harinikumar & Bagyaraj, 1988). Root and AMF hyphal fragments, which are important for early colonisation of the host plant, only survive for around 6 months in the soil (Tommerup & Abbot, 1981) and so during the growth of a non mycorrhizal crop, propagule numbers decline and AM colonisation of the subsequent crop will be both delayed and reduced in quantity. Miller (2000) examined AM colonisation of maize and showed that when maize followed non-mycorrhizal canola AM colonisation was reduced along with early season P uptake, which in some cases resulted in yield reductions. Karasawa *et al*. (2002) also showed that AM colonisation and yield of maize was decreased by growing it in soil following mustard (*Sinapis* spp.)(a non-AMF crop), compared with sunflower (*Helianthus annuus*) (an AMF crop). Gavito and Miller (1998) showed that previous cropping with non-mycorrhizal canola had a greater detrimental impact upon subsequent AM colonisation of maize, than either P fertilisation or soil tillage. Even crops which are weakly mycorrhizal can have a significant effect on subsequent AMF crops. Douds *et al*. (1997) showed consistently lower spore numbers in soil and lower AMF infectivity following spinach (*Spinacea oleraceae*) or pepper, compared with maize, wheat or oats (*Avena sativa*). Miller and Jackson (1998) showed that weed hosts were important in maintaining AMF in non-mycorrhizal crops, forming a mycorrhizal bridge between mycorrhizal

crops. However, intensive agricultural practice aims for the elimination of all weeds, effectively removing this mycorrhizal bridge, exacerbating the effect of non-mycorrhizal crops, especially where non-mycorrhizal crops are grown consecutively. However, despite the apparently severe effect of non-AMF crops in a rotation, there are instances where use of non-mycorrhizal crops has had little effect on AMF (Ocampo & Hayman, 1981; Powell, 1982b) or even resulted in an increase in yield of the subsequent mycorrhizal crop (Ryan & Graham, 2002). The reasons for which are unclear.

As AMF propagules have a limited lifespan (Tommerup & Abbot, 1981) and propagule number has a direct effect on AM colonisation (Al-Karaki & Clark, 1999, Nagesh *et al*., 1999), including bare fallow periods in the rotation can also prove severely detrimental to AMF. Hamilton *et al*. (1993) showed that AM colonisation of bean and Zn uptake was reduced if it followed a fallow as opposed to maize, bean or wheat, maize being most effective at boosting colonisation and Zn uptake. Kabir *et al*. (1997) examined overwinter survival of AMF in Canada. Extraradical hyphal survival was higher where they were attached to roots of maize than where roots were absent. Disturbance further reduced hyphal survival; suggesting bare fallow periods should be left uncultivated to minimise their effect. Such is the strength of the detrimental affect of bare fallows on AMF that in Queensland, Australia, where soils have a low index of phosphorus availability, long bare fallows result in a recognised condition known as *long fallow disorder* (see ***section 3.1***), in which crops exhibit P and Zn deficiency. The severity of which is shown to be directly related to AMF inoculum density and P deficiency (Thompson 1987, 1991). The adverse effect of fallow periods on AMF inoculum potential can be avoided by growing a winter cover crop, such as wheat (Dodd & Jeffries, 1986; Galvez *et al*., 1995; Boswell *et al*., 1998). Boswell *et al*. (1998) found that not only did winter wheat increase AM inoculum potential, but the growth and yield of maize in the following growing season was directly correlated with AM colonisation of the roots. Alternatively, if a bare fallow period is unavoidable keeping it as short as possible will minimise the negative impact on AMF (Kabir *et al*., 1999).

3.5.5 Organic amendments

In the UK around 14% of cropped land and 44% of grassland receive organic manures (Chalmers, 2001) with other organic amendments, such as sewage sludge and food processing waste being locally important. The effect of such organic amendments on AMF is generally positive. Kabir *et al*. (1998) measured increased densities of total and viable AMF hyphae on a clay soil amended with dairy slurry compared with soil fertilised with inorganic (N and P) fertiliser. Harinikumar and Bagyaraj (1989) found that farmyard manure (FYM) increased AM colonisation in a range of crops, while Baby and Manibhushanrao (1996) showed that various organic amendments, especially green manures, increased the degree of association of mycorrhiza with rice plants, while reducing the incidence of sheath blast disease caused by *R.solani*. Miller and Jackson (1998) also showed that various organic amendments were positively correlated with AM colonisation and spore numbers in 18 intensive lettuce production systems. Though Kahiluoto and Vestberg (1999) found that incorporation of clover residues inhibited AMF. The nature of the organic amendment appears to have an influence on the response of AMF. Douds *et al*. (1997) examined the effect of leaf compost and chicken and cattle manure on AMF. Spore populations of two groups of

Glomus were increased by manure in combination with leaf compost but not by raw manure. Gaonker and Sreenivasa (1994) concluded that organic amendments with a narrow C:N ratio were more effective in promoting AMF that those with a wide C:N ratio after testing a range of organic amendments in combination with AMF on wheat.

The mechanism by which organic amendments improve AMF activity is unclear. As many organic amendments improve nitrogen availability within the soil, which in turn improves P mobilising activity and biomass of mycorrhiza, there may be a synergistic effect between increased soil N availability and increased P availability to the plant (Dar *et al*., 1997). However, there is also evidence to suggest that AMF possess some saprophytic ability, and therefore organic matter may simply provide an extra source of carbon and nutrients for the AMF to exploit. Many studies have shown that AMF proliferate extensively within organic materials, both under experimental and field conditions (Mosse, 1959; Nicolson, 1959; St John *et al*., 1983a,b; Joner & Jakobsen, 1995). Hodge *et al*. (2001) using a ^{15}N technique showed that *Glomus hoi* both enhanced decomposition of, and extracted N from, a complex organic material (*Lolium perenne* shoots) added to soil. Moreover, *G. hoi* appeared to transfer N to the host plant, as there was a direct relationship between levels of ^{15}N in the plant and hyphal length density within the organic material patch (Hodge *et al*., 2001). The same fungus also enhanced N capture (^{15}N) from organic material by *Plantago lanceolata* and *Lolium perenne* plants, though only when grown together in interspecific competition (Hodge, 2003). It is possible that the AMF mycelia within the organic matter enhance decomposition by stimulating other microbial decomposers in soil, because of the large transfer of host carbon to the soil via AMF rather than decomposing the organic matter directly (Hodge *et al*., 2001; Johnson *et al*., 2001, 2002), though more work is required to fully understand the mechanisms involved (Read & Perez-Moreno, 2003).

The reason that the nutrients in organic amendments do not suppress AMF in the way that N and P fertilisers can do, may be related to the rate of release of nutrients. Joner (2000) looked at applications of both mineral and organic fertiliser and found that moderate quantities of FYM have less adverse effects on AMF than equivalent amounts of nutrients in NPK fertilisers, concluding that this was most likely due to the gradual release of P from FYM, in contrast to the rapid release from phosphate fertilisers. However, organic amendments can impact negatively on AMF if too much P is supplied, allowing concentrations to build up. Pasolon *et al*. (1993) measured reduced AM colonisation of rice where 27t ha^{-1} yr^{-1} of FYM (equivalent to 59kg ha^{-1} P) had been applied for 9 years. Indeed Jordan *et al*. (2000) considered FYM in general, to be detrimental to AMF.

Common farming practices such as cultivations, use of fertilisers and biocides and crop selection, can all impact on both AM diversity and activity. Generally speaking, agricultural practices have a negative impact on AMF, especially on diversity. The result being that the more intensive the production system the greater the negative impact on AMF. Modern high intensity production systems, with high fertiliser and biocide usage, and low crop diversity are probably the least conducive to AMF. Though this probably has little effect on crop productivity, it may impact on crop quality and long term system sustainability.

3.6 *Mycorrhiza in organic agriculture*

It is one of the central paradigms of organic and other low input systems of agriculture that a highly active soil microbial community is required for effective functioning of the soil system. A part of this concept assumes that AMF will play a larger role in plant nutrition than in conventionally managed systems and will compensate for the reduced use of phosphorus fertilisers (Galvez *et al*., 2001). Jordan *et al*. (2000) described the conditions that suit mycorrhizal development as:

- Low input systems i.e. low tillage, lower synthetic fertilisers and P containing animal manures.
- Avoidance of fallowing.
- Minimal rotations of crops that are a poor host to or do not provide hosts to AMF.

They claim that conventional farming systems do not meet these requirements. Organic farming in contrast, incorporates a number of practices which fit the criteria espoused by Jordan *et al*. (2000), such as reduced P fertiliser use and avoidance of bare fallows, as well as other practices, such as generally more diverse rotations than those found in conventional agriculture, use of ley periods and a virtual absence of biocides, all of which are likely to encourage AMF. Other practices used in organic farming may however, impact negatively on AMF, notably weed control. Though organic systems tend to tolerate a higher level of weeds (which can provide a mycorrhizal bridge during non-mycorrhizal crops) than conventional systems. Extensive cultivation for weed control, common in some organic systems, will severely disrupt the AM hyphal network, resulting in delayed AM colonisation of crops and a reduced overall level of colonisation. Another feature of organic systems that could impact negatively on AMF is the extensive use of animal manure. Though stocking densities are in most cases lower in organic systems, so excessive use of manures is less likely than in conventional systems.

A number of studies have now been conducted on AMF activity in organic and other low input systems. Most have indeed found higher numbers of mycorrhizal spores and higher rates of AM colonisation in organically managed or low input systems, when compared with equivalent conventionally managed systems, though this does not always translate into increased growth or P uptake. Ryan *et al*. (1994) found significantly higher levels of root colonisation by AMF in wheat on an organic farm compared with an adjacent conventional farm in Australia. In a glasshouse trial, the inoculation potential of the organic soils was also significantly higher, both for wheat and for clover, though shoot dry weight was higher on the conventional soils. Ryan *et al*. (2000) found significantly higher levels of AM colonisation in white clover, perennial ryegrass (*Lolium perenne*) and paspalum (*Paspalum dilatatum*) from 10 biodynamic farms than from 10 conventional farms, the main determinant being soil and shoot P concentration. Mäder *et al*. (2000) measured AMF root colonisation in wheat, ryegrass and clover grown in conventional and organic systems in a long-term experiment (rotations and tillage were identical). AM root colonisation was 30-60% higher in the organic systems, most of the variation being explained by differences in the soil chemistry. In a glasshouse experiment, soils from the organic system had a greater capacity to initiate AM colonisation. Galvez *et al*. (2001) found higher numbers of mycorrhizal spores and greater propagule potential in soils from low input systems compared with conventional systems. Phosphorus use efficiency was also greater in the low input systems, though there was no yield benefit of higher AM

colonisation. Similar results have been obtained by Gompel *et al*. (1990), Sattlemacher *et al*. (1991), Kurle and Pfleger (1994) and Kahiluoto and Vestberg (1998).

Which of the features of organic systems account for these and similar results is unclear. Many authors have reported that differences between conventional and organic systems can be explained by differences in soil P (Scullion *et al*., 1998; Mäder *et al*., 2000; Ryan *et al*., 2000). Phosphorus fertiliser use is generally lower on organic farms, which in itself will encourage AMF. What is used, is in the form of low solubility products such as rock phosphate. Unlike superphosphate and other soluble phosphate fertilisers used in conventional systems, rock phosphate does not reduce AMF activity (Ryan *et al*., 1994; Dann *et al*., 1996). Indeed rock phosphate may actually encourage AMF mediated growth and P uptake (Alloush & Clark, 2001). P added in FYM also has less of an impact on AMF than equivalent amounts of nutrients added as soluble fertilisers (Joner, 2000), most likely due to a temporal difference in P availability, resulting from the gradual release of P from FYM. Use of other fertilisers, particularly N, which have been reported to have a negative impact on AMF (Miller & Jackson 1998; Liu *et al*., 2000; Burrows & Pfleger, 2002) is also either prohibited under organic production regulations or else they are of a slow release nature and are thus much less likely to impact negatively on AMF.

Other aspects of organic systems, which could account for higher activity of AMF, include reduced biocide use, diverse rotations and use of organic amendments. Though biocides are used in organic systems there are only a small number permitted and their use is restricted. The only ones to cause concern are copper based fungicides, used in the production of grapes, fruit and potatoes. Copper oxychloride is particularly detrimental to AMF, even below recommended application levels (Sreenivasa & Bagyaraj, 1989). However the use of copper based fungicides in organic systems is to be phased out in the near future.

Diverse rotations, required to prevent carry-over of pests and disease, are a further feature of organic systems likely to encourage AMF. Organic systems usually include grassland for animal fodder alternating with cash crops, on at least part of the holding, further increasing crop diversity. Even where animals are absent or there are only small numbers, short-term grass and clover leys are used to boost soil fertility. Greater crop diversity encourages AMF diversity. This can be important for ecosystem functioning (van der Heijden *et al*., 1998, 2003). van der Heijden *et al*. (1998) showed that reduced AMF diversity resulted in a reduction in productivity of some species in simple microcosms. More AMF species led to more hyphae in soil, more P in plants and so more efficient use of resources. Menéndez *et al*. (2001) measured diversity of AMF in different crops. Continuous wheat or barley, which is common in conventional systems, but prohibited in organic systems, had reduced AMF diversity compared with grassland and clover plots. While AMF diversity and spore numbers increased during three years of clover, a common crop in organic systems. Oehl *et al*. (2003) also found significantly reduced AMF diversity under monocropping (maize) compared with a diverse arable rotation and mixed species grassland. Zhu *et al*. (2000) measured AMF diversity in mixed grass/clover systems, ryegrass and clover showed different preferences for AMF species, giving at least a partial explanation for the greater diversity of AMF under grass/clover leys compared with monoculture cereals. Within organic rotations, bare fallow periods, which are detrimental to AMF

(Thompson 1987, 1991; Hamilton *et al*., 1993; Kabir *et al*., 1997) are also less common. They can lead to leaching of nitrogen, which is difficult to replace in an organic system where fertiliser use is limited. It is more normal in organic systems to grow a cover crop to take up the nitrogen. This can provide a mycorrhizal bride between cash crops, helping to maintain AM colonisation levels (Galvez *et al*., 1995; Boswell *et al*., 1998). However where the cover crop is a brassica (mustard or rape and stubble turnips are a common choice) the opposite will be the case.

Use of organic amendments; FYM, compost and crop residues, is also more common in organic systems and in most cases will favour AMF (Kabir *et al*., 1998; Harinikumar & Bagyaraj, 1989; Baby & Manibhushanrao, 1996; Douds *et al*., 1997; Miller & Jackson, 1998).

Despite the apparently favourable conditions for AMF found in organic and other low input farming systems, higher levels of AM colonisation are not always present and even where they are, this may not translate into superior crop growth or increased nutrient uptake. Eason *et al*. (1999) examined AM colonisation and effectivity from organic and low input grassland systems compared with high input conventional systems. Root colonisation and AMF spore numbers were significantly higher in the organic and low input systems and in glasshouse trials there was generally a greater yield response of leek (*Allium porrum*) and clover to inoculation with AMF from the organic and low input systems. However, not all organic and low input systems had effective AMF, while some high input systems had highly effective AMF. Similarly, Scullion *et al*. (1998) obtained inoculum from three organic and three conventionally managed soils and used it to inoculate leek and clover grown on sterilised organic soils in the glasshouse. There was a complex response, but generally leek was more responsive to AMF than clover and the response was stronger on less fertile soils. However, plant growth response to AMF and P uptake was only stronger with inocula from the organic soil on the lowest fertility soil and AMF actually decreased growth of clover on the most fertile soil. Further evidence that AMF in organic systems may not be more effective than AMF from conventional systems comes from Ryan *et al*. (2000), who measured the level of AM colonisation in clover and grasses in permanent pastures on 10 biodynamic farms and 10 adjacent conventional farms in Australia. Although the level of AM colonisation was higher in the biodynamic farms (the difference being explained by differences in soil phosphorus concentrations), there was a negative correlation between AM colonisation level and P concentration in shoots. A subsequent glasshouse experiment on the soils (Ryan & Ash, 1999), showed that the biodynamic soils did not differ in their response to soluble N and P fertiliser and did not appear to have developed a different process to enhance plant nutrient uptake. Other authors have also found that AMF communities from organic systems do not appear to be either different or more effective than those from conventional systems. Mäder *et al*. (2000) added propagules of *Glomus mosseae* to conventional and organic soils, the degree of colonisation of wheat was increased by almost 3 times in the organic soil as well as in the conventional soil, suggesting some native AMF communities from organically managed soils may have low colonisation efficiency, even when the system has been established for an extended period. Further evidence that AMF from organic systems may not be effective comes from work examining the effect of fertilisers in organic systems. Scullion *et al*. (1998) showed that rock phosphate was at least as effective as AMF from organic soils in increasing growth of *Trifolium* and in some cases more effective, suggesting AMF cannot

compensate for reduced fertiliser use in organic systems. Dann *et al*. (1996) also showed that AMF could not compensate for loss of P fertiliser use in an organic system. Replacing superphosphate fertiliser with insoluble rock phosphate on an organic farm had increased AM colonisation of wheat, but had resulted in lower yields compared with an adjacent conventional farm. The response to wheat grown on soils from each farm to phosphate fertilisers showed that AMF from the organic soils did not give greater access to soil P, maximum yields were produced with superphosphate on both soils.

The reason for the apparently poor performance of AMF from some organic systems is likely to be multifold. In some cases, soil P concentrations may remain too high for an effective AMF community to develop, even after some time in organic management (Scullion *et al*., 1998), particularly where P fertilisers continue to be used. Where there is a moderate level of P fertiliser use after conversion to organic management, which maintains soil P concentration, AMF may even have a negative impact on crop growth (Scullion *et al*., 1998). AM fungal species clearly show functional diversity and niche differentition, and building-up species diversity may be critical to ensuring the effectiveness of AMF communities following conversion from conventional to organic farming. However the time taken for recolonization of agricultural land by AM fungal species, the mechanisms by which it is achieved, and the potential contributions of management practice and inoculation to re-colonization are unclear.

Another reason for the poor performance of AMF in some organic systems may be that modern crop cultivars, which are used in organic systems but which were bred for high input conventional systems, are not responsive to AMF and therefore prevent maximal exploitation of AMF. There is evidence that many modern cultivars show a relatively small or even negative response to AMF in comparison with older varieties, even though colonisation may be similar (Manske 1990; Hetrick *et al*., 1996). Aguilera *et al*. (1998) showed that AMF inoculation caused a large increase dry weight and reproductive effort in less improved maize varieties, though P uptake was lower in the least improved variety. However, Hetrick *et al*. (1993, 1996), found a wide degree of variation in AMF dependency in wheat varieties, both modern and old. None the less, if organic systems are to make best use of the benefits of mycorrhizal colonisation crop breeding needs to take AMF into account.

There is also a question of propagule availability. Long-term conventional, high input management with few crop species seems not only to reduce AMF numbers but also to favour less efficient AMF (Johnson *et al*., 1992; Johnson, 1993). After conversion to organic management the AMF population may have been too depleted to provide effective AMF. In which case inoculation with appropriate AMF may be required.

3.7 *Manipulation of AMF in agriculture*

AM colonisation of an agricultural crop seems to offer multiple benefits: enhanced nutrient uptake, increased pest and disease resistance and improved water relations, as well as improved soil structure. As a result, it is not surprising that there has been a great deal of research aimed at manipulating the fungal host relationship to improved crop production. The problem with attempting to manipulate AMF is that the interaction between the fungi, its plant host and their environment is not well characterised. Many experiments looking at the role of AMF, particularly in pathogen

resistance, are laboratory or glasshouse based. They often only look at one species of AMF and concentrate on the most beneficial species, or those that are easiest to culture. The result is that the transferral of an experiment to the field often proves less than successful. Experiments must look at combinations of organisms that are known to co-occur in the field if they are to provide useful information about the field performance of AMF (Mozafar *et al*., 2000; Gange & Brown, 2001). In the field, roots will be colonised by multiple fungi and attacked by multiple pathogens. The simplified laboratory conditions used may miss essential factors governing the relationships between host plant, fungi and pathogenic organisms in the field (Hussey & Roncadori, 1982). Besides factors such as P availability and cultivation, even apparently minor factors can have an impact. Grange (2000) showed that varying the population of a single mycophagous organism, Collembola (springtails), could affect AM colonised plant growth, both negatively and positively, depending on the conditions of the experiment. Plant density can even have an effect, as planting density increases the effect of AMF declines (Koide & Dickie, 2002).

Another problem is in determining what plant growth factors to measure. For example, Izaguirre-Mayoral *et al*. (2000) examined the effect of AM colonisation on bean. Some factors were enhanced by colonisation, such as nodulation, and chlorophyll content, but others, shoot and root mass, P content and seed yield were not increased. Al-Karaki and Clark (1999) examined the effect of AMF on durum wheat; colonisation increased seed dry weight, but reduced protein content. Yield is the obvious factor to look at in an agricultural context, but this may miss subtle but important effects. For example, higher seed P resulting from AM colonisation can increase growth of plants produced by that seed, even if seed size is the same (Koide & Dickie, 2002). This has important implications for farm saved seed. However despite these difficulties, there have been successful attempts to boost crop growth using AMF in agricultural and more particularly horticultural situations and research is ongoing.

3.7.1 Direct inoculation or native AMF?

There are two approaches to encouraging AMF in agriculture. One is to use management techniques to encourage native AMF, the other is to inoculate either the soil or the plants with an introduced AMF species. It is the latter of these which has received the greatest attention.

Direct inoculation of either the host plant or the soil seems an obvious way to manipulate the plant-fungus relationship to gain maximum benefit from AMF. Kahiluoto and Vestberg (1998) increased growth of leek by 62% and P uptake by 73% on soil previously monocropped with cereal by direct inoculation with AMF, while Vosatka (1995) demonstrated that pre-inoculation of onion transplants was more effective than relying on native soil AMF to boost growth and P uptake. There are two problems with this approach however; there is little information to indicate which AMF species will be most effective with which crop species and there is the problem of competition with native AMF.

It has been traditionally thought that there is very little specificity shown by a host plant for AMF species. However, there is increasing evidence that this is not the case and that different AMF species will produce very different growth responses in the host plant. But, the response is rarely predictable or even consistent. Hernández *et al*.

(2000) compared *Glomus mosseae* and *Scutellespora fulgida* inoculation of cowpea (*Vigna luteola*). *G. mosseae* was more efficient at promoting growth of cowpea despite similar degrees of colonisation. Though when the two fungi were inoculated together there was a complex interaction. Ortas *et al*. (2002) examined the effect of five *Glomus* species on the growth and nutrient uptake of citrus in a glasshouse experiment. There were significant differences in the growth, nutrient uptake and degree of AM colonisation between the *Glomus* sp. but the relative effectiveness of the different AMF differed between two experiments. Charron *et al*. (2001a,b) also found differences in the effectiveness of different species of *Glomus*, in this case on the growth and quality of onions. The concentration of N, P, and Zn was higher with *G. versiforme*, but Mn concentration was higher with *G. intraradices*. Xavier and Germida (1997) found a significant effect of P fertilisation on the effectiveness of added AMF on the growth of lentil (*Lens culinaris*) and two wheat cultivars. Plants were either inoculated with *Glomus clarum* or native soil AMF. Dry weight, and AM colonisation were significantly higher in lentil with *G. clarum* inoculation compared with native soil AMF, at two lower levels of P fertilisation, but at the highest level, shoot dry weight was reduced by inoculation with *G. clarum*. In contrast, wheat cultivar Neepawa, showed no positive effect on root or shoot growth or AM colonisation with *G. clarum* inoculation at the two lower levels of P fertilisation, but at the highest P fertilisation level inoculation increased yield by 20%. Cultivar Laura showed no positive effect of *G. clarum* inoculation at any soil P fertilisation level.

With such large differences in the response of not only different plant species but also different cultivars of the same species on a single soil, it is clear that making effective use of added inoculum is very difficult. If host plants do have a preference for AMF then the additions of AMF inocula need to be carefully selected to ensure a compatible host/fungus/substrate combination is used (Azcón-Aguilar & Barea, 1997). A task which is complicated by the fact that the most effective AMF species is likely to be different, depending on whether the main aim is nutrient uptake, increased pathogen resistance or improved water relations. Further complications arise because different sources of inoculum, spores, extraradical hyphae and infected roots are not equally suitable propagules, but which are and which are not varies from one AMF species to another (Charron *et al*., 2001a; Klironomos & Hart, 2002). Failure to find the most appropriate AMF/host/inoculation method may explain why some inoculants used thus far have failed to have a beneficial effect, even though degree of colonisation may be high. With this in mind, it is not surprising that attempts to inoculate AMF in combination with other "beneficial" micro organisms have also generally met with failure. Belimov *et al*. (1999) found that co-inoculation of *Glomus* sp., N-fixing bacteria and P solubilising bacteria did not produce any synergistic or additive effect on the growth and uptake of nutrients in barley, while Dubey (1999) reported no significant differences between different treatments of bio-fertilisers containing bacteria and AMF on growth of soybean with and without nitrogen, phosphorus and potassium fertilisers.

In cases where an effective AMF host combination has been identified, there remains the problem of native soil AMF. As they are indigenous, native AMF will be more suited to the soil environment and as a result, may out-compete added AMF. Alternatively, the native mycorrhizal population may be as effective as the introduced inoculum, thereby negating any benefit of inoculation (Izaguirre-Mayoral *et al*., 2000). An introduced inoculum may even depress yield if the native AMF population

is effective (Kahiluoto & Vestberg, 1998). Even in cases where the introduced AMF is effective, it may not persist in competition with native AMF. Harinikumar and Bagyaraj (1996) introduced *G. intraradices* into plots in the field for 1, 2 or 3 consecutive seasons. Results showed that the introduced fungus persisted in the field for only one season.

Use of direct inoculation can be more successful in combination with a sterilised soil, where there is no competition from native AMF or other soil micro organisms. Garlic propagules have to be produced under nematode free conditions. This is traditionally done by soil sterilisation with methyl bromide, but this results in stunted growth. Koch *et al*. (1997) applied AMF inoculum to the soil after sterilisation; garlic bulbs from inoculated beds were generally larger, 51g compared with 27g in un-inoculated beds. However, such uses are limited. For the efficient use and manipulation of AMF for long-term agricultural stability and productivity, our understanding of their physiology and function and their interactions with crops and environmental conditions need to be improved. Many of the reported yield improvements from inoculation with AMF come from tropical areas (e.g. El Fiel *et al*., 2002; Kumar & Murugesh, 2002; Rakesh *et al*., 2002) and it may be that soil type and climate are key factors in determining the benefits from AMF inoculants. The effect of different agronomic practices, such as application of chemical fertilisers and biocides on the ecology and function of AMF also needs to be elucidated before their successful utilisation in agriculture (Aryal & Xu 2000; Berg *et al*., 2001).

The alternative to direct inoculation with AMF is to encourage native AMF populations. Aikawa *et al*. (2000) have succeeded in identifying two growth stimulants which improve in vitro hyphal growth of the AMF *Gigaspora margarita.* However, this type of manipulation is in its infancy. Much can be done in the field to encourage native AMF through use of appropriate rotations and tillage and minimising the use of fertilisers and detrimental biocides. Though this approach does rely on there being species of AMF appropriate to the crops grown, which may not be the case in systems where long-term use of fertilisers, limited crop diversity and/or other management practices have reduced AMF diversity. In such cases, inoculation of the soil with a wide range of AMF species may be required to maximise the benefits of AMF.

Despite the apparently multitudinous benefits of AMF to crops, some authors have questioned whether it is useful to encourage AMF in agriculture at all, particularly in high input types of agriculture, such as horticulture. In such systems, soil phosphorus concentrations tends to be kept high to maximise yield of high value crops, resulting in suppression of AMF. Reducing phosphorus inputs to encourage AMF is likely to reduce yields because of the high carbon drain of AMF on the host plant (up to 20%), with little economic saving in terms of reduced fertiliser inputs in return (Ryan & Graham, 2002). Even with arable crops, where soil P concentrations are usually lower, requiring less adjustment to the system to encourage AMF, greater AM colonisation does not necessarily result in higher yields. For example Miller (2000) reports no yield benefit resulting from higher AM colonisation and phosphorus uptake in maize in an arable system. Where phosphorus inputs have been historically high, it may take many years for AM colonisation to increase after cessation of fertiliser use. Dekkers and van der Werff (2001) demonstrated that even 10 years after high P fertiliser inputs had ceased, AM colonisation was still significantly lower than where

the previous P fertilisation rate had been low or zero, though despite this, there was no yield reduction.

In established organic farming systems, soil nutrient concentrations are generally lower than in conventional systems. Encouragement of AMF to compensate seems a logical choice. In many cases, such systems already have a high level of native AMF diversity and activity and so the addition of AMF inoculant may be superfluous (Kahiluoto & Vestberg, 1995). However, there is evidence that not all organic soils contain effective AMF (Eason *et al*., 1999), or there may be instances where the native AMF is effective at improving nutrient uptake, but not at enhancing resistance to pathogens, or vice versa. This scenario is especially likely in the period of conversion from conventional to organic farming, when AMF diversity is likely to be low. Arable systems particularly those based on monoculture, are highly denuded of AMF diversity, which is likely to impact on the functioning and reliability of the AMF community (Helgason *et al*., 1998, van der Heijden *et al*., 1998). Although there is some evidence of enhanced AMF function with increasing time in organic management (Scullion *et al*., 1998), where AMF diversity and/or activity is shown to be particularly low before conversion to organic management, it may be useful to establish a more diverse population through inoculation. After which, organic practices, such as reduced fertiliser use and diverse rotations should maintain a higher level of AMF diversity and activity. The beneficial effects of organic agriculture on AMF can then be further enhanced by minimising cultivations, careful management of manures and phosphorus fertilisers and the minimal use of non-AMF crops such as brassica cover crops.

3.7.1.1 Commercial availability

If direct inoculation of crops or soils is to be commercially viable, inoculum needs to be available in large quantities at low cost. The first AMF inocula to be made available commercially was of *Glomus deserticola*, in the late 1970s. The diversity of inocula available has increased immensely since then. In recent years the number of biological control agents available for soil-borne pathogens for instance has doubled (Paulitz, 2000), along with the number of organisations who sell them. This improved availability is owed partly to improved culture methods. However, despite this improved availability AMF still only occupy a small portion of the market of agents for use against soil-borne pathogens and their use to boost nutrient uptake is also limited to specialised, mostly horticultural situations. Attempts to use commercial inoculum to boost AM colonisation and yield on a large scale generally meet with little success. For instance, Bull *et al*. (2000) used six commercially available inoculants on strawberry plants grown in the field. They had no effect on percentage colonisation or yield. This situation is likely to continue until there is a more thorough understanding of the interaction of host and its environment, allowing more targeted and therefore more successful use of AMF in the field. Other problems include expense. Though the cost of mycorrhizal inoculum is relatively low, even to administer in large dosages, so is the cost of biocides and fertilisers. If AMF brings little yield benefit it will not be economic compared with the tried and tested use of agrochemicals. There is also the fact that there is no certification of brands and so no guarantee of their effectiveness. This discourages growers from buying them because they cannot be sure that a certain formulation, will work in their soil.

Alternative commercial products include those designed to encourage native AMF to be more effective. Bell and Kopp-Holtwiesche (2001) reported improved disease resistance and Mularwaman *et al*. (2000) increased nematode resistance in the field from a commercial product of this type. Whilst Aikawa *et al*. (2000) have demonstrated *in vitro* changes in fungal hyphae using a stimulant extracted from the citric fruit Satsuma. However, their efficacy has yet to be demonstrated in a range of conditions and their use has yet to be adopted.

3.7.1.2 Biotechnology

Although no genetically modified mycorrhiza have been created, the idea has been suggested, possibilities include:

- Modify host plants to increase their susceptibility to AMF.
- Improve the adaptability of mycorrhiza to changing soil conditions.
- Optimise the association by increasing expression frequency of pathogen antagonistic genes in AMF.

Plants harbour genes that support beneficial interactions made by mycorrhiza. Biotechnology could be used to increase the expression of these genes or even transfer them to non-mycorrhizal crops (Smith & Goodman, 1999). Such modified AMF are still only theoretical however, and their acceptability, particularly in organic systems, is questionable, commercial use is a long way ahead.

4: Recommendations

AMF play a very important role in plant-soil interactions and the mycorrhizal condition is the norm rather than the exception. Mycorrhizal association provides many benefits to the plant. Of most importance from an agricultural point of view, is improved nutrition that enhances growth and fitness of the plant and improved resistance to soil-borne pests and diseases resulting from antagonistic processes associated with mycorrhiza. For these reasons, mycorrhiza have been highlighted as plant growth promoters and possible biological control agents. However the transition from showing these effects in the laboratory or glasshouse to demonstrating them in the field has proved difficult. Conventional systems, particularly high input systems, probably have little to gain from encouraging AM colonisation, as the carbon drain on the crop by the fungi may be substantial, while the benefits which the plant gains such as greater access to nutrients and reduced disease pressure, can be achieved at lower cost through inputs of fertiliser and biocides. Though this situation may change as a result of increasing pressure to reduce the use of inputs and develop more sustainable systems of food production. Organic and other low input systems potentially have more to gain from encouraging AM colonisation of crops, and changes to tillage and cropping could easily made to encourage AM fungal establishment. However, though AMF activity has frequently been shown to be higher in organic systems, this has not always been shown to be beneficial, particularly where soil phosphorus concentrations are moderate to high.

Direct inoculation of crops with AMF, particularly for use as biological control agents, occupies only a very small portion of the market compaed with other methods of soil-borne pathogens control. Although currently mycorrhizal inocula are easily

obtainable commercially, there is not at the moment a high demand, this small demand maybe due to unconvincing literature concerning their efficacy in field situations. Successful field trials are rarely found in journals and commercial advertisements do not provide evidence of mycorrhiza working in field conditions, usually only giving examples of their effects when grown under controlled glasshouse or laboratory conditions. A better understanding of their physiology, ecology, function and interactions with existing crops and environmental conditions is needed if this situation is to change in the near future.

In organic farming the functions of AMF most likely to bring enhanced productivity are:

- the increased protection of AM colonised roots against pathogen attack
- increased potential for nutrient capture by AM colonised roots
- the established practice of bi-cropping (where a commercial crop and legume are grown together) could be made more effective if the N nutrition of the commercial crop could be improved by transferral of N-containing compounds via the common mycelial network (CMN)
- increased drought resistance conferred by AM colonisation could be of benefit in the long term if drought risk increases as a result of global warming

To achieve these improvements a better understanding in field scale UK Organic Farming conditions is required of:

- New methods to characterise the diversity of AMF communities
- The role of AMF species diversity in determining the responses of crop plants and rotations to AMF
- Mechanisms and time-scales involved in re-colonization of agricultural land by AMF species following conversion from conventional to organic management
- The role of management practice in controlling the structure and functioning of AMF communities
- Host/AMF specificities, infectivity and effectivity
- AMF community interactions with livestock manures, composts and green manures
- AMF community interactions with legumes
- AMF community interactions with cover crops
- AMF community interactions with existing pest/weed/disease control measures such as stale seedbeds

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