Chapter 3
Use of Mycorrhiza Bioassays in Ecological Studies

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3.1 Introduction

It is well known that arbuscular mycorrhizal (AM) fungi are ubiquitous in agricultural and natural ecosystems (Brundrett 1991, 2002) and that most plant species form symbiotic associations with these fungi (Newman and Reddell 1987). The symbiosis can increase the growth and development of plants by enhancing nutrient uptake, especially phosphorus (Ortas 1996; Smith et al. 2004), into plants and by improving water relations of plants (Auge 2001; Boomsma and Vyn 2008; Jacobson 1997; Kyllo et al. 2003; Mohammad et al. 1995). Plants differ in their growth response to mycorrhizal colonization (Monzon and Azcon 1996; Ortas et al. 2002; Pankova et al. 2008). Explanations for differences in growth responses to mycorrhizal colonisation over the full life cycle of plants are complex (Gazey et al. 1992) and new explanations of mycorrhizal function are emerging (Li et al. 2008). Mycorrhizal dependency (Menge et al. 1978; Gerdemann 1975) varies among plant species (Abbott et al. 1995; Pankova et al. 2008), and may influence the abundance and community structure of AM fungi in soil and their infectivity (Crush 1978; Egerton-Warburton et al. 2007; Püschel et al. 2007; Zangaro et al. 2000). AM fungi can also contribute to improving soil physical fertility by enhancing soil aggregation (e.g. Moreno-Espíndolaa et al. 2007; Rillig and Mummery 2006) and protect crops against plant disease (see Douds and Johnson 2003). The soil environment, particularly factors that control soil fertility, strongly influences mycorrhizal function (Abbott and Robson 1982, 1991b; Cardoso and Kuyper 2006).

The diversity of AM fungi is generally low in agriculture compared with indigenous plant communities (Boerner et al. 1996; Helgason et al. 1998; Sieverding

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1991). Crop and rotation history also influence the abundance and diversity of AM fungi in agricultural soil (Johnson and Gehring 2007; Douds and Johnson 2003). Therefore, the capacity of AM fungi to form mycorrhizas that contribute substantially to plant nutrition, plant health or soil fertility depends on soil management. Within this context, calibrated bioassays of infectivity have the potential to enable interpretation of mycorrhizal function. However, due to the complexity, diversity and dynamics of mycorrhizal associations in space and time, measurements of mycorrhizal status of roots at one point in time is unlikely to enable full understanding of the interactions between AM fungi and host plants over their life cycle.

3.2   Assessment of Infectivity of AM Fungi in Soil

The term "soil infectivity" was used to define the ability of soil containing propagules of pathogens to initiate infection of host plants (Hornby 1990). Based on this plant pathology concept, "infectivity" of AM fungi in soil has been used with reference to the ability of AM fungi to colonise roots of a host plant (Abbott and Robson 1981) and explored further within the framework of mycorrhiza bioassays (e.g. Gianinazzi-Pearson et al. 1985; Plenchette et al. 1989). The infectivity of AM fungi can differ among host plants even when grown together in the same soil (Abbott et al. 1995). It is also important to be aware that bioassay measurements of infectivity of AM fungi can vary with soil environment, including differences in soil fertility, soil disturbance, host plant type and architecture, temperature, and water availability (Abbott et al. 1995).

Knowledge of the abundance and distribution of AM fungi in soil is acquired from measurements of the length of mycorrhizal root, the proportion of the root length colonised, the length of hyphae in soil, the number and types of spores and other propagules in soil, and the infectivity of the community as a whole (Abbott and Robson 1991a). Colonisation of roots by AM fungi can be estimated directly using microscopic observation of roots and indirectly using bioassays (Abbott and Robson 1981) or molecular assays (Jacquot et al. 2000; Stukenbrock and Rosendahl 2005).

3.2.1   Direct Assessment of AM Fungi

Direct measurement of mycorrhizal colonisation of roots by length of root colonised and percentage of root length colonised is usually based on staining to distinguish the fungi from root tissues (see Brundrett et al. 1984). Colonisation can also be observed in unstained roots (Arias et al. 1987), but most histological methods have limitations because some fungi are not easily visible even after roots are cleared and the fungi stained (Saito et al. 1993). Most assessments of AM fungi

in roots do not take into account changes in the intensity of fungal colonisation within the root, but McGonigle et al. (1990) overcame this limitation to some extent by developing a magnified intersection method for assessing the colonisation of roots. This procedure assesses the proportion of root length containing mycorrhizal fungal structures (arbuscules, vesicles and hyphae). In-depth microscopic analysis of colonisation intensity is necessary for more complete understanding of relationships between root colonisation and mycorrhizal function (Gazey et al. 1992), and this will differ for plants with different root structures (Merryweather and Fitter 1998; van der Heijden et al. 1998).

   None of the measurements mentioned above provide information about the activity of the fungi present inside roots or in soil, so histochemical procedures are required to determine whether the fungi are living or dead. Hyphae and vesicles can be observed inside roots, even in dead roots, and enzyme assays can be used to determine whether the fungi are alive (e.g. MacDonald and Lewis 1978; Saito 1995; Saito et al. 1993).

   As root systems develop, changes occur in the abundance of fungi in the root system either as a whole or in terms of the relative abundance of different AM fungi; sequential sampling may be necessary. As suggested above, the architecture of the root system also needs to be considered when interpreting colonisation data. For example, calculation of the proportion of root length colonised for the whole of a woody root system is of little significance compared with a measurement that is restricted to the fine (most active) roots of the same plant. Similarly, calculations of the proportion of root length colonised on root systems of plants with highly differentiated root systems might be of little value without quantifying the relative abundance of different types and ages of roots. Mycorrhizal colonisation of plants that shed roots extensively may be difficult to determine accurately.

   Morphological structures of AM fungi inside roots can be distinctive within some communities of fungi (Abbott 1982; Merryweather and Fitter 1998) but overall the fungi are structurally diverse (Dickson et al. 2007; Dodd et al. 2000; Smith and Smith 1997). The recognition of morphological similarities among AM fungi occurring in a soil community requires extensive experience based on observations of mycorrhizas formed in pure pot cultures of fungi isolated from one location and studied on a standard host plant (Abbott 1982). The morphology of a fungus within roots of different host plants can differ (Lackie et al. 1987; Smith and Smith 1997), so, while morphological characters may provide a tool for understanding the dynamics of colonisation of some root systems by distinct morphotypes (e.g. Abbott and Robson 1982; Merryweather and Fitter 1998; Scheltema et al. 1987), molecular characterisation is required to distinguish among fungi within morphotypes (e.g. Jacquot et al. 2000; Stukenbrock and Rosendahl 2005).

   Combined with methods for determining physiological activity within hyphae (e.g. MacDonald and Lewis 1978; Saito 1995), direct morphological and molecular features of AM fungi can be used to determine the abundance, dynamics, diversity and activity of AM fungi inside roots (Solaiman and Abbott 2004; Wubet et al. 2004).

3.2.2 Indirect Assessment: Mycorrhiza Bioassay

Mycorrhiza bioassays involve use of bait plants grown in a soil sample; roots are examined for the presence of AM fungi after a set time. Glasshouse bioassays have been used to determine the infectivity of AM fungi in a range of situations (Table 3.1).

Table 3.1 Examples of studies using bioassays to assess the infectivity of AM fungi (after Djuuna 2006)

Purpose	Bioassay technique	Example references
Infectivity and effectiveness of AM fungi	Infectivity of AM fungi was assessed from field soil using subterranean clover	Abbott and Robson (1981)
Infectivity and effectiveness of AM fungi	Glasshouse trial using bait plants	Gianinazzi-Pearson et al. (1985)
The concept of soil infectivity and a method for its determination as applied to AM fungi	Soil infectivity was assessed by a standard bioassay method using Leek (*Allium porum*) as host plant	Plenchette et al. (1989)
Seasonal variation in the infectivity of AM fungi in an annual pasture in a Mediterranean environment	Undisturbed soil cores sown with *Trifollium subterraneum* L. in the glasshouse. Colonisation by AM fungi was measured 3 and 6 weeks after sowing	Scheltema et al. (1987)
The total inoculum potential of AM fungi	The rate of colonisation by AM fungi assessed by growing bait plants in intact cores of soil	Brundrett (1991)
Spatial variation in inoculum potential of AM fungi in mine site and agricultural soil	Infectivity of AM fungi in intact cores soil from disturbed and undisturbed sites	Jasper et al. (1991)
Abundance and frequency of mycorrhizal propagules	AM fungi assessed by growing bait plants in intact soil cores	Brundrett et al. (1996)
Infectivity of AM fungi exposed to long term saline conditions	Infectivity of AM fungi in saline soils studied after trap pot culture and multiple and single spore inoculation	Rafique (2005)
Infectivity of AM fungi in jarrah forest rehabilitated site after bauxite mining	Infectivity of AM fungi in intact soil cores collected from jarrah forest	Solaiman and Abbott (2003)

Generally, bioassay plants are grown in defined conditions using soil collected from the field, and the extent of mycorrhizal colonisation is assessed after a predetermined period. Bioassays of this type have been used to show differences among soils in the infectivity of AM fungi present (e.g. Abbott and Robson 1982; Gianinazzi-Pearson et al. 1985). Few studies have evaluated the relationship between the infectivity assessed in a bioassay and the development of mycorrhizas *in situ* at the site from which the bioassay soil was collected (see Abbott and Robson 1991a). Steps in each mycorrhiza bioassay (soil sampling, bioassay conditions, calibration and testing field predictions) all need to be selected with the purpose of the analysis well defined in advance (Abbott et al. 1995).

Soil sampling: Soil samples can be collected as bulk soil (disturbed soil samples) or as soil cores (undisturbed soil). Sampling needs to be representative of each sampling point. Soil samples should be used quickly or stored at low temperature until used. Consideration should be given to whether or not to allow the soil to dry, and this will depend on the biology of the AM fungi expected to be present. Prior understanding of the fungi present is therefore advantageous.

Bait plants: Host plants such as grass or legume species are good bait plants for bioassays, but other species are suitable if they are highly mycorrhizal. Plants with fast-growing roots in the soil and environmental conditions used in the bioassay, and plants with roots which are easy to clear and stain for AM fungi are most useful as bait plants (Brundrett et al. 1996).

Duration of bioassays: Brundrett and Abbott (1995) noted that the duration of bioassays should not exceed the growth stage required for an adequate level of mycorrhizas to form. If a longer time is chosen before the bait plants are assessed, the plateau level of colonisation may mask differences in rate of colonisation (Abbott and Robson 1981; Sanders et al. 1977). While it is necessary to stop the bioassay before colonisation reaches the plateau, this time might depend on the communities of AM fungi, soil conditions, growing conditions and species of bait plant, highlighting the need for customised bioassays.

Predicting mycorrhizal colonisation in the field: An additional value of mycorrhiza bioassays is that they may be used to predict mycorrhiza development in agricultural or horticultural plants (Abbott et al. 1995). However, management practices imposed after collection of the soil can influence the subsequent field colonisation (Abbott and Robson 1991b). Therefore, if the intention is to use the bioassay to predict colonisation later in the season, a calibration is needed to take into account any effect of management practices imposed after soil is collected for the bioassay on expected colonisation in the field.

A bioassay for predicting mycorrhiza formation could use soil collected before the start of the season combined with (1) information about the proposed agricultural practice, (2) soil conditions, and (3) knowledge of how the AM fungi in this soil are expected to respond to these conditions and practices. Furthermore, in order to consider using a mycorrhiza bioassay to predict the rate and extent of colonisation of roots by AM fungi, the bioassay needs to be conducted under defined conditions.

Mycorrhiza bioassays have been used to quantify seasonal and spatial variations in inoculum potential of AM fungi (Brundrett and Abbott 1995; Brundrett et al. 1996; Jasper et al. 1991; Scheltema et al. 1987). Bioassay plants grown in undisturbed soil cores are likely to closely represent field conditions, but mixed soil samples can be used as part of a standardized bioassay procedure. However, if field soil is disturbed, infectivity of the fungi present may be altered (Miller et al. 1995), so interpretation of the data could be complicated by the conditions under which the assessment is conducted.

Quantification of the rate of colonisation of bioassay plants by different morphotypes or genetically distinguishable groups of AM fungi in field soil could be used to estimate the potential of the components of the community to develop mycorrhizas (Abbott et al. 1995). Alternatively, it is possible that this may not represent the true potential infectivity of each group of fungi if there were no competing fungi present. Observations can be complicated by competition among the AM fungi during colonisation of roots (Solaiman and Abbott 2003).

3.3   Purpose of Bioassays for Infectivity Assessment of AM Fungi

The majority of ecological studies of AM fungi include a measure of mycorrhizal colonisation. However, colonisation of roots by AM fungi is a complex process and it is difficult to represent such a dynamic process in a single measurement made at one point in time. The complex characteristics of mycorrhizal associations depend on root growth and architecture as well as fungal diversity in the soil and inside roots (Abbott and Robson 1984).

The infectivity of AM fungi changes with spore maturation or dormancy (Tommerup 1983), in response to the presence of roots of different forms and age (e.g. associated with the nature and quantity of root exudates) and as the fungi proceed through stages in their life cycle (Pearson and Schweiger 1993). In the latter case, the infectivity of AM fungi associated with mycorrhizal roots paralleled the pattern of sporulation (and transfer of energy resources), despite the presence of fungi in the roots detected by staining. In natural ecosystems with little physical disturbance of soil, roots may be colonised by hyphae associated with adjacent plant species or by new hyphae from germinated spores. When soil is disturbed (e.g. with farming practices), the infectivity of AM fungi can be altered (Evans and Miller 1990). The extent of change may depend on the abundance of propagules of AM fungi present in the soil (Jasper et al. 1989). Soil disturbance in natural ecosystems can reduce the infectivity of AM fungi, especially where propagule abundance is low (Jasper et al. 1987).

A direct measurement of the presence of AM fungi in roots at a particular time results from colonisation events that have taken place during the life of the plant. In contrast, a measure of infectivity of AM fungi using a bioassay bait plant represents

the capacity of the hyphae to colonise roots at a particular point in time. A measure of infectivity of AM fungi assessed in a bioassay can be made at different stages in the plant's growth cycle and interpreted in terms of the ability of the hyphae in soil or in the roots to colonise new roots.

3.4 Conclusions

Mycorrhiza bioassays measure the infectivity in soil at a point in time, representing the potential of AM fungal propagules present in soil to colonise roots. However, the conditions of the bioassay may not be the same as those under field conditions; therefore, it is possible that fungi are present in soil in an infective state but not able to colonise roots for some reason, perhaps related to the physiological state of the roots or due to competition from other AM fungi. Bioassays of infectivity of AM fungi have potential to assist in predicting how roots might become colonised, but calibrations are necessary that take into account an understanding of the relationships between mycorrhizal colonisation under the expected soil conditions (i.e. that take into account intervening management practices such as fertilizer addition, crop or tillage practice). Therefore, several factors need to be considered before conducting a mycorrhizal bioassay, including the time of soil sampling, choice of bait plant, handling of soil samples, and length of the bioassay, and precautions are necessary when interpreting mycorrhiza bioassay data.

References

Abbott LK (1982) Comparative anatomy of vesicular–arbuscular mycorrhizas formed on subterranean clover. Aust J Bot 30:485–499

Abbott LK, Robson AD (1981) Infectivity and effectiveness of five endomycorrhizal fungi: competition with indigenous fungi in field soils. Aust J Soil Res 32:621–630

Abbott LK, Robson AD (1982) Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils. Aust J Agric Res 33:1049–1059

Abbott LK, Robson AD (1984) Colonisation of the root system of subterranean clover by three species of vesicular–arbuscular fungi. New Phytol 96:275–281

Abbott LK, Robson AD (1991a) Field management of VA mycorrhizal fungi. In: Kelster DL, Cregan PB (eds) The Rhizosphere and Plant Growth. Kluwer, Norwell, MA, pp 355–362

Abbott LK, Robson AD (1991b) Factors influencing the occurrence of vesicular–arbuscular mycorrhizas. Agric Ecosyst Environ 35:121–150

Abbott LK, Robson AD, Scheltema MA (1995) Managing soils to enhance mycorrhizal benefits in mediterranean agriculture. Crit Rev Biotechnol 15:213–228

Arias I, Sainz MJ, Grace CA, Hayman DS (1987) Direct observation of vesicular–arbuscular mycorrhizal infection in fresh unstained roots. Trans Br Mycol Soc 89:128–131

Auge RM (2001) Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Boerner REJ, DeMars BG, Leight PN (1996) Spatial patterns of mycorrhizal infectiveness of soils along a successional chronosequence. Mycorrhiza 6:79–80

Boomsma CR, Vyn TJ (2008) Maize drought tolerance: potential improvements through arbuscular mycorrhizal symbiosis? Field Crops Res 108:14–31

Brundrett M (1991) Mycorrhizas in natural ecosystems. Adv Ecol Res 21:171–313

Brundrett M (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154: 275–304

Brundrett M, Abbott LK (1995) Mycorrhizal fungus propagules in the jarrah forest. 2. Spatial variability in inoculum levels. New Phytol 131:461–469

Brundrett MC, Piché Y, Peterson RL (1984) A new method for observing the morphology of vesicular–arbuscular mycorrhizae. Can J Bot 62:2128–2134

Brundrett MC, Ashwath N, Jasper DA (1996) Mycorrhizas in the Kakadu region of tropical Australia. 2. Propagules of mycorrhizal fungi in disturbed habitats. Plant Soil 184:173–184

Cardoso IM, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agric Ecosyst Environ 116:72–84

Crush JR (1978) Changes in effectiveness of soil endomycorrhizal fungal populations during pasture development. N Z J Agric Res 21:683–685

Dickson S, Smith FA, Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? Mycorrhiza 17:375–393

Djuuna IAF (2006) The infectivity of arbuscular mycorrhizal fungi in relation to soil characteristics and agricultural land use history. PhD thesis, School of Earth and Geographical Sciences, The University of Western Australia, Perth, Australia

Dodd JC, Boddington CL, Rodriguez A, Gonzalez-Chavez C, Mansur I (2000) Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. Plant Soil 226:131–151

Douds DDJ, Johnson NC (2003) Contributions of arbuscular mycorrhizas to soil biological fertility. In: Abbott LK, Murphy DV (eds) Soil biological fertility — a key to sustainable land use in agriculture. Kluwer, Dordrecht, pp 129–162

Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecol Monogr 77:527–544

Evans DG, Miller MH (1990) The role of the external mycelial network in the effect of soil disturbance upon vesicular–arbuscular mycorrhizal colonisation of maize. New Phytol 114: 65–72

Gerdemann JW (1975) Vesicular–arbuscular mycorrhizae. In: Torrey JG and Clarkson DT (eds) The development and function of roots. Academic, London, pp 575–591

Gazey C, Abbott LK, Robson AD (1992) The rate of development of mycorrhizas affects the onset of sporulation and production of external hyphae by two species of Acaulospora. Mycol Res 96:643–650

Gianinazzi-Pearson V, Gianinazzi S, Trouvelor A (1985) Evaluation of the infectivity and effectiveness of indigenous vesicular–arbuscular fungal populations in some agricultural in Burgundy. Can J Bot 63:1521–1524

Helgason T, Daniel TJ, Husband R, Fitter AH, Young BD (1998) Ploughing up the wood-wide web. Nature 394:431

Hornby D (1990) Biological control of soil-borne plant pathogens. CAB International, Wallingford

Jacobson KM (1997) Moisture and substrate stability determine VA-mycorrhizal fungal community distribution and structure in an arid grassland. J Arid Environ 35:59–75

Jacquot E, van Tuinen D, Gianinazzi S, Gianinazzi-Pearson V (2000) Monitoring species of arbuscular mycorrhizal fungi in planta and in soil by nested PCR: application to the study of the impact of sewage sludge. Plant Soil 226:179–188

Jasper DA, Robson AD, Abbott LK (1987) The effect of surface mining on the infectivity of vesicular–arbuscular mycorrhizal fungi. Aust J Bot 35:641–652

Jasper DA, Robson AD, Abbott LK (1989) Soil disturbance reduces the infectivity of external hyphae of vesicular–arbuscular mycorrhizal fungi. New Phytol 112:93–99

Jasper DA, Abbott LK, Robson AD (1991) The effect of soil disturbance on vesicular arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol 118:471–476

Johnson NC, Gehring CA (2007) Mycorrhizas: symbiotic mediators of rhizosphere and ecosystem processes. In: Cardon ZG, Whitbeck JL (eds) The rhizosphere: an ecological perspective. Academic, Amsterdam

Kyllo DA, Velez V, Tyree MT (2003) Combined effects of arbuscular mycorrhizas and light on water uptake of the neotropical understory shrubs, Piper and Psychotria. New Phytol 160:443–454

Lackie SM, Garriock ML, Peterson RL, Bowley SR (1987) Influence of host plant on the morphology of the vesicular–arbuscular mycorrhizal fungus *Glomus versiforme* (Daniels and Trappe) Berch. Symbiosis 3:147–158

Li HY, Smith SE, Ophel-Keller K, Holloway RE, Smith FA (2008) Naturally occurring arbuscular mycorrhizal fungi can replace direct P uptake by wheat when roots cannot access added P fertiliser. Funct Plant Biol 35:124–130

McDonald RM, Lewis M (1978) The occurrence of some acid phosphatases and dehydrogenases in the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae*. New Phyto 80:135–140

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonizarion of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol 115:495–501

Menge JA, Johnson ELV, Platt RG (1978) Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytol 81:553–559

Merryweather JW, Fitter AH (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* – I. Diversity of fungal taxa. New Phytol 138:117–129

Miller MH, McGonigle TP, Addy HD (1995) Functional ecology of vesicular–arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. Crit Rev Biotechnol 15:241–255

Mohammad M, Pan WL, Kennedy AC (1995) Wheat responses to vesicular–arbuscular mycorrhizal fungal inoculation of soils from eroded toposequences. Soil Sci Soc Am J 59:1086–1090

Monzon A, Azcon R (1996) Relevance of mycorrhizal fungal origin and host plant genotype to inducing growth and nutrient uptake in *Medicago* species. Agric Ecosyst Environ 60:9–15

Moreno-Espíndolaa IP, Rivera-Becerrilb F, Ferrara-Guerrerob MDJ, de Leon González F (2007) Role of root-hairs and hyphae in adhesion of sand particles. Soil BiolBioch 39:2520–2526

Newman EI, Reddell P (1987) The distribution of mycorrhizas among families of vascular plants. New Phytol 106:745–751

Ortas I (1996) The influence of use of different rates of mycorrhizal inoculum on root infection, plant growth, and phosphorus uptake. Commun Soil Sci Plant Anal 27:2935–2946

Ortas I, Ortakci D, Kaya Z (2002) Various mycorrhizal fungi propagated on different hosts have different effect on citrus growth and nutrient uptake. Commun Soil Sci Plant Anal 33:259–272

Pankova H, Munzbergova Z, Rydlova J, Vosatka M (2008) Differences in AM fungal root colonization between populations of perennial Aster species have genetic reasons. Oecologia 157:211–220

Pearson JN, Schweiger P (1993) *Scutelospora calospora* (Nicol. and Gerd.) Walker and Sanders associated with subterranean clover: dynamics of colonisation, sporulation and soluble carbohydrates. New Phytol 124:215–219

Plenchette C, Perrin R, Duvert P (1989) The concept of soil infectivity and a method for its determination as applied to endomycorrhizas. Can J Bot 67:112–115

Puschel D, Rydlova J, Vosatka M (2007) Mycorrhiza influences plant community structure in succession on spoil banks. Basic Appl Ecol 8:510–520

Rafique R (2005) Salinity influences arbuscular mycorrhizal fungi in agricultural soil. PhD thesis, School of Earth and Geographical Sciences, The University of Western Australia, Perth, Australia

Rillig MC, Mummery DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

Saito M (1995) Enzyme activities of the internal hyphae and germinated spores of an arbuscular mydorrhizal fungus, *Gigaspora margarita* Becker and Hall. New Phytol 129:425–431

Saito M, Stribley DP, Hepper CM (1993) Succinate dehydrogenase activity of external and internal hyphae of a vesicular–arbuscular mycorrhizal fungus, *Glomus mosseae* (Nicol. and Gerd.) Gerdmann and Trappe during mycorrhizal colonisation of roots of leek (*Alliu porrum* L.), as revealed by *in situ* histochemical staining. Mycorrhiza 4:59–62

Sanders FE, Tinker PB, Black RLB, Palmerley SM (1977) The development of endonycorrhizal root systems. I. Spread of infection and growth-promoting effects with four species of vesicular–arbuscular endophytes. New Phytol 78:257–268

Scheltema MA, Abbott LK, Robson AD (1987) Seasonal-variation in the infectivity of VA mycorrhizal fungi in annual pastures in a Mediterranean environment. Aust J Agric Res 38:707–715

Sieverding E (1991) Vesicular–arbuscular mycorrhizae management in tropical agrosystems. Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ) GmbH, Eschboran, p 371

Smith FA, Smith SE (1997) Structural diversity in (vesicular)–arbuscular mycorrhizal symbiosis. New Phytol 137:373–388

Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. New Phytol 162:511–524

Solaiman MZ, Abbott LK (2003) Phosphorus uptake by a community of arbuscular mycorrhizal fungi in jarrah forest. Plant Soil 248:313–320

Solaiman MZ, Abbott LK (2004) Functional diversity of arbuscular mycorrhizal fungi on root surfaces. In: Varma AK, Abbott LK, Werner D, Hampp R (eds) Plant surface microbiology. Springer, Berlin, pp 331–349

Stukenbrock EH, Rosendahl S (2005) Development and amplification of multiple co-dominant genetic markers from single spores of arbuscular mycorrhizal fungi by nested multiplex PCR. Fungal Genet Biol 42:73–80

Tommerup IC (1983) Spore dormancy in vesicular arbuscular mycorrhizal fungi. Trans Br Mycol Soc 81:37–45

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Wubet T, Weiss M, Kottke I, Teketay D, Oberwinkler F (2004) Molecular diversity of arbuscular mycorrhizal fungi in *Prunus africana*, an endangered medicinal tree species in dry Afromontane forests of Ethiopia. New Phytol 161:517–528

Zangaro W, Bononi VLR, Trufen SB (2000) Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil. J Trop Ecol 16:603–621