

Mycorrhizas in agriculture, horticulture and forestry

Introduction

The widespread occurrence of mycorrhizal fungi of all types on crops and trees in natural ecosystems, together with effects on their mineral nutrition and growth, led to the early recognition that the different mycorrhizal symbioses might be manipulated to increase crop yields in different types of primary production systems. Most plants used in agriculture and horticulture, as well as some forest species, form arbuscular mycorrhizas (AM), but other mycorrhizal types are important in particular situations: ectomycorrhizas (ECM) for forest production and in reafforestation programmes, ericoid mycorrhizas (ERM) for fruit crops such as blueberries and orchid mycorrhizas for enhanced propagation particularly for conservation. As components of the soil biota, all mycorrhizal types are potentially important in restoration of sites degraded by mining or by forestry operations. In consequence, the effects of such disturbance on communities of mycorrhizal fungi and outcomes for productivity are receiving increasing attention. Furthermore, the multifaceted roles of mycorrhizas in soil aggregation and stabilization, in disease tolerance and in mobilizing forms of nutrients that are not directly available to roots (see Chapters 5, 9, 10 and 15) have attracted attention in the areas of biological farming and sustainable management of production systems. Techniques to enhance the yields of edible fruit bodies of ectomycorrhizal fungi, many of which command very high prices, are being actively pursued. This chapter will review selected examples of the application of arbuscular and ectomycorrhizas in managed environments and discuss possible avenues for future work.

Arbuscular mycorrhizas in agriculture and horticulture

There are many instances where crop productivity is influenced by AM symbioses, but there are still rather few examples where inoculation or management to increase AM colonization are carried out as part of normal commercial practice. Attempts have received most publicity in developed and highly mechanized agricultural and horticultural systems. In these, the application of large amounts of fertilizers and pesticides is often routine (Abbott and Robson, 1982, 1991; Menge, 1982; Miller *et al.*, 1986; Hall, 1988; Larsen *et al.*, 2007), which may reduce the potential of the fungal

symbionts to have a significant net effect on plant nutrition and growth. However, increasing emphasis on more sustainable and ecologically oriented production systems may at least partially reduce this trend. In recent years, more attention has been paid to the possibility that arbuscular mycorrhizas may increase tolerance of plants to toxic elements or reduce disease (Plenchette *et al.*, 2005; Larsen *et al.*, 2007). Furthermore, the potential of making money from sale of inoculum continues to attract interest from biotechnology companies (Gianinazzi and Vosátka, 2004). The difficulties and costs of producing high quality inoculum of the obligate symbionts, both in terms of AM infectivity and absence of pathogens, must not be underestimated and, in consequence, application may well be effectively limited to high value crops well into the future.

The emphasis of commercial applications of either inoculation with selected fungi or management of indigenous populations has generally been on increasing yields, with relatively less attention being paid to establishment or maintenance of production systems that are 'sustainable' in preserving (or improving) soil resources (Bethlenfalvay and Linderman, 1992; Gianinazzi and Schüepp, 1994; Ryan and Graham, 2002). In these environments, and in less developed systems with low inputs (Sieverding, 1987, 1991; Plenchette *et al.*, 2005), naturally occurring AM populations may play important roles in crop nutrition that is not always appreciated. Indeed, demonstrations that the AM pathway of P uptake operates in colonized plants in the field, even when there are no net benefits in terms of yield, net nutrient uptake or food quality (Chapter 5), means that the AM fungi are indeed integral to the function of root systems and must not be ignored in programmes aimed at maximizing use of nutrients in soil.

Wherever costs of production and application of fertilizers are important, or minimum input or organic agriculture is practised, the contribution of biological processes and organisms (including AM fungi) in nutrient dynamics deserves to receive more attention (Oberson *et al.*, 1993; Plenchette *et al.*, 2005; Jakobsen *et al.*, 2005). Recognition that rock phosphate deposits are limited and that reserves of high quality for fertilizer manufacture could run out before the end of this century, must lend increased urgency to the search to harness plant adaptations that maximize the use of P accumulated in soil. The 'non-nutritional' effects of mycorrhizas in modifying water relations (Chapter 5), reducing the severity of some plant diseases and in stabilizing soil structure are also potentially important (Chapter 16). These have received less emphasis than increases in production, probably because the environmental and economic benefits are less easily quantified. The functions and activities of AM symbioses have rarely been included in integrated systems for pest management, but there is an argument that this should be considered. There is an even stronger argument for their inclusion in programmes for the management of nutrient or water use, particularly where soils are highly P-fixing, fragile or subject to erosion or leaching of nutrients. The possible economic benefits of managing AM populations in agriculture and horticulture need to be critically assessed in the context of the ecology of the systems, not simply the growth of the crops (Miller *et al.*, 1994).

Mycorrhizal involvement in crop nutrition and growth in the field

Unequivocal proof that AM colonization contributes to crop nutrition, growth or yield in the field is difficult to obtain, because roots are normally colonized and appropriate

non-mycorrhizal controls are hard to produce (see Chapters 4, 5 and 16) (Abbott and Robson, 1982; Fitter, 1985, 1990; Hall, 1988). Chemical or heat treatments of soil that eliminate the AM population may alter the levels of available nutrients or release toxic compounds. They also eliminate other members of the soil biota which may themselves have direct positive or negative effects on the plants or interact with AM fungi.

Despite these problems, there are many situations where the effects of soil treatments on plant growth and yield are best explained by activities of AM fungi, because either fumigation decreased growth or inoculation increased it. McGonigle (1988) evaluated 78 field trials with AM fungi and found that inoculation (either in sterilized or untreated field soil) resulted in an average yield increase of 37%. At the time he was doubtful that nutrient uptake via AM fungi was involved, because the magnitude of the increases were not correlated with per cent colonization. We now know that the extent of colonization is not necessarily a good predictor of nutrient uptake via AM fungi (see Chapter 5). However, the possibilities remain that benefits stemmed, at least in part, from lower disease or other non-nutritional effects.

Soil fumigation or steam treatments are often used for high value crops, to reduce losses caused by plant pathogens. These processes sometimes lead to 'stunting', poor yields and variable growth of many plant species including: *Citrus*, *Persea* (avocado), *Capsicum*, *Cassava*, *Vitis*, *Allium*, *Malus* (apple), *Prunus* (peach), *Tamarillo*, *Liquidambar*, *Liriodendron*, *Elaeis* (oil palm), *Manihot* (cassava), *Cacao* and many woody ornamentals. Sometimes stunting can be reversed or reduced by applications of fertilizer, but many species that are highly AM responsive (see Chapter 4) are unable to make effective use of P and other immobile nutrients unless their roots are colonized by AM fungi. This effect is most noticeable in P-fixing soils and varies with plant species (Yost and Fox, 1979; Haas *et al.*, 1987; Li *et al.*, 2005).

Plenquette *et al.* (1983a, 1983b) investigated the effects of fumigation on the growth of 22 species grown in the field under temperate conditions, in a soil of relatively high P availability. The crops fell into three major groups: those which formed arbuscular mycorrhizas and grew better in non-fumigated soil comprised 16 species, including corn (*Zea mays*), carrot (*Daucus carota*), tomato (*Solanum esculentum*), potato (*S. tuberosum*) and a number of legumes; those which formed arbuscular mycorrhizas, but whose growth was unaffected by fumigation, including oats (*Avena sativa*) and wheat (*Triticum aestivum*); and those non-host species, cabbage (*Brassica olearacea*) and garden beet (*Beta vulgaris*), which actually grew better in fumigated soil. These groups would reasonably have been predicted from the discussion on variations in AM responsiveness (see Chapters 4 and 5) and confirm earlier findings of pot experiments with many species. Yost and Fox (1979) explored the mycorrhizal response and P uptake of a number of tropical crops grown in an acid, P-fixing soil. Ten levels of P were applied to establish solution P concentrations in the range 0.012–1.0 µg P/ml. Fumigation had little effect on bicarbonate-extractable P and caused a small but insignificant increase in inorganic N. *Brassica chinensis* grew better on fumigated than unfumigated plots and was non-mycorrhizal in both situations. The other crops all formed arbuscular mycorrhizas in non-fumigated soil and grew much better in this treatment, although the levels of soil P at which fumigation ceased to exert an effect on P concentration in the tissues differed. The order of responsiveness (together with the critical solution P concentration) was *Manihot esculenta* and *Stylosanthes hamata* (>1.6 µg/ml), *Leucaena leucocephala* (1.6 µg/ml), *Allium cepa* (0.8 µg/ml), *Vigna unguiculata* (0.2 µg/ml) and *Glycine max* (0.1 µg/ml).

As highlighted by Menge (1982) and shown subsequently in many situations, the influence of soil type, particularly with respect to P supply and P-fixation, is also important in determining responses.

Fumigation as a technique to eliminate AM fungi for experimental purposes has been criticized because it eliminates other soil organisms (including pathogens) and may also release nutrients, with consequent difficulties in interpretation of data simply in terms of AM involvement (see Chapter 16). Jakobsen discussed these difficulties in the context of the growth of several temperate field crops, including *Pisum* and *Hordeum*, and came to the conclusion that, in the cropping system he was investigating, the AM contributions to P uptake and growth were important and were underestimated by the fumigation technique (Jakobsen and Neilsen, 1983; Jakobsen, 1987). It is worth noting that some cultivars of barley and of wheat respond to AM colonization, with those that are responsive to fertilizer application also being responsive to colonization (Baon *et al.*, 1993; Hetrick *et al.*, 1993a, 1993b; Zhu *et al.*, 2003; Li *et al.*, 2005). Thus, not all cereals fall into the non-responder group (Plenchette *et al.*, 1983a) and, again, there will be significant effects of identity of fungal symbionts and soil conditions (Graham and Abbott, 2000).

Application of more selective biocidal treatments can also reduce AM populations, root colonization and cause stunting of plants (Menge, 1982). Although there are few examples of this causing major problems in production, usually because high levels of fertilizer are used simultaneously, the risk is significant in situations where there is an AM contribution to net nutrient uptake. The systemic fungicide benomyl certainly reduces AM colonization in both pots and in the field (Fitter, 1985; Fitter and Nichols, 1988; Koide *et al.*, 1988b; Sukarno *et al.*, 1993; West *et al.*, 1993b). Together with other fungicides, benomyl has been used experimentally to eliminate or reduce colonization by AM symbionts (Schweiger and Jakobsen, 1999b; Schweiger *et al.*, 2001). In controlled conditions and low P soil, the result is much reduced plant growth that can be directly attributed to lack of AM P uptake (Sukarno *et al.*, 1993; Schweiger and Jakobsen, 1999b; Schweiger *et al.*, 2001). However, in field situations, the occurrence of fungal pathogens, which might also be eliminated, confuses the picture in relation to AM effects on nutrition. Nevertheless, the technique certainly highlighted the possible importance of AM fungi in reducing the effects of pathogens and hence acting as biocontrol agents (West *et al.*, 1993a; Fitter and Garbaye, 1994; Hooker *et al.*, 1994; Graham, 2001). With the phase-out of the fumigant methyl bromide, alternative, low input approaches to eliminate pathogens have been sought. In Florida, Sylvia *et al.* (2001) investigated a strip tillage system in which tomatoes were transplanted into bahagrass (*Paspalum notatum*) pasture. The system was designed to reduce effects of pathogens and improve soil conservation, but tests showed that competition between crop and grass actually reduced tomato yields. Pot experiments revealed that tomato was highly responsive to AM colonization in the low P and low pH soil used and bahagrass was less so. AM colonization increased the ability of the tomato to compete with the grass, when they were grown together. However, as shown in a second experiment, application of P reduced the relative competitive ability of tomato. The authors stressed that development of low input systems, such as the one investigated, requires careful development of strategies to maximize nutrient uptake by the crop. They suggested that reduced P application might allow tomatoes to take advantage of their relatively high mycorrhizal responsiveness in this soil type. The involvement of mycorrhizas

in productivity during intercropping has scarcely been explored, but deserves investigation.

Severe soil disturbance by tillage, mining or natural causes can also reduce plant nutrient uptake and hence crop yield. A major effect in this case is the disruption of the network of mycorrhizal hyphae in soil, with consequent reductions in colonization, nutrient acquisition and growth. In the field, this has been shown to be important for growth and nutrition of *Zea mays*, particularly with relatively low fertilizer P applications (Evans and Miller, 1988, 1990; McGonigle and Miller, 1993) and a number of species from native vegetation in soil disturbed by mining (Powell, 1980; Jasper *et al.*, 1989, 1992). Variations in susceptibility of different AM fungi to such damage and consequent effects on plant nutrition have been observed (McGonigle *et al.*, 2003).

Crop rotations involving long periods of bare fallow (1 year or more) have sometimes been adopted, with the aim of accumulating moisture and mineral N in the soil profile. In Queensland, Australia, this practice led to severe stunting and P and Zn deficiency in a wide range of taxonomically unrelated crops (Thompson, 1987). This so-called 'Long-fallow Disorder' was tracked down to a deficiency in AM propagules in the soil, with consequent decreases in the rate and extent of AM colonization and uptake of nutrients (Thompson, 1990, 1994). The problem was overcome by eliminating long fallow periods from rotations and adopting management practices that maintain AM fungal populations. In this case, as in many other examples, the data from soil tests used to determine appropriate rates of P and Zn application were obtained in situations where the fungal symbionts normally made substantial contributions to P and Zn uptake by the plants. Reduction in AM colonization, by whatever means, reduced or eliminated the fungal contribution to nutrition, with the consequence that the response to fertilizer was also reduced and the data from the soil tests were no longer good predictors of crop response to P application (Haas *et al.*, 1987; Thompson, 1987). A note of caution is required here, because field experiments on similar soils in south-eastern Australia found that increasing AM colonization by management of rotations rarely resulted in any increases in yield or nutrition of wheat. The discrepancy was attributed to differences in P fertilizer applications between the two regions (Ryan *et al.*, 2002).

A novel experimental approach to determining the potential contribution of AM fungi to tomato production in the field was recently adopted by Cavagnaro *et al.* (2006). AM and non-mycorrhizal treatments were established without soil fumigation or fungicide application, by using a mutant tomato with reduced AM colonization (*rmc*) and its wild-type progenitor, planted in an organically managed field at the same time as a normal commercial tomato crop. Naturally-occurring AM fungi colonized the wild-type tomato normally (24% of root length), but were only able to invade the epidermal cells of the mutant over 4% of root length, with no cortical development. This pattern of colonization does not support any P uptake via the AM pathway (Poulsen *et al.*, 2005). At the final harvest, there were no significant differences in shoot or fruit biomass between the genotypes, a similar finding to several pot experiments with these plants grown in neutral or slightly alkaline soils (Cavagnaro *et al.*, 2004a; Poulsen *et al.*, 2005). However, the wild-type (AM) plants had considerably higher concentrations of both P and Zn in shoots and fruits than the mutant (non-mycorrhizal). Not only do these data indicate that the AM fungal populations were contributing to nutrition of the colonized wild type, but they highlight an increase in food quality in terms of nutrient densities. Similar results have

occasionally been observed in highly colonized wheat, compared with wheat grown in field rotations that resulted in low AM colonization (Ryan *et al.*, 2002). Effects of arbuscular mycorrhizas on nutritional value of crops deserve ongoing research, particularly in the light of concerns that use of highly purified fertilizers and other modern agricultural practices are reducing micronutrient densities below those required for human health (Welch, 2002; Welch and Graham, 2002). Possibilities that AM colonization may increase concentrations of nutritionally beneficial compounds like antioxidants are currently being actively explored.

Positive effects of high AM colonization on yields are by no means always observed. Growth depressions have most often been attributed to C drain caused by AM colonization, as discussed for citrus (see Chapter 4). Graham and Abbott (2000) reported a wide range of responses of wheat in a pot experiment, with growth depressions associated with rapid and high colonization by what they referred to as 'aggressive' AM fungi. In the field, high populations of *Glomus macrocarpum* have been linked to tobacco stunt disease, with the poor growth alleviated by soil fumigation that effectively eliminated AM fungi (Modjo and Hendrix, 1986; Hendrix *et al.*, 1992). The mechanism of inhibition of growth was not fully worked out, but reduced root growth of AM plants may have been a contributory factor (Jones and Hendrix, 1987). More recently, Ryan *et al.* (2002) carried out a series of five experiments, examining the effects of cropping sequence on AM colonization, P and N nutrition, growth and yield of wheat. The soils were alkaline vertisols, similar to those on which reduced AM fungal populations had been shown to be associated with Long-fallow Disorder. Low AM colonization following brassicas did not affect early crop growth, P and Zn uptake or yield. In only one of the experiments was there a positive correlation between AM colonization and grain P and Zn concentrations. Findings such as these led Ryan *et al.* (2002) to conclude that farmers need not consider the effects of cropping sequence on extent to which wheat becomes colonized on these soils. They went further to question more generally any positive roles of AM fungi in crop production and suggest that, for some normally non-responsive crops like wheat, the C drain to the fungal symbionts may have significant effects in reducing yields. However, these suggestions may require re-evaluation in the light of demonstration of the involvement of AM fungi in nutrition, independent of net growth benefits (see Chapters 4 and 5).

The work discussed so far has evaluated potential AM effects in terms of net positive or negative effects on plant growth and nutrition. The demonstration that the AM pathway of nutrient uptake may replace that of roots has not yet received much attention in cropping situations. However, several field experiments have demonstrated considerable uptake of ^{32}P into crops and pastures from buried hyphal compartments (HCs) which successfully prevented P uptake by plant roots and root hairs from inside the HCAs. In the experiment of Schweiger and Jakobsen (1999b) with wheat, the rate of P uptake per unit length of AM hyphae was similar to that observed in pot experiments. Application of the fungicide carbendazim to the HCAs, at concentrations known to inhibit AM fungal P transport, effectively eliminated P uptake via the fungal pathway. The results demonstrate the considerable contribution of native AM fungi to overall P uptake of field-grown winter wheat, even at typical field soil fertility levels of $28\mu\text{g NaHCO}_3$ -extractable P/g. A similar approach was used to investigate the AM contribution to nutrition of peas also grown in a relatively fertile soil (Schweiger *et al.*, 2001). Again, the native AM fungi made a

considerable contribution to plant P uptake, which was reduced or eliminated at high levels of application of commercial fungicides. Interestingly, at recommended field application rates, hyphal P uptake and transfer were slightly increased. This effect was attributed to relatively greater negative effects on other members of the soil microbial community and highlights the importance of establishing appropriate protocols that not only control unwanted pathogens but also maintain the function of potentially beneficial organisms. AM contributions to nutrition of grassland and pasture plants growing in undisturbed soil cores have also been assessed using $^{32/33}\text{P}$ -labelled soil buried in HCs. Johnson *et al.* (2001) showed that there was considerable movement of ^{33}P from soil in the HC to a mixture of grassland species growing in the main pot compartment, which was greatly reduced if the external mycelium of the AM fungal assemblage was severed by rotating the cores. Jakobsen *et al.* (2001) used a similar approach to show that the contribution of native AM fungi to P transfer to *Trifolium subterraneum* was very variable, depending on soil type and on the growth of external mycelium into the labelled soil cores. Inhibition of AM fungal activity with the benomyl applied to the HCs resulted in reduced P concentrations and reduced ^{32}P uptake into plant shoots in several of the soils. However, in one soil, plants grew very badly regardless of fungicide application, the external AM hyphae did not grow into the HCs and there was little or no ^{32}P transfer to the plants. Taken together these experiments clearly demonstrate the importance of the external mycelium of field assemblages of AM fungi and the AM pathway of P uptake to a range of plants of varying responsiveness in several soils of different P status.

It is highly likely that AM fungi make similar contributions to uptake of P, Zn and possibly other nutrients in a variety of crops and pastures in the field, even when net benefits in terms of yield or nutritional quality are not apparent. It may be thought that these 'hidden' contributions are unimportant. However, crop improvement for enhanced efficiency of nutrient uptake increasingly depends on understanding the details of the uptake processes and their genetic control. If crops are normally and unavoidably colonized in the field, then P uptake via AM fungi must be taken into account in research programmes. Modern crop varieties have certainly been selected and bred without taking AM symbiosis into account and the lack of responsiveness of many crop plants may be a consequence of this (Smith *et al.*, 1992; Hetrick *et al.*, 1996). At least two investigations indicate that AM responsiveness may be an inherited trait in wheat and barley and closely related to P responsiveness (Baon *et al.*, 1993; Hetrick *et al.*, 1996). In the main, plant breeders have ignored potentially beneficial AM symbioses, but future genetic research and breeding could usefully capitalize on these findings, particularly bearing in mind that AM fungi are integral to root function of both responsive and non-responsive AM plant species and that colonization in the field and contribution to P uptake may be very high (Ryan *et al.*, 2002; Li *et al.*, 2005, 2006) and hard to eliminate without drastic management. We need to understand fully the pathways and mechanisms of nutrient uptake, including coordinated regulation of direct and AM symbiotic P uptake pathways in order to manipulate them to improve P efficiency of crops either by breeding, genetic manipulation or management.

Evaluation and management of AM fungal populations

Most of the examples given above show that nutrient uptake and hence crop production in the field can involve the naturally occurring assemblages of AM fungi.

The composition of these communities, in terms of species and propagule densities, is not usually well known and the way in which differences in species richness may affect plant productivity is not at all clear. Indeed, the fungal attributes that result in a large contribution to plant nutrient uptake and growth are not fully worked out, so that selection of 'efficient' fungi still remains largely empirical. It is known that an extensive external mycelium is necessary and that different species of fungi vary in the way that this mycelium develops (see Chapters 2 and 5). Rapid and early colonization of the roots and the production of numerous arbuscules are also important and may be functions both of the innate characteristics of the fungal species, the propagule density and other conditions in the soil. The picture is further complicated by differences in the way different plant-fungus combinations function in terms of nutrient transfer and by difficulties in relating physiological effects (e.g. nutrient uptake, growth response) to per cent colonization of the root system, which is frequently measured rather late in the growth period when the density and activity of arbuscules may be relatively low.

In the field, many different AM fungal species are likely to be present and will colonize crops to varying extents, depending on plant-fungus selectivity, as well as relative propagule densities (see Chapter 1). We now recognize that the efficiency of particular fungi may vary when associated with different plant species, but we have little idea whether those fungi which are most efficient with respect to nutrient uptake are the same as those which play a major role in reducing the effects of pathogenic organisms or stabilizing soil. There are essentially two approaches to establishing and maintaining high infectivity of AM fungi in soils used for agriculture or horticulture. These are inoculation (and subsequent management) of selected AM fungi and adoption of field practices which increase the infectivity of indigenous assemblages. The relative merits of these approaches in different situations have been reviewed many times (Abbott and Robson, 1982, 1991; Menge, 1983, 1984; Hall, 1988; Gianinazzi *et al.*, 1990b; Sieverding, 1991; Bethlenfalvay, 1992b; Bethlenfalvay and Linderman, 1992; Wood and Cummings, 1992; Dodd and Thomson, 1994; Brundrett *et al.*, 1996; Ryan and Graham, 2002; Larsen *et al.*, 2007).

There is general consensus that before any approach is adopted a number of key factors need to be evaluated. These include: the responsiveness of the crops to be grown, the assemblage of indigenous AM fungi present, particularly with respect to their infectivity and effectiveness, the possible effects of soil management (e.g. tillage, P and N application) on their AM assemblages and the characteristics of the soil, as they affect both nutrient availability, crop responsiveness and fungal survival. To these need to be added information on the incidence of pathogens and methods that are used to reduce their effects (e.g. fumigation, application of fungicides) and an evaluation of whether AM mycelium in soil may have a significant effect on the establishment and stabilization of soil structure (Tisdall, 1994; Miller and Jastrow, 2002; Rillig and Mumey, 2006) (see Chapter 16). The latter may be very important in fragile environments that are being farmed more intensively in attempts to maintain outputs (Plenchette *et al.*, 2005). Finally, the economic costs and benefits of any management practices are vital considerations and need to be incorporated into long-term plans for sustainable use of soil resources (Miller *et al.*, 1994).

The effects of agricultural practices on AM fungi have recently been comprehensively reviewed (Jansa *et al.*, 2006; Larsen *et al.*, 2007). The factors rank (in decreasing order) crop rotation, soil tillage, fungicide application and application of fertilizers

Table 17.1 Positive and negative influences on arbuscular mycorrhizal assemblages and colonization of subsequent crops by different agricultural management practices.

Management factor	Positive influence	Negative influence
Plant species	Host species High colonization High spore production High mycorrhizal root length density	Non-host species
Bare fallow	None	Reduces populations
Pasture	Increased propagule densities	
Disturbance–tillage–rotation	Minimum tillage pasture phase	Conventional tillage compaction
Management	Organic, biodynamic	Conventional
Fertilizer application	Drip feeding Slow release rock phosphate	High applications of soluble P and N
Fumigation	None	Reduces propagules
Fungicides	Variable effects	Variable effects
Low light (glasshouse)	None	Colonization and growth decreased

in importance in modifying or reducing AM fungal populations. Other factors such as irrigation, burning and grazing, pollutants and topsoil removal are also relevant (Table 17.1). Of course, the most extreme examples of low populations result from soil sterilization, fumigation or use of soil-less media for production of high value crops. Here, the greatest potential for successful inoculation with AM fungi exists, including the production of micropropagated plants (Vestberg and Estaún, 1994; Lovato *et al.*, 1995, 1996). However, few, if any, commercial production systems use inoculation because of the difficulties of producing and applying inoculum and of introducing modifications in cultural practices (Menge, 1984; Wood and Cummings, 1992; Lovato *et al.*, 1995; Gianinazzi and Vosátka, 2004).

Many high value glasshouse crops are increasingly being grown in soil-less media, such as rockwool. The systems have advantages in that such rooting media are light and nutrient supplies can be closely controlled. However, it has been found that pathogen attack can produce significant losses and some research is now being directed towards introducing an element of biological balance to improve plant health into these systems. In one recent investigation of tomato and cucumber production in Denmark, Ravnskov and Larsen (2005) tested the effects of application of commercial AM inoculants in relation to nutrient supply. Tomatoes responded positively to application of P fertilizer over the whole range tested, up to 100% of concentrations recommended to growers. AM colonization was relatively low and none of the inoculants gave any benefits in terms of vegetative biomass under experimental conditions. In contrast, non-mycorrhizal cucumber showed increased growth only up to 50% of recommended P fertilizer concentrations and also showed a positive response to inoculation. A grower trial showed no benefits of inoculation of cucumber in terms of growth

or yield, but one of the cultivars tested produced almost 4% more first class fruit. This increase may seem rather small, but it was significant and represented an increased profit to the grower involved of €50 000 (£33 500) in spring production (2005 prices). In evaluating the findings, Ravnskov and Larsen (2005) concluded that there was potential for ongoing investigations of AM applications for cucumber production, but not for tomatoes. The plants did not suffer from disease either under experimental conditions or in the grower trial, so potential benefits of AM inoculation in disease tolerance could not be assessed in this case, but would be beneficial in future.

In field situations, evaluation of composition of AM fungal assemblages requires both identification of the species present and quantification of propagule densities and infectivity (see Chapter 2). The assemblages are usually described in terms of spore types and numbers, whereas bioassays of various types are used to evaluate the infectivity of the soil based on all the functional propagules. Spore isolation, unfortunately, cannot show which fungi are active in roots and soil. The limitations are clearly appreciated and a number of different methods are being developed to identify AM fungi in the absence of spores. Techniques used with varying success and sensitivity include DNA-based fingerprinting, isozyme banding patterns and fatty acid methyl ester profiles. DNA-based methods, in particular, have the potential to be sufficiently precise to distinguish different strains of a single species but will need further development to make them satisfactorily quantitative. Until we have precise and rapid methods of identification and evaluation of AM fungi which are present as vegetative stages and contribute to soil–plant processes, we are not likely to make much progress in understanding and managing the fungi in agro-ecosystems. Even then uncertainties in relating taxonomic position or extent of colonization to function are likely to remain significant.

If populations are low or ineffective, then inoculation or management to increase propagule densities can be considered. Conversely, and as cautiously advocated by Ryan and Graham (2002), there may be advantages in managing soils to reduce AM populations for specific crops and on particular soils, if negative effects on yield are such as to have significant effects on crop profitability. This could be achieved by extensive tillage or rotations with non-hosts such as brassicas, but advantages would have to be very carefully offset against the advantages of minimum tillage, which is frequently and effectively adopted to minimize soil erosion. Furthermore, until we know more about the ways in which plant and fungal nutrient uptake processes are integrated and operate in field situations, the practice certainly should not be widely adopted. Additionally, there are many who would argue that conserving biodiversity of potentially beneficial soil microbial populations, including AM fungi, is crucial to soil health.

Inoculation

As Wood and Cummings (1992) predicted, the unculturability of the fungi continues to be a major barrier to the development of cheap and easy inoculation techniques. AM inoculum currently has to be grown in symbiosis with plants. Production costs are high, the product often bulky and quality control to maintain infectivity and exclude pathogens is a significant concern (Menge, 1984; Gianinazzi and Vosátka, 2004). Nevertheless, over 20 companies worldwide are reportedly producing AM inoculum (Gianinazzi and Vosátka, 2004), capitalizing in part on the

desire of many producers to use 'biological' methods. Symbiotic production does have the important advantage of automatically monitoring the ability of the fungi to colonize roots, at least during the production stage.

The plant-based inocula now available are quite diverse and require different methods of application. As outlined by Gianinazzi and Vosátka (2004), they are produced in different ways ranging from nursery plots to *in vitro* monoxenic root organ cultures. The resulting materials (spores, hyphae, root fragments etc.) are added to different carriers, resulting in a wide range of formulations. The different systems have a range of advantages and disadvantages in terms of ease of use, quality and cost. The suitability of these inocula for different applications depends on the identity of the main AM propagules and on their ability to retain infectivity during storage and to persist in soil or roots from year to year, as well as on the methods available for application. One promising approach is encapsulation of AM roots, containing high densities of fungal vesicles, in alginate beads. The resulting inoculum retained infectivity for at least 3 years (Plenchette and Strullu, 2003). It is a matter for discussion whether 'generic products', containing several AM fungi and potentially suitable for a range of applications, are more appropriate for the market than those with precise formulations and AM fungi specifically tuned to particular end-uses.

Quality control criteria have not yet been set for AM inoculum, but are urgently required so that the products meet reasonable standards. At the very least, the inoculum must initiate colonization in the root systems of plant species that are able to form mycorrhizas, at the doses recommended by the suppliers, it must not contain pathogens or other agents that could reduce plant growth and it must have a reasonable shelf-life when stored under recommended conditions. Although outcomes are difficult to prove without direct experimentation, the products should also meet the expectations and requirements of purchasers including decreasing the need for fertilizer applications, increasing plant growth, flowering, yields or tolerance to disease or pollutants. Not all available inocula meet these criteria, an important issue both with respect to maintaining confidence of the market and meeting critical quarantine standards. No doubt the DNA fingerprinting methods now under development will find application both at the level of identification of wanted and unwanted organisms in the inoculum and survival in roots and soil following application. Further considerations and progress towards regulation of products are documented in two recent reviews (Gianinazzi and Vosátka, 2004; Plenchette *et al.*, 2005).

At present, routine inoculation in broad scale, highly developed farming systems is not realistic, because of the expense of production and application and uncertainties relating to the competitive ability of inoculant fungi in field situations. Management of indigenous populations is the only currently viable option. This was demonstrated to have some potential in bell pepper (*Capsicum annuum* L.) production in Australia (Olsen *et al.*, 1999). The field site was subjected to two cycles of crops which are highly mycorrhizal, establishing a well-developed mycelial network with very high density of infective propagules. AM fungal populations were effectively eliminated by fumigation to provide non-mycorrhizal control plots and P was applied as milled superphosphate at five levels (P0–P5), from zero to 135 kg P/ha. *Capsicum* seedlings were transplanted into the plots, according to normal horticultural practices. Phosphate application did not affect the percentage colonization of the plants. Phosphate nutrition was monitored in the youngest mature leaves and showed that plants in the AM treatments had adequate P concentrations at all P

applications except P0, but the non-mycorrhizal plants only achieved adequate nutrition at P5. At harvest, the yields of AM plants were higher than controls, except at P5. Gross margin of non-mycorrhizal plants at P5 was AUD 3440/ha (1999 prices). All AM treatments except P1 showed similar profitability. The authors concluded that AM had only limited potential as a substitute for P at that time, because fertilizer made a low contribution to the overall costs of production. However, this situation will change if P fertilizer costs rise significantly.

In relatively small-scale, high value operations such as nursery production, routine inoculation is certainly feasible and likely to be highly advantageous in increasing growth rates and uniformity of the product. In these situations, potting media are frequently sterilized to eliminate pathogens, so that reintroduction of AM fungi specifically selected for the particular application has potential. Work in this high-tech area continues actively, including production of high quality inoculum for research purposes (Gianinazzi *et al.*, 1990a; Lovato *et al.*, 1995; Gianinazzi and Vosátka, 2004).

Inoculation of seedlings is potentially a good method for establishing selected fungi in roots, before potting on or planting-out into the field. It is appropriate where transplanting is part of the normal production system. An advantage is that the inoculant fungus is established in the root systems at an early stage and may consequently have a competitive advantage over soil-borne species. The method has been tested with a number of different crop species in the field. Sasa *et al.* (1987) inoculated *Allium porrum* in pots, so that at transplanting the roots were about 80% colonized by a mixture of AM fungi. These plants grew better than uninoculated controls after transplanting, with 5.7 and 1.5 fold increases, in fumigated and unfumigated soil, respectively. Similar increases in growth have been observed for such diverse species as chilli (Bagyaraj and Sreeramulu, 1982), apple (Plenchette *et al.*, 1981) and guayule (*Parthenium argentatum*, Bloss and Pfeiffer, 1984). Snellgrove and Stribley (1986) adapted normal commercial methods in their work with *A. cepa*, but had difficulties establishing colonization in the peat modules used for transplanting. The approach deserves more extensive evaluation and the results highlight the need to fit inoculation procedures to acceptable production methods, as well as to soil-type and plant species. Inoculation with two or more fungi needs to be considered as it could reduce the variation in response that might be expected with different soils, plant species and growing conditions, following inoculation with single species (Sieverding, 1990; Bethlenfalvay and Linderman, 1992).

At the other end of the scale, manufacture of 'home-grown' inoculum of highly colonized roots and soil that is applied to plots immediately before planting a crop could make a valuable contribution to food production in many relatively small, low input systems. Its potential should not be under-rated because it does not make large profits in monetary terms. In most cases, experiments have been carried out with annual crops grown in monoculture. However, tree crops are also important and, particularly in developing countries, are sometimes cultivated in plantations or gardens with considerable species diversity. Examples include coffee and cacao, which are grown with mycorrhiza-responsive shade trees (Wibawa *et al.*, 1995) and many tropical fruits, grown in mixed agroforestry systems (Janos, 1980). It is known that both coffee and cacao, as well as citrus, cashew and many other tree crops of tropical origin, are responsive to AM colonization (Janos, 1987; Alexander, 1988; Sieverding, 1991; Smits, 1992; Smith *et al.*, 1998). Both cultivation and monoculture

appear to change the species composition of the fungal populations and reduce their diversity, but the impact of these changes on crop production has not been adequately evaluated (Black and Tinker, 1979; Johnson *et al.*, 1992; Johnson, 1993; Allen *et al.*, 1995; Hendrix *et al.*, 1995; Helgason *et al.*, 1998).

In those situations where the cost and availability of phosphate fertilizers is very significant, AM fungi may increase the accessibility of relatively cheap fertilizers, such as rock phosphate (RP) when used directly on acid soils. Again, the responses will vary with the plant under consideration. In the work of Wibawa *et al.* (1995) on shade trees used in coffee and cacao plantations, *Sesbania grandiflora* appeared to be relatively efficient at acquiring P from deficient soil and did not respond significantly to triple superphosphate (TSP) or RP, regardless of AM inoculation. Two other species grew poorly when unfertilized, responded to TSP but not RP in the absence of inoculation and responded to RP when inoculated with *Gigaspora margarita*. Colonization of the roots was not determined, but spore production was significantly influenced by treatment for all species. In general, inoculation increased spore production to a much greater extent with RP than with TSP, an important consideration in the context of management of AM fungal populations.

The same practices that are used to manage AM fungi for crop production can be used to enhance AM effects on soil structure and structural stability and have the potential to make an important contribution to agricultural and horticultural ecosystems, particularly where erosion is a serious concern. It has also been suggested that an extensive mycelial network that ensures effective removal of P from soil will prevent off-site losses to streams, rivers and groundwater (e.g. Jakobsen *et al.*, 2005b). This possibility has not been extensively tested, but it was recently shown that the main effect of AM colonization in reducing the mobility of P in repacked soil columns was associated with increases in plant growth in low P soils. When available soil P was increased, P losses in leachate were much higher and not reduced if plants were AM (Asghari *et al.*, 2005).

Management

The continuing (though not continuous) presence of host plants is essential for inoculum build-up. Growth responses of plants to colonization are not important; rather the mycorrhizal root length-density in soil and the production of extraradical hyphae and spores will contribute most to the population of propagules. Pasture, which combines production of colonized root and low disturbance, has a high potential for inoculum build-up, as well as production of water-stable soil aggregates, stabilized by hyphae of AM fungi (see Chapter 16). Other management strategies that might maintain fungal populations include sequential cropping, where two or more crops are grown each year, or intercropping, where two crops are grown simultaneously. In either case, if at least one of the crops is potentially mycorrhizal, an adequate inoculum level is likely to be maintained (Andrews and Kassam, 1976; Tisdall and Adem, 1990). Both bare fallow and cropping with non-hosts will, sooner or later, reduce mycorrhizal populations, or may delay re-establishment of a pool of infective propagules (Ocampo and Hayman, 1980; Thompson, 1987; Ryan *et al.*, 2002).

Tillage, other types of disturbance and stockpiling of soil reduce the populations of viable propagules (Jasper *et al.*, 1987, 1989, 1992; Miller and Jastrow, 1992b, 2002) and should be minimized to allow maintenance or build-up of AM populations, as

well as to maintain soil structural stability. Soil compaction also has negative effects on root colonization, as well as on root growth itself. Mulligan *et al.* (1985) reported reduced per cent colonization as bulk density increased due to trafficking by agricultural vehicles. It might be expected that restricted root growth would result in lower mycorrhizal root length density in soil, even if there were no effects on the ability of the fungi to colonize the roots. In fact, reported effects of increasing compaction on colonization are variable, with both increases and decreases recorded (Nadian *et al.*, 1996, 1997; Li *et al.*, 1997; Yano *et al.*, 1998). The findings are likely to have been influenced by the extent to which root growth was reduced under compaction, in conjunction with infectivity of the soils. Hyphal extension in soils from colonized roots has also been shown to be reduced, but not to the same extent as the roots (Nadian *et al.*, 1996). Mycorrhizal plants grew better than non-mycorrhizal at all levels of compaction, but there were consistent decreases in responsiveness as compaction increased. Different AM fungi have been shown to respond differently to compaction and to variations in pore size in soil, both with respect to root colonization and ability to extend out into the compacted soil and absorb P or colonize new plants (Drew *et al.*, 2003, 2005).

Biodynamic and organic farm management results in higher per cent colonization of roots of pasture and annual crops than conventional management (Ryan *et al.*, 1994, 2000; Ryan and Ash, 1999). The effects are at least partly the result of lower P applications and use of less available fertilizer sources. The interactions between application of P and N, growth responses and maintenance of soil populations of propagules are of major importance. So far as growth responses are concerned, the form and timing of fertilizer application, as well as the sensitivity of the particular plant-fungus combinations needs to be taken into account. For most crops, P (frequently as superphosphate) is applied once, before sowing, and may have large effects in reducing per cent colonization and growth response. Other forms of P, such as RP, do not have the same effects on colonization and may be much more compatible with maximizing the contribution of arbuscular mycorrhizas in plant nutrition. Drip feeding of nutrients in irrigation water (fertigation) is sometimes practised in intensive vegetable production. In one field investigation with *Capsicum* on a highly P-fixing soil, the practice maintained soil solution P at a relatively low concentration, permitting both extensive mycorrhizal colonization and good growth and yield (Haas *et al.*, 1987). Application of slow-release fertilizers might be expected to have the same effect. Selection of P-tolerant fungi has also been canvassed, with the possibility of using them in inoculation programmes where high P application is practised.

Biological control of pathogens is now an accepted component of pest management programmes. Success in this area will be reflected in the reduced use of pesticides, including fungicides. The consequences in terms of AM function are likely to be positive. Damage to non-target AM fungi will be minimized and the effects of these on plant nutrition, on soil structure and on root-infecting pathogens themselves maximized. The potential for including AM fungi in pest control packages as biocontrol agents has not been widely explored, although some potential certainly exists. In the horticultural industry in particular, it is possible to envisage an integrated package in which AM fungi (possibly in association with other beneficial microorganisms) are applied with the aim of making most effective use of fertilizer and minimizing losses due to disease. Such strategies are likely to become more attractive as the use of chemicals for fumigation and disease control is progressively

discouraged and fertilizer becomes a proportionally higher component of the cost of production. Future management of soil microbial populations to maximize the effective use of P reserves stored in various soil fractions will need to take all potential benefits and interactions into account (Jakobsen *et al.*, 2005b).

Ectomycorrhizas and forest production

In native forests managed for timber production, ectomycorrhizas are an accepted part of the ecosystem and their potential significance in tree nutrition has been recognized since the earliest experiments (Frank, 1885). Less attention has been paid to arbuscular mycorrhizas, despite the fact that a number of significant timber trees form this mycorrhizal type. Timber extraction, and especially clear-cut logging, have major impacts on forest ecosystems, not only on the plant species that regenerate in clear cuts, but also on the communities of ECM fungi that survive and colonize regenerating or transplanted seedlings. The composition and diversity of these fungal assemblages is attracting increasing attention because of their potential impacts on forest productivity and development of site management strategies (Jones *et al.*, 2003).

Plantation forestry is increasing in many parts of the world as the demand for timber and pulp increases and in response to calls to increase sequestration of CO₂ through increases in forest cover. Although outplanted or naturally regenerating seedlings can develop ECM associations from any naturally occurring mycorrhizal inoculum, the potential advantages of nursery stock being mycorrhizal before out-planting are clear. Grove and Le Tacon (1993) provide a comprehensive outline of the imperatives to developing successful strategies to maximize the benefits of ectomycorrhizas in forestry. Many commercial practices, particularly those employed to improve hygiene in tree nurseries, are inimical to the growth of all but a few ruderal species of ECM fungi. In consequence, special techniques have been developed which enable selected fungi to colonize plants prior to outplanting. The application of these techniques has facilitated superior performance in tree crops in many parts of the world, particularly those which lack natural local sources of inoculum (see Marx *et al.*, 2002). At the same time, interest has grown in the possibility of harvesting edible fruit bodies of ECM fungi which have been used as commercial inoculum both to supplement diet and revenue.

Inoculation can have benefits at two stages of the timber production systems; in the nursery itself and after outplanting to the field. Much of the experimental work has focused on advantages to be gained from the production of well-developed seedlings that, with their fungal symbionts, will become successfully established in the field. However, rapid growth in nurseries also results in direct savings by increasing the rate of throughput. Experience of the use of inoculated seedlings has indicated that responses to ECM colonization are often greatest under the most extreme conditions, particularly those involving exposure to drought, metal contamination and pathogens. Such observations have led to analysis of the functional basis of the ameliorative effects of ECM fungi. In addition to the well-documented effects on plant nutrition and growth, considerable advances have been made towards understanding the roles of the symbioses in providing resistance to these stresses which, though they also occur in natural ecosystems, are often locally increased by previous land-use practices or by the afforestation process itself. Indeed, many of

the discrepancies between nursery and field performance following inoculation may stem from different impacts of soil and other environmental conditions on the individual mycorrhizal partnerships established by inoculation. Forest productivity is threatened by aspects of global change such as direct inputs to soils of nitrogen and sulphur and concomitant decreases in pH, which may have adverse effects on both symbionts. Such effects are thought to be contributory factors in the forest decline syndrome experienced in Europe and north-eastern USA. There are also indications that base-cation availability may come to limit forest productivity, so that any positive effects of ectomycorrhizas on uptake will be beneficial (see Chapter 10).

Inoculum production and inoculation practice

The use of defined inoculum consisting of ECM fungi that were physiologically and ecologically appropriate for the planting site, with a view to improving performance of the crop, was pioneered by Moser (1958) in Austria, Takacs (1967) in Argentina and Theodorou and Bowen (1973) in Australia. Prerequisites for the widespread use of ECM inoculation programmes are the selection of fungal symbionts and the development of methods for the large-scale production of inoculum (Grove and Le Tacon, 1993; Brundrett *et al.*, 1996). The requirements are interrelated because, in addition to being functionally compatible with and enhancing performance of the inoculated crop, the selected fungus must be able to withstand the physical, chemical and biological stresses involved in the production and storage of the inoculum, as well as those imposed by the soil and other site characteristics, first of forest nurseries and later at the sites to which the seedlings are outplanted (Kropp and Langlois, 1990; Grove and Le Tacon, 1993).

One of the most widely used and successful early inoculation programmes employed *Pisolithus tinctorius*. Interest in this fungus was prompted by its apparently wide geographic distribution, broad host range (Marx, 1977) and the knowledge that it became prominent on adverse sites, particularly those subject to drought, high temperature or contamination (Schramm, 1966). It is a striking feature of the programme of inoculum production using *P. tinctorius* that only one vegetative isolate of the fungus was used throughout. This strain was originally obtained from a sporophore found under *Pinus taeda* in Georgia, USA (Marx and Bryan, 1969). Its aggressive traits and ability to enhance growth of plant partners have apparently been enhanced by annual re-isolation over 30 years from seedlings growing in inoculation trials.

In order to eliminate weeds, pathogens and other symbiotic fungi which are potential competitors, seed beds or potting mixes are routinely fumigated before inoculation. Even so, re-invasion of fumigated soil by spores of naturally occurring ECM fungi, particularly *Thelephora terrestris*, normally occurs within days and it is a requirement of the inoculant fungus that it has the ability to colonize roots quickly. *T. terrestris* appears to be the dominant ECM fungus of forest nursery soils worldwide (Mikola, 1970; Ivory, 1980; Marx *et al.*, 1984a) and, whether as a result of re-invasion after fumigation or natural occurrence, its presence as a potential colonist of roots must be recognized in the majority of nursery studies. Because of the ubiquitous occurrence of *T. terrestris*, experiments designed to evaluate the influence of an inoculant fungus are complicated by the fact that most of the uninoculated 'control' plants, as well as some of those in the inoculated treatments, are frequently colonized from natural sources. There may also be other 'casual' colonists, among

which E-strain fungi (see Chapter 7) and *Laccaria* species are common. Such trials are therefore comparisons of performance between *T. terrestris* (or other contaminant fungus) and the inoculated symbiont. One exception to this generalization (Xu *et al.*, 2001) is discussed below. Experience with *P. tinctorius* as the introduced organism strongly suggests that large numbers of mycorrhizas must be produced consistently on the roots of the seedlings if maximum promotion of growth is to be achieved when they are outplanted to reforestation sites. In these situations, Marx *et al.* (1976, 1988) showed that, if less than half of all mycorrhizas are formed by *P. tinctorius*, no growth promotion occurs, relative to that seen in *Thelephora*-colonized plants. Outplanting trials in several regions of the world indicate that increased growth in nurseries may not be correlated with improved performance in the field and that inoculant fungi may persist only a few years after outplanting, before being supplanted by naturally occurring fungi. Nevertheless, it is quite likely that early benefits accrue from dependence of young trees on uptake of nutrients from soil via their ECM symbionts. Later, internal recycling of nutrients within large trees plays an increasingly important role, while direct uptake declines (Grove and Le Tacon, 1993).

Various commercial inoculum formulations and inoculation techniques have been developed for use in seedling production systems (Marx and Bryan, 1975; Marx, 1980, 1991; Marx and Kenney, 1982; Sieverding, 1990; Bethlenfalvay and Linderman, 1992; Brundrett *et al.*, 1996). Spore-based inoculum, including pellets or seed coating, is appropriate and convenient for fungi that produce sporocarps with copious spores. Mycelial inoculum types include slurries and alginate bead formulations (Brundrett *et al.*, 1996). Some of the most successful have involved the growth of vegetative mycelium in vermiculite-peat mixtures moistened with liquid nutrient medium (Marx and Kenney, 1982). Vermiculite provides a well-aerated laminated substrate within which the mycelium is protected and addition of peat in different ratios enables adjustment of pH to the required range, usually 4.8 to 5.5. Nutrient solutions commonly have a C:N ratio of between 50 and 60, added in amounts sufficient to ensure that all free C is utilized by the mycorrhizal fungus in the course of its development in the medium. The presence of available C at the time of inoculation leads to competitive exclusion of the mycorrhizal fungus by saprotrophs. Details of inoculum production procedures and methods for screening isolates are available in Brundrett *et al.* (1996).

Development of inoculation procedures commercially presents a number of major challenges. One is the scale of the operation required. Even in 1985, Marx reported that 1.5 billion seedlings of *Pinus* spp. were produced per year in nurseries of the southern USA (Marx, 1985). Since then, worldwide increases in plantation forestry as well as in revegetation programmes have increased the requirement for stock of a wide range of broadleaf and conifer species. In Australia, the forestry department in the state of New South Wales alone produced 7.5 million seedlings and 2 million cuttings in 2006 (S. Sullivan, Forests New South Wales, personal communication). Problems with the use of solid substrates for inoculum production include the large space required for storage, difficulties in maintaining homogeneity of conditions within and between batches and the inability to control physicochemical conditions in the medium in the absence of water. Because of these difficulties, there have been various attempts to use liquids or gels as culture media. The main advantages of submerged, liquid culture are the homogeneity of the medium and the control which can be obtained over physical and chemical conditions. Vessels suitable for

large-scale axenic production of fungal inoculum have been developed for other purposes in the chemical and pharmaceutical industries. They are designed to facilitate careful regulation and optimization of culture conditions for particular organisms, reducing the period of culture compared with solid substrates (Le Tacon *et al.*, 1985; Boyle *et al.*, 1987). Inoculum produced in this way can be applied directly as a slurry (Boyle *et al.*, 1987; Gagnon *et al.*, 1988), requiring some form of fragmentation. Unfortunately, this treatment greatly reduces the vigour of many ECM fungi. Attempts have been made to retain viability of fragmented inoculum by incorporation in a protective carrier medium. Sodium alginate has been successfully used, either applied as a gel directly to the bare roots (Deacon and Fox, 1988), granulated (Kropacek *et al.*, 1989) or as beads (Le Tacon *et al.*, 1985; Mauperin *et al.*, 1987). The susceptibility of many fungi to fragmentation damage, even when protected in this way, has led to a search for alternative culture methods. One approach which has considerable promise involves the production of the inoculum inside hydro-gel beads, which can be applied directly, circumventing the fragmentation phase (Jeffries and Dodd, 1991; Kuek *et al.*, 1992). Several ECM fungi, including species of *Descocolea*, *Hebeloma*, *Laccaria* and *Pisolithus*, have been successfully grown as inoculum in this way and it has been shown that viability can be retained after storage for up to 7 months at low temperature (Kuek *et al.*, 1992).

Basidiospore inoculum of *P. tinctorius*, either as a suspension sprayed on the soil or encapsulated on seed with clay, has been used on an experimental basis in the USA and elsewhere. This can yield growth responses, but rarely produces as many mycorrhizas per plant as does the 'super-strain' of vegetative inoculum and so is less effective (Marx *et al.*, 1984b, 1991). A delay in production of mycorrhizas from spores might be expected because, as described in Chapter 6, colonization would not normally take place from monokaryotic mycelia. Only after hyphal fusion and the formation of dikaryons does ECM colonization occur.

Good though the responses to inoculation with *P. tinctorius* have been in warmer and more drought susceptible parts of the world, this fungus has proved less successful in cooler climates. In the Pacific north-west of the USA, for example, the 'super-strain' of *P. tinctorius* performed less well than did local isolates of the fungus (Perry *et al.*, 1987). In this region, the US Forest Service developed a spore inoculation programme based upon the use of ECM fungi known to be important in local ecosystems, including species of *Laccaria*, *Hebeloma*, *Rhizopogon* and *Suillus* (Castellano and Molina, 1989). Spores were applied to seed beds through the nursery irrigation system or to container-grown plants using mist-propagation units. Poor colonization was obtained with *Rhizopogon* and *Suillus* spp. (Perry *et al.*, 1987). In contrast, several strains of *Laccaria* produced abundant mycorrhizas in container-grown plants (Molina, 1982). One strain, subsequently referred to as *L. bicolor* S238, was found to have particular promise as an inoculant. Some *Laccaria* and *Hebeloma* strains have been developed as commercial inoculum, producing high levels of colonization on *Pseudostuga menziesii* in containers and under nursery conditions (Hung and Molina, 1986).

It appears, however, that despite success in achieving colonization by vigorous strains of fungi, outplanting performance of the seedlings improved rather little (Perry *et al.*, 1987). This was also the experience in Europe. Le Tacon *et al.* (1988, 1992) describe a number of experiments in France, Spain and Britain in which the performance of nursery inoculated plants of *P. menziesii* and *Pinus sylvestris* has been

followed for several years after outplanting. The fungi used were mostly strains of *Thelephora*, *Hebeloma* and *Laccaria*, including the vigorous Oregon strain S238 of *L. bicolor*, originally isolated by Molina. The extent of success in obtaining colonization by the inoculant fungus varied from nursery to nursery, being apparently determined largely by the rate of re-invasion and vigour of indigenous *Thelephora* strains. Even where high levels of colonization by inoculant fungi were achieved, improvements in performance of the outplanted trees were rarely observed. Inoculation of *P. menziesii* with *L. bicolor* S238 provided significant increases of height growth and a doubling of wood volume at one site in central France six years after outplanting, but at the remaining sites, differences between control plants colonized by *T. terrestris* and those that were inoculated were small. Jackson *et al.* (1995) reported a similar experience with container-grown *P. menziesii* and *Picea sitchensis* which were inoculated with a wider range of fungi and transplanted, after colonization, to six nursery sites across the UK.

A second major challenge is the selection of appropriate fungi for inoculation programmes, based on their performance as symbionts for the plant species in nursery production and on their likely survival and competitiveness at field sites. The huge diversity of fungi now shown to occur in different forest ecosystems makes this a daunting task and may well contribute to the observation that extensive work on methods of inoculum production has not always been matched by demonstration of efficacy in forest productivity. As a result, while there are theoretical studies of the economic advantages to be gained from use of the new inoculation technologies (Kuek, 1994), prospects for their extensive application are not particularly promising.

Nevertheless, a number of targeted programmes have had success, especially in reafforestation projects on degraded sites mainly in SE Asia. In China, eucalypt plantations have been established on sites that are both low in available P and lack appropriate ECM fungi; in consequence establishment of early plantations was poor. Xu *et al.* (2001) investigated the potential of both P fertilizer application and ECM inoculation in the nursery to improve growth and after outplanting. They found that application of superphosphate generally improved seedling survival and stand volume three years after outplanting. The effect of nursery inoculation with ECM fungi was also tested. In these experiments, all inoculated seedlings formed ectomycorrhizas and the non-inoculated controls remained non-mycorrhizal, so that comparisons with seedlings inoculated with several different ECM fungi could be attributed to the effects of these fungi and not to weedy fungal species. There were no differences in growth of inoculated and non-inoculated seedlings at the time of outplanting. Inoculation had variable effects on growth in plantations, depending on P application and the identity of the inoculant fungus. As shown in Figure 17.1, after three years growth in the field, seedlings of *Eucalyptus euophylla* inoculated with *Hebeloma westraliense* showed decreases in stand volume relative to controls, whereas those inoculated with either *Laccaria lateritia* or *Pisolithus albus* showed increases, at least at some levels of P application. The negative effect of *Hebeloma* was related to very poor seedling survival, possibly because of poor survival of the fungus after outplanting. The conclusions were that optimum growth of plantations could be achieved by appropriate fertilizer management, coupled with inoculation with effective ECM fungi. The results bring into focus the need for careful site evaluation with respect to nutrient status and requirement for nutrient applications, as

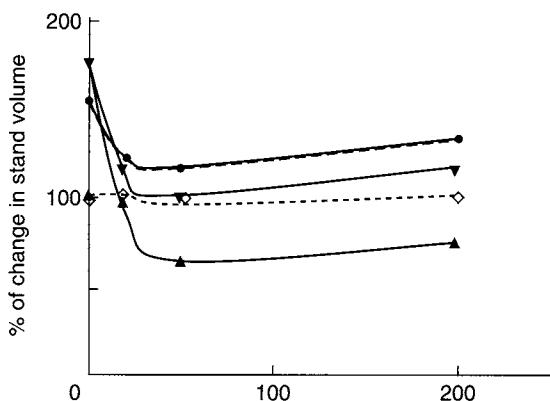


Figure 17.1 Effect of fungal inoculation in the nursery with three ectomycorrhizal fungi on stand volume of *Eucalyptus europhyllo* averaged across three years of growth after outplanting. Values are per cent stand volume in relation to uninoculated control at each level of P fertilizer application. Control, \diamond ; *Pisolithus*, ∇ ; *Laccaria*, \bullet ; *Hebeloma*, \blacktriangle . Redrawn from Xu et al. (2001).

well as for testing of inoculant fungi with respect to survival and compatibility with the tree species, as well as effects on timber production (see Chapters 9 and 10).

Unfortunately, it appears that many of those fungi selected to achieve optimal colonization in the nursery are often poor competitors in the field, especially when outplanting sites contain indigenous populations of mycorrhizal fungi. McAfee and Fortin (1986) preinoculated seedlings of *Pinus banksiana* with *L. bicolor*, *P. tinctorius* and *R. rubescens* before transplanting them to denuded, burned or natural pine stands. After two months in the natural stand, colonization by *L. laccata* and *P. tinctorius* had declined significantly, whereas that of *Rhizophagus rubescens* showed modest increase. *P. tinctorius* showed an ability to colonize new roots in a denuded site which lacked competition from an indigenous mycorrhizal population. These are the circumstances in which the greatest successes have been achieved in inoculation programmes involving this fungus. The failure of *L. bicolor* to compete with indigenous fungi is in line with the observation of Bledsoe et al. (1982) that the closely related *L. laccata* failed to persist on seedlings of *P. menziesii* when challenged by native fungi on outplanting sites in Washington.

There are a number of possible explanations for the common failure of inoculation to produce beneficial effects at outplanting sites. Probably among the most important of these is the inability of introduced inoculum to persist on the roots of planting stock after transfer from the nursery to the field, as shown for *Hebeloma* in the study outlined above. In addition to the fact that soil conditions experienced by nursery and container-grown plants are very different from those in most outplanting sites, the lifting, storage and transport of seedlings, especially those raised in bare-root nurseries, can be expected to reduce the vigour of fine roots and their fungal associates. These treatments are likely to favour replacement of introduced fungi by those resident in soil of the replant site. It is noteworthy in this context that the most strongly beneficial effects of inoculation have been observed where plants are transferred to disturbed or treeless sites in which inoculum potential of any indigenous fungi is likely to be low. Here, in contrast to the situation so often reported in

Table 17.2 Per cent increase in survival and volume growth of pine seedlings after 2 to 4 years with *Pisolithus tinctorius* ectomycorrhizas over controls with naturally occurring ectomycorrhizas on various adverse sites.

Pinus species	Site	Adversity	% Increase in seedling	
			Survival	Volume
<i>P. resinosa</i>	Coal spoil	pH 3.0	214	60
<i>P. echinata</i>	Coal spoil	pH 4.1	5	400
<i>P. virginiana</i>	Coal spoil	pH 3.1	87	444
<i>P. virginiana</i>	Coal spoil	pH 3.8	480	422
<i>P. rigidae</i>	Coal spoil	pH 3.8	0	420
<i>P. rigida</i>	Coal spoil	pH 3.4	57	215
<i>P. rigida</i>	Coal spoil	pH 4.3	8	180
<i>P. taeda</i>	Coal spoil	pH 3.3	20	415
<i>P. taeda</i>	Coal spoil	pH 3.4	14	750
<i>P. taeda</i>	Coal spoil	pH 4.1	41	400
<i>P. taeda</i>	Coal spoil	pH 3.4	96	800
<i>P. taeda</i>	Coal spoil	pH 4.3	16	380
<i>P. taeda</i>	Kaolin spoil	Low fertility	0	1100
<i>P. taeda</i>	Fullers' earth	Low fertility	0	47
<i>P. taeda</i>	Copper basin	Eroded	0	45
<i>P. virginiana</i>	Copper basin	Eroded	0	88
<i>P. taeda</i>	Borrow pit	Droughty	17	412

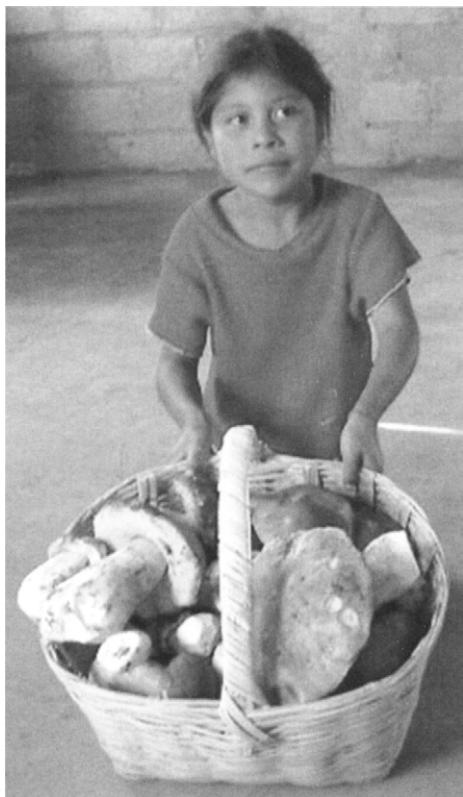
From Marx, 1975; Marx et al., 1989.

soils with a pre-existing vegetation cover, responses to inoculation can be quite dramatic (Table 17.2) involving improvements in survival as well as increases in yield (Marx, 1991) and they appear to be most marked where the soil is contaminated with metal ions. In this context, the ability of ectomycorrhizal *Betula* spp. to spontaneously colonize mine spoils is widely recognized.

The natural formation of ectomycorrhizas in nurseries means that some forest production systems do not consider inoculation necessary or worthwhile. Furthermore, in some parts of the world, forest regeneration following clear cut is allowed to occur naturally, sometimes after site preparation that may disturb the soil or remove the organic horizons. There has been considerable interest in the effects of such practices on the composition and diversity of assemblages of ECM fungi and the possible impacts of any changes on the ECM-development on and performance of the regenerating seedlings. Jones *et al.* (2003) classify changes into those which result in shifts in the amount or type of inoculum and those which are related to changes in the soil environment. They come to the conclusions that the second group of changes are at least as important as the first and, consequently, that the fungi that do colonize the seedlings are likely to be better adapted to cope with the changed environmental conditions. They do, however, stress that data for diversity are at present based on taxonomic and genetic criteria and that future work on changes in community composition and effects on tree growth must be based on physiological and ecological attributes. In any event, the conclusions are important in informing policy on the way clear-cut sites are prepared and managed for maximum future productivity.

Edible mycorrhizal fungi

While most of the emphasis in applied research on ectomycorrhizas has concentrated on improvement of tree production, there is an increasing awareness of the uses to which fruit bodies of ECM fungi are put as food and medicines. In many countries, fungi, many of them ectomycorrhizal, are gathered in the wild and used directly as food and also to provide an important source of income (Figure 17.2a, b; Colour Plate 17.1). Interestingly, the subsistence uses of edible fungi in general have often been ignored in the context of development projects, probably because harvesting has largely been perceived as for personal use (Boa, 2004). In Mexico, unofficial estimates suggest that hundreds of tonnes of fresh mushrooms are sold annually in markets and that, in rural areas with a traditional knowledge of wild mushrooms, as much as 100% of family income comes from mushroom sales in the season (Jesus Perez-Moreno, personal communication). This contrasts with strong interest in development of industries based on the luxury trade in edible ECM fungi, such as truffles (*Tuber* spp.) and matsutake (*Tricholoma matsutake*), as well as species with perceived medicinal potential



(a)



(b)



(c)

Figure 17.2 Edible mushroom production in Mexico. (a) Girl selling *Boletus edulis* in a market. (b) and (c) Baskets of ECM fungi of which the main ones are: *Gomphus flocculosus*, *Lactarius salmonicolor*, *Helvella crispa* and *Amanita aspera* var *franchetii*. Photographs courtesy Jesus Perez-Moreno. See also Colour Plate 17.1.

(Hall and Wang, 1998; Boa, 2004). Harvesting highly sought-after species in the wild can add substantially to the income of rural communities, such that 60% of porcini (*Boletus edulis*) imported into Italy come from China. Exports of matsutake from China, Bhutan, Korea and both USA and Canada help to supply the huge demand for this delicacy in Japan, where availability in the forests where it was traditionally gathered has declined substantially. Such is the demand for and potential contribution of ECM fruit bodies that there are serious dangers that environmental damage will result from harvesting activities. Furthermore, in some places, loss of native woodlands and replacement with plantation crops (such as introduced eucalypts colonized by non-edible ECM fungi) has reduced the production and ease of harvesting of traditional fungal crops, with impact on traditional gatherers (Boa, 2004).

The possibilities of exploiting the commercial value of the fruit bodies produced by ECM fungi, particularly truffles, is being explored in a number of countries. At present, a small number of mycorrhizal species are particularly prized for their gastronomic quality and are hence of very high value (Table 17.3). In 2005, the price for black truffles (*Tuber melanosporum*) reached £1100 per kg. Edible fungi of this type are still mostly collected from natural stands and make only a small contribution (~4%) of the total global production of edible fungi (Figure 17.3), most of which are saprotrophs grown relatively easily under controlled conditions. Production of ECM fruit bodies has apparently declined in native ecosystems for a range of social and environmental reasons (Wang and Hall, 2004). In order to increase supply of ECM fruit bodies, the current demands for which far outstrip supply, three approaches are being adopted: extension of harvesting from the wild to non-traditional regions (see above), attempts to develop management strategies that will enhance or restore production in traditional harvesting areas, and development of new plantations of tree species, specifically to produce fruit bodies rather than timber. Numerous

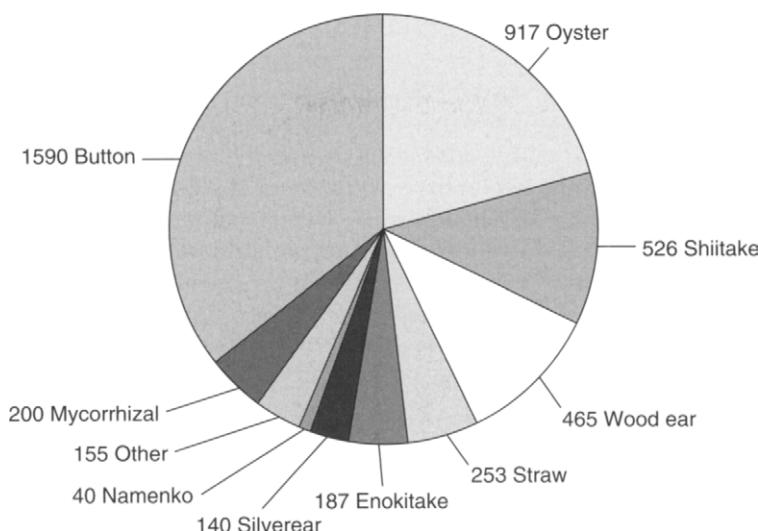


Figure 17.3 The contribution of mycorrhizal fungi to the approximate world production of edible mushrooms in 1991; Values are tonnes \times 1000. From Hall et al. (1994), with permission.

Table 17.3 Prices of selected edible fungi for the luxury market.

Common name	Latin binomial	How sold	£ per kg	USD per lb
Black truffle	<i>Tuber melanosporum</i>	Fresh	425	900
Bianchetto truffles	<i>Tuber albidum</i>	Fresh	700	
	<i>T. magnatum</i>	Fresh	1700	
Summer truffles	<i>T. aestivum</i>	Fresh	105	
Porcini	<i>Boletus edulis</i>	Sliced and dried	95	
Morel	<i>Morcella elata</i>	Sliced and shredded	255	
Chanterelle	<i>Cantharellus cibarius</i>		15	
Matsutake	<i>Tricholoma matsutaki</i>	Dried		76

Source: various web pages, August 2006.

commercial organizations are involved in planting trees which have been pre-colonized by inoculation with appropriate fungi. Particular emphasis has been placed on truffles (*Tuber* spp.) because of their extremely high economic value, the most important of these being the black truffle, *T. melanosporum*. Indeed, according to Wang and Hall (2004), this species, together with *T. uncinatum*, is among the few to have been successfully cultivated commercially. Others include desert truffles, *Lactarius deliciosus* and the tubers of the mycoheterotrophic orchid *Gastrodia elata* (Chapter 13).

Techniques for the germination of the ascospores of *T. melanosporum* and for the aseptic production of mycorrhizas by a number of *Tuber* spp. pioneered in France (Grente *et al.*, 1972; Chevalier and Desmas, 1975; Chevalier and Grente, 1978) and Italy (Palenzona, 1969; Fontana and Bonfante-Fasolo, 1971) are widely used and remain the most popular (Wang and Hall, 2004). *T. melanosporum* has a broad host range and can be successfully grown on calcareous soils with the hardwood genera *Corylus*, *Quercus*, *Carpinus* and *Castanea*, as well as softwoods such as *Pinus*. Commercial production of colonized seedlings, particularly of *Quercus* and *Corylus*, now takes place in a number of centres in both the northern and southern hemispheres. In France alone, about 160000 plants colonized by *T. melanosporum* were earlier reported to be produced annually, some being exported to the USA (Hall *et al.*, 1994).

Truffières have been established, usually as mixed plantings of *Quercus* and *Corylus* on potentially favourable sites, often at some distance from any other ECM communities to reduce competition between pre-existing and introduced fungi. The possibility of replacement of the truffle fungi with 'weedy' ECM fungal species has given some impetus for the development of molecular markers to monitor establishment and maintenance of truffle fungi and their competitors in plant roots. Many truffières were developed with *T. melanosporum* in the USA in the 1980s and began producing fruit bodies around 8–10 years later. Truffle production was commenced somewhat later in New Zealand in a programme pioneered by Hall, involving the introduction of both fungus and plant as exotic species. These truffières again began production after around 5 or more years, with the successful sites being in the warmer regions north of Christchurch. A second southern hemisphere initiative in Tasmania, Australia, is apparently adopting similar strategies and the establishment of truffières is extending into southern parts of the mainland of Australia, with about 90 or more hectares planted altogether (Wang and Hall, 2004). These ventures aim to capture the benefits of production in the southern hemisphere for sale when traditional supplies from the northern hemisphere are seasonally unavailable.

Commercial truffle production appears set to expand to appropriate sites, with ventures reported from Israel and several Asian countries including Taiwan.

Efforts have been made for some time to establish commercially productive systems for other valuable edible fungi such as *Boletus edulis*, *Cantharellus cibarius*, *Tricholoma matsutake* and *Tuber magnatum*. Seedlings have been successfully colonized under sterile laboratory conditions, but no success in the field has been reported (Wang and Hall, 2004). Despite advances in science and technology, which provide the prospect of large-scale production of a number of edible ECM fungi, the commercial success of the ventures is not yet fully assured. To a large extent the value of the commodities (especially of truffles) is based upon their limited availability, so that prices will certainly drop if large-scale production is achieved. However, especially in crops that can be used for timber, harvesting of edible fruit bodies could be an additional source of revenue or food, especially in developing countries. The large-scale establishment of eucalypt plantations in China, using planting stock pre-inoculated with edible fungi has considerable potential to provide an important dietary supplement (B. Dell and N. Malajczuk, personal communication).

In addition to fruit bodies, symbiotic mycorrhizal orchid tubers have been used in traditional medicine for over 2000 years (Xu and Mu, 1990; Kim and Ko, 1995; Xu and Guo, 2000). These practices continue to the present, so that there is an increasing demand which may threaten natural populations of some species. In order to maintain supplies, considerable research has been directed to the propagation of *Gastrodia elata*, which has as its fungal symbionts *Mycena osmundicola* in early protocorm stages, followed by *Armillaria mellea*. The latter fungus supports growth of the orchid with organic C derived saprotrophically from large logs of wood. This forms the basis for the current production systems (see Chapter 13).

Conclusions

Biological activities in soil are widely recognized as playing a vital part in nutrient cycling and in developing and maintaining soil structure and contributing to 'soil health'. Sustainable land-use, either in highly developed or subsistence economies requires that soil degradation ceases and that soil management practices are adopted to conserve and augment soil resources. Mycorrhizal fungi comprise just one of the functional groups of organisms that are important in soil ecosystems, but their position in forming direct links between roots of plants and the soil fabric means that they play key roles in soil-plant interactions, particularly in nutrient uptake and deposition of organic C. This applies as much to crops as it does to plants in undisturbed ecosystems and the difficulties in experimental demonstration of 'benefits' of mycorrhizas in the field are very similar. In both situations it is clear that mycorrhizas influence nutrient uptake, but benefits of crop inoculation will always depend on the resident mycorrhizal microflora and soil nutrient status.

Enormous efforts have been made to harness the potential benefits of mycorrhizal symbioses in commercial production systems, whether in horticulture, agriculture or forestry. The effects of mycorrhizal associations on production are almost all potentially beneficial, with only a few reports of growth depressions in agricultural field situations that remain imperfectly explained. There is an increasing awareness that mycorrhizal symbionts contribute to nutrient uptake in all types of crops, even

when direct benefits of inoculation or management are difficult to demonstrate in field situations. Mycorrhizal inocula have been successfully developed for both AM and ECM fungi. Direct inoculation of AM fungi is currently limited to relatively small-scale, high value systems or subsistence farming. This picture may change when inoculum can be readily produced in a form that is convenient for wider application and contains fungi that are both effective and persist in the highly modified horticultural or agricultural environments. Similarly, in forestry it has often been the experience, particularly in sites with a well-established resident flora of ECM fungi, that inoculant species have had little impact. It thus appears that natural selection, operating over many generations, has produced stable populations of symbionts that are resistant to invasion by alien organisms. The assemblages of fungi may be modified by the agricultural and forestry practices applied, but are, nevertheless, adapted to the production systems in which they are now found. This may, in fact, be a desirable feature otherwise inoculant fungi, artificially selected for their vegetative vigour and now introduced with little legislative restriction to so many parts of the world, would come to dominate natural ecosystems. The consequences of the loss of biodiversity which would result from such invasion cannot as yet be quantified but, clearly, until such time as the roles of an extensive gene pool in sustaining ecosystems is evaluated, it would be prudent to protect and return as much diversity as possible.

It is therefore important to view mycorrhizal associations as integral components of the complex soil ecosystems and to manage those systems in order to maximize the contributions that mycorrhizas most certainly make to soil processes and growth of plants. Research directed towards understanding the activities of mycorrhizal fungi in production systems is valuable both in determining appropriate management strategies and as a background against which effective inoculation techniques may be developed in future. There remains enormous scope for investigation of both fundamental and applied aspects of mycorrhizal symbioses. These studies, if pursued at the cellular, whole plant and community levels, will enrich our understanding of the role of mycorrhizas in extant ecosystems both natural and man made. The need for reliable data on the impacts of disturbance, fragmentation of natural ecosystems and climate change introduces an element of urgency to the quest for knowledge.

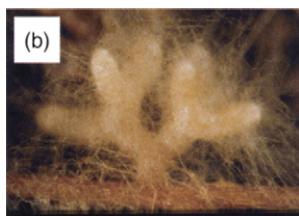


Plate 16.1 Roots of *Pinus sylvestris* used in studies of the protozoan populations associated with (a) non-mycorrhizal short roots (NM), (b) ectomycorrhizal short roots (MR) and (c) extraradical mycelium of *Paxillus involutus* (see Figure 16.11).



Plate 17.1 Edible mushroom production in Mexico. (a) Girl selling *Boletus edulis* in a market. (b) and (c) Baskets of ECM fungi of which the main ones are: *Gomphus flocculosus*, *Lactarius salmonicolor*, *Helvella crispa* and *Amanita aspera* var *franchetii*. Photographs courtesy Jesus Perez-Moreno.