Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms?[1]

Melanie D. Jones and Sally E. Smith

Abstract: Mycorrhizas are considered to be classic mutualisms. Here, we define mutualism as a reciprocal increase in fitness of the symbionts, and we review the evidence for mycorrhizal mutualism at the community, whole-plant, and cellular scales. It is difficult to use results of most mycorrhizal studies because (*i*) fungal contribution to nutrient uptake is not accurately estimated, (*ii*) increased growth is not necessarily correlated with increased plant fecundity or survival, especially in communities, and (*iii*) benefits that occur only at certain times of year, or under specific extreme conditions, may not be detected. To produce the nonmycorrhizal controls required to study mutualism in the field, soil microflora and fauna must be severely perturbed; therefore, it is virtually impossible to evaluate effects of mycorrhizas on plant fitness under realistic conditions. Using the evidence available, we conclude that mycorrhizas can occupy various positions along the continuum from parasitism to mutualism, depending on the specific plant and fungal genotypes and their abiotic and biotic environments. Although we discuss the possibility of defining mycorrhizas by some physiological characteristic, we conclude that mycorrhizas should be defined on a structural or developmental basis and that any requirement to demonstrate mutualism be eliminated.

Key words: mycorrhiza, mutualism, parasitism, physiology, fitness, community.

Résumé : On considère généralement les mycorhizes comme un cas de mutualisme classique. Les auteures décrivent le mutualisme comme une augmentation réciproque de l'adaptation des symbiotes, et elles revoient les preuves de mutualisme mycorhizien à l'échelle de la communauté, de la plante entière et de la cellule. Il est difficile d'utiliser les résultats de la plupart des études, parce que (*i*) la contribution du champignon dans l'apport de nutriments n'est pas suffisamment documentée, (*ii*) une croissance accrue n'est pas nécessairement corrélée avec une augmentation de la fécondité ou de la survie, surtout dans les communautés et (*iii*) les bénéfices qui peuvent survenir seulement à certains moments de l'année, ou sous certaines conditions extrêmes, peuvent ne pas être décelés. Afin de produire les témoins non mycorhiziens requis dans les études sur le mutualisme, conduites aux champs, la microflore et la microfaune du sol doivent être profondément perturbées, et il est conséquemment impossible d'évaluer les effets des mycorhizes sur l'adaptation de la plante sous des conditions réalistes. En utilisant les preuves disponibles, les auteurs concluent que les mycorhizes peuvent occuper diverses positions le long du continuum allant du parasitisme au mutualisme, selon les génotypes spécifiques des plantes et des champignons et selon leurs environnements abiotiques et biotiques. Bien que les auteures discutent la possibilité de définir les mycorhizes par certaines caractéristiques physiologiques, elles concluent que les mycorhizes devraient être décrites sur la base des structures et de leur développement, et que toute obligation d'en démontrer le mutualisme devrait être éliminée.

Mots clés : mycorhizes, mutualisme, parasitisme, physiologie, adaptation, communauté.

[Traduit par la Rédaction]

Introduction

Mycorrhizas are considered to be classic examples of mutualistic symbioses. The mutualism is attributed primarily to carbon (C) gain by the fungus from the plant, with reciprocal nutrient transfer from fungus to plant, although improved water relations and pathogen tolerance can also contribute to plant fitness (Smith and Read 1997). Mycorrhiza formation appears to be obligate for vegetative growth and sporulation of arbuscular mycorrhizal (AM) fungi and for fruit-body production by ectomycorrhizal (EcM) fungi, leading to the assumption that, for these mycorrhizal types, fungal fitness is completely reliant on C supplied by autotrophic plants. This relationship is less clear for ericoid mycorrhizas and appears not to be the case at all for fungi associating with orchids and other myco-heterotrophs.

For plants, benefits of the symbioses are much less clear and, furthermore, should be considered at different scales,

Received 31 October 2003. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 20 August 2004.

M.D. Jones.[2] Biology Department, Okanagan University College, 3333 University Way, Kelowna, BC V1V 1V7, Canada.
S.E. Smith. Centre for Plant Root Symbioses, Soil and Land Systems, School of Earth and Environmental Sciences, Waite Campus, The University of Adelaide, South Australia, 5005 Australia.

[1]This article is one of a selection of papers published in the Special Issue on Mycorrhizae and was presented at the Fourth International Conference on Mycorrhizae.

[2]Corresponding author (e-mail: mjones@ouc.bc.ca).

 doi: 10.1139/B04-110

from community to cellular levels. Community interactions are as yet little understood, particularly with respect to cost–benefit considerations. So far, the findings vary, with activity and (or) diversity of AM fungi sometimes associated with an increased diversity and (or) productivity of reconstructed plant communities and sometimes not (see Hartnett and Wilson 2002; Urcelay and Diaz 2003). At the whole-plant level some plants are considered to be obligately mycorrhizal in nature, whereas others are facultatively so and others constitutively nonmycorrhizal (NM; see Molina et al. 1992). The literature is replete with reports of increased growth of single plants associated with inoculation by mycorrhizal fungi in pot experiments. Increased uptake of N, P, Ca, Mg, Zn, Cu, Fe, and other nutrients has been detected, with the most important being P and Zn for AM plants and with the addition of N in particular for EcM and ericoid mycorrhizal (ErM) species. Reports of decreased growth and nutrient uptake are less frequent and have been largely ignored (or even suppressed), although some authors acknowledge that, functionally, mycorrhizal associations may range from parasitism to mutualism and that symbiotic outcomes are likely to vary with environmental conditions (Smith 1980; Smith and Smith 1996; Johnson et al. 1997; Zhou and Sharik 1997). At the cellular level, there is evidence that nutrients can be absorbed and translocated by the fungi to the plants (Melin et al. 1958; Jakobsen et al. 1992; Bending and Read 1995; Janos et al. 2001; Hodge 2003; Smith et al. 2003*b*) and (particularly in arbuscular mycorrhizas) that cellular processes, including gene expression and localization of transport-related proteins, may be markedly altered during colonization and establishment of symbiotic interfaces (Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002).

More attention is now being paid to studying the developmental and functional diversity of interactions between symbionts in mycorrhizal associations (van der Heijden et al. 1998*a*; Bever et al. 2002; Hart and Klironomos 2002; Peterson and Massicotte 2004). It has long been recognized that there are thousands of EcM fungi, with a range of physiological attributes and host specificities (Molina et al. 1992). However, the possibility of greater fungus–plant specificity and genetic as well as physiological variability in AM interactions has been recognized only recently (e.g., Helgason et al. 2002; Sanders 2002). We acknowledge that there are a great many plant genotype – fungal genotype combinations for the major types of mycorrhizas and that we should therefore expect a range of interactions beyond mutualism, including parasitism and cheating. The accompanying paper by Egger and Hibbett (2004) discusses evolutionary aspects of this range of interactions. Increased attention to the diversity of responses is crucial if we are to understand how the various symbioses function, especially in communities.

The debate about where mycorrhizas fall along the continuum of symbioses as originally described by de Bary (1887) has been going on since mycorrhizas were first described. According to Melin (1925, as translated by Stickel 1930), a common view in the 1880s and 1890s was that they were "harmful to the higher plants under all conditions". Scientists such as Henschel had reported a negative correlation between the vigour of *Picea excelsa* and the extent of colonization (Henschel 1887, as cited by Harley 1984). However, it is clear that Frank, the man who first coined the term "mycorrhiza", viewed the symbiosis as beneficial to both plant and fungus (Frank 1885, as translated by Trappe 1984). He describes the fungi as "parasitic" because he hypothesized that they received C from the tree, but he also hypothesized that the "mycelium performs a wetnurse function and takes over the entire nourishing of the tree from the soil". He based this view on observations that mycorrhizal fine roots were healthy, but totally enclosed by the fungi and, therefore, not directly in contact with the soil. Nevertheless, Melin (1925) reported situations in pure culture where colonization by EcM fungi had detrimental effects on seedlings. He concluded that this was most likely to occur under conditions where growth of the seedlings was "weak" but where growth of hyphae was "strong". Although he had not observed any such situations in nature, he expected that they occurred.

Through the early decades of the 20th century positive correlations between the presence or abundance of ectomycorrhizas and the vigour of conifer trees were increasingly reported. Melin observed this in trees growing in bogs (Melin 1917, as cited by Harley 1984). Rayner (1938) reported that low vigour of conifer seedlings had been observed in exotic locations throughout the British Commonwealth and that this could be relieved by the addition of raw humus, with the resulting formation of ectomycorrhizas. Similar problems had occurred in some conifer nurseries in the United States. In these cases, any groups of vigorous seedlings were typically mycorrhizal, while the remainder of the seedlings was generally NM (Mitchell et al. 1937). The evidence for the importance of ectomycorrhizas was only circumstantial until Hatch (1937) observed positive responses of conifer seedlings to inoculation with pure cultures of EcM fungi. With the exception of Burges, who described both EcM and "endophytic" mycorrhizal fungi as "potential parasites" (Burges 1936) and felt that there was insufficient evidence to conclude that they decomposed soil organic matter and thereby released nutrients for plants, by the late 1930s most people studying mycorrhizas had become convinced that "in soils of moderate nutrient deficiency mycorrhizal seedlings grew faster in height and weight" (Harley 1984).

By the 1990s the idea that mycorrhizas were mutualisms was accepted implicitly by most researchers in the mycorrhiza field (Trappe 1994), and mutualism was considered "a critical character" of a mycorrhiza (Allen 1991). Mycorrhizas came to be labelled as classic cases of mutualism in general biology textbooks. There were only rare papers that discussed other possibilities. For example, when Garrett (1960) classified root-inhabiting fungi into three groups, "primitive soil-inhabiting pathogens, specialized pathogenic root-inhabiting fungi, and symbiotic fungi", he stated that "further research may demonstrate a continuous gradation of behaviour rather than sharp boundary lines between these three groups". Lewis (1973) pointed out that the more narrow view of mycorrhizal functioning might have been due, in part, to inconsistent use of the term "symbiosis". This term had originally been broadly defined by de Bary (1887) but had come to be used synonymously with "mutualism", especially amongst botanists. Smith and Read (1997) continued to emphasize the place of mycorrhizas along a contin-

uum of responses: "Most mycorrhizal symbioses are now clearly recognized…as being common and significant representatives of the mutualistic end of the symbiotic spectrum…However,…not all…mycorrhizas have been shown to be mutualistic by experimental analysis of nutritional interactions or fitness". In their evaluation of where mycorrhizas fit along this continuum Johnson et al. (1997) concluded that mycorrhizal associations are "generally mutualistic, with occasionally commensal and parasitic excursions from this norm".

To evaluate whether all mycorrhizas should be classified as mutualisms, we must be clear in our terminology. We will consider a mutualism to be "a positive reciprocal relationship between two individuals of different species which results in increased fitness for both parties" (Mackenzie et al. 1998). This is consistent with the view of Stanier et al. (1971) that whether a symbiosis is mutualistic must be "evaluated by comparing the fitness of the two members when living independently with their fitness when living in association". We will consider "fitness" to refer to the "contribution of an individual to future generations, relative to other members of the same species growing in the same environment" (Krebs 2001). Flower and pollen production, seed production, survival and growth of offspring, as well as measures of vegetative reproduction such as stolon branching, have been used as indicators of fitness in studies comparing mycorrhizal and NM plants; (Bryla and Koide 1990; Carey et al. 1992; Merryweather and Fitter 1995*b*; Shumway and Koide 1995; Streitwolf-Engel et al. 1997; Philip et al. 2001).

In this paper we will discuss different approaches to determining benefit to plant and fungal components of the symbioses and, thus, of determining whether a specific plant–fungus association is mutualistic. We highlight recent results that demonstrate the variability in functional responses of plants to mycorrhiza formation. Much of the discussion is necessarily at the level of single plants in symbiosis with single fungal species or mixed soil communities, but we also address issues of defining mutualism at cellular and community levels. This paper builds on excellent previous reviews on this topic by Smith and Smith (1996), Johnson et al. (1997), and Smith et al. (2003*a*) and will focus on insights provided by recently generated data. Finally, we consider whether it is feasible or even productive to try to define mycorrhizas on a functional basis, given the diversity of outcomes. During this discussion, it is important to remember three points. Firstly, demonstration that a mycorrhizal fungus is capable of utilizing organic C from soil when grown in axenic culture does not mean that this is the main source of C when in symbiosis; it may mean simply that the fungus is a facultative symbiont. Secondly, benefit to either symbiont must be considered to be the net impact of the benefit and cost of the association (Koide and Elliott 1989; Tinker et al. 1994; Smith and Smith 1996). Thirdly, what constitutes a cost and a benefit must be carefully evaluated. As stated by Johnson et al. (1997): "Carbon allocated to a fungus is only a cost [to the plant] if it could otherwise have been allocated to increase plant fitness, and resources gained through the activities of a fungal symbiont are only beneficial if those resources are in limiting supply". Similar statements could be made for the fungi.

Do all mycorrhizal fungi benefit from symbiosis?

The benefit of the symbiosis to mycorrhizal fungi, with the exception of those associated with myco-heterotrophic plants, is generally not questioned. Arbuscular mycorrhizal fungi, in particular, appear to be obligate symbionts. Germ tubes extend only a short distance from spores in the absence of the plant (Bécard and Piché 1989), and AM fungi have been grown in vitro only in root-organ culture (Pfeffer et al. 1999; Fortin et al. 2002). The finding that spore production by AM fungi varies with species of phytobiont is further evidence that fitness of AM fungi is heavily influenced by the plant partner (Bever et al. 2002 and references therein). Although some EcM fungi may be able to grow vegetatively in the absence of a symbiotic partner, they produce mycelium at a faster rate when in symbiosis (Melin 1925; Erland et al. 1990). Furthermore, many mycorrhizal macrofungi appear unable to produce fruiting bodies without being connected to a photosynthetically active plant (Romell 1938; Last et al. 1979; Lamhamedi et al. 1994). All of the above evidence supports the conclusion that EcM and AM fungi rely on an active association with a plant to grow and (or) reproduce and, thus, benefit to them from the symbiosis is without question.

There are two groups of mycorrhizal fungi where total dependence on the plant is not so well established. Ericoid mycorrhizas are formed on autotrophic plants in the Ericaceae and Epacridaceae by a diverse, poorly characterized group of fungi (Perotto et al. 1996; Berch et al. 2002), and therefore little is known about their absolute reliance on plant partners for fixed carbon. Pearson and Read (1973) and Stribley and Read (1974) demonstrated that ErM fungi receive photosynthate from their plant symbionts by detecting ^{14}C, from $^{14}CO_2$ supplied to *Vaccinium macrocarpon* or *Calluna vulgaris*, in the hyphae of mycorrhizal plants. The extent to which ErM fungi also survive and reproduce as active saprophytes is still under investigation. In a recent study, *Oidiodendron maius*, one of the most common ErM fungi isolated from roots, sporulated in 99.6% of 288 natural peat samples placed in moist chambers without plant symbionts (Rice and Currah 2002). This indicates both that *O. maius* is widely distributed in the soils of Canadian boreal peatland systems and that it can reproduce when growing saprotrophically. It is quite possible that other ErM fungi will be widely detected in the free-living state if appropriate sampling techniques are used (*O. maius* was detected at very low frequencies in peat using traditional isolation media). However, a fungus may be able to complete its life cycle as a saprotroph and yet still undergo an increase in fitness when symbiotic. A saving in metabolic energy would be expected if C is received in the form of simple sugars from the plant rather than from complex soil organic compounds. The extra energy available to the fungus could then be used in reproduction. Soil organic matter is not only a source of C, however, but also of nutrients such as N, P, and S. This is especially true in peaty soils, where most ErM plants of the Northern Hemisphere are found (Abuarghub and Read 1988*a*, 1988*b*). Therefore, ErM fungi continue to secrete degradative enzymes, even in symbiosis (Bajwa and Read 1985; Bajwa et al. 1985; Leake and Read 1989), to release

soluble forms of N and P. The necessity for enzyme secretion would reduce the benefit of the association to the fungus. Because the C/N ratio of peats is higher than that of fungal tissue (Brady 1974; Killham 1994), C in excess of fungal requirements would be released during the mineralization of P and N. Under such conditions, the plant might, therefore, be considered to be parasitic on its mycobiont.

It has long been appreciated that orchid mycorrhizas (and other mycorrhizas formed by myco-heterotrophic plants (see Leake 1994; Taylor et al. 2002; Peterson and Massicotte 2004) are physiologically distinct from those mycorrhizas in which the plant partner is autotrophic. Seeds of these plants are often extremely small and all pass through a stage of dependence on an outside source of organic C, and probably also mineral nutrients such as P, supplied by their fungal symbionts (Smith and Read 1997). This continues in adult achlorophyllous species, with the fixed C sometimes originating from soil organic matter, delivered by a saprophytic symbiont such as a *Rhizoctonia* spp. or from an EcM or AM plant that is connected to the myco-heterotroph via a mycorrhizal fungus (McKendrick et al. 2000; Bidartondo et al. 2002; Taylor et al. 2002). In adult photosynthetic orchids, it is still unclear whether the fungi receive any fixed C from the plant (Alexander and Hadley 1985; Leake 1994; Smith and Read 1997). Thus, mycorrhizas formed in heterotrophic plants are clearly different from others, and at this stage is seems highly unlikely that the symbioses are mutualistic; rather, the plants appear to be parasitic on the fungi, at least for part of their life histories (Smith and Read 1997; Gardes 2002; Taylor et al. 2002). Because the physiology of these associations has been studied much less than other types of mycorrhizas, and because they appear to function in a very different way, we will not discuss them further in this paper.

Do mycorrhizal plants always benefit from the symbiosis?

Benefits to plants from mycorrhizal associations can accrue from a number of factors: increased nutrient supply, tolerance to pathogens, improved water relations or drought tolerance, reduced uptake of heavy metals, and effects on shoot or root architecture that can influence nutrient uptake or vegetative reproduction (Newsham et al. 1995; Grange et al. 1997; Smith and Read 1997; Streitwolf-Engel et al. 1997). However, it is not always easy to determine the extent of benefit or the appropriate criteria to use to evaluate it, particularly as benefit can be investigated at a number of temporal and spatial scales, from the cellular to whole-plant to population or community levels and at different stages of plant ontogeny or community development. Although ecologists and evolutionary biologists consider the ultimate measure of benefit to be an increase in fitness (see Introduction), the effect of mycorrhiza formation on the relative contribution of a plant to the gene pool in the next generation is not easy to measure, even in annual plants. When it has been done, aspects of flower and seed production (fecundity by individual plants), as well as the growth of plants that develop from these seeds, have been the most common variables measured (Bryla and Koide 1990; Koide and Lu 1992; Carey et al. 1992; Newsham et al. 1994; Heppell et al. 1998; Philip et al. 2001), although aspects of vegetative reproduction have also been considered (Strietwolf-Engel et al. 1997). In long-lived plants, comparing the reproductive output of mycorrhizal and NM plants is essentially impossible. Such species may require a decade or more to become reproductively mature, and it would be an enormous logistical challenge to keep a plant growing in an NM state for such a period. For this reason, in mycorrhizal investigations benefit has been quantified most commonly by comparing nutrient accumulation or biomass of mycorrhizal and NM plants under different environmental conditions, usually related to nutrient supply. This may be a reasonable approach for herbaceous crop plants or ruderal plant species, if seed production is correlated with shoot biomass, but it is not necessarily appropriate for plants growing in undisturbed, natural environments or for long-lived perennials (Johnson et al. 1997). For plant species that take many years to reach sexual maturity, lifetime fitness may be determined more by the ability to survive through occasional environmental extremes, such as nutrient or water limitation, than by rapid growth rates during more common, moderate conditions (Grime 1979; Chapin 1980). If mycorrhiza formation increases tolerance to extreme conditions and these occur infrequently or heterogeneously, there may be a temporal separation of costs and benefits to the plant. In other words, there may be a net benefit to a plant during one year, or one time of year, and a net cost at another time. Finally, benefit may depend on the biological context in which the plant is growing, including plant and fungal assemblages, density, and so on. We will discuss these complicating factors in the following sections, but first, we will review the range of responses to mycorrhiza formation, both in terms of nutrient uptake and growth.

Are mycorrhizal fungi always involved in plant nutrient acquisition?

Increased growth rates in mycorrhizal plants have most often been attributed to increased nutrient uptake. For example, careful pot studies have demonstrated that positive plant growth responses to colonization by AM or EcM fungi under P-limiting conditions can be directly linked to increased P inflow (P uptake per unit length; note that this can be calculated either for the entire root system or for the mycorrhizal fungus only, but see following text for comment on the latter approach) (Stribley et al. 1980*b*; Smith 1982; Bougher et al. 1990; Jones et al. 1990). Mycorrhizas appear to increase nutrient acquisition, especially for elements that are immobile in soils, either because inorganic ions diffuse slowly (such as P, Cu, Zn, and Ca) or when (like both N and P) they are present in complex organic forms (Melin 1925; Abuzinadah and Read 1989*a*, 1989*b*; Tinker and Nye 2000). Mycorrhizal stimulation of nutrient uptake is attributed to (*i*) uptake by fungi from soil beyond the depletion zones that can develop around roots, (*ii*) production of degradative extracellular enzymes or organic acids by the fungi, and (or) (*iii*) the ability of fungi to translocate nutrients faster than they could diffuse through soil (Bolan et al. 1983; Smith and Read 1997; Wallander 2000; Simard et al. 2002).

In ectomycorrhizas the fungus must influence uptake, because the mantle completely surrounds absorbing rootlets and nutrients can only reach the root cells via the fungus. Whether this movement is apoplastic or symplastic (as

Table 1. Accumulation of ^{15}N over 24 or 72 h by *Picea engelmannii* seedlings from ^{15}N-nitrate injected into soil at a subalpine site.

Dominant type of mycorrhiza	Dry mass of shoot + roots (mg)	^{15}N concentration in tissues (μg $N \cdot g^{-1}$)	^{15}N accumulated per seedling (μg)
Wilcoxina (Estrain)	105 ± 52	113 ± 28a	6.9 ± 3.2a
Amphinema	56 ± 20	65 ± 12ab	1.2 ± 0.8b
Mycelium radicis atrovirens	85 ± 52	42 ± 13b	–0.5 ± 0.7b
Nonmycorrhizal	44 ± 12	62 ± 8b	0.3 ± 0.4b
P (one-factor ANOVA)	0.2	0.01	0.0006

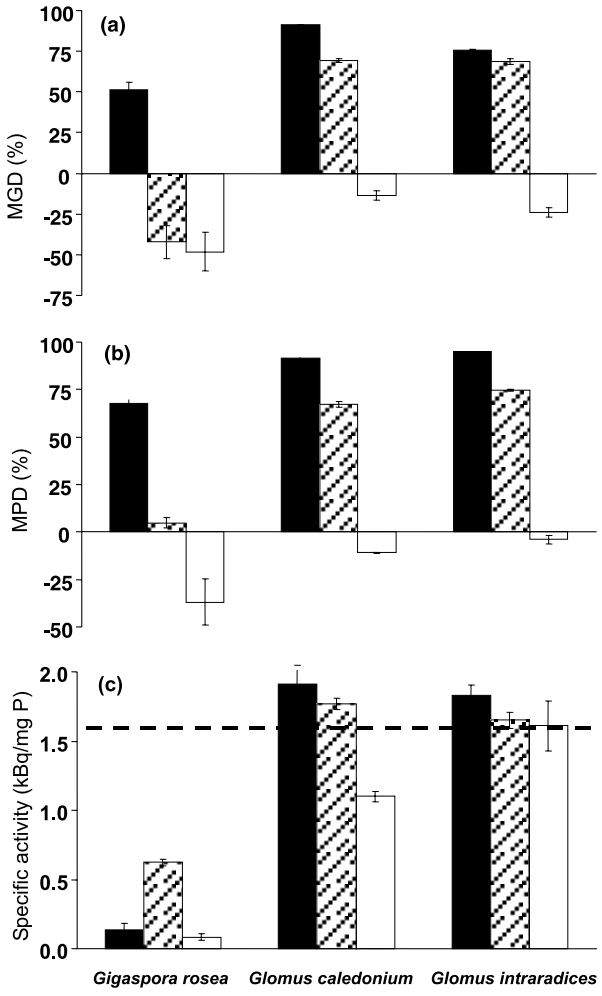
Note: Nonmycorrhizal seedlings had been planted 12 months earlier and allowed to become mycorrhizal in situ. Some seedlings remained nonmycorrhizal, and some formed only one morphologically distinct type of mycorrhizal. Data from only these seedlings are presented. Values shown are means ± SE, as back-transformed from $\log_{10}$ data. Means followed by different letters differ from others in the column according to a Tukey–Kramer honestly significant difference test at $\alpha = 0.05$ (M.D. Jones, F. Grenon, H. Peat, L.J. Philip, and R. Bradley, unpublished data). Seedlings were classified as nonmycorrhizal when no root tips were covered with a mantle. Seedlings were classified as being colonized primarily by one fungus if at least 30% of root tips were colonized and at least 80% of those were colonized by the same fungus.

clearly demonstrated in some EcM symbioses; Ashford et al. 1989; Vesk et al. 2000; Bücking et al. 2002), there is obvious opportunity for marked and direct fungal influence on the uptake processes, and uptake by an ectomycorrhiza may be quite different from uptake by NM rootlets. Nevertheless, EcM fungi differ in their effects on nutrient uptake by plants. In a recent field experiment Jones et al. (M.D. Jones, F. Grenon, H. Peat, L.J. Philip, and R. Bradley, unpublished data) injected ^{15}N-labelled nitrate or ammonium into soil surrounding 14-month-old *Picea engelmannii* seedlings to compare N uptake both between mycorrhizal and NM seedlings and amongst seedlings colonized by different EcM fungi. Interestingly, similar amounts of ^{15}N were accumulated by NM and mycorrhizal seedlings (approximately 20% of seedlings remained colonized at this subalpine site after 12 months of growth in the field); however, there were significant differences amongst the mycorrhizal seedlings, depending on the fungal symbiont (Table 1). In particular, under these conditions, where soil was not disturbed and a natural soil microflora was present, seedlings colonized primarily by *Wilcoxina* sp. absorbed significantly more ^{15}N from nitrate than did seedlings froming primarily *Amphinema* or *Mycelium radicis atrovirens* mycorrhizas. Seedlings forming primarily *Mycelium radicis atrovirens* mycorrhizas accumulated less ^{15}N, on average, that did NM seedlings, even though their mean dry mass was higher. The mutualistic nature of the fungi forming *Mycelium radicis atrovirens* mycorrhizas has frequently been questioned (e.g., Jumpponen 2001), even when, as in this case, the root tips colonized by these fungi had a mantle (albeit a thin one) and a Hartig net. Perhaps this is an example where an EcM fungus falls within the commensal portion of the parasitism–mutualism continuum.

The special opportunity for EcM fungi to influence nutrient uptake because of their location between root tissues and the soil has long been appreciated (Harley 1969). However, in arbuscular or ericoid mycorrhizas, where fungus–plant interfaces are "intracellular" (see Peterson and Massicotte 2004), there are two potentially independent pathways of P uptake: through the epidermis and via the fungal pathway to the root cortex. It is generally assumed, despite several suggestions to the contrary (Jakobsen 1995), that mycorrhizal colonization (either directly or via changes in P nutrition) has no effect on uptake by the root and that the two pathways act strictly additively. Hence, the AM fungal contribution to uptake has been determined by subtraction (uptake by AM fungus = nutrient content of AM plant – nutrient content of NM plant) and used to determine mycorrhizal influences, including "inflow" via the mycorrhizal pathway. Under conditions where nutrient supply is high, as well as in some unresponsive plant–fungus combinations (see following section), nutrient content may be no greater in AM than in NM plants, leading to the conclusions that the AM fungi make no contribution to uptake (Smith et al. 1986) and that there is no nutritional benefit of the mycorrhizal condition. Use of tracers, particularly ^{33}P and ^{32}P, supplied to AM fungi (but not roots) in compartmented pots has provided direct evidence that this simplistic view is incorrect. The mycorrhizal uptake pathway has been shown to make highly significant contributions to P uptake in nonresponsive cucumber (Pearson and Jakobsen 1993) and barley (Zhu et al. 2003) grown in pots and wheat grown in the field (Schweiger and Jakobsen 1999). In these examples the fungal contribution could not be accurately quantified, because the specific activity of available P in the labelled soil was not determined and the significance of the findings has not been widely appreciated. However, recently Smith et al. (2003*b*) have quantified the fungal contribution to P uptake in nine plant–fungus combinations of varying responsiveness with respect to growth and P uptake (Fig. 1). As expected, the fungi made substantial contributions in plants that responded to colonization at the whole-plant level, but the contributions determined from transfer of ^{33}P were, in several cases, higher than predicted from P contents. More surprisingly, in two other combinations, the fungus took up a high proportion of the P, but with no overall increase in plant P content. This implies that transfer of P via AM hyphae to the plant can severely suppress or eliminate P uptake from the soil directly by roots.

In these two cases, growth was depressed compared with NM plants. The results illustrate several important points: (*i*) fungi may make higher contributions to P uptake than previously appreciated, (*ii*) transfer of P from fungus to plant does not necessarily mean that the plant will accumulate more P or be able to grow more, (*iii*) a great range of interactions between plants and mycorrhizal fungi can be detected at the physiological level, and (*iv*) results using the "subtractive" approach to determining P inflow via mycor-

Fig. 1. Mycorrhizal growth dependency (*a*, % MGD), mycorrhizal P dependency (*b*, % MPD) and specific activity of ^{33}P in plants (*c*) of flax (black bars), medic (cross hatched bars), and tomato (open bars) grown with three arbuscular mycorrhizal (AM) fungi, as shown. % MGD was calculated as follows: % MGD = $100(DM_M - DM_{NM})/DM_M$, where DM_M and DM_{NM} are total plant dry masses, of mycorrhizal and nonmycorrhizal plants, respectively. % MPD was calculated in the same way, substituting total plant P content for dry mass. The horizontal dotted line in (*c*) is the theoretical maximum specific activity in plants if hyphae of the AM fungi were evenly distributed in root and hyphal compartments and all the P in the plants was obtained via the hyphal pathway. See text for explanation of experimental approach (after Smith et al. 2003*b*).



rhizal fungi are likely to lead to serious underestimates of fungal activity. Importantly, the results show that there can be reciprocal movement of nutrients (in this case P and organic C) in the absence of growth or nutrient responses at the whole-plant level. The reciprocal movement certainly falls within some definitions of mutualism, and these findings will be discussed in more detail later.

Does mycorrhiza formation always result in increased plant growth?

As indicated previously, there is increasing recognition (particularly for arbuscular mycorrhizas) of the enormous diversity in outcomes of symbiosis in different plant–fungus combinations. Growth depressions associated with mycorrhizal colonization have been regularly, but infrequently, reported both in carefully controlled pot experiments and in the field. For example, they have been observed in reinoculated AM plants grown in sterile soil (Fig. 2; Son and Smith 1988; Clapperton and Reid 1992; van der Heijden et al. 1998*b*; Hart and Klironomos 2002; Klironomos 2003), EcM conifers grown in nurseries (Stenström et al. 1990), AM citrus in fertilized agricultural soils (Graham and Eissenstat 1998), and AM crops in nutrient-poor tropical soils (Khasa et al. 1992). It is important to remember, however, that it is difficult to establish adequate controls for AM fungal treatments and that some of these results could be due to confounding effects of other microbes (Koide and Li 1989). Sometimes greenhouse experiments are conducted in sterile soil with AM roots added to mycorrhizal treatments

Fig. 2. A frequency distribution of the biomass response of 64 herbaceous plant species from an Ontario old-field site to inoculation with *Glomus etunicatum*, an arbuscular mycorrhizal (AM) fungus, relative to nonmycorrhizal controls. Percent difference in biomass was calculated as $[1 - (DM_{NM}/DM_M)] \times 100\%$, for species where mean dry mass of mycorrhizal plants (DM_M) was greater than than the mean dry mass of nonmycorrhizal plants (DM_{NM}). When mean dry mass of mycorrhizal plants was less than the mean dry mass of nonmycorrhizal plants, it was calculated as $[-1 + (DM_M/DM_{NM})] \times 100\%$. Plants were grown in a sterile mixture of sandy-loam soil and silica sand (1:1) for 16 weeks and fertilized once per week with half-strength Long-Ashton solution. See Klironomos (2003) for list of plant species (Hart and Klironomos 2002; Klironomos 2003). (Reproduced from *Mycorrhizal ecology*, Vol. 157, Hart and Klironomos, Diversity of arbuscular mycorrhizal fungi and ecosystem functioning, p. 236, © 2002, with permission of Springer-Verlag GmbH & Co.)

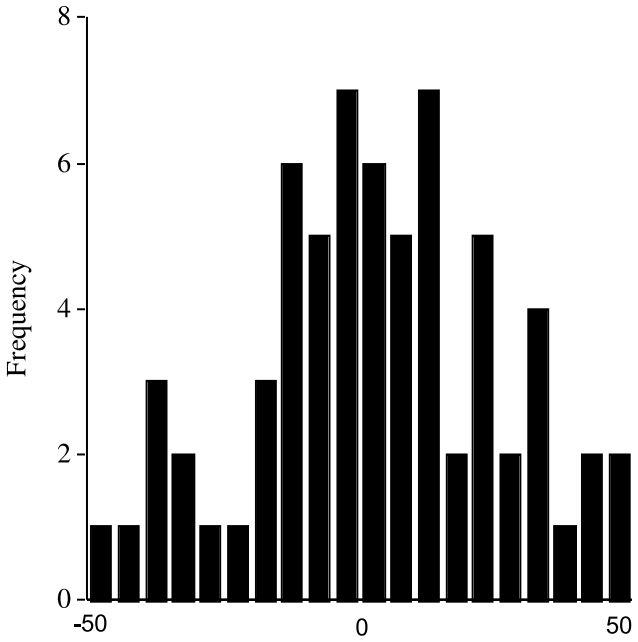
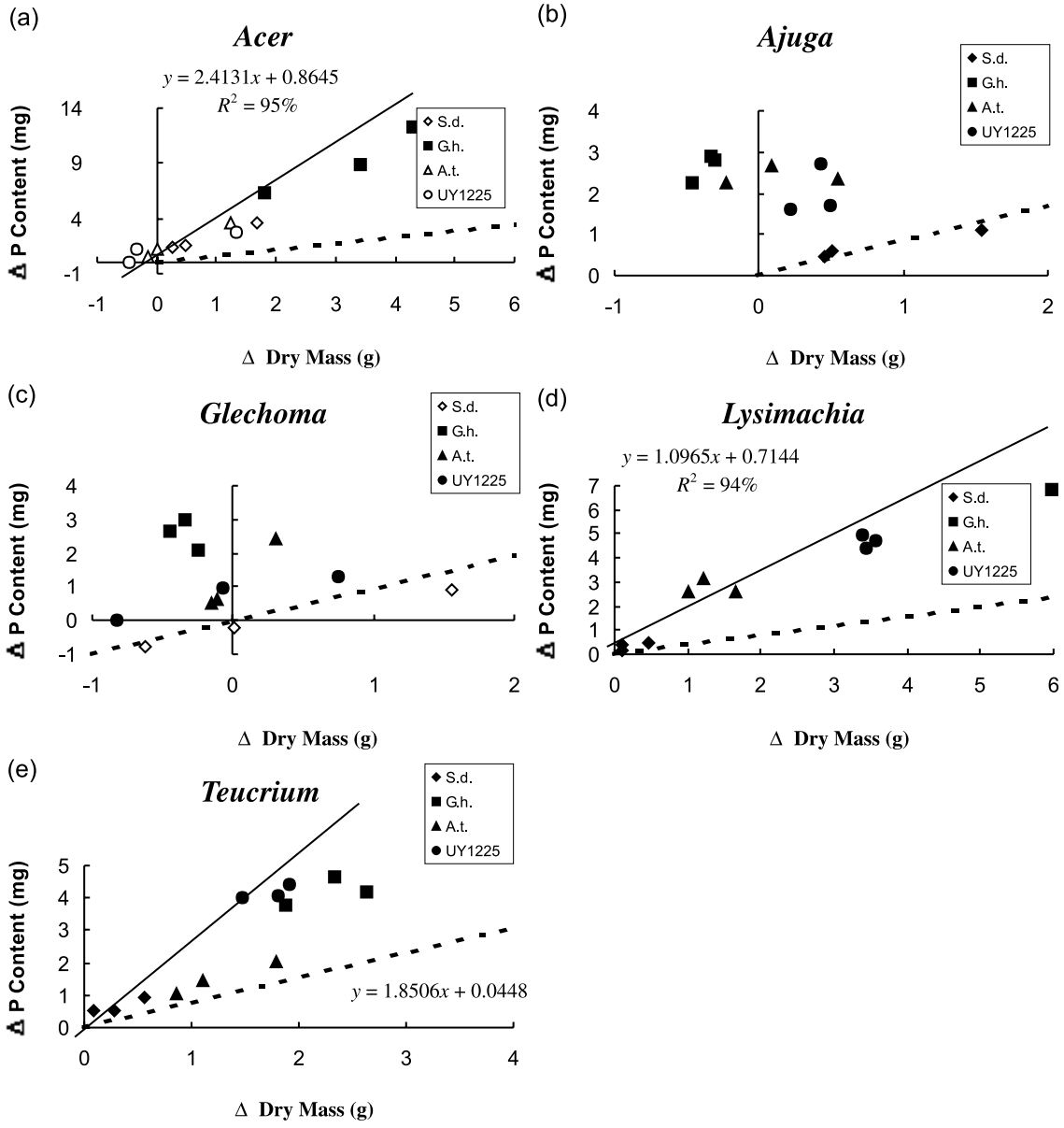


Fig. 3. The relationship between the change in P content and the change in plant dry mass, over the course of the experiment, following inoculation of *Acer pseudoplatanus*, *Ajuga reptans*, *Glechoma hederacea*, *Lysimachia mummularia*, and *Teucrium scorodonia* with *Scutellospora dipurpurescens* (S.d.), *Glomus hoi* (G.h.), *Archaeopsora trappei* (A.t.), or *Glomus* sp. (UY1225). Plants were grown in a 4:1 mixture of sterilized sand and woodland soil (Ah horizon). The three points for each plant–fungus combination are the means of five plants grown with either 0, 3, or 27 $\mu g \cdot g^{-1}$ P added to the substrate as bonemeal. The solid lines are regression lines for data from all four inoculation treatments (shown only if significant). Broken lines are based on mean P concentrations for nonmycorrhizal controls. (From Helgason et al. 2002, reproduced with permission of J. Ecol., Vol. 90, p. 377, © 2002 Blackwell Scientific Publications Ltd.).



and NM or autoclaved roots added to control treatments. With this approach, it is possible that pathogenic fungi could be introduced along with the mycorrhizal inoculum. A common approach in field experiments is to reduce AM colonization in some treatments by sterilizing or applying fungicide to the soil. This treatment reduces both AM and pathogenic fungal inoculum, and hence any negative plant growth response must be interpreted carefully to determine whether it is due to the presence of AM or pathogenic fungi (Newsham et al. 1994). In spite of these concerns, it is quite clear that colonization by EcM or AM fungi sometimes causes growth reductions in plants.

Even in fungus–plant combinations where mycorrhiza formation increases P uptake, plant growth rate does not always increase (e.g., Oliver et al. 1983; Abbott and Robson 1984; Adjoud et al. 1996). The range of possible responses, both in terms of P uptake and growth response, is illustrated by the results of Helgason et al. (2002), who inoculated five native AM plants with four of their co-occuring AM fungi. They found that some AM fungi increased the growth of some

plants in direct proportion to the increase in P accumulation (Fig. 3). The most common response, however, was an increase in plant biomass that did not match the increase in P content (milligrams per plant), thereby resulting in increasing P concentrations in plant tissues. This response to colonization has been observed frequently since the very first physiological investigations of AM symbioses and was one of the clues that led to the identification of P nutrition as one of the major potential benefits of AM colonization (Mosse 1973). As outlined by Stribley et al. (1980*a*), the most likely explanation is that some of the potential gain in plant biomass due to improved nutrition is "lost" because of the transfer of C to the fungus and the plants are C rather than P limited. For some of the plant–fungus combinations in the Helgason et al. (2002) experiment, increased P acquisition did not result in any increase in plant biomass, and this mechanism was probably responsible.

Under conditions of adequate irradiance, both AM and EcM plants frequently have higher photosynthetic rates than NM plants, and although this has sometimes been ascribed to improved nutrition, it can occur even when both groups of plants receive adequate nutrients (Koch and Johnson 1984; Brown et al. 1988; Dosskey et al. 1990; Eissenstat et al. 1993). In these cases, higher photosynthetic rates seem to be driven by increased sink strength induced by the fungus and therefore do not necessarily result in increased plant growth (Wright et al. 1998; Staddon et al. 1999; Miller et al. 2002). For example, Wright et al. (1998) found that if nutrients were supplied to *Trifolium repens* so that NM plants had the same N and P content as AM plants, then photosynthetic rates increased, but no growth increase was observed. Staddon et al. (1999) found that mycorrhiza formation caused higher photosynthetic rates in *Plantago lanceolata* grown under elevated CO_2, even though NM plants were supplied with adequate soluble P. Much of the additional fixed C did not end up in increased plant biomass, and the authors attributed the higher photosynthetic rates to higher root or fungal respiration or to dark respiration by the shoot. Miller et al. (2002) observed higher net C gains (assimilation rate multiplied by plant size) in AM than in NM *Andropogon geradii* plants, but with no increase in plant biomass. Because, in this case, AM and NM plants were matched in terms of N/P ratios in their tissues, the authors concluded that the increased C gain was driven strictly by C demands of the fungus rather than by a nutritional effect. All these studies illustrate that transfer of C from the plant to the fungus can occur in the absence of a growth benefit to the plant, although it would not be considered actually detrimental if the plant can increase its photosynthetic rate to match the demand (Tinker et al. 1994; Johnson et al. 1997). Any reduction in plant size, such as that observed by Son and Smith (1988) under low irradiance, could affect the ability of the plant to compete for light and other resources, but it may be that the high nutrient capitals (independent of growth responses) could be used in later vegetative or reproductive growth (Quoreshi and Timmer 2000; Helgason et al. 2002). Indeed, increased seed P status in mycorrhizal plants may be an important contributor to fitness (see later section).

Alterations in biomass allocation between different plant parts further complicate discussions of plant growth response. Some plant species show a highly plastic response to increased nutrient status, decreasing root biomass in favour of photosynthetic shoot tissue. Similar changes are frequently observed in AM and EcM plants, although responses vary (Harley 1984; Jones et al. 1990, 1991; MacFall and Slack 1991; van der Heijden et al. 1998*b*). We presume that hyphae provide a more economical (in terms of biomass) means of exploiting soil resources (Simard et al. 2002). Even so, shoot growth may not always be greater in mycorrhizal than in NM plants. Arbuscular mycorrhizal fungi can also influence the growth form of aboveground biomass (Streitwolf-Engel et al. 1997), making the interpretation of plant growth response difficult.

Whether a plant grows faster after becoming mycorrhizal may also depend on the nutrient deficit of the plant. Nutrient deficit, as defined by Koide (1991), is the combination of nutrient supply by the soil and nutrient demand by the plant. Because of slow growth rates, long-lived tissues, and efficient nutrient reallocation, some plants have inherently low nutrient demands (Chapin 1980; Smith et al. 2003*a*). Hence, these plants may show little whole-plant response to either added fertilizer or mycorrhizal colonization, particularly in situations when nutrient uptake is the main benefit of colonization (Janos 1987; Koide 1991; Smith and Smith 1996). However, there are fast-growing species (such as cultivated cereals) that show little or no mycorrhizal responsiveness, and it might equally be argued that "inherent slow growers should show large mycorrhizal responsiveness because slow growth of NM roots will result in a greater probability of nutrient depletion" (Smith et al. 2003*a*). In summary, whether a negative or positive growth response occurs will depend on the environment in which the mycorrhizal plant is found (particularly with potentially limiting factors such as nutrients or light), on the genotype of the plant (and hence on the root architectural and physiological strategies), and on the genotype of the fungus (and hence factors contributing to "efficiency"). We review the importance of these variables in the following sections. Smith et al. (2003*a*) provide a summary of additional factors that may influence mycorrhizal responsiveness of autotrophic plants (see Table 11.3 in Smith et al. 2003*a*).

Variation in growth response depending on abiotic environment

In pot studies, positive growth responses of plants to colonization by AM, EcM, or ErM fungi typically disappear once available nutrients in soil reach concentrations that no longer limit plant growth (e.g., Oliver et al. 1983; Abbott and Robson 1984; Bajwa and Read 1985; Jones et al. 1990; MacFall et al. 1991), although this is not always the case (e.g., MacFall and Slack 1991). This is frequently, but certainly not always, associated with reduced colonization (e.g., Zhou and Sharik 1997). When colonization is maintained it is not unusual to find that mycorrhizal plants are equal in size or even smaller than NM plants (Oliver et al. 1983; Stenström et al. 1990; Graham 2000 and references therein). These growth reductions can be sizeable enough to be of concern in agronomic or forestry applications. For example, citrus grew 9%–14% faster in the field after benomyl application, which reduced AM colonization but had no apparent effect on pathogens (Graham and Eissenstat 1998). Such growth reductions are attributed to decreased benefit com-

bined with no decrease in cost of the association to the plant (Johnson et al. 1997; Graham 2000). In these situations the fungus may be considered to be parasitic on the plant, but again the distinction between whole-plant and cellular processes needs to be taken into consideration.

Growth responses to formation of arbuscular mycorrhizas are lower, and actual depressions are more likely to occur, in poor light (e.g., Son and Smith 1988; Facelli et al. 1999). This is consistent with the C demand of the fungus discussed previously. Although, formation of ectomycorrhizas often declines at low irradiance, no consistent interactions between ectomycorrhiza formation and irradiance on plant growth have been found (Reid et al. 1983; Zhou and Sharik 1997). Interestingly, Simard et al. (1997) found increased photosynthetic rates associated with colonization of Douglas-fir by certain EcM fungi, even in deeply shaded forests. After finding that ectomycorrhiza formation on *Quercus rubra* was highest at intermediate levels of forest canopy cover, Zhou and Sharik (1997) characterized the symbiosis as "fungal parasitism" on the plant at low irradiances and "plant parasitism" on the fungus at high irradiances. Clearly, light is an important environmental factor that influences the response of plant and fungus to the symbiosis. The same is true for soil moisture status (Duddridge et al. 1980; Kothari et al. 1990; Augé 2001) and, undoubtedly, other components of the abiotic environment.

Differential benefit depending on biological environment

Whether a plant exhibits a positive or negative response to colonization also depends on the biological environment of the plant, including both soil microbes and other plants. For example, it is clear that ectomycorrhizas sometimes do not form without the appropriate complement of bacteria in the rhizosphere (Garbaye 1994). Mycorrhizal fungi can also interact in complex ways with symbiotic N-fixing bacteria. In a pot study where inoculation with two AM fungi had no overall effect on growth or nutrient uptake by *Pisum sativum*, there was a strong interaction between a *Rhizobium* strain and an AM fungal isolate (Xavier and Germida 2003). One AM fungal isolate significantly decreased the growth and N nutrition of plants inoculated with an effective *Rhizobium* strain, whereas a second isolate significantly enhanced the growth of these plants. There was no effect of AM inoculation on pea plants colonized with less effective *Rhizobium* strains. Egerton-Warburton and Allen (2001) found that different types of mycorrhizal fungi can interact to affect growth of *Quercus agrifolia*. Colonization by EcM or AM fungi alone increased plant survival, uptake of N and P, and growth, but none of these responses occurred when seedlings were colonized jointly by both types of mycorrhizal fungi. Because dual colonization is typical of young seedlings in the field, the authors hypothesize that mycorrhizal benefit is unlikely at this growth stage. It is likely to increase as the trees age, however, because they lose arbuscular mycorrhizas and become exclusively ectomycorrhizal. Although mycorrhiza formation can reduce damage caused by some root pathogens (e.g., Sylvia and Sinclair 1983; Duchesne et al. 1989), it can stimulate colonization by other parasites (see Dehne 1982 and references therein). Sanders et al. (1993) found increased infection by dodder in AM plants, although in this case, biomass production was still higher in AM than in NM plants, in spite of the presence of dodder. Schönbeck and Dehne (1979) found increased fungal parasitism on aboveground parts of AM plants. Soil microfauna such as earthworms, nematodes, and collembola can influence the nature of the interaction between mycorrhizal fungi and plants by disrupting hyphae, increasing hyphal turnover, providing sources of nutrients, or acting as plant pathogens, among other things (Larsen and Jakobsen 1996; Jothi and Sundarababu 2001; Bakonyi et al. 2002; Founoune et al. 2002; Tuffen et al. 2002).

Most experiments have examined the impact of mycorrhiza formation on plants growing individually in pots. As pointed out by Johnson et al. (1997), the challenge of quantifying net benefit to plants is to do so at the appropriate scale. In some contexts, it is the performance of individual plants as members of populations and communities that may be of interest, because this will affect the species composition and diversity of communities. This has been addressed by varying the density and species composition of plants in pot or microcosm studies. Several such studies have found that AM colonization is less likely to stimulate plant growth when seedling density is high, even if concentrations of soil P are low (Facelli et al. 1999; D.P. Schroeder and M.S. Janos, unpublished data). The variation in seedling growth response was also more variable at higher densities. Along similar lines, Kytöviita et al. (2003) found that inoculation with some AM fungi stimulated growth of seedlings of four native plant species grown at high density, but not when they were competing with larger, established seedlings. By contrast, Shumway and Koide (1995) found that weak AM colonization had no effect on the biomass–density relationship during self-thinning of *Abutilon threophrasti*, although it did influence fitness (see below). Taken together with the study of Egerton-Warburton and Allen (2001), these studies suggest that mycorrhiza formation could contribute to self-thinning in some plant populations and may benefit plants in these populations only once they are larger and the density has decreased. Another study (D.P. Schroeder and M.S. Janos, unpublished data) points out that plants in less dense populations more closely resemble plants in pot studies, for which positive plant growth responses have been found repeatedly.

Many studies have shown that the presence of AM inoculum can alter the balance between competing species, which frequently have different strategies for nutrient acquisition, thereby making mechanistic comparisons difficult (Grime et al. 1987; see Smith and Read 1997 and references therein; Bever et al. 2002). A recent study by Cavagnaro et al. (2004) has shown that mycorrhiza-induced increases in plant growth may only be apparent at the community level. Using wild-type tomato (AM) and an NM mutant derived from it to simulate competition between an AM and nonhost species with the same growth and nutrient uptake rates, they showed that there was no difference in growth of the two genotypes when grown separately, either singly or at four plants per pot; the AM wild type was unresponsive to mycorrhizal colonization and therefore apparently received no "benefit" at the whole-plant level. However, when the two genotypes were grown together in a replacement series, the wild-type plants responded positively to inoculation in terms of biomass per plant, and the extent of the increase

was related to the intensity of the competition from the NM (mutant) plants. As AM tomato does receive considerable P via the mycorrhizal uptake pathway, despite lack of P or growth response (Fig. 1; Smith et al. 2003*b*), the results suggest that increased ability to compete with the NM plant for limited soil P resources is the basis for the growth increase and that mutualism was expressed only in a community situation. Similarly, Hodge (2003) found that *Glomus hoi* stimulated N uptake by both *Plantago lanceolata* and *Lolium perenne* when they were grown together in mixtures, but not when they were grown in monocultures. There has been only one equivalent study with EcM plants. *Pinus ponderosa* and *Pseudotsuga menziesii* (Douglas-fir) mutually inhibited each other in an addition series when both were colonized with *Thelephora terrestris*, but when inoculated with several other EcM fungi this mutual inhibition disappeared (Perry et al. 1989). Instead, growth of Douglas-fir was stimulated because of increased uptake of P and N at the expense of luxury uptake by ponderosa pine. The net effect of mycorrhiza formation on plant competition may depend on whether the dominant plant in the microcosm studies is mycorrhiza responsive or not (O'Connor et al. 2002; Urcelay and Diaz 2003).

Variation in growth response depending on plant and (or) fungus genotypes

It is well established that there is considerable variation in the mycorrhizal responsiveness of different plants, whether they are crops or native species. In the last few years there has been an increasing realization that mycorrhizal fungi are also highly diverse with respect to effectiveness. Figure 1 illustrates these points, showing not only marked differences in responsiveness of different plant species, but also that *Gigaspora rosea* was, for all three plant species tested, a less effective symbiont than either *Glomus caledonium* or *Glomus intraradices*.

Recently, there have been several studies that have ambitiously attempted to determine the range of responses of native plant communities to colonization by AM fungi. For example, when communities of 11 herbaceous plants were established in sterile, unfertilized microcosms of calcareous grassland soil from Switzerland and inoculated with four AM fungi, either singly or as a group, 37 plant–fungus combinations resulted in larger mean plant size than in uninoculated communities, and 17 combinations resulted in mean growth depressions (van der Heijden et al. 1998*a*, 1998*b*). The overall mean biomass of the inoculated communities was the same as or lower than that of the uninoculated communities, depending on the fungus. Of 110 combinations of 10 plant species and 11 AM fungal species isolated from an old field in southern Ontario, Canada, and grown individually in fertilized, sterile field soil, 47 resulted in growth stimulations for the plant and 53 resulted in growth depressions (Klironomos 2003). Seventy-three of the combinations resulted in biomass changes of less than 25% but the range was from a growth depression of 49% to a growth increase of 46%. Interestingly, when 64 different herbaceous plants were inoculated with an isolate of *Glomus etunicatum* from the same site, a normal curve of growth responses resulted (Fig. 2), with a median biomass that was the same as uninoculated plants (Hart and Klironomos 2002; Klironomos 2003). This means that most plants exhibited minimal growth responses, and equal numbers of species showed positive or negative growth responses. Other studies that compared native plants and their co-occuring AM fungi found only a few (e.g., Wilson and Hartnett 1998) or no growth reductions relative to NM plants, even with added P (e.g., Helgason et al. 2002). Nevertheless, taken as a whole, these studies indicate that a wide range of responses to AM fungi is possible and that parasitic responses, at least over the short term (see also Smith 1980), may be more common than acknowledged even by Johnson et al. (1997). They also indicate that plant and fungal genotypes interact strongly in determining the response of the plant to colonization by the fungus, although the mechanisms underlying the variation have not yet been elucidated. These studies were all performed on sterilized soils in greenhouses, and in some cases the soils were fertilized, which would be expected to shift plant response to inoculation in a negative direction; therefore, the results must be treated with caution. Furthermore, mycorrhizal responsiveness in pots may not predict the response of a plant species to colonization when it is growing in a community (O'Connor et al. 2002).

There have been no equivalent studies comparing the response of a large number of EcM or ErM plants to colonization by a range of mycorrhizal fungi, but it seems clear from inoculation trials of EcM fungi for plantation forestry that a range of postive and negative plant growth responses are likely, even to different isolates of the same fungal species (Burgess et al. 1994; see Jones et al. 2003 and references therein).

Based on their comparisons of a large number of plant – AM fungal combinations, both van der Heijden et al. (1998*b*) and Klironomos (2003) reject the idea that any plant species has a consistent mycorrhizal dependency. Instead, growth responses of a plant will vary with the fungal isolate involved as well as with soil nutrient status, competitive interactions, etc. In unsterilized soil, plants are colonized by a large number of AM species simultaneously, and the combination of different strategies in P uptake and indeed in C demand would be expected to modify the outcomes (see Jakobsen et al. 2002 and references therein). Nevertheless, there are plant species that (in single-species pot experiments) do not appear to respond to mycorrhizal colonization, and several plant characteristics seem to be linked with a reduced positive response or indeed a negative response. Native plants that exhibit negative or no responses to AM fungi tend to (*i*) have lower tissue P concentrations when NM (Adjoud et al. 1996); (*ii*) be ruderals (Philip et al. 2001), annual plants, or perennial cool-season grasses (Hetrick et al. 1991; Wilson and Hartnett 1998); or (*iii*) have fine, highly branched roots (Baylis 1975; Bryla and Koide 1990; Smith and Smith 1996; and see Smith et al. 2003*a*). Citrus varieties that grow more slowly than others after AM colonization tend to have higher nonstructural carbohydrates in the roots and higher respiration rates than other varieties when NM (Peng et al. 1993; Graham et al. 1997). Johnson et al. (1997) hypothesized that native plants would be more likely than agricultural plants to show positive responses to mycorrhizal fungi, because the two symbionts had evolved together. However, Koide and coworkers have found the reverse to be true when they compared wild versus cultivated varieties of

tomato and oats (Koide et al. 1988; Bryla and Koide 1990). They hypothesize that native genotypes have adaptations for growing in nutrient-deficient soils that have been lost during the breeding of cultivated varieties. The likelihood that these and other traits, such as root/shoot ratio, growth rate, root hairs, and tissue nutrient concentrations can be used to predict mycorrhizal responsiveness is discussed in detail in Smith and Smith (1996).

We can also ask whether some mycorrhizal fungi are more likely than others to cause growth depressions in plants, given appropriate conditions (Johnson et al. 1997). In the studies by Klironomos (2003) and van der Heijden et al. (1998*a*), no fungus stimulated or inhibited the growth of all plant species. Nevertheless, Graham (2000) suggests that fungi that exhibit rapid rates of colonization in high-nutrient soils are those most likely to inhibit plant growth under these conditions. Interestingly, Helgason et al. (2002) found that *Glomus hoi*, the most aggressive colonizer amongst four naturally co-occuring AM fungi, stimulated growth and P content of *Acer pseudoplatanus* to the greatest extent, regardless of whether 0 or 27 ppm P was added to pots. Other fungal traits that may influence mycorrhizal responsiveness to a particular fungus include extent of external hyphae, colonization rate, and the rates at which nutrients and C are translocated and delivered (see Smith and Smith 1996).

The complexity of the interactions amongst plants and mycorrhizal fungi in the field is enormous because of the high diversity of both plant and fungal communities. In EcM communities, high fungal diversity is viewed positively, with the hypothesis being that association with a number of physiologically diverse fungi will benefit an individual tree by maximizing access to nutrients from a range of soil substrates (Amaranthus and Perry 1994; Hagerman et al. 1999; Baxter and Dighton 2001; Jonsson et al. 2001). For example, Ogawa (1985) describes a range of foraging strategies by EcM fungi growing in a Japanese forest. Similarly, a combination of hyphal foraging strategies by AM fungi colonizing the same plant may increase the effectiveness of an AM root system to scavenge nutrients at different distances from the root (Koide 2000). When we consider that different plants vary in growth response to the same fungus, as discussed above, and that mycorrhizal fungi also vary in their responses to different plants (see Bever et al. 2002), then predicting the response of the entire community becomes complex. For example, in some cases a fungus will increase productivity of one plant species more than that of another and yet have higher fitness (as measured by spore production) when associated with the plant that shows the lesser growth response (Bever et al. 2002). In these cases of negative feedback, a fungus would be expected to have higher fitness in a diverse plant community than in a community comprising primarily the plant species whose growth it stimulates the most (Bever et al. 2002).

The importance of determining fitness

Almost all of the studies discussed previously evaluated benefit to mycorrhizal plants in terms of biomass production or nutrient content, often in single species grown alone. This is understandable given that these variables are easy to measure. For crop plants, they may even be important in their own right. Nevertheless, ecological and evolutionary definitions of mutualism invoke fitness as the appropriate measure of benefit to symbionts. Fitness is also the most effective variable for integrating the multiple effects that symbioses can have on plants, including non-nutritional effects, which can be difficult to evaluate.

Evidence of increased fitness in mycorrhizal plants

Plant fitness can be evaluated by many methods, but the most common approaches are to quantify the survival of plants, their subsequent fecundity, and the growth and survival of their offspring. Fecundity has occasionally been measured on AM plants, but rarely in the field. This is partly because it can be difficult to maintain control plants in an NM state after outplanting. Two major approaches have been used in field experiments: (*i*) fumigation or steam sterilization of plots followed by reintroduction of AM fungi or (*ii*) fungicide application to reduce colonization. Both have their drawbacks, which are discussed by Hartnett and Wilson (2002). Fumigation kills the soil microflora and fauna in both treatments and thus eliminates any interactions between AM fungi and other soil organisms. Fungicides reduce other active fungi, in addition to AM fungi, in the sprayed plots and tend to reduce AM colonization rather than eliminating it altogether (Carey et al. 1992). Although these field approaches are to be commended because they are more realistic than greenhouse studies, it must be emphasized that these methodological constraints prevent us from determining the effects of mycorrhiza formation on plant fitness under truly realistic soil conditions. Furthermore, because EcM plants typically require a very long period to reach maturity, it is effectively impossible to compare the fecundity of EcM and NM plants; therefore, our evaluation of fitness is restricted to the effects of ectomycorrhiza formation on survival of young plants.

Early indication of the importance of ectomycorrhizas to the survival of conifer seedlings came from observations of conifer nurseries in the 1930s. Under the conditions used at the time, growth and survival of most seedlings was poor, with little mycorrhiza formation; however, patches of larger seedlings with healthy foliage and higher nutrient contents were often observed and these were usually mycorrhizal (McComb 1938). When nursery beds were inoculated with small amounts of forest soil, such that red and white pine (*Pinus rubra* and *Pinus strobus*) formed ectomycorrhizas but soil nutrient contents were not increased, seedling growth improved substantially (Mitchell et al. 1937). Although specific data on seedling survival is not presented in any of the early papers, references to "forest nursery and plantation failures" (Mitchell et al. 1937) and the lack of any P uptake in the absence of mycorrhizas (Hatch 1937; Stone 1949) make it clear that NM seedlings were not developing normally. Unfortunately, even recent studies on the survival of EcM or AM seedlings in the field are primarily correlative, like these earlier examples, and do not allow us to separate cause and effect. For example, there is circumstantial evidence that formation of mycorrhizas allowed conifer seedlings germinating on volcanic ash to survive over the winter period (Allen 1987), lodgepole pine seedlings to survive in hot, dry soils (Miller et al. 1998), and cotton to survive at low temperatures (Zak et al. 1998).

The vast majority of published studies on the effect of mycorrhizas on seedling survival have been conducted under laboratory conditions on micropropagated plants during the acclimation phase, when they are transplanted into pots. In most cases, inoculation with AM (e.g., Naqvi and Mukerji 1998; Gaur and Adholeya 1999), EcM (Martins et al. 1996), or ErM (Starrett et al. 2001) fungi increased both growth and survival. Although these studies occurred under highly artificial conditions, they may have some relevance to natural settings in that the acclimation phase can be a time of high stress for plantlets. In a more realistic microcosm study, plants from calcareous grassland (Grime et al. 1987) were grown from seed in trays with *Festuca ovina* (the grassland dominant), colonized or not by naturally occurring AM inoculum. There were marked differences, not only in growth of the different plant species, but also in their survivorship. All the AM species survived better in the inoculated treatments, whereas the reverse was found for plant species that are constitutively NM.

A few controlled studies have confirmed that mycorrhizas influence seedling survival in the field. For example, inoculation of nursery beds with pure cultures of EcM fungi produced normal seedling growth (Hatch 1936; Young 1936; Rayner 1938), thereby providing the first strong evidence that ectomycorrhizas were directly responsible for the differences in growth amongst seedlings. Much more recently, Roldan-Fajardo (1994) found that inoculation of six native plants with *Glomus aggregatum* substantially increased survival in an arid field. As discussed above, most of these studies required some kind of soil perturbation to establish the mycorrhizal and NM treatments and, as such, cannot be considered to represent truly realistic soil conditions. Nevertheless, the body of evidence from the published literature is that formation of arbuscular or ectomycorrhizas increases seedling survival under many conditions.

Work on the effect of mycorrhiza formation on fecundity occurred later. For example, Daft and Okusanya (1973) showed that petunia flowered earlier and produced more flowers, maize produced more pollen, and strawberry produced more flowers and fruits when inoculated with AM fungi in fertilized sand culture. Koide and his coworkers also found increased survival and fecundity of several AM plant species along with increased growth rates and fecundity of their offspring (Stanley et al. 1993; Shumway and Koide1994; Koide and Lu 1995; Heppell et al. 1998). By contrast, Wright et al. (2000) found that colonization of one *Holcus lanatus* genotype by *Glomus hoi* depressed flower biomass, and Philip et al. (2001) found no effect of AM colonization on overall reproductive output per plant of *Lythrium salicaria* (purple loosestrife). Thus, greenhouse studies demonstrate no consistent response of fecundity to formation of arbuscular mycorrhizas.

Only a few studies have compared the fecundity of mycorrhizal and NM plants in the field, especially under natural conditions. Carey et al. (1992) found that suppression of AM fungi, through the application of benomyl, increased fecundity of *Vulpia ciliata* subsp. *ambigua* at one site, but decreased it at another. The study of Shumway and Koide (1995) is especially interesting because it was conducted at different densities, under field conditions (albeit on fumigated soil). In this case, AM *Abutilon theophrasti* plants from early cohorts, or planted at low densities, produced seeds with larger individual masses than those of NM plants. There was no difference in fecundity between AM and NM plants at high density. One highly significant investigation studied the growth and P budget of the woodland plant *Hyacinthoides non-scripta* (English bluebell) over its entire life cycle (Merryweather and Fitter 1996). This is an obligately mycorrhizal geophyte, which can only maintain a positive P budget when AM, as it loses a great deal of P during annual death of leaves and roots. Interestingly, field-grown plants drenched with benomyl, which reduced AM colonization, showed reduced P concentrations in vegetative parts, but maintained seed P at the same concentrations as AM controls for 1 year. The plants were apparently protecting their reproductive structures by selective P allocation but could not maintain this for a second year when NM; the reduced P concentration in the flowers of benomyl-treated plants suggested that AM colonization is important to fecundity in *Hyacinthoides non-scripta*. In summary, and bearing in mind the methodological constraints mentioned previously, it is clear that mycorrhiza formation can increase the fecundity (and therefore fitness) of AM plants, although the results depend on plant genotype and environmental conditions. There is also good evidence that mycorrhiza formation affects fitness of EcM plants by increasing initial establishment of seedlings.

Growth response and fitness are not always positively correlated

Given that most experiments measure plant growth and not fitness, it is important to realize that these two variables are not necessarily correlated. It is possible for some mycorrhizal fungi to have no effect on or to suppress growth under some conditions and yet increase fitness over the long term. For example, (Copeman et al. 1996) found that AM fungi isolated from a saline site depressed growth of tomato compared with NM plants or with plants inoculated with AM fungi isolated from a nonsaline site, even when grown under saline conditions. However, the smaller AM plants were expected to have the highest survival under field conditions, because they accumulated chloride at the slowest rate when exposed to conditions of moderate salinity (salinity stress at this high-Ca site was expected to come from chloride, not sodium). Inoculation of micropropagated cuttings of *Pinus pinaster* or *Pinus sylvestris* with some isolates of *Hebeloma cylindrosporum* depressed seedling height by up to 40%, but increased survival by 17% during the period when the seedlings were being acclimated to the open air (Normand et al. 1996). Fecundity can also be affected differentially from growth. For example, when *Avena fatua* (wild oats) plants became mycorrhizal, they produced more biomass and accumulated more N than NM plants, but flowered for a shorter length of time and tended to produce seeds with a reduced individual mean mass (Koide et al. 1988). Colonization by *Glomus aggregatum* depressed the biomass of plants of the long morph of *Lythrium salicaria* (purple loosestrife) under fertilized greenhouse conditions but without an overall effect on pollen or ovule production per plant (Philip et al. 2001). Helgason et al. (2002) found that *Archaeospora trappei* was the only one of four AM fungi to stimulate flowering in *Lysimachia nummularia* over the course of their experiment,

even though two other AM fungi stimulated accumulation of P and plant dry mass to a much greater extent. They also speculated that their slowest growing treatments might be expected to have high vegetative reproductive rates in the following spring, fuelled by higher shoot P concentrations. Streitwolf-Engel et al. (1997) also found that the effect of AM fungi on vegetative reproduction could be independent of their effect on overall plant biomass. In general terms, therefore, it appears that the response of overall biomass production to mycorrhiza formation is not a reliable predictor of fitness, as measured either by survival, fecundity, or asexual reproduction.

Temporal separation of nutrient flow to plant and fungus

Another factor that reduces the usefulness of short-term experiments in determining mutual benefit to mycorrhizal symbionts is that maximum C transfer to the fungus (possible cost to the plant) and maximum inflow of nutrients (typical measurement of benefit) may be offset in time. Jones et al. (1991) found that maximum P inflow occurred shortly after colonization in EcM willow growing in pots, whereas the amount of C transferred below ground did not increase until several weeks later. This meant that the cost/benefit ratio varied substantially over the course of the experiment. Under natural conditions, where nutrient uptake may occur in flushes throughout the year (Sanders and Fitter 1992), the presence of mycorrhizas may be a net cost to the plant at certain times of year (unless the plant can increase its photosynthetic rate sufficiently to compensate; see Miller et al. 2002) and a net benefit at other times. One obvious example of this is the extra C required by EcM fungi in the fall to produce large fruit bodies (Vogt et al. 1982) or extramatrical hyphae (Wallander et al. 2001). Högberg and Högberg (2002) estimated that such extramatrical hyphae could constitute 30%–40% of microbial biomass in northern coniferous forests. A second example is the high nutrient demand of decidous trees as their buds flush, this being at a time when the photosynthetic capacity of plants to meet C demands of mycorrhizal fungi is restricted.

Three studies give some suggestion that this phenomenon occurs in the field. First, the detailed calculation of P budgets and life history of *Hyacinthoides non-scripta* growing under natural conditions suggest that there is likely temporal separation between maximum C allocation to the AM fungi and maximum P inflow into the plant. The data of Merryweather and Fitter (1995*a*, 1995*b*) indicate that the highest C demand by the fungi is likely to be from September through January, when the roots grow from the bulb and become colonized, whereas the highest P inflow into the plant was in February and March, during the photosynthetic phase. In a second study, fumigation of soil in the fall caused an increase in the growth of winter wheat over the winter, but a decrease in growth in the spring (Trent et al. 1989). This could be attributed to a difference in the cost and benefits of arbuscular mycorrhizas in the two seasons, that is, mycorrhizas played an important role in nutrient uptake in the spring but imposed a C drain over the winter. With this kind of experimental design it is impossible, however, to rule out a role for pathogenic fungi in the growth suppression observed in the winter. Carey et al. (1992) also found differences in plant response to soil fumigation depending on the season. *Vulpia ciliata* subsp. *ambigua* had lower fecundity at one site after benomyl was applied from March through June; colonization by AM fungi was significantly reduced at this site. At a second site, when benomyl was applied from late August through May, fecundity was increased. AM colonization and P content were low at the latter time and site, even without benomyl application, and therefore the result may have been due to a reduction in pathogens, as shown later by West et al. (1993). Sanders and Fitter (1992) found no relationship between the percentage of root length colonized in six grassland species and P, Cu, or Mn inflow over the course of 2 years. This suggests that AM fungi may be present and consuming C from the plant even during periods when nutrient uptake rates are not high. During these periods, the fungi have the potential to act as parasites (Sanders and Fitter 1992) but could still increase overall plant fitness when integrated over the life-span of the plant. All of these results reinforce the need to calculate nutrient and C budgets over the entire year when trying to determine whether a relationship is mutualistic.

When interpreting nutrient uptake and growth response in the field, it is also important to realize that mycorrhizal fungi may not stimulate nutrient uptake continuously throughout the growing season. Rather, they may facilitate storage and later release of nutrients and thereby dampen fluctuations in nutrient supply (Bücking and Heyser 2001). This kind of storage and delayed transfer has been observed using several different experimental approaches in EcM plants and varies from fungus to fungus. In the 1950s, Harley and coworkers established that a large proportion of ^{32}P absorbed by excised beech mycorrhizas is stored in the EcM mantle, largely as polyphosphate, and subsequently transferred to the root tissue (Harley and McCready 1952; although see comments on the use of excised roots in Harley (1969)). More recently, Bücking and Heyser (2001) used microautoradiography to show accumulation of ^{33}P in the mantle and Hartig net of one type of ectomycorrhiza of intact *Pinus sylvestris* seedlings, with no further transfer of this P to root cells. In another experiment, colonization by *Suillus bovinus* increased the concentration of P in shoots of *P. sylvestris* growing at low P, relative to NM plants, whereas the situation was reversed at high P supply (Bücking and Heyser 2000). This is consistent with the results of Morrison (1954), who found that ^{32}P previously taken up into EcM roots of pine seedlings continued to be translocated to the shoots even after ^{32}P was removed from the nutrient solution, but that no further transfer to shoots occurred in NM plants. There is some evidence that AM roots may maintain a steady P supply (inflow) to *Trifolium subterraneum* plants from soil of different P status over 10 weeks, but that in NM plants inflow declined to very low levels after an initial rapid uptake stage (Smith et al. 1986). The evidence for P storage in AM roots is less substantial than for ectomycorrhizas, although many studies have shown higher P concentrations in AM than NM roots, which has sometimes been interpreted as P sequestration (storage) in the fungi (e.g., Smith et al. 2000). Use of ^{31}P nuclear magnetic resonance spectroscopy and other techniques certainly shows the accumulation of P, both as soluble P_i and sometimes as polyphosphate, in AM roots under conditions of high P supply (Rasmussen et al. 2000; T. Cavagnaro and

T. Ezawa, unpublished data). The retention of P under high P supply might be considered to be detrimental in a typical short-term experiment where nutrient supply is kept constant, but this may be a considerable advantage under field conditions with seasonal fluctuations in nutrient supply. In consequence, a clear understanding of the outcomes of reciprocal C and nutrient flows can only be achieved if measurements are taken throughout one or more growing seasons, as in the study of bluebell by Merryweather and Fitter (1995*a*, 1995*b*).

Benefit may be apparent only under acute conditions

Another problem with determining net benefit or cost to the plant from short-term growth experiments is that the conditions under which mycorrhizal fungi benefit the plant may be quite specific and may not be simulated or encountered during the experiment. These conditions might occur only during specific stages of the life cycle, seasons of the year, or years of acute environmental conditions. Survival through such stresses may be important for long-term fitness, particularly for perennial plants, but should not be ignored, even for annual plants. Some short-lived plants may never experience stress conditions during their individual life-spans; however, if the stress occurs frequently enough, the gene pool of the population as a whole will be influenced. For natural selection to favour mycorrhizal associations under conditions where symbiosis sometimes results in reduced growth, fecundity, or nutrient uptake, the benefit in terms of survival and fecundity over the life-span of a plant would need to be positive, in spite of a reduction in competitive ability during certain periods. Detection of net benefit would be very difficult, even in field experiments, unless they are of very long duration, as shown by the following example. Inoculation of *Quercus robur* with *Paxillus involutus* stimulated growth in height over 7 years, due exclusively to higher growth increments during dry years (Garbaye and Churin 1997). During years of average rainfall, no difference in height increase occurred. For a tree species that does not reach reproductive maturity for 10 or 15 years, survival during extreme conditions during one of those years would make the difference between successful reproduction or not.

Considering mycorrhizal mutualism at a finer scale: Should we redefine "mutualism" for mycorrhizas at a cellular or physiological scale?

We have shown that methodological constraints make it impossible to determine whether mycorrhizal associations are mutualistic at the whole-plant or community levels. However, recent work is providing a much clearer picture of the way cellular processes in both symbionts are integrated in the symbioses. Given this, it is tempting to ask whether mycorrhizal mutualism could be defined and detected at the cellular or molecular levels. As stated in the Introduction, tracer studies 50 years ago established both that mycorrhizal hyphae can act as conduits for mineral nutrients from the soil into mycorrhizal roots and that they are sinks for C fixed from the shoot, but accurate quantification was lacking and still is, to a very great extent. The finding that tracer moves from one organism to another does not provide clear evidence for net or quantitatively significant movement in either direction. Studies at the cellular level have focused on the structure and development of the fungus–plant interfaces (see Peterson and Massicotte 2004); on visualizing evidence for active transport processes (e.g., ATPases) in the membranes associated with arbuscules, intercellular hyphae, or Hartig net (e.g., Lei and Dexheimer 1988; see Smith and Smith 1990; Gianninazi-Pearson et al. 1991); on detecting changes in the regulation and localization of genes that could be involved in P uptake by plant or fungal cells at the specialized interfaces (Maldonado-Mendoza et al. 2001; Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002); on following metabolism of key substrates using nuclear magnetic resonance spectroscopy (Shachar-Hill et al. 1995; Pfeffer et al. 1999; Rasmussen et al. 2000; Bago et al. 2002); and on quantifying mineral nutrient transfer using electron microscopy (Bücking and Heyser 2001) or tracer measurements (Joner et al. 2000; Nielsen et al. 2002; Smith et al. 2003*b*). It is very significant that three unrelated plant species (*Medicago truncatula*, *Solanum tuberosum*, and *Oryza sativa*) have been shown to possess phosphate transporter genes that are only expressed in AM roots and that expression is localized in arbuscule-containing cells, the presumed site of P transfer to the plant (Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002). It seems highly likely that other AM plants will be found to possess similar genes. The microarray approaches currently being used to study gene expression during the formation of ectomycorrhizas will likely turn up similar genes in these systems. The results may allow us to define mycorrhizas as mutualisms on a functional basis at the level of reciprocal transfer of nutrients or regulation of genes that reflect symbiotic processes.

For both AM and EcM symbioses we take it as axiomatic that the fungi, growing in symbiosis, receive organic C from autotrophic partners (see previous section). This may also be true for ErM, but not for myco-heterotrophs, and the latter must be described as parasitic interactions unless, in the future, fungal benefit can be proved. We could define mycorrhizas in autotrophic plants on a functional basis as associations where we can detect some mineral nutrient absorbed from the soil by hyphae and subsequently transferred to plant cells (or identify a cellular change closely correlated with transfer). We could modify this, as discussed previously, to take into account seasonal and ontogenetic changes. We need to ask several questions: Does this approach encompass the whole range of possible benefits conferred by the symbionts on each other? Is it practical to determine reciprocal nutrient movements or related changes, rather than attempt to establish mutual increase in fitness? Would identification of mutualism at the cellular level for a particular situation have value in predicting likely outcomes at other scales? What measurements are appropriate?

Although it would be technically challenging to obtain evidence for reciprocal nutrient flows in the field, it is more workable for tree species than the current definition, which requires demonstration of increased fitness. It is certainly feasible for relatively small plants, and coordinated studies of gene regulation and nutrient movements might very well allow us to verify the proposal. A disadvantage of a very

precise physiological definition is that it removes the requirement that the transfer of nutrients result in increased growth or fitness to either symbiont and, therefore, avoids the strict definition of mutualism. It would not directly detect mutualism expressed only at community levels or in competition, although it might provide evidence that benefits at that level were a possibility. It also means that if either symbiont benefits from non-nutritional outcomes of symbiosis (such as increased resistance or tolerance to pathogens, or improved water relations) then these would not be included in the definition unless markers for these effects could be identified. Nevertheless, we believe that function at the cellular level is worth exploring. The demonstration on the one hand that even nonresponsive plants may receive nutrients directly from their fungal symbionts, and that at the same time mycorrhiza-specific genes encoding transport related molecules are likely to be expressed, does provide compelling evidence that highly regulated and coordinated symbiotic processes in both organisms come into play as mycorrhizas develop.

Conclusions

This paper describes how any attempt at a functional definition of a mycorrhiza is fraught with difficulties. If we consider a definition at the cellular level that relates to a specific function such as nutrient transfer, we run into the problem that the transfer from the fungus to the plant may not increase whole-plant nutrient uptake (Smith et al. 2003*b*). Furthermore, increased nutrient uptake does not always result in increased growth, and growth response is not always correlated with a response in fitness, either at the single-species or even perhaps at the community levels. An increase in fitness is the ultimate test if we are to continue to define mycorrhizas as mutualisms. An alternate approach is to accept that benefits to both plant and fungus are likely to vary extensively depending on the genotypes of the fungus and plant and on the environment in which they live. It is highly likely that some plants or fungi are cheaters (Smith and Smith 1996; see Egger and Hibbett 2004), but it is probably impossible to determine whether a specific association benefits the symbionts under field conditions, especially if the plant is a long-lived perennial. Mycorrhizal symbioses may benefit the symbionts at different times during their lifespan, and possibly, for very short periods. This takes the question full circle. If increased nutrient uptake does not ultimately result in improved fitness, the symbiosis should not be considered a benefit to the plant. The solution may be to define mycorrhizas on a structural or developmental basis and to abrogate the requirement that mutualism be present in order for a symbiosis to be considered mycorrhizal.

In any event, mycorrhizal symbioses are acknowledged as being widespread both in diverse biomes and diverse plant species. To persist for what seems likely to be the whole evolutionary history of life on land (Nicolson 1975; Redecker et al. 2000) argues strongly for selective advantages of the symbioses in terms of fitness for both partners. One has only to compare the stable mycorrhizal state with the situation in rapidly evolving interactions between plants and their fungal pathogens, leading both to resistance and to specificity, to realize that we are dealing with very different symbioses indeed. And finally, we should consider some words of wisdom, for which we have only anecdotal memories: Jack Harley would have said, "it does not matter how you define [a mycorrhiza], what matters is how it functions at cellular, whole plant and ecosystem levels". Our challenge is to determine those functions.

Acknowledgements

We thank Jim Graham, David Janos, Iver Jakobsen, John Klironomos, Michelle Schroeder, Andrew Smith, and Tim Cavagnaro for supplying preprints or unpublished data and David Eissenstat, David Janos, Roger Koide, David Read, and Andrew Smith for helpful discussions. The authors wish to thank the Natural Sciences and Engineering Research Council of Canada (NSERC; M.D.J.) and the Australian Research Council (S.E.S.) for financial support.

References

Abbott, L.K., and Robson, A.D. 1984. The effect of VA mycorrhizae on plant growth. *In* VA mycorrhiza. *Edited by* C.L. Powell and D.J. Bagyaraj. CRC Press, Inc., Boca Raton, Fla. pp. 113–130.

Abuarghub, S.M., and Read, D.J. 1988*a*. The biology of mycorrhiza in the Ericaceae XI. The distribution of nitrogen in soil of a typical upland callunetum with special reference to the free amino acids. New Phytol. **108**: 425–432.

Abuarghub, S.M., and Read, D.J. 1988*b*. The biology of mycorrhiza in the Ericaceae XII. Quantitative analysis of individual free amino acids in relation to time and depth in the soil profile. New Phytol. **108**: 433–442.

Abuzinadah, R.A., and Read, D.J. 1989*a*. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. IV. The utilization of peptides by birch (*Betula pendula* L.) infected with different mycorrhizal fungi. New Phytol. **112**: 55–60.

Abuzinadah, R.A., and Read, D.J. 1989*b*. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. V. Nitrogen transfer in birch (*Betula pendula*) grown in association with mycorrhizal and non-mycorrhizal fungi. New Phytol. **112**: 61–68.

Adjoud, D., Plenchette, C., and Halli-Hargas, R. 1996. Response of 11 eucalyptus species to inoculation with three arbuscular mycorrhizal fungi. Mycorrhiza, **6**: 129–135.

Alexander, C., and Hadley, G. 1985. Carbon movement between host and mycorrhizal endophyte during development of the orchid *Goodyera repens* Br. New Phytol. **101**: 657–665.

Allen, M.F. 1987. Re-establishment of mycorrhizas on Mount St. Helens: migration vectors. Trans. Br. Mycol. Soc. **88**: 413–417.

Allen, M.F. 1991. The ecology of mycorrhizae. Cambridge University Press, Cambridge, UK.

Amaranthus, M.P., and Perry, D.A. 1994. The functioning of ectomycorrhizal fungi in the field: linkages in space and time. Plant Soil, **159**: 133–140.

Ashford, A.E., Allaway, W.G., Peterson, C.A., and Cairney, J.W.G. 1989. Nutrient transfer and the fungus–root interface in sheathing mycorrhizas. J. Plant Physiol. **16**: 85–97.

Augé, R.M. 2001. Water relations, drought and vesicular–arbuscular mycorrhizal symbiosis. Mycorrhiza, **11**: 3–42.

Bago, B., Pfeffer, P.E., Zipfel, W., Lammers, P.J., and Shachar-Hill, Y. 2002. Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Plant Soil, **244**: 189–197.

Bajwa, R., Abuarghub, S., and Read, D.J. 1985. The biology of mycorrhiza in the Ericaceae. X. The utilization of proteins and the production of proteotypic enzymes by the mycorrhizal endophyte and by mycorrhizal plants. New Phytol. **101**: 469–486.

Bajwa, R., and Read, D.J. 1985. The biology of mycorrhiza in the Ericaceae. IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytol. **101**: 459–467.

Bakonyi, G., Posta, K., Kiss, I., Fabian, M., Nagy, P., and Nosek, J.N. 2002. Density-dependent regulation of arbuscular mycorrhiza by collembola. Soil Biol. Biochem. **34**: 661–664.

Baxter, J.W., and Dighton, J. 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (*Betula populifolia*) seedlings in host–symbiont culture conditions. New Phytol. **152**: 139–149.

Baylis, G.T.S. 1975. The magnoloid mycorrhiza and mycotrophy in root systems derived from it. *In* Endomycorrhizas. *Edited by* F.E. Sanders, B. Mosse, and P.B. Tinker. Academic Press, London. pp. 373–384.

Bécard, G., and Piché, Y. 1989. Fungal growth stimulation by carbon dioxide and root exudates in vesicular–arbuscular mycorrhizal symbiosis. Appl. Environ. Microbiol. **55**: 2320–2325.

Bending, G.D., and Read, D.J. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behaviour and translocation of nutrients from exploited litter. New Phytol. **130**: 401–409.

Berch, S.M., Allen, T.R., and Berbee, M.L. 2002. Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Plant Soil, **244**: 55–66.

Bever, J.D., Pringle, A., and Schultz, P.A. 2002. Dynamics within the plant–arbuscular mycorrhizal fungal mutualism: testing the nature of community feedback. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 267–292.

Bidartondo, M.I., Redecker, D., Hijri, I., Wiemken, A., Bruns, T.D., Dominguez, L., Sersic, A., Leake, J.R., and Read, D.J. 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. Nature (Lond.), **419**: 389–392.

Bolan, N.S., Robson, A.D., and Barrow, N.J. 1983. Plant and soil factors including mycorrhizal infection causing sigmoidal response of plants to applied phosphorus. Plant Soil, **73**: 187–201.

Bougher, N.L., Grove, T.S., and Malajczuk, N. 1990. Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. New Phytol. **114**: 77–85.

Brady, N.C. 1974. The nature and properties of soils. 8th ed. Macmillan, New York.

Brown, M.S., Thamsurakul, S., and Bethlenfalvay, G.J. 1988. The *Glycine–Glomus–Bradyrhizobium* symbiosis. VIII. Phosphorus-use efficiency of CO_2 and N_2 fixation in mycorrhizal soybean. Physiol. Plant. **74**: 159–165.

Bryla, D.R., and Koide, R.T. 1990. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. II. Eight wild accessions and two cultivars of *Lycopersicon esculentum* Mill. Oecologia, **84**: 82–92.

Bücking, H., and Heyser, W. 2000. Subcellular compartmentation of elements in non-mycorrhizal and mycorrhizal roots of *Pinus sylvestris*: an X-ray microanalytical study. I. The distribution of phosphate. New Phytol. **145**: 311–320.

Bücking, H., and Heyser, W. 2001. Microautoradiographic localization of phophate and carbohydrates in mycorrhizal roots of *Populus tremula* × *Populus alba* and the implications for transfer processes in ectomycorrhizal associations. Tree Physiol. **21**: 101–107.

Bücking, H., Kuhn, A.J., Schroeder, W.H., and Heyser, W. 2002. The fungal sheath of ectomycorrhizal pine roots: An apoplastic barrier for the entry of calcium, magnesium, and potassium into the root cortex? J. Exp. Bot. **53**: 1659–1669.

Burges, A. 1936. On the significance of mycorrhiza. New Phytol. **35**: 117–131.

Burgess, T., Dell, B., and Malajczuk, N. 1994. Variation in mycorrhizal development and growth stimulation by 20 *Pisolithus* isolates inoculated on to *Eucalyptus grandis* W. Hill ex Maiden. New Phytol. **127**: 731–739.

Carey, P.D., Fitter, A.H., and Watkinson, A.R. 1992. A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. Oecologia, **90**: 550–555.

Cavagnaro, T.R., Smith, F.A., Hay, G., Carne-Cavagnaro, V.L., and Smith, S.E. 2004. Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytol. 161: 485–494.

Chapin, F.S. 1980. Mineral nutrition of wild plants. Annu. Rev. Ecol. Syst. **11**: 233–260.

Clapperton, M.J., and Reid, D.M. 1992. A relationship between plant growth and increasing VA mycorrhizal inoculum density. New Phytol. **120**: 227–234.

Copeman, R.H., Martin, C.A., and Stutz, J.C. 1996. Tomato growth in response to salinity and mycorrhizal fungi from saline or nonsaline soils. Hortscience, **31**: 341–344.

Daft, M.J., and Okusanya, B.O. 1973. Effect of *Endogone* mycorrhiza on plant growth. VI. Influence of infection on the anatomy and reproductive development in four hosts. New Phytol. **72**: 1333–1339.

de Bary, A. 1887. Comparative morphology and biology of the mycetozoa and bacteria. Clarendon Press, Oxford.

Dehne, H.W. 1982. Interactions between vesicular–arbuscular mycorrhizal fungi and plant pathogens. Phytopathology, **72**: 1115–1119.

Dosskey, M.G., Linderman, R.G., and Boersma, L. 1990. Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas. New Phytol. **115**: 269–274.

Duchesne, L.C., Peterson, R.L., and Ellis, B.E. 1989. The time-course of disease suppression and antibiosis by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol. **111**: 693–698.

Duddridge, J.A., Malibari, A., and Read, D.J. 1980. Structure and function of mycorrhizal rhizomorphs with special reference to their role in water transport. Nature (Lond.), **287**: 834–836.

Egerton-Warburton, L., and Allen, M.F. 2001. Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization and effects on seedling growth. Mycorrhiza, **11**: 283–290.

Egger, K.N., and Hibbett, D.S. 2004. The evolutionary implications of exploitation in mycorrhizas. Can. J. Bot. **82**: 1110–1121.

Eissenstat, D.M., Graham, J.H., Syvertsen, J.P., and Drouillard, D.L. 1993. Carbon economy of sour orange in relation to mycorrhizal colonization and phosphorus status. Ann. Bot. **71**: 1–10.

Erland, S., Söderström, B., and Andersson, S. 1990. Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. New Phytol. **115**: 683–688.

Facelli, E., Facelli, J.M., Smith, S.E., and Mclaughlin, M.J. 1999. Interactive effects of arbuscular mycorrhizal symbiosis, intraspecific competition and resource availability in *Trifolium subterraneum* cv. Mt. Baker. New Phytol. **141**: 535–547.

Fortin, J.A., Becard, G., Declerck, S., Dalpe, Y., St-Arnaud, M., Coughlan, A. P., and Piche, Y. 2002. Arbuscular mycorrhiza on root-organ cultures. Can. J. Bot. **80**: 1–20.

Founoune, H., Duponnois, R., and Bâ, A.M. 2002. Ectomycorrhization of *Acacia mangium* Willd., and *Acacia holosericea*, A. Cunn. ex G. Don in Senegal. Impact on plant growth, populations of indigenous symbiotic microorganisms and plant parasitic nematodes. J. Arid Environ. **50**: 325–332.

Frank, B. 1885. On the root-symbiosis-depending nutrition through hypogeous fungi of certain trees. Translated by J.M. Trappe 1984. In Proceedings of the 6th North American Conference on Mycorrhizae, Bend, Oregon, 25–29 June 1984. *Edited by* R. Molina. Forest Research Laboratory, Corvallis, Ore. pp. 18–25.

Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. **128**: 197–210.

Garbaye, J., and Churin, J.-L. 1997. Growth stimulation of young oak plantations inoculated with the ectomycorrhizal fungus *Paxillus involutus* with special reference to summer drought. For. Ecol. Manage. **98**: 221–228.

Gardes, M. 2002. An orchid–fungus marriage: physical promiscuity, conflict and cheating. New Phytol. **154**: 4–7.

Garrett, S.D. 1960. The biology of root infecting fungi. Cambridge University Press, Cambridge, UK.

Gaur, A., and Adholeya, A. 1999. Mycorrhizal effects on the acclimatization, survival, growth and chlorophyll of micropropagated *Syngonium* and *Draceana* inoculated at weaning and hardening stages. Mycorrhiza, **9**: 215–219.

Gianinazzi-Pearson, V., Smith, S.E., Gianinazzi, S., and Smith, F.A. 1991 Enzymatic studies on the metabolism of vesicular–arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolysing enzyme activities in plant–fungus interfaces? New Phytol. **117**: 61–74.

Graham, J.H. 2000. Assessing costs of arbuscular mhycorrhizal symbiosis in agroecosystems. *In* Current advances in mycorrhizal research. *Edited by* G.KI. Podila and D.D. Douds, Jr. APS Press, St. Paul, Minn. pp. 127–140.

Graham, J.H., and Eissenstat, D.M. 1998. Field evidence for the carbon cost of citrus mycorrhizas. New Phytol. **140**: 103–110.

Graham, J.H., Duncan, L.W., and Eissenstat, D.M. 1997. Carbohydrate allocation patterns in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency. New Phytol. **135**: 335–343.

Grange, O., Bartschi, H., and Gay, G. 1997. Effect of the ectomycorrhizal fungus *Hebeloma cylindrosporum* on in vitro rooting of micropropagated cuttings of arbuscular mycorrhiza-forming *Prunus avium* and *Prunus cerasus*. Trees (Berl.), **12**: 49–56.

Grime, J.P. 1979. Plant strategies and vegetation processes. Wiley Chichester, New York.

Grime, J.P., Mackey, J.M.L., Hillier, S.H., and Read, D.J. 1987. Floristic diversity in a model system using experimental microcosms. Nature (Lond.), **328**: 420–422.

Hagerman, S.M., Jones, M.D., Bradfield, G.E., Gillespie, M., and Durall, D.M. 1999. Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Can. J. For. Res. **29**: 124–134.

Harley, J.L. 1969. The biology of mycorrhiza. 2nd ed. Leonard Hill, London.

Harley, J.L. 1984. Mycorrhiza: the first 65 years; from the time of Frank until 1950. *In* Proceedings of the 6th North American Conference on Mycorrhizae, Bend, Oregon, 25– 29 June 1984. *Edited by* R. Molina. Forest Research Laboratory, Corvallis, Ore. pp. 26–33.

Harley, J.L., and McCready, C.C. 1952. The uptake of phosphate by excised mycorrhizal roots of the beech. II. Distribution of phosphorus between beech and fungus. New Phytol. **51**: 56–64.

Harrison, M.J., Dewbre, G.R., and Liu, J.Y. 2002 A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell, **14**: 2413–2429.

Hart, M., and Klironomos, J.N. 2002. Diversity of arbuscular mycorrhizal fungi and ecosystem functioning. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 225–242.

Hartnett, D.C., and Wilson, G.W. 2002. The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. Plant Soil, **244**: 319–331.

Hatch, A.B. 1936. The role of mycorrhizae in afforestation. J. For. **34**: 22–29.

Hatch, A.B. 1937. The physical basis of mycotrophy in *Pinus*. Black Rock For. Bull. **6**: 1–168.

Helgason, T., Merryweather, J.W., Denison, J., Wilson, P., Young, J.P.W., and Fitter, A.H. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occuring fungi and plants from a temperate deciduous woodland. J. Ecol. **90**: 371–384.

Heppell, K.B., Shumway, D.L., and Koide, R.T. 1998. The effect of mycorrhizal infection of *Abutilon theophrasti* on competitiveness of offspring. Funct. Ecol. **12**: 171–175.

Hetrick, B.A.D., Wilson, G.W.T., and Leslie, J.F. 1991. Root architecture of warm- and cool-season grasses: relationship to mycorrhizal dependence. Can. J. Bot. **69**: 112–118.

Hodge, A. 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. New Phytol. **157**: 303–314.

Högberg, M.N., and Högberg, P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol. **154**: 791–795.

Jakobsen, I. 1995. Transport of phosphorus and carbon in VA mycorrhizas. *In* Mycorrhiza: structure, function, molecular biology and biotechnology. *Edited by* A. Varma and B. Hock. Springer-Verlag, Berlin. pp. 297–324.

Jakobsen, I., Abbott, L.K., and Robson, A.D. 1992. External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of ^{32}P over defined distances. New Phytol. **120**: 509–516.

Jakobsen, I., Smith, S.E., and Smith, F.A. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 75–92.

Janos, D.P. 1987. VA mycorrhizas in humid tropical ecosystems. *In* VA mycorrhizae: an ecophysiological approach. *Edited by* G. Safir. CRC Press, Boca Raton, Fla. pp. 107–134.

Janos, D.P., Schroeder, M.S., Schaffer, B., and Crane, J.H. 2001. Inoculation with arbuscular mycorrhizal fungi enhances growth of *Litchi chinensis* Sonn. trees after propagation by air-layering. Plant Soil, **233**: 85–94.

Johnson, N.C., Graham, J.H., and Smith, F.A. 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol. **135**: 575–585.

Joner, E.J., Ravnskov, S., and Jakobsen, I. 2000. Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. Biotechnol. Lett. **22**: 1705–1708.

Jones, M.D., Durall, D.M., and Tinker, P.B. 1990. Phosphorus relationships and production of extramatrical hyphae by two types of willow ectomycorrhiza along a soil phosphorus gradient. New Phytol. **115**: 259–267.

Jones, M.D., Durall, D.M., and Tinker, P.B. 1991. Fluxes of carbon and phosphorus between symbionts in willow ectomycorrhizas and their changes with time. New. Phytol. **119**: 99–106.

Jones, M.D., Durall, D.M., and Cairney, J.W.G. 2003. Tansley review: ectomycorrhizal fungal communities in young stands regenerating after clearcut logging. New Phytol. **157**: 399–422.

Jonsson, L.M., Nilsson, M.-C., Wardle, D.A., and Zackrisson, O. 2001. Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. Oikos, **93**: 353–364.

Jothi, G., and Sundarababu, R. 2001. Management of root-knot nematode in brinjal by using VAM and crop rotation with green gram and pearl millet. J. Biol. Control, **15**: 77–80.

Jumpponen, A. 2001. Dark septate endophytes — Are they mycorrhizal? Mycorrhiza, **11**: 207–211.

Khasa, P., Furlan, V., and Fortin, J.A. 1992. Response of some tropical plant species to endomycorrhizal fungi under field conditions. Trop. Agric. **69**: 279–283.

Killham, K. 1994. Soil ecology. Cambridge University Press, Cambridge, UK.

Klironomos, J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology, **84**: 2292–2301.

Koch, K.E., and Johnson, C.R. 1984. Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and nonmycorrhizal root systems. Plant Physiol. **75**: 26–30.

Koide, R.T. 1991. Tansley Review No. 29. Nutrient demand and plant response to mycorrhizal infection. New Phytol. **117**: 365–386.

Koide, R.T. 2000. Functional complementarity in the arbuscular mycorrhizal symbiosis. New Phytol. **147**: 233–235.

Koide, R.T., and Elliott, G. 1989. Cost, benefit and efficiency of the vesicular–arbuscualr mycorrhizal symbiosis. Funct. Ecol. **3**: 252–255.

Koide, R.T., and Li, M. 1989. Appropriate controls for vesicular–arbuscular mycorrhiza research. New Phytol. **111**: 35–44.

Koide, R.T., and Lu, X. 1992. Mycorrhizal infection of wild oats: maternal effects on offspring growth and reproduction. Oecologia, **90**: 218–226.

Koide, R.T., and Lu, X. 1995. On the cause of offspring superiority conferred by mycorrhizal infection of *Abutilon theophrasti*. New Phytol. **131**: 435–441.

Koide, R., Li, M., Lewis, J., and Irby, C. 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants I. Wild vs. cultivated oats. Oecologia, **77**: 537–543.

Kothari, S.K., Marschner, H., and George, E. 1990. Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. New Phytol. **116**: 303–311.

Krebs, C.J. 2001. Ecology. 5th ed. Benjamin Cummings, San Francisco.

Kytöviita, M.M., Vestberg, M., and Tuomi, J. 2003. A test of mutual aid in common mycorrhizal networks: established vegetation negates benefit in seedlings. Ecology, **84**: 898–906.

Lamhamedi, M.S., Godbout, C., and Fortin, J.A. 1994. Dependence of *Laccaria bicolor* basidiome development on current photosynthesis of *Pinus strobus* seedlings. Can. J. For. Res. **24**: 1797–1804.

Larsen, J., and Jakobsen, I. 1996. Effects of a mycophagous Collembola on the symbiosis between *Trifolium subterraneum* and three arbuscular mycorrhizal fungi. New Phytol. **133**: 295–302.

Last, F.T., Pelham, J., Mason, P.A., and Ingleby, K. 1979. Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp. Nature (Lond.), **280**: 168–169.

Leake, J.R. 1994. The biology of myco-heterotrophic ("saprophytic") plants. New Phytol. **127**: 171–216.

Leake, J.R., and Read, D.J. 1989. The biology of mycorrhiza in the Ericaceae. XIII. Some characteristics of the extracellular proteinase activity of the ericoid endophyte *Hymenoscyphus ericae*. New Phytol. **112**: 69–76.

Lei, J., and Dexheimer, J. 1988. Ultrastructural localization of ATPase activity in the *Pinus sylvestris*/*Laccaria laccata* ectomycorrhizal association. New Phytol. **108**: 329–334.

Lewis, D.H. 1973. Concepts in fungal nutrition and the origin of biotrophy. Biol. Rev. **48**: 261–278.

MacFall, J.S., and Slack, S.A. 1991. Effects of *Hebeloma arenosa* on growth of red pine seedlings in high-fertility nursery soil in Wisconsin. Can. J. For. Res. **21**: 482–488.

MacFall, J.S., Slack, S.A., and Iyer, J. 1991. Effects of *Hebeloma arenosa* and phosphorus fertility on growth of red pine (*Pinus resinosa*) seedlings. Can. J. Bot. **69**: 372–379.

Mackenzie, A., Ball, A.S., and Virdee, S.R. 1998. Instant notes in ecology. Bios, Oxford.

Maldonado-Mendoza, I.E., Dewbre, G.R., and Harrison, M.J. 2001. A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol. Plant–Microbe Interact. **14**: 1140–1148.

Martins, A., Barroso, J., and Pais, M.S. 1996. Effect of ectomycorrhizal fungi on survival and growth of micropropagated plants and seedlings of *Castanea sativa* Mill. Mycorrhiza, **6**: 265–270.

McComb, A.L. 1938. The relation between mycorrhizae and the development and nutrient absorption of pine seedlings in a prairie nursery. J. For. **36**: 1148–1154.

McKendrick, S.L., Leake, J.R., and Read, D.J. 2000. Symbiotic germination and development of myco-heterotrohic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytol. **145**: 539–548.

Melin, E. 1925. Investigations of the significance of tree mycorrhiza. An ecological– physiological study. Translated by P.W. Stickel, 1930. Edwards Brothers, Ann Arbor, Mich.

Melin, E., Nilsson, H., and Hacskaylo, E. 1958. Translocation of cations to seedlings of *Pinus virginiana* through mycorrhizal mycelium. Bot. Gaz. **119**: 243–246.

Merryweather, J., and Fitter, A. 1995*a*. Arbuscular mycorrhiza and phosphorus as controlling factors in the life history of *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. New Phytol. **129**: 629–636.

Merryweather, J., and Fitter, A. 1995*b*. Phosphorus and carbon budgets: mycorrhizal contribution in *Hyacinthoides non-scripta* (L.) Chouard ex Rothm. under natural conditions. New Phytol. **129**: 619–627.

Merryweather, J., and Fitter, A. 1996. Phosphorus nutrition of an obligately mycorrhizal plant treated with the fungicide benomyl in the field. New Phytol. **132**: 307–311.

Miller, S.L., McClean, T.M., Stanton, N.L., and Williams, S.E. 1998. Mycorrhization, physiognomy, and first-year survivability of conifer seedlings following natural fire in Grand Teton National Park. Can. J. For. Res. **28**: 115–122.

Miller, R.M., Miller, S.P, Jastrow, J.D., and Rivetta, C.B. 2002. Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. New Phytol. **155**: 149–162.

Mitchell, H.L., Finn, R.F., and Rosendahl, R.O. 1937. The relation between mycorrhizae and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock For. Pap. **1**: 58–73.

Molina, R., Massicotte, H.B., and Trappe, J.M. 1992. Ecological role of specificity phenomena in ectomycorrhizal plant communities: potential for interplant linkages and guild development. *In* Mycorrhizas in ecosystems. *Edited by* D.J. Read, D.H. Lewis,

A.H. Fitter, and I.J. Alexander. CAB International, Wallingford, UK. pp. 106–112.

Morrison, T.M. 1954. Uptake of phosphorus-32 by mycorrhizal plants. Nature (Lond.), **174**: 606–607.

Mosse, B. 1973. Advances in the study of vesicular arbuscular mycorrhiza. Annu. Rev. Phytopathol. **11**: 171–196.

Naqvi, N.S., and Mukerji, K.G. 1998. Mycorrhization of micropropagated *Leucaena leucocephala* (Lam.) de Wit. Symbiosis, **24**: 103–114.

Newsham, K.K., Fitter, A.H., and Watkinson, A.R. 1994. Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. J. Ecol. **82**: 805–814.

Newsham, K.K., Fitter, A.H., and Watkinson, A.R. 1995. Multifunctionality and biodiversity in arbuscular mycorrhizas. Trends Ecol. Evol. **10**: 407–411.

Nicolson, T.H. 1975. Evolution of vesicular–arbuscular mycorrhizas. *In* Endomycorrhizas. *Edited by* F.E. Sanders, B. Mosse, and P.B. Tinker. Academic Press, London. pp. 25–34.

Nielsen, J.S., Joner, E.J., Declerck, S., Olsson, S., and Jakobsen, I. 2002. Phospho-imaging as a tool for visualization and noninvasive measurement of P transport dynamics in arbuscular mycorrhizas. New Phytol. **154**: 809–819.

Normand, L., Bartschi, H., Debaud, J.-C., and Gay, G. 1996. Rooting and acclimatization of micropropagated cuttings of *Pinus pinaster* and *Pinus sylvestris* are enhanced by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. Physiol. Plant. **98**: 759–766.

O'Connor, P.J., Smith, S.E., and Smith, F.A. 2002. Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. New Phytol. **154**: 209–218.

Ogawa, M. 1985. Ecological characters of ectomycorrhizal fungi and their mycorrhizae. Jpn. Agric. Res. Q. **18**: 305–314.

Oliver, A.J., Smith, S.E., Nicholas, D.J.D., Wallace, W., and Smith, F.A. 1983. Activity of nitrate reductase in *Trifolium subterraneum*: effects of mycorrhizal infection and phosphate nutrition. New Phytol. **94**: 63–79.

Paszkowski, U., Kroken, S., Roux, C., and Briggs, S. P. 2002. Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc. Natl. Acad. Sci. U.S.A. **99**: 13324–13329.

Pearson, J.N., and Jakobsen, I. 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants measured by dual labelling with ^{32}P and ^{33}P. New Phytol. **124**: 489–494.

Pearson, V., and Read, D.J. 1973. The biology of mycorrhiza in the Ericaceae. II. The transport of carbon and phosphorus by the endophyte and the mycorrhiza. New Phytol. **72**: 1325–1331.

Peng, S., Eissenstat, D.M., Graham, J.H., Williams, K., and Hodge, N.C. 1993. Growth depression in mycorrhizal citrus at high-phosphorus supply. Plant Physiol. **101**: 1063–1071.

Perotto, S., Actis-Perino, E., Perugini, J., and Bonfante, P. 1996. Molecular diversity of fungi from ericoid mycorrhizal roots. Mol. Ecol. **5**: 123–131.

Perry, D.A., Margolis, H., Choquette, C., Molina, R., and Trappe, J.M. 1989. Ectomycorrhizal mediation of competition between coniferous tree species. New Phytol. **112**: 501–511.

Peterson, R.L., and Massicotte, H. 2004. Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. Can. J. Bot. **82**: 1074–1088.

Pfeffer, P.E., Douds, D.D., Bécard, G., and Shachar-Hill, Y. 1999. Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol. **120**: 587–598.

Philip, L.J., Posluszny, U., and Klironomos, J.N. 2001. The influence of mycorrhizal colonization on the vegetative growth and sexual reproductive potential of *Lythrum salicaria* L. Can. J. Bot. **79**: 381–388.

Quoreshi, A.M., and Timmer, V.R. 2000. Early outplanting performance of nutrient-loaded containerized black spruce seedlings inoculated with *Laccaria bicolor*: a bioassay study. Can. J. For. Res. **30**: 744–752.

Rasmussen, N., Lloyd, D.C., Ratcliffe, R.G., Hansen, P.E., and Jakobsen, I. 2000. ^{31}P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. Plant Soil, **226**: 245–253.

Rausch, C., Darram, P., Brunner, S., Jansa, J., Laloi, M., Leggewie, G., Amrhein, N., and Bucher, M. 2001. A phosphate transporter expressed in arbuscule-containing cells in potato. Nature (Lond.), **414**: 462–470.

Rayner, M.C. 1938. The use of soil or humus inocula in nurseries and plantations. Emp. For. J. **17**: 236–243.

Redecker, D., Kodner, R., and Graham, L.E. 2000. Glomalean fungi from the Ordovician. Science (Washington, D.C.), **289**: 1920–1921.

Reid, C.P.P., Kidd, F.A., and Ekwebelam, S.A. 1983. Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. Plant Soil, **71**: 415–432.

Rice, V., and Currah, R.S. 2002. New perspectives on the niche and holomorph of the myxotrichoid hyphomycete, *Oidiodendron maius*. Mycol. Res. **106**: 1463–1467.

Roldan-Fajardo, B.E. 1994. Effect of indigenous arbuscular mycorrhizal endophytes on the development of six wild plants colonizing a semi-arid area in south-east Spain. New Phytol. **127**: 115–121.

Romell, L.-G. 1938. A trenching experiment in spruce forest and its bearing on problems of mycotrophy. Sven. Bot. Tidskr. **32**: 89–99.

Sanders, I.R. 2002. Specificity in the arbuscular mycorrhizal symbiosis. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 416–437.

Sanders, I.R., and Fitter, A.H. 1992. The ecology and functioning of vesicular–arbuscular mycorrhizas in co-existing grassland species I. Seasonal patterns of mycorrhizal occurrence and morphology. New Phytol. **120**: 517–524.

Sanders, I.R., Koide, R.T., and Shumway, D.L. 1993. Mycorrhizal stimulation of plant parasitism. Can. J. Bot. **71**: 1143–1146.

Schönbeck, F., and Dehne, H.W. 1979. The influence of endotrophic mycorrhiza on plant diseases. 4. Fungal parasitism on aerial plant parts *Opidium brassicae* Tobacco Mosiac Virus. Z. Pflanzenkr. Pflanzenschutz, **86**: 103–112.

Schweiger, P.F., and Jakobsen, I. 1999. Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. Agron. J. **91**: 998–1002.

Shachar-Hill, Y., Pfeffer, P.E., Douds, D., Osman, S.F., Doner, L.W., and Ratcliffe, R.G. 1995. Partioning of intermediary carbon metabolism in vesicular–arbuscular mycorrhizal leek. Plant Physiol. **108**: 7–15.

Shumway, D.L., and Koide, R.T. 1994. Within-season variability in mycorrhizal benefit to reproduction in *Abutilon theophrasti* Medic. Plant Cell Environ. **17**: 821–827.

Shumway, D.L., and Koide, R.T. 1995. Size and reproductive inequality in mycorrhizal and nonmycorrhizal populations of *Abutilon theophrasti*. J. Ecol. **83**: 613–620.

Simard, S.W., Perry, D.A., Smith, J.E., and Molina, R. 1997. Effects of soil trenching on occurrence of ectomycorrhizas on *Pseudotsuga menziesii* seedlings grown in mature forests of *Betula papyrifera* and *Pseudotsuga menziesii*. New Phytol. **136**: 327–340.

Simard, S.W., Jones, M.D., and Durall, D.M. 2002. Carbon and nutrient fluxes within and between mycorrhizal plants. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 33–74.

Smith, S.E. 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev. **55**: 475–510.

Smith, S.E. 1982. Inflow of phosphate into mycorrhizal and non-mycorrhizal plants of *Trifolium subterraneum* at different levels of soil phosphate. New Phytol. **90**: 293–303.

Smith, S.E., and Read, D.J. 1997. Mycorrhizal symbiosis. Academic Press, San Diego.

Smith, S.E., and Smith, F.A. 1990. Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytol. **114**: 1–38.

Smith, F.A., and Smith, S.E. 1996. Mutualism and parasitism: diveristy in function and structure in the "arbuscular" (VA) mycorrhizal symbiosis. Adv. Bot. Res. **22**: 1–43.

Smith, S.E., St. John, B.J., Smith, F.A., and Bromley, J.L. 1986. Effects of mycorrhizal infection on plant growth, nitrogen and phosphorus nutrition of glasshouse-grown *Allium cepa* L. New Phytol. **103**: 359–373.

Smith, F.A., Jakobsen, I., and Smith, S.E. 2000. Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. New Phytol. **147**: 357–366.

Smith, F.A., Smith, S.E., and Timonenen, S. 2003*a*. Mycorrhizas. *In* Root ecology. Ecol. Stud. **168**: 257–295.

Smith, S.E., Smith, F.A., and Jakobsen, I. 2003*b*. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol. **133**: 16–20.

Son, C.L., and Smith S.E. 1988. Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. New Phytol. **108**: 305–314.

Staddon, P.L., Fitter, A.H., and Robinson, D. 1999. Effects of mycorrhizal colonization and elevated atmospheric carbon dioxide on carbon dioxide fixation and below-ground carbon partitioning in *Plantago lanceolata*. J. Exp. Bot. **50**: 853–860.

Stanier, R.Y., Doudoroff, M., and Adelberg, E.A. 1971. General microbiology. 3rd ed. Macmillan Education, London.

Stanley, M.R., Koide, R.T., and Shumway, D.L. 1993. Mycorrhizal symbiosis increases growth, reproduction and recruitment of *Abutilon theophrasti* Medic. plants grown for two generations in the field. New Phytol. **128**: 219–224.

Starrett, M.C., Blazich, F.A., Shafer, S.R., and Grand, L.F. 2001. In vitro colonization of micropropagated *Pieris floribunda* by ericoid mycorrhizae. II. Effects on acclimatization and growth. Hortscience, **36**: 357–359.

Stenström, E., Ek, M., and Unestam, T. 1990. Variation in field response of *Pinus sylvestris* to nursery inoculation with four different ectomycorrhizal fungi. Can. J. For. Res. **20**: 1796–1803.

Stone, E.L., Jr. 1949. Some effects of mycorrhizae on the phosphorus nutrition of Monterey pine seedlings. Soil Sci. Soc. Am. Proc. **14**: 340–345.

Streitwolf-Engel, R., Boller, T., Wiemken, A., and Sanders, I.R. 1997. Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. J. Ecol. **85**: 181–191.

Stribley, D.P., and Read, D.J. 1974. The biology of mycorrhiza in the Ericaceae. III. Movement of carbon-14 from host to fungus. New Phytol. **73**: 731–741.

Stribley, D.P., Tinker, P.B., and Rayner, J.H. 1980*a*. Relation of internal phosphorus concentration and plant weight in plants infected by vesicular–arbuscular mycorrhizas. New Phytol. **86**: 261–266.

Stribley, D.P., Tinker, P.B., and Snellgrove, R.C. 1980*b*. Effect of vesicular–arbuscular mycorrhizal fungi on the relations of plant growth, internal phosphorus concentration and soil phosphate analyses. Soil Sci. **31**: 655–672.

Sylvia, D.M., and Sinclair, W.A. 1983. Suppressive influence of *Laccaria laccata* on *Fusarium oxysporum* and on Douglas-fir (*Pseudotsuga menziesii*) seedlings. Phytopathology, **73**: 384–389.

Taylor, D.L., Bruns, T.D., Leake, J.R., and Read, D.J. 2002. Mycorrhizal specificity and function in myco-heterotrophic plants. *In* Mycorrhizal ecology. *Edited by* M.G.A. van der Heijden and I. Sanders. Springer-Verlag, Berlin. pp. 375–413.

Tinker, P.B., and Nye, P.H. 2000. Solute movement in the rhizosphere. Oxford University Press, New York.

Tinker, P.B., Durall, D.M., and Jones, M.D. 1994. Carbon use efficiency in mycorrhizas: theory and sample calculations. New Phytol. **128**: 115–122.

Trappe, J.M. 1994. What is a mycorrhiza? *In* Proceedings of the Fourth European Symposium on Mycorrhizas, Granada, Spain, 11–14 July 1994. *Edited by* C. Azcon-Aguilar and J.M. Barea. The European Commission, Luxembourg. pp. 3–6.

Trent, J.D., Svejcar, T.J., and Christiansen, S. 1989. Effects of fumigation on growth, photosynthesis, water relations and mycorrhizal development of winter wheat in the field. Can. J. Plant Sci. **69**: 535–540.

Tuffen, F., Eason, W.R., and Scullion, J. 2002. The effect of earthworms and arbuscular mycorrhizal fungi on growth of and ^{32}P transfer between *Allium porrum* plants. Soil Biol. Biochem. **34**: 1027–1036.

Urcelay, C., and Diaz, S. 2003. The mycorrhizal dependence of subordinates determines the effect of arbuscular mycorrhizal fungi on plant diversity. Ecol. Lett. **6**: 388–391.

van der Heijden, M.G.A., Boller, T., Wiemken, A., and Sanders, I.R. 1998*a*. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology, **76**: 2082–2091.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., and Sanders, I.R. 1998*b*. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature (Lond.), **396**: 69–72.

Vesk, P.A., Ashford, A.E., Markovina, A.-L,., and Allaway, W.G. 2000. Apoplasmic barriers and their significance in the exodermis and sheath of *Eucalyptus pilularis – Pisolithus tinctorius* ectomycorrhizas. New Phytol. **145**: 333–346.

Vogt, K.A., Grier, C.C., Meier, C.E., and Edmonds, R.L. 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington USA. Ecology, **63**: 370–380.

Wallander, H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ecto-mycorrhizal fungi. Plant Soil, **218**: 249–256.

Wallander, H., Nilsson, L.O., Hagerberg, D., and Bååth, E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. New Phytol. **151**: 753–760.

West, H.M., Fitter, A.H., and Watkinson, A.R. 1993. The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* ssp. *ambigua* under natural conditions. J. Ecol. **81**: 345–350.

Wilson., G.W.T., and Hartnett, D.C. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. Am. J. Bot. **85**: 1732–1738.

Wright, D.P., Scholes, J.D., and Read, D.J. 1998. Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. Plant Cell Environ. **21**: 209–216.

Wright, W., Fitter, A., and Meharg, A. 2000. Reproductive biomass in *Holcus lanatus* clones that differ in their phosphate uptake kinetics and mycorrhizal colonization. New Phytol. **146**: 493–501.

Xavier, L.J.C., and Germida, J.L. 2003. Selective interactions between arbuscular mycorrhizal fungi and *Rhizobium leguminosarum* bv. *viceae* enhance pea yield and nutrition. Biol. Fertil. Soils, **37**: 261–267.

Young, H.E. 1936. A mycorrhiza-forming fungus of *Pinus*. Aust. Inst. Agr. Sci. **2**: 32–34.

Zak, J.C., McMichael, B., Dhillion, S., and Friese, C. 1998. Arbuscular-mycorrhizal colonization dynamics of cotton (*Gossypium hirsutum* L.) growing under several production systems on the Southern High Plains, Texas. Agric. Ecosyst. & Environ. **68**: 245–254.

Zhou, M., and Sharik, T.L. 1997. Ectomycorrhizal associations of northern red oak (*Quercus rubra*) seedlings along an environmental gradient. Can. J. For. Res. **27**: 1705–1713.

Zhu, Y.G., Smith, F.A., and Smith, S.E. 2003. Phosphorus efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. Mycorrhiza, **13**: 93–100.