8

Growth and carbon allocation of ectomycorrhizal symbionts

Introduction

Frank (1894) conducted experiments on the effects of ectomycorrhizal (ECM) colonization on the growth of seedlings of *Pinus*. Those which were grown in unsterilized soil developed mycorrhizas and grew faster than those in sterilized soil. Although this experimental design is faulty, because heat sterilization may release both nutrients and toxic substances, the results have in principle been repeatedly confirmed. Refinements aimed at obviating the effects of heat sterilization have been used and the results can be accepted with confidence. In addition, observations on the establishment of exotic species of ECM trees in many parts of the world have shown that artificial inoculation is usually essential to success, although non-mycorrhizal seedlings may be grown if provided with adequate fertilizers (see Chapter 17).

Surprisingly, while there have been innumerable reports of growth enhancement as a result of ECM colonization, relatively few reports have provided unequivocal evidence, in the form of nutrient response curves, of the benefits accruing to a plant from colonization when its growth is limited by a single nutrient. Some such studies are now available (e.g. Heinrich and Patrick, 1986; Bougher *et al.*, 1990; Jones *et al.*, 1990, 1998).

The study of Bougher *et al.* (1990) is particularly instructive in the overall context of growth response. *Eucalyptus diversicolor* plants, whose small seeds have little initial nutrient reserve, were grown in a sandy soil of low organic content which was given mild sterilization unlikely to produce unnatural side effects. Furthermore, the experiments examined responses to inoculation with four fungi (two isolates, A and B, of *Descolea maculata* and one each of *Laccaria laccata* and *Pisolithus tinctorius*) and to one element, P, the deficiency of which was known to be a major factor limiting growth in soils supporting the plant in nature. Ecological relevance was thus added to good experimental design and the results provide a useful example of the type of growth response that might be expected in the field.

The growth response curves of non-mycorrhizal plants in relation to soil P were of the sigmoid type, as seen typically in other coarse-rooted plants (Bolan *et al.*, 1983; Figure 8.1a). In these, there is a threshold P concentration in soil below which growth will not occur. Colonization by *D. maculata* A and B and *L. laccata* modified the sigmoidal growth curve by removing the threshold effect and allowing plants to

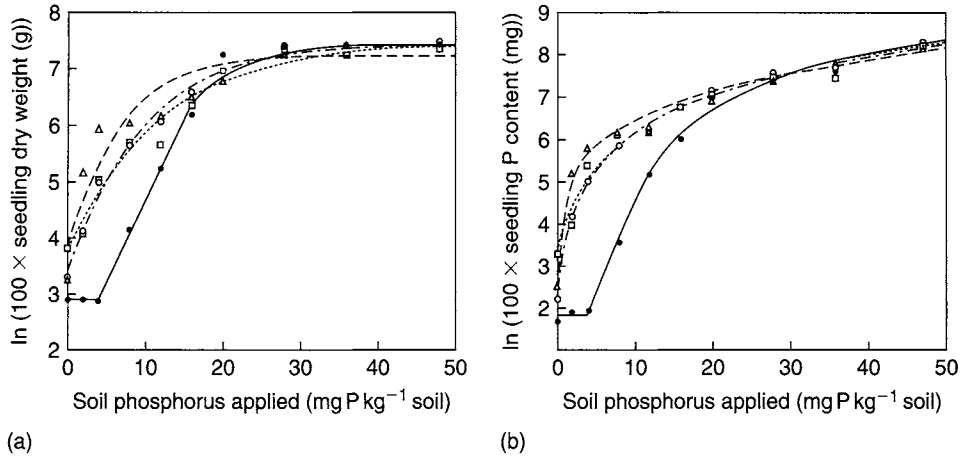

Figure 8.1 Growth and P uptake of *Eucalyptus diversicolor* in response to P application and mycorrhizal colonization by two strains of *Descolea maculata*, A (□) and B (○) or by *Laccaria laccata* (△), compared with uninoculated controls (●). (a) Dry weight; (b) P content. From Bougher *et al.* (1990), with permission.

grow in extremely nutrient-poor soil. This effect is believed to result from the ability of extraradical mycelium to exploit nutrients, in this case P, beyond depletion zones surrounding the root (see Chapter 5). The fungi may also have a lower threshold concentration for absorption of the element, or may have the ability to release P associated with complex substrates which would add to their ability to increase nutrient uptake (see Chapters 9 and 10).

In the range of P supply in which a growth response to ECM colonization occurred (2–12 mg P/kg soil) fungal isolates differed in their ability to promote seedling growth (Figure 8.1a). Dry weight of seedlings inoculated with *L. laccata* was significantly greater than that of plants colonized by *D. maculata* at 2 and 4 mg P/kg soil. At the latter concentration, biomass of *L. laccata* seedlings was nine times that of seedlings ECM with *D. maculata* and 21 times non-mycorrhizal plants. The pattern of P accumulation by plants in relation to soil P supply was similar to that of dry weight (Figure 8.1b) and again ECM colonization removed the threshold seen in uncolonized plants. However, seedling dry weight reached a maximum at around 28 mg P/kg soil, whereas P content increased linearly above this level of P application. ECM plants, in addition to showing no threshold, had higher tissue P concentrations than non-mycorrhizal plants even with no added P.

This example, in addition to demonstrating that ECM colonization has the potential to enhance growth, shows that it can fundamentally change the nature of growth response curves of plants at low nutrient concentrations. It further emphasizes that there may be considerable differences both between and within fungal species in their ability to acquire nutrients and promote growth. This biologically important observation also indicates that response curves comparing the performance of different ECM fungi can be used to predict more accurately their potential for application and effectiveness for nursery and afforestation practices (see Chapter 17). However, it must be borne in mind that this selected example describes responses to P when other nutrients were supplied in adequate amounts. Depending upon

Table 8.1 Growth of *Picea sitchensis* in axenic culture with *Lactarius rufus* over 14 weeks.

	Control	Inoculated	Significance of difference P<
Mycorrhizal colonization (%)	0	58.2 ± 9.9	
Shoot height (cm)	5.8	9.2	0.05
Shoot dry weight (mg)	47.1	100.2	0.01
Root dry weight (mg)	25.5	74.0	0.001
Total dry weight (mg)	72.6	174.2	0.001
Root–shoot ratio	0.59	1.76	NS
Number of lateral buds	4.4	5.9	NS
Length of lateral roots (cm)	98.2	219.0	0.01
Total number of root tips	60.8	236.7	0.05
Short root/cm lateral roots	0.64	1.17	NS
Short roots/mg weight	2.43	3.31	NS

Data from Alexander (1981). Substrate: vermiculite-peat with nutrient medium. The roots were kept below 25°C in a greenhouse. Midday irradiance 25W/m^2 approximately. NS, not significant.

the soil or ecosystem in which a plant occurs, other elements, in particular N (see Chapter 9), may be more important as growth limiting factors, and that often P and N may be co-limiting. Experiments investigating dose response curves in these different situations would be extremely instructive.

The difficulties of carrying out experiments with trees under controlled conditions have precluded extensive research except during a small part of their early life. In consequence, comparisons between ECM plants and controls have been made over one or a few seasons at most. Much work has also been done in open beds of soil, sometimes inoculated with soil, humus, or chopped mycorrhizas, with controls treated with sterilized inoculum (examples are given by Harley, 1969). In some cases, the soil has been sterilized by drenching or fumigation before planting. The published conclusions are simple: ECM seedlings are usually taller and have larger root systems, both shoots and roots are of greater dry weight but the ratios of root weight to shoot weight are very frequently smaller.

The change in the root:shoot ratio needs further investigation, for irrespective of mycorrhiza formation, the ratio diminishes during the early growth of the young seedling and is lower in soils of high nutrient availability, especially of N, as well as being sometimes reduced in conditions where photosynthesis is reduced. Although a lower ratio might be expected in mycorrhizal plants, both because of a greater ability to absorb nutrients and an increased concentration of nutrients in their tissues, it would be of great value to know more about the extent to which greater size and age themselves contribute to carbon (C) allocation in the seedlings. Alexander (1981) grew *Picea sitchensis* seedlings in monoxenic culture with *Lactarius rufus* for 14 weeks. The results are shown in Table 8.1. The usual increases of height and dry weight of both shoots and roots were observed, but the root:shoot ratio was greater (although below $P = 0.05$ significance) in ECM plants. In discussing this, Alexander observed that, if the fungal sheath were to comprise 20% of the weight of the roots, which is not impossible, the slight rise in root:shoot ratio might be explained by the fungal colonization directly. Bougher *et al.* (1990) also provided data relating root: shoot ratio of *E. diversicolor* to total seedling dry weight, which showed no differences in response to the fungi they employed and further, no differences between

ECM and non-mycorrhizal plants. This again emphasizes that effects on root:shoot ratio may be related less to colonization than to plant size.

Other observations of Alexander (1981) seem to confirm the much older results (e.g. McComb, 1938) that colonization increases the total number of short roots per seedling. In this case, the form of the root system changes, for there are more short roots both per centimetre of root length and per milligram of root weight on the ECM seedlings. There is a great need to disentangle the normal changes in form due to increase in size, those dependent on changes in nutrient supply and absorption, and those arising from ECM colonization and its physiological consequences. Moreover, although ECM colonization is known to increase growth rates of plants supplied with suboptimal levels of a major nutrient such as P (Bougher *et al.*, 1990), relatively few studies have examined the consequences of ECM development in plants receiving optimal supplies of nutrients. On theoretical grounds it is evident that under these circumstances, the benefits of colonization in the form of increased nutrient capture would be eliminated, yet the costs to the plant in terms of C supplied to the fungus for growth and respiration (quantified below) would remain. In fact, several studies (Molina and Chamard, 1983; Ingestad *et al.*, 1986; Rousseau and Reid, 1991) indicate that, while ECM plants suffered little or no growth reduction at adequate or supra-optimal nutrient supply, such reductions were experienced under lower nutrient regimes. A possible explanation for this apparent anomaly was sought by Rousseau and Reid (1991). They point out that plants in the higher nutrient regimes generally have lower root:shoot ratios relative to those under low regimes. By definition, plants with lower root:shoot ratio have more tissues that are able to gain rather than consume C. Therefore, even if equal quantities of root tissue were colonized in seedlings with different root:shoot ratios, those with the higher ratio would have proportionately more mycorrhizal development per plant than their counterparts with a low ratio of root to shoot. Thus, if an ECM fungus constituted 20% of the root system of a seedling with a 1:1 ratio of shoot to root, it would make up 10% of the dry weight; in cases where that ratio was 3:1, the weight of fungus would contribute only 5%. The relative C costs of supporting the fungus in a seedling with a 1:1 shoot root ratio could thus be double that of a seedling with a 3:1 ratio, even though the fungal biomass was the same in both cases. The data show that, in reality, the extent of C costs imposed by the fungus under high nutrient regimes may be further reduced by inhibition of development of the extraradical mycelium (Jones *et al.*, 1990; Wallander and Nylund, 1992; and see Chapters 4 and 5).

Results which show no effect of ECM colonization or even a decrease of growth rate may be found scattered through the literature, but are not often emphasized. They are important for two reasons. First, they indicate that ECM colonization does not invariably increase growth of the plant in size or weight, and that there is frequently an initial phase, sometimes inexplicably prolonged when the growth of ECM plants is similar or slower than non-mycorrhizal ones. Secondly, they often show that different combinations of plant and fungus are differently effective in growth, as already discussed for *Eucalyptus diversicolor*. Although different species of fungus were compared on a single species of plant, there is good evidence that different strains of a single fungal species also differ in their effects on a plant. Table 8.2 gives one example from the work of Marx (1979b) of the effect of different strains of *Pisolithus tinctorius* on *Quercus rubra* seedlings. Variability of this kind is exactly what would be expected from the genetic, physiological and biochemical variability

Table 8.2 Growth of *Quercus rubra* seedlings with different strains of *Pisolithus tinctorius*.

Fungal strain	Height (cm)	Fresh weight (g)	Percentage of ectomycorrhizal short roots
138	13.3	8.5[a]	72[a]
136	13.5	6.5[c]	15[b]
145	11.5	7.0[b]	1[c]
Control	12.5	6.7[c]	0

Data from Marx (1979b). Day temperature 28°C, night 22°C. Day length 14.5 h. Growth period four months. Height differences insignificant at $P = 0.05$. Within other columns common letters denote insignificance. Percentage of ectomycorrhizas estimated visually.

of ECM fungi. Similarly, different biotypes of a plant may react differently with a single strain of a fungal species (Marx and Bryan, 1971; Marx, 1979b), although research on this aspect has not gone far. Clearly, further information on both these topics is essential to the full elucidation of the physiological relationships between the symbionts and to the determination of the most efficient combinations of fungus and plant for the production of forest crops. Experimentation should aim at describing in detail the structure and function of ECM which appear to be associated with greater or lesser growth rate. Information concerning Hartig net development, sheath thickness and, particularly, the quantity and extent of extraradical mycelium needs to be collected from plants grown in near-natural conditions, in order to calculate the C demand of the fungi (see below). Some advances in this area have been discussed in Chapter 6. Equally important are physiological properties of the fungal strains, in particular economic coefficients such as mass of C used per mass of mycelium formed. By such analyses the recognition of effective symbionts could be put on a firm basis, as has been done for single plant–fungus combinations by Jones *et al.* (1991) and Rygiewicz and Anderson (1994; and see below).

In all this work, the growth of the plant was the main preoccupation of the experimenters because they have been concerned, however distantly, with its ecology or its use for human benefit. From the point of view of understanding the physiology of symbiosis, much more knowledge is needed concerning the physiological activities of the fungi in symbiosis and in culture and factors that affect them.

The decreases in dry weight of the plant which sometimes follow colonization appear to be exactly similar to those observed with arbuscular mycorrhizal (AM) plants (see Chapter 4). A decrease in growth might be expected in a system where there is mutual dependency of one symbiont on the other for C compounds and for nutrients essential to support growth and photosynthesis. Decreases in growth rate would be expected in conditions of low irradiance that limit the rate of photosynthesis, but not the intensity of mycorrhizal colonization, or where the supply of soil-derived nutrients is adequate for growth but is not high enough to decrease the intensity of colonization. Similar examples where colonization results in no increase or sometimes a decrease in growth rate of the plant are also found in ericoid mycorrhizas (see Chapter 11).

Having discussed in general terms the growth of ECM plants, normally regarded as being a process of C accumulation, it is now appropriate to consider details of C

requirements of the fungi before returning later to examine C distribution in intact ECM symbioses.

Carbon supplies for ectomycorrhizal fungi

Unlike the glomeromycotan AM fungi, many of the fungi of ectomycorrhizas have been isolated into culture and the physiology of their growth has been studied. However, the results have been disappointing. Much has been learned about factors which affect mycelial growth but little about intermediary metabolism or biochemistry. No positive characteristic that might explain the ECM habit has been discovered. Few of the early experiments even considered intermediary metabolism, respiration, secretion of metabolites or similar activities except in the sphere of the production of auxin-like substances. More recently, production of enzymes, organic anions, siderophores and metallotheionins has been highlighted, especially in relation to their roles in nutrition and heavy metal tolerance (see Chapters 9 and 10).

Frank (1894) assumed from the first that the source of organic C for ECM fungi was the photosynthetic plant, although he recognized that these might be supplemented by supplies from soil. Much later, Melin (1925) began to examine experimentally the requirements of the fungi for organic C. He found that most strains had little ability to grow on complex polymers, such as might be found in litter and humus, and that they could not use lignin or cellulose. They therefore seemed to be dependent on simple sugars such as might be produced by or released from the roots with which they were associated. Rommell (1938, 1939a) showed that a number of ECM fungal species depended upon association with living roots to produce fruit bodies, a reasonable expectation if they depended on the roots for C supplies. The positive relationship between irradiance and carbohydrate concentration in the root system and the intensity of mycorrhiza development in *Picea* and *Pinus* shown by Bjorkman's experiments (1942–1956) further appear to be consistent with this view.

From a survey of much work on growth of ECM fungi in culture, Harley and Smith (1983) concluded that most of the ECM fungi have at most, a limited ability to use lignin and cellulose as substrates for growth. Despite reports of slight lignase and cellulase activity, the ability to degrade polymers of this kind is much less than that of wood decomposing fungi or even of some ericoid mycorrhizal fungi (Trojanowski *et al.*, 1984; Haselwandter *et al.*, 1990; Cairney and Burke, 1994; see Chapter 11). Abilities vary within and between species in the ease with which starch, glycogen and insulin, the simpler oligosaccharides and the disaccharides sucrose and trehalose are used. The monosaccharides glucose, mannose and fructose are usually good sources of C for growth, whereas pectic substances can be used for growth by some ECM fungi but not others. This information has proved important in studies of the mechanisms of transfer of C from plant to fungus (see below).

The potential to use different sources of C clearly varies between fungal species and strains, and could possibly be related to fungal survival in soil. However, cellulose- and pectin-degrading abilities might be related not to use of litter as a source of organic C, but rather to penetration of root tissues, where enzyme production need only be localized and the degradation linked to softening the cell walls during Hartig net development. Much lower activity would be required for penetration than for

releasing monosaccharides from complex polymers in amounts likely to affect the growth of the fungi. This point was clearly appreciated by Lindeberg and Lindeberg (1977) and research directed towards clarifying the extent of production of enzymes within plant roots and their roles in colonization processes is discussed in Chapter 6. The abilities of the fungi when free-living to use organic C-sources in soil has only limited relevance to nutritional interactions of the associated symbionts.

Björkman's conclusions (1970) that high internal carbohydrate supply in the roots favoured fungal colonization, although subject to more recent criticism (Nylund, 1988), seem to have been upheld by Marx *et al.* (1977). However, the fact that the root system is more susceptible to colonization when it contains high concentrations of soluble carbohydrates (such as sucrose) is not in itself a proof that the fungal symbiont derives its supply of C in whole or in part from the plant. Proof that this did occur was given by Melin and Nilsson (1957) who fed $^{14}CO_2$ to the leaves of axenically-grown *Pinus sylvestris* seedlings in combination with either *Suillus variegatus* or *Rhizopogon roseolus*. Photosynthetic products were translocated through the seedlings and were found in the roots and in their fungal mantles. Although this experiment demonstrated very clearly that the products of photosynthesis were translocated directly and rapidly to the fungal symbiont in ectomycorrhizas, it did not show anything about the quantities involved. Nor did it prove that all C compounds in the fungus were derived from the plant. Moreover, the controls in these experiments were decapitated ECM plants which also accumulated ^{14}C in their tissues. The quantities at the end of the experiment amounted to 6%, 11% and 15% of that in the intact photosynthesizing plants in stem, non-colonized roots and mycorrhizas, respectively. Lewis (1963) pointed out that this was explicable in terms of dark fixation of CO_2 which commonly occurs in plant material.

Much was learned about the C metabolism of ectomycorrhizas of *Fagus* using excised roots and some of this work is certainly relevant to a discussion of the supply of organic C to the fungal sheath by the plant (Harley and Jennings, 1958; Lewis, 1963; Lewis and Harley, 1965a, 1965b, 1965c). The monosaccharides glucose and fructose are readily absorbed by excised mycorrhizas from aerated solutions, but glucose is selected preferentially from mixtures. In mycorrhizas, sucrose is hydrolysed by an invertase attached to the plant wall and glucose preferentially absorbed from the products. The rate of absorption of hexoses is temperature- and oxygen-dependent and inhibited by metabolic inhibitors of the cytochrome oxidase pathway and of oxidative phosphorylation. The analysis of the tissues after uptake shows that the change in concentration of glucose and other monosaccharides is small and absorbed hexoses are rapidly converted to other compounds. Among the storage carbohydrates, mycorrhizas contain those characteristic of both the plant and the fungus (Table 8.3). Glucose and fructose are common to both. Trehalose, mannitol and glycogen are fungal, whereas sucrose and starch are from the plant. It may be assumed that sucrose is a plant sugar. The typical fungal carbohydrates, trehalose and mannitol, are absorbed by non-mycorrhizal roots at rates only one-tenth and one-twentieth of that of mycorrhizas (Table 8.4). The disaccharides, sucrose and probably trehalose, are hydrolysed before absorption and glucose and fructose have different destinations in the tissues. Since they competed in uptake it was thought that they probably had a common transporter on the plasma membrane. However, after absorption glucose was mainly converted to trehalose and fructose to mannitol among the sol-uble carbohydrates of the ECM mantle.

Table 8.3 Carbohydrate content of excised mycorrhizas and changes after storage for 20 h at 20°C in water or in 0.5% (w/v) solutions of glucose, trehalose, sucrose, fructose or mannitol.

	Initial sample	Changes after storage in Water	Mannitol	Fructose	Sucrose	Trehalose	Glucose
Total soluble	14.49	−5.36	−5.66	−1.53	−0.10	+2.02	+2.91
Total reducing sugars	4.86	−1.02	−0.22	+0.34	−0.43	−0.69	+0.06
Sucrose	5.07	−2.34	−3.20	−0.99	−0.80	−0.06	+0.28
Trehalose	4.74	−2.00	−2.24	−0.88	+1.13	+2.77	+2.57
Mannitol	Nil	Nil	+7.69	+6.36	+3.86	+2.27	+3.86
Insoluble (glucose units)	25.94	−2.84	+3.87	+6.83	+5.20	+8.10	+11.14
Total carbohydrate present	40.63	32.43	38.84	45.93	45.73	50.75	54.68

Values as mg per g fresh weight (= 150 mg dry weight approximately). Data from Lewis and Harley (1965a).

Table 8.4 Relative rate of uptake of carbohydrate from solution by mycorrhizal and non-mycorrhizal roots of *Fagus*.

Sugar supplied	Ratio of uptake rate mycorrhizal:non-mycorrhizal
Glucose	3.1
Fructose	2.7
Sucrose	2.3
Trehalose	11.8, 10.8
Mannitol	19.6, 23.6

Data from Lewis and Harley (1965b, c).

Lewis and Harley (1965c) conducted experiments in which they fed the cut stumps of *Fagus* mycorrhizas with ^{14}C-sucrose by placing agar blocks on them (Figure 8.2) in order to stimulate C supply in intact roots. The analysis of the apical region of the mycorrhizas after separation of the sheath and core tissue by dissection (Figure 8.2c) showed movement of C into the sheath. Between 55% and 76% of the ^{14}C that had been translocated through the plant tissues to the tip region and not released as CO_2 was found in the fungal sheath, where it was present mainly as trehalose, mannitol and glycogen.

The uptake and destination of monosaccharides has been confirmed in intact systems (Söderström *et al.*, 1988). The inhibitory effect of fructose on glucose absorption was confirmed and has been suggested, together with regulation of apoplastic invertase activity, to be a factor important in the control of transfer of C from plant to fungus (Salzer and Hager, 1993).

Amanita muscaria, Hebeloma crustuliniforme and *Pisolithus tinctorius* are unable to use sucrose in culture because they lack a wall-bound invertase that would enable them to hydrolyse the disaccharide to glucose and fructose (Taber and Taber, 1987; Salzer and Hager, 1991; Schaeffer *et al.*, 1995). Protoplasts of *A. muscaria* can absorb both these monosaccharides, but whereas the uptake of fructose is strongly inhibited by glucose, the converse was not found and nor did sucrose inhibit uptake of either

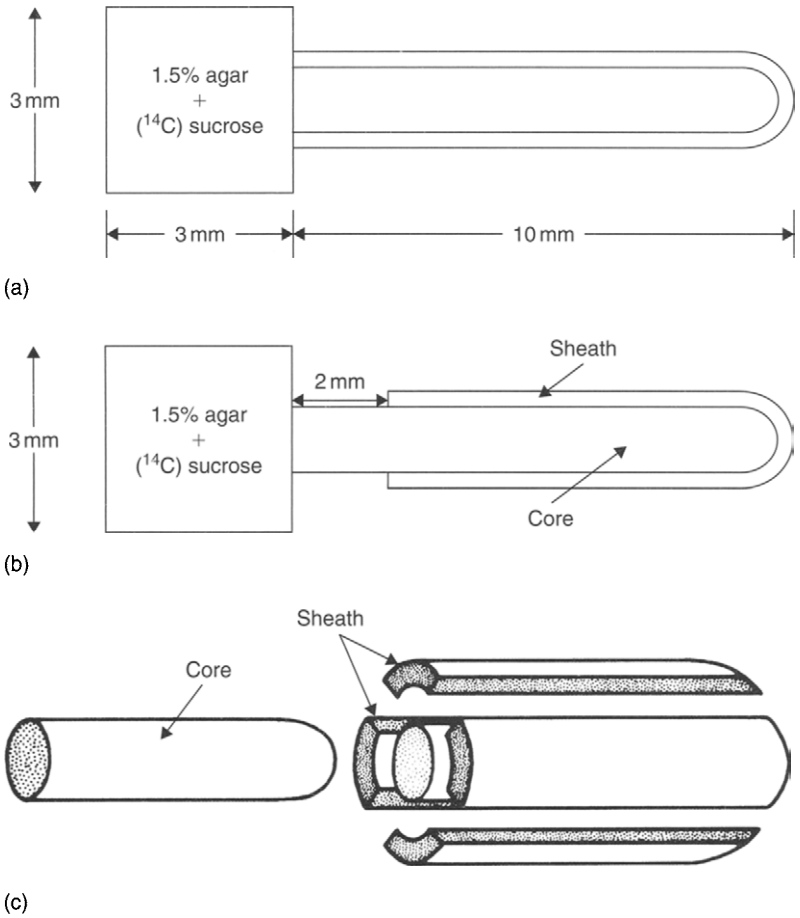

Figure 8.2 Schematic drawings to show the method of feeding mycorrhizal roots with ^{14}C-labelled sugars in agar blocks in studies of ^{14}C transfer from plant to fungus (see text). (a) The agar block abuts on both sheath and core; (b) a collar of sheath tissue has been removed to prevent contact between the fungus and the agar block, so that direct transfer of ^{14}C-sucrose to the fungus is also prevented; (c) dissection of the fungal sheath from the root core at the end of the feeding period. Modified from Lewis and Harley (1965c), with permission.

monosaccharide. The uptake system had a much higher affinity for glucose than for fructose (K_m 1.25 and 11.3 mM and V_{max} 18 and 30 pmoles per 10^6 protoplasts per minute, for glucose and fructose respectively; Chen and Hampp, 1993). It appears that the only way that *A. muscaria* can use sucrose is in symbiosis when the disaccharide is hydrolysed by an apoplastic or wall-bound invertase derived from the plant (Salzer and Hager, 1991).

Two hexose transporter genes have now been identified in *A. muscaria* (Nehls *et al.*, 1998). Of these, *AmMst1* and *AmMst2* encode proteins of 520 and 519 amino acids, respectively. *AmMst1* has a high sequence homology with a hexose transporter from the basidiomycotan fungus *Ruomyces fabae*, while *AmMst2* has closest homology to transporter *Stll* from the ascomycete *Saccharomyces cerevisae*.

When expressed in yeast, *AmMst1* had K_m values of 0.4 mM for glucose but 4.0 mM for fructose (Wiese *et al.*, 2000). The much higher affinity for glucose is reflected in culture studies which show that hyphal uptake of glucose is strongly favoured, even in the presence of large excesses of fructose (Nehls *et al.*, 2001a). At glucose concentrations above 0.5 mM, uptake of fructose is inhibited. The expression of these genes is upregulated by a threshold response mechanism determined by the external concentration of monosaccharides (Nehls *et al.*, 1998). Up to a monosaccharide concentration of 2 mM both of the transporters show a basal level of expression, but concentrations above this threshold trigger a fourfold increase in transcript accumulation. This increase takes place slowly over a period of up to 24 hours.

In contrast to the *AmMst1* gene that exhibits slow sugar-dependent enhancement of expression, Nehls *et al.* (1999) identified a further gene in *A. muscaria*, *AmPAL*, that showed rapid sugar-dependent repression. Transcripts of this gene encode a key enzyme of secondary metabolism, phenylalanine ammonia lyase (PAL), which is involved in phenol biosynthesis. Transcripts accumulated to high levels in free-living mycelia grown at low glucose concentrations and it was suggested that they may be involved in production of defence-related compounds, protecting hyphae from fungivores as they extend through the soil.

Working on the same fungus growing in ECM association with spruce, Nehls *et al.* (1998) found an increase of *AmMst1* expression similar to that found in free-living mycelia when grown at elevated monosccharide concentrations. It was suggested that both the extended lag phase for enhanced *AmMst1* expression and its threshold response to elevated monosaccharide concentrations are hexose-regulated adaptations to the homeostatic conditions found at the fungus–plant interface. Here the hexose-enriched ambient environment would contrast strongly with that of the soil which is largely free of available monosaccharides. However, not only *AmMst1* but also *AmPAL* was strongly expressed in intact mycorrhizas. Since these genes were differentially expressed in a hexose-dependent manner in pure culture, it could only be suggested that the regulation of the genes was affected by their location in the mycorrhizal system. To address this question, ectomycorrhizas were dissected and gene expression was investigated separately in the hyphal, fungal mantle and Hartig net compartments (Nehls *et al.*, 2001b). As in pure culture, *AmMst1* was expressed only at the basal level in hyphae of the fungal mantle. In contrast, *AmPAL* showed a high transcript level in this part of the mycorrhiza. In Hartig net hyphae the opposite expression pattern was observed. As for hyphae in pure culture, in the presence of high external hexose, the transcript level of *AmMstl* was enhanced sixfold, while that of *AmPAL* was barely detectable. The opposing patterns of gene expression in hyphae of the fungal sheath and Hartig net (the latter resembling that of genes in pure culture situations) led Nehls *et al.* (2001b) to postulate that there must be a hexose gradient between the apoplast of the root–fungus interface (i.e. the Hartig net where the hexose concentration must be assumed to be above 2 mM) and the fungal sheath (Figure 8.3). The mechanisms that maintain gradients remain to be elucidated.

The question of the extent to which, if at all, the plant partner may be able to control the movement of sugars to the fungus has been little studied. However, a recent analysis of monosaccharide transporter gene expression in roots of *Populus tremula* showed that one of three such transporters, *PttMST3.1*, showed a 12-fold increase

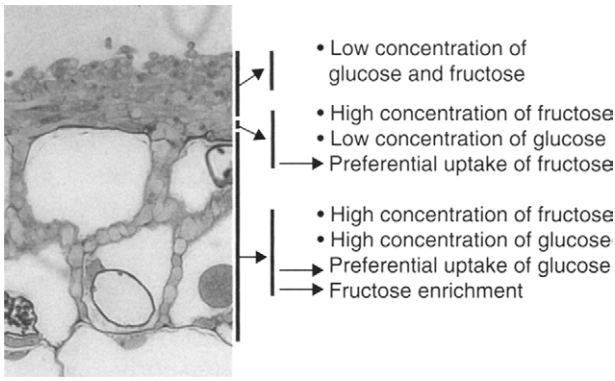

Figure 8.3 Hypothetical model showing the spatial distribution of hexose uptake by the fungal hyphae in ectomycorrhizas. Sucrose hydrolysis in the apoplast of the Hartig net results in high glucose and fructose concentrations. In this compartment, glucose is preferentially taken up since the uptake of fructose is inhibited (by glucose concentrations above 0.5 M). In the innermost one or two layers of the fungal sheath, glucose concentration is low due to efficient uptake by fungal hyphae of the Hartig net. Thus, mainly fructose is taken up. In the apoplast of other layers of the fungal sheath, glucose as well as fructose concentrations are low due to the efficient hexose uptake by hyphae of the Hartig net and the inner layers of the sheath. From Nehls *et al.* (2001b).

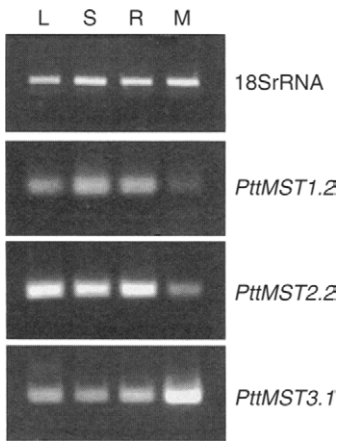

Figure 8.4 Expression profiles of *Populus tremula* × *tremuloides* monosaccharide transporter genes *PttMST1.2*, *PttMST2.2* and *PttMS3.1* in leaves (L), stems (S), non-mycorrhizal (R) and mycorrhizas (M), showing upregulation of *PttMST3.1* in mycorrhizal roots. From Grunze *et al.* (2004).

of transcript accumulation in ECM relative to non-mycorrhizal roots (Figure 8.4; Grunze *et al.*, 2004). Upregulation in response to the presence of the fungus suggests that root cells are able to compete with fungal hyphae for hexoses present in the common apoplast of the Hartig net. On the basis of these observations, Grunze *et al.* (2004) suggested that (through its ability to control expression of its own hexose

transporters) an autotroph could restrict the flux of sugars towards the fungus under circumstances in which mineral nutrient supply from the heterotroph was restricted. By such mechanisms the plant would be in a position to regulate the carbon balance of the symbiosis.

The characteristics of acid invertases have been investigated in *Picea abies* in relation to their possible role in nutrient supply to ECM fungi. Using cell-suspension cultures, Salzer and Hager (1993) showed that there were two important acid invertases associated with the apoplast. Both had relatively low pH optima, with the ionically bound form showing high activity between pH 3.5 and 4.5 and the tightly bound form having a sharp optimum at pH 4.5. Above pH 6 neither form showed significant activity. Both invertases had relatively high K_ms with respect to sucrose (16 and 8.6 mM for the ionically and tightly bound forms, respectively) and were competitively inhibited by fructose but not by glucose. These authors suggested that control of carbohydrate supply might be regulated by the fungus, via changes in apoplastic pH and uptake of fructose. Using whole *Picea abies* roots, Schaeffer *et al.* (1995) showed an acid invertase with a pH optimum of around 4.0 and a K_m (sucrose) of 5.7 mM, which is rather less than for the suspension cultures. By comparing different segments of ECM and non-mycorrhizal long roots it was shown that, although overall the activity of invertase was lower in ECM roots (in agreement with findings of Lewis and Harley, 1965b), when the weight of fungal tissue was taken into account, invertase production by the plant was unaffected by fungal colonization. Around 75% of the invertase activity was associated with cells of the root cortex rather than the stele and was thus in the tissue accessible to the fungal symbiont. Interestingly, Schaeffer *et al.* (1995) could find no evidence for increase in plant invertase activity associated with fungal colonization and they highlight the difference between this situation and that in some parasitic symbioses.

Figure 8.5 shows a hypothetical scheme for sugar transfer from plant to fungus, based on current knowledge. The overall picture is one in which, following sucrose efflux to the apoplast and hydrolysis by plant invertase, glucose would be preferentially absorbed by the fungus via the monosaccharide transporter *AmMst1*. Fructose released by the invertase would exert feedback inhibition on invertase activity, whereas glucose would inhibit fructose uptake by the fungus. As the concentration of glucose fell, the fungus would absorb fructose, releasing invertase inhibition and permitting further sucrose hydrolysis. One important gap appears in this reasoning and that is the possible need to reduce or eliminate uptake by the plant of both sucrose and the hexoses from the interfacial apoplast. Here again proton-symports are likely to operate and net flux in the direction of the fungus could only be maintained by a mechanism favouring net efflux (as sucrose) from the plant and net uptake (of monosaccharides) by the fungus. Perhaps fructose also competes with reabsorption of glucose by the plant or perhaps plant hexose transporters are downregulated. This area clearly requires continued investigation. Monosaccharide uptake by the fungus might be passive, with a concentration gradient maintained by metabolic conversion within the fungus. However, it seems just as likely that active proton co-transport would drive the inward flux, especially as low apoplastic pH controlled by the membrane-bound H^+-ATPase (of either organism) is necessary to maintain invertase activity. ATPase activity has been demonstrated cytochemically on both fungal and plant plasma membranes in the Hartig net zone of *Pinus sylvestris–Laccaria laccata* mycorrhizas and shown by its DES sensitivity to be, in all probability, an H^+-ATPase (Lei and Dexheimer, 1988).

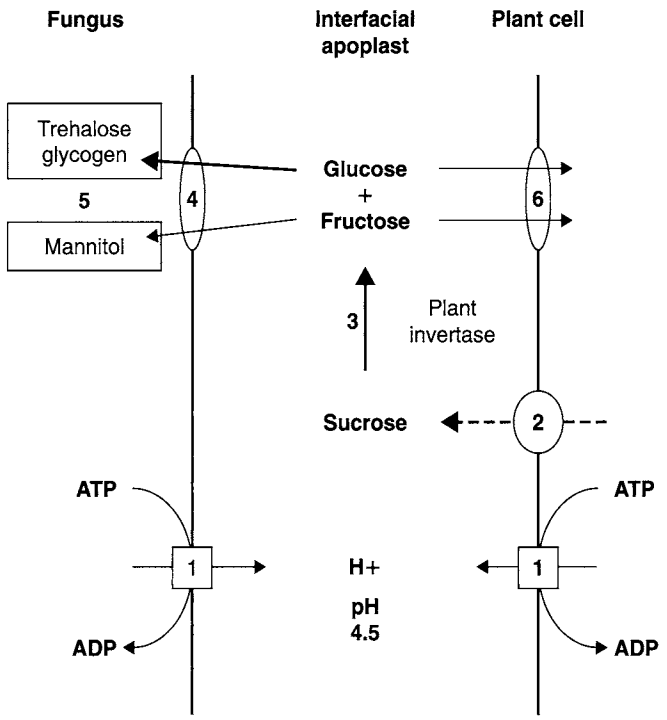

Figure 8.5 Diagrammatic representation of the plant–fungus interface in an ectomycorrhiza, showing processes likely to be important in sugar transfers between plant and fungus. (1) Activity of both plant and fungal H^+-ATPases transfers protons to the interfacial apoplast, lowering the pH and creating proton motive force necessary for active transport. (2) Sucrose, delivered to the roots is exported from the plant cells and hydrolysed by a plant wall-bound invertase (acid pH optimum) to glucose and fructose (3). (4) Glucose is preferentially absorbed by the fungal partner via a hexose transporter (*AmMst1* and *2* in *Amanita muscaria*), which may be a proton hexose symporter. Fructose can also be absorbed, but is outcompeted by glucose. (5) Glucose is used by the fungus to synthesize large amounts of glycogen and lesser amounts of trehalose. Fructose is converted to mannitol. (6) Upregulation of a plant monosaccharide transporter *PttMST3.3* in ECM poplar roots suggests that this plant may have a means of reabsorbing some of the sugar lost to the apoplast (see Figure 8.4) (Grunze *et al.*, 2004). However, no such upregulation of plant sugar transporter activity was found in *Betula* (Wright *et al.*, 2000).

The importance of maintaining a net flux of organic C in favour of the fungus has been the subject of considerable discussion. Since trehalose and mannitol were only very slowly absorbed by non-mycorrhizal roots, it was suggested that they, with glycogen, constituted a sink in the ECM mantle into which carbohydrates were accumulated in a form not readily available to the adjacent plant tissues even if they were present in the apoplast. This idea was elaborated in a review by Smith *et al.* (1969) in which they consider allied features of carbohydrate movement between the partners in lichens, pathogenic associations and other symbioses. The hypothesis of a 'biochemical valve' ensuring movement in the direction of the fungus has not yet been effectively superseded.

Although sugars from the plant certainly move to the fungus, movement of organic C is not exclusively in that direction. Harley (1964) showed, again using excised roots, that the rate of dark fixation of CO_2 by the mycorrhizas of *Fagus* was greatly increased if NH_4^+ was being absorbed. Using ^{14}C-bicarbonate he was able to show, and Carrodus (1967) later confirmed, that the main destination of the increased fixation was into glutamine. Since this method provided a simple way of labelling glutamine, Reid and Lewis (see Lewis, 1976) used it to observe the movement of glutamine from the fungal sheath to the plant, demonstrating how C compounds may return to the plant as the C-skeletons of amino compounds. Subsequently, the pathways for assimilation of inorganic N and the mobilization of organic N sources in soil received considerable attention (see Chapter 9). There is as yet little evidence on the extent to which C-skeletons of the organic N sources utilized by the fungi contribute to the C budgets of the plants. Abuzinadah and Read (1989a) calculated that, in ECM seedlings of *Betula pendula* supplied with N exclusively in the organic form, as much as 8% of total plant C could be derived from the fungus. The movement of C and N compounds synthesized in the fungus certainly provides a route for 'cycling' of organic C between the symbionts. The relative fluxes in the two directions have not been measured and this will certainly be an important challenge in assessing the potential of the 'cycling' of organic N through the interface to support net interplant transfer of C (see Chapters 9 and 15).

Carbon distribution in intact plant–fungus systems

In their study of distribution of ^{14}C-labelled photosynthetic products between ECM and non-mycorrhizal roots on the same plants of *Pinus radiata*, Bevege *et al.* (1975) found that 15 times more C was allocated to the colonized roots, 45–50% of the label being in trehalose and 1–22% in mannitol. A similar study using individual plants of *Eucalyptus pilularis*, some roots of which were colonized by *Pisolithus tinctorius*, showed that the ratio of ^{14}C-accumulation in ECM versus non-mycorrhizal roots was 18:1 (Cairney *et al.*, 1989). The ability of these ectomycorrhizas to attract photosynthate was greatest soon after their formation and there was a progressive reduction in the amount translocated to them with age so that 90 days after inoculation all translocation had ceased. Cairney and Alexander (1992) compared allocation of ^{14}C to younger and older mycorrhizas of *Picea sitchensis* formed by *Tylospora fibrillosa* growing on peat. Although some ^{14}C-labelled compounds were translocated to older mycorrhizas in all plants, the ratio of activity in young to older mycorrhizas, which was initially around 2:1, became progressively greater as the whole root system aged, reaching 54:1 38 weeks after transfer of the newly colonized seedlings to the peat substrate.

In the studies quoted above, the extent of onward transport of fixed C from root to extraradical mycelium was not determined. The importance of this mycelium as a potential sink for fixed C is evident from studies in which intact systems develop over semi-natural substrates (see Chapters 6, 9 and 15). Autoradiographic techniques have shown clearly that ^{14}C fed to assimilating shoots of *Pinus* is rapidly transported to the extraradical network (see Figures 6.27a, b and Colour Plate 6.2a, b) which, in addition to exploring the soil, provides interconnection between individual mycorrhizal short roots in the same and on adjacent compatible plants (Finlay and Read, 1986a) (Figure 6.26). Thus the fungus supplies a series of potential pathways for

the flow of C from structures of small or decreasing sink strength, such as old roots, to younger tissues.

A question of fundamental importance concerns the amount of C allocated by the plant to support the growth and maintenance of its ECM fungi. The first studies of respiratory activity specifically of the mycelial phase of the ECM association (Söderström and Read, 1987) demonstrated that approximately 30% of total respiration was attributable to the ECM mycelium. This respiration was shown to be highly dependent upon the supply of current assimilate and, if mycelial connections to the roots were severed, there was a reduction in respiration rate of at least 50% within 24 hours (Figure 8.6). A similar dependence upon current assimilate for production of sporophores has been demonstrated in an association of *Pinus strobus* with *Laccaria bicolor* (Lamhamedi *et al.*, 1994) (Figure 8.7).

Rygiewicz and Anderson (1994) fed *P. ponderosa* seedlings, either colonized by *Hebeloma crustutiniforme* or non-mycorrhizal, with $^{14}CO_2$ and examined the distribution of label in different fractions after incubating the plants in microcosms for 72 hours. Both sets of seedlings retained around 40% of labelled C in their shoots, but

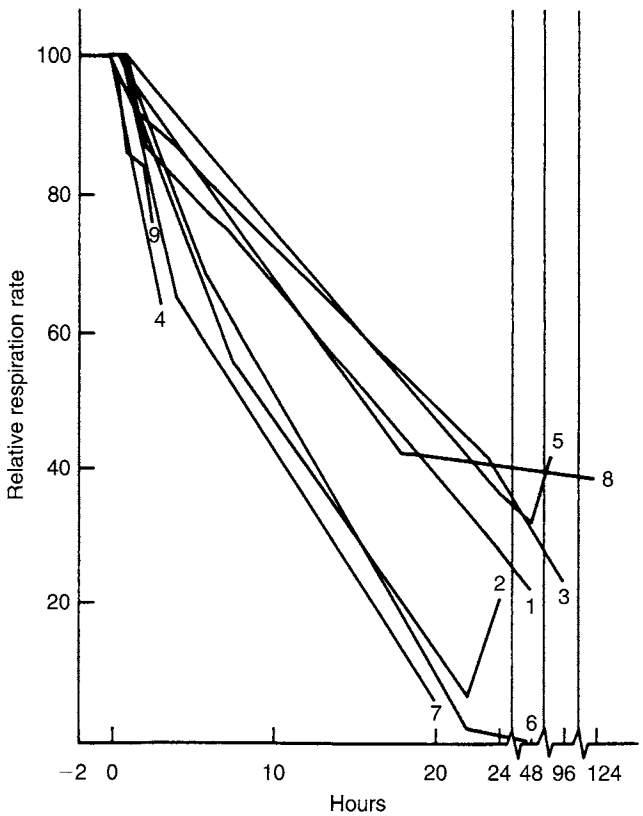

Figure 8.6 Effect of cutting the mycelium (and hence detaching it from a source of carbohydrate from the plant) on respiration of ectomycorrhizal mycelium. The curves represent the relative respiration rate (per cent of value before cutting) for nine separate combinations of plant and fungal species. From Söderström and Read (1987), with permission.

the presence of the fungus (representing only 5% of total seedling weight) increased the below-ground allocation of ^{14}C by 23% relative to that in non-mycorrhizal plants. Dry matter allocation to roots and mycorrhizas was similar, the difference being due to increased respiration by ECM roots and mycelium. The greater allocation below ground led to a small reduction of seedling biomass in the ECM plants.

Jones *et al.* (1991) considered C allocation to mycorrhizas as a cost to the plant. They examined efficiency in terms of amount of P taken up per unit of C expended below ground, using cuttings of *Salix viminalis* either colonized by *Thelephora terrestris* or grown in the non-mycorrhizal condition. Efficiency was calculated according to the formula $\Delta P/\Delta C_b$ of Koide and Elliott (1989) (see Chapter 4), where ΔP is the amount of P taken up by the plants over a defined interval, and ΔC_b is the total amount of C allocated to the below-ground system during the same interval. C_b includes the C incorporated into root or fungal tissue, lost in respiration and deposited in the soil. C_b was calculated for both ECM and non-mycorrhizal plants, which had been pulse-labelled prior to each harvest, for the intervals up to the first harvest (0–50 days) and from the first harvest to the final harvest (50–98 days).

Two methods were used to obtain C_b.

In the first method, it was calculated as:

$$C_{b(Pn)} = P_n \frac{\%C_{BG}}{100 - \%C_{SR}} 57\,600$$

where $C_{b(Pn)}$ is the amount of C allocated below ground in mMol C in a 24 hour period; P_n is the net rate of photosynthesis as mmol C/s for a whole shoot system;

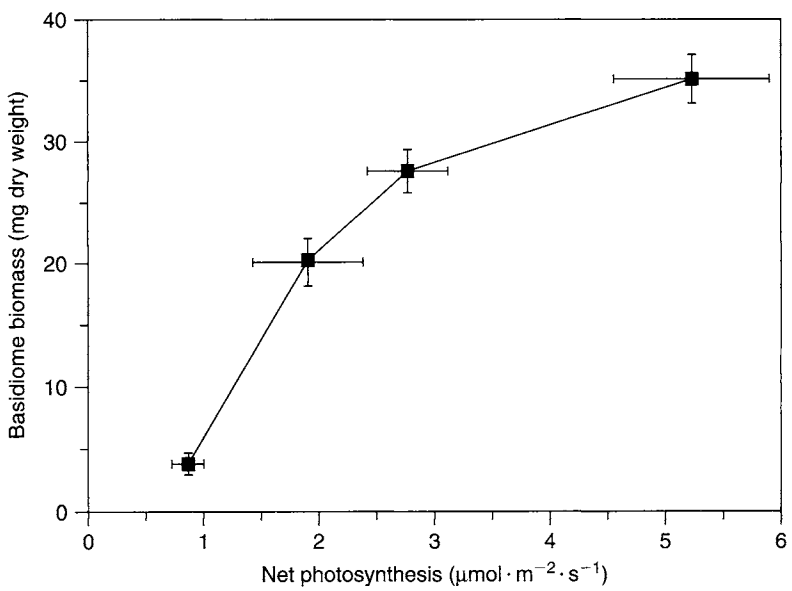

Figure 8.7 The influence of rate of net photosynthesis of *Pinus strobus* seedlings on the biomass of fruit bodies produced by associated *Laccaria bicolor* after 20 days. Bars are standard errors of means. From Lamhamedi *et al.* (1994), with permission.

$\%C_{BG}$ is the percentage of the total $^{14}CO_2$ absorbed allocated below ground over a 9 day period; $\%C_{SR}$ is the percentage of the absorbed $^{14}CO_2$ released as shoot respiration; and 57600 is the length of the daily light period in seconds. The term $100–\%C_{SR}$ is a correction for the fact that P_n is exclusive of shoot respiration. A curve was then constructed of $C_{b(Pn)}$ against time and the resulting equation integrated over the interval between each harvest to give the total amount of C allocated below ground over these intervals ($\Delta C_{b(Pn)}$).

In the second method, shoot weight was used as an integrated measure of the amount of C deposited in tissue over a harvest interval. The relationship between C deposited in shoot tissue and C allocated below-ground as determined by the ^{14}C labelling was then used to calculate ΔC_b directly:

$$\Delta C_{b(Wt)} = \Delta W_s \frac{\%C_{BG}}{\%C_{ST}}$$

where ΔW_s is the mean increase in shoot weight over the interval and $\%C_{ST}$ and $\%C_{BG}$ are the mean percentages of the ^{14}C fixed allocated to shoot tissue, and to the below-ground compartments, respectively. For the first interval, $\%C_{ST}$ and $\%C_{BG}$ from the first harvest were used. For the second interval, a weighted (i.e. based on the length of time between harvests) average of the $\%C_{ST}$ and $\%C_{BG}$ value for harvests two, three and four were used. Dry weight was converted to g C using a correction factor of 0.5 g C/g dry weight.

The amount of C allocated below ground was consistently greater in ECM than non-mycorrhizal plants throughout the experimental period (Figure 8.8).

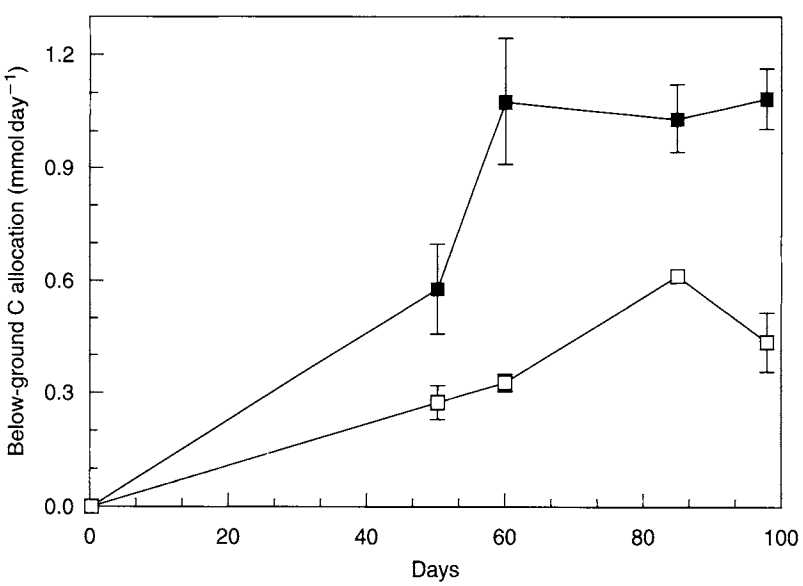

Figure 8.8 Below-ground carbon allocation ($C_{b(Pn)}$) (mmol C/day) of mycorrhizal (■) and non-mycorrhizal (□) *Salix viminalis* during a 98-day growth period. Values are means of 3 replicates at 50, 60 and 85 days and 4 (M) or 5(NM) at 98 days ± standard error of means. From Jones *et al.* (1991), with permission.

Table 8.5 P uptake, C translocated below ground and P acquisition efficiency of mycorrhizal *Salix viminalis* over a 98-day growth period (see text).

Mycorrhizal treatment	Growth period (days)	ΔP(μmol)	$\Delta C_{b(Pn)}$ † (mmol)	$\Delta C_{b(Wt)}$ ‡ (mmol)	$\Delta P/\Delta C_{b(Pn)}$ † (μmol P/mmol C)	$\Delta P/\Delta C_{b(Wt)}$ ‡ (μmol P/mmol C)
Mycorrhizal	0–50	50.1	14.2	17.0	3.52	2.94
Non-mycorrhizal	0–50	17.1	5.7	9.7	2.97	1.75
Mycorrhizal	50–98	25.6	46.6	69.8	0.55	0.37
Non-mycorrhizal	50–98	17.9	22.0	21.2	0.82	0.84

From Jones *et al.* (1991). ΔC_b is the amount of C translocated below ground during a given interval, including that deposited in root and fungal tissues, that respired by roots and soil and that deposited in the soil. ΔP is the amount of P taken up during a given interval. † Values were calculated using photosynthetic measurements. ‡ Values were calculated using weight measurements. Data based on the 3–5 plants labelled with ^{14}C prior to each harvest.

When the data shown in Figure. 8.6 were integrated, it was shown (Table 8.5) that over the interval up to the first harvest ECM plants allocated 2.5 times more C ($\Delta C_{b(Pn)}$) below ground than did those not colonized by *T. terrestris*. Expressed on a weight basis, C allocation ($\Delta C_{b(Wt)}$) was 1.75 times greater in ECM plants. Since colonized plants absorbed three times as much P from soil over the same period, they had a higher P use efficiency according to both methods of calculation. Because, over the second half of the experiment the difference in P uptake between ECM and non-mycorrhizal plants was less, the efficiency of P acquisition was higher in the non-mycorrhizal than ECM plants. Cost-benefit analysis shows the costs, expressed as C required to produce and maintain the nutrient absorbing structures, are lowest at the earliest stage of growth while the benefits, in the form of P acquisition, are highest. This is a pattern which Jones *et al.* (1991) suggest will match the requirements of field-grown perennial plants where, in temperate and boreal climates, maximum demands for nutrients may be experienced in spring.

Jones *et al.* (1998) subsequently analysed the growth, C and P relations of *Eucalyptus coccifera* when colonized by ECM or AM fungi, comparing the performance of the plants with these mycorrhizal associates and without mycorrhizas. Growth promotion was significantly greater in the ECM than in the AM or uncolonized states but, when the plants were fed with $^{14}CO_2$ after 89 days growth no significant differences were detected between treatments in the quantities of C allocated below ground. This is surprising in view of the fact that the ECM plants produced three to seven times more extraradical mycelium and acquired significantly more P than their AM counterparts (see also Chapter 10). The authors propose that failure to detect effects of ECM on below-ground C allocation was attributable to the small size and low root weight fractions (0.21–0.27) of their young plants. In an earlier experiment, differences in below-ground C allocation between *Salix viminalis* grown in the ECM and AM conditions increased with time but were not significant until the root weight fraction of the ECM plants exceeded 0.35 (Durall *et al.*, 1994).

The seasonality of C allocation is generally overlooked. The literature (e.g. Shiroya *et al.*, 1966; Gordon and Larson, 1968; Ursino *et al.*, 1968; Glerum and Balatinecz, 1980) suggests that the main surge of C allocation to below-ground systems occurs not in spring but towards the end of the growing season after stem elongation and

bud set are complete. This is also the time at which the main flush of root growth occurs in many of the tree species which are characteristically ECM (Lyr and Hoffman, 1967) and it coincides with the late summer flush of fruit body production by epigeous ECM-forming mushrooms. Since these are, as shown above, dependent upon current assimilate for their development, it is implicit in such observations that this is also a time during which the extraradical mycelium must be particularly active. There is evidence (Langlois and Fortin, 1984) for a distinct seasonality in the pattern of absorption of P (see Chapter 10) by ECM roots of *Abies balsamea*, with maximum rates again observed in August, after bud set. This feature may also be a reflection of greater below-ground C allocation. A requirement for enhanced nutrient inflow in the autumn would be expected in boreal and temperate systems because winter-hardening involves significant augmentation of cellular components, in particular membrane phospholipids (Siminovitch *et al.*, 1975) which are remobilized to support the spring flush of above-ground growth.

Environmental factors, in particular availability of soil moisture, may be superimposed on the inherent seasonality of ECM processes, but it is important to bear in mind, whether carrying out experiments under constant conditions of day length and irradiance in the laboratory or collecting ECM roots in the field, that the results obtained at any one time may not reflect accurately the situation as it prevails in the field. There is scope for much more work on seasonality in function in ECM systems, though it appears likely that the main phase of activity is at the end rather than the beginning of the growing season as surmised by Jones *et al.* (1991).

Non-nutritional effects upon carbon assimilation

Formation of ECM symbioses can influence the C balance of the plant via a number of interrelated processes but, in particular, through its effects on net photosynthetic rate and mineral nutrition. These influences can be detected both in the leaves which are the C sources and in the roots and their symbionts which are the C sinks. The impacts of ECM colonization can be detected in the source leaves. The regulation of sucrose synthesis in the leaf cytosol mainly occurs through the activities of sucrose phosphate synthase (SPS) and fructose 2,6-bisphosphatase (FBPase), the latter being inhibited by an effector metabolite, fructose 2,6-bisphosphate (F26BP) (Quick and Schaffer, 1996). Loewe *et al.* (2000) reported increased activation of SPS and decreased levels of the inhibitor FBPase in ECM seedlings of spruce (*Picea abies*). This is indicative of an increased capacity for sucrose synthesis in source tissues of ECM plants. This effect of ECM formation is almost certainly linked to the role of the fungal partners as sinks for assimilates produced in the source tissues. The importance of sink strength in determining the rates of C assimilation in leaves is now well established (Herold, 1980; Gifford and Evans, 1981; Sonnewald *et al.*, 1994; Quick and Shaffer, 1996). In the context of mycorrhizas, the assumption is that, through their ability to maintain a net flux of C in their favour (Lewis and Harley, 1965a; Nehls *et al.*, 2001) and to increase the overall C demand of the root (Finlay and Söderström, 1992), the fungal partners exert direct impacts upon C assimilation by the plant. It remains necessary to determine the relative importance of enhanced provision of nutrients by the ECM fungi and the increased sink strength arising from their presence in determining the observed shifts of plant carbon balance.

Whereas several earlier studies showed that photosynthetic rates are enhanced in ECM plants relative to those grown in the non-mycorrhizal condition (Reid *et al.*, 1983; Nylund and Wallander, 1989; Loewe *et al.*, 2000), the confounding influence of changed mineral nutrient balance in the foliage of colonized plants makes it impossible to discriminate between the effects of nutritional and non-nutritional factors in these experiments. The necessary discrimination can be achieved by adding nutrients to non-mycorrhizal plants so as to compensate for the gains normally associated with colonization by the fungi. Experiments using plants nutritionally matched in this way (Dosskey *et al.*, 1990; Rousseau and Reid, 1990; Conjeaud *et al.*, 1996; Wright *et al.*, 2000) have confirmed that ECM sink strength alone can have major impacts both upon assimilatory activities in the source tissues and upon fluxes to the roots. Rousseau and Reid (1990) grew ECM and non-mycorrhizal *Pinus taeda* seedlings that were nutritionally matched to varying extents by exogenous supply of P to the uncolonized plants. The photosynthetic rates of the ECM plants, some of which were lightly, some moderately and some heavily colonized by the fungus, were then compared with those of uncolonized plants from each of the P treatments, before harvests were taken to determine tissue P status.

Relationships between mycorrhiza development, net photosynthetic rate and foliar P (Figure 8.9) indicate that, with low and medium colonization, assimilation

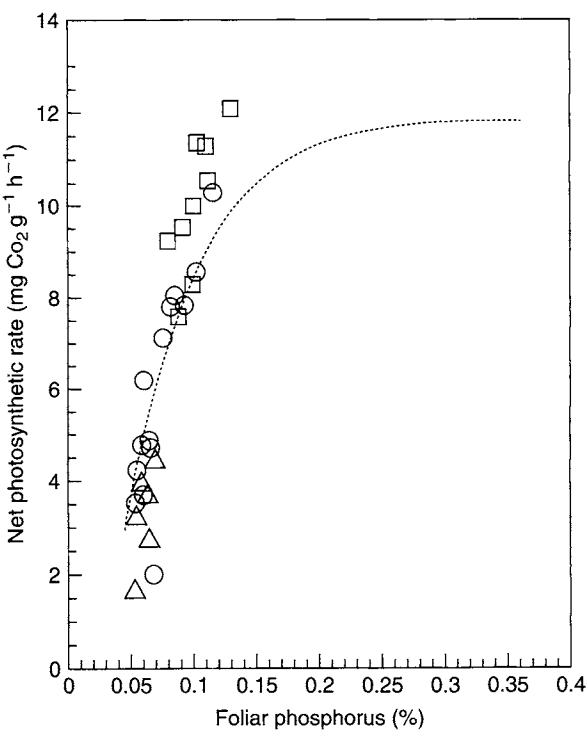

Figure 8.9 Net rate of photosynthesis (mg CO_2/g/h) versus foliar P concentration (percent dry weight) for ectomycorrhizal seedlings. The different symbols indicate seedlings with different concentrations of glucosamine (mg/g) derived from the fungal symbiont associated with their roots: △, 0–7.99 mg; ○, 8–15.99 mg; □, 16–24 mg. The dashed line shows the regression obtained from non-mycorrhizal seedlings. From Rousseau and Reid (1990), with permission.

of seedlings responded to increasing foliar P concentration in the same way as uncolonized plants, suggesting that the rate was responding to increased P accumulation. However, at high levels of colonization net rates of photosynthesis in ECM plants were significantly greater (up to 17%) than those seen in non-mycorrhizal plants of the same foliar P concentration. Clearly, this suggests an effect of colonization upon photosynthesis not related to P status. The likelihood that the increased sink strength imposed by the highest level of colonization was responsible for the enhancement of assimilation was stressed by Rousseau and Reid (1990), though alternative explanations, including changes of hormone balance induced by the fungus, were also discussed. The possibility that nutrients other than P had become limiting for the highly colonized plants, despite the apparently adequate nutrient supplies, also deserves attention.

Increased rates of photosynthesis in ECM plants can certainly occur independently of nutrient enrichment (Dosskey *et al.*, 1990). Colonization of *Pseudotsuga menziesii* by *Rhizopogon vinicolor* led to significant increases of net photosynthesis, whereas two other fungi, *Hebeloma crustuliniforme* and *Laccaria laccata* had no effect. Despite the increased net assimilation rate, *Rhizopogon*-colonized seedlings were smaller than the controls. The same type of result was observed in seedlings of *Pinus pinaster* when colonized by *H. cylindrosporum* (Conjeaud *et al.*, 1996). Despite significant increases of photosynthetic rate over non-mycorrhizal plants, with the same or even greater tissue N and P concentrations, there was an accompanying 35% reduction of growth. In these cases, stimulation of photosynthetic rate arising from increases in sink strength are not sufficient to compensate for the costs of production of mycorrhizas and associated extraradical mycelium. A similar effect was observed by Wright *et al.* (2000) using *Betula pendula* colonized by *Paxillus involutus*. Analysis of diurnal carbon budgets of ECM and nutritionally matched non-mycorrhizal plants revealed that the net amount of C assimilated per unit dry weight was 29% higher in the colonized than in the uncolonized plants (Table 8.6). This further confirms the important role of sink strength in determining C assimilation rates. However, because more C was respired by the root systems of ECM than non-mycorrhizal plants, the net C gain in both sets of plants was similar. Overall, the total biomass of the ECM plants was again significantly lower than that of their

Table 8.6 Diurnal carbon budgets of non-mycorrhizal (NM) birch plants and birch plants ectomycorrhizal with *Paxillus involutus* (M).

	CO_2 exchange			
	μmol CO_2/plant/day		μmol CO_2/g DW/day	
	Non-mycorrhizal	Mycorrhizal	Non-mycorrhizal	Mycorrhizal
Net CO_2 assimilation	690 ± 158	444 ± 28	2233 ± 394	2870 ± 437
Dark shoot respiration	123 ± 18	79 ± 3*	355 ± 21	516 ± 95
Root system respiration	225 ± 67	252 ± 43	593 ± 33	1029 ± 136**
Carbon gain of whole plant (NM) and plant and fungus (M)	342 ± 94	113 ± 44	1394 ± 257	1325 ± 326

From Wright *et al.* (2000). Results are expressed either per unit dry weight (μmol CO_2/g DW/day) or on a per plant basis (μmol CO_2/plant/day). Values are means ± SE (n = 4). The data for each parameter for M and NM plants were compared by one-way ANOVA. *$P < 0.05$, ** $P < 0.01$.

uncolonized counterparts, indicating that the increases in C assimilation were insufficient to compensate for the demands of both the plant and fungal partners.

Community level patterns of carbon allocation

Rommell (1939b) provided probably the first estimate of the C demand of ECM fungi under field conditions, based upon fruit body production and showed that the C required was 10% of that invested by the plants in annual timber production. The calculation assumed a fruit body yield of 180 kg/ha/year which some workers have suggested is a high value. Fogel and Trappe (1978) quote values of dry weight of epigeous fruit bodies for a number of forest types in the Northern Hemisphere in the range 3 to 180 kg/ha/year. However, Vogt *et al.* (1982) recorded 380 kg/ha/year of hypogeous and 30 kg/ha/year of epigeous fruit bodies in a 180-year-old stand of *Abies amabilis*. In addition, *Cenococcum* sclerotia yield 2700 kg/ha/year in these forests. By combining all such values with estimated fungal biomass in mantles and Hartig nets, Vogt *et al.* (1982) estimated that 15% of net primary production was allocated to the fungi, a value which still did not take account of vegetative mycelium in the soil, nor the possibility of increased C allocation due to turnover of the ECM rootlets.

It is a prerequisite for determination of the carbon costs of ECM symbioses that the annual production of vegetative mycelium of the system be calculated. It is, of course, difficult to determine the biomass of ECM hyphae growing as they do in mixed populations with other fungi in forest soils. However, some estimates are now available. On the basis of regression relationships between mycelial respiration and biomass, Finlay and Söderström (1989) estimated that in a Swedish pine forest soil dominated by *Lactarius rufus* there were 200 m/g soil of ECM hyphae. This figure is remarkably similar to that calculated by Read and Boyd (1986) for mycelial systems of *Suillus bovinus* growing in peat in observation chambers (see Chapter 6) and is not dissimilar to values of 100–700 m/g presented earlier by Söderström (1979) for lengths of active hyphae (stained with fluoroscein diacetate) in the FH horizon of Swedish forests. When converted to fungal biomass, the value of 200 m/g dry soil is equivalent to 3.5 kg of living mycelium per ha/year. Assuming a turnover time of one week throughout a five-month growing season, the hyphal production would be 70 kg/ha/year. This is more than double the estimated dry matter yield of fruit bodies of *L. rufus* which, in Swedish forests, is considered to be around 30 kg/ha/year (Richardson, 1970). Based on results obtained using the mesh bag ingrowth method (Chaper 6), Wallander *et al.* (2001) calculated an annual production of ECM mycelium of 125–200 kg/ha/year in a mixed conifer forest and 590 kg/ha/year in a spruce forest (Wallander *et al.*, 2001, 2004). This represents 80% of the total ECM fungal biomass. Nevertheless, according to a more recent assessment (Hendricks *et al.*, 2006), even these high values may be considerable underestimates.

Determination of total ECM biomass in the system also requires estimates of fine root production and turnover of fine roots, all of which are likely to be mycorrhizal in boreal forest systems (Taylor *et al.*, 2000). Unfortunately, considerable discrepancies have arisen between estimates of these parameters. Some studies based upon soil coring methods (e.g. Persson, 1978; Vogt *et al.*, 1982; Gill and Jackson, 2000) indicate that the population of fine roots (<2 mm diam) turns over several times per year in boreal and temperate forests. The high values obtained by these approaches

uggest that fine root production and turnover is the major short-term fate of plant C that is allocated below ground. In contrast, results obtained from recently developed direct methods of measuring C allocation have led to these estimates being challenged (Högberg and Read, 2006). In a Free Air Carbon Dioxide Enrichment (FACE) facility (Andrews *et al.*, 1999), which enabled $^{13}CO_2$-labelling of photosynethetic products, much slower rates of fine root turnover were suggested, the median value being about 4 years. Measurements using the ^{14}C signature left as a legacy of nuclear weapons testing (Gaudinski *et al.*, 2000, 2001) support those based on the FACE technology, suggesting fine-root turnover times of 3–18 years.

New observations on the life span of fungal components of the mycorrhiza indicate that, in contrast to the fine roots, they have a short life span. Using the bomb-^{14}C method, Hobbie *et al.* (2002) showed the ages of ECM sporophores to be of the order of weeks, which is consistent with visual observations. A further feature indicating that the production of fine roots cannot be the major sink for photosynthate allocated below ground is the decline of soil respiratory CO_2 flux, by 56% within 14 days, following girdling of trees late in the growing season (Figure 8.10; Högberg *et al.*, 2001). This was considered to be a conservative estimate of the respiratory contribution of mycorrhizas because root starch reserves were shown to be used following curtailment of C supplies from the canopy. Together with almost immediate cessation of sporophore production of the ECM fungi following girdling (Table 8.7), the findings show that, at this time of year, the major short-term sink for current assimilate allocation below ground is the fungal mycelium and its associated reproductive structures. Godbold *et al.* (2006) have shown that the external ECM mycelium of popal can provide the dominant pathway by which C enters the soil organic matter pool, far exceeding the inputs from leaf litter and fine root turnover. These observations are consistent with the losses of respiratory activity seen following hyphal severance

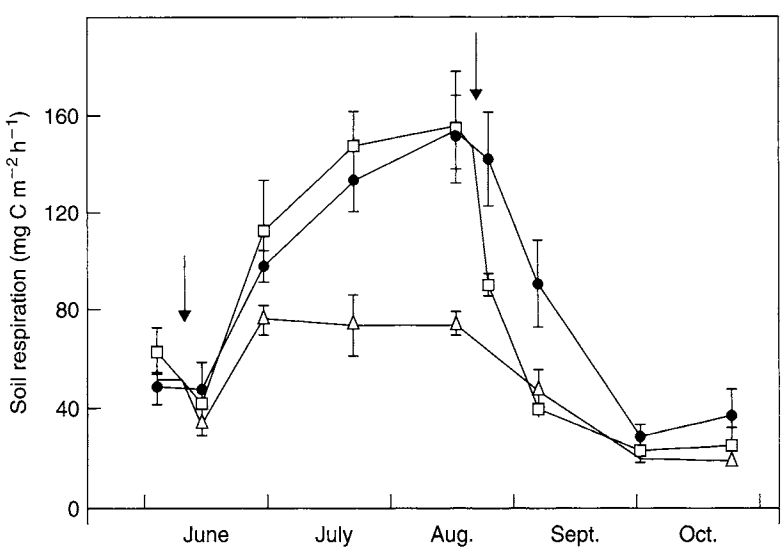

Figure 8.10 Respiratory soil CO_2 efflux from ungirdled control (●) early girdled (△) and late girdled (□) plots of *Pinus sylvestris*. Arrows indicated times of early (June) and late (August) girdling. After the early girdling, soil respiration was reduced by ~54% within one month, and after the late girdling it was reduced by 37% within 5 days. Högberg *et al.* (2001).

Table 8.7 The numbers of species and of sporocarps and their dry weights collected from early (June) and late (August) girdled and from control (ungirdled) plots in a 50-year-old stand of *Pinus sylvestris* at Aheden, Sweden.

Treatment	No. of species	No. of sporocarps	Biomass (g dry wt)
Control	11 ± 3	252 ± 116	84.4 ± 24.4
Early girdling	1 ± 1 (72)	4 ± 4 (72)	0.5 ± 0.5 (72)
Late girdling	10 ± 1 (2–3)	108 ± 48 (2–3)	40.9 ± 8.5 (2–3)

From Högberg *et al.* (2001). Figures in brackets are the number of days since the girdling treatment. Sporocarp production was virtually eliminated 72 days after early girdling and more than halved within 2–3 days of late girdling.

(Söderström and Read, 1987; see Figure 8.6) and the collapse of sporocarp production following canopy shading (Lamhamedi *et al.*, 1994; see Figure 8.7).

The girdling study revealed two further features that emphasize the importance of seasonality in determining the C balance of the ECM community. First, the decline of respiration at 5 days following girdling early in June was, at 27%, considerably less than that in September. This is probably in part attributable to the fact that temperate forest conifers preferentially allocate C to support shoot, needle and bud expansion growth early in the season (Hansen *et al.*, 1997). Another contributory factor is that, at this time of year, the roots contain significantly more starch as a potential respiratory substrate (Högberg *et al.*, 2001). Second, the observation that the highest levels of respiratory activity were recorded in August and September, well after the period of maximum solar irradiance and highest temperature in June and July, eliminate these factors as being the primary drivers of the respiratory events. Högberg and Högberg (2002) showed a decline of microbial biomass in the girdled plots of around 32% 1–3 months after the treatment was applied and calculated that one third of the total soil microbial biomass was attributable to the ECM mycelial component. These authors stress that the possible contribution of the ECM biomass to soil respiration is likely to be underestimated because respiration of the heterotrophic community was probably stimulated by substrate released from the decomposing ECM structures. In a follow-up study two years following girdling, it was shown that respiratory output in the treated plots had reduced by 65% (Bhupinderpal *et al.*, 2003). This can be expected to be a more precise measure of the contribution of the mycorrhizal roots and mycelium to soil respiration because, by this stage, the alternative substrates released as a result of the girdling activity, and hitherto being used to support the heterotrophic community, would have been exhausted.

Two scenarios emerge for below-ground C allocation. The first predicts that fine roots are a major and rapidly turning over destination for current assimilate. The second shows that the roots are a less important sink and that the mycelial and fruiting components of the ECM systems are the primary C sinks, their production and activities being tightly coupled to current assimilate supply. Calculations based upon the first scenario indicate that fine root production in pine forest of the kind studied by Finlay and Söderström (1989) is of the order of 2030 kg/ha/year (Persson, 1978). With the conservative estimate that 90% of these roots are ECM and that 40% of an ECM root is fungal (Harley and McCready, 1952b; Vogt *et al.*, 1982), 730 kg of fungal sheath would be produced per year. Thus, total annual fungal production (mycelium = 70 kg, sporophores = 30 kg, sheath = 730 kg, see above)

would be 830 kg/ha. If the C content of the fungal material is taken to be 40% and the respiratory efficiency is 60%, the C demand of ECM fungi in the forest would be 830 kg C/ha/year. Photosynthetic production in the same forest has been estimated at 5800 kg C/ha/year (Linder and Axelsson, 1982). On this basis, the ECM fungi use around 15% of assimilated C. This value, as Finlay and Söderström (1992) point out, is strikingly similar to those presented by Vogt *et al.* (1982) and, indeed, by Rommell (1939b).

Clearly, these values may need to be revised in the light of the suggestion that fine root and hence fungal mantle production and turnover are lower, perhaps by at least four times, than those indicated by the coring studies. Set against the possible reductions in estimates of C allocation required by a lower value of fine root production, are the observations that both mycelial (Wallander *et al.*, 2001, 2004) and fruit body production (Högberg *et al.*, 2001) in these types of ECM forest can be considerably greater than earlier estimates suggest. Accordingly, while values for production of biomass of mantles may need to be reduced by four times, those for mycelial biomass and fruit body production could require sixfold and fourfold increases, respectively. Repeating the earlier calculation assuming the same C conversion and efficiency factors the values, based on 172 kg/ha/year for fine root turnover, 420 kg/ha/year for mycelium and 12 kg/ha/year for fruit bodies is equivalent to a carbon allocation of 722 kg/ha/year. This would still be around 12.5% of annual primary production of the stand.

Conclusions

The construction of nutrient response curves has gone some way to providing an understanding of the influence of ECM colonization on plant growth. When plants face a shortage of a mineral element such as P, there is a threshold of availability below which growth will not occur. By enhancing access to the growth-limiting nutrient, colonization can significantly reduce the threshold. The extent of plant response varies with the fungus and its pattern has been shown to be distinctive at inter- and even at intraspecific level, both in the effectiveness with which the nutrient resource is exploited and in the C demands imposed upon the plant. It is now recognized that relationships between effectiveness of resource exploitation and extent of C demand can usefully be explored by means of cost-benefit analysis. Costs and benefits of ECM associations are again shown to differ with fungus and experimental circumstances.

Further progress has been made towards understanding of the molecular-biochemical mechanisms involved in C transfer from autotroph to heterotroph. While not changing our perceptions concerning the nature and localization of the biochemistry of transfer at the interface between the partners, the new research has begun to identify specific sugar transporters and to elucidate some of the under-lying mechanisms that control the transfer.

We are now better aware of the extent of coupling between the flow of current assimilate, the activities of the extraradical mycelium and the production of sporocarps. It had been calculated from laboratory experiments that between 10 and 20% of photosynthate C was allocated to sustain the vegetative mycelium of ECM fungi, but major advances have now been achieved by extending these studies to the field.

Elimination of photosynthate supply by tree stem girdling in pine, leads rapidly to major reductions of soil respiratory CO_2 release and almost instantaneously to cessation of sporocarp production. Calculations based upon this type of field experiment indicate that ECM mycelia constitute at least one third and probably a good deal more of total soil microbial biomass. As a major sink for plant C these components of the mutualism directly influence assimilation rates in the leaves, giving the potential for positive feedbacks on allocation of C below ground. There is now a need for field experiments involving manipulation of source-sink relations to be carried out in different types of forest in order to determine the relative importance of mycorrhizal communities as C sinks in a range of ecosystems.