

Phosphorus and Carbon Relationships in Willow Ectomycorrhizae*

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Abstract

The growth response of a *Salix viminalis* L. clone to a range of soil P concentrations, and carbon allocation at the lowest soil P concentration, were determined on ectomycorrhizal (*Laccaria proxima* or *Thelephora terrestris*) and non-mycorrhizal plants. Plants responded to mycorrhizal inoculation and P addition up to, but not above, 10 mg P kg⁻¹ soil. It was concluded that the growth response was due to increased P uptake. Using pulse-chase ¹⁴C labelling, mycorrhizal plants were found to have diverted 7% more of their assimilated carbon belowground than non-mycorrhizal plants in one experiment; in a second experiment 12% more was allocated belowground.

Introduction

Major increases in the growth of higher plants may follow their infection by mycorrhizal fungi. This growth enhancement is usually due to an improvement in the nutritional status of the plant, and is accompanied by a flow of fixed carbon from the plant to the fungus. The growth enhancement, in spite of the carbon drain occurs because total photosynthesis is commonly increased by mycorrhizal infection. These conclusions have been well established for the vesicular-arbuscular (VA) mycorrhizae, where growth enhancement is almost always due to an increase in phosphorus supply. The amount of carbon diverted from the higher plant into the VA mycorrhizal fungus has been estimated as approximately 5-10% of the total amount fixed by the plant (Smith and Gianinazzi-Pearson 1988).

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These concepts have been less seriously studied with the ectomycorrhizae. There is only one published experiment in which a full response curve with varying levels of phosphorus in the soil has been determined both with and without mycorrhizal infection (Bougher et al., 1990). That carbon drain by ectomycorrhizae can be substantial is suggested by the growth suppression sometimes seen in young mycorrhizal seedlings, especially when they are grown under nutrient-sufficient conditions. Only one detailed experiment quantifying this carbon cost has been reported (Reid et al., 1983), but field measurements on adult trees have led to estimates as high as 50% of the total stand throughput passing through mycorrhizae (roots plus mantles) (Fogel and Hunt 1983). If correct, this would be extremely important for forest productivity, soil ecology and our understanding of the carbon cycle.

The overall assessment of the carbon cost of a mycorrhizal association is most simply made in terms of the allocation of carbon between shoot and root. This depends upon the assumption that the rate-limiting step in plant growth is light interception. Consequently any carbon which could have formed additional photosynthetic tissue, but is instead diverted to the root, can be seen as causing a reduction in the potential rate of growth of the plant. Thus, as a simple first estimate, the difference between the percentage of carbon fixed which is allocated belowground in mycorrhizal and non-mycorrhizal plants can be considered as the mycorrhizal cost.

The situation is somewhat more complex than this, however, because soil and plant nutrient status influences the relative distribution of carbon between root and shoot systems. When grown under the low nutrient conditions where growth stimulations are observed, non-mycorrhizal plants are phosphorus deficient. Thus, part of the difference in carbon allocation pattern could be due to a difference in physiology between the phosphorus-sufficient mycorrhizal plants and the phosphorus-deficient non-mycorrhizal plants. In the present experiment, carbon allocation in non-mycorrhizal plants of low phosphorus status was compared with that of mycorrhizal plants and with non-mycorrhizal plants matched for size and phosphorus status with mycorrhizal plants.

Materials and Methods

Five replicate cuttings per treatment of *Salix viminalis* L. Bowles Hybrid were surface sterilized, rooted, and planted in a sandy loam soil mixed 1:2 (v/v) with sterile washed sand. Peat/vermiculite spawn of *Laccaria proxima* (Boud.)Pat or *Thelephora terrestris* (Ehrh.)Fr. or autoclaved spawn was combined 1:5 (v/v) with the soil:sand mixture. This final substrate contained 4, 6, 10, 21, 60 or 90 mg bicarbonate-extractable P kg⁻¹ soil through the addition of KH₂PO₄. After 12 weeks, the plants were harvested, dried and weighed, and a sub-sample of the roots stained (Philips and Hayman, 1970). The grid-intersect method was used to assess the proportion of the root length colonized by the fungi (Giovannetti and Mosse 1980).

The carbon demand of two ectomycorrhizal fungi upon willow was determined in two pulse-chase experiments. In the first experiment, three uninoculated and three plants inoculated with *L. proxima* from the above experiment, grown at 4 mg P kg^{-1} for 18.5 weeks, were used. They were sealed into two-compartment chambers which allowed the atmospheres around the shoots and roots to be separately sampled (Snellgrove et al., 1982). The root compartment consisted of a PVC sleeve through which CO_2 -free air was drawn. The shoots were pulse labelled (1 h) with $1.1 \text{ MBq } ^{14}\text{CO}_2$. Air from the shoot and root compartments, containing respired $^{14}\text{CO}_2$, was bubbled through separate traps containing 100 ml 1N NaOH. The NaOH was sampled and replaced at 12, 36, 60, 84, 108, and 202 h following the initial introduction of $^{14}\text{CO}_2$. The plants were harvested after 202 h and the roots and shoots separated from the soil. Ground samples of soil and plant tissue were combusted in a sample oxidizer before liquid scintillation counting. The total fixed ^{14}C was separated into five fractions: shoot tissue, shoot respiration, root tissue, root respiration and soil.

In the second experiment, three groups of willow were grown for 13 weeks: non-mycorrhizal willow plants and plants infected with *T. terrestris*, grown at 6.1 mg P kg^{-1} soil, and non-mycorrhizal plants grown at 21 mg P kg^{-1} soil. All other methods were as described above. The inoculated plants had $33.3 \pm 2.6\%$ of their root length colonized by the mycorrhizal fungus in experiment 1 and $39.7 \pm 2.3\%$ in experiment 2.

Results and Discussion

1. Responses to phosphorus, and the origin of the mycorrhizal effect

The plants responded to mycorrhizal inoculation by 10 weeks after planting and inoculation, indicating that mycorrhizal infection had occurred before this time. Growth responses occurred at the lowest three soil phosphorus levels only (Table 1). Above 21 mg P kg^{-1} , there were no significant effects either from further phosphate additions or from infection.

The way in which ectomycorrhizal infection is best quantified is debatable. In this case, the length of infected root could easily be measured by standard intercept techniques (Giovannetti and Mosse 1980). The percentage infection by *L. proxima* was constant up to 10 mg P kg^{-1} , then diminished regularly with increasing soil P, being virtually eliminated at 60 mg P kg^{-1} (Table 1). Percentage infection with *T. terrestris* was less affected by high levels of added phosphorus.

Response curves to phosphorus obtained with mycorrhizal and non-mycorrhizal willow thus followed the pattern found previously for VA mycorrhizae. Mycorrhizal inoculation stimulated growth at soil P concentrations below 21 mg P kg^{-1} where phosphorus was limiting. Above this concentration, infection diminished. Thus, it is

Table 1. Shoot dry weight and percent infection of *Salix viminalis* cuttings grown at a range of P concentrations

Fungus	Soil Phosphorus Concentration (mg kg ⁻¹)					
	4	6	10	21	60	90
<i>Shoot dry weight (g)</i>						
<i>L. proxima</i>	0.64b	0.76b	1.34b	1.76a	2.49a	2.42a
<i>T. terrestris</i>	0.47b	0.69b	0.94a	1.60a	1.95a	1.99a
Non-mycorrhizal	0.30a	0.39a	0.66a	1.96a	2.27a	1.94a
P†	0.0007	0.0001	0.003	0.35	0.38	0.28
<i>Root length colonized (%)</i>						
<i>L. proxima</i>	46 ± 5	46 ± 3	46 ± 4	34 ± 6	1 ± 0	0 ± 0
<i>T. terrestris</i>	55 ± 4	46 ± 6	45 ± 4	48 ± 6	8 ± 4	0 ± 0

Means ± standard errors; n=3 for mycorrhizal treatments and n=5 for the uninoculated treatment except for shoot weight where n=5 and n=10 for the mycorrhizal and uninoculated treatments, respectively. Values within a column followed by different letters differ significantly according to a Fishers Protected Least Significant Difference Test at $P=0.05$.

†Probability values of one factor ANOVAs between inoculation treatments for each level of phosphorus. Percent infection did not differ between fungi at any phosphorus level.

concluded that the growth response is solely due to increased phosphate supply by the mycorrhizae.

2. Carbon allocation in mycorrhizal and non-mycorrhizal plants

The total allocation of photosynthesized carbon in mycorrhizal and non-mycorrhizal plants can be found by short term labelling via fixation of ¹⁴CO₂, followed by a long chase period such that the evolution of respired ¹⁴CO₂ is almost completed. At this stage, the majority of the label has been incorporated into non-labile pools (Dickman and Kozlowski, 1970). Thus, an appreciable further change in ¹⁴C allocation between shoot and root is unlikely. In the present experiment, the evolution of radioactive CO₂ from both shoot and root plus soil was measured continuously over the long chase period. The percentage allocation of carbon to these components plus that retained in root and shoot tissue and in soil is shown in Table 2.

The allocation belowground in the two experiments were similar even though different mycobionts were present (Table 2). In experiment 1, mycorrhizal plants diverted 7% more of the ¹⁴C fixed belowground than did low-P non-mycorrhizal plants. Twelve percent more of the total fixed carbon was allocated belowground by mycorrhizal plants in the second experiment. The larger difference was possibly because plants in the second experiment were younger and growing more vigorously. The belowground carbon allocation of the high-P non-mycorrhizal plants of the second experiment was intermediate between the other two treatments.

This difference in belowground carbon allocation can be taken as one measure of the additional carbon cost of a mycorrhizal symbiosis because this carbon was not

Table 2. Percent distribution of ^{14}C assimilated by non-mycorrhizal willow of low (NM-P) and high (NM+P) phosphorus status, and by mycorrhizal (M) willow, after 1 h pulse labelling with $^{14}\text{CO}_2$ and a 202 h chase period

Compartment	Experiment 1 [†]		Experiment 2 [‡]		
	NM	M	NM-P	NM+P	M
Shoot Tissue	46.8	48.2	50.5	46.2	40.4*
Shoot Respiration	15.1	6.6	10.7	9.1	8.7**
Root Tissue	17.9	27.3	16.1	20.8	24.1**
Root Respiration	16.7	14.0	19.4	20.6	22.6
Soil	3.6	3.9	3.3	3.3	4.3
Total Belowground	38.1	45.2	38.8	44.7	50.9*

Values are percent of total ^{14}C assimilated by plants and are means of three replicate plants, except for experiment 1, NM where there were two replicates only.

[†]20-week-old plants infected with *Laccaria proxima*.

[‡]13-week-old plants infected with *Thelephora terrestris*.

Note: Experiment 2 treatments differed significantly at $P=0.10$ (*) or $P=0.05$ (**) as indicated by a one-way ANOVA.

available for manufacture of photosynthetic structures. In the present experiment, roughly 7–12% less of the total fixed carbon was allocated aboveground in ecto-mycorrhizal plants than in the low phosphorus non-mycorrhizal plants. Nevertheless, because the total photosynthesis by mycorrhizal plants was substantially higher (data not shown), the benefit of a mycorrhizal association for these plants was not far short of a doubling of their shoot growth.

Additional insight into the question of mycorrhizal cost can be gained by comparing carbon allocation in mycorrhizal plants with non-mycorrhizal plants matched to the mycorrhizal plants in size and phosphorus status. As shown above (Table 1), the increased plant size, and consequently the amount of carbon fixed by mycorrhizal plants, is due to their improved phosphorus status. Approximately half of the increase in belowground carbon allocation was apparently due to the differences in physiology caused by higher phosphorus status (Table 2). The remaining 6% can be considered to be a carbon drain due directly to the fungus, and that the shoot, at this level of phosphorus supply, could have grown even larger if this carbon had not been diverted to the roots and thence to the fungus.

The additional carbon initially translocated to the roots of mycorrhizal plants can be deposited within fungal tissue associated with the root, or in the soil; it can be used for the formation of additional root; it can leak in soluble form or as sloughed-off cells into the soil; or it can be respired, either directly from the fungal tissue or as increased respiration by the host root tissues. In the present experiments, the increased belowground ^{14}C was found in the root tissue fraction (=root plus internal fungal

tissue), rather than in respiration or in the soil. This is in contrast to some earlier studies with VA mycorrhizae where it was the root respiration fraction that was increased in mycorrhizal plants (Snellgrove et al., 1982). Our results are consistent with the rapid growth of the mycorrhizal root systems (root/shoot ratios were 0.69 ± 0.07 and 0.48 ± 0.05 for mycorrhizal and low phosphorus non-mycorrhizal plants respectively, in experiment 2). Rapid growth rates would increase the amount of carbon in both the tissue and respiration fractions. In non-mycorrhizal plants, however, less new root tissue was being produced and therefore a higher proportion of the belowground ^{14}C appeared as respiration.

This increase of 6–12% in belowground carbon allocation caused by ectomycorrhizal infection is in excellent agreement with published data, from VA mycorrhizal studies (Pang and Paul 1980, Snellgrove et al., 1982). It also falls within the range of cost values found by Reid et al. (1983) in which 6–36% more of the total fixed carbon was diverted from above to belowground in ectomycorrhizal plants. These data suggest that the costs of the symbiosis are appreciable, but they are less than some field estimates, which range from 15–24% of net primary production (Vogt et al., 1982). Our estimates are far less than the 50% of total stand throughput suggested by earlier field work as supporting mycorrhizae (Fogel and Hunt, 1983). These field estimates do not necessarily include the extramatrical hyphae and respiration components. Further collaboration between field and laboratory researchers is needed so that detailed physiological measurements can be used to augment estimates of dry matter turnover. Thus a more complete picture of the effect of ectomycorrhizae on the carbon physiology of their hosts could be achieved.

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