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Mycorrhizal C costs and nutritional benefits in developing grapevines

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Abstract Arbuscular mycorrhizal (AM) C-costs in grapevines were investigated. Dormant vines rely on stored C for initial growth. Therefore AM colonisation costs would compete with plant growth for available C reserves. One-year-old grapevines, colonised with *Glomus etunicatum* (Becker and Gerdemann), were cultivated under glasshouse conditions. The C-economy and P utilisation of the symbiosis were sequentially analysed. AM colonisation, during the 0–67 day growth period, used more stem C relative to root C, which resulted in lower shoot growth. The decline in AM colonisation during the period of 67–119 days coincided with stem C replenishment and higher shoot growth. Construction costs of AM plants and root C allocation increased with root P uptake. The efficiency of P utilisation was lower in AM roots. The reliance of AM colonisation on stem C declined with a decrease in colonisation, providing more C for the refilling of stem carbohydrate reserves and shoot growth. Once established, the AM symbiosis increased P uptake at the expense of refilling of root C reserves. Although higher root C allocation increased plant construction costs, AM roots were more efficient at P utilisation.

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Introduction

Marschner and Dell (1994) showed that arbuscular mycorrhizal (AM) fungi can supply 80% of P and 25% of N to the host plants. The enhanced P nutrition of AM plants growing in phosphate-limited soils, usually leads to higher plant growth rates than non-AM plants (Sanders and Tinker 1971; Smith 1982; Bolan 1991; Orcutt and Nilsen 2000). The dependency of the host plant on AM is balanced by the costs of maintaining the relationship. The costs of the symbiosis are in the form of organic carbon (C) derived from the host, which is transported below ground due to the sink effect of the fungus (Snellgrove et al. 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990). The C-costs of the fungus can be considerable and the fungus can receive up to 23% of the plant's photosynthetically fixed carbon (Snellgrove et al. 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990).

The C taken up by an AM fungus is incorporated into the growth and development of new fungal structures and spores. More than 90% of plant roots can be colonised by an AM fungus (Motosugi et al. 2002), which can constitute up to 20% of the root dry mass (Harris and Paul 1987). Respiration of AM roots was found to be between 6.6% and 16.5% (depending on fungal species) higher than uncolonised roots in cucumber plants (Pearson and Jakobsen 1993). The increased respiration rate contributes to the sink effect of the fungus, and indicates that colonised roots have a higher metabolic activity than uncolonised roots.

The majority of studies to date have concentrated on quantifying the effects of AM and the C-costs of the symbiosis using photosynthetically fixed C (Koide and Elliot 1989; Peng et al. 1993; Fay et al. 1996; Wright et al. 1998a; Black et al. 2000). Few research projects have focused on the mobilisation and utilisation of C from storage tissue

for the development of the AM symbiosis. Merryweather and Fitter (1995) showed the reallocation of stored C for the growth of new roots and shoots in the geophyte *Hyacinthoides non-scripta* (L) Chouard ex Rothm. This latter study found that the C needed for the new season's growth was derived from C stored within the bulb. However, as the plant's photosynthetic tissue developed, its reliance on stored C declined, allowing the C reserves to be replenished.

A number of factors will determine a plant's dependency on AM for nutrients, one being the type of root system. This is seen with coarse-rooted plant species, such as vines, which are more dependent on AM for nutrient uptake than finer rooted species (Eissenstat 1992; Motosugi et al. 2002). In deciduous plants like vines, which rely on stored C for the growth of new tissues in spring, the additional C-drain for AM fungal growth could also tap into the plant C reserves (Buttrose 1966; McArtney and Ferree 1998). AM fungal growth and nutrient acquisition also present a considerable C-drain on host plant reserves (Kucey and Paul 1982; Peng et al. 1993; Johnson et al. 1997; Black et al. 2000) and could therefore influence C reserve mobilisation during the initial growth stages of the vine. The aim of this study was to assess the host C economy during AM development and the subsequent C-costs of N and P uptake once root colonisation is established.

Materials and methods

Plant growth and AM inoculation

One-year-old grafted grapevine cuttings (*Vitis vinifera* L. cv. Pinotage, grafted onto Richter 99 rootstocks) were planted in 20 l pots containing river sand, between May and August 2002. The average sand grain size was 0.51 mm, and the pH was 7. The sand was sterilised in an autoclave for 1 h at a temperature of 120°C and a pressure of 200 kPa. The pots were placed in a north-facing glasshouse at the University of Stellenbosch, Stellenbosch, South Africa. The maximum daily photosynthetically active irradiance was between 600 and 700 μmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 23/15°C and 35/75% respectively. The plants were watered with distilled water and, every 2nd week, with a standard Long Ashton nutrient solution. The standard Long Ashton solution was modified to contain nitrate as the N source, 100 μM phosphate and pH 6. The P concentration was based on soil nutrient analyses from a study on a Western Cape vineyard (Meyer 2002). The inoculum consisted of spores and hyphae from *Glomus etunicatum* (Becker and Gerdemann) (accession number J100092, Moss Herbarium, University of the Witwatersrand) and host root fragments in an inert clay-based granular support substrate. The inoculum was produced from host roots of 6-week-old sorghum plants, grown in pot culture with an inert clay-based granular substrate. The experimental plants were inoculated in the sand-culture pots, by layering 20 g inoculum at 1 cm below the level of the rootstock. This was to ensure that as the developing roots grow through the sand, they would come into contact with the inoculum below. The control plants received a filtered inoculum solution, which was prepared by filtering the inoculum through a 37 μm mesh to remove mycorrhizal fungal material.

Harvesting and nutrient analysis

The vines were pruned back to one-bud and allowed to grow until the respective harvests, which took place after 21, 43, 67, 95 and 119 days. Upon harvesting, the plants were separated into different components, which consisted of new shoot tissue, woody scion tissue, stem and roots (new and old roots), and the fresh weight of each component was recorded. Sub-samples of new root segments were obtained by cutting the new root material into 1 cm strips and then randomly selecting samples. These samples were stored in 50% ethanol in order to determine percentage AM fungal colonisation at a later stage. The harvested material was then placed in an oven at 80°C for 2 days and dry weights (DW) were recorded. The dried plant material was milled using a 0.5 mm mesh (Arthur H Thomas, Philadelphia, Pa.). In addition, a bulk sample was also made up by combining proportional sub-samples of each component, based on the percentage of each component in the whole plant (e.g. where roots were 10% of the plant DW, then the bulk samples comprised 10% of the root material). Milled samples were analysed for their respective C, N and P concentrations by a commercial laboratory (BemLab, De Beers Rd, Somerset West, South Africa), using inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with Spectrascan standards (Drobak, Norway).

Determination of percentage AM colonisation

Roots were harvested at 21, 43, 67, 95 and 119 days. Non-woody roots were cut into 1 cm segments and rinsed and cleared with 10% KOH for 5 min in an autoclave at 110°C under steam pressure of 200 kPa. The KOH was rinsed off and the segments acidified with 2 *N* HCl for 10 min. Thereafter, the roots were stained with 0.05% (w/v) aniline blue for 10 min in an autoclave at 110°C under steam pressure of 200 kPa and then destained in lactic acid overnight. Root segments were placed on slides and the colonisation components were determined according to Brundrett et al. (1994).

Calculation of C-costs and AM efficiency

Root and stem C fluxes (mmolC tissue $type^{-1}$ day^{-1}) represent the C fluxes of the root and stem tissues for a given growth period. C flux is expressed as the rate of movement of an absolute amount of C in a specific tissue, and is calculated by dividing the change in C content of a specific tissue over time.

Root construction costs (mmolC gDW^{-1}) were calculated from the tissue construction cost, modified from the equation used by Peng et al. (1993): Cw = [C + kN/14×180/24] (1/0.89)(6,000/180), where Cw is the construction cost of the tissue (mmolC gDW^{-1}), C is the carbon concentration (mmolC/g), k is the reduction state of the N substrate (NO_3 was used, therefore k is +5) and N is the organic nitrogen content of the tissue (g gDW^{-1}) (Williams et al. 1987). The constant (1/0.89) represents the fraction of the construction cost that provides reductant that is not incorporated into biomass (Williams et al. 1987; Peng et al. 1993) and (6,000/180) converts units of g glucose gDW^{-1} to mmolC gDW^{-1}.

Efficiency of P utilisation was expressed using the equation proposed by Koide and Elliott (1989) to calculate the quantity of C accumulated divided by the quantity of P accumulated for a given period of time: $\Delta C^r/\Delta P^r$, where ΔC^r is the C accumulated in the roots over a given time period and ΔP^r is the total P accumulated in the roots over the same time period. Similarly, the efficiency of shoot P utilisation was calculated using the C and P values of the shoots. It should be noted that a low efficiency value indicates that less C is required for the given amount of P utilised by the plant or plant component.

Growth respiration (mmol CO_2 gDW^{-1}) representing the C respired for the biosynthesis of new tissue was calculated as proposed by Peng et al. (1993): Rg(w) = Rg (t)/root gr, Rg(w) represents growth respiration based on dry weight, root gr is the root growth rate (gDW day^{-1}) and Rg(t) is the daily growth respiration (μmol CO_2 day^{-1}): $R_{G(t)} = C_t - \Delta W_c$, C_t (μmol CO_2 day^{-1}) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (gDW day^{-1}) by tissue construction cost (C_w). ΔW_c (μmol day^{-1}) is the change in root C content and was calculated by multiplying the root C content and the root growth rate.

Statistical analysis

The differences in percentage AM colonisation between harvests (n=6 for each treatment) were separated using a post hoc Student Newman Kuels (SNK), multiple comparison test ($P \leq 0.05$) (SuperAnova). Different letters indicate significant differences between treatments. The percentage data were arcsine transformed (Zar 1999). For each harvest, the difference between the means of AM and non-AM plants was separated using a Student's t-test (Statistica 6.0, StatSoft, Tulsa, Okla.) for independent samples by groups ($P \leq 0.05$). Different letters indicate significant differences between treatments.

Results

Growth and C-fluxes

Uninoculated plants remained non-mycorrhizal for the duration of the experiment. The percentage and rate of AM fungal colonisation (Fig. 1, Table 1) increased over days 0–67 and declined during days 67–119. This coincided with a loss of C from the above-ground components of AM plants, (Table 1) relative to non-AM plants. During the 0–67 day period of colonisation and above ground C loss, the shoot growth rate (Table 1) was lower in the AM plants. Furthermore, there was also a similar loss of C

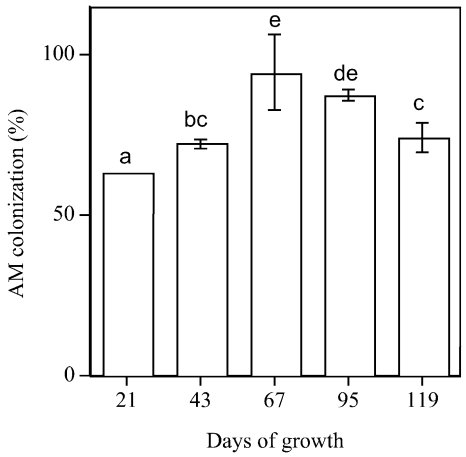

Fig. 1 Percentage arbuscular mycorrhizal (AM) (n=6) colonisation of grapevines root systems by *Glomus etunicatum*. Grapevines were grown under glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. The differences between harvests (n=6) were separated using a post hoc Student Newman Kuels (SNK), multiple comparison test (P<0.05). Significant differences (P<0.05) between each harvest are indicated by different letters

Table 1 Colonisation rate of AM roots (% root length infected day^{-1}), above-ground (mmol C shoot and stem $tissue^{-1}$ day^{-1}) and root C fluxes (mmol C $root^{-1}$ day^{-1}), and new root and shoot growth rates [g dry weight (DW) day^{-1}] of grapevines infected with *Glomus etunicatum*. Grapevines grown under glasshouse conditions were harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and uninoculated (−AM) (n=6) plants were determined by Student's t-test for independent samples by groups. Significant differences (P<0.05) between inoculated and uninoculated plants for each harvest, are indicated by different letters

Parameters	Growth period (days)	
	0–67	67–119
Colonisation rate (% root length infected day^{-1})		
+ AM	1.41 b	−0.31 a
Above ground C flux (mmol C shoot and stem $tissue^{-1}$ day^{-1})		
+ AM	−5.61 a	4.12 a
−AM	11.6 b	2.03 a
Root C flux (mmol C $root^{-1}$ day^{-1})		
+ AM	−1.57 a	−3.37 a
−AM	−1.53 a	3.11 b
New root growth rate (gDW day^{-1})		
+ AM	0.024 a	0.131 b
−AM	0.043 b	0.102 a
Shoot growth rate (gDW day^{-1})		
+ AM	2.87 a	5.26 b
−AM	5.2 b	2.86 a

Table 2 DW (g) of grapevines infected with *Glomus etunicatum*. Grapevines grown under glasshouse conditions were harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and uninoculated (−AM) (n=6) plants were determined by Student's t-test for independent samples by groups. Significant differences (P<0.05) between inoculated and uninoculated plants for each harvest are indicated by different letters

Component DW (g)	Days of growth				
	21	43	67	95	119
Shoot					
+AM	0.36 a	1.87 a	2.70 a	7.23 a	9.08 a
−AM	0.41 a	2.01 a	5.35 b	8.83 a	7.78 a
Stem					
+AM	8.68 a	10.73 a	11.46 a	10.67 a	11.69 a
−AM	6.18 a	11.53 a	9.64 a	10.72 a	11.45 a
Scion					
+AM	1.99 a	1.35 a	2.01 a	1.87 a	2.36 a
−AM	1.28 b	1.73 a	2.16 a	2.50 a	1.95 a
Root					
+AM	8.00 a	7.04 a	10.72 a	7.02 a	10.26 a
−AM	7.20 a	6.85 a	6.76 b	11.48 a	8.55 a
Bulk					
+AM	32.73 a	38.02 a	45.93 a	39.03 a	36.63 a
−AM	24.26 a	43.28 a	38.58 a	40.62 a	46.45 a

from AM and non-AM roots during this period. For the 0–67 day phase, new root growth rate was lower in AM plants (Table 1).

As the percentage and rate of AM fungal colonisation declined during the 67–119 day period, refilling of above-ground C reserves occurred in AM plants along with an increase in new root and shoot growth rates (Table 1). During the same period the non-AM roots had an increase in C flux, whilst the AM roots maintained a negative C flux (Table 1).

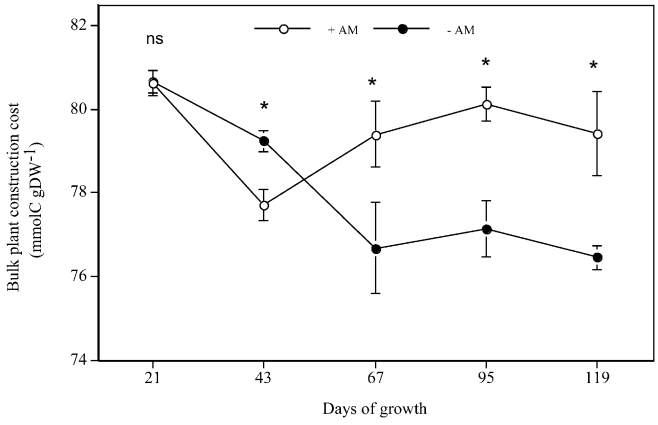

Fig. 2 The bulk construction costs (Cw) of glasshouse-cultivated grapevines (mmol C gDW^{-1}). Grapevines colonised by *G. etunicatum* were harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and uninoculated (−AM) (n=6) plants were determined by t-test for independent samples by groups. * Significant differences (P<0.05) between inoculated and non-inoculated plants for each harvest, *ns* non-significance

Table 3 Shoot N (mmol N g DW^{-1}), shoot P (mmol P g DW^{-1}), bulk N (mmol N g DW^{-1}) and bulk P (mmol P g DW^{-1}) concentrations of grapevines infected with*Glomus etunicatum*.Grapevines grown under glasshouse conditions were harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and uninoculated (−AM) (n=6) plants were determined by Student's t-test for independent samples by groups. Significant differences (P<0.05) between inoculated and uninoculated plants for each harvest, are indicated by different letters

Nutrient concentration	Days of growth				
	21	43	67	95	119
N (mmol N gDW^{-1})					
+AM shoot	2.50 a	1.71 a	1.19 a	1.08 a	0.95 a
−AM shoot	2.46 a	1.76 a	1.26 a	0.94 a	0.88 a
+AM bulk	0.48 x	0.46 x	0.51 x	0.53 x	0.53 x
−AM bulk	0.52 x	0.44 x	0.48 x	0.53 x	0.51 x
P (mmol P gDW^{-1})					
+AM shoot	0.16 a	0.10 a	0.07 a	0.04 a	0.03 a
−AM shoot	0.16 a	0.10 a	0.05 a	0.03 a	0.03 a
+AM bulk	0.04 x	0.03 x	0.03 x	0.02 x	0.02 x

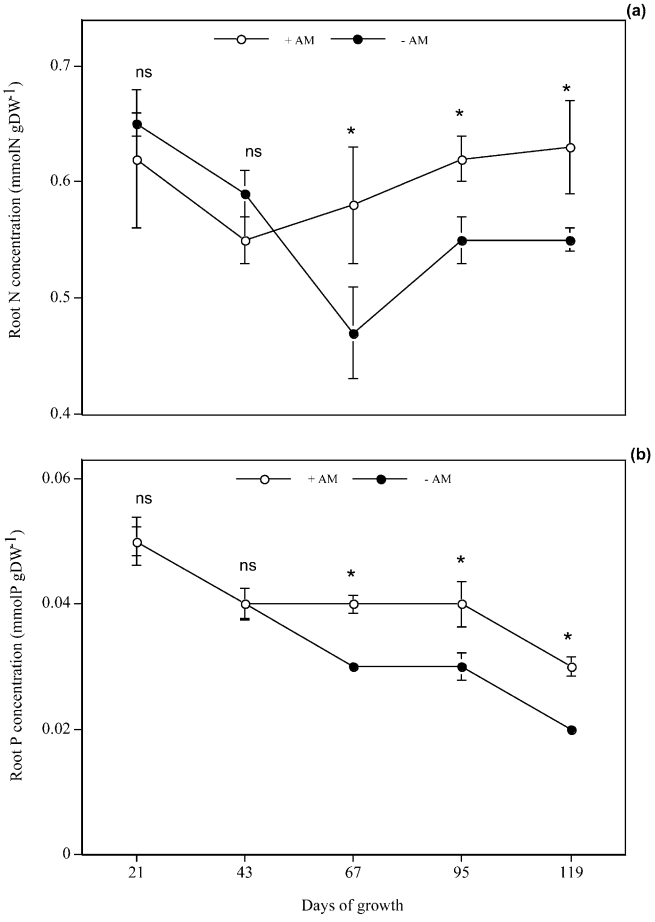

Fig. 3 a Root N concentration (mmol N gDW^{-1}) and **b** root P concentration (mmolP gDW^{-1}) of grapevines colonised by *G. etunicatum*. Grapevines were grown under glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and non-inoculated (−AM) (n=6) plants were determined by t-test for independent samples by groups. * Significant differences (P<0.05) between inoculated and non-inoculated plants for each harvest, *ns* non-significance

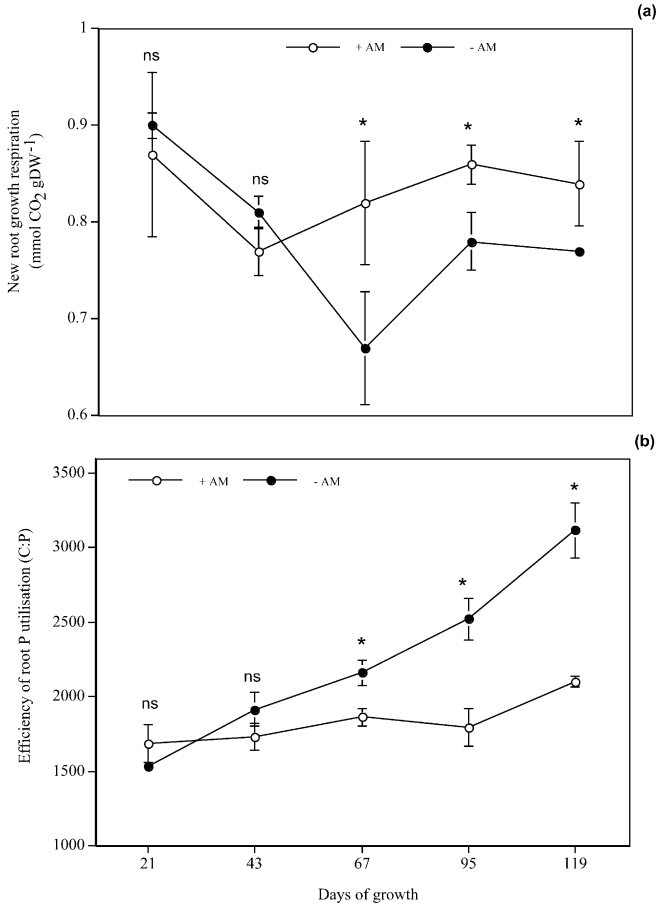

Fig. 4 a New root growth respiration (mmol CO_2 gDW^{-1}) and **b** efficiency of P utilisation (C:P). Glasshouse-cultivated grapevines were colonised by *G. etunicatum* and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n=6) and non-inoculated (−AM) (n=6) plants were determined by t-test for independent samples by groups. * Significant differences (P<0.05) between inoculated and non-inoculated plants for each harvest, *ns* non-significance

At day 67, when AM fungal colonisation was at a maximum (Fig. 1), the AM shoot DW was lower, whilst the AM root DWs were higher than the non-AM plants (Table 2). As AM fungal colonisation declined after day 67, there were no further differences between AM and non-AM DWs for all the components (Table 2). However, the AM plants had a lower tissue construction cost (Cw) from 0–43 days, and a higher Cw from day 67 onwards (Fig. 2).

Nutrient assimilation

No overall differences were found in the N and P concentrations of the shoots or the bulked plants between AM and non-AM plants for the duration of the experiment (Table 3). There were also no differences prior to day 67 in the root N and P concentrations between AM and non-AM plants, but subsequently the root N and P for AM plants were higher (Fig. 3a, b). Concomitant with these increases (67–119 days), the growth respiration of new root tissue was higher in AM plants (Fig. 4a). In spite of the higher growth respiration of new roots, the efficiency of P utilisation in AM roots was lower than non-AM roots (Fig. 4b). This means that less C was used for P incorporation. It is likely that AM fungal tissue, rather than the new roots, accounted for the lower efficiency of root P utilisation, as found in the positive correlation (y=7.718x +1,187.379, r^2=0.983) between efficiency P utilisation and AM fungal colonisation (data not shown).

Discussion

The roots and stem are considered as regions of C storage for young developing grapevines (Buttrose 1966; McArtney and Ferree 1998), but the source of C for the new growth of a developing AM symbiosis has been unclear. The current findings show that above-ground C contributes significantly to the C budget during the 0–67 day period of AM development, which concurs with the phase of rapid root colonisation. This is probably due to the combined activity of new root growth and AM fungal colonisation requiring more C than is available in the root reserves alone, necessitating the above-ground C drain. The rapid loss of C from the colonised roots can be attributed to the relatively higher growth rate of new fungal structures compared to the growth of new root tissue in non-colonised plants (Jakobsen and Rosendahl 1990). The AM C-drain from host reserves concurs with other findings of the sink effect of AM, albeit from photosynthetically fixed C that is supplied to the AM fungus and not C from stored plant reserves (Snellgrove et al. 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990).

The C drain imposed by the rapid phase of AM fungal colonisation had a negative impact on host growth, as evidenced by the lower new root and shoot growth rates. This may indicate that AM fungal colonisation was the preferential below-ground sink, since colonised roots have higher metabolic rates (Pearson and Jakobsen 1993). The current data are inconsistent with other findings of higher root and shoot growth rates in AM vines (Linderman and Davis 2001). This possibly resulted from the current measurements being taken during the rapid phase (0–67 days) of AM development, whilst other studies on woody plants (Estrada-Luna et al. 2000; Linderman and Davis 2001) had data from vines aged 102 days (Linderman and Davis 2001), and guava plants at 116 days (Estrada-Luna et al. 2000), i.e. probably during the plateau phase of AM development.

Once the plateau phase of AM development was reached (67–119 days), the established symbiosis no longer drained the above-ground C reserves, allowing refilling of these reserves. A similar pattern of refilling was found in the bluebell (*Hyacinthoides non-scripta* (L.) Chouars ex Rothm); however, the refilling occurred towards the end of the growth season (Merryweather and Fitter 1995). Once the AM symbiosis was established, the increased new root and shoot growth rates may have resulted from two major factors affecting the AM C-economy. Firstly, at the plateau phase of

AM development, the decline in AM fungal growth rate would have reduced the C-sink in the AM roots and secondly, the AM roots were more efficient at using C for P utilisation during this period.

The further depletion of root C reserves in AM plants during the plateau phase of AM development (67–119 days) may reflect the C requirement of AM associated metabolism, as reported by Peng et al. (1993), that AM roots have higher below-ground respiration rates. This is congruent with the current findings of higher growth respiration in new roots of AM plants. The higher shoot growth rates of AM vines at the plateau phase of AM colonisation are likely to have resulted from the more efficient use of C for P utilisation, since there were no differences in the above-ground C-flux and the shoot N and P concentrations between AM and non-AM plants.

During the plateau phase of AM development (67–119 days) the root nutritional benefits of the AM symbiosis also became apparent in the higher N and P concentrations of AM roots. This may be because, during the established symbiosis, the hyphal network of the AM fungus is more developed than in the former phase, thus providing a greater surface area for nutrient absorption (Jakobsen et al. 1991; Smith and Read 1997). The cost of nutrient uptake is a significant C drain. Baas et al. (1989) found that 13% of fungal C was used for increased nutrient uptake and the remaining 87% for fungal respiration in the AM root symbiosis. However, the fungal respiratory costs may be further subdivided into maintenance costs and growth costs, both of which indirectly affect nutrient uptake by increasing and maintaining the hyphal network (Peng et al. 1993). Therefore the percentage C used for nutrient acquisition may be larger than 13%. During the phase of rapid development a greater percentage C will be used for the growth of new fungal structures and only once the symbiosis is functional will more C be used for nutrient acquisition. In this regard, AM fungi are able to absorb P more efficiently than their hosts by increasing the absorptive area of the plant's root system and accessing P sources unavailable to the host roots (Bolan 1991; Smith and Read 1997). The AM fungal hyphae also have a higher affinity (lower K_m) for P than the host plants and the fungi are more efficient at competing with other soil microbes for nutrients (Bolan 1991; Smith and Read 1997).

The AM C drain and subsequent nutrient uptake costs may have resulted in the higher Cw of AM plants. These higher Cw values did not negatively impact the bulk DW of hosts. It has been shown when AM costs exceed the nutritional benefits to the host, negative growth responses can ensue (Graham and Eissenstat 1998). This would indicate that, in the present study, the C-drain imposed by the AM fungus was possibly compensated for by increased photosynthetic rates, as previously reported (Fitter 1991; Wright et al. 1998a, 1998b). Furthermore, it is concluded that the initial costs of AM fungal colonisation were borne by the above-ground C reserves in young grapevines. These C costs were offset once the plateau phase of AM development was reached.

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