Arbuscular Mycorrhizal Colonisation Modifies the Water Relations of Young Transplanted Grapevines (*Vitis*)

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Submitted for publication: June 2002

Accepted for publication: August 2004

Key words: Water relations, photosynthesis, mycorrhizas, transplantation

The effect of arbuscular mycorrhizal (AM) colonisation on the alleviation of transplantation shock in young grapevines was investigated. One-year-old grapevines (Sauvignon blanc on Richter 99), colonised with *Glomus etunicatum* (Becker and Gerdemann), were cultivated in an atmosphere-controlled tunnel. Water relations, leaf photosynthetic parameters and growth characters were evaluated. AM colonisation enhanced the photosynthetic performance of host plants, but had no influence on biomass and mineral nutrition of the transplanted hosts. The increased photosynthetic rates of the AM plants were related to improved water relations. Stomatal conductance, transpiration rate and midday xylem water potential were higher in the AM hosts during the transplanted period. These results indicate that AM inoculation can influence the water relations of transplanted grapevine rootstocks, thereby improving photosynthetic performance and potential survival during the initial growth stages of the host plants.

The role of arbuscular mycorrhiza (AM) in enhancing plant growth and yield of crops has been previously reported (Bolan, 1991). In this regard one of the greatest potential benefits of AM fungi for host plants is the increase in biomass and growth of the host plants. This increase in growth and biomass may be caused by the host plant's increased ability to acquire essential nutrients and water (Ruiz-Lozano & Azcon, 1995). The beneficial effect of mycorrhizae is of special importance to plants such as grapevines that have a coarse and poorly branched root system. Grapevines appear to be reliant on AM fungal colonisation for normal growth and development (Menge *et al.,* 1983; Karagiannidis *et al.,* 1995; Biricolti *et al.,* 1997; Linderman & Davis, 2001). Furthermore, it was found that coarse-rooted species, such as vines (Motosugi *et al.,* 2002) are more reliant on AM colonisation than fine-rooted species (Bolan, 1991; Eissenstat, 1992).

The fungal species and the rootstock cultivar will determine many of the benefits attributed to the symbiosis (Menge *et al.,*1983; Schubert *et al.,*1988; Karagiannidis *et al.,* 1997). Schubert *et al.* (1988) inoculated different rootstock cultivars with different AM fungi and found that certain fungal species combined with specific rootstocks increased plant growth to a greater extent than other combinations did. The percentage colonisation, degree of growth response and nutritional benefits of AM colonisation of vine roots will vary according to the AM fungal species and the rootstock cultivar involved (Linderman & Davis, 2001; Schreiner, 2003).

Vineyards infected with soil pathogens such as phylloxera often require fumigation treatments. However, the fumigant clears the soil of both desired and undesired soil microbes, including AM fungi (Menge *et al.,* 1983; Linderman & Davis, 2001). Therefore the inoculation of vines before planting in fumigated soils is needed to ensure AM fungal colonisation of the vine roots. Menge *et al.* (1983) reported that non-AM vines planted in fumigated soils had stunted growth compared to the inoculated vines. This transplantation shock of the vines may be related to root-system damage (Waschkies *et al.,* 1993), which may impair water and nutrient uptake. The phenomenon of transplantation shock has been found to be reduced by inoculating the vines with AM fungi (Linderman & Davis, 2001). It is currently not known how AM colonisation could mediate an alleviation of the transplantation shock in young grapevines, but the evidence of root-system damage (Waschkies *et al.,* 1993) suggests that AM fungi may improve water relations and nutrient access of host plants. The objective of this study was therefore to assess the contribution of a single-strain AM inoculum on the host-water relations and the consequent impact of this on mineral nutrition, photosynthesis and growth of transplanted grapevine rootstocks. The rootstocks were inoculated during transplantation and the host performance was assessed after 90 days of growth.

MATERIALS AND METHODS

Growth conditions

One-year-old grafted grapevine cuttings (*Vitis vinifera* L. cv. Sauvignon blanc, grafted onto Richter 99 rootstocks) were plant-

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ed in 20-litre pots, containing irradiated (20 kGy) filter sand with a grain size of 0.51 mm and pH of 7. Registered, commercial *Vitis vinifera* L. cv. Sauvignon blanc grafted onto Richter 99 rootstocks were selected to have similar shoot thickness and root development. The plants were grown for 90 days in a north-facing tunnel at the University of Stellenbosch, Western Cape, South Africa. The maximum daily photosynthetically active irradiance was between 700 and 800 µmol m^{-2} s^{-1} and the average day/night temperatures were 23/19°C. The transplanted grapevines were watered with a standard Long-Ashton nutrient solution modified to contain NO_3^-/NH_4^+ as N source and 100 µM P. The solution was added every seven days at field capacity (2 l) of the sand.

Inoculation

The inoculum consisted of a single-strain pure culture (spores and fragments of roots and hyphae in an inert carrier, collected from pot culture) of an AM species, *Glomus etunicatum* (supplied by Dr C. Straker from University of the Witwatersrand). The spore density of the inoculum was 12 spores/g. The AM grapevines were inoculated and the control plants received a filtered inoculum solution, which was prepared by filtering the inoculum through a 37 µm mesh to remove the mycorrhizal fungal material. Inoculation was conducted by spreading the inoculum around the roots in the planting hole. The pot was then filled up with the sand covering the roots and the inoculum. This ensured that the propagated roots were immediately in contact with the AM fungal propagules.

Plant harvest

At 90 days of pot culture the physiological measurements were taken and the plants were harvested. The leaf areas of the plants were measured with a leaf area meter (Licor, model LI-3000, Lambda Instruments Corporation, USA) and the water potential was determined with a pressure chamber (PNS intruments Co. Oregon USA). The roots were carefully blotted dry, root pieces of the root were randomly cut off, weighed and stored in a vial with 50% ethanol (v/v) solution for estimation of mycorrhizal colonisation. The components were dried at 80°C for more than 72 h and weighed to determine the dry weight.

The biomass parameters were calculated as follows. Leaf-area ratio is the ratio of total leaf area relative to total plant mass. Specific leaf mass describes the density of leaves, expressed as leaf mass per leaf area (kg/m^2). Relative growth rate is the specific growth (mg) for an existing plant mass (g) over time (mg/g/day). Growth rate is the average growth for a given period (g/day). The biomass N and P use efficiencies express the ratio of total plant dry matter accumulated per amount of total plant N or P (g dw/mmol N or P).

Mycorrhizal colonisation

During plant harvest, the thin lateral roots were removed and stored in 50% ethanol. Root segments were cleared in 2.5% KOH in an Autoclave for 6 minutes (hot clearing). Afterwards, the KOH was rinsed from the segments and acidified with 1% HCL for 24 h at room temperature. The roots were stained with 0.05% Analine blue in 70% acidified glycerol for 48 h at room temperature. Roots were cut into 1 cm pieces and randomly selected to be packed on slides. They were subsequently examined at x 400 magnification under a light microscope. Infection was determined according to the methods described by Brundrett *et al.* (1994).

Photosynthesis

The youngest fully expanded leaf for each plant was used for the photosynthetic determinations. The photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (E) were determined at midday, using a portable infrared gas analyser (LiCor). Photosynthetic nitrogen-use efficiency (PNUE) and photosynthetic phosphorus-use efficiency (PPUE) were obtained by dividing Pn by either the leaf N or P concentration, respectively. Photosynthetic water-use efficiency (PWUE) was calculated from measurements of Pn and transpiration rate. Intercellular CO_2 response curves were determined using the facility on the infrared gas analyser, by manually adjusting the CO_2 concentration in the leaf chamber. The CO_2 response curves were used to calculate electron transport capacity and RUBISCO activity, using the equations of Watanabe *et al.* (1994).

Chemical analyses

Chlorophyll analyses were performed on leaf discs taken from the same leaves which were used for the gas exchange measurements. Chlorophyll was extracted at 4°C in acetone. The resulting extract was centrifuged at 3000 g for 3 minutes, and the chlorophyll concentration was determined according to the method of Arnon (1949) by measuring the absorbance at 646, 663 and 710 nm in a spectrophotometer. The oven-dried (72 h, 80°C) plant material of each treatment was milled in a Wiley mill (A.H. Thomas, Philadelphia, Pa, USA) using a 60 mesh screen, for leaf, stem and root material. The plant material was analysed by BEMLABS (Somerset West, RSA) for N and P.

Xylem water potential

Xylem water potentials (XWP) were taken at midday, using a pressure chamber (PNS Instruments Co. Oregon, USA). A terminal branch bearing the first fully expanded leaf was placed in the pressure bomb, with the leaf inside the chamber and cut surface of the stem protruding from the chamber. The pressure was gradually increased, until the xylem sap evenly covered the cut surface. At this point the pressure was turned off and recorded as the water potential.

Statistical analyses

Plants were spaced in a random block design. The percentage data were arcsine transformed (Zar, 1984). The influence of the factor, mycorrhizal inoculation, was tested with a one-way analysis of variance (1-way ANOVA) and the differences between treatments were separated using a *post hoc* Student Newman Kuels (SNK), multiple comparison test (SuperANOVA version. 6.11 for Macintosh). Different letters indicate significant differences between treatments ($P \leq 0.05$), n= 5.

RESULTS

Roots inoculated with live AM had 61% colonisation, whilst the control plants remained non-mycorrhizal for the duration of the trial (Table 1). The presence of AM colonisation did not influence the biomass of the host plants and there were no differences in growth parameters between AM and non-AM plants (Table 1). N and P nutrition of the host plants also remained unaffected by AM colonisation (Table 2).

In spite of the absence of differences between AM and non-AM biomass and nutrition, the net photosynthetic (Pn) gas exchange and leaf water relations were significantly influenced by AM

TABLE 1

Biomass-parameters of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between +AM and -AM treatments in each row (P≤ 0.05).

Parameters	-AM		+AM	
Leaf data				
leaf dry mass (g)	1.33	±0.12 a	1.62	±0.13 a
leaf fresh mass (g)	4.54	±0.44 a	5.87	±0.62 a
leaf area (m^2)	291.73	±30.53 a	378.20	±31.84 a
leaf number	24.33	±1.67 a	25.67	±2.91 a
leaf area ratio	0.15	±0.01 a	0.13	±0.01 a
specific leaf mass (kg/m^2)	4.58	±0.14 a	4.29	±0.03 a
Biomass				
root dry mass (g)	18.07	±2.75 a	20.80	±1.93 a
shoot dry mass (g)	23.44	±3.12 a	24.31	±4.61 a
total plant dry mass (g)	44.72	±6.12 a	49.35	±6.84 a
root:shoot	0.77	±0.04 a	0.90	±0.13 a
plant relative growth rate (mg/g/day)	0.981	±1.129 a	0.844	±3.391 a
plant growth rate (g/day)	0.497	±0.068 a	0.548	±0.076 a
Arbuscular mycorrhizal colonisation				
root colonisation with AM (%)	0	±0.0 a	61.10	±7.72 b

TABLE 2

N and P concentrations of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between +AM and -AM treatments in each row (P≤ 0.05).

Parameters	-AM		+AM	
N concentration (mmol N/g dw)				
root N	9.28	±1.37 a	1.62	±0.13 a
stem N	5.90	±0.90 a	5.87	±0.62 a
leaf N	20.06	±1.14 a	22.09	±0.51 a
biomass N-use efficiency (g dw/ mmol N)	0.08	±0.01 a	0.06	±0.005 a
P concentration (mmol P/g dw)				
root P	1.58	±0.24 a	1.87	±0.43 a
stem P	1.05	±0.16 a	1.18	±0.04 a
leaf P	3.26	±0.33 a	3.23	±0.27 a
biomass P-use efficiency (g dw/mmol P)	0.47	±0.08 a	0.38	±0.04 a

TABLE 3

Phototsynthetic parameters of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between +AM and -AM treatments in each row (P≤ 0.05).

Parameters	-AM		+AM	
Photon yield	0.231	±0.050 a	0.206	±0.019 a
Rubisco activity (µmol CO_2 $m^{-2}.s^{-1}$)	29.885	±0.749 a	34.486	±3.825 a
Electron transport activity (µmol CO_2 $m^{-2}.s^{-1}$)	124.607	±2.665 a	131.155	±15.519 a
Chlorophyll a (µmol.m^{-2})	393.938	±52.841 a	397.483	±19.378 a
Chlorophyll b (µmol.m^{-2})	130.658	±20.391 a	166.839	±28.851 a
Chlorophyll a:b	3.056	±0.200 a	2.492	±0.321 a
Total Chlorophyll (µmol.m^{-2})	524.596	±72.215 a	564.322	±48.218 a
Photosynthetic N-use effciency (µmol CO_2 $mmol^{-1}$ N $m^{-2}.s^{-1}$)	0.018	±0.003 a	0.012	±0.001 a
Photosynthetic P-use effciency (µmol CO_2 $mmol^{-1}$ P $m^{-2}.s^{-1}$)	0.003	±0.001 a	0.002	±0.000 a

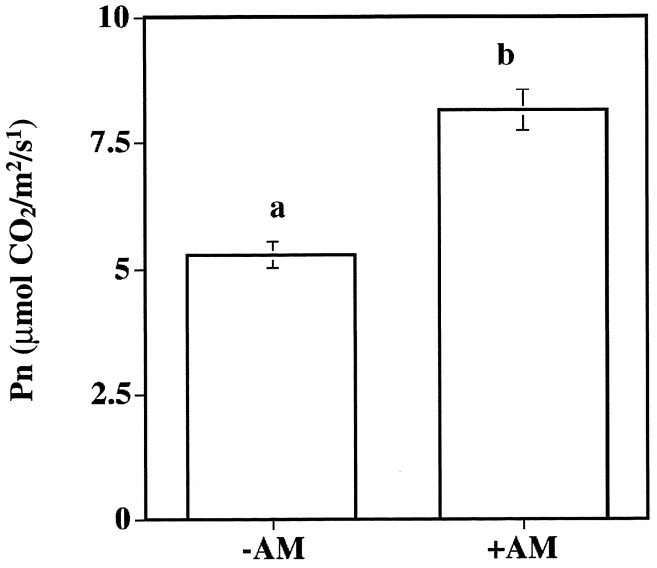

FIGURE 1

Photosynthetic rates (Pn) of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 µM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between the two treatments (P≤0.05).

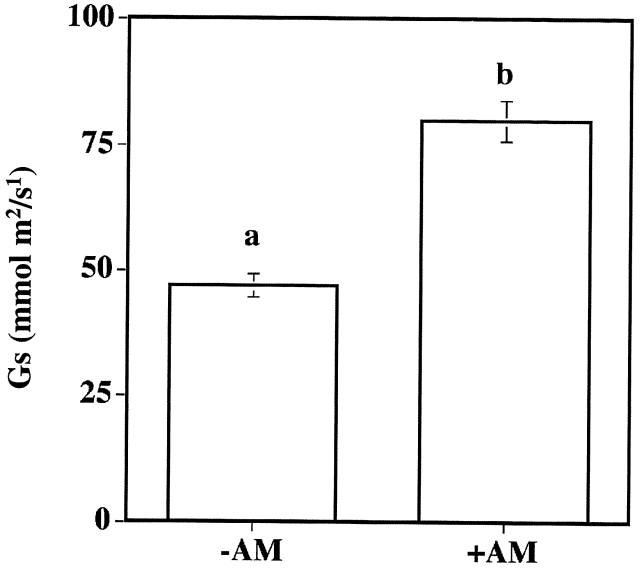

FIGURE 2

Stomatal conductance (GS) of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between the two treatments ($P \leq 0.05$).

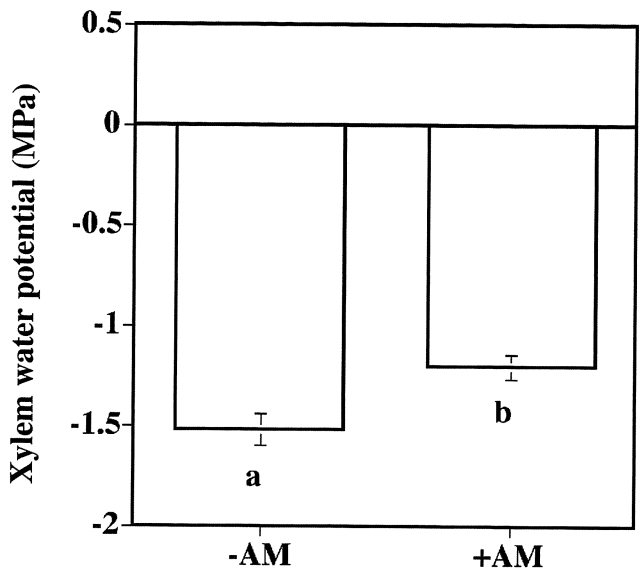

FIGURE 3

Xylem sap pressure potential (XWP) of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between the two treatments ($P \leq 0.05$).

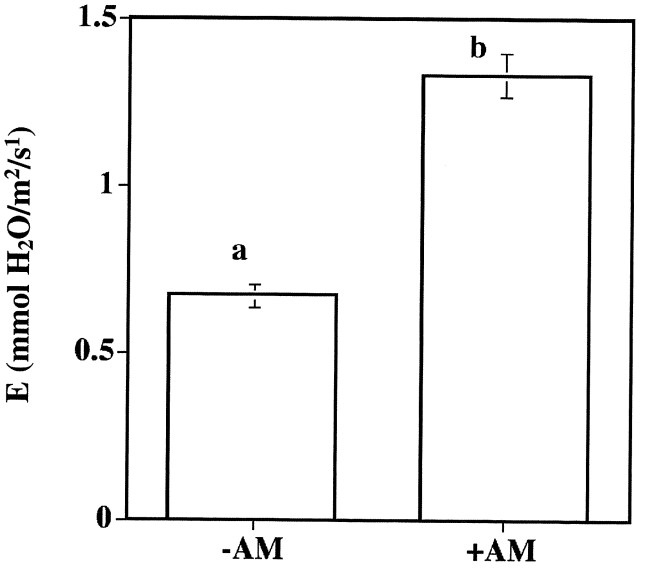

FIGURE 4

Transpiration rate (E) of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between the two treatments ($P \leq 0.05$).

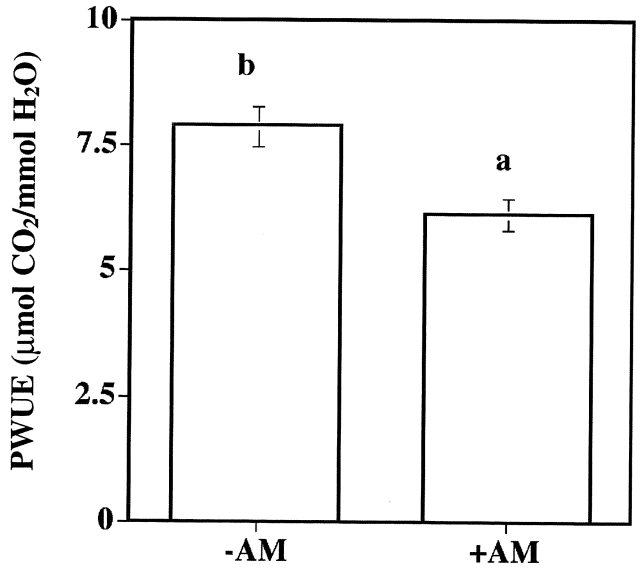

FIGURE 5

Photosynthetic water use efficiency (PWUE) of 90-day-old +AM and -AM grapevines, grown in pots containing sterilised filter sand. The +AM inoculum was live *Glomus etunicatum*, whilst the -AM inoculum was an irradiated *Glomus etunicatum* sample, containing a filtrate of the non-mycorrhizal microbes present in the live inoculum. The plants received a standard Long-Ashton nutrient solution in which the N concentration was modified to 2 mM NO_3/NH_4 and 100 µM P. The values represent averages with a standard error (±). The different letters indicate significant differences between the two treatments ($P \leq 0.05$).

colonisation. The Pn rate was higher in the AM leaves compared to the non-AM leaves (Fig. 1), but this was not related to other leaf photosynthetic parameters (Table 3). In this regard, RUBISCO activity, electron transport capacity, chlorophyll levels, photosynthetic nutrient-use efficiencies and photon yield were unaffected by AM colonisation (Table 3). Instead, the increased Pn corresponded with enhanced stomatal conductance (Gs) (Fig. 2) and transpiration rates (E) (Fig. 4) in the AM leaves. Midday xylem water potentials (XWP) were lower (less negative) in the AM plants, implying less water stress than in the non-AM controls (Fig. 3). However, the higher photosynthetic water-use efficiency (PWUE) of the AM plants indicates that AM host plants lost more water than non-AM plants during photosynthetic CO_2 fixation (Fig. 5).

DISCUSSION

The absence of increases in biomass and mineral nutrition of AM colonised grapevines contradicts earlier findings of mycorrhizal benefits to host grapevines (Possingham & Obbink, 1971; Menge *et al.,* 1983). However, the improved rate of photosynthesis (Pn) in AM plants is congruent with other studies of herbaceous hosts (Allen *et al.,* 1981; Levy & Kirkun, 1980; Valentine *et al.,* 2001; 2002), as well as grapevine hosts (Nikolaou *et al.,* 2003b). In particular the present study concurs with Nikolaou *et al.,* (2003b), who also demonstrated that AM-colonised vines had higher CO_2 assimilation rates than uncolonised vines, but with no increase in the biomass of the host plants. Since the increase of Pn in the AM plants occurred in the absence of improved nutrient status or the derived photosynthetic parameters, it is therefore proposed to be related to an AM sink effect or an AM-induced change in water relations.

The association of the increase in Pn and the percentage of AM colonisation suggests a relationship between AM colonisation and carbohydrate availability, as proposed by Valentine *et al.* (2001; 2002). It is well known that the demand for photo-assimilates can stimulate the rate of Pn (Neales & Incoll, 1968; Herold, 1980; Foyer, 1987) and since a large proportion of photosynthetic product is allocated to the root of AM plants (Marschner, 1995; Jacobsen & Rosendahl, 1990), this is one potential mechanism for the observed increases in Pn of AM plants.

The improved water relations, as another potential mechanism of the AM stimulation of host Pn, have been causally related to Pn via increases in the stomatal conductance (Gs) of the AM plants (Allen *et al.,* 1981; Brown & Bethelenfalvay, 1987; Fitter, 1988; Valentine *et al.,* 2001; 2002). In the present study the improved Gs, transpiration rate and xylem water potential in AM grapevines, provide sufficient evidence that the water status was increased by AM colonisation. Previous studies (Giovanetti & Mosse, 1980; Graham & Syverston, 1984) found that the altered water status of the AM plants was closely associated with an improved host nutrition, particularly P. These findings do not concur with the current study, where host nutrition was not affected by AM status. However, the present work does concur with other findings (Read, 1992; Sylvia & Williams, 1992; Koide, 1993) where it was proposed that mycorrhizal infection can facilitate a significant increase of water flux independent of changes in the nutrient status of the host. Water uptake by root tissue may therefore be a result of the presence of AM fungi on these roots, as was found by Ruiz-Lozano & Azcon (1995). Motosugi *et al.* (2002) also reported that AM-colonised roots were more efficient in the uptake of water compared to uncolonised roots. Nikolaou *et al.* (2003a) determined that AM vines have an improved water status and drought-sensitive rootstocks showed greater growth when colonised by an AM fungus under non-irrigated conditions. AM fungi can therefore aid in the uptake of water and contribute to an improved water status in vines, enabling the vines to grow under low irrigation or survive water-stressed conditions. Interestingly, the improved water status by the AM fungi may also have caused the AM plants to allow greater water loss during photosynthetic CO_2 assimilation. This less efficient PWUE suggests that the host plants are able to spend more water under conditions of sufficient water supply by AM fungal symbionts.

CONCLUSIONS

It is concluded that inoculation of transplanted grapevines with AM fungi may improve the plant water status and thereby increase the potential alleviation of transplantation shock. The AM mechanism of action on water relations appears to be independent from an improved nutrient status. Although AM may contribute to a healthier plant in pot culture, further studies are required to assess whether the same benefits are possible under field conditions. The potential of such future work is supported by the suggestion of Allen & Allen (1986) that AM fungi are important in sustainable agriculture because they improve plant-water relations and drought resistance of host plants.

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