Plant and Soil 99, 291–302 (1987). Ms. 6751

Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress*

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Received 14 February 1986. Revised September 1986

Key words Carbohydrate content Drought Glomus Mycorrhiza Phosphorus *Rosa hybrida* Water relations

Summary Shoot water relations and carbohydrate levels were compared for droughted nonmycorrhizal and vesicular-arbuscular (VA) mycorrhizal *Rosa hybrida* L. cv 'Samantha' plants grown with high and low phosphorus fertilization. Leaf diffusive conductance (g_l) of plants colonized by *Glomus intraradices* Schenk and Smith and *Glomus deserticola* Trappe, Bloss and Menge were 2 × and 1.5 × greater, respectively, than in nonmycorrhizal plants. Regardless of P fertilization, leaf osmotic and bulk water potentials were 0.5 to 1.1 MPa higher in mycorrhizal than in nonmycorrhizal plants. Leaf starch, chlorophyll and water contents were higher in *G. intraradices*-colonized plants than in the high-P nonmycorrhizal plants, while fructose, glucose and total soluble carbohydrates were lower. Level of P fertilization had no effect on water relations or soluble carbohydrate content of nonmycorrhizal roses. The water status of droughted rose was improved more by *G. intraradices* than by *G. deserticola*.

Introduction

Recent examinations of vesicular-arbuscular (VA) mycorrhizal symbioses have indicated that VA fungi may improve host resilience to drought stress[2,5,18,28]. In some instances recovery from drought may also be hastened[13,23,37].

Whether or not the fungal influence is through direct hyphal water uptake and transport, through altered hormonal or other biochemical relations, or through increased mineral uptake in dry soils remains unclear[10,34]. It is possible that different mechanisms predominate between different symbionts.

In some VA mycorrhizal associations, enhanced P nutrition apparently accounts for the altered host water status[11,20,27,33]. In others, mycorrhizal effects are not attributable simply to increased plant P levels, and some other mechanism must prevail[4,5,14,23,24]. This paper summarizes the impact of drought and P nutrition on shoot water relations of VA mycorrhizal rose. Because cultivated rose is commonly mycorrhizal even under optimum P fertilization, it was selected for study of mycorrhizal influences beyond enhanced mineral nutrition.

* Washington State University College of Agriculture and Home Economics Research Center Scientific Paper No. 7375.

Materials and methods

Plant and fungal culture

Five-month old *Rosa hybrida* L. cv 'Samantha' plants, which were colonized by either *Glomus intraradices* Schenk and Smith or *Glomus deserticola* Trappe, Bloss and Menge[4], or were nonmycorrhizal, were used in this study. These plants had been examined earlier under well-watered conditions[4], and the current report records the effects of drought stress administered immediately after the previous experiments. When water relations measurements began, root development had occurred throughout the pots but plants were not yet pot-bound. Inoculation procedure, growth room conditions, plant culture and statistical analysis were as described earlier[4]. Plants were provided with a full nutrient regime in soluble form, with low phosphorus (LP) and high phosphorus (HP) treatments receiving 23 and 92 mg P l^{-1}, respectively, with each fertilization.

Leaf water status

Leaf water potential (Ψ_l) and leaf diffusive conductance (g_l) were sampled as before[4]. Pots were brought to field capacity, then allowed to dry, and each parameter was assessed at 20–25% relative soil water content (θ; field capacity = 100% relative θ, oven-dry soil = 0% relative θ). Due to the low water diffusivity of the medium, particularly at low soil θ, and consequent Ψ_{soil} variations within pots, relative soil θ was selected as a measure of soil water status, for consistency and repeatability. Drying periods ranged from 4–11 d, except in the case of the stunted, low-P nonmycorrhizal plants, which took 18 d to dry down.

The average transpiration rate (E_{avg}) during the entire drying cycle was measured gravimetrically. Total water loss was the difference between pot-plus-plant weight at field capacity and at low soil θ. Nontranspirational water loss, estimated from the daily weight loss from three unplanted pots containing a soil mixture identical to that used for the roses, was subtracted from total water loss for a measure of E_{avg}. Leaf area was measured with a leaf area meter (Li-Cor, Lincoln, NE) following the droughting period.

Leaf water content (*LWC*) = [(Fresh weight leaf − dry weight leaf)/fresh weight leaf] × 100%[22].

After Ψ_l was determined, leaves were immediately frozen in liquid N_2, and the osmotic potential (Ψ_o) determined with a vapor pressure osmometer (Wescor 5100C, West Coast Scientific, Seattle, WA) on sap expressed from quickly warmed leaves. Osmotic potentials were corrected for the dilution of cell sap with apoplastic water that occurs during freezing-thawing by determining the apoplastic water percentage of 20 leaves using pressure-volume relations, as described elsewhere[5]. Solute content of xylem solution expressed with the pressure chamber from cut petiole ends was measured with the osmometer and found to be negligible ($\Psi_o > -0.01$ MPa). Apoplastic water percentages were also estimated using the relative dry weight (*RDW*) of leaves. *RDW*, calculated on the 20 rehydrated leaves used in the above P-V work, = [(dry weight)/(rehydrated leaf weight − dry weight)] × 100%.[32].

Leaf carbohydrate status

For carbohydrate determinations, a composite sample of three leaves was excised from each plant at the described low soil θ, weighed and immediately placed into liquid nitrogen. Leaves were lyophilized and a 100 mg sample was extracted 2 × with 7 ml of 80% ethanol, followed by 7 ml of water.

Soluble carbohydrate and starch contents were analysed as described previously[25,29].

For histochemical analysis, leaf strips approximately 1 × 6 mm were removed (7–9 h into the photoperiod) from the three leaves to be used in carbohydrate determinations, immediately before their excision, from three replicates per treatment. Strips were obtained from terminal leaflets, 2–3 mm from the midrib and parallel to it. The samples were fixed in 4% glutaraldehyde in a phosphate buffer, pH = 7.2, dehydrated through an ethanol series and embedded in acrylic (LR White) resin. A modified periodic acid-Schiff's procedure[7], which specifically stains polysaccharides, was performed on sections 1 μm in thickness for the observation of starch grains.

Leaf concentrations of total chlorophyll, chlorophyll a and b were measured spectrophotometrically[16] directly after the drying period (12 days after onset of drying, except in the case of the low-P nonmycorrhizal plants, which required 18 days for all replicates to reach the specified low soil θ). Three leaf discs from each of eight replicates were used in the analysis. Plants were well-watered before determinations were made.

Results

Plant growth and nutrient status

Levels of mycorrhizal colonization and tissue dry weights, areas, P contents and growth ratios are listed in Tables 1 and 2 of Augé *et al.*[4]. All plants were of similar size and P nutrition except for the low-P nonmycorrhizal plants, which were smaller and P-deficient. Plant P levels in all high-P roses ranged from 1.5 to 1.6 mg g^{-1} dry weight, and from 1.1 to 1.3 mg g^{-1} in low-P mycorrhizal roses. Nonmycorrhizal low-P plants had 0.7 mg P g^{-1}.

Leaf water status

At low soil water content, nonmycorrhizal roses had comparable g_l, E_{avg}, Ψ_l, Ψ_o, and *LWC*, regardless of P fertilization (Table 1). Ψ_l and g_l were greater in the low-P mycorrhizal roses than in the high-P nonmycorrhizal roses (Table 1). The high-P mycorrhizal plants, though colonized to a lesser degree, were also able to maintain higher g_l, Ψ_l and Ψ_o than the high-P uncolonized plants. The g_l of *Glomus intraradices*-colonized plants was similar at both P regimes, and was twice that of nonmycorrhizal roses. VA mycorrhizas had a substantial effect on host water potential: the Ψ_l of high-P nonmycorrhizal roses was 1.0 MPa lower than plants colonized by *G. intraradices*. The linear contrast between high and low P mycorrhizal treatments indicated no significant difference in any parameter measured, except for E_{avg}.

The two fungal species differed in their effect on droughted rose. In roses colonized by *G. intraradices*, g_l, Ψ_l and Ψ_o were all greater (55%, 20% and 16%, respectively) than in the *G. deserticola*-colonized roses. E_{avg} and *LWC* were similar in the two fungal treatments. The average transpiration rate during the drying cycle was 67% greater in the highly colonized mycorrhizal plants than in the high-P uncolonized plants, and *LWC* differed between these two groups by 11% (Table 1).

Apoplastic water percentage of rose leaves estimated using P-V relations was 50%, SE = 2.9, and 49%, SE = 0.8, using *RDW*. A value of 50% was used for Ψ_o correction: measured Ψ_o = .5(Ψ_o of apoplast) + .5(Ψ_o of symplast).

Table 1. Effect of phosphorus fertilization and VA mycorrhizae on shoot water relations of *Rosa hybrida* cv 'Samantha' under water deficit stress (HP = high phosphorus; LP = low phosphorus)

Treatment		g_l^b (mm s^{-1})	Ψ_l^c (MPa)	Ψ_o^d(MPa)	E_{avg}^c (mg m^{-2} s^{-1})	LWC^c (%)	Mean relative soil θ^c (%)
VAM	Phosphorus						
Nonmycorrhizal	High	0.7	−2.92	−4.38	7.7	52.5	24.0
	Low	0.8	−2.56	−4.60	8.6	49.5	22.4
G. deserticola	High	0.9	−2.44*[e]	−4.18	8.7	55.0	24.4
	Low	1.1	−2.27**	−3.82	13.6**	58.0	24.7
G. intraradices	High	1.5**	−1.96***	−3.42***	8.9	57.0	23.7
	Low	1.6***	−1.82***	−3.26***	12.1*	58.2	20.3*
$LSD_{0.05}$		0.5	0.42	0.28	4.1	6.2	2.8
Linear contrast[a]							
Nonmycorrhizal HP *vs* mycorrhizal HP		+	+++	+	NS	NS	NS
Nonmycorrhizal HP *vs* mycorrhizal LP		++	+++	+++	++	+	NS
Nonmycorrhizal *vs* *G. deserticola*		NS	NS	++	+	+	NS
Nonmycorrhizal *vs* *G. intraradices*		+++	+++	++	NS	+++	NS
G. deserticola vs *G. intraradices*		++	++	+++	NS	NS	+

[a] Nonsignificant (NS), or signficiant at the 5% (+), 1% (++), or 0.1% (+++) level

[b] Values represent the means of 24 observations each

[c] Values represent the means of eight replications each

[d] Values represent the means of five to eight replications each

[e] Significantly different from the high-P nonmycorrhizal control at the 5% (*), 1% (**), or 0.1% (***) level

Leaf chlorophyll and carbohydrate content

Chlorophyll content following the drought period was correlated with drought strain*; the more highly strained, high-P and low-P nonmycorrhizal roses possessed less total chlorophyll than any of the mycorrhizal roses (Table 2). The low-P mycorrhizal plants colonized by *G. intraradices* exhibited levels 40% above high-P controls (Table 2), even though their leaf specific weight was the same or less than in the controls (Table 1[4]). The low-P nonmycorrhizal roses had less chlorophyll b than their high-P counterparts, and therefore a higher a/b ratio. Drought effects on chlorophyll level have been demonstrated before[17,36].

The uncolonized roses had 96% more fructose and 100% more glucose on a dry weight basis than the roses colonized by *G. intraradices* (Table 3). *G. deserticola*-colonized roses, intermediate in degree of drought strain, also had intermediate levels of the two hexoses. The linear contrasts indicated that levels of sucrose and the two polyols did not differ significantly between low-P or high-P mycorrhizal plants, or between mycorrhizal and nonmycorrhizal plants, though the trend was toward lesser amounts in the more highly strained, high-P nonmycorrhizal plants. Total sugar content was 18% greater in the uncolonized plants and 17% greater in the *G. deserticola*-colonized plants than in plants colonized by *G. intradices*, which is consistent with the lower Ψ_o observed in the former treatments.

Starch content of low-P nonmycorrhizal rose leaves was 60% higher than in leaves from high-P nonmycorrhizal plants (Table 3). Leaves from *G. intraradices*-colonized roses possessed 38% more foliar starch than leaves from *G. deserticola*-colonized roses and 60% more starch than leaves from the high-P nonmycorrhizal roses (Table 3 and Fig. 1).

Discussion

As in well-watered rose[4], both *Glomus* species increased water movement into droughted rose, with *G. intraradices* having the greater influence. The effects of the fungi were not associated with promotion of P uptake, as nonmycorrhizal and mycorrhizal plants had similar P content. *G. intraradices*-colonized plants experienced less drought strain at a given low soil θ, and this was reflected in each of the parameters measured (Table 1). This is consistent with previous findings, where *G. intraradices*-colonized citrus seedlings sustained less drought strain than uncolonized controls[18]. In this work mycorrhizal plants contained greater P levels even under nonstress conditions, so mycorrhizal effects

* The authors have adopted the terminology of Levitt throughout this paper in distinguishing an environmental limitation ("stress") from the related plant response to the limitation ("strain")[22].

Table 2. Effect of phosphorus fertilization and VA mycorrhizae on leaf chlorophyll content of *Rosa hybrida* cv 'Samantha' under water deficit stress (HP = high phosphorus; LP = low phosphorus)

Treatment		Chlorophyll content[b] (mg dm^{-2})			
VAM	Phosphorus	a	b	Total	a/b
Nonmycorrhizal	High	2.69	1.08	3.77	2.5
	Low	2.94	0.75**[c]	3.69	3.9***
G. deserticola	High	3.62**	1.36*	4.98**	2.7
	Low	3.45*	1.34*	4.79*	2.6
G. intraradices	High	2.89	1.19	4.08	2.4
	Low	3.87***	1.40**	5.27***	2.8
$LSD^{.05}$		0.69	0.11	0.85	0.8
Linear contrast[a]					
Nonmycorrhizal HP *vs* mycorrhizal HP		NS	NS	+	NS
Nonmycorrhizal HP *vs* mycorrhizal LP		++	++	+++	NS
Nonmycorrhizal *vs* *G. deserticola*		++	+++	+++	NS
Nonmycorrhizal *vs* *G. intraradices*		+	+++	+++	+
G. deserticola vs *G. intraradices*		NS	NS	NS	NS

[a] Nonsignificant (NS), or significant at the 5% (+), 1% (++), or 0.1% (+++) level
[b] Values represent the means of 24 observations each
[c] Significantly different from the high-P nonmycorrhizal control at the 5% (*), 1% (**), or 0.1% (***) level

derived strictly from enhanced P nutrition could not be ruled out. VA mycorrhizal plants have also been shown to recover more quickly from drought[23,37]. In the present study, g_l, Ψ_l and Ψ_o were highest in *G. intraradices*-colonized plants at either P fertilization rate, and in conjunction with corresponding leaf carbohydrate and chlorophyll contents, revealed that the *G. intraradices*-colonized roses experienced less strain than did the uncolonized, high-P controls (Table 2 and 3; Fig. 1). These effects were achieved in spite of the fact that soil moisture levels were actually significantly lowest in the low-P *G. intraradices*-colonized plants (Table 1).

It is interesting to note that under dry soil conditions the influence of the fungus became more pronounced than under moist conditions[4], at least in the case of the high-P *G. intraradices*-colonized roses. These plants were able to maintain leaf water potentials and leaf conductances as high as the plants colonized by this species at low P (Table 1), even though their roots were colonized to a lesser degree[4]. The same trend was apparent in the *G. deserticola*-colonized plants, and was reflected for both fungal species in *LWC* (Table 1).

Table 3. Effect of phosphorus fertilization and VA mycorrhizae on leaf carbohydrate content of *Rosa hybrida* cv 'Samantha' under water deficit stress (HP = high phosphorus; LP = low phosphorus)

Treatment		Soluble carbohydrates[b] (μmol g^{-1} dry weight)						Starch[c] (mg g^{-1} dry weight)
VAM	Phosphorus	Glucose	Fructose	Sucrose	Sorbitol	Inositol	Total	
Nonmycorrhizal	High	85	93	116	91	51	435	1.59
	Low	75	85	134	113	55	463	2.55*[d]
G. deserticola	High	64	71	149	106	47	438	1.82
	Low	58	64	138	136	54	450	1.86
G. intraradices	High	44*	48**	124	120	52	388	2.27
	Low	36***	43***	133	113	48	373	2.70*
$LSD_{.05}$		25	28	81	42	17	88	0.90
Linear contrast[a]								
Nonmycorrhizal HP *vs* mycorrhizal HP		+++	++	NS	NS	NS	NS	NS
Nonmycorrhizal HP *vs* mycorrhizal LP		++	++	NS	NS	NS	NS	NS
Nonmycorrhizal *vs* *G. deserticola*		+	+	NS	NS	NS	NS	NS
Nonmycorrhizal *vs* *G. intraradices*		+++	+++	NS	NS	NS	+	NS
G. deserticola vs *G. intraradices*		+	+	NS	NS	NS	+	+

[a] Nonsignificant (NS), or significant at the 5% (+), 1% (++), or 0.1% (+++) level
[b] Values represent the means of six replications each
[c] Values represent the means of five to eight replications each
[d] Significantly different from the high-P nonmycorrhizal control at the 5% (*), 1% (**), or 0.1% (***) level

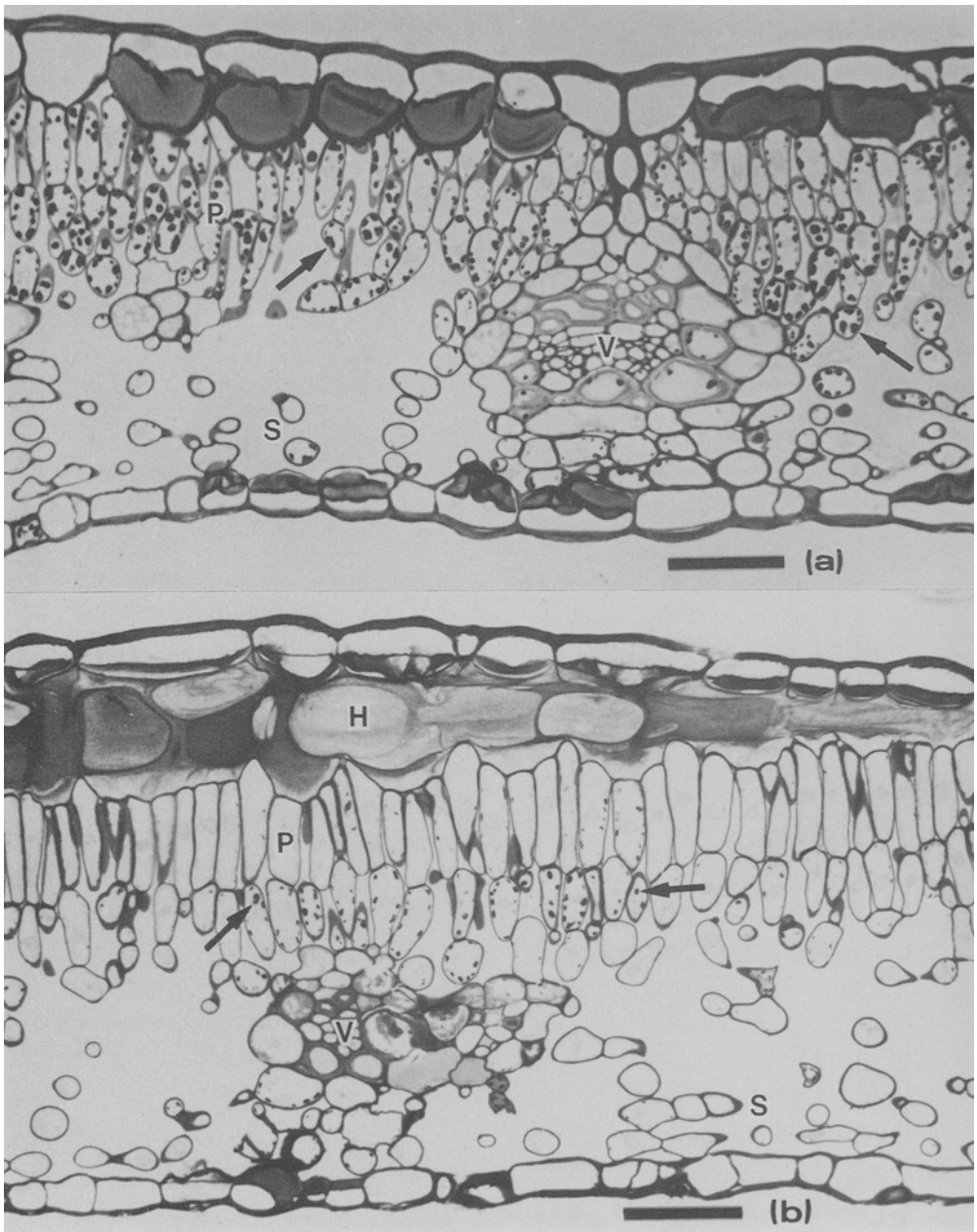

Fig. 1. Light micrographs of leaf cross-sections from droughted *Rosa hybrida* cv. 'Samantha' plants, stained for polysaccharide using the periodic acid-Schiff's procedure. Note the difference in chloroplastic starch content (*arrows* denote starch grains). (a). Low-P *Glomus intraradices* treatment. × 320. (b). High-P nonmycorrhizal treatment. × 320. Scale: 50 μm. H, hypodermis, occasionally observed in high-P nonmycorrhizal rose leaves; P, palisade mesophyll; S, spongy mesophyll; V, vascular bundle.

It has often been demonstrated that VA mycorrhizal fungal species can differ in their influence on a particular host[2,30,35]. Introduced, 'exotic' species often have greater effects than do the indigenous species[19,28], although this was not the case in the present study. Under well-watered situations *G. deserticola* and *G. intraradices* exerted a comparable influence on rose Ψ_l and g_l^4, yet the native species, *G. intraradices* (isolated from a commercial cut-rose greenhouse), improved water movement more than *G. deserticola* during drought. Interestingly, this occurred in spite of the fact that plants colonized by *G. deserticola* had greater relative root surface areas and tended to possess more root surface area per gram dry weight root than plants associated with *G. intraradices*[4].

It is noteworthy that root and plant P content increased with increased P fertilization in mycorrhizal roses[4], yet produced no effect on most water status parameters (Tables 1 and 2). High-P *G. intraradices*-colonized roses had leaf and root P contents comparable to the high-P nonmycorrhizal group, and yet they experienced substantially less strain than the nonmycorrhizal roses. Transpiration was diminished more during the drought cycle in the lesser-colonized, high-P mycorrhizal plants, and was thus correlated with degree of fungal colonization. It is apparent, then, that in this mycorrhizal association the effect of the fungus is unrelated to enhanced P uptake. This is in contrast to droughted onion colonized by *G. etunicatum*, in which mycorrhizal influence was attributed to elevated plant P levels[28]. *Rosa hybrida* and *R. manetti* produce coarse, rather scanty root systems and so may be more reliant upon their VA symbionts than more profusely rooting plants[6].

The lack of a role of P nutrition in altering rose water status during drought was corroborated by the fact that water-stressed nonmycorrhizal plants manifested no differences in Ψ_l, Ψ_o, g_l, E_{avg}, sugar or chlorophyll content between the two P treatments. Similar to other species[3], the P-deficient roses had root/shoot ratios double that of the adequately fertilized plants, which may have allowed comparable water uptake and transport. Moreover, both root dry weight/leaf area and relative root surface area/leaf area ratios were more than twice as great for the P-deficient plants than in high-P controls[4].

Drought strain often causes a decrease in foliar starch content and sometimes an increase in sugar[9,21]. This study revealed similar trends in rose plants. Uncolonized roses given adequate P experienced a high degree of strain (low Ψ_l and g_l) and possessed the lowest starch content (Table 2). The low g_l values at low soil θ indicated substantial stomatal closure, and it appeared that CO_2 fixation was reduced (Fig. 1). Since leaves possess only a small capacity to store carbon in labile forms[12], this may have necessitated the breakdown (and/or diminished the formation) of chloroplastic starch to sustain the plants' metabolic requirements. The

low-P *G. intraradices*-colonized plants displayed ample starch storage throughout the palisade mesophyll, whereas in the high-P nonmycorrhizal plants visible starch grains were present in much smaller quantities, and only in the lower palisade layer, near vascular bundles (Fig. 1). Leaf conductance of the P-deficient, uncolonized roses was also only half that of *G. intraradices*-colonized roses, yet foliar starch levels remained relatively high. This effect is in accord with prior data on carbon partitioning, in which low cellular Pi levels have often been shown to lead to starch accumulation, as phosphate influences the distribution of fixed carbon between starch and sucrose[1,15,31].

All these parameters describe a greater state of strain at low soil θ in the nonmycorrhizal roses. As Ψ_l declines and stomates close, CO_2 diffusion into leaves is inhibited[8]. If low stomatal conductances are sustained, carbon-starvation effects on leaf metabolism could, within hours, influence the metabolism of other organs[12]. If VA mycorrhizas allow the maintenance of greater leaf water potentials and diffusive conductances at a given low soil moisture level, then CO_2 fixation may be greater, as well. This may result over time in enhanced growth rates even in adequately P-nourished plants subjected to drought stress. The influence of VA mycorrhizas during chronic water stress warrants investigation.

Acknowledgements The authors acknowledge the very able assistance of Dr Vincent Franceschi and Alexis Lansing with microtechnique and photography.

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