Symbiosis, 12 (1992) 1–17
Balaban, Philadelphia/Rehovot

Symplastic Molality of Free Amino Acids and Sugars in *Rosa* Roots with Regard to VA Mycorrhizae and Drought

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Received April 10, 1991; Accepted June 18, 1991

Abstract

Mycorrhizal symbiosis can alter amino acid concentrations of plant roots, but little is known about the extent to which this occurs in mycorrhizal roots of droughted plants. Our objective was to determine if symplastic molalities of free amino acids or sugars are affected similarly in mycorrhizal and nonmycorrhizal roots with exposure to drought. Comparably sized rose plants (*Rosa hybrida* L. cv. Love) were produced by colonizing them with *Glomus intraradices* Schenck & Smith or giving uncolonized plants supplemental P fertilization. Plants were kept well-watered or were exposed to four to five drought cycles for a total of 21 days. Mycorrhizal colonization and drought interacted in modifying free amino acid and sugar pools in roots. Regardless of treatment, amino acid pools of roots consisted primarily of asparagine. Asparagine and total measured amino acids were almost three times as high in nonmycorrhizal than in mycorrhizal roots. Compared with droughted nonmycorrhizal roots, droughted mycorrhizal roots had more fructose and glucose but less sucrose, asparagine, aspartic acid, glutamic acid, glutamine, glycine + arginine (which co-eluted), serine, threonine and lysine. These amino acids and sugars accounted for one-third to two-thirds of total symplastic osmolality.

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Keywords: carbohydrates, cations, *Glomus intraradices*, starch, solutes, water stress

Abbreviations: NW = nonmycorrhizal, well-watered, MW = mycorrhizal, well-watered; ND = nonmycorrhizal, droughted; MD = mycorrhizal, droughted; ROWC = relative osmotic (or symplastic) water content; RWC = relative water content; SE = standard error; VA = vesicular-arbuscular, Ψ = water potential, Ψ_π = osmotic potential, Ψ_p = turgor potential.
Amino acids: ALA = alanine; ASN = asparagine; ASP = aspartic acid; GLU = glutamic acid; GLN = glutamine; GLY = glycine; LYS = lysine; PRO = Proline; SER = serine; THR = threonine; VAL = valine; TRP = tryptophan.

1. Introduction

The quantity and composition of free amino acids and sugars may differ in nonmycorrhizal roots and roots colonized by VA mycorrhizal fungi (Young et al., 1972; Baltruschat et al., 1973; Coxwell and Johnson, 1985; Thompson et al., 1986; Pacovsky, 1989; Amijee et al., 1990). Studies are insufficient, however, to judge whether such differences represent stable changes in plant metabolite availability and whether they are host-, fungus- or environment-specific (Pacovsky, 1989). How solute pools differ in mycorrhizal and nonmycorrhizal roots with exposure to soil moisture stress is also unknown. Our objective was to determine how mycorrhizal colonization and drought affect concentrations of free amino acids and sugars in roots from rose plants that were grown under P regimes producing plants of similar size. As we are concerned with the living, metabolizing portions of roots — cell solutions within plasmalemmae — we determined relative partitioning of water between root symplast and apoplast and expressed concentrations on the basis of symplast molality.

2. Materials and Methods

Plant culture and fungal inoculation

Stem cuttings of rose (*Rosa hybrida* L. cv. Love), rooted for eight weeks under mist in vermiculite, were planted in 20 cm pots in calcined montmorillonite clay (Turface; IMCore, Mundelein, IL), which facilitates fungal growth (Caron et al., 1985) and root excavation. At planting, mycorrhizal treatments received inoculum of *Glomus intraradices* Shenck & Smith consisting of fresh pot culture (Turface and mycorrhizal root pieces) of *Vigna unguiculata* L. Walp. (cowpea), incorporated throughout the pot at a rate of 1 inoculum:

8 fresh Turface (v/v). Nonmycorrhizal treatments received an inoculum wash sieved free of mycorrhizal propagules (final sieve = 25 μm).

Plants were grown under natural light in a greenhouse for 12 months before the experiment and during the three-week drought treatment. Plants were well-watered until the drought treatment began, receiving Peter's 15-0-15 soluble fertilizer at a concentration of 10 mM N and 3 mM K with each irrigation. Rates of P fertilization were adjusted to assure adequate P for all plants under drought conditions; uninoculated plants received 16.1 mM P and mycorrhizal plants 3.2 mM as KH_2PO_4, weekly. Rose plants form a coarse, magnolioid type of root system (Bayliss, 1975), which had not fully ramified the pot at the time of sampling.

The experiment consisted of four treatments, designed as a completely randomized 2 × 2 factorial, replicated four times. Factors were soil moisture (well-watered and droughted) and mycorrhizal colonization (mycorrhizal or nonmycorrhizal). Root water relations of the plants described above have been reported previously (Augé and Stodola, 1990).

Drought procedure

Plant drought strain was imposed for 21 days as previously described (Augé et al., 1986b) when plants were of similar size, by allowing half of the mycorrhizal and nonmycorrhizal plants to dry until leaf conductance declined to $\leq$ 1 mm s^{-1} before rewatering. Lengths of the drought cycles and leaf conductance of well-watered plants and of droughted plants at rewatering are given in Table 1 of Augé and Stodola (1990). Leaf conductance was measured daily at 0800 hr with a dynamic diffusion porometer (Mk3, Delta-T Devices, Cambridge, England) on the abaxial surface of two exposed, recently matured leaves from all droughted plants and two well-watered plants. Leaf temperatures and greenhouse relative humidity throughout the experiment at the time of porometry measurements ranged from 17 to 29°C and 30 to 60%, respectively.

Harvest

Plants were harvested for pressure-volume (Augé and Stodola, 1990) and root solute analysis at 0800 hr on days 21 and 22 (droughted plants) and days 22 and 23 (well-watered plants) following drought initiation. Fine, non-woody roots were quickly excavated, rehydrated to saturation for 15 min in H_2O at room temperature, lyophilized (amino acids and carbohydrates) or oven-dried (inorganic ions; 70°C for 24 hr) and ground with a Wiley mill.

Symplastic water percentages at full turgor of live roots of the plants examined in the current study were estimated previously by pressure-volume analysis (Augé and Stodola, 1990).

Amino acids

Amino acids were extracted as described by Foster (1990). Approximately 0.5 g of lyophilized tissue was extracted with 90 ml of 35% (v/v) aqueous ethanol for 90 min using Soxhlet extractors. One ml of 5 mM S-(4-pyridylethyl)-DL-penicillamine (Pierce Chemical Co., Rockford, IL) was added as an internal standard. Sample extracts were concentrated to dryness under nitrogen at 40°C, and the remaining residue was resuspended in 10 ml of extraction medium. A 0.25 ml aliquot of this preparation was loaded onto a Sep-Pak C_{18} column (Waters Associates, Milford, MA) and eluted successively with 0.5 ml water and two 0.75 ml aliquots of methanol. The combined eluates were adjusted to a final volume of 2.5 ml with water.

Free amino acids (except proline) in extracts were derivatized with *o*-phthalaldehyde (OPA, Pierce) prepared by dissolving 50 mg of OPA in 1 ml of HPLC-grade methanol, adding 0.05 ml of 2-mercaptoethanol (Bio-Rad Laboratories, Richmond, CA) and bringing the solution to a final volume of 10 ml with 0.40 M sodium borate-KOH, pH 9.5 containing 0.1% (v/v) Brij 35 (polyoxyethylene lauryl ether, Fisher Scientific, Pittsburgh, PA). Freshly prepared OPA stock solution was stored overnight at 0 to 5°C before use. A 100 μl aliquot of the solution was mixed with 20 μl of Sep-Pak-prepared extract 90 s before injection onto the HPLC column.

Derivatized samples were analyzed using a Beckman model 344 binary gradient HPLC system equipped with an Altex 4.6 $\times$ 45 mm, 5 μm Ultrasphere-ODS octadecylsilane analytical column maintained at 45°C following the protocol of Foster (1989). Amino acid derivatives were detected using a Beckman model 157 fluorescence detector equipped with a 9 μl flow cell and filters for excitation at 305 to 395 nm and emission at 430 to 470 nm. Detector range and time constant settings were 0.02 relative fluorescence units and 0.5 s, respectively. Amino acids were identified by comparing their retention times to those of pure amino acid standards (Sigma) and by coinjection of known amino acids. Peak areas were determined using a Nelson Analytical (Cupertino, CA) model 4416 chromatography data system. Amino acids were quantified using standard curves for each amino acid generated over the concentration range found to occur in the tissues examined.

For proline derivatization, 250 μl of the Sep-Paked sample was lyophilized

and the residue was resuspended in 100 μl of coupling buffer composed of acetonitrile:ethanol:triethylamine:water (10:5:2:3 v:v:v:v). Five μl of phenyl isothiocynate (PITC, Sigma P-1034) was added and the mixture was incubated for 5 min at 25°C. After lyophilization, the derivatized sample was resuspended in 125 μl of 50 mM ammonium acetate buffer (pH 6.5) containing 10 μl of methanol. A 20 μl aliquot was used for HPLC analysis of proline, performed using the HPLC system described above with a Beckman Model 160 UV detector at 254 nm. The mobile phase was composed of mixtures of (A) 50 mM ammonium acetate, pH 6.5 and (B) 10 mM ammonium acetate, pH 6.5: acetonitrile (50:50 v:v) pumped at a rate of 2.0 ml min^{-1}. The overall gradient consisted of a series of seven isocratic and linear gradient steps: first an isocratic step of 5% B for 1 min, then a linear gradient from 5% to 10% B over 5 min, followed by a linear gradient from 10% to 14% B over 1 min, a linear from 14% to 16% B over 11 min, a linear gradient from 16% to 100% B over 1 min, an isocratic step of 100% B for 10 min, and a linear gradient from 100% to 0% B over 1 min. The column was maintained at 50°C. Quantification was accomplished as described for the OPA procedure.

Carbohydrates

Lyophilized and ground tissues (100 mg) were extracted three times with 80% ethanol (7 ml + 3.5 ml + 3.5 ml) to remove soluble carbohydrates. Seven ml each of water and chloroform were added to the combined extracts. The aqueous phase was collected and taken to dryness in vacuo at 40°C and the residue was resuspended in 2 ml pyridine containing 30 mg ml^{-1} hydroxylamine monohydrochloride and B-phenyl-D-glucoside as an internal standard. The resultant oximes were derivatized to trimethylsilyl ethers (Sweeley et al., 1963) and analyzed by gas liquid chromatography (Loescher et al., 1982).

The pellet remaining after extraction of soluble carbohydrate was assayed for starch. Pellets were boiled for 1 hr in 0.1 M sodium acetate buffer, pH 5, then incubated for 16 hr at 55°C with amyloglucosidase (EC 3.2.1.3; from Boehringer) (Oakley, 1983). The resulting glucose was assayed colorimetrically using glucose oxidase (Sigma Tech. Bull. 510; EC 1.1.3.4).

Inorganic ions

Inorganic ion concentration of roots was assayed spectrographically by the Soil Testing and Plant Analysis Laboratory of the University of Georgia, Athens, with an inductively coupled argon plasma (ICP) spectrophotometer (Thermo Jarrell-Ash, Franklin, MA). Phosphorus was assayed as previously

described (Chapman and Pratt, 1961) on samples dry-ashed at 750°C with magnesium nitrate for 2 hr and digested in nitric acid.

Colonization

Root pieces removed randomly from the root system were fixed in FAA, cleared in 10% KOH, bleached in H_2O_2, infiltrated with HCl, stained with trypan blue and assessed for degree of mycorrhizal colonization as described before (Augé et al., 1986a).

3. Results

Symplastic water percentages of roots of these rose plants were determined previously (Augé and Stodola, 1990): symplastic water percentage was 74% in MD roots and 52–56% in NW, ND and MW roots. Water content of nonmycorrhizal roots at full turgor was reduced 6.7% by drought treatment, from 85.3% to 78.6%. Water content of mycorrhizal roots at full turgor was reduced 11.3% by drought treatment, from 87.5% to 76.2%.

Nonmycorrhizal roots had much higher concentrations of ASN and total measured amino acids than mycorrhizal roots (Fig. 1A, Table 1). NW roots had nearly twice as much ASN as MW roots and ND roots nearly 3× as much ASN as MD roots. ASN increased by 43% with drought in nonmycorrhizal roots but was unchanged with drought in mycorrhizal roots. Similarly, ASP and GLU, which were equal in NW and MW plants, nearly doubled in nonmycorrhizal roots after drought but remained unchanged in mycorrhizal roots after drought (Fig. 1B). Roots of well-watered plants had no detectable PRO, but ND and MD roots had symplastic concentrations of 5.0 and 3.7 mmoles kg^{-1} PRO, respectively. ND roots had greater symplastic molality than MD roots of almost every amino acid: more ASN, twice as much ASP and GLU, almost 4× as much GLN, more GLY + ARG, over twice as much THR and SER, 75% more LYS and 35% more PRO (Fig. 1B–C). Total measured amino acids were 72% greater in NW than MW roots and 164% greater in ND than MD roots (Fig. 1A).

Total soluble sugar molality was about 40% higher in NW than MW roots (Fig. 2). Sucrose concentrations were similar in well-watered roots, but fructose was 50% higher and glucose 40% higher in NW than MW roots. In response to drought, total soluble sugars increased 2.6× in mycorrhizal roots and 1.7× in nonmycorrhizal roots, compared to well-watered controls (Fig. 2). Sucrose accounted for all of this increase in ND roots (over a 9-fold increase in sucrose compared with NW roots) and about 60% of the increase in MD roots

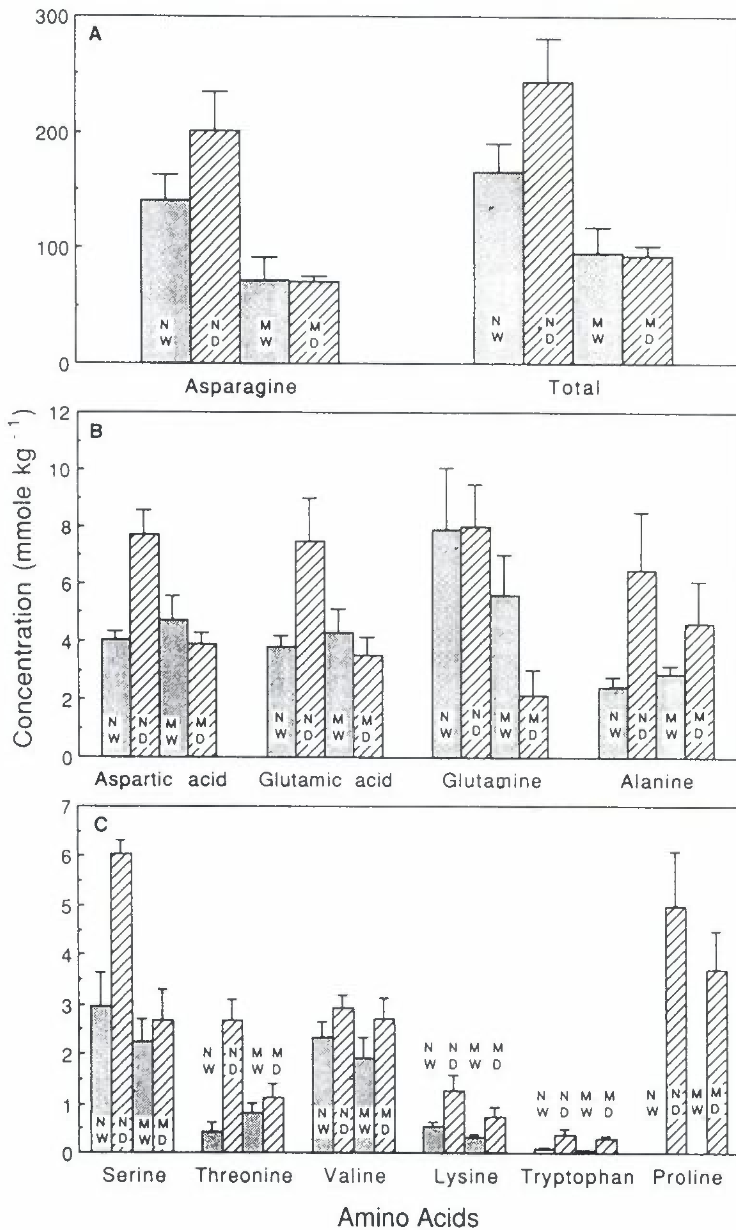

Figure 1. The influence of mycorrhizal colonization and drought on symplastic molality or concentration (mmoles amino acid kg^{-1} symplastic water) of several amino acids in *Rosa* roots at full turgor. "Total" depicted in (A) is the sum of all amino acids measured. Values are means and SE for 4 (droughted) or 3 (well-watered) replicates.

Table 1. Analysis of variance linear contrasts, showing main effects and interaction of mycorrhization and drought on concentrations of amino acids, carbohydrates and mineral elements in roots of *Rosa hybrida* plants. Values indicate probability of significance ($P \leq 0.05$). Probability values above 0.05 considered nonsignificant (NS).

	Linear contrasts		
	Mycorrhizal vs. Nonmycorrhizal	Well-watered vs. Droughted	Mycorrhization × Drought
Amino Acids			
Asparagine	0.005	NS	NS
Aspartic acid	0.05	NS	0.02
Glutamic acid	NS	NS	NS
Glutamine	0.04	NS	NS
Alanine	NS	NS	NS
Serine	0.008	0.02	0.06
Proline	NS	0.0009	NS
Threonine	NS	0.007	0.03
Valine	NS	NS	NS
Lysine	NS	0.05	NS
Tryptophan	NS	0.02	NS
Total	0.005	NS	NS
Carbohydrates			
Fructose	NS	NS	0.03
Glucose	NS	NS	0.04
Sucrose	0.04	0.0000	0.06
Total solubles	NS	0.0005	NS
Starch	NS	NS	NS
Total carbohydrate	NS	0.004	NS
Mineral elements			
P	0.0000	0.002	0.008
K	0.03	0.003	NS
Ca	0.004	NS	NS
Mg	NS	NS	NS
B	NS	NS	NS
Cu	NS	NS	NS
Zn	NS	NS	NS
Mn	NS	NS	NS
Sr	NS	NS	NS
Ba	0.0001	NS	NS

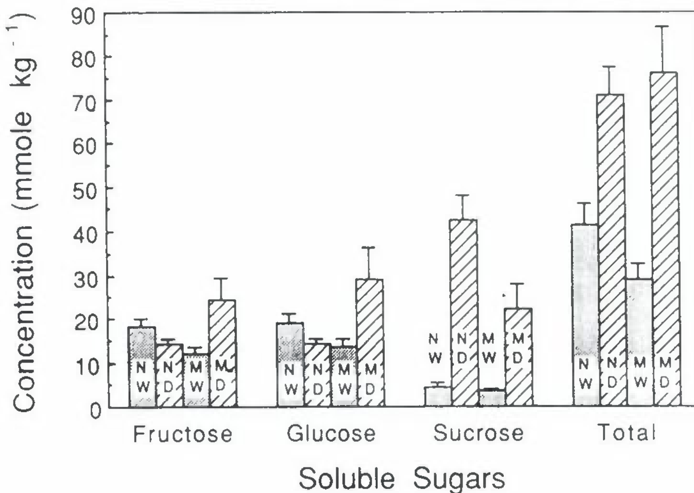

Figure 2. The influence of mycorrhizal colonization and drought on symplastic molality or concentration (mmoles sugar kg^{-1} symplastic water) of sugars in *Rosa* roots at full turgor. Values are means and SE for 4 replicates.

(over a 6-fold increase in sucrose compared with MW roots). Fructose and glucose were each doubled by drought in mycorrhizal roots but were reduced in nonmycorrhizal roots to about three-quarters that of well-watered controls. Root inositol content (data not shown) ranged from 0.35 to 0.53 millimolar (0.01 to 0.03% dry weight) and was not affected by either mycorrhizal colonization or drought. ND roots contained 58% more starch than MD roots (on a dry weight basis) and 74% more starch after drought than before drought (Fig. 3D). Starch levels were similar in well-watered plants whether or not roots were mycorrhizal and similar in mycorrhizal plants before and after drought.

After drought, the relative proportions of solutes were changed in nonmycorrhizal vs. mycorrhizal roots. ASN and total measured amino acids were still greater in nonmycorrhizal roots, but fructose and glucose were each greater in mycorrhizal roots, as was the fraction of the total osmolality represented by unassayed solutes.

Nonmycorrhizal roots had about three times the P content of mycorrhizal roots. MW roots had 75% as much K and half as much Ca as NW roots (Fig. 4). MD roots had about two-thirds the K and Ca content of ND roots. Drought did not affect Ca but did decrease K in each mycorrhizal treatment. Zn content of MD roots was 74% that of ND roots. Mg, B, Cu, Sr and Mn contents were not affected by mycorrhizal colonization.

Colonization was 33% and 24% for MW and MD roots, respectively, and 0%

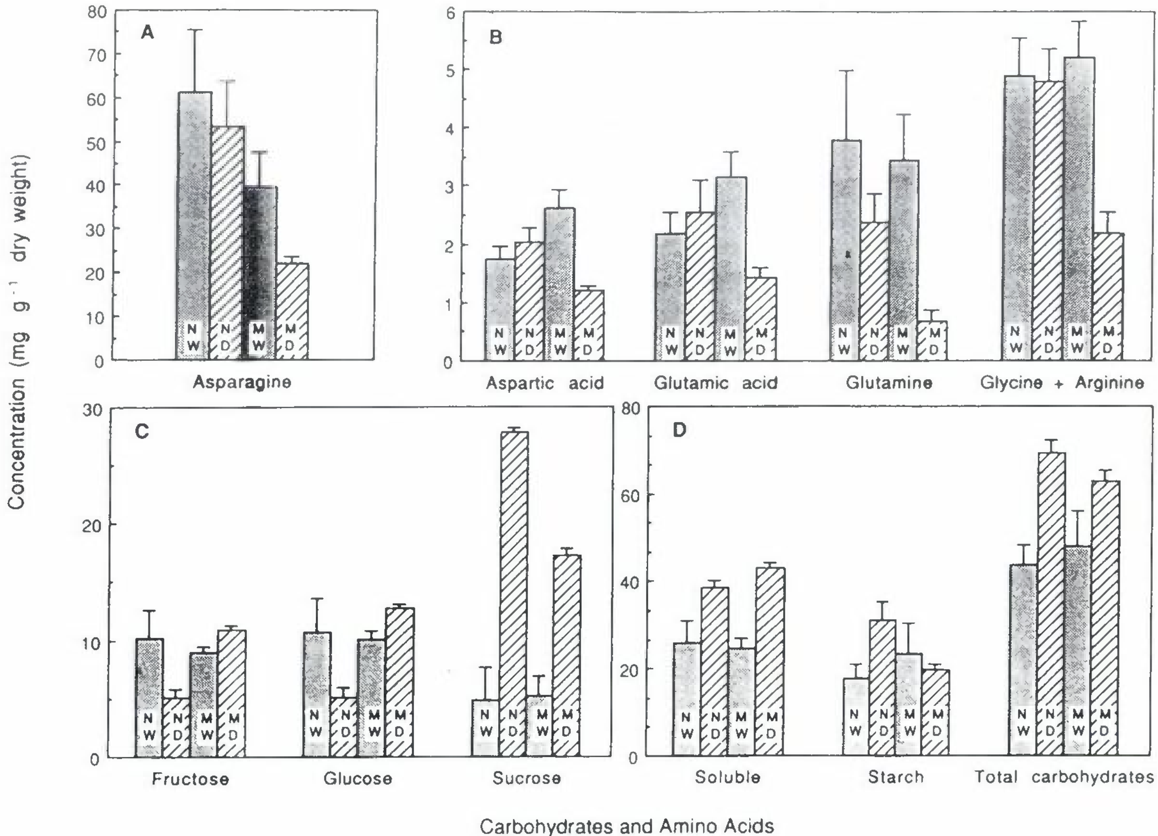

Figure 3. The influence of mycorrhizal colonization and drought on free amino acid and carbohydrate concentration of *Rosa* roots, expressed as mg g^{-1} dry root weight. Values are means and SE for 4 (carbohydrates; amino acids in droughted roots) or 3 (amino acids in well-watered roots) replicates.

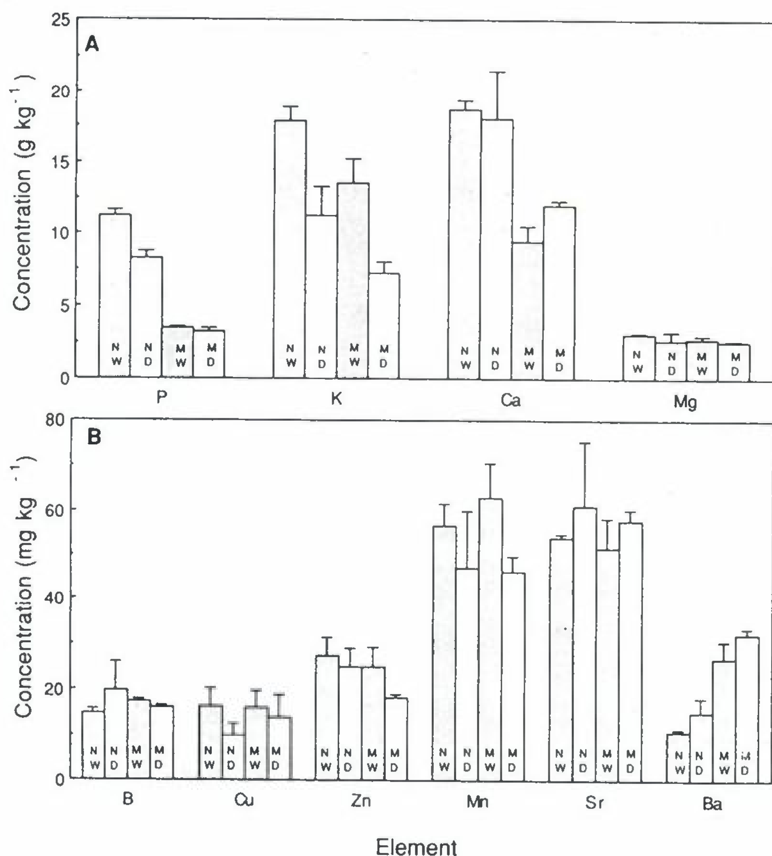

Figure 4. The influence of mycorrhizal colonization and drought on phosphorus and cation concentration of *Rosa* roots, expressed as g kg^{-1} dry weight (A) or mg kg^{-1} dry weight (B). Values are means and SE for 4 replicates.

for each of the nonmycorrhizal treatments. These colonization percentages are normal for *R. hybrida* and *G. intraradices* under high P fertilization (Augé et al., 1986a).

4. Discussion

As we are concerned primarily with the living, metabolizing portion of roots, it is probably more meaningful to express tissue solute concentration on the basis of symplastic molality rather than weight per dry weight. Such expression changes the interpretation of results considerably (compare Figs. 1–2 with Fig. 3), as treatments affected partitioning of water between symplast and apoplast as well as fresh/dry weight ratios of roots. The relative abundance of

GLN, for example, is the same in the symplasm of NW and ND roots (Fig. 1B). Yet NW roots had 60% more GLN than ND roots on a per dry weight basis (Fig. 3B), not because NW roots actually had more GLN but because they had less dry weight per unit amount of symplasm. Likewise, mycorrhizal roots had substantially less ASN on a dry weight basis after drought than before drought. When expressed on the basis of symplastic water at full turgor, however, no differences in ASN were observed between well-watered and droughted mycorrhizal roots, owing to differing symplastic water percentages. This analysis assumes that organic solutes are located exclusively or at least primarily in the symplastic or living portions of the plant organ, which for practical purposes is a fair assumption for many organs under many circumstances (Tyree and Jarvis, 1982). Expressing solute content as molality at full turgor normalizes the data at a common tissue relative water content and gives insight into how symplastic solute pools actually respond to mycorrhization or drought.

The contribution of ASN, other measured amino acids and total sugars to overal symplast Ψ_π at full turgor is depicted in Fig. 5. ASN represented a substantial amount of total symplast osmolality, regardless of treatment; high ASN content of roots is common (e.g. Shen et al., 1989; Shen et al., 1990). Tissues of many plants accumulate ASN in response to water deficits (Stewart and Larher, 1980; Drossopoulos et al., 1985); drought-induced ASN increases were observed in nonmycorrhizal roots but not in mycorrhizal roots. Higher total amino acid and sugar concentrations in the NW vs. MW roots in this study is consistent with previous reports of greater osmolality (lower Ψ_π) of NW roots of *Rosa* (Augé and Stodola, 1990) and NW roots of *Vigna unguiculata* (Augé, unpublished), compared to MW roots colonized by *G. intraradices.*

It is interesting to note that drought, which did not change full turgor Ψ_π in nonmycorrhizal roots (Augé and Stodola, 1990), resulted in increases in total sugars and most amino acids in nonmycorrhizal roots. Unassayed solutes accounted for about two-thirds of total osmolality in NW roots but only about a third of total osmolality in ND roots (Fig. 5). In mycorrhizal roots, where drought gave an osmotic adjustment of −0.4 MPa (a 0.16 osmolal increase), sugars increased with drought but predominant amino acids remained similar or decreased. Solutes other than the sugars and amino acids assayed here accounted for about three-fourths of the osmotic adjustment in mycorrhizal roots (Fig. 5). Possibilities for those solutes include various organic acids, inorganic ions, quaternary ammonium compounds and polyols; all may increase in tissues subjected to water deficits (Borowitzka, 1981).

Cation data are presented to indicate relative nutritional status of nonmycorrhizal and mycorrhizal roots. As noted previously (Augé and Stodola,

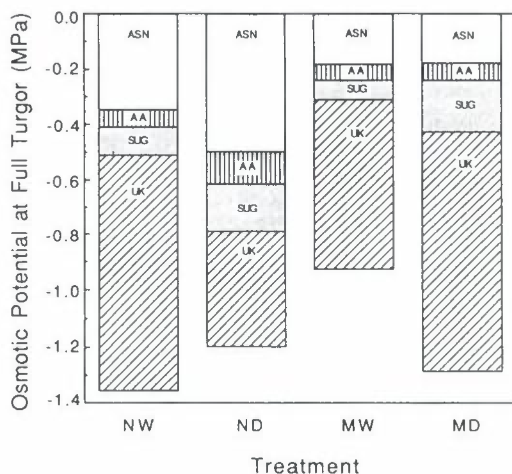

Figure 5. The contribution of ASN, all other measured amino acids, sugars and unassayed solutes to total Ψ_π of root symplasm at full turgor. UK = osmotic contribution of unknown solutes. Number of replicates (n) = 4 for amino acids after drought and for sugars in either watering treatment, n = 3 for amino acids in well-watered treatments and n = 12 for root Ψ_π.

1990), root P contents were much higher in nonmycorrhizal than in mycorrhizal plants. Phosphate uptake can be decreased in soils of low moisture (Nelsen and Safir, 1982), so we deliberately used high rates of P fertilization to preclude the possibility of P deficiency in nonmycorrhizal plants exposed to drought. It is very difficult to produce mycorrhizal and nonmycorrhizal plants having equal contents of P and other elements (Pacovsky, 1989), particularly under drought, so investigators often tailor fertilization schemes to obtain nonmycorrhizal controls having as much or more P than mycorrhizal plants (e.g. Bethlenfalvay et al., 1987 and 1988). Root P content, adequate in all treatments, was not correlated with Ψ_π or other root water relations characteristics in these plants (Augé and Stodola, 1990). We know of no reports on the impact of luxury P fertilization on composition of carbohydrates or amino acids in roots. Previously, relatively high P fertilization and tissue P contents had no adverse effects on growth of roses (Sadasivaiah and Holley, 1973) or water relations of other species (Fitter, 1988).

The literature contains varying reports regarding the influence of VA mycorrhizal colonization on free amino acid and soluble carbohydrate status of roots. Because mycorrhizal fungi can alter fresh/dry weight ratios or symplastic/total water ratios (Augé and Stodola, 1990), direct comparisons of root solute contents expressed as symplastic osmolality and weight per dry or fresh

weight root are not always appropriate. Most studies have provided reports of well-watered plants, however, and we did not observe differences between MW and NW roots in dry matter or symplastic water partitioning. Increases or decreases in free amino acids in soybean roots colonized by *G. fasciculatum* have been reported to follow transient changes in N content, with a tendency for elevated ASP and ASN in mycorrhizal roots (Pacovsky, 1989). Differences in individual amino acids between nonmycorrhizl and *G. mosseae*-colonized *Pittosporum* roots have been linked to relative abundance of NH_4^+ and NO_3^- in applied fertilizers (Coxwell and Johnson, 1985). Regardless of fertilization ratio, ASP levels were much higher than other free amino acids in both mycorrhizal and nonmycorrhizal roots in that study. Contrary to our findings in *Rosa*, ASN was present in lower concentrations than GLN, GLU or ASP and was generally higher in mycorrhizal than in nonmycorrhizal *Pittosporum* roots (Coxwell and Johnson, 1985). Young et al. (1972) also found greater ASN levels in mycorrhizal than in nonmycorrhizal corn roots. In a tobacco study (Baltruschat and Schoenbeck, 1975), mycorrhizal roots were observed to contain higher quantities of GLN and GLU than nonmycorrhizal roots. GLN and GLU were similar in nonmycorrhizal and mycorrhizal *Rosa* roots with sufficient moisture but with drought were lower in mycorrhizae. Other reports show no changes in roots in free amino acid content as a consequence of mycorrhizal colonization (Schwab et al., 1983; Thompson et al., 1986).

Concentrations of soluble carbohydrates in roots may be positively correlated with mycorrhizal colonization (Amijee et al., 1990; Thompson et al., 1986; Same et al., 1983), negatively correlated with colonization (Koch and Johnson, 1984), uncorrelated with colonization (Nemec and Guy, 1982; Schwab et al., 1983) or correlated only at certain plant ages (Pacovsky, 1989; Ocampo and Azcón, 1985) or nutritional states (Nemec and Guy, 1982; Pacovsky, 1989). In wheat/ *Glomus mosseae* associations, for example, sugar content was higher in mycorrhizal roots of young plants (Ocampo and Azcón, 1985) but lower in older plants with developed sheaves (Azcón and Ocampo, 1985). The actual percentage of root length colonized or frequency of hyphal penetration may (Thompson et al., 1986; Same et al., 1983; Jasper et al., 1979) or may not (Ocampo and Barea, 1982; Ocampo and Azcón, 1985) correlate with soluble sugar concentrations of roots. The above reports concern well-watered plants. The total sugar concentrations of roots of well-watered rose plants in our study were unaffected by mycorrhizal colonization; after drought mycorrhizal roots had somewhat higher total sugar levels than nonmycorrhizal roots. Relative abundance of reducing and non-reducing sugars in nonmycorrhizal vs. mycorrhizal *Rosa* roots was inverted by drought.

Our data favor Pacovsky's (1989) suggestion, that effects of mycorrhizal

fungi on carbohydrate and free amino acid levels may be transitory, host- or fungus-specific. The interesting finding is that mycorrhizal colonization and environmental conditions, in this case low soil moisture, interacted in modifying sugar and free amino acid pools in roots.

REFERENCES

Amijee, F., Stribley, D.P., and Tinker, P.B. 1990. Soluble carbohydrates in roots of leek (*Allium porrum*) plants in relation to phosphorus supply and VA mycorrhizas. *Plant Soil* **124:** 195–198.

Augé, R.M., Schekel, K.A., and Wample, R.L. 1986a. Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. *New Phytol.* **103:** 107–116.

Augé, R.M., Schekel, K.A., and Wample, R.L. 1986b. Osmotic adjustment in leaves of VA mycorrhizal and nonmycorrhizal rose plants in response to drought stress. *Plant Physiol.* **82:** 765–770.

Augé, R.M., Schekel, K.A., and Wample, R.L. 1987. Leaf water and carbohydrate status of VA mycorrhizal rose exposed to water deficit stress. *Plant Soil* **99:** 291–302.

Augé, R.M. and Stodola, A.J.W. 1990. An apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted *Rosa* plants. *New Phytol.* **115:** 285–295.

Azcón, R. and Ocampo, J.A. 1981. Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytol.* **87:** 677–685.

Baltruschat, H., Sikora, R.A., and Schoenbeck, F. 1973. Effect of VA-mycorrhiza (*Endogone mosseae*) on the establishment of *Thielaviopsis basicola* and *Meloidogyne incognita* in tobacco. *2nd Int. Congr. Plant Pathol.*, Minneapolis, Minnesota, Abstract 661.

Baltruschat, H. and Schoenbeck, F. 1975. Untersuchungern über den Einfluss der endotropen Mycorrhiza auf den Befall von Tabak mit *Thielaviopis basicola. Phytopathol. Z.* **84:** 172–188.

Bayliss, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: *Endomycorhizas.* F.E. Sanders, B. Mosse and P.B. Tinker, eds. Academic Press, New York, pp. 373–389.

Bethlenfalvay, G.J., Brown, M.S., Ames, R.N., and Thomas, R.S. 1988. Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphorus uptake. *Physiol. Plantarum* **72:** 565–571.

Bethlenfalvay, G.J., Brown, M.S., Mihara, K.L., and Stafford, A.E. 1987. *Glycine-Glomus-Rhizobium* symbiosis. V. Effects of mycorrhiza on nodule activity and transpiration in soybeans under drought stress. *Plant Physiol.* **85:** 115–119.

Borowitzka, L.J. 1981. Solute accumulation and regulation of cell water activity. In: *Physiology and Biochemistry of Drought Resistance in Plants.* L.G. Paleg and D. Aspinall, eds. Academic Press, New York, pp. 97–130.

Caron, M., Fortin, J.A., and Richard, C. 1985. Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* on tomatoes. *Plant Soil* **87:** 233–239.

Chapman, H.D. and Pratt, P.F. 1961. *Methods of Analysis for Soils, Plants and Waters.* University of California, Riverside, CA, 1961, pp. 161–174.

Coxwell, M.A. and Johnson, C.R. 1985. Effects of vesicular-arbuscular mycorrhizae and nitrogen source on growth and transport amino acid composition of *Pittosporum tobira. J. Amer. Soc. Hort. Sci.* **110:** 800–803.

Drossopoulos, J.B., Karamanos, A.J., and Niavis, C.A. 1985. Changes in free amino compounds during the development of two wheat cultivars subjected to different degrees of water stress. *Ann. Bot.* **56:** 291–305.

Fitter, A.H. 1988. Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. *J. Exper. Bot.* **39:** 595–603.

Foster, J.G. 1990. Optimal extraction procedure for high performance liquid chromatographic determination of diaminobutyric acid in flatpea (*Lathyrus sylvestris* L.). *J. Agric. Food Chem.* **38:** 748–752.

Foster, J.G. 1989. High performance liquid chromatographic analysis of diaminobutyric acid in flatpea extracts. *J. Liq. Chromatogr.* **12:** 3033–3050.

Jasper, D.A., Robson, A.D., and Abbott, L.K. 1979. Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biol. Biochem.* **11:** 501–505.

Koch, K.E. and Johnson, C.R. 1984. Photosynthate partitioning in split-root citrus seedlings with mycorrhizal and non-mycorrhizal root system. *Plant Physiol.* **75:** 26–30.

Loescher, W., Marlow, G.C., and Kennedy, R. 1982. Sorbitol metabolism and sink-source interconversions in developing apple leaves. *Plant Physiol.* **70:** 335–339.

Nelsen, C.E. and Safir, G.R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. *Planta* **154:** 407–413.

Nemec, S. and Guy, G. 1982. Carbohydrate status of mycorrhizal and non-mycorrhizal citrus rootstocks. *J. Amer. Soc. Hort. Sci.* **107:** 177–180.

Oakley, E.T. 1983. Enzymatic determination of starch in fresh green, lyophilized green, and cured tobacco. *J. Agric. Food Chem.* **31:** 902–905.

Ocampo, J.A. and Azcón, R. 1985. Relationship between the concentration of sugars in the roots and VA mycorrhizal infection. *Plant Soil* **86:** 95–100.

Ocampo, J.A. and Barea, J. 1982. Depressed metabolic activity of VA mycorrhizal fungi by photosynthesis inhibitor herbicides. In: *Les Mycorrhizes: Biologie et Utilization.* INRA Publ. no. 13. INRA, pp. 267–270.

Pacovsky, R.S. 1989. Carbohydrate, protein and amino acid status of *Glycine-Glomus-Bradyrhizobium* symbioses. *Physiol. Plant.* **75:** 346–354.

Same, B.I., Robson, A.D., and Abbott, L.K. 1983. Phosphorus, soluble carbohydrates and endomycorrhizal infection. *Soil Biol. Biochem.* **15:** 593–597.

Sadasivaiah, S.P. and Holley, W.D. 1973. Ion balance in nutrition of greenhouse roses. *Joseph H. Hill Memorial Foundation Research Report*, November.

Schwab, S.M., Menge, J.A., and Leonard, R.T. 1983. Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass roots in relation to vesicular-arbuscular mycorrhiza formation. *Plant Physiol.* **73:** 761–765.

Shen, L., Foster, J.G., and Orcutt, D.M. 1989. Composition and distribution of free amino acids in flatpea (*Lathyrus sylvestris* L.) as influenced by water deficit and plant age. *J. Exp. Bot.* **40:** 71–79.

Shen, L., Orcutt, D.M., and Foster, J.G. 1990. Influence of drought on the composition and distribution of 2,4-diaminobutyric acid and other free amino acids in tissues of flatpea (*Lathyrus sylvestris* L.). *Environ. Exper. Bot.* **30:** 497–504.

Stewart, G.R. and Larher, F. 1980. Accumulation of amino acids and related compounds in relation to environmental stress. In: *The Biochemistry of Plants*, Vol. 5. Academic Press, New York, pp. 609–635.

Sweeley, C.C., Bentley, R., Makita, M., and Wells, W.W. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. *J. Amer. Chem. Soc.* **85:** 2497–2507.

Thompson, B.D., Robson, A.D., and Abbott, L.K. 1986. Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. *New Phytol.* **103:** 751–765.

Tyree, M.T. and Jarvis, P.G. 1982. Water in tissues and cells. In: *Encyclopedia of Plant Physiology 12B: Physiological Plant Ecology II.* O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler, eds. Springer-Verlag, Berlin, pp. 35–77.

Young, J.L., Ho, I., and Trappe, J.M. 1972. Endomycorrhizal invasion and effect on free amino acids content of corn roots. *Agronomy Abstracts*, p. 102.