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Water Stress- and Nutrient Solution-mediated Changes in Water Relations and Amino Acids, Organic Acids, and Sugars in Xylem Fluid of *Prunus salicina* and *Lagerstroemia indica*

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Abstract. **The effects and interactions of water stress and nutrient solution on water relations and concentrations of amino acids, organic acids and sugars in xylem fluid of 'Methley' plum *(Prunus salicina* Lindl.) and 'Carolina Beauty' crape myrtle *(Lagerstroemia indica* L.) during midday were determined. Container-grown plants were irrigated with water or nutrient solution (i.e., osmolarity = 138 mM) for 15 days, then irrigation was either continued or terminated for the next 5 days. The experiments were analyzed as factorial designs for each species separately, with the nutrient solution and irrigation status the last 5 days as the main factors. Xylem fluid tension increased ≈ 2- to 3-fold and leaf conductance to water vapor and transpiration were reduced ≈ 10-fold by withholding irrigation for both species; plant water relations of *L. indica* were also influenced by the nutrient solution. For both species, the osmolarity of xylem fluid was not altered by withholding irrigation. The predominant organic compounds quantified in both species were amides (i.e., glutamine and asparagine), arginine, and citric and malic acids. Sugars represented a small proportion (i.e., generally ≤ 1%) of total osmolarity. Irrigation altered the chemical profile of amino acids and organic acids to a greater degree than the nutrient solution. Water stress induced a 3-fold increase in total organic acids in xylem fluid of both species. The osmolarity and the concentration of most organic compounds in xylem fluid of *P. salicina* were not significantly affected by the nutrient solution. Arginine increased markedly in concentration by withholding irrigation or with the application of nutrient solution for *L. indica.* The concentration of most organic compounds did not vary greatly in response to variations in soil water or nutrient status. In conclusion, soil water-or nutrient-mediated changes in plant water relations exceeded changes in xylem fluid chemistry.**

Xylem vessels serve as a major conduit for the delivery of inorganic ions and certain organic compounds to plant tissues. Xylem fluid is extremely dilute (i.e.,>95% water) and consists primarily of amino acids, organic acids and inorganic ions (Andersen and Brodbeck, 1989a-c, 1991; Andersen et al., 1989, 1992; Clark et al., 1986). Although the concentration of solutes in xylem fluid is low, a large quantity of solute is transported in xylem fluid due to the large amount of water typically transpired by mesophytic plants. Despite the importance of xylem fluid to plant nutrient status and to leaf and shoot physiology, there have been relatively few attempts to characterize the influence of environmental stresses on the chemistry of xylem fluid.

Soil water and soil fertility status may drastically alter the concentration of organic compounds in foliar tissue (Boggess et al., 1976; Good and Zaplachinski, 1994; Rabe 1990; van Rensburg et al., 1993; Voetberg and Sharp, 1991). Rabe (1990) reviewed the influence of numerous abiotic and biotic stresses on the composition of N-containing compounds in plants. The amino compounds most often accumulated in foliar tissue as a function of stress include glutamine, asparagine, arginine, proline, citrulline, and ornithine. It is not known, however, to what extent the chemical compounds derived from xylem fluid contribute to stress-induced changes in the chemical profile of foliar tissue.

Evidence derived from plants exuding xylem fluid under positive pressure has been consistent with the concept that the composition of xylem fluid is regulated by the plant and is not greatly influenced by the composition of the rhizosphere. Fertilization during the dormant season had a minimal influence on the chemistry of xylem fluid during budbreak of several *Vitis* species. (Andersen and Brodbeck, 1991; Roubelakis–Angelakis and Kliewer, 1979) and kiwi (Clark et al., 1986). Andersen and Brodbeck (1991) found no difference in the osmolarity of xylem fluid of bleeding *V. rotundifolia* despite irrigation with 3x Hoagland's solution, although total water and solute flux were increased 3-fold compared to plants irrigated with water. Few differences in the concentration of organic and inorganic constituents in xylem fluid of *Vitis* spp. before budbreak occurred after the application of fertilizer in the field (Andersen and Brodbeck, 1991; Roubelakis–Angelakis and Kliewer, 1979). The potential contribution of solutes derived from the xylem fluid during periods of growth and positive root pressure is relevant to studies involving nutrient partitioning or stress-induced osmotic adjustment in plant tissue. These data may also be useful for developing and understanding C and N budgets.

Our objectives were to test the effects and the interaction of water stress and of soil applied nutrients on plant water relations and the concentration of amino acids, organic acids, and sugars in the xylem fluid of two woody species *Prunus salicina* and *Lagerstroemia indica,* which were chosen since they are in different families and have xylem fluid with markedly different profiles of organic compounds (Andersen et al., 1989).

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Materials and Methods

Plant material. In March 1989, *P. salicina* 'Methley' and *L. indica* 'Carolina Beauty' were potted in 12-liter containers with media consisting of 3 pine bark : 1 Canadian sphagnum peat : 1 sand. One cubic meter of medium was amended with 6.1 kg dolomite, 2.2 kg superphosphate, 0.9 kg Micromax (12S–0.lB–0.5 Cu-12Fe–2.5 Mn-.5Mo-12Zn), and 5.9 kg Osmocote 18-N-2.6P–10K (Grace Sierra Corp., Milpitas, Calif.); initial medium pH was 5.6. Plants were supplied with overhead irrigation twice daily until 14 July, at which time they were transported to a rainproof shelter covered with 6 ml clear polyethylene. Twenty-eight plants of each species were selected for uniform size. From 14 to 28 July, one-half of the plants was irrigated with 500 ml tap water or with triple-strength Peters soluble fertilizer 20N–8.2P–16.6K (Grace Sierra Corp.) twice daily. The nutrient solution was composed of ammonia (30 mM), nitrate (47 mM), urea (38 mM), superphosphate (14 mM), and potash (30 mM). Total osmolarity was 138 mM. On July 28, irrigation was terminated for one-half of the plants for 5 days. On 2 Aug., plant water status was determined and xylem fluid was extracted for quantification of amino acids, organic acids, and sugars.

Thus, the four treatments were 1) irrigation with water for 20 days (well-watered treatment); 2) irrigation with nutrient solution for 20 days (nutrient solution treatment); 3) irrigation with nutrient solution for 15 days which was then discontinued for 5 days (nutrient solution/water-stress treatment); 4) irrigation with water for 15 days which was then discontinued for 5 days (water-stress treatment).

Plant water relations. Leaf conductance to water vapor (g,) and transpiration rates (E) were measured on two recently expanded leaves of each plant with a steady-state porometer (model 1600M; LI–COR, Lincoln, Neb.) between 1100 and 1400 HR. Air temperature was 29 to 33C and relative humidity was 70% to 80% during the measurement period.

Leaves were placed in the leaf chamber for 1 minor until near steady-state conditions of H_2O vapor exchange were achieved. Water vapor exchange was measured on abaxial leaf surfaces since adaxial g_1 was negligible. Measurements were recorded when photosynthetic photon flux exceeded 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Several minutes after measurements of leaf H_2O vapor exchange, xylem fluid tension was measured on one or two 25- to 35-cm stem segments of each of seven plants per treatment with a pressure chamber apparatus (model 600; Plant Moisture Stress Corp., Corvallis, Ore.) (Scholander et al., 1965). Tissue exterior to the xylem was removed from the 2- to 4-cm stem section protruding from the chamber before measurement of xylem tension and collection of xylem fluid.

Collection of xylem fluid and chemical analyses. After recording the xylem tension, the pressure was increased 0.5 MPa higher than the balance pressure for 90 sec (Andersen et al., 1993; Berger et al., 1994). Xylem fluid was collected in 1.5-ml Eppendorf tubes, sealed, and placed on ice. Total osmolality of xylem fluid was determined with a vapor-pressure osmometer (model 5500; Wescor, Logan, Utah). Osmolality and osmolarity are virtually identical for dilute samples; hence, to facilitate comparison, concentrations of organic compounds will be expressed as osmolarity (mM). Preliminary experiments conducted on shoots of *P. persica* (L.) Batsch and *L. indica* have shown that the osmolarity of xylem fluid was not significantly altered in samples collected from 0 to 2 min after pressurization. Samples were stored at –20C for several months before chemical analyses. Samples were thawed and centrifuged at 1200× g through a 10,000 MW filter (Millipore Corp., Bedford, Mass.). Filtrate was divided into two subsamples for determination of amino acids and of organic acids and sugars (Andersen et al., 1993). The quantity of sample was insufficient for a comprehensive analysis of the chemical profile of inorganic ions.

Amino acids were analyzed following hydrolysis with constant boiling (110C) 6 M HC1 for 24 h under N_2. Samples were lyophilized and one hundred@ of 2 ethanol: 2 triethanolamine (TEA) :1 H_2O were added to each sample before drying under vacuum. Precolumn derivatization was completed by adding 20 µl of 7 ethanol : 1 TEA : 1 H_2O : 1 phenylisothiocyanate under N_2 atmosphere for 20 min. Amino acids were analyzed via a Waters high-performance liquid chromatography (HPLC) gradient system equipped with an ultraviolet (UV) detector and Pico Tag column (Waters Division Millipore Corp., Milliford, Mass.). The eluent consisted of 5 mM sodium phosphate buffer and 6% acetonitrile. Detection was at 254 nm.

Organic acids were analyzed on a Waters HPLC system and a variable-wavelength UV detector (Beckman Corp., San Roman, Calif.). Samples in 0.015 mM H_2SO_4 buffer were run isocratically at 37C through an Ion-300 polymeric cation exchange column (Interaction Corp., San Jose, Calif.). Detection was at 214 nm.

Sugars were analyzed with the HPLC system and detected with a Dionex Pulse Amperometric detector equipped with a gold electrode (Dionex Corp., Sunnyvale, Calif.). Pulse settings were

Table 1. The influence of irrigation and nutrient solution on plant water relations and the concentration of some organic compounds in xylem fluid of *Prunus salicina.* Treatment designation. t = irrigated with water for 20 days (well-watered treatment); ++= irrigated with nutrient solution for 20 days (nutrient solution treatment),+= nutrient solution for 15 days which was terminated for the final 5 days (nutrient solution/water-stress treatment), -- = irrigated with water for 15 days which was terminated for the final 5 days (water-stress treatment).

Dependent variable	Irrigation (I) / Nutrient solution (N)	+ / –	+ / +	– / +	– / –	Significance[z] T	N	I × N
Xylem tension (MPa)		0.66	0.74	1.60	2.14	*	NS	NS
Leaf conductance ($mol \cdot m^{-2} \cdot s^{-1}$)		0.203	0.198	0.041	0.021	***	NS	NS
Transpiration ($mmol \cdot m^{-2} \cdot s^{-1}$)		6.58	5.24	1.44	0.83	***	NS	NS
Total osmolarity (mM)		26.0	28.0	40.0	31.0	NS	NS	NS
Amino acids (mM)		2.32	2.67	3.09	3.17	NS	NS	NS
Organic acids (mM)		1.18	0.93	2.47	3.44	***	NS	NS
sugars (mM)		0.15	0.16	0.26	0.23	NS	NS	NS
Total organic compounds (mM)		3.64	3.75	5.81	6.84	**	NS	NS
Organic N (mM)		*3.69*	4.42	5.13	5.17	NS	NS	NS
Organic N : C		0.258	0.308	0.210	0.186	***	NS	NS

[z]NS,** *,''* Nonsignificant or significant at $P \leq 0.05, 0.01$, or 0.001, respectively.

Table 2. The influence of irrigation and nutrient solution on the concentration of amino acids, organic acids and sugars in xylem fluid of *Prunus salicina* 'Methley'. Treatment designation:+-= irrigated with water for 20 days (well-watered treatment);++ = irrigated with nutrient solution for 20 days (nutrient solution treatment); -+= nutrient solution for 15 days which was terminated for the final 5 days (nutrient solution/water-stress treatment); = irrigated with water for 15 days which was terminated for the final 5 days (water-stress treatment).

Irrigation (I)	+	+	—	—			
Nutrient solution(N)	–	+	+	—	Significance		
Dependent variable	Concn (μM)				I	N	I × N
Amino acid							
ASN	937	1191	1317	1350	NS	NS	NS
ASP	234	118	180	381	- *	**	NS
GLU	49	57	4599	NS	NS	*	
GLN	87	139	182	148	NS	NS	NS
SER	14	26	44	63	*	NS	NS
GLY	1	3	2	3	NS	NS	NS
HIS	47	67	9	1 116	*	NS	NS
ARG	122	130	171	121	NS	NS	NS
ALA	485	559	481	374	NS	NS	NS
THR	90	84	122	125	NS	NS	NS
PRO	84	94	116	111	NS	NS	NS
TYR	14	15	32	14	NS	NS	NS
VAL	56	57	160	115	NS	NS	NS
CYS	26	8	19	15	NS	NS	NS
MET	20	28	24	20	NS	NS	NS
ILE	17	28	43	36	NS	NS	NS
LEU	17	27	31	43	NS	NS	NS
PHE	4	7	9	12	**	NS	*
LYS	11	28	19	24	NS	NS	NS
Organic acid							
OX	9	10	20	23	NS	NS	NS
CIT	165	157	419	358	***	NS	NS
MAL	908	658	1810	2972	***	*	NS
SUC	74	91	169	50	NS	*	NS
FUM	17	12	28	29	*	NS	NS
sugar							
SUCR	11	9	13	42	NS	NS	NS
FRUC	9	75	116	85	NS	NS	NS
GLUC	73	73	131	99	NS	NS	NS

[NS,*,**,***]Nonsignificant or significant at $P \leq 0.05, 0.01$, or 0.001, respectively.

established at 0.6, 0.07, and -0.8 mV for 120, 120, and 300 milliseconds, respectively. Sugars were analyzed using 100 mM sodium phosphate buffer run isocratically through a Dionex Ion–Pac ion-exchange column.

Subsets of nonhydrolyzed samples were used for two additional analyses. Since hydrolysis resulted in delamination, the first subset was used for the quantification of the ratio of the amides to their respective acids. The second subset was used for a more accurate quantification of succinic acid since coelution with an unknown compound occurred with normal UV detection procedures. In this case, quantification was facilitated with a Beckman 168 diode array detector scanning from 200 to 320 nm.

Organic N and C were calculated from the concentration of each amino acid, organic acid, and sugar detected. Total organic compounds represent the sum of amino acids, organic acids, and sugars quantified.

Statistics. The experiment was designed and analyzed as a 2 × 2 factorial analysis of variance with water and nutrient solution as the two main factors. Species were analyzed separately. All seven plants of each treatment for each species were used for the analyses of plant water relation variables; however, only four replications from each treatment were analyzed chemically and subjected to statistical analyses of xylem fluid chemistry.

Results

Plant water relations were more greatly influenced by irrigation than concentration of organic compounds in xylem fluid of both species (Tables 1-4). Application of nutrient solution to *P. salicina* did not alter plant water relations, total osmolarity, or any class of organic compounds (Table 1). The nutrient solution affected plant water relations, osmolarity, and the concentration of organic N in xylem fluid of *L. indica*, although the chemical variables were more strongly influenced by withholding water (Tables 3 and 4).

Xylem tension of both species was ≈ 0.65 to 2.15 MPa for the well-watered and water-stress treatments, respectively (Tables 1 and 3). The 3-fold increase in xylem tension resulted in wilting of expanding leaves, yet at the termination of the experiment all plants recovered and resumed growth. The water-stress treatment was associated with a leaf conductance (g_l) of only 0.021 $mol \cdot m^{-2} \cdot s^{-1}$ compared to >0.20 $mmol \cdot m^{-2} \cdot s^{-1}$ for the well-watered treatment; transpiration also declined by an order of magnitude for both species. The nutrient solution/water-stress treatment resulted in g_l and E ≈ 1.5- to 2-fold greater than those of the water-stress treatments.

Osmolarity of xylem fluid was not significantly influenced by withholding irrigation from either species (Tables 1 and 3). How-

Table 3. The influence of irrigation and nutrient solution on plant water relations and the concentration of some organic compounds in xylem fluid of *Lagerstroemia indica.* Treatment designation:+-= irrigated with water for 20 days (well-watered treatment);++ = irrigated with nutrient solution for 20 days (nutrient solution treatment);-+= nutrient solution for 15 days which was terminated for the final 5 days (nutrient solution/water-stress treatment); -- = irrigated with water for 15 days which was terminated for the final 5 days (water-stress treatment).

Dependent variable	Irrigation (I)	+	+	−	−	Significance		
	Nutrient solution(N)	−	+	+	−	I	N	I × N
Xylem tension (MPa)		0.64	1.01	1.71	2.15	***	***	***
Leaf conductance ($mol \cdot m^{-2} \cdot s^{-1}$)		0.251	0.080	0.030	0.021	***	***	***
Transpiration ($mmol \cdot m^{-2} \cdot s^{-1}$)		6.48	2.27	0.85	0.44	***	***	***
Total osmolarity (mM)		27.0	32.0	55.0	25.0	NS	**	*
Amino acids (mM)		2.21	2.34	4.74	2.14	NS	*	NS
organic acids (mM)		0.71	1.22	2.04	2.48	***	NS	NS
Sugars (mM)		0.24	0.22	0.44	0.38	**	NS	NS
Total organic compounds (mM)		3.17	3.77	7.22	5.00	**	*	NS
Organic N (mM)		3.92	4.47	9.92	4.45	*	*	NS
Organic N : C		0.267	0.253	0.286	0.174	NS	NS	*

^{NS,*,**,***}Nonsignificant or significant at $P \leq 0.05, 0.01$, or 0.001, respectively.

ever, application of nutrient solution increased the osmolarity of xylem fluid of *L. indica.* For both species, the sum of the concentration of amino acids, organic acids, and sugars corresponded to 12% to 15% of the respective values of total osmolarity with the exception of the water stress treatment (22% and 25% in *P. salicina* and *L. indica*, respectively) (Tables 1 and 3). Organic acids were the compounds that increased most in xylem fluid from the water stress treatment (Tables 1-4). The concentrations of individual and total amino acids were less influenced by withholding irrigation.

The 2- to 3-fold increases in citric and malic acids for *P. salicina* in the water-stress treatment compared to the well-watered treatment (Table 2) accounted for much of the increase in total organic acids, the increase in total organic compounds quantified, and the reduction in the ratio of organic N to organic C (Table 1). By contrast, irrigation did not influence the concentration of total amino acids (Table 1); however, concentrations of aspartic acid, serine, histidine, and phenylalanine increased in response to water stress (Table 2). Arginine was the only amino acid that increased significantly in concentration with application of nutrient solution. The concentrations of sucrose, fructose, and glucose were low (i.e., ≈ 1% of total osmolarity) and were not significantly altered by irrigation or nutrient solution. Irrigation x nutrient solution interactions were not significant for any of the variables listed in Table 1, and few of the individual compounds listed in Table 2.

Plant water relations of *L. indica* were greatly influenced by irrigation, nutrient solution, and irrigation × nutrient solution interactions (Table 3). Unlike *P. salicina, L. indica* receiving nutrient solution for the duration of the experiment manifested considerably lower g_s and E compared to the well-watered treatment. Xylem tension, g_s, and E for *L. indica* were significantly influenced by irrigation × nutrient solution interactions.

Concentration of total organic acids, total sugars, total organic compounds, and organic N in xylem fluid of *L. indica* all increased by withholding irrigation (Table 3). Increased concentrations of amino acids associated with application of nutrient solution was responsible for the increase in total organic compounds (and organic N) in xylem fluid. Concentration of total amino acids and organic N increased 2-fold in the nutrient solution–water-stress treatment (Table 3). The concentration of sugars in xylem fluid of plants not irrigated for 5 days also increased ≈ 2-fold compared to plants irrigated daily with water or nutrient solution. The ratio of organic N to organic C in xylem fluid was dependent on irrigation x nutrient solution interactions. The N to C ratio was particularly low for the water stress treatment.

The concentration of glutamic acid, arginine, alanine, isoleucine, citric acid, malic acid, succinic acid, and glucose in xylem fluid of *L. indica* were influenced by withholding irrigation (Table 4). Malic acid increased from 335 to 2017 μM in the water stress treatment. A 6-fold increase in arginine concentration in xylem fluid occurred in plants in the nutrient solution–water-stress treatment. This increase in total amino acids and organic N can be ascribed mainly to increased N-rich arginine. Irrigation × nutrient solution interactions occurred for asparagine, aspartic acid, glutamine, serine, and malic and fumaric acid; all compounds except malic acid occurred in low concentration.

Discussion

The osmolarity of xylem fluid of *P. salicina* was maintained between 26 and 40 mM, despite the application of 138 mM nutrient solution or with the imposition of severe drought stress that induced nearly complete stomatal closure. The concentration of major plant nutrients in the soil solution is usually in the range of 10^{-6} to 10^{-3} M (Clarkson, 1985), or at least two orders of magnitude less than the concentration of soluble nutrients applied in the current study. The osmolarity of xylem fluid of *L. indica* was influenced by nutrient solution, but not by irrigation. For both species, the proportion of the total organic compounds quantified divided by total osmolarity was also relatively constant (12% to 15%) for all except the water-stress treatment (22% to 25% for *P. salicina* and *L. indica*, respectively). This regulation of xylem fluid chemistry is consistent with studies showing a minimal influence of fertilization on the chemistry of xylem fluid collected from bleeding plants (Andersen and Brodbeck, 1991; Cooper and Clarkson, 1989; Clark et al., 1986; Roubelakis-Angelakis and Kliewer, 1979). The low concentrations of sugars, particularly sucrose, in xylem fluid of *P. salicina* and *L. indica* are also consistent with previous analyses obtained from bleeding plants, and suggest minimal contamination from phloem fluid or ruptured cells.

Several mechanisms may be responsible for maintaining xylem fluid chemistry within narrowly defined limits. Electrogenic ion pumps, one located at the epidermis-root interface and one located at the plasmalemma–xylem vessel interface (deBoer et al., 1983) regulate nutrients entering xylem vessels (Clarkson, 1985). Perumalla (1986) surveyed 213 angiosperm species from 52 families and found that 88% of the species had Casparian bands between cortex and stele (endodermis) and in the hypodermis (exodermis). In these species, transport of nutrients from the

Table 4. The influence of irrigation and nutrient solution on the concentration of amino acids, organic acids and sugars in xylem fluid of *Lagerstroemia indica*. Treatment designation: +– = irrigated with water for 20 days (well-watered treatment); ++ = irrigated with nutrient solution for 20 days (nutrient solution treatment); -+ = nutrient solution for 15 days which was terminated for the final 5 days (nutrient solution/water-stress treatment); -- = irrigated with water for 15 days which was terminated for the final 5 days (water-stress treatment).

Irrigation (I)	+	+	—	—			
Nutrient solution (N)	–	+	+	—	Significance		
Dependent variable	Concn (uM)				I	N	I × N
Amino acid							
ASN	108	40	104	85	NS	NS	**
ASP	72	26	69	57	NS	NS	**
GLU	16	18	54	49	*	NS	NS
GLN	793	875	1293	358	NS	NS	*
SER	54	25	54	30	NS	NS	**
GLY	3	4	9	15	NS	NS	NS
HIS	65	25	4	4 52	NS	*	NS
ARG	314	574	1843	851	**	*	NS
ALA	232	246	502	219	*	NS	NS
THR	99	37	113	61	NS	NS	*
PRO	52	105	171	26	NS	NS	NS
TYR	20	43	26	18	NS	NS	NS
VAL	103	85	172	87	NS	NS	NS
CYS	12	11	14	11	NS	NS	NS
MET	10	27	13	16	NS	NS	NS
ILE	59	40	37	30	*	NS	NS
LEU	67	92	36	75	NS	NS	NS
PHE	10	15	20	2	NS	NS	NS
LYS	40	12	27	32	NS	NS	NS
Organic acid							
OX	64	50	50	64	NS	NS	NS
CIT	224	134	378	335	*	NS	NS
MAL	335	926	1552	2017	**	NS	*
SUC	52	78	40	34	*	NS	NS
FUM	19	29	18	32	NS	NS	*
Sugar							
SUCR	31	32	24	61	NS	NS	NS
FRUC	111	84	218	158	NS	NS	NS
GLUC	101	100	205	163	**	NS	NS

“’* ’**Nonsignificant or significant at $P \leq 0.05, 0.01$, respectively.

epidermis to xylem vessels is symplastic (Peterson, 1988). Cycling of nutrients occurs from shoot to root and from root to shoot via the phloem and xylem, respectively (Cooper and Clarkson, 1989; van Bel, 1990). Cooper and Clarkson (1989) showed that >60% of the amino-N in xylem fluid of *Triticum aestivum* L. represented recycled N. They proposed that a single regulatory pool of amino-N common to both roots and shoots controls N uptake at the whole plant level. The cytoplasmic concentration of nutrients is also well-buffered by the release–sequestration of nutrients (i.e., K+, NO_3^-, Mg^{2+}, amino acids) in vacuoles (Bhat, 1982; Clement et al., 1978; Huber–Wachli and Wiemken, 1979; Letey et al., 1982).

Reductions in g_l or E greatly predominated over changes in xylem chemistry when irrigation was withheld. Given that mass flow of water in xylem vessels can be approximated by measurements of transpiration (Steinberg et al., 1990), for similarly sized plants it follows that the 10-fold reduction in g_l or E would correspond to an approximate 10-fold reduction in volume flux to the leaves of water-stressed plants via xylem vessels. Regulation of stomatal aperture during water stress facilitates the maintenance of leaf turgor and reduces water and solute flux in xylem vessels. We propose that the reduction in volume flux in xylem vessels may ameliorate the potential adverse effects of altered chemical profiles of xylem fluid on foliar tissue.

The greatest change in chemical composition in response to water stress occurred in the concentration of organic acids, whereas the amino acids were more stable. Concentrations of organic acids increase in foliar tissue as a consequence of water deficit (Osmond, 1978; Timpa et al., 1986; Venekamp and Koot, 1988). Organic acids accumulate during nitrate reduction (Blevins et al., 1974; Kirkby and Knight, 1977; Wallace et al., 1971) and facilitate ionic balance during excess cation uptake (Triplett et al., 1980; Wallace et al., 1971).

The concentration of free amino acids (particularly proline) often increases markedly in leaves or other plant tissue with exposure to many biotic and abiotic stresses (Boggess et al., 1976; Good and Zaplachinski, 1994; Handa et al., 1986; Naidu et al., 1990; Rabe, 1990; Rainieri et al., 1989; Stewart, 1981; van Rensburg et al., 1993; Voetberg and Sharp, 1991). Proline may represent 1% of leaf dry matter in many plant species subjected to water stress. For example, 68-and 100-fold increases in proline, were recorded in drought-stressed leaves *of Brassica napus* (Good and Zaplachinski, 1994) and roots of *Zea mays* subjected to a water deficit (Voetberg and Sharp, 1991). However, the contribution of proline derived from xylem fluid has seldom been considered. In our study, the concentration of proline in xylem fluid represented a very small fraction of the amino acid profile and did not vary

significantly as a function of water or fertilization.

Organic acids may be the principal C source for proline accumulation in drought-stressed plants. Venekamp and Koot (1988) calculated that most of the proline accumulating with drought stress in *Vicia faba* was via de novo synthesis from organic acids, and not from the hydrolysis of proteins or from preexisting amino acids. Venekamp et al. (1989) demonstrated that organic acids (citric and malic acids, etc.) served as the C source in the synthesis of proline via glutamate dehydrogenase. The synthesis of proline from organic acids consumes H^+ and is an efficient mechanism to control cytosolic pH (Venekamp et al., 1989). The importance of the increased concentration of organic acids in xylem fluid occurring in response to water stress to proline synthesis in foliar tissue has not been determined.

The concentration of organic N in *L. indica* varied with irrigation and nutrient solution. Arginine, the amino acid with the highest N to C ratio, increased 6-fold in xylem fluid of plants in the nutrient solution–water-stress treatment. Rabe (1990) proposed that any stress that results in glucose depletion or reduced growth results in increases in N-containing compounds and that the primary role for the accumulation of N-containing compounds reported for numerous environmental stresses (mineral, drought, salinity, temperature, anoxia, pH) is NH_4^+ detoxification. Andersen et al. (1989) did not detect NH_4^+ in xylem fluid of *L. indica* (limits of detection= 6 μM) planted in the container medium described in materials and methods, indicating that virtually all NH_4^+ was converted to amino compounds before loading in xylem vessels. Application of nutrient solution to *L. indica* was associated with increased water stress as evidenced by increased xylem tension and reduced g_l and E. These data are consistent with the hypothesis that *L. indica* was experiencing nutrient toxicity. Thus, increased arginine synthesis may have resulted in the detoxification of NH_4^+.

In conclusion, the chemistry of xylem fluid was well-buffered against changes in water or nutrient content of the rhizosphere. Reductions in solute transport via xylem vessels exceeded changes in the chemical profile with water stress or the application of nutrient solution. Water stress imposed over a 5-day period induced marked reductions in g_l and E of both species; however, the influence of the nutrient solution was species dependent. Withholding irrigation and/or the application of nutrient solution (i.e., osmolarity = 138 mM) to *P. salicina* did not significantly alter the total osmolarity of xylem fluid. Water stress induced marked changes in the concentration of malic and citric acid in xylem fluid, yet had a relatively minor influence on the concentration of most amino acids. Irrigation and nutrient status influenced plant water relations and the chemical profile of xylem fluid of *L. indica.* The concentration of malic acid, citric acid, and arginine increased most markedly as a function of water stress, or by the application of nutrient solution. Overall, a substantial degree of regulation of xylem fluid chemistry occurred in response to large changes in soil water or nutrient status.

Literature Cited

Andersen, P.C. and B.V. Brodbeck. 1989a. Diurnal and temporal the chemical profile of xylem exudate from *Vitis rotundifolia*. Physiol. Plant. 75:63–70.

Andersen, P.C. and B.V. Brodbeck. 1989b. Chemical composition of xylem exudate from bleeding spurs of *Vitis rotundifolia* Noble and *Vitis* hybrid Suwannee in relation to pruning date. Amer. J. Enol. Viticult. 40:155-160.

Andersen, P.C. and B.V. Brodbeck. 1989c. Chemical composition of xylem exudate from bleeding spurs of *Vitis rotundifolia* Noble and *Vitis* hybrid Suwannee in relation to pruning date. Amer. J. Enol. Viticult. 40:155-160.

Andersen, P.C. and B.V. Brodbeck. 1991. Influence of fertilization on xylem fluid chemistry of *Vitis rotundifolia* Noble and *Vitis* Hybrid Suwannee. Amer. J. Enol. Viticult. 42:245–251.

Andersen, P.C., B.V. Brodbeck, and R.F. Mizell, 111.1989. Metabolism of amino acids, organic acids and sugars extracted from the xylem fluid of four host plants by adult *Homalodisca coagulata*. Entomol. Expt. Applied 50:149–159.

Andersen, P.C., B.V. Brodbeck, and R.F. Mizell, III.1992. Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. J. Insect Physiol. 38:611-622.

Andersen, P.C., B.V. Brodbeck, and R.F. Mizell, III. 1993. Diurnal variations of amino acids and organic acids in xylem fluid from *Lagerstroemia indica:* An endogenous circadian rhythm. Physiol. Plant. 89:783–790.

Berger, A., R. Oren, and E–D Schulze. 1994. Element concentrations in the xylem sap of *Picea abies* (L.) Karst seedlings by various methods under different environmental conditions. Tree Physiol. 14:111–128.

Bhat, K.K.S. 1982. Nutrient inflows into apple roots. II. Nitrate uptake rates measured on intact roots of mature trees under field conditions. Plant Cell Environ. 5:461-469.

Blevins, D., A.J. Hiatt, and R.H. Lowe. 1974. The influence of nitrate and chloride uptake on expressed sap pH, organic acid synthesis, and potassium accumulation in higher plants. Plant Physiol. 54:82–87.

Boggess, S.F., C.R. Stewart, D.Aspinall, and L.G. Paleg. 1976. Effect of water stress on proline synthesis from radioactive precursors. Plant Physiol. 58:398-404.

Clark, C.J., P.T. Holland, and G.S. Smith. 1986. Chemical composition of bleeding xylem sap from kiwifruit vines. Ann. Bet. 58:353–362.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annu. Rev. Plant Physiol. 36:77–115.

Clement, C. R., M.J. Hopper, and L.H.P. Jones. 1978. The uptake of nitrate by *Lolium perenne* from flowing culture solution. I. Effect of NO_3^- concentration. J. Expt. Bet. 29:453-464.

Cooper, H.D. and D.T. Clarkson. 1989. Cycling of amino-nitrogen and other nutrients between shoots and roots in cereals—A possible mechanism integrating shoot and root in the regulation of nutrient uptake. J. Expt. Bet. 40:753–762.

deBoer, A.H., H.B.A. Prins, and P.E. Zanstra. 1983. Biphasic composition of trans-root potential in roots of *Plantago* species: Involvement of spatially separated electrogenic pumps. Planta 157:259–266.

Good, A.G. and S.T. Zaplachinski. 1994. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol. Plant. 90:9–14.

Handa, S., A.K. Handa, P.M. Hasegawa, and R.A. Bressan. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol. 80:938–945.

Huber–Wachli, V. and A. Wiemken. 1979. Differential extraction of soluble pools from the cytosol and the vaculoes of yeast *(Candida utilis)* using DEAE-dextran. Arch. Microbiol. 120:141–149.

Kirkby, E.A. and A.H. Knight. 1977. Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation, and cation–anion balance in whole tomato plants. Plant Physiol. 60:349–353.

Letey, J., W.M. Jarrell, and N. Valoras. 1982. Nitrogen and water uptake patterns and growth of plants at various minimum solution nitrate concentrations. J. Plant Nutr. 5:73–89.

Naidu, B. P., L.G. Paleg, D. Aspinall, A.C. Jennings, and G.P. Jones. 1990. Rate of imposition of water stress alters the accumulation of nitrogen-containing solutes by wheat seedlings. Austral. J. Plant Physiol. 17:653–664.

Osmond, C.B. 1978. Crassulacean acid metabolism: A curiosity in context. Annu. Rev. Plant Physiol. 29:379-414.

Perumalla, C.J. 1986. Studies on the hypodermic of roots and rhizomes of various angiosperm species. PhD diss. Univ. of Waterloo, Waterloo, Ontario, Canada, Univ., Microfilms Internat., Ann Arbor, Mich.

Peterson, C.A. 1988. Exodermal Casparian bands: Their significance for ion uptake by roots. Physiol. Plant. 72:204-208.

Rabe, E. 1990. Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. J. Hort. Sci. 65:231–243.

Rainieri, A., R. Bernardi, P. Lanese, and G.F. Soldatini. 1989. Changes in free amino acid content and protein pattern of maize seedlings under water stress. Environ. Expt. Bet. 29:351–357.

Roubelakis–Angelakis, K.A. and W.M. Kliewer. 1979. The composition of bleeding sap from Thompson Seedless grapevines as affected by nitrogen fertilization. Amer. J. Enol. Viticult. 30:14-18.

Scholander, P.F., H.T. Hammel, E.D. Bradstreet, and E.A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339–346.

Steinberg, S. L., M.J. McFarland, and J.W. Worthington. 1990. Comparison of trunk and branch sap flow with canopy transpiration in pecan. J. Expt. Bet. 41:653–659.

Stewart, C.R. 1981. Proline accumulation: biochemical aspects, p. 243–249. In: L.G. Paleg and D. Aspinall (eds.). The physiology and biochemistry of drought resistance in plants. Academic Press, New York.

Timpa, J.D., J.J. Burke, J.E. Quisenberry, and C.W. Wendt. 1986. Effects of water stress on the organic acid and carbohydrate compositions of cotton plants. Plant Physiol. 82:724-728.

Triplett, E.W., N.M. Barnett, and D.G. Blevins. 1980. Organic acids and ionic balance in xylem exudate of wheat during nitrate or sulfate absorption. Plant Physiol. 65:610-613.

van Bel, A.J.E. 1990. Xylem–phloem exchange via the rays: The undervalued route of transport. J. Expt. Bet. 227:631-644.

van Rensburg, L., G.H.J. Kruger, and H. Kruger. 1993. Proline accumulation as drought-tolerance selection criterion: Its relationship to membrane integrity and chloroplast ultrastructure in *Nicotiana tabacum* L. J. Plant Physiol. 141:188–194.

Venekamp, J.H. and J.T.M. Koot. 1988. The sources of free proline and asparagine in field bean plants, *Vicia faba* L., during and after a short period of water withholding. J. Plant Physiol. 132:102–109.

Venekamp, J. H., J.E.M. Lampe, and J.T.M. Koot. 1989. Organic acids as sources for drought-induced proline synthesis in field bean plants, *Vicia faba* L.J. Plant Physiol. 133:654-659.

Voetberg, G.S. and R.E. Sharp. 1991. Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol. 96:1125–1130.

Wallace, A., A.M. Abou–Zamzam, and E. Motoyama. 1971. Cation and anion balance in the xylem exudate of tobacco roots. Plant and Soil 35:433-438.