# Effects of salinity on the establishment and early growth of *Prosopis argentina* and *Prosopis alpataco* seedlings in two contrasting soils: Implications for their ecological success

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Abstract In arid zones, water availability is the most important factor limiting seedling establishment and plant distribution. However, within a region with a defined water regime, the physical and chemical properties of the soil could be the cause of the spatial pattern of plant communities. Prosopis argentina Burk. and Prosopis alpataco Phil. are sympatric at a regional scale but at the local scale they occupy clearly differentiated edaphic niches. Prosopis argentina reaches its ecological optimum in the sandy soils of active dunes, whereas P. alpataco achieves it in heavy, clayish, saline and periodically flooded soils. We studied the effect of salinity, and its interaction with soil type, on the establishment and early growth of these species in order to evaluate their adaptive mechanisms, and to analyse how this was related to the ecological success of these species. Salinity affected emergence and early growth of *P. argentina* and *P. alpataco* seedlings differently. Higher salinity led to decreased height, total biomass and shoot and root biomass of plants in both species but the effect was stronger in *P. argentina* than in *P. alpataco*, and greater in clayish than in sandy soils. These results would indicate that exclusion of *P. argentina* from clayish and saline soils would occur during emergence and the first stages of seedling establishment as a consequence of salinity. In P. alpataco other edaphic limitations, like texture or fertility in sandy soils, appear to be always more important. The osmotic effect evidenced by decreasing water and osmotic potentials of plants under saline conditions may be associated with growth reduction in both *P. argentina* and *P. alpataco*. However, this factor cannot explain differences in growth between species under salinity. The higher contents of  $Ca^{++}$  and  $K^{+}$ , and the lower contents of Na<sup>+</sup> in *P. alpataco* suggest that the greater tolerance of this species depends on its ability to regulate and control absorption and transport of ions.

Key words: ion content, Monte desert, osmotic adjustment, Prosopis, salinity, soil texture, water relation.

## INTRODUCTION

In arid zones, water availability is the most important factor limiting seedling establishment and plant distribution. However, within a region with a defined water regime, the physical and chemical properties of the soil could be the cause of the spatial pattern of plant communities through modification of the water regime (Noy-Meir 1973) and the different amount, quality and spatial and temporal distribution of nutrients and salts (Van Auken & Bush 1989; Jarrel & Virginia 1990). Moreover, texture can act as a mechanical factor that controls root penetration (Miller 1986; Brar & Palazzo 1995). Therefore occupation of different edaphic environments by plants implies the presence of adaptations that allow species to reproduce and grow in the particular conditions of each soil type. Among physiological processes, germination and establishment constitute the most critical periods in the life cycle of desert plants (Solbrig & Cantino 1975; Rathcke & Lacey 1985; Sosebee & Wan 1987). Consequently, the presence of adaptations in plants during these stages can determine their natural distribution.

Soluble salts affect seedling establishment and growth by osmotic effect or by toxicity of certain ions present in the soil solution (Greenway & Munns 1980). Salts can produce decreased water uptake, disturbances in metabolism and nutrient intake and reduced growth (Parman & Moore 1966; Mayer & Poljakoff-Mayber 1982; Volkmar *et al.* 1998). The relative importance of these effects depends not only on the level of salinity but also on the time that plants are exposed to salinity (Munns 2002). Besides, the effect of salinity can be modified by soil characteristics, especially texture, because ion distribution

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within the profile changes as soil changes (Noy-Meir 1973). Some mechanisms like osmotic potential regulation, control of stomatal opening, regulation of ion uptake and transport, and induction of anatomical changes in roots and stems are considered to be very important in plant adaptations to salinity stress (Levitt 1980; Zhao & Harris 1992; Catalán et al. 1994; Maun 1994; Serrano 1996; Serrato Valenti et al. 1996; Volkmar et al. 1998; Munns 2002). A greater knowledge of the effects of interactions between salinity and other edaphic factors, like texture, on the establishment and growth of desert species would contribute to the understanding of the adaptive mechanisms and niche differentiation of these species as well as the consequences upon the spatial pattern of plant communities.

The expansion over evolutionary time of species in the genus *Prosopis* (Fabaceae, Mimosoideae) from the Argentinean-Paraguayan Chaco to areas under more adverse conditions towards the south and west may have involved several adaptive processes, such as the change from tree to shrub form, leaf size reduction, increased thorniness and several physiological mechanisms (Burkart 1976; Burkart & Simpson 1977; Roig 1993).

In this genus, tree species show different levels of salt tolerance (Felker et al. 1981; Jarrell & Virginia 1984, 1990; Khan et al. 1987; Ansari et al. 1991; Catalán et al. 1994). Some of them such as Prosopis flexuosa (Catalán et al. 1994), Prosopis articulata, Prosopis tamarugo (Felker et al. 1981) and Prosopis farcta (Bazzaz 1973) are regarded as highly tolerant to salinity, at the same level as some halophytic species like Chenopodium spp., Atriplex spp. and Suaeda spp. Some authors found higher tolerance in Prosopis plants originated from seeds collected in more highly saline zones (Bazzaz 1973; López Villagra & Galera 1992). In contrast, others suggested no relation between salt tolerance and the origin of seeds (Dafni & Negbi 1978). The study of the tolerance of *Prosopis* species to salinity is considered important for both theoretical and applied considerations (Velarde et al. 2003).

*Prosopis argentina* Burk. and *P. alpataco* Phil. (Fabaceae, Mimosoideae) are good examples of species adapted to extreme and contrasting edaphic conditions, and could represent adaptive extremes in the evolution of the genus. At the regional scale, both species are sympatric within the Monte desert, located in the arid zone of western Argentina (average annual rainfall <350 mm). However, at the community or local scale, these species occupy clearly differentiated edaphic niches. *Prosopis argentina* reaches its ecological optimum, with the highest densities and the largest individuals, in the loose sandy soils of active dunes; whereas *P. alpataco* achieves its ecological optimum in heavy, clayish, saline and periodically flooded soils. No communities have been found with both species coex-

isting (Villagra & Roig 2002). In the area where these species are sympatric the climate is extremely arid. Mean annual rainfall ranges between 80 and 200 mm and mean annual temperature is between 15°C and 17°C (Villagra & Roig 2002). Aside from texture, the main difference between soils is the higher concentration of salts and nitrogen observed in clayish soils where *P. alpataco* is found. Electrical conductivities over 60 000  $\mu$ S cm<sup>-1</sup> have been recorded in some parts of its distribution. In addition to xeric climatic conditions, both edaphic environments present adverse conditions for the establishment and growth of most plant species, including the mechanical impedance for root growth in clayish soils and plant burial and extremely high surface temperatures in sandy soils (Miller 1986; Danin 1991).

In a previous study (Villagra & Cavagnaro 2000) we found that soil type can explain the exclusion of *P. alpataco* from sandy soils but not the exclusion of *P. alpataco* from clayish soils. The growth of *P. alpataco* strongly decreased in sandy soils as expected, but that of *P. argentina* was higher in clayish than in sandy soils (Villagra & Cavagnaro 2000). This suggested that other factors, such as the interaction of soil type with other physical or biological factors, could limit the distribution of *P. argentina* in clayish soil.

This paper investigates the effect of salinity and its interaction with soil type on the establishment and early growth of *P. argentina* and *P. alpataco*, in order to evaluate the adaptive mechanisms of each species and to analyse their relation with the species ecological success.

### MATERIALS AND METHODS

We performed two experiments to determine the effects of salinity and soil type on the establishment and growth of *P. argentina* and *P. alpataco* seedlings (experiment 1) and on their water relations and ion contents (experiment 2).

### **Experiment 1**

Experiment 1 was conducted in the experimental field of the Regional Center of Scientific and Technological Researches of Mendoza (CRICYT) (32°53'S, 68°57'W) between 4 January and 11 April 1996.

We used *P. argentina* seeds collected from the Telteca Reserve (Lavalle, Mendoza, Argentina) and *P. alpataco* seeds collected from Asunción (Lavalle, Mendoza, Argentina) in January and February of 1994. Equivalent numbers of seeds were collected from 10 plants and mixed in a pool for each species. Seeds were manually separated from their pods and preserved following the methodology proposed by Cony (1993) for other species of the genus. Broken and insect-

Origin	Asunción Clayish soil	Telteca Reserve
Environment	Periodically flooded	Sand dunes
Dominant species	Г. аграгасо	1: argentina
Soil classification	Typic torrifluvent entisol	Typic torripsament entisol
Texture	Clayish	Sandy
Sand (%)	21.31	47.10
International silt (%)	30.18	18.15
American silt (%)	41.60	27.50
Clay (%)	6.43	2.86
Field capacity (% weight)	33.00	8.50
Actual electrical conductivity (µS cm <sup>-1</sup> )	2551-7280	321–382
$Ca^{++} (meq L^{-1})$	29.80-44.40	2-2.50
$Mg^{++}(meq L^{-1})$	4.40-6.80	0.20-0.30
$Na^+ (meq L^{-1})$	13.20-14.50	0.63-1.20
Sodium absorption ratio	2.86-4.20	1.03-1.14
pH	7.02-7.50	8.03-8.15
N ( $\mu g g^{-1}$ )	392-735	84-171
$P(\mu g g^{-1})$	5.53-8.36	6.01-7.96
K ( $\mu$ g g <sup>-1</sup> )	236-626	230-782
Organic matter (%)	0.52–1.25	0.16-0.39

**Table 1.** Physical and chemical properties of sandy and clayish soils where *Prosopis argentina* and *Prosopis alpataco* reach their greater development (Lavalle, Mendoza, Argentina)

Values are minima and maxima for all four samples except for the texture analysis where a pool from the four samples was used.

damaged seeds were discarded. Seeds were scarified with sandpaper to allow water absorption, and then disinfected by immersion in 70% ethanol for 7 min, followed by 7 min in commercial hypochloride (60 g  $L^{-1}$  active Cl) diluted to 15% (Villagra 1995). Finally they were washed repeatedly with distilled water.

The two types of soil for the experiment were obtained from the sites where the seeds were collected: sandy soil (classified as Typic Torripsament Entisols, Moscatelli 1990) from dunes in the Telteca Reserve, where the dominant species is *P. argentina*, and clayish soil (classified as Typic Torripfluvent Entisols, Moscatelli 1990) from the locality of Asunción where *P. alpataco* is the dominant species. Table 1 shows the physical and chemical characteristics of these soils obtained from composite samples taken at four different sites within the area where seeds and soil were collected. At each site we took several samples from the first 0.25-m depth. Soil texture was determined by granulometry in a pool including all four samples. The pH was determined in a 1:1 suspension of soil in water using an Orion 501 digital pH meter (Orion Research Inc., Cambridge, MA, USA). Electrical conductivity (EC) was determined on a saturation extract. The sodium absorption ratio (SAR) was estimated using Na<sup>+</sup> concentration (estimated by flame photometry) and Mg++ and Ca++ concentrations (determined by EDTA tritation) (Jackson 1976). Organic matter was estimated by oxidization with

dichromate in presence of  $H_2SO_4$ . Total nitrogen was determined using the microKjeldahl digestion technique. Potassium was determined with Pratt's method, and phosphorous with Jackson's technique (Jackson 1976).

Pots of 10 cm in diameter and 85 cm in depth, with six 5-mm holes at the bottom for drainage, were filled with 9 L of air-dried, sieved (5-mm mesh) soil (14.5 kg for sandy soil and 11.5 kg for clayish soil), and distributed under a 100- $\mu$ m-thick yellow polyethylene rain shelter. Light level was 70–85% of the outside light intensity. Two seeds were planted in each pot and, once seedlings became established, pots were thinned to one plant per pot.

A completely randomized experimental design with three fixed factors was followed. Factors were species (P. argentina and P. alpataco), type of soil (clayish and sandy) and type of irrigation solution (tap water, 0.1 M NaCl and 0.2 M NaCl). For each of the 12 treatment combinations, we used seven replicates of 12 plants each, that is, a total of 84 plants per treatment and 1008 plants in all. As logistical constraints prevented us from measuring all variables in all plants, each variable was measured in only some (and different) plants in each replicate. We used the mean value of these measurements as the single entry representing the replicate (n = 7 in all cases). Each replicate of 12 plants was randomly distributed in the experimental area. To avoid edge effects in the pots located on the outer edge of the design a complete line of pots were used as border plants that were not included in measurements.

Pots were watered twice a week to maintain the water content near field capacity with a volume enough to ensure drainage at each irrigation time. The EC of the irrigation solution was between 800 and 900  $\mu$ S cm<sup>-1</sup> in tap water, 8800  $\mu$ S cm<sup>-1</sup> in the 0.1 M NaCl solution and 17 700 µS cm<sup>-1</sup> in the 0.2 M NaCl solution. Salt solutions were prepared on every irrigation date. To evaluate the degree of salinization for different soils, we determined EC on a saturation extract in two pots of each treatment, randomly selected, at the start and at the end of the experiment. Electrical conductivity at the end of the experiment in clayish soils was two to three times higher than in irrigation solutions, whereas in sandy soils EC was lower than in irrigation solutions (Table 2). This situation in sandy soils can be explained by the method used to determine EC. In this method, each soil sample is hydrated to the saturation point with distilled water. The soil solution becomes diluted by distilled water in the saturation paste. As sandy soils have low water retention capacity and practically no accumulation of ions because of the absence of colloidal particles, this dilution is approximately two times greater in sandy soils than in clayish soils with the consequent lower value in EC measurements (Richards 1954).

Seedling emergence, defined as the development of the first true leaf, was recorded daily in every pot throughout the first 10 days, and expressed as the percentage of seeds sown in each of the seven replicates. Seedling emergence rate was estimated through the Maguire index (Naylor 1981).

$$M = n1/t1 + n2/t2 + \dots + n7/t7$$

 $n1, n2, \ldots, n7$  being the number of seedlings emerged at times  $t1, t2, \ldots, t7$  (time in days).

Seedling height was measured periodically (every 6– 15 days) in six randomly selected plants per replicate. At the end of the experiment (100 days after sowing) leaf, stem and root dry matter, the number of leaves and leaf area were registered in one randomly selected plant for each of the seven replicates. In order to measure root biomass each pot was divided into four 20-cm-long cylinders from which roots were obtained by washing. Root and shoot dry weights were recorded after oven drying at  $60^{\circ}$ C for 15 days. Leaf area was determined with a leaf area meter LI-COR model A3000 (LI-COR Inc., Lincoln, NB, USA), and the specific leaf area was estimated as the ratio between foliar area and leaf biomass.

To compare the effect of salinity on both species and to evaluate their salt tolerance, we calculated 'proportional growth' as the ratio between the biomass reached in each saline treatment and that of the control for all species—soil combinations.

Data were subjected to a three-way analysis of variance (ANOVA) with species, soils and salinity as factors. Tukey's test was used for comparison of means (Zar 1984). We analysed variance homogeneity using the Levene Test. As some data did not meet the ANOVA assumptions, they were transformed before analysis according to the following criteria:  $\arcsin \sqrt{x}$  transformation was applied to emergence percentages, and  $\log(x + 1)$  transformation was applied to seedling height and root biomass (Zar 1984). Mean differences were considered to be significant at P < 0.05.

## **Experiment 2**

Experiment 2 was performed in the experimental field of CRICYT, Mendoza (32°53'S, 68°57'W) between 9 October 1996 and 1 April 1997.

One-year-old plants of *P. argentina* and *P. alpataco*, originated from seeds obtained at the same places and cultivated in the same way as in experiment 1, were used. They were periodically irrigated with tap water until the experiment started.

A completely randomized experimental design with three factors was followed. Factors were species (*P. argentina* and *P. alpataco*), type of soil (clayish and sandy) and type of irrigation solution (water and 0.2 M NaCl). Every treatment had five replicates with five plants per replicate, that is, a total of 25 plants per treatment and 200 plants in all. Plants were irrigated in the same way as in experiment 1.

Plant water potential ( $\psi_a$ ) was measured in a small branch with a pressure chamber. Predawn and midday measurements of one plant per replicate were taken on 13 February, 20 February and 6 March 1997, in all cases 3 days after irrigation. We used a different

**Table 2.** Electrical conductivity ( $\mu$ S cm<sup>-1</sup>) of the irrigation solution and the soil at the beginning and on day 100 of the assay

	Beginning of assay	Water	100 days 0.1 M NaCl	0.2 M NaCl
Irrigation solution		800–900	8800	17 700
Sandy soil	321-382	405-710	3430-3830	4970-6250
Clayish soil	1281-6610	1895–4680	15 880–19 110	40 800–57 900

Values represent maxima and minima.

plant for each measurement. Leaves from the same plants were sampled to measure leaf osmotic potential  $(\psi_0)$ . These leaves were maintained at  $-18^{\circ}$ C in 2-mL Eppendorf tubes until  $\psi_0$  measurements were performed. Osmotic potential was determined, after thawing material at room temperature, using a Wescor psychrometer Mod. HR-33T (Wescor Inc., Logan, UT, USA; Turner 1981). Turgor pressure  $(\psi_t)$  was calculated with the equation  $\psi_t = \psi_a - \psi_0$ .

Chemical analyses were measured for obtaining Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>++</sup> concentration in leaves. Sodium and potassium were determined by flame photometry, and calcium by complexometry (Association of Official Analytical Chemists 1970). Attributed to the small amount of material we used a single compound sample from 10 plants per treatment, so statistical analyses could not be performed.

Data on water relations were subjected to a threeway analysis of variance for repeated measures (ANOVA) with species, soils and salinity as factors. Tukey's test was used for comparing means (Zar 1984). Mean differences were considered to be significant at P < 0.05.

### Emergence percentages Emergence rate 80 Emergence percetages 60 : (Magu 3 40 2 20 0 0.0 0.1 0.2 0.0 0.1 0.2 NaCl concentration (M) NaCl concentration (M)

**Fig. 1.** Effect of salinity on the percentage and rate (Maguire index) of emergence of *Prosopis argentina* and *Prosopis alpataco* in clayish and sandy soils. Different letters indicate significant differences at P < 0.05 among all data for each variable. ( $-\Phi$ -) *Prosopis argentina*-clayish; ( $-\bigcirc$ -) *P. argentina*-sandy; ( $-\nabla$ -) *P. alpataco*-clayish; ( $-\bigtriangledown$ -) *P. alpataco*-sandy.

### RESULTS

### **Experiment 1**

Salinity negatively affected both the final percentage and the rate of seedling emergence in *P. argentina* and *P. alpataco* (Fig. 1). This effect was stronger in clayish than in sandy soils, and greater on *P. argentina* than on *P. alpataco*. In sandy soils, salinity only affected the emergence rate, expressed as Maguire index, but not the final percentage of emergence in either species; in clayish soils, instead, these two parameters were affected.

Salinity also influenced the growth of *P. argentina* and *P. alpataco* seedlings during the first 100 days of their lives. It decreased plant height (Fig. 2, Table 3) and above-ground and root biomass (Table 4). Plant



**Fig. 2.** Effect of salinity on plant height of *Prosopis argentina* and *Prosopis alpataco* in different soil types. (a) *P. argentina*. (b) *P. alpataco*. Different letters among treatment combinations and species indicate significant differences at P < 0.05. (...O...) Sandy-water; (- $\Phi$ -) clayishwater; (... $\Box$ -..) sandy-0.1 M NaCl; (... $\Delta$ -..) sandy-0.2 M NaCl; (- $\Phi$ -) clayish-0.2 M NaCl.

**Table 3.** Results from the analysis of variance performed on final emergence percentage, emergence rate, seedling height on day 36 and final height

		Emergence		Emergence rate		Height on day 36		Final height	
Effects	d.f.	F	P	F	P	F	P	F	P
Species (A)	1	32.42	< 0.001	94.14	< 0.001	4.80	0.030	11.64	0.001
Type of soil (B)	1	67.79	< 0.001	95.04	< 0.001	69.48	< 0.001	854.21	< 0.001
Irrigation solution (C)	2	24.59	< 0.001	53.23	< 0.001	93.34	< 0.001	74.92	< 0.001
A×B	1	3.47	< 0.001	6.04	0.016	59.90	< 0.001	79.90	< 0.001
$A \times C$	2	1.23	0.296	0.67	0.51	0.32	0.720	6.87	0.002
$B \times C$	2	12.64	0.001	10.57	0.001	29.23	< 0.001	2.72	0.073
$A \times B \times C$	2	1.23	0.299	1.53	0.220	0.69	0.500	0.08	0.920
Error		d.f. MS =	= 72; 0.0320	d.f. MS =	= 72; 0.0097	d.f. MS = 2	= 69; 238.9000	d.f. = MS =	= 68; 0.0040

Table 4. Sali	nity effects (	on the biomass (mg) of .	Prosopis argentina and	d Prosopis al	<i>pataco</i> seedlings groups back	owing in two	types of soil			
			Leaves		Stems		Root		Total	
Species	Soil	Irrigation solution	Biomass (mg)	PG	Biomass (mg)	PG	Biomass (mg)	PG	Biomass (mg)	PG
P. argentina	Sand	0 M NaCl	47.8	1.00	177.9	1.00	239.5	1.00	465.2	1.00
	Sand	0.1 M NaCl	30.4	0.63	96.4	0.54	143.0	0.59	269.9	0.58
	Sand	0.2 M NaCl	22.9	0.47	66.1	0.37	103.4	0.43	192.4	0.41
P. argentina	Clay	0 M NaCl	297.4	1.00	1120.5	1.00	578.1	1.00	1996.0	1.00
I	Clay	0.1 M NaCl	98.6	0.33	334.7	0.29	168.5	0.29	601.1	0.30
	Clay	$0.2 \text{ M } \text{NaCl}^{\dagger}$	I	I	I	I	I	I	I	I
P. alpataco	Sand	0 M NaCl	28.8	1.00	61.9	1.00	153.8	1.00	244.5	1.00
I	Sand	0.1 M NaCl	27.4	0.95	58.6	0.94	103.7	0.67	174.9	0.71
	Sand	0.2 M NaCl	13.4	0.47	32.6	0.52	76.4	0.50	122.3	0.50
P. alpataco	Clay	0 M NaCl	1152.9	1.00	2795.3	1.00	1675.2	1.00	5623.4	1.00
	Clay	0.1 M NaCl	686.0	0.60	1207.5	0.43	1048.1	0.63	2941.6	0.52
	Clay	0.2 M NaCl	381.4	0.33	566.4	0.20	348.9	0.20	1296.7	0.23
ANOVA results		d.f.	F	Ρ	F	Ρ	F	Ρ	F	Р
Species-soil	interaction	1	79.4	<0.001	88.50	<0.001	47.30	<0.001	82.90	<0.001
Species-salir	nity interacti	on 2	2.75	0.070	3.96	0.023	3.85	0.030	3.16	0.050
Soil-salinity	interaction	2	4.17	0.020	7.20	0.002	1.88	0.160	3.71	0.030
Species-soil-	-salinity inte	traction 2	0.71	0.490	1.53	0.22	2.88	0.060	2.56	0.080
Error			d.f. = 63	3;	d.f. = 6	4;	d.f. = 6	3;	d.f. = 6	4;
			MS = 16.0	050	MS = 0.1	027	MS = 0.3	230	MS = 0.0	028
<sup>†</sup> This treatm	ent was not	included because surviv	orship was very low	(four plant	s).					
PG, proport	ional growth	the ratio between the	biomass reached in e	s are cuscuss each saline 1	treatment and the r	espective con	itrol).			

Species	Soil	Irrigation sol	ution	Shoot-ro	ot ratio	Leaf a	rea (cm <sup>2</sup> )	Number of	of leaves	Specific le	eaf area
P. argentina	Sand	0 M NaCl		0.9	3		5.79	6.2	29	0.1	5
	Sand	0.1 M NaC	21	1.0	5		3.74	5.1	17	0.1	2
	Sand	0.2 M NaC	21	0.9	1		2.45	4.4	57	0.1	1
P. argentina	Clay	0 M NaCl		2.8	6	3	34.28	14.7	71	0.1	2
-	Clay	0.1 M NaC	21	2.8	5	1	1.55	8.0	57	0.1	2
	Clay	0.2 M NaC	C1 <sup>†</sup>	_			_	_		_	
P. alpataco	Sand	0 M NaCl		0.6	6		4.40	12.0	00	0.1	6
-	Sand	0.1 M NaC	21	0.8	3		4.73	9.5	57	0.1	6
	Sand	0.2 M NaC	21	0.7	1		1.92	6.33		0.1	4
P. alpataco	Clay	0 M NaCl		2.4	5	16	5.30	64.1	4	0.1	4
•	Clay	0.1 M NaC	21	1.9	4	8	39.10	41.8	35	0.1	3
	Clay	0.2 M NaC	21	3.3	0	4	3.10	30.85		0.13	
ANOVA resul	ts		d.f.	F	Р	F	Р	F	Р	F	Р
Species-sc	oil interact	tion	1	0.22	0.64	110	< 0.001	61	< 0.001	0.56	0.46
Species-salinity interaction		raction	2	0.90	0.38	2.5	0.090	1.50	0.240	0.06	0.94
Soil-salinity interaction		2	0.70	0.47	2.5	0.002	1.90	0.150	1.02	0.36	
Species-sc	oil–salinity	interaction	2	1.20	0.30	0.5	0.600	0.21	0.810	0.34	0.71
Error	5			d.f. = MS = 0	63; .0100	d.f MS =	£ = 65; = 0.0250	d.f. MS =	= 65; 0.0130	d.f. MS =	= 64; 0.0013

**Table 5.** Shoot-root ratio, leaf area (cm<sup>2</sup>), number of leaves and specific leaf area of *Prosopis argentina* and *Prosopis alpataco* seedlings growing in two types of soil

<sup>†</sup>This treatment was not included because survivorship was very low (four plants).

Measurements were taken 100 days after sowing. Mean comparisons are discussed in the text.

height of each species responded in a different way to soil type and salt concentration, showing significant species-soil (F = 79.9; P < 0.001) and species-salinity interactions (F = 6.87; P = 0.002) (Fig. 2, Table 3). Comparing both species, *P. argentina* showed greater height than *P. alpataco* in sand for all salt concentrations. In contrast, in clayish soil *P. alpataco* was taller than *P. argentina*, this difference increasing as salt concentration rose.

The effect of soil-salinity interactions on seedling height changed over the 100 days of the experiment: it was significant until day 36 (F = 29.2; P < 0.001) but not on day 100 (F = 2.72; P = 0.07) (Table 3).

Aerial and root biomass of both species were affected by salt concentration and soil type (Table 4). Speciessalinity interactions were significant for total biomass, and for stem and root biomass, indicating that the detrimental effect of salinity was stronger in *P. argentina* than in *P. alpataco*, especially in clayish soil. The proportional growth of *P. argentina* in each soilsalinity combination was always lower than that of *P. alpataco* (Table 4). Diminution of total biomass by salts was greater in clayish than in sandy soils, although biomass was always greater in clay than in sand. The treatment of *P. argentina* irrigated with 0.2 M NaCl in clayish soil was excluded from this analysis because plant survival was very low (four plants).

The only factor affecting the shoot/root ratio was the type of soil (F = 135.5; P < 0.001), with a larger ratio

in clay than in sand for both species (Table 5). No significant differences among salt concentrations or between species were found. None of the interactions among factors was significant.

The biomass percentage allocated to root, stems and leaves was not affected by salinity. But allocation to different organs changed between species: P. argentina put more biomass in root and stems than P. alpataco, whereas the latter partitioned a larger proportion to leaves (Table 4). Salinity diminished leaf number and leaf area of plants (Table 5). In all treatments *P. alpataco* showed higher values in both variables than P. argentina. Leaf area, leaf biomass and the number of leaves were several times greater in clayish than in sandy soils. In addition, leaf area was more affected by salinity in clayish than in sandy soils, though leaf number decreased in the same proportion in both soils. No significant leaf mortality was observed in either of the species. The specific leaf area only differed between species (Table 5).

### **Experiment 2**

Predawn water and osmotic potentials were lower under the salt treatment, but no differences were found between species in either of the two soils (Fig. 3). At midday, lower water potential was observed only in the salt treatment in clayish soil. None of the other parameters showed differences among treatments (Fig. 4).

Prosopis alpataco had lower Na<sup>+</sup> and higher K<sup>+</sup> than *P. argentina* in all treatments (Table 6). In both species Na<sup>+</sup> content increased and K<sup>+</sup> content decreased under saline conditions, although this effect was lower in *P. alpataco*. Calcium content was higher in *P. argentina* than in *P. alpataco* in the control treatment, but lower under salt conditions (Table 6). *Prosopis alpataco* showed an increment in calcium content under salt conditions, whereas in *P. argentina* calcium content decreased (Table 6).



**Fig. 3.** Effect of salinity on predawn water relations in oneyear-old plants of *Prosopis argentina* and *Prosopis alpataco* in different soil types. Different letters among treatments indicate significant differences at P < 0.05. ( $-\Phi$ -) *Prosopis argentina*-water; ( $-\bigcirc$ -) *P. argentina*-0.2 M NaCl; ( $-\Psi$ -) *P. alpataco*-water; ( $-\bigtriangledown$ -) *P. alpataco*-0.2 M NaCl.

### DISCUSSION

In a previous study we observed that both species grew better in clayish than in sandy soils without salinity or water stress (Villagra & Cavagnaro 2000). This suggests that exclusion of *P. argentina* from clayish soil should be related to the interaction of soil type with another factor. Results from this paper indicated that salinity had stronger effects on the emergence and early growth of *P. argentina* than on those of *P. alpataco*. In addition, the effects of salinity increased on clayish soil suggesting that interaction between both factors could



**Fig. 4.** Effect of salinity on midday water relations in oneyear-old plants of *Prosopis argentina* and *Prosopis alpataco* in different soil types. Different letters among treatments indicate significant differences at P < 0.05. ( $-\Phi$ -) *Prosopis argentina*-water; ( $-\Box$ -) *P. argentina*-0.2 M NaCl; ( $-\Psi$ -) *P. alpataco*-water; ( $-\nabla$ -) *P. alpataco*-0.2 M NaCl.

Table 6. Effects of salinity on the ion contents of one-year-old plants of *Prosopis argentina* and *Prosopis alpataco* growing in different types of soil

Species	Soil	Irrigation solution	Na <sup>+</sup> (µg g <sup>-1</sup> dry weight)	$K^+$ (µg g <sup>-1</sup> dry weight)	Ca <sup>++</sup> (µg g <sup>-1</sup> dry weight)
P. argentina	Sand	Water	1 486	15 900	9 964
-	Sand	0.2 M NaCl	4 238	10 100	6 392
P. argentina	Clay	Water	1 564	12 500	10 945
	Clay	0.2 M NaCl	10 490	9 400	5 747
P. alpataco	Sand	Water	748	21 600	6 935
-	Sand	0.2 M NaCl	2 216	19 000	8 084
P. alpataco	Clay	Water	831	19 550	8 170
-	Clay	0.2 M NaCl	5 051	17 900	10 592

be important in the niche differentiation of both species.

Without salinity the type of soil did not affect final seedling emergence (Villagra & Cavagnaro 2000). With the incorporation of salinity as an additional factor, seedling emergence was affected in different ways according to soil type. In clayish soils salinity had stronger adverse effects than in sandy soils. These findings are consistent with the postulations of Noy-Meir (1973) and can be critical for the exclusion of saltsensitive species under saline conditions. There was almost no emergence of *P. argentina* in clayish soil when it was watered with 0.2 M-NaCl solution. The emergence rate was more sensitive to salinity than the final emergence percentage in sandy soils. This suggests that the first effect of salinity was to decrease seedling vigour, which could induce emergence failure when seedlings cannot overcome the mechanical impedance of clayish soil. The higher vigour shown by P. alpataco seeds (Villagra 1997) could be the cause of the weaker effects of salinity on seedling emergence in this species.

In this experiment, salinity affected emergence at lower salt concentrations than it affected germination under laboratory conditions (Villagra 1997). Especially in clayish soil the emergence percentages of P. argentina only reached 17% in 0.1 M NaCl and 3% in 0.2 M NaCl, whereas in the laboratory test, in 0.2 M NaCl it rose to 67–90% germination depending on temperature. This indicates that although seeds can germinate under certain saline concentrations, lower seedling vigour is responsible for emergence failure. However, it is important to note that the final salt concentration in laboratory assays is not really comparable to that obtained in soils. This is because the total amount of salts in soils depends on the proportion of salts directly contributed by soils, on the accumulation of salts attributed to evaporation of water from the soil surface and on the differential capacity for leaching. Thus, EC measurements made at the end of the experiment showed that in clayish soils salt concentration was twice the irrigation solutions, which did not happen in sandy soil (Table 2).

Both soil type and salinity affected the early growth of these species. The difference in growth between soils could be attributed to the differences in nitrogen content, as suggested by the decreased number of leaves, leaf area and leaf biomass observed in sandy soils in both species and in all saline treatments. Several authors have indicated that these responses are typical of plants growing under conditions of nitrogen deficiency (Radin & Boyer 1982; Onillon *et al.* 1995). The effects of soil on plant growth were stronger for *P. alpataco* than *P. argentina*, indicating a higher tolerance of the latter to sandy soil. *Prosopis argentina* shows features typical of plants from low-resource environments, such as leaf reduction, a higher proportion of stem biomass and a deep root system (Villagra & Cavagnaro 2000).

The response of plant growth to salinity differed between the two species, as suggested by the fact that species-salinity interactions were significant for seedling height and biomass. The criterion used to analyse the effects of salinity on each species was to compare growth under saline conditions with growth under control conditions (proportional growth). In this respect, *P. argentina* showed lower proportional growth for most variables as a result of salinity, which indicates that this species was more affected than *P. alpataco*. These results suggest that *P. alpataco* would be more tolerant to salt stress than *P. argentina* during seedling establishment, which would represent an adaptive advantage of *P. alpataco* to environmental conditions at the sites where it naturally grows.

The soil–salinity interaction was significant for plant height on day 36 of the cycle (P < 0.00001) and for final biomass (P < 0.03), that is, the salinity effect was stronger in clayish than in sandy soils. This can be explained by a higher ion exchange capacity, a larger mechanical impedance and the effect of matric potential under water stress, although the matric potential in this experiment was negligible as treatments were maintained near field capacity.

The effects of salinity on *P. argentina* in clayish soil were greater at the beginning of the cycle, suggesting later acclimation of plants (Fig. 2) as observed by Arce and Balboa (1988) for *Prosopis chilensis*. According to these results during initial stages, the growth of *P. argentina* would be limited mainly by salinity, but other factors related to soil type, like nutrients, could later become more important limiting factors. This would indicate that the exclusion of *P. argentina* from clayish and saline soils would occur during seedling emergence, and during the first stages of seedling establishment. In *P. alpataco* other edaphic limitations, like fertility in sandy soils, appear to be always the major limiting factors.

Some authors have observed similar effects of salinity on plant growth, although they reported great variability among species and provenances (Felker *et al.* 1981; Jarrell & Virginia 1984; Arce & Balboa 1988; Catalán *et al.* 1994). The injurious action of salt results from osmotic effects as well as from the toxic effects of certain ions, especially Na<sup>+</sup> and Cl<sup>-</sup>, on plant cells. These two factors are interrelated and they co-occur under saline soil conditions (Greenway & Munns 1980; Levitt 1980; Serrano 1996). The ability of species to tolerate salinity is related to the possibility they can counteract each of these effects.

The osmotic effect shown by reduced water and osmotic potential of plants under saline conditions may be associated with growth decrease in *P. argentina* and *P. alpataco*. In addition, reduction of leaf number and leaf area under salinity is similar to that caused by water stress in the same species (Villagra 1998). Thus, salinity would exert an indirect control on plant growth through an osmotic effect. However, as no differences were found between the water relations of both species under saline conditions, this factor cannot explain growth differences between species under salinity. Moreover, according to Munns (2002), the osmotic effect is important over short time scales (minutes-hours) but over longer time (days or weeks), like in this experiment, the toxicity effect appears to be more important.

The differences between salt-sensitive and salttolerant species are mainly linked to their capacity to avoid the arrival of toxic ions at leaf level (Volkmar et al. 1998; Munns 2002). Regulation of Na<sup>+</sup> contents and ability to maintain high K<sup>+</sup> concentrations in leaves have been proposed as important mechanisms in plant adaptation to salinity, by decreasing the toxic effect of salts (Greenway & Munns 1980; Catalán et al. 1994; Serrano 1996; Volkmar et al. 1998). The higher rate of Na<sup>+</sup> uptake and the lower K<sup>+</sup> concentration observed in *P. argentina* suggest that the lower salinity effect observed in *P. alpataco* could be related to its ability to regulate and control absorption and transport of ions. Similar results were found by Jarrel and Virginia (1990) for *P. glandulosa*, another salt-tolerant species. In addition, P. alpataco showed increased calcium content in leaves whereas *P. argentina* showed a diminution of this cation. Ca<sup>++</sup> reduces the adverse effect of Na<sup>+</sup> by controlling the intake of toxic ions through the selectivity of the cell membrane (Volkmar et al. 1998; Munns 2002). The capacity to increase Ca<sup>++</sup> contents could be one of *P. alpataco*'s adaptive mechanisms to cope with salinity.

The high concentration of Na<sup>+</sup> and the low concentration of Ca<sup>++</sup> and K<sup>+</sup> in the leaves of *P. argentina*, and the fact that this species has shown water stress tolerance (Villagra 1998) suggest that exclusion of this species from saline soils may be caused by the toxic effect of Na<sup>+</sup> and not by the osmotic effects of salt. Zhao and Harris (1992) arrived at a similar conclusion for *P. chilensis*. In contrast, *P. alpataco* appears to be a halophyte that can tolerate both the osmotic and toxic effects of salinity and can grow at an EC over 50 000 µS cm<sup>-1</sup>. These data suggest that an interaction between soil type and salinity constitutes an important factor in niche separation between species of arid lands. The development of mechanisms of tolerance, such as the ability to control ion uptake, appears to be a major feature in the evolution of arid land species allowing them to colonize highly stressful environments.

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