

## **Sapling growth, water status and survival of two native shrubs from the Monte Desert, Mendoza, Argentina, under different preconditioning treatments**

### **Crecimiento, estado hídrico y supervivencia de plantines de dos arbustos nativos del Desierto del Monte, Mendoza, Argentina, sometidos a diferentes tratamientos de acondicionamiento**

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#### **ABSTRACT**

Revegetation in arid zones poses numerous challenges, due particularly to drought, extreme temperatures, high irradiance, and infertile soils, factors which threaten the survival of installed saplings. Nursery techniques to avoid transplant shock focus on manipulating the watering regime so as to favor the acclimation of seedlings to unfavorable field conditions. In order to analyze the suitability of drought preconditioning treatments, analyzed the effects of water stress on sapling growth, water status and survival on two native shrub species of the Monte Desert. It was used a randomized experimental design with one fixed factor, with three levels of water supply: control (T1), moderate stress (T2), and severe stress (T3). Preconditioning treatments had a detrimental effect on the growth of saplings of both species, as it was clearly shown in the reduction of leaf area, sapling height, stem diameter, and biomass. Shoot-to-root ratio decreased significantly under T3 in both species, and they showed highly negative water potentials and low stomatal conductance under this treatment. Sapling survival decreased only under T3. Moderate water stress seems more suitable for preconditioning this species since it reduces growth, leaf area and leaf conductance without seedling mortality and maintaining a good growth rate.

#### **Keywords**

*Capparis atamisquea* • *Larrea cuneifolia* • water potential • stomatal conductance • Monte biogeographic region • preconditioning treatments

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## RESUMEN

La revegetación de zonas áridas presenta numerosos desafíos, debido a las condiciones de sequía, temperaturas extremas, elevada irradiación y baja fertilidad de los suelos. Las técnicas de vivero que evitan el shock del trasplante se enfocan en manipular el riego para favorecer la aclimatación de los plantines a las condiciones desfavorables del campo. Para evaluar la utilidad del precondicionamiento, se analizó los efectos del estrés hídrico en el crecimiento, estado hídrico y sobrevivencia de plantines de dos especies arbustivas nativas del Desierto del Monte. Se utilizó un diseño experimental al azar con un factor fijo, con tres niveles de riego: control (T1), estrés moderado (T2) y estrés severo (T3). Los tratamientos de precondicionamiento tuvieron un efecto negativo en el crecimiento de los plantines, lo que se evidenció por la reducción del área foliar, altura, diámetro del tallo y biomasa. La relación vástago/raíz disminuyó bajo T3 en ambas especies, y presentaron potenciales hídricos y conductancias estomáticas menores a las plantas con mayor disponibilidad hídrica. La supervivencia disminuyó solamente bajo T3. El estrés hídrico moderado parece más adecuado para precondicionar estas especies ya que reduce su crecimiento, área foliar y conductancia foliar sin que haya mortandad de plantines, manteniendo buenas tasas de crecimiento.

### Palabras clave

*Capparis atamisquea* • *Larrea cuneifolia* • potencial hídrico • conductancia estomática • región biogeográfica del Monte • tratamientos de precondicionamiento

## INTRODUCTION

Arid lands occupy 70% of the total area of Argentina and it stretches over three-biogeographic Provinces: Patagonia, Puna, and Monte (6). The Monte region is located in the western area of Argentina, covering approximately 460.000 km<sup>2</sup> (45). This region presents water deficit most of the year and it has an average annual rainfall ranging from 30 to 350 mm, with a mean temperature between 13 and 18°C (30, 45). Some areas present moderate to severe degree of native ecosystem degradation. Human activities have been suggested as the main causes of degradation processes (14, 43). To reverse these processes, a revegetation program would be the starting point to reach ecosystem restoration.

As restoration actions carried out using seeds had little success (11), the most common revegetation method consists in the introduction of one-or two-year-old saplings (19, 38). These activities in arid zones pose numerous challenges (1, 50), due particularly to drought conditions, extreme temperatures, high irradiance, and infertile soils, along with grazing, all of which threaten the survival of installed saplings (20, 26).

Shrub species play an important role in arid land revegetation, since in several cases, they act as "nurse plants" improving microclimatic conditions, increasing water and nutrient availability, and offering protection against herbivory (4, 7, 47).

These conditions may benefit survival and growth of other species under their canopies (5, 13, 21, 28, 36, 41), and thus, will facilitate the natural revegetation of these areas.

Revegetation with native evergreen shrubs is desirable, but techniques for successful establishment of these species are not fully developed. Transplant shock is one of the major obstacles for plantation success. This shock is the initial short-term stress experienced by saplings since they are transferred from favorable nursery conditions to the adverse field environment (9, 51).

Soil water availability is the main environmental factor inducing transplant shock in arid zones; hence, selecting native species capable of surviving to extreme water scarcity is a key step in planning the revegetation.

The application of a drought-preconditioning treatment during the last months of nursery culture is a potential technique for reducing transplant shock (9, 51). This treatment has four main objectives: to manipulate seedling morphology and to induce dormancy, to acclimate seedlings to the natural environment, to develop stress resistance, and to improve seedling survival and growth after outplanting (24).

Nursery techniques to avoid transplant shock focus on manipulating the watering regime so as to favor the acclimation of seedlings to unfavorable field conditions. The intensity, duration and time for optimal irrigation should be adjusted to the plant species and the seedling characteristics, particularly their stress resistance (51). During the application of the drought period it is important to avoid intense drought conditions that can damage the seedlings irreversibly (*i.e.*, loss of xylem conductivity due to cavitation processes).

In general, mild water stress is known to reduce growth to a higher extent than carbon fixation, resulting in an accumulation of carbohydrates and nutrients (9, 32).

In order to analyze the responses of seedlings to the preconditioning treatments, many studies evaluate different physiological (photosynthetic rates, stomatal conductance, chlorophyll concentration, water relations) and morphological changes (size of the plant, some part of them or the proportion between them through some quality indices) on seedlings (9, 51, 56).

Also some quality indices may help to analyze these responses, such as the sturdiness quotient (SQ), which is calculated by length: diameter stem ratio and shoot: root dry weight ratio (S:R). Lower values of these indices indicate higher survival ability. One of the most commonly used indices is Dickson quality index (DQI) (14), which is determined by the equation:  $\text{total dry weight}/(\text{SQ}+\text{S:R})$ . In this case, higher values indicate greater survival ability of plants under field conditions. No information is available about the application of these treatments on shrub species from the Monte Desert.

This study aims to analyze the suitability of drought preconditioning treatments in the nursery as a practice to improve the drought resistance of two native shrub species of the Monte Desert -*Capparis atamisquea* and *Larrea cuneifolia*-. The specific objective is to evaluate morpho-physiological changes on the seedlings delivered by each treatment. Specifically, it is postulate that hardening treatments exert some morpho-physiological changes on *C. atamisquea* and *L. cuneifolia*, improving their sapling quality, such as lower leaf conductance, DQI, S/R ratio, and higher survival.

## MATERIAL AND METHODS

### Species and seed collection

*C. atamisquea* Kuntze (Capparaceae) is a 2-3 m high forage shrub with small leathery leaves, densely pubescent on their abaxial face (42). It has a facultative phreatophytic behavior (23), and its seedlings tend to establish in closed, canopied microsites, with less incident light and better moisture and nutrient conditions (8, 37, 44) than open areas. *L. cuneifolia* Cav. (Zygophyllaceae) is a microphyllous shrub with resinous cuticles and small stomata showing high resistance and closure at midday during dry seasons (2, 29). It can tolerate very hot environments by physically evading the midday sun and intercepting early morning and late afternoon light with its erect, east-facing leaves and branches. It colonizes the hottest and driest parts of the Monte Desert (17).

Seeds of *C. atamisquea* were collected from the Telteca Natural Reserve, NE Mendoza (32°14'56" S; 67°49'05" W) and *L. cuneifolia* from a natural shrubland located in Agrelo, SW Mendoza (33°07'01" S; 68°52'46" W). They were stored in paper bags and kept at room temperature until experiments were performed. To obtain the seeds, fruits of both species were rubbed between two rubber sheets, and only well-formed seeds were used in the assays. Seeds of *L. cuneifolia* were washed with running water for 48 h in order to break dormancy (18). Prior to sowing, both types of seeds were disinfected with a 15% solution of commercial hypochlorite (60 g Cl<sup>o</sup>/l) for 7 minutes and then repeatedly washed with sterile water.

### Experimental set-up

The assay was performed in the glass-house of IADIZA (Instituto Argentino de Investigaciones en las Zonas Áridas) (32°53' S; 68°57' W), Mendoza, Argentina, from January to April (summertime) 2012 for *L. cuneifolia* and 2013 for *C. atamisquea*, both over a period of 106 days.

Seeds were sown in a terrine and then transplanted to plastic pots 25 cm in diameter and 10 liters of capacity filled with air-dried sandy loam soil (7 kg for *C. atamisquea* and 7.5 kg for *L. cuneifolia*). Field capacity (FC) of the soil was 20.3% for *C. atamisquea* and 20% for *L. cuneifolia*.

Pots were watered as necessary until the start of the experiment. Maximum and minimum mean daily temperatures during 2012 ranged between 36.5° and 19°C, and during 2013 between 35.4° and 19.9°C; relative humidity ranged between 18.7% and 52% during 2012 and between 20% and 54% during 2013, and mean PAR at midday was 1100  $\mu\text{mol m}^{-2}\text{s}^{-1}$  on both years. A foam pad was placed around all pots to avoid overheating from the sun. The experiment was started with plants 1 year old.

### Water treatments

The assay was set up in a completely randomized experimental design with one-factor of water supply with three levels: T1 (control) was watered when soil water content decreased by 50% from the FC (*C. atamisquea* being watered 16 times, and *L. cuneifolia* 14 times), T2 (moderate water stress) when it decreased by 70% (*C. atamisquea* and *L. cuneifolia* being watered 8 and 7 times respectively), and T3 (severe water stress) when it decreased by 85% (both species being watered 3 times).

The amount of water to be replenished to FC at each irrigation date was determined from the gross weight of pots. We used 200 plants of each species distributed among the three treatments.

#### **Leaf water status**

Water potential ( $\Psi$ , MPa) was measured on the main shoot immediately after being excised at predawn (pd), 4:00 to 5:00 h, and midday (md), 12:30 to 13:30 h, before re-watering. Measurements were made using a pressure chamber (Biocontrol, Buenos Aires, Argentina), based on Scholander *et al.* (1965). For these measurements, ten plants per treatment were randomly selected on a monthly basis.

#### **Stomatal conductance to water vapor**

It was adaxial and abaxial stomatal conductance ( $G$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) to water vapor with a steady-state diffusion porometer (SC-1, Decagon Devices, Pullman, WA, USA). Since conductance did not differ significantly between both leaf faces in *L. cuneifolia*, the measurement was made only on the abaxial face (in accordance with Barbour *et al.* (1974), who found equal stomatal density on both leaf faces for this species. Midday  $G$  was measured on the same 10 plants as used for water status determination. In all cases are measured four expanded leaves per plant from different positions.

#### **Growth**

Ten plants per treatment were randomly selected to determine sapling height and stem basal diameter every 20-30 days.

On each sampling date, after measuring  $\psi$  and  $G$ , we separately determined leaf, stem and root dry matter after oven-drying them at 60°C for 72 h.

Leaf area (LA) was determined according to a linear regression equation derived from the relationship between the dry weight of a sample of leaves and their surface area measured on a digitalized image of the leaves generated by a scanner (hp psc 1210), and then treated with an image analyzer (IMAGEJ- National Institute of Health- USA). We used 30 replicates of five leaves to adjust the regression equation. The linear regression model was highly significant for both species ( $p \leq 0.001$ ;  $r^2=0.99$  for *C. atamisquea* and  $r^2=0.97$  for *L. cuneifolia*).

#### **Indices and survival**

Using data from growth parameters, we calculated quality indices such as sturdiness quotient (SQ) determined by the ratio of the length and diameter of the stem; the shoot-to-root ratio by the dry weight of shoot and root (S:R); the Dickson quality index (DQI) determined by the ratio of total dry weight (TDW) with the sum of SQ and S:R, according to equation of Dickson *et al.* (1960):  $\text{DQI} = [\text{TDW} / (\text{SQ} + \text{S:R})]$ , and the Relative Growth Rate,  $\text{RGR} = (\text{Ln}(\text{TDW}_t) - \text{Ln}(\text{TDW}_0)) / \text{time}$  (Porter & Garnier, 2007---Functional plant ecology). Also, in order to compare the effect of water stress on seedlings, we calculated the "proportional growth" as the ratio between the mean biomass reached in each water stress treatment and that of the control treatment (31).

Sapling survival was recorded at the end of the assay, in four replicates of ten saplings, per treatment and specie.

#### **Statistical Analysis**

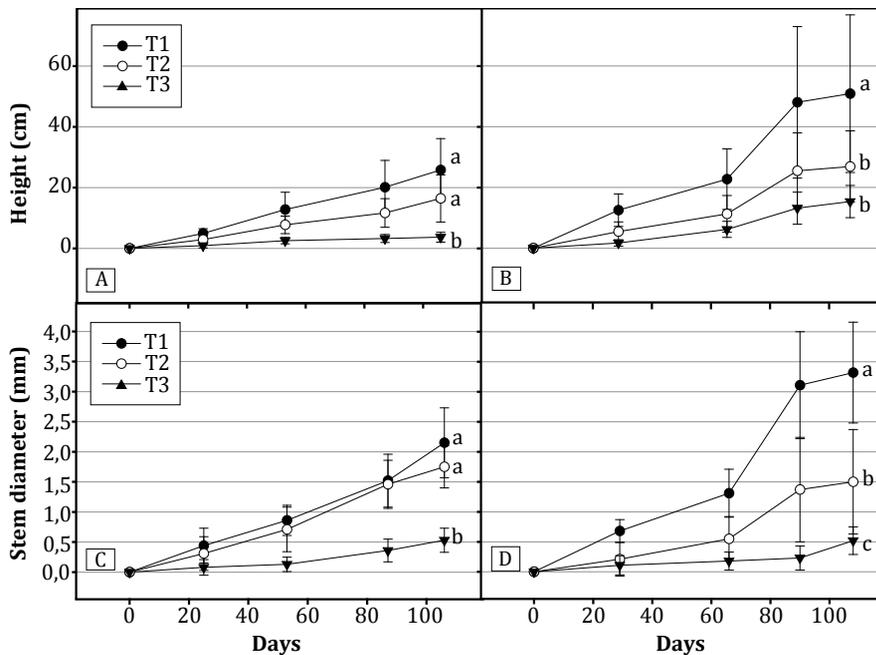
Growth, water potential and stomatal conductance data were subjected to a one-way analysis of variance (ANOVA).

Tukey's test was used for comparison of means. Variation in G as a function of  $\Psi$  md or soil water content was evaluated with linear regression. Infostat/L (16) was used for statistical analysis.

Because the data on sapling height, dry weight and G did not meet the ANOVA assumption of variance homogeneity, an  $\ln(x+1)$  transformation was applied to data on sapling height and dry weight, and a  $\log_{10}$  transformation on G.

## RESULTS

The severe water stress treatment (T3) significantly reduced both growth parameters in *C. atamisquea* (height:  $F= 38.89$ ,  $P <0.0001$ , diameter:  $F= 59.48$ ,  $P <0.0001$ ), while in *L. cuneifolia* T2 and T3 reduced height and stem diameter (height:  $F= 11.25$ ,  $P =0.0003$ , diameter:  $F= 39.68$ ,  $P <0.0001$ ). Under the highest water stress conditions, sapling height in *C. atamisquea* and *L. cuneifolia* was respectively 25.3% and 35.7% lower than in T1, while stem basal diameter was respectively 27% and 30.1% lower than in T1 (figure 1).



Different letters among treatments indicate significant differences at  $p < 0.05$ .

Letras distintas entre tratamientos indican diferencias significativas a nivel  $p < 0,05$ .

**Figure 1.** Growth curves of average sapling height and stem basal diameter for *C. atamisquea* (A – C) and *L. cuneifolia* (B – D) saplings imposed by different irrigation levels.

**Figura 1.** Curvas de crecimiento en altura y diámetro basal de tallo promedio de plantines de *C. atamisquea* (A – C) y *L. cuneifolia* (B – D) bajo diferentes niveles de riego.

Plants of both species (table 1), under water stress, showed a concomitant decrease in dry weight of leaves, stems and roots, and leaf area, although the difference between T2 and T1 was not significant for roots in *L. cuneifolia*. In T2 and T3, total dry weight decreased by 30% and 48% in *L. cuneifolia*, and by 26% and 54% in *C. atamisquea* in relation to their respective controls (T1).

No significant difference was found between SQ and RGR determinations. Measurements of RGR on saplings under T3 were not included in the analysis because almost all the values were 0.

The DQI was higher in saplings under T1 in both species, and the Proportional Growth showed similar values on both species and was lower in T3 (no statistical analysis was done). All saplings under T1 and T2 survived. Under severe water stress conditions (T3), some saplings died, but the percentage of survival was rather high in both species (table 1).

Shoot-to-root ratio decreased in both species under T3 (table 1).

*C. atamisquea* saplings under T3 allocated a lower proportion of dry weight to leaves ( $F= 7.84, P= 0.0021$ ), with a correspondingly increased allocation to roots ( $F= 17.42, P<0.0001$ ) (figure 2, page 40).

**Table 1.** Effects of the three levels of water supply (T1, T2 and T3) on: shoot-to-root ratio, sturdiness quotient (SQ), Dickson quality index (DQI), Relative Growth Rate (RGR), Proportional growth, leaf area and survival of *C. atamisquea* and *L. cuneifolia* saplings at the end of the assay (S.E. between parentheses).

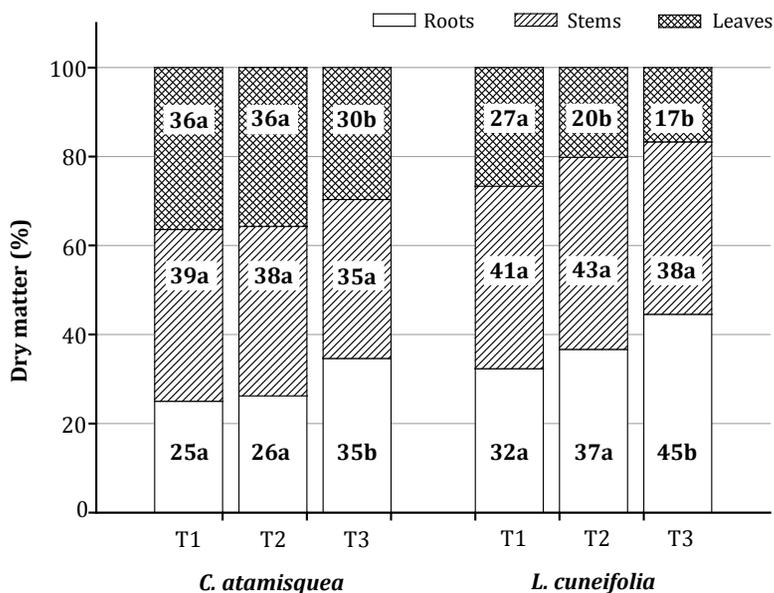
**Tabla 1.** Efectos de los tres niveles de irrigación (T1, T2 y T3) en: relación vástago/raíz, cociente de robustez (SQ), Índice de Calidad de Dickson (SQI), Tasa de Crecimiento Relativo (RGR), Crecimiento proporcional, área foliar y supervivencia de plantines de *C. atamisquea* y *L. cuneifolia* al finalizar el ensayo (E.E. entre paréntesis).

	<i>C. atamisquea</i>			
	T1	T2	T3	P value
Shoot/root ratio	3.04 a (0.44)	2.9 a (0.49)	1.9 b (0.45)	<0.0001
SQ	11.77 (1.75)	11.99 (2.64)	12.14 (2.81)	0.943
DQI	1.69 a (0.04)	1.29 b (0.05)	0.83 c (0.02)	<0.0001
RGR (mg/g día)	8	5	---	0.0557
Proportional Growth		0.46	0.74	----
Leaf area (mm <sup>2</sup> )	448.5 a (93.55)	325.3 b (45.4)	169.5 c (35.67)	<0.0001
Survival (%)	100 a	100 a	89 b	<0.0001

	<i>L. cuneifolia</i>			
	T1	T2	T3	P value
Shoot/root ratio	2.1 a (0.37)	1.8 a (0.38)	1.3 b (0.29)	<0.0001
SQ	9.61 (2.73)	9.81 (2.37)	9.23 (2.13)	0.863
DQI	2.38 a (0.09)	1.73 ab (0.07)	1.34 b (0.03)	0.0026
RGR (mg/g día)	6	7	----	0.35
Proportional Growth		0.53	0.73	----
Leaf area (mm <sup>2</sup> )	592.2 a (104.23)	326.3 b (96.94)	200.3 c (71.3)	<0.0001
Survival (%)	100 a	100 a	92 b	<0.0001

Different letters among treatments for each species indicate significant differences at  $p<0.05$ .

Letras distintas entre tratamientos para cada especie indican diferencias significativas a nivel  $p<0,05$ .



Different letters among treatments for each species and each biomass portion indicate significant differences at  $p < 0.05$ .

Letras distintas entre tratamientos para cada especie y cada región morfológica indican diferencias significativas a nivel  $p < 0.05$ .

**Figure 2.** Percentage of dry biomass allocated to leaves, stems, and roots at the end of the assay by *C. atamisquea* and *L. cuneifolia* saplings growing under three different irrigation levels: T1, T2, and T3.

**Figura 2.** Porcentaje de materia seca asignado a hojas, tallos, y raíces al finalizar el ensayo en plantines de *C. atamisquea* y *L. cuneifolia* creciendo bajo tres niveles de irrigación: T1, T2 y T3.

In *L. cuneifolia*, under both water stress conditions, dry matter allocation was lower to leaves ( $F = 15.11$ ,  $P < 0.0001$ ) and higher to roots ( $F = 15.01$ ,  $P < 0.0001$ ). No significant difference was found between stems of both species in any of the three treatments (*C. atamisquea*  $P = 0.2971$ , *L. cuneifolia*  $P = 0.2583$ ).

As it was expected, under high stress conditions (T3), both species maintained

highly negative water potentials, which were measured until  $-9$  MPa for safety reasons. Because of this, they were not included in the statistical analysis in table 2 (page 41). These extreme values apparently did not cause any injury in the plants. Water potential at predawn and midday measured at the three harvest dates were significantly lower in T2 for both species (table 2, page 41).

**Table 2.** Water potential at predawn ( $\Psi_{pd}$ ) and midday ( $\Psi_{md}$ ) and leaf conductance ( $G$ )<sup>a</sup> in *C. atamisquea* and *L. cuneifolia* saplings measured in T1 and T2.**Tabla 2.** Potencial hídrico al pre-amanecer ( $\Psi_{pd}$ ) y a mediodía ( $\Psi_{md}$ ), y conductancia estomática ( $G$ )<sup>a</sup> en plantines de *C. atamisquea* y *L. cuneifolia* medidos en T1 y T2.

	<i>C. atamisquea</i>			<i>L. cuneifolia</i>		
	T1	T2	P value	T1	T2	P value
-January						
$\Psi_{pd}$ (MPa)	-2.6 a (0.23)	-4.2 b (0.54)	<0.0001	-2.2 a (0.35)	-3.7 b (0.68)	<0.0001
$\Psi_{md}$ (MPa)	-4.8 a (0.33)	-6.3 b (0.22)	<0.0001	-3.5 a (0.59)	-4.9 b (0.75)	0.0003
G (mmol/m <sup>2</sup> s)	46.2 a (16.56)	12.8 b (5.42)	<0.0001	33.3 a (15.5)	11.6 b (5.9)	0.0006
-February						
$\Psi_{pd}$ (MPa)	-3 a (0.38)	-5.6 b (0.34)	<0.0001	-2.7 a (0.3)	-4.8 b (0.49)	<0.0001
$\Psi_{md}$ (MPa)	-5.8 a (0.48)	-6.5 b (0.31)	0.0007	-3.5 a (0.29)	-5.8 b (0.47)	<0.0001
G (mmol/m <sup>2</sup> s)	44.4 a (20.1)	11.2 b (4.2)	<0.0001	21.8 a (9.47)	11.1 b (4.39)	0.0047
-March						
$\Psi_{pd}$ (MPa)	-3.2 a (0.25)	-4.2 b (0.55)	<0.0001	2.8 a (0.39)	-4.7 b (0.68)	<0.0001
$\Psi_{md}$ (MPa)	-5.2 a (0.5)	-6 b (0.5)	0.0025	3.6 a (0.37)	-5.6 b (0.81)	<0.0001
G (mmol/m <sup>2</sup> s)	72.43 a (16.7)	26.5 b (9.69)	<0.0001	38.9 a (15.75)	17 b (5.1)	0.0001

Different letters between treatments for each species indicate significant differences at  $p < 0.05$  (S. E. between parentheses).

Letras distintas entre tratamientos para cada especie indican diferencias significativas a nivel  $p < 0,05$ . (E. E. entre paréntesis).

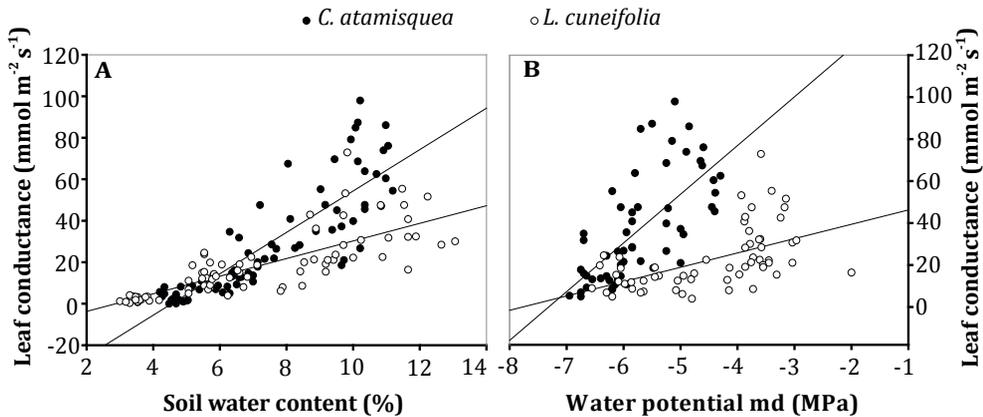
<sup>a</sup> Values of G are from the abaxial leaf face of both species.

<sup>a</sup> Los valores de G son de la cara abaxial en ambas especies.

Likewise, measurements of G on saplings under T3 were not included in the analysis because almost all the values were 0 mmol/m<sup>2</sup>s. Both species showed higher G in T1 throughout the three harvest times. In the case of *C. atamisquea*, G values measured in March were higher than those for January and February. Since this specie has sunken stomata in the adaxial leaf face, which were observed with the leaf surface imprint method (data not shown), values of G were low

in the three treatments at the three measurement dates (T1=7.23; T2= 2.27; and T3= 0 mmol/m<sup>2</sup>s).

Midday G varied significantly and positively with Soil Water Content and  $\Psi_{md}$  on both species, although this correlation was higher between G and soil water content (*C. atamisquea*  $r^2 = 0.71$ ,  $P < 0.0001$ ; *L. cuneifolia*  $r^2 = 0.67$ ,  $P < 0.0001$ , figure 3A, page 42, and *C. atamisquea*  $r^2 = 0.51$ ,  $P < 0.0001$ ; *L. cuneifolia*  $r^2 = 0.33$ ,  $P < 0.0001$ , figure 3B, page 42).



Symbols represent single measurements; straight line is the trend of the linear regression.

Los símbolos representan medidas únicas; la línea recta es la tendencia de la regresión lineal.

**Figure 3.** (A) Leaf conductance versus Soil water content, and (B) Leaf conductance versus Midday leaf water potential.

**Figura 3.** (A) Conductancia estomática versus Contenido hídrico del suelo, y (B) Conductancia estomática versus Potencial hídrico a mediodía.

## DISCUSSION

Nearly all morphological characters varied significantly between seedlings under control and both hardening treatments, on both species. Only height and stem diameter did not differ between control and T2 on *C. atamisquea*.

The RGR of both species was low in all treatments, as expected for shrub species from arid environments (40). This rate did not decrease significantly under the moderate water stress treatment, and the proportional growth, also showed that seedlings under T2 were less affected than under T3. Usually, restoration practitioners tend to look for small seedlings because they spend less water than seedling with big canopies, and they have a better water economy (56).

However, many studies show that seedling with a big aerial part tend to have

greater survival and growth faster after transplanting to the field (10, 55).

The sturdiness quotient did not differ between treatments on both species, but *L. cuneifolia* seedlings showed lower values. A low SQ value in adverse areas is recommended since this index is associated with more robust plants and therefore potentially more resistant to stress caused by planting (35).

Control saplings of both species showed higher DQI, suggesting greater survival ability under field conditions, although it was expected that preconditioned seedlings have a greater DQI. Probably, this is due to the lack of significant variation in SQ, and therefore DQI values were more influenced by the total dry weight. These indices have been used more extensively in forest species

(3, 12, 27), so it is important to make some variation in these ones or test other indices more suitable for species from desert areas.

The decreasing shoot-to-root ratio calculated under T3 may actually be induced by water stress, which can affect leaf expansion and reduce carbon and energy uptake. Therefore, a greater proportion of the plant's assimilates can be distributed to the root system, where they can support further root growth while maintaining a better water status (49). The shoot-to-root ratios of these species are in line with those reported by other authors for shrubs of the Monte region (39, 52, 53). This morphological attribute is important for restoration practitioners, because it has been positively correlated with sapling survival in some species from Mediterranean shrublands (25). None of the two species, under the mild water stress treatment showed a significantly reduction in the shoot-to-root ratio.

Both shrubs showed low stomatal conductance under all three treatments. Hence, these saplings have a small area of stomatal opening whereby they minimize water loss to adjust to a limited water supply. We found out that, *C. atamisquea* and *L. cuneifolia* saplings under moderate water stress treatment maintained their stomata open at midday while leaf water potential was -6.5 and -5.8 MPa respectively. G was affected under both preconditioning treatments. It was reduced in 70% and 56% under T2, on *C. atamisquea* and *L. cuneifolia* respectively, while total stomatal closure occurs under the severe water stress treatment. These responses might help drought-conditioned seedlings to expend less water and maintain better water status when transplanted to the field (9, 57). This was also seen in the positive corre-

lation between G and soil water content on both species. *C. atamisquea* seedlings were more sensitive to the decrease of soil water content than *L. cuneifolia*, but they maintain their stomata open at lower water potential. The acclimation of preconditioned seedlings to drought in terms of stomatal conductance has been widely described in the literature and has been considered an important regulatory mechanism to enhance better performance of seedlings (51).

Studies of the effects of soil moisture on plant growth indicate a strong relationship between water potential and growth (49). Thus, water potential appears to be one of the most suitable indices of water stress, and in many species, such as in *L. tridentata*, it is the most important factor controlling phenological events, photosynthesis and productivity (34).

Our data show that *C. atamisquea* and *L. cuneifolia* did not stop growing at a predawn plant water potential of - 4.2 to -5.6 MPa, which corresponds to a soil moisture content of 6%. Barbour *et al.* (1974) found similar water potential values in 6-8 month old *L. cuneifolia* saplings. Other shrubs like *A. lampa* continue to grow at -5.7 to -6.9 MPa -soil moisture content of 5%- with a 30% reduction in biomass (Passera, unpublished results), and *L. tridentata* maintained stomatal opening and net photosynthesis down to - 6 MPa (33, 34, 48). It must be noted that crop plants like alfalfa (*Medicago sativa* L.) ceases to grow when plant water potential declines to less than -1.5 MPa (22).

Under severe water stress conditions (3% of soil water content) a high percentage of saplings (89-92%) of both species survived. Although environmental conditions in the field are usually harder than in nursery, these results show a great species capability to cope with water

stress. Irrigation of saplings under T3 was done approximately every 30 days, with low sapling mortality and very low water potentials (lower than -9 MPa), and under T2 irrigation was done every 8-10 days with no sapling mortality. It is important to notice that most of saplings under the severe water stress treatment died during the first drought cycle, suggesting the importance of the preconditioning treatments before transplanting to the field.

## CONCLUSION

Moderate water stress treatment seems more suitable for preconditioning these species since it reduces growth, leaf area and leaf conductance without

seedling mortality and maintaining a good growth rate. In fact, several studies have observed that mild or moderate drought levels perform better than very intense drought conditions (54, 57). These results could be a consequence of surpassing certain limits of stress resistance during the process of desiccation (51).

However, it is necessary in future studies to evaluate seedlings response after transplanting to the field, in order to compare and analyze the suitability of these treatments.

The water stress treatments (T2 and T3) applied in this study, are a starting point to propose some preconditioning treatments for other native shrubs species from the Monte Desert.

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