

## Leaf Water Potential of Three Browse Species of a Semiarid Ecosystem in Northern of Mexico Potencial Hídrico Foliar de Tres Especies Arbustivas de un Ecosistema Semiárido en el Norte de México

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### SUMMARY

Climate change is a global phenomenon, its repercussions disrupt the functioning and integrity of ecosystems. The most marked impact of climate change is water stress, hence the importance of plant selection studies and their strategies based on the availability of water in the soil. The current study aimed to establish how the xylem water potential ( $\Psi$ ) in three native shrubs, such as *Condalia warnockii* (Rhamnaceae), *Flourensia cernua* (Asteraceae), and *Larrea tridentata* (Zygophyllaceae), is influenced by soil water content, air temperature, relative humidity, and rainfall. The study was carried out in an area located the municipality of Galeana, state of Nuevo Leon. Using a Scholander pressure bomb, the  $\Psi$  were estimated at 15-day intervals between June 3 and November 18, 2022, in four different plants per species at 06:00 h (predawn) and 14:00 h (midday). At the wettest period,  $\Psi$  oscillated from -1.13 to -4.27 MPa (*C. warnockii*) at predawn. In contrast, at midday, *L. tridentata* and *C. warnockii* achieved the highest (-1.46 MPa) and lowest (-3.75 MPa)  $\Psi$  values, respectively. Soil water content at different soil depths, and relative humidity were significantly and positively correlated with  $\Psi$  at predawn of *C. warnockii* and *F. cernua*; whereas air temperature was correlated with *F. cernua* and *L. tridentata* at midday *F. cernua* and *L. tridentata* maintained high values in  $\Psi$  at predawn and midday under water stress conditions, so these species may be considered as drought tolerant species.

**Index words:** arid, drought, hydric, shrubs, stress.

### RESUMEN

El cambio climático es un fenómeno global, sus repercusiones alteran el funcionamiento y la integridad de los ecosistemas. El impacto más marcado del cambio climático es el estrés hídrico, de ahí la importancia de los estudios de selección de plantas y sus estrategias en función de la disponibilidad de agua en el suelo. De ahí que el objetivo del presente estudio fue establecer el potencial hídrico del xilema ( $\Psi$ ) en tres arbustos nativos como son *Condalia warnockii* (Rhamnaceae), *Flourensia cernua* (Asteraceae), *Larrea tridentata* (Zygophyllaceae). El estudio se realizó en el ejido la Hediondilla municipio de Galeana, Nuevo León. Utilizando la bomba de presión Scholander, se estimaron los  $\Psi$  a las 06:00 h (antes del amanecer) y a las 14:00 h (mediodía) en intervalos de 15 días entre el 3 de junio y el 18 de noviembre de 2022 en cuatro individuos por especie. En el período más húmedo,  $\Psi$  osciló de -1,13 a -4,27 MPa (*C. warnockii*) antes del amanecer. Por el contrario, al mediodía, *L. tridentata* y *C. warnockii* alcanzaron los valores de  $\Psi$  más altos (-1,46 MPa) y más bajos (-3,75 MPa), respectivamente. El contenido de agua del suelo a diferentes profundidades y la humedad relativa se correlacionaron significativa y



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positivamente con  $\Psi$  antes del amanecer de *C. warnockii* y *F. cernua*; mientras que la temperatura del aire se correlacionó con *F. cernua* y *L. tridentata* al mediodía. *F. cernua* y *L. tridentata* mantuvieron valores altos de  $\Psi$  antes del amanecer y al mediodía en condiciones de estrés hídrico, por lo que estas especies se consideran tolerantes a la sequía.

**Palabras clave:** árido, sequía, hídrico, arbustos, estrés.

## INTRODUCTION

Climate change is a global phenomenon of growing scientific, political and social interest, and its repercussions affect and alter practically all human activities, disturbing the functioning of the biosphere and the integrity of ecosystems (Schewe *et al.*, 2019).

The most marked impact of climate change is water stress, causing longer and drastic periods of drought in most regions of northeastern Mexico (Ayala-Meza, 2020<sup>1</sup>; Cervantes-Gaeta, 2021<sup>2</sup>; Cantú-Martínez, 2022) and changes in the different stages of plant life, such as budding dates and shortening of phenological periods, changes in your performance and movement of species vertically, changing the altitudinal breadth of their distribution (Van Leeuwen and Darriet, 2016).

Therefore, studies are carried out to select the most efficient plants in the use of water, the basic mechanisms that they develop to conserve and distribute the little humidity that reaches their structures and scaling from what happens in a plant to what happens in an ecosystem, as well as modeling to propose various future scenarios in the face of climate change (González-Rodríguez *et al.*, 2018; Lustre-Sánchez, 2022).

The xerophilous scrub is the best represented type of vegetation in northern Mexico (Rzedowski, 2006). In fact, in some entities it occupies the largest vegetation cover, such as the state of Nuevo León, where the community occupies 54.6% of its total surface, comprises an area of 1 056 046 ha on which the vegetation of arid zones grows (González-Delgado, Foroughbakhch, Rocha, Guzmán, and González, 2017)

The microphyllous desert scrubland brings together communities where the plants that print the physiognomic character of the vegetation correspond to small-leaf shrub elements, which almost always include *Larrea tridentata* (Sessé & Moc. ex DC.) Coville and *Flourensia cernua* DC (González-Delgado *et al.*, 2017; Zyalalov, 2004). which *Larrea tridentata* probably plays a relevant role as a nurse plant (Ugalde-Ávila, Granados y Sánchez, 2008).

The physiological study of plants in arid or semiarid areas contributes to understanding the strategies and processes carried out by species based on the availability of water in the soil. For example, the plant species of arid and semiarid regions develop in challenging environmental conditions, according to the water requirements, they are considered mesophytes if they respond to an intermediate amount of water (<-20 bars) and have characteristics that allow them to adapt to various environmental conditions without their growth being conditioned. In contrast, xerophytic plants have adaptations to survive extreme environments (<-40 bars) (Nilsen and Orcutt, 1996). To survive the low availability of water in the soil they make temporary changes in their anatomy, morphology and physiology (Bucci *et al.*, 2008) For example, have developed morphological characteristics like the reduced size of its leaves and deep root and physiological attributes such as stomatal closure, CAM photosynthesis and adjustment of the water from the zones of higher to lower water potential that allow them to adapt to the low availability of water in the soil (Larcher, 2003; Zyalalov, 2004).

In particular, the study of water potential tells us what strategies plants follow to survive in the presence or absence of humidity. It allows us to understand the function of each species and the behavior and resilience of the ecosystem to environmental factors, as well as the lack of water availability.

However, few studies (Himmelsbach, 2009<sup>3</sup>; González-Rodríguez *et al.*, 2018; Gebrekirstos, van Noordwijk, Neufeldt, and Mitlöhner, 2011) have evaluated the water potential of plant species in these environments; these studies have used the pressure chamber to measure the pressure of the plant sap (Rodríguez-Domínguez *et al.*, 2022; Turner, 1988). The method involves increasing the pressure around a shoot to the xylem sap, which spreads outside the exposed chamber and is at atmospheric pressure; the pressure necessary to maintain this condition represents the existing negative pressure in the stem. The amount of pressure needed to force the water out of the leaf cells in the xylem is a function of the water potential of leaf cells. Water travels from areas where the water potential is higher (less negative) to areas where it is lower (more negative).

<sup>1</sup> Ayala-Meza, C. D. J. (2020). *Evaluación fisiológica y de compuestos bioactivos en maíces nativos ante estrés de temperatura y sequía*. Tesis para obtener el grado de Doctor en Ciencias. Universidad Autónoma de Nuevo León. Disponible en: <http://eprints.uanl.mx/21831/1/1080315074.pdf>

<sup>2</sup> Cervantes-Gaeta, H. M. (2021). *Análisis de la presencia histórica de sequía meteorológica en la cuenca del río Nazas*. Tesis para obtener el grado de Doctor en Ciencias. Colegio de Postgraduados. Disponible en: <http://hdl.handle.net/10521/4933>

<sup>3</sup> Himmelsbach, W. 2009. *Caracterización de bosques mixtos de pino-encino en la Sierra Madre Oriental en México considerando el factor limitante hídrico*. Tesis para obtener el grado de Doctor en Ciencias. Universidad Autónoma de Nuevo León. Disponible en: <http://eprints.uanl.mx/1967/6/1080191371.pdf>

Considering as hypothesis that under adverse conditions the more drought-resistant species tend to reduce water potentials more rapidly than less resistant species, the purpose of this research was to evaluate the foliar water potential of three browsing species of a semi-arid environment in northern Mexico, in periods of high temperatures and little precipitation and to use these relationships to characterize the specific water use strategy of each species, which will allow to propose various future scenarios in the face of climate change.

## MATERIALS AND METHODS

### Research Site

Fieldwork was conducted in an area of 11 988 ha of microphyllous desert scrubland in the Northeast of Mexico, at an altitude of 1880 m in the Galeana municipality. The sampling site was selected at La Hediondilla between 24° 58' 45" N and 100° 41' 49" W (Figure 1). The local climate corresponds to the dry or temperate type BSok(x'), subtype of the driest with a P/T ratio lower than 22.9; Average annual temperature of 12 °C to 18 °C, of the coldest month of 3 °C to 18 °C. The dominant soils are of the Haplic xerosol type and rich in calcium, which support a vegetation resistant to high concentrations of gypsum (Pando-Moreno, Reyna, Scott, and Jurado, 2013) (Table 1)

### Plant Material

In a previously delimited plot under disturbance (400 m) were randomly selected three plants. The plant species were: *Condalia warnockii* M.C. Johnst. (Rhamnaceae; Warnock's snakewood, with microphyllous compound leaves), *Flourensia cernua* DC. (Asteraceae; American tarwort, American tarbush, tarbush, with microphyllous compound leaves) and *Larrea tridentata* (DC.) Coville (Zygophyllaceae; creosote Bush, perennial evergreen shrub with microphyllous compound leaves). They were selected based on a previous inventory carried out and the choice of the three plant species of most significant ecological importance in the site, individuals with a height of 3 meters and a coverage of 4 meters during the flowering season were selected.

In a previously delimited and disturbed plot (400 m), the three plant species with the greatest ecological importance were selected based on homogeneous height and coverage (González-Delgado *et al.*, 2017). The plant species was: *Condalia warnockii* M.C. Johnst. (Rhamnaceae; Warnock's snakewood, with microphyllous compound leaves), *Flourensia cernua* DC. (Asteraceae; American tarwort, American tarbush, tarbush, with microphyllous compound leaves) and *Larrea tridentata* (DC.) Coville (Zygophyllaceae; creosote Bush, perennial evergreen shrub with microphyllous compound leaves).

### Sample Measurements

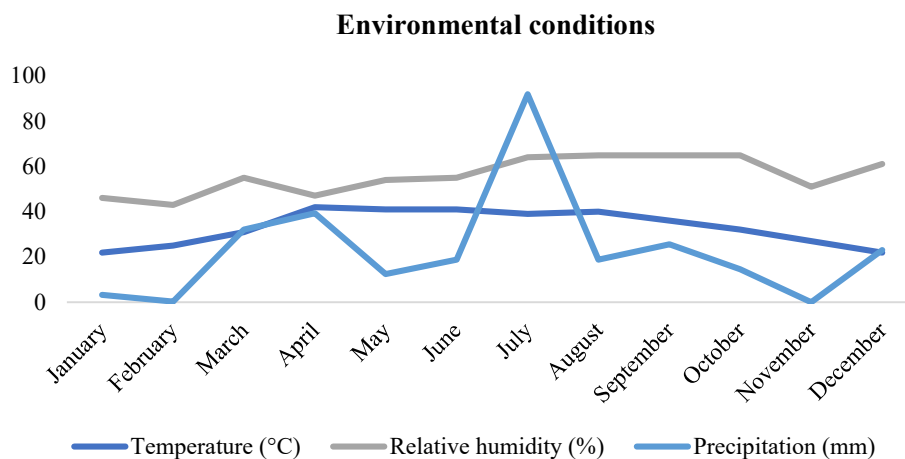
From the experimental plot were randomly chosen three samples per species (repetitions), which were evaluated for xylem water potentials ( $\psi$ , MPa) throughout the experimental period with a frequency of two weeks. The measurement period covers from June 3 to November 18, 2018.

### Water Potential Determination

Terminal branches with fully expanded leaves were sampled from each selected plant. The water potential was evaluated 10-25 seconds after sample collection, with a Scholander pressure pump (Model 3005, Soilmoisture Equipment Corp., Santa Barbara, CA, EE.UU.) before dawn (6:00) and at noon (12:00) the pressure supplied to the chamber was at a rate of 0.05 (Ritchie and Hinckley, 1975).

**Table 1. Results of apparent density, pH, electrical conductivity, and organic matter at profile depths 0-30 and 30-60 centimeters.**

Soil depth profile	Apparent density	Percentage			pH	Electric conductivity	Organic material
		Sand	Silt	Clay			
cm						$\mu\text{S cm}^{-1}$	%
0-30	0.9	25.9	47.6	26.6	8.04	2.641.5	2.73
30-60	1.5	12.6	39.3	48.1	8.24	2.396.0	1.08



**Figure 1. Environmental conditions of January to December 2022 at the research site.**

To characterize changes in  $\psi$  during the day under high soil water content ( $>0.20 \text{ kg kg}^{-1}$ ) in the 0-30 cm soil layer, measurements were carried out during the month with the most stable climatic variables in September 2022, at 6:00, 10:00 a.m., 2:00, and 6:00 p.m.

### Environmental Data

Temperature ( $^{\circ}\text{C}$ ), relative humidity, and precipitation (mm) were recorded daily (Rosenberg, Blad, and Verma, 1983). Data obtained from the San José de Raíces meteorological station through the National Water Commission.

The gravimetric content of soil water (%) on each sampling date was determined in soil samples taken at 10 cm depth intervals from 0 to 50 cm using a soil sampling tube. Each sample was replicated four times, and the samples were placed at  $105^{\circ}\text{C}$  for 72 hours until they reached a constant weight and were then dried to obtain the dry weight.

### Statistical Analyses

A completely randomized analysis of variance was performed to identify significant differences in soil depth profiles. Differences were validated using Tukey's test ( $P < 0.05$ ) (Brown and Forsythe, 1974; Steel and Torrie, 1980; Ott, 1993). The Kolmogorov-Smirnov test was performed to analyze the assumptions of normality and homogeneity of variances. The non-parametric Kruskal-Wallis test was used because in most of the data collection dates, the analysis of variance did not present equal assumptions (Ott, 1993). A correlation analysis was carried out between the xylem water potentials at pre-dawn and at noon with the moisture content in the soil, temperature, and relative humidity. Statistical analyzes were performed with the SPSS® (Statistical Package for the Social Sciences) software package (standard released version 22.0 for Windows) (IBM SPSS Statistics, 2013)

## RESULTS AND DISCUSSION

### Evaluation of Environmental Variables

The values of temperature, relative humidity, and precipitation are presented in Figure 1. During the study period, a precipitation accumulation of 280 mm with an average temperature of  $33^{\circ}\text{C}$  and 56% of relative humidity was recorded (Figure 1).

The values of the gravimetric soil water content different depth profiles, by the 12 sampling dates, showed that the maximum content was recorded in June 03 profile 40-50 cm depth with  $0.011 \text{ kg kg}^{-1}$ , the minimum content in November 04 profile 0-10 cm depth with  $0.001 \text{ kg kg}^{-1}$ , are presented in Figure 2. Results indicate that moisture content in the soil varied according to the incidence or absence of precipitation.

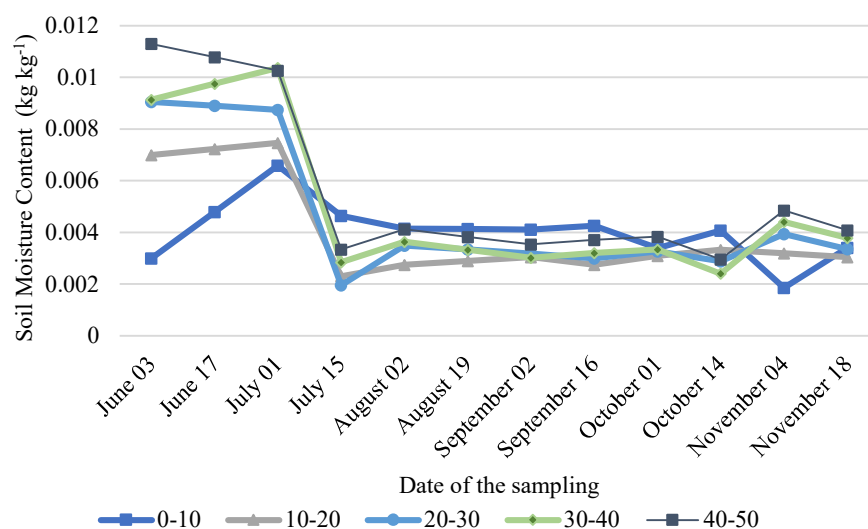


Figure 2. Gravimetric soil water content at five soil profile depths by the 12 sampling dates.

### Regulation of Stem Water Potential by the Sampling Dates

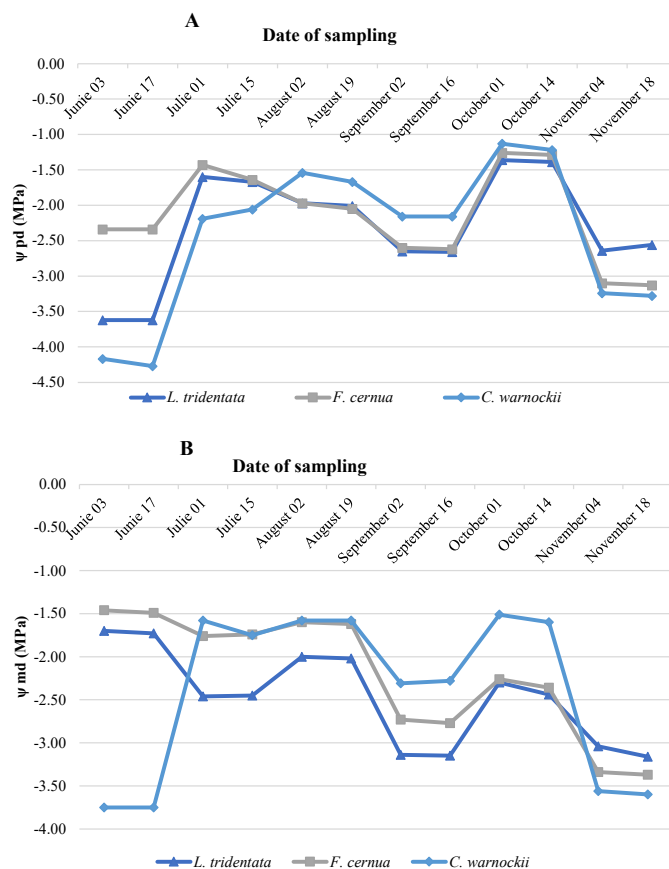
We found differences in the 12 sampling dates for the studied species (Figure 3 A and B; Table 2). During the wettest sampling dates, July to October, the highest values in the  $\Psi_{pd}$  (-1.13 MPa, respectively) were recorded in *C. warnockii*, however *F. cernua* and *L. tridentata* also maintained their  $\Psi_{pd}$  (-1.26 MPa and -1.36 MPa) and during the months with less rainfall (February, June and November), the shrub species faced severe water stress, for example, values of -2.4 MPa were detected in the  $\Psi_{pd}$  in *L. tridentata*, while the lowest values in the  $\Psi_{pd}$  were observed at -4.7 MPa *Condalia warnockii* and *Flourensia cernua* -3.2 MPa, this indicates that these species are more sensitive to the absence of water in the soil and the recovery of their potential is related to precipitation.

In July, the month of highest precipitation, the water potential at the midday presented the highest values and the the moisture content in the soil at depth 0-50 cm was 16% (Table 4), an average the  $\Psi_{md}$  was of -1.95 MPa of all the species; on the other hand, on the low rainfall sampling date (June) the species *C. warnockii* showed hydric potential -3.75 MPa at noon, with respect to *F. cernua*, which reached a maximum of -1.46 MPa and *L. tridentata* with a maximum of -1.70, while in November, in the absence of precipitation and a 7% of soil moisture, the species on average manifested potential hydric -3.35 MPa at noon (Table 3).

Table 2.  $\chi^2$  dates and results of Kruskal-Wallis test and  $\Psi_{pd}$  for each data collection day.

Data collection day	$X^2$	Significance	<i>L. tridentata</i>	<i>F. cernua</i>	<i>C. warnockii</i>
06-03-22	11.00	**	-3.62	-2.34	-4.17
06-17-22	12.26	**	-3.62	-2.34	-4.27
07-01-22	12.50	**	-1.60	-1.43	-2.19
07-15-22	9.40	**	-1.67	-1.64	-2.06
08-02-22	6.62	*	-1.97	-1.97	-1.54
08-19-22	9.38	**	-2.01	-2.05	-1.67
09-02-22	6.70	*	-2.65	-2.60	-2.16
09-16-22	9.40	**	-2.66	-2.62	-2.16
10-01-22	9.82	**	-1.36	-1.26	-1.13
10-14-22	6.05	*	-1.39	-1.29	-1.22
11-04-22	10.36	**	-2.64	-3.10	-3.24
11-18-22	10.22	**	-2.56	-3.13	-3.28

\* = statistical significance at 5%; \*\* = statistical significance at 1%.



**Figure 3. Documentation of water potential A ( $\Psi_{pd}$ ) and B ( $\Psi_{md}$ ) in three browse species.**

**Table 3.  $\chi^2$  dates and results of Kruskal-Wallis test and  $\Psi_{md}$  for each data collection day.**

Data collection day	$\chi^2$	Significance	<i>L. tridentata</i>	<i>F. cernua</i>	<i>C. warnockii</i>
06-03-22	12.02	**	-1.70	-1.46	-3.75
06-17-22	12.50	**	-1.73	-1.49	-3.75
07-01-22	12.02	**	-2.46	-1.76	-1.58
07-15-22	9.42	**	-2.45	-1.74	-1.75
08-02-22	9.56	**	-2.00	-1.60	-1.58
08-19-22	9.98	**	-2.02	-1.62	-1.58
09-02-22	12.50	**	-3.14	-2.73	-2.31
09-16-22	12.50	**	-3.15	-2.77	-2.28
10-01-22	9.88	**	-2.30	-2.26	-1.51
10-14-22	9.78	**	-2.44	-2.36	-1.60
11-04-22	11.29	**	-3.04	-3.34	-3.56
11-18-22	11.52	**	-3.16	-3.37	-3.60

\*\* = statistical significance at 1%.

On the sampling dates such as June 3 and 17 and August 2 and 19, an unusual response in  $\Psi$  was found, especially in the species *F. cernua* and *C. warnockii*, resulting in that the water potential  $\Psi$  was higher at noon than at pre-dawn, for a high content of solutes in the xylem which reduces the evapotranspiration of water in high ambient temperatures (Mirfenderesgi *et al.*, 2016) check in similar data an imbalance of the soil moisture content at pre-dawn and the leaves and stem in various mesophytic and xerophytic plants due to the apoplastic accumulation of solutes.

### Connection of the Leaf Water Potential and Environmental Conditions

*C. warnockii* showed correlated ( $P < 0.05$ ) between the relative humidity and soil moisture in the depth profiles of 20 to 50 cm, compared with the  $\Psi_{pd}$  of *F. cernua* and *L. tridentata* that showed a correlation not significant ( $P \geq 0.05$ ); similar results of adaptation of native shrubs to drought stress have been documented by other authors (Adhikari and White, 2014; González-Rodríguez, *et al.*, 2016; González-Rodríguez *et al.*, 2018).

This relation indicates that the higher the moisture in the soil, the browse species in the study raised the water potential values in the early morning, decreased at midday, and recovered at night due to the reduction in the rate of evapotranspiration.

Since the environmental conditions were the same, the differences presented in the water potential at noon can be attributed to the particular morphological and physiological characteristics of the species (Table 4) (Stienen, Smits, Reid, Landa, and Boerboom, 1989; Castro-Díez, Puyravaud, and Cornelissen, 2000; Bussotti *et al.*, 2002).

**Table 4. Spearman's correlation coefficients (n=12) for xylem water potential values before pre-dawn ( $\Psi_{pd}$ ) and at midday ( $\Psi_{md}$ ) with environmental variables.**

Environmental variable	Species		
	<i>L. tridentata</i>	<i>F. cernua</i>	<i>C. warnockii</i>
Water potential pre-dawn ( $\Psi_{pd}$ )			
Temperature	-0.37 ns	0.12 ns	-0.12 ns
Relativity humidity	0.49 ns	0.56 *	0.70 **
Precipitation	0.16 ns	0.36 ns	0.34 ns
Soil moisture content			
Depth of 0 to 10 cm	0.05 ns	0.25 ns	-0.03 ns
Depth of 10 to 20 cm	-0.08 ns	0.13 ns	-0.46 ns
Depth of 20 to 30 cm	-0.44 ns	-0.23 ns	-0.67 *
Depth of 30 to 40 cm	-0.33 ns	-0.21 ns	-0.68 *
Depth of 40 to 50 cm	-0.43 ns	-0.22 ns	-0.67 *
Total	-0.33 ns	-0.18 ns	-0.63 *
Water potential midday ( $\Psi_{md}$ )			
Temperature	0.65 *	0.80 **	-0.03 ns
Relativity humidity	0.02 ns	0.10 ns	0.80 **
Precipitation	0.07 ns	0.28 ns	0.51 ns
Soil moisture content			
Depth of 0 to 10 cm	0.03 ns	0.31 ns	0.20 ns
Depth of 10 to 20 cm	0.29 ns	0.19 ns	-0.25 ns
Depth of 20 to 30 cm	0.44 ns	0.42 ns	-0.38 ns
Depth of 30 to 40 cm	0.26 ns	0.28 ns	-0.30 ns
Depth of 40 to 50 cm	0.42 ns	0.41 ns	-0.37 ns
Total	0.34 ns	0.34 ns	-0.26 ns

\* = statistical significance at 5%; \*\* = statistical significance at 1%; ns = not significant.

Tree and shrub species that thrive in the world's major arid type ecosystems have been observed to acclimate to defined dry seasons. Presenting characteristics and elements inside the wood, such as the presence of pores, tracheids, and size and quantity of vessels that allow high transport efficiency when water is available, ensuring parallel maintenance of a hydraulic system during periods of drought (Lambers and Oliveira, 2019). In addition, authors document the connection of the water potential before dawn with the presence of the stomata in the leaves as a key role in the regulation of the water status of the plant (Litvak, McCarthy, and Pataki, 2012). The authors suggest that the presence of values away from one in the water potential of the leaf is due to the osmotic adjustment and connections with the water of the apoplast (Binks *et al.*, 2016). Hence, the importance of studying the water status of the plant species of the xerophytic scrub as a key step for the ecophysiological knowledge of the plants of the arid zones.

This indicates under humid conditions in the environment *L. tridentata* and *F. cernua* they are plants that have adapted to the little or no presence of moisture in the soil and maintain hydration of their tissues, on the other hand, adaptation of *Condalia warnockii* with the dry environment it depends on the strategies it develops to deal with the loss of internal moisture, hence the presence of more negative values in the xylem water potential (Mirfenderesgi *et al.*, 2016).

In the case of *L. tridentata*, studies show that the small leaves have a high surface-to-volume ratio, as well as a resinous layer that optimizes water conservation. When water availability is high, the leaflets are opened in the morning. Under these conditions, the plant consumes a lot of water, as exposure to photosynthetically active direct light increases. Therefore, the plants of this species can regulate, through the movement of the leaves, the use of water and the strategy of light interception, and allow the gooseberry to function as a water-conserving plant or as a plant. Consumer, without losing its leaves. This gives the species great plasticity in arid zones: a wide leaf opening can allow it to intercept abundant photosynthetically active radiation during periods of high water availability, while the closure of leaflets during hot and dry seasons allows it to reduce the direct radiation capture (Barbour, Diaz, and Breidenbach, 1974; Mirfenderesgi *et al.*, 2016) as well as the ability to reduce the number of stomata, which, as has been documented, is an important mechanism for reducing transpiration and regulating in the xylem water potential (Barbour *et al.*, 1974; Tognetti, Minnocci, Penuelas, Raschi, and Jones, 2000) the presence of resin in its leaves that allows it to make more efficient use of water and behave as an antiperspirant and remain dormant even in a desiccated state (Shultz, Meinzer, Wisdom, Gonzalez, and Rundel, 2016) in addition to a root system 170 cm down, branching laterally up to 4 m (Brinker, 1996).

## CONCLUSIONS

The species *L. tridentata* and *F. cernua* have adapted to low availability of water in the soil, tend to avoid tissue dehydration, while the adaptation of *C. warnockii* depends on morphological strategies that allow it to cope with mechanisms such as the reduction of the leaf surface or its deciduousness (Whittaker *et al.*, 1995). In conclusion, *L. tridentata* is classified as a species that withstands drought, *F. cernua* as a resistant species, and *C. warnockii* is the most susceptible species as a drought avoider (Shantz and Zon, 1956) because it cannot exert limited control over water that is lost through perspiration, and it has a shallow root system.

Even though the species behave differently during the drought period, they play an important role in the maintenance of the productivity of semiarid ecosystems, and have adapted to the low availability of soil water, so they should be considered potential species for xerophytic scrub restoration practices.

## ETHICS STATEMENT

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF SUPPORTING DATA

Not applicable.



## COMPETING INTERESTS

The authors declare that they have no competing interests.

## FINANCING

Not applicable.

## AUTHORS' CONTRIBUTIONS

Conceptualization: M.G. Methodology: M.G., L.R., and H.G. Investigation: M.G., and L.R. Resources: M.G., L.S., and H.G. Writing, original draft preparation: M.G., and L.R. Writing, review and editing: M.G., N.M., and L.S.

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