Some Secrets of *Argania spinosa* Water Economy in a Semiarid Climate

Mari Cruz Díaz Barradas[a], María Zunzunegui[a], Mari Paz Esquivias[a], Said Boutaleb[b], Javier Valera-Burgos[a], Tarek Tagma[b] and Fátima Ain-Lhout[c]

[a]*Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Spain*

[b]*Département de Géologie, Faculté des Sciences, Université Ibn Zohr, Morocco*

[c]*Faculté Polydisciplinaire de Taroudant, Université Ibn Zohr, Morocco*

diaz@us.es

Argania spinosa is an example of an avoider tree growing under semi-arid conditions in Morocco. To assess what are the physiological strategies of this species, different variables were measured through an annual cycle in two populations located in the species' main distribution area. Results show the expected decrease of leaf water potential (Ψ) with an increase of water-use efficiency (A/g_s) with the onset of the dry season. In summer, leaf conductance (g_s) was sensitive to vapour pressure deficit (VPD), and stomatal closure occurred over 30 mbar of VPD. Surprisingly, carbon isotope discrimination ($\delta^{13}C$) maintained very low values over the year, with almost no relationship with any physiological or morphological variable. Hence *Argania spinosa* presents a complex set of mechanisms to avoid water deficit, but $\delta^{13}C$ cannot be used as an ecological tracer of long term WUE.

Keywords: Drought adaptation, Carbon isotope discrimination, Water use efficiency (WUE).

Dryland forests and woodlands account for some 6% of the world's forests. The argan tree [*Argania spinosa* (L.) Skeels] is an example of a sustainable resource in a semiarid climate [1]. Argan oil, obtained from the seeds, is manufactured by women cooperatives and has become famous in the last two decades for its cosmetic virtues [1-3]. Goats and sheep graze on argan leaves as elevated grasslands, especially during the dry season [4]. Manures produced from animals' wastes are used as a fertilizer for cereal crops growing under the canopy of the argan trees [5]. In addition, as argan wood is very hard, it can be used as fuel or building of agricultural tools. Furthermore, old trees have spiritual value and are linked to sacred places [6].

On the other hand, biodiversity conservation, the regulation of the hydrological cycle, soil protection and carbon fixation are examples of services generated by these ecosystems and can be defined as cultural landscapes.

A. spinosa is endemic to Morocco and grows in areas ranging from lower than 100 to 300 mm of annual rainfall, maintaining an elevated productivity, only shedding leaves for a variable time under extremely dry conditions [4]. The aim of this contribution is to explain some of the water economy peculiarities of this tree in its natural habitat. For this purpose we have used some physiological measurements and carbon isotope discrimination ($\delta^{13}C$) that is widely accepted as an index of long-term WUE [7]. In particular, we try to answer the following questions: (1) what are the seasonal and daily changes in plant and leaf water status and stomatal behaviour in *A. spinosa*? and (2) whether carbon isotope discrimination could be used as an integrative ecological tracer to evaluate long term WUE in this semiarid species?

In two *A. spinosa* populations in the neighbourhood of Agadir, (main distribution area of the species) detailed seasonal-ecophysiological measurements were performed during the 2010 annual cycle. One was a coastal population with a rocky soil facing

the Atlantic Ocean, and the other was located inland, 5 km to the south of Agadir, in an area traditionally managed for livestock raising, with a well developed soil belonging to the Souss river basin [8].

The climate is temperate, due to marine influence, with maximum temperatures only seldom surpassing 30ºC, and minimum temperatures never below 7ºC. Annual rainfall is scarce, 232 mm, and concentrated from October to April, with a long summer drought. Relative humidity is elevated, with frequent fogs during the summer period.
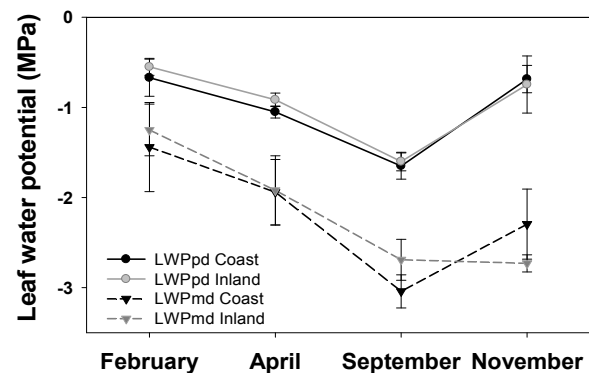

Figure 1: Average predawn and midday leaf water potential of *A. spinosa* trees in the two sampling zones throughout the study period. Each bar represents ±sd.

Leaf water potentials (Ψ) of trees were high during the mild winter conditions; figures decline through spring and markedly in summer, reaching the lowest values in September. Water potentials recovered in autumn, except for Ψ_{md} in the inland zone, exhibiting similar low values in November as in September (Figure 1). The coastal population showed lower Ψ_{md} values than the inland population in February and September, but with no significant differences ($P>0.05$).

In the coastal zone, intrinsic water use efficiency (WUE$_i$=A/g$_s$) was elevated in April and September, while in the inland population the highest values were reached in September. However, significant difference between the two populations was only found in April ($P<0.001$) (Figure 2).
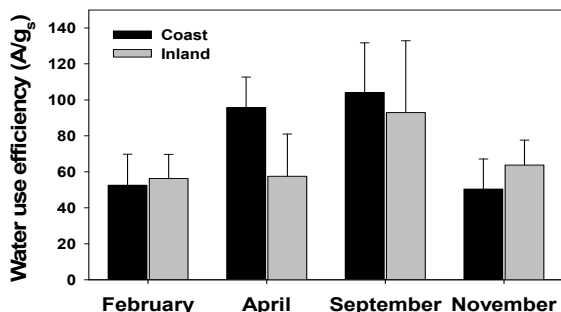

Figure 2: Average intrinsic WUE (A/g$_s$) in the two sampling zones throughout the study period. Each bar represents ±sd.

A/g$_s$ was correlated with c$_i$/c$_a$ (r=-0.91, $P<0.001$), which indicated a strong dependence of stomatal control. Under summer conditions, leaf conductance seemed to respond to the increase in VPD; when it reached values over 20 mbar, there was a fast decrease in stomatal conductance, with a complete stomatal closure over 30 mbar of VPD (Figure 3).
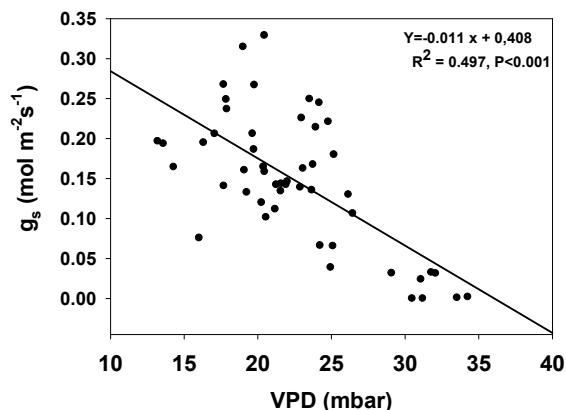

Figure 3: Relationship between Vapour Pressure Deficit (VPD in mbar) and stomatal conductance (g$_s$ in mol m^{-2}s^{-1}) in a summer day, and linear regression between both variables. The points indicate measurements of individual trees in the morning and midday.
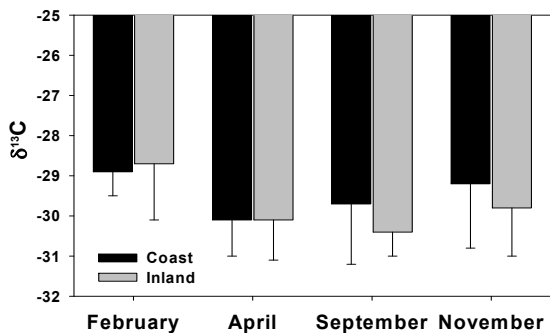

Figure 4: Average carbon isotope discrimination (δ^{13}C in ‰) in the two sampling zones throughout the study period. Each bar represents ±sd.

The large seasonal changes in water availability were not clearly reflected in seasonal changes in δ^{13}C. In both populations, values

ranged between -28.5 ‰ in February to -30 ‰ for the rest of the year. Significant differences were found between February and April, and February and September values in the inland population ($p<0.05$), while in the coastal population significant differences only occurred between the February and April data ($P<0.01$) (Figure 4).

Leaf Mass Area (g m^{-2}) showed a clear increase during the study period, from February (70-100 g m^{-2}) to November, (165-170 g m^{-2}), which might be associated with leaf longevity. The coastal population presented significantly higher values than the inland population throughout the study period ($P<0.05$), except for November (Figure 5).
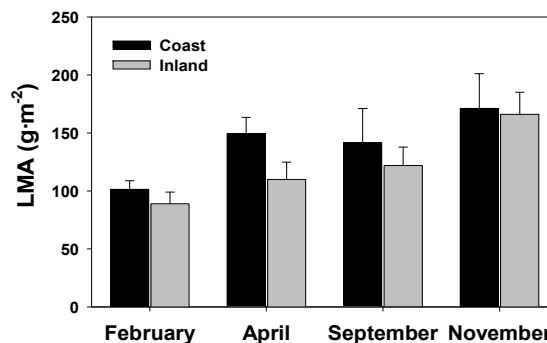

Figure 5: Average leaf mass area (LMA in g m^{-2}) in the two sampling zones throughout the study period. Each bar represents ±sd.

Only a weak correlation was found between Ψ_{pd}, Ψ_{md} and δ^{13}C ($P<0.05$ in inland population). In all other cases, no significant relationships were found between either physiological or morphological variables and δ^{13}C, with only partial relations in a specific month (Table 1). For example, δ^{13}C was correlated with N content (%) in February ($P<0.05$).

Table 1: Pearson correlation coefficients between δ^{13}C and different physiological (Ψ_{pd}, Ψ_{md}, N%, A/g$_s$) and morphological (LMA) variables in coastal and inland *A. spinosa* populations.

	Coastal	Inland
Ψ_{pd}	0.231	0.434*
Ψ_{md}	0.174	0.442*
N%	0.302	0.266
A/g$_s$	-0.281	-0.264
LMA	-0.027	-0.154

*Significant correlation ($P<0.05$).

The results of this study show that *Argania spinosa* adjusts its physiological attributes according to environmental conditions, both on a daily and seasonal scale.

Over the course of the day, following radiation and temperature maxima, Ψ showed more negative values in all months at midday, as has been reported in Mediterranean species [9-10].

Leaf water potential values exhibited minimum values in summer, as shown in a previous study [4]. Even though the absence of rainfall and the elevated summer temperatures occurring at the beginning of September (due to air masses from the Sahara), Ψ_{md} never decreased below -3 MPa, while in a mountain population summer Ψ_{md} could reach -6 MPa [4]. Our results of Ψ_{pd} and Ψ_{md} in this study are similar to those reported for *O. europaea* without irrigation under Mediterranean climate [11].

The seasonal increase of WUEi in spring and summer might be associated with either an increase in photosynthetic assimilation or

a decrease in stomatal conductance. Previous results have shown a linear relationship between A and g_s in *A. spinosa* [4], but in summer the increase in WUEi is mainly associated with stomatal control, which is evidenced by the strong relation between A/g_s and c_i/c_a. Our results suggest that the stomata were responsive to VPD, although we cannot discard a possible role of chemical or hydraulic signals coming from the roots. In olive trees it is widely accepted that VPD is the main driving mechanism for g_s control [12].

The results of carbon isotope discrimination suggested differences in the process of leaf maturation; young *A. spinosa* leaves in February had a less negative $\delta^{13}C$ than mature leaves in the rest of the year; similar results have been reported for *Quercus pubescens* [7]. The existence of a significant correlation between $\delta^{13}C$ and N content in February supports the hypothesis that high leaf N concentrations lead to increase photosynthetic capacity during the period of leaf development [13].

In mature leaves, most leaf carbon is formed during periods of good water status, presenting in all cases elevated isotope discrimination. On the other hand, leaves are always very soft, with LMA lower than 170 g m^{-2} in summer, which indicated high internal leaf conductance and subsequently high discrimination at the site of carboxylation [14]. Hence the leaves of *A. spinosa* are not very hard, but maintain elevated stomatal control, a high photosynthetic activity during favourable periods [4], and probably a short lifespan.

In Mediterranean scrub species [15] found a higher correlation between carbon isotope discrimination and leaf water potential in drought semi-deciduous species that present a shorter leaf lifespan than in evergreen sclerophylls. In *A. spinosa* we found soft leaves, in which most carbon is assimilated during the favourable season, but not showing a clear relationship with leaf water potential, although this tree is able to assimilate carbon during the early hours of the dry season, maintaining an elevated productivity.

Experimental

General: In both populations, 10 well developed trees were permanently marked, and measurements of gas-exchange and leaf water potential were taken in the field and leaf samples were collected for leaf mass area and carbon and nitrogen content and stable isotopes measurements. Sampling was carried out during different phases of the seasonal cycle and under contrasted environmental conditions in February, April, September and November.

Measurements: Measurement of net photosynthetic rates (A), stomatal conductance (g_s) and transpiration rates (E) were made using a portable compact CO_2/H_2O system (LCi-portable photosynthesis ADC system, UK). Intrinsic water use efficiency (WUEi) was calculated as the ratio of net photosynthesis to stomatal conductance (WUEi=A/gs). Leaf surface was computed in the laboratory (Midebmp, Ordiales, 2000), and gas exchange measurements were recalculated for leaf surfaces. Leaf Mass Area was calculated as the ratio between leaf dry weight and leaf surface (g m^{-2}). Gas-exchange measurements were made between 08.30 and 10.30 hours solar time, and repeated between 12 and 12.30 h to evaluate possible midday depression of leaf conductance. VPD values were calculated using air temperature and moisture content data from our LCi, following the method of Coombs *et al.* [16].

Predawn (Ψ_{pd}) and midday leaf water potential (Ψ_{md}) were determined in the field with a Scholander type pressure chamber (Manofrigido, Lisbon, Portugal) using terminal shoots. Sun exposed leaf samples were collected from the same trees, oven-dried at 60ºC for 48 h, and milled to fine powder for isotopic analysis using a ball mill (Retsch, Haan, Germany). Leaf N and C concentrations and $\delta^{15}N$ and $\delta^{13}C$ signatures of the ground leaf material were determined in separate runs for N and C using an elemental analyzer, and coupled with a continuous-flow isotope ratio mass spectrometer. For detailed methodology see [15].

Statistical evaluation: Normal distribution of the variables was tested by the Kolmogorov-Smirnov test. One-way ANOVAs were applied to compare significant differences between sites for any of the studied variables. Bivariate correlation tests were made between the different variables. Regression analyses were performed between correlated variables. All statistical tests were made with SPSS 18 for Windows.

References

[1]   Charrouf Z, Guillaume D. (**2008**) Argan oil, functional food, and the sustainable development of the argan forest. *Natural Product Communications*, *3*, 283-288.
[2]   Charrouf Z, El Hamchi H, Mallia S, Licitra G, Guillaume D. (**2006**) Influence of roasting and seed collection on argan oil odorant composition. *Natural Product Communications*, *1*, 399-403.
[3]   Lybbert TJ, Magnan N, Aboudrare A. (**2010**) Household and local forest impacts of Morocco's argan oil bonanza. *Environment and Development Economics*, *15*, 439-464.
[4]   Díaz Barradas MC, Zunzunegui M, Ain-Lhout F, Jáuregui J, Boutaleb S, Alvarez-Cansino L, Esquivias MP. (**2010**) Seasonal physiological response of *Argania spinosa* tree from Mediterranean to semi-arid climate. *Plant Soil*, *337*, 217-231.
[5]   de Waroux Y, Lambin EF. (**2012**) Monitoring degradation in arid and semi-arid forests and woodlands: the case of the argan woodlands (Morocco). *Applied Geography*, *32*, 777-786.
[6]   Simenel R. (**2004**) De la fôret du santau pâturage de chrétiens: Perception du paisaje et gestión du couvert vegétal chez les Ait Barman du Sud Marocain. *Cahiers Recherche du CJB*, *1*, 119-133.
[7]   Damesin C. (**1998**) Seasonal and annual changes in leaf $\delta^{13}C$ in two co-occurring Mediterranean oaks: relations to leaf growth and drought progression. *Functional Ecology*, *12*, 778-785.
[8]   Dindane K, Bouchaou L, Hsissou Y, Krimissa M. (**2003**) Hydrochemical and isotopic characteristics of groundwater in the Souss Upstream Basin, southwestern Morocco. *Journal of African Earth Science*, *36*, 315–327.
[9]   Gratani L, Varone L. (**2004**) Adaptive photosynthetic strategies of the Mediterranean maquis species according to their origin. *Photosynthetica*, *42*, 551–558.
[10]   Alvarez-Cansino L, Zunzunegui, M., Díaz Barradas, MC, Esquivias, MP. (**2010**) Physiological performance and xylem water isotopic composition underlie gender-specific responses in the dioecious shrub *Corema album*. *Physiologia Plantarum*, *140*, 32-45.
[11]   Torres Ruiz JM. (**2012**) Influencia del régimen hídrico en la funcionalidad hidráulica del olivo. Ph D Thesis, University of Seville.
[12]   Fernández JE, Moreno F. (**1999**) Water use by the olive tree. *Journal of Crop Production*, *2*, 101-162.

[13] Sparks JP, Ehleringer JR. (**1997**) Leaf carbon isotope discrimination and nitrogen content for riparian trees along elevational transects. *Oecologia 109*, 362-367.

[14] Warren CR, Adams MA. (**2006**) Internal conductance does not scale with photosynthetic capacity: implications for carbon isotope discrimination and the economics of water and nitrogen use in photosynthesis. *Plant, Cell and Environment*, *29*, 192-201.

[15] Máguas C, Werner C. (**2010**) Carbon isotope discrimination as a tracer of functional traits in a Mediterranean macchia plant community. *Functional Plant Biology*, *37*, 467-477.

[16] Coombs J, Hind G, Leegood RC, Tieszen LL, Vonshak A. (**1985**) Analytical techniques. In *Techniques in bioproductivity and photosynthesis*. Coombs J, Hall DO, Long SP, Scurlock JM. (Eds). Pergamon Press, Oxford. 223–224.