DOI: 10.1007/s11099-018-0810-z PHOTOSYNTHETICA 56 (4): 1177-1187, 2018

Relationship between photosynthetic rate, water use and leaf structure in desert annual and perennial forbs differing in their growth

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Abstract

Specific leaf area (SLA) is a key trait to screen plants for ecological performance and productivity; however, the relationship between SLA and photosynthesis is not always up-scalable to growth when comparing multiple species with different life cycles. We explored leaf anatomy in annual and perennial species of *Physaria*, and related it to photosynthesis and water loss. The annual *Physaria gracilis* had higher SLA, thinner leaves, and lower investment in protective tissues, than perennial *P. mendocina. Physaria angustifolia* (annual), and *P. pinetorum* (perennial) showed intermediate values. Both perennials had a thicker palisade and high photosynthesis, relative to annuals. The larger leaf veins of perennials should allow high water availability to the mesophyll. The thicker palisade should determine high resistance to water flow and help explain their high water-use efficiency. These leaf functions reflect the construction of long-lived leaves that efficiently use resources under environmental limitations of arid environments.

Additional keywords: leaf anatomy; *Physaria*; relative growth rate; specific leaf area; total biomass; xylem anatomy.

Introduction

Leaf structure and morphology is characterized by traits, such as SLA (the ratio between leaf area and leaf dry mass), and plays an important role in leaf and plant functioning including processes, such as gas exchange, relative growth rate, plant survival, and litter decomposability (Lambers and Poorter 1992, Shipley *et al.* 2005, Poorter *et al.* 2009). Specific leaf area has, therefore, often been used as a tool to screen species for their productivity (White and Montes 2005) or ecological performance (Diaz *et al.* 2004). Besides, the SLA of a species is a good indicator of the position of that species along an axis based on resources acquisition (Westoby *et al.* 2002, Wright *et al.* 2004). This fact is a consequence of multiple trade-offs between the requirements of a leaf to perform its primary function, photosynthesis, and a suite of ¨constraints¨, such as the need for mechanical support, prevention of water loss, regulation of energy exchange, and defense (Givnish 1986, Niklas 1999), which lead to suboptimal returns on C fixation (Shipley *et al.* 2005).

Leaves of multiple species can be classified along a continuum between high SLA at one end, which are those capable of fast resource acquisition, fast metabolic rate (high rates of photosynthesis and respiration), and plant growth (high relative growth rate, RGR), and low SLA at the other, which provides resource conservation and persistence (low RGR; Wright *et al.* 2004, Poorter *et al.* 2009). However, these links between anatomy and performance still lack a thorough understanding of the functional relationships between morphological features, such as SLA and anatomical structure, and physiological traits. Despite its physiological and ecological relevance, the traits, which underlie the variation in SLA in forbs species, are still poorly understood.

Within the genus *Physaria* (*syn Lesquerella*; Brassicaceae), it has been found that total biomass production, a key character for reproductive effort, is determined mainly by the structure of its leaves or specific leaf area (González-Paleo and Ravetta 2011a, 2015). The annual species *P. gracilis*, which showed an acquisitive strategy and high biomass production, grew faster and had higher SLA, but had lower photosynthetic capacity per area (P_{Narea}) than the perennial *P. mendocina*, which had higher photosynthesis but lower biomass production. This higher photosynthetic capacity in leaves of perennial species coupled with a conservative growth strategy has been reported in other experimental systems in which

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Received 9 August 2016, *accepted* 26 June 2017, *published as online-first* 12 April 2018.

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Abbreviations: *E* – transpiration rate; P_{Narea} – photosynthetic rate per unit leaf area; P_{Nmass} – photosynthetic rate per unit leaf mass; RGR – relative growth rate; SLA – specific leaf area; WUE – water-use efficiency.

Acknowledgements: This study was funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT 2011 Nº 0598) and CONICET (PIP 11220110100780). The first author held a postdoctoral scholarship from CONICET during the time this work was done.

co-generic annual and perennial species have been tested together (Ploschuk *et al.* 2001, Jaikumar *et al.* 2013). However, other studies on grass species have shown that leaves of fast-growing annuals were slightly thinner, had higher photosynthetic capacity, and were less protected than those of less productive perennial species (Garnier 1992, Garnier and Laurent 1994, Garnier *et al.* 1997). These contrasting results show the lack of consistency in studies that test the relationship among anatomical and morphological features of leaf and whole plant growth.

The analysis of how variations in SLA are related to changes in leaf anatomy, and how this determines carbon gain (CO_2 assimilation rate, P_N) and water loss (transpiration rate, E) is an active area of research in ecophysiology (Castro-Díez *et al.* 2000, Meziane and Shipley 2001, Mediavilla *et al.* 2001, Niinemets 2015, de la Riva *et al.* 2016). For example, a thick leaf (low SLA) can be the result of a large fraction of mesophyll, or a high proportion of lignified tissues, that may be interpreted as having different consequences for leaf productivity and thereby for plant growth and survival (Reich *et al.* 1999, Meziane and Shipley 2001, Alvarez-Clare and Kitajima 2007). If a leaf is thick (low SLA) because of a high proportion of mesophyll, the relationship between SLA and photosynthesis could be negative.

Within groups of diverse plants, negative interspecific relationships have sometimes been observed between leaf mass area (the inverse of SLA), and photosynthetic rate (P_{Nmass}; Enríquez *et al.* 1996, Niinemets 1999, Garnier *et al.* 2001) and plant growth (Poorter 1989, Nielsen *et al.* 1996, Westoby *et al.* 2002). Some authors, therefore, have proposed that SLA is a good proxy to select for high-yielding genotypes (White and Montes 2005, Long *et al.* 2006, Zhu *et al.* 2010). However, the relationships between leaf structural and functional variables reported in the literature are contradictory (*i.e.*, positive, negative or null relationships between photosynthetic rate and SLA have been reported; Niinemets 1999). As a result, features, such as tissue-specific rates of photosynthesis, have undergone relatively little change with selection to increase plant productivity and crop yield (Evans 1993, Jackson and Koch 1997).

An additional morphological feature, little explored in forbs, is the degree of coordination between photosynthetic systems and hydraulic capacity (Brodribb *et al.* 2002). Water transport from the soil to the leaves is crucial for plant performance, as CO_2 fixation is largely dependent on the supply of water to the leaves. Thus, species with a higher leaf water loss need a larger capacity to supply water (Sperry 2000, Maseda and Fernández 2006). The understanding of hydraulic properties of the xylem may help explain the variations in leaf physiology (CO_2 assimilation rate, evapotranspiration rate, and water-use efficiency, WUE; Meinzer and Goldstein 1996), and variations in a suite of leaf-level traits reflecting how plants balance photosynthetic carbon gain with water loss (Santiago *et al.* 2004).

Here, we explored leaf and xylem anatomical traits in two annual and two perennial species of *Physaria* that conform a gradient between acquisitive and conservative carbon-use strategies (*P. gracilis*, *P. angustifolia*, *P. pinetorum*, and *P. mendocina*, from the most acquisitive to most conservative strategy). Some species of this genus, including these two perennials species, are in the incipient process of domestication as potential oilseed crop for arid lands (Dierig *et al.* 1993, González-Paleo and Ravetta 2011b, Masnatta and Ravetta 2011, Pastor-Pastor *et al.* 2015). In these low-resource environments, yields are often highly variable, and so, the main breeding objective is seed-yield stability and plant survival to minimize the likelihood of crop failures (Ceccarelli *et al.* 1991). In this context, additional eco-physiological criteria to the seed yield would be useful to provide a conservative use of nutrients and water, and a greater stress tolerance.

We related anatomical and structural traits to differences in carbon gain (P_{Narea} and P_{Nmass}) and water loss (E and WUE), and leaf life span. We proposed and discussed a functional model as a framework to link leaf anatomy with leaf physiology and whole plant carbon and water economy, in the context of plant productivity in arid lands, and chances for its improvement. We expected that leaves of annual, acquisitive species (with high total biomass and high SLA) should have a lower investment in protective tissues (epidermis, cuticle, and sclerenchyma), when compared to leaves of perennial, conservative species (with low total biomass and SLA) with high proportion of epidermis, cuticle, and sclerenchyma. These characters are major determinants of longer leaf longevity. Also, differences in carbon-use strategies should be related to leaf physiology and anatomy. Species with high CO_2 assimilation rate should have a higher proportion of mesophyll, and lower water-use efficiency.

Materials and methods

Species and growth conditions: We carried out a field experiment in Chubut, Argentina (43º14´S, 65º18´O). The environment, where experiment was carried out, is semiarid with a Mediterranean climate (FAO, 2010). The Mediterranean-type climate has a notable wet season during fall and winter, while summers are hot and dry. During the year of our experiment, the mean annual precipitation was 130.7 mm, the mean temperature was 13.9ºC, the mean maximum temperature was 21.4ºC, and the mean minimum temperature was 6.3ºC. The PPFD in this environment is between 2,100–2,500 μmol(photon) m^{-2} s^{-1}.

Four species of wild *Physaria* (syn *Lesquerella,* Brassicaceae), two perennials: *P. mendocina* (Phil.) Kurtz (La Pampa, Argentina) and *P. pinetorum* Wooton & Standley (Arizona), and two annuals: *P. angustifolia* Wats

(Oklahoma) and *P. gracilis* Wats (Oklahoma), were included in the experiment. This set of species differs in their C-use strategies. The more acquisitive annual, *P. gracilis* has a plant strategy characterized by high productivity and leaves with high SLA. The most conservative perennial, *P. mendocina*, shows a set of attributes, which provide drought tolerance (high allocation to belowground, and long-lived leaves with low SLA). The annual *P. angustifolia* and the perennial *P. pinetorum* have intermediate plant strategies (*i.e.*, intermediate values of traits typically found in plants with either annual or perennial life cycles; González-Paleo and Ravetta 2011a, 2015). The genus *Physaria* has been used as a model for the understanding of the domestication of new oil-seed crops for arid lands (Dierig *et al.* 1993, Ravetta and Soriano 1998). The comparison of annual and perennial species differing in C-use strategies is not only relevant for the understanding of intrinsic differences in leaf economic traits between annual and perennial desert forbs, but could also help sort out a current debate on the agroecological usefulness of morpho-physiological criteria for the selection of perennial species in low resource ecosystems (Pimentel *et al.* 2012).

For this experiment, seeds were sown on 3 February 2011 in germination trays filled with top soil and peat moss (1:1, v/v) and maintained in a greenhouse until they were transplanted to the field (after 60-d germination). The experimental design had one factor (species) that was randomized completely in the experimental units. Experimental units within this common garden consisted in 24 plots (1.5 m × 0.9 m) each of 10 individual plants. We randomly assigned six plots to each of the four species (six replicates per species). Plant density was 23 plants m^{-2} (0.3 m between rows, 0.15 m between plants). Density was low enough to avoid intraspecific competition and detrimental effects on final individual biomass and reproductive output (Brahim *et al.* 1998). Weed control was done by hand pulling. The top soil is clay–loam with less than 0.5% of organic matter.

Gas exchange: Photosynthetic (P_{Narea}) and transpiration (E) rates were measured before plant harvest (12 October 2011) with an *ADC-LCA4* portable gas-exchange system (Hodegsdon, UK), at ambient CO_2 concentrations, and saturating PPFD. Mean air temperature and humidity were 25.3ºC and 76%, respectively. CO_2 concentration was 395 ppm. Measurements were taken from 10:00 to 14:00 h on completely expanded, current-year leaves on six individual plants of each species (one plant per plot, six replicates). Plants were at the stage of fructification. The ratio P_{Narea}/E (assimilation per unit area/transpiration per unit area) was taken as an estimate of water-use efficiency (WUE). Plants used for gas-exchange measurements were harvested and transported to the laboratory for measurement of leaf area, and for anatomical determinations. With this procedure, the anatomy of each individual leaf could be related to the gas-exchange rates of the same individual.

Total biomass and specific leaf area: Plants were harvested when they reached the ontogenetic stage of fructification (late October; one plant per plot; six replicates per species). Total leaf area per plant was determined using *UTHSCSA Image Tool for Windows, version 2.02*. After scanning, plant samples were dried at 70°C for 48 h and weighed to obtain total biomass and leaf dry mass. These data were used to calculate specific leaf area (SLA, cm^2 of total leaf area per g of leaf dry mass).

Roots were sampled using a core of similar diameter of the above-ground rosette, and only the tap root was used for anatomical measurements.

Leaf longevity was measured after the winter dormancy, on one leaf per plant of one plant per plot. Recently emerged leaves (within one day of emergence) were marked in late winter (15 September), and monitored weekly. A leaf was considered dead when more than 50% of leaf lamina was chlorotic (dark yellow). Leaf longevity was expressed in weeks, from leaf appearance to leaf senescence.

Anatomical measurements: Of each harvested plant (six individual plant per species for leaf anatomy and four individual plant per tap root anatomy), three completely expanded leaves per plant, and three tap root samples from the same plant were fixed in formamide:acetic acid: alcohol (1:1:8, v/v) mixture and used for anatomical measurements. The dehydration and embedding protocol was performed according to Jackson (1991). Tissue dehydration was carried out in an ascending alcohol series (50% alcohol for 1/2 h, 70% alcohol for 1/2 h, 90% alcohol for 1 h, and 100% for 1 h), then an ascending alcohol–xylol series. Dehydrated leaves were included in *Paraplast Plus* ®, sectioned with a rotation microtome (10–12 μm), and stained with safranin-G and fast green. The anatomical parameters were measured on cross-sections, in the position of the leaf main vein under the microscope (*Nikon Photolab II*, Japan; Fig. 1S, *supplement available online*). The following anatomical parameters were measured: total thickness (excluding hairs) and thickness of palisade, spongy mesophyll, sclerenchyma, mean vein leaf, cuticle and epidermal layers, diameter of xylem vessel root and central cylinder.

The mean values of all the anatomical measurements were calculated per leaf or root, and subsequently averaged for each individual plant of each plot. The mean values of each tissue were expressed in two ways: the absolute volume of each tissue per unit leaf area (VA, also denoted as the thickness of each tissue) and the volume fraction (the proportion of the leaf section occupied by the different tissues).

Statistical analysis: One way-analysis of variance (*ANOVA*) was performed to determine significant differences between species (factor) for all measured anatomical and physiological traits. *Tukey*′s test was used for post-hoc comparisons of means. Using the data of six individual plants (one per plot) of each of the four species ($n = 24$), a path analysis was performed to evaluate the direct and indirect functional relationships between anatomical traits, specific leaf area, and physiological parameters. The full path model was constructed using as a base the models proposed by Meziane and Shipley (1999), Evans and Poorter (2001), and Shipley *et al.* (2005), and is shown in Fig. 1. Specific leaf area can be broken into the average leaf thickness and the tissue density of the leaf. Particularly, leaf thickness is negatively related to SLA, and plays an important role in leaf and plant functioning and is related to species' strategies of resource acquisition and use (Shipley *et al.* 2005). Also, Field and Mooney (1986) reported interspecific positive relations between SLA and CO_2 assimilation rate and transpiration. On the other hand, to understand functional variations in SLA it is necessary to consider the different anatomical tissues that shape the leaves. Specific leaf area has been found to be positively correlated with the proportional thickness of mesophyll, and negatively related with the thickness of the epidermis, vascular tissues or sclerenchyma (Meziane and Shipley 1999). We also considered the trade-off between productive (palisade and spongy mesophyll) and protective tissues (epidermis and sclerenchyma) that are shown in the model through a double arrow (Garnier and Laurent 1994). Under this background theory, the full path model in Fig. 1 assumes that tissue components, such as sclerenchyma, palisade mesophyll, spongy mesophyll, and epidermis, could be the direct cause of differences in SLA and in leaf physiology (P_{Narea} and E), or that they could cause indirect changes in SLA through their effects on leaf thickness. The full model tested using this model includes every one of the 21 paths shown in Fig. 1. This Path model was tested using the maximum likelihood Chi-squared statistic with the program package *AMOS* (Arbuckle and Wothke 1999). Data that contradict the predicted patterns of covariance, and therefore the hypothesized causal structure of the data, produces a significant Chi-squared value, indicating that the model must be rejected. A well-fitting model produces a non-significant Chi-squared value (Shipley 2002a).

We also tested the relationships of SLA with total leaf thickness, thickness of each tissue component (*i.e.*, epidermis, spongy, palisade, and sclerenchyma) and gas-exchange traits (*i.e.*, P_{Narea} and E) using linear regressions. We evaluated the relationships between the conductive capacity of the xylem, and the water-use-efficiency using linear regression.

Results

Morpho-physiological and anatomical differences between species: We found differences in leaf morphology and biomass between species. The perennial *Physaria mendocina* had lower total biomass and lower SLA than the annual *P. gracilis*. The other two species, *P. angustifolia* and *P. pinetorum*, had intermediate values of total biomass and SLA, between these two extremes (Table 1). Both perennial species had higher CO_2 assimilation rate per unit area (P_{Narea}), lower E, and higher WUE than the two annual species (Table 1). Carbon gain on a mass basis (P_{Nmass}) did not differ between species. The leaves of both annuals species had lower longevity in relation to that of the perennials (Table 1).

Cuticle and epidermis thickness followed a similar pattern as the one found for SLA: *P. mendocina* had the thickest cuticles and epidermis and *P. gracilis* the thinnest, while cuticle and epidermis thickness were intermediate for the other two species (Table 2). The thickness of the mesophyll varied between species, and was thinner in *P. gracilis* and *P. angustifolia* than that in *P. pinetorum* and *P. mendocina*. The variation observed in mesophyll thickness was mainly due to the proportion of palisade

Table 1. Total biomass, leaf longevity, specific leaf area (SLA), leaf gas exchange (P_{Narea} – CO_2 assimilation rate per unit area; P_{Nmass} – CO_2 assimilation rate per unit leaf mass), transpiration rate (E), and water-use efficiency (WUE) of four species of *Physaria*, two annuals (*P. gracilis* and *P. angustifolia)*, and two perennials (*P. pinetorum* and *P. mendocina)*. Data are means ± SE. $n = 6$, $\mathrm{df}_{\mathrm{error}} = 20$. For each trait *different letter* indicate that means of species are statistically different at ** $p<0.01$ and *** $p<0.001$; ns – insignificant differences.

Parameter	Annuals *P. gracilis*	*P. angustifolia*	Perennials *P. pinetorum*	*P. mendocina*	F value
Total biomass [g]	11.2 ± 0.3^{a}	9.4 ± 0.5^{ab}	8.8 ± 0.4^{ab}	6.2 ± 0.3^{b}	8.3^{**}
Leaf longevity [week]	9.6 ± 1.5^{b}	11.3 ± 2.3^{b}	23.8 ± 2.8^{a}	25.3 ± 3.2^{a}	21.8^{**}
SLA [cm^2 g^{-1}]	115.9 ± 5.5^{a}	102.9 ± 3.7^{ab}	94.9 ± 14.9^{ab}	61.4 ± 4.5^{b}	7.5^{**}
P_{Narea} [µmol(CO_2) m^{-2} s^{-1}]	6.8 ± 0.52^{b}	6.93 ± 0.48^{b}	9.24 ± 1.25^{a}	10.24 ± 1.25^{a}	5.2^{**}
P_{Nmass} [nmol(CO_2) g^{-1} s^{-1}]	78.7 ± 7.8^{ns}	69.8 ± 15.1^{ns}	86.5 ± 19.1^{ns}	61.3 ± 13.9^{ns}	0.56^{ns}
E [mmol(H_2O) cm^{-2} s^{-1}]	6.1 ± 0.7^{a}	6.5 ± 1.3^{a}	2.3 ± 2.0^{b}	3.4 ± 2.3^{b}	11.3^{***}
WUE [µmol(CO_2) mmol^{-1}(H_2O)]	1.13 ± 0.5^{b}	1.21 ± 0.4^{b}	4.5 ± 1.2^{a}	3.9 ± 1.0^{a}	6.05^{***}

Table 2. Leaf and root anatomical traits of four species of *Physaria*, two annuals *P. gracilis* and *P. angustifolia*, and two perennials, *P. pinetorum* and *P. mendocina*. Data are means ± SE. $n = 6$, $\mathrm{df}_{\mathrm{error}} = 20$. [a] For root anatomical traits $n = 4$ and $\mathrm{df}_{\mathrm{error}} = 12$. Means with the *different letter* are statistically different at * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.001$. ns – insignificant differences.

Parameter	Annual species		Perennial species		F value
	P. gracilis	*P. angustifolia*	*P. pinetorum*	*P. mendocina*	
Leaf anatomical traits: thickness					
Total leaf [µm]	399.7 ± 115.2^{b}	478.8 ± 49.9^{ab}	437.6 ± 29.5^{ab}	702.5 ± 24.6^{a}	6.10^{**}
Mesophyll [µm]	346.9 ± 25.3^{b}	332.6 ± 14.4^{b}	384.7 ± 25.1^{a}	512.2 ± 32.1^{a}	4.98^{*}
Spongy mesophyll [µm]	153.2 ± 34.9^{ns}	222.8 ± 33.8^{ns}	145.1 ± 22.2^{ns}	186.5 ± 39.6^{ns}	1.05
Palisade mesophyll [µm]	193.7 ± 49.7^{b}	119.7 ± 11.7^{b}	239.6 ± 31.2^{a}	278.1 ± 12.5^{a}	6.1^{**}
Epidermis [µm]	21.4 ± 2.4^{b}	33.4 ± 2.4^{ab}	29.6 ± 0.9^{ab}	43.5 ± 1.7^{a}	18.03^{***}
Cuticle [µm]	2.9 ± 0.3^{c}	3.8 ± 0.1^{b}	4.2 ± 0.2^{b}	5.2 ± 1.1^{a}	22.2^{***}
Mean vein [µm]	93.4 ± 7.3^{b}	89.7 ± 1.1^{b}	159.5 ± 16.9^{ab}	189.5 ± 21.9^{a}	9.12^{***}
Sclerenchyma [µm]	30.6 ± 2.6^{b}	38.1 ± 2.8^{b}	43.9 ± 1.2^{b}	63.9 ± 7.3^{a}	8.02^{**}
Fraction of spongy [$\mu m^2\ \mu m^{-2}$]	0.41 ± 0.08^{a}	0.44 ± 0.06^{a}	0.36 ± 0.03^{b}	0.24 ± 0.04^{b}	4.33^{*}
Fraction of palisade [$\mu m^2\ \mu m^{-2}$]	0.39 ± 0.04^{b}	0.38 ± 0.03^{b}	0.42 ± 0.04^{a}	0.50 ± 0.04^{a}	7.42^{**}
Fraction of epidermis + cuticle [$\mu m^2\ \mu m^{-2}$]	0.06 ± 0.01^{b}	0.07 ± 0.01^{b}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	4.78^{*}
Fraction of mean vein+sclerenchyma [$\mu m^2\ \mu m^{-2}$]	0.14 ± 0.05^{ns}	0.11 ± 0.02^{ns}	0.12 ± 0.04^{ns}	0.16 ± 0.03^{ns}	1.19
Root anatomical traits: diameter [a]					
Xylem vessel root [µm]	54.4 ± 3.4^{a}	40.4 ± 2.9^{a}	16.74 ± 1.2^{b}	14.1 ± 1.1^{c}	65.1^{***}
Central cylinder [µm]	473.43 ± 24.9^{a}	344.53 ± 10.9^{a}	220.83 ± 1.4^{b}	220.90 ± 2.2^{b}	13.1^{***}

mesophyll, since the spongy mesophyll thickness did not differ between species (Table 2). The sclerenchymatous and vascular tissues in the leaf were thicker in perennial *P. mendocina* in relation to the other species.

We also analyzed the anatomical differences by means of the proportion of area of each tissue in a cross section of the leaves. Independently of thickness differences between species, the proportion of vascular tissue and sclerenchymatous tissue in the mean vein did not differ between species (Table 2). The only significant differences were a slightly higher proportion of palisade mesophyll and protective tissues (epidermis plus cuticle) in perennials at the expense of a lower proportion of spongy mesophyll, when compared to the two annuals (Table 2).

When considering the anatomy of the roots, differences in root xylem between species were also evident. Both perennial species (*P. pinetorum* and *P. mendocina*) had a smaller xylem vessel diameter and central cylinder than that of the annual species (Table 2).

Anatomical basis for differences in physiological performance: The model shown in Fig. 2 was not rejected ($X^2 = 90.9$, $p=0.24$, df = 6) given our experimental data, and provided a good fit of the data. The model showed that leaf anatomical traits affected P_{Narea} indirectly through changes in leaf thickness and a negative relationship with SLA (Fig. 2). This indicates that leaves with a thicker sclerenchyma and palisade mesophyll had lower SLA and higher assimilation rates (Fig. 2). *E* was positively linked to the thickness of the spongy mesophyll and negatively to the epidermis thickness. All the other relationships proposed in the full model of Fig. 1, had either not direct or indirect (through leaf thickness) effects on SLA, nor on photosynthesis or transpiration.

SLA was negatively correlated with total leaf thickness (Fig. 3*C*), palisade mesophyll thickness (Fig. 3*E*), and sclerenchyma thickness (Fig. 3*D*). Variation in SLA among annual and perennial species was not correlated with thickness of the spongy mesophyll (Fig. 3*F*) or epidermis (Fig. 3*G*). SLA was negatively correlated with photosynthetic rate (Fig. 3*A*), but was not correlated with *E* (Fig. 3*B*).

We found a negative relationship between root xylem diameter (xylem vessel root and central) and WUE at the leaf level (Fig. 4*A*, 4*B*). Annual species had lower WUE and larger xylem vessel diameter and central cylinder, than both perennial species (Tables 1 and 2).

Discussion

Differences in leaf anatomy, morphology, and physiology within the group of annual and perennial *Physaria* evaluated were found to be in accordance with the gradient in carbon-use strategies previously found in this group of species (from acquisitive to conservative; González-Paleo and Ravetta 2011a, 2015). As expected, *P. gracilis,* a species, which had both high total biomass and high SLA, had thinner leaves, with a low investment in protective tissues, such as epidermis, cuticle, and sclerenchyma, when compared to *P. mendocina*, a perennial species with low total biomass and low SLA (Table 1). The other two species included in our study had intermediate values for protective tissues as well as for total biomass and SLA.

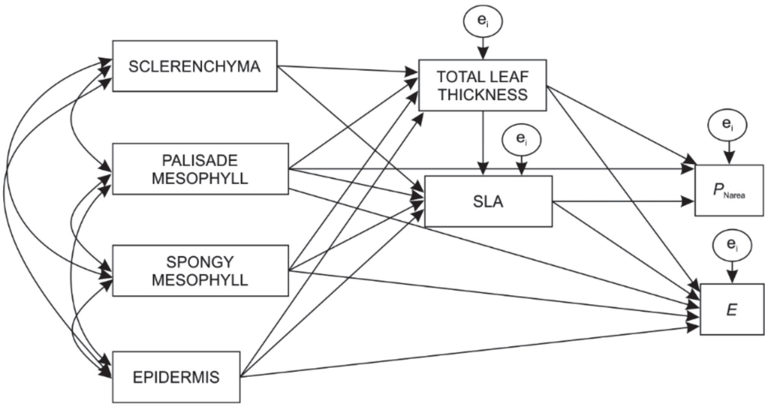

Fig. 1. Theoretical path model to be tested using *AMOS* program. *Single arrows* indicate the relationships between X and Y variables, and are characterized by a regression coefficient; double arrows indicate covariance between X variables, and e_i represent the residual variance or error. This diagram shows the theorethical functional relationships between leaf anatomical traits (thickness of different tissues: epidermis, spongy mesophyll, palisade mesophyll, and sclerenchyma) and leaf physiology SLA – specific leaf area [$cm^2 g^{-1}$]; P_{Narea} – CO_2 assimilation rate [$\mu mol(CO_2) m^{-2} s^{-1}$]; E – transpiration rate [$mmol(H_2O) m^{-2} s^{-1}$].

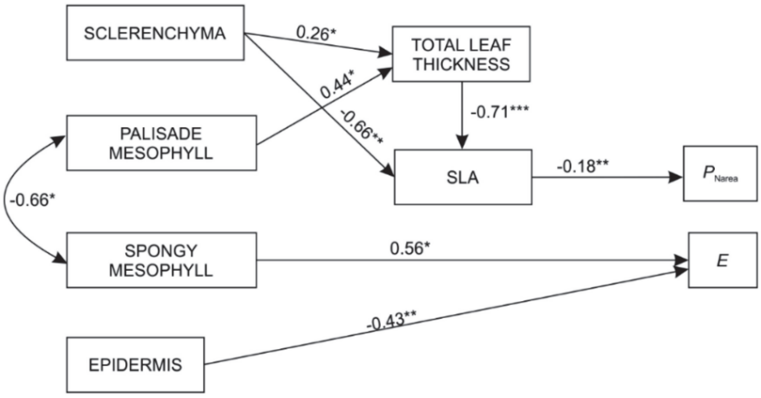

Fig. 2. Path diagram shows the functional relationships between leaf anatomical traits (thickness of different tissues) and leaf physiology. SLA – specific leaf area [$cm^2 g^{-1}$]; P_{Narea} – CO_2 assimilation rate [$\mu mol(CO_2) m^{-2} s^{-1}$]; E – transpiration rate [$mmol(H_2O) m^{-2} s^{-1}$]. The final path model only showed the predicted and dependent variables, and the relationships that contributed significantly to explain differences in photosynthesis and transpiration between species. *Solid arrows* show significant pathways with their path coefficient (* – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$). Path coefficients are the standardized slopes of the multiple linear regressions. The relationships between X and Y variables are shown by the *single-headed arrows* and are characterized by a regression coefficient that measure the effect of each X variable on the dependent variable Y. The X variables are not necessarily independent; thus we allow them to freely covary (this is shown by *double-headed arrows*).

It is expected that the physiological trade-off that favors either productivity or persistence (Reich *et al.* 1992, Poorter *et al.* 2009) should translate into an anatomical trade-off (investment in photosynthetic *vs.* protective tissues). Generally, in highly productive annuals, the trade-off is shifted towards a higher proportion of mesophyll and a lower proportion of protective tissues (Garnier and Laurent 1994, Garnier 1992, Garnier *et al.* 1997). In relative terms, we did not find differences in the proportion of total mesophyll between annual and perennial *Physaria*. However, we found a greater proportion of palisade mesophyll, epidermis, and cuticle, in low SLA, well protected leaves of the slower-grown perennials, at the expense of the proportion of spongy mesophyll. Both anatomical traits (more protective tissues and palisade parenchyma) are common characters found in plants that are stress-tolerant (Nobel and Walker 1985) and allow for high P_N in high-radiation environments (Terashima *et al.* 2011).

Even when both perennial species had more protective tissues, longer-lived leaves, and less growth than the two annuals, they also had the highest P_{Narea}, relative to both annual species (Tables 1, 2; Fig. 2). In general, it has been reported that a thick leaf structure carries the cost of a lower nitrogen allocation and lower P_{Nmass} (Reich *et al.* 1986, Field and Mooney 1986). However, a thick leaf could also result in a productive leaf since higher photosynthetic capacity per leaf area can be achieved by an extra layer of palisade parenchyma, a larger amount of enzymes, and more light- harvesting complexes per unit of leaf area (Nobel *et al.* 1975, Björkman 1981, Terashima *et al.* 2001).

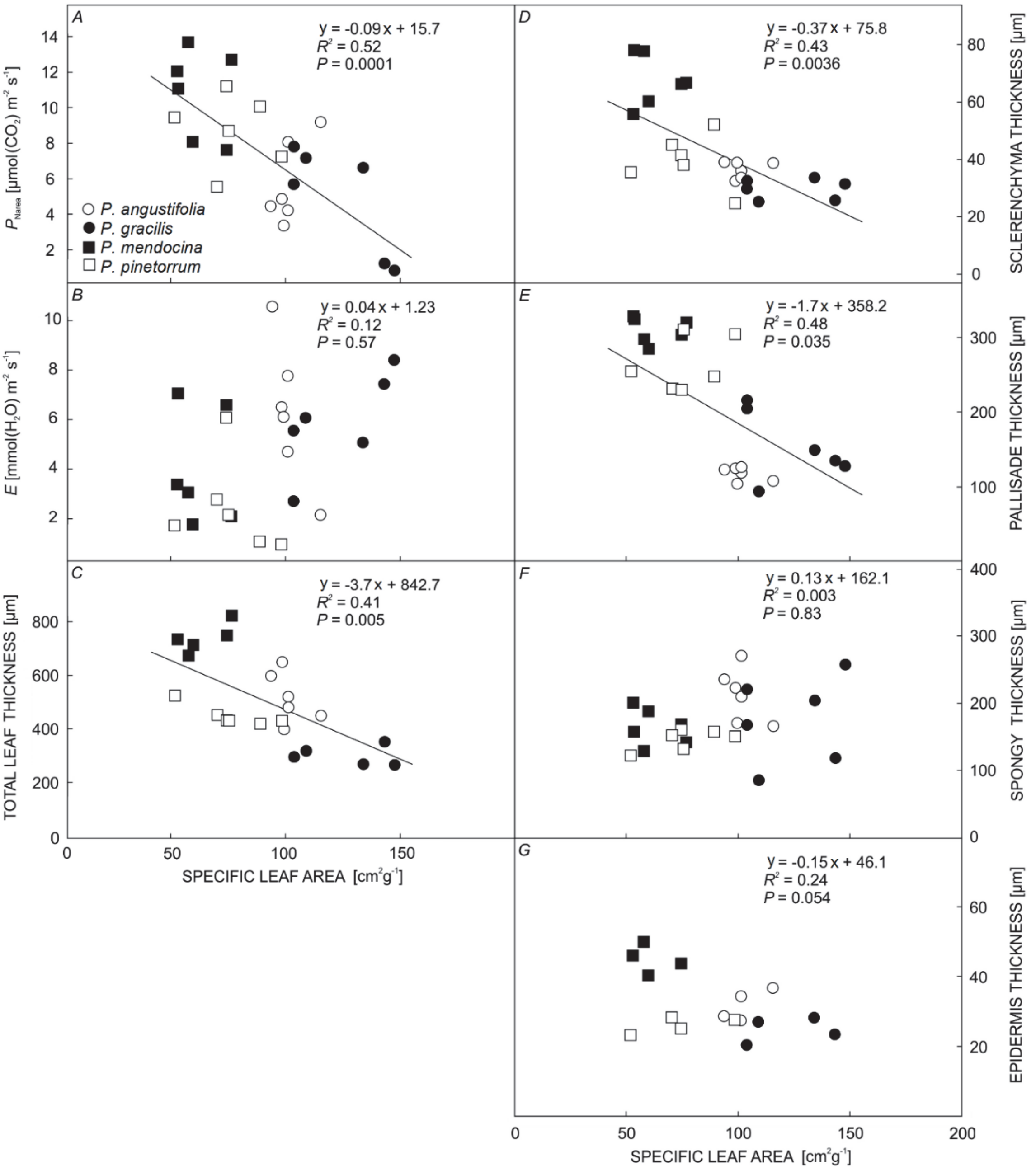

Fig. 3. Relationships between specific leaf area (SLA) and (*A*) photosynthetic rate at saturated PPFD (P_{Narea}), (*B*) transpiration rate (*E*), (*C*) total leaf thickness, (*D*) sclerenchyma thickness, (*E*) palisade mesophyll thickness, (*F*) spongy mesophyll thickness, and (*G*) epidermis thickness. The *square symbols* are perennial species (black, *Physaria mendocina*; white, *Physaria pinetorum*); the *circular symbols* are annual species (black, *Physaria gracilis*; white, *Physaria angustifolia*). Six individual plants of different plots of each species were used for the analysis of regression ($n = 24$). Equation of regression, R^2 and *p*-values are shown for each relationship.

Few studies have contributed to the question of how life history (annual *vs*. perennial life cycle) is related to differences in photosynthetic physiology. Jaikumar *et al.* (2013) found a higher carbon gain (P_{Narea}) in perennial cereal crops (accessions of perennial wheat, intermediate wheat grass, and perennial rye) compared to close annual relatives (wheat and rye), which was related to a higher leaf soluble protein and chlorophyll content. Perennial and annual cereals differed in P_{Nmass}, when this value was integrated over the expected leaf lifespan. However, most studies do not consider that fitness should be closely related to net C fixation over the lifespan of the leaf, and mostly do not include leaf longevity. The understanding of the cause of this decoupling between leaf carbon fixation and whole-plant growth may need to use models that incorporate traits such as leaf longevity and construction costs.

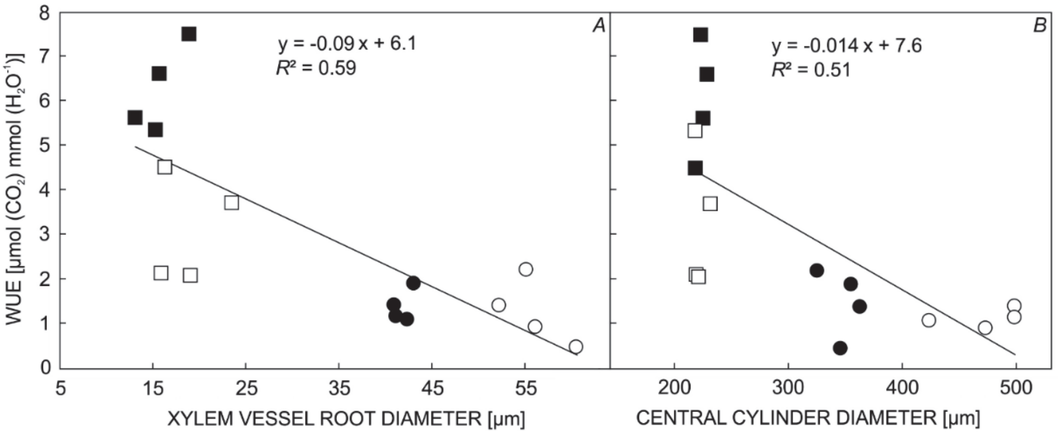

Fig. 4. Relationships between xylem characteristics: (*A*) xylem vessel diameter and (*B*) central cylinder diameter, and water-use efficiency (WUE). The *square symbols* are perennial species (black, *Physaria mendocina*; white, *Physaria pinetorum*); the *circular symbols* are annual species (black, *Physaria gracilis*; white, *Physaria angustifolia*). Four individual plants of each species (one per plot) were used for the analysis of regression (n = 12).

Differences in anatomical and structural features may be interpreted as having different consequences in the way leaves perform. On one side, a larger proportion of sclerenchyma and vascular tissues is expected to be found when leaves have low SLA; this should also result in leaves with a higher longevity (Reich *et al.* 1992). The construction of smaller, thicker, and tougher leaves (a set of characters considered as xeromorphic) would impose a high leaf construction cost (Garnier and Laurent 1994), but is beneficial for plants to withstand drought by reducing water loss, excessive irradiance, and heat loads (Sobrado and Medina 1980), and appears to be an important mechanism of nutrient conservation in low-resource environments (Aerts 1996, Pastor-Pastor *et al.* 2015, for *Physaria*). Furthermore, the strong relationship between leaf life span and SLA across species suggests that structural reinforcement of leaves plays a major role in determining their longevity; up to 40% of the variation in leaf life span can be explained by a combination of two traits: leaf thickness and leaf toughness (Wright and Cannon 2001). In this context, the larger allocation of carbon to structural tissues related to the increase in leaf thickness in the perennials *P. pinetorum* and *P. mendocina* appears to explain their high leaf longevity observed.

On the other hand, high photosynthetic capacity has also been linked to the ratio palisade/spongy tissue thickness; many desert-adapted species have a high palisade/spongy ratio, which would confer leaves an adaptation to environments with high radiation and allow to reach higher light-saturated rates of photosynthesis (Shields 1950, Oguchi *et al.* 2003). A high assimilation rate per leaf area can be achieved by a large amount of enzymes and light-harvesting complexes per unit of leaf area, and possibly an extra layer of palisade parenchyma, all of which decrease SLA (Poorter 1989, Evans and Poorter 2001). In our experiment, we found an indirect positive relationship between palisade mesophyll thickness and photosynthesis, through leaf thickness and SLA (Fig. 1). Still, we found no link to the plant's growth.

The lack of relationship between photosynthesis and growth is consistent with the idea proposed by Poorter (2002) that differences in photosynthesis per unit of leaf area are not of overriding importance in explaining variation in whole plant growth, and that structural differences in leaves are an important factor explaining the interspecific variation in growth capacity. In fact, the cost of investing a high proportion of carbon in structural tissues could be the cause of the lack of link between photosynthesis and annual growth rate (Lambers and Poorter 2004, Osunkoya *et al.* 2010). In our experiment, we found that perennial *Physaria* diverted energy that could be used for growth and reproduction (they have low RGR and a seed yield) to the construction of long-lived, well protected leaves. On the other side, annuals, in spite of their lower P_N per unit of area, would assimilate more carbon per unit of energy invested in leaf construction, allowing for a higher growth capacity. In addition, a thick palisade has been found to fulfill a protective function, providing leaves with mechanical stiffness, in a way analogous to that of the epidermis (Garnier *et al.* 1999); this could be the case in the two perennial species of *Physaria* we studied, and would allow for a high photosynthetic rate together with a long leaf life span.

Another group of structural plant characteristics, which have played a fundamental role in linking leaf structure with photosynthetic capacity, are the hydraulic properties of the root and leaf tissues (Brodribb *et al.* 2007). The leaves are a major bottle-neck of plant hydraulic conductivity and contribute about 30% of the whole plant hydraulic resistance (Sack *et al.* 2003). The hydraulic conductance of a leaf is determined by vascular pathways, such as the leaf bundle diameter, density of bundles, and features of the xylem conduits within the bundles (Sack and Frolle 2006), and by extra-vascular pathways of transpired water, such as mesophyll thickness (Aasamaa *et al.* 2005). In our experiment, both perennial species had a larger leaf bundle diameter compared to the annuals (Table 2). This wider bundle should provide a higher capacity for water transport in the leaf veins and should enable perennial *Physaria* to maintain a high water potential and sustain leaf performance (Meinzer 2003, Santiago *et al.* 2004, Gago *et al.* 2014). However, perennials also had a thicker palisade mesophyll, which generally accounts for high resistance to water flow (Brodribb *et al.* 2007). Further evaluations of the relative influence of bundle diameter and the thickness and compactness of the palisade mesophyll are needed to understand the apparent mismatch in hydraulic conductivity found between roots and leaves in our experiment and how they influence water movement and transpiration. The length of the mesophyll tissue that must be traversed as the transpiration stream passes from a vein ending at the site of evaporation reflects the resistance to water flow. Since a thicker palisade mesophyll determines both, a lower E (through the indirect pathway with spongy mesophyll thickness) and higher photosynthesis (through the indirect pathway with leaf thickness and SLA; Fig. 2), perennial *Physaria* should have, and had higher WUE than the two annuals (Table 1).

In conclusion, we found that the main anatomical cause of variation in SLA across this group of co-generic species was the difference in leaf thickness, which resulted from variation of the degree of sclerification in the central midrib of the lamina, and the thickness of the palisade mesophyll. Traits are functionally co-ordinated if they are co-selected in a given environment, and provide a better performance (Givnish 1986, Niklas 1999). Although we found a negative relationship between SLA (leaf structure) and photosynthesis (leaf function) in *Physaria*, this link could not be further followed to explain differences in growth between these species. Leaf structure and function in perennial species of *Physaria* appears to be co-ordinated for the construction of long-lived leaves, reflecting a co-selection of leaf traits that bear on the ability of plants to efficiently use and conserve resources, such as water and nitrogen, in desert environments. Long-lived leaves of perennial *Physaria* had the higher water-use efficiency, a trait that has been related to drought tolerance (Blum 2005). Also, long-lived leaves should have more prolonged mean nutrient residence time in the plant, higher nitrogen-use efficiency, and a lower dependence on external resources (Ekstein *et al.* 1999, Dawson *et al.* 2008). Although not fully responsible for differences in growth capacity, specific leaf area appears as suitable criteria for selection of new *Physaria* perennial crops for low resource environments or low-input agricultural systems.

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