Plant Soil (2008) 310:235–243
DOI 10.1007/s11104-008-9653-x

REGULAR ARTICLE

Sex-related differences in leaf traits in an androdioecious shrub under contrasting levels of soil salinity

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Received: 18 February 2008 / Accepted: 13 May 2008 / Published online: 12 June 2008

Abstract This study explores whether male and hermaphrodite plants of *Phillyrea angustifolia* (Oleaceae) show physiological and structural differences at the leaf level under severe water stress driven by drought and soil salinity. Leaf traits were measured in summer, at the height of the summer drought period, in male and hermaphrodite plants from two adjacent sites under contrasting soil salinity levels. Male plants from the saline site had significantly higher leaf proline content compared to males from the nonsaline site. By contrast, leaf proline levels were similarly low in hermaphrodite plants from both sites. On the other hand, hermaphrodite plants from the saline site had higher leaf stomatal frequency than hermaphrodites from the nonsaline site, whereas this parameter did not differ for male plants across sites. Such differences could be interpreted as the result of two different solutions to the same selective pressure in the androdioecious shrub *P. angustifolia*.

Keywords Divergent responses · Proline · Resource allocation · Sexual dimorphism · Stomatal frequency

Responsible Editor: Tibor Kalapos.

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Introduction

Summer soil water deficit is one of the main limiting factors for plant productivity in Mediterranean-type ecosystems (Tenhunen et al. 1987). In woody plants, this seasonal drought stress is first manifested at the leaf level, usually by a decrease in leaf relative water content (RWC) and/or chlorophyll content (see e.g. Gratani and Varone 2004), which results in a subsequent seasonal reduction in growth rate (Orshan et al. 1989; Cherubini et al. 2003). Soil salinity also causes stress in salt-sensitive plants or plants of low salt tolerance by disrupting water osmotic adjustment and ion homeostasis at both the cell and the whole plant levels (Zhu 2001; Munns 2002). Apart from enhancing drought stress, soil salinity may cause oxidative stress, leading to severe molecular and cell damage, to decreases in growth rate and, ultimately, to plant death (Zhu 2001).

Biosynthesis and accumulation of soluble organic compounds in the leaves has been extensively reported in plants as a way to withstand osmotic water stress imposed by either drought or salinity (Hare et al. 1998; Chen and Murata 2002). Their accumulation as compatible osmolytes has been mainly associated with cell osmoregulation and osmoprotection of cellular macromolecules against cell dehydration. Free amino acids (e.g. proline), soluble carbohydrates (e.g. fructans) and sugar alcohols (e.g. mannitol, sorbitol) have been identified among these compatible osmolytes in plants (Chen and Murata 2002). The biosynthesis of proline, in addition to its osmoregulation and osmoprotectant roles, also confers protection against oxidative damage, supporting the pentose phosphate pathway activity under stress (Hare and Cress 1997; Chen and Murata 2002; Kavi-Kishor et al. 2005). This would, in turn, help satisfy the demand for increased secondary metabolite production (e.g. sugar alcohols) as compatible osmolytes against water stress. Therefore, despite proline not always being the most abundant compatible osmolyte (e.g. see Rejšková et al. 2007), leaf proline biosynthesis is of paramount importance for plants as a key physiological response to severe osmotic water stress imposed by drought and/or salinity (Hare and Cress 1997; Kavi-Kishor et al. 2005). Nonetheless, there is a high cost involved in proline biosynthesis, in terms of both energy and nutrients, particularly nitrogen (Raven 1985).

Plant reproduction is also highly costly in terms of energy and nutrients and should compromise other aspects of the plant's life-history, such as growth and/or stress-response mechanisms (Obeso 2002). Most analyses of the costs of reproduction have been carried out on dioecious species, where nutrient allocation to reproduction is notably different between genders (Dawson and Geber 1999; Obeso 2002). Fruiting is particularly demanding in terms of nitrogen allocation (Nicotra 1999). Hence, there should be a strong resource (nitrogen) conflict between leaf proline biosynthesis to keep plant metabolism during water stress periods and the female function (i.e. fruiting). In fact, functional differences at the leaf level reported so far in dioecious species have been interpreted as the outcome of underlying compensating mechanisms against potential asymmetries in reproductive allocation (e.g. Nicotra 1999; Nicotra et al. 2003; see also Case and Ashman 2005).

Leaf stomatal parameters (e.g. stomatal conductance, stomatal frequency) are also important components of the response of woody plants to drought stress (Kozlowski and Pallardy 1997). The control of stomatal conductance is governed by stomatal closure dynamics at both daily and seasonal time scales. While it contributes to the avoidance of water loss, it also limits photosynthesis, particularly in C_3 plants (Flexas and Medrano 2002). This temporary limitation of photosynthesis would account for the seasonal (summer) decrease in growth rate characteristic of Mediterranean woody plants (Cherubini et al. 2003). Lower stomatal frequencies are often associated with higher water use efficiencies through a decrease in transpirational water loss by a decrease in stomatal conductance (e.g. Kloeppel and Abrams 1995). Unlike the variable nature of stomatal conductance (both daily and seasonal), stomatal frequency does not change appreciably once leaves have matured (Royer 2001). They should thus provide valuable information on plant water relations integrated over extended periods.

In this study we present a comparative analysis of leaf traits in male and hermaphrodite plants of the androdioecious shrub *Phillyrea angustifolia* L. (Oleaceae) under soil water deficit driven by summer drought and moderate levels of soil salinity. We tested for differences in leaf physiological (e.g. proline) and/or structural parameters (e.g. stomatal frequency) between sex-morphs as a consequence of their allegedly strong differences in reproductive allocation (Vassiliadis et al. 2002). Our study was conducted in the middle of the summer drought period, approximately at the height of fruit development. Specifically, we tested the predictions that (1) male individuals will have higher levels of leaf proline than hermaphrodites under severe stress conditions, as a consequence of a strong resource (nitrogen) conflict with the female function (i.e. fruiting) in hermaphrodites, and (2) leaf stomatal frequency values will be lower under more pronounced water stress conditions driven by soil salinity (with no differences between sex-morphs expected).

Material and methods

Study species and area

Phillyrea angustifolia (Oleaceae) is a long-lived, evergreen shrub species distributed throughout the western Mediterranean Basin. It is relatively common in Mediterranean shrublands on sandy or loamy soils (Pannell and Ojeda 2000), and may also be found in salt-marsh verges and coastal habitats (Bassett 1978). Leaves in this species are entire, simple, and sclerophyllous, having numerous small stomata restricted to the abaxial surface (Rotondi et al. 2003). Leaves also have glandular trichomes, mainly found in the abaxial surface (Rotondi et al. 2003), which have been thoroughly described in the sister species

Phillyrea latifolia (Gravano et al. 1998), and for which a role in conferring tolerance against moderate salinity has been suggested (Gucci et al. 1997).

This species is androdioecious (Vassiliadis et al. 2002), and both male and hermaphrodite individuals flower profusely every other year in early to middle spring (J.L. Medina-Gavilán, unpublished data). Hermaphrodite plants produce drupes which finish ripening in late summer (August–September; see Traveset 1994 for a comprehensive account of the reproductive biology of this species).

The study area was located at "Pinar de la Algaida", in the Bay of Cádiz, SW Spain (36° 32′ 00″ N, 6° 12′ 34″ W), where a large population of *P. angustifolia* ($N>500$ plants) occurs. This area lies on the shore of the San Pedro river, a sea channel that runs inland from the Atlantic ocean. It is dominated by Quaternary, sandy soils, and it has a coastal Mediterranean climate. The study was conducted in July 2006, at the height of summer drought period. The study area consisted of two contrasting patches or sites separated by a dirt road. One site is adjacent to a shallow, saline, tidal lagoon connected to the San Pedro river by a narrow channel; this site is strongly affected by salinity (hereafter, *saline site*). The other site is farther from the saline tidal lagoon and hence somewhat protected from the salt water influence (hereafter, *nonsaline site*). In order to verify the existence of contrasting differences in soil salinity between the two sites, soil electrical conductivity (EC)—as indicative of soil soluble salts—was measured in August 2006 using a 1:1 soil:water ratio ($EC_{1:1}$). $EC_{1:1}$ at the saline site was 3.14 ± 2.6 dS m^{-1} (mean±SD; $n=66$), whereas $EC_{1:1}$ at the nonsaline site was about six times lower (0.47 ± 0.14 dS m^{-1}; $n=63$; $P<0.001$; Student's *t* test). The former mean value actually corresponds to moderate soil salinity conditions, whereas the latter fits in the range of nonsaline soils (Whitney 1998).

These contrasting differences in soil salinity are also reflected in the vegetation structure of the two neighbouring sites, as well as in their floral composition. In the saline site, *P. angustifolia* co-occurs with salt and drought tolerant species (e.g. *Tamarix canariensis* Willd., *Limonium algarvense* Erben, *Thymelaea hirsuta* (L.) Endl.), and *Pistacia lentiscus* L. as the only other Mediterranean sclerophyllous shrub. In the nonsaline site, by contrast *P. angustifolia* co-occurs with many other Mediterranean shrub species such as *Rhamnus lycioides* L., *Cistus salvifolius* L., *Chamaerops humilis* L., *Juniperus phoenicea* L., and *P. lentiscus*. Relative abundance of *P. angustifolia* is lower in the saline site (5%; line-cover transect) than in the nonsaline (14%). The proportion of male plants was 0.29 and 0.35 in the saline and nonsaline site, respectively; these proportions were not significantly different from one another ($P=0.37$; chi-square test), and are within the bounds of theoretical predictions for androdioecious species (Pannell 2002).

Since the treatment effect (saline *vs.* nonsaline sites) could not be replicated in this "quasi-experimental" field design, our study design is technically pseudoreplicated (Hurlbert 1984). However, valid ecological inferences of the treatment effect can still be made (for a discussion in this topic see Oksanen 2001, 2004; Cottenie and De Meester 2003; Hurlbert 2004), particularly considering that site differences in soil salinity were prominent.

Leaf physiological parameters

Leaf water status, total chlorophyll content, and proline concentration were recorded in mid-summer (July 2006) for 20 randomly selected adult individuals (ten males and ten hermaphrodites) in the saline, and 19 (nine males and ten hermaphrodites) in the nonsaline site. In all instances, mature healthy-looking leaves (i.e. non-chlorotic leaves with no signs of herbivory), were chosen from east-facing, sunny exposed twigs.

Leaf water status was expressed in terms of leaf relative water content (RWC), an appropriate surrogate for this parameter (Kramer and Boyer 1995; see also Kalapos 1994). RWC was calculated as (fresh weight−dry weight)/(turgid weight−dry weight), and expressed as a percentage. In each site, ten fully expanded leaves per plant were taken at mid-day, in non-cloudy summer days, placed in zip-lock plastic bags and returned immediately to the laboratory (5–20 min) where fresh weight was obtained. Turgid weight at water saturation was obtained by immersing leaves with the cut end in distilled water at 4°C in darkness for 5 h, until turgid weight became constant.

Leaf chlorophyll content was quantified on 2-year-old leaves, at a late stage of their life-span (which ranges from 28 to 36 months; Pérez-Latorre and Cabezudo 2002), but with no signs of senescence. It was measured using a Minolta SPAD 502 portable chlorophyll-meter (Minolta Camera Co. Ltd., Osaka, Japan), which provides an adequate estimation of the total chlorophyll content in the leaves (Kapotis et al. 2003). Thirty leaves per plant were measured after cleaning surface dust, and total leaf chlorophyll content, expressed as SPAD readings, was then obtained as an average value for each plant.

For proline measurements, ten fully expanded, 1-year-old leaves were collected per plant, immersed in liquid nitrogen, and stored at −80°C until analysis. Each plant's ten leaves were pooled and ground in liquid nitrogen. Four 0.25 g replicates per plant of this ground leaf tissue were used for proline quantification following the spectrophotometric analytical procedure described by Bates et al. (1973) after homogenization in 10 cm^3 of 3% sulfosalicylic acid. Proline concentration was then calculated using L-proline (Sigma-Aldrich, Steinheim, Germany) as a standard.

Leaf structural parameters

Leaf area, glandular trichome density, and stomatal density on the abaxial leaf surface were recorded in other healthy-looking, fully expanded leaves from east-facing, sunny exposed twigs from the same 39 plants used for the physiological analyses.

Leaf area was measured for 30 leaves per plant. To do so, the one-sided projected area of fresh, non-damaged leaves were recorded using a flatbed scanner and measured using Sigmascan Pro 5.0 (SPSS, Chicago, IL, USA; measurement error approximately 3%).

Glandular trichome density and stomatal density were estimated on the abaxial face of five leaves per plant. Each leaf abaxial face was coloured with a permanent blue ink pen, whose ink was found to stain cell boundaries very clearly, unequivocally revealing epidermal cells, stomata guard cells, and glandular trichomes when viewed under the microscope. Three individual images per leaf, located at the base, middle, and tip, were captured using an inverted microscope (Leitz, Fluovert) connected to a video camera CCD (Kappa, CF15/2), avoiding midrib and margins. Images were then displayed on a high resolution monitor (Mitsubishi), whose screen corresponded to a 0.25 mm^2 leaf surface field. This allowed us to count salt glands and stomata in each field and thus to obtain their corresponding density values.

Finally, the stomatal index or proportion of stomata to epidermal cells was calculated for each of the ten individuals that showed the five highest and five lowest values of stomatal density. To do this, stomata and epidermal cells were counted on seven randomly selected 0.027 mm^2 fields per leaf (variance stabilized with sample sizes higher than five) and five leaves per plant, and stomatal index (SI) was calculated as stomatal density/(stomatal density + epidermal cell density) and expressed as percentage (see Royer 2001). This leaf parameter allowed us to ascertain whether differences in stomatal density were due to a change in the total number of leaf stomata, or just an allometric consequence of variations in epidermal cell expansion.

Statistical analyses

A series of full-factorial, two-way ANOVAs were performed to determine the effect of soil salinity (saline vs. nonsaline site) in the existence of differences between male and hermaphrodite plants in each of the leaf parameters. Both 'sex-morph' and 'site' were considered as fixed effects. When appropriate, subsequent planned pair-wise contrasts were conducted through Bonferroni-adjusted *t*-tests to further examine differences between sites within each sex-morph, and/or between sex-morphs within each site.

Additionally, in order to verify that differences in stomatal density were due to changes in the number of leaf stomata, rather than the result of changes in epidermal cell size, a Student's *t* test was used to compare the stomatal index between the five plants with the highest and the five plants with the lowest stomatal densities. In all instances, data were checked for normality and homogeneity of variances prior to analyses, and no transformations were needed.

Results

Leaf physiological parameters

Values of leaf RWC were similar in hermaphrodite and male plants and, for both sex morphs, this parameter was slightly lower in the saline site (Fig. 1a), although differences between sites were only marginally significant (P=0.08; Table 1).

At the nonsaline site, total leaf chlorophyll content in male plants was significantly higher than in

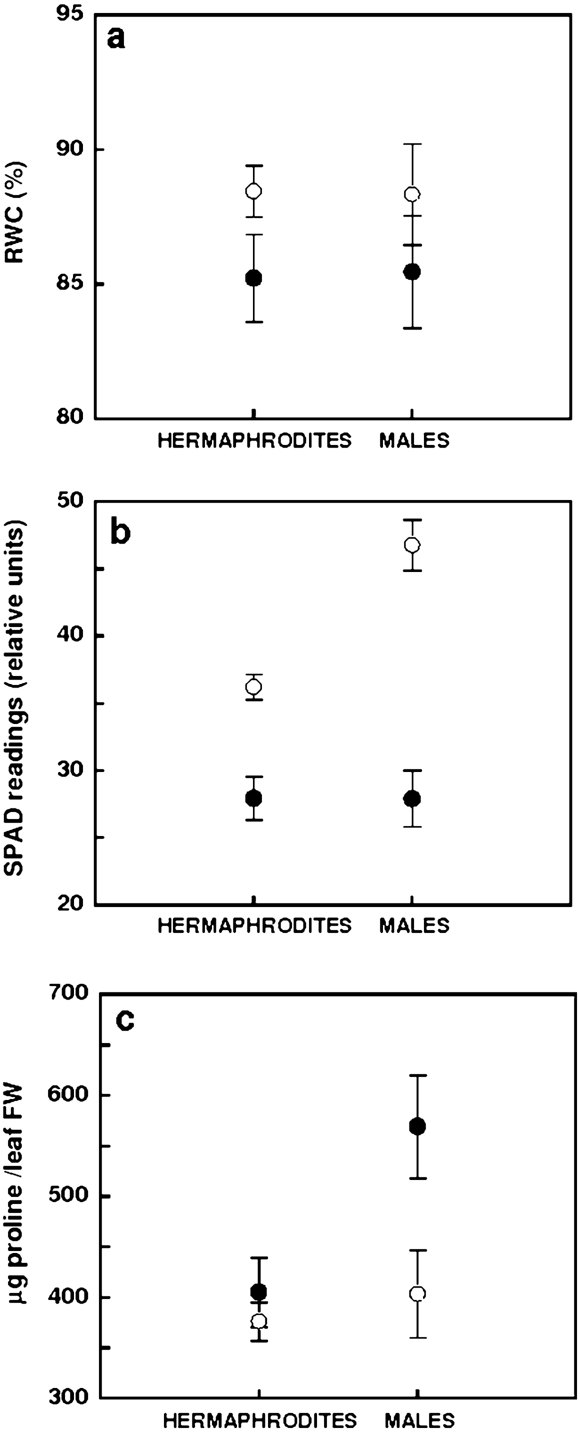

Fig. 1 Effects of soil salinity on **a** leaf relative water content, **b** total leaf chlorophyll content (as measured by the portable SPAD chlorophyll-meter), and **c** leaf proline concentration across sexual morphs in *P. angustifolia*. *Open* and *filled circles* refer to the nonsaline and saline sites, respectively. *Error bars* indicate ±1 standard error

hermaphrodites (Fig. 2b; $P<0.025$, Bonferroni *t*-test). However, leaf chlorophyll content dropped significantly in the two sex morphs at the saline site, and differences between sex-morphs vanished (Fig. 1b; Table 1).

Regarding leaf proline, the response of male and hermaphrodite plants to soil salinity was different (Fig. 1c), although the sex-morph × site interaction was only marginally significant ($P=0.07$; see Table 1). There was virtually no difference in proline concentration between hermaphrodite plants from the nonsaline and the saline sites (Fig. 1c; $P=0.64$, Bonferroni *t*-test), whereas leaf proline was markedly higher in male plants from the saline site (Fig. 1c; $P=0.005$, Bonferroni *t*-test). Therefore, while no between-morph differences in leaf proline concentration existed at the nonsaline site ($P=0.61$, Bonferroni *t*-test), they were prominent and significant at the saline site ($P=0.006$, Bonferroni *t*-test).

Leaf structural parameters

Both male and hermaphrodite plants had slightly smaller leaves at the saline site (Fig. 2a), although such slight differences were non-significant (Table 2).

Hermaphrodite plants had an overall lower glandular trichome density than males (Fig. 2b; Table 2). The site effect was similar for male and hermaphrodite plants (nonsignificant sex-morph × site interaction; Table 2), with both sex-morphs showing higher densities at the saline site (Fig. 2b; Table 2; $P=0.037$ for hermaphrodites, and $P=0.047$ for males, Bonferroni *t*-tests).

Table 1 Summary ANOVA results for comparisons of leaf physiological parameters in nonsaline and saline sites across sexual morphs

	RWC (%)			Total chlorophyll (relative units)			Proline[a] ($\mu g\ g^{-1}$ DW)		
	df	MS	*P*	*df*	MS	*P*	*df*	MS	*P*
Morph	1	0.0	0.97	1	324.7	0.07	1	84,827	0.02
Site	1	91.1	0.08	1	1,649.4	0.0001	1	81,877	0.022
Morph × site	1	0.3	0.92	1	329.2	0.07	1	41,866	0.095
Error	35	27.6		35	94.9		32	14,110	

[a] No data of one hermaphrodite from the nonsaline site and two hermaphrodites from the saline site

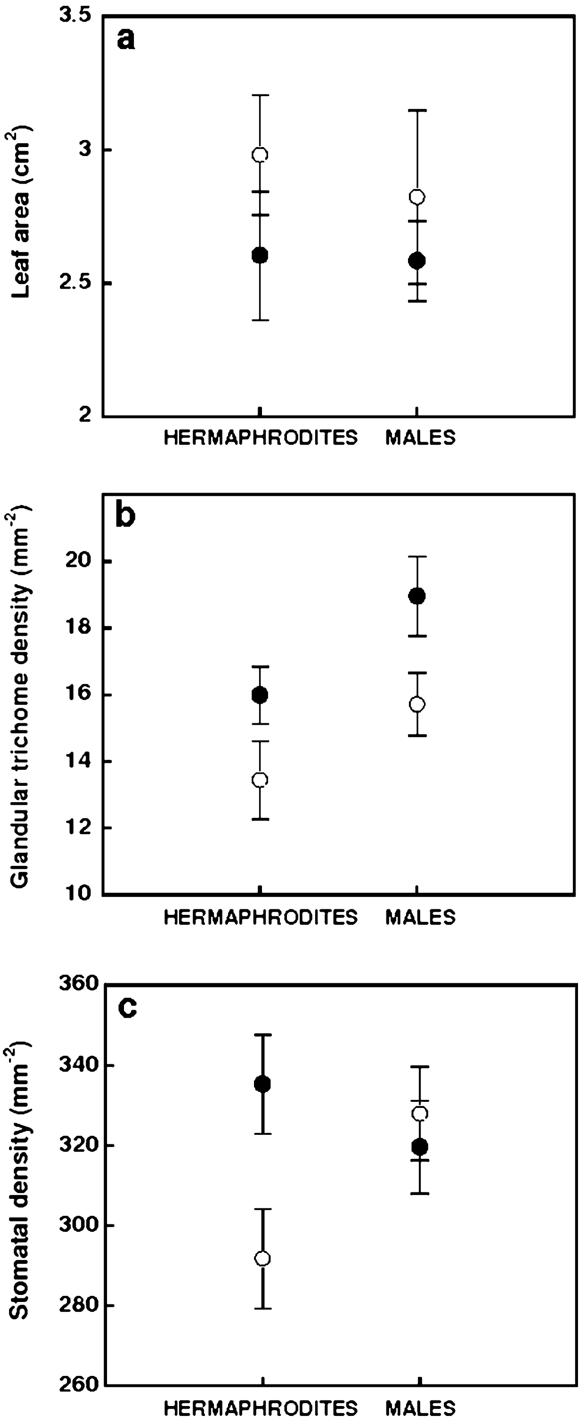

Fig. 2 Effects of soil salinity on **a** leaf area, **b** glandular trichome density, and **c** stomatal density across sexual morphs in *P. angustifolia*. *Open* and *filled circles* refer to the nonsaline and saline sites, respectively. *Error bars* indicate ±1 standard error

At the nonsaline site, hermaphrodites had lower leaf stomatal densities than males (Fig. 2c; P=0.044, Bonferroni t-test), but the site effect was different between sexes (significant sex-morph × site interaction; Table 2). Stomatal density increased significantly in hermaphrodite plants at the saline site (Fig. 2c; P= 0.014, Bonferroni t-test), whereas it did not in males (Fig. 2c; P=0.63, Bonferroni t-test). As a result, stomatal density of hermaphrodite plants at the saline site was not significantly different from males (Fig. 2c; P=0.36, Bonferroni t-test).

The five plants from the nonsaline site having the lowest stomatal density values also had significantly lower SI values (16.3±1.2; mean±SD) than the five ones from the saline site having the highest (SI= 19.7±0.7; t_8=-5.37; P<0.001; Student's t test), all of them being hermaphrodites. Therefore, between-site differences in stomatal density within hermaphrodite plants (see Fig. 2c) were determined by an actual increase in the number of stomata rather than by plastic variation in leaf area and/or in epidermal cell size.

Discussion

Although leaf parameters in this study were measured only once, the results presented are reliable since (1) leaf proline concentration was measured at the height of fruit development, which determines the differences in reproductive allocation between sex-morphs in dimorphic species when one of the morphs does not present the female function (see e.g. Guitián 1995; Nicotra 1999); and (2) stomatal frequency, particularly stomatal index, is a rather consistent leaf parameter within individual plants (Royer 2001).

There were no apparent differences between the two sexual morphs of *P. angustifolia* in their resistance to severe soil water stress driven by summer drought plus soil salinity, as indicated by their leaf RWC values and their total leaf chlorophyll content at the saline site. However, as expected, male plants showed increased levels of free proline in the leaf tissue at the saline site, as a plausible response to severe osmotic stress, whereas hermaphrodites did not.

Free proline biosynthesis and accumulation in the leaf tissue has been extensively reported as a physiological response against water stress in Mediterranean woody plant species (e.g. Ain-Lhout et al. 2001; Sofo et al. 2004), but this is the first time, to

Table 2 Summary ANOVA results for comparisons of leaf structural parameters in nonsaline and saline sites across sexual morphs

	Leaf area[a] (cm^2)			Glandular trichome density (mm^{-2})			Stomatal density (mm^{-2})		
	df	MS	*P*	df	MS	*P*	df	MS	*P*
Morph	1	0.07	0.71	1	65.62	0.019	1	1,021	0.402
Site	1	0.84	0.20	1	96.84	0.005	1	3,024	0.153
Morph × site	1	0.04	0.77	1	0.01	0.973	1	6,568	0.038
Error	32	0.49		35			35		

[a] No data of two males from the nonsaline site and one male from the saline site

our knowledge, that sexual dimorphism has been considered explicitly. There are two possible interpretations to explain the differences in the levels of leaf proline between sex morphs shown in this study. First, soil salinity levels at the saline site may not have been high enough for hermaphrodites so as to trigger a physiological response against osmotic stress. Second, assuming that male and hermaphrodite plants are equally sensitive to soil salinity, there may have been a strong functional trade-off between nutrient (nitrogen) allocation to the female function of hermaphrodites (e.g. fruiting) and leaf proline biosynthesis. In fact, berries and drupes of fleshy-fruited plant species present high concentrations of nonprotein nitrogen compounds (Izhaki 1993; Stines et al. 1999; Al-Shahib and Marshall 2003), acting as strong nitrogen sinks (Nicotra 1999). This nitrogen sink effect might also account for the lower values of leaf chlorophyll in hermaphrodite plants compared to males at the nonsaline site.

Some evidence of the functional trade-off between leaf proline biosynthesis and fruiting may actually be extracted from the agricultural biotechnology literature. For instance, Hsieh et al. (2002) showed that transgenic lines of tomato, whose plants presented high levels of leaf proline even in the absence of water stress, produced about 70% fewer fruits per plant, and about 85% fewer seeds per fruit than the wild-type. Hence, it may be argued that leaf proline biosynthesis is strongly penalized in hermaphrodite *P. angustifolia* plants, since it would counter their female reproductive success.

Regarding leaf structural parameters, we detected an overall higher density of glandular trichomes on the abaxial leaf surface of males, and a similar response of trichome density increase with soil salinity in the two sex morphs. The role of these glandular trichomes in withstanding moderate salinity in *Phillyrea* species has been already reported (Gucci et al. 1997), although possible sex-related differences were not then considered. By contrast, when focusing on leaf stomatal frequency, our results differed from what we had initially predicted. The lower leaf stomatal density values of hermaphrodite plants compared to those of males in the nonsaline condition might be interpreted as an intrinsic sex-morph difference, which would result from a stronger need in hermaphrodite plants to minimize water loss through stomatal conductance (Chaerle et al. 2005). However, while leaf stomatal density in male plants did not seem to change from the nonsaline to the saline condition, hermaphrodite plants at the saline site presented higher leaf stomatal density values than hermaphrodites from the nonsaline site.

At first thought, these results might seem counter-intuitive, since lower stomatal frequency values are often associated with a decrease in water loss by stomatal conductance and a subsequent higher water use efficiency (e.g. Kloeppel and Abrams 1995). However, it should be emphasized that most studies supporting such an association have been carried out on woody deciduous plant species, characterized by a poor control of stomatal conductance (Duhme and Hinckley 1992; Beerling et al. 1996). By contrast, evergreen, sclerophyllous woody species from Mediterranean-type biomes present a highly effective control of transpiration through stomatal closure (Lange et al. 1982; Nardini et al. 1999). During the dry season, Mediterranean sclerophyllous species open their stomata only during cool morning hours, thus minimizing desiccation (Lange et al. 1982). However, this desiccation avoidance through stomatal closure poses an additional stress on C_3 plants, as it strongly limits CO_2 diffusion to the mesophyll and, hence, photosynthesis (Flexas and Medrano 2002). Higher stomatal frequency might then counterbalance the reduction in daily opening time of the stomata by enhancing the amount of CO_2 entering the leaves during the short stomatal opening periods. This would increase the intercellular partial pressure of CO_2 in the leaves and, thus, extend the photosynthetic activity for longer into the hot, dry period. Such a gain in stomatal frequency would not be paralleled by a proportional increase in water loss through stomatal conductance because of the above-mentioned confinement of stomatal daily openings to favourable conditions characteristic of Mediterranean sclerophyllous species. The increment of leaf stomatal density reported for South African evergreen, sclerophyllous tree species from temperate forest (low summer stress) to Mediterranean-like (i.e. high summer stress) *fynbos* ecosystems (Hlwatika and Bhat 2002) seems to support this argument.

The higher leaf stomatal density values of hermaphrodite plants at the saline site were largely explained by differences in stomatal index between hermaphrodite plants from both sites. This latter leaf structural parameter has a strong genetic basis and is heritable (Ferris et al. 2002), and it shows little variation within individual plants (Royer 2001). Hence, our results may be interpreted as the outcome of strong selection for plants with high stomatal index values in hermaphrodites of *P. angustifolia* so as to overcome a plausible daily reduction in stomatal opening time under conditions of severe osmotic stress. A parallel selective response would not be found in males because a reduction in stomatal opening time would be less pronounced due to the alleviation of drought and/or salinity stress driven by the biosynthesis of leaf proline.

Therefore, the reported variation of leaf proline concentration in males, and of leaf stomatal frequency in hermaphrodites of the androdioecious *P. angustifolia* across contrasting soil salinity conditions could be interpreted as the result of two different adaptive solutions to the same selective pressure (e.g. see Meagher 1984), but this deserves further investigation.

Acknowledgements We thank Francisco Bravo, director of *Bahía de Cádiz* Natural Park, for kindly providing facilities for field work; Ana Cruz for helping with leaf proline measurements, and Fernando Ramírez-Pousa and Antonio Nieto for helping with leaf glandular trichome and stomata counting; and Miguel Verdú, Roberto Salguero, John Pannell, and Juan Arroyo for providing useful comments on the manuscript. This study was funded by research projects BOS2002-00609 and AGL2005-07440-C02-02/FOR from the Spanish *Ministerio de Educación y Ciencia*, and by a FPI-MEC scholarship to JLMG.

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