ORIGINAL PAPER

Limited capacity to cope with excessive light in the open and with seasonal drought in the shade in Mediterranean *Ilex aquifolium* populations

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Abstract Climate change is expected to involve more-frequent and intense summer droughts in the Mediterranean region. This represents a threat for long-term persistence of woody species, such as European holly (*Ilex aquifolium*), that originated under humid climates during the Tertiary period. The capacity of this species to persist under increased water stress, both in gaps and in the understory of an oak-dominated woodland, was assessed by quantifying phenotypic plasticity in response to drought and shade. Physiological responses in plant-water relations and gas exchange were used as performance indicators under the different environments. Phenotypic plasticity of drought-stressed holly trees in response to changes in the light environment was low relative to the known response of co-occurring forest trees. Differences between morphological traits (e.g. specific leaf area and leaf: sapwood ratio in twigs) of sun- and shade-grown trees were small but significant while physiological traits were largely unresponsive to light availability. This supports the hypothesis that late-successional shade-tolerant species exhibit greater morphological than physiological plasticity. Sapling acclimation capacity through physiological mechanisms such as osmotic adjustment was insufficient to protect from summer drought. Holly mainly inhabits oceanic climates where extreme temperatures and droughts are unusual. Our results suggest that the species occupies a narrowing niche in continental Mediterranean habitats, and may lack the capacity to persist under more-severe future climate scenarios because of its low phenotypic plasticity in response to light and drought stresses.

Keywords Water stress · Plasticity · Acclimation · Holly · Climate change

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Introduction

European holly (*Ilex aquifolium*) has a wide distribution across Europe. It is an arcto-tertiary relic species, and is unusual as an evergreen angiosperm often growing in the understory of temperate forests (Boufford and Spongberg 1983; Lavin and Luckow 1993). As such, it fulfils a unique ecological role as a persistent understory- or mature-canopy tree, and provides important habitat and food to woodland birds and grazers (Oria de Rueda 1992). The Iberian Peninsula represents the southernmost limit of

holly distribution (Arrieta and Suarez 2006). Future scenarios of a harsher Mediterranean climate are expected to impose more-frequent droughts and warmer temperatures (IPCC 2001), increasing water stress on holly and on the beech and oak woodland that provides shade facilitating its establishment. Separate studies have indicated that heavy shade (Arrieta and Saurez 2006), and at least 600-mm annual precipitation (Oria de Rueda 1992) are required for holly to survive in central Spain.

Holly, like other late-successional species, employs a conservative growth strategy, which enables it to establish and persist under heavy shade (Peterken and Lloyd 1967; Valladares et al. 2005). This strategy involves high investment in long-lived foliage and tough unpalatable spiny leaves (Potter and Kimmerer 1986; Walter and Reich 1999; Lusk 2002). But, by physiologically optimising light capture under heavy shade, species like holly are thought to compromise their capacity to respond to an increase in light availability (e.g. gap opening) (Bazazz 1979; Bazzaz and Carlson 1982; Givnish 2002). Such low phenotypic plasticity in response to a change in light availability has been proposed as the basis for niche partitioning among co-occurring forest tree species (Valladares et al. 2000). However, the physiological limitations behind this compromise are not completely understood (Kitajima 1994; Walters and Reich 1999; Wright et al. 2003). Furthermore, in the case of holly, some physiological traits differ between males and females and may lead to gender-specific niche partitioning (Obeso et al. 1998; Retuerto el al. 2000; Obeso and Retuerto 2002), although both genders are relatively sensitive to drought (Valladares et al. 2005).

It is insufficient to interpret the reaction of shade-tolerant species to gap opening with respect only to an increase in light availability, since concomitant changes in other factors, such as water and temperature, will contribute to their response under the new light environment (Niinemets et al. 2003; Niinemets and Valladares 2004). Increased heat load and stimulation of photosynthesis effected by the transition from shady to sunny conditions will result in higher transpiration, with the potential to cause a decrease in tissue hydration (Abrams and Mostoller 1995; Niinemets 2001), reduced functionality of the hydraulic system due to cavitation (Maherali et al. 1997; Cochard et al. 1999), and an increase in photoinhibition (Valladares and Pearcy 1997).

The aim of this study was to assess the ability of natural populations of mature holly trees to respond to drought stress (1) when receiving full sunlight in a gap, which increases plant transpiration, compared with drought stress (2) under heavy shade induced by competition for water from canopy trees, in an oak-forest understory. By measuring the physiological and morphological traits of holly under these two environments we attempted to address specific hypotheses that: (a) plasticity in the response to full sunlight of those physiological traits controlling water relations and gas exchange is low in holly; (b) photosynthetic up-regulation is inadequate to prevent significant photoinhibition (reduced F_v/F_m); (c) drought stress exacerbates the negative effect of full sunlight, further restricting the photosynthetic acclimation response, and reduced leaf water potential and hydraulic conductivity result in still less photosynthetic adjustment; (d) morphological traits, such as specific leaf area (SLA) and leaf sapwood ratio (LA : SA), respond to changes in light and water availability to a similar extent in holly and in other co-occurring tree species.

Materials and Methods

Study Site

Our experiment was conducted in the beech-oak forest of Montejo de la Sierra. The studied stand is one of the most south-westerly beech populations in Europe (41°7′ N 3°30′ W). It is situated in the "Sistema Central" of the Iberian Peninsula at 1,250–1,400 m above sea level. The local climate is continental, with frequent summer droughts during the mid-late growing season (Aranda et al. 2001, 2005). The study was carried out during 2001, a year that was characterised by low summer rainfall reflected in the low predawn water potential in September (see below).

The forest is comprised of three dominant deciduous tree species, beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* Matt [Liebl]), and melojo oak (*Quercus pyrenaica* Willd.), as well as European holly (*I. aquifolium* L.). Holly is present in the understory of mature beech and oak stands and can make up 30–40% of the basal area, though its presence in the experimental plots was slightly lower. More details of the Montejo de la Sierra forest composition are given in Pardo et al. (1997), Gil et al. (1999) and Aranda et al. (2000).

Two plots were selected representing distinct light environments (Table 1). An old firebreak was used to provide a treatment in almost full sunlight (1), whilst a closed-canopy forest within 100 m of the firebreak gave the heavy-shade treatment (2). Measurements were performed during the spring and summer of 2001 in the mid-canopy at two distal orientations (Fig. 1) on leaves of three trees from each treatment (3–8 m height, and basal diameter of 11–88 cm).

Hemispherical photographs were taken to characterise the light environment. These were digitised with a scanner (Olympus ES-10, Olympus Optical Co., Europe GMBH) and analysed with the commercial software, HemiView (Hemiview 2.1, Canopy Analysis Software, Delta-T

Table 1 Microclimatic conditions in the gap (sun) and understory (shade)

|	Gap	Understory
GSF (%)	61.8 ± 6.4	9.1 ± 1.4
$PPFD_{max}$ ($\mu mol\ m^{-2}\ s^{-1}$)	$1,838 \pm 58$	692 ± 166
VPD_{max} (kPa)	3.59 ± 0.09	3.02 ± 0.02

Global site factor (*GSF* %) is the irradiance relative to full sunlight assessed using digitised hemispheric photographs. $PPFD_{max}$ maximum photosynthetic photon flux density and VPD_{max} maximum vapour pressure deficit, were recorded at the same time as gas exchange measurements

Devices Ltd.). Photosynthetic photon flux density (PPFD) was also recorded concurrently with each gas exchange measurement (Table 1). The global site factor (GSF) was used as a surrogate of relative irradiance at each sampling point.

Physiological traits: gas exchange and chlorophyll fluorescence

Gas exchange was always measured on cloud-free days in the early morning and at mid-day under saturating PPFD.

Fig. 1 Environmental variables at the time of gas exchange measurements (see Fig. 4) in the early morning (*left panels*) and at mid-day (*right panels*). Leaves from the east and west side of holly trees in a gap and in the understory were measurement through the growing season. *PPFD* photosynthetic photon flux density ($\mu mol\ m^{-2}\ s^{-1}$), *VPD* vapour pressure deficit (kPa) and, T_{leaf} leaf temperature (°C). Mean $\pm$ 1 SD
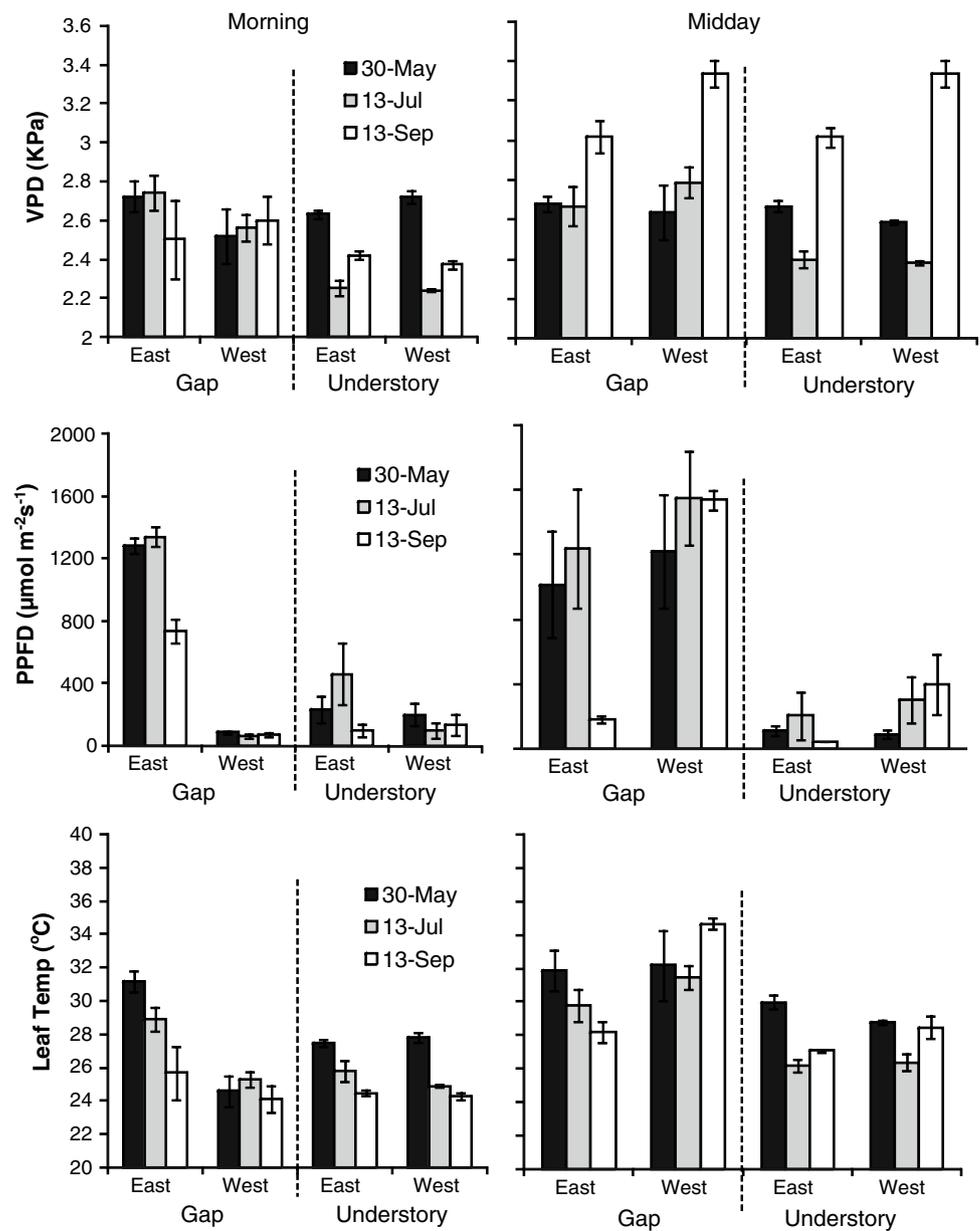
The same leaves were selected for measurement from each tree on three occasions during the field season.

An infra-red gas analyser (IRGA, LCA–4, Analytical Development Corporation, UK), open system differential mode, was used to make gas exchange measurements of light-saturated net photosynthesis (A_{sat}). The chamber was lightly shaded for 30 s between every measurement to avoid overheating. A quantum sensor mounted on top of the chamber measured photosynthetic photon flux density (PPFD). Net photosynthesis on a per area basis ($A_{net-area}$), stomatal conductance of water vapour (g_{wv}) and sub-stomatal CO_2 concentration (C_i) were calculated, following von Caemmerer and Farquhar (1981).

Chlorophyll fluorescence, at light saturated (F_m), and ground (F_0) states, was determined at dawn in the same, 30-min dark-adapted, leaves used to measure gas exchange (PSM Chlorophyll Fluorimeter, Biomonitor S.C.I. AB Umea, Sweden). The F_v/F_m ratio was considered as a surrogate of maximum quantum efficiency of PS II (Kitajima and Butler 1975; Demmig-Adams et al. 1989). Variable fluorescence was calculated from $F_v = F_m - F_o$.

Morphological traits

Those leaves used for physiological trait measurements were later harvested for determination of SLA and leaf nitrogen content. Following excision of the petiole, leaves were scanned (Image Analyser, Delta-T) and leaf area calculated. These leaves were dried to a constant weight at 65°C, weighed for dry mass and analysed for nitrogen content using the Kjedhal Method.

Water relations

Water relations in mature trees were assessed by recording predawn- and mid-day- leaf water potentials (Ψ_{pd} and Ψ_m) in the same leaves used for gas exchange measurements. As were the parameters derived from pressure-volume (P-V) curves, obtained by recording the time-coarse change in leaf water potential against mass loss due to transpiration.

To construct P-V curves, two leaves per tree were collected from the south-facing branches of trees in each light treatment. One-year old leaves were removed at dawn during late spring prior to the unfolding of new leaves. The dehydration of leaves overnight, did not adversely affect pressure-volume relationships, as consistent with previous studies (Dreyer et al. 1990; Kubiske and Abrams 1990, 1991). The air-drying method was used to establish water potential isotherms (Hinckley et al. 1980), by taking concurrent measurements of leaf mass and Ψ at regular intervals. Osmotic potential at full turgor ($\Psi\pi^{100}$) and zero turgor ($\Psi\pi^0$), relative water content at zero turgor (RWC), and maximum bulk elastic modulus (E_{max}), were estimated from the P-V curves (Robichaux 1984).

Two 1-year-old shoots were sampled from south- and north-facing branches of the same trees under each light treatment. The proximal end of each shoot was re-cut under water immediately after collection. Leaves from both ends of these shoots (LA) were measured with a Digital Image Analyser (Delta-T), and shoot length (L) was recorded. Maximum hydraulic conductivity (k_{max}) was evaluated after the refilling of any embolisms, achieved by flushing the shoot segments with degassed water (filtered to 0.20 μm) for 1 h at a pressure of 0.8 MPa (Sperry and Tyree 1988). Hydraulic conductivity was recorded by measuring the water flux (F) with a pressure gradient (ΔP) of 6.5 kPa. The outflow solution from segments was collected in an analytical balance every minute until their weight stabilised. That is, when coefficient of variation was lower than 5%. $k_{max,}$ calculated from:

$$k_{max} = FL/\Delta P$$

The sapwood area of segments (SA) was estimated from the diameter of the proximal end after bark stripping, which allowed calculation of the leaf : sapwood ratio (LA/SA). The leaf-specific hydraulic conductivity (K_L) and specific hydraulic conductivity (K_S) were calculated from:

$$K_L = k_{max}/LA$$

$$K_S = k_{max}/SA$$

K_L provides information about the hydraulic sufficiency of a shoot in relation to the leaf area it supports. K_S deals with the hydraulic efficiency of sapwood.

Statistical analysis

Physiological variables were analysed by three-way nested ANOVA. Date, time-of-day, and microsite were considered as the main fixed effects, and orientation as a factor nested within microsite. All statistical analyses were performed with the open-source software package Statgraphics 4.1. Differences between mean values were tested by LSD test. When orientation was not significant data were pooled.

Acclimation of all parameters to the light and water availabilities was assessed by quantifying phenotypic plasticity using the approach of Valladares et al. (2000). Plasticity for each trait was calculated as the difference between the minimum and maximum mean response along a standardised gradient of light. To obtain an index between 0 and 1 this difference was divided by the maximum mean value for each trait.

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Our two light treatments are at opposite extremes of the natural radiation gradient where holly trees can be found at our study site. For most traits, plasticity indices can be adversely influenced by ontogeny (Gedroc et al. 1996), and confounding environmental factors, such as drought. We attempted to avoid these problems by using trait data from 1-year old leaves of similar-sized individuals in late spring, just prior to new leaf unfolding, to obtain the plasticity estimates.

Results

Water relations

Holly leaf water potential (Ψ_{pd} and Ψ_m) was highest at the beginning of the season under both microenvironments. Minimum values of Ψ_{pd} were reached on 13 September when drought was most acute. Ψ_{pd} was not significantly different between sites during June and July, but was higher in the leaves of trees growing in full sun than in the oak-forest understory during September (Fig. 1). Despite very different relative irradiance and vapour pressure deficit (VPD) between the two micro-sites (Fig. 1), Ψ_m was only slightly lower overall in trees growing under full sun than those in the understory (Fig. 2).

Water-related parameters derived from P-V curves showed high seasonal variation (Fig. 3). This was due to the ontogenic differences between leaves sampled in June and those from July and September. This is highlighted by the proportion of dry weight to total weight (DW/TW) of leaves, an index of scleriphycation, which was lowest in July just after the unfolding of new leaves. There were treatment differences in all traits related to the maturity of leaves (Fig. 3).

The low responsiveness of $\Psi\pi_{100}$ and $\Psi\pi_0$ to the light environment and to drought in September was unexpected (Figs. 2, 3). Leaves from trees growing in full sun had similar $\Psi\pi_{100}$ to those from the understory. This low acclimation potential to light environment, rendered a low index of plasticity for most traits affecting water relations. Likewise, there was little response of leaf-turgor loss to drought, with no differences in $\Psi\pi_0$ measured at the end of spring and summer, although there was a significant seasonal decrease in Ψ_p (Fig. 3).

In contrast to water relations derived from P-V curves, specific hydraulic-(K_S) and leaf-specific hydraulic (K_L) conductivities were higher in shoots of trees growing in full sun than in the understory (Table 2). However, leaf to sapwood area ratio (LA/SA) was lower for trees in the open, and, as such, exhibited a more plastic response than other morphological and physiological traits measured (Table 2). The decreased LA/SA and increased K_S reflect
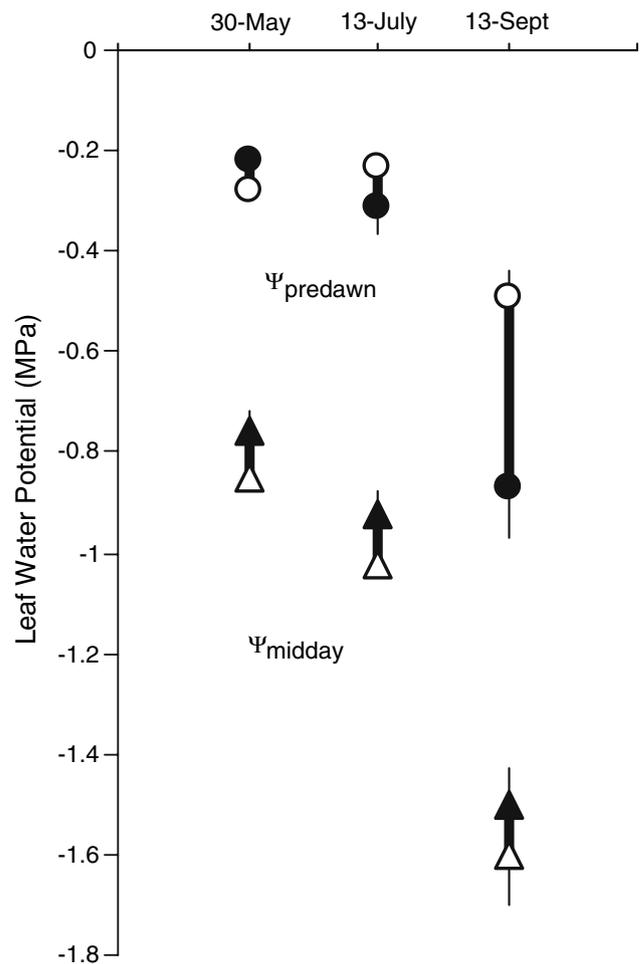

Fig. 2 Change in predawn (*circles*) and mid-day (*triangles*) leaf water potential (Ψ) during the field season. Gap: *Open circles/triangles*, understory: *Closed circles/triangles*. Mean $\pm$ 1 SE (*pencil line*)

an increase in hydraulic sufficiency, expressed by K_L, which was higher in full sun (0.56 plasticity index) than the understory.

Morphological traits and chlorophyll content

SLA was highly correlated with irradiance (data not shown), and much lower in the gap than the understory. It exhibited the most plastic trait response to the light environment (Table 2), and appeared to drive the other morphological changes. Leaf nitrogen only differed between light treatments when considered on an area basis (N_a), not a mass basis (N_m), and mirrored changes in SLA (Table 2). There was less investment in chlorophyll per unit of dry mass (Chl$_{mass}$) in leaves from the gap than the understory. However, when the difference in SLA with light environment was taken into account, this trend was reversed
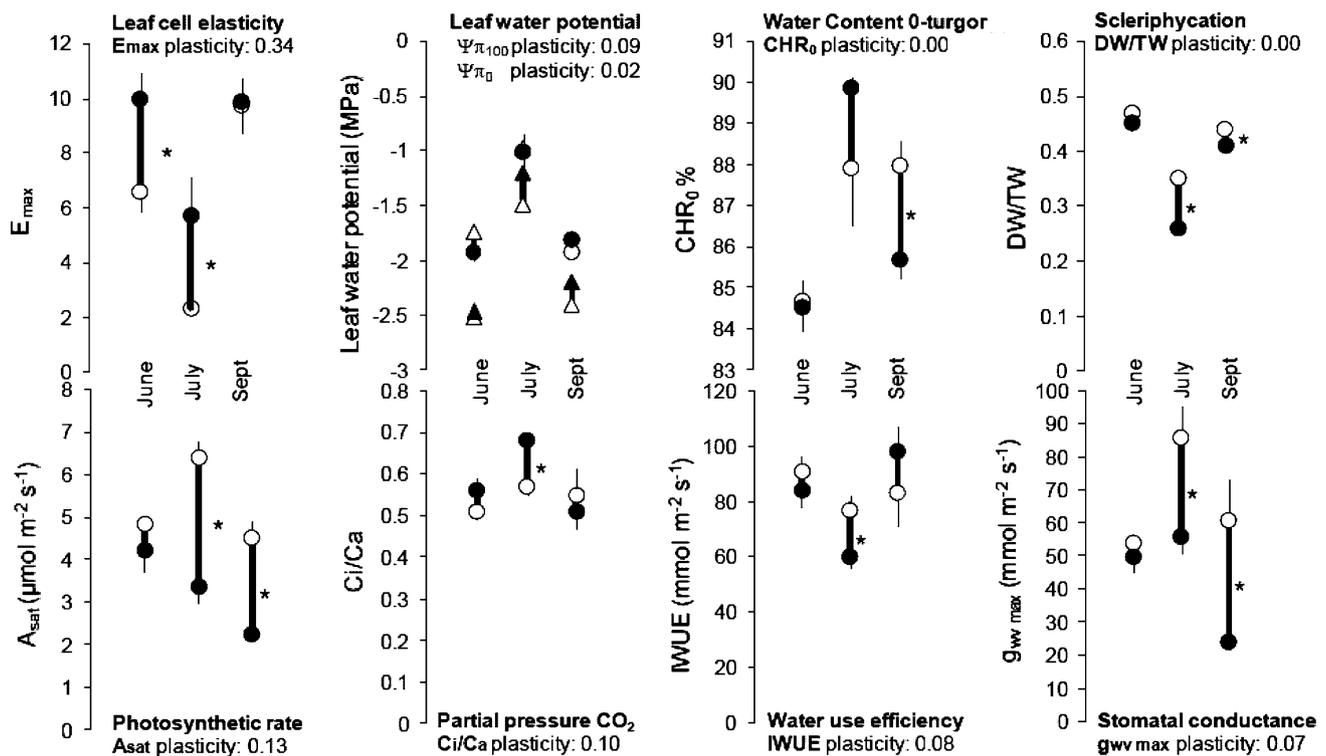
Fig. 3 Plasticity of traits involved in water relations and gas exchange, measured on three dates through the field season. *Open circles* gap, *filled circles* understory. * indicates a significant difference ($P < 0.05$) in trait values between the two light environments. Measurements of 1-year old leaves in early June, and new leaves in July and September

Table 2 Plasticity of leaf morphological traits, pigmentation and chlorophyll fluorescence

Leaf trait	Plasticity index	Gap	Understory
K_L (kg m^{-1} s^{-1} MPa^{-1})	0.56*	4.47×10^{-5}	1.95×10^{-5}
N_a (g m^{-2})	0.37*	2.78 ± 0.10	1.76 ± 0.09
LA/SA (m^2 m^{-2})	0.37*	3.74×10^{-4}	5.98×10^{-4}
SLA (cm^2 g^{-1})	0.36	39.21 ± 0.78	61.72 ± 1.91
F_m	0.30*	2.02 ± 0.15	2.89 ± 0.11
K_S (kg m^{-1} s^{-1} MPa^{-1})	0.24	7.89×10^{-2}	5.38×10^{-2}
Chl$_{mass}$ (mg g^{-1})	0.21*	2.84 ± 0.17	3.58 ± 0.13
Chl$_{area}$ (µg cm^{-2})	0.20*	71 ± 4	57 ± 2
F_o	0.19*	0.42 ± 0.02	0.52 ± 0.01
F_v/F_m	0.04*	0.788 ± 0.01	0.819 ± 0.004
N_m (mg g^{-1})	0	10.82 ± 0.32	10.77 ± 0.33

*Indicates a significant difference ($P < 0.05$) in the trait value between the two light environments

since chlorophyll content cm^{-2} leaf area (Chl$_{area}$) was slightly higher in leaves from holly trees growing in full sun. The plasticity of chlorophyll content was intermediate between that of SLA and other parameters involved in water relations and the vascular system (Table 2).

Gas exchange and chlorophyll fluorescence

There was little variation between treatments in the rates of gas exchange of 1-year old holly leaves at the end of May, just before the flush of new leaves (Fig. 4). Changes in $A_{net-area}$ with orientation can largely be explained by fluctuations in PPFD during the measurements, though this effect was more important in the gap than the understory (Fig. 4).

During July, there was a strong ontogenic effect on gas exchange, similar to that on water relations (Figs. 2, 3). As such, higher values of A_{sat} and g_{wv-max} were obtained in the gap where leaves reached maturity sooner than in the understory. The flush of new leaves took place 10-days earlier in the gap than the understory, as confirmed by lower DW/TW and $\Psi_{\pi 0}$-values (Fig. 3). There were no differences in Ci/Ca and intrinsic water use efficiency (IWUE) between the two light environments, except in July, when the quotient between $A_{net-area}$ and g_{wv} was proportionally higher in leaves of trees growing in the gap than in the oak understory, causing a lower maximum Ci/Ca and higher IWUE in leaves from the gap (Fig. 3). During September, there was a decrease in $A_{net-area}$ and g_{wv} at both sites (Fig. 4). Gas exchange tended to be lower during the mid-day measurement due to late summer drought. The plasticity of gas exchange rates was very low,

Fig. 4 Trends in net photosynthesis ($A_{net-area}$) and stomatal conductance of water vapour (g_{wv}) in gap and understory holly through the growing season. Comparison of leaves from the east and west sides of the trees, in the early morning (*left panels*) and at mid-day (*right panels*) measurements. Mean ± 1 SE
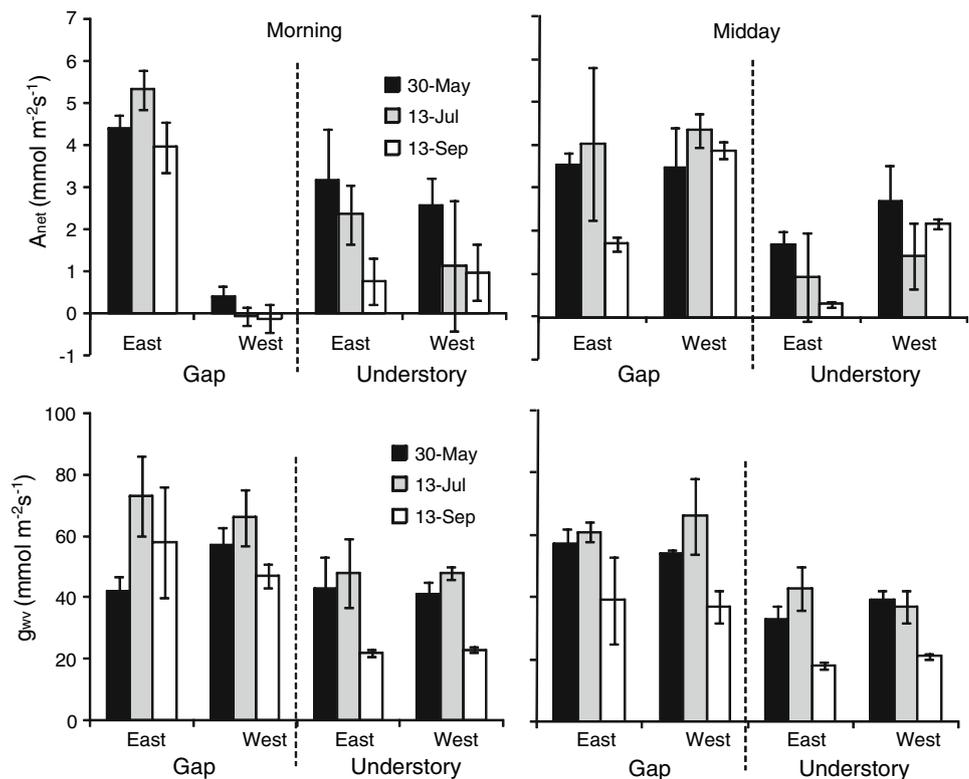
and similar to that of water relations parameters. The most plastic (0.13) was A_{sat}. (Table 3; Fig. 3g, h).

Hollys in the gap had a slightly lower F_v/F_m, as well as lower F_m and F_0, than those from the understory (Table 2). The degree of plasticity in F_m and F_0 was slightly higher than that of the gas exchange parameters.

Discussion

Holly growing under increased solar radiation in the gap exhibited stress responses in most of the traits measured. Additionally, late summer drought was sufficiently severe to lower predawn leaf water potential (Ψ_p) to extreme

Table 3 Relative plasticity of holly, beech and oak leaves from sun and shade in the central Iberian Peninsula

	I. aquilifolium	*Q. petraea*[a]	*F. sylvatica*[a]
Morphological traits			
SLA	0.36	0.35	0.48
N_m	0.01	0.01	0.01
N_a	0.37	0.34	0.38
Physiological traits			
g_{wv-max}	0.07	0.22	0.60
$A_{satarea}$	0.13	0.12	0.51
$A_{satmass}$	0.13	0.16	0.26

[a] Plasticity calculated from data in Aranda et al. (2001, 2004)

values in both sun and shade. Contrary to our expectation about the influence of drought on holly, the drying effect of full sunlight in the gap was less severe than the stress in the understory, which we attribute to competition for water with established canopy trees. This agrees with previous studies of other temperate and Mediterranean forests (Abrams and Mostoller 1995; Aranda et al. 2004).

The morphological traits, SLA and LA/SA ratio, were much more responsive to the change in light environment than the physiological traits measured. This inequality in the plasticity of response of morphological versus physiological traits is frequently associated with shade-tolerant species (Valladares et al. 2000; Niinemets and Valladares 2004). The most responsive trait was SLA, demonstrating the typical decrease that occurs with acclimation to a sunny environment (Givnish 2002). Decreased LA/SA in the sun caused an increase in K_L, similar to that reported for beech (Cochard et al. 1999), maple (Maherali et al. 1997) and ponderosa pine (Maherali and DeLucia 2001). However, the combination of low plasticity of the response of K_S, together with the small difference in g_{wv}, of holly leaves between shade and sun may indicate low capacity to activate these drought-tolerance mechanisms to maintain leaf turgor when under severe water stress.

Leaf flush in the gap was 10-days ahead of understory holly. Because of this ontogenic disparity, the photosynthetic capacity of new leaves in July was greater in the gap than the understory. Physiological leaf traits associated with

gas exchange and water relations, responded with very low plasticity to full sunlight, and, as predicted, did not prevent significant photoinhibition (reduced F_v/F_m) in the gap.

Low maximum modulus of elasticity for holly leaves in the sunny compared to the shady site was the only water-related trait to demonstrate plasticity. Low modulus of elasticity is sometimes associated with a low leaf epidermal cell density (Niinemets et al. 2003), which together can reduce water loss (low g_{wv}). This strategy would limit the daily decrease in leaf water potential and subsequent loss of turgor (Abrams 1986; Augé 1998; Aranda et al. 2000). Previous studies in Montejo de la Sierra have reported that mid-day water potential (Ψ_m) can reach as low as -2.5 MPa in beech and -2.7 MPa sessile oak (Aranda et al. 2000, 2004), whereas in holly Ψ_m was only -1.6 MPa in the gap and -1.5 MPa in the understory. The plasticity of stomatal conductance (g_{wv}) in holly also compared poorly with co-occurring beech and oak under both light environments (Table 3). But, despite generally low plasticity in water relations, the turgor loss point of holly was higher than these co-occurring species (Table 4). This is consistent with a strategy to cope with drought, resulting in a conservative pattern of water use (Pockman and Sperry 2000; Bhaskar and Ackerly 2006), through low g_{wv} and high $\Psi\pi_{100}$ accompanied by inefficient hydraulic conductance supplying minimal water to the leaves. Despite this conservative strategy, drought-avoidance mechanisms had insufficient capacity to ameliorate the effects of late-season drought, such as a decrease in pre-dawn water potential.

Late-season drought further reduced the already low photosynthetic capacity of holly. Drought-stress responses such as low g_{wv-max}, tended to interfere with rather than compliment to low light, increasing the stomatal constraint of carbon assimilation. This supports the idea that universal trade-offs in physiological stress-tolerance mechanisms preclude that a species can be both shade tolerant and drought avoiding (Niinemets and Valladares 2006; Sánchez-Gomez et al. 2006). Evolutionary processes favour adaptation towards a particular functional strategy, leading to niche partitioning and compromising the possibilities of tolerance to various stresses (Niinemets and Valladares 2006). For example, holly is thought to have evolved in sub-tropical conditions during the arcto-tertiary period, where little selection for drought tolerance would have occurred under the prevailing humid Mediterranean climate (Peterken and Lloyd 1967; Palamarev 1989).

Both the morphological and physiological features of holly leaves seem to impose limits on the maximum photosynthetic capacity. Low A_{sat} despite high N_a is consistent with the conservative strategy of evergreen leaves that allocate N to non-photosynthetic compounds involved in herbivory defence and longevity (Cornelissen et al. 1997; Castro-Díez et al. 2000). Low photosynthetic capacity in both sun and shade may be responsible for the high osmotic potentials recorded here, which would preclude drought avoidance through osmotic adjustment. The rate of carbon gain per investment is low compared with co-occurring shade tolerant species (Aranda et al. 2004), also reflected in lower SLA and N_m although this could be partially compensated by a long leaf life span in holly.

Conclusions

Our study supports the idea that holly has a reduced phenotypic plasticity in response to light and that when subject to a combination of stress factors (e.g. shade plus drought) the acclimation potential of holly is further reduced. This was illustrated by the poor responses of holly compared with co-existing woody species that employ similar growth strategies. Niche partitioning in shady environments seems to be the key factor in ensuring the long-term survival of shade-tolerant species particularly for those species whose competitive ability is low (Pacala et al. 1994), as is the case of holly (Niinemets et al. 2003). With climate change, the increasing occurrence of high intensity drought in the mountains of the Mediterranean region (Peñuelas et al.

Table 4 Comparison of maximum rates of gas exchange ($A_{net-area}$, net photosynthesis on an area basis; $A_{net-mass}$, net photosynthesis on a mass basis, g_{wv-max}, stomatal conductance to water vapour) and minimum values of leaf water potential ($\Psi\pi_{100}$, water potential at full turgor, $\Psi\pi_0$ water potential at turgor loss) in holly and co-occurring oak and beech species in mature sun leaves

	I. aquifolium	*F. sylvatica**	*Q. petraea**
$A_{net-area}$ (µmol CO_2 m^{-2} s^{-1})	6.41 ± 0.37[a]	10.93 ± 0.23[b]	16.87 ± 0.31[c]
$A_{net-mass}$ (nmol CO_2 g^{-1} s^{-1})	44.16 ± 2.55[a]	129.66 ± 2.73[b]	173.02 ± 3.18[c]
g_{wv-max} (mmol H_2O m^{-2} s^{-1})	86 ± 9[a]	271 ± 11[b]	528 ± 26[c]
$\Psi\pi_{100}$ (MPa)	-1.92 ± 0.03[a]	-2.15 ± 0.03[b]	-2.24 ± 0.07[b]
$\Psi\pi_0$ (MPa)	-2.40 ± 0.04[a]	-2.69 ± 0.10[b]	-2.67 ± 0.06[b]

Different letters, a, b, c, segregate significant differences among species in each trait

*F. sylvatica and *Q. petraea* data elaborated from Aranda et al. (1996, 2000)

) would be expected to further constrict the niche available for holly in central Spain. Of the two most shade-tolerant strategists, holly exhibits lower plasticity than beech to cope with recurrent cycles of drought. As such we anticipate reduced survival of holly in central Spain under predicted climate change scenarios.

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