

## Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Ilex aquifolium* in continental Mediterranean sites

FERNANDO VALLADARES,<sup>1,2</sup> SAGRARIO ARRIETA,<sup>3</sup> ISMAEL ARANDA,<sup>4</sup> DAVID LORENZO,<sup>4</sup> DAVID SÁNCHEZ-GÓMEZ,<sup>1</sup> DAVID TENA,<sup>1</sup> FRANCISCO SUÁREZ<sup>3</sup> and JOSÉ ALBERTO PARDOS<sup>5</sup>

<sup>1</sup> Centro de Ciencias Medioambientales, CSIC, Serrano 115 dpdo, E-28006 Madrid, Spain

<sup>2</sup> Corresponding author (valladares@ccma.csic.es)

<sup>3</sup> Departamento de Ecología, Facultad de Ciencias, Universidad Autónoma de Madrid, E-28049 Madrid, Spain

<sup>4</sup> INIA, Ctra. de La Coruña km 7.5, E-28040 Madrid, Spain

<sup>5</sup> Escuela Técnica Superior de Ingenieros de Montes, Universidad Politécnica de Madrid, E28040 Madrid, Spain

Received July 14, 2004; accepted December 4, 2004; published online June 1, 2005

**Summary** Shade tolerance, plastic phenotypic response to light and sensitivity to photoinhibition were studied in holly (*Ilex aquifolium* L.) seedlings transported from the field to a greenhouse and in adult trees in the field. All plants were growing in, or originated from, continental Mediterranean sites in central Spain. Seedlings tolerated moderate but not deep shade. Mortality was high and growth reduced in 1% sunlight. Survival was maximal in 12% sunlight and minimal in full sunlight, although the relative growth rate of the seedlings surviving in high light was similar to that of plants in moderate shade. Maximum photochemical efficiency at predawn was significantly lower in sun plants than in shade plants in the field, revealing chronic photoinhibition that was most pronounced in winter. Plasticity in response to available light varied according to the variable studied, being low for photosynthetic capacity and stomatal conductance, and high for specific leaf area, root:shoot ratio and leaf area ratio, particularly in seedlings. Differences in water relations and hydraulic features between sun and shade plants in the field were marginal. High water potential at the turgor loss point of field-grown plants suggested that holly is sensitive to drought during both the seedling and the adult stage. Low relative growth rates in both high and low light with low physiological plasticity in response to light indicate the existence of a stress-tolerance mechanism. We conclude that holly is a facultative understory plant in areas of oceanic and relatively mild climate, but an obligate understory plant in dry continental areas such as the study site. The impact of abandonment of traditional management practices and climate change on these Mediterranean populations is discussed.

**Keywords:** acclimation, holly, high light stress, photosynthesis, relative growth rate, seedlings, sun and shade, survival, water relations.

### Introduction

Plant species typically segregate across light gradients in the field, giving rise to the light partitioning hypothesis that explains species coexistence in forest ecosystems (Poorter and Aerts 2003, Valladares 2003). Species are thus classified into obligate or facultative shade plants and obligate or facultative sun plants (Damascos and Rapoport 2002). However, extensive comparative studies indicate that there are a few extremely shade-tolerant and a few extremely light-demanding species, with most species having intermediate and thus overlapping light preferences (Wright et al. 2003). Our understanding of the shade tolerance of most plants relies on classifications based largely on observational and anecdotal evidence, and the assignment of a given species to one category or another is commonly based on qualitative estimates of the natural light environment in which the species most often occurs (Reich et al. 2003). The restriction of adult plants to the understory may be because dispersion of the propagules is limited to the understory, or because of the poor survival of seedlings or saplings or adult plants in high light, or a combination of these factors (Givnish 1988, Henry and Aarssen 1997). Determining light requirements of plants and their ecophysiological adaptations to light conditions is particularly difficult in the case of long-lived species that may be exposed to different light environments at different stages of their life cycle, and the difficulty is compounded when other stresses such as drought co-occur (Valladares 2003, Niinemets and Valladares 2004, Sánchez-Gómez et al., unpublished results).

Holly (*Ilex aquifolium* L.) is a widespread shrub or small tree that is found mainly in the understory of a range of temperate forests and woodlands (Peterken and Lloyd 1967). Vegetative dispersion is limited despite vigorous sucker formation, so that the regeneration of holly relies heavily on seedlings, which are sensitive to drought (Peterken and Lloyd 1967). In oceanic regions, holly can tolerate either sun or

shade, although it performs best in partial shade, but in arid and hot environments, holly is an obligate shade plant (Brickeell and Zuk 2002). Consistent with this characterization, Ellenberg et al. (1991) found holly seedlings largely restricted to shaded sites (5–10% of full sun). But quantitative estimates of the shade tolerance of holly and ecophysiological information on the acclimation responses of holly to light are scarce (Karlic and Richter 1983, Groom et al. 1991, Del Hierro et al. 2000, Schmidt et al. 2000, Martínez-Vilalta et al. 2002). Despite detailed information on reproductive biology, costs of reproduction and the functional implications of dioecy of holly (Obeso 1997, Obeso et al. 1998), there is little information on its regeneration niche and ecophysiological field performance.

The distribution of holly and its habitat preferences indicate sensitivity to low temperatures (Rütten and Santarius 1988). Holly is a poor competitor in temperate oceanic habitats where it is subject to replacement by *Rhododendron ponticum* L. (Peterken and Lloyd 1967, Niinemets et al. 2003). It has been suggested that holly is especially susceptible to replacement by other species as a result of increasing atmospheric CO<sub>2</sub> concentrations (Hattenschwiler and Körner 2003). At the southern limit of its distribution, holly experiences a range of adverse conditions, particularly in dry, cold continental areas. In some of these areas (e.g., central Spain), there are dense, mono-specific holly formations of great ecological value, which are the result of long-term management (Oria de Rueda 1992, Arrieta 2002, Arrieta and Suárez 2005).

Our objective was to acquire a better understanding of the effects of sun and shade on the performance of holly, by combining the information available for populations in temperate Europe with our research on populations in continental, Mediterranean locations where high solar irradiance is associated with other environmental stresses such as drought and high temperatures. We focused on characterizing the light environment of seedlings and adults, and on estimating phenotypic plasticity for a range of morphological and physiological variables. Phenotypic plasticity in response to variation in resource availability is central to the environmental adaptations of sessile organisms exposed to changing environmental conditions (West-Eberhard 2003), and plant species have been shown to exhibit significant differences in their plastic response to light (Valladares et al. 2000b). We tested three hypotheses: (1) continental, Mediterranean conditions impose multiple stresses on holly populations which should, therefore, exhibit low phenotypic plasticity (Balaguer et al. 2001 and Valladares et al. (2002a); (2) as a shade-tolerant species, holly should display greater phenotypic plasticity in morphological variables than in physiological variables (Valladares et al. (2002b); and (3) Mediterranean populations of holly should be sensitive to photoinhibition, particularly when high solar irradiance is coupled with other stresses according to the findings of Groom et al. (1991). To test these hypotheses, we studied shade tolerance of seedlings and the effects of high light on adult plants, as well as the plastic phenotypic responses to light of both seedlings and adults. In one set of studies, we assessed the survival of seedlings exposed to different shade treatments, and in another set of studies we determined the effects of sun

and shade on photoinhibition and water relations of adult plants in the field.

## Materials and methods

### *Study sites, vegetation and natural light environments*

We studied shade tolerance and plastic responses to light of holly (*Ilex aquifolium*) seedlings and adult plants at three field sites in central Spain: Oncala (41°57' N, 2°20' W) in Soria, and Robregordo (41°10' N, 3°30' W) and Montejo de la Sierra (41°7' N, 0°11' W) in Madrid. Elevation was 1425 ± 50 m a.s.l. at each site. The climate of the study sites is continental Mediterranean, characterized by a prolonged summer drought and contrasting diurnal and seasonal temperatures (Instituto Nacional de Meteorología 2003). Winter temperatures below -10 °C occur frequently, particularly on clear days. Oncala is slightly wetter and colder, and the summer drought less pronounced (annual rainfall of 704 mm and mean temperature of 8.5 °C, means for the last 20 years in the nearby stations of Arévalo de la Sierra and Barriomartín, Soria) than at the two Madrid sites (annual rainfall of 673 mm and annual temperature of 9.8 °C, means for the last 12 years in Montejo de la Sierra). At all sites, species-rich woodlands are interspersed with grasslands. *Ilex aquifolium* forms dense patches at Oncala and Robregordo (Arrieta 2002, Arrieta and Suárez 2005), whereas individuals are mainly isolated or in small groups in the mixed oak forest at Montejo de la Sierra (Lorenzo 2001). Soils are slightly acidic (pH ≤ 6), relatively sandy, with a low fraction of clay, a poor water retention capacity, and moderate to low organic matter (1.3 ± 0.2%) and nutrient contents (50 ± 12 mg kg<sup>-1</sup> of P, 650 ± 42 mg kg<sup>-1</sup> of K and 8 ± 2 mg kg<sup>-1</sup> of N).

Twenty-six species of trees, shrubs and climbers were observed at Oncala, where *I. aquifolium* formed thick patches 5–8 m tall with a high stem density (13,100 stems ha<sup>-1</sup> measured in a 100 m<sup>2</sup> plot), leading to a basal area of 167 m<sup>2</sup> ha<sup>-1</sup>, of which 92% was *I. aquifolium*. The mixed forest at Montejo de la Sierra was 15–20 m in height and comprised *Quercus petraea* L. ex Liebl., *Q. pyrenaica* Willd. and *Fagus sylvatica* L. in order of decreasing dominance, with *I. aquifolium*, *Prunus avium* L. and *Sorbus aucuparia* L. in the understory. This forest had a mean basal area of 25 m<sup>2</sup> ha<sup>-1</sup> and 967 stems ha<sup>-1</sup>, of which 520 were > 10 cm in diameter at breast height. Holly-dominated formations at Robregordo had a high stem density (22,400 stems ha<sup>-1</sup> in a 100 m<sup>2</sup> plot) and a basal area of 140 m<sup>2</sup> ha<sup>-1</sup>, of which 52% was *I. aquifolium*. More information on the field sites can be found in Arrieta and Suárez (2005).

In the interior of the dense holly-dominated patches, photosynthetic photon flux (PPF) was only 0.4 ± 1.4% of the PPF in the open, with an absolute maximum of 17.8% and a minimum of 0.1% (results from 110 open-understory pairs of measurements taken with a HD 9021 PPF quantum photometer, Delta OHM, Italy) at midday ± 1 h during June and July 1999. In the understory of the mixed oak forest, PPF was estimated to be 9.1 ± 1.4% of full sunlight (based on four hemispherical photographs analyzed with the software Hemiview

(Delta T, Burwell, U.K.). Although indirect estimates of PPF in the understory (e.g., from hemispherical photographs) likely overestimated the light availability, the difference is, in general, no more than 10% (Kull et al. 1999). Thus, PPF in the understory of the mixed oak forest was probably about 8% of the PPF available in the open.

#### *Plant material and experimental design*

Seedlings were sampled at Oncala and Robregordo, and adult plants were studied in situ at Montejo de la Sierra. Seedlings were collected in the field at the cotyledon stage about 1 week after germination in May 1999. Each seedling was carefully potted together with the substratum surrounding the root system in a 200-ml pot filled with top soil from the site and transported immediately to a greenhouse at the Autonomous University of Madrid. Plants that exhibited symptoms of water stress or mechanical damage to roots, stems or cotyledons during the first 2 weeks after transportation to the greenhouse were discarded. After 2 weeks, seedlings were distributed at random in six light environments produced with neutral shade cloth. One group of 70 seedlings was grown in the open, and the other five groups, each of 70 seedlings, were grown at 71, 48, 24, 12 and 1% of full sunlight. Daily incident PPF in Madrid was  $56.9 \text{ mol m}^{-2} \text{ day}^{-1}$  during the primarily clear summer days of the experiment. Seedlings were watered every other day to full soil capacity, and survival was checked weekly. Thirty-three additional seedlings were collected and dried to estimate initial plant mass. Relative growth rate and plant allometry and morphology were measured at the end of the experiment, after 5 months of growth in each light treatment. The experiment was repeated in 2000 following the same protocol with the same number of seedlings, but with only three light environments (100, 32 and 3% of full sunlight). Chlorophyll and carotenoid contents and maximum rates of photosynthetic electron transport were measured in seedlings in the second experiment. Data from the two populations of seedlings exhibited no significant differences for any of the variables studied and were pooled for statistical analyses.

The ecophysiological response to light availability of adult plants was explored in individuals of *I. aquifolium* growing in the oak forest understory and in the open at Montejo de la Sierra. The open site was the result of a firebreak created in 1995, so the individuals studied had been exposed to high solar irradiance for at least 6 years before the measurements.

#### *Morphology and leaf biochemistry*

Holly seedlings were harvested and separated into leaves, stems and roots for the different analyses at the end of the experiments. Leaf areas of seedlings and of shoots of adult plants were determined in fresh samples within 6 h of collection with a conventional scanner and Sigmascan and Leica Qwin image analysis software (Jaendel Scientifics, San Diego, CA). Leaves were kept in dark plastic bags to prevent desiccation during transport and manipulation. Plant material was dried for 48 h at 65 °C and weighed. From these data, we calculated specific leaf area (SLA;  $\text{m}^2 \text{ kg}^{-1}$ ) in both seedlings and adult

plants, and root:shoot ratio, leaf area ratio (LAR;  $\text{m}^2 \text{ kg}^{-1}$ ), and relative growth rate (RGR;  $\text{week}^{-1}$ ) in seedlings.

Chlorophylls were extracted from leaf disks from both seedlings and adult plants with dimethyl sulfoxide (DMSO) for 2.5 h at 65 °C and the chlorophyll and carotenoid concentrations of the extracts determined spectrophotometrically as described by Barnes et al. (1992). Total foliar nitrogen was determined by Kjeldahl analysis with  $\text{SeSO}_4$  and  $\text{K}_2\text{SO}_4$  as catalyzers in a 1016 Digestion System 12 (Tecator, Höganäs, Sweden) and a Kjelte System 1026 (Tecator) distilling unit. Photosynthetic nitrogen-use efficiency was estimated as maximum net photosynthetic rate per g of N.

#### *Fluorescence and gas exchange measurements*

Maximum photochemical efficiency or the quantum efficiency of open photosystem II centres (Maxwell and Johnson 2000) was estimated by the fluorescence parameter  $F_v/F_m$ :

$$F_v/F_m = F_m - F_o/F_m \quad (1)$$

where  $F_o$  is dark-adapted minimum fluorescence,  $F_v$  is variable fluorescence and  $F_m$  the maximum fluorescence yield of a dark-adapted sample. Chlorophyll fluorescence was determined at different times of the day with a PMS Mark II fluorometer (Biomonitor SCIAB, Umeå, Sweden), after 30 min of dark adaptation of the leaves with a leaf clip. Measurements were made on adult plants, with a total of 12 leaves from each of the two light environments at the field site at Montejo de la Sierra throughout 2001. Only predawn values of  $F_v/F_m$  are shown because they are the best descriptors of chronic photo-inhibition (Rohacek 2002, Werner et al. 2002).

A pulse-amplitude modulated fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) with a leaf clip holder (Model 2030-B) was used to estimate apparent light-saturated rate of photosynthetic electron transport (ETR;  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) (Schreiber et al. 1994):

$$\text{ETR} = 0.5\Phi_{\text{PSII}} \Theta \text{PPF} \quad (2)$$

where  $\Theta$  is leaf absorptance and  $\Phi_{\text{PSII}}$  is the effective quantum yield, estimated as:

$$\Phi_{\text{PSII}} = \frac{F_m' - F}{F_m'} \quad (3)$$

where  $F$  is the fluorescence yield in actinic light and  $F_m'$  is the maximum fluorescence yield of the light-adapted sample. Actinic irradiance was provided by an external halogen lamp, and the measurements were conducted at seven PPF values from 0 to  $2200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Starting with the lowest PPF, pulses of white light of  $8000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  were applied to determine  $F_m'$ . During these fluorescence measurements in the greenhouse, mean leaf temperature across individual leaves was  $25.1 \pm 2$  °C. The ETR responses to PPF of all seedlings belonging to the same light treatment were fitted by nonlinear regression analysis. Maximum ETR obtained from the regression of each curve was used in the comparisons of seedlings

from different light environments.

Maximum net photosynthetic rate and maximum stomatal conductance were determined in adult plants under the optimum field conditions with a portable open gas exchange system (Model LCA4, Analytical Development, Hoddesdon U.K.). Diurnal gas exchange measurements were made on clear days in spring 2001 on a total of 12 fully developed leaves from 4–5 adult plants from each of the two light environments in Montejo de la Sierra. The central unit recorded the incident PPF and the temperature of the cuvette simultaneously.

#### *Water relations of adult plants*

Water relations of adult individuals were determined from pressure–volume ( $P$ – $V$ ) curves made in the laboratory with fresh material brought directly from the field. Two leaves were collected from southern branches of each of three mature trees from each light environment. One-year-old leaf samples were taken at dawn at the end of spring before new leaves unfolded. Leaf samples were first rehydrated overnight. No artifacts as a consequence of the rehydration were observed in the  $P$ – $V$  relationships (Dreyer et al. 1990, Kubiske and Abrams 1990). The air-drying method was used to establish the water potential isotherms (Hinckley et al. 1980, Kubiske and Abrams 1991) so that pair measurements of leaf mass and water potential ( $\Psi$ ) were taken concurrently at regular intervals. Samples were weighed before and after the leaf water potential measurements. Osmotic potential at full ( $\pi^{100}$ ) and zero turgor ( $\pi^0$ ), relative water content at zero turgor ( $\text{CHR}_0$ ), and maximum bulk elastic modulus ( $\epsilon_{\text{max}}$ ) were estimated from the  $P$ – $V$  curves according to Robichaux (1984).

Two 1-year-old shoots were sampled from the same trees. Proximal ends of the shoots were recut under water just after collection in the field. Leaves attached to the shoots (SF) from the proximal to the distal segment were measured with a Digital Image Analyser (Delta-T, Cambridge, U.K.). Shoot length ( $L$ ) was also recorded. Maximum hydraulic conductivity ( $k_{\text{max}}$ ) was evaluated after full reversal of native embolism in the laboratory by flushing the stem segments with degassed water previously filtered to 0.20  $\mu\text{m}$  for 1 h at a pressure of 0.8 MPa (Sperry and Tyree 1988). Hydraulic conductivity was estimated by measuring water flux ( $W$ ) with a pressure gradient ( $\Delta P$ ) of 6.5 kPa. Outflow solution from segments was collected in vial on an analytical balance every minute until stability in weight was reached, i.e., when the coefficient of variation was < 5%. We calculated  $k_{\text{max}}$  as:

$$k_{\text{max}} = WL/\Delta P \quad (4)$$

Sapwood area of the shoot was estimated from the diameter at the base measured with a caliper after bark stripping, which allowed for the calculation of the leaf:sapwood ratio ( $S_F/S_A$ ). Leaf-specific hydraulic conductivity, which provides information about the hydraulic sufficiency of a shoot in relation to the leaf area supported ( $K_L$ ), and specific hydraulic conductivity

( $K_S$ ), a measure of hydraulic efficiency on a sapwood area basis, were calculated as:

$$K_L = k_{\text{max}}/S_F \quad (5)$$

$$K_S = k_{\text{max}}/S_A \quad (6)$$

#### *Statistical analyses and plasticity index*

Analysis of variance (ANOVA) was conducted to evaluate the differences among light treatments in seedlings, or among field light environments in adult plants. Variables of seedling morphology were logarithmically transformed and biomass was used as a covariate as recommended by Coleman et al. (1994). In all cases, the data met the assumptions of normality and homoscedasticity. The relationship between ETR and PPF was analyzed by nonlinear regression. The effect of light availability on seedling survival was analyzed by the Mantel-Haenszel test. Differences in seedling survival among groups of light treatments were tested with a log-rank test on the survival curves obtained for each treatment, and Bonferroni's correction for multiple comparisons was applied. Statistical analyses were performed with SYSTAT 9.0 Windows Version 1999 (SPSS, Chicago, IL).

We defined phenotypic plasticity as the fraction of the total phenotypic variation observed in a given variable that is explained by differences between environments (Valladares et al. 2000a, 2002a). An index of phenotypic plasticity ranging from 0 to 1 was calculated for each variable as the difference between the minimum and the maximum mean values of each light treatment (or light environment in the field) divided by the maximum mean value (Valladares et al. 2000b). This phenotypic plasticity index, which has been used in previous studies (Balaguer et al. 2001, Niinemets et al. 2003, Valladares et al. 2000a, 2002a, 2002b), has the advantage that changes in variables expressed in different units and with contrasting variation ranges can be compared. In practice, it yields similar results to other more complex indices such as that used by Robinson and Rorison (1988).

## **Results**

#### *Shade tolerance and response to light of holly seedlings*

Survival of holly seedlings was significantly affected by light availability (Mantel-Haenszel test  $P < 0.001$ ). Log-rank tests on the survival curves revealed that survival was maximal at 12% sunlight, minimal at both 1 and 100%, and intermediate at 24, 48 and 71% ( $P < 0.001$ ; Figure 1). Initial relative growth rate of the seedlings was generally low, particularly at 1% PPF (Figure 1). Specific leaf area and leaf area ratio decreased significantly with increasing PPF, whereas root:shoot ratio significantly increased with increasing PPF, although it decreased again in the full sun treatment (Figure 2). Chlorophyll concentration on a dry mass basis was lower in full sun than in intermediate and deep shade, whereas the reverse was true for maximum ETR and carotenoid:chlorophyll ratio (Figure 3).

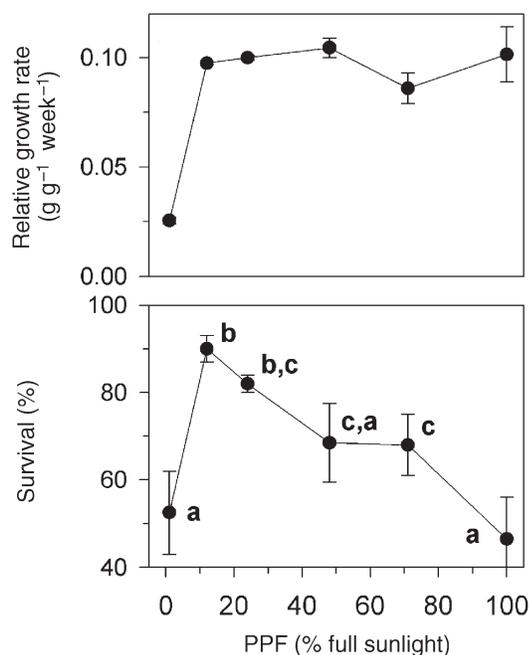


Figure 1. Survival and relative growth rates of *Ilex aquifolium* seedlings in six light treatments (1, 12, 24, 48, 71 and 100% of full sunlight) after 5 months. Data are means  $\pm$  SE for the two populations studied; initially,  $n = 70$  plants per treatment. Letters indicate significant differences among means (log-rank test of survival curves  $P < 0.01$ , after Bonferroni's correction for multiple comparisons).

#### Field performance and response to light of adult holly

Maximum photochemical efficiency at predawn was significantly lower in sun plants than in shade plants in the field, and particularly low during the winter months (Figure 4). Chlorophyll concentration was lower in adult plants than in seedlings, although the sun–shade pattern was similar to that observed in seedlings, with significantly lower values in sun plants than in shade plants throughout the seasons (Figure 4). Maximum net photosynthetic rate, maximum stomatal conductance and leaf nitrogen concentration were unaffected by light availability in the field (Figure 5), and the same was true for most of the parameters derived from the  $P$ - $V$  curves (Figure 6). However, specific leaf area and photosynthetic nitrogen-use efficiency (Figure 5) and modulus of elasticity (Figure 6) exhibited significant differences between plants from different light environments, with lower values in the sun than in the shade.

Hydraulic architecture was similar between sun and shade plants as revealed by the absence of significant differences in either hydraulic conductivity or specific hydraulic conductivity (Figure 7). The only difference in hydraulic architecture between sun and shade plants was found in the foliar to sapwood area ratio, which was higher in shade plants (Figure 7).

#### Analysis of phenotypic plasticity

High values of the phenotypic plasticity index (PI) correlated with highly significant differences between sun and shade plants across the different variables studied (Table 1). Variables ranged from highly plastic (PI > 0.6) to non plastic (PI

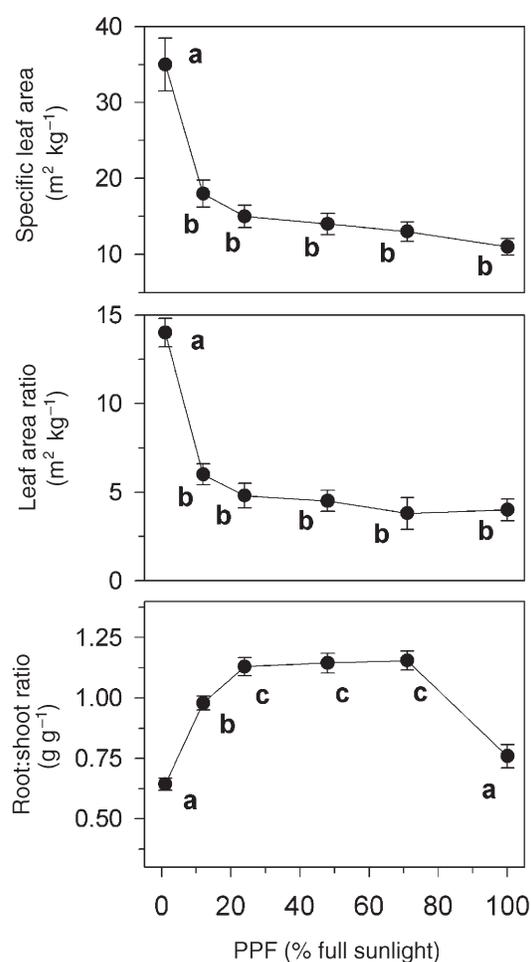


Figure 2. Root:shoot dry mass ratio, leaf area ratio and specific leaf area of *Ilex aquifolium* seedlings after 5 months in six light treatments. Data are means  $\pm$  SE;  $n = 30$ –60 plants per treatment. Letters indicate significant differences among the means (ANOVA,  $P < 0.01$ , Bonferroni's  $t$  test).

about 0) and were grouped into three types: very plastic (e.g., most morphological variables measured in seedlings and adult plants), not plastic (e.g., many water relations variables measured in adult plants), and of variable plasticity depending on the reference units, i.e., plasticity of a given variable buffered by plasticity in other variables (Table 2). An example of the latter was photosynthetic plasticity on a dry mass basis, which was counteracted by plasticity in SLA, resulting in a homeostatic response of photosynthesis on an area basis. For the variables measured in both seedlings and adults (specific leaf area and chlorophyll content and concentration), seedlings had higher values of PI than adults (Table 1).

## Discussion

#### Shade and high light tolerance of holly

Holly seedlings from the two populations studied tolerated moderate but not deep shade (1% sunlight), in agreement with

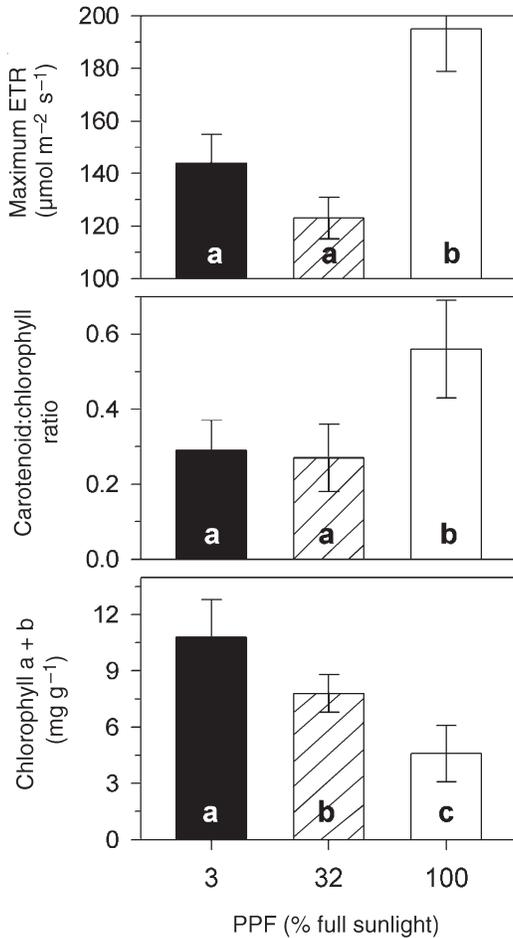


Figure 3. Chlorophyll a + b concentration, carotenoid:chlorophyll ratio and maximum photosynthetic electron transport rate per unit area (ETR, estimated by PSII chlorophyll fluorescence) of leaves of *Ilex aquifolium* seedlings grown in three light environments for 5 months. Data are means  $\pm$  SE;  $n = 20$  plants per treatment. Letters indicate significant differences among the means (ANOVA  $P < 0.01$ , Bonferroni's  $t$  test).

earlier observations (Peterken 1966). Thus, even though these seedlings are able to survive in the understory of the typical mixed oak forests of the area (about 8% sunlight; Lorenzo 2001) or at the edges of dense monospecific holly patches (1.5–8.5% sunlight; Arrieta 2002, Arrieta and Suárez 2005), light availability in the understory of dense holly patches was too low ( $< 1\%$ ) for holly seedling establishment, which supports the findings of Arrieta and Suárez (2005) based on field monitoring of seedling survival. Thus, within dense holly formations, only vegetative reproduction is possible. The intolerance of holly to heavy shading may not prevent regeneration of the species at the southern limit of its distribution, however, because canopies are relatively open and understory light is mostly well above 10% of full sunlight (Valladares 2003, Gómez et al. 2004). However, the poor performance of holly at high irradiances may hamper natural regeneration in open habitats. Thus, natural establishment of holly seem to be restricted to bright or moderately shaded forest understories in

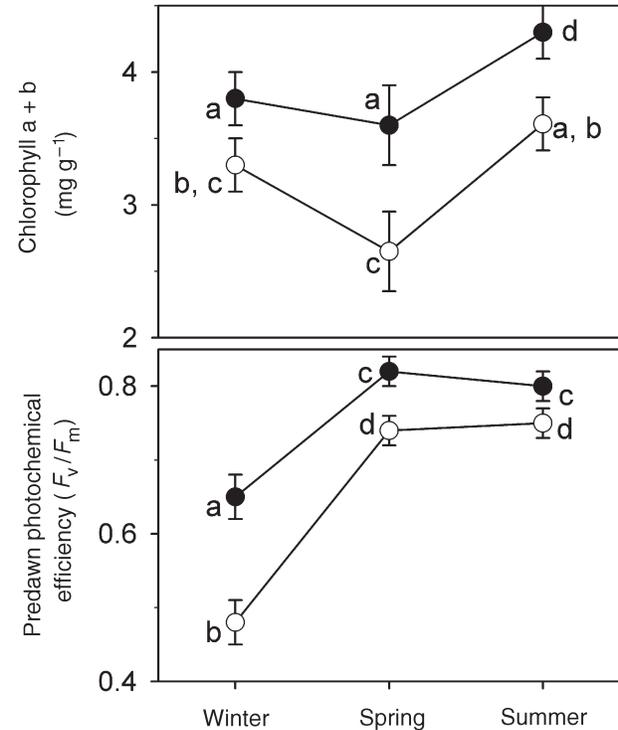


Figure 4. Seasonal patterns of predawn photochemical efficiency ( $F_v/F_m$ ) and chlorophyll a + b concentration of sun ( $\circ$ ) and shade ( $\bullet$ ) adult field plants of *Ilex aquifolium*. Data are means  $\pm$  SE and were collected at Montejo de la Sierra;  $n = 12$  leaves from each light environment. Letters indicate significant differences among the means (ANOVA  $P < 0.01$ , Bonferroni's  $t$  test).

continental Mediterranean areas.

Different variables, however, showed different tolerances to high light. Although both survival and relative growth rate (RGR) were maximal at 12% sunlight, survival decreased in full sunlight, whereas RGR remained constant. This finding challenges our understanding of plant tolerance to extreme light conditions and complicates the definition of shade and high-light tolerance (see discussion in Sack and Grubb 2003). High-light-tolerant species are classified as those that survive and grow only in high-light conditions, and shade-tolerant species are defined as those that can survive in shaded understories receiving 1–2% of full sunlight (Kitajima 1994, but see also Smith and Huston 1989, Walters and Reich 1996, Henry and Aarssen 1997). Accordingly, holly would be classified as an intermediate species because it does not fulfill any of the requirements for either a true high-light or a true low-light species. We found that individual plants that survived in high light had a similar RGR to plants in low light. This constancy of RGR under both high- and low-light conditions agrees with the results of Kitajima (1994) and indicates RGR may be a poor indicator of tolerance of high light environments.

#### Inter- and intraspecific analyses of phenotypic plasticity

Holly seeds are dispersed primarily by birds, so seedling emergence takes place largely below bird perches, which reduces

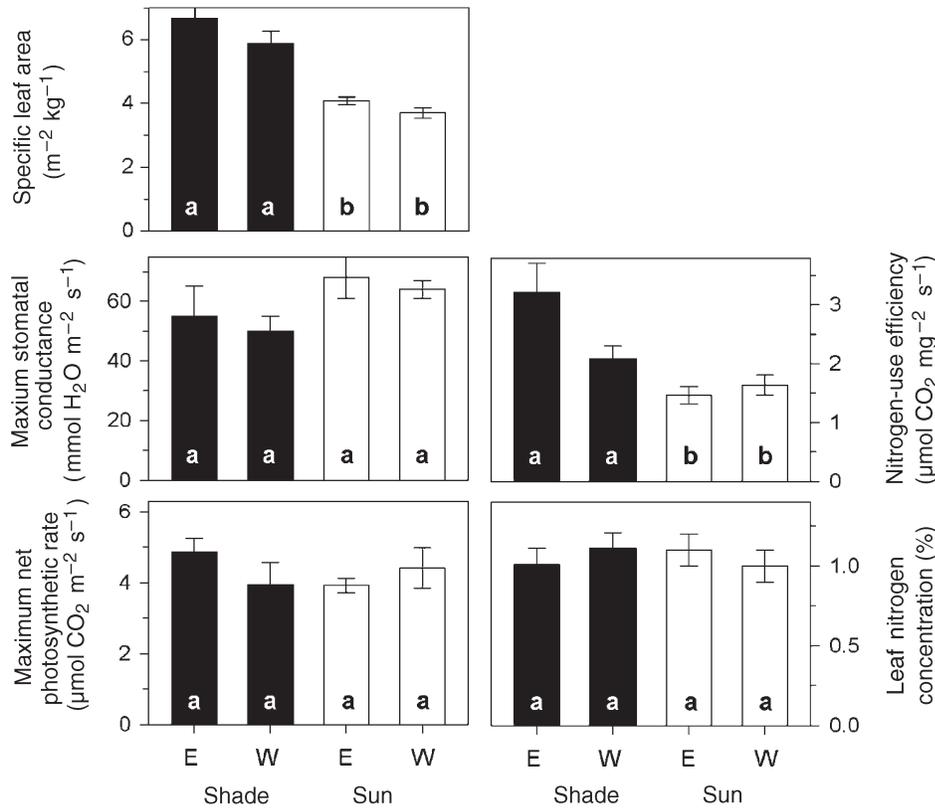


Figure 5. Maximum stomatal conductance, net photosynthetic rate, leaf nitrogen concentration, photosynthetic nitrogen-use efficiency and specific leaf area of sun (open bars) and shade (closed bars) adult plants of *Ilex aquifolium* in Montejo de la Sierra. Samples were taken in spring (May 30–June 6, 2001) from two orientations (east and west) in each plant. Data are means  $\pm$  SE;  $n = 6$  leaves from each orientation and light environment. Letters indicate significant differences among the means (ANOVA,  $P < 0.01$ , Bonferroni's  $t$  test).

the range of light conditions experienced by seedlings (Gómez et al. 2004). Thus, we expected only a limited plastic response to light in this species. What we found was that the phenotypic response of holly to light varied with the parameter studied, but it was low for certain key physiological variables, including photosynthetic rate, stomatal conductance and most water relation and hydraulic characteristics. Phenotypic inertia (i.e., lack of responsiveness) was particularly high in adult plants

and is part of the suite of features characterizing stress-tolerant woody plants (Brzeziecki and Kienast 1994) that invariably includes low relative growth rates (Kitajima 1994, Valladares et al. 2000b). Based on the findings of Balaguer et al. (2001) and Valladares et al. (2002a) and the multiple constraints imposed by the continental, Mediterranean conditions of the study sites, we predicted that our holly populations would exhibit lower phenotypic plasticity than populations in more temper-

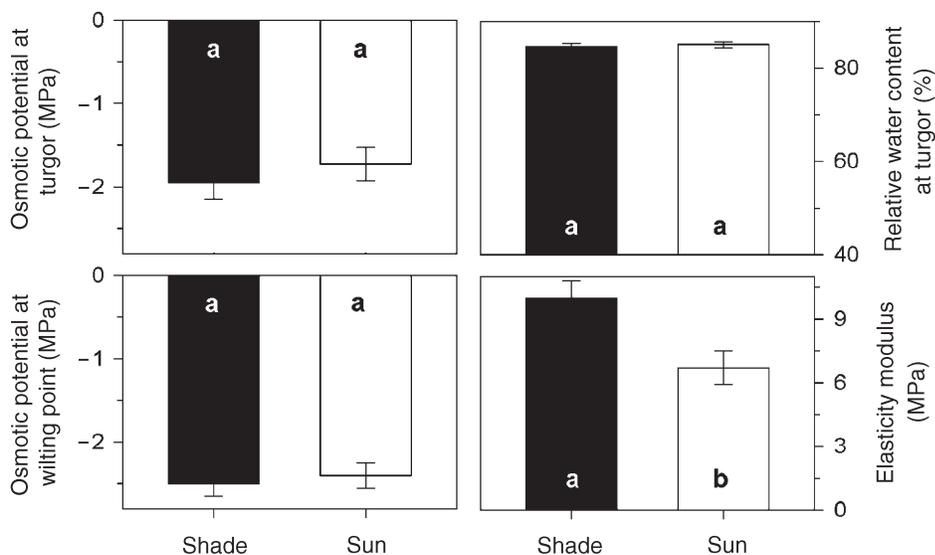


Figure 6. Water relation variables from pressure–volume curves (osmotic potential at full turgor and at wilting point, modulus of elasticity, and relative water content at turgor loss point) of sun (open bars) and shade (closed bars) adult plants of *Ilex aquifolium* in Montejo de la Sierra. Data are means  $\pm$  SE;  $n = 12$  shoots from each light environment. Letters indicate significant differences among the means (ANOVA  $P < 0.01$ , Bonferroni's  $t$  test).

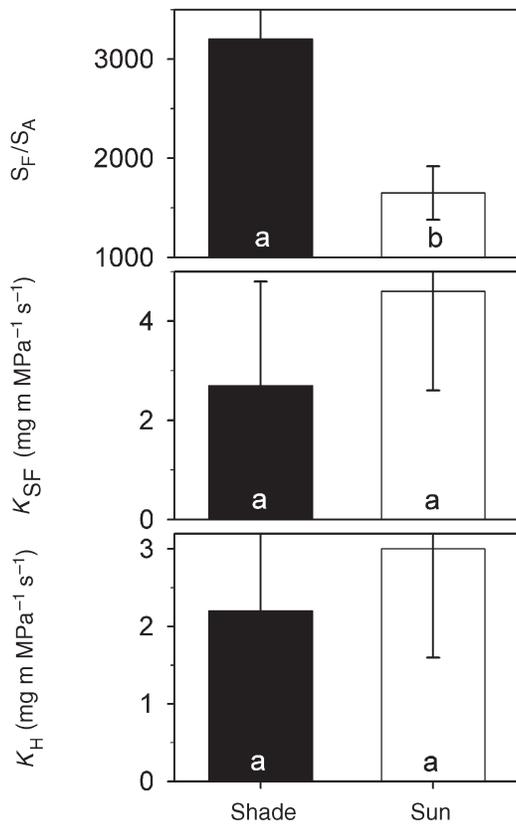


Figure 7. Hydraulic architecture variables (hydraulic conductivity ( $K_H$ ), leaf specific hydraulic conductivity ( $K_{SF}$ ) and foliar:sapwood surface area ratio ( $S_F/S_A$ )) of sun- (open bars) and shade-grown (closed bars) adult plants of *Ilex aquifolium* in spring (May 30–6 June 6, 2001) at Montejo de la Sierra. Data are means  $\pm$  SE;  $n = 12$  shoots from each light environment. Letters indicate significant differences among the means (ANOVA,  $P < 0.01$ , Bonferroni's  $t$  test).

ate, oceanic habitats. Based on a reappraisal of the data of Peterken and Lloyd (1967), we found that the plastic light response of British holly populations was higher with respect to the photosynthetic capacity (PI 0.39 versus 0.19) and specific leaf area (PI 0.58 versus 0.44 of adult plants) than the Mediterranean populations we studied. Plasticity of the Belgian populations of holly studied by Niinemets et al. (2003) was also higher than the plasticity of our populations with respect to leaf chlorophyll (PI 0.18 versus 0.05) and electron transport rate (IP 0.50 versus 0.37), but not for specific leaf area (PI 0.43 versus 0.44). Although this comparison across different studies must be viewed with caution, it supports the hypothesis that phenotypic plasticity decreases as habitat adversity increases.

We obtained support for our hypothesis that phenotypic plasticity is higher for morphological variables than for physiological variables in shade-tolerant species. The low plasticity for physiological traits agrees with previous studies of the species (Schmidt et al. 2000, Niinemets et al. 2003) and seems to be characteristic of shade-tolerant trees, such as *F. sylvatica* (Valladares et al. 2002b) and *Abies alba* Mill. (Robakowski et al. 2003) and shrubs (Valladares et al. 2000b). Holly exhibited a moderate to highly plastic response to light in morphological

and allometric variables such as specific leaf area, leaf area ratio and root:shoot ratio, which is common in shade-tolerant species (Niinemets and Valladares 2004). Shade tolerance typically translates into a low plasticity for photosynthetic energy conversion and a high plasticity for variables involved in light capture (Valladares et al. 2002b, Yamashita et al. 2002), which commonly lead to low rates of carbon accumulation in shade-tolerant species in both low- and high-light environments. Kitajima (1994) argued that maximization of whole-plant carbon gain in low light is not an essential part of shade tolerance, but may be associated with resilience to herbivores and pathogens. This argument holds for holly, a species with spiny and sclerophyllous leaves loaded with defensive compounds (Potter and Kimmerer 1986).

A physiologically plastic response to high light has been interpreted as a mechanism underlying invasiveness (Durand and Goldstein 2001, Yamashita et al. 2002). The relatively low photosynthetic plasticity of holly contrasts with that of the invasive *R. ponticum* coexisting with holly in Belgium (Niinemets et al. 2003), and replacing holly in temperate, oceanic Europe (Peterken and Lloyd 1967).

#### *Ecophysiology of the response of holly to light*

Holly plants exhibited significant photoinhibition under high-light conditions in the field, and the photoinhibition was significantly more pronounced in winter. This finding agrees with a report by Groom et al. (1991) that emphasized the importance of combined high light and low temperature for the reduction of  $F_v/F_m$  in British populations of holly. Chronic winter photoinhibition has been reported for several evergreen species in the Mediterranean region despite the mildness of the winters (García-Plazaola et al. 1999, Oliveira and Peñuelas 2000, Martínez-Ferri et al. 2004). Photoinhibition is a photo-protective mechanism (Oquist and Huner 2003) and has been observed in Mediterranean evergreens exposed to exceptionally cold weather (García-Plazaola et al. 2003). Photoinhibition of holly in high light is related to reduced photosynthetic capacity, and can be attributed to both the sensitivity of holly to high light, and low nitrogen availability. The holly populations we studied grew on nitrogen-poor soils and exhibited lower leaf nitrogen concentrations than reported for other populations (Niinemets et al. 2003, Aranda et al. 2004). It is well documented that low leaf nitrogen contents can limit the capacity for acclimation to high light and for adapting to high irradiances (Johnson et al. 1997, Skillman and Osmond 1998, Valladares et al. 2000a).

The high chlorophyll concentrations that we measured in shade plants are in accordance with classical sun–shade acclimation responses (Lambers et al. 1998) and with results for seedlings of other temperate trees (Valladares et al. 2002b). The low chlorophyll concentrations in our field plants could be a result of both enhanced photodegradation of chlorophylls and limited chlorophyll synthesis due to nutrient limitations. Maximum rates of net photosynthesis did not differ between sun and shade populations of adult plants in the field and limited nitrogen availability could explain this result, because soil nitrogen concentration was high in our seedling experiment

Table 1. Plasticity index (PI = (maximum – minimum)/maximum, after Valladares et al. 2000b) of morphological (1), biochemical (2), photosynthesis and gas exchange (3) and water relations (4) variables in seedlings and adult plants of *Ilex aquifolium*. Variables that were transformed into other variables by multiplying by specific leaf area or that were directly affected by the leaf area ratio of the shoot (i.e., specific hydraulic conductivity) are indicated by the letter b. Variables are arranged in decreasing order of PI. Variables exhibiting significant differences between light environments are indicated by an asterisk.

Plasticity index	Type of variable	Variable	Plants sampled
0.76	1	Leaf area ratio (LAR) *	Seedling
0.69	1	Specific leaf area (SLA) *	Seedling
0.57	2	Chlorophyll a + b concentration (dry mass basis) *	Seedling
0.52	2	Carotenoid to chlorophyll ratio *	Seedling
0.50	3b	Photosynthetic electron transport rate (dry mass basis) *	Seedling
0.44	1	Root:shoot ratio *	Seedling
0.37	3	Photosynthetic electron transport rate (area basis)	Seedling
0.06	2b	Chlorophyll a + b content (leaf area basis)	Seedling
0.55	3b	Photosynthetic nitrogen use efficiency *	Adult
0.48	1	Leaf area to sapwood area ( $S_F/S_A$ ) *	Adult
0.44	1	Specific leaf area (SLA) *	Adult
0.44	2b	Nitrogen content (leaf area basis) *	Adult
0.44	3b	Maximum net photosynthesis (dry mass basis) *	Adult
0.44	4b	Specific hydraulic conductivity *	Adult
0.35	2	Chlorophyll a + b concentration (dry mass basis) *	Adult
0.33	4	Modulus of elasticity *	Adult
0.26	3	Maximum stomatal conductance	Adult
0.22	4	Hydraulic conductivity	Adult
0.19	3	Maximum net photosynthesis (area basis)	Adult
0.13	4	Osmotic potential at wilting point	Adult
0.10	2	Nitrogen concentration (dry mass basis)	Adult
0.05	2b	Chlorophyll a + b content (leaf area basis)	Adult
0.04	4	Osmotic potential at turgor	Adult
0.01	4	Relative water content at turgor	Adult

and in the Belgian population studied by Niinemets et al. (2003), and low for our adult plants in the field.

Many tree species alter their water relations and hydraulic properties in response to available light (Abrams 1986, Abrams and Mostoller 1995, Aranda et al. 2001). However, adult holly plants growing in two contrasting light environments showed only minor differences in water relations. Holly showed a limited capacity for osmotic adjustment, with higher

water potential at the turgor loss point than any other co-occurring tree species (Aranda et al. 1996). Given that  $\pi^{100}$  has often been taken as a drought tolerance index (Bahari et al. 1985, Abrams 1990, Tschaplinski et al. 1998), holly can be regarded as a drought-sensitive species despite its moderate resistance to cavitation (Martínez-Vilalta et al. 2002). Holly plants exhibited no change in hydraulic conductance with change in available light, although they exhibited changes in foliar:sapwood area ratios, as has been reported for other species (Maherali et al. 1997). The low responsiveness of the water relations properties of holly to environmental conditions may indicate an evolutionary constraint on changes in vascular structure as argued for *Rhododendron* species (Cordero and Nilsen 2002); the similarity in stomatal conductance of sun and shade plants of holly may be associated with the limited capacity for acclimation of the hydraulic system to varying evaporative demands.

#### *Mediterranean holly populations in a changing environment*

Based on our findings, holly can be considered a facultative understory plant in areas of oceanic and relatively mild climate but an obligate understory species in dry, continental areas such as those studied here. In a global change scenario, holly could be replaced by more drought-tolerant species in open habitats and by invasive, highly competitive species in the

Table 2. Mean plasticity index (PI) in response to the light environment for the different groups of variables (grouped according to Table 1). The letter code indicates significantly different groups of variables (Post hoc Bonferroni's *t* test,  $P < 0.05$ ).

PI	Type of variable	Post-hoc test
0.54	1. Morphology	a
0.50	3b. Photosynthesis and gas exchange (dry mass basis)	a
0.44	4b. Specific hydraulic conductivity	a
0.38	2. Biochemical	a
0.26	3. Photosynthesis and gas exchange	b
0.18	2b. Biochemical	b, c
0.15	4. Water relations	c

understory. However, autochthonous holly populations may respond differently to populations originating from commercial varieties, given that the latter are listed as invasive in USA (Randall and Marinelli 1996). The dense holly forests found in certain Mediterranean areas are the result of human management (Oria de Rueda 1992, Arrieta 2002, Arrieta and Suárez 2004) and are, therefore, highly vulnerable to abandonment of the traditional practices, which sustained them, particularly if coupled with a predicted increase in temperature and decrease in precipitation (IPCC 2001). Holly present in the Mediterranean region is a relict of the Tertiary tropical flora (Suc 1984) and depends increasingly on the microclimatic conditions arising from the presence of other tree species to survive. Its limited phenotypic plasticity and competitive ability in the Mediterranean region may compromise its long-term natural regeneration in the understory. This contrasts with the expansion and ecological success of other tropical woody relicts inhabiting the Mediterranean region, e.g., *Pistacia lentiscus* L., which may be associated with its tolerance to high irradiance and severe drought (Verdú and García-Fayos 2002, Vilagrosa et al. 2003, Valladares et al. 2004).

#### Acknowledgments

Analysis of data and preparation of this manuscript were made possible by the Spanish Ministry for Science and Technology and the Spanish Ministry of Education and Science (Grants ECOFIARB REN2000-0163-P4 and RASINV CGL2004-04884-C02-02/BOS). The autonomous government of Madrid provided a Ph.D. research grant to S.A., and authorized the field work at the Robregordo and Montejo sites. Environmental Council of Soria (the autonomous government of Castilla-León) is acknowledged for allowing field work at Oncala.

#### References

- Abrams, M.D. 1986. Physiological plasticity in water relations and leaf structure of understory versus open grown *Cercis canadensis* in northeastern Kansas. *Can. J. For. Res.* 16:1170–1174.
- Abrams, M.D. 1990. Adaptations and responses to drought in *Quercus* species of North America. *Tree Physiol.* 7:227–238.
- Abrams, M.D. and S.A. Mostoller. 1995. Gas exchange, leaf structure and nitrogen in contrasting successional tree species growing in open and understory sites during a drought. *Tree Physiol.* 15:361–370.
- Aranda, I., L. Gil and J. Pardos. 1996. Seasonal water relations of three broadleaved species (*Fagus sylvatica* L., *Quercus petraea* (Mattuschka) Liebl. and *Quercus pyrenaica* Willd.) in a mixed stand in the center of the Iberian Peninsula. *For. Ecol. Manage.* 84:219–229.
- Aranda, I., L. Gil and J. Pardos. 2001. Effects of thinning in a *Pinus sylvestris* L. stand on foliar water relations of *Fagus sylvatica* L. seedlings planted within the pinewood. *Trees.* 15:358–364.
- Aranda, I., F. Pardo, L. Gil and J.A. Pardos. 2004. Anatomical basis of the change in leaf mass per area and nitrogen investment with relative irradiance within the canopy of eight temperate tree species. *Acta Oecol.* 25:187–195.
- Arrieta, S. 2002. Dinámica de la regeneración de las acebedas (*Ilex aquifolium* L.) en el Centro Peninsular. Ph.D. Departamento de Ecología. Universidad Autónoma de Madrid, Spain, 280 p.
- Arrieta, S. and F. Suárez. 2001. Seedling diversity and spatially related regeneration dynamics in holly woodlands and surroundings habitats. *Web Ecol.* 2:38–46.
- Arrieta, S. and F. Suárez. 2005. Spatial patterns of seedling emergence and survival as a critical phase in holly (*Ilex aquifolium* L.) woodland recruitment in central Spain. *For. Ecol. Manage.* 205:267–282.
- Bahari, Z.A., S.G. Pallardy and W.C. Parker. 1985. Photosynthesis, water relations and drought adaptation in six woody species of oak–hickory forest in Central Missouri. *For. Sci.* 31:557–569.
- Balaguer, L., E. Martínez-Ferri, F. Valladares, M.E. Pérez-Corona, F.J. Baquedano, F.J. Castillo and E. Manrique. 2001. Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Funct. Ecol.* 15:124–135.
- Barnes, J.D., L. Balaguer, E. Manrique, S. Elvira and A.W. Davison. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll a and b in lichens and higher plants. *Environ. Expt. Bot.* 32:85–100.
- Brickell, C. and J.D. Zuk. 2002. American horticultural society A–Z encyclopedia of garden plants. DK Publishing, New York, 575 p.
- Brzeziecki, B. and F. Kienast. 1994. Classifying the life-history strategies of trees on the basis of the Grimian model. *For. Ecol. Manage.* 69:167–187.
- Coleman, J.S., K.D.M. McConnaughay and D.D. Ackerly. 1994. Interpreting phenotypic variation in plants. *Trends Ecol. Evol.* 9:187–191.
- Cordero, R.A. and E.T. Nilssen. 2002. Effects of summer drought and winter freezing on stem hydraulic conductivity of *Rhododendron* species from contrasting climates. *Tree Physiol.* 22:919–928.
- Damascos, M.A. and E.H. Rapoport. 2002. Differences in the herb and shrub flora growing under canopy gaps and under closed canopies in a *Nothofagus pumilio* forest of Argentina. *Rev. Chil. Hist. Nat.* 75:465–472.
- Del Hierro, A.M., W. Kronberger and H. Richter. 2000. Chloroplast movements in leaves of two evergreen sclerophyllous plants (*Ilex aquifolium* and *Prunus laurocerasus*). *Plant Biosystems* 134:297–304.
- Dreyer, E., F. Bousquet and M. Ducrey. 1990. Use of pressure volume curves in water relation analysis on woody shoots—influence of rehydration and comparison of four European oak species. *Ann. Sci. For.* 47:285–297.
- Durand, L.Z. and G. Goldstein. 2001. Photosynthesis, photoinhibition and nitrogen use efficiency in native and invasive tree ferns in Hawaii. *Oecologia* 126:345–354.
- Ellenberg, H., H.E. Weber, R. Düll, V. Wirth and W. Werner. 1991. Indicator values of plants in Central Europe—Zeigerwerte von Pflanzen in Mitteleuropa. Erich Goltze KG, Scripta Geobotanica 18 Göttingen, 350 p.
- García-Plazaola, J.I., U. Artetxe, M.K. Dunabeitia and J.M. Becerril. 1999. Role of photoprotective systems of holm-oak (*Quercus ilex*) in the adaptation to winter conditions. *J. Plant Physiol.* 155:625–630.
- García-Plazaola, J.I., J.M. Olano, A. Hernandez and J.M. Becerril. 2003. Photoprotection in evergreen Mediterranean plants during sudden periods of intense cold weather. *Trees* 17:285–291.
- Givnish, T.J. 1988. Adaptation to sun and shade: a whole-plant perspective. *Aust. J. Plant Physiol.* 15:63–92.
- Gómez, J.M., F. Valladares and C. Puerta-Piñero. 2004. Differences between structural and functional heterogeneity caused by seed dispersal. *Funct. Ecol.* 18:787–792.
- Groom, Q.J., N.R. Baker and S.P. Long. 1991. Photoinhibition of Holly (*Ilex aquifolium*) in the field during the winter. *Physiol. Plant.* 83:585–590.

- Hättenschwiler, S. and C. Körner. 2003. Does elevated CO<sub>2</sub> facilitate naturalization of the non-indigenous *Prunus laurocerasus* in Swiss temperate forests? *Funct. Ecol.* 17:778–785.
- Henry, H.A.L. and L.W. Aarssen. 1997. On the relationship between shade tolerance and shade avoidance strategies in woodland plants. *Oikos* 80:575–582.
- Hinckley, T.M., F. Duhme, A.R. Hinckley and H. Richter. 1980. Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. *Plant Cell Environ.* 3:131–140.
- Instituto Nacional de Meteorología. 2003. Calendario meteorológico. 2003. Ministerio de Medio Ambiente, Madrid.
- IPCC. 2001. Climate change, 2001: the scientific basis. *In* Third Assessment Report of the Intergovernmental Panel on Climate Change. Eds. J.T. Houghton, Y. Ding, J. Griggs, M. Noguer, P.J. Van der Linden, X. Dai, K. Maskell and C.A. Johnson. Cambridge University Press, Cambridge, 384 p.
- Johnson, J.D., R. Tognetti, M. Michelozzi, S. Pinzauti, G. Minotta and M. Borghetti. 1997. Ecophysiological responses of *Fagus sylvatica* seedlings to changing light conditions. 2. The interaction of light environment and soil fertility on seedling physiology. *Physiol. Plant.* 101:124–134.
- Karlic, H. and H. Richter. 1983. Developmental effects on leaf water relations of two evergreen shrubs (*Prunus laurocerasus* L. and *Ilex aquifolium* L.). *Flora.* 173:143–150.
- Kitajima, K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–428.
- Kubiske, M.E. and M.D. Abrams. 1990. Pressure–volume relationships in non-rehydrated tissue at various water deficits. *Plant Cell Environ.* 13:995–1000.
- Kubiske, M.E. and M.D. Abrams. 1991. Rehydration effects on pressure–volume relationships in 4 temperate woody species—variability with site, time of season and drought conditions. *Oecologia* 85:537–542.
- Kull, O., M. Broadmeadow, B. Kruijt and P. Meir. 1999. Light distribution and foliage structure in an oak canopy. *Trees* 14:55–64.
- Lambers, H., F.S. Chapin, III, and T.L. Pons. 1998. *Plant physiological ecology.* Springer-Verlag, York, PA, 485 p.
- Lorenzo, D. 2001. Adaptaciones fisiológicas y morfológicas del acebo (*Ilex aquifolium* L.) a la radiación solar bajo una cubierta de robles (*Quercus petraea* (Mattuschka) Liebl.) y a plena exposición, en el hayedo de Montejo de la Sierra (Madrid). Master Thesis. Proyecto Fin de Carrera Escuela Técnica Superior de Ingenieros de Montes. Universidad Politécnica de Madrid, Madrid, 221 p.
- Maherali, H., E.H. DeLucia and T.W. Sipe. 1997. Hydraulic adjustment of maple saplings to canopy gap formation. *Oecologia* 112: 472–480.
- Martínez-Ferri, E., E. Manrique, F. Valladares and L. Balguer. 2004. Winter photoinhibition in the field involves different processes in four co-occurring Mediterranean tree species. *Tree Physiol.* 24: 981–990.
- Martínez-Vilalta, J., E. Prat, I. Oliveras and J. Piñol. 2002. Xylem hydraulic properties of roots and stems of nine Mediterranean woody species. *Oecologia* 133:19–29.
- Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.* 51:659–668.
- Niinemets, U. and F. Valladares. 2004. Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. *Plant Biol.* 6:254–268.
- Niinemets, U., F. Valladares and R. Ceulemans. 2003. Leaf-level phenotypic variability and plasticity of invasive *Rhododendron ponticum* and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites. *Plant Cell Environ.* 26:941–956.
- Obeso, J.R. 1997. Costs of reproduction in *Ilex aquifolium*: effects at tree, branch and leaf levels. *J. Ecol.* 85:159–166.
- Obeso, J.R., M. Alvarez-Santullano and R. Retuerto. 1998. Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). *Am. J. Bot.* 85:1602–1608.
- Oliveira, G. and J. Peñuelas. 2000. Comparative photochemical and phenomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) Mediterranean woody species. *Acta Oecol.* 21:97–107.
- Oquist, G. and N.P.A. Huner. 2003. Photosynthesis of overwintering evergreen plants. *Annu. Rev. Plant Biol.* 54:329–355.
- Oria de Rueda, J.A. 1992. Las acebedas de Castilla y León y La Rioja: origen, composición y dinámica. *Ecología* 6:79–91.
- Peterken, G.F. 1966. Mortality of holly (*Ilex aquifolium*) seedlings in relation to natural regeneration in the New Forest. *J. Ecol.* 54: 259–269.
- Peterken, G.F. and S. Lloyd. 1967. Biological flora of the British Isles, *Ilex aquifolium* L. *J. Ecol.* 55:841–858.
- Poorter, L. and E.J.M.M. Aerts. 2003. Light environment and tree strategies in a Bolivian tropical moist forest: an evaluation of the light partitioning hypothesis. *Plant Ecol.* 166:295–306.
- Potter, D.A. and T.W. Kimmerer. 1986. Seasonal allocation of defense investment in *Ilex opaca* Aiton and constraints on a specialist leafminer. *Oecologia* 69:217–224.
- Randall, J.M. and J. Marinelli. 1996. *Invasive plants, weeds of the global garden.* Brooklyn Botanic Garden, New York, 224 p.
- Reich, P.B., I.J. Wright, J. Cavender-Bares, J.M. Craine, J. Oleksyn, M. Westoby and M.B. Walters. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *Int. J. Plant Sci.* 164 (3 Suppl.):S143–S164.
- Robakowski, P., P. Montpied and E. Dreyer. 2003. Plasticity of morphological and physiological traits in response to different levels of irradiance in seedlings of silver fir (*Abies alba* Mill). *Trees.* 17: 431–441.
- Robichaux, R.H. 1984. Variation in tissue water relations of two sympatric Hawaiian *Dubautia* species and natural hybrids. *Oecologia* 65:75–81.
- Robinson, D. and I.H. Rorison. 1988. Plasticity in grass species in relation to nitrogen supply. *Funct. Ecol.* 2:249–257.
- Rohacek, K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning and mutual relationships. *Photosynthetica* 40:13–29.
- Rütten, D. and K.A. Santarius. 1988. Cold acclimation of *Ilex aquifolium* under natural conditions with special regard to the photosynthetic apparatus. *Physiol. Plant.* 72:807–815.
- Sack, L. and P.J. Grubb. 2003. Crossovers in seedling relative growth rates between low and high irradiance: analyses and ecological potential (reply to Kitajima and Bolker 2003). *Funct. Ecol.* 17: 276–287.
- Schmidt, J., F. Batic and H. Pfanz. 2000. Photosynthetic performance of leaves and twigs of evergreen holly (*Ilex aquifolium*). *Phyton* 40:179–190.
- Schreiber, U., W. Bilger and C. Neubauer. 1994. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. *In* *Ecophysiology of Photosynthesis.* Eds. E.-D. Schulze and M.M. Caldwell. Springer-Verlag, Berlin, pp 49–70.
- Skillman, J.B. and C.B. Osmond. 1998. Influence of nitrogen supply and growth irradiance on photoinhibition and recovery in *Heuchera americana* (Saxifragaceae). *Physiol. Plant.* 103: 567–573.
- Smith, T.M. and M.L. Huston. 1989. A theory of the spatial and temporal dynamics of plant communities. *Vegetatio* 83:49–69.
- Sperry, J.S. and M.T. Tyree. 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiol.* 88:581–587.

- Suc, J.P. 1984. Origin and evolution of the mediterranean vegetation and climate in Europe. *Nature* 307:429–432.
- Tschaplinski, T.J., G.M. Gebre and T.L. Shirshac. 1998. Osmotic potential of several hardwood species as affected by manipulation of throughfall precipitation in an upland oak forest during a dry year. *Tree Physiol.* 18:291–298.
- Valladares, F. 2003. Light heterogeneity and plants: from eco-physiology to species coexistence and biodiversity. *In* *Progress in Botany*. Eds. K. Esser, U. Lüttge, W. Beyschlag and F. Hellwig. Springer-Verlag, Heidelberg, pp 439–471.
- Valladares, F., E. Martínez-Ferri, L. Balaguer, E. Perez-Corona and E. Manrique. 2000a. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol.* 148:79–91.
- Valladares, F., S.J. Wright, E. Lasso, K. Kitajima and R.W. Pearcy. 2000b. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81:1925–1936.
- Valladares, F., L. Balaguer, E. Martínez-Ferri, E. Perez-Corona and E. Manrique. 2002a. Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytol.* 156:457–467.
- Valladares, F., J.M. Chico, I. Aranda, L. Balaguer, P. Dizengremel, E. Manrique and E. Dreyer. 2002b. Greater high light seedling tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees* 16:395–403.
- Valladares, F., I. Dobarro, D. Sánchez-Gómez and R.W. Pearcy. 2004. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. *J. Exp. Bot.* 56:483–494.
- Verdú, M. and P. García-Fayos. 2002. Reproductive ecology of *Pistacia lentiscus* L. (Anacardiaceae): an evolutionary anachronism in the mediterranean shrubland. *Rev. Chil. Hist. Nat.* 75: 57–65.
- Vilagrosa, A., J. Bellot, V.R. Vallejo and E. Gil-Pelegrin. 2003. Cavitation, stomatal conductance and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. *J. Exp. Bot.* 54:2015–2024.
- Walters, M. and P. Reich. 1996. Are shade tolerance, survival and growth linked? Low light and nitrogen effects on hardwood seedlings. *Ecology* 77:841–853.
- Werner, C., O. Correia and W. Beyschlag. 2002. Characteristic patterns of chronic and dynamic photoinhibition of different functional groups in a Mediterranean ecosystem. *Funct. Plant Biol.* 29: 999–1011.
- West-Eberhard, M.J. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York, 816 p.
- Wright, S.J., H.C. Muller-Landau, R. Condit and S.P. Hubbell. 2003. Gap-dependent recruitment, realized vital rates and size distributions of tropical trees. *Ecology* 84:3174–3185.
- Yamashita, N., N. Koike and A. Ishida. 2002. Leaf ontogenetic dependence of light acclimation in invasive and native subtropical trees of different successional status. *Plant Cell Environ.* 25: 1341–1353.