Leaf-level phenotypic variability and plasticity of invasive *Rhododendron ponticum* and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites

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ABSTRACT

To understand the role of leaf-level plasticity and variability in species invasiveness, foliar characteristics were studied in relation to seasonal average integrated quantum flux density (Q_{int}) in the understorey evergreen species *Rhododendron ponticum* and *Ilex aquifolium* at two sites. A native relict population of *R. ponticum* was sampled in southern Spain (Mediterranean climate), while an invasive alien population was investigated in Belgium (temperate maritime climate). *Ilex aquifolium* was native at both sites. Both species exhibited a significant plastic response to Q_{int} in leaf dry mass per unit area, thickness, photosynthetic potentials, and chlorophyll contents at the two sites. However, *R. ponticum* exhibited a higher photosynthetic nitrogen use efficiency and larger investment of nitrogen in chlorophyll than *I. aquifolium*. Since leaf nitrogen (N) contents per unit dry mass were lower in *R. ponticum*, this species formed a larger foliar area with equal photosynthetic potential and light-harvesting efficiency compared with *I. aquifolium*. The foliage of *R. ponticum* was mechanically more resistant with larger density in the Belgian site than in the Spanish site. Mean leaf-level phenotypic plasticity was larger in the Belgian population of *R. ponticum* than in the Spanish population of this species and the two populations of *I. aquifolium*. We suggest that large fractional investments of foliar N in photosynthetic function coupled with a relatively large mean, leaf-level phenotypic plasticity may provide the primary explanation for the invasive nature and superior performance of *R. ponticum* at the Belgian site. With alleviation of water limitations from Mediterranean to temperate maritime climates, the invasiveness of *R. ponticum* may also be enhanced by the increased foliage mechanical resistance observed in the alien populations.

Key-words: invasiveness; light acclimation; light harvesting; nitrogen use-efficiency; phenotypic variability; plasticity; support costs.

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INTRODUCTION

Many introduced plant species have become particularly competitive and invasive in new environments (Mooney 1999), constituting a danger to local biodiversity. Although such biological invasions have become increasingly frequent, plant ecophysiological and ecological traits underlying these invasions are still poorly understood. Global change phenomena such as elevated carbon dioxide concentrations as well as high nitrogen (N) deposition rates, and associated increases in the area of habitats that certain species can colonize, apparently provide one explanation for enhanced dispersal of plants that may be of limited competitive ability if the resource availability is limited (Dukes & Mooney 1999). High resource use efficiency as well as enhanced phenotypic plasticity have been hypothesized as decisive plant traits allowing the species to become invasive in newly colonized habitats (Abbott 1992; Dukes & Mooney 1999; Davis, Grime & Thompson 2000; Huxman & Smith 2001), but there are few ecophysiological comparisons of species growing in invaded and native stands as well as between native and invasive plant species (Yamashita *et al.* 2000; Durand & Goldstein 2001).

In the British Isles and in Belgium, *Rhododendron ponticum* L. is an alien, widely naturalized and very invasive species (Cross 1975; Rotherham 1983). It is considered as a threat to local biodiversity and as a forest weed that is difficult to control (Brown 1953; Tabbush & Williamson 1987). The primary native range of *R. ponticum* extends north and south to the Black Sea, in the Caucasus and northern Turkey. However, there are a few isolated populations in Lebanon, Portugal and southern Spain (Cross 1975). These isolated populations are relicts of Tertiary subtropical conditions in Europe, while most of the range of *R. ponticum* in the Mediterranean Basin has vanished with the onset of typical summer-dry climates (Palamarev 1989). Nowadays these populations are restricted to mountain ranges that mitigate the Mediterranean drought, and function as ecological refugiae for many Tertiary relicts (Hampe & Arroyo 2002). The isolated native populations of *R. ponticum* are currently declining due to land use changes (Madueño 1991; Blanca *et al.* 2000), and also possibly due to long-term changes in water availability. The plant has

been included in the recent Red List of Threatened Vascular Plants of Andalucía (Blanca *et al*. 2000). According to latest genetic studies, the invasive populations of *R. ponticum* in the British Isles, and possibly also in Belgium primarily originate from the relict southern Spanish population (Milne & Abbott 2000).

The enhanced invasive potential of introduced individuals of *R. ponticum* in the British Isles and Belgium is still not entirely understood. High competitive potential of introduced *R. ponticum* has been related to its ability to tolerate shade while still maintaining large growth rates in high light, to its effective vegetative dispersal, as well as to its high concentrations of toxic protective compounds such as andromedotoxin and rhododendrin that essentially avoid herbivory of *R. ponticum* foliage (Cross 1975). It has been suggested that disturbance and selective grazing of non-toxic co-occurring species such as *Ilex aquifolium* L. are the major factors aiding to the invasion of this species (Cross 1981; Rotherham 1983).

The presence of defence compounds deters herbivores (Jela 1997; Jela & Lawton 1997), but it also brings about low litter decomposition rates in *Rhododendron* species (Cross 1975; Benfield & Webster 1991) due to antibacterial and antifungal activities of these protective chemicals (Walker *et al*. 1999; Reddy *et al*. 2001). Slow litter decomposition rates, in turn, may lead to limited nutrient availability for foliage construction. Thus, over a long term, high nitrogen immobilization in soil organic matter may potentially constrain the amount of light intercepted by the *Rhododendron* canopy, and allow establishment of seedlings of competing tree species. Given that the maximal height of *R. ponticum* canopy is approximately 4–6 m (Cross 1975), this may mean that long-term competitive success of *R. ponticum* is limited by low soil nitrogen availability, facilitating the establishment of overstorey tree seedlings. This may partly explain the non-invasiveness of this species in its native habitats. However, during the past few decades there has been a dramatic increase in N-deposition rates in many forest ecosystems. Such increases in N availability may allow leaf formation under lower irradiance levels, thereby leading to important increases in plant light-harvesting efficiency, and enhanced plant competitive potential in lower irradiances. The rise in N-deposition rates has also been paralleled by a sharp increase in the rate of *Rhododendron* invasion during the last 30 years (Tabbush & Williamson 1987). To gain insight into the enhanced N deposition loads on species-competitive potentials, we studied a native declining *R. ponticum* population from southern Spain, and an introduced very invasive population in Belgium. The atmospheric N deposition rates are low in the habitat of the native population, but high in the invasive range of *R. ponticum*. We asked whether the light-harvesting efficiencies differ between the various populations of *R. ponticum*, and whether part of the invasiveness of *R. ponticum* is associated with enhanced N availability in the western European forests.

There are indications that high phenotypic plasticity, and in particular, a highly plastic response to light, is involved

in the invasive capacity of certain species (Yamashita *et al*. 2000; Durand & Goldstein 2001). To further understand the role of plasticity in species competitive potential, we have compared leaf-level plasticity in response to canopy light gradients between the native and the introduced populations of *R. ponticum*. We hypothesized that the leaf-level plasticity was higher in the Belgian population, allowing for a more efficient acclimation to various forest understorey and gap conditions in the alien population. As a contrast to the plastic response of *R. ponticum,* we included in the study the native species *Ilex aquifolium,* another evergreen, understorey shrub. The local abundance of *I. aquifolium* has dramatically decreased in Belgium and in the British Isles due to the greater competitiveness of *R. ponticum*, which has occupied the microsites of *I. aquifolium*. Even though there is detailed information on dispersal and reproduction of these two species in forest understoreys (Rotherham 1983; Obeso 1997; Esen 2000), comparative data of leaf- and canopy ecophysiology are scant. We hypothesized that the greater competitive ability of *R. ponticum* over *I. aquifolium* results from a more efficient N use in photosynthesis and an improved light-harvesting efficiency in low irradiance in *R. ponticum*, and that these species differences are specifically amplified by enhanced N deposition rates.

MATERIALS AND METHODS

The Spanish site

The native population of *Rhododendron ponticum* L. was investigated in the Alcornocales Natural Park, Sierra del Aljibe, Cádiz province (Sierra del Cabrito, Llanos del Juncal area, 36°20′ N, 5°30′ E, elevation approximately 300–600 m). The study area supports Mediterranean sclerophyllous evergreen and semi-deciduous forests dominated by cork oak (*Quercus suber* L.), accompanied by *Arbutus unedo* L., *Quercus canariensis* Willd., *Phillyrea latifolia* L.*, Rhamnus alaternus* L. and *Erica arborea* L. (Ojeda, Marañón & Arroyo 2000). These stands are 5–12 m tall, and have leaf area indices (LAI) of 2–5 $m^2 m^{-2}$. However, *R. ponticum* is primarily restricted to wetter patches in the understorey of the riparian gallery forests extending in narrow strips along small creeks (Aparicio, Porras & Ceballos 2001). In certain especially humid areas used for cattle grazing, it may also disperse onto open slopes, but in such locations is generally associated with springs. Shrubs or small trees of *Ilex aquifolium* are also restricted to more humid areas, which support vigorous understorey vegetation and stand LAI higher than 4 $m^2 m^{-2}$. Yet, *I. aquifolium* tolerates lower water availability than *R. ponticum* (authors' observations), and may occasionally reach the overstorey to form mixed stands with *A. unedo* and *Quercus* spp.

The sandy soil is derived from Oligo-Miocenic sandstones, and is a dystric regosol according to FAO classification. The pH measured in the distilled water was 5.82 ± 0.14 (mean $\pm$ SE, $n = 8$) for the A horizon below the *R. ponticum* shrubs, and 5.22 ± 0.01 below the *I. aquifolium* shrubs

(means were significantly different at $P < 0.02$ according to a *t*-test). Total N content of the A horizon was $0.49 \pm 0.05\%$ below *R. ponticum*, and $0.65 \pm 0.09\%$ below *I. aquifolium*, and C/N molar ratio was 21.9 ± 0.5 below *R. ponticum*, and 19.4 ± 0.6 below *I. aquifolium* (means were not significantly different for both comparisons, $P > 0.1$).

The climate is Mediterranean subhumid with average long-term yearly temperature of $16.7\,°C$ (measured at Alcalá de los Gazules, approximately 10 km from the study site at elevation 221 m; Hampe & Arroyo 2002). The yearly average precipitation is 880 mm, and it is primarily concentrated in the autumn and winter months. Summer drought is alleviated by local fogs produced by the uplift of oceanic winds that are frequent in the area of the Strait of Gibraltar (Sinamba Difusión 1998). The moisture availability within the gorges of the Sierra del Aljibe is larger and seasonally less variable due to additional water supplied as fog throughout the year (Hampe & Arroyo 2002).

The Belgian site

The introduced invasive population of *R. ponticum* was studied in the understorey of an open *Pinus sylvestris* L. forest in Brasschaat, the province of Antwerpen (51°18′ N, 4°31′ E, elevation 16 m). The 73-year-old *P. sylvestris* plantation with a dominant height of 22–24 m and stocking of 556 trees ha^{-1} had an overstorey LAI of 1.8–2.4 m^2 m^{-2} (Gond *et al.* 1999). The understorey woody layer was dominated by the exotic species *Prunus serotina* Ehrh. (height 3–9 m), and *R. ponticum* (height 1–5 m). Due to partial removal of the woody understorey, the shrub layer was non-uniformly distributed, such that LAI ranged from 1 to 5 m^2 m^{-2} in different understorey patches (Nadezhdina, Tatarinov & Ceulemans 2003). In more open locations, *R. ponticum* often formed a thick monospecific canopy. In the denser parts of the understorey, taller trees of *P. serotina* provided extensive shading to shorter individuals of *R. ponticum*. *Ilex aquifolium* was present throughout the site, but generally as a minor understorey associate.

The stand is on a deep moderately wet Umbric Regosol (FAO classification) that has a distinct humus or iron B horizon. The litter layer (A$_0$) thickness varies from 0 to 6 cm, being occasionally even thicker under the shrubs of *R. ponticum*. The pH determined in distilled water is moderately acidic (3.8–4.1) throughout the entire soil profile (Van Ranst *et al.* 2002). The area is characterized by high atmospheric nitrogen deposition rates of up to 30–45 kg N ha^{-1} year^{-1} (Neirynck, Roskams & Lust 1999; Neirynck *et al.* 2002), and the total N content of the litter layer is $1.11 \pm 0.19\%$, and of the A horizon $0.91 \pm 0.11\%$ (C/N molar ratio 20.4 ± 0.7) (Janssens *et al.* 1999; Van Ranst *et al.* 2002). Foliar analyses indicate development of moderate soil cation and phosphorous limitations (Roskams, Sioen & Overloop 1997).

The temperate maritime climate is characterized by high yearly average precipitation of 767 mm that exceeds the yearly potential evaporation of 670 mm. According to 30-year records, the mean temperature is $3\,°C$ for the coldest month and $18\,°C$ for the warmest month, and the yearly average temperature is $9.8\,°C$ (Janssens *et al.* 1999).

Foliage sampling for morphological and chemical analyses

Current-year foliage was sampled during 10–11 July 2002 at the Spanish site, and during 22–23 July at the Belgian site from a total of 42 individual plants of *R. ponticum* and *I. aquifolium*. Leaves were taken from the top to the bottom along the light gradient in the shrubs' canopy. On each individual plant, one to ten canopy locations were selected, and four to six leaves from each canopy location were collected. Leaves were enclosed in plastic bags and maintained on ice at $0–5\,°C$ until analysis in the laboratory on the next day. Sheets of wet filter paper were enclosed in the plastic bags with the leaves to avoid leaf transpiration and allow the leaves to reach full hydration (Gucci, Lombardini & Tattini 1997).

Leaf morphological measurements

For each leaf, lamina thickness (T) was measured in 5–14 (on average 8.1) leaf locations across both sides of the leaf mid-rib by digital precision callipers, and averages were calculated. Leaf area and leaf perimeter were measured using a photographic technique. Because the leaves were rarely completely flat, we measured both the horizontal projection of a leaf with its natural three-dimensional shape (silhouette area, A_S), and after pressing the leaf to a flat section (projected area, A_P). For photographing, the leaves were laid on a light table, and digital photographs were taken from a distance of 1.6 m using Nikon Coolpix 990 camera equipped with Tele Converter TC-E2 2x (Nikon Corporation, Tokyo, Japan). Objects of known area were photographed together with the leaves, and each image file was calibrated separately to obtain an appropriate pixel to centimetre conversion ratio. Repeated measurements of these calibration objects in different image files indicated that the error in area measurements was less than 1%. UTHSCSA Imagetool 2.00alpha (C. Donald Wilcox, S. Brent Dove, W. Doss McDavid and David B. Greer, Department of Dental Diagnostic Science, The University of Texas Health Science Center, San Antonio, TX, USA; ddsdx.uthscsa.edu) was used to measure all estimates of foliar variables from the digital images.

Leaf laminas and petioles were separately weighed after oven drying at $70\,°C$ for 48 h, and lamina (F_L) and petiole (F_P) dry to fresh mass ratios, and lamina dry mass per unit area (M_A) were calculated. Lamina density (mass per unit volume) was determined as M_A/T.

Seasonal integrated irradiance

Hemispherical photographs were taken above each leaf cluster using the Nikon Coolpix digital camera equipped with a Nikon Fisheye Converter FC-E8 0.21x. The hemispherical images were analysed with HemiView 2.1 (Delta-

T Devices, Cambridge, UK), and the fractions of penetrating diffuse solar radiation of open sky (indirect site factor, I_D) and of potential penetrating direct solar radiation of open sky (direct site factor, I_B) were determined. Diffuse light distribution was calculated for uniformly overcast sky conditions. Calculations were carried out for eight azimuth classes and 20 zenith angle classes, giving 160 sky regions. I_B was calculated separately for each month of the year. The mean I_B for the months relevant for leaf development according to phenological observations at the study sites (March to June in Spain, April to July in Belgium) were calculated from the monthly values.

The relative irradiance indices determined from the hemispherical photographs were used to calculate average (1 March–30 June for the Spanish site, and 20 April–20 July for the Belgian site) daily integrated incident quantum flux densities during leaf growth and development (Q_{int}, mol m^{-2} d^{-1}). For the Spanish site, the hemispherical technique was calibrated using the monthly average values of above-canopy global solar radiation (R_G, MJ m^{-2} d^{-1}) measured in Cádiz (36°32′ N, 6°17′ E, Secretaría General Técnica, Ministerio de Medio Ambiente 2001):

$$Q_{int} = \gamma R_G [I_D p_D + I_B (1 - p_D)] \qquad (1)$$

where γ (mol MJ^{-1}) is the conversion factor for the global solar radiation, and p_D is the proportion of diffuse quantum flux density (Niinemets & Kull 1998). Monthly values of γ were obtained from measurements in Almeria (36°83′ N, 2°41′ W, Alados, Foyo-Moreno & Alados-Arboledas 1996), yielding an average (March–June) above-canopy quantum flux density (γR_G) of 40.0 mol m^{-2} d^{-1}. An average value of p_D of 0.263 was derived from the number of overcast and partly overcast days in Cádiz (Secretaría General Técnica, Ministerio de Medio Ambiente 2001) and measurements of atmospheric transmissivity and sky irradiance in Almeria (Alados-Arboledas et al. 2000).

At the Belgian site, the above-canopy quantum flux density was measured with a quantum sensor (190SA; Li-Cor, Inc., Lincoln, NE, USA), and half-hour averages were stored. These measurements yielded an average (20 April–20 July) above-canopy Q_{int} of 29.9 mol m^{-2} d^{-1}. A p_D of 0.513 was derived from the occurrence of overcast and partly overcast sky conditions, and using an estimate of 0.3 for p_D on clear days.

Determination of leaf chlorophyll, carbon and nitrogen contents

An estimate of leaf chlorophyll content was determined *in situ* for each leaf by Minolta SPAD 502 Chlorophyll Meter (Minolta Camera Co., Ltd, Osaka, Japan). SPAD readings were obtained in five to eight different leaf locations, and an average was stored. To calibrate the SPAD values, we selected for each species from each site 12 to 15 leaves of contrasting SPAD readings for destructive chlorophyll content estimations. For these leaves, a SPAD estimate was taken again, and leaf discs were punched from the immediate location of the SPAD measurement. Leaf chlorophylls were extracted from 5 mm-diameter leaf discs with dimethyl sulphoxide (DMSO) for 2.5 h at 65 °C as in Barnes *et al.* (1992). Absorptance of the extracts was determined with a spectrophotometer Spectronic 2000 (Bausch & Lomb, Rochester, NY, USA), and leaf chlorophyll concentrations were calculated according to Barnes *et al.* (1992). These data were used to derive non-linear relationships between leaf chlorophyll content and SPAD values. As observed previously (Richardson, Duigan & Berlyn 2002), best fits to the data were obtained by exponential regressions (Fig. 1). For each leaf, chlorophyll content was calculated from the SPAD value using species- and site-specific regressions (Fig. 1).

Foliar nitrogen (N_M) and carbon (C_M) contents were measured using a gas chromatograph after flash-combustion of the sample in oxygen by a NC 2100 elemental analyser (Carlo Erba Institute, Milan, Italy).

Chlorophyll fluorescence measurements

In the Belgian site, a pulse-amplitude modulated fluorometer (PAM-2000; Heinz Walz GmbH, Effeltrich, Germany) with leaf clip holder (model 2030-B) was used to estimate the *in situ* light-saturated rate of linear photosynthetic electron transport (J_{max}, μmol m^{-2} s^{-1}; Schreiber, Bilger & Neubauer 1994):

$$J_{max} = 0.5 \Phi_{PSII} \Theta Q \qquad (2)$$

where Q is the photosynthetically active quantum flux density, Θ is leaf absorptance, and Φ_{PSII} is the effective quantum yield of photosystem II. Φ_{PSII} is given as:
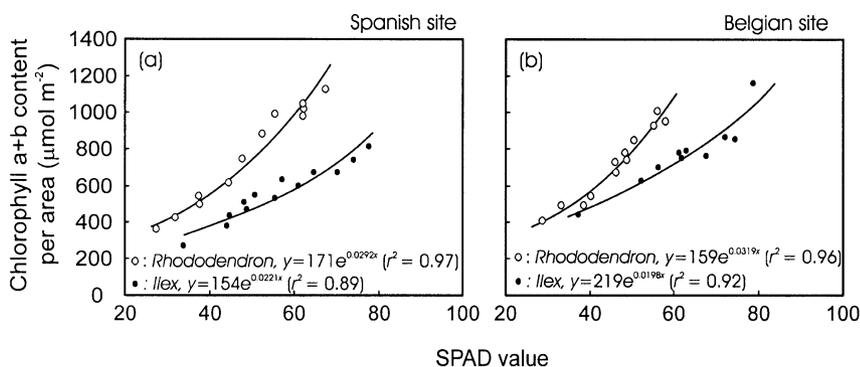
Figure 1. Calibration of Minolta Chlorophyll Meter (SPAD 502; Minolta Camera Co., Ltd, Osaka, Japan) readings for *Ilex aquifolium* (filled symbols) and *Rhododendron ponticum* (open symbols) leaves sampled at the Spanish (a) and Belgian (b) sites. Chlorophyll content was determined spectrophotometrically after extraction with dimethyl sulphoxide (DMSO). The data were fitted by non-linear regressions.

$$\Phi_{PSII} = (F_m' - F)/F_m' \qquad (3)$$

where F is the fluorescence yield in actinic light, and F_m' is the maximum fluorescence yield of the light-adapted sample. For these estimates, we determined leaf absorptance from leaf chlorophyll content using an empirical equation of Evans (1993). Overall, Eqn 2 provides a reliable measure of foliar photosynthetic electron transport (Edwards & Baker 1993) that is only moderately affected by partitioning of absorbed light between the two photosystems (Laisk *et al.* 2001).

During the measurements, low natural background diffuse irradiance of 5–100 μmol m^{-2} s^{-1} was supplemented by the actinic irradiance from the internal halogen lamp of PAM-2000, and the measurements were conducted at Q values of 200–300, 700–800, 1200–1400, 1700–1800 μmol m^{-2} s^{-1}. Starting with the lowest Q, pulses of white light of 8000 μmol m^{-2} s^{-1} were applied to determine F_m'. Φ_{PSII} was computed online, and the measurements were continued at each light level until the steady-state quantum yield was achieved. For each leaf, J_{max} was defined as the average of the three highest measurements. During the fluorescence measurements, average leaf temperature across individual leaves varied from 19.3 to 24.9 °C (mean ± SD = 21.5 ± 1.2 °C).

Statistical analyses

Because the relationships between Q_{int} and leaf morphological and structural characteristics were generally non-linear (see insets in Fig. 2a & b for the sample fits), Q_{int} was transformed before the statistical analysis using log$_{10}$-transformation. Thus, linear regression analyses could be employed to explore the relationships between the studied foliar variables and Q_{int}. Species and site differences were separated by covariance analyses (ANCOVA). First, a separate slope ANCOVA analysis was carried out to test for the slope differences. When differences in the slopes were statistically insignificant, the intercepts were compared by a common slope ANCOVA analysis (Sokal & Rohlf 1995).

Total phenotypic variability in the samples and variability within and among plants were estimated by the coefficient of variation, namely the standard deviation (SD) divided by the mean of the characteristic (see Valladares *et al.* 2002a and references therein). Phenotypic plasticity was defined as the fraction of total phenotypic variability that was unambiguously related to environmental variability; in our case to the variability in Q_{int}. By examination of the 25, 50 and 75 percentiles of Q_{int} distribution, leaf samples were separated in four categories: deep shade (<3.5 mol m^{-2} d^{-1}), shade (3.6–6.7 mol m^{-2} d^{-1}), moderate shade (6.8–11 mol m^{-2} d^{-1}), and open or partial shade (>11 mol m^{-2} d^{-1}). Values for *R. ponticum* corresponding to Q_{int} lower than 1.4 mol m^{-2} d^{-1}, which were only obtained in the Belgian population, were discarded to keep equivalent ranges of environmental variation for the two populations. Phenotypic plasticity was estimated by both the coefficient of variation between the means of these four groups and a plasticity index calculated as the difference between the minimum and the maximum mean values among these four light levels divided by the maximum mean value (Valladares *et al.* 2000a, b). Only key foliar structural variables were included in the latter analysis.
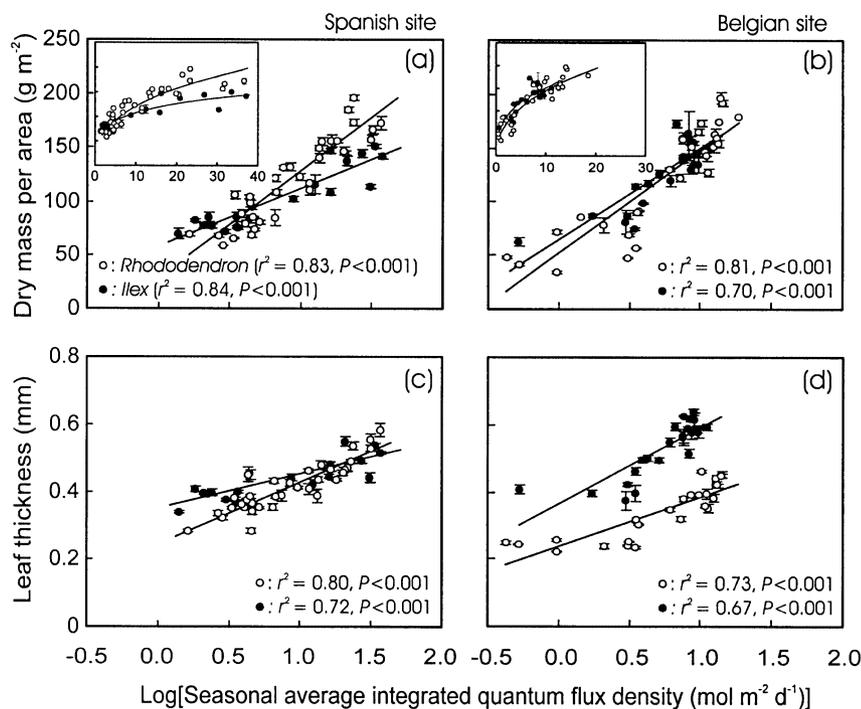
Figure 2. Effects of seasonal average integrated quantum flux density (Q_{int}) on (a, b) leaf dry mass per unit area (M_A), and (c, d) leaf thickness (*T*) in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols) at the Spanish (a, c) and the Belgian site (b, d). Error bars give ± SE. Log(Q_{int}) versus M_A and *T* relationships were fitted by linear regressions (Table 1). Insets in (a) and (b) demonstrate the relationships between non-transformed Q_{int} and M_A.
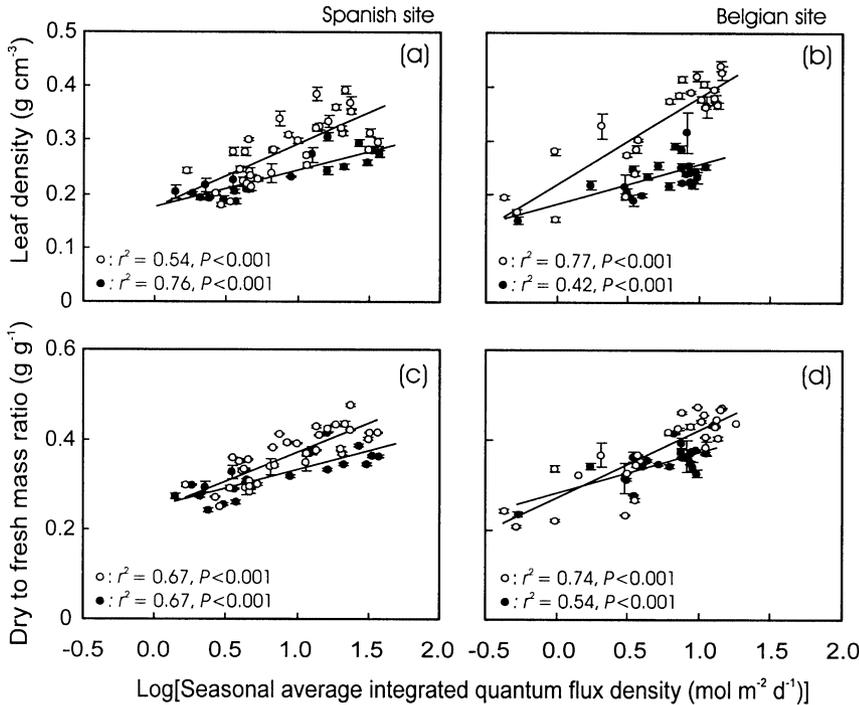
Figure 3. Leaf density (a, b) and dry to fresh mass ratio (c, d) in relation to Q_{int} in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols) in both sites. Data presentation as in Fig. 2.

RESULTS

Plastic morphological response to light

Lamina dry mass per unit area (M_A) scaled positively with seasonal average integrated quantum flux density (Q_{int}) in both *I. aquifolium* and *R. ponticum* and at both the Spanish and the Belgian site (Fig. 2a & b). This positive relationship resulted from changes in lamina thickness (T, Fig. 2c & d) and density (D, Fig. 3a & b) with Q_{int} ($M_A = D \cdot T$). Simi-

larly to D, lamina dry to fresh mass ratio was positively correlated with irradiance in both species (Fig. 3c & d).

Average projected area per leaf (A_P) as well as the silhouette leaf area with its natural three-dimensional shape (A_S) was independent of Q_{int} in all cases ($P > 0.1$). However, the A_S/A_P ratio was negatively related to Q_{int} in *R. ponticum* at the Spanish site (Fig. 4a), and in *I. aquifolium* at the Belgian site (Fig. 4b), indicating that the leaves were more curled and rolled at higher irradiance in these cases. Leaf
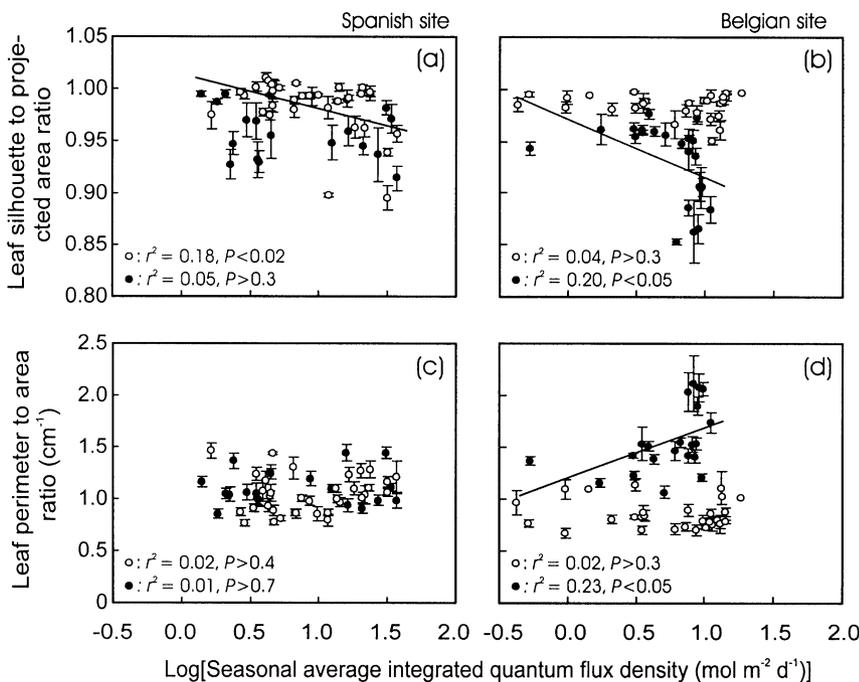
Figure 4. Leaf silhouette to projected area ratio (a, b) and perimeter to projected area ratio (c, d) in dependence on Q_{int} in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols) in both sites. Silhouette area is the leaf horizontal projection with its natural three-dimensional shape, whereas the projected area was estimated after pressing the leaf to a flat section. Non-significant regressions ($P > 0.05$) are not drawn.
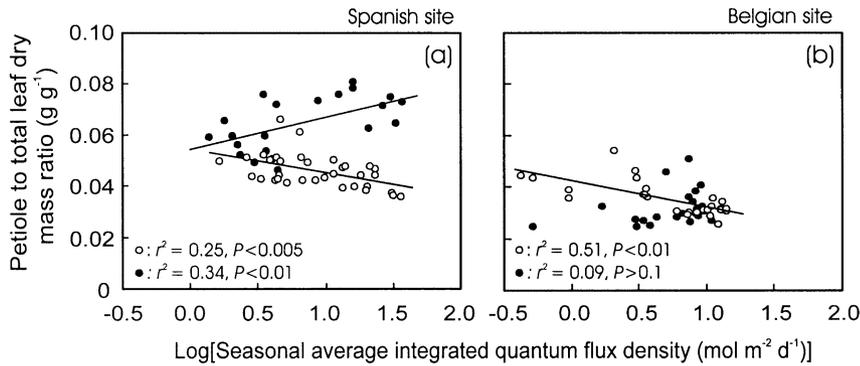
Figure 5. Correlations between Q_{int} and petiole to total leaf dry mass ratio in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols) in both sites. Data presentation as in Fig. 4.

perimeter to A_P ratio was positively associated with Q_{int} in *I. aquifolium* at the Belgian site (Fig. 4d). Thus, the leaves of this species became more spiny with increasing irradiance.

Petiole to total leaf dry mass ratio was negatively associated with Q_{int} in *R. ponticum* in both sites (Fig. 5a & b), whereas in *I. aquifolium*, this variable increased with increasing Q_{int} at the Spanish site (Fig. 5a), and was independent of Q_{int} at the Belgian site (Fig. 5b).

Variation in foliar chemistry along the light gradients

Leaf nitrogen content per unit dry mass (N_M) was independent of irradiance in both species at the Spanish site (Fig. 6a), whereas it was negatively related to Q_{int} at the Belgian site (Fig. 6b). Increases in M_A with irradiance (Fig. 2a & b) also led to a positive relationship between leaf nitrogen content per unit leaf area (N_A) and irradiance
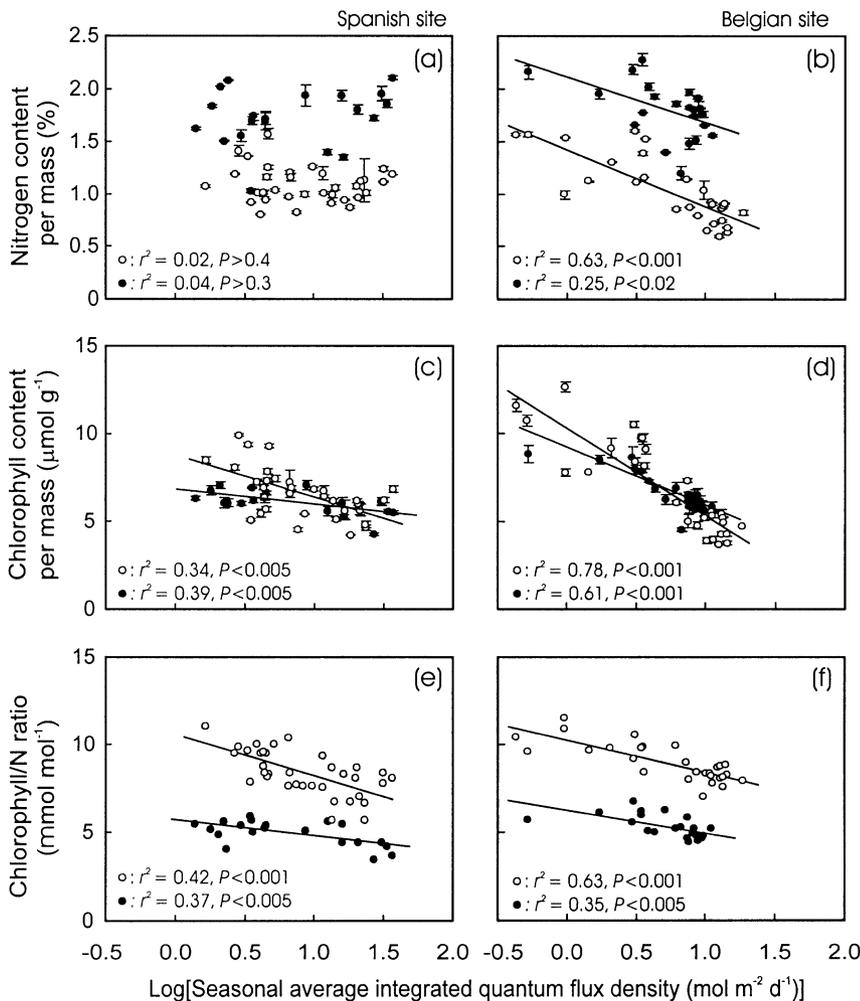
Figure 6. Leaf nitrogen (a, b), and chlorophyll contents (c, d) per unit dry mass, and chlorophyll to nitrogen ratio (e, f) relative to Q_{int} in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols) in both sites. Data presentation as in Fig. 4.

$(N_A = M_A N_M)$ with r^2 varying from 0.56 to 0.81 ($P < 0.001$ for all) for different site and species combinations.

Chlorophyll content per unit dry mass (C_M; Fig. 6c & d) and chlorophyll to nitrogen ratio (Fig. 6e & f) increased with decreasing irradiance in all cases, indicating an enhanced investment of resources to improve light harvesting in low irradiance. However, positive effects of light on M_A were larger than the negative effects on C_M, resulting in positive correlations between Q_{int} and chlorophyll content per unit area (C_A; Table 1).

Interspecific differences in plastic morphological response to light

The slope of the M_A versus Q_{int} relationship was significantly larger ($P < 0.01$) in *R. ponticum* than in *I. aquifolium* in the Spanish site (Fig. 2a; Table 1), but the slopes and intercepts ($P > 0.4$ for both comparisons) were not different between the species at the Belgian site (Fig. 2b). A larger slope in *R. ponticum* at the Spanish site resulted from a greater plasticity in both lamina thickness (Fig. 2c; $P < 0.001$) and density (Fig. 3a). At the Belgian site, the slope of leaf density versus Q_{int} relationship was also larger in *R. ponticum* (Fig. 3b; Table 1; $P < 0.005$), but the slope of thickness versus Q_{int} dependence (Fig. 2d; Table 1) was larger in *I. aquifolium* ($P < 0.05$). This led to similar plasticity in M_A ($T \cdot D$) in both species at the Belgian site. As a general pattern, leaf density was larger in *R. ponticum* than in *I. aquifolium* over most of the understorey light gradient in both sites (Fig. 3a & b). Greater plasticity in D was also paralleled by more extensive changes in lamina dry to fresh mass ratio in *R. ponticum* in both sites (Fig. 3c & d; $P < 0.001$ for the species difference at the Spanish, and $P < 0.05$ at the Belgian site).

Leaf silhouette to projected area ratio tended to be lower in *I. aquifolium* than in *R. ponticum* at both sites (Fig. 4a & b), indicating lower light-harvesting efficiency in *I. aquifolium*. Although the leaves of *I. aquifolium* were wider and shorter than those of *R. ponticum*, leaf perimeter to projected area ratio was not significantly different ($P > 0.1$) between the species at the Spanish site (Fig. 4c). At the Belgian site, this ratio was even larger over most of the irradiance range in *I. aquifolium* (Fig. 4d). These results provide indirect evidence of a large foliar investment in spines, that is the structural defence in *I. aquifolium*.

Foliar dry mass investment in petioles was larger in *I. aquifolium* at the Spanish site (Fig. 5a), whereas contrasting patterns were observed at the Belgian site (Fig. 5b).

Species differences in foliar nitrogen and chlorophyll contents

Leaf nitrogen content per unit dry mass was significantly larger at a common irradiance ($P < 0.001$) in *I. aquifolium* than in *R. ponticum* in both sites (Fig. 6a & b). This difference also resulted in a greater N_A in *I. aquifolium* ($P < 0.001$, cf. Figs 2a & b and 6a & b).

Leaf chlorophyll content per unit dry mass increased

more with decreasing Q_{int} in *R. ponticum* than in *I. aquifolium* at the Spanish site ($P < 0.05$; Fig. 6c). The same pattern was also evident at the Belgian site ($P = 0.06$; Fig. 6d). At the Spanish site, chlorophyll content per unit area also increased more with increasing irradiance in *R. ponticum* ($P < 0.001$; Table 1), but not at the Belgian site ($P > 0.3$). Chlorophyll to nitrogen ratio was consistently larger in *R. ponticum* at both sites (Fig. 6e & f).

Species differences in photosynthetic capacity in relation to foliar structure and nitrogen

The light-saturated photosynthetic electron transport rate (J_{max}) per unit area ($J_{max;A}$) was positively associated with M_A (Fig. 7a) and with N_A (Fig. 7b) in both species at the Belgian site. The correlation between J_{max} per unit dry mass ($J_{max;M}$) and M_A was not significant (Fig. 7c). $J_{max;M}$ was strongly related to N_M, indicating that the positive relationship between $J_{max;A}$ and nitrogen content per unit area was determined both by changes in leaf nitrogen concentration and accumulation of photosynthetic biomass per unit area with M_A.

The slopes of $J_{max;A}$ versus M_A ($P > 0.6$; Fig. 7a), $J_{max;A}$ versus N_A ($P > 0.05$; Fig. 7b) and $J_{max;M}$ versus N_M ($P > 0.8$; Fig. 7d) relations were not significantly different between the species. However, the intercepts of $J_{max;A}$ versus N_A (Fig. 7b) and $J_{max;M}$ versus N_M (Fig. 7d) relations were significantly larger in *R. ponticum* than in *I. aquifolium* ($P < 0.001$ for both comparisons), demonstrating that the photosynthetic nitrogen use efficiency is higher in *R. ponticum*.

General trends and species–site interactions in phenotypic variability and plasticity

Although phenotypic variability among leaves of a given cluster (either whorls in *R. ponticum* or adjacent leaves in a stem or branch in *I. aquifolium*) was very low, variability among plants of the same species and site was quite high, accounting for the largest fraction of the total mean variability observed for eight foliar variables (Table 2). The Belgian population of *R. ponticum* exhibited significantly larger phenotypic variability than that of the Spanish one and than those of both the Belgian and the Spanish populations of *I. aquifolium* (Table 2). Mean phenotypic plasticity, defined as the fraction of total phenotypic variability unambiguously linked to variability in Q_{int}, was significantly larger in the Belgian population of *R. ponticum* than in the Spanish population (Table 3).

Ilex aquifolium was significantly more plastic at the Belgian than at the Spanish site with respect to increases in M_A ($P < 0.02$ for slope differences, Table 1), leaf thickness ($P < 0.005$), and for decreases in C_M ($P < 0.001$) with increasing Q_{int}. The slopes were not different, but leaf dry to fresh mass ratio ($P < 0.001$) and C_A ($P < 0.001$) were larger at a common Q_{int} in this species at the Belgian site. Neither slopes nor intercepts of leaf density and chlorophyll to N ratio versus Q_{int} relations of *I. aquifolium*

Table 1. Effects of seasonal average integrated quantum flux density [Log(Q_{int}, mol m⁻² d⁻¹] on foliar morphology and chemistry in *Ilex aquifolium* and *Rhododendron ponticum*: linear regression analyses[a]

Independent variable	Spanish site				Belgian site			
	I. aquifolium		*R. ponticum*		*I. aquifolium*		*R. ponticum*	
	Intercept[b]	Slope	Intercept	Slope	Intercept[b]	Slope	Intercept	Slope
Leaf dry mass per unit area (g m⁻²)	56.2***	54.8***	27.9***	98.9***	63.7***	85.1***	51.7***	96.8***
Leaf thickness (mm)	0.345***	0.104***	0.244***	0.183***	0.365***	0.224***	0.237***	0.148***
Leaf density (g cm⁻³)	0.176***	0.0685***	0.176***	0.116***	0.182***	0.0734***	0.219***	0.161***
Leaf dry to fresh mass ratio (g g⁻¹)	0.252***	0.0811***	0.245***	0.128***	0.283***	0.0913***	0.273***	0.151***
Leaf projected to silhouette area ratio	0.973***	-0.0122 ns	1.01***	-0.0325*	0.972***	-0.0559*	0.988***	-0.0046 ns
Leaf perimeter to projected area ratio (cm⁻¹)	1.08***	0.0290 ns	0.975***	0.075 ns	1.21***	0.482*	0.881**	-0.038 ns
Petiole to total leaf dry mass ratio (g g⁻¹)	0.0544***	0.0127**	0.0541***	-0.0090**	0.0269***	0.0068 ns	0.0424***	-0.0104***
Leaf nitrogen content (%)	1.63***	0.117 ns	1.14***	-0.058 ns	2.10***	-0.420*	1.42***	-0.536***
Leaf chlorophyll content (µmol g⁻¹)	6.80***	-0.871**	8.69***	-2.35***	9.17***	-3.26***	10.3***	-4.97***
Leaf chlorophyll content (µmol m⁻²)[b]	398***	240***	348***	420***	632***	256***	562***	190***
Chl/N ratio (mmol mol⁻¹)	5.66***	-0.900**	10.5***	-2.31***	6.19***	-1.23***	10.3***	-1.85***

[a]The explained variances of the regressions (r^2) are reported in Figs 2–6. For Chl/area versus log(Q_{int}) relationships, the values of r^2 were 0.70 (*I. aquifolium*, Spanish site), 0.68 (*R. ponticum*, Spanish site), 0.55 (*I. aquifolium*, Belgian site), 0.40 (*R. ponticum*, Belgian site).
[b]****$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.05$.
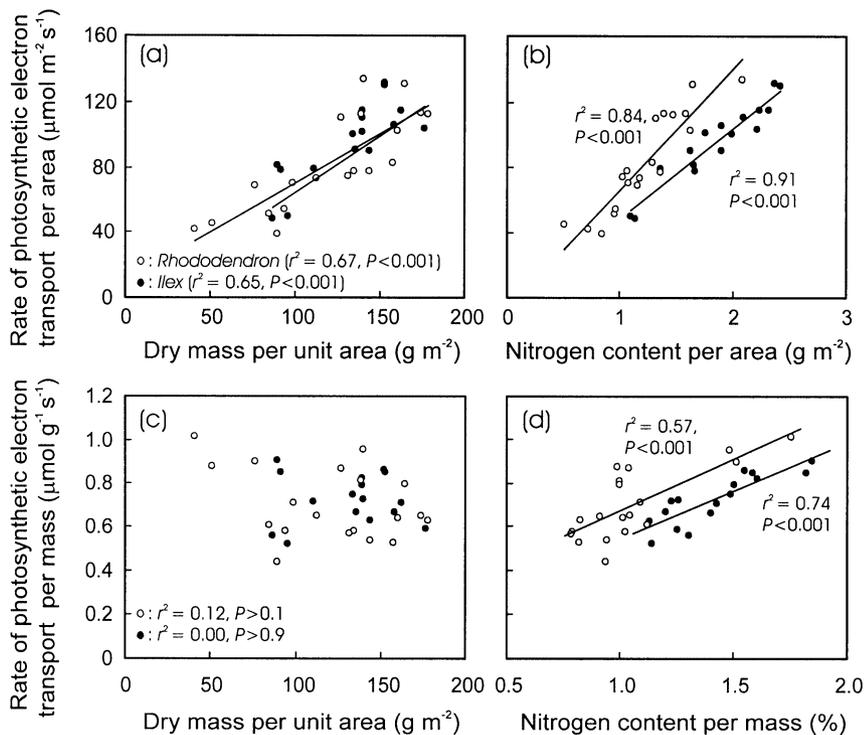
Figure 7. Relationships of photosynthetic electron transport rate per unit area (a, b) and per unit dry mass (c, d) with leaf dry mass per unit area (a, c) and leaf nitrogen content per unit area (b) and dry mass (d) in *I. aquifolium* (filled symbols) and *R. ponticum* (open symbols). The measurements were conducted at the Belgian site. The data were fitted by linear regression.

differed between the sites. Thus, site differences in C_M were primarily modified by site differences in N_M versus Q_{int} relations (Fig. 6a & b).

In *R. ponticum*, C_M decreased significantly more ($P < 0.005$) and C_A increased significantly less with increasing Q_{int} at the Belgian site. Again, chlorophyll to N ratio was not different between the sites in this species ($P > 0.4$), indicating that site differences in foliar chlorophyll versus irradiance relations resulted from contrasting variation patterns in foliar N availability for chlorophyll formation. There were no other site differences in slopes in *R. ponticum*. However, M_A, D and dry to fresh mass ratio were significantly larger at a common irradiance at the Belgian site ($P < 0.001$; Table 1), whereas T and petiole to total leaf mass ratio were larger at the Spanish site ($P < 0.001$; Table 1) in this species.

DISCUSSION

Plastic morphological responses to light

Both *R. ponticum* and *I. aquifolium* (Figs 2 & 3) exhibited a significant morphological plasticity to seasonal average integrated irradiance (Q_{int}). Increases in leaf dry mass per unit area (M_A) with Q_{int} allow accumulation of photosynthetic compounds per unit leaf area, and provide the primary explanation for the scaling of leaf photosynthetic capacities with irradiance (Niinemets & Tenhunen 1997). In turn, decreases in M_A lead to formation of larger foliar area, and greater plant light interception with common biomass investment in foliage, which is a relevant mechanism for a

vast number of plant species with contrasting life strategies (Valladares, Skillman & Pearcy 2002).

In our study, changes in M_A resulted from modifications in both thickness (T; Fig. 2c & d) and leaf density (D, Fig. 3a & b). An increase in D with light may either indicate a larger relative contribution of tightly packed palisade mesophyll at the expense of spongy parenchyma, and/or smaller mesophyll cells with thicker and more lignified cell walls and lower fraction of intercellular air space (Niinemets 1999). This is an important difference, because the first modification increases leaf photosynthetic capacity, whereas the second one decreases it (Niinemets 1999). Larger D generally also results in a higher leaf elastic modulus and allows lower leaf water potentials for a common water loss. In fact, parallel changes in D and leaf dry to fresh mass ratio with Q_{int} support the hypothesis of a larger structural investment at higher irradiance (Garnier *et al.* 1999). Given that foliar water limitations often increase with increasing Q_{int} (Niinemets *et al.* 1999), such modifications in foliar structure may provide an important way to cope with high light environments. Thus, greater D (Fig. 3a & b) and dry to fresh mass ratio (Fig. 3c & d) at high irradiance in *R. ponticum* than in *I. aquifolium* in both sites may partly explain the larger competitive potential of *R. ponticum* in open habitats. However, leaf morphology versus Q_{int} relationships were curvilinear in all cases (Fig. 2a & b), hinting at general limitations associated with the use of high irradiances in both species. As a rule, M_A versus irradiance relationships are linear in true sun species (Niinemets & Kull 1998).

Leaf density of both *I. aquifolium* and *R. ponticum* was

Table 2. Total, among, and within plant variability of eight foliar variables in *Ilex aquifolium* and *Rhododendron ponticum* at the two sites. Variability was estimated by the coefficient of variation (SD/mean)

Site and group		Coefficient of variation								Mean	Significance
		Leaf dry mass per unit area	Leaf thickness	Leaf density	Leaf dry to fresh mass ratio	Petiole to total leaf dry mass	Leaf N content per dry mass	Leaf chlorophyll content per area	Chl/N molar ratio		
Total variability (*n* = 503 samples)											
I. aquifolium	Spain	0.282	0.139	0.169	0.147	0.160	0.155	0.234	0.144	0.179	a
	Belgium	0.260	0.163	0.166	0.123	0.171	0.170	0.152	0.140	0.168	a
R. ponticum	Spain	0.326	0.184	0.202	0.154	0.170	0.183	0.257	0.176	0.206	a, b
	Belgium	0.424	0.235	0.257	0.223	0.192	0.317	0.216	0.122	0.248	b
Variability among plants (*n* = 42 plants)											
I. aquifolium	Spain	0.182	0.057	0.120	0.134	0.176	0.035	0.113	0.025	0.105	a
	Belgium	0.245	0.146	0.156	0.110	0.216	0.150	0.128	0.101	0.157	a
R. ponticum	Spain	0.164	0.110	0.090	0.079	0.060	0.030	0.097	0.091	0.090	a
	Belgium	0.416	0.250	0.210	0.193	0.200	0.292	0.182	0.097	0.230	b
Variability within plants (*n* = 104 leaf clusters)											
I. aquifolium	Spain	0.068	0.039	0.061	0.030			0.066		0.053	ns
	Belgium	0.078	0.052	0.069	0.045			0.084		0.066	ns
R. ponticum	Spain	0.063	0.054	0.061	0.028			0.064		0.054	ns
	Belgium	0.056	0.041	0.045	0.028			0.068		0.048	ns

Significant (*P* < 0.05, ANOVA, Tukey test) differences among the means are indicated by a letter code; ns: no significant differences.

Table 3. Phenotypic plasticity (i.e. variability related to differences in light availability) for eight foliar variables in *Ilex aquifolium* and *Rhododendron ponticum* at the two sites. Plasticity is estimated by both the coefficient of variation and by a plasticity index [(Maximum−minimum)/maximum], Valladares *et al.* (2000a). Only samples within equivalent ranges of light availability were considered

Species	Site	Leaf dry mass per unit area	Leaf thickness	Leaf density	Leaf dry to fresh mass ratio	Petiole to total leaf dry mass	Leaf N content per dry mass	Leaf chlorophyll content per area	Chl/N molar ratio	Mean	Significance
Coefficient of variation											
I. aquifolium	Spain	0.254	0.106	0.143	0.141	0.124	0.084	0.216	0.082	0.144	a, b
	Belgium	0.236	0.161	0.091	0.082	0.109	0.108	0.094	0.092	0.122	b
R. ponticum	Spain	0.326	0.153	0.189	0.112	0.066	0.065	0.187	0.120	0.152	a
	Belgium	0.391	0.269	0.176	0.159	0.198	0.198	0.135	0.107	0.204	c
Plasticity index											
I. aquifolium	Spain	0.418	0.205	0.267	0.277	0.224	0.186	0.323	0.182	0.260	a, b
	Belgium	0.437	0.309	0.188	0.170	0.222	0.223	0.184	0.196	0.241	a
R. ponticum	Spain	0.528	0.298	0.332	0.230	0.138	0.138	0.348	0.212	0.278	b
	Belgium	0.589	0.465	0.332	0.309	0.374	0.325	0.273	0.195	0.358	c

Significant (*P* < 0.05, ANOVA, Tukey test) differences among the means are indicated by a letter code.

relatively low, being comparable with *D* estimates for temperate species (0.3–0.5 g cm^{-3}, Niinemets 2001). In Mediterranean sclerophylls, *D* is considerably larger (0.6–0.8 g cm^{-3}, Niinemets 2001). Given that low density is associated with low elastic modulus, and accordingly with a lower ability for water extraction from drying soil with a common leaf water loss (Niinemets 2001), this may explain the low tolerance of low soil water availability in both species. Thus, limited tolerance of water shortage may be the primary reason of constrained dispersal of both species at the Spanish site. Distribution of many Tertiary relicts in Mediterranean climates is curbed by the high sensitivity of these species to water stress (Gulías *et al.* 2002).

The species difference in *D* was also paralleled by higher dry to fresh mass ratio in *R. ponticum* in both sites. Furthermore, both of these variables were larger at a common irradiance in *R. ponticum* at the Belgian site. Thus, this species may be more drought tolerant in the naturalized habitat than at the native site, possibly as a result of hybridization with other introduced *Rhododendron* species (Milne & Abbott 2000).

Foliage light-harvesting efficiency

Leaf silhouette to projected area was generally larger in *R. ponticum* than in *I. aquifolium* (Fig. 4a & b), in particular, in higher irradiance. This difference implies higher effective leaf area for light interception and greater light-harvesting efficiency in *R. ponticum*, further contributing to higher competitive potential of this species.

Leaf chlorophyll contents per unit dry mass (C_M) increased with decreasing Q_{int} in all situations (Fig. 6c & d). However, there was evidence of greater C_M, and accordingly, greater light absorptance per unit leaf mass in lower irradiance in *R. ponticum* in both cases (Fig. 6c & d). Furthermore, nitrogen partitioning to chlorophyll and associated proteins of leaf light-harvesting apparatus was significantly larger in *R. ponticum* (Fig. 6e & f), demonstrating that this species achieved higher light-harvesting efficiency in limited irradiance with lower nitrogen investments in leaves.

Chlorophyll to N ratio differs little among deciduous broad-leaved temperate forest species with values varying from approximately 9 to 4 mmol mol^{-1} along the canopy irradiance gradient (Niinemets *et al.* 1998). These values are comparable with those in *I. aquifolium* in high and intermediate light, whereas the chlorophyll to N ratios were higher in *R. ponticum* than in co-existing broad-leaved deciduous trees. This may mean that inherently high nitrogen partitioning to light harvesting provides a further explanation for the invasiveness of this species.

According to previous studies, *R. ponticum* may exist in forest understoreys at less than 2% of incident irradiance (Cross 1975; Tabbush & Williamson 1987). However, it generally becomes less competitive at irradiances below 5–10% of full light (Tabbush & Williamson 1987), and its growth rates are the largest in the highest light treatments (Cross 1975). This may be linked to limited N availability for new foliage formation and increase in leaf chlorophyll concentrations at low irradiance. Therefore, it is important that the foliar nitrogen concentrations were higher in low Q_{int} at the Belgian site (Fig. 6a & b), possibly because of enhanced N deposition rates. The hypothesis of N and chlorophyll controls on species dispersal to understorey patches with low irradiance is supported by the existence of leaves in both species at lower irradiances at the Belgian site: 0.43 mol m^{-2} d^{-1} (1.4% of above-ground irradiance) for *R. ponticum* and 0.53 mol m^{-2} d^{-1} (1.8%) for *I. aquifolium*, than at the Spanish site: 1.65 mol m^{-2} d^{-1} (4.1%) for *R. ponticum* and 1.39 mol m^{-2} d^{-1} (3.5%) for *I. aquifolium* (Fig. 2).

High N depositions at the Belgian site only enhanced foliar N concentrations in low irradiance (Fig. 6a & b). However, there is evidence of considerably higher leaf area indices of *R. ponticum* in the Belgian (approximately 1.3 m^2 m^{-2} scaled up to the entire stand, Nadezhdina *et al.* 2003) than at the Spanish site (approximately 0.2 m^2 m^{-2}), and the overstorey trees of *P. sylvestris* at the Belgian site have high N concentrations on the order of 2% (Janssens *et al.* 1999). Thus, N availability is apparently higher at the Belgian site, but *R. ponticum* increased foliar area rather than N of single leaves in response to increased N loads. Enhanced foliar area formation is compatible with greater plant light interception, and with superior plant performance in forest understorey. Given that nitrogen concentration of *R. ponticum* leaves was lower (Fig. 6a & b), this species can form a larger foliar display with equal total foliar N content. This indicates that the invasive *R. ponticum* can more efficiently capitalize on enhanced nutrient availability than the co-existing native species *I. aquifolium*.

Photosynthetic nitrogen use efficiency

We observed higher foliar photosynthetic capacity at a common leaf nitrogen content in *R. ponticum* than in *I. aquifolium* (Fig. 7b & d). Thus, both species had a similar rate of net photosynthesis per unit area (Fig. 7a), but *R. ponticum* achieved this with lower N investments in leaves. Given that the specific activities of the enzymes participating in the dark and light reactions of photosynthesis are believed to be very conserved among C$_3$ plants (Niinemets & Tenhunen 1997; Evans 1998; Evans & Poorter 2001), species differences in photosynthetic nitrogen use efficiency may directly result from inherently lower investments of foliar N in photosynthesis apparatus in *I. aquifolium*.

Species do significantly differ in N partitioning in photosynthetic apparatus (Evans & Poorter 2001; Le Roux *et al.* 2001). As our study suggests, high fractional N investments in photosynthetic machinery may be an important feature of invasive plant species. Similarly to *Rhododendron*, the photosynthetic nitrogen use efficiency is high and may vastly be enhanced by low site nutrient availability in evergreen dwarf-bamboo *Sasa senanensis* that is a strong invader in temperate broad-leaved Japanese forests (Lei & Koike 1998; Wang *et al.* 2001).

Evergreen leaves of *Rhododendron* may fix a large

amount of nutrients and make them unavailable to other species (Monk, McGinty & Day 1985). Combined with the allelopathy and slow turnover of *Rhododendron* litter (Nilsen *et al*. 1999; Walker *et al*. 1999; Wright & Coleman 2000), the ecosystems dominated by *Rhododendron* are generally low in nutrients. This is similar in *S. senanensis* that also produces litter with a low decomposition rate due to the occurrence of allelopathic compounds (Eyin, Jayakumar & Pannirselvam 1989). Thus, inherently high photosynthetic nitrogen use efficiency may be a general trait of invasive species with ample production of toxic secondary compounds, effectively compensating for slow litter turnover rates. It is further important that increased N availability may significantly enhance the competitive potential of these species via enhanced foliage production, which allows them to shade out the competitors.

The role of phenotypic variability and plasticity in species invasiveness

Introduced species often hybridize with native species (Strefeler *et al*. 1996; Milne & Abbott 2000), leading to an increased genetic diversity in the alien plant populations. In fact, introduced populations of *R. ponticum* frequently contain a number of hybrids (Tabbush & Williamson 1987) and there is molecular genetic evidence of hybridization of *R. ponticum* with North-American relatives in the British Isles (Milne & Abbott 2000). The higher phenotypic variability of the alien, Belgian population of *R. ponticum* in comparison with the native, Spanish population seems to reflect differences in the genetic variability of each population, which is likely to be higher in the Belgian population than in the relict and receding Spanish one. The presence of hybrids and the increased genotypic variability could have some bearing on the invasive vigour of alien *R. ponticum* populations.

It has been hypothesized that enhanced phenotypic plasticity plays an important role in successful colonization of new habitats by alien species (Abbott 1992; Williams, Mack & Black 1995; Yamashita *et al*. 2000; Durand & Goldstein 2001). In agreement with this hypothesis, our results indicate enhanced plasticity in response to light in *R. ponticum,* especially in the invasive population (Table 3). The relatively high phenotypic plasticity of *R. ponticum,* a shade-tolerant species, does not agree with the general trend of low plasticity in shade-tolerant species (Valladares *et al*. 2000b). However, a distinction must be made between plasticity in morphological versus physiological variables as discussed in Valladares *et al*. (2000b). It has been shown that shade-tolerant understorey species have low photosynthetic rates and low photosynthetic plasticity to irradiance (Valladares *et al*. 2000b; Yamashita, Koike & Ishida 2002), whereas the photosynthetic plasticity is particularly large in high light species (Valladares *et al*. 2000b). The reverse seems to be true for morphological and light capture variables, at least in species like the European beech (Valladares *et al*. 2002b). A physiologically plastic response to high light has been interpreted as

a mechanism of invasive species to be more efficient at utilizing light than native species in high light environments associated with high levels of disturbance (Yamashita *et al*. 2000; Durand & Goldstein 2001; Yamashita *et al*. 2002). The relatively low plasticity of *I. aquifolium*, which agrees with the low photosynthetic plasticity in response to changes in growth irradiance observed by Schmidt, Batic & Pfanz (2000) in this species, suggest a conservative use of resources (Valladares *et al*. 2000a). This contrasts with the more opportunistic resource use of the invasive populations of *R. ponticum*. Yet, studies also demonstrate that nitrogen availability may be the critical factor limiting photosynthetic acclimation (Ferrar & Osmond 1986). In our study, the rate of photosynthetic electron transport varied along the light gradient by a factor of three in both species at the Belgian site that has high N depositions, demonstrating similar photosynthetic plasticity in both species (Fig. 7). However, in comparison with the native *I. aquifolium*, the alien population of *R. ponticum* had higher nitrogen investments in light harvesting (Fig. 6e & f) and in photosynthetic machinery (Fig. 7), allowing it to produce more foliar area with equal photosynthetic and light-harvesting characteristics.

As suggested by Dukes & Mooney (1999), invasive plant species possess a set of traits that allow them to capitalize on increasing nitrogen availability. As our work shows, enhanced nitrogen investment in light-harvesting and photosynthetic compounds combined with high rates of leaf area production could partly explain the invasive nature of *R. ponticum*. Paralleling the increase in nitrogen deposition rates, there has been a general increase in the coverage of *Rhododendron* species in several places in the world (Nilsen *et al*. 1999; Esen 2000), providing indirect evidence of the role of nitrogen in the invasion potential of *Rhododendron* species. The high nitrogen use efficiency of *R. ponticum* together with its relatively high plastic response to light, despite its shade tolerance, and its capacity to hybridize and enhance its genetic and phenotypic variability, are the new features to be added to our understanding of the success of *R. ponticum* as an invading species in many temperate, humid and subhumid regions of Europe.

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