The Ratio of Leaf to Total Photosynthetic Area Influences Shade Survival and Plastic Response to Light of Green-stem Leguminous Shrub Seedlings

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Different plant species, and organs within a plant, differ in their plastic response to light, which influences their performance and survival in relation to the light environment, which may range from full sunlight to deep shade. Plasticity, especially with regard to physiological features, is linked to a greater capacity to exploit high light and is usually low in shade-tolerant species. Among photosynthetic organs, green stems, which represent a large fraction of the total photosynthetic area of certain species, are hypothesized to be less capable of adjustment to light than leaves, due to biomechanical and hydraulic constraints. The response to light of leaves and stems of six species of leguminous, green-stem shrubs from dry and high-light environments was studied by growing seedlings in three light environments: deep shade, moderate shade and sun (4, 40 and 100 % of full sunlight, respectively). Survival in deep shade ranged from 2 % in *Retama sphaerocarpa* to 74 % in *Ulex europaeus*. Survival was maximal at moderate shade in all species, ranging from 80 to 98 %. The six species differed significantly in their ratio of leaf to total photosynthetic area, which influenced their light response. Survival in deep shade increased significantly with increasing ratio of leaf to total photosynthetic area, and decreased with increasing plasticity in net photosynthesis and dark respiration. Responses to light differed between stems and leaves within each species. Mean phenotypic plasticity for the variables leaf or stem specific mass, chlorophyll content, chlorophyll *a/b* ratio, and carotenoid to chlorophyll ratio of leaves, was inversely related to that of stems. Although mean plasticity of stems increased with the ratio of leaf to total photosynthetic area, the mean plasticity of leaves decreased. Shrubs with green stems and a low ratio of leaf to total photosynthetic area are expected to be restricted to well-lit habitats, at least during the seedling stage, due to their inefficient light capture and the low plasticity of their stems.   © 2003 Annals of Botany Company

Key words: *Coronilla juncea*, *Cystisus scoparius*, *Genista scorpius*, green stems, leguminous shrubs, light acclimation, phenotypic plasticity, *Retama sphaerocarpa*, shade tolerance, *Spartium junceum*, *Ulex europaeus*.

INTRODUCTION

Green stems make important contributions to the overall carbon gain of many species of plant (Pfanz and Aschan, 2001). They contribute up to 50 % of the carbon gain of plants in deserts, tropical dry or thorn woodlands and Mediterranean-type ecosystems (Nilsen, 1992a, *b*), and are particularly important in many leguminous shrubs characteristic of arid habitats (Bossard and Rejmanek, 1992; Pugnaire *et al*., 1996; Nilsen and Sharifi, 1997).The extension of the period of carbon gain in environments with periodic droughts (Smith and Osmond, 1987; Nilsen, 1992a), heat (DePuit and Caldwell, 1975; Smith and Osmond, 1987) or excessive irradiance (Valladares, 1999; Valladares and Pugnaire, 1999), and an increased tolerance to herbivory (Bossard and Rejmanek, 1992) are the principal benefits to plants with green, photosynthetic stems. It has been argued that stem photosynthesis could

explain the invasive nature of certain leguminous shrubs, e.g. *Cytisus scoparius* and *Spartium junceum* (Bossard and Rejmanek, 1992; Nilsen *et al*., 1993). There is great variability in the relative contribution of leaves and stems to carbon gain between species with green stems. For instance, in the leguminous shrub *Retama sphaerocarpa*, where leaves have a very brief life, carbon is fixed almost entirely by stems (Haase *et al*., 1999). However, in other widespread leguminous shrubs, such as *Cytisus scoparius* and *Spartium junceum*, green stem area is only 1·5–1·7 times the area of leaves and contributes only 30–50 % of whole plant carbon gain (Nilsen, 1992b; Nilsen *et al*., 1993). Since leaves are better oriented for light capture and generally have larger photosynthetic rates per unit area than stems (Nilsen, 1992*b*), the relative contribution of these two organs to whole plant carbon gain may not be linearly related to their surface area.

Plants, as sessile organisms, exhibit a remarkable capacity to modify their physiology and morphology according to the prevailing environment, so-called pheno-

typic plasticity (Valladares, 2000; Pigliuci, 2001). Even though a phenotypically plastic response to light can enhance survival under extreme or variable light conditions, the ecological and evolutionary implications of phenotypic plasticity in plants are not well established (Pigliuci, 2001). We still need to elucidate general trends among species regarding their phenotypic plasticity before robust conclusions can be drawn. Plastic responses of leaves and stems of leguminous shrubs to light are particularly unclear, despite the studies of Nilsen and coworkers (Nilsen, 1992a, b; Nilsen *et al.* 1993), and the mechanisms involved are poorly known. Analyses of plants with other growth forms cannot be directly extrapolated to green stem shrubs due to the functional peculiarities conferred by their crown architecture. Plasticity of phenotypic response to light is low in species from dry ecosystems (Valladares *et al.*, 2000a, b; Balaguer *et al.*, 2001), but whether this applies to leguminous shrubs, some of which are invasive and spread during disturbances (and thus they are likely to exhibit high phenotypic plasticity; see Grime *et al.*, 1986) is uncertain. The picture is further complicated by the great differences in the light environment experienced by adult and juvenile plants: while the former are usually exposed to high light, the latter can experience a range of light, from abundant in clearings and gaps in vegetation to severe limitations in the understorey. Thus, shade tolerance and plasticity in response to light may play an important role in survival and performance of leguminous shrubs from arid sites, particularly at the seedling stage.

In this study, we explored shade tolerance and phenotypic plasticity in response to light of six leguminous, green-stem shrub species by growing seedlings in three light environments, deep shade, moderate shade and full sunlight. Species were chosen to comprise a range of morphologies within the green stem habit, with large differences in the ratio of leaf to total photosynthetic area. Variables studied covered three main categories involved in the responses of plants to light: morphology, biochemistry (photosynthetic pigment concentration) and gas exchange (photosynthesis and respiration). The objectives of this study were two-fold: (1) to quantify the influence of morphological and physiological factors on the survival capacity of the species under different light environments; (2) to explore the differential, plastic response of green stems and leaves to light in these six species. Specific hypotheses underlying each objective were: (1) since leaves are better oriented for light capture than stems (Valladares, 1999), a high ratio of leaf to total photosynthetic area could enhance survival under low light conditions, while a low ratio could be favourable under high light where stem light harvesting can prevent photoinhibition (Valladares and Pugnaire, 1999); (2) stems are less plastic than leaves mainly due to biomechanical constraints, as suggested by the low physiological responsiveness of *Spartium junceum* stems (Nilsen, 1992). Since high plasticity, specially in physiological variables, has been linked to a greater capacity to exploit high light (Yamashita *et al.*, 2000; Valladares *et al.* 2000b, 2002*a*), and shade tolerators are phenotypically less plastic than light-demanding species in their responses to light (Henry and Aarssen, 1997), we

TABLE 1. *Photosynthetic photon flux (PPF) in the three experimental light environments for 10 consecutive, clear days during July 1999*

	Sun	Moderate shade	Deep shade
Maximum midday PPF (μmol m^{-2} s^{-1})	2210 ± 240	1356 ± 392	353 ± 93
Total PPF per day (mol m^{-2} day^{-1})	56·9 ± 10	18·2 ± 7·9	1·9 ± 0·2
Mean available PPF (% of full sunlight)	100	32	3

Each value is the mean ± s.d. of four sensors.
Means differed significantly (ANOVA, Tukey test $P < 0.0001$).

expected a negative relationship between shade tolerance and phenotypic plasticity in the species studied.

MATERIALS AND METHODS

Species and experimental design

The species studied were: *Coronilla juncea* L., *Spartium junceum* L., *Genista scorpius* L., *Cytisus scoparius* (L.) Link. and *Retama sphaerocarpa* (L.) Boiss, which are native and widespread in the Mediterranean region, and *Ulex europaeus* L., which is characteristic of more oceanic and northern areas in Europe. All have conspicuously green stems (cladodes), which can photosynthesize, but they differ in the relative contributions of leaves and stems to the photosynthetic surface area of the crown. Leaves are small to very small, and are shed 2–4 months after expansion under normal environmental conditions. The species are typically 2–4 m in height, although they can easily reach 5 m in favourable locations. Seeds from these six leguminous species were obtained commercially (Semillas Zulueta, Pamplona, Spain). The seeds were boiled in water for 1 min to break dormancy and sown in 0·5-l pots filled with washed river sand at the end of March 1999. The pots were watered frequently to maintain pot capacity. Once a week, 2 ml of a liquid fertilizer (N$^:$P$^:$K, 7$^:$5$^:$6; KB Universal Fertilizer; Rhone-Poulenc, Lyon, France) were added with the water so a total of 2·2 mg of N, 1·6 mg of P and 1·9 mg of K were given to each pot throughout the experiment. Emerged plants were maintained in moderate shade during the first month and then transferred to the different light treatments.

Three different light environments (sun, moderate shade and deep shade) were created in the nursery of the University Complutense of Madrid using layers of neutral shade cloth supported by metal frames, each 30 m^2 and 2·5 m tall to facilitate ventilation. The photosynthetic photon flux (PPF) within each enclosure was measured at 2-min intervals for 10 consecutive, clear days of July with home-made sensors cross-calibrated under a range of natural irradiances with a quantum sensor (Li-Cor, Lincoln, NB, USA) attached to data-loggers (Hobo, Onset, USA). One data-logger with four sensors was placed in each light environment and the mean values are in Table 1. These three

TABLE 2. *Leaf area as percentage of total photosynthetic surface area, and relative number of leaves (no. cm⁻¹ stem length), for seedlings of six leguminous shrub species in moderate shade and full sunlight*

Species	Light treatment	Leaf area (%)	No. of leaves (cm⁻¹)
Retama sphaerocarpa	Moderate shade	35·0 ± 12·5[a]	0·9 ± 0·9[a]
	Sun	2·1 ± 0·1[b]	1·7 ± 1·3[b]
Spartium junceum	Moderate shade	74·1 ± 5·7[c]	0·8 ± 0·3[a]
	Sun	35·2 ± 6·4[a]	1·4 ± 0·8[b]
Genista scorpius	Moderate shade	52·4 ± 9·8[d]	2·6 ± 2·0[c]
	Sun	48·5 ± 3·3[d]	3·3 ± 1·2[cd]
Cytisus scoparius	Moderate shade	75·8 ± 29·4[c]	2·1 ± 0·9[c]
	Sun	52 ± 11·3[d]	2·8 ± 1·7[c]
Coronilla juncea	Moderate shade	22·6 ± 9·8[a]	4·0 ± 1·0[d]
	Sun	85·2 ± 13·8[c]	3·8 ± 1·3[d]
Ulex europaeus	Moderate shade	81·3 ± 23·1[c]	10·5 ± 2·1[e]
	Sun	85·1 ± 22·7[c]	10·7 ± 2·0[e]

Values are the mean ± s.d. for three to seven individuals.

The letter code indicates significant differences between species and treatments (ANOVA, Tukey test, $P < 0.005$)

light environments spanned the natural range of light experienced by seedlings of these species in the field: sparse vegetation in semi-arid sites reduce total daily irradiance by about 20–40 %, while light penetration through dense stands of *Ulex europaeus* is less than 5 % (Pugnaire *et al*., 1996; Ojea *et al*., 1998). Twenty pots with a minimum of 40 seeds each were used for each combination of species and light treatment.

Survival, morphological and physiological measurements

At the end of May, seedlings were thinned to three per pot; competition was considered negligible due to the smallness of individual plants in relation to the pot during the whole experiment, so there was no mutual shading and roots did not fill the pots. Survival of each species in each light treatment was measured twice a month until the end of the experiment in September 1999. The number of leaves, the length of the stem and the photosynthetic surface area of five individual seedlings were recorded after 3 months' growth in each light and the ratio of leaf to total photosynthetic area was calculated. Stem diameter was measured with a calliper (accuracy 0·01 mm) and the stem area was calculated assuming it was cylindrical (projected area of the stem multiplied by $0.5\,\pi$). Leaf area was measured with a PC-compatible desktop scanner and an image analyser software (Sigmascan; Jaendel Scientifics, USA). All stems and leaves were scanned and finally dried in an oven at 80° C for a minimum of 48 h, and leaf and stem specific mass (LSM and SSM, respectively) were calculated from fresh area and dry mass.

Chlorophyll and total carotenoids were measured in 0·05-g portions of stems and leaves. Each portion was incubated in 3 ml of dimethyl sulfoxide for 2 h in a dark oven at 65° C. Optical density of the extracts was measured spectro-photometrically at 663·0, 646·8, 480·0, 435·0 and 415·0 nm and concentrations calculated according to Wellburn (1994).

Light-saturated, maximum rate of net photosynthesis (A_{max}) and dark respiration (DR) were measured *in situ*

during the morning of consecutive, clear days in August and September 1999 with a portable infrared gas analyser (LCA-4; ADC Bioscientific Ltd, UK). Portions of stems with a varying number of leaves were placed in the cuvette and the steady state A_{max} measured under natural incident PPF $\geq$ 1800 µmol m⁻² s⁻¹. Care was taken to avoid mutual shading by organs. Steady-state DR was measured after a minimum of 5 min in the dark, with the cuvette covered with thick, black fabric. Leaf and stem area were determined and A_{max} and DR expressed per unit total surface area.

Data analysis

ANOVA (followed by Tukey test) by Sigmastat 2.0 Windows version (Jaendel Scientifics, USA) tested for differences between species and treatments. In all cases, the data met the assumptions of normality and homocedasticity. An index of phenotypic plasticity was calculated for each variable and species as the difference between the minimum and the maximum mean values among the different treatments divided by the maximum mean value. This phenotypic plasticity index has been used in previous studies (Valladares *et al*., 2000a, *b*; Balaguer *et al*., 2001), and it has the advantage that changes in variables expressed in different units and with contrasting ranges, can be compared since it scales from 0 to 1. Linear and non-linear regression protocols of Sigmastat were used to test for significant dependences among key variables. Comparisons for most variables were restricted to sun *vs*. moderate shade due to low survival and few leaves in deep shade.

RESULTS

Species significantly ($P < 0.01$) differed in the ratio of leaf to total photosynthetic area (Table 2). This ratio was larger in the moderate shade than in the sun for three of the six species, was the same in *Ulex europaeus* and *Genista scorpius* and smaller in *Coronilla juncea*, which had elongated stems and very small leaves in the shade (Table 2). Survival in deep shade ranged from 2 % in

TABLE 3. *Maximum rates of net photosynthesis and dark respiration (A_{max} and DR, respectively, in $\mu mol\ m^{-2}\ s^{-1}$) of six species of leguminous shrubs grown from emergence in moderate shade and in the sun*

	R. sphaerocarpa		*S. junceum*		*G. scorpius*		*C. scoparius*		*C. juncea*		*U. europaeus*	
Variable	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
A_{max}												
Sun	10·9[a]	1·3	8·6[a]	2·8	12·0[a]	3·8	9·0[a]	0·0	9·3[a]	3·6	4·5[a]	3·3
Moderate shade	1·2[b]	0·5	6·7[a]	0·8	2·7[b]	1·2	23·4[c]	8·9	5·3[a]	4·0	7·5[a]	6·2
PI for A_{max}	0·90		0·21		0·77		0·61		0·43		0·40	
DR												
Sun	1·1[a]	0·2	1·0[a]	0·6	0·6[a]	0·6	0·8[a]	0·4	2·2[b]	0·6	0·7[a]	0·7
Moderate shade	0·4[c]	0·1	0·5[c]	0·4	0·3[c]	0·5	0·4[c]	0·3	1·1[a]	0·5	0·4[c]	0·5
PI for DR	0·65		0·74		0·50		0·30		0·50		0·40	

Phenotypic plasticity index [PI = (maximum – minimum)/maximum] for each variable is also shown.
Measurements were made in representative portions of stems with the leaves attached.
Values are the mean ± s.d. of five individual plants.
The letter code indicates significant differences between species and treatments (ANOVA, Tukey test, $P < 0.005$).
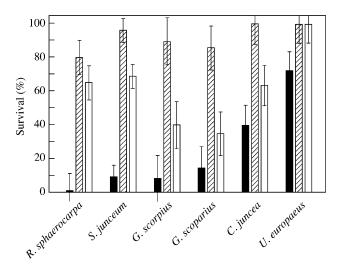

FIG. 1. Percentage survival of seedlings of six species of leguminous shrubs grown in the sun (open bars), moderate shade (hatched bars) and deep shade (black bars). The species were ordered according to their ratio of leaf to total photosynthetic area from left to right. Bars represent the mean ± s.d. ($n = 5$)
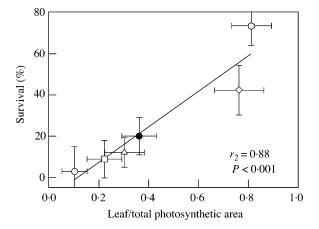

FIG. 2. Percentage survival in deep shade of seedlings of six leguminous shrubs versus their ratio of leaf to total photosynthetic area. Each value represents the mean ± s.d. ($n = 5$). Symbols for the species are: circle, *R. sphaerocarpa*, square, *S. junceum*, triangle, *G. scorpius*, closed circle, *C. scoparius*, diamond, *C. juncea*, hexagon, *U. europaeus*.

Retama sphaerocarpa to 74 % in *Ulex europaeus*, but was always smaller than in other light treatments (Fig. 1). Survival of all species was greater (>80 %) in moderate than deep shade; the latter significantly increased with increasing ratio of leaf to total photosynthetic area (Fig. 2).

Whilst maximum photosynthetic rates (A_{max}) of sun plants did not differ significantly between species, values of A_{max} in moderate shade were significantly different between species (Table 3). *Retama sphaerocarpa* and *Genista scorpius* had significantly greater A_{max} in the sun than moderate shade, and had the largest values of phenotypic plasticity for this variable. *Cytisus scoparius*, with a very plastic A_{max}, exhibited the reversed pattern, with higher rates in moderate shade than in the sun (Table 3). Dark respiration was higher in the sun than in moderate shade in all species, and the phenotypic plasticity index for this variable ranged from 0·30 to 0·74 (Table 3).

Specific mass (g m^{-2}) of stems was larger than that of leaves in all species, both in the sun and shade (Table 4). Specific mass of leaves, but not of stems, was significantly larger in the sun than in moderate shade in *G. scorpius*, *C. scoparius* and *U. europaeus* (Table 4). Chlorophyll content per unit area differed between sun and shade leaves in most of the species, being larger in the sun than in the shade in *S. junceum*, *G. scorpius* and *U. europaeus*, while that of stems increased in the sun in *R. sphaerocarpa*, *G. scorpius*, *C. juncea* and *U. europaeus* (Table 4). The chlorophyll *a/b* ratio was higher in stems than in leaves in the shade but not in the sun (Table 4). The carotenoids/chlorophyll ratio increased in the sun in most species both in leaves and stems (Table 4).

Phenotypic plasticity differed between species and organs (leaves *vs*. stems), and the trends were different for each particular variable (Table 5). Mean plasticity (i.e. the mean for phenotypic plasticity indexes for the variables of Table 4) of leaves and stems exhibited a very different pattern: while

TABLE 4. *Specific mass (g m^{-2}), chlorophyll content (mg m^{-2}), chlorophyll a/b ratio and carotenoids/chlorophylls ratio of leaves and stems of six species of leguminous shrubs grown from emergence in moderate shade and in the sun*

Variable	Organ	Light environment	*R. sphaerocarpa*		*S. junceum*		*G. scorpius*		*C. scoparius*		*C. juncea*		*U. europaeus*	
			Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Specific mass	Leaf	Sun	–	–	28·9[a]	20·7	117·0[b]	5·3	143·1[b]	70·8	28·8[a]	8·2	107·0[b]	0·8
		Moderate shade	–	–	36·7[a]	2·3	69·2[a]	13·3	47·3[a]	5·9	30·7[a]	22·1	44·9[a]	4·9
Specific mass	Stem	Sun	1183·1[a]	306·6	261·4[b]	231·2	439·5[b]	121·8	446·7[b]	228·7	427·7[b]	166·1	624·4[b]	0·0
		Moderate shade	1198·9[a]	622·0	209·7[b]	126·7	310·0[b]	62·8	245·7[b]	41·9	110·7[c]	3·6	493·7[b]	224·7
Chlorophyll content	Leaf	Sun	–	–	410·4[a]	87·7	644·4[b]	87·0	149·7[c]	23·1	92·0[c]	27·8	417·3[a]	33·4
		Moderate shade	–	–	184·5[c]	118·6	384·2[ac]	58·0	188·5[c]	44·1	117·6[c]	73·8	294·0[c]	68·1
Chlorophyll content	Stem	Sun	605·4[a]	84·5	364·8[b]	79·0	970·6[c]	88·2	125·8[d]	51·0	766·5[a]	365·1	489·3[b]	168·2
		Moderate shade	454·9[b]	26·5	416·8[b]	163·5	517·8[b]	102·7	314·2[b]	101·7	97·9[d]	22·9	254·7[c]	59·4
Chlorophyll *a/b*	Leaf	Sun	4·0[a]	0·6	4·1[a]	0·7	2·6[b]	0·6	4·5[a]	1·0	3·0[b]	0·9	3·6[ab]	0·5
		Moderate shade	–	–	1·8[c]	0·1	2·8[b]	1·0	4·1[a]	0·9	3·4[b]	1·0	3·3[b]	0·7
Chlorophyll *a/b*	Stem	Sun	3·2[b]	0·3	3·5[b]	1·6	4·4[a]	0·7	3·9[ab]	1·4	3·8[ab]	0·5	5·2[d]	1·1
		Moderate shade	2·7[b]	0·2	4·6[a]	0·5	5·4[d]	1·4	4·1[a]	1·1	4·6[a]	1·5	4·3[a]	0·9
Carotenoids/chlorophyll	Leaf	Sun	–	–	0·26[a]	0·09	0·20[a]	0·01	0·30[a]	0·10	0·15[b]	0·10	0·23[a]	0·10
		Moderate shade	–	–	0·16[b]	0·10	0·08[b]	0·20	0·10[b]	0·10	0·13[b]	0·10	0·16[b]	0·03
Carotenoids/chlorophyll	Stem	Sun	0·11[c]	0·06	0·17[b]	0·03	0·20[ab]	0·06	0·25[a]	0·04	0·16[b]	0·06	0·21[a]	0·05
		Moderate shade	0·13[c]	0·03	0·14[bc]	0·06	0·12[c]	0·06	0·17[ab]	0·06	0·11[c]	0·04	0·12[c]	0·06

Values are the mean ± s.d. of five individuals.
Not enough leaves (less than three) for reliable measurements of most variables were obtained in *Retama sphaerocarpa*.
The letter code indicates significant differences between species and treatments (ANOVA, Tukey test, $P < 0.005$).

TABLE 5. *Phenotypic plasticity index [PI = (maximum − minimum)/maximum] for specific mass (g m^{-2}), chlorophyll concentration (mg m^{-2}), chlorophyll* a/b *ratio and carotenoids/chlorophylls ratio of leaves and stems of six species of leguminous shrubs*

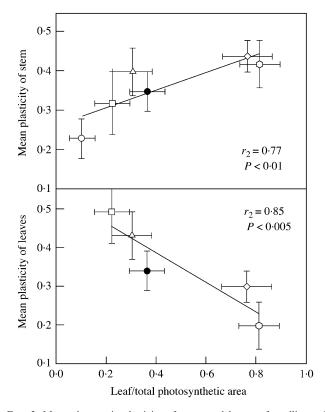| Variable	R. sphaerocarpa	S. junceum	G. scorpius	C. scoparius	C. juncea	U. europaeus
Specific mass LEAF	–	0·76	0·41	0·67	0·06	0·58
Specific mass STEM	0·01	0·20	0·30	0·45	0·74	0·21
Chlorophyll content LEAF	–	0·55	0·40	0·70	0·22	0·30
Chlorophyll content STEM	0·25	0·12	0·80	0·60	0·87	0·48
Chlorophyll *a/b* LEAF	–	0·61	0·48	0·01	0·26	0·23
Chlorophyll *a/b* STEM	0·13	0·14	0·40	0·13	0·23	0·30
Carotenoids/chlorophyll LEAF	–	0·38	0·60	0·55	0·13	0·30
Carotenoids/chlorophyll STEM	0·15	0·17	0·40	0·32	0·31	0·43
Mean PI LEAF	–	0·58	0·47	0·48	0·17	0·35
Mean PI STEM	0·13	0·16	0·48	0·38	0·54	0·36

FIG. 3. Mean phenotypic plasticity of stems and leaves of seedlings of six leguminous shrub species [plasticity was estimated as the mean of the values of the phenotypic plasticity index, PI = (maximum − minimum)/maximum, calculated for each variable of Table 4] versus their leaf to total photosynthetic area ratio. Each value represents the mean ± s.d. (n = 5). Symbols for species as in Fig. 2.
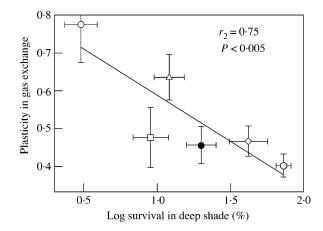

FIG. 4. Mean phenotypic plasticity for gas exchange variables (maximum net photosynthetic rate and DR) of seedlings of six leguminous shrub species versus their survival in deep shade (log of percentage survival). Each value represents the mean ± s.d. (n = 5). Symbols for species as in Fig. 2.

that for leaves decreased, that for stems increased with increasing ratio of leaf to total photosynthetic area (Fig. 3). Plasticity in gas exchange variables (mean PI for A_{max} and DR) decreased with increasing survival in deep shade (Fig. 4).

DISCUSSION

Survival in deep shade generally involves maximization of light capture, which in turn involves the proper arrangement of the photosynthetic surfaces, primarily on a horizontal plane (Valladares and Pearcy, 1998; Valladares *et al.*, 2002b). Light harvesting by green stems is generally small, because they tend to be vertical, nevertheless, it can be advantageous in arid habitats with high irradiance (Valladares and Pugnaire, 1999). Our study has shown that the ratio of leaves to total photosynthetic area, which, in contrast to stems, can be efficiently arranged for maximal light capture, determines the short-term survival of leguminous shrub seedlings in deep shade (Fig. 2). Thus, species with green stems and a low ratio of leaf to total photosynthetic area seem to be restricted to well-lit habitats. It must be noted, however, that survival of all species studied here was higher at intermediate light intensities, in agreement with the notion that both extremes of the light gradient are stressful (Evans *et al.*, 1988; Valladares, 2000). Morphological features, such as the ratio of leaf to total photosynthetic area, are important for shade survival since they increase efficiency of light capture and enhance survival in shade as discussed in Valladares *et al.* (2002b). Physiological plasticity, on the other hand, frequently enhances high-light tolerance (Valladares *et al.*, 2002a).

Species tolerant of deep shade had low plastic response of gas exchange variables to light (Fig. 4), which agrees with the idea that a better exploitation of high light involves physiological plasticity, while shade tolerance involves phenotypic stability, as deduced from comparative studies of invasive and non-invasive species, and of plants of different successional status or light requirements (Valladares *et al*., 2000b; Yamashita *et al*., 2000).

Stem elongation in the shade, which is part of the shade avoidance syndrome (Smith and Whitelam, 1997), is another morphological feature that can affect light capture, since it generally implies that less biomass is available to form leaves resulting in a low efficiency of light interception. Henry and Aarssen (1997) argued that shade avoidance and tolerance are quite different strategies and, while plasticity can be adaptive in very dynamic light environments, phenotypic stability is more likely to be selected in uniformly shaded environments. In our comparative study, only *Coronilla juncea* significantly elongated its stems in the shade, whereas other species had a greater ratio of leaf to total photosynthetic area increased in the shade. Thus, the six leguminous species could be grouped in three categories: shade intolerant (*R. sphaerocarpa, S. junceum, G. scorpius* and *C. scoparius*), shade avoiding (*C. juncea*) and shade tolerant (*U. europaeus*). However, these results should only be taken as indicative, since the red/far red ratio, which is a key elicitor of the shade avoidance response, was not modified in this experiment.

Most of the changes found here in the photosynthetic rates, leaf area and pigment concentrations associated with light availability (e.g. increased DR, specific leaf mass and carotenoid/chlorophyll ratio in the sun) conformed with expectations from typical sun–shade comparisons (Evans *et al*., 1988; Young, 1991; Valladares, 2000). Carotenoids, primarily photoprotective pigments (Young, 1991), were at higher concentrations in leaves than in stems, in agreement with the different exposure to high light and risk of photoinhibition associated with their different mean elevation angle (Ehleringer and Cooper, 1992; Valladares and Pearcy, 1992). Leaves and stems responded differently to the light environment, and correlations in their responses were either negative, suggesting a compensating mechanism, or absent. Mean phenotypic plasticity of leaves exhibited a reverse pattern to that of stems: while mean plasticity of the stem increased with increasing ratios of leaf to total photosynthetic area, the mean plasticity of leaves decreased (Fig. 3). We hypothesized in the Introduction that when compared with leaves, the multiple functions of green stems impose constraints on maximizing light capture and photosynthesis so their plastic response to light should be smaller than that of leaves. In agreements with this, photosynthesis of stems was less responsive than that of leaves to environmental factors such as light, temperature and relative humidity (Nilsen, 1992*a*), and carbon gain from stem assimilation was rather constant throughout the seasons in *Spartium junceum* and *Cytisus scoparius* (Nilsen *et al*., 1993). However, mean plasticity of leaves was similar to that of stems. Only the patterns of plasticity associated with the ratio of leaf to total photosynthetic area were significantly different between stems and leaves.

The leguminous shrubs studied dwell in open, high-light environments. However, their growth and establishment at the seedling stage, and thus recruitment into the population may take place in quite different light environments. *Retama sphaerocarpa,* a species from semi-arid habitats, has a loose crown that only reduces total daily PPF by about 20–40 % (Pugnaire *et al*., 1996; Moro *et al*., 1997), and the same applies to other shrub species occurring in the same area. Thus, *R. sphaerocarpa* seedlings are not likely to experience deep shade during establishment, either in the open or below the moderate shade of adult shrubs. On the contrary, *Ulex europaeus,* a species from relatively more humid, oceanic habitats, generates a very dense canopy, due (at least in part) to a remarkable accumulation of woody and dead phytomass (Ojea *et al*., 1988). This results in substantially decreased light penetration, so that less than 5 % of the PPF reaching the canopy penetrates to the soil surface (Puentes, 1995). Intraspecific competition for light increases rapidly with stand age and density in this species (Hely and Forgeard, 1998). Thus, *U. europaeus* seedlings are likely to experience deep shade when recruiting under the canopy of either the adults or the surrounding vegetation. Light environment in the understorey of the other species studied is less well documented, but personal observations indicate that they fall within the extremes of the relatively high-light understorey of *R. sphaerocarpa* and the deep shade understorey of *U. europaeus*. The ranking of species according to their deep shade tolerance (Fig. 1) then suggests that these differences in the light transmission through the canopy of the adult shrubs correlate with the relative shade tolerance of each species at the seedling stage.

Most leguminous shrubs studied here did not tolerate deep shade as seedlings. The plastic response of gas exchange to shade i was inversely related to their tolerance to deep shade, which was enhanced by a large ratio of leaf to total photosynthetic area. Responses of leaves were quite different from those of stems, although mean plasticity was similar. Our results confirm that, while morphological adaptations and responses to light enhance survival in deep shade, physiological plasticity enhances high light tolerance, and shows the importance of studying light as an ecological factor, too frequently neglected for plants of arid habitats.

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