

Species-specific Variation in the Importance of the Spectral Quality Gradient in Canopies as a Signal for Photosynthetic Resource Partitioning

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• **Background and Aims** Plants adjust the distribution of photosynthetic capacity and chlorophyll to canopy density. The importance of the gradient in the red : far-red ratio (R : FR) relative to the irradiance gradient was studied for its perception with respect to this partitioning of photosynthetic resources. Whether the relative importance of these two signals varied between six species of different growth habit (*Phaseolus vulgaris*, *Lysimachia vulgaris*, *Hedera helix*, *Ficus benjamina*, *Carex acutiformis* and *Brachypodium pinnatum*) was investigated further.

• **Methods** Single leaves of plants were shaded in daylight by a spectrally neutral filter or a leaf. In another experiment, leaves were treated with supplemental FR. In most cases, treatment effects were evaluated after 2 weeks.

• **Key Results** Nitrogen and photosynthetic capacity (A_{\max}) per leaf area, parameters pertaining to between-leaf resource partitioning, were strongly reduced in neutral shade but not additionally by spectral leaf shade. Supplemental FR reduced these parameters also, except in *Carex*. Acceleration of induction of senescence was observed in spectral leaf shade in primary bean leaves. A_{\max} per unit chlorophyll, a parameter pertaining to within-leaf resource partitioning, was reduced in neutral shade, but not in spectral leaf shade or supplemental FR.

• **Conclusions** Signalling mechanisms associated with perception of the R : FR gradient in canopies were less important than those associated with the irradiance gradient for between-leaf and within-leaf partitioning of photosynthetic resources. The relative importance of the signals differed between species because *Carex* was the only species for which no indications were found for an involvement of the spectral gradient in perception of canopy density.

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Key words: Chlorophyll, herbaceous dicotyledonous species, irradiance, leaf canopies, light gradient, monocotyledonous species, photosynthetic capacity, photosynthetic resource partitioning, red : far-red ratio, spectral quality, shade acclimation, woody species.

INTRODUCTION

The partitioning of photosynthetic resources between leaves is adjusted to canopy density in many plants in such a way that upper leaves in high irradiance have high leaf nitrogen content (N_{LA}) and photosynthetic capacity (A_{\max}) per unit area (Hirose *et al.*, 1988; Schieving *et al.*, 1992; Pons *et al.*, 1993; Rousseaux *et al.*, 1996; Anten *et al.*, 1998; Pons and Jordi, 1998; Löttscher *et al.*, 2003). Plants are also plastic in the partitioning of resources within their leaves between two main functions of the photosynthetic apparatus, capacity and photon absorption (Anderson *et al.*, 1995; Hikosaka, 1996; Murchie and Horton, 1998). Adjustment to canopy density of between-leaf partitioning of resources for photosynthetic capacity and within-leaf partitioning of photosynthetic resources in chloroplasts can both contribute to efficient utilization of these resources for whole-plant photosynthesis (Evans, 1993b; Anten *et al.*, 2000; Pons and Anten, 2004). The question addressed here is what environmental signals are involved in the perception of canopy density with respect to both forms of partitioning?

The light gradient in canopies has a quantitative and a qualitative component. The total irradiance and the red : far-red ratio (R : FR) decrease with canopy depth (Holmes and Smith, 1977; Barkman and Stoutjesdijk, 1992). Plants can potentially use both components as a signal for canopy density. Multiple signalling pathways may be involved in

the response to a single environmental factor. A relevant example is the morphological shade avoidance response in canopies where several photoreceptors act as irradiance or spectral quality detectors (Ballaré, 1999; Gyula *et al.*, 2003; Pierik *et al.*, 2004). It is likely that there are also multiple signalling pathways involved in density effects on resource partitioning because these can be induced by spectrally neutral irradiance gradients and spectral quality gradients independent of each other (Barreiro *et al.*, 1992; Pons *et al.*, 1993; Hikosaka *et al.*, 1994; Rousseaux *et al.*, 1996; Vos and van der Putten, 2001; Frak *et al.*, 2001).

Perception of variation in R : FR in leaf canopies occurs through phytochrome. The involvement of this family of photoreceptors in morphogenetic shade responses is well known (Smith and Whitelam, 1997; Ballaré, 1999), but it has received relatively little attention as a possible signal involved in resource partitioning in canopies. However, processes that are relevant in this study, allocation, photosynthetic protein synthesis and particularly leaf senescence can also be under phytochrome control (Stoddart and Thomas, 1982; Rousseaux *et al.*, 1997; Kappers *et al.*, 1998; Pons and Jordi, 1998). It has been shown for annual dicots that the R : FR in the canopy light gradient is indeed involved in between-leaf photosynthetic resource partitioning and in the induction of senescence in lower leaves (Guaiamet *et al.*, 1989; Barreiro, 1992; Rousseaux *et al.*, 1996, 1997, 2000). However, this leaves the possibility open that the importance of spectral signal may be different

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for other growth forms. Most experiments dealing with R : FR effects on plants have been carried out not with light gradients, but with a homogeneous distribution of light over a plant. Several species showed a decrease in A_{\max} and the chlorophyll *a/b* ratio in homogeneously low R : FR, but others did not respond (Corré, 1983; Barreiro *et al.*, 1992; Smith *et al.*, 1993; Tinoco-Ojanguren and Percy, 1995; Walter and Horton, 1995; Murchie and Horton, 1998). These species-specific R : FR effects on photosynthetic parameters indicate that the spectral gradient in canopies may be used differently by species for density perception with respect to photosynthetic resource partitioning.

Further evidence of possible species-specific differences in the relative importance of the R : FR gradient in canopies was obtained from the comparison of N_{LA} and A_{\max} distributions in canopies and in experimental irradiance gradients. *Lysimachia vulgaris*, *Phaseolus vulgaris* and *Carex acutiformis* showed strong effects in both conditions (Hirose *et al.*, 1988; Schieving *et al.*, 1992; Pons *et al.*, 1993; Pons and Bergkotte, 1996; Pons and Jordi, 1998; Pons *et al.*, 2001), which suggests that the R : FR gradient would not be important in these species. However, canopy gradients in *Brachypodium pinnatum* and *Ficus benjamina* could not be satisfactorily explained from responses to spectrally neutral effects, which lead to the hypothesis that the R : FR component would be more important for density perception in these species (Pons *et al.*, 1993; Pons and Jordi, 1998).

This study was an investigation into the relative importance of the R : FR gradient as a signal for the partitioning of photosynthetic resources in canopies compared with the irradiance gradient. The above-mentioned five species were included for an evaluation of species-specific variation. Two levels of resource partitioning were investigated – the between-leaf re-allocation from shaded parts and the within-leaf partitioning between photosynthetic functions. Fully expanded leaves were shaded with a tobacco leaf or with a spectrally neutral filter in daylight conditions in a glasshouse. Leaves were also irradiated with supplemental FR in a growth chamber. Between-leaf resource partitioning was evaluated on the basis of measurements of leaf nitrogen and A_{\max} , and within-leaf partitioning between photosynthetic functions on the basis of A_{\max} per unit chlorophyll and chlorophyll *a/b* ratio. The species that were included have widely different growth habit and life history: two herbaceous dicotyledons (*Phaseolus* and *Lysimachia*), two monocotyledons (*Brachypodium* and *Carex*) and the evergreen woody climber *Hedera helix* was added so that together with the hemi-epiphytic tree *Ficus*, there were two evergreen woody species.

MATERIALS AND METHODS

Plant material

A climbing bean cultivar *Phaseolus vulgaris* L. ‘Mechelse markt’ was grown from seed and used for the experiment at 2 weeks after planting. The other species were propagated vegetatively and used for the experiments after several months of growth in a glasshouse until a suitable vegetative

growth stage was achieved (see below). *Lysimachia vulgaris* L. was grown from over-wintering rhizomes. The evergreen woody climber *Hedera helix* L. was grown from cuttings obtained from the Botanical Garden of Utrecht University. Small plants of the tropical rainforest hemi-epiphyte *Ficus benjamina* L. ‘Exotica’ were supplied by a commercial grower. The monocotyledonous *Carex acutiformis* Ehrh. and *Brachypodium pinnatum* L. were grown from cuttings taken from fens north of Utrecht and chalk grassland in South Limburg, respectively. The plants were grown in the glasshouse in 1-L pots with potting soil and 3 g of slow release complete fertilizer (Osmocote plus; release time of 3–4 months).

The experiments

The light gradient in canopies is best simulated in quantitative as well as qualitative terms with green leaves in daylight. Two experiments were carried out in a glasshouse in natural daylight. There was no supplemental lighting, light from other compartments of the glasshouse was kept out by black curtains and full sunlight was admitted. Air temperature was thermostatically regulated at 20 °C during the day and 16 °C at night. This was the actual temperature during cloudy days, but it increased during warm sunny days up to 26 °C. Relative humidity was kept at 70 % by means of water dispersers. About 40 % of diffuse daylight was transmitted to the plants, but peak irradiances in direct sunlight (up to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were about 85 % from outside. Attached tobacco leaves were used to shade single leaves of the experimental plants, and they were compared with the effect of a spectrally neutral filter of the same transmission of photosynthetically active radiation (PAR).

In the first experiment carried out in May and June 2001 effects of shading single leaves were compared in the six species. The entire leaf was shaded, except in the case of *Carex*, where shading was applied to the upper 25 cm of the 40–50 cm long leaf blade. All leaves were removed from the tobacco plants except the one used for the shade treatment. The tobacco leaves were placed on top of the horizontally held target leaf of the experimental plants. Occasionally damaged or senescing tobacco leaves were replaced by fresh ones. The spectrally neutral shading device consisted of two layers of white paper of which the lower one was printed a shade of grey that gave the combination the same transmission as the tobacco leaf (7 % of PAR). The target leaves of the experimental plants, the covering tobacco leaves and the neutral shading screens were supported by wires that kept them in a more or less horizontal orientation. The use of white reflective paper resulted in similar leaf temperatures in direct sunlight of the shaded and unshaded leaves, which was about 4 °C above air temperature. Leaf temperature under the tobacco leaf was also similar. Daylight in the glasshouse had an R : FR photon ratio (R = 655–665 nm; FR = 725–735 nm) of 1.05 under overcast conditions as measured with a spectroradiometer (Licor LI-1800). Light under the neutral filter was not significantly different and light transmitted through the tobacco leaves had a R : FR

of about 0.08. Target leaves also received some reflected light of high R : FR from below, but that was not evaluated. Three similar branches, shoots or tillers per plant were selected for the three treatments. Each treatment was applied to a just fully expanded leaf on a shoot. This scheme was applied to all species except *Phaseolus*. One treatment was applied per plant on one of the pair of primary leaves of that species. The treatment duration was 1 week for *Phaseolus* and 2 weeks for the other species as in earlier experiments (Pons and Bergkotte, 1996; Pons and Jordi, 1998; Pons *et al.*, 2001). There were six replications for all species. After the treatment, A_{\max} , area, dry mass, nitrogen concentration and chlorophyll were measured.

The experiment on spectral leaf shading effects on induction of senescence of primary *Phaseolus* leaves was carried out 1 year later in a way similar to the previous one. A mosquito screen was fixed underneath the spectrally neutral and leaf-shade devices in a way that the target *Phaseolus* leaf could easily be removed for non-destructive chlorophyll measurements. The treatment was maintained until abscission and withering of the shaded leaves.

The experiment on the effect of supplemental FR was carried out in a growth cabinet at a PFD of $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by a mixture of fluorescent and incandescent lamps. Plants were grown in the glasshouse and taken to the cabinet at least 2 weeks in advance, except *Phaseolus* that was fully grown in the cabinet. FR was irradiated from below on the abaxial side of the horizontal leaf. The FR source was made of a halogen narrow beam spotlight with aluminium reflector (Philips; Halotone B15d, 20 W) with a cut-off filter transmitting $\lambda > 715 \text{ nm}$ (Schott, RG-715) above it. Increase in leaf temperature ($<1^\circ\text{C}$) was prevented by putting a 7-cm water filter between lamp and filter, which blocked the longer wavelengths. Target leaves, including controls, were clamped between two circular wires and the FR spot of about 3 cm diameter was focused on the target leaf from below. FR (725–735 nm) intensity was $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ as measured with a spectroradiometer. The R : FR of growth cabinet light was 3–6. The combination of the two light sources resulted in an R : FR of 0.05 at the spot of the leaf, combining the white light from above and the FR from below. Treatment durations and number of replications were the same as in the leaf shading experiment. After the treatment, measurements were taken of A_{\max} , dry mass, nitrogen concentration and chlorophyll of the irradiated part of the leaves and control leaves that were clamped but not irradiated.

Leaf analysis and gas exchange

The light and CO_2 saturated rate of photosynthesis (A_{\max}) was determined at 120 Pa partial pressure of CO_2 in air at a PFD of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. The leaves were clamped in a Parkinson leaf chamber with a circular window of 18 mm diameter. An infra-red gas analyser (Licor LI-6262) was used to measure the difference in CO_2 and H_2O partial pressure. Further measurement conditions were: leaf temperature 25°C and a leaf-to-air vapour pressure difference of about 1 kPa. Other details of the set-up were described by Pons and Welschen (2002).

Chlorophyll was determined spectrophotometrically after extraction of fresh leaf material with dimethylformamide (Inskeep and Bloom, 1985). Non-destructive chlorophyll measurements were carried out with a chlorophyll meter (SPAD-502, Minolta) that was calibrated with destructive samplings. Dried and homogenized leaf material was analysed for nitrogen with an elemental analyser (Carlo Erba, Model EA NA 1110, Milan, Italy). Nitrate was analysed in the same material using salicylic acid as a reagent (Cataldo *et al.*, 1975). Nitrate concentrations were subtracted from total nitrogen in the case of *Phaseolus*, *Ficus* and *Carex* that had up to 10 atom% nitrogen as nitrate. The other species had insignificant amounts of nitrate.

RESULTS

Leaf shade

The spectrally neutral shading of a leaf in the glasshouse reduced nitrogen per leaf area (N_{LA}) and photosynthetic capacity (A_{\max}) relative to unshaded control leaves by 33 % and 49 %, respectively, as an average across all species (Fig. 1). Shading other leaves on the same plant by a tobacco leaf with the same transmittance for PAR had the same effect as the neutral filter on these parameters in all species (Fig. 1). This suggests that the spectral quality of shade light is not important for between-leaf resource partitioning. The shading treatments also caused a reduction in leaf mass per area (LMA) of 26 %, which contributed substantially to the effect on N_{LA} (Fig. 1). Nitrogen per leaf mass (N_{LM}), the other factor contributing to variation in N_{LA} was also reduced somewhat (average 9 %) by the shading treatments in most species except *Hedera* and *Carex*.

Chlorophyll per leaf area was not reduced by neutral shading in all species except *Phaseolus*, and leaf shading caused small additional reductions in *Lysimachia* and *Hedera* only. The strong effects on A_{\max} combined with small or absent effects on chlorophyll resulted in strong effects of shading on A_{\max} per unit chlorophyll (A_{\max}/chl) in all species, but spectral effects were also absent (Fig. 1). This was different for the chlorophyll *a/b* ratio, because a shift in favour of chlorophyll b occurred in response to the spectral shade of a tobacco leaf, in addition to the decreased in chlorophyll *a/b* observed in neutral shade in four of the six species (Fig. 1). There were thus spectral leaf shade effects on one of the two within-leaf partitioning parameters.

Phaseolus showed a tendency of a spectral effect on between-leaf partitioning in the treatment period of 1 week, but that was not statistically significant (Fig. 1). A period of 2 weeks, as in the other herbaceous species, would have resulted in senescence of the primary leaves and thus have prohibited the estimation of all partitioning parameters. However, the sensitivity of these leaves for induction of senescence by shade makes them suitable for investigating spectral effects on that process. The shading devices were kept over one of the primary leaves until full senescence. Abscission of the leaves took on average 22 d in

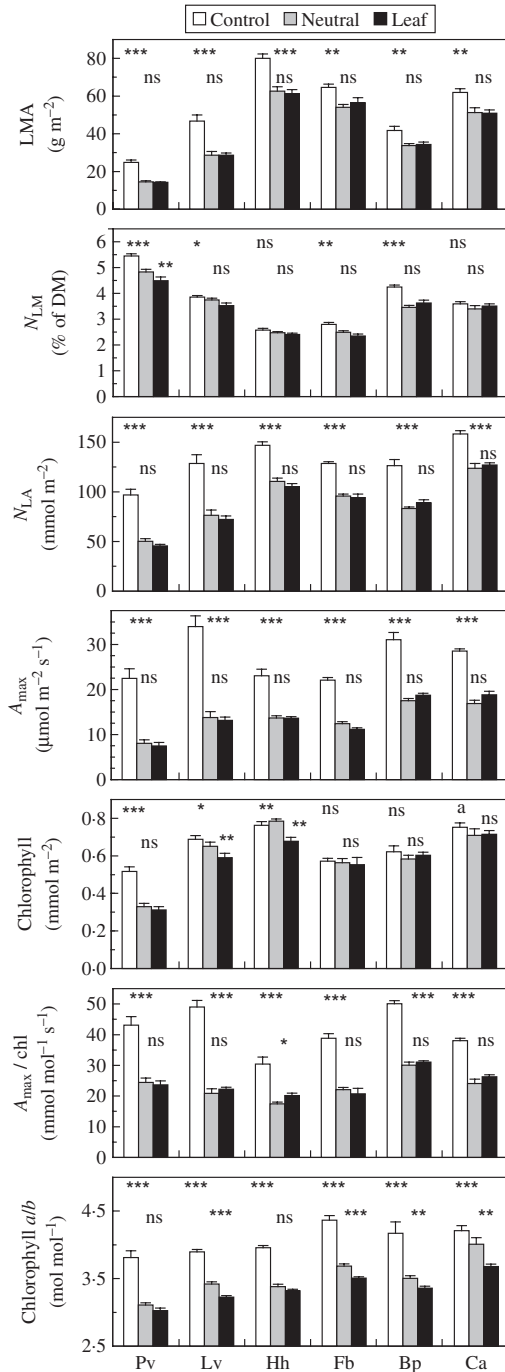


FIG. 1. The effect of shading one leaf by a spectrally neutral filter or a tobacco leaf on parameters pertaining to between-leaf resource partitioning and within-leaf chloroplast organization. Both shading devices had the same PAR transmittance. Six species were compared: *Phaseolus vulgaris* (Pv), *Lysimachia vulgaris* (Lv), *Hedera helix* (Hh), *Ficus benjamina* (Fb), *Brachypodium pinnatum* (Bp) and *Carex acutiformis* (Ca). Parameters (means \pm s.e.) are: leaf mass per area (LMA), nitrogen per leaf mass (N_{LM}), nitrogen per leaf area (N_{LA}), photosynthetic capacity per area (A_{max}) and per chlorophyll (A_{max}/chl), total chlorophyll per area and chlorophyll a/b ratio. The upper row of statistical notations refers to the results of a one-way ANOVA on the three treatment means per species. The lower row refers to the significance of the differences between the two shading treatments based on a Student's t -test performed on data expressed relative to unshaded control leaves on the same plant. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s., not significant.

the case of neutral shade, which was significantly accelerated to 17 d by spectral leaf shade (Table 1). The spectral effect only became evident after 1 week when chlorophyll declined in the senescence phase and went more rapidly under leaf shade (Fig. 2). Ultimate re-allocation of resources from senescing leaves was not differently affected as LMA, N_{LM} and N_{LA} of senesced leaves were the same for the two shading treatments (Table 1).

Supplemental FR

The set-up of the leaf shade experiment implies a large effect of reduction in irradiance, which may have masked a possible separate effect of low R : FR. It may thus have resulted in underestimation of the importance of the spectral component of the canopy light gradient for resource partitioning. A strong beam of supplemental FR in the growth light conditions incident on the abaxial side of the leaves over a sufficiently large area that allowed photosynthesis measurement was used to reduce R : FR independent of PAR. This treatment reduced N_{LA} and/or A_{max} in all species except *Carex* by an average of 15 % and 21 %, respectively (Fig. 3). Chlorophyll was similarly reduced, hence, A_{max}/chl remained constant (Fig. 3). The chlorophyll a/b ratio was also reduced under low R : FR from on average 4.2 to 3.7 (Fig. 3). This indicates that at constant PAR between-leaf resource partitioning was altered by a low R : FR in most species but not in *Carex*. Aspects of chloroplast organization had changed in all species including *Carex* as is evident from chlorophyll a/b , but not the within-leaf partitioning between light harvesting and photosynthetic capacity.

DISCUSSION

Between-leaf resource partitioning

The large effect of a reduction of irradiance incident on one leaf of a plant irrespective of the use of either a spectrally neutral screen or a leaf as a shading device (Fig. 1) suggests that the irradiance gradient in canopies is the dominant environmental signal used by all six species for neighbour detection with respect to between-leaf resource partitioning. That is consistent with the many experiments in which canopy light gradients were experimentally simulated in a spectrally neutral manner (Pons *et al.*, 1993; Hikosaka *et al.*, 1994; Pons and Percy, 1994; Pons and Jordi, 1998). However, the way the experiment on spectral leaf shading was carried out includes inevitably that irradiance is the main experimental factor and that the spectral effect is added as a secondary factor. This may have precluded the expression of a spectral effect superimposed on the already large irradiance effect, because these two factors may drive parallel signalling pathways and one may evoke the full response on its own. To further investigate the possible involvement of the spectral component as a separate signal perceived by phytochrome in canopy density responses, the R : FR was reduced with supplemental FR at moderate irradiance. This treatment affects only phytochrome, and not other possible signalling pathways that are associated with irradiance such as UV and blue light photoreceptors,

TABLE 1. Effect of shading one leaf of the pair of primary *Phaseolus vulgaris* leaves by a tobacco leaf and a spectrally neutral filter with the same PAR transmittance

| | Initial | No shade | Neutral shade | Leaf shade | Spectral effect |
|-----------------------------------|-------------|-------------|---------------|-------------|-----------------|
| Days until abscission | — | — | 22 (1) | 17 (1) | * |
| LMA (g m^{-2}) | 14.3 (0.3) | 23.8 (0.4) | 10.2 (0.4) | 9.9 (0.2) | n.s. |
| N_{LM} (% of DM) | 6.13 (0.05) | 3.20 (0.12) | 1.90 (0.08) | 1.95 (0.09) | n.s. |
| N_{LA} (mmol m^{-2}) | 62.8 (1.3) | 54.3 (2.4) | 13.8 (0.8) | 13.8 (0.8) | n.s. |
| Leaf area (cm^2) | 74.4 (6.3) | 96.0 (6.0) | 74.2 (4.1) | 72.4 (3.4) | n.s. |

Leaf dry mass per area (LMA) and nitrogen per leaf dry mass (N_{LM}) of shaded leaves were determined after abscission.

Leaf area was measured at 12 d after the start of the treatments and used for the calculation of nitrogen per leaf area (N_{LA}). Control leaves on separate plants were harvested at 21 d after start of treatment.

Differences between means of the two shading treatments were tested with a *t*-test, for statistical notation see legend to Fig. 1.

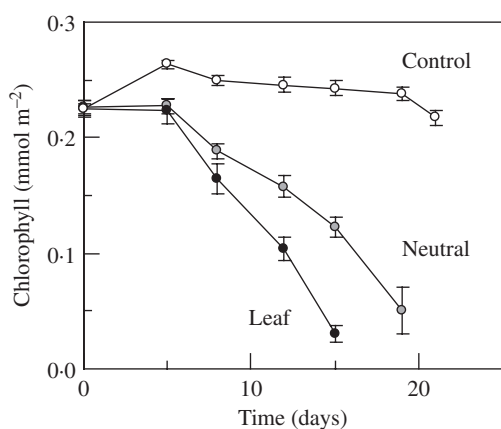


FIG. 2. Effect of shading one leaf of the pair of primary *Phaseolus vulgaris* leaves by a tobacco leaf and a spectrally neutral screen with the same PAR transmittance on the time course of chlorophyll per area (means \pm s.e.). Chlorophyll was measured non-destructively on the same leaves until abscission.

transpiration rate and photosynthetic activity (Ono *et al.*, 2001; Pons *et al.*, 2001). The possible temperature effect of the increased total irradiance was ruled out. Supplemental FR reduced N_{LA} and A_{max} in most species (Fig. 3), but the effect was smaller compared with the effect of irradiance reduction alone (Fig. 1). Hence, it is concluded that in five out of the six species, there are at least two signalling pathways involved in the perception of canopy density with respect to between-leaf resource partitioning, but that the spectral component of the light gradient is less important than the irradiance component.

The above conclusion pertains to the first week (*Phaseolus*) or 2 weeks (other species) of shading. However, a clear effect of spectral leaf shade in between-leaf resource partitioning appeared in addition to the irradiance effect in the experiment that was taken to full senescence of primary *Phaseolus* leaves. Although spectral effects were not yet clear after 1 week, later on induction of senescence was accelerated under a leaf compared with a neutral filter (Fig. 2 and Table 1). Spectral leaf shade effects might thus also have emerged in the other species when the duration of the treatment would have been taken to the senescence

phase. However, that would take much longer than with *Phaseolus*, particularly in the evergreens. Effects of manipulative changes of R : FR incident on single leaves were also most evident towards senescence in *Glycine max* (Guiamét *et al.*, 1989) and *Helianthus annuus* (Rousseaux *et al.*, 1996, 2000). Results of the many studies on irradiance and spectral treatments of whole plants may be less relevant with respect to whole-plant resource partitioning in canopy gradients because source–sink relationships are differently affected. Leaf senescence was indeed not induced in whole-plant shading, whereas it was induced in single-leaf shading or darkening (Pons and Pearcy, 1994; Weaver and Amasino, 2001). The data on *Glycine* and *Helianthus* of Guiamét *et al.* (1989) and Rousseaux *et al.* (1996, 2000), together with the present results for *Phaseolus*, suggest that, at least in annual dicots, a spectral effect on phytochrome is not so much involved in early responses of resource reallocation to canopy density, but more towards the later stages when leaf senescence is induced. This contrasts with the role of phytochrome in early neighbour detection with respect to morphological shade avoidance in herbaceous plants (Morgan *et al.*, 1980; Ballaré *et al.*, 1990; Ballaré, 1999). The situation may be somewhat different in woody plants, because senescence was not induced even after almost 3 months of exposure to a low R : FR in *Juglans* trees (Frak *et al.*, 2001). Nevertheless, spectrally modified daylight reduced N_{LA} and A_{max} after that treatment period in *Juglans*, whereas this was not the case with the woody *Hedera* and *Ficus* in our experiments of shorter duration (Fig. 1). This suggests that phytochrome may also become progressively more important for neighbour detection after canopy closure with respect to between-leaf resource partitioning in woody plants without the induction of leaf senescence.

In contrast to the other species, *Carex acutiformis* has erect leaves that span the whole depth of the canopy in the vegetative phase. The term ‘between-leaf’ partitioning may thus be confusing, because the partitioning of resources for photosynthetic capacity is between parts of leaves in different canopy positions rather than between leaves. Still that term is maintained for this form of partitioning. *Carex* did not show a reduction in N_{LA} , A_{max} or chlorophyll in the experiment with supplemental FR (Fig. 3) and also not in a preliminary experiment (data not shown). The lower part in

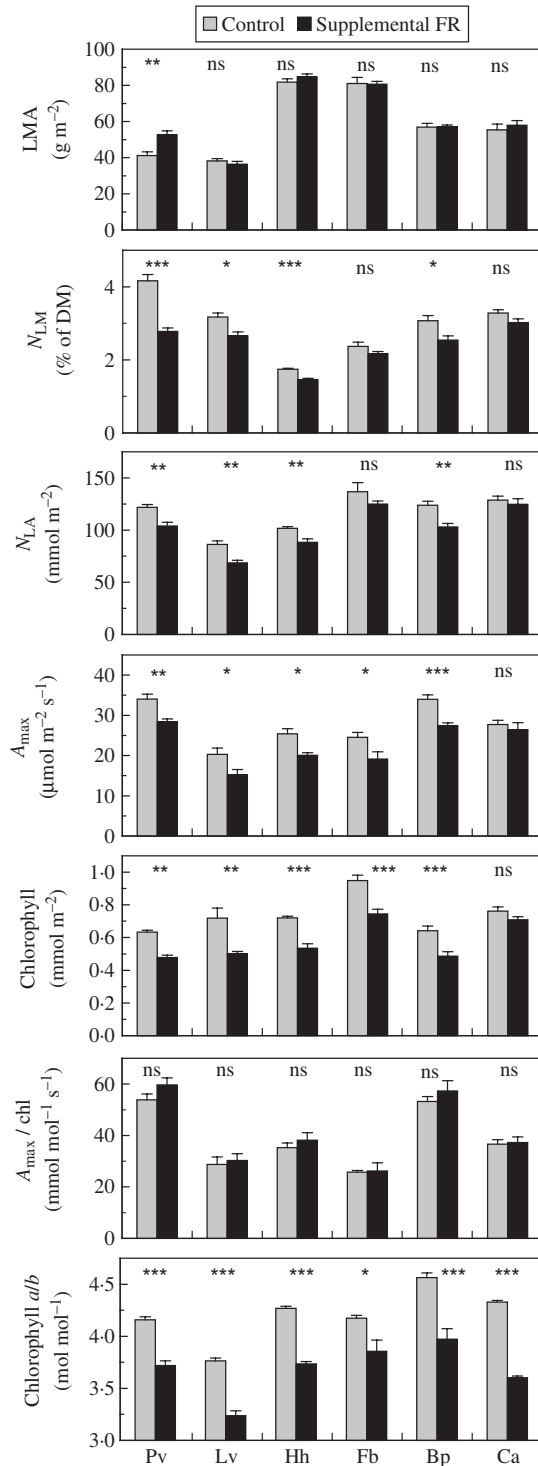


FIG. 3. The effect of supplemental FR applied on a 3-cm-diameter spot of a leaf on parameters pertaining to between-leaf resource partitioning and within-leaf chloroplast organization. Six species were compared: *Phaseolus vulgaris* (Pv), *Lysimachia vulgaris* (Lv), *Hedera helix* (Hh), *Ficus benjamina* (Fb), *Brachypodium pinnatum* (Bp) and *Carex acutiformis* (Ca). Parameters (means \pm s.e.) are: leaf mass per area (LMA), nitrogen per leaf mass (N_{LM}), nitrogen per leaf area (N_{LA}), photosynthetic capacity per area (A_{max}) and per chlorophyll (A_{max}/chl), total chlorophyll per area and chlorophyll *a/b* ratio. Differences between means were tested for significance with a *t*-test, for notation see legend to Fig. 1.

low light of the erect leaves that span the whole light gradient supports the upper part (Hirose *et al.*, 1989; Schieving *et al.*, 1992; Pons *et al.*, 1993). That lower part has thus not only a photosynthetic function, but also a support function for the upper part that may be jeopardized when senescence is induced there. It is therefore possible that plants of this growth form lack a low R : FR-induced senescence altogether.

Within-leaf partitioning between photosynthetic functions

The chlorophyll *a/b* ratio was clearly reduced as a result of a lower irradiance in all species (Fig. 1). This phenomenon was consistently found in many other studies and is ascribed to the relative increase of the chlorophyll *b*-containing light-harvesting complex II (LHCII), which is associated with the decrease in A_{max}/chl (Anderson *et al.*, 1995). A low R : FR further reduced the chlorophyll *a/b* ratio, although not significantly in all species in response to spectral leaf shade (Fig. 1), but clearly in all species under supplemental FR (Fig. 3). This phenomenon is found in many studies and is ascribed to an increase in the PSII : PSI ratio and thus in relative abundance of LCHII (Chow *et al.*, 1990a; Anderson *et al.*, 1995; Tinoco-Ojanguren and Percy, 1995; Walters and Horton, 1995; Murchie and Horton, 1998). The imbalance in excitation of the two photosystems as a result of the FR absorption by PSI is re-established by this altered stoichiometry of the photosystems, which maximizes quantum yield (Chow *et al.*, 1990b). The low chlorophyll *a/b* ratio in the lower ranges of leaf canopies will thus be the combined result of the reduced irradiance and the lower R : FR.

The strong effect of reduced irradiance incident on a leaf on A_{max}/chl (Fig. 1) indicates that the partitioning between the capacity and light-harvesting functions within the photosynthetic apparatus acclimated to the local light conditions. This was true for all species and is consistent with the results of many earlier studies (Terashima and Evans, 1988; Evans, 1989; Anderson *et al.*, 1995; Hikosaka, 1996; Murchie and Horton, 1998). In most of these studies, the light treatment was applied to whole plants instead of parts of their foliage as in the present experiments, but there are no arguments to suppose different effects on chloroplast organization (Pons and Percy, 1994). The acclimation of stoichiometry between PSI and PSII to a low R : FR that caused the decrease in the chlorophyll *a/b* ratio is, however, not necessarily accompanied by an altered A_{max}/chl . Spectral leaf shade and supplemental FR had, indeed, contrary to irradiance, no significant effects on A_{max}/chl in all species except *Hedera*, where it caused a small increase in the leaf shade treatment only (Figs 1 and 3). Absence of an effect of R : FR on this parameter was also found in three tropical trees (Tinoco-Ojanguren and Percy, 1995), increases and decreases in A_{max}/chl were found in four wild herbaceous species (Murchie and Horton, 1998) and it also increased under low R : FR in *Pisum sativum* (Chow *et al.*, 1990a). Effects of R : FR were thus absent or small in these studies. Similar ranges in A_{max}/chl were found in canopies compared with spectrally neutral gradients (Percy and Seemann, 1990; Burkey and Wells, 1991; Evans,

1993a; Pons and Jordi, 1998). On the basis of these literature data and the present results, it is concluded that the partitioning between photosynthetic functions within leaves acclimates mainly to the local irradiance and that the spectral component is either not involved or insignificant.

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