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RESEARCH PAPER

Effects of elevated CO_2 on intra-specific competition in *Sinapis alba*: an examination of the role of growth responses to red:far-red ratio

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Keywords
Asymmetric competition; elevated CO_2; light quality; red:far-red ratio; shade avoidance; size variability; stand structure.

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ABSTRACT

Response of plants to elevated CO_2 differs markedly between individually- and competitively-grown plants, both in terms of mean size and variation about the mean. Using *Sinapis alba*, we explored whether these contrasting effects are a consequence of the effect of competition on the red:far red (R:FR) light ratio. Plants were grown at both ambient and elevated (700 $\mu l \cdot l^{-1}$) CO_2 in competitive stands, and as individuals at either a low (0.7) or high (1.25) R:FR ratio at a constant photosynthetic photon fluence rate. Elevated CO_2 increased stand biomass by enhancing the growth of canopy dominants, but not the subordinates. As a consequence, elevated CO_2 increased the coefficient of variation in size within the stands. Elevated CO_2 did not enhance the growth of individually-grown plants at the low R:FR ratio, but did at the high R:FR ratio. Both the poor response of subordinate plants to elevated CO_2 and the increased size inequalities of individuals within the stand can be explained in terms of the effect of the R:FR ratio on CO_2 responsiveness. The effect of the R:FR ratio on CO_2 response may be related to its effect on allocation patterns and nutrient uptake.

INTRODUCTION

Significant increases in global atmospheric CO_2 concentration in the recent past, along with the predicted increases in atmospheric CO_2 concentration in the next 100 years have the potential to impact plant growth and physiology and, in turn, affect community structure and composition (Bazzaz 1990; Körner 2000; Ramseir *et al.* 2005). Elevated CO_2 directly influences plants by increasing photosynthetic rate and decreasing transpiration rate at the leaf level through changes in stomatal conductance (Bowes 1993). Elevated CO_2 also influences respiration, leaf morphology and anatomy, rate of senescence, branching patterns, stem elongation and time of flowering (Murray 1995; Reekie 1996; Ward & Strain 1999). However, the effects of CO_2 are highly variable, depending upon environmental conditions (*e.g.* nutrients, light, moisture and temperature), developmental stage and the species of interest (Bazzaz 1990; Ward & Strain 1999; Jablonski *et al.* 2002; Poorter & Navas 2003).

The responses of plants growing at elevated CO_2 in competition are often very different from plants grown as individuals (Thomas & Bazzaz 1993; Wayne & Bazzaz 1995; Poorter & Navas 2003). In general, growth responses to CO_2 tend to decrease as stand density increases. This change in CO_2 response is often attributed to increased limitation by light and nutrients as density increases (Bazzaz & McConnaughay 1992; Wayne & Bazzaz 1995). Although there is often a poor correlation between the response of individually-grown plants to elevated CO_2 and their response when grown in the presence of intra-specific competitors, the latter often correlates well with the CO_2 response of the same species when grown in multi-species mixtures (Poorter & Navas 2003), suggesting that it is competition *per se*, rather than the identity of the competitors that affects response to CO_2. In addition to affecting mean plant size, elevated CO_2 also has marked effects on size distribution within competitive stands. This in spite of the fact that elevated CO_2 has little effect on size distribution in individually-grown plants (Poorter & Navas 2003).

Elevated CO_2 has been shown to either increase or decrease size hierarchies within monospecific stands (Bazzaz & McConnaughay 1992; Morse & Bazzaz 1994; Wayne & Bazzaz 1997; Hikosaka *et al.* 2003; Nagashima *et al.* 2003). The reasons for these changes in size hierarchy and stand structure in elevated CO_2 are not entirely clear. Size hierarchies develop when competition is asymmetric, such that large individuals capture a disproportionate share of the resources. Asymmetric competition occurs when the resource for which the plants are competing is supplied from a single direction, such as light, whereas symmetric competition occurs when readily diffusible resources (*e.g.* water, soil nutrients) are accessible to all individuals in a stand in proportion to their size (Weiner 1990). As CO_2 is a readily diffusible resource, changes in the level of CO_2 are unlikely to have any direct effect on size hierarchy; rather, the effect is likely to be indirect through its impact upon the availability of other resources (Hikosaka *et al.* 2005). Elevated CO_2 may affect the availability of light, water and mineral nutrients through its effects on growth and stomatal conductance. However, only light availability is likely to impact size hierarchy within the stand, as both water and mineral nutrients are also readily diffusible resources. Further, decreases in light availability might explain why elevated CO_2 may increase size inequalities in a stand (Hikosaka *et al.* 2003), but cannot explain why elevated CO_2 would decrease size inequalities unless one assumes elevated CO_2 preferentially favours the growth of plants at low-light levels (Wayne & Bazzaz 1997), or that self-thinning of the smaller individuals has occurred (Morse & Bazzaz 1994).

An alternative explanation for contrasting effects of elevated CO_2 on individually- *versus* competitively-grown plants, and on size inequalities in stands is that the response to elevated CO_2 varies with the R:FR ratio of the light environment. Although it is well known that the response of plants to elevated CO_2 can vary substantially depending upon a number of different environmental factors (Poorter & Navas 2003), there is little information on the effect of R:FR ratio on CO_2 response (Arnone & Körner 1993; but see Hoddinott and Scott 1996a,b). Given that R:FR ratios within plant canopies are lowered by the selective removal of red light by chlorophyll (Smith 1982; Franklin & Whitelam 2005), this is a critical gap in our understanding of how and why the CO_2 response of plants grown in competition is so different from that of individually-grown plants. Further, if CO_2 response does vary with R:FR ratio, the CO_2 response of the canopy dominants would differ from that of the subordinates and this, in turn, would affect size inequalities within the canopy and stand structure.

Our objective in the present study was to determine whether the changes in R:FR ratio that occur in plant canopies can potentially explain the differential response of canopy dominants *versus* subordinates to elevated CO_2 and the effects this has on stand structure. To do this, we conducted two experiments with the shade-intolerant annual, *Sinapis alba* L. The first experiment grew plants in competitive arrays at ambient *versus* elevated CO_2 and examined the effects of CO_2 on stand biomass, leaf area index, R:FR ratio and size inequalities within the stand. The second experiment grew plants as individuals at one of two R:FR ratios at either ambient or elevated CO_2, and the interactive effects of CO_2 and R:FR ratio on growth over time were examined using functional growth analysis.

MATERIALS AND METHODS

Competition experiment

The first experiment was carried out in controlled environment glasshouse compartments at the K.C. Irving Environmental Science Center (Wolfville, NS) from May 25 to July 12 2004. Temperature, humidity and CO_2 composition of the air were maintained using a computerised control system (Argus Controls, White Rock, BC), with temperature set to mimic external conditions (±0.5 °C). External temperature was measured with a thermistor installed in a standard Stevenson screen at a height of 1.5 m above the ground surface. Relative humidity was maintained at levels no lower than 65% (using high-pressure fogging), and plants were exposed to the natural photoperiod and light levels. Carbon dioxide levels were monitored by an infrared gas analyser (Vaisala GMM1 1A) that sampled air once every 12 min, and adjusted CO_2 levels as needed by injecting pure CO_2 (Praxair, Mississauga, ON, USA). Four individual glasshouse compartments were used for the experiment; two replicate glasshouses for each of the two CO_2 treatments. The ambient CO_2 compartments were maintained at external conditions (approximately 370 $\mu l \cdot l^{-1}$), while the elevated CO_2 level was set at 700 $\mu l \cdot l^{-1}$.

Sinapis alba seeds were planted in 25-cm diameter (12.5-cm deep) Kord traditional round pans filled with ASB Greenworld Original Grower Mix soil (Pointe Sapin, NB). Seeds were evenly spaced using a planting grid to obtain a density of 632 plants$\cdot m^{-2}$ (31 plants$\cdot pot^{-1}$). Three seeds were planted per hole (thinned to one seedling per location 5 days post-emergence) and pots were randomly assigned to one of the four glasshouse compartments. Plants were watered as needed and given a fertilizer treatment on day 31, using Plant Prod Chelated Micronutrient Mix (Plant Products Co. Ltd., Brampton, ON) at a rate of 3 $g \cdot l^{-1}$ (2.1, 0.6, 0.12, 0.03, 0.39 and 0.018 ppm Fe, Mn, Zn, Cu, B and Mo, respectively). Once seedlings grew above the lip of the pot, 60% green shade cloth was attached around the circumference of the pot and raised daily with the top of the plant canopy to simulate shade from neighbouring plants outside the pot. Red:far-red ratios were measured at the base of the plant canopy in the centre of the pots on days 23, 34, 42 and 48 using a Skye SKR 110 R:FR sensor that measures the ratio of red to far-red quanta in a 30-nm bandwidth centred at 660 nm and a 20-nm bandwidth centred at 730 nm (Skye Instruments, Powys, UK).

One pot per treatment per replication was harvested on days 20 and 48. Only shoots were harvested due to the difficulty of separating roots of individual plants. Plants were dried to a constant weight at 55 °C before weighing.

Light quality experiment

The second experiment was carried out in four Conviron model E15 growth chambers (Controlled Environments Limited, Winnipeg, MB). There were four treatments, two R:FR ratios (0.7 *versus* 1.25), crossed with two CO_2 levels (ambient *versus* 700 $\mu l \cdot l^{-1}$) in a factorial treatment design. Each treatment was randomly assigned to one of the four chambers. There were two replicates of each of the four treatments in that the experiment was repeated over time. In the second replicate, treatments were assigned to different chambers from those used in the first replicate to avoid confounding possible chamber differences with treatment effects.

Temperature was set at 25 °C and humidity maintained between 60 and 80%. Atmospheric CO_2 levels were maintained at either ambient (*ca.* 370 $\mu l \cdot l^{-1}$), or at 700 $\mu l \cdot l^{-1}$ for the elevated CO_2 treatment. All plants were provided with an 18-h photoperiod with a photosynthetic photon fluence rate (PPFR) of 110 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over the waveband 400–700 nm. This light level was chosen to approximate the relatively low-light levels experienced by subordinate plants in a plant canopy. Light levels were checked weekly with a LI-189 Light Meter (Li-Cor Inc., Lincoln, NE) and the PPFR was maintained by adjusting the height of the light bank accordingly. Desired R:FR ratios were created by using different combinations of incandescent and fluorescent lights. The high R:FR ratio was produced using two Philips 160 W cool white fluorescent bulbs in combination with four Sylvania 100 W frosted incandescent bulbs, while low R:FR ratios were produced using 12 Sylvania 100 W frosted incandescent bulbs. R:FR ratios were measured with a Skye SKR 110 R:FR sensor (Skye Instruments, Powys, UK).

Sinapis alba seeds were planted in 10-cm square (10-cm deep) pots filled with Turface MVP (Applied Industrial Materials Corp., Deerfield, IL). Turface MVP is composed of compressed clay particles that come from Blue Mountain, Mississippi. Four seeds were planted per pot and thinned to one seedling per pot at emergence. Each treatment replication had 48 pots randomly assigned to one of four growth chambers. Plants were watered once a day with Plant Prod 20-20-20 (N-P-K) hydroponic fertiliser with micronutrients (Plant Products Co. Ltd., Brampton, ON) at concentrations of 100 ppm N ($0.5\ g \cdot l^{-1}$) for the first 11 days and 300 ppm N for the remainder of the experiment ($1.5\ g \cdot l^{-1}$ until day 20).

Harvests were carried out daily from day 5 (emergence) until day 20. Three plants were randomly selected and harvested from each treatment and replicate on each harvest day (*i.e.* six plants per treatment), and harvested plants had their roots washed from the Turface. Stem length and leaf area were measured as in the competition experiment. Relative chlorophyll content was assessed on freshly-harvested plants with a SPAD-502 Chlorophyll Meter (Minolta Camera Co. Ltd., Japan), using the newest leaf of each plant that was at least 1 cm in length. Three measurements were taken on each leaf and averaged. Relative chlorophyll measurements were converted to quantitative values using a standard curve (Equation: mg chlorophyll per $cm^2 = 1.3946 \times$ SPAD measurement-6.4857; $R^2 = 0.9316$) produced by correlating the SPAD measurements with quantitative spectrophotometric determinations of chlorophyll (Witham *et al.* 1986) for 30 *S. alba* leaves covering the range of experimental chlorophyll measurements. Plants were subsequently dried to a constant weight at a temperature of 55 °C. Dry weights of the root, leaf and stem tissues were measured to the nearest 0.00001 g for tissue harvested up to day 12, and to the nearest 0.0001 g for plants harvested after day 12.

Statistical analysis

As glasshouse compartments were the unit of replication for CO_2 treatments in the competition experiment, differences among treatments in stand biomass and the coefficient of variation for size were assessed using a one-way analysis of variance with two replicates. As biomass data for the individual plants had a log-normal distribution, data were transformed by taking the natural logarithm of the raw values prior to calculating the coefficient of variation for each stand. Separate analyses were carried out for plants harvested at the two different dates. A repeated measures analysis of variance was used to examine the effects of CO_2 level on R:FR within the stands using sampling days as the within-subject factor.

In the light quality experiment, analysis of covariance was used to fit quadratic regressions to the natural logarithm (ln) transformed dry weight and ln transformed leaf area data as a function of time. A preliminary analysis indicated that adding a cubic term to the regression models did not significantly improve the fit. To avoid pseudoreplication, the mean data from each growth chamber, rather than the data for individual plants, were used in this analysis. Level of CO_2 and R:FR were categorical factors in this analysis of covariance, and the impact of these treatments on the fitted relationships was determined by examining their interactions with the linear and quadratic terms in the statistical model. The resulting regressions for each of the four treatments were used to estimate relative growth rate (RGR), leaf area ratio (LAR) and unit leaf rate (ULR), along with their 95% confidence intervals following the procedures of Hunt & Parsons (1974).

A split plot factorial analysis of variance was used to examine the effect of CO_2, R:FR and time on specific leaf area (SLA), chlorophyll content and biomass allocation to the stem, root and leaf. Chambers were the main plots and individual plants were the sub-plots in this analysis. The main plot error term was used to test the significance

of the CO_2 and R:FR terms as well as their interaction, while the residual error term was used to test the effect of time and its interactions with CO_2 and R:FR.

The General Linear Model (GLM) procedure of the Statistical Analysis Software (SAS) program (Windows version 5.1., 1999–2001 by SAS Institute Inc., Cary, NC) was used for all analyses.

RESULTS

The initial harvest of the competitive array experiment was timed to coincide with canopy closure; *i.e.* bare ground was no longer visible when viewed from above. At this time, there were no differences between CO_2 levels in stand biomass or the coefficient of variation in plant size. Stand biomass was 2.53 g at low CO_2 compared to 3.45 ± 0.29 g at high CO_2 ($F = 5.13$, $P = 0.1518$). The corresponding values for the coefficient of variation in plant size were 15.8 and 15.9 ± 1.3 ($F = 0.01$, $P = 0.9497$). At the final harvest, which was timed to coincide with the end of the vegetative growth phase, stand biomass was greater at elevated (18.20 g) than at ambient (14.77 ± 0.28 g) CO_2 ($F = 75.15$, $P = 0.0130$). The coefficient of variation in individual plant size was also greater at elevated (92.9) than at ambient (76.2 ± 1.4) CO_2 ($F = 76.12$, $P = 0.0129$). The greater coefficient of variation at elevated CO_2 was due to an increase in size of the larger plants; the smaller plants at elevated CO_2 were similar in size to the smaller plants at ambient CO_2 (Fig. 1). The R:FR ratio at ground level did not differ among the four sampling days ($F = 1.09$, $P = 0.408$), or between CO_2 levels ($F = 2.16$, $P = 0.1803$), and there was no interaction between sampling day and CO_2 level ($F = 0.07$, $P = 0.9735$). The average R:FR ratio across sampling days and CO_2 levels was 0.48 ± 0.04.

Both level of CO_2 and R:FR ratio affected the pattern of leaf area and dry mass accumulation over time. This was seen in the significant interactions between these effects and either the linear or quadratic terms in the regressions of leaf area/dry mass with time (Fig. 2). There were also significant interactions between CO_2 and R:FR ratio for both leaf area and dry mass accumulation over time. The essence of these effects is that elevated CO_2 increased total biomass and leaf area at a high R:FR ratio in the latter part of the experiment, but had no effect at a

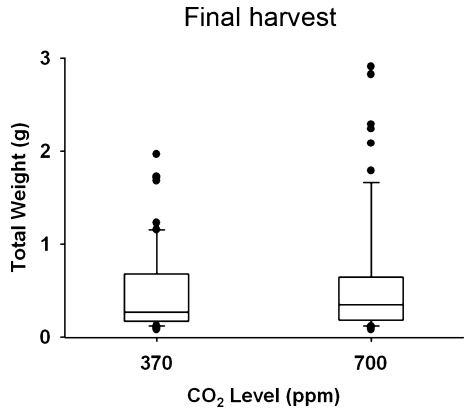

Fig. 1. Final size distribution of plants grown within competitive canopies in either low (370 $\mu l \cdot l^{-1}$) or high (700 $\mu l \cdot l^{-1}$) CO_2 conditions (n = 62 for each treatment). Each graph plots the 10th, 25th, 50th, 75th and 90th percentiles. Individual symbols represent outliers.

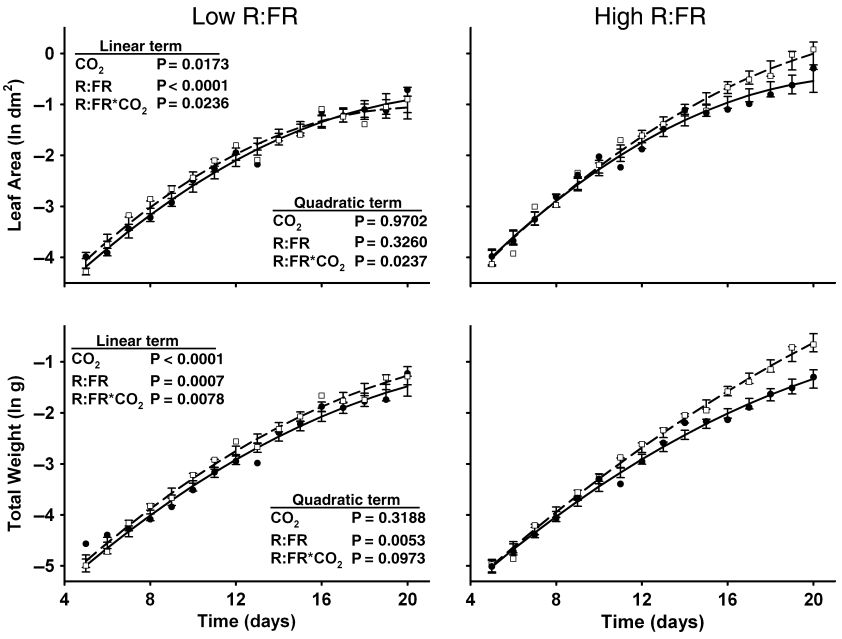

Fig. 2. Effect of CO_2 concentration on total weight and leaf area of individually-grown plants exposed to either a low (0.7) or high (1.25) R:FR ratio. Shaded circles and open squares represent the mean values for low (370 $\mu l \cdot l^{-1}$) and high (700 $\mu l \cdot l^{-1}$) CO_2 conditions respectively. The solid (low CO_2) and dashed (high CO_2) lines represent the quadratic relationships fitted to the raw data for growth analysis. Probability (P) values give the level of significance for the effect of R:FR ratio, CO_2 and their interaction on either the linear or quadratic term of the fitted relationship. Error bars represent the 95% confidence intervals for the predicted values.

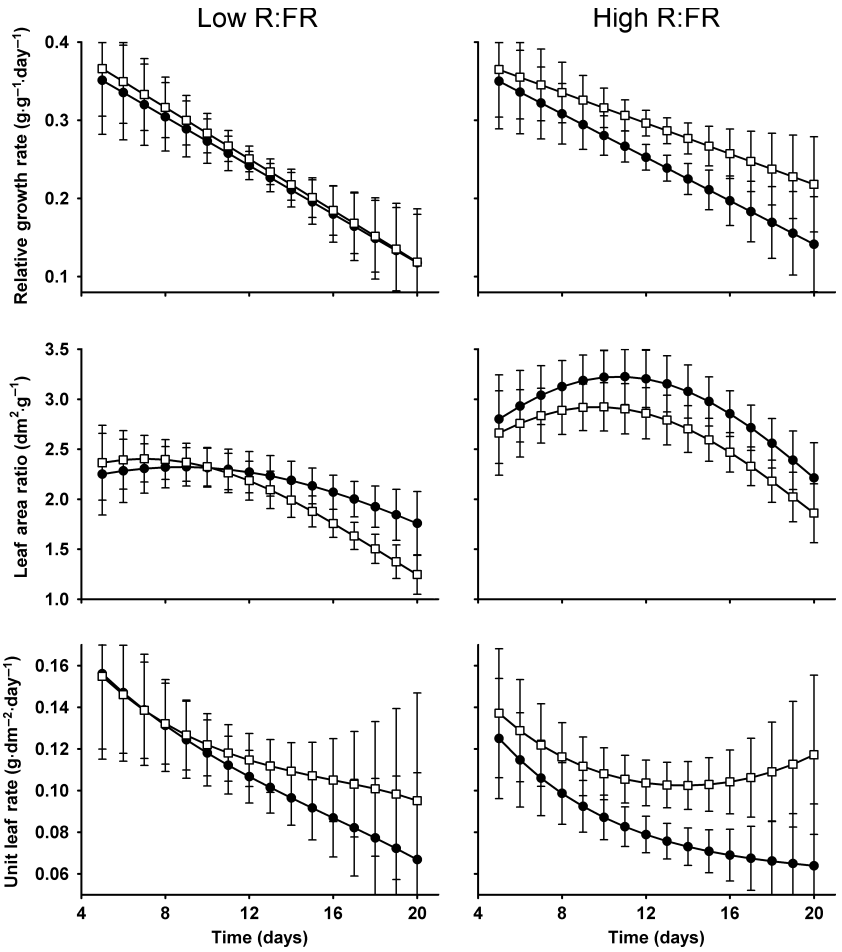

Fig. 3. Effect of CO_2 concentration on relative growth rate, leaf area ratio and unit leaf rate of individually-grown plants exposed to either a low (0.7) or high (1.25) R:FR ratio. Shaded circles and open squares represent low (370 $\mu l \cdot l^{-1}$) and high (700 $\mu l \cdot l^{-1}$) CO_2 conditions, respectively. Error bars represent the 95% confidence intervals.

low R:FR ratio (Fig. 2). Similarly, elevated CO_2 had no effect on RGR when plants were grown at a low R:FR ratio, but increased RGR from days 12 to 15 when plants were grown at a high R:FR ratio (Fig. 3). Elevated CO_2 had no effect on LAR at a high R:FR ratio, and depressed LAR from day 17 to 19 in the low R:FR treatment. In general, LAR was higher in the high R:FR treatment than it was in the low treatment. Elevated CO_2 had no significant effect on ULR in the low R:FR treatment and increased ULR from day 11 to day 17 in the high R:FR treatment.

A high R:FR ratio reduced biomass allocation to stems and increased allocation to leaves and roots (Fig. 4). The effects of R:FR ratio on stem and root allocation were more pronounced in the latter half of the experiment. In general, leaf allocation increased over time, while root allocation decreased. Level of CO_2 had no effect on allocation to leaves, stems or roots.

A high R:FR ratio decreased the height of the plants; this effect was more marked towards the end of the experiment when the plants were taller (Fig. 5). Although level of CO_2 had relatively little effect on height, there was some evidence that elevated CO_2 increased height marginally towards the end of the experiment. The R:FR ratio and level of CO_2 had relatively little effect on specific leaf area, but SLA was marginally lower at elevated CO_2. This weak effect was most noticeable in the latter half of the experiment in the low R:FR treatment. Specific leaf area in all treatments increased with time up to approximately day 9, then declined to day 15, after which it remained more or less constant. Chlorophyll content was increased by a high R:FR ratio, but was not affected by CO_2. Chlorophyll levels decreased steadily over time until day 12, then remained relatively constant.

DISCUSSION

Although elevated CO_2 increased stand biomass in the competitive arrays, this increase in biomass was not equally distributed among the various individuals in the stand. The larger individuals in the stand responded more positively to elevated CO_2 than the canopy subordinates, markedly increasing the size hierarchy within the canopy. One possible explanation for why the canopy subordinates did not respond as well as the canopy dominates to elevated CO_2 is that the environment experienced

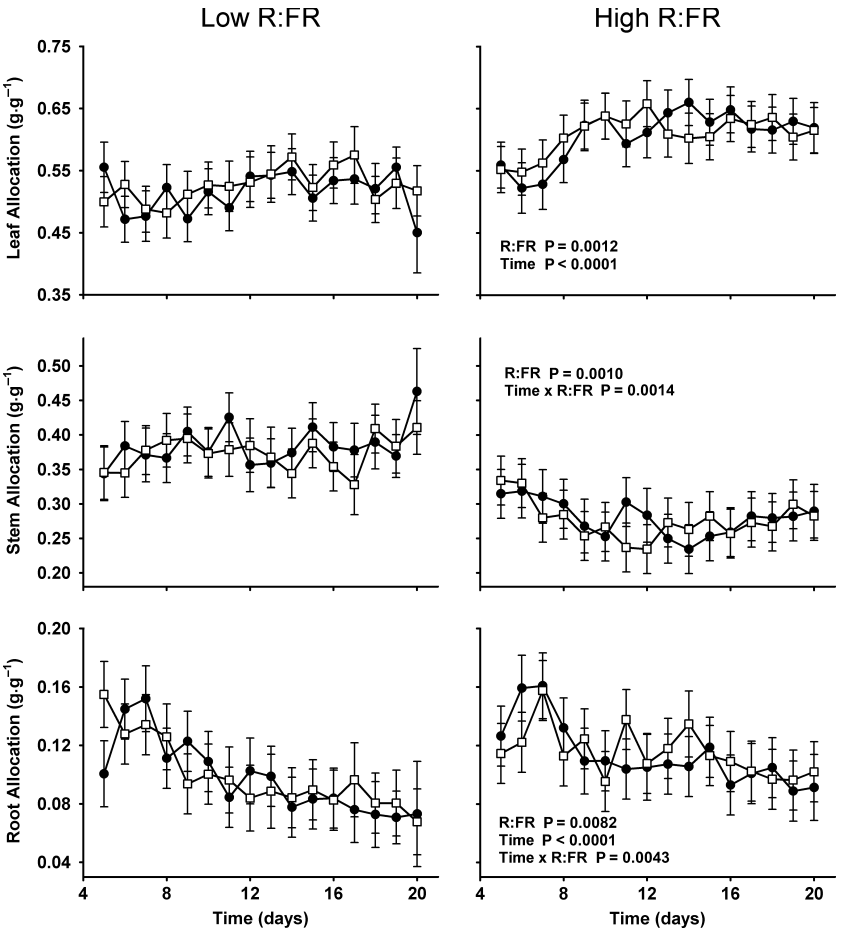

Fig. 4. Effect of CO_2 concentration on proportion of total biomass allocated to leaves, stems and roots in individually-grown plants exposed to either a low (0.7) or high (1.25) R:FR ratio. Shaded circles and open squares represent low (370 $\mu l \cdot l^{-1}$) and high (700 $\mu l \cdot l^{-1}$) CO_2 conditions, respectively. Probability (P) values give the level of significance for R:FR ratio, CO_2, time and their interactions. Only those effects that were significant at the 0.05 level are listed. Error bars represent the 95% confidence intervals.

by the dominant plants allows them to respond to increases in level of CO_2, while the environment of subordinate plants inhibits their response. In this respect, it should be noted that the mean R:FR ratio measured at the base of the canopy was 0.5, indicating that, depending upon position within the canopy, individual plants would experience a R:FR ratio anywhere between 0.5 and 1.2 (full sunlight).

The experiment with the individually-grown plants demonstrated that the capacity of *S. alba* to respond to elevated CO_2 was strongly influenced by light quality when plants were grown at the same irradiance. Plants grown at a low R:FR ratio, simulating light quality conditions of subordinate plants in a canopy, did not increase their growth in response to elevated CO_2. On the other hand, plants grown at high R:FR, simulating light quality conditions of dominant plants in a canopy, responded very positively to elevated CO_2. These results suggest that differences in R:FR ratio contribute, at least in part, to the contrasting responses of dominant *versus* subordinate plants to elevated CO_2 and the resulting increase in size inequalities in competitive arrays of *S. alba* when grown at elevated CO_2.

Growth analysis revealed that elevated CO_2 increased the relative growth rate of the solitary plants at a high R:FR ratio because of an increase in ULR rather than an increase in LAR. Leaf area ratio actually declined slightly at elevated CO_2, but this decline was more than compensated for by the increase in ULR in the high R:FR treatment. This response of ULR and LAR to elevated CO_2 is common in C3 plants (Poorter & Navas 2003). Increasing the level of atmospheric CO_2 enhances CO_2 uptake by the leaves and inhibits photorespiration, thus increasing ULR. The decrease in LAR is a consequence of the increased allocation of photosynthate to organs other than leaves, storage of carbohydrate within leaves and increases in leaf thickness (Poorter & Navas 2003). The reasons for the lack of any effect of elevated CO_2 on ULR at a low R:FR ratio however, needs some explanation.

The R:FR ratio affected both morphology and biomass allocation patterns in the individually-grown plants. The high R:FR ratio decreased shoot height, allowing for a shift in allocation from stems to leaves and roots. This increase in leaf allocation at high R:FR was responsible for the increase in LAR. These changes in morphology and allocation in response to R:FR are typical of plants adapted to relatively high-light levels (*i.e.* shade-avoiding plants) and allow plants growing in dense stands to compete for light more effectively through increases in height,

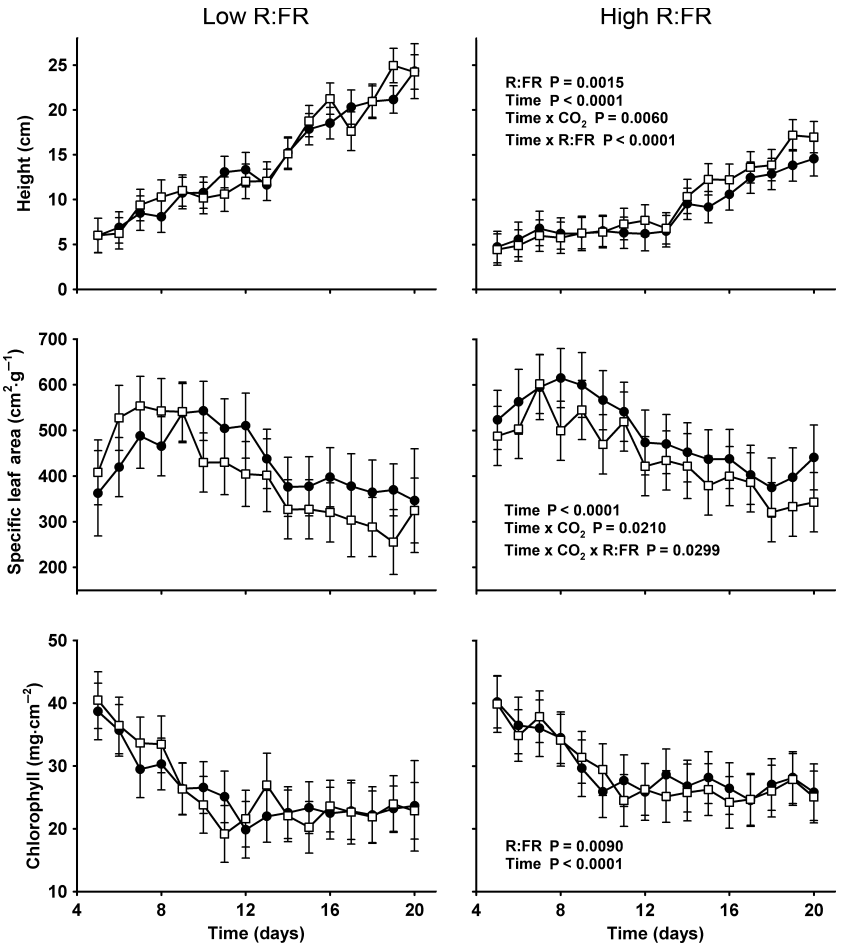

Fig. 5. Effect of CO_2 concentration on plant height, specific leaf area and chlorophyll content of individually-grown plants exposed to either a low (0.7) or high (1.25) R:FR ratio. Shaded circles and open squares represent low (370 $\mu l \cdot l^{-1}$) and high (700 $\mu l \cdot l^{-1}$) CO_2 conditions, respectively. Probability (P) values give the level of significance for R:FR ratio, CO_2, time and their interactions. Only those effects that were significant at the 0.05 level are listed. Error bars represent the 95% confidence intervals.

while minimising height growth (and the resources it requires) when plants do not have to compete for light (Smith 1982; Franklin & Whitelam 2005). It also means that when competing for light (*i.e.* under low R:FR conditions), low-root allocation may limit the capacity of the plants to exploit soil resources. In particular, it may limit the uptake of mineral nutrients such as nitrogen. This is seen in the present study in the low-chlorophyll content per unit leaf area at a low R:FR ratio. Chlorophyll content is a sensitive indicator of the nitrogen status of a leaf and suggests that plants growing at low R:FR were more limited by nitrogen than plants growing at high R:FR. Low-nutrient availability has been repeatedly shown to limit plant response to elevated CO_2 (see literature reviewed in Poorter & Perez-Soba 2001; Poorter & Navas 2003). Aside from other pollutants, it is the single most important environmental factor affecting the response of plants to elevated CO_2, more important than temperature and either water or light availability (Poorter & Navas 2003). Given that the extent to which a plant can increase the rate of CO_2 assimilation at elevated CO_2 concentrations will be limited by the light harvesting capacity of the photosynthetic machinery (Hikosaka *et al.* 2005), it stands to reason that decreases in chlorophyll content, as well as the other nitrogen-rich molecules that are part of the light harvesting complex, will have a negative impact on CO_2 response. Therefore, the low-root allocation of plants growing at low R:FR and the effect this has on nutrient uptake may be the ultimate reason why these plants failed to respond to elevated CO_2. Maintenance of adequate root growth at elevated CO_2 has been shown to be critical in avoiding nitrogen limitation and allowing plants to respond positively to elevated CO_2 (Norby & Iversen 2006).

Although the effect of R:FR ratio on allocation patterns and nutrient uptake may help explain the poor response of canopy subordinates to elevated CO_2 in *S. alba* and other species where elevated CO_2 increases size inequality within the canopy, there is at least one report of elevated CO_2 decreasing size inequality by favouring the growth of canopy subordinates over that of the canopy dominants (Wayne & Bazzaz 1997). It is worth noting that the species in which elevated CO_2 is reported to increase size inequality within the canopy are shade-intolerant annuals: *Sinapis alba* (the present study), *Chenopodium album* (Hikosaka *et al.* 2003; Nagashima *et al.* 2003), *Amaranthus retroflexus* (Morse & Bazzaz 1994) and *Abutilon theophrasti* (Morse & Bazzaz 1994). In contrast, the species

in which elevated CO_2 favoured the growth of the canopy subordinates was *Betula allegenensis*, a tree with intermediate shade tolerance. In shade-tolerant species, a low R:FR ratio does not enhance stem elongation, nor increase allocation to stem growth at the expense of other functions (Smith 1982). This implies that shade-tolerant species should have a very different response to elevated CO_2 in low *versus* high R:FR environments than shade-intolerant species. If this is true, this could help explain why elevated CO_2 has contrasting effects on size hierarchies in canopies of different species.

There is little information on the response of plants to elevated CO_2 at different R:FR ratios, making it difficult to draw firm conclusions regarding the response of shade-tolerant and -intolerant species. Hoddinott & Scott (1996a,b) examined the effects of both R:FR ratio and level of CO_2 on growth in *Pinus banksiana*, *Picea mariana* and *Picea glauca*. Although these three tree species differ in their level of shade tolerance, all three tree species are likely more shade tolerant than an annual herb such as *S. alba*. In contrast to our study, they found that decreases in R:FR did not increase stem allocation, and the effect of CO_2 on growth did not differ between the R:FR treatments. Interestingly, they did find a decline in root allocation at a low R:FR ratio in *Pinus banksiana*, the most shade intolerant of their three species, and this decline was associated with a decrease in leaf chlorophyll content, as in our study. However, the decline in leaf chlorophyll content was more than compensated for by an increase in leaf allocation (*i.e.* biomass was allocated to leaves at the expense of roots), and growth was highest at a low R:FR ratio. Arnone & Körner (1993) constructed two-storied canopies of the shade-intolerant herb, *Ricinus communis*, by establishing a first cohort of seedlings, and 4 weeks later, planting a second cohort in the shade of the first. They observed a differential response to CO_2 enrichment between overstorey and understorey plants, with an increase in stem biomass of overstorey plants and an increase in height without a corresponding increase in biomass of understorey plants. They suggest the differential response of the overstorey *versus* understorey plants to elevated CO_2 was a function of their contrasting R:FR environments, but as the environment of the overstorey and understorey plants differs in a number of other respects aside from R:FR ratio, it is difficult to draw firm conclusions.

This present study has demonstrated that the R:FR environment of a plant can have a marked effect on its response to elevated CO_2. Further, the contrasting responses of canopy dominants *versus* subordinates to elevated CO_2 that has been observed in a number of studies may be a function, at least in part, of the R:FR gradient in plant canopies. The resultant changes in size hierarchies at elevated CO_2 have important implications for plant populations. Large individuals contribute disproportionately to the gene pool of the next generation; therefore, an increase in size inequality will decrease effective population size (Jurik 1991; Thomas & Bazzaz 1993; Wayne & Bazzaz 1997), as will a decrease in survivorship with increasing size inequality (Morse & Bazzaz 1994). Conversely, a decrease in size inequality will have the opposite effect. Changes in effective population size will likely impact the relative importance of natural selection *versus* genetic drift and therefore will affect the capacity of these populations to evolve in response to changes in the environment (Thomas & Bazzaz 1993). Changes in survivorship resulting from size inequalities also have the potential to affect long-term carbon storage in ecosystems (Körner 2004). In the case of economically important species, whether CO_2-induced changes in biomass are spread equally or unequally among all individuals may affect harvesting practices (Wayne & Bazzaz 1997).

Given the importance of understanding how elevated CO_2 will impact plants growing in competition, the marked effect of competition on light quality, and the paucity of information on how light quality impacts the CO_2 growth response, further work on how the response to elevated CO_2 is modified by R:FR ratio in both shade-tolerant and -intolerant species is warranted.

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