

The influences of increased CO₂ and water supply on growth, biomass allocation and water use efficiency of *Sinapis alba* L. grown under different wind speeds

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Abstract. We examined how independent and interactive effects of CO₂ concentrations, water supply and wind speed affect growth rates, biomass partitioning, water use efficiency, diffusive conductance and stomatal density of plants. To test the prediction that wind stress will be ameliorated by increased CO₂ and/or by unrestricted water supply we grew *Sinapis alba* L. plants in controlled chambers under combinations of two levels of CO₂ (350 ppmv, 700 ppmv), two water regimes and two wind speeds (0.3 ms⁻¹, 3.7 ms⁻¹). We harvested at ten different dates over a period of 60 days. A growth analysis was carried out to evaluate treatment effects on plant responses. Plants grown both in increased CO₂ and in low wind conditions had significantly greater stem length, leaf area and dry weights of plant parts. Water supply significantly affected stem diameter, root weight and leaf area. CO₂ enrichment significantly increased the rate of biomass accumulation and the relative ratio of biomass increase to leaf area expansion. High wind speed significantly reduced plant growth rates and the rate of leaf area expansion was reduced more than the rate of biomass accumulation. Regression analysis showed significant CO₂ effects on the proportion of leaf and stem dry weight to total dry weight. A marked plant-age effect was dependent on water supply, wind speed and CO₂ concentration. A reduced water supply significantly decreased the stomatal conductance, and water use efficiency significantly increased with a limited water supply, low wind and increased CO₂. We found significant CO₂ × wind effects for water diffusion resistance, adaxial number of stomata and water use efficiencies and significant wind × water effect for water use efficiency. In conclusion, wind stress was ameliorated by growing in unrestricted water but not by growing in increased CO₂.

Key words: Allocation – CO₂ – Growth analysis – Relative growth rates – Stomatal density – Water stress

There is substantial evidence that anthropogenic activities such as the combustion of fossil fuels and the clearing of forests are increasing the atmospheric CO₂ concentration of the Earth (Clark 1982; Houghton et al. 1983; Rotty and Marland 1986; Houghton 1988; Woodwell 1988). The present CO₂ concentration of 350 cm³ m⁻³ is rising at the rate of 1.2 cm³ m⁻³ per year and is expected to have doubled by the mid- to late 21st century (Conway et al. 1988; Watson et al. 1990). Even if emissions of CO₂ could be kept at current rates atmospheric CO₂ would increase to about 450 ppmv by the year 2050 and 520 ppmv by the year 2100 (Watson et al. 1990). The predicted increase in atmospheric CO₂ concentration may have direct effects on individual plants, populations, communities and ecosystems (reviewed in Strain 1987; Bazzaz 1990; Woodward et al. 1991) and indirect effects, by changing climatic conditions, through an enhanced greenhouse effect above that due to natural greenhouse gas concentrations (Ramanathan 1988).

The positive and negative biological feedbacks in the responses to CO₂ and the ways in which other environmental factors interact with CO₂ are not well known. Ackerly et al. (1992) concluded that plant responses to CO₂ are not easily predictable since they depend on levels of other environmental factors and on the species, emphasizing the importance of studying multiple factors simultaneously. The knowledge of the responses of the biota to these interactions will be of the greatest value in predicting the ecological consequences of the expected change in atmospheric CO₂ levels, since although there are many uncertainties with regard to the timing, magnitude and regional patterns of climate change, the biota will be living in an environment richer in CO₂, regardless of how the climate will change.

Plant responses to elevated CO₂ atmospheric concentrations have been extensively investigated (see Strain

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and Bazzaz 1983; Bazzaz 1990; Woodward et al. 1991, for relevant literature). Many of these investigations have sought to examine the interactions of different levels of CO₂ and water availability (Sionit et al. 1980; Bazzaz and Carlson 1984; Morison and Gifford 1984a, b; Warwick et al. 1986; Nijs et al. 1988; Prior et al. 1991). This literature has established that enhancement of growth under restricted water conditions still occurs in enriched CO₂ environments. Reduced stomatal conductance (by about 40% for a CO₂ doubling) and to a lesser extent reduced transpiration rates have also been reported. All this results in an increased water use efficiency with increased CO₂ concentration. Thus, in water-limited environments drought stress in plants will be ameliorated by CO₂ enhancement (Sionit et al. 1980; Bazzaz and Carlson 1984; Jones et al. 1985).

Little or no work has been designed to investigate the interactions of CO₂ with wind and water. Wind is an important climatic factor, mainly in coastal and mountain areas where it can explain the observed patterns of distribution of some species (Griggs 1938). Its complex effect on vegetation acts both via mechanical effects and by influencing the turbulent transfer of heat, water vapour and CO₂. Therefore, important interactive effects with different levels of CO₂ and water supply are expected. Conflicting reports on the effects of wind on transpiration and stomatal conductance (see Grace and Russell 1977; Kozłowski et al. 1991; Retuerto and Woodward 1992) make it difficult to predict with any certainty whether the established plant responses to CO₂ and water supply will continue to occur at higher wind speeds. Grace and Russell (1977) demonstrated that leaves growing in high-wind conditions were increasingly prone to water loss. Since plant water use could be reduced by the increased water use efficiency that results from growth at elevated CO₂ concentrations, we hypothesized that plants growing in enriched-CO₂ environments would be more able to compensate for water lost due to wind stress. If, as suggested by Kalma and Kuiper (1966), water stress could be an important cause of reduced growth in wind we also expected a reduced effect of wind on plants grown in well watered conditions. That is, we expect that the effect of wind stress will be ameliorated by growing in increased-CO₂ environments and/or well-watered conditions. To test these predictions and examine whether wind speed affected growth rates, biomass partitioning and some of the components of water use efficiency (diffusive conductance and stomatal density) plants of *Sinapis alba* were grown in controlled growth chambers under different combinations of two wind speeds, two water regimes and two levels of CO₂.

Material and methods

Plant material, growth conditions and experimental design

Selected seeds of *Sinapis alba* L. (white mustard, from Mr. Fothergill's Seeds Ltd., Newmarket, UK) of similar size and bigger than 2 mm were germinated on Petri dishes on two layers of filter paper.

Seeds were moistened with distilled water and left to germinate at a mean temperature of about 20° C for 48 h under natural light. Once the cotyledons had appeared four seedlings were planted in 1268-ml (6.5 cm by 6.5 cm by 30 cm high) black PVC pipe tubes mounted on plastic pots to allow drainage and filled with an equal amount of 9:1 mixture of John Innes no. 3 potting compost, which has a high NPK content (N: 5%, P₂O₅: 7.5%, K₂O: 10%) and perlite (Silvaperl Products Ltd.). This pot size was chosen to minimise the problems of a limited rooting space and soil-resource supply (Coleman and Bazzaz 1992). For the following 2 days the tops of the tubes were covered with glass plate to create a humid environment and facilitate the establishment of the seedlings. Then, all but one of the seedlings per tube were removed, the remaining plants being selected for uniformity of size and stage of development. After 2 days, when *Sinapis* plants were on average 3.63 cm ± 0.11 (SE) tall and leaf area was 1.9 cm² (plant⁻¹) ± 0.08, the treatments began.

Sinapis plants were grown in four 75 cm by 75 cm by 105 cm high environmentally monitored glass-top chambers. A total of 240 pots were randomly assigned to four individual glass-top growth chambers. The plants were kept far enough apart to avoid competition for light. The chambers, equipped with CO₂ concentration controls, were initially assigned either ambient (350 ± 20 cm³ m⁻³) or enriched (700 ± 40 cm³ m⁻³) CO₂ concentrations in air. The CO₂ concentrations chosen represent present-day and the mid-21st century predicted level (see Conway et al. 1988). Chambers with the same CO₂ concentration in air were joined by two 12 cm diameter PVC tubes with a small fan incorporated inside to ensure uniform mixing of the atmosphere within the two chambers. Two chambers, one for each CO₂ treatment, were equipped with large fans to produce a turbulent flow (Grace and Wilson 1976), at a mean velocity of 3.74 ms⁻¹ and 3.63 ms⁻¹, respectively, for the two chambers. These fans were turned on for 9 h during the light period (from 8 a.m. to 5 p.m.). This treatment will be referred to as "high wind speed". The mean wind speeds registered in each of the adjacent, low wind speed chambers were 0.31 ms⁻¹ and 0.37 ms⁻¹. This treatment will be referred to as the "low wind speed" treatment. All speeds were measured by a temperature-compensated heated thermistor anemometer (type AVM 501 TC, Prosser Scientific Instrument LTD.) and were the average of 36 measurements taken at 6 different positions and 6 heights within each growth chamber.

The 60 plants in each chamber were randomly assigned to two movable trays placed in the chambers. Plants growing on one of the trays in each chamber were supplied with as much tap water as was consumed. Water use was determined gravimetrically. To know the average water consumed by these plants four pots from these trays in each growth chamber were weighed every day and the average water lost was supplied to all the plants in these trays. This treatment will be referred to as the "well-watered" treatment. Plants growing on the other tray in the same growth chamber received just half of the water supplied to the plants growing in the well-watered treatment. This treatment will be referred to as the "low water" treatment. Because of the impossibility of replicating the growth chambers and in order to reduce chamber and pseudoreplication effects, the positions of the trays and the positions of the pots on each tray were daily randomized within each chamber and every 3 or 4 days pots were rotated between the growth chambers, after CO₂ levels were appropriately reprogrammed.

The experiment was conducted between 29 August and 3 November 1990 in the Botanical Garden of Cambridge (UK). The growth chambers located in a glasshouse received natural photoperiod (≈ 14 h) and light regimes. All the plants were grown at uniform radiation levels by raising the trays containing shorter plants so that all plants received equal PAR. Temperature and relative humidity in the chambers were monitored throughout the experiment. The chambers showed closely matching environmental conditions. Differences among the chambers were always less than 0.6° C for temperatures and less than 5% for relative humidity. Mean growing conditions for the period of the experiment were the following: day/night temperatures were 25.6° C ± 0.52 (SE)/15.1° C ± 0.38,

day/night relative humidity was $50.3\% \pm 1.35/72.6\% \pm 0.85$ and irradiance was $8.31 \text{ MJ m}^{-2} \text{ day}^{-1} \pm 0.55$.

Plant performance analysis

To evaluate the effects of the treatments on plant growth responses, a full growth analysis was carried out over 60 days during which the plants were growing in the different treatments. The optimal growth analysis for the functional approach (Hunt 1982) is achieved by several harvests with few replicates, rather than the reverse. There were ten harvest dates (0, 10, 14, 18, 25, 32, 39, 46, 53, 60 days after the beginning of treatments) with three replicates randomly chosen for each harvest and treatment, so that 240 plants had been harvested by the end of the experiment. The initial harvest just before beginning the treatments (harvest day 0) was made to compute growth rates in the first days of the treatments but it was not considered in the statistical analyses. At each harvest, plants were separated into roots, stems and leaves. Roots were washed and all plant material was oven-dried at 80°C for 48 h and weighed.

At each harvest the following plant attributes were measured for each plant: number of leaves, total leaf area of the plant, which included cotyledons and leaves more than 3 mm in length using a leaf-area meter (Delta-T Devices, Burwell, Cambridge, UK), stem length and dry weights of leaves, stems and roots. These attributes were used to estimate the instantaneous values of relative growth rates (leaf area, RlaGR; leaf dry weight, RlvGR; stem dry weight, RstGR; root dry weight, RrtGR; overall index of plant performance, RGR) and unit leaf rate (ULR) (Hunt 1982). Biomass allocation patterns were assessed by calculating: leaf area ratio (LAR), root/shoot ratio (RS), specific leaf area (SLA) and leaf, stem and root weight ratios (LWR, SWR and RWR respectively).

On day 53, adaxial and abaxial stomatal densities were measured on the third leaf of *Sinapis* plants on the assumption that these leaves had developed in full irradiance and had completed expansion. Stomatal densities were measured by the analysis of microscopic sections of the leaves, with ten random fields selected on each surface of peels from five plants per treatment. On days 28 and 52, leaf temperatures and water diffusion resistances were taken at two different times (1000 and 1600 hours) with the automatic porometer MK3 (Delta-T Devices) on the youngest fully expanded leaf of five plants in each treatment.

Because plant water use has been shown to change with different levels of CO_2 , wind speed and water availability, we evaluated the water use efficiency of plants (WUE). WUE was evaluated gravimetrically as the ratio of dry weight or leaf area gain to integrated water loss (Morison and Gifford 1984b).

Statistical analyses

The distribution of the variables was examined for outliers, non-normality and heteroscedasticity and when significant violations were found data were \log_e or arcsin transformed to ensure that the assumptions of parametric statistics were met. Satisfactory normality of residuals were examined by normal probability plots. Cochran's C , Bartlett-Box's F (univariate homogeneity of variance test), and Pillais and Hotellings (multivariate tests of significance) statistics were computed concurrently with all analyses of variance to test for heterogeneity of variance.

Data on stomatal density were subjected to a three-way analysis of variance (CO_2 , wind speed, water supply) to look for differences in stomatal density as function of the different treatments and their interactions. To determine whether leaf temperature and water diffusion resistance data differed as a function of the different treatments (CO_2 , wind speed, water supply), we performed analysis of covariance (ANCOVA) with time of the day and plant age at which the measurements were taken as covariates. In this way, we controlled for the time of day and age effect before performing an analysis of variance on the treatment effects. To examine age-

related changes and interactive effects of treatments with plant age, data were subjected to multivariate analysis of variance (MANOVA) with CO_2 , wind speed, water supply and plant age as attribute variables. Analysis of covariance (ANCOVA), with plant age fitted as covariate, was used to assess the overall effects of the different treatments and their interactions on plant growth, water use efficiency and biomass allocation. With the use of ANCOVA the effects of disturbing variables (plant age in this case) were removed and so homogeneity is tested for the variable dependent means after they are adjusted for the groups' differences in the covariate. Thus, the changes detected are not due to inherent ontogenetic drift. When relevant, we used least-square means comparisons on general linear modelling (LSM-GLM) of SAS to make individual comparisons of the effects of the different combinations of treatment levels.

To reflect biomass partitioning, dry weight was used in comparisons between biomass of the different organs and total or shoot biomass. To analyse how the dry weight of a plant part scales to total biomass, we fitted by the technique of principal axis (Model II regression) the equation $\log y = a + b \log x$, where x denotes total biomass (or shoot biomass to examine the root/shoot ratio) and y the different biomass of plant parts. The slope b , or allometric coefficient, indicates how biomass in plant parts changes in proportion to total biomass, with $b < 1$ indicating a decrease (negative allometry), $b = 1$ indicating independence (isometry) and $b > 1$ indicating an increase of proportional plant part biomass. SLA was plotted as leaf area versus leaf biomass and their relationships were also analysed by the principal axis method. For logarithmically transformed data, when there is error in both variables measured and it is difficult to determine which variable is dependent and which is independent, the principal axis method is preferable for finding the line of best fit (Sokal and Rohlf 1981; Pagel and Harvey 1988).

Results

Growth parameters

ANCOVA analysis specifically removed the effect of plant age so that the response of all the plants harvested during the 60 days of the experiment could be jointly compared to look for significant effects of the treatments. The effect of the covariate was significant for all the dependent variables considered ($P < 0.013$, df 1, 207) which means that controlling for the plant age effect incorporated relevant information for the analysis of the differences between the means of the treatments.

Effects of CO_2 . The analysis showed considerable differences in the number of leaves, stem length and diameter and leaf area between CO_2 treatments (in all the cases $P < 0.001$, df 1, 207). Mean values over the 60 days of the treatments (Table 1) were always greater for those plants grown in elevated CO_2 concentrations than for plants grown in ambient CO_2 atmospheres (on average, stem length and leaf area were 23.7% and 18.4% greater). On day 32, stem lengths were 60.6% and leaf area 48.1% greater in those plants grown in increased CO_2 concentrations than in those at ambient CO_2 levels, but at the end of the experiment (day 60) these differences were reduced to 11% and 4%, respectively (Fig. 1) and the number of leaves was greater in those plants exposed to ambient levels of CO_2 . Plants grown at elevated CO_2 concentrations also had significantly greater leaf, stem, root and total dry weights (in all the cases $P < 0.001$, df

Table 1. ANCOVA cell mean values (± 1 SE) of growth attributes of *Sinapis alba* plants grown for 60 days under two different CO₂ concentrations, two wind speeds and two different levels of water supply

	Ambient CO ₂				Elevated CO ₂			
	Low wind		High wind		Low wind		High wind	
	Low water	Well-watered	Low water	Well-watered	Low water	Well-watered	Low water	Well-watered
No. leaves	9.67 ± 0.75	10.07 ± 0.96	7.70 ± 0.40	8.33 ± 0.72	10.19 ± 0.69	11.04 ± 0.82	8.67 ± 0.53	9.30 ± 0.56
Stem length (cm)	15.09 ± 2.00	20.47 ± 3.35	10.07 ± 0.92	11.96 ± 1.45	18.85 ± 2.28	29.77 ± 4.39	11.77 ± 1.14	15.91 ± 1.79
Stem diameter (cm)	3.31 ± 0.34	4.18 ± 0.51	2.61 ± 0.19	3.10 ± 0.32	4.21 ± 0.37	4.83 ± 0.52	2.85 ± 0.19	3.31 ± 0.25
Leaf area (cm ²)	132.99 ± 23.34	178.77 ± 37.10	42.73 ± 5.33	71.16 ± 14.88	162.59 ± 24.39	235.76 ± 40.71	52.16 ± 6.98	71.32 ± 12.27
Leaf biomass (mg)	319.97 ± 63.24	352.83 ± 82.95	143.79 ± 19.87	205.09 ± 48.33	481.12 ± 81.46	507.09 ± 99.53	182.94 ± 27.69	233.96 ± 44.95
Stem biomass (mg)	172.94 ± 45.59	287.46 ± 90.50	60.98 ± 4.34	104.23 ± 32.38	333.68 ± 74.50	532.34 ± 129.29	86.60 ± 16.37	189.04 ± 50.96
Root biomass (mg)	136.15 ± 39.83	117.07 ± 37.97	46.15 ± 7.70	65.16 ± 19.68	197.44 ± 43.51	176.76 ± 45.55	66.17 ± 14.54	82.24 ± 27.93
Shoot biomass (mg)	492.92 ± 107.97	640.29 ± 172.24	204.77 ± 29.97	309.31 ± 79.87	814.81 ± 154.61	1039.43 ± 228.0	269.54 ± 90.31	423.00 ± 137.86
Total biomass (mg)	629.06 ± 146.09	757.36 ± 209.47	250.93 ± 37.37	374.47 ± 99.34	1012.25 ± 194.9	1216.19 ± 272.2	335.66 ± 57.64	511.24 ± 116.19
RGR (day ⁻¹)	0.101 ± 0.019	0.103 ± 0.017	0.069 ± 0.017	0.081 ± 0.021	0.109 ± 0.015	0.109 ± 0.019	0.084 ± 0.016	0.091 ± 0.020
RLaGR (day ⁻¹)	0.088 ± 0.015	0.094 ± 0.015	0.059 ± 0.014	0.066 ± 0.027	0.093 ± 0.015	0.094 ± 0.018	0.072 ± 0.018	0.075 ± 0.020
RlwGR (day ⁻¹)	0.096 ± 0.018	0.099 ± 0.019	0.067 ± 0.017	0.078 ± 0.020	0.105 ± 0.017	0.102 ± 0.021	0.081 ± 0.017	0.081 ± 0.022
RStGR (day ⁻¹)	0.108 ± 0.021	0.110 ± 0.025	0.068 ± 0.022	0.085 ± 0.025	0.118 ± 0.017	0.124 ± 0.024	0.088 ± 0.016	0.106 ± 0.020
RRlGR (day ⁻¹)	0.104 ± 0.027	0.100 ± 0.020	0.077 ± 0.017	0.083 ± 0.026	0.104 ± 0.019	0.102 ± 0.026	0.087 ± 0.018	0.086 ± 0.028
ULR (mg cm ⁻² day ⁻¹)	0.372 ± 0.069	0.332 ± 0.056	0.290 ± 0.066	0.330 ± 0.087	0.449 ± 0.056	0.360 ± 0.069	0.407 ± 0.082	0.412 ± 0.114

RGR = Relative growth rate; RLaGR = Relative leaf area growth rate; RLwGR = Relative leaf dry weight growth rate; RStGR = Relative stem dry weight growth rate; RRlGR = Relative root dry weight growth rate; ULR = Unit leaf rate

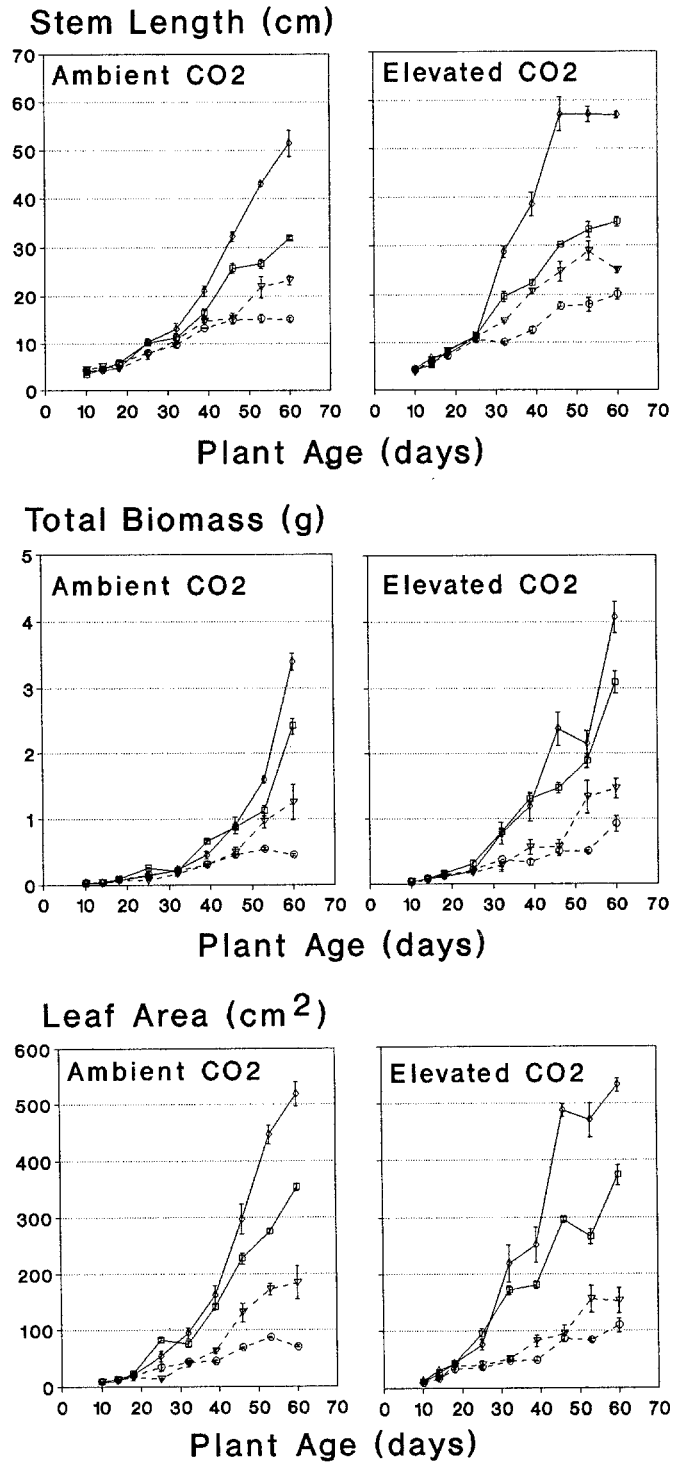


Fig. 1. Stem elongation, biomass accumulation and leaf area of *Sinapis* plants (mean \pm SE) as a function of plant age, CO₂ concentration, wind speed and water supply. Squares and solid line high wind speed-high water plants; rhombs and solid line, high wind speed-well watered plants; triangles and dashed line, low wind speed-well watered plants; circles and dashed line, low wind speed-low water plants

1, 207). On average, leaf, stem and root dry weights were 27.2%, 45.2% and 31% greater, respectively, for plants grown in enriched CO₂ environments than for plants grown in ambient CO₂ levels. On day 32 these differences were 58.2%, 72.6% and 64.5% for leaf, stem and root dry

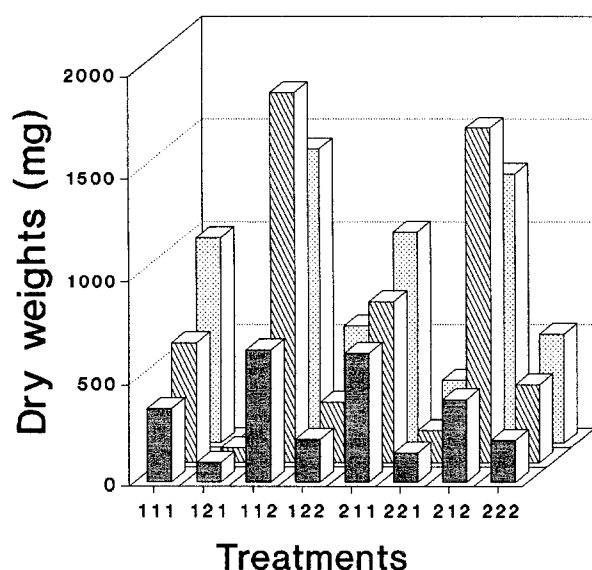


Fig. 2. Dry weights at day 60 of the different plant organs as a function of CO₂ concentration, wind speed and water supply. Bars (from front to back); solid, root mass; hatched, stem mass; dotted, leaf mass. Treatments are represented by figures of three numbers: first number indicates ambient (1) or elevated (2) CO₂, second number indicates low wind speed (1) or high wind speed (2) and the third number indicates low water (1) or well-watered (2) treatment

weights, respectively, but by the end of the experiment, the differences were notably reduced to 13.7%, 32.1% and 12.3%, respectively (Fig. 2). There were no significant CO₂ treatment effects on the values of unit leaf rate nor relative growth rates (minimum $P=0.159$, df 1,207), although mean values for these rates over the 60 days of the treatments were always higher in plants grown exposed to elevated CO₂ concentrations. Growth rates of plants in their early life (by day 18) were always greater in plants which grew in high CO₂ levels (Fig. 3).

Effects of wind. High wind speed significantly reduced the number of leaves, stem length and diameter, leaf area, and dry weights of leaf, stem, root and total plant (maximum $P<0.001$, df 1,207). On average, cell mean values (Table 1) for leaf area and stem length were 67% and 41% greater, respectively, in plants grown in low wind conditions than in plants grown in high wind speeds. Plant dry weights were also reduced by increased wind speeds. Leaf, stem and root dry weights from plants grown in low wind speeds were, on average, 53.9%, 66.8% and 57.8% greater, respectively, than those from plants which grew in high wind conditions. RGR, RlaGR and RlvGR were also significantly reduced by high wind speeds (maximum $P=0.047$, df 1,207). Plants were less responsive to the wind effect in their early life. On day 32, stem length, leaf area, and dry weights of leaves, stems and roots were, respectively, 38.1%, 67.5%, 43.4%, 59.2% and 47.1% greater in plants which grew in low wind conditions than in plants grown at high wind speeds. These differences were notably increased on day 60, when stem length, leaf area and dry weights of leaves, stems and roots were 52.4%, 71.0%, 64.3%, 72.7% and 68.2% greater, respectively, in plants grown in low wind conditions than in those grown in high wind speeds (Fig. 2).

Effects of water. Mean values for growth parameters were greater in plants which grew in well watered conditions than in those grown in low-water conditions except for root dry weight, relative root growth rate and unit leaf rate (Table 1). We only detected significant water effects on the values of stem length and diameter, root dry weight (maximum $P=0.002$, df 1,207) and leaf area ($P=0.05$, df 1,207). Plants grown in well-watered conditions had, on average, 22.1% greater total plant dry weight, 29.9% greater leaf area and 28.6% higher stem length than plants grown at low watered conditions. On day 32, stem length and leaf area were, respectively,

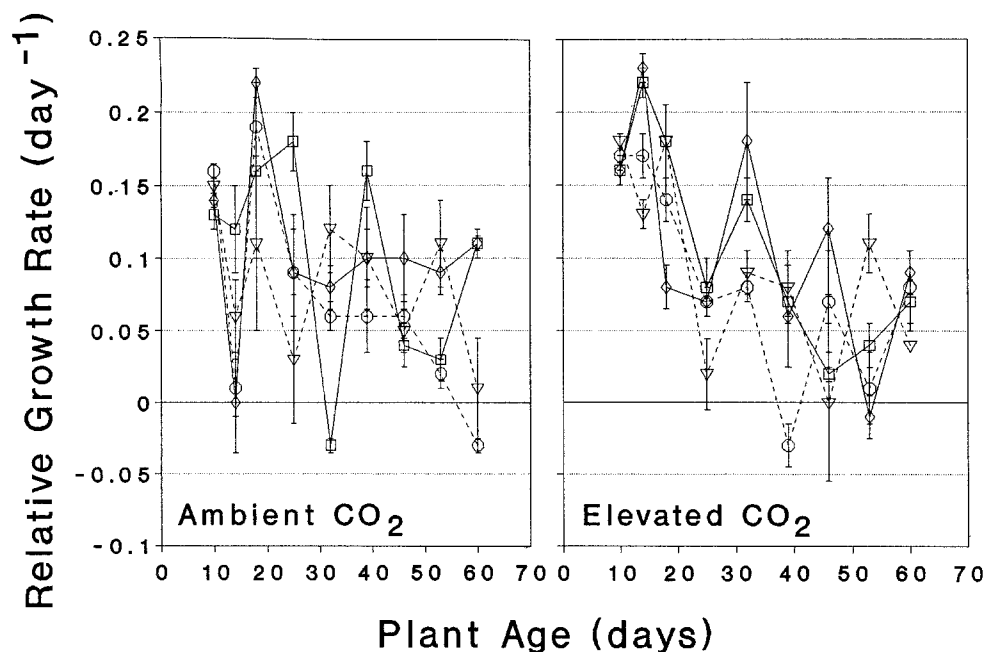


Fig. 3. Change over time in relative growth rates (mean \pm SE) of the whole plants as affected by CO₂, wind speed and water supply. Squares and solid line, high wind speed-low water plants; rhombs and solid line, high wind speed-well watered plants; triangles and dashed line, low wind speed-well watered plants; circles and dashed line, low wind speed-low water plants

24.6% and 16.3% greater in plants grown in well-watered conditions than in plants grown in low-water conditions. In contrast, total dry weights were 9% greater in plants grown in low-water conditions. On day 60 the differences were notably increased for stem length and leaf area which were 34.8% and 34.7% greater, respectively, in plants grown in well-watered conditions and reversed for total dry weight which were 32.6% greater in plants grown at well-watered conditions (Fig. 2).

Interactive effects. We did not detect any significant interactive effect of any order on the values of the growth parameters analysed, but some interesting joint effects were observed. Plants in low-water conditions and high wind had significantly reduced leaf area by 67.9% and leaf, stem and root dry weights by 59.2%, 70.9% and 66.3%, respectively (maximum $P < 0.0001$, for LSM comparisons). The reductions due to high wind were also significant (maximum $P < 0.0002$, for LSM comparisons) but lower when plants grew in well watered conditions. On average, leaf area was reduced by 65.6% and leaf, stem and root dry weights by 47.8%, 48.9% and 64.2%, respectively. In contrast to expectations wind stress was not ameliorated by growing in enriched CO_2 environments. In plants which experienced ambient CO_2 concentrations wind stress significantly reduced leaf area by 63.5% while leaf, stem and root dry weights were reduced by 56.0%, 47.3% and 64.1%, respectively (maximum $P < 0.0014$ for LSM comparisons). In plants which experienced enriched CO_2 atmospheres wind stress produced more significant (maximum $P < 0.0001$, for LSM comparisons) and even larger reductions on leaf area (on average, 69.0%) and leaf, stem and root dry weights (on average, 58.7%, 57.8% and 68.2%, respectively). We also observed for some variables that the effects of wind were not totally independent from the effect of water. Thus, irrespective of the CO_2 treatments, plants in low wind speeds developed greater root dry weights and unit leaf rates (for root dry weights $P < 0.0039$, for LSM comparisons) when growing in low-water conditions than those which grew in well watered conditions (on average, 11.9% greater).

Plant age significantly affected all dependent variables considered (maximum $P = 0.013$, df 1, 207). The response of plants to each level of CO_2 depended on the age of the plant for the number of leaves, stem length and diameter, leaf area, total and plant parts dry weights (maximum $P = 0.001$, df 1, 207), but the analysis did not detect dependence for the relative growth rates nor unit leaf rate. The wind \times plant age interaction term in the MANOVA was statistically significant for all the growth parameters analysed ($F = 4.074$, $P = 0.045$, df 1, 207 for RGR, maximum $P < 0.0001$, df 1, 207 for the other variables) except unit leaf rate. The water \times plant age interaction term in the analysis showed that the effect of water was not independent of plant age for number of leaves, stem length and diameter, leaf area and for stem and root dry weights (maximum $P = 0.030$, df 1, 207). This interaction term was not significant for leaf and total plant dry weights, relative growth rates or unit leaf rate (minimum $P = 0.152$, df 1, 207). MANOVA did not detect any other

higher order interaction term which significantly affected the growth parameters considered.

Allocation and architectural parameters

ANCOVA showed that CO_2 effects were significant for LAR, SLA, LWR and SWR (maximum $P = 0.001$, df 1, 207). Cell mean values (Table 2) in the ANCOVA analysis showed that plants grown in ambient CO_2 had greater LAR, SLA, LWR, RWR and root/shoot ratio and smaller SWR than plants which grew in increased CO_2 atmospheres. This tendency in the mean values was maintained at the end of the experiment. LAR, SLA, LWR and SWR were significantly affected by wind speed (minimum $P = 0.01$, df 1, 207) but neither RWR ($F = 0.183$, $P = 0.669$, df 1, 207) nor root/shoot ratio ($F = 0.101$, $P = 0.751$, df 1, 207) were affected. Cell mean values showed that only LWR and RWR were greater in those plants exposed to high wind speed. This tendency in the mean values was maintained to the end of the experi-

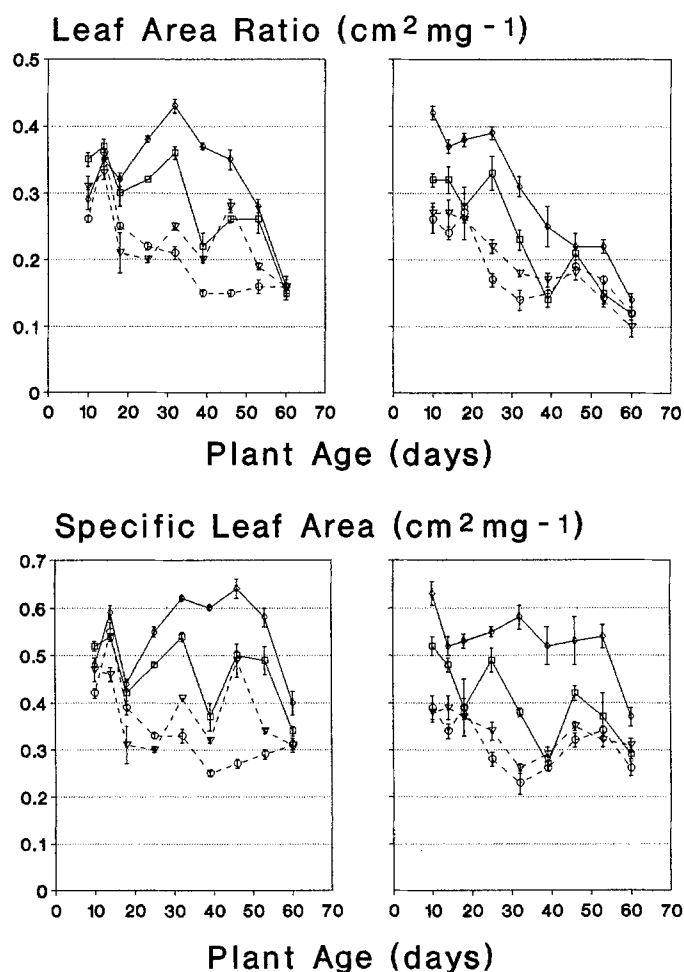


Fig. 4. Time course of leaf area ratio and specific leaf area (mean \pm SE) of *Sinapis* plants grown under two different CO_2 concentrations, two wind speeds and two levels of water supply. Squares and solid line, high wind speed-low water plants; rhombs and solid line, high wind speed-well watered plants; triangles and dashed line, low wind speed-well watered plants; circles and dashed line, low wind speed-low water plants

Table 2. ANCOVA cell mean values (± 1 SE) of measured allocation parameters of *Sinapis alba* grown for 60 days under two different CO₂ concentrations, two wind speeds and two different levels of water supply

	Ambient CO ₂				Elevated CO ₂			
	Well-watered		High wind		Well-watered		High wind	
	Low water	Well-watered	Low water	High wind	Low water	Well-watered	Low water	High wind
Root: shoot ratio	0.215 \pm 0.016	0.184 \pm 0.021	0.225 \pm 0.014	0.188 \pm 0.015	0.230 \pm 0.020	0.150 \pm 0.011	0.213 \pm 0.011	0.161 \pm 0.015
LAR (cm ² mg ⁻¹)	0.288 \pm 0.015	0.385 \pm 0.016	0.214 \pm 0.014	0.237 \pm 0.013	0.232 \pm 0.017	0.301 \pm 0.020	0.190 \pm 0.014	0.199 \pm 0.013
SLA (cm ² mg ⁻¹)	0.467 \pm 0.017	0.543 \pm 0.018	0.347 \pm 0.018	0.380 \pm 0.019	0.401 \pm 0.020	0.531 \pm 0.027	0.313 \pm 0.018	0.335 \pm 0.012
LWR	0.611 \pm 0.018	0.595 \pm 0.022	0.609 \pm 0.013	0.622 \pm 0.015	0.566 \pm 0.021	0.557 \pm 0.020	0.600 \pm 0.015	0.586 \pm 0.027
SWR	0.216 \pm 0.012	0.256 \pm 0.021	0.210 \pm 0.010	0.223 \pm 0.012	0.253 \pm 0.018	0.315 \pm 0.026	0.226 \pm 0.011	0.279 \pm 0.025
RWR	0.174 \pm 0.010	0.149 \pm 0.013	0.181 \pm 0.009	0.155 \pm 0.010	0.181 \pm 0.013	0.129 \pm 0.007	0.174 \pm 0.007	0.135 \pm 0.011

LAR = Leaf area ratio; SLA = Specific leaf area; LWR = Leaf weight ratio; SWR = Stem weight ratio; RWR = Root weight ratio

Table 3. Results of allometric analysis of dry weight partitioning and leaf area versus leaf dry weight of *Sinapis alba* plants grown under two different CO₂ concentrations, two wind speeds and two different levels of water supply

Treatments	In leaf dry weight versus		In stem dry weight versus		In root dry weight versus		In leaf area versus	
	In total dry weight		In total dry weight		In total dry weight		In leaf dry weight	
	a	b \pm CI	a	b \pm CI	a	b \pm CI	a	b \pm CI
350 CO ₂ , Low wind, low water	-0.09	0.910 \pm 0.03c	-2.51	1.171 \pm 0.04ab	-2.38	1.110 \pm 0.07	-2.38	1.142 \pm 0.10
350 CO ₂ , Low wind, well-watered	-0.11	0.922 \pm 0.03a	-2.75	1.242 \pm 0.04	-1.78	0.960 \pm 0.11	-2.90	1.203 \pm 0.12
350 CO ₂ , High wind, low water	-0.22	0.943 \pm 0.05d	-2.68	1.219 \pm 0.07	-1.91	1.034 \pm 0.12	-1.77	1.048 \pm 0.14
350 CO ₂ , High wind, well-watered	-0.15	0.941 \pm 0.02bc	-2.37	1.173 \pm 0.05c	-2.33	1.081 \pm 0.10	-2.25	1.105 \pm 0.13
700 CO ₂ , Low wind, low water	0.02	0.905 \pm 0.04	-2.89	1.242 \pm 0.04	-2.06	1.057 \pm 0.11	-1.91	1.064 \pm 0.13
700 CO ₂ , Low wind, well-watered	0.27	0.857 \pm 0.02abcd	-2.93	1.272 \pm 0.04bc	-2.35	1.049 \pm 0.08	-2.26	1.051 \pm 0.10
700 CO ₂ , High wind, low water	0.06	0.891 \pm 0.03	-2.62	1.218 \pm 0.05	-2.43	1.121 \pm 0.09	-2.44	1.170 \pm 0.09
700 CO ₂ , High wind, well-watered	0.34	0.840 \pm 0.07e	-2.94	1.296 \pm 0.07a	-2.85	1.145 \pm 0.16	-2.85	1.172 \pm 0.20

Intercepts (a) and slopes (b) were obtained from regressions fitted by the technique of principal axis. All coefficients of determination, $r^2 > 0.95$, $P < 0.001$; 95% Confidence intervals (CI), are given for the slopes. For each column, slopes are significantly different if they are followed by the same letter

Table 4. Water use efficiencies (± 1 SE), expressed as the ratio of increase in growth attributes to water consumed, of *Sinapis alba* plants grown under two different CO₂ concentrations, two wind speeds and two different levels of water supply

	Ambient CO ₂				Elevated CO ₂			
	Well-watered		High wind		Well-watered		High wind	
	Low water	Well-watered	Low water	High wind	Low water	Well-watered	Low water	High wind
Stem length (cm/g H ₂ O)	0.690 \pm 0.04	0.042 \pm 0.01	0.031 \pm 0.09	0.017 \pm 0.22	0.083 \pm 0.13	0.057 \pm 0.12	0.034 \pm 0.10	0.021 \pm 0.05
Stem diameter (cm/g H ₂ O)	0.018 \pm 0.00	0.010 \pm 0.00	0.009 \pm 0.00	0.005 \pm 0.00	0.022 \pm 0.00	0.012 \pm 0.00	0.009 \pm 0.00	0.005 \pm 0.00
Leaf area (cm ² /g H ₂ O)	0.465 \pm 0.07	0.279 \pm 0.06	0.113 \pm 0.01	0.076 \pm 0.01	0.586 \pm 0.06	0.390 \pm 0.06	0.929 \pm 0.01	0.083 \pm 0.01
Leaf mass (mg/g H ₂ O)	1.071 \pm 0.18	0.529 \pm 0.13	0.349 \pm 0.04	0.210 \pm 0.05	1.623 \pm 0.23	0.789 \pm 0.13	0.428 \pm 0.03	0.258 \pm 0.03
Stem mass (mg/g H ₂ O)	0.473 \pm 0.14	0.352 \pm 0.15	0.131 \pm 0.02	0.095 \pm 0.03	0.934 \pm 0.24	0.683 \pm 0.21	0.173 \pm 0.03	0.161 \pm 0.05
Root mass (mg/g H ₂ O)	0.376 \pm 0.12	0.153 \pm 0.06	0.103 \pm 0.01	0.060 \pm 0.02	0.579 \pm 0.13	0.237 \pm 0.06	0.134 \pm 0.02	0.080 \pm 0.03
Shoot mass (mg/g H ₂ O)	1.542 \pm 0.31	0.882 \pm 0.27	0.481 \pm 0.06	0.305 \pm 0.08	2.553 \pm 0.46	1.472 \pm 0.34	0.600 \pm 0.05	0.419 \pm 0.07
Total mass (mg/g H ₂ O)	1.918 \pm 0.42	1.035 \pm 0.33	0.584 \pm 0.07	0.365 \pm 0.09	3.132 \pm 0.58	1.709 \pm 0.40	0.734 \pm 0.07	0.500 \pm 0.10

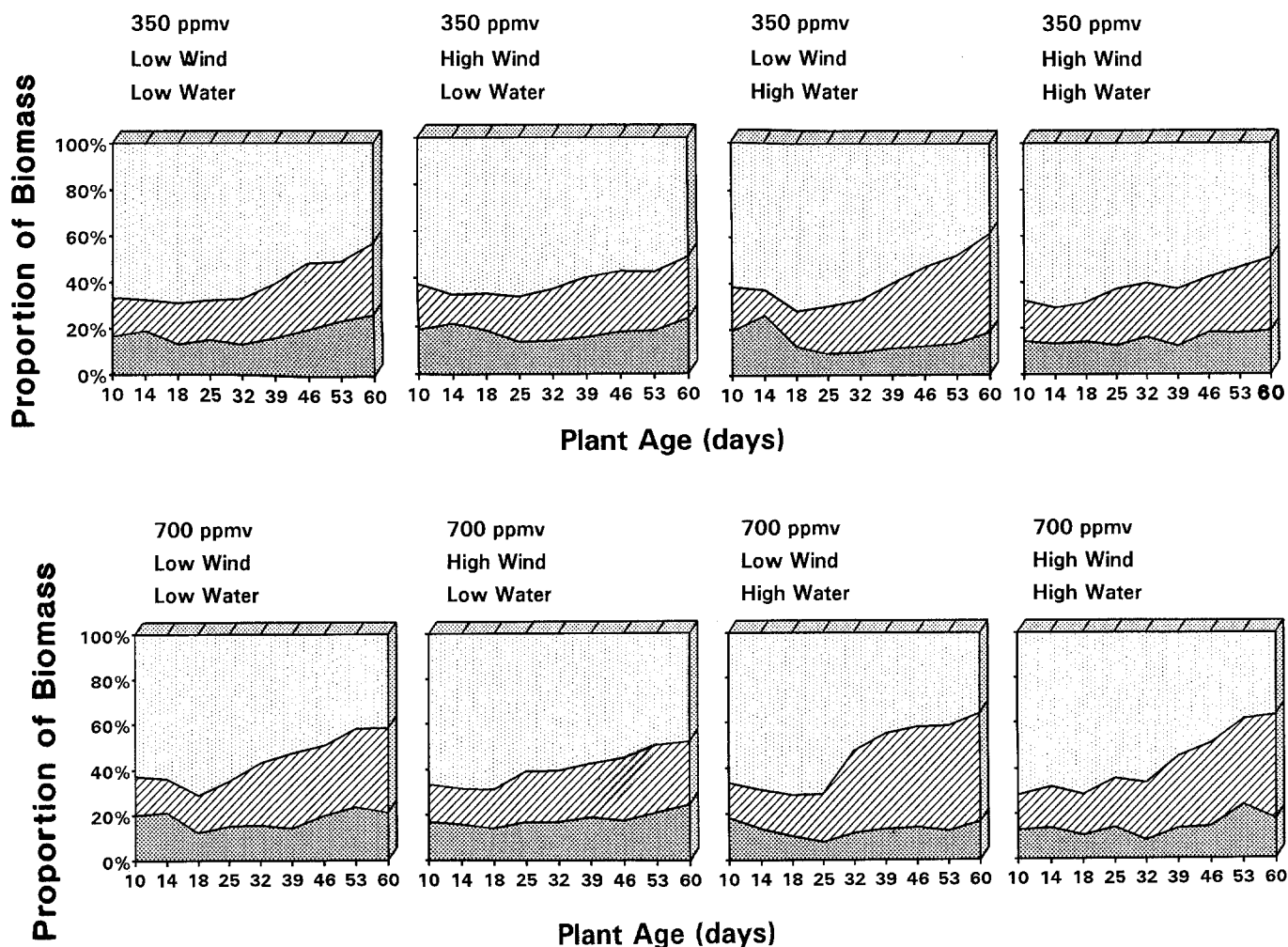


Fig. 5. Mean proportion of biomass allocated to roots, stems and leaves as a function of plant age, CO₂ concentration, wind speed and water supply. *Solid area*, root mass; *hatched area*, stem mass; *dotted area*, leaf mass

ment. Water supply significantly affected RS, LAR, SLA, SWR and RWR (in all cases $P < 0.001$, df 1, 207). Cell means in the ANCOVA showed that LAR, SLA and SWR were greater in plants grown in well watered conditions than in plants which grew in low-water conditions while RS, RWR and LWR were greater in plants which grew in low-water conditions. This tendency in the mean values was maintained to the end of the experiment (on day 60). ANCOVA also showed that plant responses to wind were significantly affected by the level of water supply for LAR ($F = 5.576$, $P = 0.019$, df 1, 207) and for SLA ($F = 10.549$, $P = 0.001$, df 1, 207) (Fig. 4). No other interactive effect was detected by the ANCOVA analysis.

In all treatments, the relative proportion of stem and root dry weight increased with total dry weight ($b > 1$, "positive allometry", Table 3). The same was true for the relationship between root dry weight and shoot dry weight. In contrast, the proportion of leaf dry weight to total dry weight declined, showing a negative allometry ($b < 1$). CO₂ significantly affected the slope of the relationship between leaf dry weight and total plant dry weight which was significantly lower ($P < 0.001$, df 1, 106) in plants grown in high CO₂ levels than in those grown at ambient CO₂ concentrations. In contrast, the slope of

the equation relating stem dry weight and total plant dry weight was significantly ($P \leq 0.05$, df 1, 106) higher in plants grown at elevated CO₂ than in those grown at ambient CO₂ concentrations. We did not detect any water or wind effect on the slopes of these allometric relationships. The study of these relationships for the plants growing in each of the eight different combinations of treatments only showed statistically significant differences in the slopes of the equations relating leaf dry weight with total dry weight and stem dry weight with total dry weight (Table 3). The differences detected always were between plants growing at two different CO₂ concentrations. Whichever the treatment, the proportion of leaf area to leaf dry weight declined, showing a negative allometry ($b < 1$). We did not detect any main effect on the relationship between leaf area and leaf dry weight but some significant differences were detected when we analysed this relationship for each of the eight different combinations of treatments.

Results of MANOVA for plant age and interactive effects of plant age with the different factors showed that plant age had significant effects on all dependent variables (maximum $P = 0.010$, df 1, 207) (Fig. 5). CO₂ × plant age interactive effects were significant for LAR, SLA,

LWR and SWR ($P < 0.003$, df 1, 207 in all the cases) but not for root/shoot ratio ($F = 0.610$, $P = 0.436$, df 1, 207) nor for RWR ($F = 0.686$, $P = 408$, df 1, 207). The wind \times plant age interactive effect was significant for LAR, SLA, LWR and SWR (maximum $P < 0.001$, df 1, 207). The water \times plant age interactive effect was significant for RS, LAR, SLA, SWR and RWR ($P < 0.001$, in all the cases) but did not for LWR ($F = 3.545$, $P = 0.061$, df 1, 297). Higher order interactive terms were not significant except for the wind \times water \times plant age term for LAR ($F = 3.956$, $P = 0.048$, df 1, 207) and for SLA ($F = 7.922$, $P = 0.005$, df 1, 207) and the CO₂ \times water \times plant age term for SWR ($F = 8.230$, $P = 0.005$, df 1, 207).

Leaf temperature, diffusion resistance and number of stomata

For leaf temperature and water vapour diffusion resistance both covariates, time and plant age, had a significant effect (maximum $P = 0.019$, df 2, 150). Analysis of covariance showed that differences among the plants grown in the different treatments were not statistically significant for leaf temperatures. Diffusion resistances were higher in those plants grown in high CO₂ levels, in low wind conditions and in those plants suffering water restrictions. Analysis of covariance detected statistically significant water diffusion resistance differences for the water effect ($3.51 \text{ s/cm} \pm 0.16$ (SE) for low-water plants and $2.32 \text{ s/cm} \pm 0.08$ for well-watered plants, $P = 53.754$, $P < 0.001$, df 1, 150), for interactive CO₂ \times wind effect ($F = 11.663$, $P = 0.001$, df 1, 150) and for the CO₂ \times wind \times water effect ($F = 6.933$, $P = 0.009$, df 1, 150).

MANOVA did not detect any significant effect of treatments on the total (adaxial+abaxial) number of stomata or on the abaxial number of stomata. MANOVA detected a significant CO₂ \times wind interactive effect on the adaxial stomatal density ($F = 8.587$, $P = 0.006$, df 1, 32). The adaxial stomatal density in plants grown at low wind conditions decreased with increasing CO₂ concentrations (from 73.2 at 350 cm³ m⁻³ CO₂ concentration to 57.8 at 700 cm³ m⁻³ CO₂ concentration in the air) but this tendency was reversed in plants grown in high wind conditions (from 64.8 at 350 cm³ m⁻³ CO₂ concentration to 88.5 at 700 cm³ m⁻³ CO₂ concentration in the air).

Water use efficiency

WUE values expressed as the ratio of plant-part dry weights or leaf area gain to integrated water loss (Table 4) were significantly affected by the age of the plant (maximum $P = 0.004$, df 1, 63) which justified to fit it as covariate and thus removed its effect before the analysis of the differences between the means of the treatments. ANCOVA for WUEs showed significant water treatment effects (in all the cases, $P < 0.001$, df 1, 63) except for WUE measured as the ratio of stem dry weight gain to integrated water loss ($F = 3.475$, $P = 0.067$, df 1, 63). WUE was increased in low watered conditions. On av-

erage and expressed in terms of leaf area and dry weights of leaf, stem and root, WUE were 35.9, 48.4%, 55.4% and 24.5% greater, respectively, in plants grown in low-water conditions than in plants which grew in well-watered conditions.

Wind also significantly affected the efficiency in the use of water ($P < 0.001$, df 1, 63, in all the cases) and its effect was stronger than that of water or CO₂. Leaf area calculated as the ratio of leaf area gain to water uptake by the plant was 76.6% greater in the low wind speed treatment than in the high wind speed treatment. Leaf, stem and root dry weights were 68.9%, 77.1% and 71.9% greater, respectively, in plants which grew in low wind conditions than in those grown in high wind conditions.

As expected, plants which grew in enriched CO₂ atmospheres showed improved water use efficiencies. Water use efficiencies were affected significantly by CO₂. The effect of CO₂ was significant for leaf area, root dry weight ($P = 0.010$, df 1, 63, in both cases), stem length, stem dry weight ($P = 0.001$, df 1, 63, in both cases), leaf, shoot and total plant dry weights ($P < 0.001$, df 1, 63, in all the cases), but did not affect stem diameter ($F = 3.167$, $P = 0.080$, df 1, 63). On average, WUE expressed in terms of leaf area, leaf, stem and root dry weights, were 21.5%, 30.3%, 32.8% and 46.1% greater, respectively, in plants grown in high CO₂ levels than in those grown in ambient CO₂ atmospheres.

The analysis also showed significant CO₂ \times wind and wind \times water interactive effects. CO₂ \times wind interactions significantly affected stem length ($F = 5.133$, $P = 0.027$, df 1, 63), leaf area ($F = 4.262$, $P = 0.043$, df 1, 63) and leaf, stem, shoot and total plant dry weights (maximum $P = 0.032$, df 1, 63). For plants experiencing the same wind speeds, WUE was always greater in plants grown at high CO₂ levels than in plants grown at ambient CO₂ concentrations. The effect of CO₂ in improving WUE was greater for plants growing at low wind speeds than in plants at high wind speeds. In low wind conditions WUE was increased with a doubling of CO₂ by 23.8% for leaf area and by 33.6%, 48.9% and 35.0% for leaf, stem and root dry weight, respectively. CO₂ sensitivities of WUE for plants at high wind speed were 14.3% for stem length, 10.4% for leaf area and 18.4%, 32.3% and 23.3% for leaf, stem and root dry weights, respectively, with a doubling of CO₂. In spite of the higher WUE of plants at elevated CO₂, the reduced WUE at high wind speed was not ameliorated by growing under CO₂ enrichment. WUE expressed in terms of leaf area, leaf, stem and root dry weights is reduced by effect of wind stress in 74.6%, 65.0%, 72.6% and 69.2%, respectively, when plants grew at ambient CO₂ concentrations and in 78.3%, 71.5%, 79.4% and 73.8%, respectively, when plants grew at high CO₂ levels. Wind \times water interactions were significant for stem length and diameter, leaf area and dry weight of leaves, roots, shoots and total plant (in all the cases, $P < 0.026$, df 1, 63). For plants that experienced the same wind conditions, WUE was always greater in plants grown under low-water conditions than those grown in well-watered conditions. In low-wind conditions WUEs in terms of leaf area, leaf, stem and root dry weights were, respectively, 36.3%, 50.9%, 26.4% and 59.22% greater for

plants grown in low-water conditions than in plants grown under well watered conditions. At high wind speeds WUEs, expressed in terms of leaf area, leaf, stem and root dry weights were, respectively, 33.9%, 39.8%, 15.8% and 41.2% greater for plants grown under low-water conditions than in plants grown in well watered conditions. The effect of wind stress on WUE is slightly ameliorated by growing at well watered conditions. Thus, WUEs expressed in terms of leaf area, leaf, stem and root dry weights were reduced by effects of high wind speed in 77.0%, 71.1%, 78.4% and 75.3%, respectively, when plants were grown in low-water conditions and in 76.3%, 64.5%, 75.3% and 64.1%, respectively, when plants were grown in well-watered conditions.

Discussion

Growth parameters

Marked and positive effects of increased CO₂ concentrations on plant growth responses reported here in the early stages of plant growth are consistent with previous results which have shown that plants are more responsive to CO₂ enrichment when young (Tolley and Strain 1985; Garbutt et al. 1990; Ryle et al. 1992). This strong initial enhancement of growth in response to CO₂ enrichment declined in time, another response which has been commonly reported (Tolley and Strain 1984; Bazzaz et al. 1989; see also Cure and Acock 1986 and Bazzaz 1990). The cause of this response has not been well established, although in addition to limited enzyme capacity the most common explanation is an imbalance in the supply and demand of carbohydrates resulting in feedback inhibition or physical damage of the chloroplasts due to starch accumulation (Herold 1980; Ehret and Joliffe 1985; Jarvis 1989). A range of explanations for the decline has also been suggested by Bazzaz (1990). In this experiment, under natural daylight, the effects of a declining daylength cannot be discounted. The differential growth rates between CO₂ treatments, although not significant, have a noticeable effect on long-term biomass accumulation, which show, as with previous observations, that quite small differences in RGR have appreciable effects on final plant weight and size (Russell and Grace 1978; Coleman and Bazzaz 1992).

Of all of the treatments, increased wind has the most drastic effect on plant growth and plants were sturdier and smaller, features which have been previously reported (Whitehead 1962, 1963; Russell and Grace 1978, 1979; Grace and Russell 1977; Telewski and Jaffe 1986; Braam and Davis 1990; Retuerto and Woodward 1992). In the experiment reported here, wind speed did not alter the dry weight gain per unit of leaf area and so the reductions in dry weight with increased wind speed (Table 1) were a consequence of reduced rates of leaf expansion (Whitehead 1962). Like Russell and Grace (1978) and Retuerto and Woodward (1992) we reject the possibility that the small differences in leaf temperatures associated with the changes in wind speed were enough

to account for the observed decline on leaf expansion. It is also unlikely that the decline could have been caused by an adverse tissue water status when water is freely available as in the study of Retuerto and Woodward (1992) or in our well-watered treatment. Therefore, the suggestion by Russell and Grace (1979) that a mechanical stimulus might account for the most of the observed reduction in the leaf expansion rate of the wind exposed plants is possible. This is consistent with the findings of Braam and Davis (1990) who reported that in *Arabidopsis* a variety of mechanical stimuli, including wind, regulated the expression of at least five genes which could be involved in developmental growth alterations. Drought has also been suggested as an important cause of reduced growth in wind (Morse and Evans 1962; Kalma and Kuiper 1966). However, Russell and Grace (1978), Retuerto and Woodward (1992) and our present results suggest that wind reduces growth rates even when plants are not affected by drought.

Although ANCOVA did not detect any significant interactive effects on the growth parameters analysed we observed some interesting simultaneous effects of various treatments. The effect of high wind on growth was ameliorated by growth in well-watered conditions (Grace and Russell 1982). However a doubling in the CO₂ concentration did not counteract the effect of high wind (Table 1) and the CO₂ stimulation of growth was proportionally greater in plants grown in the low wind speed. The joint effects of CO₂ and water supply were statistically independent but the greater dry weights and leaf areas with CO₂ enrichment were more pronounced in the well-watered plants. As reported (Bazzaz and Carlson 1984) water limitation can greatly diminish growth stimulation by CO₂.

The significant interactive effect of plant age indicates that plant responses to environmental changes vary throughout the distinct morphological, physiological and developmental phases of ontogeny. This is an important point since the examination of plant responses to treatments at different times could lead to quite different interpretations of experiments. Coleman and Bazzaz (1992) have previously reported that harvest date may significantly affect the interpretations of CO₂ experiments and Retuerto and Woodward (1992) have made the same observation for wind experiments. The fact that the root system had not filled the pots allowed us to reject the possibility that root growth, and the age effect, could have been constrained by pot size and nutrient availability (Coleman and Bazzaz 1992).

Allocation and structural parameters

Eamus and Jarvis (1989) have argued that changes in allocation reflect a changed timing of development and some evidence supports this assertion (Sionit et al. 1987; Bazzaz et al. 1989). The analysis of the allometric relations using Model II regressions shows that under most of the treatments all points are on one line, that is, plants follow the same developmental track, in terms of biomass allocation. This suggests that most of the changes ob-

served in ratios could be an artificial effect of plant size (see Samson and Werk 1986). However, because growth rates differ with treatments, plants may be at different points along the same developmental track. Therefore, it is appropriate to compare plants both in terms of stages of development (biomass allocation) and in terms of growth and ratios (e.g. LAR, SLA) at a particular time.

Our results showed non-significant slightly higher root to shoot ratios and RWR in plants which grew at ambient CO₂ than in those grown in increased CO₂ levels. These results and the changes in root:shoot ratios and RWR from one harvest to another support the suggestion of no effect of atmospheric CO₂ on root/shoot allocation, even when water is limiting. No significant differences in root to shoot ratios were found for plants grown at different wind speeds, which is consistent with results from Retuerto and Woodward (1992) but contrary to many other results reported in the literature (Whitehead 1962; Whitehead and Luti 1962; Russell and Grace 1978; Grace and Russell 1982). Root:shoot ratios and RWR were, as expected, significantly higher in plants grown in low watered plants. Aronson et al. (1992) have reported that water stress leads to increased allocation of biomass to root growth in plants of desert and Mediterranean populations and Vartinian (1971) has found that during drought, the growth of *S. alba* taproot is accelerated. The fact that a larger proportion of assimilates was deployed to root production is particularly significant in maintaining an adequate water balance.

LAR was significantly higher at ambient CO₂ than at elevated CO₂ concentrations, as previously reported (Garbutt et al. 1990; Ryle et al. 1992). LAR also was significantly higher in plants grown in low wind speed than in plants grown in higher wind speed. Similar observations have been reported by Whitehead (1962), Retuerto and Woodward (1992) and Russell and Grace (1978) who suggested that most of the variation on LAR could be due to large reductions in SLA. LAR increased with water supply.

The reduced SLA under elevated CO₂ has been previously reported (DeLucia et al. 1985; Garbutt et al. 1990; Ryle et al. 1992, but see papers cited in Woodward et al. 1991). It has been suggested that plants with high SLA would be more wind-sensitive than plants with lower SLA (Woodward 1983) and that the reduction of SLA may be due to an increase in the bulk density of the leaf rather than an increased thickness (Grace and Russell 1982). The significantly higher SLA found in the well-watered plants is consistent with the suggestion of Woodward (1983) that high SLA plants would be more drought sensitive than plants with lower SLA. Grace and Russell (1977) and Pugnaire and Chapin (1992) have also found that water stress reduces SLA. The wind × water term in the ANCOVA showed that the positive effect of water supply on leaf area per unit of dry weight was significant at low wind but did not at high wind speeds, where plants did not increase their leaf areas with increasing water supply. If, as suggested above, most of the variation on LAR could be due to large reduction in SLA then the interactive wind × water effect detected on LAR could also be due to large reduction on SLA.

Leaf temperature, water use efficiency and stomatal density

ANCOVA did not detect any significant effect on leaf temperatures although, at least under low wind-speed conditions, higher leaf temperatures at increased levels of CO₂ has been suggested as result of lower transpiration rates (Surano et al. 1986; Idso et al. 1987). The uniform aerodynamic mixing within the chambers reduced the likelihood of significant differences in leaf temperatures among the treatments.

The experiment demonstrated improved water use efficiency for *Sinapis* grown at elevated CO₂ as a result of increased photosynthetic rates (Table 4). The mechanism by which CO₂ interacts with the process of stomatal opening is unclear but some possible mechanisms have been suggested (see Woodward et al. 1991). The commonly reported increased conductance and transpiration in plants grown in high wind speeds (Grace and Russell 1977, 1982; Russell and Grace 1978; Leite and Alvim 1978) is consistent with lower water diffusion resistances we observed in plants grown in high wind, although we did not find significant differences. It has also been reported that the cooling effect of wind on leaves can decrease the vapour pressure gradient and transpiration (Sena Gomes and Kozlowski 1989), a feature which is supported by results reported in other studies (Whitehead and Luti 1962; Dixon and Grace 1984). We also found lower water diffusion resistances in plants grown at low levels of water availability, a feature which is consistent with the reported high sensitivity of conductance to water vapour during drought (Grace and Russell 1977; Ticha 1982, cited by Woodward 1987). Chaves (1991) stated that this response is a quicker and more flexible process than alternatives such as changes in life cycle or in allocation patterns.

A number of studies have shown that stomatal density increases markedly as the CO₂ partial pressure is reduced below the current level but that stomatal density does not respond to CO₂ levels exceeding the current level (Woodward 1987; Woodward and Bazzaz 1988; Peñuelas and Matamala 1990). Insensitivity of the stomata to increased CO₂ has also been reported by Madsen (1973), and Thomas and Harvey (1983). Woodward (1988) has suggested that stomatal density may either increase or remain constant as the partial pressure of CO₂ is raised above 35 Pa for mature trees and shrubs. In contrast, Oberbauer et al. 1985 observed a reduction in stomatal density at levels of CO₂ higher than ambient. We observed this same effect in plants grown at low wind speeds but this tendency was reversed when plants grew at high wind speeds. That is, the effect of CO₂ on stomatal density is not independent of the wind speed conditions affecting the plants. Our findings that high wind speed exerts a positive but non-significant effect on the adaxial stomatal density is in agreement with results from Whitehead and Luti (1962) and Grace and Russell (1977) who have reported that the number of stomata per unit area rose with increased wind speed and with the corresponding failure of the leaf to expand. We did not detect a significant effect of water supply on the number of stoma-

ta which is somewhat in contrast to the more frequent and smaller stomata per unit area reported by Grace and Russell (1977). However, Woodward (1987) has noted that in many cases where drought seems to influence stomatal density, it does so by affecting the expansion of leaf area, causing the stomata to be packed more densely. Thus, when the effects of epidermal cell size are taken into account the drought effect may disappear.

We detected significant wind \times water and CO₂ \times wind effects on WUE. As hypothesized, the WUE of plants grown at high wind was ameliorated by growing in well watered conditions, although the small effect of water supply in improving the performance of plants grown under high wind confirms that drought is not the main cause of reduced growth in high wind and that mechanical stimulation could be the important cause. Although increased CO₂ improved WUE of plants grown both in low and in high wind, the beneficial effect of elevated CO₂ on WUE was proportionally greater in plants grown in low wind. This result is in contrast to expectations that wind stress will be ameliorated more in plants grown in enriched CO₂, than in plants grown in ambient CO₂, again suggesting that water stress was not the main cause of reduced growth in high wind.

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