

## Transpiration, Intercellular Carbon Dioxide Concentration and Carbon-isotope Discrimination of 24 Wild Species Differing in Relative Growth Rate

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### Abstract

Various aspects of the water economy were investigated for a range of herbaceous species varying in relative growth rate. Plants were grown in a growth chamber with a non-limiting supply of water and nutrients, and the rate of transpiration, short-term intercellular CO<sub>2</sub> concentration and long-term carbon isotope discrimination ( $\Delta$ ) in the leaves were determined. No correlation was found between the relative growth rate of these species, and the transpiration rate per unit leaf area, the intercellular CO<sub>2</sub> concentration and the <sup>13</sup>C-discrimination. There was a positive correlation, however, with the rate of water uptake per unit root weight. From these observations we infer that the previously observed differences in photosynthetic nitrogen-use efficiency, the rate of photosynthesis per unit leaf nitrogen, can not be explained by variation in intercellular CO<sub>2</sub> concentration.

These data were also used to analyse correlations between  $\Delta$  and both growth parameters and chemical composition. Apart from parameters related to the water economy,  $\Delta$  only correlated (positively) with the fractional biomass allocation to the roots (root weight ratio) and the specific root length (root length divided by root weight), and not with any other investigated growth parameters.

### Introduction

When a range of plant species from a variety of habitats is grown under identical, close to optimum conditions, a large interspecific variation in growth rate is found, with species naturally occurring in nutrient-rich habitats showing much higher relative growth rates than species from nutrient-poor sites (Grime and Hunt 1975; Poorter and Remkes 1990). Generally, these differences in relative growth rate (RGR) are associated more with differences in the leaf area ratio (LAR, leaf area:total plant weight) than with variation in the growth parameter net assimilation rate (the increase in plant weight per unit leaf area and time; Lambers and Poorter 1992). In the few cases where interspecific variation in RGR was analysed at the physiological level often no correlation was found at all between RGR and the rate of photosynthesis per unit leaf area (Evans and Dunstone 1970; Poorter *et al.* 1990). This is not to say that leaves of all species function in an exactly similar manner. In an analysis of the growth of 24 herbaceous species, the fast-growing species achieved this rate of photosynthesis with a lower investment of organic nitrogen per unit leaf area than the slow-growing ones. Consequently, their photosynthetic nitrogen-use efficiency (PNUE, the rate of photosynthesis per unit organic leaf nitrogen) was higher (Poorter *et al.* 1990).

A difference in PNUE between species can be achieved in several ways (Evans 1989; Lambers and Poorter 1992). One explanation for the higher PNUE of fast-growing species might be that they operate at higher intercellular CO<sub>2</sub> concentration and therefore (a) suppress oxygenation to a larger extent and (b) supply substrate at a higher rate (cf. Farquhar and von Caemmerer 1982). However, as their rate of photosynthesis per unit leaf

area does not differ systematically from that of slow-growing species, this implies that fast-growing species would have a higher leaf conductance and, consequently, a lower short-term water-use efficiency ( $WUE_p$ , the ratio of photosynthetic carbon gain over water loss; Farquhar *et al.* 1989). The first goal of this paper is to test whether this hypothesis holds. To this end we measured the rate of transpiration, the intercellular  $CO_2$  concentration relative to the ambient one ( $c_i/c_a$ ) and the carbon-isotope composition (Farquhar *et al.* 1982) of the leaves of the same 24 species that have formerly been described with respect to growth, carbon and nitrogen economy, and chemical composition (Poorter and Remkes 1990; Poorter *et al.* 1990; Poorter *et al.* 1991; Poorter and Bergkotte 1992).

Carbon-isotope discrimination is frequently used not only to estimate the  $c_i/c_a$  ratio, but also as a probe of long-term water-use efficiency ( $WUE_g$ , the total amount of biomass accumulated over the amount of water transpired during a time interval; Farquhar *et al.* 1989; Condon *et al.* 1990; Hubick 1990). By doing so, emphasis shifts from the gas-exchange at the level of the leaf to that of the physiology of the whole plant. Consequently, other plant attributes like leaf and root morphology, biomass allocation and chemical composition may modulate the relationship between  $\Delta$  and  $WUE_g$ . Aiming at a more thorough understanding of plant performance, relations have therefore been sought between carbon isotope discrimination and a range of parameters like yield, LAR, specific leaf area, root length density and leaf mineral content (Masle and Farquhar 1988; Virgona *et al.* 1990; White *et al.* 1990; Masle *et al.* 1992; Mayland *et al.* 1993). These correlations have been obtained by varying environmental conditions or comparing genotypes within a species or genus. Most of these experiments have been conducted in the field, or in pots in glasshouses, where both the availability of light, nutrients and water vary with time and with the size of the plants. A second goal of this paper is to investigate whether inherent differences in discrimination measured for a wide range of herbaceous species correlate with other plant characteristics, for plants grown under conditions of non-limited nutrient and water supply and a constant light intensity and vapour pressure deficit.

#### List of Variables Used in the Text

<i>Variable</i>	<i>Definition</i>	<i>Units</i>
$c_a$	ambient $CO_2$ concentration	$\mu\text{mol mol}^{-1}$
$c_i$	intercellular $CO_2$ concentration	$\mu\text{mol mol}^{-1}$
LAR	leaf area ratio (leaf area: total plant weight)	$\text{m}^2 \text{kg}^{-1}$
LWR	leaf weight ratio (leaf weight: total plant weight)	$\text{g g}^{-1}$
NAR	net assimilation rate (growth parameter, rate of dry matter increase per unit leaf area)	$\text{g m}^{-2} \text{day}^{-1}$
NP	nitrogen productivity (rate of dry matter increase per unit organic plant nitrogen)	$\text{g (mol N)}^{-1} \text{day}^{-1}$
PNUE	photosynthetic nitrogen-use efficiency (rate of photosynthesis per unit organic leaf nitrogen)	$\mu\text{mol (mol N)}^{-1} \text{s}^{-1}$
RGR	relative growth rate (rate of dry matter increase per unit dry matter)	$\text{mg g}^{-1} \text{day}^{-1}$
SLA	specific leaf area (leaf area: leaf dry weight)	$\text{m}^2 \text{kg}^{-1}$
SRL	specific root length (root length: root dry weight)	$\text{m g}^{-1}$
$TR_a$	transpiration rate per unit leaf area	$\text{mmol m}^{-2} \text{s}^{-1}$
VPD	vapour pressure deficit	kPa
$WUE_g$	water-use efficiency at growth level (biomass accumulated over water transpired)	$\text{g kg}^{-1}$
$WUE_p$	water-use efficiency of photosynthesis ( $CO_2$ fixed per unit water transpired)	$\text{mmol mol}^{-1}$
$\Delta$	carbon isotope discrimination	‰

## Material and Methods

### Growth of the Plants

Plants of 24 wild species, from a wide range of habitats in western Europe, were grown in nutrient solution in a growth chamber. These species were the monocotyledons *Brachypodium pinnatum* (L.) Beauv., *Briza media* L., *Corynephorus canescens* (L.) Beauv., *Cynosurus cristatus* L., *Dactylis glomerata* L., *Deschampsia flexuosa* (L.) Trin., *Festuca ovina* L., *Holcus lanatus* L., *Lolium perenne* L., *Phleum pratense* L. and *Poa annua* L., and the dicotyledons *Anthriscus sylvestris* (L.) Hoffm., *Galinsoga parviflora* Cav., *Geum urbanum* L., *Hypericum perforatum* L., *Lysimachia vulgaris* L., *Origanum vulgare* L., *Pimpinella saxifraga* L., *Plantago major* ssp. *major* L., *Rumex crispus* L., *Scrophularia nodosa* L., *Taraxacum officinale* Weber, *Trifolium repens* L. and *Urtica dioica* L.

Details about collection of the seeds and germination conditions are given in Poorter and Remkes (1990). After germination the seedlings were placed in a growth room with the following conditions: Day: 14 h, photosynthetic photon flux density  $315 \pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ , temperature  $20 \pm 0.5^\circ\text{C}$ , vapour pressure deficit 0.8 kPa. Night: 10 h, temperature  $20 \pm 0.5^\circ\text{C}$ . Light was provided by fluorescent lamps (Philips TL-33-RS, 215 W) and incandescent bulbs (Philips, 40 W) in a ratio of 4:1. Plants were grown without mutual shading. The nutrient solution, a modified Hoagland solution with a  $\text{NO}_3^-$  concentration of 2 mM (Poorter and Remkes 1990), was replenished at least once a week. With this frequency of replenishment the  $\text{NO}_3^-$  concentration in the nutrient solution never dropped below 1 mM. Plant dry weight was determined six times (eight replicates per harvest) during a period of 17 days, starting when plants had a fresh weight of approximately 100 mg. Full details are given in Poorter and Remkes (1990).

### Gas Exchange

Whole shoot gas exchange was measured on intact plants (eight individuals per species), placed in a cuvette with shoot and roots in separate compartments (Poorter and Welschen 1993). The photosynthetic photon flux density and temperature were the same as in the growth room.  $\text{CO}_2$  and  $\text{H}_2\text{O}$  exchange were measured differentially with infrared gas analysers (ADC, model 225 MK3, Hoddesdon, UK) in an open system. Calculations of the rate of photosynthesis, transpiration and shoot respiration were made according to Caemmerer and Farquhar (1981), with the correction of the IRGA output suggested by Bunce and Ward (1985). As it was not possible to arrive at exactly the same VPD in all cuvettes, we standardised all transpiration rates to a VPD of 0.8 kPa. In the calculation of the internal  $\text{CO}_2$  concentration,  $c_i$ , the average leaf temperature over the whole shoot was assumed to be equal to the air temperature.

### Isotope Discrimination

Carbon isotope discrimination was determined on two independent bulk samples of leaf material, collected during the growth experiment. Each sample was ground and a subsample of 5–10 mg was combusted to  $\text{CO}_2$ . Carbon isotope composition ( $\delta_p$ ) of the purified  $\text{CO}_2$  was determined with a VG SIRA 24 ratio mass spectrometer, using PDB as a standard. The discrimination against  $^{13}\text{C}$ ,  $\Delta$ , was calculated as:

$$\Delta = (\delta_a - \delta_p) / (1 + \delta_p),$$

with the isotopic composition of the air ( $\delta_a$ ) taken -8‰ relative to PDB (Mook *et al.* 1983; Farquhar *et al.* 1989).

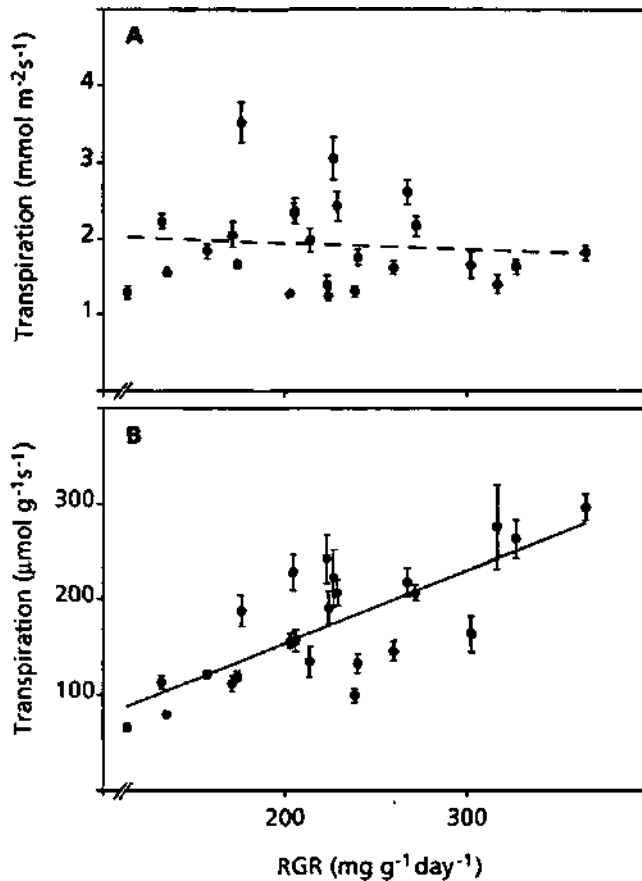
### Statistical Analysis

Relations between variables were calculated from linear regression equations and tested for significance with the SPSS statistical program with the  $H_0$ -hypothesis of no relationship.

## Results

Relative growth rates, calculated over a common dry weight trajectory of 30–100 mg to correct for possible size-dependency, varied between species, the fastest growing species (*Galinsoga parviflora*) having a more than three times higher RGR than the slowest growing one (*Corynephorus canescens*). No significant differences in the rate of transpiration per

unit leaf area were found between fast- and slow-growing species (Fig. 1A). However, the rate of water uptake per unit root dry weight increased 3-fold with RGR (Fig. 1B,  $P < 0.001$ ).



**Fig. 1.** Rate of transpiration (A) per unit leaf area, and (B) per unit root dry weight of 24 species differing in relative growth rate (mean values  $\pm$  s.e.,  $n = 8$ ). The linear regression line fitted through the points is not significant in (A) ( $P > 0.5$ ,  $r^2 = 0.01$ ) and significant in (B) ( $P < 0.001$ ,  $r^2 = 0.59$ ).

Species with a high rate of transpiration per unit leaf area had a higher  $c_i/c_a$  ratio, and showed larger discrimination against  $^{13}\text{C}$ , and as expected, a lower  $\text{WUE}_p$  (Table 1). Furthermore, there was a significant positive correlation between the  $c_i/c_a$  ratio, as determined in the short-term gas-exchange measurements, and the  $\Delta$  of the leaves, although the correlation was not very tight ( $P < 0.05$ ,  $r = 0.41$ ). No significant relationship was found between the RGR of the different species and their  $\text{WUE}_p$  (Fig. 2) or with their  $c_i/c_a$  ratio (Fig. 3A). Similarly, there was no correlation between RGR and the carbon-isotope discrimination (Fig. 3B).

**Table 1. Correlation diagram (Pearson product moment correlation coefficients) between transpiration per unit leaf area ( $TR_a$ ,  $c_i/c_a$ ,  $WUE_p$  and  $\Delta$ , and a range of physiological and morphological growth parameters as well as the dry matter % and the nitrogen and mineral content of leaves and whole plants**

Absence of any  $r$ -value indicates a correlation with a  $P$ -value larger than 0.1. Data are from Poorter and Remkes (1990), Poorter *et al.* (1990) and Poorter and Bergkotte (1992)

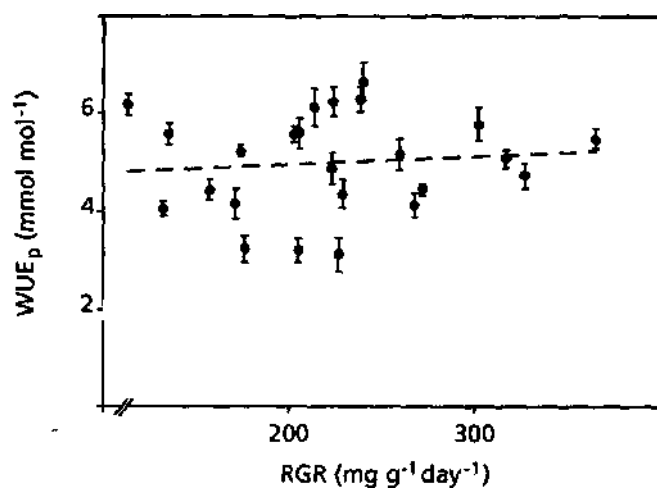
	$TR_a$	$c_i/c_a$	$WUE_p$	$\Delta$
$TR_a$		0.77 <sup>D</sup>	-0.75 <sup>D</sup>	0.58 <sup>C</sup>
$c_i/c_a$	0.77 <sup>D</sup>		-0.99 <sup>D</sup>	0.41 <sup>B</sup>
$WUE_p$	-0.75 <sup>D</sup>	-0.99 <sup>D</sup>		-0.41 <sup>B</sup>
$\Delta$	0.58 <sup>C</sup>	0.41 <sup>B</sup>	-0.41 <sup>B</sup>	
RGR				
NAR	0.36 <sup>A</sup>			
LAR				
SLA				
LWR	-0.51 <sup>B</sup>			-0.47 <sup>B</sup>
RWR	0.59 <sup>C</sup>	0.46 <sup>B</sup>	-0.45 <sup>B</sup>	0.54 <sup>C</sup>
Photosynthesis/leaf area	0.53 <sup>B</sup>			
PNUE				
Nitrogen productivity				
Specific root length				0.47 <sup>B</sup>
N leaf/leaf area	0.36 <sup>A</sup>			
Minerals leaf/leaf weight	0.50 <sup>B</sup>			
Minerals plant/plant weight				
Dry matter % leaf				
Dry matter % plant				

<sup>A</sup>0.05 <  $P$  < 0.10.

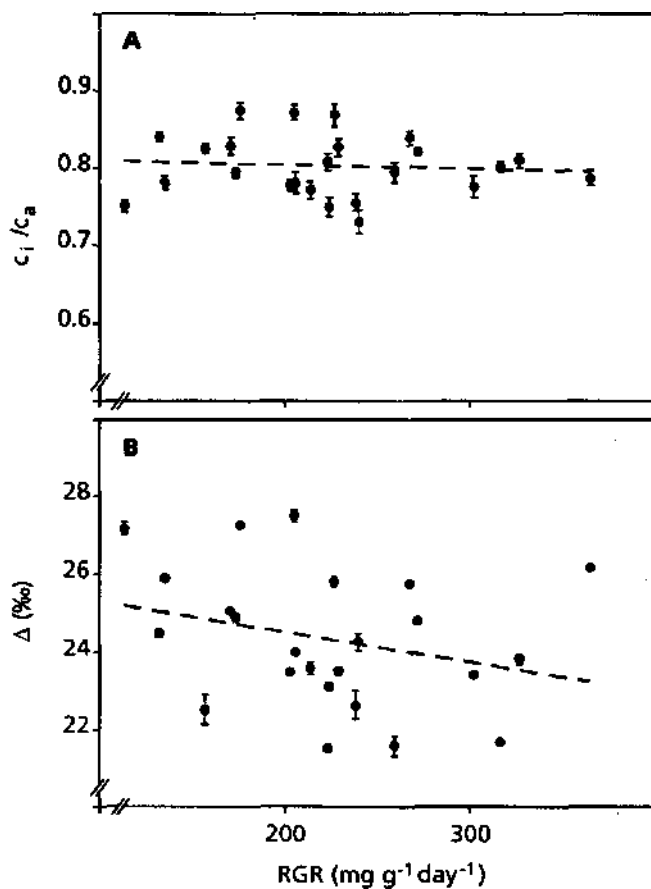
<sup>B</sup> $P$  < 0.05.

<sup>C</sup> $P$  < 0.01.

<sup>D</sup> $P$  < 0.001.



**Fig. 2.** Photosynthetic water-use efficiency (mmol CO<sub>2</sub> fixed in photosynthesis per mol of H<sub>2</sub>O transpired) of 24 species differing in relative growth rate (mean values  $\pm$  s.e.,  $n = 8$ ). The regression line fitted through the points is not significant ( $P > 0.5$ ,  $r^2 = 0.01$ ).



**Fig. 3.** (A) Ratio of intercellular over ambient CO<sub>2</sub> concentration in leaves of 24 species differing in relative growth rate (mean values  $\pm$  s.e.,  $n = 8$ ). (B) Discrimination against <sup>13</sup>C as determined by the <sup>13</sup>C/<sup>12</sup>C ratio of the leaves of the 24 species. Values are the average  $\pm$  s.e. of two independent bulk samples of leaves collected during the experiment. The regression lines fitted through the points are not significant in both (A) ( $P > 0.5$ ,  $r^2 = 0.01$ ) and (B) ( $P > 0.1$ ,  $r^2 = 0.08$ ).

There were hardly any other significant correlations between WUE<sub>p</sub>,  $\Delta$  or  $c_i/c_a$  ratio on one hand, and a suite of growth and allocation characteristics on the other (Table 1). A high transpiration rate,  $\Delta$ ,  $c_i/c_a$  ratio and a low WUE correlated with a high allocation of biomass to the roots. Species with a high specific root length had higher values for  $\Delta$  and species with a high mineral concentration in the leaves were those that had a high transpiration per unit leaf area. However, neither of these last two correlations was very strong. No correlations at all were found with LAR, SLA, nitrogen productivity (the increase in dry weight per unit nitrogen in the plant), the PNUE and the dry matter percentage (dry weight: fresh weight  $\times 100$ ) of the plants or its composing organs.

### Discussion

No differences were found in the rate of transpiration per unit leaf area between fast- and slow-growing species, grown with an ample supply of nutrients and water (Fig. 1A).

As no correlation between RGR and the rate of photosynthesis per unit leaf area was found for the same species either (Poorter *et al.* 1990) it is not surprising to find no systematic differences in  $\text{WUE}_p$  (Fig. 2).

No differences were observed in the  $c_i/c_a$  ratio of fast- and slow-growing species, either when calculated from short-term gas exchange or when derived from the carbon-isotope composition of leaf dry matter bulked over the whole experiment. Two complications may arise from the use of the carbon-isotope composition of the leaves as a long-term integrated indicator of  $c_i/c_a$ . Firstly, the  $^{13}\text{C}$  contents of stems and roots do not necessarily match those of the leaves. In the few cases where  $\Delta$  of stem and roots were investigated, they were generally lower than those of leaves (Yoneyama and Ohtani 1983; Hubick *et al.* 1986). Hubick *et al.* (1986) concluded that, although  $\Delta$  values of the three organs were well correlated,  $\Delta$  of the whole plant showed a better correlation with  $\text{WUE}$  than did  $\Delta$  of leaves only. As the slow-growing species allocate more biomass to roots and stems than fast-growing species, they might be expected to have a lower overall  $\Delta$ . Secondly,  $\text{CO}_2$  released in respiration is enriched in  $^{13}\text{C}$  relative to the plant biomass itself (Park and Epstein 1961; Troughton *et al.* 1974, cited in Yoneyama and Ohtani 1983, but see Caemmerer and Evans 1991). Consequently, due to respiration,  $\Delta$  of the remaining biomass will increase. Thus, species that respire a larger fraction of the  $\text{CO}_2$  fixed daily might have a relatively higher  $\Delta$  in their biomass than expected from short-term measurements during photosynthesis. For the 24 species investigated here, slow-growing ones respired proportionally more of the carbon fixed daily than the fast-growing ones (Poorter *et al.* 1990). Therefore, both differences in biomass allocation and respiration are expected to decrease the estimated discrimination at the site of Rubisco more in slow-growing species than in faster-growing ones, implying that we might have overestimated the average  $c_i/c_a$  in the slow-growing species by using  $\Delta$  values of leaves. However, assuming  $\Delta$  in stems and roots to be 1% lower than that in leaves (which is at the high side of the values reported by Yoneyama and Ohtani (1983) and Hubick *et al.* (1986)), and a  $\Delta$  in the  $\text{CO}_2$  derived from respiration 5% lower than that in the plant's biomass (Park and Epstein 1961; Troughton *et al.* 1974 cited in Yoneyama and Ohtani 1983), and using the observed allocation patterns (Poorter and Remkes 1990) and rates of photosynthesis and respiration (Poorter *et al.* 1990) still no significant differences in discrimination emerge.

Another complication in the use of carbon isotope discrimination in the present experiment is that physical space in the growth chamber did not allow us to grow all species at the same time. Therefore, we cannot be entirely sure that the isotopic composition of the air in the growth chamber was the same for all species. However, care was taken that conditions were similar throughout the experiment and that species which were expected to have similar RGRs were distributed equally over the whole experimental period. Indeed, no correlation was found between the RGR of the various species and the order in which they were investigated, and the same applied to  $\Delta$ . Thus, if any relationship between  $\Delta$  and RGR were to exist, the correlation observed might be less tight due to possible background variation in  $^{13}\text{C}$ , but we expect the general trend not to be affected. As there is no trend at all (Fig. 3B), we assume no relationship at all. As an additional check we grew two of the grass species of this experiment, the slow-growing *Deschampsia flexuosa* and the fast-growing *Holcus lanatus* again, but now at the same time rather than 3 months apart as during the large experiment. The plants in this second experiment showed exactly the same difference in  $\Delta$  as the first time.

From the evidence presented above, we conclude that there are no systematic differences in  $c_i/c_a$  between the fast- and slow-growing herbaceous species, neither when measured directly nor when inferred from carbon isotope discrimination. Therefore, the previously observed differences in PNUE (Poorter *et al.* 1990) cannot be explained by variation in intercellular  $\text{CO}_2$  concentration. Alternative explanations for the observed variation in PNUE could be that fast-growing species do invest proportionally more of their organic nitrogen in the photosynthetic apparatus than slow-growing species, that slow-growing species

do suffer from feed-back inhibition of photosynthesis, or that slow-growing species, which have higher concentrations of chlorophyll and organic nitrogen per unit leaf area, suffer from internal shading within the leaves (e.g. Evans 1989; Lambers and Poorter 1992; Pons *et al.* 1994).

Although the rate of transpiration per unit leaf area was similar between fast- and slow-growing species (Fig. 1A), the rate of water uptake per unit root weight is *ca* 3 times higher for the fast-growing ones (Fig. 1B). This is due to the much larger leaf area:root weight ratio for those species compared to the slow-growing ones (cf. Poorter and Remkes 1990). Under conditions of unlimited water supply and low VPD, this apparently poses no problems for the fast-growing species. It would be of interest to analyse whether roots have an overcapacity to absorb water anyway (cf. Brouwer 1963), or whether this rather large difference has brought about anatomical or morphological adaptations in roots of the fast-growing species. Furthermore, if these plants would have grown under water-limiting conditions or at a higher VPD, the water uptake rate might be restricted in an earlier phase for fast- than for slow-growing species. This would imply either a stronger decrease in the leaf area: root weight ratio or a stronger decrease in  $c_i/c_a$  or photosynthesis for the former group under such circumstances.

A second goal of this paper was to explore correlations between  $\Delta$  and growth and allocation characteristics. Most studies on the relation between  $\Delta$  and productivity have focused on grain yield or final biomass of drought-stressed plants. Interestingly, Condon *et al.* (1987) also found a positive correlation between above-ground biomass and grain yield on one hand, and  $\Delta$  on the other, in field-grown *Triticum aestivum* which was well-watered. However, above-ground biomass, although relevant from an agronomic perspective, is not necessarily linked to physiological or morphological growth parameters, but might also be the result of variation in seed biomass, germination time, or duration of the period of growth. Masle and Farquhar (1988) observed a relationship between RGR and  $\Delta$ , but this correlation was environmentally induced rather than genotypical. Virgona *et al.* (1990) investigated carbon-isotope discrimination of genotypes of *Helianthus annuus* and concluded that the highest discrimination was in genotypes with the highest leaf area ratio (LAR, leaf area:total plant weight). This was at least partly due to the correlation of  $\Delta$  with the specific leaf area (SLA, leaf area:leaf dry weight). Such a correlation contrasts with results on *Phaseolus* (White *et al.* 1990), where no relation was observed between SLA and  $\Delta$ . Masle *et al.* (1992) observed a strong positive correlation between  $\Delta$  and ash content of the leaves of genotypes of four crop species, and concluded that, at least for these species, mineral content may even be an easier criterion to select for high  $WUE_g$  than carbon isotope composition. These results were confirmed by Mayland *et al.* (1993).

All of the above correlations have been made within a range of genotypes of a particular species. If a general and causal relationship were to exist between the various growth characteristics and  $\Delta$ , we expect them to show up in the present experiment as well. To check this, we constructed a correlation diagram, assuming linear relationships between variables (Table 1). As can be seen in this table, there are not many correlations anyway between  $\Delta$  on the one hand, and a suite of growth characteristics on the other. In fact, apart from parameters directly linked with transpiration ( $c_i/c_a$ ,  $WUE_p$ ),  $\Delta$  only showed a significant correlation with biomass allocation to roots and the specific root length. Thus, we conclude that most of the above-mentioned correlations, observed within a species, do not imply a general and causal relationship valid across species. However, it should be borne in mind that the present experiment was carried out with hydroponically-grown plants at a non-limiting supply of nutrients and a relatively low VPD and light intensity. Thus, water stress is not likely to have occurred at all, in contrast with plants grown in pots in the glasshouse or in the field. There, plants will always experience some water stress, even when watered daily. Therefore, in the environments where breeding and selection for efficient genotypes occurs, relations between  $\Delta$  and growth parameters may show up more readily.



## Conclusions

In this experiment there is no systematic difference in transpiration per unit leaf area,  $c_i/c_a$  or carbon isotope concentration between potentially fast- and slow-growing species. The previously observed positive correlation between PNUE and potential RGR can therefore not be due to variation in the intercellular CO<sub>2</sub> concentration. Hardly any correlations were found between  $\Delta$  and growth and allocation characteristics in this set of species with widely contrasting relative growth rates.

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