Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C$_4$ species *Sorghum bicolor* in the glasshouse and the field

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Abstract. Transpiration efficiency, W, the ratio of plant carbon produced to water transpired and carbon isotope discrimination of leaf dry matter, Δ_d, were measured together on 30 lines of the C$_4$ species, *Sorghum bicolor*, in the glasshouse and on eight lines grown in the field. In the glasshouse, the mean W observed was 4.9 mmol C mol^{-1} H$_2$O and the range was 0.8 mmol C mol^{-1} H$_2$O. The mean Δ_d was 3.0‰ and the observed range was 0.4‰. In the field, the mean W was lower at 2.8 mmol C mol^{-1} H$_2$O and the mean Δ_d was 4.6‰. Significant positive correlations between W and Δ_d were observed for plants grown in the glasshouse and in the field. The observed correlations were consistent with theory, opposite to those for C$_3$ species, and showed that variation in Δ_d was an integrated measure of long-term variation in the ratio of intercellular to ambient CO$_2$ partial pressure, p_i/p_a. Detailed gas exchange measurements of carbon isotope discrimination during CO$_2$ uptake, Δ_A, and p_i/p_a were made on leaves of eight *S. bicolor* lines. The observed relationship between Δ_A and p_i/p_a was linear with a negative slope of 3.7‰ in Δ_A for a unit change in p_i/p_a. The slope of this linear relationship between Δ_A and p_i/p_a in C$_4$ species is dependent on the leakiness of the CO$_2$ concentrating mechanism of the C$_4$ pathway. We estimated the leakiness (defined as the fraction of CO$_2$ released in the bundle sheath by C$_4$ acid decarboxylations, which is lost by leakage) to be 0.2. We conclude that, although variation in Δ_d observed in the 30 lines of *S. bicolor* is smaller than that commonly observed in C$_3$ species, it also reflects variation in transpiration efficiency, W. Among the eight lines examined in detail and in the environments used, there was considerable genotype x environment interaction.

Introduction

In C$_3$ species, genetic variation in carbon isotope discrimination of leaf dry matter, Δ_d, has been linked to variation in transpiration efficiency, W, the ratio of dry matter produced to water transpired by the plant (for review see Farquhar *et al*. 1989*b*). This correlation occurs because of independent linear relationships of Δ_d and W to the ratio of intercellular to ambient partial pressures of CO$_2$, p_i/p_a, in leaves (Farquhar and Richards 1984). As whole-plant W is difficult to measure on a large scale, the association between Δ_d and W has proved interesting to plant breeders in selecting lines of C$_3$ species with increased W (e.g. Hubick *et al*. 1988; Hall *et al*. 1989, 1994). In this paper we examine the usefulness of Δ_d as a measure of W in C$_4$ species.

CO$_2$ fixation in C$_4$ species is characterised by a CO$_2$-concentrating mechanism that requires the coordinated function of mesophyll and bundle-sheath cells within a leaf. CO$_2$ is initially assimilated into C$_4$ acids by phospho*enol*pyruvate (PEP) carboxylase in the mesophyll cells. These acids are transported to and decarboxylated in

the bundle sheath, where the CO$_2$ is refixed by ribulose bisphosphate carboxylase-oxygenase (Rubisco). Carbon-isotope discrimination in C$_4$ species reflects these two different carboxylations. It is dependent not only on the ratio of intercellular to ambient partial pressure of CO$_2$, p_i/p_a, but also on leakiness, ϕ (Farquhar 1983), the proportion of CO$_2$ released into the bundle sheath by the C$_4$-acid cycle which is not fixed by Rubisco but leaks back to the mesophyll. It is a measure of the extent to which PEP carboxylations exceed Rubisco carboxylations. The value of ϕ determines the magnitude of the slope of the relationship between Δ and p_i/p_a (Farquhar 1983).

Various estimates of ϕ have been made employing a variety of techniques (Hatch *et al*. 1995). In a study of on-line carbon isotope discrimination, Henderson *et al*. (1992) estimated ϕ to be constant at 0.2 under a range of environmental conditions of different irradiances, temperatures and ambient partial pressures of CO$_2$ for *S. bicolor* (L.) Moench and *Amaranthus edulis* Speg. They also found ϕ to be 0.2 in a number of C$_4$ species of the various C$_4$-decarboxylation types. Since there was little

variation in ϕ, carbon isotope discrimination was, as in C_3 species, linearly related to p_i/p_a. Hence the possibility exists that Δ_d can be used as a tool for estimating transpiration efficiency, W, in C_4 species. The major disadvantage is the much reduced signal, as the slope of the relationship between Δ_d and p_i/p_a is much less for C_4 species than C_3 species, which may make it difficult to differentiate between lines.

Genetic variation in W and Δ_d has been reported in separate studies of C_4 species. For instance, Owonubi *et al*. (1982), Onken *et al*. (1986) and Hammer *et al*. (1990) found significant differences in W for *S. bicolor* lines. A greater number of studies has found genetic variation in p_i/p_a, CO_2 assimilation rate or stomatal conductance which, in turn, could result in genetic variation in W (for *S. bicolor*, Henzell *et al*. 1976; Krieg 1983; Blum and Sullivan 1986; Kidambi *et al*. 1990; Peng *et al*. 1991; Donatelli *et al*. 1992; for *Zea mays*, Duncan and Hesketh 1968; Gaskel and Pearce 1981; for sugarcane, Irvine 1975; and *Pennisetum americanum*, Blum and Sullivan 1986). Genetic variation in Δ_d has also been reported in C_4 species; for example, for lines of *Zea mays* (Samejima 1984), *Panicum coloratum* (Ohsugi *et al*. 1988), *S. bicolor* (Hubick *et al*. 1990) and *Saccharum* spp. (Meinzer *et al*. 1994). O'Leary (1988), however, found no significant variation in Δ_d among 120 lines of *Z. mays*.

To test whether variation in Δ_d is correlated with variation in W, we grew 30 *S. bicolor* lines in the glasshouse in large sealed pots and measured W as well as Δ_d. The same lines were also grown in the field at Emerald, Central Queensland, to determine variation in Δ_d in the field. In the subsequent 2 years, further field measurements were made of Δ_d and W on a selected set of eight lines. These eight lines were also grown in the glasshouse in Canberra for detailed measurements of carbon isotope discrimination during CO_2 uptake, Δ_A, in the laboratory.

Materials and methods

Experiment 1

Thirty lines of *S. bicolor* (L.) Moench. were grown in the glasshouse in Canberra in sealed pots to measure transpiration efficiency, W, and Δ_d. The experiment was conducted in November and December 1988 in an air-conditioned glasshouse with mean day and night temperatures of $33/20^{\circ}C$, a mean relative humidity of 60% and a midday irradiance of 1500 µmol quanta m^{-2} s^{-1}.

Before seeds were sown, pots were flushed with a nutrient solution containing 12 mM nitrate (Hewitt and Smith 1975). They were allowed to drain overnight. They were then sealed with rubber stoppers and weighed with an electronic load cell mounted on a gantry device, with a hand-winch, which was used to lift the pots. The soil was assumed to be at field capacity, and the weight of the pots was recorded as the initial weight.

Seeds were obtained from R. Henzell, Queensland Department of Primary Industries (Table 2). Four seeds were planted per pot. Pots were 1 m high and 15 cm in diameter and contained approximately 20 kg of sterilised soil which consisted of one part sand and four parts sandy loam.

A day after emergence, seedlings were thinned to one per pot. Four replicate plants were grown for each line and pots were well spaced and arranged in a randomised design. One week after seedlings had emerged,

the tops of the pots were covered with a layer of gravel 7 cm thick to minimise soil evaporation. The pots were weighed regularly and water lost from the pots was replaced every 2–3 days with known amounts of water or nutrient solution. Water loss was also measured in pots with no plants and from this soil evaporation was calculated to be approximately 10% of the total water loss. At harvest, pots were weighed and water use was calculated as initial weight minus final weight (taking into account the gravel added to the pots) plus the volume of water and nutrient added during the experiment, minus soil evaporation.

Plants were harvested at approximately 30 days after emergence. Shoots were separated from roots at the soil surface, separated into leaves and stems, and soil was washed from the roots. Leaves, stems and roots were dried in an oven at $80^{\circ}C$ for at least 72 h before weighing. In all experiments, transpiration efficiency was calculated as grams dry matter produced per kg of water used and was converted to mmol C produced per mol H_2O used by assuming that dry matter in *S. bicolor* consists of 40% carbon (McCree 1986). In fact the ash content varies inversely with transpiration efficiency in sorghum (Masle *et al*. 1992) and this makes the C content vary systematically. However, the errors involved are small.

To measure Δ_d, leaves were ground finely. Samples of 0.6 ± 0.2 mg were combusted in a Carlo Erba (Model 1108, Italy) elemental analyser and the CO_2 was then analysed by static dual inlet measurements using a VG Isogas SIRA 24 ratio mass spectrometer. The standard deviation on replicated sucrose samples was 0.1‰. The standard deviation on a dry matter sample, determined on 10 replicates, was 0.12‰. Δ_d was calculated as described by Farquhar and Richards (1984) from the carbon isotope composition of the samples with respect to the standard Pee Dee Belemnite (PDB), δ_p, and the carbon isotope composition of the glasshouse air, δ_a, as $\Delta_d = (\delta_a - \delta_p)/(1 + \delta_p)$.

Experiment 2

Lines of *S. bicolor* (Trump, Trojan II, 623/430, 378/430, Tx610SR, White Charger, Hylan 4x8 and Success 40W) were grown in an air conditioned glasshouse in Canberra from January to March 1991. The glasshouse conditions were as in Experiment 1. Plants were grown in 5 L free-draining pots containing sterilised garden soil, with one plant per pot. Plants were watered twice daily and received a complete nutrient solution containing 12 mM nitrate, three times a week (Hewitt and Smith 1975).

The youngest fully expanded leaves were used for gas exchange measurements (leaves 4 to 6). Measurements of gas exchange and concurrent measurements of carbon isotope discrimination during CO_2 uptake were made as described by von Caemmerer and Evans (1991) and Henderson *et al*. (1992) at a CO_2 partial pressure of 350 µbar and 21% O_2. Leaf temperature was maintained at $29^{\circ}C$. Leaf-to-air vapour pressure difference, V, was varied between 10 and 20 mbar and irradiance between 500 and 2000 µmol quanta m^{-2} s^{-1}, to obtain variation in the ratio of intercellular to ambient partial pressure of CO_2, p_i/p_a. Additional gas exchange measurements without concurrent trapping were carried out at 1600 µmol m^{-2} s^{-1} and $V = 13$–17 mbar, to characterise possible differences in gas exchange properties among the lines.

After gas-exchange measurements, the leaf used for gas exchange was oven dried at $80^{\circ}C$ for later analysis of Δ_d. During this time period δ_a was measured as –8.8‰ (Henderson *et al*. 1992).

Experiment 3

Seeds of the 30 lines used in Experiment 1 were also sown in the field at Emerald on 20 January 1989, to assess variation in Δ_d. Each plot had dimensions of 4x12 m and there were three replicate plots grown per line in a randomised complete block design. In all the field experiments the plants were grown in a uniform, black cracking clay soil which is typical of the soils in this region. The plots were fertilised prior to sowing. The crop grew well and was free of weeds. Furrow irrigation was used to water the plots at three times during the growth period. The crop also received 260 mm of rain

during the growth period. Other meteorological data for the growth period are shown in Table 1.

Plants were harvested from a block of size 1 m^2 when 50% of them were flowering which was between 56 and 62 days after planting. The plants were separated into leaves and stems and dried in an oven at 80°C. The dried leaf samples were ground for carbon isotope analysis. Δ_d was calculated as before, assuming δ_a was –7.8‰ in the field, which is the average global value in the absence of industrial activity (Farquhar *et al.* 1989*b*).

Experiment 4

The lines Trump, Trojan II, 623/430 and Tx610SR were grown in the field at Emerald between October and December 1989 and Δ_d and W were measured. To measure W in the field, intact soil cores were excavated from the plots (Wright *et al.* 1988) and were placed in plastic pots (30 cm in diameter and 80 cm long), which were then returned to the holes from which the cores had been removed. The holes were lined with 1 mm sheets of galvanised iron around the circumference to prevent loose soil falling back into the hole. Six pots, one plant per pot, were grown this way as part of a canopy in plots of 5 m by 6 m with a 60 cm row spacing and a 30 cm plant spacing. There were four replicate plots for each line and these were arranged in a randomised block design. Movable shelters were used to exclude rain. Plants in the pots were watered by hand with known amounts of water to maintain the soil at field capacity. Soil evaporation from pots was minimised by covering the tops of the pots with thin plastic. Other plants were watered with a drip irrigation system.

Plants were sown on 23 October 1989 and harvested at 35 days (panicle initiation) and 45 days after sowing (DAS). All pots were weighed at three times and eight pots (two pots per plot) for each line were harvested. Shoots were separated from roots and roots were washed. Leaves, roots and stems were oven dried at 80°C and then weighed. Leaf dry matter was ground finely for later analysis of Δ_d.

Transpiration efficiency was calculated from the dry matter produced between 35 and 45 DAS divided by the water used in the same time period. Meteorological data from the site are shown in Table 1.

Experiment 5

Experiment 4 was repeated in 1990 with a different set of lines. These were New Tropic, White Charger, Hylan 4x8 and Success 40W. Sowing date was 16 October and plants were harvested at 35 (panicle initiation) 44, 51 and 58 (mid-flowering) days after sowing (DAS). Pots were weighed on these days. Transpiration efficiency was calculated as before for the periods from 35 to 44 DAS (Harvest 2), 35 to 51 DAS (Harvest 3) and 35 to 58 DAS (Harvest 4). Meteorological data for this experiment are also shown in Table 1.

Theory

The relationship between transpiration efficiency and carbon isotope discrimination

Farquhar and Richards (1984) derived an equation which links the ratio of intercellular to ambient partial pressure of CO_2, p_i/p_a, and transpiration efficiency, W, as follows

$$W\left(\text{mol C mol}^{-1}\,H_2O\right)=\left(1-p_i/p_a\right)\bullet\frac{p_a}{1.6V}\bullet\frac{\left(1-\phi_r\right)}{\left(1+\phi_w\right)} \quad,\quad(1)$$

where ϕ_r is the ratio of CO_2 respiration occurring at night and in non-photosynthetic tissue during the day to assimilation rate. The symbol ϕ_w denotes the ratio of night-time and non-stomatal water loss to daytime transpiration and V is the leaf-to-air vapour pressure difference. For Experiment 1, where *S. bicolor* lines were grown in the glasshouse, ambient CO_2 pressure, p_a, was measured as 375 μbar and the average V was 21 mbar. We assumed $\phi_r = 0.30$ for *S. bicolor*, and we took $\phi_w = 0.10$, which was determined by Rawson and Clarke (1988) for a wheat crop. On substitution, this gives the following numerical equation:

$$W \text{ (mol C mol}^{-1}\,H_2O) = 7.1\,(1 - p_i/p_a) \times 10^{-3}. \quad(2)$$

In the field experiments (Experiments 4 and 5) temperatures were greater and we took $\phi_w = 0.2$, $\phi_r = 0.30$, the mean V was assumed to be 30 mbar and ambient CO_2 partial pressure was lower at 340 mbar in open fields. With these values, the value of 7.1 in Equation 2 is replaced by 4.1.

Farquhar (1983) summarised the relation between Δ_A, p_i/p_a and ϕ for C_4 species by (modified by Henderson *et al.* 1992) :

$$\Delta_A = a + (b_4 + \phi(b_3 - s) - a)\,p_i/p_a, \quad(3)$$

where a is the fractionation during diffusion of CO_2 in air through stomata (4.4‰), b_4 is the combined fractionation of PEP carboxylation and the preceding isotopic equilibrium during dissolution and hydration of CO_2 (−5.2‰ at 30°C), b_3 is the fractionation by Rubisco (30‰). A fractionation of 1.8‰ during leakage was introduced to

Table 1. Mean meteorological data for different experimental periods
The data were collected at Emerald Research Station

Expt. No.	Experimental period (days after sowing)	Pan evaporation (mm day^{-1})	Relative humidity (9 am)	Max. temp. (°C)	Min. temp. (°C)
3	0–62	5.9	70	31.0	19.4
4	0–35	6.8	75	30.8	18.6
4	35–45	7.4	71	31.3	18.7
5	0–35	9.5	63	34.0	18.1
5	35–44	7.8	64	34.0	19.3
5	45–51	9	67	36.0	21.4
5	52–58	10.0	57	36.0	21.3

the equation by Henderson *et al.* (1992). There is a known temperature dependence of the isotopic equilibrium during dissolution and conversion of CO_2 to bicarbonate (Mook *et al.* 1974), and the temperature dependence of b_4 was given by Henderson *et al.* (1992). The other fractionation constants are not known to be temperature dependent (O'Leary 1992). Henderson *et al.* (1992) showed that ϕ is constant at 0.21 under a range of short-term changes in environmental conditions and in a number of C_4 species. In particular, for *S. bicolor*, a value of 0.21 was measured. Substituting this value of ϕ and the above values into Equation (3) and rearranging, one obtains the following expression:

$$p_i/p_a = 1.2 - 272\,\Delta_A, \qquad (4)$$

(with Δ_A typically having values of 3‰, i.e. 3×10^{-3}) which, combined with (2), gives:

$$W \text{ (mol C mol}^{-1}\text{ H}_2\text{O}) = 1.9\,\Delta_A - 1.4 \times 10^{-3}, \qquad (5a)$$

for Experiment 1.

For the field experiments, the relationship was taken as:

$$W \text{ (mol C mol}^{-1}\text{H}_2\text{O}) = 1.1\Delta_A - 0.8 \times 10^{-3}. \qquad (5b)$$

The analysis presented above assumes that the carbon isotope discrimination of leaf dry matter, Δ_d, is the same as Δ_A. However our results suggest that there was a constant offset of approximately 1‰ between the Δ_A values and the measurements of Δ_d, i.e. Δ_A is about 1‰ less than Δ_d.

Variation amongst lines of C_3 species compared with S. bicolor

To determine how repeatable the differences among lines are, we have calculated line mean repeatability (Fehr 1987), a measure of the proportion of the phenotypic variance due to genotypic variance, for different species.

The models adopted to calculate line mean repeatability were: $Y_{ij} = m + g_i + e_{ij}$ for completely randomised designs in Experiment 1, and $Y_{ij} = m + g_i + b_j + e_{ij}$ for the randomised complete block design, where Y_{ij} is the phenotypic observation j on line i ($i = 1, …, k$ and $j = 1, …, n$, and k and n are the numbers of genotypes and replicates, respectively); m is the grand mean; g_i is the effect of line i, $N(0, \sigma^2_g)$; b_j is the effect of replicate j, $(N(0, \sigma^2_b)$; and e_{ij} is the error effect associated with observation j on genotype i, $N(0, \sigma^2_e)$.

Genotypic and error variances were calculated by equating the expected and observed mean squares from the analysis of variance and solving for the components of variance. The line mean repeatability (R^2) was calculated from the variance components as follows:

$$R^2 = \frac{\sigma^2_g}{\sigma^2_g + \sigma^2_e / n}, \qquad (6)$$

where n is the number of replicates used (Fehr 1987). The line-mean repeatability will be low if the error variance is high relative to the genotypic variance in that environment.

Results

Experiment 1

W, and Δ_d were measured on 30 *S. bicolor* lines grown in the glasshouse (Table 2). The lines are ranked from the highest to lowest W. Significant variation was found in both parameters. The mean W was 4.93 mmol C mol^{-1} H_2O and the range was 0.81 mmol C mol^{-1} H_2O. The mean Δ_d was 3‰ and the range was 0.43‰.

A weak but significant correlation was found between mean W and Δ_d for lines and the relationship was positive, with W increasing as Δ_d increased (Fig. 1). The observed relationship is consistent with the relationship predicted from theoretical considerations which is shown on the figure as both have positive slopes (the theoretical and regression equations are given in the Legend). Dry matter accumulation did not correlate with W or Δ_d, although there was a tendency for it to be less in plants with greater Δ_d and greater transpiration efficiency, W.
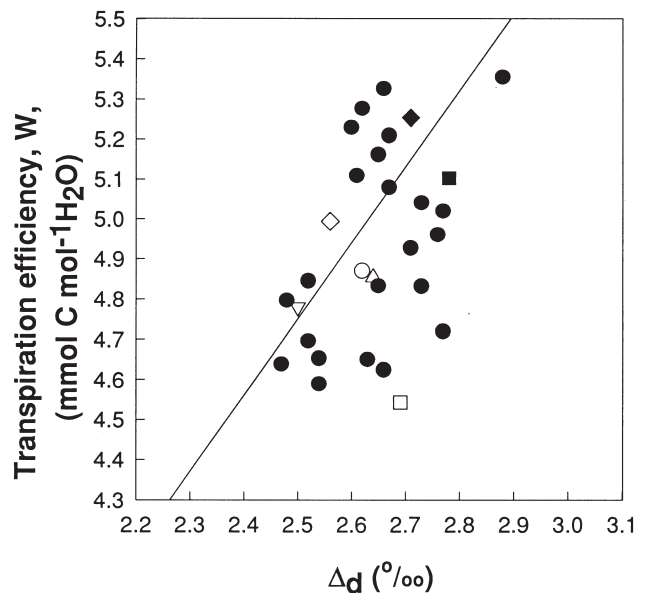
Fig. 1. Mean transpiration efficiency, W, versus mean leaf dry matter discrimination, Δ_d, for 30 *S. bicolor* lines grown in sealed pots in the glasshouse in Expt 1. The linear regression for the data was $W = 1.0\Delta_d + 1.93 \times 10^{-3}$ ($r^2 = 0.18$, $P<0.05$) (not shown). The theoretical equation is $W = 1.9\Delta_A - 1.4 \times 10^{-3}$. We have separately denoted those of the 30 leaves which were used in subsequent experiments (2, 4, 5): Trump (□), Tx610SR (○), 623/430 (◇), Trojan II (■), White Charger (◆), NewTropic (▽), and Hylan 4x8 (△).

Table 2.   Mean transpiration efficiency, *W*, leaf dry matter discrimination, Δ_d, and water transpired for 30 *S. bicolor* lines grown in the glasshouse in Experiment 1 and Δ_d for the same lines grown in the field in Experiment 3
The lines marked with * were grown in the field in Experiment 4 and those marked by ** were grown in the field in Experiment 5

Line	Experiment 1			Experiment 3
	W (mmol C per mol H$_2$O transpired)	Water transpired (L)	Δ_d (‰)	Δ_d (‰)
Guardian MR	5.35	2.29	2.89	3.91
QL33/QL36	5.33	2.78	2.66	4.02
622/16-6	5.28	2.62	2.62	3.74
White Charger**	5.25	2.75	2.70	3.91
DK55+	5.23	3.59	2.60	3.43
QL35/QL36	5.21	2.92	2.68	3.59
623/16-6	5.16	2.77	2.64	3.92
Prize	5.11	2.62	2.60	3.66
Trojan II*	5.10	2.82	2.78	3.77
E57+	5.08	2.98	2.67	3.79
378/16-6	5.04	2.90	2.73	3.80
QL38/QL39	5.02	2.50	2.77	3.92
623/430*	4.99	2.86	2.55	3.65
CS310	4.96	2.49	2.76	3.98
QL39/QL36	4.93	2.11	2.71	3.75
Tx610SR*	4.87	3.27	2.65	3.79
Hylan 4x8**	4.85	2.92	2.63	3.71
Goldrush	4.85	2.97	2.51	4.08
622/430	4.83	3.10	2.64	3.60
DK370	4.83	2.78	2.73	3.66
399/430	4.80	3.77	2.48	3.66
New Tropic**	4.78	3.73	2.49	3.49
Pacific 810	4.72	2.98	2.77	3.85
6990/430	4.70	3.00	2.51	3.48
52203-4/QL36	4.65	3.13	2.54	3.65
Nugget	4.65	2.45	2.62	3.79
Pride	4.64	2.12	2.46	3.80
Goldmine	4.62	2.75	2.65	3.87
6990/16-6	4.59	2.93	2.54	3.65
Trump*	4.54	2.48	2.69	4.1
LSD (*P*=0.05)	0.39		0.17	0.3

Experiment 2

From Experiment 1, eight lines were grown again in the glasshouse in Canberra in free draining pots and the youngest fully expanded leaves were used for simultaneous measurements of CO$_2$ assimilation, *A*, the ratio of leaf intercellular to ambient CO$_2$ partial pressure, p_i/p_a, and carbon isotope discrimination.

Fig. 2 shows the results of concurrent measurements of p_i/p_a and Δ_A. To obtain variation in p_i/p_a, measurements were made at a range of irradiances and various leaf-to-air vapour pressure differences, *V*. Most extensive measurements have been made on the line TX610SR and the data on this line were previously presented by Henderson *et al.* (1992). The line in Fig. 2 depicts the theoretical relationship (Equation 3) when the CO$_2$ leakiness of the C$_4$ pathway is taken as $\phi = 0.21$. The regression is significant with discrimination decreasing as p_i/p_a increases and this agrees closely with the theoretical relationship.

Leakiness, estimated from Δ_A and p_i/p_a for the 8 lines from Equation (3), varied between 0.19 and 0.25 with an average value of 0.23 (Table 3). Average differences in leakiness for leaves were not significant. The leaves all had high CO$_2$-assimilation rates at high irradiance, the average being 54 μmol m^{-2} s^{-1} (Table 4). The average p_i/p_a was 0.33 (Table 4). There were significant differences in both *A* and p_i/p_a when lines were measured at constant irradiance and near-constant *V*. Although variation in *g* was not significant, variation in p_i/p_a was positively related to *g* (r^2=0.58, *P*<0.05), and not to *A*.

Experiment 3

The 30 lines used in Experiment 1 were also grown in the field at Emerald. Δ_d was determined on bulk leaf
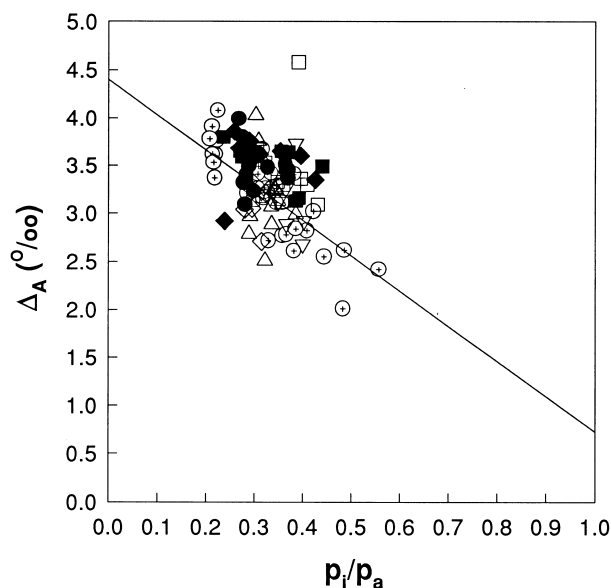
Fig. 2. Individual measurements of carbon isotope discrimination, Δ_A, during CO_2 uptake as a function of p_i/p_a for eight lines of *S. bicolor*. Measurements were made at a range of irradiances between 800 and 1600 μmol quanta m^{-2} s^{-1} and, at V of between 10 and 20 mbar, leaf temperature was 29°C and p_a was 350 μbar. The line is the theoretical relationship (Equation 3) where Δ_A =[4.4 + (28.2ϕ–9.6) p_i/p_a] x 10^{-3} with ϕ = 0.21. The linear regression for the data is Δ_A= [4.34 – 3.16 p_i/p_a] x 10^{-3} (r^2 = 0.25, $P<0.05$. Symbols are Trump (□), Tx610SR (⊕), 623/430 (◇), Trojan II (■), White Charger (◆), NewTropic (▽), Hylan 4x8 (△) and Success 40 (●).

material from three replicate plots. and significant genetic variation in Δ_d was found (Table 2). The range was 0.7‰, compared to 0.43‰ in the glasshouse in Experiment 1. Δ_d was, on average, greater by 0.8‰ than in Experiment 1, partly because of the value of δ_a used to calculate Δ_d. We used –8.43‰ in Experiment 1 in the glasshouse and –8.00‰ for the field (see Materials and methods). If we

Table 3. Genotypic means of carbon isotope discrimination during CO_2 uptake, Δ_A, p_i/p_a, and leakiness, ϕ, for the data shown in Fig. 2
No significant differences in leakiness were found when leaf means were considered in an analysis of variance

Line	No. of leaves measured	Δ_A (‰)	p_i/p_a	Leakiness, ϕ
White Charger	3	3.59	0.31	0.25
Trojan II	2	3.51	0.34	0.24
623/430	2	3.15	0.30	0.19
Tx610SR	6	3.13	0.35	0.21
Hylan 4x8	3	3.31	0.33	0.22
New Tropic	4	3.21	0.33	0.21
Trump	2	3.41	0.36	0.24
Success 40W	3	3.55	0.30	0.24

Table 4. The mean rate of CO_2 assimilation, leaf conductance to water vapour and p_i/p_a for leaves of eight *S. bicolor* lines measured at irradiances of 1600 μmol quanta m^{-2} s^{-1}, leaf to air vapour pressure difference between 13 and 17 μbar, ambient CO_2 partial pressure of 350 μbar and a leaf temperature of 29°C
The p_i/p_a and CO_2 assimilation rates followed by a different letter are significantly different from each other (LSD = 0.05, $P<0.05$). No significant differences in leaf conductance were found

Line	Rate of CO_2 assimilation (μmol m^{-2} s^{-1})	Leaf conductance to water vapour (mol m^{-2} s^{-1})	p_i/p_a
Tx610SR	59.7a	0.40	0.34ab
623/430	56.8ab	0.34	0.30bc
Trump	55.5ab	0.39	0.36ab
Success 40W	54.9ab	0.37	0.33ab
Hylan 4x8	54.8ab	0.38	0.34ab
New Tropic	52.4ab	0.35	0.32abc
Trojan	48.2b	0.34	0.36a
White Charger	46.5b	0.29	0.27c

had used the same value for both experiments, the difference would have been greater. Transpiration efficiency was not measured in this experiment. The negative relationship between shoot dry matter production per hectare and Δ_d in the field reported by Hubick *et al.* (1990) was not observed.

Experiment 4

Four lines (Trojan, 623/430, Tx610SR, Trump) were grown in the field at Emerald and both W and Δ_d were measured. Water use was measured for the individual plants in pots from 35 days after sowing (DAS — panicle initiation) to 45 DAS. At 35 DAS plants had on average acquired 23% of the total dry matter produced by 45 DAS. Transpiration efficiency was calculated as total dry matter accumulated during this period divided by the water used. Significant differences were found in both W and Δ_d (Fig. 3). W was lower in the field than in the glasshouse but V may have been higher which would explain some of the difference. Δ_d was again 0.8‰ larger for plants grown in the field (Fig. 3). The lines ranked differently in mean Δ_d and W in this experiment, compared to Experiments 1 and 3 (Fig. 3).

For individual plants, a significant correlation existed between W and Δ_d (Fig. 4). The relationship had a positive slope, as in Experiment 1 and as predicted by theory (Equation 5b).

Experiment 5

The four lines used in these experiments were White Charger, Hylan 4x8, New Tropic and Success 40W. The last line had not been included in a previous experiment but was assumed to be similar to 623/430. As before, water use was
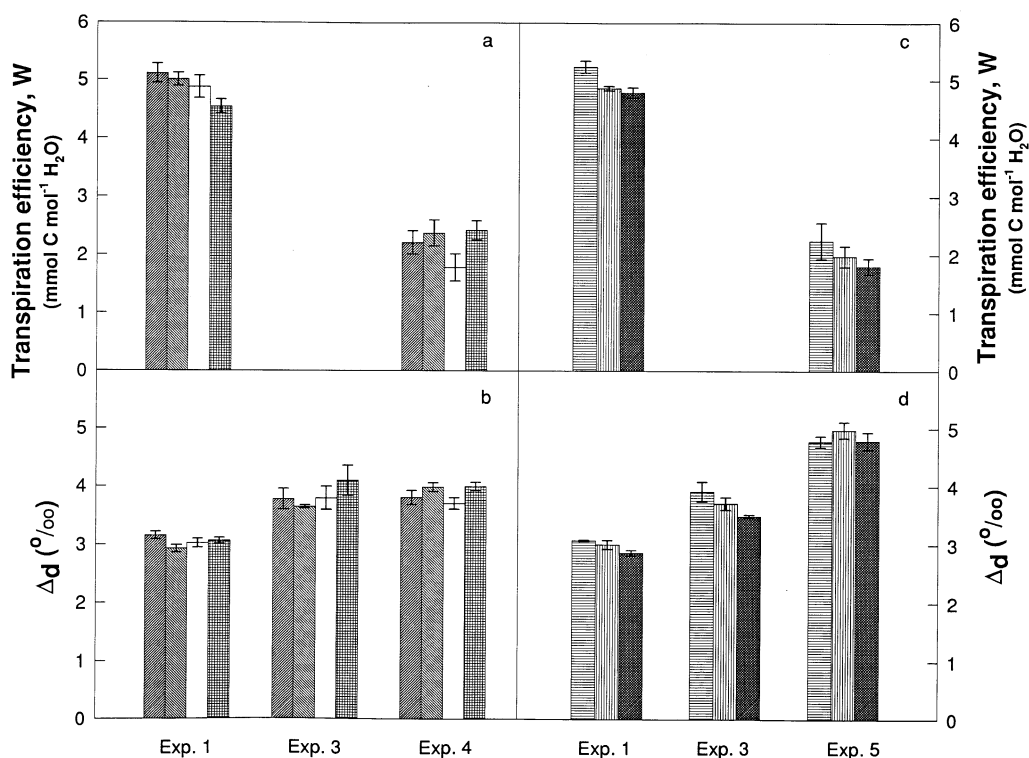
Fig. 3. Comparisons of genotypic means of transpiration efficiency, W, and Δ_d across experiments. Error bars show standard errors.

monitored for individual plants in pots immersed in a canopy. Water use was monitored over three separate time periods of 35 to 44 DAS (Harvest 2), 35 to 51 DAS (Harvest 3) and 35 to 58 DAS (Harvest 4). The plants grew faster between 0 to 35 DAS than in the previous experiment, probably as a result of warmer weather (Table 1). At 35 DAS, dry matter formed was 51%, 32% and 30% of the total dry matter formed at 41, 51 and 58 DAS, respectively.

Table 5 shows W and Δ_d measured at the different harvests. The ranking of the lines varied with harvest and

there was no difference between lines in either W or Δ_d at the final harvest (Harvest 4). Overall, W decreased with successive harvests, whereas Δ_d increased.

As in the previous experiments, there was a significant positive correlation between W and Δ_d for individual plants, at all harvests. In Fig. 5, results are shown for Harvest 2 and Harvest 4. Δ_d was on average 1.3 ‰ higher than in Experiment 1 (at Harvest 2) and there was an average increase in Δ_d of 0.6‰ between Harvest 2 and Harvest 4.

Table 5. Mean transpiration efficiency, W (mmol C mol^{-1} H$_2$O) , and leaf dry matter discrimination, Δ_d (‰), for *S. bicolor* lines at three different times after sowing (DAS) in Experiment 5

W was measured for Harvest 2 from 35 to 44 days after sowing (DAS) for Harvest 3 from 35 to 51 DAS for Harvest 4 from 35 to 58 DAS. The number in round brackets gives the rank of each line from lowest to highest for each parameter at the different harvest. The numbers in square brackets are the number of replicates of each line at each harvest

| Line	Trait	Harvest 2	Harvest 3	Harvest 4
Success 40W	W	3.48 (3) [7]	3.00 (1) [4]	1.94 (2) [8]
New Tropic	W	3.73 (4) [4]	3.22 (2) [8]	1.80 (1) [6]
White Charger	W	2.79 (2) [8]	3.35 (3) [4]	2.24 (4) [8]
Hylan 4x8	W	2.26 (1) [8]	3.42 (4) [6]	1.98 (3) [8]
Success 40W	Δ_d	4.4 (4) [7]	4.6 (2) [4]	4.9 (3) [8]
New Tropic	Δ_d	4.2 (1) [4]	4.5 (1) [8]	4.8 (2) [6]
White Charger	Δ_d	4.2 (2) [8]	4.7 (3) [4]	4.8 (1) [8]
Hylan 4x8	Δ_d	4.2 (3) [8]	4.8 (4) [6]	5.0 (4) [8]
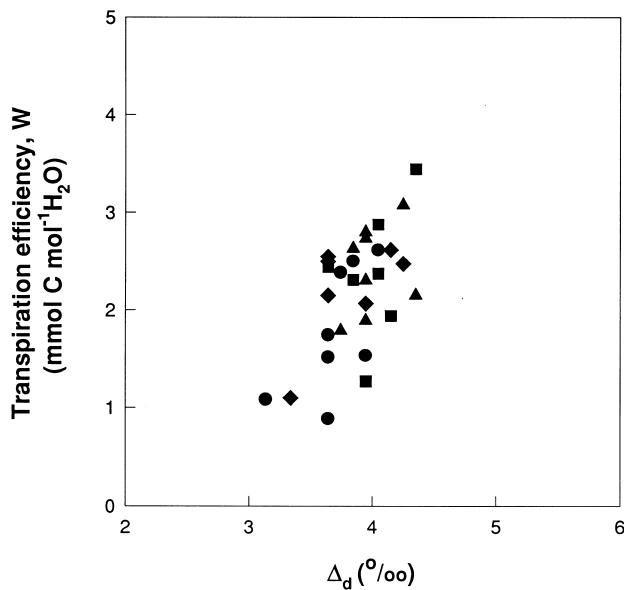
Fig. 4. Transpiration efficiency, *W*, for individual plants grown in a canopy in the field versus carbon isotope discrimination of leaf dry matter, Δ_d, for four *S. bicolor* lines of Experiment 4. Lines were Trump (▲), Tx610SR (●), 623/430 (■) and Trojan II (◆). *W* was measured between 35 and 45 DAS. The correlation is significant with $W = 1.32 \, \Delta_d - 2.93 \times 10^{-3}$ ($r^2 = 0.36$, $P<0.05$). The theoretical equation is $W = 1.2\Delta_A - 0.9 \times 10^{-3}$ (Equation 5b).

Discussion

Relationship between W, p_i/p_a, g and D

The relationship between whole plant transpiration efficiency, *W*, and carbon isotope discrimination of leaf dry matter is well established for C_3 species (for review, see Farquhar *et al.* 1989a). We have demonstrated, with the use of *S. bicolor* lines grown in the glasshouse and the field, that a relationship also exists between *W* and Δ_d in C_4 species (Figs 1, 4 and 5).

The relationship between *W* and p_i/p_a is common to C_3 and C_4 species (Equation 1), although C_4 species typically have lower ratios of p_i/p_a than are commonly observed for C_3 species at *V* values of approximately 20 mbar (Ludlow and Wilson 1972; Wong *et al.* 1979). We measured an average value of $p_i/p_a = 0.33$ (Table 3) which compares to average values of 0.7 in C_3 species (Wong *et al.* 1979). In accordance with the low values of p_i/p_a, we also observed greater transpiration efficiencies in the 30 *S. bicolor* lines grown in the glasshouse than have been observed for C_3 species grown under similar conditions (Hubick *et al.* 1986; Hubick and Farquhar 1989; Virgona *et al.* 1990). These differences in *W* between C_4 and C_3 species under well-watered conditions have also been documented previously (Shantz and Piemeisel 1927; Black *et al.* 1969; Slatyer 1971).
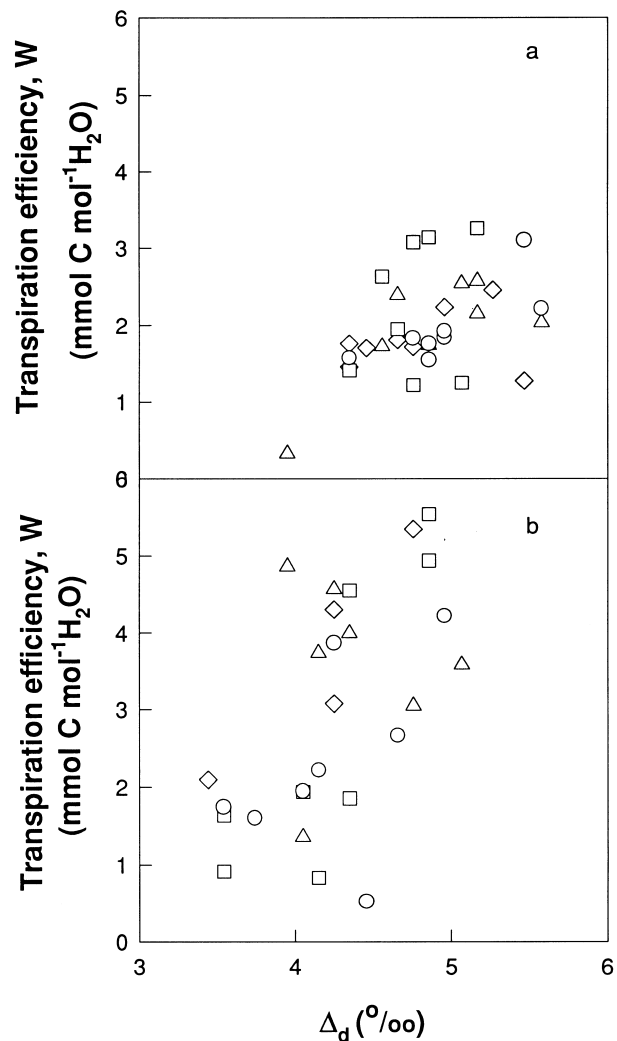
Fig. 5. Transpiration efficiency, *W*, for individual plants grown as part of a canopy in the field versus leaf dry matter discrimination, Δ_d, for four *S. bicolor* lines. These were White Charger (□), Success 40W (△), 378/430 (New Tropic) (◇) and Hylan 4x8 (O). The theoretical relationship between Δ_A and *W* is given by the equation $W = 1.1\Delta_A - 0.8 \times 10^{-3}$ (Equation 5b). (*a*) *W* and Δ_d measured at Harvest 4. The correlation is significant, with $W = 0.76\Delta_d - 1.72 \times 10^{-3}$ ($r^2 = 0.21$, $P<0.05$). (*b*) *W* and Δ_d measured at Harvest 2. The correlation is significant with $W = 2.05\Delta_d - 5.44 \times 10^{-3}$ ($r^2 = 0.36$, $P<0.05$).

The relationship between Δ_A and p_i/p_a is different in C_3 and C_4 species. In C_3 species, Δ_A and p_i/p_a are linearly related with a positive slope of approximately 22.6‰ per unit change in p_i/p_a (Farquhar *et al.* 1989a). In C_4 species, Δ_A and p_i/p_a are also linearly related but the slope of the relationship is dependent on the leakiness, ϕ, of the CO_2 concentrating mechanism (Farquhar 1983). Concurrent measurements of p_i/p_a and Δ_A made during gas exchange measurements are shown for 8 of the 30 lines, together with the theoretically predicted relationship when $\phi = 0.21$

(Equation 3, Fig. 2). We detected only small genotypic differences in ϕ amongst these eight lines and the value of ϕ was similar to that previously determined for a number of other C_4 species (Table 3, Henderson *et al*. 1992). The linear relationships between Δ_A and p_i/p_a for C_4 species has a negative slope. At $\phi = 0.21$, the slope is less than in C_3 species, with only 3.7‰ change in Δ_A per unit change in p_i/p_a. Henderson *et al*. (1992) also showed that there was little variation in ϕ with short-term variation in irradiance, temperature or ambient CO_2 partial pressure. Thus our gas exchange measurements suggest that most of the variation in Δ_d reflects variation in p_i/p_a and, therefore, in *W*. In the gas exchange measurements reported in Table 4, the variation in p_i/p_a is in turn largely related to that in stomatal conductance, and such a possible relationship was one we speculated about earlier (Hubick *et al*. 1990; Farquhar *et al*. 1994). However, there was a great deal of x environment interaction here in the various experiments when gas exchange characteristics were not measured, and so we are cautious about the result.

The negative relationship between Δ_A and p_i/p_a for C_4 species translates into a positive relationship between *W* and Δ_d. We observed the expected positive relationship between *W* and Δ_d when *W* was measured for 30 lines in the glasshouse (Fig. 1) and in the field (Figs 4 and 5). The close agreement between theoretical expectations (Equations 5*a* and 5*b*) and data show that the difference observed in *W* is that expected from the difference observed in Δ_d (regression equations are given in the figure captions).

The significant correlations between *W* and Δ_d found in the field are of particular interest. Low crop boundary layer conductances and effects of advection could confound the theory underlying our analysis (Jarvis and McNaughton 1986; Cowan 1988). As stomata open, the air in the canopy becomes moister and cooler, which could also change the relationship between *W* and Δ_d. If the boundary layer conductance of the canopy is low, this may result in reduced sensitivity of transpiration rate to stomatal conductance. Nevertheless, like us, Wright *et al*. (1988) also found relationships between *W* and Δ_d in the field for peanut regardless of whether the plants were in the canopy or above the canopy.

Environmental variation in W and Δ_d

Transpiration efficiency was lower for plants grown in the field than for plants grown in the glasshouse and also decreased during the growing season (Figs 4 and 5). There are several factors which may cause these large differences in *W*. (1) The 10 mbar greater *V* in the field can account for a reduction in *W* of approximately 30%. (2) Night respiration increases with temperature in *S. bicolor* (Gerik and Eastin 1985) and this would lead to an increase in the respiration ratio, ϕ_r, in Equation 1. (3) Fraction of water loss

that is non-stomatal may increase at higher temperatures and higher *V*. In the theoretical relationships (Equations 5a and 5b), ϕ_r has been increased from 0.25 in the glasshouse to 0.30 in the field, and ϕ_w, the water loss factor, has been increased from 0.10 to 0.20.

Δ_d was, on average, 1‰ greater in the field than in the glasshouse and increased during the growth season in Experiment 5. It is possible that these differences are artefacts of differences in δ_a in the two environments. There can be diurnal and seasonal variation in δ_a resulting from industrial activity in local areas. Inoue and Sugimura (1984) found 2‰ variations in δ_a at a field site 60 km from Tokyo and this could explain why Δ_d exceeds 4.4‰ when p_i/p_a is zero. However, Emerald is much more isolated from industrial activity than is Tokyo. Another possible complication is the variation of the discrimination factor, b_4, with temperature due to changes in the discrimination during dissolution and hydration of CO_2. The temperature dependence of b_4 was given by Henderson *et al*. (1992) and we calculate that a shift in temperature from 25 to 30°C could increase Δ_d by 0.35.

The relationship between Δ_d of leaf dry matter and carbon isotope discrimination during CO_2 uptake is also affected by possible fractionations occurring during synthesis of chemical constituents of plant dry matter after photosynthesis; for example, lipids are depleted in [13]C (O'Leary 1981). With available evidence at the time of review, Farquhar *et al*. (1982) and Evans *et al*. (1986) suggested that intrinsic fractionation associated with respiration was small. However, Henderson *et al*. (1992) have found that Δ_d was not a good indicator of Δ_A when a diverse set of C_4 species was compared. It may be that post-photosynthetic fractionations are more important in C_4 species where variations in Δ_d caused by variations in p_i/p_a are smaller than in C_3 species.

Genetic variation in W and Δ_d

Significant genetic variation in *W* and Δ_d was found for these lines when they were grown in the field. The seven lines selected for gas exchange measurements also showed small but significant differences in p_i/p_a. It is clear from the results and the theory that in C_4 species the variation in Δ_d for a particular change in *W* is much smaller than in C_3 species. On the other hand, precision should also be improved because temporal or microenvironmental variations in p_i/p_a give smaller variations in Δ_d than in C_3 species.

Tables 6 and 7 provide an overview of genetic variation and precision observed in Δ_d and *W* for some C_3 species, *S. bicolor* and *Panicum coloratum*. The standard deviations for Δ_d (a measure of genetic variability) among the *S. bicolor* genotypes were approximately a quarter or less of those observed for C_3 means. This lower standard deviation

Table 6. **Genotypic variation in Δ_d for C_3 and C_4 species, given as standard deviation amongst the means for Δ_d and the least significant difference (LSD, P=0.05), the ratio of these two parameters and the line mean repeatability (calculated from the LSD and genotype means)**

The number given in brackets after the LSD is the number of replicates per genotype

Researchers	Environment	Line mean repeatability	Least significant difference (P=0.05)	Standard deviation (σ_g)	Ratio of σ_g to LSD (P=0.05)
Barley: Hubick and Farquhar (1989)	Well watered, glasshouse	0.92	0.43 (6)	0.51	1.2
Barley: Crauford *et al.* (1991)	Cambridge, irrigated	—	—	0.53	—
Coffee: Meinzer *et al.* (1990)	San Ramon, irrigated	—	—	0.62	—
Cotton: Hubick and Farquhar (1987)	Well watered, glasshouse	—	—	0.39	—
Cowpea: Hall *et al.* (1990)	Well watered	0.71	0.71 (6)	0.47	0.7
Beans: White *et al.* (1990)	Palmira, irrigated	0.75	0.6 (4)	0.42	0.7
Peanut: Hubick *et al.* (1986)	Well watered	0.99	0.64 (4)	0.83	1.3
Peanut: Wright *et al.* (1988).	Kingaroy				
In-ground pots		—	—	0.44	—
Above-ground pots		—	—	0.71	—
Potato: Vos and Groenwold (1991)	Netherlands, well watered	—	—	0.40	—
Rice: Dingkuhn *et al.* (1991)	Philippines, moderate water deficit	—	—	0.44	—
Sunflower: Virgona *et al.* (1990)	Well watered, glasshouse	—	0.32 (6)	0.66	2.1
Wheat: Condon *et al.* (1990)	Well watered, glasshouse	0.85	0.6 (3)	0.53	0.9
	Field, Australia	0.81	0.5 (4)	0.50	1.0
Wheat: Ehdaie *et al.* (1991)	Well watered, glasshouse				
1987		—	—	0.74	—
1988		—	—	1.40	—
	Well watered field				
1987		—	—	0.76	—
1988		—	—	0.49	—
Sorghum: Hubick *et al.* (1990)	Well watered, field	0.99	0.13 (3)	0.18	1.4
Experiment 1	Well watered, glasshouse	0.65	0.17 (4)	0.10	0.6
Experiment 3	Well watered, field	0.61	0.33 (3)	0.17	0.5
Panicum coloratum (C_4)	Well watered, glasshouse	—	—	0.54	—

in sorghum Δ_d is, however, accompanied by a lower LSD showing that there is less variability in the replicates. The ratio of the standard deviation to LSD is lower in the experiments reported here, compared with the C_3 lines. However, the experiment of Hubick *et al.* (1989) which was also on sorghum, has a ratio which is comparable with the C_3 ratios. Furthermore the line mean repeatabilities are comparable with those of the C_3 species when the experiments of Hubick *et al.* are included. The usefulness of Δ_d as a tool for selecting for variation in W remains somewhat uncertain.

For W there was a similar standard deviation for the present sorghum results as for the C_3 species but the line

mean repeatability was lower, indicating a lower genetic contribution to phenotypic variance which may reflect more variation amongst replicates. The least significant difference was also higher for the sorghum lines than the C_3 lines.

A problem for selection in sorghum is that lines rank differently in different environments (Fig. 3), and across time (Table 5) suggesting line x environmental interactions for both Δ_d and W. In many studies of C_3 species, W and Δ_d appear to be under strong genetic control as ranking of lines is often maintained across different environments (Hubick *et al.* 1986, 1988; Condon *et al.* 1987, 1990; Hubick and Farquhar 1989; Hubick 1990;

Table 7. Genotypic variation in *W* for C$_3$ and C$_4$ species, given as standard deviation amongst the means for *W* and the least significant difference (LSD, *P*=0.05), the ratio of these two parameters and the line mean repeatability where the LSD and genotype means are included in the paper

If *W* is given in g dry matter kg^{-1} H$_2$O it has been converted to mmol C per mol H$_2$O assuming a carbon content of dry matter of 40%. The number given in brackets after the LSD is the number of replicates per genotype

Researchers	Environment	Line mean repeatability	Least significant difference (*P*=0.05)	Standard deviation (σ_g)	Ratio of σ_g to LSD (*P*=0.05)
Barley: Hubick and Farquhar (1989)	Well watered, glasshouse	0.93	0.17 (6)	0.22[B]	1.3
Cotton: Hubick and Farquhar (1987)	Well watered, glasshouse	—	—	0.15[A]	—
Peanut: Hubick *et al*. (1986)	Well watered, glasshouse	0.94	0.19 (4)	0.30[A]	1.6
Peanut: Wright *et al*. (1988)	Kingaroy				
	In-ground pots	—	—	0.22[A]	—
	Above-ground pots	—	—	0.23	—
Potato: Vos and Groenwold (1991)	Netherlands, Well watered	—	—	0.36[A]	—
Sunflower: Virgona *et al*.	Well watered, glasshouse	—	—	0.32[B]	—
Wheat: Ehdaie *et al*. (1991)	Well watered, glasshouse				
	1987	—	—	0.44[A]	—
	1988	—	—	0.40	—
Sorghum — Experiment 1	Well watered, glasshouse	0.66	0.39 (4)	0.24[A]	0.6

[A] Originally given in g dry matter kg^{-1} H$_2$O. [B] Originally given in mmol C mol^{-1} H$_2$O.

Virgona *et al*. 1990; Johnson *et al*. 1990; Johnson and Bassett 1991; Ehdaie *et al*. 1991). However in other experiments, genotypic ranking for $\triangle_d$ changed in different environments (White *et al*. 1990; Hall *et al*. 1989; Crauford *et al*. 1991). Masle *et al*. (1993) found that for *Arabidopsis* $\triangle_d$ was dependent upon the combination of leaf irradiance and vapour pressure differences which the plant had been exposed to during growth and this caused ecotype x environment interaction. In our experiment, the ranking of the 30 lines from glasshouse to the field was not maintained indicating some line x environment interaction (Fig. 4).

The question of genetic control may have been easier to address with a more diverse germplasm than that contained in this set of 30 *S. bicolor* lines. For instance, Samejima (1984) found 1% difference amongst five inbred lines of *Zea mays* and, with genetic crosses, was able to show that lines with greater $\triangle_d$ values had the more dominant genes.

Conclusion

Genetic variation in $\triangle_d$ and $\triangle_A$ was negatively related to changes in p_i/p_a, and positively to *W* as expected. The sensitivity of discrimination to changes in p_i/p_a is intrinsically smaller for C$_4$ species then for C$_3$ species, meaning that both genetic variation and environmental 'noise' in p_i/p_a have smaller effects. The ranking of sorghum genotypes for $\triangle_d$ varied across environments. Some of the line x environment interaction and experimental error in $\triangle_d$ may be associated with variation in chemical composition, and techniques which enable the examination of isotopic composition of an individual component may prove useful in reducing this variation. However, there was also line x environment interaction in *W* itself, which suggests that the interaction was also in p_i/p_a.

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