Correlation between carbon isotope discrimination and transpiration efficiency in lines of the C₄ 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, Δp, were measured together on 30 lines of the C₄ 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 H₂O and the range was 0.8 mmol C mol⁻¹ H₂O. The mean Δp 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 H₂O and the mean Δp was 4.6‰. Significant positive correlations between W and Δp were observed for plants grown in the glasshouse and in the field. The observed correlations were consistent with theory, opposite to those for C₃ species, and showed that variation in Δp was an integrated measure of long-term variation in the ratio of intercellular to ambient CO₂ partial pressure, p/i. Detailed gas exchange measurements of carbon isotope discrimination during CO₂ uptake, Δ, and p/i, were made on leaves of eight S. bicolor lines. The observed relationship between Δ and p/i was linear with a negative slope of 3.7‰ in Δ for a unit change in p/i. The slope of this linear relationship between Δ and p/i in C₄ species is dependent on the leakiness of the CO₂ concentrating mechanism of the C₄ pathway. We estimated the leakiness (defined as the fraction of CO₂ released in the bundle sheath by C₄ acid decarboxylations, which is lost by leakage) to be 0.2. We conclude that, although variation in Δp observed in the 30 lines of S. bicolor is smaller than that commonly observed in C₃ 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 C₃ species, genetic variation in carbon isotope discrimination of leaf dry matter, Δp, 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. 1989b). This correlation occurs because of independent linear relationships of Δp and W to the ratio of intercellular to ambient partial pressures of CO₂, p/i, in leaves (Farquhar and Richards 1984). As whole-plant W is difficult to measure on a large scale, the association between Δp and W has proved interesting to plant breeders in selecting lines of C₃ species with increased W (e.g. Hubick et al. 1988; Hall et al. 1989, 1994). In this paper we examine the usefulness of Δp as a measure of W in C₄ species.

CO₂ fixation in C₄ species is characterised by a CO₂-concentrating mechanism that requires the coordinated function of mesophyll and bundle-sheath cells within a leaf. CO₂ is initially assimilated into C₄ acids by phosphoenolpyruvate (PEP) carboxylase in the mesophyll cells. These acids are transported to and decarboxylated in the bundle sheath, where the CO₂ is released by ribulose bisphosphate carboxylase-oxygenase (Rubisco). Carbon-isotope discrimination in C₄ species reflects these two different carboxylations. It is dependent not only on the ratio of intercellular to ambient partial pressure of CO₂, p/i, but also on leakiness, φ (Farquhar 1983), the proportion of CO₂ released into the bundle sheath by the C₄-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 (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 CO₂ for S. bicolor (L.) Moench and Amaranthus edulis Speg. They also found φ to be 0.2 in a number of C₄ species of the various C₄-decarboxylation types. Since there was little
variation in $\phi$, carbon isotope discrimination was, as in $C_3$ species, linearly related to $p/p_a$. Hence the possibility exists that $\Delta$ 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 $\Delta$ and $p/p_a$ 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 $\Delta$ 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 $p/p_a, CO_2$ 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 $\Delta$ also has been reported in $C_3$ species; for example, for lines of Zea mays (Samejima 1984), Panicum coloratum (Ohsgui 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 $\Delta$ among 120 lines of Z. mays.

To test whether variation in $\Delta$ 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 $\Delta$. The same lines were also grown in the field at Emerald, Central Queensland, to determine variation in $\Delta$ in the field. In the subsequent 2 years, further field measurements were made of $\Delta$ 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, $\Delta_a$, 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 $\Delta$. The experiment was conducted in November and December 1988 in an air-conditioned glasshouse with mean day and night temperatures of 33/20°C, a mean relative humidity of 60% and a midday irradiance of 1500 $\mu$mol quanta m$^{-2}$ s$^{-1}$.

Before seeds were sown, pots were flushed with a nutrient solution containing 12 m$m$ 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 weighted 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°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$_2$O 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 $\Delta$, 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%. $\Delta$ 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), $\delta_p$, and the carbon isotope composition of the glasshouse air, $\delta_a$, as $\Delta = (\delta_a - \delta_p)/(1+\delta_p)$.

Experiment 2

Lines of $S. bicolor$ (Trump, Trojan II, 623/430, 378/430, Txs610SR, White Charger, Hylan 4×8 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 $\mu$bar and 21% O$_2$. Leaf temperature was maintained at 29°C. Leaf-to-air vapour pressure difference, $V$, was varied between 10 and 20 mbar and irradiance between 500 and 2000 $\mu$mol 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 $\mu$mol 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°C for later analysis of $\Delta$. During this time period $\delta_a$ 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 $\Delta$. 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 m² 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. \( \Delta_a \) was calculated as before, assuming \( \delta_1 = -7.8\% \) in the field, which is the average global value in the absence of industrial activity (Farquhar et al. 1986).

**Experiment 4**

The lines Trump, Trojan II, 623/430 and Tcx610SR were grown in the field at Emerald between October and December 1989 and \( \Delta_a \) 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 \( \Delta_a \).

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 4x8T 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 \( \text{CO}_2 \), \( p_i/p_a \), and transpiration efficiency, \( W \), as follows

\[
W (\text{mol C mol}^{-1} \text{H}_2\text{O}) = \left(1 - p_i/p_a\right) \cdot \frac{p_a}{1.6V} \cdot \phi_a \cdot (1 - \phi)\cdot (1 - \phi)
\]

where \( \phi_a \) is the ratio of \( \text{CO}_2 \) respiration occurring at night and \( \phi \) in non-photosynthetic tissue during the day to assimilation rate. The symbol \( \phi_w \) 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 \( S. \text{bicolor} \) lines were grown in the glasshouse, ambient \( \text{CO}_2 \) pressure, \( p_a \) was measured as 375 µbar and the average \( V \) was 21 mbar. We assumed \( \phi = 0.30 \) for \( S. \text{bicolor} \), 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:

\[
W (\text{mol C mol}^{-1} \text{H}_2\text{O}) = 7.1 (1 - p_i/p_a) \times 10^{-3}.
\]

In the field experiments (Experiments 4 and 5) temperatures were greater and we took \( \phi = 0.2, \phi_w = 0.30 \), the mean \( V \) was assumed to be 30 mbar and ambient \( \text{CO}_2 \) 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.

**Table 1.** Mean meteorological data for different experimental periods

The data were collected at Emerald Research Station

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Experimental period (days after sowing)</th>
<th>Pan evaporation (mm day(^{-1}))</th>
<th>Relative humidity (9 am)</th>
<th>Max. temp. (°C)</th>
<th>Min. temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0–62</td>
<td>5.9</td>
<td>70</td>
<td>31.0</td>
<td>19.4</td>
</tr>
<tr>
<td>4</td>
<td>0–35</td>
<td>6.8</td>
<td>75</td>
<td>30.8</td>
<td>18.6</td>
</tr>
<tr>
<td>4</td>
<td>35–45</td>
<td>7.4</td>
<td>71</td>
<td>31.3</td>
<td>18.7</td>
</tr>
<tr>
<td>5</td>
<td>0–35</td>
<td>9.5</td>
<td>63</td>
<td>34.0</td>
<td>18.1</td>
</tr>
<tr>
<td>5</td>
<td>35–44</td>
<td>7.8</td>
<td>64</td>
<td>34.0</td>
<td>19.3</td>
</tr>
<tr>
<td>5</td>
<td>45–51</td>
<td>9</td>
<td>67</td>
<td>36.0</td>
<td>21.4</td>
</tr>
<tr>
<td>5</td>
<td>52–58</td>
<td>10.0</td>
<td>57</td>
<td>36.0</td>
<td>21.3</td>
</tr>
</tbody>
</table>
the equation by Henderson et al. (1992). There is a known temperature dependence of the isotopic equilibrium during dissolution and conversion of CO2 to bicarbonate (Mook et al. 1974), and the temperature dependence of \( b_d \) 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 \( \phi \) is constant at 0.21 under a range of short-term changes in environmental conditions and in a number of C4 species. In particular, for \( S. \) bicolor, a value of 0.21 was measured. Substituting this value of \( \phi \) and the above values into Equation (3) and rearranging, one obtains the following expression:

\[
p/p_a = 1.2 - 272 \Delta A
\]

(4)

(with \( \Delta A \) typically having values of 3‰, i.e. \( 3 \times 10^{-3} \)) which, combined with (2), gives:

\[
W(\text{mol C mol}^{-1} \text{H}_2\text{O}) = 1.9 \Delta A - 1.4 \times 10^{-3}, \quad (5a)
\]

for Experiment 1.

For the field experiments, the relationship was taken as:

\[
W(\text{mol C mol}^{-1} \text{H}_2\text{O}) = 1.1 \Delta A - 0.8 \times 10^{-3}, \quad (5b)
\]

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

**Variation amongst lines of C4 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 on line \( i (i = 1, \ldots, k) \) and genotype \( j = 1, \ldots, n \), and \( k \) and \( n \) are the numbers of genotypes and replicates, respectively; \( m \) is the grand mean; \( g_i \) is the effect of line \( i, N(0, \sigma_g^2) \); \( b_j \) is the effect of replicate \( j, N(0, \sigma_b^2) \); and \( e_{ij} \) is the error effect associated with observation \( j \) on genotype \( i, N(0, \sigma_e^2) \).

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:

\[
R^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2 / n}, \quad (6)
\]

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 \( \Delta_d \) 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\(_2\)O and the range was 0.81 mmol C mol\(^{-1}\) H\(_2\)O. The mean \( \Delta_d \) was 3‰ and the range was 0.43‰.

A weak but significant correlation was found between \( W \) and \( \Delta_d \) for lines and the relationship was positive, with \( W \) increasing as \( \Delta_d \) increased (Fig. 1). The observed relationship is consistent with the relationship predicted from theoretical considerations which is shown on the figure as both having positive slopes (the theoretical and regression equations are given in the Legend). Dry matter accumulation did not correlate with \( W \) or \( \Delta_d \), although there was a tendency for it to be less in plants with greater \( \Delta_d \) and greater transpiration efficiency, \( W \).

![Fig. 1. Mean transpiration efficiency, \( W \), versus mean leaf dry matter discrimination, \( \Delta_d \), for 30 \( S. \) bicolor lines grown in sealed pots in the glasshouse in Expt 1. The linear regression for the data was \( W = 1.05 \Delta_d + 1.93 \times 10^{-3} \) (\( r^2 = 0.18, P < 0.05 \)) (not shown). The theoretical equation is \( W = 1.9 \Delta_d - 1.4 \times 10^{-3} \). We have separately denoted those of the 30 leaves which were used in subsequent experiments (2, 4, 5): Trump (\( \bigtriangleup \)), Tx610SR (\( \bullet \)), White Charger (\( \bigstar \)), NewTropic (\( \bigtriangledown \)), and Hylan 4x8 (\( \Delta \)).](image)
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 \( \text{CO}_2 \) assimilation, \( A \), the ratio of leaf intercellular to ambient \( \text{CO}_2 \) partial pressure, \( p_i/p_a \), and carbon isotope discrimination.

Fig. 2 shows the results of concurrent measurements of \( p_i/p_a \) and \( \Delta_e \). 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 \( \text{CO}_2 \) 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 \( \Delta_e \) 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 \( \text{CO}_2 \)-assimilation rates at high irradiance, the average being 54 \( \text{mmol} \text{m}^{-2} \text{s}^{-1} \) (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. \( \Delta_d \) was determined on bulk leaf
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 $\Delta_d$ 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 $\Delta_d$ 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 $\Delta_d$ (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. $\Delta_d$ was again 0.8‰ larger for plants grown in the field (Fig. 3). The lines ranked differently in mean $\Delta_d$ and $W$ in this experiment, compared to Experiments 1 and 3 (Fig. 3).

For individual plants, a significant correlation existed between $W$ and $\Delta_d$ (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
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 Δd measured at the different harvests. The ranking of the lines varied with harvest and there was no difference between lines in either W or Δd at the final harvest (Harvest 4). Overall, W decreased with successive harvests, whereas Δd increased.

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

**Table 5.** Mean transpiration efficiency, W (mmol C mol⁻¹ H₂O), and leaf dry matter discrimination, Δd (%e), for *S. bicolor* lines at three different times after sowing (DAS) in Experiment 5

<table>
<thead>
<tr>
<th>Line</th>
<th>Trait</th>
<th>Harvest 2</th>
<th>Harvest 3</th>
<th>Harvest 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success 40W</td>
<td>W</td>
<td>3.48 (3) [7]</td>
<td>3.00 (1) [4]</td>
<td>1.94 (2) [8]</td>
</tr>
<tr>
<td>White Charger</td>
<td>W</td>
<td>2.79 (2) [8]</td>
<td>3.35 (3) [4]</td>
<td>2.24 (4) [8]</td>
</tr>
<tr>
<td>Hylan 4x8</td>
<td>W</td>
<td>2.26 (1) [8]</td>
<td>3.42 (4) [6]</td>
<td>1.98 (3) [8]</td>
</tr>
<tr>
<td>Success 40W</td>
<td>Δd</td>
<td>4.4 (4) [7]</td>
<td>4.6 (2) [4]</td>
<td>4.9 (3) [8]</td>
</tr>
<tr>
<td>White Charger</td>
<td>Δd</td>
<td>4.2 (2) [8]</td>
<td>4.7 (3) [4]</td>
<td>4.8 (1) [8]</td>
</tr>
<tr>
<td>Hylan 4x8</td>
<td>Δd</td>
<td>4.2 (3) [8]</td>
<td>4.8 (4) [6]</td>
<td>5.0 (4) [8]</td>
</tr>
</tbody>
</table>

Fig. 3. Comparisons of genotypic means of transpiration efficiency, W, and Δd across experiments. Error bars show standard errors.
Discussion

Relationship between \( W \), \( \frac{p_i}{p_a} \), \( g \) 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 \( D \) in C\(_4\) species (Figs 1, 4 and 5).

The relationship between \( W \) and \( \frac{p_i}{p_a} \) is common to C\(_3\) and C\(_4\) species (Equation 1), although C\(_4\) species typically have lower ratios of \( \frac{p_i}{p_a} \) 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 \( \frac{p_i}{p_a} = 0.33 \) (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 \( \frac{p_i}{p_a} \), 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).

The relationship between \( \Delta_A \) and \( \frac{p_i}{p_a} \) is different in C\(_3\) and C\(_4\) species. In C\(_3\) species, \( \Delta_A \) and \( \frac{p_i}{p_a} \) are linearly related with a positive slope of approximately 22.6\(\%\) per unit change in \( \frac{p_i}{p_a} \) (Farquhar et al. 1989a). In C\(_4\) species, \( \Delta_A \) and \( \frac{p_i}{p_a} \) are also linearly related but the slope of the relationship is dependent on the leakiness, \( \phi \), of the CO\(_2\) concentrating mechanism (Farquhar 1983). Concurrent measurements of \( \frac{p_i}{p_a} \) and \( \Delta_A \) 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 $\phi$ amongst these eight lines and the value of $\phi$ was similar to that previously determined for a number of other C$_4$ species (Table 3, Henderson et al. 1992). The linear relationships between $\Delta_a$ 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 $\Delta_a$ per unit change in $p_i/p_a$. Henderson et al. (1992) also showed that there was little variation in $\phi$ 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 $\Delta_a$ 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 $\Delta_a$ and $p_i/p_a$ for C$_4$ species translates into a positive relationship between $W$ and $\Delta_d$. We observed the expected positive relationship between $W$ and $\Delta_d$ 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 5a and 5b) and data show that the difference observed in $W$ is that expected from the difference observed in $\Delta_d$ (regression equations are given in the figure captions).

The significant correlations between $W$ and $\Delta_d$ 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 $\Delta_d$. 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 $\Delta_d$ in the field for peanut regardless of whether the plants were in the canopy or above the canopy.

Environmental variation in $W$ and $\Delta_d$

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, $\phi_r$, 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), $\phi_r$ has been increased from 0.25 in the glasshouse to 0.30 in the field, and $\phi_w$, the water loss factor, has been increased from 0.10 to 0.20.

$\Delta_d$ 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 $\delta$ in the two environments. There can be diurnal and seasonal variation in $\delta$ resulting from industrial activity in local areas. Inoue and Sugimura (1984) found 2% variations in $\delta$ at a field site 60 km from Tokyo and this could explain why $\Delta_d$ 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, $b_x$, with temperature due to changes in the discrimination during dissolution and hydration of CO$_2$. The temperature dependence of $b_x$ was given by Henderson et al. (1992) and we calculate that a shift in temperature from 25 to 30°C could increase $\Delta_d$ by 0.35.

The relationship between $\Delta_d$ 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 $\Delta_d$ was not a good indicator of $\Delta_a$ 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 $\Delta_d$ caused by variations in $p_i/p_a$ are smaller than in C$_3$ species.

Genetic variation in $W$ and $\Delta_d$

Significant genetic variation in $W$ and $\Delta_d$ 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 $\Delta_d$ 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 $\Delta_d$ than in C$_3$ species.

Tables 6 and 7 provide an overview of genetic variation and precision observed in $\Delta_d$ and $W$ for some C$_3$ species, S. bicolor and Panicum coloratum. The standard deviations for $\Delta_d$ (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
in sorghum $\Delta_d$ 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 $\Delta_d$ 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 $\times$ environmental interactions for both $\Delta_d$ and $W$. In many studies of $C_3$ species, $W$ and $\Delta_d$ 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 $C_4$ and $C_3$ 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 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.

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Environment</th>
<th>Line mean repeatability</th>
<th>Least significant difference ($P=0.05$)</th>
<th>Standard deviation ($\sigma_s$)</th>
<th>Ratio of $\sigma_s$ to LSD $^a$ ($P=0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley: Hubick and Farquhar (1989)</td>
<td>Well watered, glasshouse</td>
<td>0.93</td>
<td>0.17 (6)</td>
<td>0.22$^b$</td>
<td>1.3</td>
</tr>
<tr>
<td>Cotton: Hubick and Farquhar (1987)</td>
<td>Well watered, glasshouse</td>
<td>—</td>
<td>—</td>
<td>0.15$^a$</td>
<td>—</td>
</tr>
<tr>
<td>Peanut: Hubick et al. (1986)</td>
<td>Well watered, glasshouse</td>
<td>0.94</td>
<td>0.19 (4)</td>
<td>0.30$^a$</td>
<td>1.6</td>
</tr>
<tr>
<td>Peanut: Wright et al. (1988)</td>
<td>In-ground pots</td>
<td>—</td>
<td>—</td>
<td>0.22$^a$</td>
<td>—</td>
</tr>
<tr>
<td>Potato: Vos and Groenwold (1991)</td>
<td>Well watered</td>
<td>—</td>
<td>—</td>
<td>0.23</td>
<td>—</td>
</tr>
<tr>
<td>Sunflower: Virgona et al. (1991)</td>
<td>Well watered, glasshouse</td>
<td>—</td>
<td>—</td>
<td>0.36$^a$</td>
<td>—</td>
</tr>
<tr>
<td>Wheat: Ehdaie et al. (1991)</td>
<td>Well watered, glasshouse</td>
<td>1987</td>
<td>—</td>
<td>0.44$^a$</td>
<td>—</td>
</tr>
<tr>
<td>Sorghum — Experiment 1</td>
<td>Well watered, glasshouse</td>
<td>0.66</td>
<td>0.39 (4)</td>
<td>0.24$^a$</td>
<td>0.6</td>
</tr>
</tbody>
</table>

$^a$ Originally given in 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 $\Delta_d$ changed in different environments (White et al. 1990; Hall et al. 1989; Crauford et al. 1991). Masle et al. (1993) found that for Arabidopsis $\Delta_d$ 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 $\Delta_d$ values had the more dominant genes.

Conclusion

Genetic variation in $\Delta_d$ and $\Delta_A$ 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 than 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 $\Delta_d$ varied across environments. Some of the line $x$ environment interaction and experimental error in $\Delta_d$ 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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References


