Do morphological changes mediate plant responses to water stress? A steady-state experiment with two C$_4$ grasses

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Summary

- We hypothesized that plant growth reduction under water stress is caused primarily by a reduction of leaf-area ratio (LAR, leaf area per unit of total plant dry mass).
- Two perennial Chihuahuan desert grass species (slow-growing *Bouteloua eriopoda* and fast-growing *Eragrostis lehmanniana*) were subjected over 6 wk to a combination of two water-supply regimes (control and drought) and two levels of atmospheric CO$_2$ partial pressure (375 and 750 µmol mol$^{-1}$).
- Drought reduced final biomass in *Bouteloua* by 60% regardless of CO$_2$ concentration. *Eragrostis* experienced a similar biomass reduction at 375 µmol mol$^{-1}$, but large plants under elevated CO$_2$ attained growth rates comparable to those of controls. Overall, for plants of similar size, drought reduced LAR in both species much more strongly than it affected net assimilation rate. This reduction in LAR was caused by reductions in both specific leaf area and leaf weight ratio.
- We conclude that reduced growth under drought can be considered as a byproduct of the same plastic, developmental responses that result in a reduced water loss.

Key words: allometry, *Bouteloua eriopoda* (blackgrama), Chihuahuan Desert, drought, *Eragrostis lehmanniana* (lovegrass), growth analysis, plasticity, water-use efficiency (WUE).

Introduction

Drought studies on crops and other herbaceous species have repeatedly shown that leaf expansion and associated morphological and allocation variables are more sensitive to water stress than leaf conductance and instantaneous carbon gain (Kalapos *et al.*, 1996; Kramer & Boyer, 1996; Sadras & Milroy, 1996). Compared with well-watered plants, water-stressed plants tend to have a smaller proportion of their biomass as leaves (i.e. reduced leaf-weight ratio, LWR) and thicker and/or heavier leaves (i.e. smaller specific leaf area, SLA). However, these well-known morphological changes have two evolutionary consequences of opposite effects: on one hand, they may effectively reduce stress at the leaf level; on the other hand, they may also reduce whole-plant growth. An examination of LWR and SLA, and other components of growth such as relative growth rate (RGR) and net assimilation rate (NAR), provides a powerful framework in which to evaluate relationships between whole-plant growth, its various components, and environmental factors (Hunt, 1982). One important generalization from such growth analyses is that the RGR of seedlings under nonlimiting conditions (RGR$_{max}$) is a key diagnostic trait for classifying species because of its correlation with a number of other ecophysiological and morphological traits. Another generalization is that the leaf-area ratio (LAR, the ‘morphological’ component of growth; Table 1) is the main determinant of RGR$_{max}$, not NAR (the ‘physiological’ component of growth) (Hunt & Cornelissen, 1997).

Mathematically, LAR is the product of LWR and SLA (Table 1, Equation 1). As explained above, these two growth components tend to decrease with increasing water stress, which suggests the intriguing possibility that plastic, morphological change within a species is the primary reason why herbaceous plants exhibit reduced growth when subjected to drought. While this possibility does not appear to have been formally explored, it has limited experimental support. Kalapos *et al.*
(1996) found that drought-induced reductions of RGR in wheat were preceded by changes in biomass partitioning; Fernández & Reynolds (2000) reported that changes in SLA and root-to-shoot ratio in response to drought in eight perennial grasses (representing a wide range of $RGR_{\text{max}}$) were as large as within-treatment differences between species.

In this paper we present a controlled-environment experiment to test two hypotheses related to plant growth under drought conditions. First, that decreased growth is due to a reduction of leaf-area ratio (LAR) and that changes in net assimilation rate (NAR), if present, play a lesser role. Second, that environmental factors with a positive influence on NAR, e.g. elevated atmospheric CO$_2$ (Roumet et al., 1996) can ameliorate the drought-induced, negative impact of reduced LAR on growth. These two hypotheses are presented in graphical form in Fig. 1, which incorporates the well-known decline in RGR during ontogeny (Lambers et al., 1998). A corollary is that very large changes in NAR are needed to offset drought-induced reductions in RGR.

We subjected two C$_4$, perennial grasses – Bouteloua eriopoda (Torr.) Torr (blackgrama) and Eragrostis lehmanniana Nees (Lehmann love-grass) – to two watering regimes (control and drought) and two levels of atmospheric CO$_2$ partial pressure (375 µmol mol$^{-1}$ and 750 µmol mol$^{-1}$). We estimated relative growth rate (RGR) and its components (Table 1) over a 6-wk growing period. Bouteloua and Eragrostis, which coexist in the northern Chihuahuan Desert of New Mexico, USA, differ substantially in their maximum RGR: Bouteloua, a native species, is inherently slow-growing whereas Eragrostis, a South African species introduced to North America during the 1930s (Anable et al., 1992), is inherently fast-growing with an $RGR_{\text{max}}$ double that of Bouteloua (Fernández & Reynolds, 2000). Adequate testing of our hypotheses requires two conditions: (1) a method for imposing water stress in a steady-state manner, and (2) a comparison of treatment effects on plants of similar size, rather than plants of similar age (Gebauer et al., 1996). The first condition was met by using a unique subirrigation method, and the second by conducting multiple harvests to gather data on plants of different sizes, and then using plant biomass as a covariate in the statistical analyses.

**Materials and Methods**

**Experimental approach and design**

Water stress was imposed by a modification of the subirrigation method proposed by Snow & Tingey (1985), as described by Fernández & Reynolds (2000). Details are given in the Experimental procedures. Briefly, plants were grown in special open-bottom pots fitted at their base with a fine mesh, allowing
nutrient solution exchange but preventing root passage. Groups of these pots were placed over columns of a uniform porous medium that were placed in containers partly filled with a nutrient solution. Keeping a constant nutrient solution height ensures a uniform and repeatable water availability in the pots (Wookey et al., 1991; Saulescu et al., 1995). Consequently, stress intensity is independent of plant size; plants have access to an unlimited volume of water that, nonetheless, is always delivered at constant water potential determined by the level of the nutrient solution.

A total of four growth chambers were randomly assigned to either doubled (750 µmol mol\(^{-1}\)) or nearly current (375 µmol mol\(^{-1}\)) atmospheric CO\(_2\) partial pressure. These conditions were maintained 24 h a day throughout the experiment. Within each chamber, six containers, each with eight pots and a single plant per pot, were established for each species (see Experimental procedures). Three of the containers were maintained as well-watered controls, with the nutrient-solution level set at 5 cm below the base of the pots (plant-available water, PAW, c. 100%); the other three received a drought treatment (nutrient solution set at 20 cm below the base of the pots; PAW c. 40%). This scheme with three subreplicates was designed to account for within-chamber variation (cf. Potvin, 1993).

Plants' water-use through transpiration was estimated by changes in container weight between successive harvests. Precautions were taken to minimize evaporative water loss (details below). Eight additional containers with no plants (four per treatment) were placed in each chamber to estimate evaporative water loss. The eight plants per container were used in seven successive harvests; the last harvest included two plants per container. Harvest intervals were selected for each combination of species and treatment to obtain data over the widest possible range of plant sizes. These intervals ranged from 3 to 8 d, with more frequent harvests for E. lehmannianna Nees (the fast grower) than for Bouteloua eriopoda (the slow grower); also, within a species, harvests were more frequent for well-watered than for droughted plants. Whole-plant water-use efficiency (WUE\(_{\text{plant}}\)) was estimated correlating the increase in dry mass of all the plants present in each container between successive harvest dates with plant-water use. This was calculated as the cumulative weight losses of plant containers minus the cumulative weight losses of blank (no-plant) containers.

By ensuring fixed soil-moisture levels regardless of any direct leaf responses to enriched CO\(_2\) levels, and by recording WUE\(_{\text{plant}}\) as a long-term indicator of stomatal behavior, we aimed to separate direct, or primary, responses of our experimental plants to elevated CO\(_2\), such as increases in photosynthetic rate and decreases in stomatal conductance (Ward & Strain, 1999), from indirect ones, such as improved soil-water conditions resulting from CO\(_2\)-decreased leaf conductance (King & Greer, 1986; Ziska & Bunce, 1997; Long, 1999; Williams et al., 2001).

### Experimental procedures

Seeds collected at the Jornada Basin Long-term Ecological Research (LTER) site, New Mexico, USA (site information at: http://jornada.nmsu.edu) were brought to the Phytotron at Duke University (Durham, NC, USA) and planted in greenhouse trays. After one leaf was fully expanded, seedlings were transplanted to sand-filled plastic pots (height 15 cm, volume 0.7 l). Each pot was fitted at its base with a thick, close-knit synthetic mesh (commercial banter cloth) that had proved to be an effective barrier to roots (checked regularly during the experiment). One seedling was transplanted to each pot, and the surface of the sand was sealed with paraffin wax (Gulf Wax, Memphis, TN, USA), except for a 2-cm circle surrounding the base of each plant. The eight pots for each replication (see previous section) were placed in 20-l containers housing a 35-cm tall column of commercial foam (No. 0140; Smithers-Oasis; Kent, OH, USA), and partly filled with quarter-strength modified Hoagland solution (Downs & Hellmers, 1975). To minimize water losses, rectangular pots were used and bound together with duct tape. Spaces between the bundled pots and the edge of the container were also sealed. The growing temperature was 30/20°C (day/night), photoperiod was 14 h, and light intensity (fluorescent + halogen) set at > 1000 µmol m\(^{-2}\) s\(^{-1}\). Vapor pressure deficit remained close to 2.5 kPa.

On the first day the drought treatments were imposed, an initial harvest of 24 plants of each species was made. These plants were never placed in containers but were grown, transplanted and wax-sealed in exactly the same way as those used for the rest of the experiment. Up to this point, all plants had been kept well watered, and placed in current-CO\(_2\) growth chambers for acclimation. At each harvest date, containers were weighed (±100 g), and pots containing plants to be harvested were removed and replaced by empty pots with waxed surfaces. The containers were then resealed and reweighed before being returned to the growth chamber. Plant material was separated into leaves (blades only), stems (including leaf sheaths), and roots. Subsamples of the leaves were immediately scanned for leaf area determination. All plant material was dried for at least 36 h at 65°C before determining weight (±0.1 mg).

### Data analyses

Data were analysed as a split-plot design, with the chambers as the main plots. JMP software (SAS Institute Inc.; Cary, NC, USA) was used to devise generalized linear models (GLMs) to assess the effects of drought and CO\(_2\) (fixed factors), as well as their interactions. Chamber was included as a random factor, taking into account its nesting within CO\(_2\) levels (Potvin, 1993). We performed functional growth analyses (sensu Hunt, 1982) by polynomial adjustment of log-transformed total biomass (ln\(W_{t}\)) and log-transformed leaf area (lnLA). We then calculated relative growth rate (RGR), leaf-area ratio...
(LAR), net assimilation rate (NAR), and allocation coefficients according to the definitions in Table 1.

Linear polynomial regressions were performed between lnW_T and time, and lnLA and time, using initial biomass (n = 24) as the y-intercept. The order of the polynomial was determined for each combination of species and treatment by the highest-order significant coefficient (Hunt, 1982). The RGR was obtained as the first derivative of the lnW_T-time function. The NAR was obtained by dividing the RGR function by the LAR function. This ratio yielded unrealistically high values of NAR for Bouteloua plants towards the end of the experiment, when RGR was very close to zero; accordingly, these data were omitted from the corresponding graphs for the largest plants of this species.

Two caveats are required. First, ‘assimilation’ in net assimilation rate (NAR) is defined in terms of growth (total biomass increase) per unit of current leaf area (Table 1, Equation 3a). Photosynthesis influences this, but two plants with identical leaf-level carbon gain can have a different NAR if, for example, each has a different proportion of biomass in leaves vs non-photosynthetic tissue (Lambers & Dijkstra, 1987). Hence, we interpret NAR as a ‘residual’, i.e. the amount of growth not explained by morphological changes. The second caveat is that growth analysis calculations always involve two types of data: primary data including variables (or combination of variables) measured at individual harvests, such as LA, W_T, W_L, W_S, W_R, and derived data, such as RGR and NAR, which require calculations based on primary data from at least two harvests. Some growth components, however, can be obtained in both ways; for example, LAR can have a point-estimation from a single harvest at time ‘i’ as LA/W_Ti, or be obtained as the continuous function that results from dividing the regression equations separately fitted for LA and W_T vs time. Both approaches were used in this study and variability of point-estimates is shown whenever possible.

Growth analysis results are presented in graphical form to facilitate a visual interpretation of responses to treatments. These results were also analysed via morphometric analyses using GLMs as follows: LAR response was assessed using lnLA as the response variable and lnW_T as the covariate; and SLA response was assessed with lnLA as the response variable and lnW_L as the covariate. Allometry (i.e. the proportional partition of biomass into leaves (LWR), stems (SWR), and roots (RWR)) was determined within the GLMs by regressing, after In–ln transformation, the dry mass of each of these organs vs. the rest of the biomass. To avoid propagation of errors, these analyses were conducted only on primary data, and not on derived data; they also never included initial biomass data (Scheiner & Gurevitch, 1993). Similar analyses were performed for tillering and water use.

Results
At current atmospheric CO_2 levels, B. eriopoda and E. lehmanniana had large differences in growth, but similar responses to drought. When well-watered, there was a four-fold difference in absolute growth between the species: from initial seedling weights of c. 7 mg for both species, Bouteloua plants reached a dry mass of about 2 g in 5 wk, whereas Eragrostis attained more than 5 g in less than 3 wk (Fig. 2). When droughted, final biomass for both species was reduced by c. 60%. By contrast, the growth response under doubled CO_2 concentration was quite different for the two species. For both well-watered and drought conditions, elevated CO_2 had no effect on Bouteloua biomass. For Eragrostis, the effect of drought was much less intense at high CO_2 (30% vs 65% reduction in final biomass, respectively; Fig. 2).

Total plant biomass was readily described by low-order polynomial regressions; these differed more between species than between treatments within a species (Table 2). Regardless of treatment, the best fit was obtained using cubic equations for Bouteloua and quadratic equations for Eragrostis (Table 2a).

In both species, treatments with a high RGR_{max} (linear term b1) tended to be those with a faster initial decrease in RGR (more negative b2). Treatment effects are most evident when comparisons are made for plants of a common size, as shown in Fig. 3. For example, the RGR of small (c. 50 mg) plants of
Table 2 Regression parameters for grass growth over time (x): (a) \( y = \ln(\text{total biomass}) \); the linear term \( b_1 \) represents the initial, exponential rate of biomass increase (RGR\(_{\text{max}}\)); the quadratic term \( b_2 \) represents RGR decrease over time. (b) \( y = \ln(\text{leaf area}) \); the linear term \( b_1 \) represents the initial, exponential rate of leaf area increase; the quadratic term \( b_2 \) represents the rate of abatement of such increase\(^a\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( b_1 )</th>
<th>Significance (^b)</th>
<th>( b_2 )</th>
<th>Significance (^b)</th>
<th>( b_3 )</th>
<th>Significance (^b)</th>
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<tbody>
<tr>
<td><strong>(a) Biomass</strong></td>
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<td>yz</td>
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<td>m</td>
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<td>yz</td>
<td>0.00006</td>
<td>m</td>
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<td>ab</td>
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<td>yz</td>
<td>0.00010</td>
<td>m</td>
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<td>ab</td>
<td>−0.010</td>
<td>yz</td>
<td>0.00011</td>
<td>m</td>
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<tr>
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<td>cb</td>
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<td>y</td>
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<td>0.532</td>
<td>d</td>
<td>−0.011</td>
<td>yz</td>
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<td><strong>(b) Leaf Area</strong></td>
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<td><em>Bouteloua</em></td>
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<tr>
<td>W–C–</td>
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<td>a</td>
<td>−0.0026</td>
<td>wx</td>
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<tr>
<td>W–C+</td>
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<td>x</td>
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<tr>
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<tr>
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<td>yz</td>
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<td>y</td>
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<td>−0.0076</td>
<td>yz</td>
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<td>z</td>
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\(^a\)All fits highly significant \( (n = 72; P << 0.001) \). Water-supply treatments: control, W+, drought, W–. Atmospheric CO\(_2\): C+, 750 µmol mol\(^{-1}\); C–, 375 µmol mol\(^{-1}\).

\(^b\)Shared letters on the same column indicate nonsignificant \( (P > 0.05) \) differences.

Both species were c. 20% higher in controls than droughted plants of similar dry mass. As plants grew larger (> 200–1000 mg), the relative difference increased to more than 100%. For example, well-watered *Bouteloua* plants had detectable increases in biomass at sizes in which droughted plants had ceased to grow (Fig. 3).

Leaf area increased twice as fast for *Eragrostis* than for *Bouteloua* (\( b_1 \) in Table 2b). During the initial, exponential growth phase, leaf expansion for both species was much more reduced by drought (74–79%) than by the CO\(_2\) (7–19%, data not shown). Regressions of ln-transformed leaf area vs time were all highly significant. Irrespective of species and treatment, the best fit to these data was obtained with quadratic models (Table 2b). As was the case for dry matter, comparisons between \( b_1 \) and \( b_2 \) shows that larger initial rates of leaf-area expansion (e.g. as under well-watered, elevated CO\(_2\) conditions) is correlated with a faster decrease with time (\( b_2 \), Table 2b).

Treatments not only influenced the trends in plant biomass and leaf area, but also the relation between them. For *Eragrostis*, there was a significant CO\(_2\)–drought interaction (Table 3, analysis 1. That is, for a given total biomass, leaf area was significantly reduced by drought under current but not elevated CO\(_2\) \( (P < 0.03) \). Moreover, for both species there was a significant lnW\(_T\)–water interaction, indicating a different slope in the lnLA–lnWT relationship for different water treatments (larger slope for well-watered plants). This slope represents LAR, the morphological component of growth, which was reduced in both species by c. 30–50% when large plants were subjected to drought (Fig. 3). The level of CO\(_2\) did not directly affect this slope or leaf area at a common biomass (Table 3, analysis 1). The NAR showed a less consistent response to the treatments: it was not always affected by drought and was enhanced by elevated CO\(_2\) in just two instances. Only small, well-watered plants of *Bouteloua* and large, droughted plants of *Eragrostis* had a higher NAR under doubled than under current CO\(_2\) levels (Fig. 3).

Reductions in LAR, as observed here, could have been caused by reductions in either SLA and/or in LWR. In this experiment, they were the result of both. In *Bouteloua*, SLA and LWR decreased with plant size for all treatments (Fig. 4, left panels), and drought intensified this decrease (analyses 2 and 3, Table 3). In *Eragrostis*, LWR changed much less with size (Fig. 4, right panels); nevertheless, both LWR and SLA were reduced in large plants by drought under current CO\(_2\) levels (interaction in analyses 2 and 3 Table 3). This means that water and CO\(_2\) levels influenced the allocation (LWR) and leaf properties (SLA) of *Eragrostis* in the same way that they influenced RGR and LAR (cf. Figures 3 and 4).

Leaf mass was the plant fraction most strongly reduced by drought in both species (cf. analyses 3–5 in Table 3). Allocation to stems was increased by drought: SWR changed from 0.46 to 0.49 in *Bouteloua*, and from 0.33 to 0.35 in *Eragrostis* (Table 3, analysis 4). However, this increase was small, and shoot–root partitioning was still significantly reduced by drought (Table 3, analysis 5). Average root-weight ratio (RWR) was almost identical for both species, and increased
under drought from c. 0.29–0.33. Root allocation was not affected by CO$_2$ and neither species had a significant CO$_2$–drought interaction (Table 3, analysis 5). The model for *Bouteloua* had a significant shoot biomass–water interaction (different slope in the lnW$_R$–lnW$_{Shoot}$ relationship between watering treatments), indicating a slight increase in RWR with ontogeny for well-watered plants, as opposed of no trend for droughted plants ($P = 0.0008$; data not shown).

We estimated plant water consumption (transpiration) following changes in the weight of large (20–25 kg) containers. These changes were relatively small, and sometimes difficult to distinguish from evaporation losses, which were substantial: between 0.7 and 1.4 kg per container (cumulative, in the absence of plants). The problem was exacerbated when trying to assess transpiration of small, slow-growing plants; so much so, that measured water use was not a good predictor of biomass production for *Bouteloua* under drought ($P = 0.43$). By contrast, *Eragrostis* biomass production was significantly correlated ($P < 0.0001$) with plant water-use estimated in this way. The regression equation had a slope of 4.97 ± 0.33 g dry matter kg$^{-1}$ of transpired water, which is a measure of whole-plant water-use efficiency ($\text{WUE}_{\text{plant}}$).

**Discussion**

*Eragrostis lehmanniana* and *Bouteloua eriopoda* respond to water stress by allocating proportionally more biomass to roots and less to leaves (lowering LWR), and by producing leaves of smaller area : weight ratio (SLA) than those under well-watered conditions (Table 3; Figs 3+4). The combined effect of these changes is to reduce LAR and therefore RGR (cf. Equation. 1 and 2b, Table 1). Consistent with our first hypothesis, this drought-induced decrease in growth rate and in LAR is not always accompanied by a reduction in NAR (Fig. 5). The impact of water shortage on both slow-growing *Bouteloua* and fast-growing *Eragrostis* is remarkably similar in both qualitative and quantitative terms. This supports previous work suggesting a lack of trade-off between growth potential and drought tolerance in desert grasses (Fernández & Reynolds, 2000).
Overall, a doubling of CO₂ concentration has little effect on growth or any of its components in these species. C₄ plant responses to elevated CO₂ are known to be variable and often weaker than C₃ plant responses; moreover, nonsaturating light levels can further constrain the responses of C₄ plants to CO₂ (Sage & Monson, 1999). Thus, we are unable to test sufficiently our second hypothesis that environmental factors with a positive influence on NAR can mitigate the drought-induced,
negative effects of reduced LAR on growth. It is true that for *Eragrostis* we found a significant CO$_2$–drought interaction, which in large plants is partly explained by a higher NAR. However, even in this case, growth enhancement by CO$_2$ was also associated with an increased allocation of biomass to leaves of high SLA, and therefore plants of higher LAR. For well-watered plants, the lack of response of the two species to elevated CO$_2$ is consistent with Roumet & Roy’s (1996) conclusion for other C$_4$ grasses of similar, relatively low SLA (see also Yoder *et al*., 2000).

Other experiments have shown that CO$_2$ stimulation of growth in droughted plants may be caused by an improved plant- and/or soil-water status (Sionit & Patterson, 1985; Knapp *et al*., 1993, 1996; Conley *et al*., 2001; but see: Barr *et al*., 1990; Ziska & Bunce, 1997). Our experimental protocol aimed to overcome such indirect effects, although it could only ensure constant soil water conditions. Thus, it is still possible that plants in the drought-elevated CO$_2$ treatment had, in comparison with plants in the drought–current CO$_2$ treatment, an improved leaf water status because of reduced stomatal conductance (Sage *et al*., 1999a). Indeed mid-afternoon porometry measurements indicated that leaf conductance was reduced in *Eragrostis* under elevated, compared with current, CO$_2$ (data not shown). However, this short-term leaf-level effect did not have an impact on whole-plant growth. Otherwise, WUE$_{plant}$ would have been improved under elevated CO$_2$ (Buchmann *et al*., 1996; Samarakoon & Gifford, 1996; Long, 1999), which is not the case (Table 3, row 7). From this, we conclude that the interactive effect of CO$_2$ and drought on *Eragrostis* growth may be a function of morphological changes (LAR and SLA: Table 3). If any direct, nonmorphological effects existed, they only became important late in the experiment, and for plants that were growing slowly.

Our estimate of WUE$_{plant}$ is within the range of reported values for other C$_4$ species (Samarakoon & Gifford, 1996; Morgan *et al*., 1998). WUE$_{plant}$ was slightly, but significantly (Table 3) improved by water availability (12%; data not shown elsewhere). This is opposite to what is expected when there are drought-induced reductions in stomatal conductance (Long, 1999), and may be explained by an increase of the CO$_2$ leakage out from the bundle sheath (Farquhar, 1983; Long, 1999; Cousins *et al*., 2001). Such stress-promoted leakage can be particularly pronounced in NAD-malic enzyme species, the C$_4$ subtype to which *Eragrostis* belongs (Sage *et al*., 1999b).

At the time of final harvest, well-watered plants of both species had a larger number of tillers than droughted ones of a similar age (*Bouteloua*: 23 vs 11, $P < 0.05$; *Eragrostis*: 51 vs 40, $P > 0.05$). An interesting difference between the species appears beyond the obvious effect of drought on total biomass. For plants of comparable mass, water stress reduced the number of tillers in *Bouteloua* and increased it in *Eragrostis* (Table 3, analysis 6); this is best illustrated by its effect on average biomass per tiller, which was slightly reduced under drought for *Eragrostis* (15%) and increased by more than 60% for *Bouteloua* (data not shown). If the number of tillers is interpreted as an index of development (Jollife *et al*., 1988), then ontogeny was accelerated with drought in *Eragrostis* but slowed in *Bouteloua*. Elevated CO$_2$ did not affect (by itself or together with) the number of tillers produced or their size (Table 3, analysis 6).

A seemingly moderate reduction in soil-water supply, when maintained over several weeks, reduced cumulative growth by more than 50% with respect to well-watered controls. Our detailed growth analyses provide an explanation: given the multiplicative nature of growth, even small initial differences in RGR produced by mild water stress, when integrated over time, can strongly reduce final biomass (Maroco *et al*., 2000). Growth analyses have repeatedly shown that RGR decreases as plants increase in size, which can be interpreted as a strong ontogeny control on RGR (Lambers *et al*., 1990). In our experiment, for each species considered separately, time
was occasionally a better predictor of RGR than biomass. For example, there were small differences between (time) coefficients in Table 2a for *Bouteloua*, compared with the obvious differences observed in the RGR–biomass plot (Fig. 3). Since absolute growth is the product of RGR and total plant biomass, any reduction in RGR for plants of similar biomass is translated into a large negative effect. This effect is not compensated by the (weaker) positive impact of small size on RGR (Table 1, Equation 2a).

If both species respond similarly to drought, why does *Eragrostis* generally grow faster than *Bouteloua* under well-watered and drought conditions? Compared with *Bouteloua*, *Eragrostis* has a similar NAR (Fig. 3) but it has a higher LAR because of a slightly larger SLA and larger LWR. Perhaps more importantly, the components of LAR do not decline as much during ontogeny as in *Bouteloua* (Fig. 4). As these differences are particularly promoted by the combination of drought and elevated CO₂, it is tempting to relate them to the changes in the relative abundance of these species in the Jornada Basin, where *Eragrostis* has been expanding and *Bouteloua* declining for a number of years. Moreover, demographic traits, such as early and profuse reproduction, may also explain the spread of *Eragrostis* perhaps better than any single physiological trait. Drought increases the proportion of reproductive (fiber- and lignin-rich) tillers in this species (Fernández & Reynolds, 2000), which could contribute to the preference of cattle for *Bouteloua* and other native species over *Eragrostis* (Freeman, 1979; Bock & Bock, 1993).

The steady-state environmental conditions imposed in this experiment permitted us to separate ontogenetic effects from treatment effects, thereby eliminating potentially confusing interactions. Direct extrapolation from these to more dynamic conditions is not possible because of important factors such as the timing and recurrence of drought and post-stress recovery. However, our study showed that the same factors (chiefly morphological) that make *Eragrostis* faster-growing than *Bouteloua* are those that determine the response of each species to drought. In their ‘stress–resistance syndrome hypothesis’, Chapin et al. (1993) argue that factors underlying evolutionary and phenotypically plastic responses to stress are the same, and that both involve a reduction in growth. Our results are consistent with this view. It remains unclear, however, whether growth rate itself is the trait subjected to selection (as suggested by Chapin, 1980 and Lambers, 1998) or it is simply a by-product of other, more basic, reactions to stress (Witkowski & Lamont, 1991; Tardieu et al., 1999). Nevertheless, our results clearly demonstrate that reduced growth is commensurate with those morphological modifications that decrease rates of resource use and, hence, potentially delay soil-water depletion.

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Research88


