

Water stress marginally increases stomatal density in *E. canadensis*, but not in *A. gerardii*

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Abstract

In order to better understand the effects of moisture levels on the balance of C_3 and C_4 prairie plants, we planted two common, co-occurring grass species, *Elymus canadensis* (C_3) and *Andropogon gerardii* (C_4), in conditions of adequate water and water stress. In our final harvest, after 27d, water-stressed *E. canadensis* exhibited marginally increased stomatal density, reflecting an attempt to use water efficiently. There were no significant effects of drought on *A. gerardii*'s stomatal density, indicating that its water use efficiency is naturally high. Neither species' root:shoot ratio, height, or biomass showed evidence of adaptation, however. The short time period available for drought-induced adaptation, as well as *A. gerardii*'s low germination rate and prolonged germination period, limited the conclusiveness of our results. Neither grass could adapt significantly to drought within a two-week stress period. But longer studies, on a variety of species, will uncover the differences between, and limits of, adaptations of C_3 and C_4 plants to water stress.

Introduction

In the tallgrass prairie's black soil, C_3 and C_4 grasses coexist, but C_4 grass is dominant. In the hot, dry summers characteristic of the Great Plains, C_4 photosynthesis is advantageous because it allows plants to close their stomata while still performing photosynthesis. During dry periods, plants close their stomata to prevent water loss through transpiration, but the resulting decreased stomatal conductance negatively affects gas exchange, causing intra-leaf oxygen levels to rise and carbon dioxide levels to drop. In C_3 plants, high oxygen levels result in photorespiration, and net loss of ATP. C_4 plants prevent this loss by employing PEP carboxylase, an enzyme with exceptionally high affinity for CO_2 (Reichmann, 1987) in their mesophyll cells (Freeman, 2002). The C_4 photosynthetic process allows plants to lengthen their growing seasons into the driest months of the year, when light availability and photosynthetic potential are highest (Bloom et al., 1992). C_3 plants cannot take advantage of plentiful light energy in hot, dry summers because they need open stomata to fix carbon.

C_4 photosynthesis facilitates water conservation but sacrifices peak carbon-fixing ability; for C_4 plants, selective pressure has not been to increase peak carbon-fixing rate but to conserve water (Gupschick, 1999; Bloom et al., 1985). The energy demanded by the C_4 cycle prevents C_4 plants from competing successfully in cool, moist conditions. While C_3 plants' peak photosynthetic rates are limited by stomatal conductance, C_4 plants' rates are limited by internal leaf factors.

This greater peak carbon-fixing ability enables C_3 plants to survive the prairie environment despite their lack of water efficiency. By growing quickly and taking advantage of moist periods, some C_3 plants can thrive even in the desert (Ehleringer, 1979). Furthermore, not all C_4 plants are more efficient than co-occurring C_3 species. Kemp and Williams (1980) found no difference in transpiration, i.e. wasting of water, and photosynthetic rates between two shortgrass prairie grasses, *Bouteloua gracilis* (C_4) and *Andropogon smithii* (C_3). This study did not, however, incorporate water deprivation. Kemp and Williams hypothesized that temperature is more important than moisture in determining species niche separation (1980); we tested whether moisture also affects niche separation.

In order to better understand the prairie's balance between C_3 and C_4 plants, determine to what extent a C_3 plant can adapt to drought, and quantify a C_4 species' disadvantage with adequate water, we compared two common inhabitants of the tallgrass prairie, *Andropogon gerardii* (Big bluestem, C_4) and *Elymus canadensis* (Canada wild rye, C_3). *A. gerardii* is widespread throughout the Great Plains and dominant on the tallgrass prairie. It grows rapidly during the warm season but tends to begin growth later than most grasses, between April 15 and May 1 on Konza Prairie in Kansas (Uchytel, 1988). It is native to North America, as is *E. canadensis*, one of 50 *Elymus* species. *E. canadensis* thrives under temperate and cool conditions and is common in North America, particularly on prairies (Anonymous "Wild rye"). It is considered "an associate" of

A. gerardii, also beginning growth in late April, but is drought-intolerant, though it competes well in unfertile soil (Simonn, 2000). We measured the effects of water stress on stomatal density, root:shoot biomass ratio, height, and total biomass for both species.

Stomatal density is positively correlated with water use efficiency (Malone et al., 1993), so we expected plants grown in the dry treatment to exhibit increased stomatal density. Particularly for the C₃ *E. canadensis*, increased stomatal density was a likely adaptation to stress: Hardy et al. (1995) found that C₃ plants often take drastic measures to save water during drought, including rolling and folding their leaves to reduce transpiration. Stomata are energetically cheap to develop and operate (Gutschick, 1999), and Malone et al. showed that elevated CO₂ can alter stomatal density within a single generation (1993). We anticipated a smaller increase in the stomatal density of *A. gerardii*, which would likely be high regardless of conditions. Its C₄ photosynthesis indicates that it has faced selective pressure for water conservation in the past. Malone observed that stomatal density of little bluestem (C₄) was unaffected by changes in CO₂.

We expected increased root:shoot ratios for both species when comparing stressed and unstressed individuals. In drought, plants allocate more energy for downward growth toward water than for upward growth toward light (Mooney et al., 1980). Kemp et al. (1980) showed that *B. gracilis* (C₄) had much higher root:shoot ratios than *A. smithii* (C₃). We investigated whether increased belowground biomass allocation could occur within a single generation, or whether such adaptation can only occur on an evolutionary time scale. Coleman and Bazzaz (1992) found no significant biomass changes in a C₄ plant subjected to water stress, but did find significant reductions in C₃ plants' biomass. Thus we expected depressed values of height and biomass for *E. canadensis* grown in dry conditions, but anticipated a less drastic effect of stress on *A. gerardii*, which naturally tolerates drought well and cannot take advantage of moist conditions as well as *E. canadensis*.

Methods

We grew individuals of *A. gerardii* and *E. canadensis* in two treatments, a control (abundant water) and a water-stressed group. The experiment was conducted over 27d, from 13 Oct.-8 Nov. in a $\approx 24^{\circ}\text{C}$ greenhouse. In addition to natural sunlight, the plants were constantly

illuminated by overhead bulbs.

We planted the samples in $\approx 4\text{cm}$ tall pots, which were grouped in quartets. We used 72 pots per treatment, with three seeds per pot, alternating species in a checkerboard pattern. Each treatment used 72 pots, with 36 pots per species. We filled the pots to $\approx 1\text{cm}$ below the top, placed three seeds in each pot, and then sprinkled soil on top of each pot.

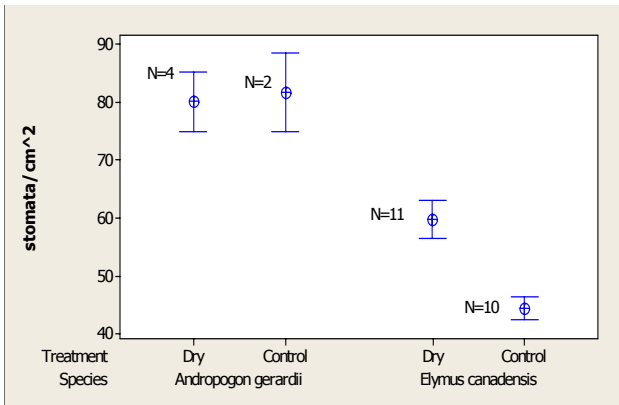
Water stress began after germination. For the first 10d, we watered both treatments daily by immersing the bottom 3cm of the flats in water. *E. canadensis* sprouted within 4-5d of planting, but the first *A. gerardii* did not appear for 10d. Its slow germination prevented us from collecting *A. gerardii* data from either treatment in the first harvest. *E. canadensis* received water throughout its early stages of growth and was already quite tall by the time we applied the water treatment. After 10 consecutive days of watering, we feared that the soil might be too wet for *A. gerardii*. Therefore, we allowed a four-day drying period before beginning the different watering regimes. After 14d, we watered the control group 5 ml/pot, every 2d, whereas the water stressed treatment received 5ml/pot every 4d.

Beginning on the 15th day, we conducted the first of three weekly harvest. In each harvest, we picked twelve plants of each species from each treatment. We measured number of individuals per pot, average plant height for each pot, and stomatal density for four individuals per treatment per species. All twelve plants were excavated, rinsed of soil, and dried for $\approx 48\text{h}$ in a 60°C oven.

To calculate stomatal density, we mounted one blade of grass from each species on a microscope slide and used a 400x magnification power. We counted the number of stomata in three haphazardly selected view fields and averaged the numbers from each individual field. We used an eyepiece micrometer to calculate field area ($.1465\text{ cm}^2$) and to derive stomatal density (cm^{-2}).

Biomass

Harvested plants were grouped in envelopes by pot. After drying, we measured total dry biomass, separated root from shoot, and massed the root to find root:shoot dry biomass ratio. To derive average individual biomass, we divided total pot biomass by number of individuals/pot for each pot. Average biomass figures were an attempt to account for differences in number of individuals/pot. However, each pot received the



	F	P
Species	46.24	0.00
Treatment	2.56	.141
Species*Treatment	4.00	.073

Figure 1: Average stomatal density ±1S.E. for *A. gerardii* and *E. canadensis* grown in wet and dry treatments. Table shows results of two-way ANOVA.

standard amount of water regardless of how many individuals were present, meaning there was less water/individual in highly populated pots.

We analyzed only data from the final harvest, in which N for *A. gerardii* was highest, in order to obtain the clearest picture possible of how differences in water availability affected development. We used ANOVA to measure species effect, water treatment effect, and species x treatment interaction.

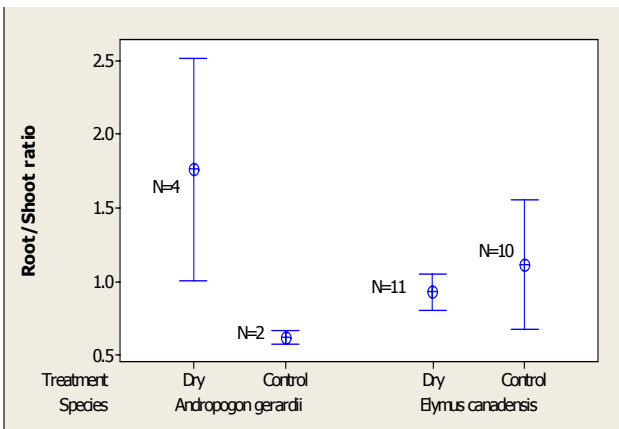
Results

Differences in stomatal density between *A. gerardii* grown in wet and dry conditions were negligible. However, in *E. canadensis*, stomatal density was 29% higher in the dry vs. wet conditions (Tukey’s pairwise comparison, $p = .071$), leading to a marginally non-significant ($p = .073$) treatment x species interaction. There was a highly significant species effect ($p < .001$), reflecting *A. gerardii*’s higher density. Root:shoot biomass ratios, heights,

and total biomasses were not affected significantly by the water treatment (Figs. 2-4). Average biomass also showed no evidence of treatment effect (Fig. 5). Species effects were significant for height and total biomass, reflecting *E. canadensis*’ greater growth rate (Figs. 3-4).

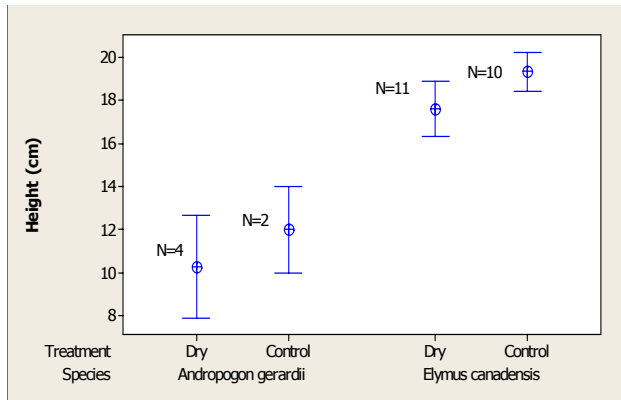
Discussion

Our most surprising observation was how vigorously *E. canadensis* grew compared to *A. gerardii*. It showed higher means for height, biomass, and especially number of germinated individuals. *E. canadensis* had only one empty pot in the dry treatment and two empty pots in the wet treatment, whereas *A. gerardii* did not germinate in eight of twelve dry pots and ten of twelve wet pots. Because *A. gerardii* is the dominant grass on the tall grass prairie, one might expect that it would outgrow a subdominant grass. It has been shown that conditions common on the tall grass prairie, high temperature and low moisture, favor C₄ grasses (Still et al., 2003).



	F	P
Species	.11	.743
Treatment	.87	.360
Species*Treatment	1.69	.206

Figure 2: Average root:shoot ratio ±1S.E. for *A. gerardii* and *E. canadensis* grown in wet and dry treatments. Table shows results of two-way ANOVA.



	F	P
Species	15.66	.001
Treatment	.87	.360
Spec*Treat	.00	.995

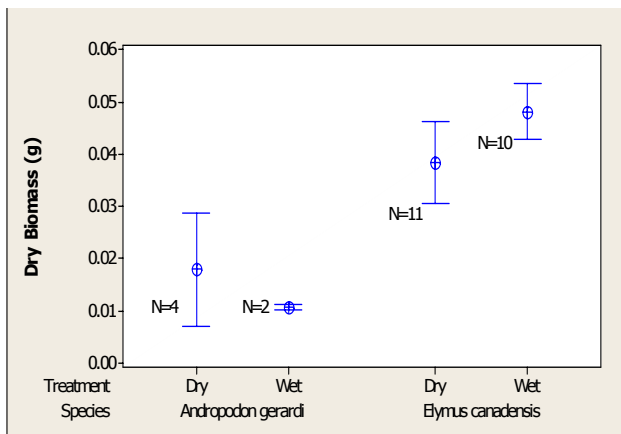
Figure 3: Average heights ±1S.E. for *A. gerardii* and *E. canadensis* grown in wet and dry treatments. Table shows results of two-way ANOVA.

However, our greenhouse growing environment differed substantially from the tallgrass prairie. Although half the sample was water stressed, ambient air was humid and this may have limited evaporation. Kemp and Williams (1980) showed that mild temperatures of 15-20°C favored C₃ species, while a 15-35°C range favored C₄ grass. Our greenhouse's ≈24°C environment helps explain why *E. canadensis* grew so vigorously. Furthermore, the mildness of our drought stress, and its mitigation by the greenhouse's high humidity, may explain why there were no significant differences in heights or root:shoot biomass ratios between the two treatments.

Stressed *E. canadensis* showed a marginally non-significant increase in stomatal density, but the difference in means did tentatively support our hypothesis of this species' adaptation to drought. In contrast, *A. gerardii*'s mean stomatal density was only 2% higher in the dry treatment. Malone et al. (1993) also found that C₄ plants did

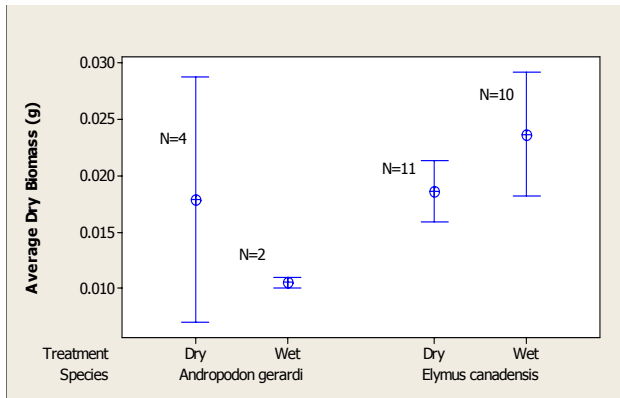
not show significant changes in stomatal density in an experiment testing the effects of CO₂. The initial saturation of the soil also may have provided a reservoir on which dry treatment plants could draw in the experiment's intermediate and latter stages, which could have decreased the magnitude of the water stress and the extent of adaptation. The limited time frame of our study may also make it difficult to see individual adaptation. A similar study by Knapp et al. (1994) that lasted the entire growing season had stomatal densities of 121 ± 5/mm² for *A. gerardii*, more than a hundred times the values we measured.

Our data's failure to show significant drought effects on root:shoot ratios contradicts earlier studies, which showed that plants will send more energy to their roots when water stressed (Mooney et al., 1980; Kemp and Williams, 1980). The initial 10d watering stage, however, may have decreased the stimulus for root growth: many *E. canadensis* already had



	F	P
Species	7.57	.011
Treatment	.01	.905
Species*Treatment	.67	.423

Figure 4: Average dry biomass ±1S.E. for *A. gerardii* and *E. canadensis* grown in wet and dry treatments. Data represent total mass from pots with one to three individuals and are from third harvest. Table shows results of two-way ANOVA.



	F	P
Species	.95	.340
Treatment	.03	.874
Species*Treatment	.75	.395

Figure 5: Average dry biomass ±1S.E. for *A. gerardii* and *E. canadensis* grown in wet and dry treatments. Data values are derived by dividing total dry biomass by number of individuals for each pot. Table shows results of two-way ANOVA.

well-developed roots by the onset of water stress. Plants from the first harvest had roots that were not proportional to shoots in length, but each successive harvest showed increased root:shoot ratios. Here, again, study length limited our findings. Had the study been continued for several more weeks, we may have been more able to conclusively determine the effects of moisture on above- and belowground biomass allocation.

We hypothesized that both height and biomass would be lower for C_3 plants in the dry treatment, and that the results would be fairly similar in both groups for *A. gerardii*. In fact, mean heights of both species were negligibly higher in the unstressed treatment, but neither group exhibited significant differences. Our low sample size prevented us from drawing firm conclusions in *A. gerardii*.

Biomass was not significantly affected by the treatment, as expected for C_4 plants. A study by Bazzaz and Carlson (2002) manipulated water, temperature, and CO_2 level, but none of these factors significantly affected dry mass in C_4 plants. The same study found that C_3 grasses were significantly affected by water stress, suffering almost 70% reductions in biomass. We did not find this in our study, possibly because of the initial watering regime or the high humidity in the greenhouse.

Aside from a longer time period, having three watering groups—a control, dry, and wet—would also yield a more complete picture of how moisture change affects development. Many studies have measured the responses of C_3 and C_4 plants' stomata to changes in CO_2 ; a deeper understanding of stomatal and intra-leaf responses to moisture variation would complement this research. Such understanding of

plants' adaptive mechanisms is important to inform management of prairies, help to answer the questions of which species can succeed in which conditions and which species can reasonably be grown on the tall-grass prairie. Study of drought adaptation should not be limited to isolated growing experiments; rather, it should analyze plants grown together and competing for resources; the chemical explanations for these results also deserve further scrutiny, particularly the problematic role of the Rubisco enzyme in C_3 photorespiration. A synthesis of this information may enable a greater and more detailed appreciation of plants' capacities to adapt to stress.

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