

ELEVATED CO₂ AND LEAF LONGEVITY IN THE C₄ GRASSLAND-DOMINANT *ANDROPOGON GERARDII*

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In central U.S. grasslands, plant and ecosystem responses to elevated CO₂ are most pronounced when water availability is limited. In a northeast Kansas grassland, responses to elevated CO₂ in leaf area, number, development, and longevity were quantified for the tallgrass prairie dominant, *Andropogon gerardii*. Plants were grown in open-top chambers (OTCs) modified to limit water availability and to maximize responses to elevated CO₂. In OTCs with elevated ($\times 2$ ambient) levels of CO₂, aboveground biomass production and leaf water potentials were increased significantly compared with those of plants in OTCs with ambient CO₂. There were no differences in leaf area or leaf number per tiller in *A. gerardii* in elevated compared with ambient OTCs. However, leaf area in adjacent unchambered plots with greater water availability was significantly higher than in the OTCs. The time required for developing leaves to achieve maximum leaf area was reduced by 29%, and the period of time until leaves senesced was increased by 20% for plants exposed to elevated compared with ambient CO₂. Thus, leaves of this C₄ grass species expanded more rapidly (6 d) and remained green longer (9 d) when exposed to elevated CO₂. Such CO₂-mediated increases in leaf longevity in the dominant species may allow this grassland to respond more opportunistically to temporally variable rainfall patterns in high-CO₂ environments. These responses should be included in leaf-based simulation models that attempt to mechanistically link physiological alterations to predicted canopy responses to increased CO₂.

Keywords: ecophysiology, elevated carbon dioxide, grassland, leaf lifespan, tallgrass prairie, water relations.

Introduction

Plant responses to increased atmospheric CO₂ concentrations range from cellular-level alterations in enzymatic activity to ecosystem-level increases in productivity (Bowes 1991; Sage 1994; Koch and Mooney 1996). Linking these data in a mechanistic framework requires knowledge of responses at a variety of intermediate scales, and it is critical to quantify these responses because they may offset or exacerbate effects at other scales. For example, leaf-level photosynthesis may increase at elevated CO₂ (Mooney et al. 1991), and the effect of this response on productivity may be enhanced by an increase in canopy leaf area (Cure et al. 1989; Lawlor and Mitchell 1991). Alternatively, other resources, such as light or nitrogen, may become limiting and may offset predicted positive responses (Wolfe et al. 1998). Simulation models can be invaluable in mechanistically linking responses in a predictive fashion (Lawlor and Mitchell 1991), but these require parameterization that is based on experimentation. In this study, we focused on two plant traits that are critically important to include in leaf-based models (Chen et al. 1994; Coughenour and Parton 1996) and that are sensitive to changes in CO₂ concentration: leaf ontogeny and longevity (Curtis et al. 1989).

Responses of the C₄-dominated tallgrass prairies of the cen-

tral Great Plains in North America to increased CO₂ concentrations have been well documented (Owensby et al. 1996, 1997). Increases in aboveground primary production occur primarily in years with below-average rainfall (Owensby et al. 1997), and this response has been attributed to improved water relations at elevated CO₂ levels. Indeed, at elevated CO₂, soil and plant water status is improved (Bremer et al. 1996; Hamerlynck et al. 1997); leaf-level photosynthesis in the C₄ grass, *Andropogon gerardii*, increases (Knapp et al. 1993); leaf-area indices increase (Owensby et al. 1993); stomatal conductance in *A. gerardii* and most other species decreases (Knapp et al. 1996); and whole-plant and ecosystem transpiration is reduced (Ham et al. 1995; Bremer et al. 1996). Finally, ecosystem-level CO₂ flux data indicate that canopy senescence may be delayed in the fall at elevated CO₂ (Ham et al. 1995). Leaf development and longevity clearly play key roles in all of these responses (Bowes 1991). The objectives of this study were to quantify responses to elevated CO₂ of two components of the life span of *A. gerardii* leaves: the time required for developing leaves to fully expand and the period of time from expansion to senescence. Because *A. gerardii* is the dominant species, both numerically and in biomass, in this grassland (Hulbert 1988), its response to elevated CO₂ should provide a predictive link to ecosystem-level phenomena.

Material and Methods

Research was conducted in native tallgrass prairie near Manhattan, Kansas, during the 1997 growing season. Sites were

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representative of the C₄-dominated grassland (*Andropogon gerardii* and *Sorghastrum nutans*) that once covered much of the central Great Plains (Knapp et al. 1998). Individuals of *A. gerardii* grew beneath open-top chambers (OTCs) that were 4.5 m in diameter and 4.0 m in height. The aluminum frames were covered with 1.5-mm-thick ultraviolet-resistant polyethylene. Four chambers that had been in place since 1993 were used: plants in two of the chambers were exposed to ambient levels of CO₂, and plants in the other two were exposed to elevated (double ambient) levels. Ambient CO₂ levels were allowed to vary naturally from 330–340 μL L⁻¹ during the day to >400 μL L⁻¹ at night. In addition, plants in two unchambered plots in adjacent undisturbed grassland were included in this study. Enrichment of CO₂ in the elevated chambers began on April 17 and continued until plant senescence in the fall. Additional details on the OTCs and their performance can be found in Ham et al. (1993) and Owensby et al. (1993).

Responses to elevated CO₂ in this grassland are primarily dependent on water availability, with significant increases in leaf-level gas exchange and ecosystem-level production occurring only in years with substantial plant water stress (Knapp et al. 1993; Owensby et al. 1997). In order to insure that significant water stress developed during the 1997 field season, the top opening of the chambers was modified with a Plexiglas disk that reduced the opening to 0.76 m in diameter. Thus, most of the rain that fell was excluded from the chambers, captured in gutters, and drained away. The chamber perimeters were trenched to 0.8 m and lined with aluminum flashing in order to reduce subsurface flow of soil water. Rain that entered the OTCs through the reduced opening was scattered evenly across the walls and vegetation by suspended fans that were used to equilibrate atmospheric pressure in the chambers. Soil water content was high at the beginning of the experiment (Bremer 1998) from spring rains, and the vegetation in the OTCs was irrigated with 50 mm of water on April 24 and July 15–16 (day of year [DOY] 196–197) to maintain physiological activity.

Plant Water Relations and Leaf-Level Responses

Plant water relations were characterized throughout the season (at 10-d intervals) by measuring leaf xylem pressure potential (Ψ) in *A. gerardii* at midday (ca. 1300 hours CDT). Mature upper-canopy leaves ($n = 5-8$) were collected from separate tillers in the four chambers and two field plots, immediately placed in humidified plastic bags, and rapidly transported to a Scholander-type pressure chamber (model 1000, Plant Moisture Stress [PMS], Corvallis, Oreg.) for Ψ determinations. In addition, the diurnal pattern of Ψ in *A. gerardii* was compared among the three treatments by sampling leaves at 2-h intervals for several days during the growing season.

Standard demographic techniques were used to quantify leaf expansion rates, leaf longevity, leaf number, and area (Sydes 1984; Aerts and de Caluwe 1995). Over the course of the growing season, 444 leaves (a minimum of 70 per chamber or plot) were monitored. Individual tillers were marked at their bases with color-coded wire rings, and new leaves were noted as they appeared. Leaves were first measured when the rolling/curling habit of the developing leaf was absent and the leaf was completely flat. At this time, the date was noted and leaf

area was determined. Leaf area was estimated from leaf length (from tip to ligule) and width (at the widest point) measurements. A regression equation ($r^2 = 0.972$; $P < 0.01$) to convert linear measurements to area was developed from 51 leaves in which length and width and area were measured. Leaf area was measured in the lab with a video leaf-area meter (Decagon Devices). Leaves were measured in the field at 2–3-d intervals until senescence was complete, which was defined as no portion of the leaf remaining green (Sydes 1984). From these data, the average area per leaf, average number of leaves per tiller, average leaf area per tiller, period of time required for leaf expansion (defined as the time from the initial leaf area measurement to the maximum leaf area measured), and the longevity of leaves were calculated. Analysis of variance at the $\alpha = 0.05$ level was used to distinguish treatment effects.

Results and Discussion

Elevated CO₂-induced alterations in soil and plant water relations play a critical role in determining responses in this C₄-dominated grassland from the leaf to the ecosystem level (Knapp et al. 1993; Owensby et al. 1997). Unfortunately, the microenvironment of the OTCs reduces evapotranspiration (Ham et al. 1993; Owensby et al. 1993; Bremer et al. 1996), effectively increasing water availability and potentially confounding the CO₂ effect. Indeed, during dry summers, the “chamber effect” alone significantly increases plant production above field levels (Owensby et al. 1993). In order to circumvent this problem, the rain-excluding modifications made to the OTCs in combination with the addition of only 10 cm of water during the season (compared with the more typical growing season precipitation of >50 cm) insured that water would limit plant production during this experiment. Indeed, aboveground plant production in the ambient OTCs was only 306 g m⁻², compared with 457 g m⁻² in the elevated CO₂ OTCs ($P < 0.05$; Bremer 1998). At the nearby Konza Prairie Research Natural Area, the 19-yr average plant production for sites with similar soils was 455 g m⁻² (Briggs and Knapp 1995), and in 1997 production was 376 g m⁻².

Because leaf water relations strongly influence leaf expansion and longevity, we documented patterns of leaf Ψ seasonally and diurnally. The seasonal course of midday Ψ in the OTCs (fig. 1) was similar to patterns noted in most years (Owensby et al. 1993; Knapp et al. 1994, 1996). Midday Ψ was higher in elevated than in ambient CO₂ plants at six of nine sampling dates, and the seasonal average was significantly higher (elevated CO₂ $\Psi = -1.56 \pm 0.11$, compared with ambient CO₂ $\Psi = -1.77 \pm 0.13$; $P < 0.05$). The midseason irrigation event before measurements at DOY 199 minimized any further seasonal reductions in Ψ in the OTCs. Midday Ψ in control plants in the field was more variable but was usually intermediate between values for elevated and ambient OTC plants (fig. 1). Midday Ψ values do not always reflect the substantial effects of elevated CO₂ on plant water relations that may occur in the morning or afternoon (Hamerlynck et al. 1997). Diurnal patterns in leaf Ψ in *A. gerardii* in early summer confirmed that plants in ambient OTCs had lower Ψ throughout most of the day, compared with elevated CO₂ or field plants (fig. 2). By late August, lower daily Ψ minima were measured in plants in all treatments, but at four of seven sampling pe-

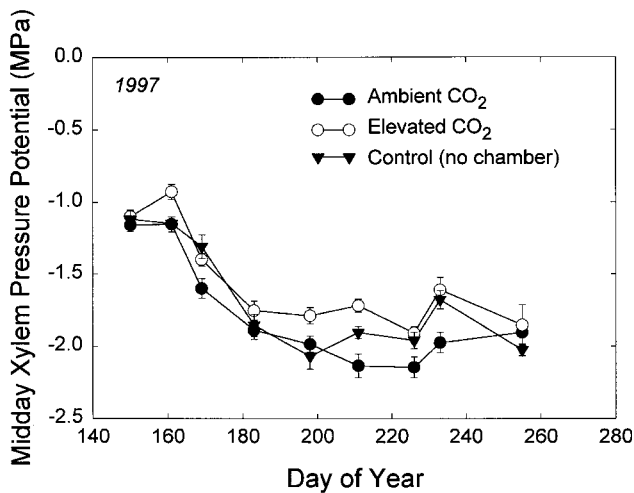


Fig. 1 Seasonal (1997) course of midday leaf xylem pressure potential (Ψ) in *Andropogon gerardii* in open-top chambers with ambient and elevated ($\times 2$ ambient) levels of CO₂ and in adjacent field plots (control) in undisturbed tallgrass prairie. Vertical bars indicate ± 1 SE of the mean. On a seasonal basis, Ψ was significantly higher ($P < 0.05$) in plants exposed to elevated CO₂ than in those exposed to ambient CO₂; control plants displayed values that were intermediate.

riods, Ψ in field plants was significantly higher than it was in elevated CO₂ plants (fig. 2). Again, Ψ in ambient CO₂ OTC plants was significantly lower throughout the day. Similar water relations responses to elevated CO₂ have been reported for other C₄ grasses (Seneweera et al. 1998).

There were no consistent patterns or differences in the mean area of leaves at elevated CO₂ compared with ambient CO₂ (mean = 8.32 ± 0.13 cm²). However, *A. gerardii* leaves in the adjacent field plots had significantly greater area (12.60 ± 0.26 cm²). A lack of response to elevated CO₂ in leaf area has been reported previously (Cure et al. 1989; Sims et al. 1998). Similarly, there were no differences among the three treatments in the number of leaves produced during the season per tiller (mean = 6.4). Thus, average leaf area per tiller was greater in the field compared with the OTCs, perhaps because of greater water availability in the field.

There were significant CO₂ effects on two key leaf development and longevity estimates in *A. gerardii*. The time required for developing leaves to achieve maximum leaf area was reduced by 29% (6 d) in leaves grown at elevated CO₂ relative to those in ambient OTCs (fig. 3). Moreover, the period of time from maximum leaf area to senescence was increased at elevated CO₂ by 20% (9 d) relative to ambient-CO₂ plants in the OTCs (fig. 3). Both of these responses are consistent with improved water relations in elevated-CO₂ plants, although changes in cell wall properties may be more important in some species (Ferris and Taylor 1994; Taylor et al. 1994; Ranasinghe and Taylor 1996). Leaves of *A. gerardii* expanded most rapidly in the field, where rainfall was not excluded, but longevity was similar in the field and ambient-CO₂ OTC leaves (fig. 3). The latter response may be a product of slightly higher temperatures in the OTCs (Ham et al. 1993; Owensby et al. 1993), which may be particularly important for delaying se-

nescence in leaves of this C₄ "warm season" grass. In addition to these season-long averages, monthly comparisons of leaf area, number, and longevity estimates were made to identify date \times treatment interactions. Although there were fewer significant differences in leaf development and longevity when subsets of the data were analyzed at monthly intervals, trends among treatments in all months were consistent with seasonal averages and, hence, no interactions were detected.

How do these leaf development and longevity responses to elevated CO₂ compare with other studies? Although techniques for quantifying leaf life spans vary considerably, Diemer et al. (1992) reported that leaf longevity in 29 herbaceous species (including grasses) ranged from 41 to 95 d; combining leaf expansion and time-to-senescence data for *A. gerardii* (fig. 3) yielded life spans (54–70 d) that were well within this range. Several studies have reported greater leaf expansion and developmental rates at elevated CO₂ (Cure et al. 1989; Ferris and Taylor 1994; Ranasinghe and Taylor 1996; Sims et al. 1998), but there are few data for grasses. However, van Oijen et al. (1998) found no evidence of accelerated leaf development in wheat, and Navas et al. (1997) reported that leaf production

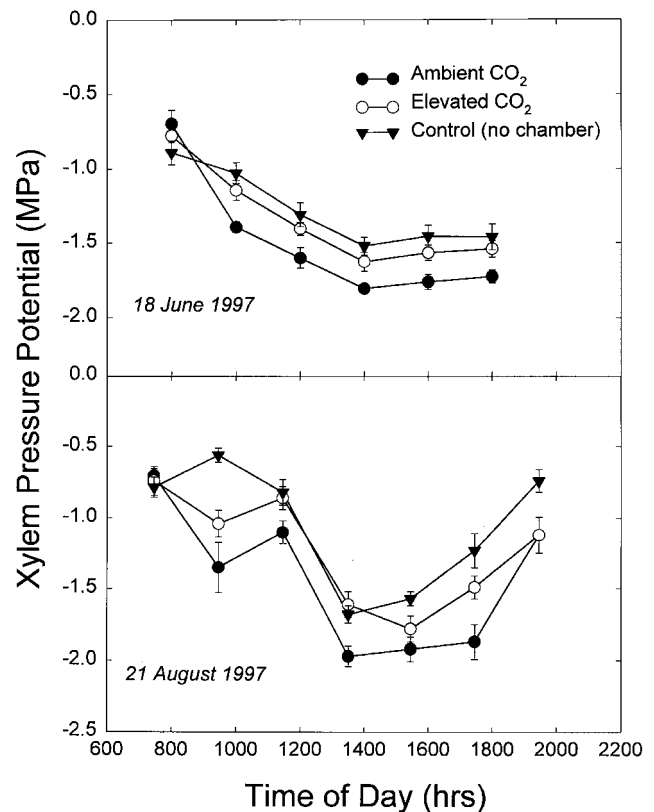


Fig. 2 Diurnal course of leaf xylem pressure potential in *Andropogon gerardii* in open-top chambers with ambient and elevated ($\times 2$ ambient) levels of CO₂ and in adjacent field plots (control) in undisturbed tallgrass prairie. Vertical bars indicate ± 1 SE of the mean. In some cases, error bars are smaller than the symbols. June data (top panel) were obtained when soil moisture was relatively high and evaporative demand low, whereas the August data (bottom panel) were obtained when soil moisture was lower and evaporative demand high (Bremer 1998).

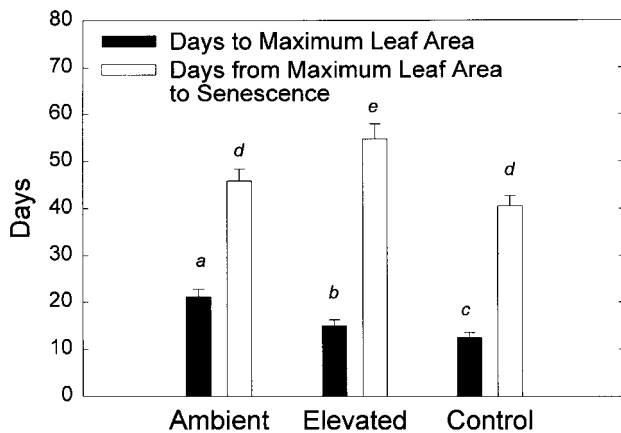


Fig. 3 The period of time required for leaves of *Andropogon gerardii* to expand to their maximum leaf area and the period of time from maximum leaf area to senescence (leaves entirely brown). Plants were in open-top chambers with ambient and elevated ($\times 2$ ambient) levels of CO_2 and in adjacent field plots (control) in undisturbed tallgrass prairie. Error bars indicate 1 SE of the mean, and the different letters above bars indicate significant differences ($P < 0.05$) over the course of the growing season.

in three grasses was not altered at elevated CO_2 . Leaf senescence has been reported to occur more rapidly in dicot species at elevated CO_2 (Pearson and Brooks 1995; McConnaughay et al. 1996; Navas et al. 1997). However, in grasses and sedges, senescence may be insensitive to, or even delayed by, elevated

CO_2 (Curtis et al. 1989; Navas et al. 1997; van Oijen et al. 1998). The significant delay in leaf senescence in *A. gerardii* (fig. 3) is, to our knowledge, the first reported for a C_4 grass.

The consequences of CO_2 -mediated increases in the rate of leaf expansion coupled with delayed senescence in this C_4 grassland dominant are substantial. Although increased leaf duration does not equate to the maintenance of photosynthetic activity (Pearson and Brooks 1995; Sicher and Bunce 1998), Ham et al. (1995) did report greater late-season CO_2 uptake in this grassland at elevated CO_2 . Greater leaf longevity combined with increased canopy leaf area indices (Owensby et al. 1993) may allow this grassland to respond more opportunistically in future high- CO_2 environments to the variable precipitation patterns characteristic of the grassland climate (Knapp et al. 1998). Moreover, global increases in nocturnal air temperatures (Easterling et al. 1997; Alward et al. 1999) may extend the growing season for the dominant C_4 grasses. A corresponding CO_2 -mediated delay in leaf senescence may significantly alter current seasonal patterns in C gain, particularly in the autumn, a critical C sink-source transition period that strongly affects annual C budgets in grasslands (Ham and Knapp 1998).

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