

Photosynthesis and fluorescence responses of C₄ plant *Andropogon gerardii* acclimated to temperature and carbon dioxide

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Abstract

Increase in both atmospheric CO₂ concentration [CO₂] and associated warming are likely to alter Earth's carbon balance and photosynthetic carbon fixation of dominant plant species in a given biome. An experiment was conducted in sunlit, controlled environment chambers to determine effects of atmospheric [CO₂] and temperature on net photosynthetic rate (P_N) and fluorescence (F) in response to internal CO₂ concentration (C_i) and photosynthetically active radiation (PAR) of the C₄ species, big bluestem (*Andropogon gerardii* Vitman). Ten treatments were comprised of two [CO₂] of 360 (ambient, AC) and 720 (elevated, EC) μmol mol⁻¹ and five day/night temperature of 20/12, 25/17, 30/22, 35/27 and 40/32 °C. Treatments were imposed from 15 d after sowing (DAS) through 130 DAS. Both F-P_N/C_i and F-P_N/PAR response curves were measured on top most fully expanded leaves between 55 and 75 DAS. Plants grown in EC exhibited significantly higher CO₂-saturated net photosynthesis (P_{sat}), phosphoenolpyruvate carboxylase (PEPC) efficiency, and electron transport rate (ETR). At a given [CO₂], increase in temperature increased P_{sat}, PEPC efficiency, and ETR. Plants grown at EC did not differ for dark respiration rate (R_D), but had significantly higher maximum photosynthesis (P_{max}) than plants grown in AC. Increase in temperature increased P_{max}, R_D, and ETR, irrespective of the [CO₂]. The ability of PEPC, ribulose-1,5-bisphosphate carboxylase/oxygenase, and photosystem components, derived from response curves to tolerate higher temperatures (>35 °C), particularly under EC, indicates the ability of C₄ species to sustain photosynthetic capacity in future climates.

Additional key words: chlorophyll fluorescence; electron transport chain; net photosynthetic rate; phosphoenolpyruvate carboxylase; photosynthetically active radiation; photosystem; respiration rate.

Introduction

Global climate change is likely to affect plant photosynthesis, growth, biomass allocation, and nutrient uptake, since atmospheric CO₂ concentration ([CO₂]) and temperature will be modified in a range where large responses can be expected (Gavito *et al.* 2001). The atmospheric [CO₂] has increased during the past 250 years from 280 to current 380 μmol mol⁻¹ and is projected to

concentrate between 540 and 970 μmol mol⁻¹ by the year 2100 (Prentice *et al.* 2001). As a consequence of increased [CO₂] and other greenhouse gases, average global air temperature increased by 0.6 °C in the 20th century (Folland *et al.* 2001), and it is projected to increase between 1.5 and 5.4 °C by the year 2100 (IPCC 2001) with an uncertainty range of 1.5–11.0 °C (Stainforth *et al.*

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Abbreviations: AC – ambient [CO₂]; C_i – internal [CO₂]; [CO₂] – CO₂ concentration; DAS – days after sowing; EC – elevated [CO₂]; ETR – electron transport rate; ETR_{sat} and ETR_{max} – maximal electron transport rate when ETR saturates to C_i and PAR, respectively; F – fluorescence; F_s – steady state Chl fluorescence detected in “actinic light”; F_{m'} – the maximal Chl fluorescence detected in “actinic light”; LCF – leaf chamber fluorometer; P_{max} – maximum photosynthesis at PAR where P_N saturates; P_N – net photosynthetic rate; P_{sat} – maximum photosynthesis at [CO₂] where P_N saturates; PAR – photosynthetically active radiation; PEPC – phosphoenolpyruvate carboxylase; R_D – dark respiration rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; T_L – leaf temperature; Φ_{CO₂} – quantum yield of CO₂ assimilation; Φ_{PS2} – maximum quantum efficiency of photosystem 2 photochemistry.

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2005). Further, global warming will likely alter synoptic weather patterns such as change in precipitation regimes and intensities, and increase air and soil temperatures, thus resulting in potentially dramatic effects on ecosystem goods and services if environmental factors exceed the tolerance range of species (Peters and Lovejoy 1992).

Resilience and plasticity of photosynthesis to growth temperatures allows plants to survive extreme climatic conditions. Of the various C₃, C₄, and CAM photosynthetic pathways, C₄ photosynthetic pathway is uniquely placed to capture excess CO₂ both in present and near future warmer climates. Response of C₄ plants to predicted climate change will play a critical role, even though they are fewer than 4% of world's higher order plants, as they contribute between 25 and 30% of global primary productivity because of the dominance of C₄ grasslands (Defries and Townsend 1994). Free-air carbon dioxide experiments (FACE) with elevated [CO₂] have shown no increase in photosynthesis or biomass of C₄ species under irrigated conditions (maize – Leakey *et al.* 2006) while an increase of biomass was observed under drought (sorghum – Ottman *et al.* 2001). A meta-analysis of C₄ and C₃ plants has shown that elevated [CO₂] stimulates growth of C₄ plants by up to 33% (Wand *et al.* 1999). This was attributed to either direct effect of higher photosynthetic carbon assimilation or indirectly through improved soil and plant water relations resulting from reduced stomatal conductance and transpiration. Several studies have shown that C₄ photosynthesis is not saturated under well-watered and current [CO₂] conditions (Wand *et al.* 1999, Ziska *et al.* 1999). However, the saturation of C₄ photosynthesis to C_i is modified by environmental variables such as temperature, irradiance, and nutrient availability (Loreto *et al.* 1995). Sage and Kubien (2003) have demonstrated that C₄ species exhibit positive response to rising [CO₂], particularly at elevated temperature and arid conditions, where they are currently common. Studies with C₃ and C₄ species have shown that under short-term (hours) high temperature conditions, photosynthesis is limited by activity of ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBPCO (Salvucci and Crafts-Brandner 2004). But, more than 2 d of heat stress results in acclimation due to accumulation of novel forms of activase polypeptide (de Jiménez *et al.* 1995, Law *et al.* 2001, Crafts-Brandner and Salvucci 2002). As [CO₂] and temperature in the atmosphere act in concert, it

Materials and methods

Plants and experimental conditions: The experiment was conducted at the Mississippi Agriculture and Forestry Experiment Station of Mississippi State (38°28'N, 88°47'W), Mississippi, USA in 2004, using controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR) units. The ten SPAR chambers are located outside and use sunlight as light source for plant growth. Each SPAR chamber consists of

is essential to understand the long-term interactive mechanistic responses of C₄ photosynthesis.

Recent advances in integration of leaf photosynthesis and fluorescence measurements have made it possible to evaluate enzyme kinetics and components of photosynthetic apparatus simultaneously in response to [CO₂] and photosynthetically active radiation (PAR) (Long *et al.* 2006, Dwyer *et al.* 2007). Yamasaki *et al.* (2002) have shown in winter wheat that if temperature is optimum for electron transport rate, then it is also optimum for RuBPCO regeneration, and, hence, for saturated net photosynthesis. This study showed that fluorescence and gas exchange are tightly linked and photosynthesis acclimates to high growth temperature. However, photosynthesis and fluorescence responses to C_i and PAR of plants acclimated to a wide range of temperatures and [CO₂] have not been studied. The response functions of intact leaves will provide valuable information for comparing and characterizing the status of the photosynthetic apparatus on the basis of gas exchange. The parameters derived can be used to improve the predictive capabilities of C₄ photosynthesis models which are rather scarce (Chen *et al.* 1994, Caemmerer and Furbank 1999) and developed under limited treatments of temperature and [CO₂]. Thus, it is essential to develop an understanding of the mechanistic responses and to quantify C₄ photosynthesis to environmental change to improve predictive ability of C₄ models.

Big bluestem (*Andropogon gerardii* Vitman), a C₄-NADP-ME type species dominating the tall grass prairies, was selected for the current controlled environment study. Potential photosynthesis of big bluestem was not achieved in the studies of Knapp *et al.* (1993) and Chen *et al.* (1994) due to water stress conditions prior to measurement period. However, Knapp *et al.* (1993) showed that photosynthetic rate was higher at EC in the temperature range of 17–35 °C. Comprehensive studies on photosynthesis and photochemical properties have not been fully investigated for C₄ species. The objective of this current study is to quantify the interactive effects of [CO₂] and temperature on various aspects of C₄ leaf photosynthesis by measuring gas exchange and fluorescence parameters in response to [CO₂] and PAR under well-irrigated, well-fertilized controlled environment conditions.

a steel soil bin (1 m deep by 2 m long by 0.5 m wide) to accommodate the root system and a Plexiglas chamber (2.5 m tall by 2.0 m long by 1.5 m wide) accommodating aerial plant system. The Plexiglas allows 97% of the visible solar radiation to pass without spectral variability in absorption. In addition, each SPAR unit consists of a heating and cooling system to maintain temperature precisely at predetermined set points for plant growth

studies under near ambient levels of PAR. Details of operation and control of SPAR chambers have been described by Reddy *et al.* (2001).

Seeds of big bluestem (*A. gerardii* Vitman) cv. Bonilla (*Sustained Horizons*, Lake Norden, South Dakota, USA) were sown in 11 equally spaced rows, across the 2 m long soil bin, in the SPAR units on 18 May 2004. Emergence was recorded 5 d after sowing. Plants were irrigated three times a day with standard Hoagland's nutrient solution delivered at 08:00, 12:00, and 17:00 h to ensure favorable nutrient and water conditions for plant growth. Irrigation with Hoagland's nutrient solution was provided through an automated computer-controlled drip system, and the amount of irrigation provided during the growing season was adjusted based on evapotranspiration measured in each unit (Reddy *et al.* 2001). Variable density black shade cloth (*Hummert Seed Co.*, St. Louis, Missouri, USA) around the border of the planted area in each SPAR unit were adjusted regularly to match plant height in order to simulate natural shading in the presence of other plants.

Treatments: The temperatures were maintained within ± 0.5 °C of treatment set points of 30/22 °C (day/night) and chamber [CO₂] were maintained within ± 6.0 $\mu\text{mol mol}^{-1}$ in all units until the seedlings had emerged and were uniformly established. At 15 d after seeding (DAS), each of the ten chambers was assigned one of ten treatments. Each of ten treatments consisting of unique combination of two [CO₂] of 360 (ambient, AC) and 720 (elevated, EC) $\mu\text{mol mol}^{-1}$ and five day/night temperatures of 20/12, 25/17, 30/22, 35/27, and 40/32 °C were assigned to each chamber until maturity, 130 DAS. The averages of [CO₂] in the EC- and AC-grown SPAR chambers were 719.0 ± 5.5 and 363.7 ± 0.9 $\mu\text{mol mol}^{-1}$, respectively. The mean temperatures recorded were 17.00 ± 0.08 , 21.50 ± 0.06 , 26.30 ± 0.08 , 30.80 ± 0.09 , and 36.10 ± 0.11 °C at AC, and 17.40 ± 0.11 , 22.10 ± 0.09 , 26.30 ± 0.08 , 30.80 ± 0.09 , and 35.00 ± 0.11 °C at EC. The chamber [CO₂] was measured with a dedicated infrared gas analyzer (*LI-COR* model *LI-6252*, Lincoln, Nebraska, USA) from the gas sample that is drawn through the lines run underground from SPAR units to the field laboratory building. Moisture was removed from the gas sample by running the sample through refrigerated water trap (4 °C) that was automatically drained and through a column of magnesium perchlorate. Chamber [CO₂] was maintained by supplying pure CO₂ from a compressed gas cylinder through a system that included a pressure regulator, solenoid, and needle valves and a calibrated flow meter (Reddy *et al.* 2001). A dedicated computer with in-house coded software monitored and controlled the environmental variables. Three sets of two topmost fully expanded leaves in each of the ten treatments were selected for photosynthesis measurements. Each set of two top most leaves was from two adjacent plants and hence a total of six plants comprised the three different

sets of leaves used for measurements. Each set of leaves selected for measuring photosynthesis was considered as one replicate. Therefore, each data point in the results represents the mean values of three replicates per treatment.

Gas exchange measurements were made on attached leaves using an open gas exchange *LI-6400* photosynthesis system (*LICOR*, Lincoln, Nebraska, USA) fitted with a *6400-40* leaf chamber fluorometer (LCF) that provides LED-based fluorescence and irradiation. Two leaf blades of similar age from adjacent plants were used to measure photosynthesis so that the 2 cm² circular leaf chamber was completely covered. Photosynthesis is driven by the "actinic light" source that uses 3 blue LEDs (470 nm) and all red LEDs. To measure steady-state fluorescence (F_s), LCF uses two red LEDs (center wavelength about 630 nm) and a detector (detects radiation at 715 nm in the photosystem 2 fluorescence band). A "flash light" (~ 7000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) achieved by 27 red LEDs is used to measure the maximal fluorescence (F_m'). The software in the instrument provides data on the fluorescence parameters and also calculates derived parameters such as electron transport rate (ETR), photochemical yield of photosystem 2 (PS2) electron transport (Φ_{PS2}), and the quantum yield of CO₂ assimilation (Φ_{CO2}). The equations used to derive these values are shown below:

$$\Phi_{\text{PS2}} = (F_m' - F_s)/F_m' \quad [\text{unitless}] \quad (1)$$

$$\Phi_{\text{CO2}} = (P_N - P_{\text{dark}})/I\alpha_{\text{leaf}} \quad [\mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{photon})^{-1}] \quad (2)$$

$$\text{ETR} = [(F_m' - F_s)/F_m']/f I \alpha_{\text{leaf}} \quad [\mu\text{mol}(\text{e}^{-1}) \text{m}^{-2} \text{s}^{-1}] \quad (3)$$

where P_N is net photosynthetic rate, P_{dark} is dark assimilation rate, both [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$], I is incident PAR [$\mu\text{mol m}^{-2} \text{s}^{-1}$], and α_{leaf} is leaf absorptance. P_{dark} is the same magnitude, but opposite sign, of dark respiration rate. In calculating ETR, f is the fraction of absorbed quanta that is used by PS2, and is typically assumed to be 0.4 for C₄ plants.

Fluorescence and net photosynthesis/internal carbon dioxide ($F-P_N/C_i$) curves: The automatic program in *LI-6400* photosynthesis system for $F-P_N/C_i$ curves was used to generate the response of P_N to C_i . Net photosynthesis and chlorophyll fluorescence characteristics were determined simultaneously. The top most recently fully expanded leaves on the main culm of 55 to 65 d old plants were used for these measurements. Measurements were taken between 10:00 and 14:00 h by changing [CO₂] in LCF in 11 steps (400, 300, 200, 100, 50, 0, 400, 400, 600, 800, and 1000 $\mu\text{mol mol}^{-1}$) under a constant PAR of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and block temperature was set to corresponding growth chamber day time temperature. Both steady-state (F_s) and maximal (F_m') fluorescence were logged along with standard photosynthetic parameters. The time allowed for the instrument to reach steady state

at each [CO₂] was 240 s. The instrument logged values when the stability or steady state indicated by total coefficient of variation was ≤1 %. Curve fitting software (*SigmaPlot for Windows 9.0*) was used to analyze the F–P_N/C_i responses using a three component exponential to maximum function of the form

$$P_N = a(1 - e^{-bx}) + c \quad (4)$$

where P_N = steady-state assimilation rate and x = C_i. Using this equation, P_{sat} was calculated as a + c and phosphoenolpyruvate carboxylase (PEPC) efficiency as the slope at P_N = 0, calculated as b[a + c]. Similarly, saturated values of ETR (ETR_{sat}) were calculated by fitting exponential to maximum function (Eq. 4) to ETR and C_i.

Fluorescence and net photosynthesis/PAR (F–P_N/PAR) curves: These measurements were made between 10:00 and 14:00 h on top most recently fully expanded leaves on plants aged 65 to 75 DAS. When measuring photosynthetic irradiance-response curves, PAR was gradually reduced from 2 000 to 0 μmol m⁻² s⁻¹ in 12 steps, the block temperature and [CO₂] inside the leaf cuvette were set to the treatment levels in the growth chamber. Artificial irradiation was applied to leaves from a red-blue LED radiation source attached to the sensor head. The photosynthetic irradiance response curves were fit using non-rectangular hyperbola least square curve fitting

Results and discussion

There were strong interactions of temperature and [CO₂] on photosynthesis and photochemical properties of big bluestem. As plants aged, the older leaves at 40 °C growth temperature turned yellow and senesced early, which was not observed at other temperatures until very end of season. No significant differences were observed between the LCF block and leaf temperature (T_L) for F–P_N/C_i curves, while the T_L during the F–P_N/PAR increased with increase in PAR. The mean T_L's were similar at both [CO₂] and measurement time did not modify the values. The mean T_L during the measurement periods were 39.4, 35.1, 29.9, 25.1, and 20.1 °C for set block temperatures of 40, 35, 30, 25, and 20 °C, respectively. The results provided evidence for sustained photosynthetic efficiency of big bluestem in a projected CO₂ enriched and hotter environment.

Leaf P_N responses to C_i: Stimulation of photosynthesis was dependent on both plant species and growing conditions. P_N response to C_i of big bluestem acclimated to different growth [CO₂] and temperatures followed an exponential rise to maximum function (Fig. 1). The P_N/C_i-response curves of leaves at both AC and EC were similar at 25 and 30 °C growth temperature. However, lower (20 °C) and the two higher (35 and 40 °C) growth temperatures caused clear difference of [CO₂] effects on

procedure (Lambers *et al.* 1998) and model described in Eq. (5):

$$P_N = \frac{\phi Q + P_{\max} - \sqrt{(\phi Q + P_{\max})^2 - 4\phi Q k P_{\max}}}{2k} - R_D \quad (5)$$

where φ is the apparent quantum efficiency, Q is the PAR, P_{max} is the PAR saturated rate of gross CO₂ assimilation, k is the convexity or curvature factor, and R_D is the dark respiration rate. *Photosyn Assistant (Dundee Scientific, Dundee, UK)* software was used to derive parameters from irradiance response curves. Maximum values of ETR (ETR_{max}) were calculated by fitting exponential to maximum function (Eq. 4) to ETR and PAR.

Statistical analysis: Nonlinear regression model of exponential rise to maximum was employed to determine relationships between P_N and PAR or C_i using curve fitting software (*SigmaPlot for Windows 9.0*). A two-way analysis of variance (ANOVA) statistical procedure (SAS Institute 1999) was carried to determine the effects of [CO₂] and temperature on measured photosynthesis parameters and those derived from F–P_N/C_i and F–P_N/PAR response curves. Additionally, the Fisher LSD test was used to determine treatment differences at p=0.05 level of significance (SAS Institute 1999). Quadratic and linear regressions were used to determine the response of derived parameters to temperature at both AC and EC.

photosynthesis, where EC resulted in higher P_N than in plants grown in AC (Fig. 1). The P_N values were significantly different when C_i values were above 30 Pa. The deviation of C_i curves at EC from those at AC was mainly due to the initial steeper slopes of the response curves and higher P_N values in the CO₂ saturation range. We did not record any down regulation of photosynthesis in the current study at EC and high temperature as big bluestem plants continuously produced sinks (tillers and seeds and only tillers at 40/32 °C; Kakani and Reddy 2007) for additional assimilates along with high rates of respiration.

In general, a doubling of AC increases photosynthesis in C₄ species between 0 and 25 %, lower than that for C₃ species (Cure and Acock 1986, Patterson and Flint 1990). However, temperature increases with increase in global [CO₂] plays an important role in regulating the kinetic properties of photosynthetic enzymes. Although C₄ plants have higher temperature optimum than C₃ plants, P_N is usually inhibited when temperature exceeds 38 °C (Edwards and Walker 1983). Two-way ANOVA analysis revealed a significant (p<0.05) [CO₂]×temperature interaction for all parameters studied, along with significant main effects of [CO₂] and temperature (Fig. 1). The initial slope (indicator of PEPC efficiency; Caemmerer 2000) and P_{sat}–CO₂ saturated rate (indicator of either

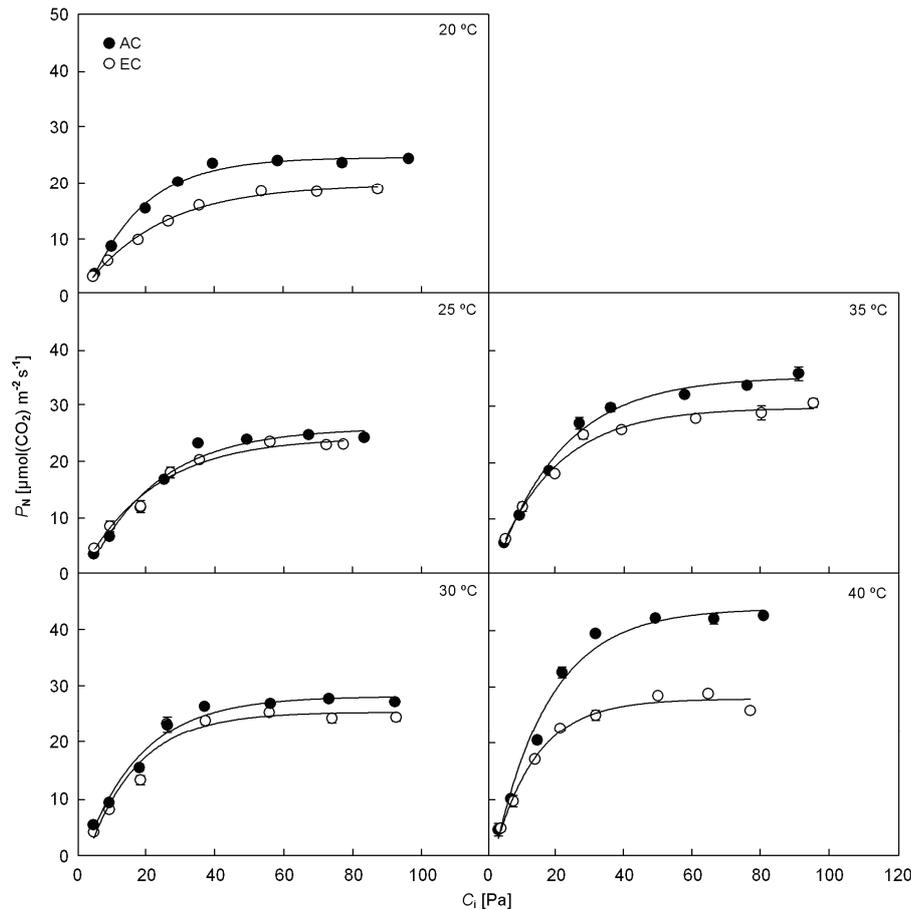


Fig. 1. Response to C_i of net photosynthetic rate (P_N) at ambient (AC) and elevated (EC) $[CO_2]$ of top most fully expanded leaves of big bluestem acclimated to five temperatures and measured between 55 and 65 DAS. Leaf cuvette was maintained at daytime temperature and $[CO_2]$ corresponding to the growth chamber and PAR was $1\ 500\ \mu\text{mol}\ \text{m}^{-2}\ \text{s}^{-1}$. Symbols are measured values and line is the exponential to maximum rise fit. Means of three replicates \pm SE.

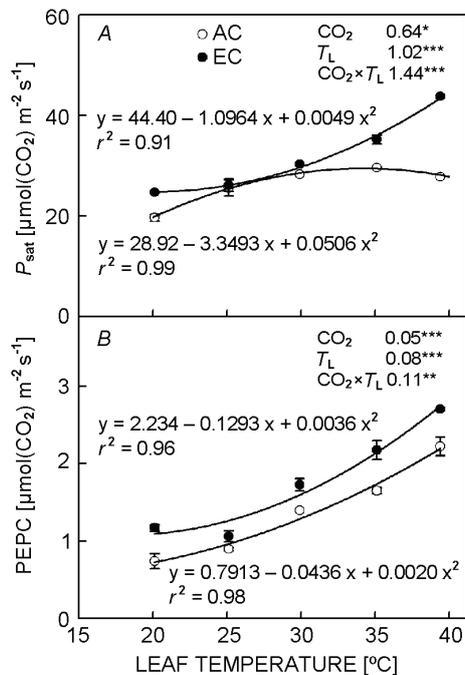


Fig. 2. Effect of leaf temperature (T_L) and $[CO_2]$ on (A) maximum net photosynthetic rate (P_{sat}) and (B) phosphoenolpyruvate carboxylase (PEPC) efficiency of top most fully expanded leaves of big bluestem. Means of three replicates \pm SE. The SED values and significance levels of two-way ANOVA analysis of $[CO_2]$ and temperature are presented.

RuBPCO activity or rate of PEP regeneration or electron transport rate or PEPC efficiency if it is very low) increased with increase in temperature at both $[CO_2]$ (Fig. 2). Both PEPC efficiency and P_{sat} were higher in plants grown at EC than at AC irrespective of temperatures. The PEPC efficiency showed a linear increase with temperature suggesting a more stable nature of this CO_2 acceptor in C_4 species. In agreement with our results, in maize, PEPC efficiency was higher at 45 °C compared to that at 30 °C and PPDK efficiency was similar at both 30 and 45 °C. However, variations in the initial slope with variation in PEPC efficiency have been demonstrated (Sage and Sharkey 1987).

The response of P_{sat} to temperature (Fig. 2A) under both $[CO_2]$ was quadratic. At AC, P_{sat} plateaued beyond T_L of 34 °C, while P_{sat} continued to increase with increase

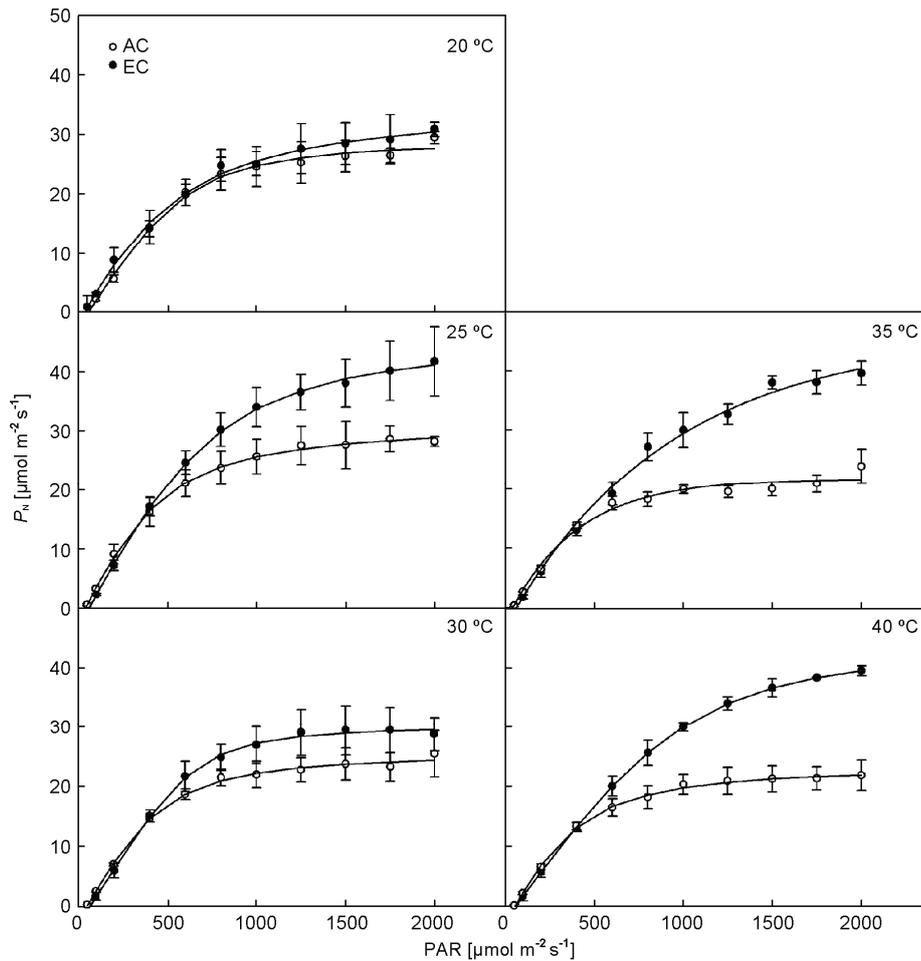


Fig. 3. Response to PAR of net photosynthetic rate (P_N) of top most fully expanded leaves acclimated to five temperatures and two $[CO_2]$ of big bluestem measured between 65 and 75 DAS. Leaf cuvette was maintained at treatment daytime temperature and $[CO_2]$ corresponding to the growth chamber. Symbols are measured values and line is the exponential to maximum rise fit. Means of three replicates \pm SE.

in temperature at EC. Maximum P_{sat} values were $29.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 35°C under AC and $43.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ at 40°C under EC. The response to temperature of PEPC efficiency (Fig. 2B), however, was linear and increased with increase in temperature. PEPC efficiency in response to temperature had a steeper slope (slope = 0.08807) at EC compared to that at AC (slope = 0.07287). An increase in P_{sat} with increase in temperature had a Q_{10} of 2.0 between 20 and 30°C in the C₄ species *Amaranthus edulis* (Kiirats *et al.* 2002). In the current study, Q_{10} values between 20 and 30°C were 1.43 at AC and 1.23 at EC. The Q_{10} trend reverted in the high temperature range of 30 – 40°C with 0.98 at AC and 1.44 at EC suggesting an increased assimilation of CO_2 . Furthermore, EC-grown leaves registered higher contents of both PEPC and P_{sat} than AC-grown leaves suggesting that RuBPCO activation state was not modified under EC and high temperature. This can be due to high temperature tolerance of RuBPCO activase which denatures at temperatures of about 40°C (Salvucci and Crafts-

Brandner 2002). Thus, photosynthesis of big bluestem is more tolerant to increasing temperature, a C₄ characteristic, in spite of it being widely adapted and grown in northern latitudes of USA.

Leaf P_N responses to PAR: The C₄ photosynthesis is characterized by irradiance response curves that saturate only at very high PAR. P_N significantly increased with increase in PAR at both $[CO_2]$ (Fig. 3). Except at temperature of 30°C , the response curves saturated at the PAR of approximately $1\ 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ at both $[CO_2]$ and showed almost constant values with further increase in the PAR. The response to PAR of P_N from plants acclimated to EC was significantly higher than those acclimated to AC, except in the low temperature treatment (20°C). Further, increase in temperature resulted in greater P_N at both $[CO_2]$ and maximum P_N was recorded at a leaf temperature of 39.1°C . Our results are in agreement with the reports by Hatch (1992) where C₄ plants exhibited higher P_N at full sunlight under tropical

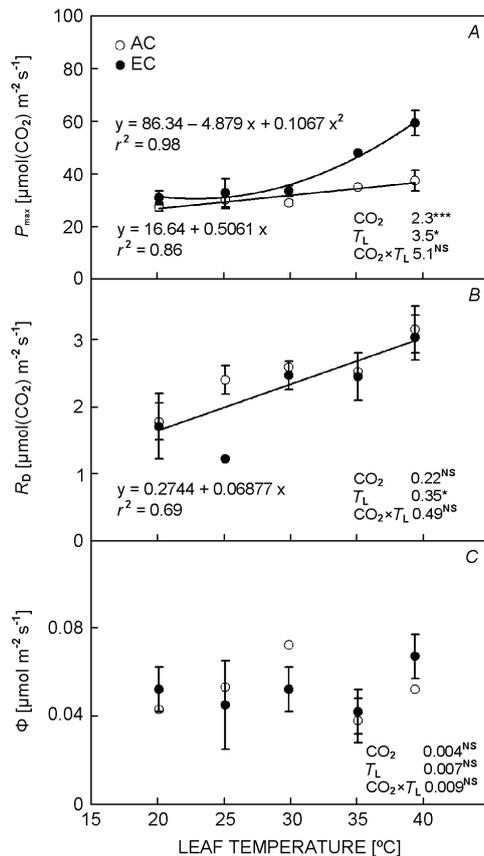


Fig. 4. Effect of leaf temperature (T_L) and $[CO_2]$ on (A) maximum net photosynthetic rate (P_{max}), (B) dark respiration rate (R_D), and (C) maximum quantum efficiency of photosystem 2 (Φ) of big bluestem. The values were derived by using irradiance response module of the *Photosyn Assistant Software* (Dundee Scientific, Dundee, UK). Means of three replicates \pm SE. The SED values and significance levels of two-way ANOVA analysis of $[CO_2]$ and temperature are presented.

conditions by avoiding photorespiration. Although, additional energy is required to assimilate CO_2 via C_4 pathway, this will become irrelevant at irradiance saturation since radiant energy will definitely be in excess of requirements (Young and Long 2000). No significant ($p > 0.05$) $[CO_2] \times$ temperature interaction was observed for the parameters derived from irradiance response curves. However, parameters differed in their statistical significance in responses to $[CO_2]$ and temperature treatments (Fig. 4). Irradiance-saturated maximum photosynthesis (P_{max}) increased linearly with the increasing temperatures irrespective of $[CO_2]$ (Fig. 4A). The rate of increase was much higher (slope = 1.473) at EC than at AC (slope = 0.506). These higher rates can be attributed to higher activities of PEPC and RuBPCO in big bluestem. Further, plants exposed to EC stimulated the rate of photosynthesis, suggesting that the current AC is insufficient to saturate RuBPCO (Drake *et al.* 1997). R_D significantly increased with increasing temperatures irrespective of $[CO_2]$ (combined slope = 0.044; Fig. 4B). Similar

observations were made by Nagy *et al.* (2000). Influence of elevated temperature on R_D was directly related to temperature effects on metabolism, because changes in R_D were significant across the temperatures.

Quantum yield is a sensitive measure of the efficiency of radiant energy utilization in plants and widely used as non-intrusive method of photorespiratory activity and photoinhibitory damage in C_3 and C_4 plants. The initial slope of irradiance-response curves in current study showed that unlike C_3 photosynthesis, quantum yield of C_4 photosynthesis was independent of $[CO_2]$, $[O_2]$, and temperature because of lack of photorespiration. Either $[CO_2]$ or temperature did not have any significant effect on quantum efficiency (Φ ; Fig. 4C). In contrast, under non-photorespiratory conditions such as ambient $[O_2]$ and EC, the quantum yield of C_4 species is less than that of C_3 species (Ehleringer and Björkman 1977). Percy and Björkman (1983) reported that a small increase in Φ may increase daily carbon gain under low irradiance. Sorghum, a C_4 species, grown at AC and EC did not show any difference in Φ (Watling *et al.* 2000). In agreement with the earlier reports, we did not observe significant interactive effect of temperature and $[CO_2]$ on Φ .

Photochemical responses: The fluorescence measurements provide evidence for tolerance of big bluestem photosynthesis to high temperature. Measured leaf fluorescence parameters, minimal fluorescence (F_0'), maximal fluorescence (F_m'), and steady-state fluorescence (F_s) responded to changes in C_i and PAR (data not shown). Strong feedback links observed between CO_2 fixation and ETR in earlier studies (Krall and Edward 1990, Yamasaki *et al.* 2002, June *et al.* 2004) were also observed in the current study. The ETR increased along with increase in P_N at both $[CO_2]$ (Fig. 5). However, the highest ETR values were achieved under high PAR, high temperature, and at EC (Fig. 5C,D). The response to temperature of ETR_{sat} (Fig. 6A) and ETR_{max} (Fig. 6B) derived from F/C_i was linear and increased with increase in temperature. However, ETR_{sat} and ETR_{max} values were higher at EC across the temperatures compared to those at AC. A linear association between $P_{sat/max}$ and $ETR_{sat/max}$ was observed in the current study due to the absence of photorespiration in C_4 species.

To assess the stability and the quantum efficiency of energy absorbed, relationships were developed between photochemical yield of PS2 electron transport (Φ_{PS2}) and the quantum yield of CO_2 assimilation (Φ_{CO2}) derived from P_N/C_i curves and those from P_N/PAR response curves (Fig. 7). High photochemical use efficiency is expected as there is no photorespiration (Krall and Edwards 1990) and alternate sinks are low in C_4 species (Loreto *et al.* 1995). The relationships derived from both the response curves were linear and the slope values suggest that Φ_{PS2} decreased more than Φ_{CO2} , suggesting the presence of alternate sinks, as observed earlier in sorghum (Loreto *et al.* 1995).

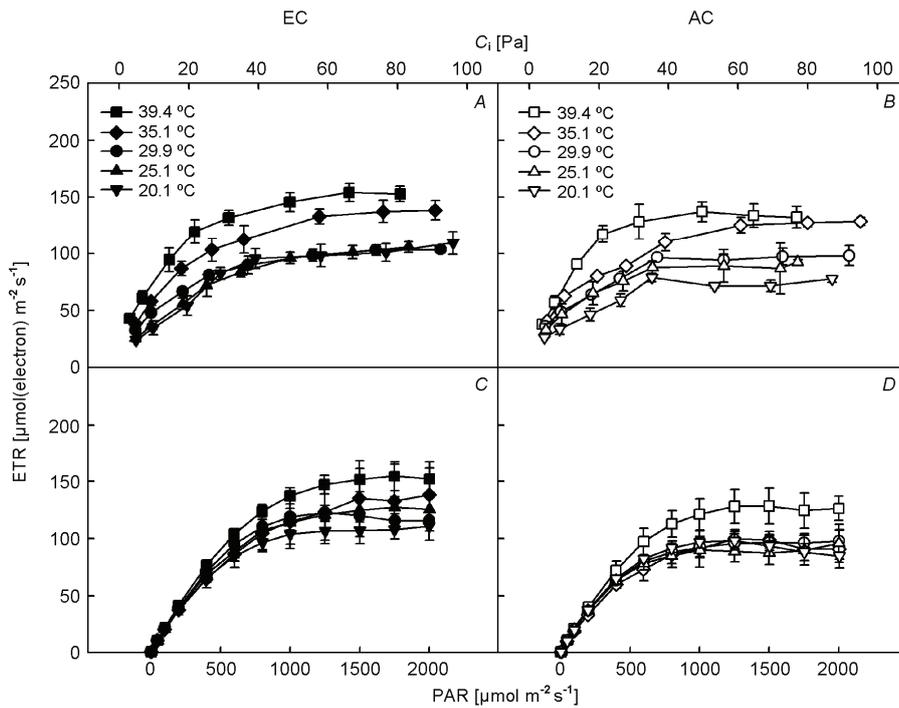


Fig. 5. Effect of temperature and [CO₂] on (A, B) electron transport rate in response to internal [CO₂] (C_i) and (C, D) photosynthetically active radiation (PAR) of top most fully expanded leaves of big bluestem. Means of three replicates ±SE.

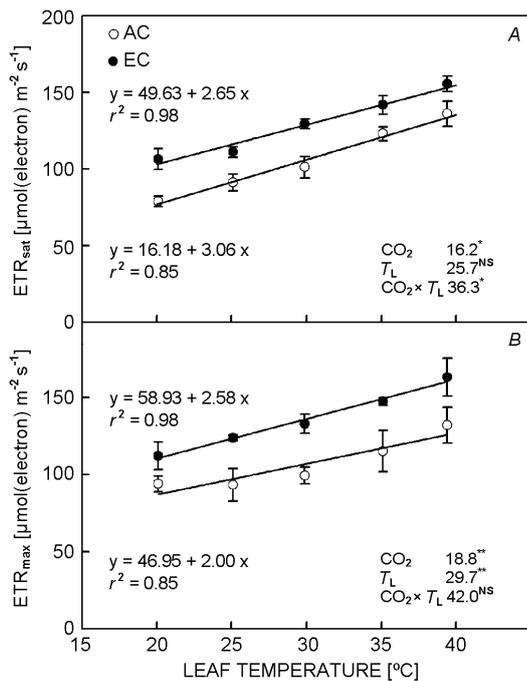


Fig. 6. Effect of leaf temperature (T_L) and [CO₂] on (A) saturated electron transport rate (ETR_{sat}) and (B) maximum electron transport rate (ETR_{max}) of big bluestem. Means of three replicates ±SE. The SED values and significance levels of two-way ANOVA analysis of [CO₂] and temperature are presented.

We found a steeper slope of ETR versus C_i at high temperatures indicating higher electron requirement with increase in temperature at low C_i . Such residual electron transport was also observed in sorghum, a C₄ species (Loreto *et al.* 1995) suggesting presence of alternate

sinks. An increase in residual electron transport was observed with increase in temperature at both [CO₂] (Fig. 8). The values recorded in this study are similar to those in other C₄ species (Loreto *et al.* 1995) and are much lower compared to those in C₃ species (Loreto *et al.* 1994). Another possibility of electron usage is CO₂ leakage from bundle sheath cells, which is temperature dependent (Henderson *et al.* 1992).

In addition, an increase of [CO₂] or PAR resulted in a decrease in electrons required to fix one molecule of CO₂. The electrons per CO₂ fixed decreased from a high of 9 to a low of 4 with increase in both C_i and PAR (data not shown). This suggests an increase in activity of photosynthetic enzymes and their ability to fix CO₂ even at high temperatures. Based on the values of P_N and ETR , the number of electrons required per CO₂ fixed (ETR/P_N) was in the range of 3.6–5.1 (Fig. 9), much lower than the range of 10.2–13.3 recorded for C₃ species, but close to the theoretical requirement for C₄ species (Krall and Edwards 1992). Thus, most of the reductive power was utilized in the assimilative process. Along with this, simultaneous increase in both PEPC and RuBPCO activities as explained by parameters derived from response curves might have contributed to continued CO₂ assimilation at high temperature.

On comparison, the total biomass produced (Kakani and Reddy 2007, data not shown) at these growth temperatures and [CO₂] had no significant correlation with P_{sat} and P_{max} . At both [CO₂], increase in growth temperature up to 30/22 °C increased total biomass; further increase in growth temperature resulted in lower biomass due to higher R_D and increased senescence. The failure of stem elongation at the two highest temperatures

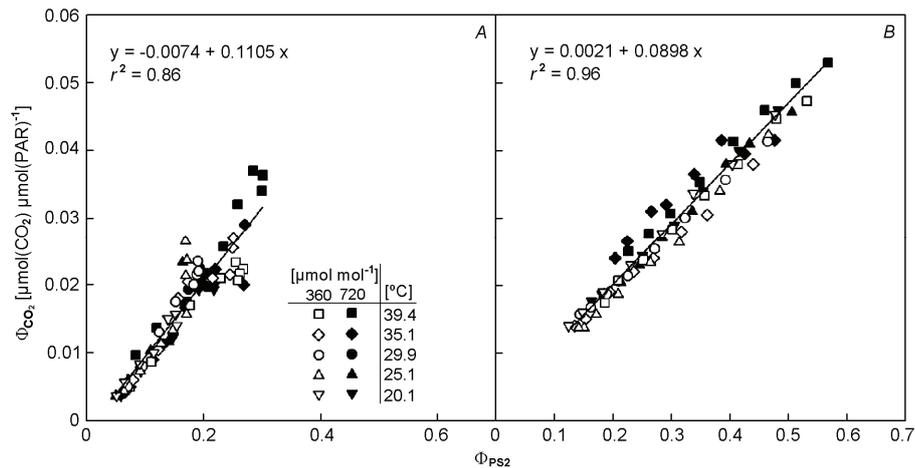


Fig. 7. Relationship between the photochemical yield of photosystem 2 electron transport (Φ_{PS2}) and the quantum yield of CO_2 assimilation (Φ_{CO2}) derived from (A) $F-P_N/C_i$ curves and (B) $F-P_N/PAR$ curves of big bluestem. The symbols used in the graphs correspond to the ten different treatments and are the same as used in previous graphs. The line is the linear fit between Φ_{PS2} and Φ_{CO2} . All data were pooled as no significant differences were observed between treatments for the relationship. Means of three replicates, SE values were not plotted for clarity.

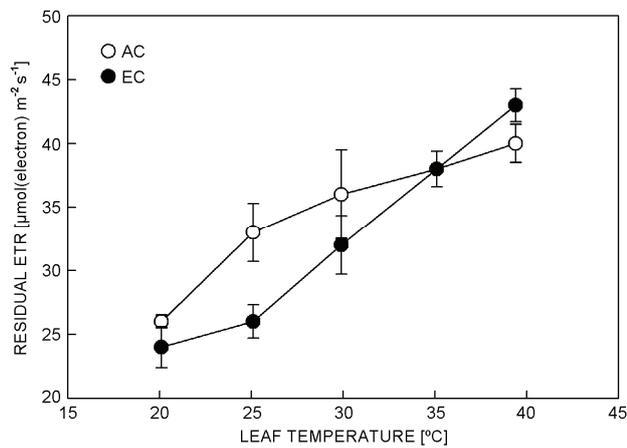


Fig. 8. Response of residual electron transport to leaf temperature of big bluestem. These values were derived from the ETR *versus* C_i response curves when photosynthesis was totally inhibited at leaf chamber $[CO_2]$ of $0 \mu mol mol^{-1}$ and $1500 \mu mol m^{-2} s^{-1}$ of PAR. Means of three replicates \pm SE.

in the study would have resulted in sink limitation contributing to lower total biomass.

In conclusion, our results demonstrate that both $[CO_2]$ and temperature interactively promote photosynthesis of C_4 species, big bluestem. The lack of photorespiration, a characteristic of C_4 species, and continued production of assimilate sinks (tillers) resulted in higher photosynthetic rates at high temperature. Overall, temperature had much greater effect on photosynthesis parameters and photosynthesis and fluorescence responses to PAR and the response to $[CO_2]$ was temperature dependent. As shown here, C_4 photosynthesis under a constant set of environmental conditions would acclimate to both $[CO_2]$ and temperature. We found also that well-controlled sunlit

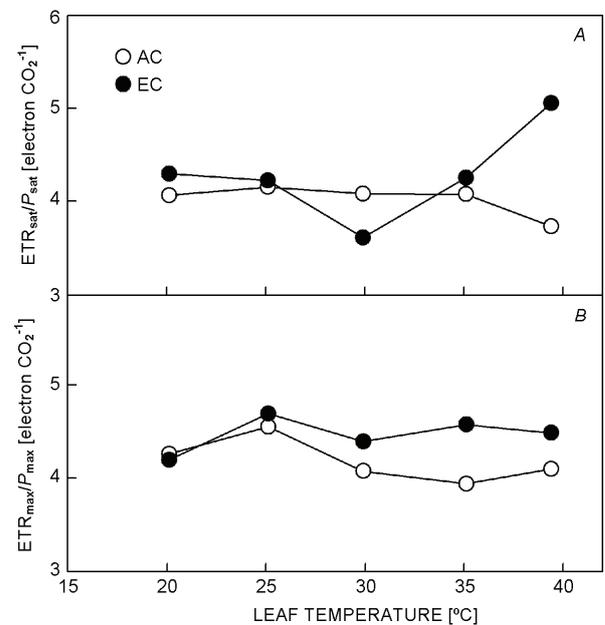


Fig. 9. Effect of leaf temperature at two $[CO_2]$ on the ratios between saturating and maximum photosynthetic rate (A - P_{sat} or B - P_{max}) and electron transport rate (A - ETR_{sat} , B - ETR_{max}) in response to internal $[CO_2]$ (C_i) of big bluestem.

chambers would provide potential data sets for developing mechanistic models. The potential of enzyme and photosystem parameters to tolerate high temperature ($>35 \text{ }^\circ C$) indicates the ability of C_4 species to sustain CO_2 assimilation in future climates but increased temperature tolerance of processes such as senescence and stem development and elongation will be essential to realize the benefits.

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