Available online at www.sciencedirect.com

ScienceDirect

Environmental and Experimental Botany 60 (2007) 412–420

Environmental and Experimental Botany

www.elsevier.com/locate/envexpbot

# Photosynthetic traits in $C_3$ and $C_4$ grassland species in mesocosm and field environments

Jesse B. Nippert [a,*], Philip A. Fay [b], Alan K. Knapp [a]

[a] *Department of Biology, 1878 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1878, USA*
[b] *USDA-ARS, 808 East Blackland Rd., Temple, TX 76502 USA*

Received 13 June 2006; received in revised form 21 December 2006; accepted 28 December 2006

## Abstract

The North American tallgrass prairie is composed of a diverse mix of $C_3$ and $C_4$ plant species that are subject to multiple resource limitations. $C_4$ grasses dominate this ecosystem, purportedly due to greater photosynthetic capacity and resource-use efficiency associated with $C_4$ photosynthesis. We tested the hypothesis that intrinsic physiological differences between $C_3$ and $C_4$ species are consistent with $C_4$ grass dominance by comparing leaf gas exchange and chlorophyll fluorescence variables for seven $C_4$ and $C_3$ herbaceous species (legumes and non-legumes) in two different settings: experimental mesocosms and natural grassland sites. In the mesocosms, $C_4$ grasses had higher photosynthetic rates, water potentials and water-use efficiency than the $C_3$ species. These differences were absent in the field, where photosynthetic rates declined similarly among non-leguminous species. Thus, intrinsic photosynthetic advantages for $C_4$ species measured in resource-rich mesocosms could not explain the dominance of $C_4$ species in the field. Instead, $C_4$ dominance in this ecosystem may depend more on the ability of the grasses to grow rapidly when resources are plentiful and to tolerate multiple limitations when resources are scarce.

*Keywords:* $A$:$C_i$ curves; Chlorophyll fluorescence; $C_3$; $C_4$; Gas exchange; Konza; Tallgrass prairie

## 1. Introduction

Central US grasslands are typically dominated by a few highly productive $C_4$ grass species (Epstein et al., 1998). These grasses are accompanied by numerous $C_3$ forb species (Freeman and Hulbert, 1985; Knapp and Seastedt, 1986; Freeman, 1998), which must compete with the dominant grasses for multiple potentially limiting resources, including nitrogen, water, and light (Schulze and Chapin, 1987; Knapp et al., 1998). Thus, in a grassland environment of high-light, warm temperatures, periodic drought, and low nitrogen availability, $C_4$ dominance over the $C_3$ species may result from a combination of mechanisms, including intrinsically higher photosynthetic capacity and resource-use efficiency of $C_4$ photosynthesis (Knapp, 1993; Tezara et al., 1998; Long, 1999) and key adaptations to major biotic and abiotic drivers and stresses in grasslands including fire, grazing by large herbivores, and climatic variability (Seastedt and Knapp, 1993; Ojima et al., 1994; Knapp and Medina, 1999).

The role of intrinsic physiological differences among $C_4$ and $C_3$ species in their relative success in tallgrass prairie assemblages remains unclear. In general, $C_4$ species have lower water and nitrogen requirements than $C_3$ species and reduced stomatal conductance and enzyme requirement per mol of $CO_2$ fixed when measured under controlled laboratory conditions (Pearcy and Ehleringer, 1984; Long, 1999; Sage, 2004). The performance of $C_3$ and $C_4$ species in the field should reflect these inherent $C_4$ advantages (Knapp, 1993). However, the expected physiological advantages of $C_4$ species are often not realized in the field. This discrepancy may result because laboratory studies often poorly reproduce field growth conditions of water, nutrient, and light limitation, or the plant material studied is unrepresentative of field populations (Evans and Seemann, 1989; Wohlfahrt et al., 1999; Gibson et al., 1999). Further, most field studies of photosynthesis have been conducted when some level of interspecific competition exists, rendering comparisons with laboratory studies difficult (Tieszen et al., 1997; Knee and Thomas, 2002; Vitale and Manes, 2005).

---

[*] Corresponding author. Present Address: Department of Ecology and Evolutionary Biology, University of Kansas, 2041 Haworth Hall, Lawrence, KS 66045, USA Tel.: +1 785 864 5229; fax: +1 785 864 5860.
*E-mail address:* gostate@ku.edu (J.B. Nippert).

0098-8472/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2006.12.012

We compared photosynthetic traits in several common tallgrass prairie grass and forb species in environments that differed in resource availability to discern if $C_4$ advantages common in laboratory studies could be replicated when performed under the prevailing mid-summer growth environment. Species traits were measured in experimental outdoor mesocosms and in upland field sites in an undisturbed annually burned prairie. The mesocosms provided abundant light, nitrogen, and water and minimal variation in these resources similar to laboratory studies, thus enabling us to estimate *potential* photosynthetic traits. By comparison, the field sites represented typical levels of resource limitation common in mid-summer in tallgrass prairie and served as a comparative indicator of *realized* photosynthetic traits. The difference between *potential* and *realized* photosynthesis may allow for a greater differentiation of photosynthetic differences between $C_3$ and $C_4$ grassland species. These traits were specifically measured in mid-July, a time that is typically dry, hot, and when the spring pulse in soil N availability has been depleted. This allowed us to test the hypothesis that $C_4$ grasses in this system have both higher *potential* and *realized* photosynthetic capacity and resource-use efficiency under typical mid-season environmental conditions.

## 2. Materials and methods

### *2.1. Study site*

Research was conducted on the Konza Prairie Biological Station (KPBS), a 3487 ha unplowed native tallgrass prairie preserve located in northeast KS, USA (39°05′N, 96°35′W). KPBS experiences a mid-continental climate of cool dry winters (−3.0 °C average) and hot summers (27 °C), with the majority of the annual precipitation (835 mm) falling between April and September. The vegetation at KPBS consists of approximately 540 species of $C_3$ herbaceous, woody, and grass species and 31 $C_4$ species, dominated by the grasses *Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, and *Panicum virgatum* L. (Freeman, 1998; Towne, 2002). We selected dominant species for this study comprising three functional groups: $C_4$ grasses *A. gerardii* and *S. nutans*, $C_3$ non-legume forbs *Aster ericoides* L., *Echinacea angustifolia* DC. var. *angustifolia*, and $C_3$ leguminous forbs *Amorpha canescens* Pursh, *Lespedeza capitata* Michx., and *Psoralea tenuiflora* Pursh var. *floribunda* (Nutt.) Rydb. All are common and widely distributed species in the central North American grasslands. $C_3$ grass species occur at KPBS, but are rare and represent a small percentage of the overall species composition on site.

### *2.2. Comparative growth environments*

#### *2.2.1. Mesocosm facility*

Plants were measured in eight 2.6 $m^3$ (1.2 m × 1.2 m × 1.8 m deep) mesocosms containing assemblages of the study species. The mesocosms provided high water, light, and nutrient availability, and favorable soil conditions for root growth. Outdoor mesocosms were chosen for this study to represent the most optimum growing conditions outdoors, for comparison to similar species growing under ambient resource limitations. Individuals were planted in late May 2003 by broadcast seeding the grasses and planting greenhouse-grown seedlings for the forbs. All seeds were from commercial sources. The legumes were not inoculated with *Rhizobium* prior to planting. Planting densities reflect natural stem densities and species relative abundances, and forbs were planted in an identical spatial arrangement in each mesocosm to avoid variation in performance from differing species associations. Mesocosms were frequently weeded to maintain the desired species composition and were watered approximately three times per week to minimize water stress. The soil profile within each mesocosm contained well-mixed A-horizon topsoil in the top 30 cm overlying B-horizon subsoil collected on site. The mesocosms were free draining to allow for natural soil moisture profiles. Average extractable inorganic soil nitrogen measured in September 2003 was $2.80 \pm 0.33$ (S.E.) $\mu g\, N\, g^{-1}$ soil.

#### *2.2.2. Field sites*

KPBS is divided into 60 watersheds of various long-term treatments of fire frequency and grazing by native herbivores. We used the upland regions of the two annually burned, ungrazed watersheds which provide typical field soil moisture, nutrient availability, and competitive interactions. Within each watershed, two separate sites were established where all seven species occurred within a 20 m-diameter circle. Watersheds were burned each April since 1982, including the study year, a typical management practice that maintains $C_4$ grass dominance and results in saturating sunlight intensities for most individual leaves. Extractable inorganic soil nitrogen from these sites has been reported previously ($0.93 \pm 0.07$ (S.E.) $\mu g\, N\, g^{-1}$ soil; Blair, 1997) from samples collected in September, the same time of year as that measured in the mesocosms.

### *2.3. Sampling procedure*

Sampling was specifically conducted in July when air temperatures were high and mid-season water stress more likely in order to distinguish these responses from previous studies conducted in early summer when water and soil N are not as limiting (Knapp, 1985). Additionally, mid-July is typically the time period for maximum rates of growth in developmentally mature plants prior to late summer senescence (Turner and Knapp, 1996). For gas exchange measurements in the mesocosms, one individual from each species was randomly selected within each mesocosm ($n = 8$ per species). For chlorophyll fluorescence and water potential measurements, 5–7 individuals per species/mesocosm combination were measured and the average value per mesocosm was used in the analysis. Based on the results of the 2003 sampling in the mesocosms, analyses were conducted in the field during the same seasonal period (early July) in 2004. This time period had a nearly identical microclimate to measurements conducted in the mesocosms the previous year (air temperature, relative humidity (RH), solar radiation, and windspeed; Table 1). Cumulative monthly precipitation in 2004 was 29% above average (Table 1), making comparisons with the mesocosms conservative with respect to water stress.