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Field measurements of photosynthesis, water-use efficiency, and growth in *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4) in the Colorado shortgrass steppe

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Summary. Field measurements of gas exchange and growth were conducted on a C_3 grass, *Agropyron smithii*, and a C_4 grass, *Bouteloua gracilis*, in order to further establish the adaptive significance of the C_4 pathway under natural conditions. Maximum rates of leaf area expansion in tillers and maximum seasonal photosynthesis rates of both species occurred during the cool, early summer month of June. The occurrence of maximum growth and photosynthesis in *B. gracilis* during this cool period was apparently related to its occupation of warm microenvironments next to the ground surface. As temperatures increased during the midsummer, photosynthesis rates decreased to 47% and 55% of the seasonal maximum in *B. gracilis* and *A. smithii*, respectively. Water-use efficiencies in both species were similar or slightly higher for *B. gracilis* during June, the period of maximum growth. By mid-July, however, leaves of the C_3 grass, *A. smithii*, exhibited water-use efficiencies approximately half as high as *B. gracilis*. These differences in water-use efficiency were the result of differences in stomatal conductance, rather than differences in daily CO_2 uptake rates which were similar in both species. The results demonstrate that in certain environments there are no offset periods of growth and maximum photosynthesis during the growing season in these C_3 and C_4 species. The greater amounts of daily water use in *A. smithii* during the midsummer might contribute to its much greater abundance in lowland sites in the shortgrass steppe. The C_4 grass, *B. gracilis*, occurs in dry upland sites in addition to the more mesic lowland sites.

Since the late 1960's numerous comparative studies of gas exchange in C_3 and C_4 plants have revealed substantial differences in their response to environmental variables (for review see Osmond et al. 1980, Pearcy and Ehleringer 1984). In general, C_4 species exhibit higher photosynthetic temperature optima, higher light saturation levels, and greater water-use efficiencies than C_3 species. On the basis of these differences it has been widely suggested that C_4 species would have an advantage over C_3 species in warm, high irradiance, arid and semi-arid environments. Most of the studies supporting this contention, however, have used growth chamber grown plants, with the gas exchange properties analyzed in response to only one environmental variable at a time.

The North American Great Plains provide ideal ecosystems for examining comparative aspects of C_3 and C_4 photosynthesis in situ. In these grasslands the C_3 and C_4 photosynthetic pathways are found in several dominant grasses, thus providing a system with variable photosynthetic function in a similar growth form. Analyses of C_3 and C_4 grasses at the community level have revealed that the two pathway types vary in abundance along environmental gradients of moisture and temperature (Teeri and Stowe 1976, Ode et al. 1980). In general, the distribution patterns are such that C_4 grasses achieve their highest relative abundance during the warmer part of the growing season and in drier topographic microsites. Once again, these patterns are in accordance with the widely accepted dogma that C_4 plants have an advantage at fixing carbon in warm, moisture-limited habitats, relative to C_3 plants. The advantage of C_4 photosynthesis in moisture-limited habitats is presumably due to its greater water-use efficiency, not a greater tolerance of low tissue water potentials (Ludlow 1976, Edwards et al. 1984).

Although these community level studies have provided a great deal of insight into the ecological importance of C_3 versus C_4 gas exchange patterns in the North American Great Plains, no comparative field studies of gas exchange in individual leaves have been conducted for corroboration. Such studies would strengthen any conclusions concerning the C_3, C_4 distribution patterns since it is unknown whether the differences in water-use efficiency and photosynthesis which have been identified in controlled growth studies are indeed expressed in situ. In the field a complex of environmental variables co-vary temporally and spatially, and can place conflicting demands on photosynthetic function. The objective of the current studies was to conduct comparative field observations of growth, photosynthesis, and water-use efficiency in a C_3 grass, *Agropyron smithii* Rydb., and a C_4 grass, *Bouteloua gracilis* (H.B.K.) Lag, in the Colorado shortgrass steppe. Emphasis was placed on examining whether the C_4 species exhibited higher water-use efficiencies than the C_3 species, and whether it demonstrated its greatest photosynthetic and growth activities during the warmer mid-summer months.

Materials and methods

Study site

All studies were conducted at the Central Plains Experimental Range (CPER) near Nunn, Colorado (40°42′N,

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104°46′W). Most of the studies were conducted during 1983 and 1984. These studies were conducted in irrigated and non-irrigated plots established in a grazing exclosure which had been free from grazing for 3 years. During 1983 studies were conducted on irrigated and non-irrigated plants; during 1984 studies were conducted on non-irrigated plants only. *Agropyron smithii* and *Bouteloua gracilis* were abundant and grew intermixed within the plots. The three irrigated plots measured 1 × 3 m and were separated by 1 m. Each plot was irrigated on days without appreciable rainfall with approximately 20 l of water. The non-irrigated plots surrounded the irrigated plots at a distance of approximately 2 m in the same lowland swale. There were no detectable differences between the irrigated and non-irrigated plots in plant species composition or community structure prior to initiating the irrigation treatments. Due to the dense nature of the vegetation, no water runoff occurred from the irrigated plots, and on the basis of differences in vegetation appearance at the end of the irrigation period, plants in the non-irrigated plots did not have access to underground water in the irrigated plots. During 1981 some studies were conducted at an upslope site, approximately 10 m upslope from a lowland swale. The 1981 studies were also conducted on non-irrigated and irrigated plants. In this case the plants were irrigated daily for four days prior to measurement.

Gas-exchange measurements

During 1983 all gas exchange rates were measured with a portable gas exchange system, similar to those previously described by Bingham et al. (1980) and Field et al. (1982). The system consisted of three principal components: a) an infrared CO_2 analyzer (ADC, model mk. III), b) an electronic control system (Bingham Interspace Co., model B-1), and c) a leaf cuvette. All dry gases were carried through copper tubing and humidified gases through teflon tubing. The system has been described in detail in a previous report (Monson 1984).

Air temperature and humidity in the cuvette were maintained at values similar to those measured within 10 cm of the experimental plants. Air temperatures in the cuvette were maintained within 0.7° C of air temperatures near the experimental plants through a feedback control circuit. In these narrow-leaved species leaf temperatures were seldom observed to be more than 2° C different from surrounding air temperatures, either within or outside the cuvette. On several occasions we checked leaf temperatures within the cuvette against those of outside leaves and always found agreement within ±2° C. Humidity in the cuvette was monitored with a capacitance-type chip (Vaisala Humicap, Weathermeasure). Ambient humidities were monitored with a second Vaisala chip located near the cuvette, and cuvette humidities were adjusted to within a maximum variability of ±10% of the ambient levels (although most measurements were conducted with cuvette humidities within 2–5% of the ambient levels). Tillers of the two species typically occupy different microclimates. Tillers of *A. smithii* in lowland sites are erect, standing up to 35–50 cm tall. Tillers of *B. gracilis*, in contrast, are densely tufted with the leaves tightly packed and occurring within 5–10 cm of the ground surface. Accordingly, on clear days midday leaf temperatures of *B. gracilis* are greater than leaves of *A. smithii*. In the determinations of daily gas-exchange patterns care was taken to track the ambient temperature at each respective height for the two species. All measurements of leaf or air temperatures outside of the gas exchange cuvette were conducted with 0.1 mm diameter copper-constantan thermocouples and a thermocouple thermometer (Wescor, model TH-65).

Typically four to five fully-expanded leaves from three or four neighboring tillers were used for the gas-exchange measurements. We encountered several problems associated with placing several leaves of these shortgrass species into the cuvette without disturbing the surrounding canopy structure. Thus, we did not attempt to make the measurements in the natural light environments of each tiller. We maintained the natural vertical leaf angle for each species, although we standardized the direction of each adaxial leaf surface to be perpendicular to the incoming solar rays. Without such standardization the results would lose their comparative value since each measurement then becomes dependent on the researcher's choice of the relative proportions of leaf area oriented in the various directions. Thus, in this study we have examined daily gas-exchange patterns as a function of differing photosynthetic physiologies and thermal microenvironments, ignoring the complexities of the light microenvironment.

CO_2 concentrations within the cuvette were maintained between 320–355 μmol/mol during the entire season's measurements, although on any given day the concentrations were maintained within a 15 μmol/mol span. Photon flux densities (400–700 nm) were measured with a quantum sensor (LiCor, model LI-1905B). Leaf areas were determined with a LiCor model LI-3000 leaf area meter or a Delta-T model AMS area meter. All gas-exchange calculations except intercellular CO_2 concentrations were conducted according to Field et al. (1982). Intercellular CO_2 concentrations were calculated according to von Caemmerer and Farquhar (1981).

The 1981 gas-exchange measurements were conducted on four days during early July. For these measurements leaves were sealed into two identical cuvettes constructed from polycarbonate on a single Peltier-regulated heat exchanger (Cambion, model 809-3040-01). In this case leaf temperatures were maintained within ±1.5° C of ambient leaf temperatures throughout the day. CO_2 and humidity differences between the air entering and leaving the cuvettes were monitored with a Beckman model 865 infrared gas analyzer, and a dew-point hygrometer (EG and G Instruments, model 880), respectively. Calibration of the CO_2 analyzer was accomplished with a Wösthoff mixing pump (model ISA 27/3F) and gases from cylinders containing 1% CO_2 in N_2 and CO_2-free air. Other specifics of the gas exchange system used in 1981 are described in Monson et al. (1982).

Growth measurements

Seasonal growth measurements were conducted during 1983 and 1984. During 1983, fifty tillers of each species were tagged in the three non-irrigated plots. The total lengths of leaf blades on each tiller were measured at intervals of 10–14 days throughout the study period. Leaf lengths were converted to calculated leaf areas using a regression of leaf length versus leaf area for both species. The regression equations were determined during the third week of June. All regressions had correlation coefficients of 0.95 or better. During 1984, sods of both species were collected from a lowland site at the CPER at two week intervals and transported to Boulder, Colorado. Within one day of collecting the sods, the total green leaf area of 20 individual tillers for each species was determined. Leaf areas were measured with the Delta-T leaf area meter described above.

Plant water potential measurements

Plant water potentials were measured on individual grass leaves with a PMS model 1000 pressure chamber using compressed nitrogen as the pressurized gas. Leaves were enclosed in a plastic sheath prior to excision from the plant and during measurement to reduce errors due to water loss. Predawn measurements were conducted within 30 min prior to sunrise. Midday measurements were conducted between 1200 h and 1300 h.

Vegetation clipping along transects

To characterize the relative positions of the two species along topographic gradients, the living aboveground tissue of each species in 0.5 m^2 quadrats was clipped at 10 m intervals along three 300 m transects. The clipped tissue was oven dried at 90° C for 48 h and weighed. The clipping studies were conducted in early June, before the site had been grazed.

Results

Abiotic variables

The 1983 growing season was characterized by spring months being cooler and wetter than the long-term average (Table 1). The months of April, May, and June were characterized by total monthly precipitation being 1.2 to 2.7 times higher than the long-term patterns (see Lauenroth and Sims 1976). Additionally, the maximum air temperature during these months averaged 1–3° C cooler than the long-term average. The midsummer months of July and August were characterized by lower than normal precipitation and average maximum air temperatures were 3–6° C higher than the norm.

In contrast to 1983, the 1984 growing season was characterized with the spring months of May and June being warmer and drier than the long-term average. Total monthly precipitation during these months was 58–88% of the long-term average, and monthly mean maximum air temperatures were 3–4° C warmer. The midsummer months of July and August were also warmer than the long-term average by approximately 4° C, with total monthly precipitation being 1.5 times higher than the long-term average during July and 1.1 times higher during August.

In order to more firmly establish the differences in thermal microclimate that the tillers of these two species occupy, we measured air temperatures at one hour intervals from 0900–1500 h at heights of 5 and 50 cm above the ground surface on 21 dates during the 1983 growing season (Fig. 1). These temperature measurements were conducted in the same non-irrigated plots used for the gas exchange measurements. Temperatures at 50 cm are representative of those that the upper leaves of *A. smithii* tillers would experience, whereas those at 5 cm are representative of air temperatures surrounding *B. gracilis* leaves. Throughout the season air temperatures at 50 cm were 2–11° C cooler than those at 5 cm. It was also apparent that day to day variability can be substantial. For example, on June 13 air temperatures at 50 cm averaged 16° C. By June 14 they had increased to 22° C. During mid- and late-July, the temperature differences at the two heights were less than earlier in the growing season. This seasonal effect could be due to drying of the shortgrass canopy and changes in the way the canopy structure captures heat and modifies the thermal microenvironment as the season progresses.

Table 1. Climatic data during the 1983 and 1984 growing seasons for the study site. Numbers in parentheses represent long-term averages obtained from Lauenroth and Sims (1976)

Month	Total monthly precipitation (mm)	Mean maximum temperature (°C)	Mean minimum temperature (°C)
1983			
April	69.8 (26)	13 (16)	−1 (−2)
May	72.6 (44)	20 (21)	5 (5)
June	73.7 (62)	25 (25)	10 (10)
July	40.9 (50)	32 (29)	14 (15)
August	44.0 (42)	33 (27)	16 (11)
1984			
April	51.3 (26)	13 (16)	−1 (−2)
May	25.7 (44)	25 (21)	7 (5)
June	54.6 (62)	28 (25)	11 (10)
July	74.9 (50)	33 (29)	15 (13)
August	46.7 (42)	32 (27)	15 (11)

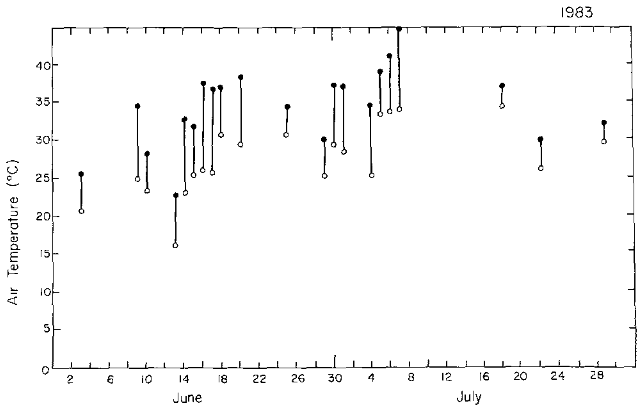

Fig. 1. Average midday air temperatures 50 cm above the ground surface (o) and 5 cm above the ground surface (●) on 21 dates during 1983. Values for the same date are connected by a solid line. Values were calculated as the mean of hourly measurements between 0900–1500 h

The influence of the warmer microclimate near the ground surface on leaf temperatures of *B. gracilis* is clearly demonstrated in the results presented in Fig. 2. On this clear day in late May when the maximum air temperature at 50 cm reached 24.7° C, leaf temperatures of the uppermost leaves on tillers of *B. gracilis* reached 32° C. In contrast, leaf temperatures of the uppermost leaves of *A. smithii* were always within 2.5° C of the 50 cm air temperature.

Growth measurements

During 1983 and 1984 plants of both species exhibited phenology patterns that are typical of long term observa-

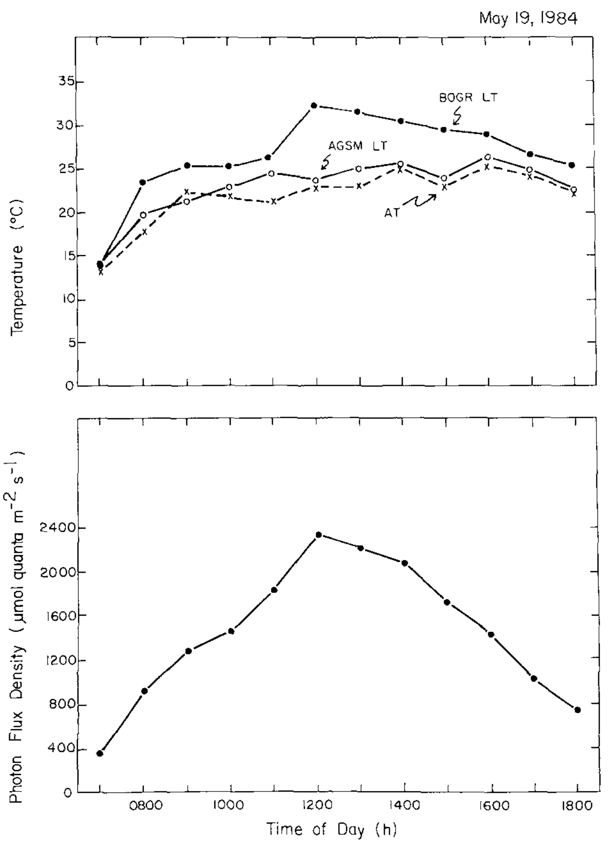

Fig. 2. Daily patterns of leaf temperature *LT*, air temperature at 50 cm above the ground surface *AT*, and photon flux density during late May. *AGSM A. smithii, BOGR B. gracilis*

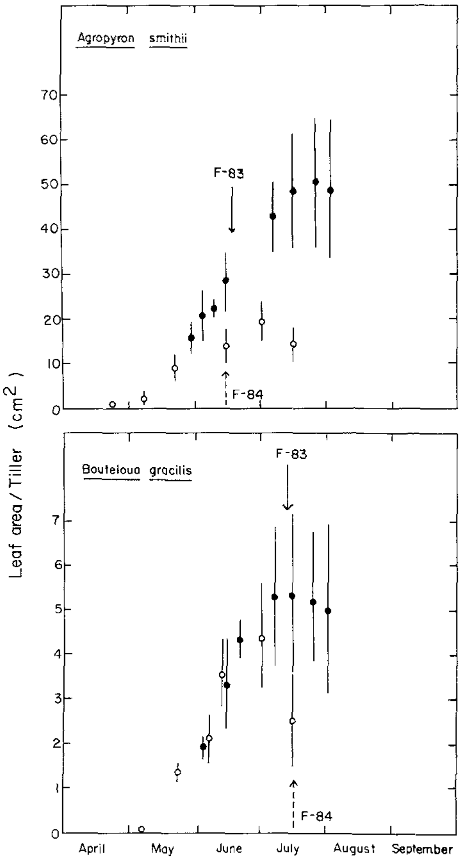

Fig. 3. Leaf area of tillers of *A. smithii* and *B. gracilis* during the initial portion of the 1983 (●) and 1984 (○) growing seasons. Vertical bars represent ±1 S.D. Arrows indicate the dates of maximum anthesis during 1983 (F-83) and 1984 (F-84)

tions (see Dickinson and Dodd 1976). *Agropyron smithii* initiated seasonal growth during the third week of April in 1983, and the second week of April in 1984. Flowering was initiated during the first week of June in both years, and appeared to reach maximum anthesis in the study area during the third week of June (Fig. 3). *Bouteloua gracilis* initiated seasonal growth during the second week of May in 1983 and the first week of May in 1984. Flowering was initiated during the last week in June, and reached apparent maximum anthesis during the second to third week in July (Fig. 3).

Despite these differences in phenology, both species exhibited similar timing in their periods of maximum increase in leaf area. *A. smithii* exhibited relatively slow rates of leaf area increase during late-April and early-May (0.40 cm^2 $tiller^{-1}$ day^{-1}) with the maximum rate occurring during late-May and the entire month of June in 1983 (0.71 cm^2 $tiller^{-1}$ day^{-1}), and only during mid-May in 1984 (0.57 cm^2 $tiller^{-1}$ day^{-1}) (Fig. 3). During 1983 mean leaf areas were maintained at ~50 cm^2/tiller throughout mid-July. In 1984, maximum mean leaf areas per tiller were much lower (20 cm^2/tiller) and values decreased during mid-July.

In *B. gracilis* the maximum rate of leaf area increase occurred during early June in both 1983 (0.16 cm^2 $tiller^{-1}$ day^{-1}) and 1984 (0.11 cm^2 $tiller^{-1}$ day^{-1}). During 1983 a maximum mean leaf area of ~5.25 cm^2/tiller was maintained throughout July. In 1984, the maximum mean leaf area (4.4 cm^2/tiller) was reached during early-July and decreased significantly (t-test, $P < 0.01$) during mid-July.

Table 2. Mean±S.E. of gas exchange parameters for *A. smithii* and *B. gracilis* determined for six replicate sets of leaves of each species on June 3–4, 1983. Environmental parameters within the leaf cuvette were as follows: photon flux density = 1,700–2,050 μmol m^{-2} s^{-1}, leaf temperature = 24.3 ± 1.2° C, $\Delta W = 18 \pm 3$ mmol H_2O mol^{-1} air, Ca = 335 ± 6 μmol mol^{-1}

Species	A (μmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	g (mmol m^{-2} s^{-1})	Ci (μmol/mol)
A. smithii	30.0 ± 1.6	7.2 ± 0.7	395 ± 16	213 ± 9
B. gracilis	31.7 ± 3.0	5.7 ± 0.5	223 ± 19	111 ± 8

Gas-exchange

The measurement of continous daily gas-exchange patterns typically precludes the use of multiple replications since the environment changes continuously during the day and it often takes a considerable period of time for steady-state conditions to be established after sealing a new leaf into the gas-exchange cuvette. In order to get an estimate of the variability we might expect between measurements, we conducted a number of replicate measurements under stan-

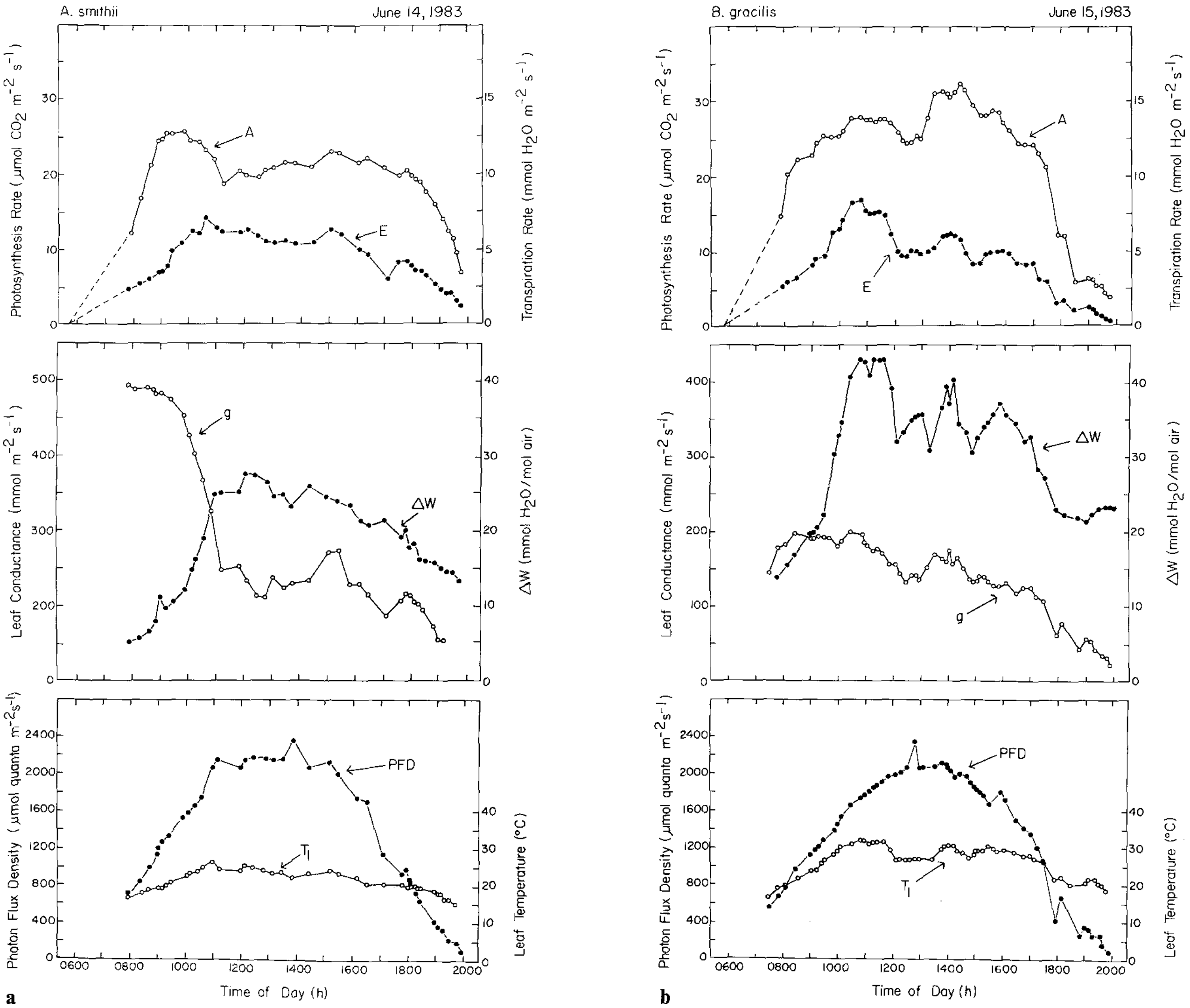

Fig. 4a, b. Daily patterns of gas exchange in non-irrigated tillers of *A. smithii* and *B. gracilis* during June. Predawn plant water potentials were −0.53±0.13 MPa and −0.38±0.08 MPa, and midday plant water potentials were −2.08±0.18 MPa and −1.84±0.15 MPa, for *A. smithii* and *B. gracilis*, respectively. *A* net photosynthesis rate; *E* transpiration rate; *ΔW* leaf to air water vapor concentration gradient; *g* leaf conductance; *PFD* photon flux density (400–700 nm); T_l leaf temperature

dardized environmental conditions on both species during early June (Table 2). The mean values reflected typical C_3,C_4 differences with the C_4 species, *B. gracilis*, exhibiting lower leaf conductances (g) and intercellular CO_2 concentrations (Ci). Mean photosynthetic rates were within the range previously measured at 25° C on plants grown in growth chambers (Monson et al. 1983). In no case was the standard error among the six replicates greater than 10% of the mean value.

Daily patterns of gas exchange were measured on 15 dates during 1983. The dictated protocol design was such that *A. smithii* was monitored on one day, and *B. gracilis* on the next. Representative patterns of these measurements are presented for non-irrigated plants during two days in June and two days in July. (Figs. 4 and 5).

On June 14 leaves of *A. smithii* exhibited their highest photosynthetic rates (A) during the morning when leaf temperatures and the leaf to air water vapor concentration gradient (*ΔW*) were relatively low (Fig. 4). Leaf conductance to water vapor (g) also reached its highest value (490 mmol m^{-2} s^{-1}) during this morning period, decreasing to midday values of 210–275 mmol m^{-2} s^{-1} as *ΔW* increased to a midday maximum of 28 mmol H_2O/mol air. This midday decrease in g kept transpiration rates (E) from increasing as *ΔW* increased, increasing midday water-use efficiencies. Leaf temperatures were 20° C during the morning photosynthetic maximum and increased to 25° C during the midday period. On June 15, the date for sampling *B. gracilis*, midday air temperatures at 50 cm above the ground were 2° C higher than the previous day. Leaf temperatures of *B. gracilis* were additionally influenced by the low plant growth pattern. Midday leaf temperatures of *B. gracilis* reached 31° C and *ΔW* reached a maximum of 42 mmol/mol (Fig. 4). The slight decrease in leaf temperature that occurred at 1200 h coincided with a short period when the area surrounding the reference thermistor outside the cuvette became accidently shaded. This discrepancy had an obvious effect on *ΔW*, with lesser effects on the gas exchange values. The maximum daily value for A occurred at 1400 h when the leaf temperature was 30.5° C. Leaf con-

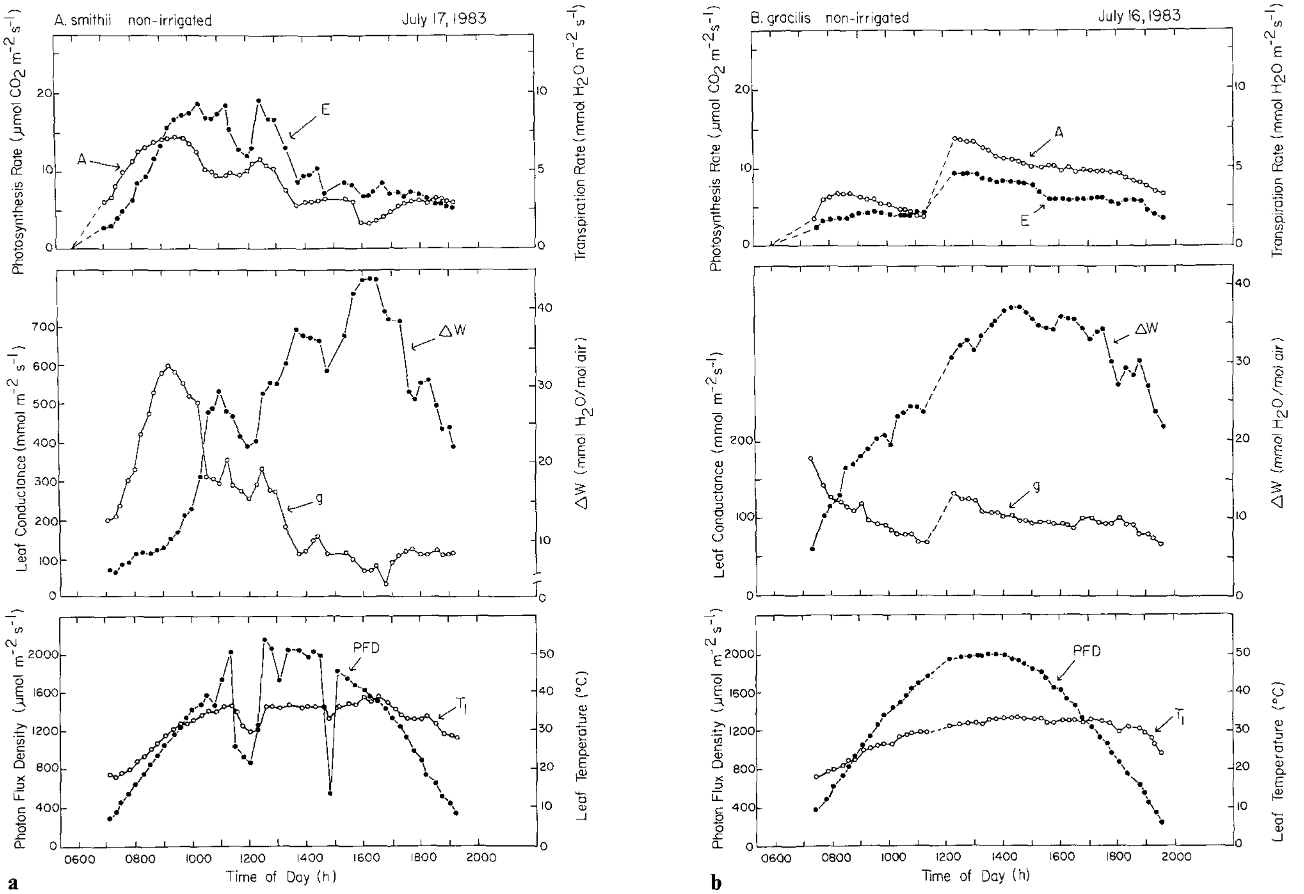

Fig. 5a, b. Daily patterns of gas exchange in non-irrigated tillers of *A. smithii* and *B. gracilis* during July. Predawn plant water potentials were -0.34 ± 0.06 MPa and -1.18 ± 0.41 MPa, and midday plant water potentials were -3.59 ± 0.28 and -3.84 ± 0.17, for *A. smithii* and *B. gracilis*, respectively. Symbols are as described in Fig. 2

ductances in *B. gracilis* were highest (~200 mmol m^{-2} s^{-1}) during the morning hours, decreasing slowly to midday values of 150–175 mmol m^{-2} s^{-1}.

By mid-July leaf temperatures of *A. smithii* reached 39.5° C during midday (Fig. 5). As in June, maximum values of A (12 μmol CO_2 m^{-2} s^{-1}) occurred during the morning, with midday rates decreasing to 6.5–11 μmol CO_2 m^{-2} s^{-1} . Leaf conductances decreased from a morning maximum of 595 mmol m^{-2} s^{-1} to a late afternoon minimum of 28 mmol m^{-2} s^{-1}. The minimum leaf conductance occurred when ΔW was 43.5 mmol/mol and leaf temperature was 39.5° C. On July 16 leaf temperatures of *B. gracilis* reached a maximum of 34.3° C and midday ΔW reached 37.2 mmol/mol (Fig. 5). Photosynthesis rates were initially measured as very low during the morning, and at 1130 h the cuvette was moved to enclose four new leaves. Photosynthesis rates in these latter leaves were 13 μmol m^{-2} s^{-1}. Leaf conductances during the midday were 90–125 mmol m^{-2} s^{-1}. The higher leaf temperatures and ΔW values observed for *A. smithii*, relative to *B. gracilis*, are due to higher midday air temperatures on July 17 (~34° C) compared to July 16 (~29° C), and a reduced microclimatic modification of *B. gracilis* leaf temperatures during this part of the summer (see Fig. 1).

The seasonal patterns in maximum A and g for these two grasses are presented in Fig. 6. The values for the months of June and July were taken as the maximum observed values during the daily patterns of gas exchange.

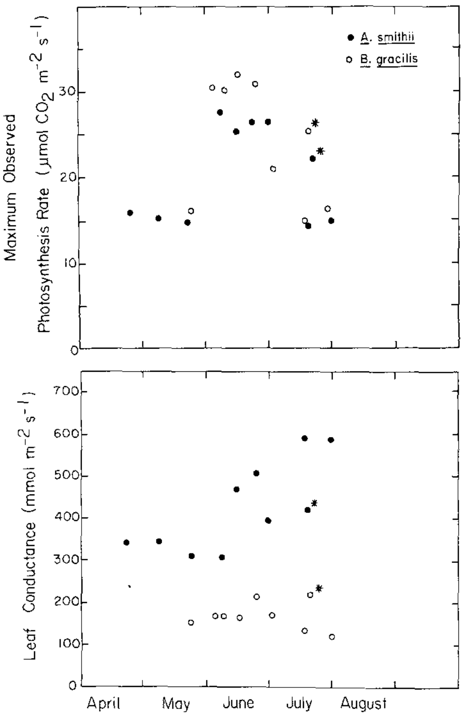

Fig. 6. Maximum observed net photosynthesis rates and the leaf conductance that occurred at those maxima during the 1983 growing season. The points marked with asterisks represent irrigated plants

The values for the months of April and May were obtained from measurements of the temperature dependence of photosynthesis. Both species exhibited their highest photosynthesis rates during June. Rates for *B. gracilis* were consistently slightly higher than those for *A. smithii.* Leaf conductances at the maximum photosynthesis rates were considerably higher for *A. smithii* throughout the season. We plotted April and May conductance values at the photosynthetic temperature optimum. For all of the April and May measurements ΔW was 17–23 mmol/mol. Maximum leaf conductances in *A. smithii* increased as the season progressed. The opposite pattern was observed for *B. gracilis* leaves, with maximum leaf conductances decreasing from early in the season to mid-July.

Daily totals for CO_2-uptake and H_2O-loss were determined by integration of the daily gas-exchange patterns (Table 3). Due to late afternoon cloud cover and thunderstorms on several of the measurement dates, we have only integrated the patterns through 1500 h. This allowed us to compare the intrinsic capacities for gas exchange in these plants during cloudless periods. *B. gracilis* exhibited its highest values for daily CO_2-uptake during early-June, with values decreasing progressively through mid-July. *A. smithii* exhibited slightly higher values for CO_2-uptake during late-June compared to early-June, although the differences between these two dates were not as great as for *B. gracilis.* By mid-July daily CO_2-uptake rates that were 31% and 41% of the maximum were observed for *B. gracilis* and *A. smithii*, respectively. Irrigation of plants resulted in increased CO_2-uptake during July, though daily totals were still only 60% and 76% of the seasonal maximum for *B. gracilis* and *A. smithii*, respectively. Rates of daily H_2O-loss were similar for the two species during early-June, but by late-June and during mid-July *A. smithii* exhibited substantially lower water-use efficiencies (CO_2 uptake/H_2O loss). The irrigated plants of *B. gracilis* responded by exhibiting proportionately higher H_2O-loss rates relative to CO_2 uptake, resulting in a lower water-use efficiency than non-irrigated plants. In irrigated plants of *A. smithii* the increase in CO_2-uptake was slightly greater than the proportional increase in water loss, resulting in a slightly increased water-use efficiency, compared to non-irrigated plants.

Intercellular CO_2 concentrations (Ci) during the midday period in leaves of *A. smithii* were significantly higher than in *B. gracilis* (t-test, $P<0.01$) throughout June and July (Table 3). Mean Ci values in *A. smithii* for the entire midday period generally fell between 200–225 µmol/mol during June, increasing to 260–290 µmol/mol during July. Mean Ci values in *B. gracilis* were 95–140 µmol/mol during June, increasing to 190–205 µmol/mol during July. In both species there were no significant differences in Ci between irrigated and non-irrigated plants during mid-July.

Two points should be made about the data of Table 3. First, leaf age was comparable during the June measurements since we only sampled the youngest fully expanded leaves. However, because new leaf production in plants of both species ceased in late June (Fig. 3), the leaves sampled during July represent progressively older leaves. Second, there was some day-to-day variability in microclimatic factors such as leaf temperature and ΔW. However, we feel that we sampled on enough different dates for each species to establish some general seasonal trends.

During July 1981, we conducted daily gas-exchange measurements on plants of both species in an upland site

Table 3. Integration of daily gas exchange patterns for plants from a lowland swale. All values represent the integration from sunrise to 1500 h. Water-use efficiencies have been multiplied by 10^3. Values for the average midday intercellular CO_2 concentration were calculated as the mean of measurements taken at 15 min intervals during the period 0900–1500 h. The values in parentheses represent the actual range observed

Date	Total net CO_2 uptake (mol/m^2)	Total H_2O loss (mol/m^2)	Water-use efficiency (CO_2 uptake/ H_2O loss)	Average midday intercellular CO_2 concentration (µmol/mol)
June 7–8, 1983				
B. gracilis	0.773	157.2	4.9	95 (76–125)
A. smithii	0.621	159.0	3.9	225 (195–280)
June 14–15, 1983				
B. gracilis	0.733	154.2	4.7	108 (77–145)
A. smithii	0.602	145.2	4.1	206 (174–248)
June 22–23, 1983				
B. gracilis	0.488	154.2	3.2	140 (102–159)
A. smithii	0.658	201.9	3.2	209 (195–243)
June 29–July 1, 1983				
B. gracilis	0.532	147.0	3.6	116 (102–127)
A. smithii	0.721	316.2	2.3	211 (185–236)
July 16–17, 1983				
B. gracilis	0.238	83.1	2.8	203 (168–240)
A. smithii	0.293	182.1	1.6	289 (265–304)
July 27–28, 1983				
B. gracilis	0.226	84.2	2.7	204 (197–242)
A. smithii	0.207	174.3	1.2	275 (257–290)
July 18–19, 1983 (irrigated)				
B. gracilis	0.531	233.4	2.3	191 (184–199)
A. smithii	0.551	311.7	1.8	260 (254–271)

Table 4. Comparison of integrated gas exchange patterns in plants from an upland site during July 7–10, 1981. All values represent the integration from sunrise to 1500 h. Water-use efficiencies have been multiplied by 10^3

Species	Total net CO_2 uptake (mol/m^2)	Total H_2O loss (mol/m^2)	Water-use efficiency (CO_2 uptake/ H_2O loss)
Non-irrigated			
B. gracilis	0.270	76.2	3.5
A. smithii	0.225	91.9	2.4
Irrigated			
B. gracilis	0.495	197.5	2.5
A. smithii	0.768	252.1	3.0

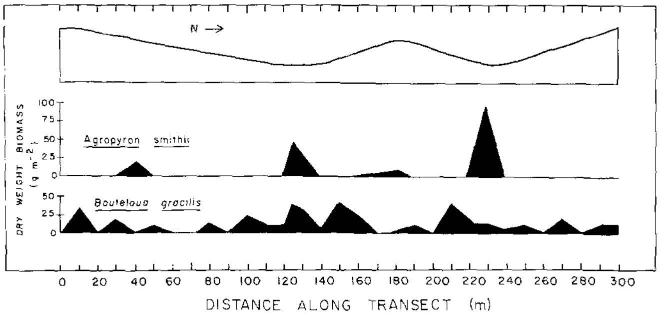

Fig. 7. Distribution of clipped aboveground biomass for *A. smithii* and *B. gracilis* along one representative transect with varying topography

(Table 4). *B. gracilis* exhibited slightly higher CO_2-uptake rates in the non-irrigated plots with a concomitant higher water-use efficiency. However, in response to irrigation the CO_2-uptake rate of *A. smithii* was substantially enhanced, and in greater proportion to the H_2O-loss rate. Thus, in a similar manner as the lowland plants, the water-use efficiency was slightly increased. *B. gracilis* responded to irrigation with proportionately higher H_2O-loss rates so that the water-use efficiency was lower. Following irrigation, CO_2-uptake rates in *A. smithii* were higher than in *B. gracilis.*

Spatial distribution of biomass

The live, aboveground vegetation of each of the two species was clipped at regular intervals during June along three transects. Each of the transects was characterized by a gently sloping topography, with at least one lowland swale present. Two of the transects had east aspects, and one had a north aspect. One representative transect is presented in Fig. 7.

Plants of *A. smithii* exhibited the greatest biomass in the lowland areas. Although it is not adequately represented in Fig. 7, when *A. smithii* did occur along the upland portion of the transects it occupied depressed microsites or areas that had been recently disturbed. A distinct pattern of biomass distribution for *B. gracilis* could not be identified. Plants of this species seemed capable of occupying any topographic position along the transects.

Discussion

Timing of seasonal growth and photosynthesis

Over the past several years numerous laboratory-based studies have described differences in the photosynthetic response to temperature in C_3 and C_4 grasses from the Colorado shortgrass steppe (Williams and Markley 1973, Williams 1974, Kemp and Williams 1980, Monson et al. 1983). From the results it was hypothesized that the two photosynthetic pathways would segregate in the field, with C_3 grasses growing primarily during the cool, early summer portion of the growing season, and C_4 grasses primarily during the warmer, midsummer part of the season.

The results of the current field-oriented study reveal that in certain environments there are no offset periods of growth and maximum photosynthesis during the growing season in these C_3 and C_4 species. Although we observed differences in phenology between *A. smithii* and *B. gracilis*, as reported previously (Dickinson and Dodd 1976), we found no evidence for their periods of maximum growth to be offset (Fig. 3). Both species exhibited maximum increases in leaf area during June. These results are consistent with previous studies that suggest the period for maximum growth for all combined species at this shortgrass steppe site is the first two weeks of June (Knight 1973, Sims and Singh 1978). Knight (1973) reported the C_4 species, *B. gracilis* to reach its maximum growth rate per unit leaf area during late May, a pattern very similar to that reported in this study. The within-species differences in maximum leaf area observed between 1983 and 1984 probably reflect the cooler and more moist conditions during June 1983 than during June 1984 (Table 1). Maximum rates of photosynthesis were also observed during June for both species, coinciding precisely with the period of maximum leaf area expansion (Fig. 6).

Field observations of maximum growth and photosynthesis by *B. gracilis* during the relatively cool period of early June was not predicted from our previous laboratory-based studies (Williams 1974, Kemp and Williams 1980, Monson et al. 1983). This lack of predictive power was due to two principal factors. First, the laboratory-based predictions failed to take adequate account of the effects of precipitation patterns on seasonal plant water stress. Leaves of both species experience significant water stress during the warmest part of the summer (July and August). Thus, even though the warm midsummer temperatures would appear to favor photosynthesis in the C_4 species *B. gracilis*, reduced leaf water potentials lead to a net reduction in photosynthetic capacity (Table 3, Fig. 6). Accordingly, both species exhibit maximum growth and photosynthesis during the month of June, which also exhibits the highest monthly precipitation (Lauenroth and Sims 1976). Second, the laboratory-based predictions used air temperatures from standard weather stations and thus failed to account for differences in the microclimates of these two species. The warm leaf temperatures we observed for *B. gracilis* early in the growing season (Fig. 4) are due to its occurrence in the warm microenvironment near the ground surface (Fig. 1). Indeed, leaf temperatures of *B. gracilis* during June were very close to the temperature optimum for photosynthesis in this C_4 species (see Monson et al. 1983). This warm microclimate presumably underlies the high photosynthetic potential of *B. gracilis* during June, despite contrary predictions based on standard weather station temperatures and the previous growth chamber studies.

Although the results of the current study did not support our previous hypothesis of offset seasonal growth activities in these two species, we do not feel that they invalidate it. The current studies on seasonal growth and photosynthesis patterns were only conducted during one growing season and at one latitudinal site in the shortgrass steppe. It is easy to envision different patterns of precipitation and temperature during the growing season eliciting different seasonal patterns of plant gas-exchange. Our results agree with other field observations of C_3 versus C_4 photosynthesis in demonstrating that clear cut predictions of seasonal patterns in photosynthesis and growth in these two pathway types cannot be adequately produced from growth chamber- and greenhouse-based studies alone (also see Caldwell et al. 1977). The complexities of microclimate and stochastic patterns in macroclimate must be taken into account.

Water-use efficiency

The results of this study confirm the greater water-use efficiency of the C_4 grass *B. gracilis* only under the warm climatic conditions of the midsummer month of July. During the month of June, however, water-use efficiencies of the two species were similar. Once again, any fitness benefit of the C_4 cycle, in this case improved water-use efficiency, does not appear to be of major importance during the period of maximum growth and carbon gain in these two species.

During June, water-use efficiencies in the two species were similar despite apparent differences in Ci during the midday period (see Table 3). The Ci values for *B. gracilis* during June (95–140 μmol/mol) are consistent with previous studies of C_4 plants in which Ci was measured as approximately 130 μmol/mol under high light, near the photosynthetic temperature optimum, with normal ambient CO_2 concentrations (e.g. Ehleringer 1983). During July, Ci increased significantly for both species and both irrigation treatments. The increased Ci reflects the disproportionate decrease in midday A relative to g during this period. The midsummer increase in Ci may be due to growth under increasingly shaded conditions during late-June and early-July as the canopy reaches its maximum leaf area index (see Knight 1973). Even leaves near the top of the tillers could receive less incident light due to the uneven nature of the canopy surface. Caldwell et al. (1983) found that older, shaded leaves within *Agropyron spicatum* canopies often exhibit higher Ci values (240–280 μmol/mol) at light saturation, relative to unshaded, younger leaves. Additional factors during July such as increased leaf temperature and ΔW could also cause stresses to the photosynthetic systems of these plants, possibly resulting in greater decreases in A relative to g.

At comparable values for ΔW and ambient CO_2 concentrations (Ca), the lower Ci values in *B. gracilis* would typically reflect greater water-use efficiencies (Farquhar et al. 1982). The similar water-use efficiencies measured in the two species during June, despite different midday Ci and g values, occurred because ΔW was not similar for the two species during the midday period. With its leaves elevated above the canopy *A. smithii* exhibited lower leaf temperatures and concomitant lower ΔW values, relative to *B. gracilis* with its leaves closer to the ground.

The differences in water-use that we observed during July, appeared to be based on differences in the magnitude of g, rather than A. Indeed, rates of daily CO_2-uptake in both species were similar. Plants of *A. smithii* exhibited greater values of g, throughout the season (Fig. 6). Several previous studies have also observed differences in g between C_3 and C_4 plants, with C_4 plants exhibiting the lower conductances (for review see Pearcy and Ehleringer 1984). The differences between the two species in July water-use efficiences were reduced in irrigated plants due to a greater proportional increase in A relative to E in *A. smithii*. This was particularly evident in the data presented in Table 4 for upland plants. Both species, however, exhibited a marked positive response in A to irrigation, suggesting an important role for midsummer afternoon rainshowers in the carbon balance of these two species. A previous study by Sala and Lauenroth (1982) also suggested an important role for small afternoon rainfall events in the Colorado shortgrass steppe.

When taken together the results of this study suggest vastly different patterns of water-use in this C_3 and C_4 species. Plants of *A. smithii* in the lowland sites exhibit a much more "extravagant" use of water than *B. gracilis* during the warm midsummer months, with water-use efficiencies in *A. smithii* being only half of those in *B. gracilis*. Different water-use patterns for these two species were also suggested by Sala et al. (1982), based on seasonal measurements of stomatal conductance and plant water potential. The greater water-use by *A. smithii* may represent a compromise to maximize nitrogen-use efficiency in this C_3 species, as suggested by Field et al. (1983) for other C_3 species. The C_4 pathway in *B. gracilis* provides for an alternative to making this compromise since high nitrogen-use efficiencies can be maintained, even at low intercellular CO_2 concentrations due to the CO_2 concentrating mechanism. The high cost of water for CO_2 assimilation in *A. smithii* appears to be met through the principal restriction of this species to lowland sites where the soils possess more organic content (Schimel et al. 1985) and higher water holding capacities, and deep root systems can reach perennial water supplies in the deeper soil horizons (see Fig. 7). The C_4 species, *B. gracilis*, is capable of occupying the drier upland sites in the shortgrass steppe. The C_4 pathway might contribute to this capacity by providing for CO_2 assimilation at much lower water costs allowing moisture reserves in the rooted zone to be extended and CO_2 assimilation to continue for longer periods between rainfall events.

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