

Photosynthetic adaptation to temperature in four species from the Colorado shortgrass steppe: a physiological model for coexistence

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Summary. Several aspects of photosynthetic adaptation to temperature were examined in four graminoid species from the Colorado shortgrass steppe. The experimental species were chosen to provide examples of a variety of in situ seasonal phenology patterns. The cool season grass, *Agropyron smithii* (C_3), exhibited higher photosynthesis rates when grown in a cool temperature regime (20/15° C), and compared to warm grown plants (35/15° C). The warm season species, *Bouteloua gracilis* (C_4) and *Buchloe dactyloides* (C_4), exhibited higher photosynthetic capacities when grown in the warmer temperature regime. The sedge, *Carex eleocharis* (C_3), which exhibits seasonal growth potential during the cool and warm portions of the growing season, exhibited a marked capacity for photosynthetic temperature acclimation. Differential effects of growth temperature on the intracellular conductance to CO_2 appeared to have a greater regulatory role in these responses for the two C_3 species, relative to stomatal conductance or photorespiration (O_2 inhibition of photosynthesis). In the two C_4 species decreases in the intracellular conductance in cool grown plants were correlated with the decreased photosynthetic capacity in normal air for *B. gracilis*, but not for *B. dactyloides*. Analysis of the Arrhenius relationship for CO_2 saturated net photosynthesis at low leaf temperatures (4.5–17° C) indicated sharp breaks in the apparent energy of activation at 5.8–9.0° C in the warm season species *B. gracilis* and *B. dactyloides*. Leaves of *A. smithii* and *C. eleocharis* exhibited no significant low temperature limitations according to this analysis. The low temperature limitations in the warm season species were partially reflected in an inhibition of the quantum yield for CO_2 uptake after 2 h at 5–6° C in the presence of high photon flux densities. Temperature dependent increases in the chlorophyll fluorescence yield at high temperatures revealed the lowest break-point values for *A. smithii*, and the highest values for *B. gracilis*. The differential patterns of temperature adaptation among the species further extend the proposal of Kemp and Williams (1980; Ecology 61:846–858) that seasonal temperature gradients in the shortgrass steppe have a regulatory role in maintaining offset patterns of resource utilization and decreasing interspecific competition.

Introduction

Photosynthetic temperature adaptation has been examined among plants which grow in different seasonal temperature regimes within the same geographical habitat (Mooney et al. 1976, Kemp and Williams 1980, Downton et al. 1980, Monson and Williams 1982); and among plants from different geographical habitats with contrasting temperature regimes (Pearcy 1977, Slatyer and Ferrar 1977). Although numerous studies have been conducted on the mechanisms underlying photosynthetic temperature adaptation, these are normally discussed in terms of individual species coping with a specific environment (for review see Berry and Björkman 1980). Only limited information is available on how temperature interacts with the photosynthetic processes of several sympatric species to influence seasonal community dynamics (Regehr and Bazzaz 1976, Mooney et al. 1977). In the current study we have examined the potential role of a seasonal temperature gradient in regulating the photosynthetic capacities of four sympatric species from the Colorado shortgrass steppe.

Photosynthetic temperature adaptation has been suggested to have an important regulatory role in maintaining niche boundaries of dominant species in the Colorado shortgrass steppe (Williams 1974, Gerwick and Williams 1978, Kemp and Williams 1980, Monson and Williams 1982). Growth activities of “cool season” species, such as *Agropyron smithii*, are greatest in the cool spring and early summer months. Additionally, photosynthetic capacities are greatest when this species is grown in a cool temperature regime (20/15° C day/night), relative to a warmer temperature regime (35/15° C, Kemp and Williams 1980). In “warm season” species, such as *Bouteloua gracilis*, growth is initiated during the late spring months and continues throughout the hotter portions of mid-summer. Photosynthesis rates in this species are highest when grown in the warmer temperature regime (Kemp and Williams 1980). Differential regulation of photosynthetic capacities through differences in the potential for temperature adaptation would result in offset patterns of resource utilization and reduced interspecific competition. An understanding of the physiological and biochemical mechanisms underlying temperature adaptation in several of these sympatric species would provide an important insight into some of the mechanisms regulating plant community function. A primary objective of the

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current study was to examine several aspects of the photosynthetic process, such as the intracellular conductance to CO_2 , the quantum yield for CO_2 uptake, oxygen inhibition of photosynthesis, and stomatal conductance limitations, with respect to temperature adaptation. Four graminoid species from the shortgrass steppe were included in these studies, all of which exhibit different seasonal phenology patterns. Two of the species which were examined in this study, *Boutelous gracilis* and *Agropyron smithii*, were also included in a previous study by Kemp and Williams (1980). In the current study we have conducted a further analysis of factors regulating photosynthetic temperature adaptation in these two species, as well as the two additional species.

Materials and methods

The species used in this study are *Bouteloua gracilis* (C_4), *Buchloe dactyloides* (C_4), *Agropyron smithii* (C_3), and *Carex eleocharis (stenophylla)* (C_3). Phenology patterns for these species are presented in a later section. All experimental plants were collected as sods from the Central Plains Experiment Range (CPER) maintained by the United States Agricultural Research Service near Nunn, Colorado, USA ($40^\circ 42' \text{ N}$, $104^\circ 46' \text{ W}$). Plants of each species were transplanted into a peat-sand mixture (1:1), and maintained in controlled environment growth chambers (model E 15, Conviron) at $20/15^\circ \text{ C}$ or $35/15^\circ \text{ C}$ day/night air temperatures (12 h at each temperature). Leaf temperatures during the day for all four species averaged 20.5 ± 1.3 (standard deviation) $^\circ \text{ C}$ and $36.1 \pm 1.8^\circ \text{ C}$, in the $20/15$ and $35/15^\circ \text{ C}$ chambers, respectively. The photoperiod during growth was 14 h. The photon flux density (400–700 nm) at plant height was $1,000 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$ in the growth chamber during the middle 12 h of the photoperiod. For a 1 h period at the beginning and end of the photoperiod, the photon flux density was reduced to $200 \mu\text{mole quanta m}^{-2} \text{ s}^{-1}$, coinciding with the lower temperature of the normal dark period. All plants were grown under these conditions for 4 months prior to experimental analysis. Plants were clipped periodically such that leaf age for the experimental plants was standardized at 35 ± 5 days.

Leaf gas exchange measurements were conducted with a gas analysis system which has previously been described (Monson et al. 1982). The system includes an infrared gas analyzer (model 865, Beckman) and a dew-point hygrometer (model 880, EG and G) for monitoring fluxes of carbon dioxide and water vapor, respectively. All gas mixtures for analysis and calibration were produced with a pair of Wösthoff mixing pumps (Model 1SA 27/3F). Single attached leaves were sealed into a temperature controlled cuvette constructed from polycarbonate which has the properties of being relatively impermeable to CO_2 and water vapor. Temperatures of enclosed leaves were measured with 0.10 mm diameter copperconstantan thermocouples appressed to the lower leaf surface. Leaf temperatures were manually adjusted by means of a Peltier-controlled heat exchanger (model 809-3040-01, Cambion) which formed the base of the cuvette. Using this apparatus leaf temperatures were controlled within $\pm 0.5^\circ \text{ C}$ of the desired temperature. The zero point of the differential CO_2 analyzer was adjusted before each gas exchange measurement. Additionally, the CO_2 analyzer was calibrated with 4–5 differential gas concentrations at the beginning of each experimental day. The

protocol which was used for measuring the photosynthetic responses to temperature, CO_2 concentration, and photon flux density has been outlined in a previous report (Monson et al. 1982).

Leaf areas were measured with a leaf area meter (model LI-3000, Lambda). Leaf transmittance and reflectance measurements were conducted on freshly cut leaf segments, at 5 nm wavelength intervals between 400–700 nm, with the integrating sphere described by Robberecht and Caldwell (1978). Absorptance was calculated by subtraction. For *Buchloe dactyloides* the leaf absorptance was assumed to be the same as for leaves of *Bouteloua gracilis*, as the leaf morphology and photosynthetic characteristics of these two species are similar.

The temperature dependence of chlorophyll fluorescence was measured with the apparatus described in Monson and Williams (1982). The high temperature fluorescence breakpoint is defined as the temperature T_2 , as described by Smillie (1979) and Monson and Williams (1982).

Results

Species phenological patterns in situ

The four species which were examined in this study exhibit a variety of seasonal growth patterns. All four species possess perennial life cycle pattern. The taxa include a cool season species (*Agropyron smithii* Rydb.), two warm season species (*Bouteloua gracilis* (H.B.K.) Lag. and *Buchloe dactyloides* (Nutt.) Englem.), and a species which grows throughout the cool and warm seasons (*Carex eleocharis* Bailey). Although we briefly describe seasonal phenology patterns in this report, they are presented in more detail in previous studies (Dickinson and Dodd 1976, Monson and Williams 1982).

Agropyron smithii (C_3) typically initiates growth during early April, and exhibits its greatest growth rates during the cool, early portion of the season (Dickinson and Dodd 1976, Boutten et al. 1980). Plants which occur in those topographic sites receiving abundant runoff water may exhibit some growth during the warm, mid-summer months (personal observation). The average date for anthesis in populations of this species is approximately mid-June.

Bouteloua gracilis (C_4) initiates growth approximately 3–5 weeks after *A. smithii* during late April or early May. A major portion of the seasonal growth activities in this species occurs during the warm, early- and mid-summer months. Flowering occurs during mid-July and early August.

Buchloe dactyloides (C_4) initiates spring growth at approximately the same time as *B. gracilis*, during late April or early May. However, the former species exhibits a slightly greater growth rate during the cool late spring and early summer months, and the mean date for anthesis is mid-June. Thus, *B. dactyloides* flowers at approximately the same time as *A. smithii* (see Dickinson and Dodd 1976).

Carex eleocharis (C_3) initiates growth during early April when air temperatures are relatively cool. Although flowering occurs during May and June, much of the leaf tissue remains green and capable of growth throughout the mid-summer months (Boutten et al. 1980, Monson and Williams 1982).

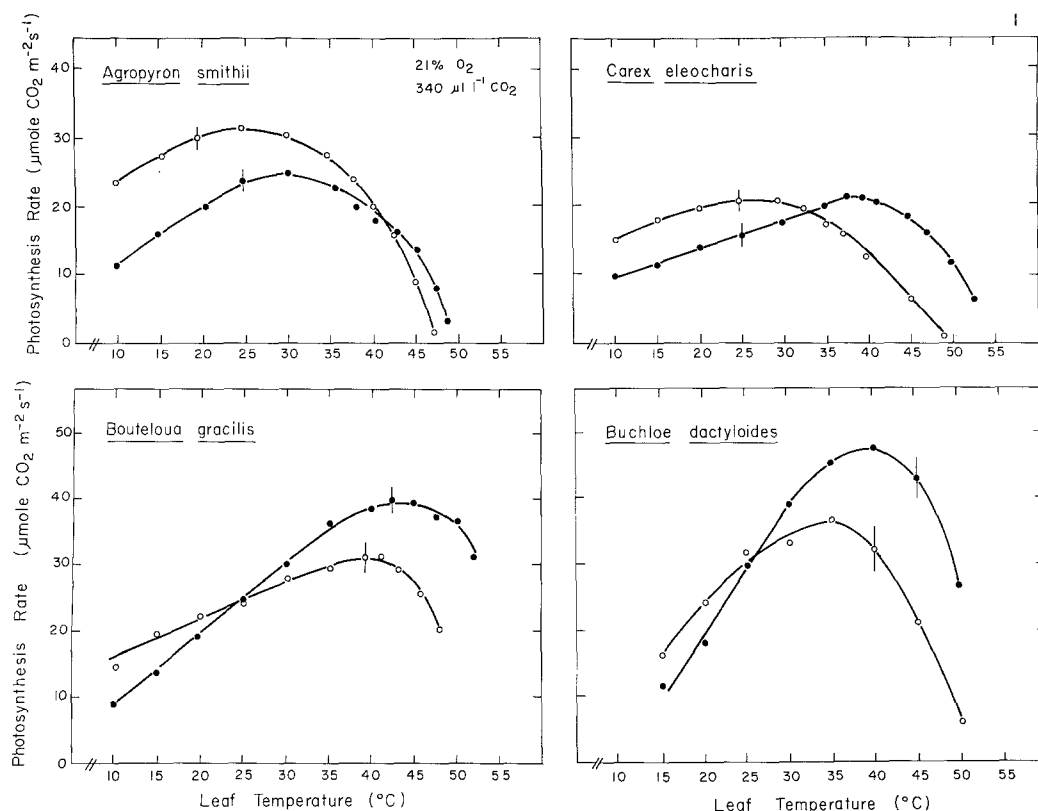


Fig. 1. The photosynthetic responses to temperature in normal air for cool grown (○) and warm grown (●) plants of the indicated species. Photon flux densities were $1,800\text{--}2,000\ \mu\text{mole quanta m}^{-2}\ \text{s}^{-1}$ (400–700 nm) and the leaf to air vapor pressure differences were less than 1.5 KPa at all analysis temperatures. Each point represents the mean of 3–4 plants. Vertical bars represent the maximum standard error observed for each experimental treatment

Effect of growth temperature on the temperature dependence of net photosynthesis

Net photosynthesis in *A. smithii* was lower at all but the highest analysis temperatures when grown in a temperature regime of $35/15^\circ\text{C}$ and analyzed in an atmosphere with 21% O_2 and $340\ \mu\text{l l}^{-1}\ \text{CO}_2$, relative to cool grown plants ($20/15^\circ\text{C}$, Fig. 1). An upward adjustment in the temperature optimum was observed from 25°C in cool grown plants to 30°C in warm grown plants. The C_3 sedge, *C. eleocharis*, demonstrated a capacity for complete photosynthetic temperature acclimation. The acclimation adjustments were characterized by a shift in the temperature optimum from $25\text{--}30^\circ\text{C}$ in cool grown plants to $37\text{--}40^\circ\text{C}$ in warm grown plants. The absolute rate of net photosynthesis was nearly equal at the respective temperature optima. The temperature dependence of photosynthesis for the C_4 grass *B. gracilis* is also sensitive to growth temperature (Fig. 1). Growth in a cool temperature regime resulted in inferior photosynthesis rates at analysis temperatures above 20°C , relative to warm grown plants. A very slight adjustment in the temperature optimum for photosynthesis occurred, being 42°C in warm grown plants and 40°C in cool grown plants. Plants of *B. dactyloides* exhibited the highest rates of photosynthesis when grown in the warmer temperature regime and compared to cool grown plants (Fig. 1). A partial acclimation to temperature occurred in plants of this species, since the temperature optimum was 35°C in cool grown plants and 40°C in warm grown plants. Additionally, photosynthesis rates at the lower analysis

temperatures ($15\text{--}25^\circ\text{C}$) were higher for the cool grown plants.

In order to eliminate growth temperature induced photorespiratory and stomatal effects on the photosynthetic response to temperature in the two C_3 species, measurements were conducted in an atmosphere containing 2% O_2 and $800\ \mu\text{l l}^{-1}\ \text{CO}_2$ (Fig. 2). Experimental plants of *A. smithii* exhibited significantly lower rates of net photosynthesis at all analysis temperatures below 35°C for the warm grown plants, relative to cool grown plants ($P < 0.05$). Differences in photosynthetic capacity at 35°C , and above, were not significant. In plants of *C. eleocharis* the acclimation adjustments to growth temperature which were reported in the previous section (Fig. 1), were also observed in the presence of 2% O_2 and $800\ \mu\text{l l}^{-1}\ \text{CO}_2$ (Fig. 2). The higher absolute rates of net photosynthesis at all analysis temperatures, and the higher temperature optima observed for both C_3 species when analyzed in the presence of 2% O_2 and $800\ \mu\text{l l}^{-1}\ \text{CO}_2$, are consistent with the elimination of O_2 inhibition of photosynthesis.

Effects of growth temperature on the intracellular conductance to CO_2 (C_i)

The initial slope of the CO_2 dependence of photosynthesis (C_i), when measured at a constant analysis temperature, was affected by growth temperature in three of the four experimental species (Fig. 3). The values for C_i of *A. smithii* were measured as 5.1 and $4.4\ \text{mm s}^{-1}$ for cool and warm

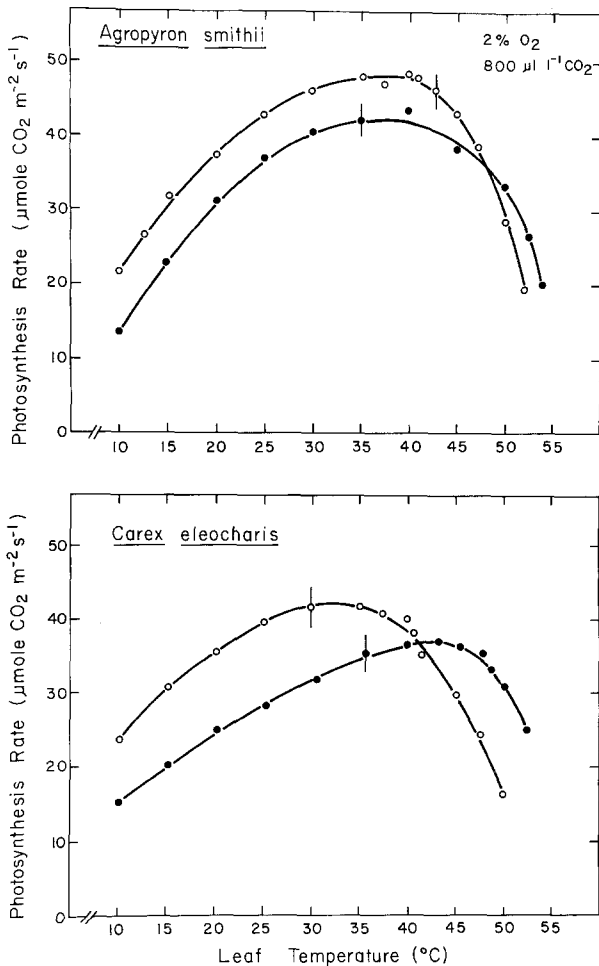


Fig. 2. The photosynthetic response to temperature in 2% O_2 and $800 \mu l l^{-1} CO_2$ for cool grown (○) and warm grown (●) plants of the two C_3 species. Other analysis conditions and symbols are the same as in Fig. 1. Points represent the mean of 3–4 plants

grown plants, respectively. An estimate of the rate of photorespiration can be obtained as the ordinate-intercept of the photosynthetic response to CO_2 . Differences in the extrapolated rate of photorespiration were apparent in *A. smithii*, being 8.1 and $7.6 \mu mole CO_2 m^{-2} s^{-1}$ for cool and warm grown plants, respectively. The C_3 sedge, *C. eleocharis* exhibited values of C_i which were lower than those for *A. smithii*, being 3.8 and $3.4 mm s^{-1}$ for the cool and warm grown plants, respectively. The extrapolated values for the photorespiration rate were $5.5 \mu mole CO_2 m^{-2} s^{-1}$ for the cool grown plants, and $6.5 \mu mole CO_2 m^{-2} s^{-1}$ for the warm grown plants. Consistent with the proposed low rates of photorespiration in C_4 plants, *B. gracilis* exhibited low CO_2 compensation points (1.8 and $4.4 \mu l l^{-1} CO_2$ for the cool and warm grown plants, respectively) relative to the two C_3 species, and low extrapolated rates of photorespiration (1.0 and $1.5 \mu mole CO_2 m^{-2} s^{-1}$ for the cool and warm grown plants, respectively). The presence of the C_4 pathway in this species was also evident in the higher values observed for C_i , being 9.6 and $6.8 mm s^{-1}$ for the warm and cool grown plants, respectively. Growth temperature had no significant influence on C_i in plants of *B. dactyloides*. The mean values for C_i was $10.4 mm s^{-1}$ for plants from both temperature regimes. The extrapolated rate of

photorespiration for this C_4 species, was also relatively low being $3.8 \mu mole CO_2 m^{-2} s^{-1}$, and the CO_2 compensation point was $9.0 \mu l l^{-1}$.

Intracellular conductances to CO_2 were also determined in the presence of 2% O_2 for the two C_3 species (Fig. 4). Enhancements of C_i were noted in all of the experimental plants, relative to the values determined in 21% O_2 . However, the apparent effects of growth temperature on C_i were similar for both species, whether assessed in the presence of 21% O_2 or 2% O_2 . In either case, values of C_i were lower for warm grown plants when compared to values for cool grown plants. In the presence of 2% O_2 values for C_i were also greater for *A. smithii* (9.8 and $7.8 mm s^{-1}$ for cool and warm grown plants, respectively), relative to *C. eleocharis* (5.6 and $5.0 mm s^{-1}$ for cool and warm grown plants, respectively).

Effect of low analysis temperatures on net photosynthesis

The response of net photosynthesis to low leaf temperatures (4.5 – $17^\circ C$) has been presented according to the Arrhenius relationship in Fig. 5. The analysis was conducted in the presence of 2% O_2 and $800 \mu l l^{-1} CO_2$ with saturating light intensities in order to insure saturation of the CO_2 substrate. Breaks in the Arrhenius relationship were not observed for any of the replicated experiments with *A. smithii*. The calculated apparent energy of activation (E_a) for photosynthesis in leaves of *A. smithii* was 12.2 – $13.9 Kcal mole^{-1}$ over the entire low temperature range. A slight break in the Arrhenius relationship was noted between 11 – $12^\circ C$ in two of three replicates in leaves of *C. eleocharis*. Above these temperatures E_a was calculated to be 14.1 – $15.6 Kcal mole^{-1}$. Below $11^\circ C$, E_a was calculated to be 22.1 – $25.0 Kcal mole^{-1}$. In the third replicate no breaks were observed over the entire low temperature range, and E_a was $15.4 Kcal mole^{-1}$. A very distinct break in the Arrhenius relationship occurred in all experimental leaves of *B. gracilis* and *B. dactyloides* between 5.8 – $9.0^\circ C$, with E_a being 19.0 to $23.6 Kcal mole^{-1}$ above this temperature range and 72.9 to $113.8 Kcal mole^{-1}$ below the temperature range.

Low temperature damage to the light-limited reactions of photosynthesis was examined in cool grown plants of *B. gracilis*, *B. dactyloides*, and *A. smithii* (Figs. 6 and 7). In leaves of *B. gracilis* the quantum yield was measured as $0.053 mole CO_2 \cdot mole^{-1}$ quanta absorbed at $25^\circ C$ (Fig. 6). After a two hour treatment at 5 – $6^\circ C$ with high light intensities ($1,900$ – $2,000 \mu mole$ quanta $PAR m^{-2} s^{-1}$) the quantum yield had been significantly reduced to $0.039 mole CO_2 \cdot mole^{-1}$ quanta absorbed ($P < 0.01$). A similar pattern was observed for leaves of *B. dactyloides* (Fig. 6). The quantum yield before the low temperature treatment was measured as $0.048 mole CO_2 \cdot mole^{-1}$ quanta absorbed. After a two hour low temperature treatment (5 – $6^\circ C$) the quantum yield had been reduced to $0.025 mole CO_2 \cdot mole^{-1}$ quanta absorbed. In leaves of *A. smithii* a two hour treatment at 4.5 – $6.0^\circ C$ did not cause a significant reduction in the quantum yield ($P < 0.05$), being $0.050 mole CO_2 \cdot mole^{-1}$ quanta absorbed before the low temperature treatment, and $0.048 mole CO_2 \cdot mole^{-1}$ quanta absorbed after the low temperature treatment (Fig. 7). However, after a two hour treatment at 1 – $3^\circ C$ with high light intensities ($1,900$ – $2,000 \mu mole$ quanta $m^{-2} s^{-1}$) a significant reduction

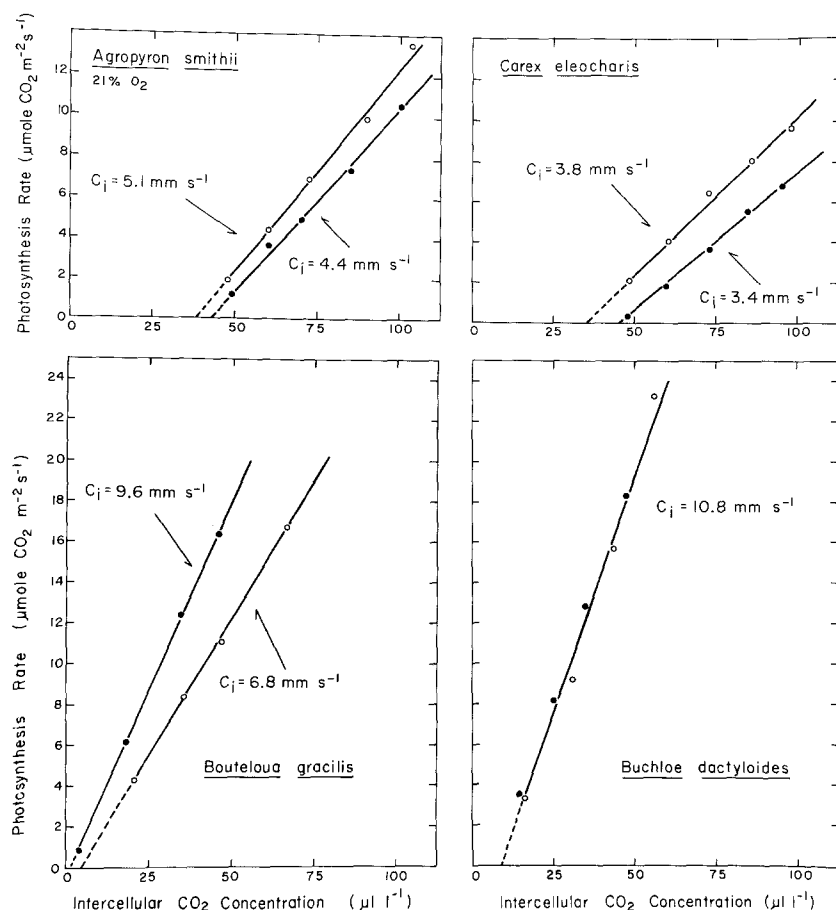


Fig. 3. The net photosynthesis rate as a function of intercellular CO_2 concentration in cool grown (○) and warm grown (●) plants of the indicated species in 21% O_2 . Leaf temperatures were 25° C for *A. smithii* and *C. eleocharis* and 30° C for *B. gracilis* and *B. dactyloides*. The intracellular conductance is represented as C_i . Each point represents the mean of 3–4 plants

in the quantum yield was observed in *A. smithii* ($P < 0.05$), being 0.041 mole $\text{CO}_2 \cdot \text{mole}^{-1}$ quanta absorbed. In a control experiment, plants of the species which were kept for two hours at 25° C with high light intensities exhibited no reductions in the quantum yield (data not shown). The irreversible reductions in quantum yield after the low temperature pretreatment in the two C_4 species, were also reflected in the light-saturated rates of photosynthesis, since the latter rates were reduced by 25–37%, with no significant change in stomatal conductance (data not shown).

High temperature damage to the photosynthetic apparatus

In order to assess the maximum temperature at which irreversible damage to the photosynthetic apparatus occurs, high temperature fluorescence breakpoints were determined in warm grown plants only (Table 1). Previous studies have indicated that the breakpoint value is correlated with the temperature at which irreversible reductions of the quantum yield occur (Schreiber and Berry 1977, Monson and Williams 1982), as well as the temperature at which light saturated whole leaf photosynthesis becomes severely inhibited (Björkman et al. 1978, Seemann et al. 1979). The breakpoint temperatures were lowest in leaves of *A. smithii*. The range of values for *C. eleocharis* and *B. dactyloides* overlapped, although leaves of the former species exhibited a slightly broader range. In *B. gracilis* the breakpoint values were extremely high, and no overlap in the range occurred with values for *A. smithii*.

Discussion

Growth temperature had a significant role in modifying the temperature dependence of photosynthesis in all four experimental species. Differential temperature adaptation of the photosynthetic processes resulted in different growth temperature optima for plants of the cool season species, *Agropyron smithii*, relative to plants of the warm season species, *Bouteloua gracilis* and *Buchloe dactyloides*. These results are in agreement with previous studies of photosynthetic temperature adaptation in *A. smithii* and *B. gracilis* (Williams 1974, Kemp and Williams 1980). In plants of *B. dactyloides* the higher maximum photosynthesis rates (particularly at leaf temperatures between 20 and 30° C), relative to *B. gracilis*, may reflect higher growth rates for the former species during the early summer. This may provide a basis for the earlier flowering response in *B. dactyloides*, and slightly offset seasonal growth activities (Dickinson and Dodd 1976). The C_3 sedge, *Carex eleocharis*, which maintains active leaf biomass throughout the entire growing season, is capable of acclimation adjustments to the prevailing growth temperature regime. Thus, photosynthetic capacities are maintained at near maximum values in a wide range of temperature regimes. Together, the results of all four species exhibit a correlation between photosynthetic adaptation to growth temperature and *in situ* seasonal phenology patterns (also see Monson and Williams 1982).

A primary goal of the current study was to identify some of the specific photosynthetic processes which contrib-

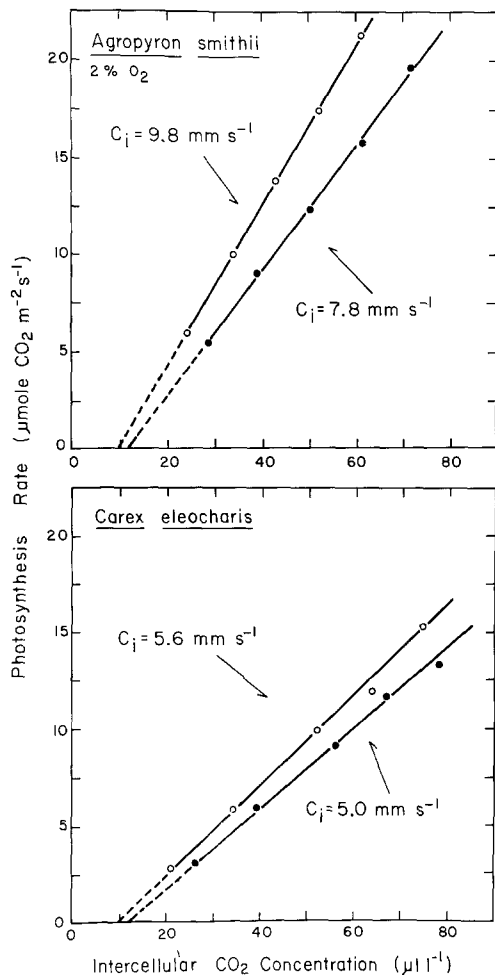


Fig. 4. The net photosynthesis rate as a function of intercellular CO_2 concentration in cool grown (○) and warm grown (●) plants of the two C_3 species in 2% O_2 . The intracellular conductance is represented as C_i . Each point represents the mean of 2–3 plants

ute to the interspecific differences in temperature adaptation described above. The absolute rates of net photosynthesis were higher for the two C_3 species when assayed in the presence of 2% O_2 and $800 \mu\text{l l}^{-1} \text{CO}_2$, and compared to the rates in normal air. The results presented in Figs. 1 and 2 indicate that the photorespiratory reactions and/or stomatal conductance limitations have a significant role in regulating the shape of the temperature response curve in normal air (also see Monson et al. 1982). However, the relative effects of growth temperature on the photosynthetic temperature responses were similar for both of the C_3 species, when assayed in high CO_2 or normal air (Fig. 2). These results indicate that stomatal diffusion limitations and differential capacities for photorespiration do not have a role in the capacity of each species to adjust to growth temperatures. In studies with *Larrea divaricata* and *Nerium oleander*, Mooney et al. (1978) and Badger et al. (1982) also concluded that photorespiration and stomatal diffusion limitations did not have a role in photosynthetic temperature acclimation.

The intracellular conductance to CO_2 (C_i) has been correlated with inherent differences in photosynthetic capaci-

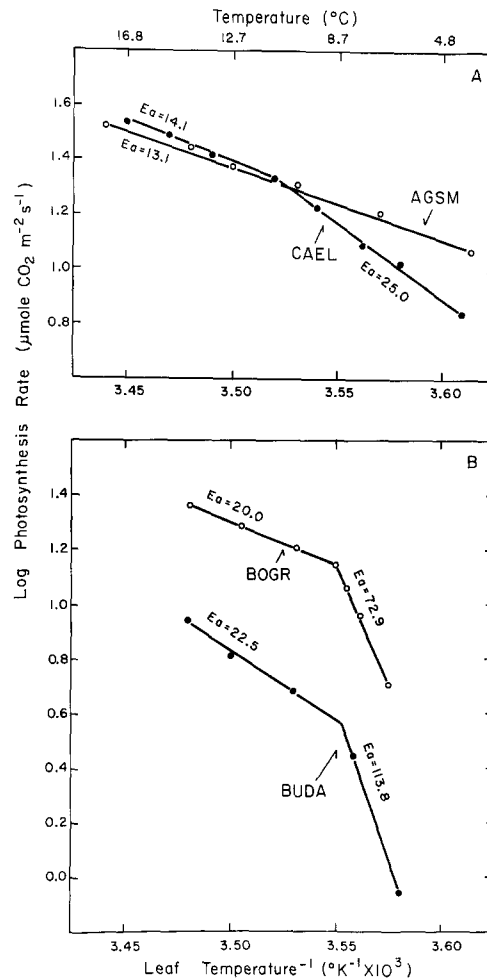


Fig. 5. Arrhenius plots of intact leaf photosynthesis rates at low temperatures. All experimental plants were grown at 20/15° C (day/night). Species names are represented as *Agropyron smithii* AGSM, *Carex eleocharis* CAEL, *Bouteloua gracilis* BOGR, *Buchloe dactyloides* BUDA. The energy of activation is represented as E_a . Points are for 1 representative leaf (see text for further descriptions)

ties among plants (Ehleringer and Björkman 1978, Longstreth et al. 1980). On a theoretical basis C_i is indicative of the carboxylation capacity of the photosynthetic enzymatic processes (von Caemmerer and Farquhar 1981, Monson et al. 1982), and has often been referred to as the "carboxylation efficiency" (Ku and Edwards 1977, Edwards and Walker 1982). An additional component of C_i occurs as the physical phase conductance to CO_2 in the mesophyll cells (see Jones and Slatyer 1972), although more recent studies suggest that this component may be small (von Caemmerer and Farquhar 1981, Seemann et al. 1981, Monson et al. 1982). In the current study the modifying influence of growth temperature on C_i (Fig. 3) was correlated with the effects of growth temperature on the photosynthesis rates at the respective temperature optima (Figs. 1 and 2) in the two C_3 species. This relationship occurred even though C_i was not always measured at the photosynthetic temperature optima. The results suggest that C_i may be most useful as an indication of the biochemical factors regulating the maximum photosynthetic capacity, and that C_i may not change in precise parallel to net photosynthesis rates, in normal air or saturating CO_2 , as leaf temperatures

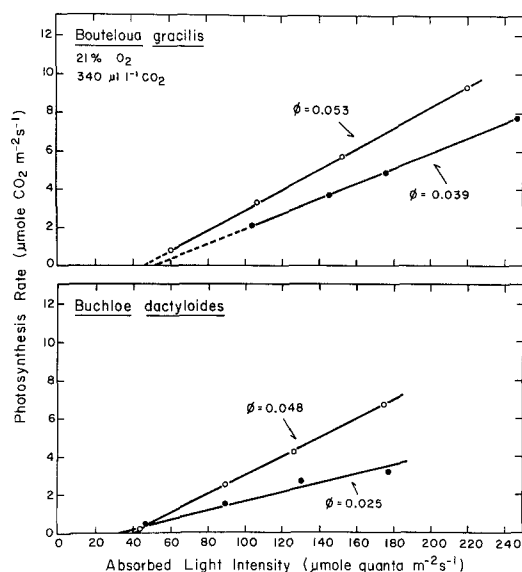


Fig. 6. The net photosynthesis rate as a function of absorbed light intensity in leaves of the two C_4 species. Measurements were conducted before (○) or after (●) a two hour cold temperature treatment at 5–6° C as described in the text. Leaf temperatures during measurement were 30° C. Leaves were allowed to equilibrate at the measurement temperature for 1 h after the low temperature treatment. The quantum yield is represented as ϕ . Points represent the mean of 3 plants

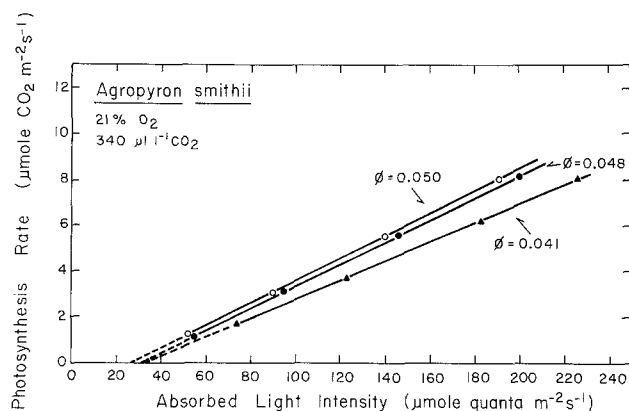


Fig. 7. The net photosynthesis rate as a function of absorbed light intensity in leaves of *A. smithii*. Measurements were conducted before (○) or after a two hour cold temperature treatment at 5–6° C (●), or 1–3° C (▲). Other analysis conditions and symbols are the same as in Fig. 6. Points represent the mean of 3 plants

Table 1. The range of fluorescence breakpoint temperatures for warm grown plants of the four experimental species

Species	Range of fluorescence breakpoint temperatures (° C)
<i>Agropyron smithii</i>	46.2–52.0 ^a
<i>Carex eleocharis</i>	51.6–54.5
<i>Buchloe dactyloides</i>	52.6–54.3
<i>Bouteloua gracilis</i>	53.8–57.0

^a Values represent the range of 5–8 measurements. All experimental plants were grown at 35/15° C (day/night)

are increased. With respect to *A. smithii*, the highest values for both C_i and the photosynthetic capacities in normal air, were observed in cool grown plants. Growth temperature had less effect on C_i in leaves of the acclimating species, *C. eleocharis*, relative to *A. smithii*. Additionally, the low photosynthesis rates of *C. eleocharis*, relative to the other four species, are correlated with correspondingly low values of C_i whether assayed in 21 or 2% O_2 . These results suggest a role for the internal conductance to CO_2 in regulating the photosynthetic response to growth temperature in these two C_3 species.

As mentioned earlier, C_i is presumably a function of RuBP carboxylase activity in C_3 plants (von Caemmerer and Farquhar 1981; Monson et al. 1982). Previous studies by Oulton (1979) have revealed decreased activity of RuBP carboxylase in warm grown plants of the C_3 species *A. smithii*, relative to cool grown plants. These results are in agreement with the lower values for C_i observed for warm grown *A. smithii* in the current study. Badger et al. (1982) and Percy (1977) have reported a correlation between activities of RuBP carboxylase and photosynthetic temperature acclimation in a C_3 and C_4 desert perennial, respectively. Activities of the enzyme fructose-1,6-bisphosphatase may also have a regulatory role in photosynthetic temperature acclimation in C_3 plants (Badger et al. 1982). All of these studies together suggest a primary relationship between growth temperature, activities of rate-limiting photosynthetic enzymes, and the intracellular conductance to CO_2 , which presumably regulates the maximum photosynthetic capacity of C_3 plants from that temperature regime.

Stomatal conductance has been suggested to have a greater role than carboxylation capacity in regulating CO_2 uptake in C_4 plants (Körner et al. 1979) although more recent discussions suggest that the evidence does not support this conclusion which is usually based on a linear resistance analysis (Farquhar and Sharkey 1982). In *B. dactyloides* stomatal conductances of cool and warm grown plants were not significantly different at all analysis temperatures below 50° C, and conductance values increased over the entire analysis temperature range (15–45° C, data not shown). The latter results suggest that stomatal conductance limitations do not have a role in the different photosynthetic capacities which were observed for cool and warm grown plants of *B. dactyloides* (Fig. 1). In the case of *B. gracilis*, Kemp and Williams (1980) suggested that differences in stomatal conductance may regulate photosynthetic temperature adaptation more than internal conductances, since the latter values were greater than the former. In the current study, the values for C_i were also greater than C_s in both cool and warm grown plants of *B. gracilis* (C_s = 5.4 and 5.1 $mm\ s^{-1}$ at 30° C for warm and cool grown plants, respectively). However, there is some question as to whether C_i represents the true internal conductance at ambient CO_2 concentrations in C_4 plants (Longstreth et al. 1980). Since the assimilation rate of C_4 plants is often CO_2 saturated in normal air, metabolic factors other than carboxylation kinetics may be rate limiting. Intuitively, those reactions involved with the regeneration of the PEP substrate, or the decarboxylation of the four-carbon carboxylation products, could limit the photosynthetic capacity of C_4 plants in normal air. Thus, we might not always expect to find a relationship between C_i and the photosynthetic capacity in normal air in C_4 plants. In the current study the dissimilarities in C_i between cool and warm grown plants of *B.*

gracilis suggest a negative effect of cool growth temperatures on the carboxylation processes. However, due to the uncertainties discussed above it is difficult to assess whether this negative effect on C_i is the cause of the lower photosynthesis rates in normal air. In *B. dactyloides* the fact that a lower maximum photosynthesis rate in cool grown plants was not correlated with a lower value for C_i , underscores the fact that we cannot generalize about these relationships in C_4 plants at this time.

The capacity for CO_2 uptake in the C_4 grasses, *B. gracilis* and *B. dactyloides*, exhibited distinct low temperature limitations relative to the two C_3 species (Fig. 5). Studies by Phillips and McWilliams (1971) have demonstrated a low temperature sensitivity (below 12°C) of PEP carboxylase in some C_4 plants. Other enzymes of the C_4 photosynthetic pathway, such as pyruvate, orthophosphate dikinase and NADP malate dehydrogenase, may also be sensitive to chilling temperatures (for review see Edwards and Huber 1981). Transport of certain metabolites across the chloroplast envelope also appear to be sensitive to low temperatures in some C_4 plants (Huber and Edwards 1977). The effect of chilling temperatures on transport processes in some C_4 plants is probably related to membrane phase transitions (Edwards and Huber 1981), which appear to be higher in plants from warm temperature environments (Pike and Berry 1980). It should be noted that these low temperature limitations in some C_4 plants appear to be associated with their phylogenetic origin in warm tropical habitats, rather than an intrinsic sensitivity of the C_4 metabolic processes. Several C_4 species are known to occur in cool, temperate environments, and analyses of photosynthetic processes have revealed no significant limitations by low temperature (Björkman et al. 1975, Sugiyama and Boku 1976, Caldwell et al. 1977).

Chilling temperatures in the presence of a high light intensity resulted in irreversible damage to the light dependent reactions of net photosynthesis in cool grown leaves of *B. gracilis* and *B. dactyloides* (Fig. 6). The light dependent photosynthetic reactions in leaves of *A. smithii* were not as sensitive to chilling temperatures. Although a required role of high light intensities in the chilling damage of these plants was not examined, the results are consistent with the low temperature, high irradiance photoinhibition observed in some C_3 and C_4 plants (Taylor and Craig 1971, Powles et al. 1980). The results are also consistent with the in situ seasonal phenology patterns described for *B. gracilis*, *B. dactyloides*, and *A. smithii*. The lower sensitivity of leaves of *A. smithii* to chilling temperatures is correlated with the observed early cool season growth activities. In contrast, the chilling sensitive processes in leaves of *B. gracilis* and *B. dactyloides* are correlated with the initial growth activities occurring later in the spring when leaf temperatures are higher.

A correlation between high temperature sensitivity of the photosynthetic apparatus and seasonal phenology patterns was also observed (Table 1), and has been described in more detail in a separate report (Monson and Williams 1982). Briefly, the cool season species *A. smithii* and the warm season species *B. gracilis* exhibited the lowest and highest photosynthetic tolerances of high temperature, respectively. The extremes in values for these species reflect their specialized adaptation to specific portions of the seasonal temperature gradient in situ. The intermediate values which were observed in *C. eleocharis* and *B. dactyloides*,

are consistent with photosynthetic activities occurring in both cool and warm portions of the growing season.

The results of the current study provide a physiological model for coexistence among the four experimental species (also see Kemp and Williams 1980). Seasonal photosynthetic activities in the cool season grass, *A. smithii*, would be offset from those of the warm season grasses, *B. gracilis* and *B. dactyloides*, through differential capacities for temperature adaptation. In the case of *C. eleocharis*, plants of this species consistently exhibited lower maximum photosynthesis rates in the current laboratory studies, as well as several measurements which were conducted *in situ* (Monson 1982). In the latter studies the maximum photosynthesis rate of *C. eleocharis* were consistently only 0.7 times as high as those for *A. smithii* and *B. gracilis* (Monson 1982). It is proposed that the low photosynthetic capacity of *C. eleocharis* may be indicative of a low rate of resource utilization in this species. Thus, the resource requirements of *C. eleocharis* would be low enough to allow its coexistence with the other species, even in the presence of interspecific competition. The irreversible high and low temperature thresholds observed for the four species (Figs. 5, 6, 7) may represent niche boundaries with respect to the seasonal temperature gradient. However, these high and low temperature limits probably do not have an active role in limiting seasonal photosynthetic capacities. Intuitively, it seems that an interaction between the internal and external processes which control a plant's phenology patterns, should allow a species to avoid the environmental extremes that result in irreversible physiological damage.

With respect to topography, productivity in the shortgrass steppe reaches its greatest rates in the lowland swales (Monson 1982). Seasonal plant water stress occurs much later in the growing season, if at all, in these lowland sites where precipitation runoff accumulates. In the upslope sites seasonal water stress appears to limit mid-summer species diversity to a few well-adapted taxa. Kemp and Williams (1980) suggested that niche separation along a seasonal temperature gradient may result in decreased interspecific competition for limiting moisture resources in the shortgrass steppe. The regulation of offset seasonal growth activities through differential temperature adaptation may have its greatest effectiveness in reducing interspecific competition in the lowland areas. The reduced interspecific competition should result in a greater overall efficiency of resource utilization. In the upslope sites mechanisms to reduce interspecific competition may not be as important as individual adaptations to water stress, in effecting the greatest efficiency of resource utilization.

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References

- Badger MR, Björkman O, Armond PA (1982) An analysis of photosynthetic response and adaptation to temperature in higher plants: temperature acclimation in the desert evergreen *Nerium oleander* L. Plant, Cell and Environment 5:85-99
- Berry J, Björkman O (1980) Photosynthetic temperature response and adaptation to temperature in higher plants. Ann Rev Plant Physiol 31:491-543

- Björkman O, Mooney HA, Ehleringer JR (1975) Photosynthetic responses of plants from habitats with contrasting thermal environments. *Carnegie Inst Wash Yearbook* 74:743–748
- Boutton TW, Harrison AT, Smith BN (1980) Distribution of biomass of species differing in photosynthetic pathway along an altitudinal transect in southeastern Wyoming grassland. *Oecologia* (Berlin) 45:287–298
- Caemmerer S von, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* 153:376–387
- Caldwell MM, Osmond CB, Nott D (1977) C_4 pathway photosynthesis at low temperature in cold-tolerant *Atriplex* species. *Plant Physiol* 60:157–164
- Dickinson CE, Dodd JL (1976) Phenological pattern in the short-grass prairie. *Amer Midl Nat* 96:367–378
- Downton WJS, Seemann JR, Berry JA (1980) Thermal stability of photosynthesis in desert plants. *Carnegie Inst Wash Yearbook* 79:143–145
- Edwards GE, Huber SC (1981) The C_4 pathway. In: *The Biochemistry of plants: a comprehensive treatise. VIII. Photosynthesis*. Stumpf PK, Conn EE (eds) pp 238–282
- Edwards GE, Walker DA Textbook on C_3 , C_4 photosynthesis. Blackwell, Oxford (in press)
- Ehleringer JR, Björkman O (1978) A comparison of photosynthetic characteristics of *Encelia* species possessing glabrous and pubescent leaves. *Plant Physiol* 62:185–190
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Ann Rev Plant Physiol* 33:317–345
- Gerwick BC, Williams GJ, III (1978) Temperature and water regulation of gas exchange of *Opuntia polyacantha*. *Oecologia* (Berlin) 35:149–159
- Huber SC, Edwards GE (1977) Transport in C_4 mesophyll chloroplasts: characterization of the pyruvate carrier. *Biochim Biophys Acta* 4:583–602
- Jones HG, Slatyer RO (1972) Estimation of the transport and carboxylation components for the intracellular limitation to leaf photosynthesis. *Plant Physiol* 50:283–288
- Kemp PR, Williams GJ, III (1980) A physiological basis for niche separation between *Agropyron smithii* (C_3) and *Bouteloua gracilis* (C_4). *Ecology* 61:846–858
- Körner CH, Schul JA, Baur H (1979) Maximum leaf diffusive conductance in vascular plants. *Photosynthetica* 13:45–82
- Ku SB, Edwards GE (1977) Oxygen inhibition of photosynthesis. II. Kinetic characteristics as affected by temperature. *Plant Physiol* 59:991–999
- Longstreth DJ, Hartstock TL, Nobel PS (1980) Mesophyll cell properties for some C_3 and C_4 species with high photosynthetic rates. *Physiol Plant* 48:494–498
- Monson RK (1982) Ecophysiological studies of three species from the Colorado shortgrass steppe: physiological factors regulating distribution in space and time. Dissertation. Washington State University p 183
- Monson RK, Williams GJ, III (1982) A correlation between photosynthetic temperature adaptation and seasonal phenology patterns in the shortgrass prairie. *Oecologia* (Berlin) 54:58–62
- Monson RK, Stidham MA, Williams GJ, III, Edwards GE, Uribe EG The temperature dependence of photosynthesis in *Agropyron smithii* Rydb. I. Factors affecting net CO_2 exchange and contribution from RuBP carboxylase measured in vivo and in vitro. *Plant Physiol* 69:921–928
- Mooney HA, Björkman O, Collatz GJ (1978) Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*. I. Carbon dioxide exchange characteristics of intact leaves. *Plant Physiol* 6:406–410
- Mooney HA, Björkman O, Ehleringer J, Berry J (1976) Photosynthetic capacity of *in situ* Death Valley plants. *Carnegie Inst Wash Yearbook* 75:410–413
- Oulton KO (1979) Growth temperature effects on carboxylase enzymes from *Agropyron smithii* and *Bouteloua gracilis*. Dissertation. Washington State University p 80
- Pearcy RW (1977) Acclimation of photosynthetic and respiratory CO_2 exchange to growth temperature in *Atriplex lentiformis* (Torr.) Wats. *Plant Physiol* 59:795–799
- Phillips PJ, McWilliam JR (1971) Thermal responses of the primary carboxylating enzymes from C_3 and C_4 plants adapted to contrasting temperature environments. In: *Photosynthesis and Photorespiration* Hatch MD, Osmond CB, Slatyer RO (eds) Wiley-Interscience, New York pp 97–104
- Pike CS, Berry JA (1980) Membrane phospholipid phase separations in plants adapted to or acclimated to different thermal regimes. *Plant Physiol* 66:238–241
- Powles SB, Berry JA, Björkman O (1980) Interaction between light intensity and chilling temperatures on inhibition of photosynthesis in chilling sensitive plants. *Carnegie Inst Wash Yearbook* 79:157–159
- Regehr DL, Bazzaz FA (1976) Low temperature photosynthesis in successional winter annuals. *Ecology* 57:1297–1303
- Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet radiation and implications for plant sensitivity to ultraviolet radiation induced injury. *Oecologia* (Berlin) 32:277–287
- Schreiber U, Berry JA (1977) Heat-induced changes of chlorophyll fluorescence in intact leaves, correlated with damage of the photosynthetic apparatus. *Planta* 136:233–238
- Seemann JR, Downton WJS, Berry JA (1979) Field studies of acclimation to high temperature: winter ephemerals in Death Valley. *Carnegie Inst Wash Yearbook* 78:159–162
- Seemann JR, Tepperman JM, Berry JA (1981) The relationship between photosynthetic performance and the levels and kinetic properties of RuBP carboxylase-oxygenase from desert winter annuals. *Carnegie Inst Wash Yearbook* 80:67–72
- Slatyer RO, Ferrar PJ (1977) Altitudinal variation in the photosynthetic characteristics of snow gum, *Eucalyptus pauciflora* Sieb. ex Spreng. II. Effects of growth temperature under controlled conditions. *Aust J Plant Physiol* 4:289–299
- Smillie RM (1979) Coloured components of chloroplast membranes as intrinsic membrane probes for monitoring the development of heat injury in intact tissues. *Aust J Plant Physiol* 6:121–133
- Sugiyama T, Boku K (1976) Differing sensitivity of pyruvate orthophosphate dikinase to low temperature in maize cultivars. *Plant and Cell Physiol* 17:851–854
- Taylor AO, Craig AS (1971) Plants under climatic stress. II. Low temperature, high light effects on chloroplast ultrastructure. *Plant Physiol* 47:719–725
- Williams GJ, III (1974) Photosynthetic adaptation to temperature in C_3 and C_4 grasses. A possible role in the shortgrass prairie. *Plant Physiol* 54:709–711

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