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Water Deficit Effects on Osmotic Potential, Cell Wall Elasticity, and Proline in Five Forage Grasses

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ABSTRACT

Physiological responses of forage grasses to water deficit are not well documented, but may be important in determining drought resistance. The objective of this study was to determine the response of osmotic potential, leaf proline concentration, and cell wall elasticity to water deficit for the C_4 (warm-season) grasses 'Nebraska 54' indiangrass [*Sorghastrum nutans* (L.) Nash], 'Pathfinder' switchgrass (*Panicum virgatum* L.), and 'Pawnee' big bluestem (*Andropogon gerardii* Vitman), and the C_3 (cool-season) grasses, 'Ioreed' reed canarygrass (*Phalaris arundinacea* L.), and 'Lincoln' smooth bromegrass (*Bromus inermis* Leyss.). Other measurements included leaf water potential, soil water content, and osmotic adjustment. A field study at Mead, NE, and a complementary greenhouse study at the University of Nebraska, Lincoln, found osmotic adjustment occurred in response to water deficit for all species, and was greater for C_4 than for C_3 grasses. Despite less osmotic adjustment, C_3 grasses had more elastic cell walls (low modulus of cell wall elasticity), which maintained turgor despite loss of water. Leaf proline concentration averaged 20 times greater in stressed compared to well-watered plants grown in the greenhouse. Proline accumulation in greenhouse-grown plants was much larger than observed under field conditions. The physiological role of proline accumulation was uncertain because even dramatic increases in leaf proline concentration were insufficient to influence osmotic potential.

THE RANGELANDS of the central USA cover approximately 100 million hectares and are commonly inhabited by indigenous C_4 forage species including indiangrass, switchgrass, and big bluestem, and introduced C_3 forage species such as reed canarygrass and smooth bromegrass (Stubbendieck et al., 1985). Total annual precipitation across the region varies from 300 to 1200 mm, and seasonal variability in precipitation often results in plant water deficits (Knapp, 1984). Mechanisms to tolerate or avoid water deficit are imperative for these perennial species to maintain forage production. Although physiological mechanisms conferring tolerance or resistance to drought, such as C_3 and C_4 metabolism, osmotic adjustment (OA), changing the modulus of cell wall elasticity (ϵ), and proline accumulation have been reviewed (Osmond et al., 1980; Turner and Jones, 1980), relatively little is known about physiological responses to water deficit in these specific forage grasses.

Grasses vary in the magnitude of osmotic potential (ψ_π) among species. The C_3 species, such as crested wheatgrass [*Agropyron cristatum* (L.) Beauv.] (Bittman and Simpson, 1989), and the C_4 species, switchgrass, big bluestem, and little bluestem [*Schizachyrium scoparium* (Michx.)] have unusually low ψ_π (Knapp, 1984). The metabolic cost in maintaining low ψ_π is offset by the benefit resulting from maintenance of leaf turgor (ψ_P) at low total water potential (ψ) (Turner and Jones, 1980). Furthermore, OA by active accumulation of solutes has been reported for smooth bromegrass (Bittman and Simpson, 1989), *Phalaris* spp. (Sambo, 1981), switchgrass, and big bluestem (Knapp, 1984).

Plants vary in the magnitude of ϵ and its response during water deficit. Cells with rigid walls (high ϵ) lose turgor rapidly with water loss, a mechanism which may be important for stomatal closure or leaf rolling and folding. Knapp (1984) argued that, in general, plants with more elastic walls (low ϵ) will have a lower ψ at zero turgor (maintain turgor longer as ψ declines). Low ϵ (high elasticity) reportedly resulted in better drought resistance of crested wheatgrass compared to smooth bromegrass (Bittman and Simpson, 1989). Conversely, however, Bowman and Roberts (1985) suggested that more rigid cell walls (high ϵ) would have lower ψ for a given change in water volume and, therefore, maintain a steeper ψ gradient for uptake of soil water. Similarly, Melkonian et al. (1982) found in wheat (*Triticum aestivum* L.) that three cycles of water deficit increased ϵ.

An increase in leaf proline concentration often has been associated with water deficit (Boggess et al., 1976), and although too small to significantly influence ψ_π, may be implicated with the synthesis of proline and glycine-rich storage and protective proteins during water deficit (Gomez et al., 1988; Singh et al. 1987). Cool- and warm-season forage grasses both exhibit similar trends in proline accumulation (Bokhari and Trent, 1985), but no information is available for the species of interest in this study.

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Abbreviations: AET, actual evapotranspiration; *B*, apoplastic water content; OA, osmotic adjustment; PVC, pressure volume curves; RWC, relative water content; RWC_{ZT}, relative water content at zero turgor; SWC, soil water content; ϵ, modulus of cell wall elasticity; ψ, water potential; ψ_{PC}, water potential measured by Scholander-type pressure chamber; ψ_{PSYC}, water potential measured by thermocouple psychrometer; ψ_P, turgor potential; ψ_π, osmotic potential; and ψ_π^{100}, osmotic potential at full turgor.

The objectives of this study were to quantify ψ_π, leaf proline concentration, and ϵ responses to water deficit in big bluestem, switchgrass, indiangrass, reed canarygrass, and smooth bromegrass.

MATERIALS AND METHODS

Experiment 1

A field study (8 June–22 July 1988) was conducted in two areas at the University of Nebraska Agricultural Research and Development Center, Mead, NE. Three-year-old, pure swards of 'Nebraska 54' indiangrass, 'Pathfinder' switchgrass, and 'Pawnee' big bluestem had been established in one area, and more than 5-yr-old, mixed swards of 'Ioreed' reed canarygrass and 'Lincoln' smooth bromegrass had been established in a second area, 200 m from the first. Swards were large areas (10 × 100 m) that were infrequently mown and never grazed. The soil was a Sharpsburg silty clay loam (Typic Argiudolls) (James et al., 1972).

A total of 15 metal rings (500-mm diam. and 200-mm depth) were placed flush with the soil surface and, beginning 8 June 1988, plants within the rings were irrigated with approximately 20 L water per ring, three times per week (watered treatment). Adjacent areas (stressed treatment) received only rainfall.

Measurements were made in two periods of stable weather, 22 to 29 June and 20 to 22 July 1988, with one species measured per day in Period 1 and two species measured per day in Period 2. Gravimetric soil water content (kg kg^{-1}) (SWC) of each experimental unit was determined from five 75-mm depth soil cores dried at 80 °C for 48 h. Rainfall was recorded 500 m from the experimental areas. Midday ψ was measured with a Scholander-type pressure chamber (ψ_{PC}) and Peltier-cooled, thermocouple psychrometer (ψ_{PSYC}) (Decagon Devices Inc., Pullman, WA)[1] in duplicate on the penultimate leaf of vegetative tillers, following procedures described by Turner (1981). Measurement of ψ_{PC} was on entire laminae, directly in the field. Measurement of ψ_{PSYC} was on 5-cm lamina sections, loaded into the psychrometer chamber in the field and transferred to an air-conditioned laboratory for 1 to 2 h equilibration prior to reading. Regression of ψ_{PC} on ψ_{PSYC} showed measurement techniques were similar (intercept 0.18 MPa, slope 0.883, r = 0.98) and only ψ_{PSYC} are presented. Osmotic potential at full turgor (ψ_π^{100}) was measured in duplicate on different sections of the same leaf as for ψ_{PSYC}. The technique and timing was similar to that used for obtaining ψ_{PSYC}, except leaf pieces were rehydrated in distilled water (15–25 h, dark, 4 °C), frozen in sealed psychrometer cups (2–3 h, −18 °C), and thawed prior to determining ψ_π^{100}. In many cases duplicate readings were repeated on the same experimental unit to allow calculation of sampling SE. Osmotic adjustment was calculated as the difference between ψ_π^{100} of watered and stressed treatments. The method for leaf proline concentration was modified from Bates et al. (1973) by decanting the supernatant following 20 min settling, rather than following centrifugation.

Both areas in Exp. 1 were a split-plot design, with species as main plots replicated three times in randomized complete blocks, and water treatments as sub-plots. Analysis of variance (ANOVA) of both measurement periods was with the Repeated Measures option of the General Linear Model (GLM) procedure of PC-SAS (SAS Inst., Inc., Cary, NC). The C_3 and C_4 species were analyzed separately because they were in different areas, resulting in relatively few numerator and denominator degrees of freedom and low power of F-tests. Means were compared by least significant differences calculated from appropriate SE.

[1]The mention of trade names is for information only and does not imply endorsement by the authors, AgResearch, USDA-ARS, or Agron. Dep., Univ. of Nebraska.

Experiment 2

A greenhouse experiment was conducted at Lincoln, NE, from 3 June 1988 until 8 Sep. 1989. Large pots (340 mm diam. and 490 mm tall) were filled with the same soil as in Exp. 1 and saturated with water. After allowing the soil water to drain for 3 d, the weight of each pot was adjusted to 80 kg by the addition or removal of soil. Two plants of the same five cultivars used in Exp. 1 were established from seed in each pot and watered from the top approximately three times per week with the nutrient solution described by Bennett and Sullivan (1981). Grasses were trimmed to 100-mm height twice during the 3 June 1988 to 17 May 1989 establishment period. Beginning 14 Apr. 1989, pots were weighed with a T63H-1K-10P1 load cell (Transducer Inc., Whittier, CA) and #833 logger (Dynamics Div., Waugh Controls Corp., Chatsworth, CA), approximately weekly, and adjusted to the same weight by the addition of variable amounts of nutrient solution. Daylight was extended to 16 h with six 1 kW metal halide lights for 3 h in the morning (10 Jan.–22 Apr. 1989) and 5 h in the evening (19 Jan.–10 May 1989). Greenhouse air temperature, recorded hourly with a CR21X integrating datalogger (Campbell Scientific Inc., Logan, UT) and appropriate sensors, averaged 25 °C and never exceeded the extremes of 36 and 12 °C. Relative humidity averaged 72%, and soil temperature at 50 mm depth averaged 31 °C.

The experiment was comprised of three 23-d drying periods (17 May–June, 10 July–2 Aug., and 15 Aug.–7 Sep. 1989), where nutrient solution was withheld from five pots (one for each species). Five control pots (one for each species) continued to receive nutrient solution within each period. Although laid out randomly, the sampling structure was in randomized complete blocks, with blocks representing each of the three drying periods. New pots were used for each period.

Measurement of ψ_π^{100} was made as in Exp. 1; however, OA was calculated by the difference between consecutive measurements of ψ_π^{100} of stressed plants only. The ψ_π^{100} of control plants did not vary appreciably with time. Leaf proline concentrations were fitted to Eq. [1] using PROC NLIN of PC-SAS. The iterative procedure METHOD = MARQUARDT was used because other methods failed to converge.

$$\text{proline } (\mu g\ g^{-1}\ DW) = (e^{-\beta \times SWC + \gamma}) - \alpha \quad [1]$$

where α, β, and γ, are arbitrary constants.

An analysis of soil water loss was made from five to nine pot weights during each dry-down period. Weights were fitted to Eq. [2] and [3] as previously described by Barker et al. (1985), using PROC NLIN of PC-SAS.

$$(w_t - w_0) = \beta_0(1 - e^{-\beta 1 t}) \quad [2]$$

$$d(w_t - w_0)/dt = AET = \beta_0\beta_1 - \beta_1(w_t - w_0) \quad [3]$$

where w_t is pot weight at day t (kg), w_0 is pot weight at day 0, β_0 is asymptote of the logarithmic function (the lower limit of plant extractable water), β_1 is the degree of curvature, t is day of dry-down (0–23), and AET is actual evapotranspiration.

Water use (kg water pot^{-1} d^{-1}) by the five species was measured throughout the experiment by the mean difference in pot weight including total water added, at the start and end of 16 periods, 4 April to 8 Sep. 1989.

Pressure volume curves (PVC) were used to estimate ψ_π^{100}, ψ_π zero turgor (ψ_π^0), relative water content at zero turgor (RWC_{ZT}), and ϵ, using the method of sap expression in a Scholander-type pressure chamber (Tyree and Hammel, 1972). Chamber pressures of a typical sample were 0.21, 0.28, 0.34, 0.52, 0.86, 1.38, 1.72, 2.07, 2.41, and 2.76 MPa, and each pressure step was held for 10 min while sap was collected on a pre-weighed filter paper. Small plastic covers and cling film were used to minimize evaporation from the filter paper and

Table 1. Mid-afternoon leaf water potential (ψ_{PSYC}), full turgor osmotic potential (ψ_π^{100}), and gravimetric soil water content (SWC) in two periods (22–29 June, and 20–22 July, 1988) for watered (W) and stressed (S) treatments, and three warm-season and two cool-season grasses in the field (Exp. 1). Data are the means of three replicates.

	ψ_{PSYC}†				ψ_π^{100}‡				SWC			
	Period 1		Period 2		Period 1		Period 2		Period 1		Period 2	
Species	W	S	W	S	W	S	W	S	W	S	W	S
	−MPa				−MPa				kg kg^{-1}			
Indiangrass	0.55	0.75	0.93	1.09	0.92	0.96	0.73	0.90	0.284	0.176	0.260	0.252
Switchgrass	1.42	1.63	1.18	1.38	1.48	1.61	-	-	0.206	0.121	0.259	0.243
Big bluestem	1.54	1.86	0.90	1.00	1.18	1.39	0.93	0.90	0.286	0.117	0.278	0.271
$P > F$ and (SE)												
period × species			0.0001 (0.124)				0.0638 (0.057)				0.0003 (0.412)	
period × water			0.7003				0.7923				0.0001 (0.505)	
species × water			0.8442				0.9526				0.2423	
species			0.0017§				0.0070§				0.0001§	
water			0.0345 (0.140)				0.0401 (0.001)				0.0256§	
period			0.1731				0.0318¶				0.0001¶	
Reed canarygrass	2.31	2.24	2.34	2.54	2.66	2.68	2.18	2.24	0.318	0.206	0.315	0.273
Smooth bromegrass	2.33	3.01	2.23	2.67	2.06	2.12	2.13	2.08	0.238	0.105	0.293	0.256
$P > F$ and (SE)												
period × species			0.1366				0.0129 (0.140)				0.0106 (1.942)	
period × water			0.7204				0.8139				0.0049 (1.587)	
species × water			0.1664				0.9452				0.5815	
species			0.2844				0.1036				0.0004§	
water			0.1147 (0.105)				0.6511				0.0037§	
period			0.6631				0.0535¶				0.0002¶	

† Sampling SE = 0.124 MPa.
‡ Sampling SE = 0.18 MPa.
§ SE not presented because of the occurrence of a significant interaction.
¶ SE not calculated.

leaf, respectively. Despite these attempts, evaporative losses from the tissue in excess of water loss to sap expression occurred; however, it was assumed this loss occurred uniformly and correction would not alter comparisons between treatments. Leaves were sampled by cutting underwater and rehydrated in distilled water (dark, 4 °C) for a mean of 20 h (range 4–44 h). Use of rehydration time as a covariate in the analysis did not significantly affect the variables ψ_π^{100} or ϵ, so it was not used. Extrapolation of the regression of leaf weight (calculated from sap loss) on pressure at 0.21, 0.28, and 0.34 MPa was used to predict leaf weight at full turgidity (ψ = balance pressure = 0) for use in calculating relative water content (RWC).

Regression of the last two pressure points was assumed to define the linear phase of each PVC. The apoplastic water content (B) was determined by extrapolation to the x-axis; however, this resulted in unreliable estimates of B (between −10 and 90%). Consequently, in subsequent analyses, the regression was forced through B = 15% (Campbell et al., 1979). Choice of the value for B had only a small effect on the regression compared to the position of incipient plasmolysis determined from PVC. Calculation of ψ_π^{100} was by extrapolation to RWC = 100%. The difference between the function of ψ_π on RWC, and balance pressure on RWC described the function of ψ_p on RWC. The slope of the linear phase of ψ_p vs. RWC (using the first three to six pressure points) gave ϵ, the change in ψ_p per unit change in RWC (Wilson et al., 1979). The RWC_{ZT} was found where this same function crossed the x-axis. Between 3 to 15 PVCs were made per species per replicate; however, for simplicity data were averaged into three periods (0–7, 8–14, and 15–23 d from withholding nutrient solution).

Measurements on the same experimental unit (i.e., as water deficit developed) were analyzed by ANOVA using the Repeated Measures option of GLM PC-SAS. Residuals were inspected for normality, and appropriate transformations used where necessary. Means were compared by least significant differences calculated from appropriate SE, and for the C_3 vs. C_4 comparison single degree of freedom (orthogonal) contrasts were used.

RESULTS AND DISCUSSION

Experiment 1

The ring technique was successful in creating differences in SWC between watered and stressed treatments, especially in the first period when no rain fell 14 d prior to measurements (Table 1). A significant period-by-water interaction for SWC ($P \leq 0.0001$) was the result of 66 mm rain in the 5 d before Period 2, which reduced the difference in SWC between watered and stressed treatments. Similarly, a period-by-species interaction was caused by rain before the second measurement period, which masked the SWC differences that were apparent at the first period (Table 1).

Differences in ψ_{PSYC} resulted from a difference between SWC of watered and stressed treatments (Table 1), especially at the first measurement. The greater (less negative) ψ_{PSYC} of indiangrass at Period 1 was probably due to greater SWC for both watered and stressed treatments; however, lower ψ_{PSYC} for cool- compared to warm-season species occurred despite similar SWC. Presumably transpiration in excess of water uptake from the soil for C_3 species allowed continued CO_2 exchange but with the result of lower ψ. The ψ_π^{100} was similarly lower for C_3 species and presumably was a potentially adaptive mechanism to allow turgor maintenance, and hence growth, despite lower ψ.

Differences between water treatments for ψ_π^{100} (Table 1) showed OA had occurred for C_4 but not C_3 species. A significant period-by-species interaction for ψ_{PSYC} showed species varied in their recovery rates from stress. Despite 66 mm of rain in the 5 d before Period 2, indiangrass ψ_π^{100} remained lower on stressed plots than watered plots. The maximum OA observed for indiangrass, switchgrass, and big bluestem was 0.17, 0.13, and 0.21 MPa, respectively. Warm-season (C_4) grasses had greater OA than cool-season (C_3) grasses, although this comparison may have been confounded with experimental site. Similarly, ψ_π^{100} of C_3 grasses appeared lower than for C_4 grasses.

No differences in leaf proline concentration between water treatments were observed for C_4 grasses (mean = 2.85 μg proline g DW^{-1}) or the C_3 grass smooth bromegrass (mean = 4.9 μg proline g DW^{-1}). A difference was observed for reed canarygrass, with the stressed treatment (10.6 μg proline g DW^{-1}) having almost twice the proline concentration of the well-watered treatment (5.4 μg proline g DW^{-1}). These concentrations were approximately 1000 times less than values previously reported for other range grasses (Bokhari and Trent, 1985), suggesting that water deficit in this experiment was relatively light.

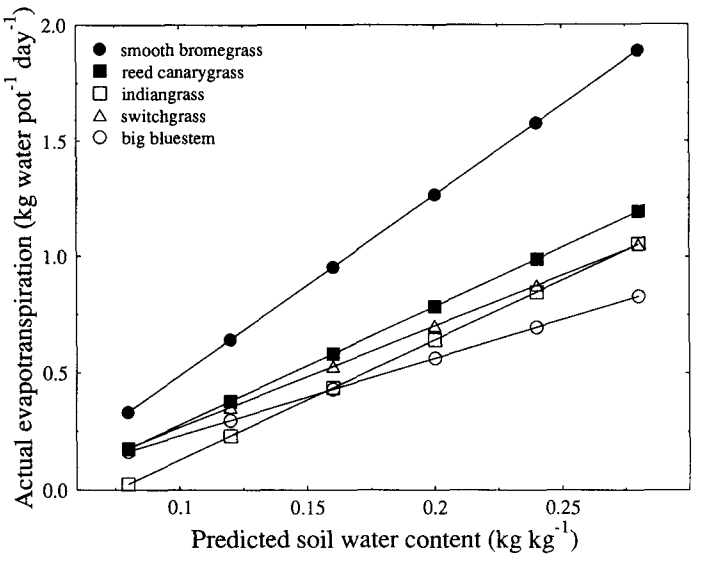

Fig. 1. Rate of water loss (actual evapotranspiration) from large pots in the greenhouse as a function of soil water content (Eq. [3]) for five forage grass species (Exp. 2).

Experiment 2

Mean water use of the five species in 16 periods prior to withholding nutrient solution (data not shown), and the parameter β_1, estimated from fitting pot weights during drying periods to Eq. [2] (0.06, 0.14, and 0.09, for reed canarygrass, smooth bromegrass, and C_4 grasses, respectively), showed significantly greater water use for smooth bromegrass. It was not determined whether this difference for smooth bromegrass resulted from a greater leaf area, a different root distribution, a greater transpiration rate per unit leaf area, or a combination of these factors. No significant difference ($P > 0.05$) was found between species for the predicted asymptote (β_0) of Eq. [2] (mean = −12.6 kg). This suggests that although species may have varied in their rate of drying, they would have dried to a constant minimum soil water content. Tukey's test for non-additivity showed a non-significant block-by-species interaction.

An exponential decline in pot weight and AET during a drying phase and the linear relationship between AET and pot weight (Fig. 1) were predicted by Eq. [2], and [3], respectively. Water content of the pots at the start of the experiment (mean = 74.44 kg) was 0.245 kg kg^{-1}, and the mean asymptote of the fitted curves (74.44 − 12.63 = 61.81 kg) was 0.027 kg kg^{-1}.

Differences in ψ_π^{100} could be attributed to species and duration of water deficit (Fig. 2). The ψ_π^{100} was significantly less (more negative) for reed canarygrass and indiangrass than for the other three species, and decreased with duration of water deficit. In contrast to Exp. 1, OA occurred for the five species but not equally between each period (Table 2). Between the first and second periods all species showed OA averaging 0.53 MPa. Between the second and third periods, however, a significant contrast between C_3 and C_4 grasses was found, with C_3 species making no further adjustments and C_4 species continuing to make further adjustment. Total OA for the C_3 species (reed canarygrass and smooth bromegrass) was 0.48 and 0.68 MPa and for the C_4 species (indiangrass, switchgrass, and big bluestem) was 1.25, 1.10, and 0.76 MPa, respectively.

The sampling SE of 23 repeated observations of ψ_π^{100} on 11 experimental units was 0.23 MPa. This source of error included differences between psychrometer cells as well as differences between leaves from the same experimental unit.

Positive correlations between ψ_π^{100} determined by PVC (data not presented) and psychrometer (r = 0.79, 0.48, and, 0.51 for successive drying periods, $P \leq 0.05$) showed agreement between the two techniques. The average value

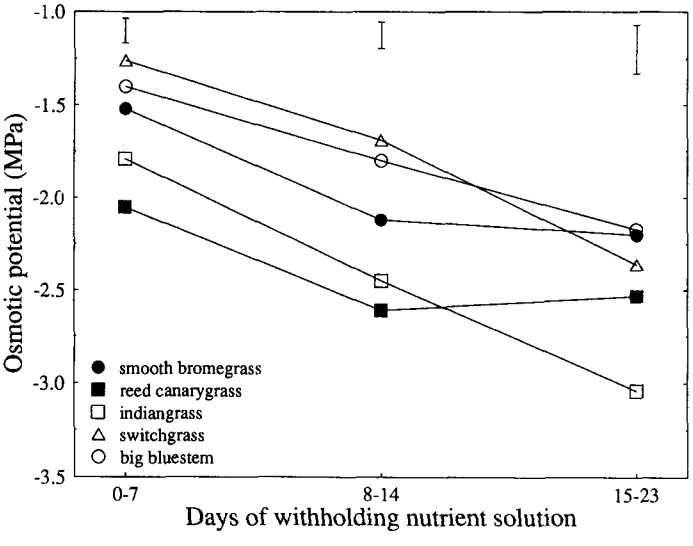

Fig. 2. Changes in mean full turgor osmotic potential (osmotic adjustment) among three observation periods for five greenhouse-grown forage grass species (Exp. 2). Vertical bars are SE.

Table 2. Osmotic adjustment between three observation periods (0–7 to 8–14, and 8–14 to 15–23 d withholding nutrient solution), and the total OA (0–7 to 15–23 d withholding nutrient solution) for five greenhouse-grown grasses (Exp. 2).

Species	Periods (days withholding nutrient solution) 0–7 to 8–14	8–14 to 15–23	Total
	MPa		
Reed canarygrass	0.56†	−0.08	0.48
Smooth bromegrass	0.60	0.08	0.68
Indiangrass	0.66	0.59	1.25
Switchgrass	0.42	0.67	1.10
Big bluestem	0.39	0.37	0.76
$P > F$ species	0.79	0.29	0.19
$P > F$ C_3 vs C_4 contrast	0.61	0.05	0.06
Mean	0.53	0.30	
$P > F$ between periods	0.28		

† Data are means of three replicates

Table 3. Modulus of cell wall elasticity calculated from pressure volume curves for five greenhouse grown forage grasses (Exp. 2).

Species	Days withholding nutrient solution 0–7	8–14	15–23	Mean
	MPa			
Reed canarygrass	36.6†	11.9	7.4	18.7
Smooth bromegrass	8.3	18.6	11.2	12.7
Indiangrass	45.6	100.2	34.5	60.1
Switchgrass	36.1	43.1	6.7	28.6
Big bluestem	39.1	43.5	33.4	38.7
$P > F$	0.25	0.06	0.21	0.03
Mean SE	11.1	18.7	10.4	8.8
Mean	33.2	43.5	18.6	interaction
$P > F$		0.02		0.27

† Data are means of 3 to 15 pressure volume curves per species per replicate.

of ψ_π^{100} determined by PVC (−1.83 MPa) was greater than that found by psychrometric determination (−2.07 MPa) despite the failure to adjust psychrometer values for the potential dilution by apoplastic water. These results were similar to those of Bittman and Simpson (1989) for smooth bromegrass. In some cases balance pressures may not have been high enough to fully determine the linear phase of each PVC or loss of apoplastic water (Cortes and Sinclair, 1985) may have biased PVC estimates of ψ_π^{100}.

Relationships between cell turgor and RWC were generally curvilinear, in contrast to the linear responses suggested by Wilson et al. (1979) and reported for wheat by Rascio et al. (1988). Treatment averages showed cool-season grasses had significantly lower ϵ values (more flexible cells) than warm-season grasses (Table 3). The value for indiangrass (60.1 MPa) was greatest. Significant drought acclimation was observed because the lowest average ϵ for almost every species occurred 16 d after withholding nutrient solution, and there was a significant time effect ($P < 0.02$). The species-by-water deficit interaction was not significant. In general, as functions of turgor on RWC approached zero, cool-season grass cell walls became more rigid (greater ϵ) whereas those of warm-season grasses became less rigid (lower ϵ) resulting in non-significant differences of RWC_{ZT} between species (Table 4).

Of some interest were negative ϵ found in some cases for C_3 species, especially smooth bromegrass lamina. Presumably the initial increase in ψ_π with decrease in RWC resulted from a particularly steep relationship between ψ_π and RWC.

Proline accumulated exponentially in leaves of all grass species as SWC decreased during each dry-down period (data not shown). Mean proline concentration at the wettest and driest observations of each dry-down were 85 and 1700 μg proline g leaf DW^{-1}, a mean increase of 20 times during the 23-d period. Proline concentration could be adequately explained by variation in SWC according to Eq. [1]; however, variation between replicates was high. Univariate and multivariate ANOVA of the three parameters from Eq. [1] showed no significant differences ($P \geq 0.2123$) between proline accumulation of the five species (mean α, β, and γ = 2.5, 0.4019, and 10.929, respectively).

Proline concentrations observed in Exp. 1 were lower than at similar SWC in Exp. 2 (Eq. [1]). Plants in the field (Exp. 1) probably accessed water from deeper in the soil profile than the 75 mm measured.

Increases in leaf proline were most dramatic for average soil water contents below 0.15 kg kg^{-1}. A moisture release curve previously prepared for the soil used, showed soil water potentials in this range were lower than −0.6 MPa. Proline responses appeared of similar sensitivity as OA, and were an indicator that plant stress had occurred.

A concentration of 1000 μg proline g leaf DW^{-1} suggests a contribution to ψ_π of only −0.005 MPa. At this level, the contribution of proline to OA was negligible; however, the contribution of proline to plant metabolism during water deficit can not be discounted.

Table 4. Relative water content at zero turgor (RWC_{ZT}) calculated from pressure volume curves for five greenhouse-grown forage grasses (Exp. 2).

Species	Days withholding nutrient solution 0–7	8–14	15–23	
	kg kg^{-1}			
Reed canarygrass	0.805†	0.831	0.826	
Smooth bromegrass	0.767	0.784	0.714	
Indiangrass	0.947	0.951	0.943	
Switchgrass	0.776	0.841	0.819	
Big bluestem	0.889	0.808	0.913	
$P > F$	0.11	0.48	0.22	
Mean SE	0.0420	0.0640	0.0640	
Mean	0.795	0.830	0.833	interaction
$P > F$		0.86		0.32

† Data are means of 3 to 15 pressure volume curves per species per replicate.

CONCLUSIONS

Osmotic adjustment occurred for all species measured, and appeared greater for C_4 than for C_3 grasses. Despite less ability for osmotic adjustment, C_3 grasses had more flexible cell walls (lower modulus of cell wall elasticity), which maintained turgor despite lower leaf water potentials. The physiological effects of proline accumulation appeared uncertain because 20-fold increases in proline concentration did not influence osmotic potential. Proline responses in the greenhouse were much larger than those observed in the field.

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