



Xylem potential- and water content-breakpoints in two wetland forbs: indicators of drought resistance in emergent hydrophytes

Brant W. Touchette^{1,2,*}, Adam R. Frank^{2,3}

¹Center for Environmental Studies, and ²Department of Biology, Elon University, Elon, North Carolina 27244, USA

³Present address: Biology Program, North Carolina State University, Raleigh, North Carolina 27695, USA

ABSTRACT: Freshwater wetlands commonly occur along the boundaries between terrestrial upland and deep open-water systems, and are consequently prone to dynamic and often highly variable hydrological regimes. Physiological adaptations to drought would be advantageous in emergent vegetation, as wetlands are often susceptible to short periods of water scarcity through seasonal declines in precipitation and/or surface water runoff. This study used 15 l microcosms to examine xylem water potential (ψ_{xylem})- and tissue water content (θ)-breakpoints in 2 wetland forbs (*Justicia americana* and *Saururus cernuus*) to determine how these plants respond to short-term (2 wk) water deficits. Overall, treatment responses by these forbs included reductions in both θ and ψ_{xylem} during the second week of drought, followed by rapid recoveries of θ and ψ_{xylem} within a few days of water repletion. Rapid tissue-water recovery, following extreme water deficits (soil moisture levels <3%), is beneficial to plants residing in areas prone to intense water fluctuations. Furthermore, while θ and ψ_{xylem} breakpoints in *S. cernuus* occurred as soil moisture levels fell below 12% (ca. -800 kPa soil water potential), breakpoints for *J. americana* occurred at considerably lower soil moisture levels (ca. 7 and 10% soil water content for θ and ψ_{xylem} , respectively; or less than -1500 kPa soil water potential). The lower breakpoints observed in *J. americana* were comparable to values reported for terrestrial grasses, including species adapted to dryer, xeric conditions. Therefore, the results from this study suggest that some wetland forbs are physiologically adapted to environments that undergo rapid changes in water hydrology, including the ability to tolerate short-term water scarcity comparable to dryer upland systems.

KEY WORDS: Wetlands · Drought · Water potential · Xylem breakpoint · Plant–water relations

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Water availability maintains a vital role in regulating plant productivity and, on larger scales, plant geographical distribution. Consequently, considerable interest has been focused on understanding physiological adaptations developed by different cultivars, populations, or closely related species to varying water conditions. While much research has centered on water relations in agriculturally important plants, stud-

ies on natural vegetation with contrasting water availability could provide further insight into how plants respond to differing water supply (Milnes et al. 1998, Touchette 2006, Romanello et al. 2008). Wetland systems are often mischaracterized as having continuously flooded and/or sustained water conditions. Nevertheless, as wetlands represent a transitional area between terrestrial and open-water systems, they often undergo considerable fluctuations in hydrology (Mitsch & Gosselink 2000, Dodds 2002). In wetlands,

*Email: btouchette@elon.edu

even slight changes in water availability can promote large changes in plant productivity and/or species composition (Mitsch & Gosselink 2000, Amlin & Rood 2002, Touchette et al. 2008). As many natural-freshwater wetlands are intermittently exposed and/or seasonally flooded, resident plants must tolerate highly unstable water conditions (Gerla 1992, Bradley 2002). For example, some seasonally flooded wetlands will undergo extensive water fluctuations as the water tables oscillate between 50 cm aboveground to 100 cm belowground over a period of weeks (Mitsch et al. 1979). Moreover, a water deficit can worsen during periods of unseasonably low precipitation or drought, often extending the duration as well as increasing the degree of water stress.

Milnes et al. (1998) evaluated physiological responses to diminishing water supply in 2 perennial grasses and noted that relative water content (θ) remained fairly constant in plant tissues until soil moisture fell below 9% (ca. -1400 kPa soil water potential). The ability to maintain high water potentials (ψ) and/or high leaf θ during periods of low water supply has been recognized as an indicator of drought resistance in some terrestrial plants (Shimshi et al. 1982, Schonfield et al. 1988, Milnes et al. 1998). In such cases, the ability to maintain high θ and/or high ψ over a wide range of soil moistures may provide some benefit by minimizing drought-induced tissue damage (Milnes et al. 1998).

Water potential- and water content-breakpoints can be useful in evaluating how plants respond to diminishing water supplies (Milnes et al. 1998). That is, by determining the point at which ψ and θ are no longer stable due to appreciable soil moisture loss, it may be possible to characterize how tolerant or intolerant plants are to short-term water deprivation and drought. In the present study, we evaluated xylem water potential (ψ_{xylem})- and θ -breakpoints in 2 obligate wetland forbs (*Justicia americana* and *Saururus cernuus*) that have contrasting water requirements. While both species have limited tolerance to prolonged drought, it has been demonstrated that *S. cernuus* can survive nearly twice as long (up to 5 wk) in low water conditions compared to *J. americana* (with high mortality rates in as little as 2 wk; Touchette et al. 2008). Therefore, the purpose of the present study was to evaluate physiological responses in these 2 wetland forbs to increasingly dryer soils. Water relation parameters that were measured over time for each plant included θ and ψ_{xylem} , and the resultant data were used to construct θ - and ψ_{xylem} -breakpoint models for both species. It was anticipated that the seemingly more drought-tolerant plant, *S. cernuus*, would be able to maintain θ and ψ_{xylem} at lower soil moistures in comparison to the less tolerant *J. americana*.

MATERIALS AND METHODS

Growth conditions. The 2 wetland forbs (*Justicia americana* L. and *Saururus cernuus* L.) used in the present study were collected from wild populations (central Piedmont region of North Carolina) in early spring and transplanted into 15 l microcosms with natural lake sediment (5 cm depth, sandy clay soil texture) and water (2 to 3 cm above the substratum). Plants were grown at densities similar to those observed in typical lake populations (70 to 100 shoots m^{-2}) and were allowed to acclimate to emerged conditions for more than 2 wk prior to the initiation of experimental treatments. At the start of the experiment, plants were approaching full size with typical dry biomasses of 0.8 and 1.2 g plant^{-1} for *J. americana* and *S. cernuus*, respectively. Water deficits were achieved by removing all surface water from drought-treated microcosms ($n = 5$ for each species), with no additional water applied to the microcosms for a period of 14 d. A 14 d drought period was selected to avoid tissue damage and elevated mortality as observed in wetland plants after > 2 wk of water deprivation (Touchette et al. 2008, Touchette & Steudler 2009). Control groups were also maintained ($n = 5$ microcosms for each species) and consisted of plants that received daily watering that sustained water levels at 2 to 3 cm above the substratum. After 2 wk of water deprivation, drought treated plants were watered at rates comparable to pre-drought/control conditions for an additional 9 to 14 d to evaluate plant recovery following water repletion.

As a general indicator of plant performance, leaf area measurements were recorded initially (Day 0), during the drought (Days 7 and 14), and 2 wk after water repletion (Day 28). Leaf areas were measured on 2 plants collected from each microcosm using a digital leaf area meter (model CI-202, CID). The mean of the 2 recorded measurements from each microcosm was treated as a single value for statistical evaluations to avoid replication within experimental units (i.e. pseudo-replication).

Plant–water relations. Plants were evaluated on 10 separate days over the 4 wk study, including pre-drought (Day 0), drought (Days 3, 5, 7, 9, 11, and 14), and post-drought periods (Days 18, 23, and 28). Leaf water content (θ) was determined according to Joly (1985) as described by the following equation:

$$\theta = (W_f - W_d)/(W_t - W_d)$$

where W_f is the fresh weight measured at the time of collection, W_t the weight at full turgor, and W_d the dry weight (dried at 60°C until constant weight). Turgid weight was determined by placing the leaf sample in 50 ml sealed containers containing deionized water

and allowing the plants to reach full turgor in darkness overnight.

Xylem water potentials (ψ_{xylem}) were measured during early morning using a Scholander pressure chamber (Scholander et al. 1965; Model 1000, PMS Instrument Company) on young, fully extended leaves enclosed within foil bags for 2 h (Meinzer et al. 2001).

Soil water content was evaluated using a gravimetric technique according to Black (1965), and expressed as the percent water by weight. Soil samples were collected using a small soil corer (1 cm in diameter) that integrated samples through the 5 cm soil profile. Water mass was determined by weighing the soil sample before and after drying (60°C until constant weight). Soil-water retention properties were also evaluated using the centrifuge method described by Khanzode et al. (2002) and modified by Reatto et al. (2008) to determine the relationship between soil moisture and soil water potential (ψ_{soil}).

Breakpoint analysis. Breakpoint models for the relationships between soil moisture and both ψ_{xylem} and θ were conducted using SigmaStat (Version 3.1, Systat Software) with the defined function as follows:

$$y = b_0 + b_1 \times (x - k) \times (x < k) + b_2 \times (x - k) \times (x \times k)$$

where k is the estimated breakpoint, b_0 is the y-intercept, and b_2 and b_1 are the linear slopes before and after breakpoint, respectively (according to Milnes et al. 1998). For this procedure, data were fitted to the model using a non-linear regression algorithm (Marquardt-Levenberg) to determine the parameters of the independent variables that provided the best fit between the equation and the experimental data. This process determined the value of each parameter that minimized the sum of the squared differences between the observed and predicted values through convergence (i.e. continues to fit new values for each parameter until the differences between the residual sum of squares no longer decrease significantly; Systat Software 2004).

Breakpoint models were constructed on all data from drought-treated plants, collectively, to provide a general model based on all observations for each species. For further statistical analyses on resultant model parameters, however, breakpoint analyses were also constructed on separate observations recorded from each individual microcosm ($n = 5$ for each species).

Data analysis. Physiological data (i.e. θ and ψ_{xylem}), leaf area, and soil moisture levels for controls and drought-treated plants were compared using a repeated-measures ANOVA (general linear model), followed by Holm-Sidak *post hoc* evaluations. Breakpoint parameters, obtained from individual microcosms ($n = 5$), were compared between the 2 species using a 1-way ANOVA. All comparisons were considered significant at $\alpha = 0.05$.

RESULTS

Over the 14 d water deprivation period, soil moisture levels significantly dropped in drought-treated microcosms ($p < 0.001$; Fig. 1). There were, however, no significant differences in the rate of water loss between the 2 plant species during that period ($p = 0.16$). In pre-drought/control conditions, water concentrations were typically $63 \pm 3\%$ (by weight); however, in treated microcosms soil moisture declined to $20 \pm 2\%$ after 7 d and $2.8 \pm 0.5\%$ after 14 d of simulated drought (Fig. 1). In general, relative to the initial water content, water loss within the microcosms was approximately $6.6 \pm 0.9\% \text{ d}^{-1}$ for the first week, then as soil moisture diminished, water loss declined to $2.5 \pm 0.9\% \text{ d}^{-1}$ in the second week.

Although there were no differences in the amount of water lost due to evapotranspiration between drought-treated *Justicia americana* and *Saururus cernuus* microcosms, there were differences in leaf area between the 2 species. The initial leaf area for *J. americana* was approximately $34 \text{ cm}^2 \text{ plant}^{-1}$, compared with $280 \text{ cm}^2 \text{ plant}^{-1}$ for *S. cernuus* (Table 1). There were no significant drought-induced changes in leaf area or any outward signs of necrosis or chlorosis in plant tissues for either species during the imposed drought; 2 wk after water repletion, however, drought-treated plants had significantly lower leaf areas in comparison to control plants (ca. 62 and 44% less leaf surface area for *J. americana* and *S. cernuus*, respectively). This difference in leaf area was not attributed to any decline in area for drought-treated plants, as leaf areas remained fairly constant in treated plants throughout the study

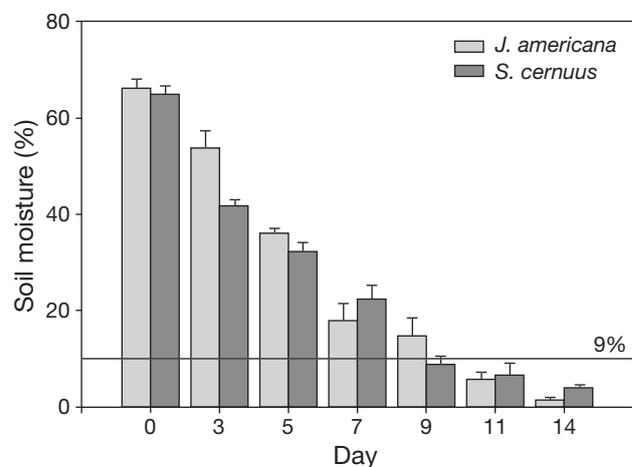


Fig. 1. *Justicia americana* and *Saururus cernuus*. Soil moisture levels in drought-treated microcosms during the 14 d water deprivation period. Soil moisture content is expressed as percent water by weight. Data are mean \pm SE. Horizontal line represents the critical breakpoint (9%) observed in perennial terrestrial grasses (Milnes et al. 1998)

Table 1. *Justicia americana* and *Saururus cernuus*. Leaf areas ($\text{cm}^2 \text{ plant}^{-1}$) measured initially (Day 0), during drought (Days 7 and 14), and following water repletion (Day 28; 2 wk after re-watering) for control and drought-treated plants. Significant differences between the control and the drought-treated plants are in **bold**. Data are mean \pm SE (n = 5)

Species (Treatment)	Day 0	Day 7	Day 14	Day 28
<i>J. americana</i>				
Control	29 \pm 4	37 \pm 3	55 \pm 8	96 \pm 12
Drought	38 \pm 9	42 \pm 5	45 \pm 3	59 \pm 18
<i>S. cernuus</i>				
Control	252 \pm 7	285 \pm 38	281 \pm 84	459 \pm 85
Drought	315 \pm 54	247 \pm 51	234 \pm 34	200 \pm 42

(although trends of increased area in *J. americana* and decreased area in *S. cernuus* were noted in treated plants). Rather, the difference between controls and drought plants for Day 28 is likely due to diminished or limited growth in treated plants in comparison to higher leaf productivities in the control. Throughout the present study, leaf areas of control plants increased by 3.3 and 1.8 times the initial value for *J. americana* and *S. cernuus*, respectively.

Low soil moistures promoted significant declines in leaf θ in both plants by Day 11 and continued through Day 14 ($p < 0.02$). During this period, θ dropped to 50 ± 5 and $32 \pm 6\%$ for *Saururus cernuus* and *Justicia americana*, respectively (Fig. 2). Nevertheless, θ in drought-treated plants recovered to levels typical of well-watered plants (i.e. between 85 and 92%) within 4 d of water repletion, indicating a comparatively rapid water recovery and rehydration.

While significant declines in θ were noted by Day 11 for *Justicia americana* and *Saururus cernuus*, drought-induced decreases in ψ_{xylem} occurred considerably sooner (Day 9) for both species (Fig. 3). In general, *J. americana* maintained lower ψ_{xylem} than *S. cernuus* during flooded and/or controlled conditions (-0.73 ± 0.03 vs. -0.43 ± 0.01 MPa, respectively), and this trend of lower ψ_{xylem} in *J. americana* continued during water deprivation. That is, the lowest ψ_{xylem} reported in *J. americana* (Day 14) was approximately 1.5 MPa lower than the values measured for *S. cernuus* (-4.3 ± 0.7 and -2.8 ± 0.5 MPa, respectively). However, unlike *J. americana*, which had similar ψ_{xylem} as the controls 4 d after water repletion, *S. cernuus* continued to have lower ψ_{xylem} 4 d after re-watering ($p = 0.04$).

Breakpoint models comparing θ with soil moisture levels between the 2 plant species reveal differences in the point at which θ sharply declined with further loss of soil water (i.e. breakpoint values, k ; Fig. 4). Not surprisingly, the models fitted with individual data had higher coefficients of determination (R^2 between 0.76

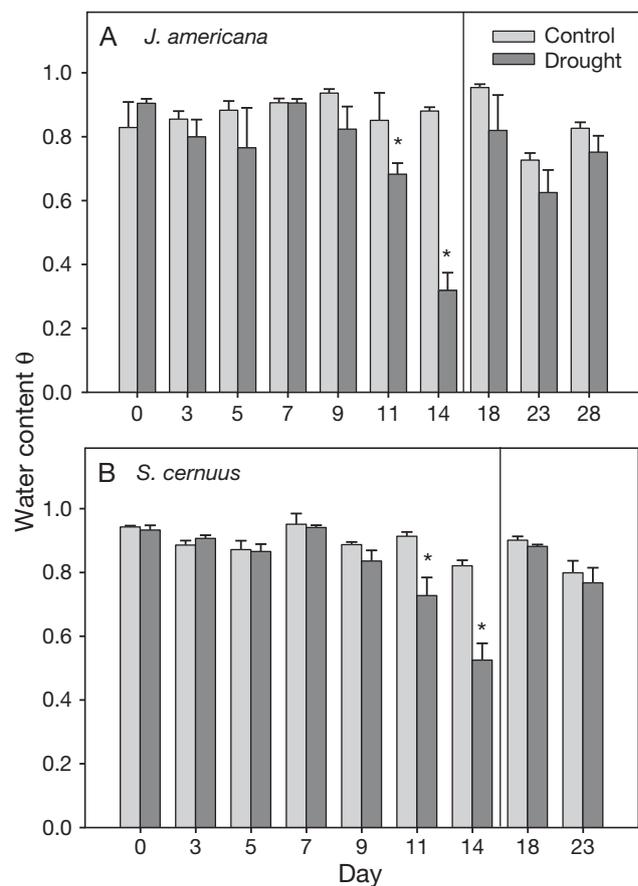


Fig. 2. *Justicia americana* and *Saururus cernuus*. Relative water content (θ) in leaf tissues for control and drought-treated plants. Water content is expressed as a proportion of full saturation. The vertical line between Days 14 and 18 represents the end of the simulated drought. Significant differences from the control are indicated by asterisks. Data are mean \pm SE

and 0.99) in comparison to models developed with all data collectively, as the collective models were likely constrained by increased variability introduced through the use of multiple replicates ($R^2 = 0.87$ and 0.79 for *Justicia americana* and *Saururus cernuus*, respectively; Table 2). For models based on individual microcosms, θ -breakpoint was significantly lower in *J. americana* ($k = 6.9 \pm 1.2\%$ soil moisture by weight) than in *S. cernuus* ($k = 11.6 \pm 1.7\%$, $p = 0.025$; Table 2). These soil moisture concentrations are equivalent to a ψ_{soil} of -3800 and -800 kPa for *J. americana* and *S. cernuus*, respectively, and suggest that, in comparison to *S. cernuus*, *J. americana* can withstand lower soil moistures without any appreciable loss of tissue water.

In contrast to the relationship for θ and soil moisture, no significant differences in k were observed with respect to ψ_{xylem} and soil water between *Saururus cernuus* and *Justicia americana* ($p = 0.417$; Fig. 5). In the present study, k values for ψ_{xylem} were 9.7 ± 1.6 and

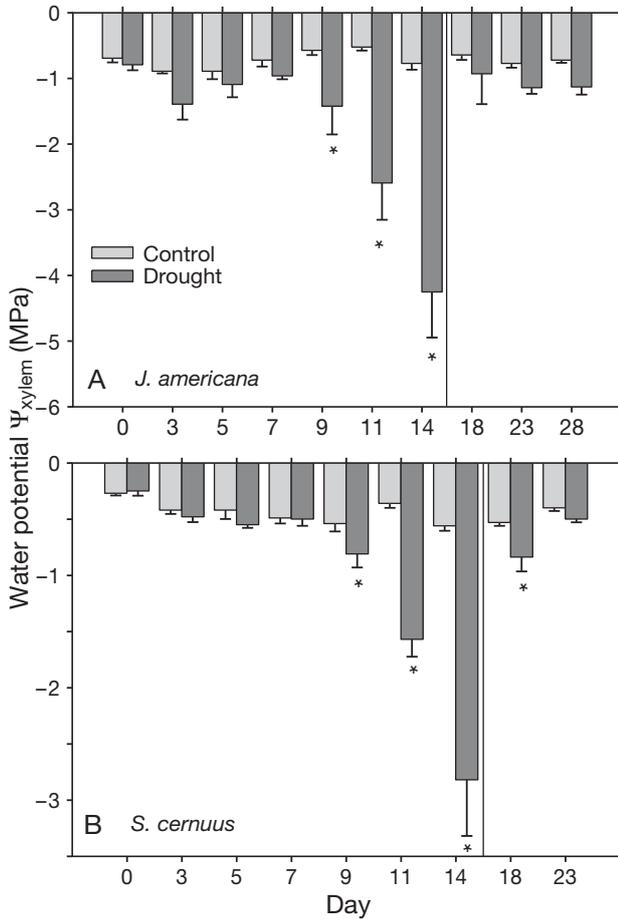


Fig. 3. *Justicia americana* and *Saururus cernuus*. Xylem water potential (Ψ_{xylem}) in leaf tissues for control and drought-treated plants. The vertical line between Days 14 and 18 represents the end of the simulated drought. Significant differences from the control are indicated by asterisks. Data are mean \pm SE

11.5 \pm 1.4% soil moisture (or -1500 and -800 kPa for Ψ_{soil}) for *J. americana* and *S. cernuus*, respectively (Table 2). Breakpoint values for θ and Ψ_{xylem} were similar in *S. cernuus* (approximately 11.5%). While it would appear that k for Ψ_{xylem} occurred prior to (i.e. at higher soil moistures) that for θ in *J. americana*, these values were not statistically different (1-way ANOVA, $p = 0.19$).

DISCUSSION

The rates of water loss from the experimental microcosms were similar between the 2 species, and represent 2 different water efflux pathways, namely, evaporation and transpiration. Although we did not measure each pathway directly, we can account for total evapotranspiration through the loss of soil moisture over

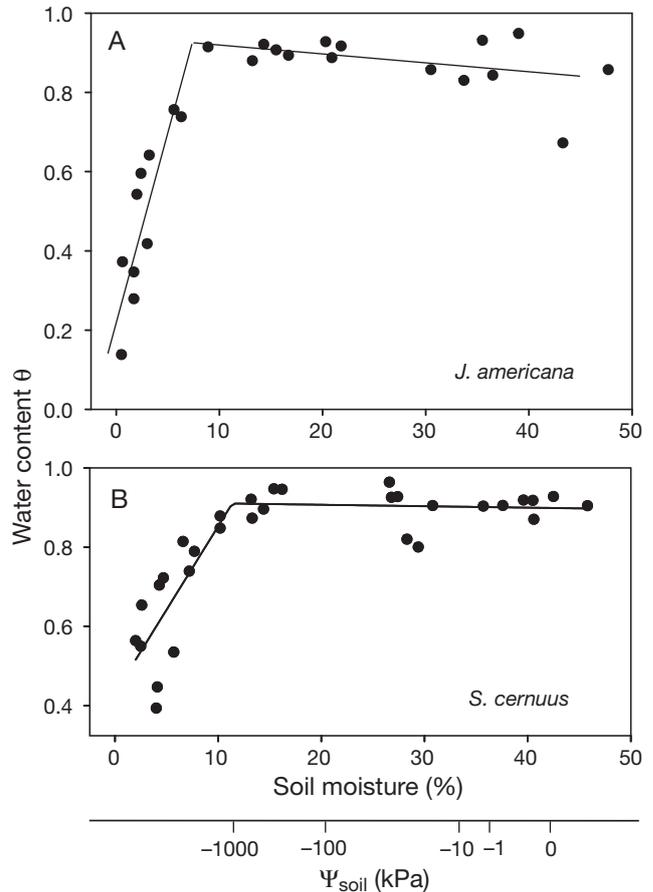


Fig. 4. *Justicia americana* and *Saururus cernuus*. Relative water content (θ) in leaf tissues, plotted against soil moisture (% by weight). Each point represents a single measurement from an individual plant. The trend lines represent the best-fit breakpoint models (fitted using a non-linear Marquardt-Levenberg regression algorithm) for the function $y = b_0 + b_1 \times (x - k) \times (x < k) + b_2 \times (x - k) \times (x \times k)$, where k represents the soil moisture breakpoint for θ (see Table 2)

time. In this case, evapotranspiration rates were comparatively high for Week 1 (ca. 6.6% d^{-1}), followed by a sharp reduction in water loss (ca. 2.5% d^{-1}) during Week 2. Lower evapotranspiration rates in Week 2 can be attributed to decreases in both soil water potential (Ψ_{soil}), making it increasingly more difficult for water loss as soil dries, and stomatal conductance (Kramer 1983). Although *Saururus cernuus* has greater leaf area and higher transpiration rates relative to *Justicia americana*, both species will substantially lower transpiration during periods of low water availability (Touchette et al. 2007). Therefore, the similar rates of water loss in microcosms containing different species suggest that evaporation is the dominant water efflux pathway in these experimental systems. If transpiration were the prevailing process, higher rates of water loss in microcosms containing *S. cernuus* (compared to *J. americana*) would likely occur.

Table 2. *Justicia americana* and *Saururus cernuus*. Estimated breakpoint parameters for the relationship between soil water content and leaf water content (θ) or xylem water potential (Ψ_{xylem}). Data are mean \pm SE for plants evaluated individually ($n = 5$) and values for plants considered collectively (see Figs. 4 & 5). Parameters were used to estimate the model: $y = b_0 + b_1 \times (\text{soil moisture} - k) \times (\text{soil moisture} < k) + b_2 \times (\text{soil moisture} - k) \times (\text{soil moisture} \times k)$, where k is defined as the breakpoint. Soil water potentials (Ψ_{soil}) at breakpoint are provided for comparison purposes. Coefficients of determination (R^2) illustrate the range in fit (for individual data) or the degree of correlation (combined data) between data and the models. Significant differences in breakpoint parameters between species are in **bold**

	b_0	b_1	b_2	k	Ψ_{soil} (kPa)	R^2
Leaf water content (θ)						
Plants individually						
<i>J. americana</i>	0.92 \pm 0.02	0.18 \pm 0.04	-0.00075 \pm 0.0007	6.86 \pm 1.24	-4160 \pm 1430	0.86 to 0.99
<i>S. cernuus</i>	0.89 \pm 0.01	0.05 \pm 0.01	0.00055 \pm 0.0003	11.64 \pm 1.68	-1010 \pm 250	0.76 to 0.94
Plants collectively						
<i>J. americana</i>	0.92	0.09	-0.00205	7.54	-3250	0.87
<i>S. cernuus</i>	0.91	0.04	-0.00036	11.36	-890	0.79
Xylem water potential (Ψ_{xylem})						
Plants individually						
<i>J. americana</i>	-1.07 \pm 0.06	0.49 \pm 0.17	-0.00007 \pm 0.0021	9.73 \pm 1.55	-1560 \pm 866	0.76 to 0.99
<i>S. cernuus</i>	-0.71 \pm 0.04	0.24 \pm 0.06	0.00938 \pm 0.0008	11.49 \pm 1.35	-1190 \pm 410	0.77 to 0.99
Plants collectively						
<i>J. americana</i>	-1.04	0.43	-0.00498	8.46	-2300	0.74
<i>S. cernuus</i>	-0.80	0.22	0.0118	10.22	-1280	0.61

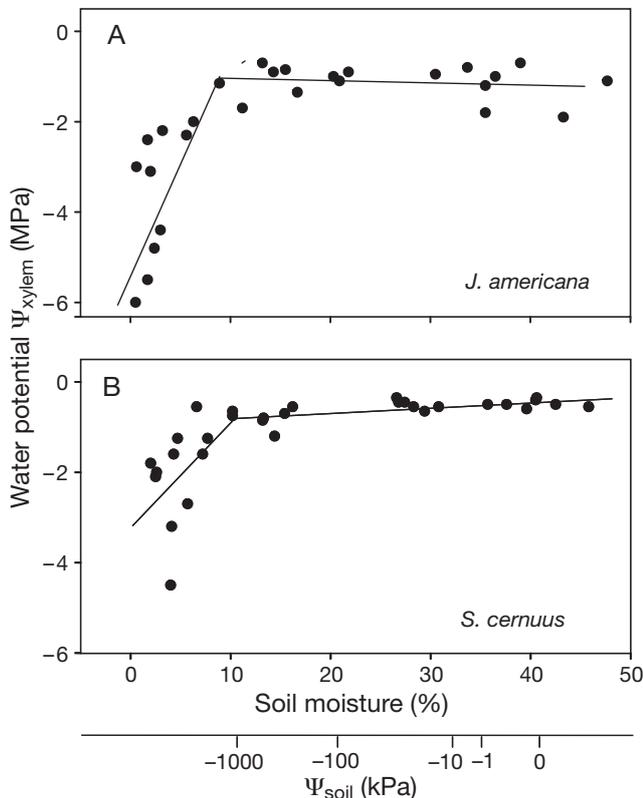


Fig. 5. *Justicia americana* and *Saururus cernuus*. Xylem water potential (Ψ_{xylem}) in leaf tissues of drought-treated plants, plotted against soil moisture (% by weight). Data points and trend lines as defined in Fig. 4

Leaf growth of treated plants during the simulated drought included either slight increases in surface area (*Justicia americana*) or no changes in total area (*Saururus cernuus*), and this response continued into the first 2 wk of re-watering. In contrast, control plants grown in flooded conditions maintained comparatively high leaf productivities. Leaf sensitivity to low water conditions is fairly common in plants (Puliga et al. 1996, Milnes et al. 1998, Touchette et al. 2008). In a microcosm study on drought and wetland macrophytes, both *J. americana* and *S. cernuus* had significant reductions in leaf area following simulated drought conditions, with *J. americana* appearing to be more sensitive than *S. cernuus*—as indicated by an 85% reduction in leaf area for *J. americana* 5 wk after experiencing a 2 wk drought (Touchette et al. 2008). However, the responses observed in the present study were not as severe, as leaf areas of drought-treated *J. americana* actually increased by 55% by the end of the investigation. In perennial terrestrial grasses, *Briza media* (L.) and the more xeric species *Koeleria macrantha* (Ledeb.), declines in leaf growth were reported as soil moisture levels fell below 15% (Milnes et al. 1998). Furthermore, while leaf tissues of the drought-treated wetland forbs in the present study appeared to recover (as indicated by dark-green tissue coloration with typical tissue water levels) within a few days of re-watering (θ declined to 31 and 52% for drought-treated *J. americana* and *S. cernuus*, respectively), recovery in the terrestrial grasses required an additional 17 d of well-

watered conditions (θ decreased to 40%). This faster recovery in wetland forbs following tissue dehydration may be associated with: (1) greater water availability (i.e. flooding conditions) during water repletion; (2) the maintenance of viable aboveground tissue in comparison to grasses where the leaves appeared dry and shriveled (Milnes et al. 1998); or (3) differences in natural habitat hydrology, wherein wetland systems can experience cyclical flooding and drying over a growing season (Gerla 1992, Bradley 2002, Thompson et al. 2007, Laitinen et al. 2008).

Physiological parameters measured on these aquatic forbs were consistent with the values reported for other plant species. In general, θ of well-watered vegetation is around 95% (although considerable variability does occur among plants; Ritchie et al. 1990, Kalapos 1994, Milnes et al. 1998, Touchette et al. 2007). In the present study, drought-treated macrophytes maintained comparable θ values through the first 9 d of water deprivation, even as soil moisture levels declined to 9%. This delay in tissue water loss is consistent with similar studies using terrestrial plants from calcicolous grasslands, *Briza media* and *Koeleria macrantha*, where significant declines in θ were not observed until after the first week (Milnes et al. 1998). Interestingly, the maintenance of high ψ and θ in plant tissues at low soil moistures has been recognized as a drought-resistant characteristic (Shimshi et al. 1982, Schonfield et al. 1988, Milnes et al. 1998, Romanello et al. 2008). The wetland forbs used in the present study may have similar drought-resistant qualities, as they appear to be able to maintain high ψ_{xylem} and θ over a wide range of water availabilities. This may confer some resistance to periodic water scarcities in emergent angiosperms that live along the transitional area between terrestrial uplands and deep-water systems. In the present study, however, the initial soil water concentrations were much higher (ca. 64%) than the levels used by Milnes et al. (1998) during an investigation on terrestrial plants (ca. 28%). Nevertheless, soil moisture levels by Day 9 were consistent with terrestrial soils with moderate to low precipitation, as well as some wetland systems during dryer periods (Callaway 1990, Castelli et al. 2000, Lookingbill & Urban 2004).

As with θ , there was an expected decrease in ψ_{xylem} when soil moisture levels diminished. The decline in ψ_{xylem} , however, was observed 2 d prior to any decline in θ , suggesting that ψ_{xylem} may be an early and sensitive indicator of drought stress in these plants. This notion is further supported by *Saururus cernuus* during water repletion, where ψ_{xylem} remained lower in drought-treated plants while θ had apparently recovered to pre-drought conditions. Although xylem cavitation could account for prolonged water stress, thereby promoting lower ψ_{xylem} in *S. cernuus* beyond

re-watering (Hacke & Sauter 1996), tissue rehydration, as indicated by a comparatively rapid θ recovery, suggests that cavitations were minimal and/or reversible (Linton et al. 1998, Tyree et al. 1999, Linton & Nobel 2001). In contrast to our findings, a study on terrestrial grasses noted significant declines in ψ_{leaf} 3 d after observing significant drops in θ (Milnes et al. 1998). This difference in timing response for θ and ψ between the 2 plant groups may, again, reflect differences in environmental hydrology between wetland and grassland systems.

In general, the models based on θ and ψ_{xylem} were quite similar between the 2 forbs with respect to the estimated breakpoint parameters, and are expected in most plants, as declines in tissue θ often promote lower ψ_{xylem} (Slavík 1974, Kramer 1983, Kramer & Boyer 1995). Nevertheless, breakpoint models of θ against soil moisture for *Justicia americana* and *Saururus cernuus* do show some notable differences between the 2 wetland forbs. For example, the breakpoint for θ was much higher in *S. cernuus* ($k = 11.6 \pm 1.7\%$ soil moisture; $\psi_{\text{soil}} = \text{ca. } -800 \text{ kPa}$) than for *J. americana* ($k = 6.9 \pm 1.2\%$ soil moisture; $\psi_{\text{soil}} = \text{ca. } -3800 \text{ kPa}$). The higher k observed in *S. cernuus* was more in line with the initial hypothesis. That is, the aquatic nature of these plants would seemingly foster adaptations that promote survival in water-saturated and/or flooded environments, and thus the capability of these plants to tolerate lower water levels, typical of dryer terrestrial systems, should be more limited. Furthermore, the observed ψ_{xylem} breakpoint for the primitive wetland monocot *Acorus americanus*, which is capable of sustained growth in <10% soil moisture for >5 wk, appears to occur between 12 and 15% soil moisture (Romanello et al. 2008). Thus, the ability of the obligate wetland plant *J. americana* to maintain stable tissue water levels at such low soil moistures (ca. 7% soil water) is in contrast to our initial expectations. The observed ψ_{xylem} breakpoint values for *J. americana* are within the range of terrestrial grasses that are naturally exposed to periodic and seasonal drought (k for grasses were between 6.3 and 8.7% soil moisture; Milnes et al. 1998).

A possible explanation for why k for θ was reached at higher soil moistures for *Saururus cernuus* in comparison to *Justicia americana* may center around dissimilarities in plant-water relations between the 2 species. *J. americana* typically maintains lower ψ_{xylem} (in comparison to *S. cernuus* during both flooded and drought conditions), and therefore is more capable of maintaining an adequate influx of water from relatively dryer soils (Blizzard & Boyer 1980, Sperry et al. 2003). In contrast, *S. cernuus* maintains higher ψ_{xylem} , which would require either an increase in solute concentrations (inorganic and/or organic solutes) or a decrease in tissue

water content (i.e. θ) to generate lower ψ_{xylem} (Edwards & Jarvis 1982, Johnson et al. 1984, Clifford et al. 1998). Thus by lowering θ , *S. cernuus* would be able to generate lower ψ_{xylem} and thereby maintain favorable plant–soil relations as soils become increasingly dryer.

In conclusion, 2 wk of water deprivation resulted in noticeable differences in both leaf productivity and/or development and plant–water relations in these 2 wetland forbs. Interestingly, the overall breakpoint parameters observed in *Justicia americana* were quite similar to those previously reported in terrestrial grasses; however, the recovery of θ following water repletion was remarkably faster in both wetland plants in comparison to that observed in upland grasses. Rapid tissue-water recovery following extreme water deficits could be beneficial to plants residing in environments that oscillate between flooded and dry conditions. Furthermore, these forbs maintained comparatively high ψ_{xylem} and θ as soil moisture diminished—a characteristic that is often attributed to drought resistance. Therefore, the results suggest that these 2 forbs are physiologically adapted to aquatic environments that undergo rapid and substantial changes in water availability, including the ability to tolerate short periods of water scarcity comparable to dry upland and/or terrestrial conditions.

Acknowledgements. Research assistance for this study was provided by G. Romanello. We appreciate the comments and insights provided by Y. Miyamoto, D. Vandermast, and 3 anonymous reviewers on earlier versions of this manuscript. This study was supported through research grants from the UNC Water Resources Research Institute and the US Geological Survey, with additional support from the Elon University Center for Environmental Studies.

LITERATURE CITED

- Amlin NM, Rood SB (2002) Comparative tolerances of riparian willows and cottonwoods to water table decline. *Wetlands* 22:338–346
- Black CA (1965) Methods of soil analysis: I. Physical and mineralogical properties. American Society of Agronomy, Madison, WI
- Blizzard WE, Boyer JS (1980) Comparative resistance of the soil and plant to water transport. *Plant Physiol* 66:809–814
- Bradley C (2002) Simulation of the annual water table dynamics of a floodplain wetland, Narborough Bog, UK. *J Hydrol (Amst)* 261:150–172
- Callaway RM (1990) Effects of soil water distribution on the lateral root development of three species of California oaks. *Am J Bot* 77:1469–1475
- Castelli RM, Chambers JC, Tausch RJ (2000) Soil–plant relations along a soil–water gradient in Great Basin riparian meadows. *Wetlands* 20:251–266
- Clifford S, Arndt S, Corlett J, Joshi S, Sankhla N, Popp M, Jones H (1998) The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). *J Exp Bot* 49: 967–977
- Dodds WK (2002) Freshwater ecology: concepts and environmental applications. Academic Press, New York
- Edwards WRN, Jarvis PG (1982) Relation between water content, potential and permeability in stems of conifers. *Plant Cell Environ* 5:271–277
- Gerla PJ (1992) The relationship of water table changes to the capillary fringe, evapotranspiration and precipitation in intermittent wetlands. *Wetlands* 12:91–98
- Hacke U, Sauter JJ (1996) Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus glutinosa* (L.) Gaertn. *Plant Physiol* 111: 413–417
- Johnson RC, Nguyen HT, Croy LI (1984) Osmotic adjustment and solute accumulation in two wheat genotypes differing in drought resistance. *Crop Sci* 24:957–962
- Joly RJ (1985) Techniques for determining seedling water status and their effectiveness in assessing stress. In: Duryea ML (ed) Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Forest Research Laboratory, Oregon State University, Corvallis, OR, p 17–28
- Kalapos T (1994) Leaf water potential–leaf water deficit relationship for ten species of a semiarid grassland community. *Plant Soil* 160:105–112
- Khanzode RM, Vanapalli SK, Fredlund DG (2002) Measurement of soil-water characteristics curve for fine-grained soils using a small-scale centrifuge. *Can Geotech J* 39: 1209–1217
- Kramer PJ (1983) Water relations of plants. Academic Press, New York
- Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, New York
- Laitinen J, Rehell S, Oksanen J (2008) Community and species responses to water level fluctuations with reference to soil layers in different habitats of mid-boreal mire complexes. *Plant Ecol* 194:17–36
- Linton MJ, Nobel PS (2001) Hydraulic conductivity, xylem cavitation, and water potential for succulent leaves of *Agave deserti* and *Agave tequilana*. *Int J Plant Sci* 162: 747–754
- Linton MJ, Sperry JS, Williams DG (1998) Limits to water transport in *Juniperus osteosperma* and *Pinus edulis*: implications for drought tolerance and regulation of transpiration. *Funct Ecol* 12:906–911
- Lookingbill T, Urban D (2004) An empirical approach towards improved spatial estimates of soil moisture for vegetation analysis. *Landscape Ecol* 19:417–433
- Meinzer FC, Clearwater MJ, Goldstein G (2001) Water transport in trees: current perspectives, new insights and some controversies. *Environ Exp Bot* 45:239–262
- Milnes KJ, Davies WJ, Rodwell JS, Francis BJ (1998) The responses of *Briza media* and *Koeleria macrantha* to drought and re-watering. *Funct Ecol* 12:665–672
- Mitsch WJ, Gosselink JG (2000) Wetlands. John Wiley, New York
- Mitsch WJ, Dorge CL, Wiemhoff JR (1979) Ecosystem dynamics and a phosphorus budget of an alluvial cypress swamp in southern Illinois. *Ecology* 60:1116–1124
- Puliga S, Vazzana C, Davies WJ (1996) Control of crops leaf growth by chemical and hydraulic influences. *J Exp Bot* 47:529–537
- Reatto A, Medrado da Silva E, Bruand A, Souza Martins E (2008) Validity of the centrifuge method for determining the water retention properties of tropical soils. *Soil Sci Soc Am J* 72:1547–1553
- Ritchie SW, Nguyen HT, Holaday AS (1990) Leaf water content and gas exchange parameters of two wheat

- genotypes differing in drought resistance. *Crop Sci* 30: 105–111
- Romanello GA, Chuchra-Zbytniuk KL, Vandermer JL, Touchette BW (2008) Morphological adjustments promote drought avoidance in the wetland plant *Acorus americanus*. *Aquat Bot* 89:390–396
- Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* 148: 339–346
- Schonfield MA, Johnson RC, Carver BF, Mornhinweg DW (1988) Water relations in winter wheat as drought resistance indicators. *Crop Sci* 28:526–531
- Shimshi D, Mayoral ML, Atsman D (1982) Responses to water stress in wheat and related wild species. *Crop Sci* 22: 123–128
- Slavík B (1974) *Methods of studying plant water relations*. Springer-Verlag, Berlin
- Sperry JS, Stiller V, Hacke UG (2003) Xylem hydraulics and the soil-plant-atmosphere continuum: opportunities and unresolved issues. *Agron J* 95:1362–1370
- Systat Software (2004) *SigmaStat 3.1 user's manual*. Systat Software, Point Richmond, CA
- Thompson Y, Sandefur BC, Miller JO, Karathanasis AD (2007) Hydrologic and edaphic characteristics of three mountain wetlands in southeastern Kentucky, USA. *Wetlands* 27: 174–188
- Touchette BW (2006) Salt tolerance in a *Juncus roemerianus* brackish marsh: spatial variations in plant–water relations. *J Exp Mar Biol Ecol* 337:1–12
- Touchette BW, Steudler SE (2009) Climate change, drought, and wetland vegetation. In: Uzochukwu GA, Schimmel K, Chang SY, Kabadi V, Luster-Teasley S, Reddy G, Nzewi E (eds) *Proc Natl Conf Environ Sci Technol*. Springer Science, New York, p 239–244
- Touchette BW, Iannacone LR, Turner GE, Frank AR (2007) Drought tolerance versus drought avoidance: a comparison of plant–water relations in herbaceous wetland plants subjected to water withdrawal and repletion. *Wetlands* 27:656–667
- Touchette BW, Frank A, Iannacone LR, Turner G (2008) Drought susceptibility in emergent wetland angiosperms: a comparison of water deficit growth in five herbaceous perennials. *Wetlands Ecol Manage* 16:485–497
- Tyree MT, Salleo S, Nardini A, Lo Gullo MA, Mosca R (1999) Refilling of embolized vessels in young stems of laurel. Do we need a new paradigm? *Plant Physiol* 120:11–21

Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany

*Submitted: December 9, 2008; Accepted: May 29, 2009
Proofs received from author(s): June 30, 2009*