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Photosynthetic performance of invasive *Pinus ponderosa* and *Juniperus virginiana* seedlings under gradual soil water depletion

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Abstract

Changes in climate, land management and fire regime have contributed to woody species expansion into grasslands and savannas worldwide. In the USA, *Pinus ponderosa* P. & C. Lawson and *Juniperus virginiana* L. are expanding into semiarid grasslands of Nebraska and other regions of the Great Plains. We examined *P. ponderosa* and *J. virginiana* seedling response to soil water content, one of the most important limiting factors in semiarid grasslands, to provide insight into their success in the region. Photosynthesis, stomatal conductance, maximum photochemical efficiency of PSII, maximum carboxylation velocity, maximum rate of electron transport, stomatal limitation to photosynthesis, water potential, root-to-shoot ratio, and needle nitrogen content were followed under gradual soil water depletion for 40 days. *J. virginiana* maintained lower L_s , higher A , g_s , and initial F_v/F_m , and displayed a more gradual decline in V_{cmax} and J_{max} with increasing water deficit compared to *P. ponderosa*. *J. virginiana* also invested more in roots relative to shoots compared to *P. ponderosa*. F_v/F_m showed high PSII resistance to dehydration in both species. Photoinhibition was observed at ~30% of field capacity. Soil water content was a better predictor of A and g_s than Ψ , indicating that there are other growth factors controlling physiological processes under increased water stress. The two species followed different strategies to succeed in semiarid grasslands. *P. ponderosa* seedlings behaved like a drought-avoidant species with strong stomatal control, while *J. virginiana* was more of a drought-tolerant species, maintaining physiological activity at lower soil water content. Differences between the studied species and the ecological implications are discussed.

Keywords: F_v/F_m , Great Plains, J_{max} , semiarid grasslands, stomatal limitation, V_{cmax} , water potential

Introduction

The Nebraska Sandhills are the largest stabilized sand dune formation in the Western Hemisphere (50,000 km², Bleed & Flowerday 1998). Although the Sandhills are dominated by grasses and managed mainly as rangelands for livestock production, the area has been witnessing changes in vegetation cover, including a significant increase in woody species expansion (Fuhlendorf et al. 2008; McKinley & Blair 2008), especially eastern red cedar (*Juniperus virginiana* L.) and ponderosa pine (*Pinus ponder-*

osa P. & C. Lawson). *P. ponderosa* is a major forest type in the western USA (Sala et al. 2005), and has expanded into adjacent grasslands from historical grassland–woodland ecotones (Steinauer & Bragg 1987; Shinneman & Baker 1997). *J. virginiana* is the most widely distributed Juniperus species in the continental USA, and can be found in every state east of the 100th meridian (McKinley & Blair 2008). It is spreading and increasing in abundance and dominance throughout the Nebraska Sandhills (Schmidt & Stubbendieck 1993) and the Great Plains (McKinley & Blair 2008; Willson et al. 2008). *J. virginiana* was character-

ized by Schmidt & Stubbendieck (1993) as “the most rapidly expanding woody species on rangelands in the Great Plains,” invading more than 20,000 ha of grasslands in western Nebraska in the last 10 years (US Forest Service), affecting approximately 7 million ha of grasslands in its western distribution of the eastern Great Plains (McKinley et al. 2008), and resulting in complete conversion to closed-canopy forests in as little as 40 years (Hoch 2000). Altered fire regimes (Bond et al. 2005; McKinley & Blair 2008), climate change (Bradley & Fleishman 2008), atmospheric nitrogen deposition, and changing land-use practices are implicated in the increased success of woody species over native grasses in semiarid grasslands in the USA and elsewhere (Scholes & Archer 1997; McCarron & Knapp 2001; Briggs et al. 2002; Fuhlendorf et al. 2008).

Soil moisture deficit has been reported to be one of the key factors limiting plant growth and ecosystem productivity worldwide (Chaves et al. 2003; Duursma et al. 2008), and is a key determinant of vegetation type, including relative abundance of grasses and woody species in semiarid grasslands like the Nebraska Sandhills (Huxman et al. 2005; Darrouzet-Nardi et al. 2006; Eggemeyer et al. 2006, 2009). A field study that examined ecophysiological and growth traits of mature *P. ponderosa* and *J. virginiana* trees and coexistent dominant grasses, Eggemeyer et al. (2006, 2009) reported that trees avoided and recovered from summer drought due in part to their deep and plastic root systems, while grasses senesced. *P. ponderosa* trees are also reported to have high stomatal control to avoid xylem cavitation induced by water stress (Law et al. 2001; Martínez-Vilalta & Piñol 2004; Sala et al. 2005; Eggemeyer et al. 2006), and *J. virginiana* trees possess inherently high water use efficiency and an ability to maintain stomatal opening at low water potentials and are, therefore, well-adapted to drought conditions (Eggemeyer et al. 2006; Willson et al. 2008).

Differences in drought resistance strategies between mature *P. ponderosa* and *J. virginiana* trees in the semiarid grasslands of the Nebraska Sandhills have been investigated (i.e., Eggemeyer et al. 2006, 2009) and, while there is a large body of literature on the ecophysiology of *P. ponderosa* and to a lesser extent on *J. virginiana* trees, it remains unclear how individuals at the seedling stage survive in areas where several years of summer drought are not uncommon (Van Auken & McKinley 2008). Seedling growth and survival is governed by dynamic interaction of biotic and abiotic factors, with water availability being a key factor in semiarid grasslands (Van Auken & McKinley 2008). Age-specific differences in plant responses to drought have been reported (e.g., Richardson 2000; Domec et al. 2004) and, given the recent history of invasiveness in these two species, understanding their resistance mechanisms to drought at different stages of development may provide important insights into their new-found encroachment into this region. This study evaluated the ecophysiological responses of *J. virginiana* and *P. ponderosa* seedlings to gradual soil water depletion – the most

common situation in natural ecosystems (Bogeat-Triboulot et al. 2007) – in a controlled environment. Our specific objectives were to examine the effects of gradual soil water depletion on photosynthesis (A), photosynthetic capacity (V_{cmax} and J_{max}), stomatal limitation to photosynthesis (L_s), PSII maximum efficiency (F_v/F_m), and water relations in *P. ponderosa* and *J. virginiana* seedlings. Recurrent measurements of these parameters provide a means to better understand species strategies to cope with water deficit in the seedling stage, and to explain species patterns of expansion in semiarid grasslands.

Materials and methods

Plant material and growth conditions

The study was conducted on 2-year-old *Pinus ponderosa* and *Juniperus virginiana* seedlings. Bare-root seedlings originating from natural populations in Nebraska National Forests and surrounding areas were obtained from the Nebraska National Forest Nursery at Halsey, Nebraska. Seedlings were individually transplanted into 32-l pots filled with greenhouse sand media fertilized with standard slow release fertilizer (NPK 30:10:10, 6 ml). Plants were left to acclimate for 8 weeks in a greenhouse before pots were transferred into a walk-in growth chamber. The chamber night:day temperatures were 20:26 °C (26 °C is the average maximum daily temperature during the growing season in the Nebraska Sandhills), and vapor pressure deficits were 1.05:1.51 kPa. Seedlings were subjected to a 12-h light:dark cycle (photosynthetic active radiation (PAR) at the foliage level was 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Seedlings were allowed to acclimate for an additional 8 weeks in the growth chamber before initiation of the water deprivation treatment. Gradual soil water depletion was initiated by withholding water for 40 days. Volumetric soil water was continuously monitored in two pots (one per species) using time domain reflectometry calibrated for sandy soils (TDR technology; CS625 Campbell Scientific Inc, Logan, UT, USA) (Figure 1). The soil volumetric water content (VWC %) progressively declined from 5.99% at field capacity to ~1.5% (25% of field capacity) at the end of the experiment (40 days).

Measured parameters

Gas exchange expressed on a leaf area basis [for *J. virginiana* based on linear regressions, Cregg (1991), and for *P. ponderosa* based on needle dimensions, Wykoff (2002)] was followed on five individuals per species with a portable photosynthetic system mounted with a fluorescence chamber (LI-6400-40; Li-Cor Inc, Lincoln, NE, USA). Measurements started at around 10:00 (~3 h after the beginning of the light period) and were completed at latest by 13:00. Maximum net photosynthesis (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) were measured at saturating PAR of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (determined from

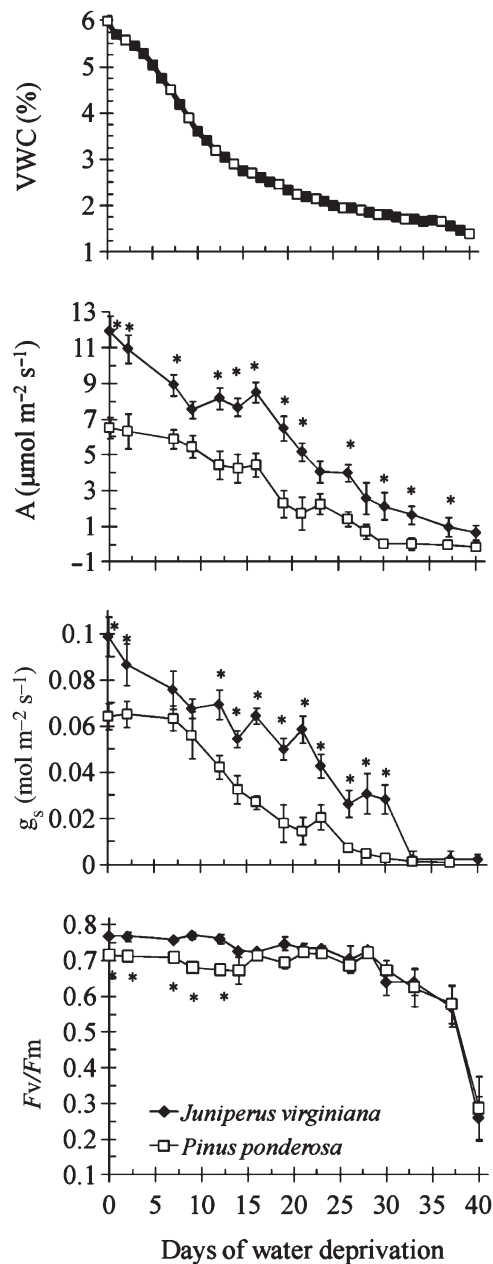


Figure 1. Volumetric water content (VWC %) under gradual soil water depletion in *Pinus ponderosa* and *Juniperus virginiana* in 32-l pots filled with sand (open symbols indicate dates of physiological measurements, SE per date averaged ± 0.0051); net photosynthesis (A) at light saturation (PAR $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$); stomatal conductance (g_s); and maximum photochemical efficiency of PSII (F_v/F_m), with standard error bars. An asterisk indicates significant differences between species within date ($P < 0.05$).

trial photosynthesis light response curves). The response of photosynthesis to CO_2 concentration (A/C_i) was determined at light saturation following the protocol described in Long & Bernacchi (2003). The chamber CO_2 concentrations used to generate A/C_i curves were 800, 600, 400, 300, 200, 100, and $50 \mu\text{mol mol}^{-1}$ (Long & Bernacchi 2003; Xu & Baldocchi 2003). Chamber CO_2 concentrations were ini-

tially increased to $1500 \mu\text{mol mol}^{-1}$ but since A saturation occurred at $\sim 600 \mu\text{mol mol}^{-1}$ and we did not record significant differences between measured and derived parameters, we decreased the maximum levels to $800 \mu\text{mol mol}^{-1}$ for time management, especially under increased water deficit. Photosynthesis was first measured at growth chamber ambient CO_2 concentration (C_a $400 \mu\text{mol mol}^{-1}$), then C_a was lowered stepwise to 300, 200, 100, and $50 \mu\text{mol mol}^{-1}$, and A was recorded at each level. After completion of measurements at $50 \mu\text{mol mol}^{-1}$, C_a was returned to $400 \mu\text{mol mol}^{-1}$ and A was measured again for validation. Once this was achieved, C_a was increased stepwise to $800 \mu\text{mol mol}^{-1}$. Maximum carboxylation velocity (V_{cmax}) and rate of electron transport (J_{max}) were calculated for each curve by non-linear regression (protocol described in Farquhar 1980; Xu & Baldocchi 2003; Manter & Kerrigan 2004). It became difficult to obtain reliable A/C_i curves with the increase in soil water deficit, especially for *P. ponderosa*, hence, the lack of data points for V_{cmax} and J_{max} on later sampling dates. Stomatal or gas phase limitation to photosynthesis (L_s), an estimate of the relative proportion of photosynthesis reduction attributable to CO_2 diffusion between the atmosphere and site of carboxylation was determined from A/C_i curves (Farquhar & Sharkey 1982; Tissue et al. 2005) as $L_s = (1 - A/A_0)\%$, where A is the rate of photosynthesis at ambient chamber C_a ($400 \mu\text{mol mol}^{-1}$) and A_0 is the rate of photosynthesis that would be achieved if gas phase limitation was eliminated (i.e., C_i equals C_a). These calculations make the assumption that mesophyll conductance is large (Farquhar & Sharkey 1982; Tissue et al. 2005).

Chlorophyll fluorescence of dark-adapted needles was measured with a leaf chamber fluorometer (6400-40) attached to the LI-6400 infrared gas analyzer (Li-Cor Inc). The variable fluorescence (F_v) is the difference between F_o and F_m . When measured in the dark, F_v/F_m is proportional to the maximum potential quantum yield of photosynthesis (system settings can be found in LI-6400-40 manual). Measurements were completed before the start of the light period; trial measurements on both species prior to treatment initiation showed that F_v/F_m was consistently underestimated in *P. ponderosa* needles covered with dark-adapting clips (provided by Li-Cor) due to needle length.

Water potential (Ψ_w) was determined on three individuals per species per sampling date with a pressure chamber (PMS Instruments, Albany, OR, USA). Different plants were sampled at each date to reduce destructive sampling pressure on seedlings.

Specific leaf area (SLA = leaf area/dry weight, $\text{cm}^2 \cdot \text{g}^{-1}$) and root-to-shoot ratio (R/S) were determined on three individuals per species that were set aside at the end of the study. Leaf area was measured and leaves were dried in a forced-air oven at 75°C until weight was constant. The R/S (g g^{-1} dry weight) was determined using weights obtained by separately drying above- and excavated belowground biomass at 75°C . Nitrogen content

in needles of five individuals per species was determined using a FP428 nitrogen determination system 601-700-300 (Leco Corporation, St Joseph, MO, USA).

Data were analyzed using the SAS statistical package (SAS Institute Inc 1998). Repeated measure analyses were used to compare species responses and to determine the effects of water depletion on measured parameters. Orthogonal contrasts were used to contrast species trends, and regression analyses were performed on A versus g_s , J_{\max} and V_{\max} trends. Data were also analyzed by date to compare species responses within dates. ANOVA in SAS was used to compare SLA, N content and R/S between species.

Results

Leaf gas exchange

Repeated measure analysis and orthogonal contrasts indicated significant differences between species A and g_s in response to water deprivation. *Juniperus virginiana* exhibited significantly higher A and g_s than *Pinus ponderosa* (Figure 1, $F_A = 49.4$, $P < 0.0001$; $F_{g_s} = 29.8$, $P < 0.0001$). Under well-watered conditions (day 0), A was 11.92 and $6.52 \mu\text{mol m}^{-2} \text{s}^{-1}$, and g_s was 0.098 and $0.064 \text{ mol m}^{-2} \text{s}^{-1}$ in *J. virginiana* and *P. ponderosa*, respectively. Significant declines in A and g_s were observed shortly after initiation of water deprivation ($F_A = 13.5$, $P < 0.0001$; $F_{g_s} = 10.8$, $P < 0.0001$), reaching minimal values after 30 days (1.8% VWC, 30% of field capacity) in *P. ponderosa*, while *J. virginiana* maintained a positive A ($\sim 1 \mu\text{mol m}^{-2} \text{s}^{-1}$) until 40 days of water deprivation (1.5% VWC, 25% of field capacity). Maximum efficiency of PSII (measured as F_v/F_m) was significantly higher in the first 12 days of water deprivation in *J. virginiana* than in *P. ponderosa* (average 0.76 versus 0.69, respectively), differences between species disappeared thereafter (Figure 1). Maximum efficiency of PSII (F_v/F_m) showed little variability in the first 28 days of water deprivation in both species before declining when VWC fell below 1.9% ($\sim 32\%$ of field capacity).

Photosynthesis was highly and positively related to g_s in both species, with r^2 exceeding 0.9 ($A_{J. virginiana} = 114.6 g_s + 0.26$, $r^2 = 0.93$, $P < 0.0001$; $A_{P. ponderosa} = 96.9 g_s + 0.30$, $r^2 = 0.95$, $P < 0.0001$, data not shown). Photosynthesis and g_s were less dependent on Ψ , which remained above -1.22 MPa for 26 days (1.95% VWC, 32.5% of field capacity) of water deprivation, before sharply declining in both species, with more negative values ($F = 4.88$, $P = 0.049$) associated with higher A and g_s in *J. virginiana* compared to *P. ponderosa* (Figure 2). Volumetric soil moisture content was found to be a better indicator of A and g_s than Ψ (Figure 2). The relationship between Ψ and F_v/F_m indicated that a decrease in F_v/F_m ($P < 0.05$) was observed at $\Psi = -2.8$ and -3.6 MPa for *P. ponderosa* and *J. virginiana*, respectively ($\sim 30\%$ of field capacity) (Figure 3).

V_{\max} , J_{\max} and L_s

The maximum carboxylation velocity (V_{\max}) and maximum rate of electron transport (J_{\max}) did not differ between *P. ponderosa* and *J. virginiana* seedlings under well-watered conditions (Figure 4). Maximum values averaged 40 and $198.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ for V_{\max} and J_{\max} , respectively. In water deprivation, both species showed a decrease in V_{\max} and J_{\max} with increasing soil water deficit. This decline was significantly more pronounced in *P. ponderosa* than in *J. virginiana* (Figure 4), and was significantly related to the decline in A in both species [for *J. virginiana*, $V_{\max} = 1.32A + 26.7$ ($r^2 = 0.71$, $P < 0.05$), $J_{\max} = 13.91A + 60.6$ ($r^2 = 0.81$, $P < 0.01$); for *P. ponderosa*, $V_{\max} = 4.07A + 14.6$ ($r^2 = 0.98$, $P < 0.01$), $J_{\max} = 31.42A + 10.5$ ($r^2 = 0.96$, $P < 0.01$), data not shown]. Stomatal limitation to photosynthesis (L_s) was two- to threefold higher in *P. ponderosa* than in *J. virginiana* (Figure 4). L_s began to rise on day 12 of water deprivation in *P. ponderosa*, reaching maximal values on day 16 (2.7% VWC, 45% of field capacity), whereas, L_s in *J. virginiana* did not reach maximal values until day 21 (2.25% VWC, 37% of field capacity) of water deprivation. L_s declined thereafter in both species.

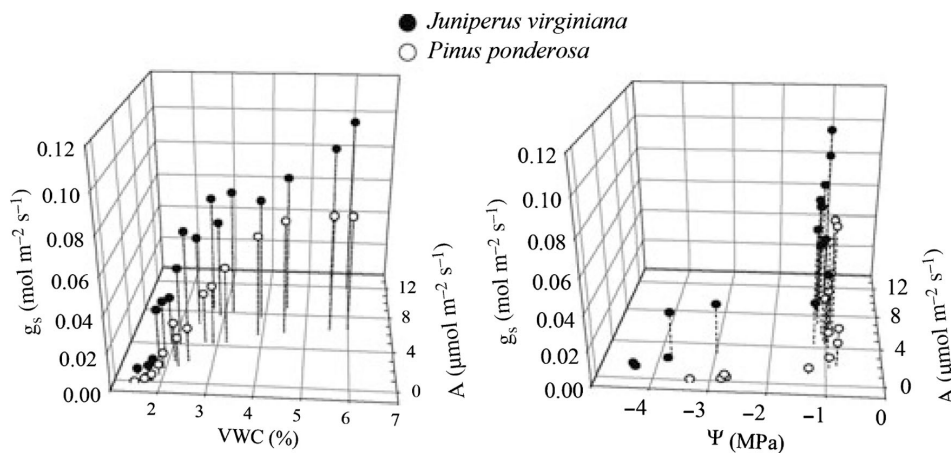


Figure 2. Net photosynthesis (A) and stomatal conductance (g_s) at light saturation ($\text{PAR } 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), as a function of volumetric water content (VWC %), or plant water potential (Ψ , MPa), in *Pinus ponderosa* and *Juniperus virginiana* seedlings under gradual soil water depletion.

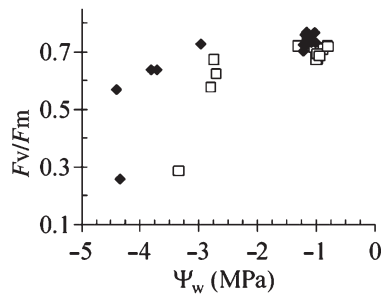


Figure 3. Maximum photochemical efficiency of PSII (F_v/F_m) as a function of plant water potential (Ψ , MPa) in *Pinus ponderosa* and *Juniperus virginiana* seedlings under gradual soil water depletion.

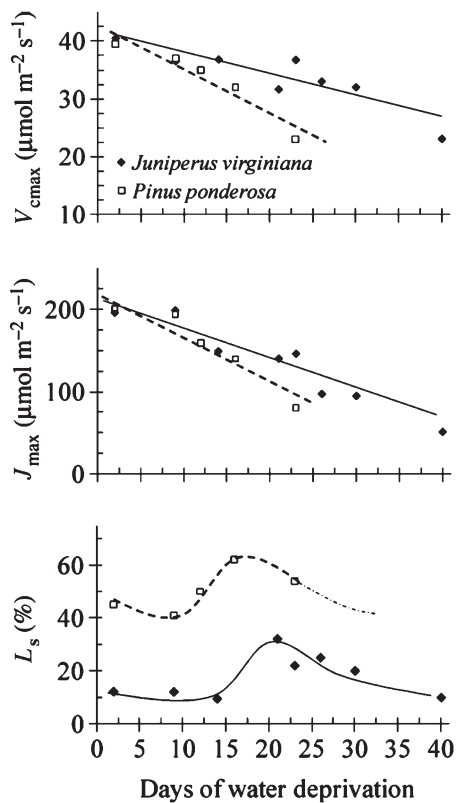


Figure 4. Maximum carboxylation rate (V_{cmax}), maximum electron transport capacity (J_{max}), and stomatal limitation to photosynthesis (L_s), in *Pinus ponderosa* and *Juniperus virginiana* seedlings under gradual soil water depletion. For *J. virginiana*, $V_{cmax} = -0.38 \text{ day} + 41.62$ ($r^2 = 0.80$, $P < 0.05$), $J_{max} = -4.06 \text{ day} + 217.84$ ($r^2 = 0.92$, $P < 0.05$), $L_s = -0.04 \text{ day}^2 + 1.6 \text{ day} + 4.4$ ($r^2 = 0.047$, $P < 0.05$). For *P. ponderosa*, $V_{cmax} = -0.784 \text{ day} + 43.044$ ($r^2 = 0.91$, $P < 0.05$), $J_{max} = -5.97 \text{ day} + 228.9$ ($r^2 = 0.92$, $P < 0.05$).

R/S, SLA, and N content

Root-to-shoot ratio and SLA were significantly higher in *J. virginiana* than in *P. ponderosa* (Table 1), indicating that *J. virginiana* invested more in below- than in above-ground biomass at the seedling stage than *P. ponderosa*. N content in needles collected at the end of the experiment was not significantly different between species (Table 1).

Table 1. Mean root-to-shoot ratio (R/S), specific leaf area (SLA), and needle nitrogen (N) concentration, with corresponding standard errors, for *Pinus ponderosa* and *Juniperus virginiana* seedlings after 40 days of water deprivation. Different letters within rows indicate that means are significantly different ($P < 0.05$).

| | <i>P. ponderosa</i> | <i>J. virginiana</i> |
|-------------------------------------|----------------------------|---------------------------|
| R/S (g g^{-1}) | $0.79 \pm 0.016 \text{ b}$ | $0.95 \pm 0.02 \text{ a}$ |
| SLA ($\text{cm}^2 \text{g}^{-1}$) | $21.2 \pm 0.9 \text{ b}$ | $26.5 \pm 0.8 \text{ a}$ |
| needle N (%) | $1.37 \pm 0.18 \text{ a}$ | $1.3 \pm 0.21 \text{ a}$ |

Discussion

Photosynthetic processes and water deprivation

The recurrent measurements of A , g_s , F_v/F_m , V_{cmax} , J_{max} , L_s , and Ψ provided a means to determine and compare limitations of photosynthesis under gradual soil water depletion. Under well-watered conditions, rates of A and g_s were within the ranges reported in the literature for both species (Gulke & Retzlaff 2001; Sala et al. 2005; Eggemeyer et al. 2006; Willson et al. 2008). Similarly, our values of F_v/F_m , V_{cmax} and J_{max} were in accordance with those reported for conifers (Marshall et al. 2001; Long & Bernacchi 2003; Panek 2004; Misson et al. 2006). Rates of V_{cmax} and J_{max} were lower than those found in several broadleaf species due to factors such as less resource allocation (i.e., N) to photosynthetic processes (Xu & Baldocchi 2003), greater amounts of inactivated Rubisco, and/or greater mesophyll resistance in conifers compared to broadleaf species (Medrano et al. 2002; Long & Bernacchi 2003). Rates of F_v/F_m were not available in the literature (to the best of our knowledge) for the studied species under severe water stress, however, our values were comparable to those reported for other woody species in semi-arid climates (Filella et al. 1998; Ogaya & Peñuelas 2003).

Reductions in g_s and A were observed shortly after the onset of water deprivation and were paralleled by declines in V_{cmax} , J_{max} and increases in L_s that were substantially higher in *Pinus ponderosa* than in *Juniperus virginiana*. On the other hand, F_v/F_m remained relatively stable for 28 days before both species exhibited significant declines. This indicates that the observed decline in A was at first a response to partial closure of stomata that led to a decrease in C_i and an increase in L_s , followed by a decrease in RuBP regeneration possibly due to impaired ATP synthesis (through ATPase impairment), leading in turn to declines in photochemistry and carboxylation efficiency. This effect was more pronounced in *P. ponderosa* due to its higher stomatal sensitivity and L_s than in *J. virginiana*. The stability of F_v/F_m (also found in *J. virginiana* trees under drought conditions; Bryan and Awada, unpublished observations) shows high PSII resistance to dehydration (Damesin & Rambal 1995), and reinforces findings that the decline in A is initially unrelated to photoinhibition in several species (Allen & Ort 2001; Hendrickson et al. 2004;

Nippert et al. 2007). Photoinhibition occurred under severe drought (von Caemmerer & Farquhar 1984; Medrano et al. 2002; Long & Bernacchi 2003), and was observed at Ψ -2.8 and -3.6 MPa in *P. ponderosa* and *J. virginiana*, respectively, indicating greater drought tolerance in the latter. Similarly, Damesin & Rambal (1995) found that F_v/F_m was stable to -4 MPa in two drought-tolerant Mediterranean oaks, and Grassi & Magnani (2005) reported that stomatal closure and increased mesophyll resistance were responsible for declines in A when g_s was greater than $0.1 \text{ mol m}^{-2} \text{ s}^{-1}$ in *Fraxinus* and *Quercus* species. However, when g_s fell below this threshold, metabolic impairment brought on by oxidative stresses was also a factor in photosynthetic limitation.

While Ψ showed a relationship with F_v/F_m , the declines in A and g_s in response to water deprivation was not linked to decline in Ψ until soil moisture fell below 32% of field capacity. Several studies have found a lack of consistent relationship between Ψ and rate of physiological processes, citing soil moisture and/or vapor pressure deficits as better predictors of gas exchange than Ψ in a number of woody, herbaceous, and grass species (e.g., Gollan et al. 1985; Reich & Hinckley 1989; Tardieu & Simonneau 1998; Thomas & Eamus 1999; Domec et al. 2004). Tardieu & Simonneau (1998) reported that plants with "isohydric behavior" are capable of maintaining Ψ constant independent of soil water content until plants are close to death. The declines in g_s and A in this case could be linked to plant chemical growth factors (e.g., abscisic acid and cytokinins), hydraulic information associated with partial loss of root hydraulic conductivity, or/and particular root water potential observed with gradual soil drying. These factors have been reported to generate a hydraulic signal that reduces g_s to maintain shoot water potential at a near constant minimum above a critical value (Gollan et al. 1985; Tardieu & Davies 1993; Domec et al. 2004). The seemingly isohydric behavior observed in the growth chamber benefits both species and improves their survival and potential success under drought. It would be worth investigating whether the isohydric behavior under controlled environment continues to be observed in the field in individuals at different growth stages, or whether other environmental and biological factors come into play.

Species differences and ecological implications

Results from this study have shown that *P. ponderosa* and *J. virginiana* seedlings differ in their responses to water depletion. Compared to *P. ponderosa*, *J. virginiana* exhibited higher A , g_s , initial F_v/F_m , more gradual declines in V_{cmax} and J_{max} , lower L_s and Ψ , and maintained physiological activity for a longer period of water deprivation. In addition, *J. virginiana* seedlings invested more in roots relative to shoots compared to *P. ponderosa*. Juniperus species are reported to be among the most resistant woody plants to drought-induced xylem cavitation (Willson et al. 2008) and maintain physiological activ-

ity at significantly more negative Ψ compared to *Pinus* species (Richardson & Rundel 1998; Tyree & Ewers 1991; Maherali & DeLucia 2000; Piñol & Sala 2000; Maherali et al. 2004; Martínez-Vilalta & Piñol 2004; Sperry 2004). *P. ponderosa* avoids drought stress, due in part to its strong stomatal control (Law et al. 2001) and high sapwood to leaf area ratio (Piñol & Sala 2000; Martínez-Vilalta & Piñol 2004). The strong stomatal control in *P. ponderosa* explains the steeper decline in V_{cmax} and J_{max} with the increase in drought stress, especially since needle N content did not differ between species (Xu & Baldocchi 2003; Misson et al. 2006).

Our results on the importance of water in the rooting zone are in agreement with those observed in the field for mature trees (Eggemeyer et al. 2006, 2009). *P. ponderosa* and *J. virginiana* appear to be suited for survival in semiarid grasslands provided that soil moisture exists, at least partially, within the rooting zone during drought periods. The two species, however, seem to follow different strategies for success in semiarid grasslands. While *P. ponderosa* seedlings behave more as a drought-avoidant species, *J. virginiana* is more of a drought-tolerant species. Differences in resistance strategies between the two confirm the drought tolerance of *J. virginiana* and might explain its widespread encroachment into the water-limited grasslands of Nebraska and the Great Plains relative to *P. ponderosa*, which is expanding into adjacent grasslands from historical grassland-woodland ecotones. Given the avoidance strategy of *P. ponderosa*, prolonged periods of drought such as those predicted under climate change scenarios for the region, might therefore negatively affect its seedling recruitment and establishment. This will have further implications on woody species distributions in the region (Mueller et al. 2005; West et al. 2007; Eggemeyer et al. 2009). Although commonly used in ecophysiological research, controlled studies of resource availability and plant response have obvious limitations (i.e., do not take into account interactions between biotic and abiotic factors observed under field conditions). Nevertheless, our results provide basic information on the ecophysiological responses of these native invasive species to water deficit in the seedling stage that is important to guide future field studies.

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