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Photosynthetic Resilience of *Elymus elymoides* and *Pseudoroegneria spicata* Seedlings Following Acute Water Stress

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ABSTRACT

Demographic studies suggest that mortality of emerging bunchgrass seedlings limits restoration success across North American cold desert rangelands, but how variation in seedling resilience to ecophysiological stress contributes to this is unclear. We measured light-saturated photosynthetic rate (A_{sat}), apparent CO₂ assimilation quantum yield (Φ_{CO_2}), and light-adapted PSII yield (Φ_{PSII}) of seedlings from two native perennial bunchgrasses (*Elymus elymoides*, *Pseudoroegneria spicata*) during and in recovery from acute water stress (6% soil moisture). All seedlings of both species survived the 14-d dry-down and recovery, and both had ~95% reduction in A_{sat} and Φ_{CO_2} by day 3 of the dry-down but had divergent recoveries from the acute water stress. *E. elymoides* took a single day to recover A_{sat} and Φ_{CO_2} to prestress levels, while it took *P. spicata* 6 d to have 50% recovery from acute water stress. Especially notable is that after reduction in Φ_{PSII} on d 7 of the dry-down, *E. elymoides* recovered within 1 d at a lower Φ_{PSII} compared with pretreatment, while *P. spicata* had an additional 1-d lag in Φ_{PSII} recovery. These results suggest that *E. elymoides* (Turkey Lake germplasm) seedlings are more physiologically resilient to acute water stress than *P. spicata* (Anatone germplasm), which has implications for restoration planning and native plant breeding.

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Introduction

Semiarid rangeland restoration has been limited by failures of seedling survival and establishment, likely due to a myriad of interacting biotic and abiotic factors (Sharp Bowman et al. 2017; Denton et al. 2018). Over the past decade, research has attempted to determine the conditions hindering bunchgrass establishment success. Bunchgrass seeds, particularly in the Great Basin, overwinter in or on top of the soil, lying dormant until soil temperatures rise above 0°C and upper soil layers are moist, as typically happens in March (Roundy et al. 2018). When bunchgrass seedlings emerge, early growing season abiotic conditions such as frozen soils or high evaporative losses from soil surfaces create drought conditions that can prevent seedling establishment (Roundy et al. 2014; Roundy et al. 2018; Shriver et al. 2019). Variations in early-season conditions have been found to differentially affect population demographics (James et al. 2012, 2019). However, little research has been conducted to address effects of biophysical condi-

tions on early-season ecophysiological performance that may modulate bunchgrass seedling survival rates.

Hamerlynck et al. (2016) examined chronic water stress effects on bunchgrass seedling photosynthesis at the two- to three-leaf stage, quite removed from the earliest seasonal growth. However, during early seedling development in the spring, plants from semiarid regions often experience acute water stress as the upper soil horizon dries rapidly because of evaporation. Because early seedling establishment presents a major demographic bottleneck for rangeland bunchgrasses (James et al., 2012), it is vital that we understand the physiological effects of acute water stress at the earliest developmental stages, as these can limit establishment success of seedlings (Larson et al. 2015).

The purpose of this study was to determine how *Elymus elymoides* and *Pseudoroegneria spicata* seedlings respond physiologically to a 7-d dry-down and recovery event. *E. elymoides* (Raf.) Swezey and *P. spicata* (Pursh) Á Löve are ubiquitous across the sagebrush steppe, are used extensively in restoration efforts, and are two target species for creating multiple germplasm lines (Larson et al. 2000; Mott et al. 2011). Specifically, we wanted to know how many days *E. elymoides* and *P. spicata* seedlings took to recover photosynthetic capacity after a rapid, acute experimental dry-down.

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Methods

We grew *E. elymoides* (Turkey Lake germplasm, Tetrazolium germination test—95%) and *P. spicata* (Anatone germplasm, Tetrazolium germination test—79%) from seed obtained from Granite Seed (Lehi, UT) in a climate-controlled grow room at the Eastern Oregon Agricultural Research Center (EOARC) in Burns, Oregon, United States (43°31.091'N, 119°1.296'W) from December 2020 to February 2021. The room was maintained at 21°C, with a relative humidity of 20%. Plants were grown under a 12-h photoperiod provided by visible spectrum LED lighting (PlatnumLED, Kailua, HI) with a quantum flux of 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ measured from the top of the conetainer soil surface. Seedlings were grown in 164-mL conetainers (3.81-cm diameter by 20.95-cm length) filled with 175 g of sifted (2-mm sieve) native Grandon series fine sandy loam soil obtained from the Northern Great Basin Experimental Range (43°27.97'N, 119°41.807'W). No additional soil substrates or nutrients were added to the soil.

The experiment was arranged in a block design (3 blocks) with 21 conetainers per species per block. Ten seeds of each species were planted to a depth of 2 cm in each conetainer, and after emergence they were thinned to two individuals per conetainer. To decrease the likelihood of locational effects within the grow room, blocks and plants were rotated every 2 d. Gravimetric soil moisture was estimated by comparing weights from a random subset of planted conetainers from each block to those of three unplanted conetainers from each block that were weighed before and after drying at 105°C for 24 h. Soil moisture was sampled on d 0, 1, 4, 7, 9, 10, and 13. Soils were watered to soil water holding capacity (22% gravimetric soil moisture) and were maintained at that level from the day seeds were planted through the first month of seedling growth. Once seedlings had two true leaves, a dry-down was initiated by withholding water and allowing the soil to dry for all conetainers. After 7 d, soil moisture had decreased from 22% to 6%. On d 8, we watered back to 22% soil moisture for the remainder of the experiment for all conetainers (Fig. 1).

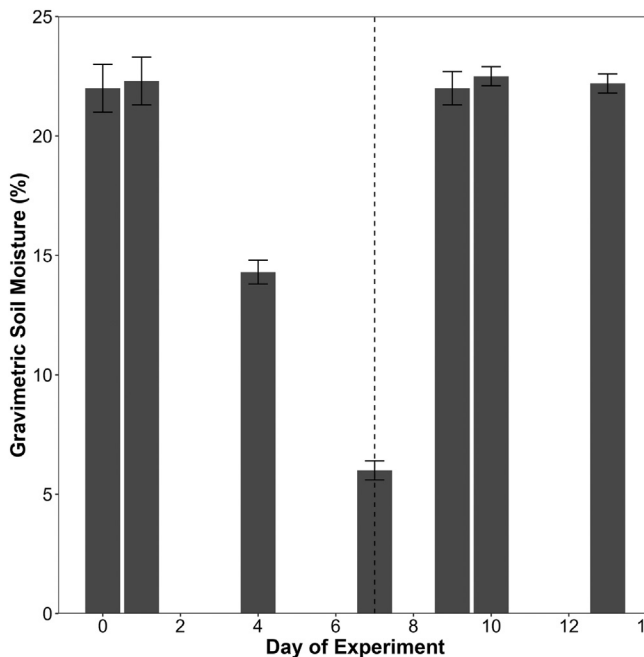


Figure 1. Gravimetric soil moisture that was sampled during the study. Soil water holding capacity for our fine sandy loam soils was 22% and complete dry-down occurred at 6%.

Light-saturated photosynthesis and assimilation yield

We generated light saturation data on fully expanded leaves at the two-leaf stage with a portable infrared gas exchange system (LI-6800, LI-COR Inc., Lincoln, NE) for three individuals from each species per block. LI-6800 operational parameters were set to factory default settings for all measurements (see Supplemental Methods). Photosynthetic light saturation occurred at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD and was achieved for all individuals measured that were not damaged from handling. We excluded from further physiological measurements any individual with leaves damaged due to the measurement. All measurements were made on one leaf from the same individuals from each species per block between 10:00 and 15:00 for each sampling period (d 0, 3, 6, 8, 9, and 13 of this study), with randomized sampling between blocks, and individuals within each block for all physiological variables of the study. We chose the individuals on the basis of which ones had the most mature expanded leaves. These data were used to determine light-saturated photosynthetic rate (A_{sat}) and apparent CO_2 assimilation quantum yield (Φ_{CO_2}) using the R package Photosynthesis (Stinziano et al. 2020). *E. elymoides* was not sampled on d 13 of the study because it had recovered on the basis of physiological measurements.

Light-adapted PSII yield

Light-adapted PSII yield (Φ_{PSII}) was determined from chlorophyll fluorescence (F) measurements made on fully expanded leaves of five different individuals from the ones previously measured with the LI-6800 of each species per block with a LI-600 fluorometer (LI-COR Inc., Lincoln, NE). Factory default multiphase fluorescence settings of the LI-600 were used to estimate Φ_{PSII} (Supplemental Methods). Measurements were made on one leaf from the same individuals from each species per block between 10:00 and 12:00 for each sampling period (d 2, 4, 6, 7, 8, 9, 10, and 13). The same five individuals of each species per block were measured at each time point over the entirety of the experiment.

Statistical analyses

To test the responses of *E. elymoides* and *P. spicata* seedlings to water stress for both A_{sat} and Φ_{CO_2} , we ran Welch's two sample *t*-tests for d 0, d 3, and d 9. If assumptions were not met, we ran a Kruskal-Wallis test (Φ_{CO_2} , d 3). D 6, 8, and 13 either did not have enough replication because of a bad gas exchange measurement or not enough sample due to mortality of measured individuals. We analyzed Φ_{PSII} using repeated measures analysis of variance (ANOVA), with species, sample day, and their interaction as our independent variables. Our random effect was individuals nested within block. The statistical model was run using the lme4 R package (Bates et al. 2015). Model data assumptions for repeated measures ANOVA were met. All statistical models were assessed at an $\alpha = 0.05$. Data processing and statistical analyses were all done in R (R Core Team 2023).

Results and Discussion

Acute soil drying induced an ~93% reduction in A_{sat} and Φ_{CO_2} by d 3 for both species (Figs. 2A and 1B). Both species maintained low rates of A_{sat} and Φ_{CO_2} with continued decreases in soil moisture for another 4 d. Upon resaturating (d 8), both species A_{sat} and Φ_{CO_2} responded positively but with distinctly different recovery trajectories. *E. elymoides* had almost complete recovery for both A_{sat} and Φ_{CO_2} 1 d following rewetting (see Fig. 2). *P. spicata* recovery was more prolonged, taking 5 d to achieve at least a 50% recovery of A_{sat} and Φ_{CO_2} from the predrying conditions (see

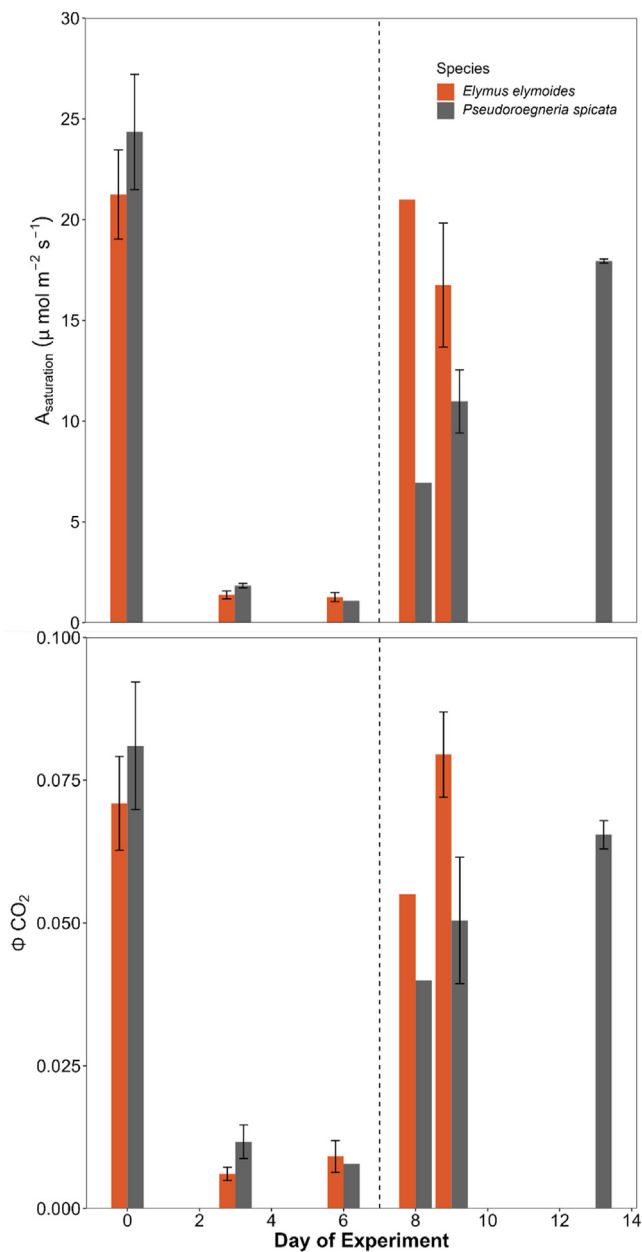


Figure 2. A, Photosynthetic saturation ($A_{saturation}$) and B, apparent CO_2 assimilation quantum yield of 1-mo-old *Elymus elymoides* (orange) and *Pseudoroegneria spicata* (gray) seedlings. The dotted line represents the last water stress day (d 7) before soils were resaturated to pot capacity (d 8). *E. elymoides* was not measured on d 13 because $A_{saturation}$ and Φ_{CO_2} recovered.

Fig. 2). Statistical analyses for A_{sat} and Φ_{CO_2} resulted in variables not being significant.

Throughout the duration of the experiment, both *E. elymoides* and *P. spicata* had similar Φ_{PSII} values. There was little change in Φ_{PSII} before d 7; however, there was an ~30% reduction in Φ_{PSII} for both *E. elymoides* and *P. spicata* at the height of soil drying on d 7 before soil rewetting (Fig. 3). After rewetting, Φ_{PSII} responses varied by species. *E. elymoides* Φ_{PSII} attained predrying levels 1 d after rewetting and then decreased over several days to a lower stabilized level (see Fig. 3). *P. spicata* Φ_{PSII} remained depressed 1 d longer before rising and recovering (see Fig. 3). These resultant Φ_{PSII} values were not consistent with the distinct patterns we observed with A_{sat} and Φ_{CO_2} between the species 5 d after stress release. The distinct pattern in responses between Φ_{PSII} , A_{sat} , and

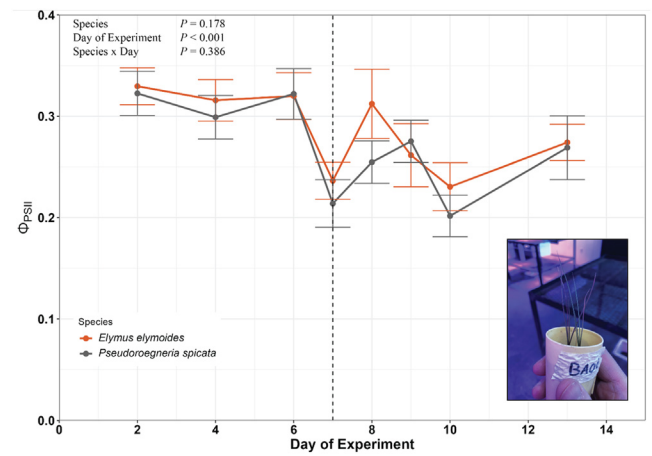


Figure 3. Mean (\pm standard of error) ambient light-adapted PSII yields (Φ_{PSII}) of 1-mo-old *Elymus elymoides* (orange) and *Pseudoroegneria spicata* (gray) seedlings during a dry-down and rehydration. The dotted line represents the last day (d 7) with acute water stress before resaturation of soils to pot capacity. Analysis of variance results presented with $\alpha = 0.05$ for significance. Insert: Photograph of *P. spicata* seedlings on d 8 showing stress-induced anthocyanin production.

Φ_{CO_2} might indicate variation in photoprotective mechanisms that these perennial bunchgrasses employed to mitigate acute water stresses (Osmond 1994).

When soils were driest, we visually observed increased expression of anthocyanins in leaf tissues concurrent with low A_{sat} , Φ_{CO_2} , and Φ_{PSII} in both species, and this continued to develop over the postwatering recovery period (see Fig. 2, inset). Although we did not directly measure anthocyanin concentrations, increased anthocyanins are considered a long-term response to a wide range of stresses, especially in developing tissue when stress is coupled with high light exposure, and impact photosynthetic performance (e.g., reduced photosynthetic capacity, decreased carbon assimilation) (Chalker-Scott 1999; Close and Beadle 2003). These differences in carbon assimilation recovery could affect overall assimilation capacity and growth as seedlings transition to established juvenile plants.

In order to establish new and effective restoration practices (Svejar et al. 2017), and as research continues to identify important demographic carry-over effects from early seedling growth stages (Larson et al. 2015; Larson et al. 2020; Quigley et al. 2023), it becomes critical to establish consequences of abiotic plant stress at fine temporal scales (hours and days) within those demographic periods (O'Connor et al. 2020) and identify the ecophysiological mechanisms allowing for survival, growth, and successful reproduction. While *E. elymoides* and *P. spicata* co-occur in similar plant communities throughout the Great Basin, *E. elymoides* is often selected for restoration efforts as it can establish under hotter and drier conditions (Blumenthal et al. 2020). The more resilient seedling ecophysiological responses to acute water stress observed here for *E. elymoides* are consistent with these previous community-based findings. *P. spicata* seedlings were observed here to recover more slowly from acute water stress than *E. elymoides*. *E. elymoides*'s ability to recover more readily from early acute water stress may reduce the cumulative stress effects associated with repeated acute drying events to a greater extent than *P. spicata*. In this study we only examined the responses of a few individuals and germplasms to a single acute water stress event over 13 d. We suggest that more studies such as this one will need to be completed using additional germplasms of *E. elymoides* and *P. spicata* with increased sample sizes to better characterize the variation in seedling responses to acute water stress.

Our study indicates that grass seedlings, at the two- to three-leaf stage, may have varied recovery responses to acute water stress, and it is important to understand how these responses translate into seedling survival. It becomes imperative to consider how many seedlings make it to the next demographic stage and also to understand the mechanisms by which some seedlings survive to juvenile and adulthood phases. Such mechanistic information about seedling survival will be important for informing predictive genotypic models of native plant breeding and help develop more biologically resilient native plant cultivars (Jones et al. 2022). Understanding the differences in the physiological recovery of native bunchgrass seedlings could also assist restoration planning efforts by creating resilient seed mixes based on physiological responses to biotic and abiotic stressors (He et al. 2018; Ledger et al. 2018; Tomlinson et al. 2022).

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Declarations of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rama.2024.03.012.

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