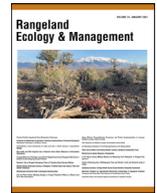




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journal homepage: www.elsevier.com/locate/ramaStomatal Conductance Relates to Sagebrush Transplant Survival Across Planting Season and Size-Class[☆]Stella M. Copeland^{1,*}, Erik P. Hamerlynck^{1,*}, Corinna M. Holfus², Emma E. Campbell³, Chad S. Boyd¹¹ USDA-Agricultural Research Service, Eastern Oregon Agricultural Research Center, Burns, OR 97720, USA² Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR 97331, USA³ Unaffiliated, Denver, CO 80210, USA

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ABSTRACT

Postfire restoration of sagebrush steppe is limited by poor sagebrush establishment from seed. Transplanting may improve seedling establishment, but it requires more labor and expense. Given variable transplant survival in big sagebrush (*Artemisia tridentata* Nutt.), establishing links between restoration practices and survival related to exposure to adverse weather, via plant response to stress, could significantly improve transplant efficacy. We tested how planting season and transplant age (size), two restoration practices likely to affect seedling survival across a range of environmental conditions, related to transplant survival and stomatal conductance, an indicator of seedling ecophysiological performance. We found low mean stomatal conductance during key periods in spring and early summer was associated with subsequent growing season mortality across all size-classes. Overall, these findings suggest managers should consider the importance of plant performance during key stressful periods in evaluating transplant survival related to restoration methods.

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Introduction

Sagebrush steppe is imperiled by invasive non-native annual grasses that accelerate and amplify interannual fire regimes (Knack et al. 2003). Largescale seedlings of the dominant shrub, big sagebrush (*Artemisia tridentata* Nutt.), are often unsuccessful (Knutson et al. 2014). Transplants are more reliable yet expensive, with high variation in survival (Dettweiler-Robinson et al. 2013; Pyke et al. 2020). Big sagebrush transplant survival is associated with site characteristics and weather (McAdoo et al. 2013; Davidson et al. 2019; Pyke et al. 2020), localized adaptation (Chaney et al. 2017), and planting methods (Minnick and Alward 2012; Dettweiler-Robinson et al. 2013). Planting season may particularly affect exposure to stressful environmental conditions. For example, fall-planted transplants can have higher first-year survival than spring

plantings (Clements and Harmon 2019) due to greater tolerance for soil drying during the transition to summer drought (Miller and Shultz 1987; Miller 1988; Romo and Haferkamp 1989).

While considerable attention has been given to environmental effects on seedling and adult big sagebrush ecophysiology (Smith et al. 1997; DiCristina and Germino 2006; Loik 2007; Reinhardt et al. 2019), studies linking big sagebrush transplant survivorship and mortality with indicators of ecophysiological performance are limited. Physiological indicators, like stomatal conductance to water vapor (g_s), reflect the trade-off and optimal regulation between photosynthesis (open stomata) and water conservation (closed stomata) that allow the plant to better maintain water balance and growth in response to environmental stressors (Smith et al. 1997). Such data may offer insight into how management factors associated with survival, such as planting season or size, are associated with environmental conditions.

To address this, we coupled survivorship observations of fall- and spring-planted Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) cohorts including seedlings of various ages (sizes) before planting with measurements of g_s over the growing season to determine if variation in g_s was associated with seedling mortality and whether this effect varied by cohort or size-class. We hypothesized that lower springtime g_s during the transition from spring to summer drought would

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be associated with mortality and that this trend would be more strongly expressed in spring-planted seedlings that had not experienced as great a range of environmental variation as fall-planted counterparts.

Methods

Site and experimental setup

The study was performed on the Northern Great Basin Experimental Range in southeastern Oregon (119°42'W, 43°29'N) at 1400 m a.s.l. in a sandy loam soil. Wyoming big sagebrush transplants were grown in a climate-controlled grow-room under high-intensity lights (seed source and procedure details: Appendix S1, available online at: [10.1016/j.rama.2021.09.008](https://doi.org/10.1016/j.rama.2021.09.008)). Seedling age ranges between 44 and 170 d were created by seeding every 14 d for 126 d to create 10 age groups (first seeding dates: fall planting season, May 1, 2019, spring, October 2, 2019). We bulked the 10 groups into 5 size-classes (28-d age range) to increase sample size for analysis, resulting in 400 total transplants and $N=40$ per size-class and planting season. The final size-classes were class 1, 44–58 d; class 2, 72–86 d; class 3, 100–114 d; class 4, 128–142 d; and class 5, 156–170 d. Seedlings were transplanted from the grow-room to a fenced (rabbit-exclusion) 25 × 25 m level area on October 18, 2019 and March 20, 2020 for fall and spring season plantings, respectively. Before planting, competing vegetation was removed with burning (September 26, 2019) and a preemergent herbicide treatment (October 15, 2019, imazapic, details: see Appendix S1) unlikely to harm sagebrush seedlings (Owen et al. 2011).

Mean daily volumetric soil moisture and temperature were calculated from hourly measurements at 5, 10, and 15 cm depths from two locations within the planting area and mean daily air temperature, relative humidity, and total precipitation were measured ≈3.5 km from the site at similar elevation and flat topography for the main monitoring period (October 2019–September 2020, US Climate Reference Network, Diamond et al. 2013; Palecki et al. 2013).

Survival and physiology measurements

Stomatal conductance to water vapor (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured on the abaxial surface of healthy, fully expanded leaves (larger than the porometer aperture) at midday (10 a.m.–1:30 p.m.) during dry high-light weather conditions (sunny or partly sunny with high clouds, SC-1 leaf porometer, Meter Group, Inc., Pullman, Washington, USA) for half of each size-class and season cohort ($N=20$ for all except for fall-planted size-class 4 for which $N=19$; one died by November 2019). We measured g_s once before winter for fall transplants (October 31–November 6, 2019) and for four growing season periods in 2020: spring (measurement periods: April 7–8, May 4–5), peak summer (July 7–8), late summer (August 3–4, August 31–September 1), and fall (September 28–29). Seedling survival, based on the presence of green leaves, was assessed on conductance measurement dates and on June 15 (peak summer) and July 22 (midsummer) in 2020 and March 2, 2021 for all individuals ($N=40$ for size-class and planting season, except $N=39$ for fall size-class 4, see earlier).

Analysis

We used a linear model to test for differences in g_s by size-class in late October to early November after planting for the fall cohort. We tested the effect of size-class and growing season measurement period (spring, peak summer, late summer, and fall) and their interaction on g_s with separate linear mixed models by planting season (random intercept term for individual, package lme4,

Bates et al. 2015). We tested whether the previous g_s measurement during the growing season predicted survival as a function of planting season and size-class using a Cox proportional hazard model that included bivariate interactions among g_s , season, and size-class and formulated for time dependence for temporal trends in g_s (package survival, Therneau 2021). We also tested the effects of mean spring and peak summer g_s (April 7–July 8) and their interaction on midsummer survival (July 22) with separate generalized linear models (binomial distribution) by planting season. We mean centered g_s when included as a predictor variable. Non-significant ($P > 0.05$) interactions and variables were removed sequentially with model comparison to arrive at final models (package car, Fox and Weisberg 2019). We calculated pairwise tests with least square means for differences between combinations of categorical variables and slopes for continuous covariates (Tukey's tests for multiple comparisons, package emmeans, Lenth 2020). All analyses were conducted in R version 4.0.3 (R Core Team 2020).

Results

Daily air temperature averaged $0.3^\circ\text{C} \pm 0.3^\circ\text{C}$ (\pm SE), with $75^\circ\text{C} < 0^\circ\text{C}$, from the fall planting date until the first spring survival measurement (October 18, 2019–April 6, 2020), $7.7^\circ\text{C} \pm 0.6^\circ\text{C}$ in the spring (April 7–May 5, 2020), $13.2^\circ\text{C} \pm 0.6^\circ\text{C}$ in peak summer (May 6–July 9, 2020), $21.0^\circ\text{C} \pm 0.4^\circ\text{C}$ in late summer (July 10–September 1, 2020), and $15.6^\circ\text{C} \pm 0.9^\circ\text{C}$ in the fall (September 2–29, 2020) measurement periods. Soil moisture was highest in the winter and declined through the 2020 growing season with punctuated increases associated with discrete precipitation events (Fig. 1). Weather during the study measurement periods was within 1 standard error of longer-term means (10 yr), except for lower precipitation in the spring (Table S1, available online at: [10.1016/j.rama.2021.09.008](https://doi.org/10.1016/j.rama.2021.09.008)).

Stomatal conductance for fall-planted, hereafter “fall,” transplants did not differ by size-class shortly after transplanting (October 31–November 6, 2019, F value = 0.72, $df=4$, $P=0.580$). However, many of the fall plants died by the first spring monitoring date (April 2020), particularly in the smallest size-class (60% mortality). Survival after the first growing season (September 2020) ranged from 33% for the fall smallest size-class to 93% for the medium (class 3) spring-planted, hereafter “spring,” transplants (class 3, Fig. S1, available online at: [10.1016/j.rama.2021.09.008](https://doi.org/10.1016/j.rama.2021.09.008)). Mortality over the subsequent fall-winter (September 2020–March 2021) was low (3/400 plants).

Spring transplant g_s significantly varied among growing season measurement periods ($\chi^2=413.2$, $df=3$, $P < 0.001$) but did not differ by size-class ($P > 0.10$, Fig. 2, Table S2, available online at [10.1016/j.rama.2021.09.008](https://doi.org/10.1016/j.rama.2021.09.008)). Specifically, spring transplant g_s was highest in peak summer and lowest in the spring but did not significantly differ between late summer and fall ($P < 0.001$, see Fig. 2, Table S2). Fall transplant g_s varied between growing season measurement periods ($\chi^2=34.8$, $df=3$, $P < 0.001$) and size-class ($\chi^2=9.4$, $df=4$, $P=0.05$, see Fig. 2). Fall transplant g_s was highest during the peak summer period compared with other periods ($P < 0.05$) and marginally higher for the smallest compared with the two largest size-classes (class 1 vs. 4, $P=0.05$, 1 vs. 5, $P=0.08$, see Fig. 2, Table S2).

Final models for growing season survival included size-class and an interactive effect of the previous g_s measurement and planting season with a slightly greater positive effect of g_s on survival for fall compared with spring transplants (Table S3, available online at: [10.1016/j.rama.2021.09.008](https://doi.org/10.1016/j.rama.2021.09.008)). Average g_s pooled across the spring and peak summer periods was positively associated with survival by midsummer (July 22) for both spring (coefficient = 0.042, $\chi^2=41.4$, $df=1$, $P < 0.001$) and fall cohorts (coefficient = 0.026, $\chi^2=27.6$, $df=1$, $P < 0.001$, Fig. 3). Size-class did not

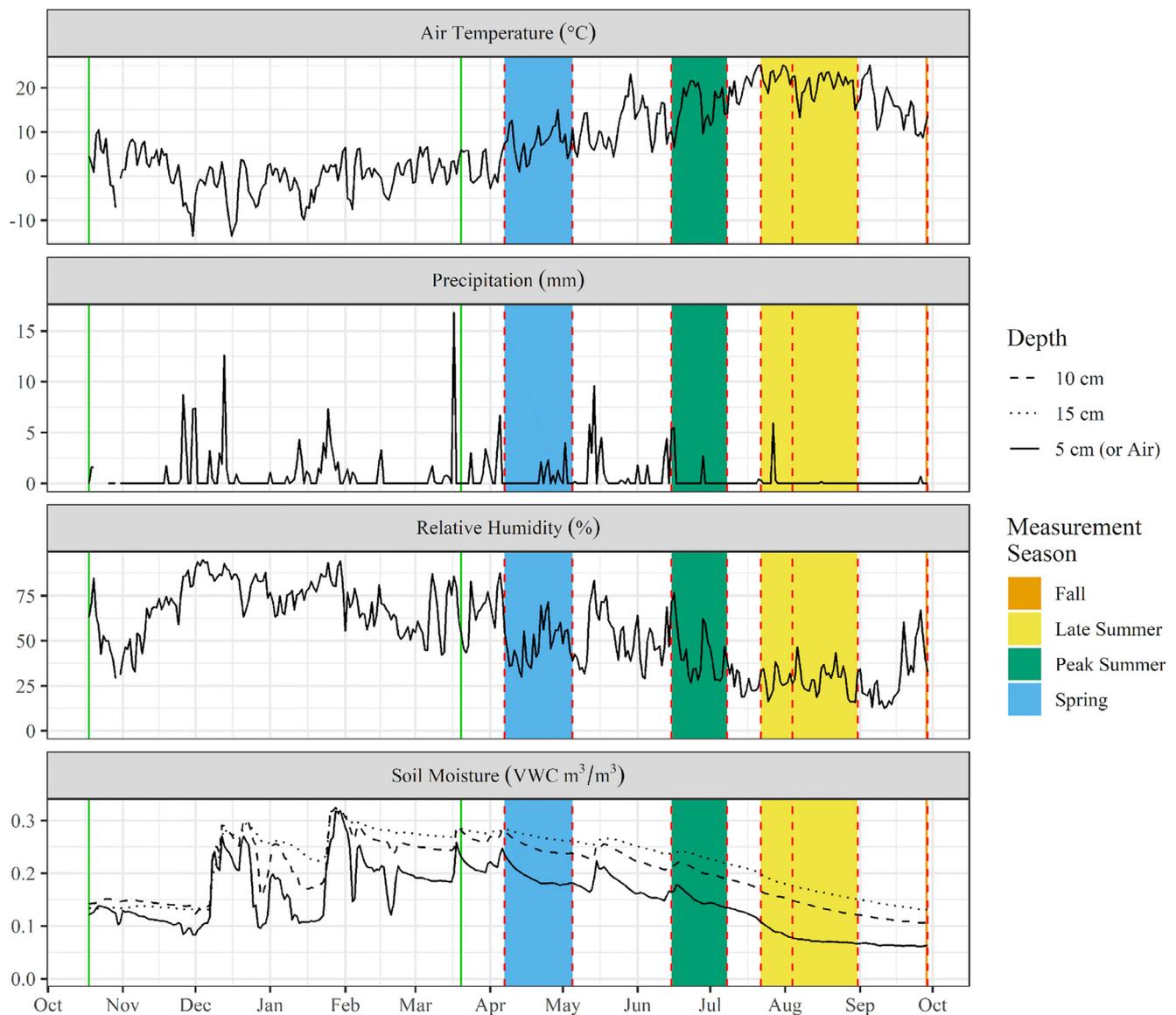


Fig. 1. Air temperature, precipitation, relative humidity, and volumetric soil moisture between the fall planting (October 18, 2019) and September 29, 2020 with green lines for planting dates and red dashed lines for measurement dates. Filled areas represent growing season measurement periods.

have significant main or interactive effects on mean g_s in these models ($P > 0.10$).

Discussion

As expected, higher spring-summer g_s was associated with survival in the spring-fall growing season across all seasonal cohorts and size-classes. Our results highlight the importance of spring and early summer, before the extended summer drought, as the period with the most variation in g_s tied to the highest rates of mortality. Overwinter mortality for fall seedlings was high, with the effect dependent upon size-class. Specifically, we observed high mortality for smaller fall seedlings by the first spring sampling. While our analysis did not allow us to pinpoint specific weather conditions related to this pattern, others have also observed high and variable sagebrush seedling mortality with freezing events (Brabec et al. 2017). Overall, we observed limited indications of higher eco-physiological performance of fall over spring transplants associated with survival during the growing season. Small fall-planted seedlings that survived through winter had higher g_s and corre-

sponding low mortality rates than their spring counterparts during the growing season, but mortality rates remained higher for larger fall individuals. While spring-planted transplants had much lower g_s than fall transplants at the beginning of the growing season, g_s was similar between both cohorts by the peak summer and fall periods.

Broadly, our results suggest that monitoring physiological activity with simple methods in planted seedlings can provide insight into poor performance and associated risk of mortality. Multiple studies have documented seasonal controls on physiological activity for this relatively well-studied native species (Miller and Shultz 1987; Miller 1988), as well as population and subspecies differences in response to weather conditions, like drought and freezing (Kolb and Sperry 1999a, 1999b; Brabec et al. 2017; Lazarus et al. 2019). More sophisticated studies linking physiological performance, growth, and survival to management factors could improve transplant success across the region (Dettweiler-Robinson et al. 2013; Pyke et al. 2020).

Although our approach showed stomatal behavior is associated with seedling susceptibility to mortality, it does not clarify

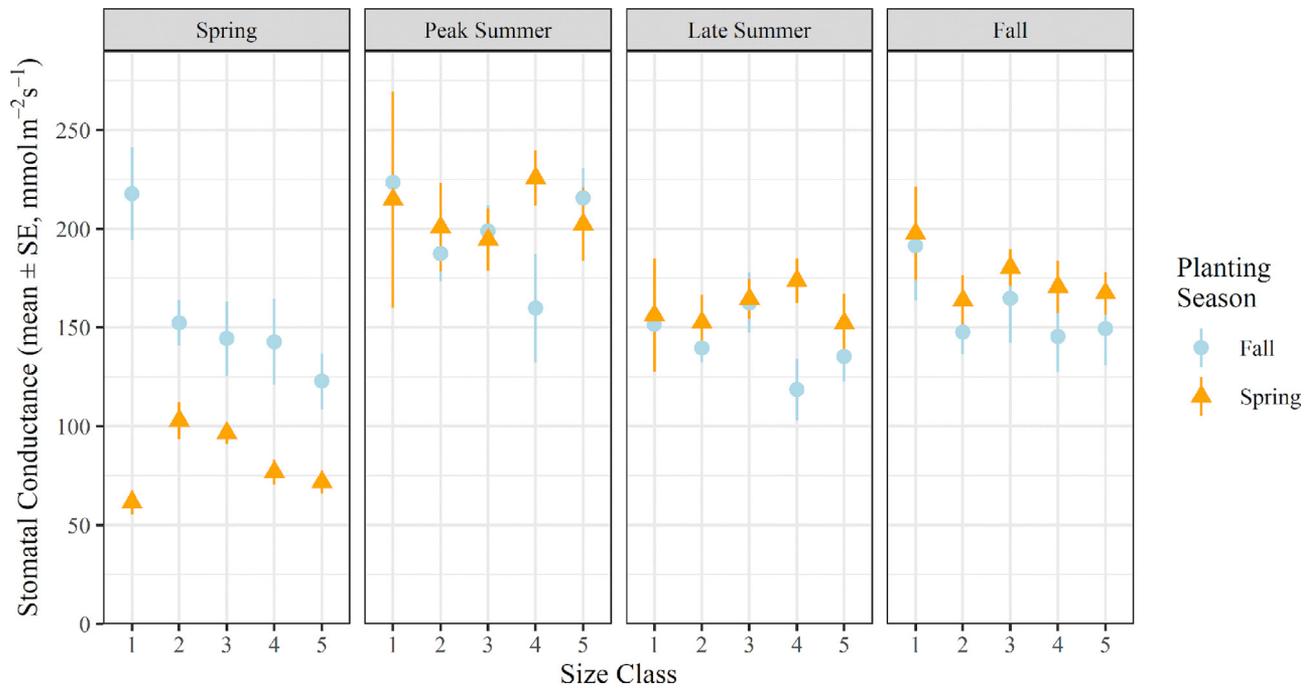


Fig. 2. Big sagebrush transplant stomatal conductance (mean ± SE) by planting season (fall: blue circles, spring: orange triangles), size-class, and growing season measurement period (spring: April 7–8, May 4–5, peak summer: July 7–8, late summer: August 3–4, August 31–September 1, and fall: September 28–29).

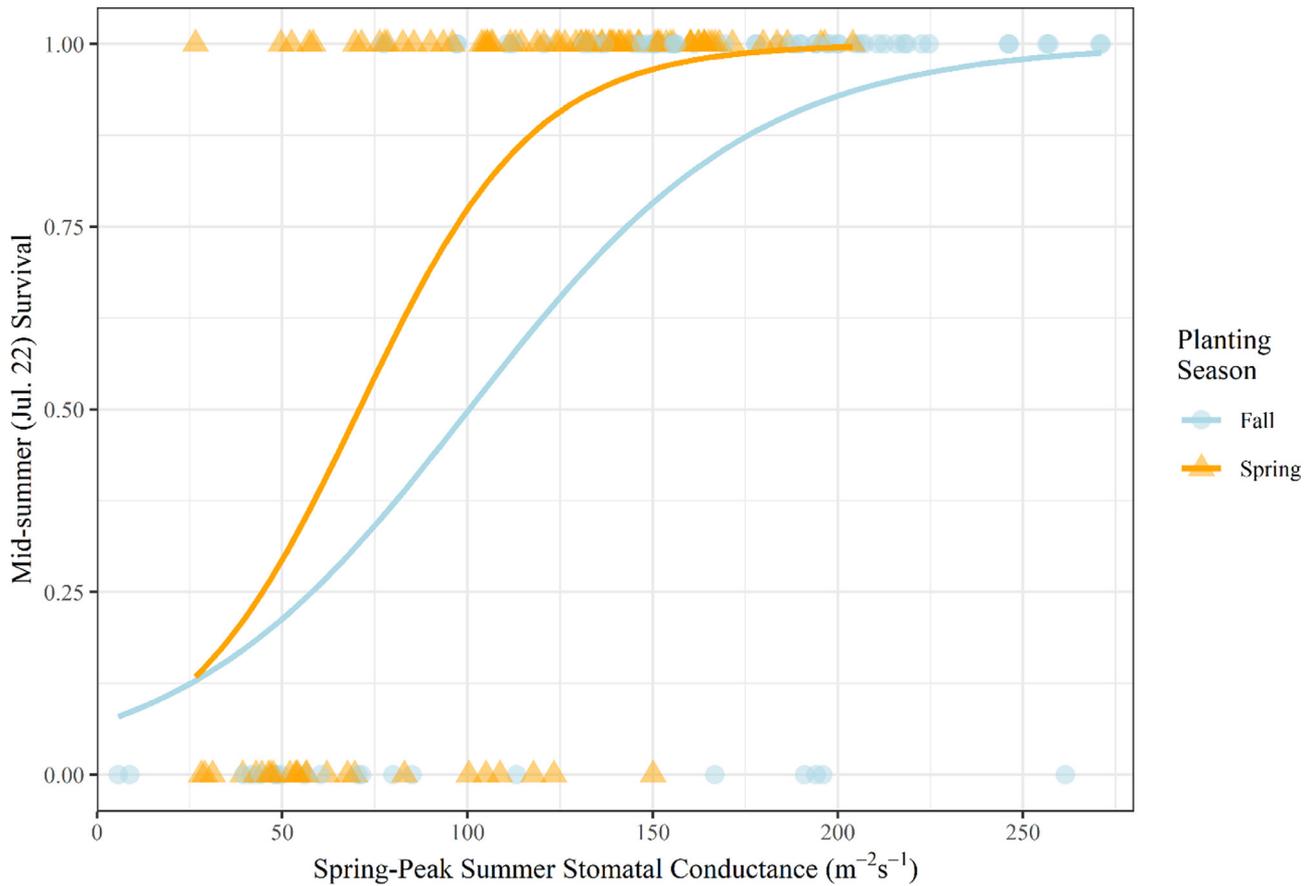


Fig. 3. Big sagebrush transplant midsummer (July 22) survival (points) and model predictions for each planting season (fitted lines for separate generalized linear models [binomial distribution] by season) associated with spring-peak summer (April 7–July 8) stomatal conductance.

mechanisms underlying seedling death. Reduced stomatal opening in plants fated to die may have limited carbon uptake needed to survive into and through summer drought or be at the onset of widespread xylem cavitation, both of which interactively determine whole-plant mortality in woody species (Hammond and Adams 2019). It is also unclear based on our results how specific aspects of the grow-out process, such as growth allocation in containers before planting, may have reduced soil water uptake needed to meet photosynthetic demand (Meinzer and Grantz 1990; Smith et al. 1997).

Implications

Our results suggest that leaf physiological activity in the early growing season is key to increasing survival of sagebrush transplants. This implies that outplanting methods like size-class and planting season are more likely to influence survival if they affect spring ecophysiological activity. Future research efforts could improve ecophysiological links with survival by identifying critical thresholds with frequent conductance measurements and/or larger sample sizes and elucidating processes associated with stomatal behavior such as xylem hydraulic function, photosynthetic carbon assimilation, photoprotective mechanisms, and nonstructural carbohydrate dynamics (Smith et al. 1997; Kolb and Sperry 1999a, 1999b; Reinhardt et al. 2019). An ecophysiological approach could also help resolve conflicting literature on transplant performance associated with restoration methods (Dettweiler-Robinson et al. 2013; Clements and Harmon 2019; Pyke et al. 2020) and potential relationships with weather conditions or the responses of different seed sources to environmental stress (Brabec et al. 2017). These plant-level ecophysiological responses could increase restoration efficiency by suggesting outplanting methods most likely to increase survival via decreased exposure to adverse weather in key time periods in highly variable sagebrush steppe landscapes.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rama.2021.09.008.

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