8-2024

Evaluating Bluebunch Wheatgrass Plant Materials for the Central Great Basin and Range

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EVALUATING BLUEBUNCH WHEATGRASS PLANT MATERIALS FOR THE CENTRAL GREAT BASIN AND RANGE

by

McKenna Delton

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Ecology

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UTAH STATE UNIVERSITY
Logan, Utah

2024

ABSTRACT

Evaluating Bluebunch Wheatgrass Plant Materials for the Central

Great Basin and Range

by

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Utah State University, 2024

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Bluebunch wheatgrass is a perennial bunchgrass native to North America's Intermountain West. This region has experienced widespread historical ecological challenges due to disturbances such as cultivation, wildfire, and grazing, leading to a decline in native perennial grass populations. While bluebunch was once dominant in numerous plant community types in this region, it often shows poor persistence and productivity compared to seeded non-native perennial grasses with greater overall adaptation to novel disturbances. Consequently, there is a demand for improved bluebunch wheatgrass materials for restoration projects.

Various plant materials originating primarily from the Columbia Plateau region have been developed. However, interest in new materials from the Central Basin and Range region is growing due to their potential adaptation to prevailing environmental conditions. Two Basin & Range materials are in development, designated as BasinSTZ3a and BasinSTZ4. They show comparable or better performance than existing cultivars in common garden settings. However, these populations have yet to be compared in a greenhouse or tested in wildland settings.

I conducted studies comparing the performance of five bluebunch wheatgrass populations, including two from the Basin & Range (BasinSTZ3a, BasinSTZ4) and three previously released materials (Anatone, Columbia, and P-7). The greenhouse study results indicate that BasinSTZ4 exhibited traits indicative of a slower growth strategy, while BasinSTZ3a resembled previous releases with higher tiller numbers and a somewhat faster growth strategy. BasinSTZ3a had slightly more vigorous growth and tiller production than BasinSTZ4 and may be suited to warm STZ3a sites with invasive annuals. The outplanting study, repeated for two years, showed limited results in its first year due to high seedling mortality. In the second cohort, with higher precipitation, preliminary results suggest that Basin STZ4 performed worse than all other populations at one of the four study sites (whereas there were no differences among populations at the other three sites). This fit with the expectation that Basin3a would fare better than BasinSTZ4 at this site due to a history of adaptation. A better understanding of the performance of these plant materials can help guide where they may be most suitable for planting and inform future development of additional materials for the Basin & Range.

(112 pages)

PUBLIC ABSTRACT

Evaluating Bluebunch Wheatgrass Plant Materials

for the Central Great Basin and Range

Mckenna Delton

Bluebunch wheatgrass is a perennial grass from North America's Intermountain West. This area has faced many ecological disturbances, including dryland farming, wildfire, and grazing. These have led to a decline in the populations of native perennial grasses. While bluebunch used to be widespread throughout the region, it often cannot compete with some non-native grasses that are better adapted to these disturbances. Therefore, there is a demand for bluebunch wheatgrass plant materials that have undergone selection for overall better performance in restoration.

Most existing plant materials for bluebunch wheatgrass come from the Columbia Plateau region, but interest is growing in new plant materials originating from the Basin and Range region because they might be better suited to the local environment. Two new materials called BasinSTZ3a and BasinSTZ4 are currently being developed. They have shown equal or better performance compared to existing materials in controlled garden settings, but they have not before been thoroughly tested in a greenhouse or a natural setting.

I conducted studies to compare the performance of five bluebunch wheatgrass populations: two from the Basin & Range (BasinSTZ3a and BasinSTZ4) and three previously released materials (Anatone, Columbia, and P-7). The greenhouse study results indicate that BasinSTZ4 exhibited traits related to a slower growth strategy, while BastinSTZ3a had traits that resembled those of previous releases with higher tiller numbers and a somewhat faster growth strategy. BasinSTZ3a had slightly more vigorous growth and tiller production than BastinSTZ4, and may be suited to warm STZ 3a sites with invasive annuals. The outplanting study, repeated for two years, had limited results in the first year because most seedlings did not survive. In the second year, with more precipitation, early results suggest that BasinSTZ4 performed worse than all other populations at one of the four study sites (whereas there were no differences among populations at the other three sites). This supported my expectation that BasinSTZ3a would perform better due to its history in similar conditions. A better understanding of the performance of these plant materials could help determine where they may be most successful in plantings and guide their future development for the Basin and Range.

ACKNOWLEDGMENTS

This project has been a collaborative effort, and I am very grateful for the support and contributions of the many people who made it all possible. First and foremost, I would like to thank my advisors, Kari Veblen and Tom Monaco, for their unwavering support, invaluable guidance, and tireless dedication throughout this process.

Thank you to Geno Schupp for his expertise and for editing the manuscript, and to Susan Durham whose help was vital to analyzing data.

I am fortunate to have had the support of my fantastic lab mates. Maria Stahl, thank you for your advice and collaboration over the years. Spencer Good, thank you for your hard work in both the field and the greenhouse, which has been instrumental in the success of this project. Josh Day, I value your diligent efforts in the greenhouse.

I am very grateful to the technicians at the USDA-ARS here in Logan, notably Cole Walton and Lia Klein, for their tireless work in the field. Without them, the plantings would not have been possible. Special thanks are due to the landowners who generously allowed us to plant native grasses on their properties and to the folks at the DNR who helped us scout sites.

I extend my heartfelt gratitude to the faculty, staff, and fellow graduate students at USU for their wealth of knowledge, encouragement, and support throughout this journey.

To my husband, whose constant support and assistance at home (and even in the field!) made the last three years possible.

Last but certainly not least, I owe a debt of gratitude to my family, whose encouragement and support have been a constant source of strength and inspiration. Your guidance and belief in me have made it possible for me to pursue a career that I am genuinely passionate about. I couldn't have done it without you!

Thank you, each one of you, for being part of this journey.

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CHAPTER 1

INTRODUCTION

The Intermountain West of North America (Appendix A.) has witnessed historical and ongoing disturbances, such as cultivation, excessive livestock grazing, and wildfires. These disturbances have resulted in a decrease in the abundance of native perennial grasses and an increase in the dominance of invasive annual grasses (Knapp, 1996; Mack, 1981; Svejcar et al., 2017). High abundance of invasive annual grasses, such as cheatgrass (*Bromus tectorum*), increase fire frequency and burn severity to levels above what is tolerable for native species to persist (Chambers, , et al., 2014). In addition, continued dominance and repeated wildfires create feedback cycles that promote continued invasion and further ecosystem degradation (et al., 2018; D'Antonio & Vitousek, 1992; Whisenant, 1992). Land managers strive to restore these ecosystems by revegetating affected areas with many species, most importantly perennial grasses. While non-native perennial grasses were more commonly used in the past for revegetation, the loss of biodiversity due to past disturbance and the heavy use of non-native species has led to policy changes where native species use is now the highest priority for restoration projects (Richards et al., 1998).

Recent decades have seen an emphasis on using native plant materials in restoration to maximize biodiversity and increase the adaptation potential of ecosystems under climate change (Vander Mijnsbrugge et al., 2010). Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve) is a formerly dominant Intermountain West native bunchgrass (Daubenmire, 2012; Eaton, 1982; Miller et al., 1994). However, bluebunch wheatgrass often has lower productivity, grazing tolerance, and establishment than non-native perennial grasses seeded in the past (Meays et al., 2000). Subsequently, there is a high demand for improved plant materials with better performance and traits that equip them to adapt to novel disturbances and climate change (Jones et al., 2015).

A thriving seed industry supplies native seed to restoration practitioners in the Intermountain West (Jones, 2019). Available plant materials are developed through plant selection and breeding programs (Larson et al., 2004; Staub et al., 2016), and the outputs of these are commonly evaluated through outplanting and seeding trials (Jones & Mott, 2016; Rigby et al., 2018; Robins, Rigby, et al., 2020). These efforts have focused primarily on comparing populations for seedling vigor and establishment, rapid germination, competitive ability, biomass production, drought tolerance, seed production, and seed quality traits (Jones et al., 2002; Jones & Mott, 2016; S. B. Monsen et al., 2003). Commonly used outputs of these programs originate primarily from the Columbia Plateau region (CP, Supplement B) and are widely seeded throughout the Intermountain West and the Central Great Basin and Range (BR) (Appendix A. & Appendix B.).

Currently, there are two populations originating from the BR under study to determine their suitability for use in restoration. Previous planting trials with these populations show equal or better performance when compared to a commonly seeded CP population, Anatone bluebunch wheatgrass (Blair Waldron, manuscript in prep 2024). These new BR materials originate from populations within two seed transfer zones (STZs) and are known as BasinSTZ3a and BasinSTZ4. Seed transfer zones are theoretical guidelines developed based on plant morphology in common garden studies. They were developed to provide land managers with guidelines for where plant materials can be transplanted with little risk of maladaptation to local conditions. The two BR materials were selected for their high fitness relative to other collections from within their STZs. These materials have been evaluated only within common gardens and not within wildland plantings. Common garden field settings differ from wildlands in that they have prior seedbed preparation and weed control. I aimed to compare the establishment and persistence of these two BR materials with previously released varieties originating mainly from the CP, namely P-7, Anatone, and Columbia, in a wildland reciprocal transplanting experiment.

I took two approaches to quantify differences among Anatone, Columbia, and P-7, which originate mainly from the CP, and BasinSTZ3a and BasinSTZ4 bluebunch wheatgrass materials from the BR. First, to establish a baseline comparison of plant materials, I compared seedlings for key functional traits, including tiller production, regrowth potential following defoliation, leaf water content, leaf mass, specific leaf area, and relative growth rate in a greenhouse setting (Chapter 2). Second, I outplanted seedlings to wildland sites to evaluate establishment and persistence within different environmental conditions. Because most studies involving bluebunch wheatgrass plant materials involve planting populations in a prepared field or common garden (et al., 2018; Massatti et al., 2018), I transplanted seedlings into wildland sites without prior site preparation such as cultivation and weed control (Chapter 3).

I expected to see higher performance of BR materials than previously released materials within STZs in the BR. The project spanned two years, with planting conducted consecutively in both years. Through this approach, I aimed to capture the nuances in growth strategies and field survival over time. Understanding potential differences between plant materials is essential for effective restoration efforts and ecosystem management, particularly in the context of increasing climate change impacts. This study facilitates informed decision-making in selecting suitable plant materials for ecosystem restoration.

CHAPTER 2

EVALUATING BLUEBUNCH WHEATGRASS PLANT MATERIALS FOR FUNCTIONAL TRAITS

Abstract

Revegetation challenges in the Intermountain West due to poor native species establishment can be better understood by characterizing functional trait variation in plant materials. Bluebunch wheatgrass *(Pseudoroegneria spicata* [Pursh] Á. Löve) is the most commonly seeded native perennial grass species, and the plant materials currently available for this species originate primarily from the Columbia Plateau (CP) and other parts of the Intermountain West outside of the Central Great Basin and Range (BR). Plant materials from the BR are currently being evaluated as potential releases, yet little is known about their functional trait expression relative to existing materials. We compared trait variability of two tentative plant material releases sourced from the BR (BasinSTZ4 and BasinSTZ3a) and three non-BR materials widely used in the Intermountain West (Anatone and Columbia (source-identified from CP), and P-7 (multi-origin polycross from multiple regions)). Using a randomized design, we grew 280 plants per material for 91 days in a climate-controlled greenhouse. Seedlings were defoliated to a 7-cm stubble height at 77 and 91 days after emergence to determine baseline and regrowth values, respectively, for tiller number, shoot fresh and dry mass (g), leaf area (cm^2), specific leaf area (SLA; g · cm^2), and percentage leaf water content (g H2O · g fresh mass · 100). We also calculated leaf area compensation (ln(regrowth-baseline), dry mass compensation (ln(regrowth-baseline), and relative change in tiller number (((regrowth –

baseline)/baseline)*100). Our results revealed significant differences in tiller number, SLA, tiller mass, and relative recovery from defoliation. BasinSTZ4 had the lowest tiller numbers, highest tiller mass, and lowest SLA, indicating potentially greater adaptation to resource-limited environments Similarly, high baseline tiller numbers and regrowth tiller numbers for BasinSTZ3a and Anatone indicate a less conservation strategy, a strategy that has been linked to improved ability to compete with invasive annual grasses because of the relation between faster growth and interference. BasinSTZ3a's traits also may confer advantages in warm, dry climates, as it evolved in dry BR climates. In contrast, trait expression for BasinSTZ4 indicates a potential advantage for growth at dry sites. BasinSTZ3a and BasinSTZ4 exhibited the highest leaf area compensation, indicating quicker regeneration of photosynthetic leaf area, which has been associated with grazing tolerance. However, these materials also demonstrated somewhat lower baseline leaf area and dry mass, indicating potentially shorter stature and traits associated with greater expression of a conservation growth strategy than the three non-BR materials. This finding suggests a potential tradeoff between growth traits and grazing tolerance, as observed in other bluebunch wheatgrass studies, but this linkage needs to be explored further. We consider these functional trait differences in the context of the potential suitability of plant materials for different restoration-site conditions to enhance seedling establishment on Intermountain West rangelands.

Introduction

Novel disturbances in the Intermountain West (Appendix A), such as cultivation and excessive historic livestock grazing, have reduced native perennial grass abundance and increased the dominance of invasive annual grasses (Knapp, 1996; Mack, 1981; Svejcar et al., 2017). Moreover, these factors are exacerbated by drought conditions and increased wildfire frequency, both of which stress native ecosystems and hinder plant recovery (Chambers et al., 2014). Concerted efforts have been deployed to offset ecosystem changes and implement restoration approaches that target the suppression of invasive annual grasses and the recovery of depleted perennial grass populations (Boyd & Davies, 2012; Monaco et al., 2017; Pilliod et al., 2017). However, native plants often have poorer establishment and productivity when used in restoration than non-native species (Robins et al., 2020), and revegetation success in the Intermountain West is unpredictable (James et al., 2013). Among the many efforts to address these challenges, one crucial component involves testing and developing native plant materials with improved traits and growth strategies as part of an overall effort to enhance the success of rangeland seedings (He et al., 2017a; Jones et al., 2010, 2015). The resulting plant materials support a thriving seed industry and help meet the high demands for better-performing materials for novel ecosystems (Jones, 2019; Jones et al., 2015).

Among the many native perennial grasses under evaluation for plant materials development, bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] Á. Löve) is highly valued and widely relied upon in restoration projects (Jones et al., 2022; Miller et al., 1994; Staub et al., 2016; Svejcar et al., 2017). Bluebunch wheatgrass exhibits a high degree of genetic diversity across its extensive range, spanning western North America, from Alaska south to New Mexico, California east to Michigan and Texas (Zlatnik, 1999; Larson et al., 2004). Despite being the most widely seeded native perennial grass in the Intermountain West (Jones et al., 2022), its seedling establishment can be highly variable

(Zlatnik, 1999; James et al., 2013). In particular, it often shows poor persistence and productivity compared to non-native perennial grasses with greater overall adaptation to novel disturbances (Jones et al., 2022; Rigby et al., 2018; Robins et al., 2020). As such, its genetic diversity and functional importance for restoration drive a tremendous demand to develop improved plant materials that ultimately yield higher establishment success and persistence following seeding efforts.

Despite the high degree of genetic diversity and broad distribution of bluebunch wheatgrass, released materials currently available for reseeding projects throughout the Intermountain West are primarily sourced from the Columbia Plateau (CP) (Jones, 2019; Jones & Mott, 2016; S. B. Monsen et al., 2003) (Supplement 2). These materials are widely seeded throughout the Central Basin and Range (BR), but they represent only a fraction of the genetic diversity within this species (Larson et al., 2004, p. 200; Massatti et al., 2018; Prive et al., 2021). Thus, the question remains whether plant materials originating from the BR exhibit distinct variations in traits commonly associated with plant establishment and survival compared to those originating from outside the BR. Such functional differences among materials may provide insights into how they may perform differently depending on prevailing environmental pressures where they are seeded or whether genetic and functional differences signify local adaptation to novel conditions and environmental change (Broadhurst et al., 2008). For example, broad, tentative seed transfer zones (STZs), based on climate and phenotypic data collected in common garden studies, have been developed for bluebunch materials across the western US to assist restoration planning and implementation (St. Clair et al., 2013). However, the underlying traits responsible for functional variation among materials have yet to be thoroughly evaluated. Additional studies on trait variation combined with ongoing plant improvement programs could help refine STZs and expedite the release of promising new materials that may express favorable traits to overcome specific site-level environmental pressures.

Numerous regional bluebunch wheatgrass materials have been developed from wildland populations. However, only two have been targeted for the BR: the northeastern Great Basin (BasinSTZ3a) and the north-central Great Basin (BasinSTZ4). These materials are currently under seed increase but have yet to be released. In particular, the BR materials have shown equal or better population persistence when compared to the most high-performing CP-released variety, Anatone, within common garden plantings at Wells, NV, and Nephi, UT (B. Waldron, personal correspondence, 2/3/2022). To augment these extensive field trials, a greater understanding of functional traits and plant growth strategies responsible for differences in seedling establishment and stand persistence is needed to expedite plant material development.

Given the broad distribution of bluebunch wheatgrass, high levels of genetic diversity among disparate populations, and variation in plant size, phenology, and leaf structure (St. Clair et al., 2013; Massatti et al., 2018), variation among materials for functional trait expression related to seedling growth and resource acquisition is also anticipated (Craine & Craine, 2009). This variation may arise due to tradeoffs between the ability of plants to grow and acquire resources rapidly and the capacity to withstand environmental stress and conserve resources (Reich et al., 2003; Wright et al., 2004). Regarding trait variation among these extremes, plants with high growth and nutrient uptake rates are typically highly productive, producing leaves with higher nutrient concentrations and specific leaf area (SLA: leaf area per dry mass) (Wright et al., 2001). Exotic annual grasses such as cheatgrass (*Bromus tectorum* L.) exemplify this end of the spectrum. By comparison, plants with slow growth and nutrient uptake rates have lower SLA and thicker and longer-lived leaves (Hoffmann et al., 2005). As a long-lived, perennial grass, bluebunch wheatgrass generally has a conservation growth strategy characterized by a slower growth rate, lower resource acquisition, and lower growth plasticity in response to environmental conditions, including soil resource availability and growth temperature compared to faster-growing perennial grasses and invasive annual grasses (Arredondo & Johnson, 2011; Meays et al., 2000). Given this strategy, bluebunch wheatgrass expresses comparatively lower overall growth plasticity (Fraser et al., 2009; Mukherjee et al., 2015).

Nevertheless, functional trait comparisons have not been made among materials from populations within the BR and previously released materials originating outside this region. In particular, evaluating variation among these materials for demographic traits (i.e., tillering dynamics), shoot biomass production and structural traits (i.e., mass, area, and SLA), and the critical physiological trait, leaf water content following a drought period) is needed (Wang et al., 2022). Such comparisons help expedite plant material development for the BR region and improve plant establishment if the favorable expression of these traits assists plants in overcoming specific obstacles encountered on restoration sites.

Some of the most challenging environmental obstacles to seedling establishment in native perennial grasses include drought, competition from invasive annuals, and herbivory (Jones et al., 2010, 2022). With these obstacles in mind, plant materials with greater expression of high SLA, more tillers, and higher biomass are expected to perform better when competing with annual grasses for temporally limiting soil resources, favoring populations with rapid emergence and accelerated resource acquisition and growth. In contrast, in exceptionally drier, more stressful prevailing site conditions, populations that express lower biomass production and SLA, as characterized by a conservation strategy, may be able to persist better than fast-growing populations in resource-poor environments (Colesie et al., 2020).

In addition, seedling defoliation presents an obstacle to the seedling establishment of bluebunch wheatgrass and similar bunchgrasses if removal occurs after internode elongation, which results in the elevation of intercalary meristems higher into the canopy (Jones & Nielson, 1997; Anderson, n.d.; Richards & Caldwell, 1985). Because regrowth following defoliation can be either rapid from intercalary meristems located below the removal point on the leaf blade and sheath structures or relatively slower from apical meristems located at the base of plants through the outgrowth of axillary buds, the developmental stage of seedlings can significantly impact their capacity to re-establish canopy biomass and leaf area (Briske & Richards, 1991). Consequently, tillering dynamics following defoliation provide insights into how differences in developmental stage influence the capacity of grasses to re-establish their canopies and the underlying mechanisms of regrowth, i.e., the relative contributions from intercalary meristems or outgrowth from axillary buds (Briske & Richards, 1991). Tiller number and biomass per tiller of regrowth biomass may also reveal differences in compensatory growth to regenerate lost canopy, relative shoot construction costs, and contribute to our understanding of variation in the relative expression of the conservation growth strategies among bluebunch wheatgrass plant materials (Coley et al., 1985; Mukherjee et al., 2015).

In this study, we evaluated baseline and regrowth (following defoliation) variation among five bluebunch wheatgrass plant materials for demographic traits (i.e., tillering dynamics), shoot biomass production and structural traits (i.e., mass, area, and SLA), and the critical physiological trait, leaf water content following a drought period. We sought to determine whether materials from outside of the CP (Anatone, Columbia, P-7) differ in expression of these traits from the two experimental materials originating from the BR (Basin-STZ3a and Basin-STZ4) (Massatti et al., 2018). We expected broad variation in baseline and regrowth trait responses among these materials and envisioned that this variation may highlight potential differences within the otherwise conservation plant growth strategies. We expected greater expression of faster growth traits from previously released materials. This expectation is based on Anatone's documented high seedling vigor, rapid development, and ability to compete with exotic annuals. Additionally, Columbia has undergone past selection for high biomass and spike numbers, and P-7 germplasm originates from across the Intermountain West. However, none of the P-7 germplasm originates from the more arid CP or the Snake River Plain regions (St. Clair et al., 2013). In contrast, based on other materials from STZs 3a and 4 that have traits associated with arid, hot climates, we expected the BR materials to demonstrate greater relative expression of the conservation-growth strategy (St. Clair et al., 2013). Finally, because St. Clair et al. (2013) showed that plants from STZ 3a are known to express earlier phenology associated with warm, dry conditions than STZ 4 plants associated with a cooler climate and potentially higher temperature seasonality (2013), we anticipated greater expression of the conservation-growth strategy in BasinSTZ4 than BasinSTZ3a. A better understanding of the functional traits and plant growth strategies of these plant materials can help guide the development of these BR materials and assist with overcoming specific obstacles to seedling establishment encountered on restoration sites.

Materials and Methods

Plant Materials

Five bluebunch wheatgrass plant materials (hereafter “materials”) were evaluated, including the following three previously released materials: Anatone, a source-identified germplasm from Anatone, WA; Columbia, a selected-class manipulated-track germplasm from eastern WA; and P-7, a multi-origin polycross from intermating 23 native populations and two cultivars from across the Intermountain West (from Washington, Oregon, Nevada, Utah, Idaho, Montana, USA, and British Columbia; Jones et al. 2002). Anatone, originating from a more mesic site (average of 508 mm annual precipitation), was selected for its high establishment and productivity compared to other bluebunch materials, and has been found to be successful at dry sites receiving a minimum of 250 mm of annual precipitation (Monsen et al., 2003). In contrast, Columbia originates from a much drier site (average annual precipitation of 250 mm) and has undergone selection for biomass production and establishment from seed (Jones & Mott, 2016). In contrast, P-7 is a multi-origin polycross created through open pollination of 25 native populations from Washington, Nevada, Idaho, Utah, and British Columbia and developed specifically to provide high genetic diversity for semi-arid to mesic sites (Jones et al., 2022).

The other two populations originate from the Central Great Basin and Range (BR) within seed transfer zones (STZs) 3a and 4 (St. Clair et al., 2013). Both were systematically evaluated for numerous years at common gardens in Wells, NV, and Nephi, Utah, USA. Multi-origin crosses from the respective STZs were made to develop the base materials, referred to as the Basin-STZ3a and Basin-STZ4 materials (Waldron et al., manuscript in prep 2024). All seeds for the study were acquired from increase fields located in Cache County, UT, USA and were stored at 4 °C at a facility operated by the US Department of Agriculture, Agricultural Research Service, Forage and Range Research (FRR) Laboratory in Logan, UT, USA.

Propagation

We started seedlings in 3.81 cm diameter X 20.96 cm length containers arranged in 98 capacity flats (Ray Leach Super Cell, SC10U cone-tainers and RL98 trays, Stuewe & Sons, Inc., Tangent, OR). Each material was planted into three replicate flats. Cone-tainers were filled with a small amount of vermiculite to block holes in the base, then filled with a 3:1 mix of Preston fine sand and peat moss. We selected large, firm seeds with clear evidence of an embryo to ensure seed quality while planting. Then, one seed was sown in each cone with the awn oriented upwards at a depth of 5 mm. Seeds that did not germinate were replaced with a new seed after one week. After seedlings reached the three-leaf stage, fertilizer was applied weekly by supplying approximately 20 ml of 20-20-20 NPK solution to each container. The fifteen flats were randomly arranged on a benchtop in a greenhouse located on the campus of Utah State University and managed by the FRR. The air temperature of the greenhouse was set to 70˚C and thermostatically controlled with both radiant heat and outside air through a fan; no supplemental lighting was provided. Soil water content was maintained at or slightly below water holding capacity (11.5% soil water content; He et al., 2017) by watering with deionized water at least every other day.

Procedure

To determine baseline, regrowth, and relative responses to defoliation among plant materials, individual plants were defoliated to a 7-cm stubble height at 77 (baseline) and 91(regrowth) days after sowing seed, respectively. In addition, the tiller number per plant was counted before defoliation events to quantify shoot production from the clonal outgrowth of axillary buds. Seedlings were not watered for three days immediately before obtaining regrowth (91-day) biomass to subject plants to a mild drought. Harvested biomass was immediately weighed on a microbalance to determine fresh (hydrated) mass. Baseline and regrowth shoot material was lyophilized (i.e., freeze-dried) under low pressure to remove water by sublimation for 28 days. This procedure retained sample volume while removing all moisture, and dry samples were weighed on a microbalance to the nearest milligram. We then calculated leaf moisture content as the (fresh mass – dry mass). Dry shoot samples were placed on a flat-bed scanner to acquire digital images at 200 DPI and analyzed with software (WinRhizo ver. 2021, Regent Instruments Inc., Quebec, Canada) to obtain leaf area (cm^2). We calculated specific leaf area (SLA; cm^2/g) from leaf area and dry mass values. We calculated the mass per tiller (g/tillers) from the dry mass values and tiller number. Finally, we calculated the relative response to defoliation as dry mass compensation, leaf area compensation, and relative change in tillers. These measures were calculated as follows: Dry mass compensation: ln(dry mass of regrowth – dry mass of baseline), and leaf area compensation: ln(leaf area of regrowth-leaf area of baseline). Relative change in tillers was calculated as 100*((tiller count of regrowth – tiller count of baseline1)/tiller count of baseline). See Table 1 for a summary of functional traits and how they were measured.

Statistical Analyses

All data were analyzed using R (v4.3.1; R Core Team 2024). Packages used include lme4, DHARMa, emmeans, glmmTMB. The alpha for all tests was 0.05.
We assessed baseline and regrowth tiller count with generalized Poisson mixed models with planting flat as a random effect and plant material (i.e., population) as a fixed effect. Model comparisons and likelihood ratio tests were employed to evaluate the model's fit. Pairwise contrasts and mean estimates were subsequently examined using the emmeans package.

Relative change in tiller number, baseline and regrowth leaf area, baseline and regrowth dry weight, baseline and regrowth mass/tiller ratio, leaf moisture content, dry mass compensation, leaf area compensation, and specific leaf area were all modeled using linear mixed models with planting flat as a random effect.

Data were transformed as needed to meet model assumptions. We applied a square root transformation to baseline leaf dry mass and log transformations to baseline SLA, baseline tiller mass, regrowth tiller mass, dry mass compensation, and leaf area compensation.

Baseline tiller number, shoot moisture, Relative change in tiller numbers, regrowth SLA, regrowth dry weight, and regrowth tiller mass analyses did not meet model assumptions and were not improved by transformations. Thus, we compared a more conservative model –averaging across flats and fitting the average values into a model – to our original models to ensure the results were qualitatively the same. We retained the more conservative model.

We removed outliers for SLA for harvest 1 and 2. We removed values that were higher than what was biologically possible, that may have been recorded in error (values were higher than 500 cm^2/g, while all other values were under 300 cm^2/g). Additionally, we removed the top three values for baseline leaf area, which were much higher than all other leaf area values, and when we inspected the leaf area, it looked like the high leaf area may have been recorded in error because it didn't correspond to a very high value.

Model comparison and likelihood ratio testing were conducted to assess the fit of the models. Pairwise contrasts and estimates of the means were also compared using the emmeans package.

Results

Baseline and Regrowth

Shoot Dry Mass

Baseline dry mass significantly differed among materials ($P = 3.057e\text{-}08$, Appendix E, Figure 1). Columbia displayed significantly higher dry mass than the two BR materials ($P < 0.05$, Appendix F) but did not significantly differ from Anatone and P-7, which had intermediate dry mass and did not themselves differ from each other ($P > 0.05$, Appendix F). BasinSTZ3a had the lowest dry mass and was significantly lower than Columbia and P-7, but not Anatone or BasinSTZ4 (Figure 1). BasinSTZ4 had the next lowest dry mass and was significantly different from Columbia ($P < 0.05$, Appendix F) but not P-7 or Anatone (*P > 0.05*, Appendix F). For regrowth, dry mass values did not significantly differ among materials (*P=0.051,* Figure 1, Appendix E).

Leaf Area

The baseline leaf area significantly differed among materials (*P* = 3.444e-10, Figure 2, Appendix E). Columbia and P-7 exhibited significantly greater leaf area than BasinSTZ3a and BasinSTZ4 (*P* < 0.05, Appendix F), while Anatone was intermediate and not significantly different than the other materials (*P* > 0.05, Appendix F). For regrowth, leaf area was not significantly different among materials (*P* = 0.07, Figure 2, Appendix E).

Tiller Number

The baseline tiller number was significantly different (*P* = 2.2e-16, Figure 3, Appendix E) among materials, with BasinSTZ3a and Anatone significantly greater than BasinSTZ4 and P-7 materials (*P* < 0.05*,* Appendix F). Columbia was next highest, significantly greater than the lowest (*P* < 0.05*,* Appendix F), BasinSTZ4. This pattern was similar for tiller regrowth (*P* = 2.2e-16; Figure 3, Appendix E). Anatone had the greatest regrowth tiller number (i.e., significantly more than all other materials except BasinSTZ3a, Appendix C.), while BasinSTZ4 had the least (i.e., significantly fewer than all other materials). Intermediate between these two were BasinSTZ3a and Columbia (which did not differ from each other), followed by P-7.

Tiller Mass

Baseline tiller mass values were significantly different among the materials (*P* = 6.68e-15, Figure 4), with BasinSTZ4 and P-7 having significantly (*P* < 0.05, Appendix F) higher tiller mass than BasinSTZ3a and Anatone. Columbia had intermediate tiller mass and was significantly higher than (P<0.05, Appendix F) than BasinSTZ3a, but not significantly different (P > 0.05, Appendix F) than BasinSTZ4. Values were also significantly different among materials for regrowth biomass (P = 2.2e-16). BasinSTZ4 had a significantly higher tiller mass value than all other materials (P > 0.05, Appendix C.). The next highest was P-7, which did not differ significantly from the next highest, Columbia and BasinSTZ3a, but was significantly greater than the lowest, Anatone (Figure 4, Appendix C.).

Specific Leaf Area

Baseline SLA was significantly different among the materials (P = 2.2e-16; Figure 5), with BasinSTZ4 significantly lower than all materials except BasinSTZ3a (P < 0.05, Appendix F), while Anatone and Columbia had significantly higher SLA than all other materials except P-7 (P< 0.05, Appendix F). P-7 and BasinSTZ3a exhibited intermediate SLA values. This pattern in SLA was similar in regrowth biomass (P = 2.2e-16; Figure 5b). Anatone had the highest SLA relative to all other materials (P < 0.05, Appendix C.), and BasinSTZ4 had the lowest relative to all other materials. P-7, Columbia, and BasinSTZ3a were all intermediate and not significantly different from each other (P > 0.05, Appendix C.).

Shoot Moisture Content

Materials exhibited significantly different shoot moisture content values (P = 0.006829, Figure 6) under mildly water-limited conditions. However, we did not detect pairwise differences between populations (P > 0.05, Appendix C.).

For a tabular summary of these rankings, see table (2).

Relative Response to Defoliation

Relative Change in Tillers

Relative change in tillers significantly differed among materials (P = 1.656e-07, Figure 3). All materials recruited new tillers following defoliation; however, BasinSTZ4 recruited significantly fewer than all other materials ($P < 0.05$, Appendix D.), including Basin 3a.

Shoot Dry Mass Compensation

No significant differences in dry mass compensation were observed among materials ($P = .05$), and all displayed similar compensation levels after defoliation (Figure 1).

Leaf Area Compensation

Leaf area compensation was significantly different among materials ($P =0.003$, Figure 2, Appendix E). BasinSTZ3a, BasinSTZ4, P-7, and Anatone exhibited similar compensation levels ($P > 0.05$, Appendix D.), while Columbia showed lower compensation than BasinSTZ3a and BasinSTZ4 ($P < 0.05$, Appendix D.) but was similar to the others ($P > 0.05$, Figure 2).

Discussion

Plants typically exhibit widely differing growth strategies to cope with exposure to multiple interacting abiotic and biotic pressures (Adler et al., 2014; Poorter et al., 2012). Understanding plant growth strategies is vital to developing restoration plant materials that must effectively perform when exposed to specific environmental regimes characterized by differing intensities of seasonal drought, grazing pressure, and competition for limiting resources (He et al., 2017b; Mukherjee et al., 2015). Gaining this understanding is particularly important in an era of rapid climatic change where the emergence of novel ecosystems lacking historical precedent may necessitate developing improved plant materials with novel adaptations (Jones et al., 2010, 2015). This is especially true for native perennial grasses that are heavily relied upon to enhance restoration site resilience and invasive weed resistance in Intermountain West rangelands (Monsen, 2004). For example, as a long-lived, native perennial grass, bluebunch wheatgrass generally exhibits slower growth rate, lower resource acquisition, and lower growth plasticity in response to environmental conditions (Arredondo & Johnson, 2011; Mukherjee et al., 2015) compared to other perennial grass species, such as the widely planted crested wheatgrass (*Agropyron cristatum* [L.] Gaertn.) that has relatively faster growth rate and resource acquisition (Meays et al., 2000). However, in our comparison of the expression of functional growth traits among two newly developed Central Basin and Range (BR) materials and three prior-released Columbia Plateau (CP) bluebunch wheatgrass materials, we found significant variability that could impact seedling establishment and material persistence in rangelands of the BR ecoregion and elsewhere in the Intermountain West. See figure 7 for a figure describing the leaf economics spectrum and how this relates to bluebunch wheatgrass, and Table 2 for results of the functional trait analysis summarized.

BasinSTZ4 exhibits slower growth and has traits that may be advantageous at dry sites with hot summers and cold winters.

Baseline measurements showed that BasinSTZ4 produced fewer tillers than the other materials, and the same was true for tiller regrowth following defoliation. Higher tiller mass in BasinSTZ4 also illustrates that it invested growth into fewer tillers at the expense of recruiting new tillers. A lower tiller number per plant indicates a more conservation approach to growth allocation because plants with a conservation resource strategy allocate more resources to individual leaves that are typically longer lived (Adler et al., 2014). Our conservation growth designation for BasinSTZ4 is fitting because the tiller number ultimately determines the number of leaves (Briske & Richards, 1991). This is related to small size, a feature associated with greater adaptation to arid, resource-poor environments in the Intermountain West (Kulpa & Leger, 2013).

Our result that BasinSTZ4 recruited the lowest number of new tillers after defoliation is consistent with previous observations of lower recovery from defoliation for plants with low tiller numbers in bluebunch wheatgrass (Mukherjee et al., 2015). Although all materials had similar dry mass compensation from defoliation, meaning they regenerated their biomass similarly, BasinSTZ4 recruited fewer new tillers, suggesting that its regrowth came from the expansion of existing intercalary meristems. It is also likely that BasinSTZ4 plants grew more slowly or had slower development, meaning intercalary meristems were less elevated into the caopy, leaving them intact after defoliation.

Additional evidence for the relatively greater expression of the resource conservation strategy for BasinSTZ4 is that it had the lowest specific leaf area (SLA), suggesting thicker leaf blades and denser leaf tissue (Wright et al., 2004). Along the plant growth strategy spectrum, plants with slow growth prioritize resource conservation and display low SLA, characteristics associated with resource conservation strategies In resource-poor environments, greater expression of the slower, conversation resource strategy may be favored (Gibson et al., 2018). Overall, these resource conservation-oriented traits may assist the persistence of BasinSTZ4 in environments where resources are limited.

BasinSTZ3a and BasinSTZ4 had higher leaf area compensation after defoliation comared to the other materials, but had very different trait expressions overall, indicating suitability for differing site types.

Both BR materials had the highest leaf area compensation after defoliation, meaning they regenerated their photosynthetic leaf area more quickly than the other materials. Faster regeneration of leaf area is a trait associated with grazing-tolerant plants (Meays et al., 2000). However, this is just one piece of the puzzle, and many other traits are related to recovery from defoliation, such as biomass compensation (Mukherjee et al., 2013), which we did not find to be significantly different among plant materials. Other studies have found a possible tradeoff between defoliation tolerance and growth in bluebunch wheatgrass (Mukherjee et al., 2013). Our results may be evidence of this tradeoff, as in our study, materials with the lowest leaf area, BasinSTZ3a, and BasinSTZ4, also had the highest leaf area compensation. Additionally, BasinSTZ3a had the lowest baseline dry mass while BasinSTZ4's was intermediate, but their dry mass compensation matched the other plant materials. However, this linkage needs to be better documented. Further study is needed to fill the gap in the knowledge surrounding traits contributing to defoliation tolerance in this species.

The overall lower baseline leaf area and lower dry mass of BR materials. indicate that these materials may be shorter in stature than P-7 and Columbia (but not Anatone). Other studies have found that bluebunch wheatgrasses from hot, dry, nutrient-poor sites tended to be smaller, shorter, and have more narrow leaves, particularly for materials originating from STZs 3a and 4 (St. Clair et al., 2013).

Despite having some similarities, we found that the expression of certain traits in the BR materials was nuanced. In particular, we found differences in SLA between the BR materials. While bluebunch typically has relatively low SLA compared to other perennial grasses (He et al., 2017b), we found that BasinSTZ3a had an intermediate baseline SLA within this conservation strategy. In contrast, BasinSTZ4 consistently had the lowest baseline and regrowth SLA values, and Anatone had the highest SLA, suggesting that BasinSTZ3a may be more adapted to dry, resource-poor sites than Anatone, as lower SLA is associated with greater expression of the resource conservation strategy. However, despite these differences, we also found that BasinSTZ3a shared some similarities with Anatone, indicating that each material may be suitable for different restoration sites.

BasinSTZ3a exhibited similarities to Anatone, which may confer benefits in warm, dry sites prone to invasion by annuals

Based on tiller traits, Basin3a was like previously released non-BR materials in several ways. First, BasinSTZ3a, Columbia, and Anatone had higher baseline tiller numbers than the other materials. Basin3a's regrowth tiller number, though lower than

Anatone, was also higher than Basin4 and P-7. Second, based on relative change in tiller number, all materials recruited new tillers; however, BasinSTZ3a and Anatone, Columbia, and P-7 recruited more new tillers than BasinSTZ4. This signifies a difference among materials in phenological development at the time of defoliation. Materials that regenerated more new tillers, thus, had faster or earlier development compared to BasinSTZ4, and as a result, their intercalary meristems were found farther from the soil surface. Faster growth may help seedlings interfere with invasive annual grasses, as resource acquisition at the critical springtime period has been linked to the ability to compete with invasive annual grasses (Arredondo & Johnson, 2011; Jones et al., 2010).

In addition to producing more tillers, BasinSTZ3a and Anatone produced tillers with the lowest mass, suggesting these materials invested less into leaf structure than other materials. Lower structural investment into leaves has been associated with shorter-lived leaves and faster growth strategies (Wright et al., 2004). Anatone was chosen for its productivity, vigor, and potential to compete against introduced annual grasses (Monsen et al., 2003). Although it originates from a relatively more mesic site, Anatone performs exceptionally well at many sites within the region (Monsen et al., 2003). The similarities between Basin3a indicate that this material may also be widely adapted and succeed at different site types in the BR. However, more studies are needed to correlate these traits with wildland performance at various sites.

Conclusion

In this study, we identified the relative expression along the conservation end of the growth strategy for two new bluebunch wheatgrass plant materials compared to previous releases. Our findings highlight the intra-specific variability in select traits among these materials. While bluebunch wheatgrass has an overall conservation growth strategy compared to other perennial grasses used regionally for ecosystem restoration (Meays et al., 2000), we found notable trait variability, with Basin4 showing greater expression of resource conservation traits. In contrast, BasinSTZ3a expressed higher values for traits linked to a faster growth strategy. BasinSTZ4 exemplifies a conservation growth strategy characterized by a low tiller number, high tiller mass, and a lower specific leaf area (SLA). On the other hand, BasinSTZ3a had a higher tiller number and relatively lower tiller mass compared to BasinSTZ4, thus aligning with the previously released material Anatone.

These materials show promise for seed transfer zones in the Basin and Range Province of the Great Basin because they originate from the BR and are expected to perform well in the BR's unique climate. BasinSTZ4 has a greater expression of a conservation growth strategy and may do well at resource-limited sites with higher temperature extremes between summer and winter (Roybal & Butterfield, 2018). On the other hand, Basin3a has many traits in common with Anatone, suggesting it may be widely suitable across the BR, yet this needs further study.

In the future, these materials will serve as base populations for further testing and cycles of selection to generate additional new plant releases. As this proceeds, field testing will need to evaluate the potential correlations between critical traits, as we have done, and field establishment and persistence under various variable environmental conditions across seed transfer zones. Additionally, these materials may undergo selection to consistently express desired traits and ensure that the traits are heritable to future generations. In the same process, key traits must be related to seed production because release materials must be capable of efficient and abundant seed for use in practical restoration settings.

Understanding these nuances in growth strategies is crucial for conservation efforts and ecosystem management in the face of climate change. By examining the intricate variations within and between bluebunch wheatgrass plant materials, our study contributes to a more comprehensive understanding of plant adaptation strategies and aids in selecting suitable plant materials for restoration.

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Tables and Figures

Table 1. Functional traits measured.

Trait name	How it was calculated	Biological significance
Tiller count baseline & regrowth	Individual tillers counted prior to defoliations	Clonal asexual growth from axillary buds - this reflects growth potential for grasses. Tillers may go on to spread laterally and create new roots.
Leaf area of baseline and regrowth	A measure of the surface area (cm^2) of defoliated leaf cuttings from baseline and regrowth.	Greater leaf area is aligned with increased available area for photosynthesis
Dry mass of baseline and regrowth	Dry mass (g) of defoliated leaf cuttings for baseline and regrowth.	Biomass is used to calculate other variables that relate to leaf construction costs.
Specific leaf area of baseline and regrowth	Quotient of surface area to mass (cm^2/ g). We calculated this for baseline and regrowth.	SLA is associated with leaf longevity and growth strategy.
Tiller mass of baseline and regrowth	This is the quotient of dry mass(g) to tiller count	This indicates the mass of individual tillers, pointing to

	(g/tillers). We calculated this for baseline and regrowth.	the structural investment and leaf construction costs among the materials.
Dry Mass Compensation	ln(leaf area of regrowth – leaf area of baseline)	This refers to the plants ability to regenerate lost biomass after defoliation
Leaf Area Compensation	ln(leaf area of regrowth – leaf area of baseline)	This refers to the plants ability to regenerate lost leaf area after defoliation
Leaf Moisture Content	The quotient of fresh mass – dry mass to fresh mass.	Water is involved in biochemical reactions and regulates metabolism and plant growth.
Relative change in tiller number	100*((regrowth – baseline1)/baseline).	A measure of whether the number of tillers relatively increased or decreased after defoliation.

Table 1: Functional traits measured.

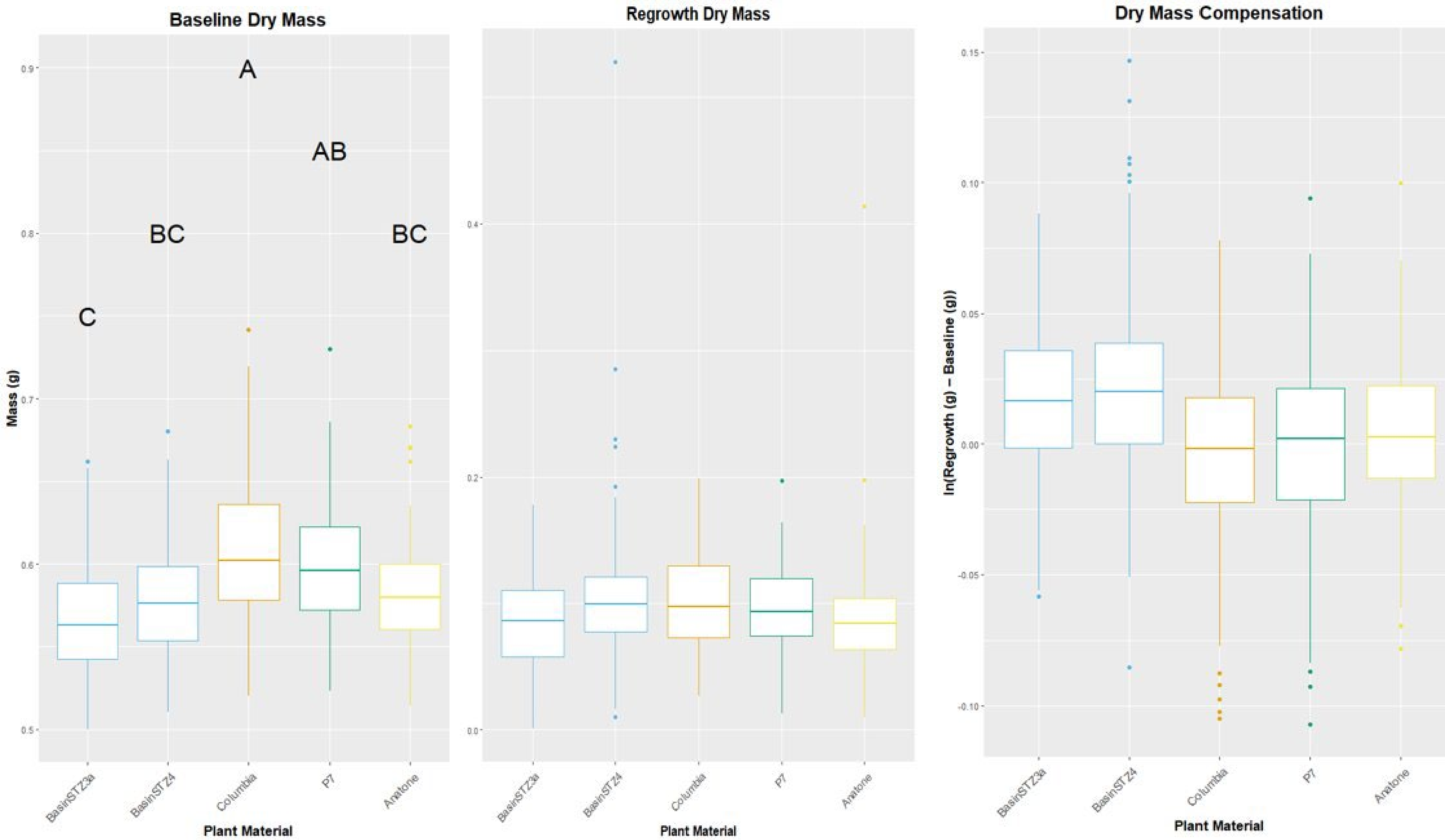

Figure 1: Dry Mass
Figure 1a, left panel: Baseline dry mass. Figure 1b, middle panel: Regrowth dry mass. Figure 1c, right panel: Dry mass compensation.

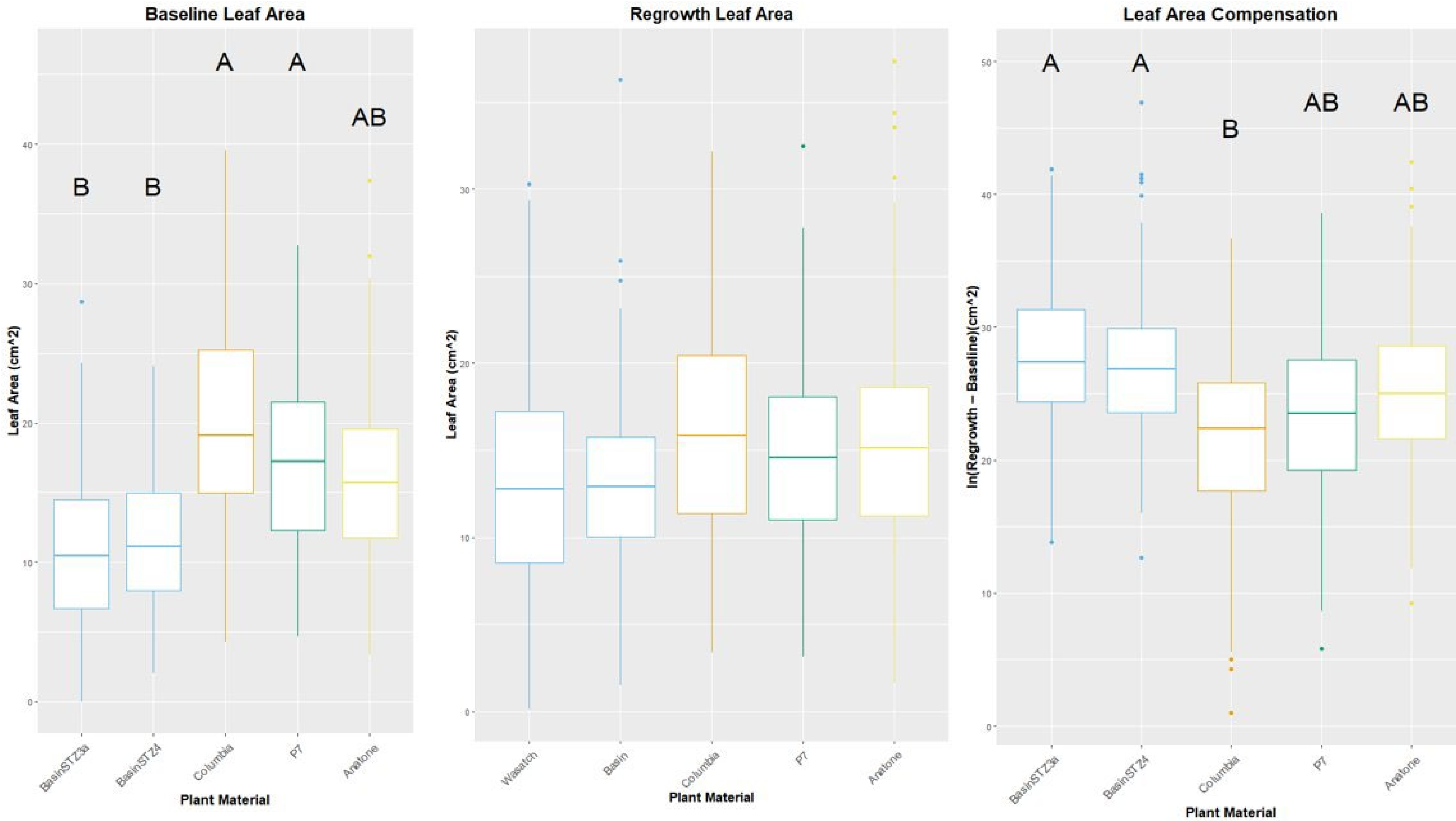

Figure 2: Leaf area

Figure 2a, left panel: Baseline leaf area. Figure 2b, middle panel: Regrowth leaf area. Figure 2c, right panel: Leaf area compensation.

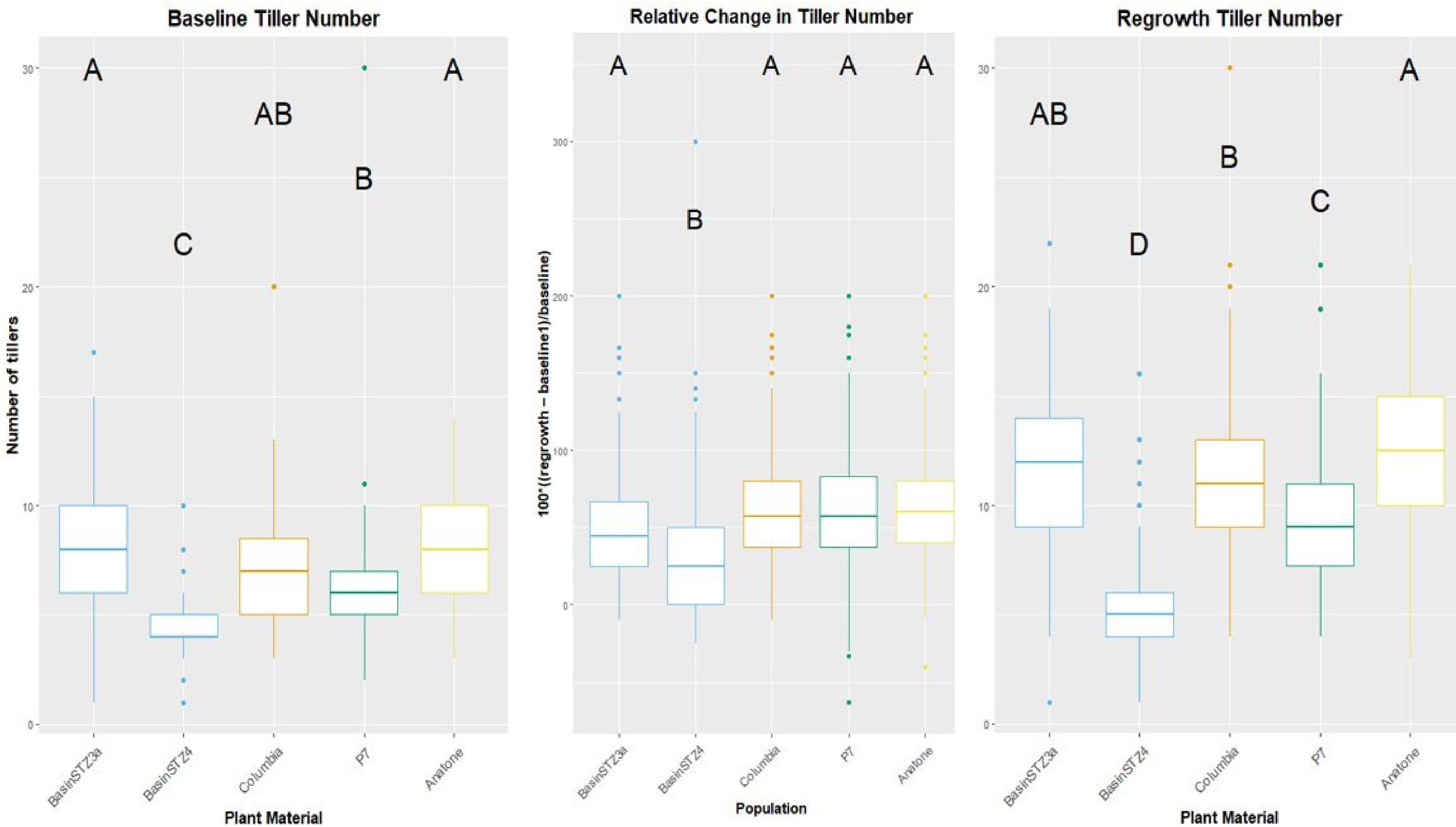

Figure 3: Tiller number

Figure 3a, left panel: Baseline tiller number. Figure 3b, middle panel: Regrowth tiller number. Figure 3c, right panel: relative change in tiller number.

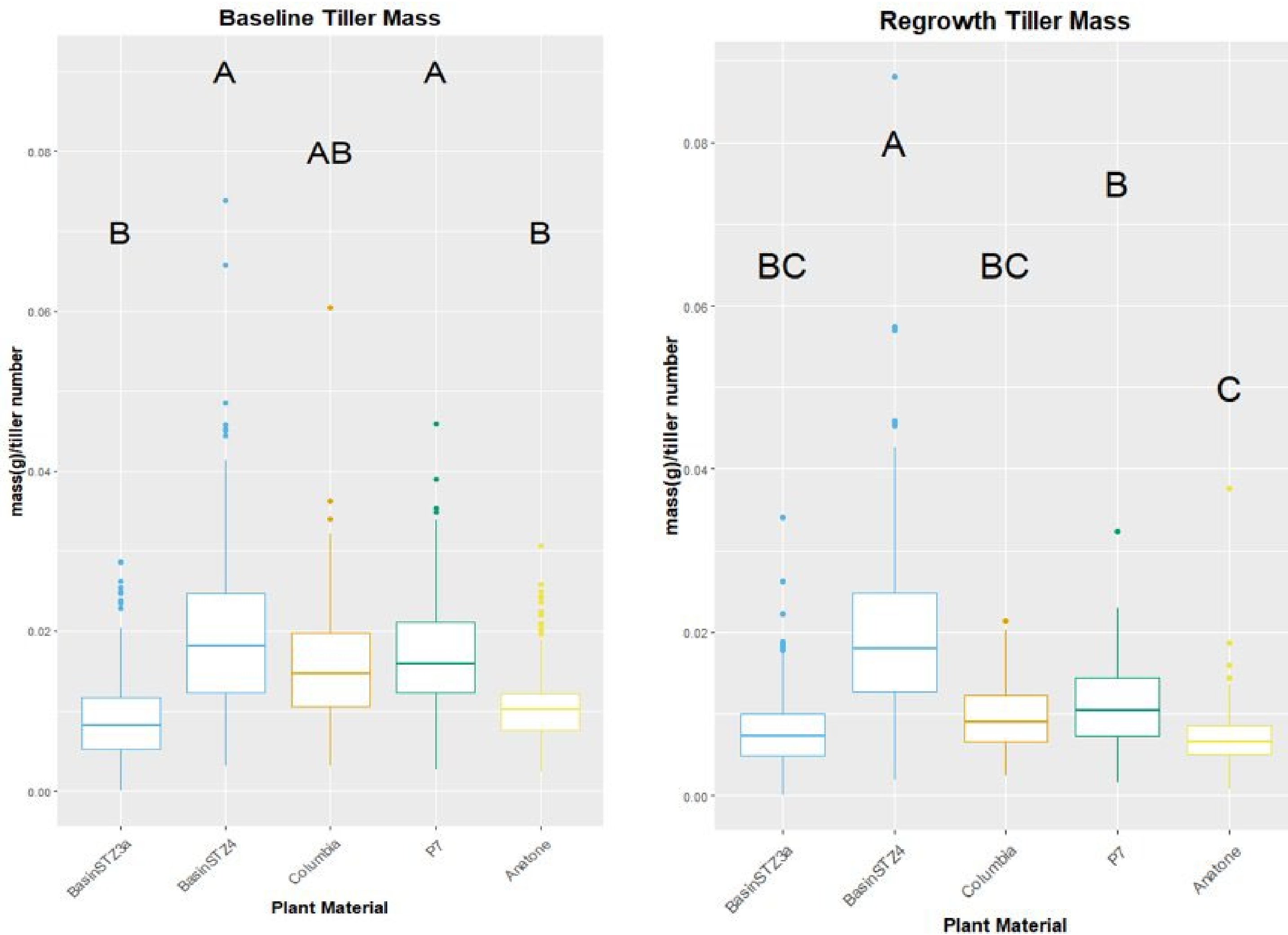

Figure 4: Tiller Mass
Figure 4a, left panel: Baseline tiller mass. Figure 4b, right panel: Regrowth tiller mass.

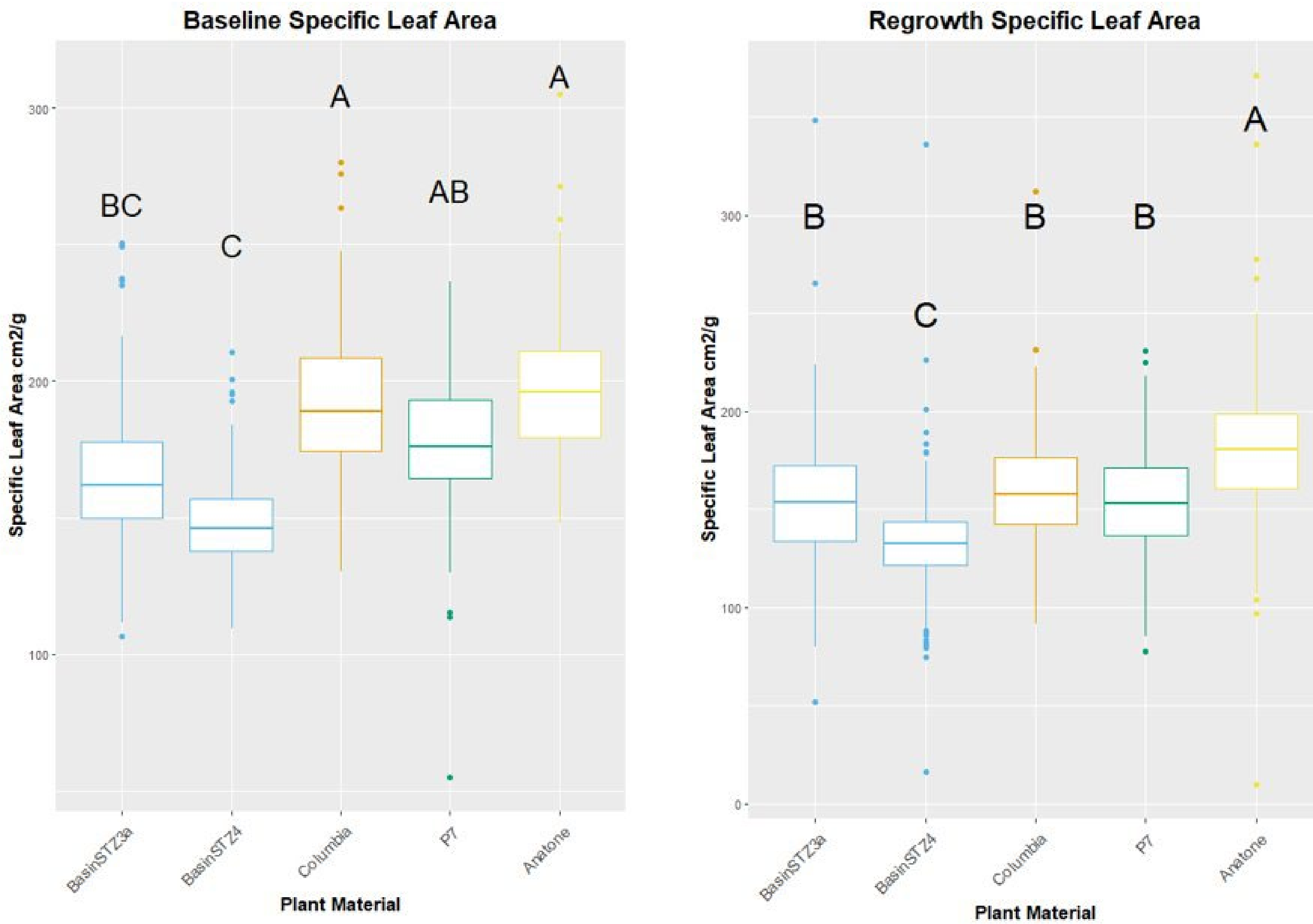

Figure 5: Specific Leaf Area
Figure 5a, left panel: Baseline tiller mass. Figure 5b, right panel: Regrowth tiller mass.

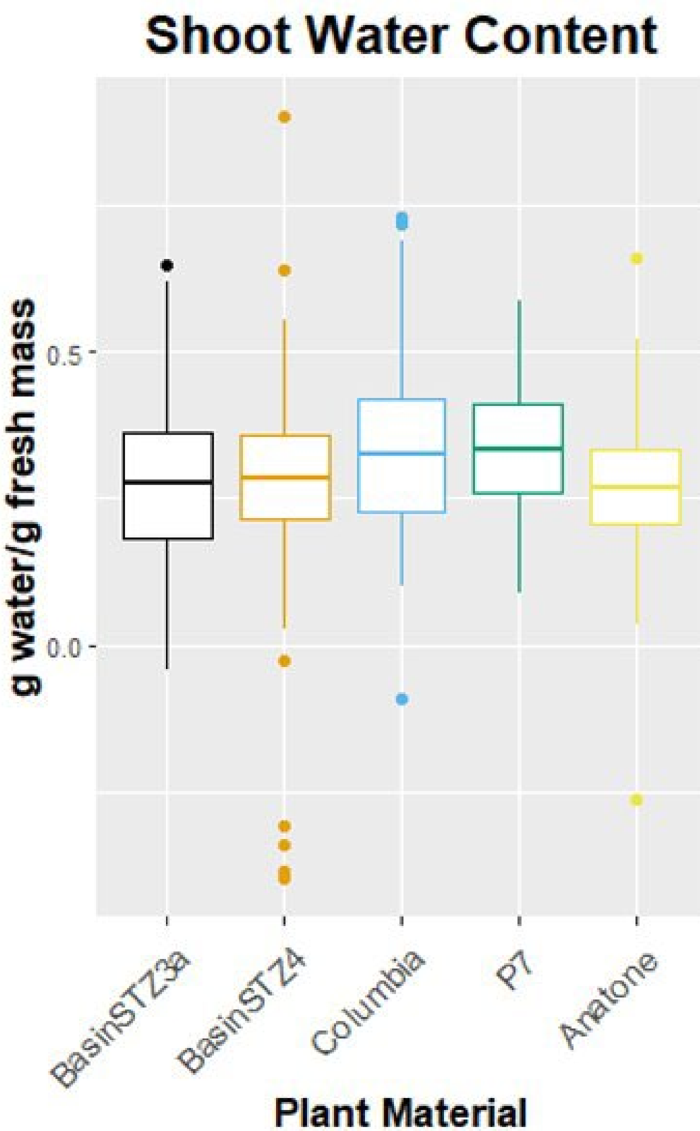

Figure 6: Shoot water content

Table 2: Results of Functional Trait Analysis

Trait	Plant Material	Rank (highest to lowest)	Leaf Economic strategy
Baseline dry mass	Columbia	A	Faster/greater generation of biomass may be associated with a resource acquisition strategy
	P7	AB	
	Anatone	BC	
	BasinSTZ3a	BC	
	BasinSTZ4	C	
Baseline leaf area	Columbia	A	Greater leaf area is aligned with increased available area for photosynthesis and is related to a resource acquisition strategy
	P7	A	
	Anatone	AB	
	BasinSTZ3a	B	
	BasinSTZ4	B	
Leaf area compensation	P7	AB	Greater leaf area compensation is
	Anatone	AB	

	Columbia	B	related to a resource acquisition strategy and may help plants compete against invasive annuals
	BasinSTZ3a	B	
	BasinSTZ4	B	
Baseline tiller number	Anatone	A	Tiller number reflects growth potential for grasses and is related to plant size. Having more tillers is related to a resource acquisition strategy.
	BasinSTZ3a	A	
	Columbia	AB	
	P7	B	
	BasinSTZ4	C	
Relative Change in Tiller Number	Columbia	A	Faster/greater generation of leaf area is associated with a resource acquisition strategy
	P7	A	
	Anatone	A	
	BasinSTZ3a	A	
	BasinSTZ4	B	
Regrowth tiller number	Anatone	A	Faster/greater generation of tillers
	BasinSTZ3a	AB	

	Columbia	B	is associated with a resource acquisition strategy
	P7	C	
	BasinSTZ4	D	
Baseline tiller mass	BasinSTZ4	A	More structural investment into tillers is related to a resoruce conservation strategy
	P7	A	
	Columbia	AB	
	Anatone	B	
	BasinSTZ3a	B	
Regrowth tiller mass	BasinSTZ4	A	More structural investment into tillers is related to a resoruce conservation strategy
	P7	B	
	Columbia	BC	
	BasinSTZ3a	BC	
	Anatone	C	
Baseline Specific Leaf Area	Anatone	A	High SLA is associated with a resource acquisition strategy
	Columbia	A	
	P7	AB	
	BasinSTZ3a	BC	

	BasinSTZ4	C	
Regrowth Specific Leaf Area	Anatone	A	Low SLA is associated with a resource conservation strategy
	Columbia	B	
	P7	B	
	BasinSTZ3a	B	
	BasinSTZ4	C	

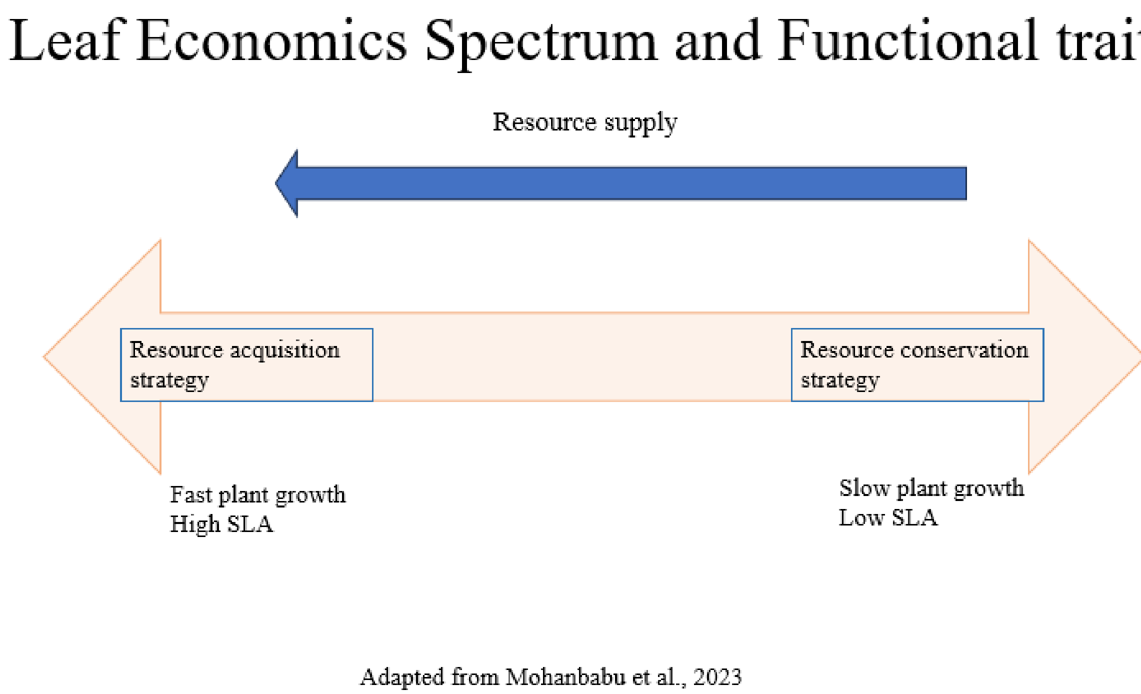

Figure 7: Leaf economics spectrum and functional traits

CHAPTER 3

EVALUATING BLUEBUNCH WHEATGRASS PLANT MATERIALS IN OUTPLANTING

Abstract

Bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve), a dominant bunchgrass of the Intermountain West, faces declining populations due to invasive species and increasing wildfire frequency. Challenges associated with restoring this grass include inconsistent or low seedling establishment due to challenging abiotic/biotic conditions. Available seed sources for restoration originate primarily from the Columbia Plateau region in Washington State and represent only a fraction of the genetic diversity in bluebunch wheatgrass. Putative seed transfer zones (STZs) have been developed where populations may be best adapted; however, there is a need to empirically test the performance of available and experimental materials within the Basin and Range (BR) region. We transplanted seedlings of three previously released (non-BR) plant materials (Anatone, Columbia, and P-7) and two experimental materials from the BR (BasinSTZ3a, BasinSTZ4) to compare patterns in establishment, survival, and percentage plant greenness in wildland settings within two seed transfer zones (3a, 4) in spring of 2022 and 2023. We expected that BasinSTZ3a and BasinSTZ4 would perform best in their respective seed transfer zones. We transplanted seedlings to two locations in STZs 3a and 4 for a total of four sites (Park Valley North (STZ4), Park Valley South(STZ3a), Holden(STZ4), and Fillmore(STZ3a)). At each site, we had 11 or 12 fenced plots, with each of five materials planted six times within each plot. For the 2022 planting, we found

plant survival did not vary among plant materials, as most seedlings died during the summer, and only 11 of the 1400 plants planted survived until spring 2023. However, for the 2023 planting, we found surviving plants after six months at both Park Valley sites. Park Valley South (3a) had 179 out of 315 survivors (57%), and Park Valley North(STZ4) had 234 survivors out of 321 (73%). At Park Valley North(STZ4), survival did not significantly differ among plant materials; however, at Park Valley South(STZ3a), BasinSTZ4 had the lowest greenness, while BasinSTZ3a, Anatone, Columbia, and P7 had similar and higher greenness. We do not yet know the potential survival from our planned spring 2024 sampling. In contrast, the first year's planting outcome shows that even with ideal planting conditions, seedlings may experience high levels of transplant shock when exposed to hot and dry conditions typical of the summer months.

Introduction

The Intermountain West (IW, Appendix A.) has faced significant ecological challenges due to disturbances such as cultivation, wildfire, and inappropriate grazing pressures, which have led to a decline in native perennial grass populations (Knapp, 1996; Mack, 1981; Svejcar et al., 2017). Consequently, there is a considerable need to investigate active restoration strategies capable of augmenting depleted perennial populations and reestablishing populations where local extinction has occurred. Historically, this has been accomplished through seeding a wide range of plant materials, with non-native perennial grasses typically performing better than native grasses (Menke, 1992). However, native materials, when possible, are preferred for restoration (Humphrey & Schupp, 1999; Knapp, 1996; Millar & Libby, 1989), because in using non-local materials, there is a risk of mal-adaption and genetic pollution (McKay et al., 2005) resulting in biodiversity losses. Native plants are recommended to conserve biodiversity, maintain ecosystem services, and enable climate adaptation (Beckwith et al., 2022).

Bluebunch wheatgrass (*Pseudoroegneria spicata*, [Pursh] A. Löve) holds significant importance as a native bunchgrass in the Intermountain West and was once a common species in many plant community types in the region (Daubenmire, 2012; Eaton, 1982; Miller et al., 1994). Despite being the most widely seeded native bunchgrass in the IW, it often shows poor seedling establishment (Elana, Zlatnik, 1999). Bluebunch also has lower productivity and establishment compared to non-native perennial grasses like crested wheatgrass (*Agropyron cristatum* (L.) Gaertn*. ssp. cristatum*) and Russian wildrye (*Psathyrostachys juncea* (Fisch.) Nevski) (Meays et al., 2000). Therefore, there is a high demand to increase establishment success by identifying and evaluating promising new materials with improved performance and traits to cope with novel disturbance regimes (Jones et al., 2015).

Considerable effort has been dedicated to understanding the genetic diversity of bluebunch wheatgrass and establishing plant selection and breeding programs (Larson et al., 2004; Staub et al., 2016). Existing bluebunch wheatgrass plant materials for restoration originate from the Columbia Plateau (CP) region (Supplement 2) and currently support a thriving seed industry that supplies the necessary seeds for rehabilitation efforts (Jones, 2019). These materials are widely seeded throughout the Central Basin and Range (BR) in rehabilitation projects but represent only a fraction of the genetic diversity that exists within the species (Larson et al., 2000, 2004; Massatti et al., 2018; Prive et al., 2021). Given the differences in climate in the CP and BR and the high levels of local adaptation in bluebunch, the question arises whether materials originating from the BR could achieve higher plant establishment and persistence than those originating from the CP. If so, it may be possible to maximize the success of BR materials by planting them in appropriate seed transfer zones (STZs), which have been developed for restoration planning and implementation (St. Clair et al., 2013). STZs are based on climate and phenotypic data collected in common garden studies and provide the spatial extent where populations of a given species can be planted with greater adaptation potential.

Several experimental bluebunch wheatgrass materials are in the final stages of development before putative release to the public for seed production and use by restoration practitioners. These materials originate from the BR region and specific seed transfer zones characterized by frequent wildfires and restoration activities. Designated as "BasinSTZ3a" and "BasinSTZ4" (with 3a and 4 indicating the STZ of origin), these materials were created through open pollination of random plants sourced from four foundational wildland collections. These collections were chosen based on their superior fitness relative to other similar origin collections/accessions at two distinct BR sites (Blair Waldron, manuscript in prep 2024). They have been assessed in the field in a common garden setting with prior seedbed preparation. These continued assessments suggest that the new materials exhibit comparable or enhanced seedling vitality and establishment compared to Anatone germplasm, which was released in 2003 (Blair Waldron, manuscript in prep 2024) and has since been widely used across the Intermountain West. However, these BR materials have yet to be tested in wildland settings, which differ from cultivated fields because they have no prior seedbed preparation. A better understanding of the wildland performance of these plant materials can help guide the further development of promising new BR bluebunch wheatgrass populations.

We conducted an outplanting study comparing already-released materials and the new materials from the Basin and Range region. We transplanted seedlings of two new materials from the BR (BasinSTZ3a, BasinSTZ4) and three previously released non-BR plant materials (Anatone, Columbia, and P-7) to compare patterns in establishment and survival at each of two sites within STZ 3a and 4 in Utah. We expected that BasinSTZ3a and BasinSTZ4 would perform best in their respective seed transfer zones and that the previously released materials would have lower survival.

Methods

Plant Materials and Origin

We utilized five distinct plant materials for this study: two originating from populations within the BR and three previously released plant materials. The BR materials were sourced from multiple origins within Seed Transfer Zones (STZs) 3a and 4 and underwent systematic evaluation in common gardens in Wells, NV, and Nephi, Utah. These populations, BasinSTZ3a and BasinSTZ4, were developed through multi-origin crosses. The released materials included P-7 (multi-origin polycross from intermating 23 native populations and two cultivars), Anatone (source-identified germplasm from Anatone, WA), and Columbia (selected-class manipulated-track germplasm from eastern WA). All seeds for the study were acquired from increase fields located in Cache County, UT, USA and were stored at four °C at a facility operated by the

US Department of Agriculture, Agricultural Research Service, Forage and Range Research (FRR) Laboratory in Logan, UT, USA.

Study Locations and Site Selection

Two wildland study locations were identified within each STZ (3a and 4), representing differing BR ecological sites and climates (Appendix G, Figure 8). The sites were selected based on the ability to obtain permissions and the presence of a sagebrush community. These locations are in Central and Northeast (NE) Utah, USA, and are either on or adjacent to private land with seasonal grazing use. All sites were at least 70% bare ground. Weedy annuals dominated two of the four sites, while the other two were dominated by seeded non-native perennial grasses (Supplements 8-11). Holden(STZ4) is a semidesert loam Wyoming big sagebrush ecological site with 80% bare ground, weedy annuals, and rodent burrows (Appendix H). Fillmore (STZ3a) is an upland stony loam Wyoming big sagebrush ecological site with 72 % bare ground with vegetation dominated by seeded introduced perennial grass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey) and weedy annual grasses (*Bromus tectorum* L.) (Appendix I). Park Valley North (STZ4) is an upland stony loam black sagebrush ecological site with 87% bare ground, seeded exotic crested wheatgrass, and native forbs (Appendix J). Park Valley South (STZ3a) is a semidesert loam Wyoming big sagebrush ecological site with 92% bare ground with seeded introduced perennials, including crested wheatgrass and forage kochia (*Bassia prostrata* (L.) A.J. Scott) (Appendix K).

A total of 12 4x4m (Appendix L) study plots each were established at the Fillmore(STZ3a) and Park Valley South(STZ3a) sites and 11 at the Park Valley North(STZ4) and Holden(STZ4) sites. Each plot was fenced to prevent livestock and large ungulate access. Fencing around each plot was approximately 1.5m tall and constructed from four cattle panels (5m length) anchored with steel t-posts on the corners at the mid-point of each panel.

Plot locations were selected based on the following criteria: avoid noticeable hills to prevent moisture runoff, avoid prominent disturbances, and select sparse portions of the sagebrush stand to allow room for transplanting in the interspace. An approximately 0.5 m buffer, in which no planting occurred, was left around the inside border of the plots to discourage herbivory through the exclosure fence.

Transplanting and Field Setup

We initiated the planting process by sowing seeds into containers measuring 3.81 cm in diameter and 20.96 cm in length, organized in flats with a capacity of 98 (utilizing Ray Leach Super Cell, SC10U cone-tainers, and RL98 trays from Stuewe & Sons, Inc., Tangent, OR). Each of the five materials was distributed across three replicate flats. Cone-tainers were prepped by adding a layer of vermiculite to cover the base holes, followed by a filling of a 3:1 blend of Preston fine sand and peat moss. Carefully selected robust seeds with visible embryos were planted to ensure high seed quality, with one seed placed in each cone, oriented with the awn facing upwards at a depth of 5 mm. Non-germinating seeds were replaced with new ones after a week. Once seedlings reached the three-leaf stage, a weekly regimen of fertilizer was introduced, administering roughly 20 ml of 20-20-20 NPK solution to each container. The fifteen flats were positioned randomly on a greenhouse benchtop located at Utah State University's campus, under the management of the FRR. The greenhouse environment was maintained at a temperature of 70˚C, regulated by radiant heat and external airflow via a fan, without additional artificial lighting. Soil moisture levels were kept at or slightly below the water holding capacity (11.5% soil water content; as per He et al., 2017) by watering with deionized water at least every other day. Plants were clipped to a 7 cm stubble height at 77 and 91 days to minimize transpiration and were transplanted to the field in May 2022. For the second planting, seedlings were clipped once before transplanting to the field in May 2023. The seedlings were placed in the interspaces between existing shrubs, ensuring minimal root disturbance. Plants were randomized within the exclosures (Figure 9), where we randomly planted six individuals of each of the five varieties: Anatone, P7, Columbia, Basin, and BasinSTZ3a. Holes were bored with either a 5-cm auger attached to a power drill or a shovel.

We backfilled the holes to avoid air pockets and ensured the transplants were slightly concave at the soil surface. The seedlings were then watered until the soil surrounding their hole was fully saturated. We watered the seedlings immediately after transplanting and again five weeks after.

Field Data Collection

To approximate plant survival, we measured plant greenness on a 5-point scale (0 = 0% green or plant was dead/absent, 1 = 1-10% green, 2 = 10-25% green, 3 = 26-50% green, 4 = 51-75% green, 5 = 76-100% green), which was intended to indicate plant survival but could not detect dormant plants. We evaluated 6-month seedling greenness in October after transplanting both years and measured one-year survival and the presence of inflorescences for the 2022 planting. The one-year survival for the 2023 planting is planned for 2024.

To evaluate the canopy and ground cover around each transplanted seedling, circular quadrats with a diameter of 35 cm were placed around each plant. Digital images were captured and analyzed for ground cover with SamplePoint (Booth et al., 2006) with a 7x7 grid. Plants were identified at the species level, and other categories included dung, moss, bare ground, organic litter, and rocks. At each site, basal gaps between perennial vegetation were measured along a 4m long transect parallel to and 1 m outside a randomly chosen side of each of the 11 or 12 plots.

Data Analyses

Data were analyzed using R (v4.3.1; R Core Team 2024). Packages used include lme4, glmmTMB, DHARMa, and emmeans).

For the 6-month greenness measurement, we used greenness as a proxy for survival (0 meaning dead, 1-5 meaning live). Greenness values from individual plants were averaged at the population level within a plot. For each site where we analyzed greenness, data were analyzed using a generalized beta family mixed model (alpha = 0.05) with a Plot as a random effect. Pairwise contrasts and mean estimates were subsequently examined using the emmeans package. We analyzed the 6-month greenness of Fillmore for the 2022 planting. We analyzed 6-month greenness for Park Valley North(STZ4) and Park Valley South(STZ3a) sites for the 2023 planting. We did not analyze the 6-month greenness for the other sites due to a lack of survivors. Likewise, we did not analyze 1-year survival for the 2022 planting due to having too few surviving plants.

Results

Weather

For the 2022 planting, all sites experienced an abnormally hot and dry summer following planting (Figures 3 & 4, Table 1). We used data from two NRCS SCAN (*Soil Climate Analysis Network | Natural Resources Conservation Service*, n.d.) stations to assess weather during the study period. These two stations are located near our four study sites: one in NE Utah near Park Valley and one in Central Utah near Holden. We found that the summer after planting was warmer than average (Figure 13a and b) except for May. May was cooler than average and with more freezing temperatures than normal at both sites (Figures 11 and 12). In the three months following planting, precipitation at the NE SCAN site was 70% of normal, while the Central Utah SCAN site was 41% of normal (Table 3).

For the three months following the 2023 planting, precipitation at the NE SCAN site was 151% of normal, while at the Central Utah SCAN site, it was 49% of normal (Table 3).

2022 Planting Greenness and Survival

In the fall after the 2022 planting, only the Fillmore(STZ3a) site had plants that appeared to have greenness values greater than 0) (Figure 14). At that site, 34% of plants (111 out of 342) had greenness scores greater than zero, and all populations performed similarly ($p = 0.11$, Appendix M). In the spring, one year after planting, we found surviving plants (3% of the total 357 planted) at only one site out of four (Park Valley North(STZ 4)).

2023 Planting greenness

In the fall of 2023 (~ 6 months after planting), both Park Valley sites had green plants with inflorescences (Figure 15). Park Valley South 3a had 179 out of 315 plants greater than 0 on the greenness scale (57%) and 9 plants with an inflorescence, and Park Valley North(STZ4) had 234 plants greater than 0 on the greenness scale out of 321 (73%) and 18 individuals with inflorescences (Figure 9). Within Park Valley North(STZ4), populations did not perform significantly differently ($P = 0.06$, Appendix M); however, within Park Valley South(STZ3a) ($P < 0.05$, Appendix M), BasinSTZ4 had the lowest greenness, while BasinSTZ3a, Anatone, Columbia, and P7 had similarly higher greenness (Supplement 14).

Discussion

The effectiveness of revegetation efforts in the Intermountain West is often uncertain (James et al., 2013), with restoration results heavily influenced by initial site conditions, historical disturbances, and fluctuating weather patterns from year to year (Hardegree et al., 2018). The BR materials had equal or better persistence than Anatone in other plantings in common garden settings (B. Waldron, personal communication), so we expected better performance from the BR materials than the previous releases. Instead, we found equally poor performance (i.e., no survival) across populations for our first cohort. The BR materials were previously evaluated in a common garden setting with prior seedbed preparation. Such site preparation may have vastly altered the soil structure, which may have led to different outcomes compared to plants grown in wildlands. Plants also face additional stressors in a wildland setting, which may explain why we had low survival across all populations. Many factors may have compounded the environmental stress experienced by outplanted seedlings, including drought, transplanting shock, freezing, and herbivory from rodents. Indeed, the western US experienced below-average to significantly reduced precipitation in 2022 (*NCEI*, 2022), while Utah saw notably higher temperatures than usual. We found similar trends using data sourced from NRCS (Natural Resources Conservation Service) scan stations (Figure 10). Simultaneously, May 2022 had more freezing events than normal. Freezing can result in seedling mortality from frost injury (Smith, 1964), which may have impacted the vulnerable seedlings directly after transplanting. However, one-year survival values from the 2023 planting are pending, and they might exceed those of the 2022 planting if the 6-month greenness values indicate spring survival.

Year of planting may have played a significant role in plant survival. The 2022 planting had almost no survival (11 of 1413 seedlings) by one year post-planting, while our fall (~6-month post-planting) assessment of the 2023 planting suggests that one-year survival may be higher than the 2002 planting. For the 2022 planting, six-month greenness values indicated plant survivors at only Fillmore(STZ3a) with an average plant greenness of 5.3%, while for the 2023 planting, the average greenness at two sites (both Park Valleys) was 34.7%. Additionally, we found 27 plants from the 2023 planting at both Park Valley sites with inflorescences, indicating that plants were robust. These results indicate that potential survivorship in the spring of 2024 may exceed what was observed for the 2022 planting.

For the 2023 planting, greenness values indicated enough surviving plants at both Park Valley sites to analyze 6-month responses. At Park Valley South (STZ3a), we found that BasinSTZ3a performed better in its STZ than did BasinSTZ4, which we had expected. We also expected BasinSTZ4 to perform the best in its own STZ (Park Valley North (STZ 4)). Although there were more survivors at this site than in Park Valley South (STZ 3a) (73% and 57%, respectively), we did not detect significant differences among populations. It is likely that under more favorable conditions in 2023 (a summer with 150% of average precipitation), population differences were less pronounced.

We also found that Anatone, Colombia, and P-7 performed similarly to BasinSTZ3a and better than Basin (STZ4) when we expected both BR materials to perform better than previously released non-BR materials. One explanation is that these previous releases may have been better equipped to compete with existing vegetation for water. The vegetation and soils differed among sites (Appendix G) and may have affected water availability throughout the summer. Plants at our field sites experienced competition for limiting soil water from other plants, including the invasive annual grass *Bromus tectorum* and the spreading perennial forb *Convolvulus arvensis.* Similarly, competition for water may also influence results at Park Valley South (STZ3a), where vegetation was dominated by forage kochia*,* which is known to suppress cheatgrass strongly (Monaco et al., 2003). We also found evidence of predation from rodents based on the presence of rabbit pellets and rodent burrows, but we are unsure how these factors may have influenced seedling mortality.

It is curious that, for our first 2022 cohort, we observed green plants in the fall (6 months post-planting) only at Fillmore(STZ3a) but found some green plants in the spring

(1 year post-planting) at Park Valley North(STZ4). It was difficult to determine if plants were alive without pulling them out of the ground to see if their root systems were intact. Our visual assessment also made it challenging to estimate survival in the late fall after plants become quiescent and typically lose greenness. Plants that we identified as 0% green may have been quiescent and still alive.

Our results reveal the limitations of transplanting seedlings as a restoration approach. Although transplanting may yield fewer established plants than seeding, it typically has greater per-plant survival in wildlands (Abella et al., 2012; Engel et al., 2019; Palma, A.C., 2015). While transplanted seedlings bypass the demographic limitations associated with germination and emergence (James et al., 2011), their success may be limited by unfavorable climatic conditions (Hardegree et al., 2018). Because plant establishment is highly dependent on temperature and timing of precipitation (Copeland et al., n.d.; James et al., 2019), insufficient soil moisture can result in poor seedling survival (Agneray et al., 2022). Thus, because our study sites experienced pronounced dry periods in the months following the 2022 planting, seedlings were undoubtedly exposed to extreme limitations to soil water availability crucial for seedling establishment.

We attribute the low survival we observed for the first 2022 cohort partially to an abnormally hot and dry summer. Given the variability in spring moisture and timing in this region, survival may have been enhanced if we had supplied additional irrigation in the months following transplanting. For example, prior transplant studies with perennial grasses near this region showed greater plant survival with continued irrigation after planting (Abella et al., 2012). In addition, similar to our results, another study found that transplants failed to survive without additional irrigation after planting (Grantz et al., 1998) and that perennial grass transplants overall had low survival rates (Abella et al., 2012). Additionally, transplanted seedlings must overcome significant transplant shock to persist, in which water is unavailable until they regrow fine root hairs (Jordan, 2010). Likely, all materials experienced physiological stress originating from transplant shock, and this may have played a role in the survival outcomes. Transplant shock may have been compounded by drought and freezing damage. Future studies should incorporate irrigation with transplants to ensure survival in dry years.

Further considerations that may play a role in the survival of transplants post-planting include container size, nursery watering regime, and duration of growth in the greenhouse (Abella et al., 2012). Container size impacts root length and volume, and a deeper container may improve plants' abilities to access soil moisture (Landis et al., 2010). We used plastic containers of all the same size. Additionally, the nursery irrigation method plays a role in outplanting performance, and subirrigation or bottom watering has been shown to improve outplanting performance (Schmal et al., 2011). We top watered all seedlings. Furthermore, seedling age is another factor that may affect outplanting performance (Landis et al., 2010); we did not include this variable in our study.

In conclusion, despite watering transplants, we could not overcome the physiological stress likely encountered under field conditions. High seedling mortality may have been caused by drought, given the dry summer following transplanting, but we cannot be sure. Transplant shock and herbivory from small mammals also likely played a role. Forthcoming results may provide evidence of the effect of year on transplant survival. Moving forward, weather-centric restoration planning could help identify ideal timeframes for outplanting because precipitation is below normal about 50% of the time in the Intermountain West (Svejcar et al., 2017). Outplanting success could be planned around periods of favorable moisture. Additionally, sites with low resistance and resilience, such as Wyoming big sagebrush sites (Chambers et al., 2014), may need higher input restoration methods, such as shading, irrigation, protection from herbivory, and weeding other established vegetation. The study underscored the importance of a comprehensive approach to overcome the challenges posed by transplanting in these ecosystems.

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Tables and Figures

Table 3. Weather Station Data

Site	Elevation (m)	Ecological sites	Latitude	Longitude	10 year normal precipitation (mm)		Cohort 1: 2022 study period precipitation (mm)		Cohort 2: 2023 study period precipitation (mm)
					Summer: May-July	annual total	Summer: May-July	Annual total	Summer: May-July
Park Valley	1553.87	Semidesert Loam (Wyoming Big Sagebrush) and Upland Stony Loam (black sagebrush)	41.77	-113.29	65 mm	223 mm	46 mm (70% of normal)	291 mm	95.8 mm (151% of normal)
Holden	1446.276	Semi Desert Loam (Wyoming big sagebrush) and Upland stony loam (Wyoming big sagebrush)	39.19	-112.4	18 .8 mm	78.2 mm	7.8 mm (41% of normal)	104 mm	8 mm (49% of normal)

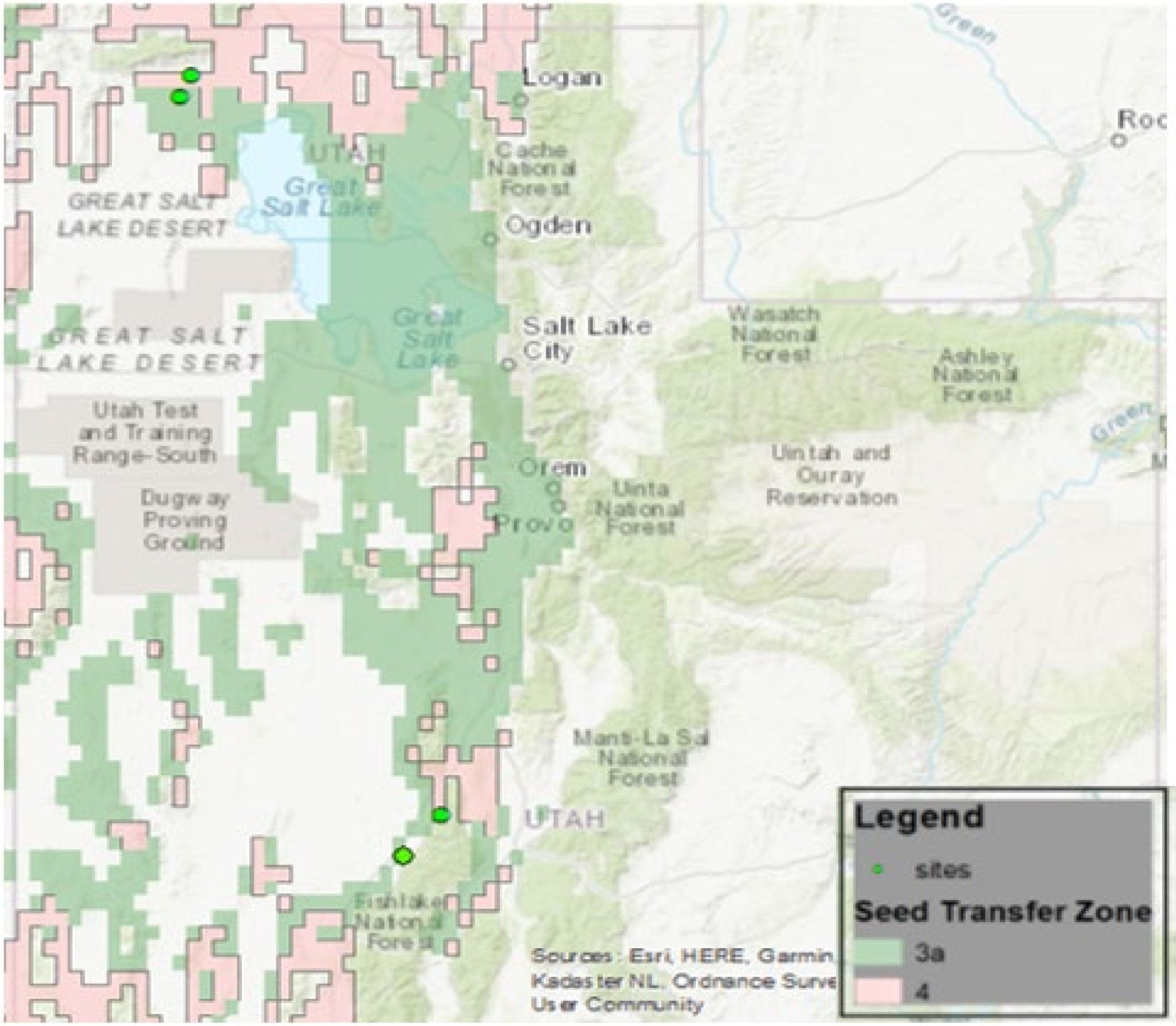


Figure 8: Map of field sites and STZs

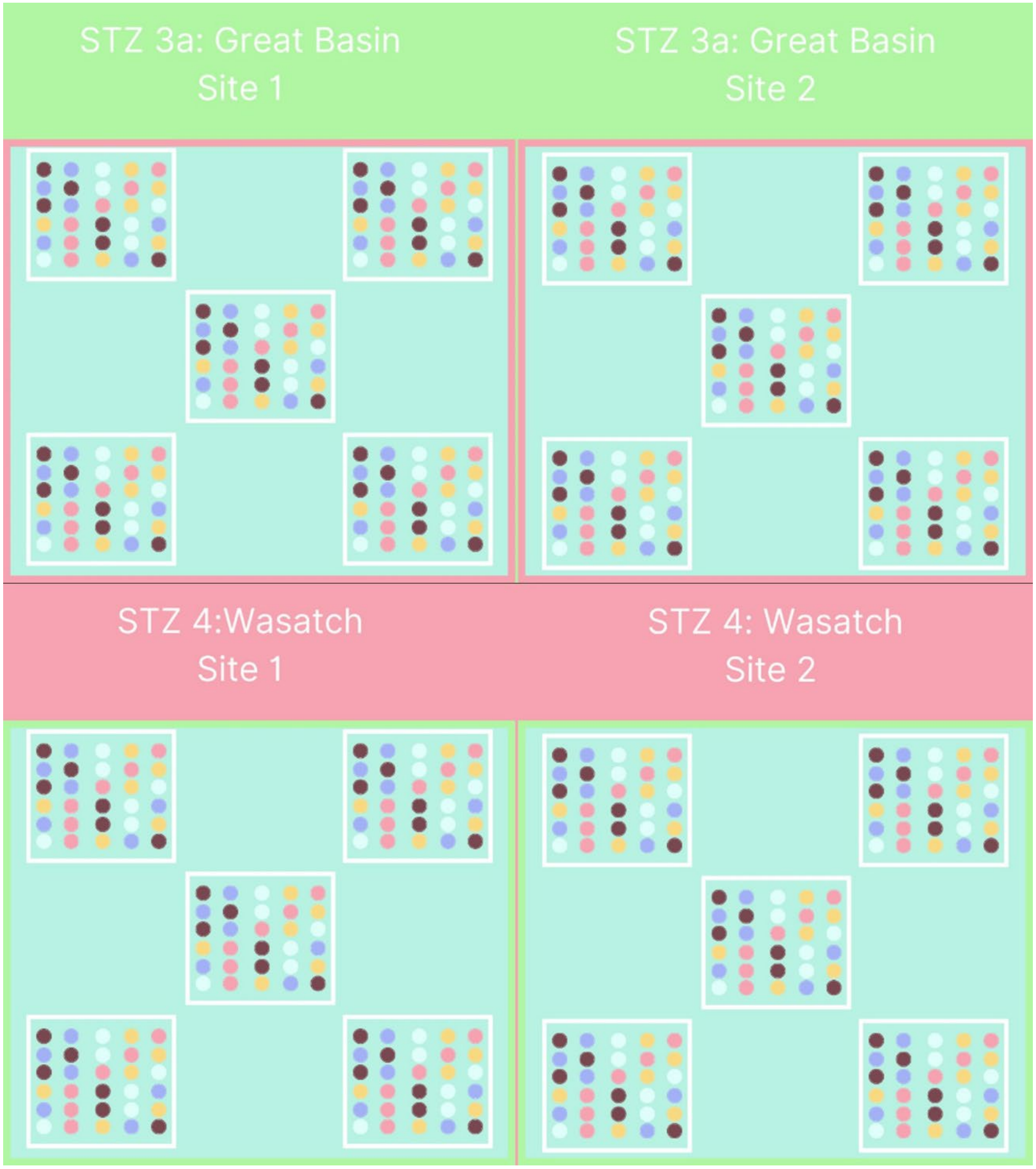


Figure 9: Randomized complete block design. Top is a diagram of the randomized complete block design with 5 of the 11 replicate exclosures shown per site for the STZ 3a. Colored dots represent the 5 different bluebunch varieties. Bottom is the same experimental design for STZ

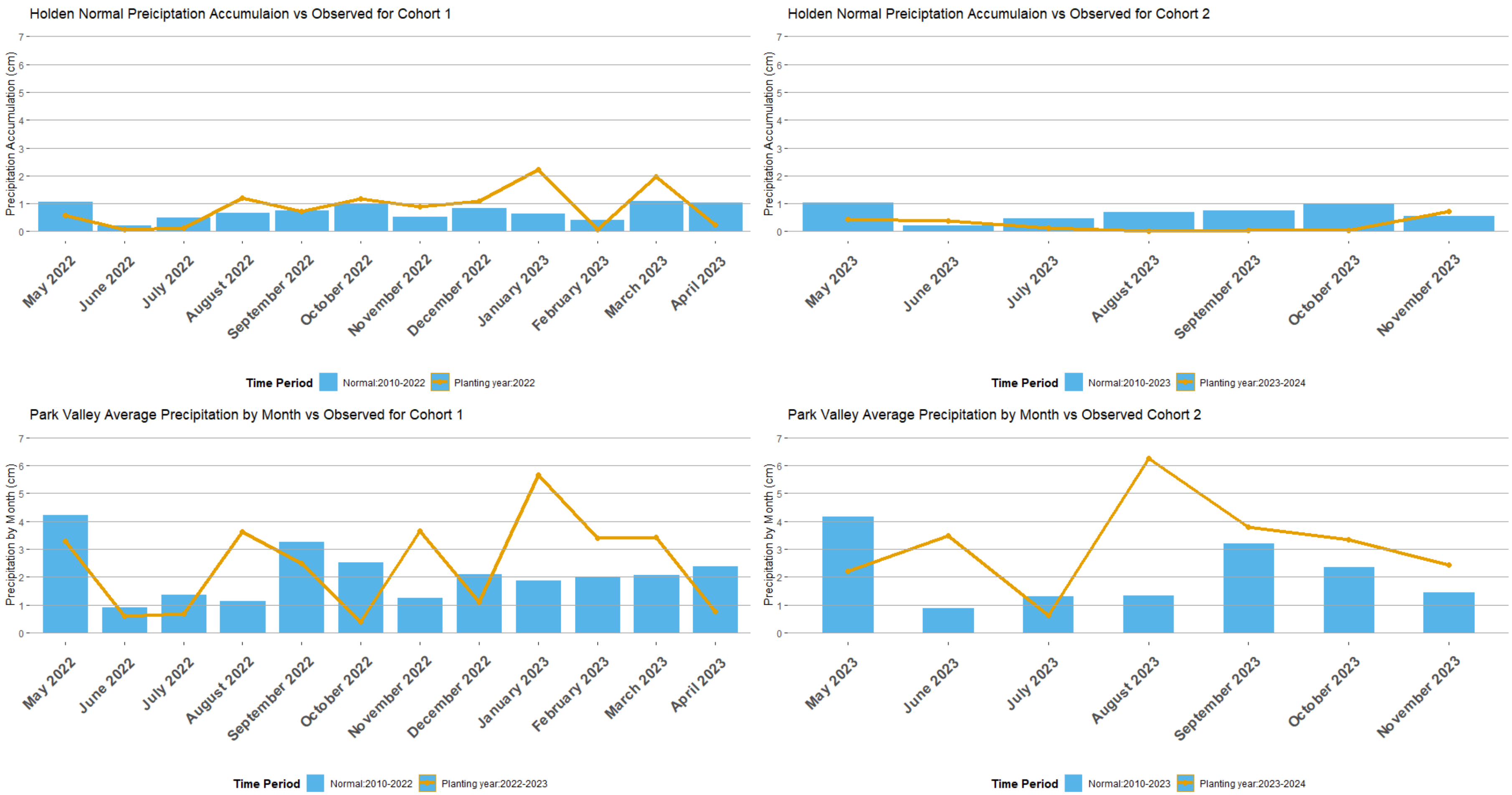


Figure 10: Normal Precipitation. Top left: Cohort 1 Holden normal precipitation vs observed. Bottom left: Cohort 1 Park Valley average precipitation vs observed.
Top right: Cohort 2 Holden normal precipitation vs observed. Bottom right: Park Valley average precipitation vs observed.

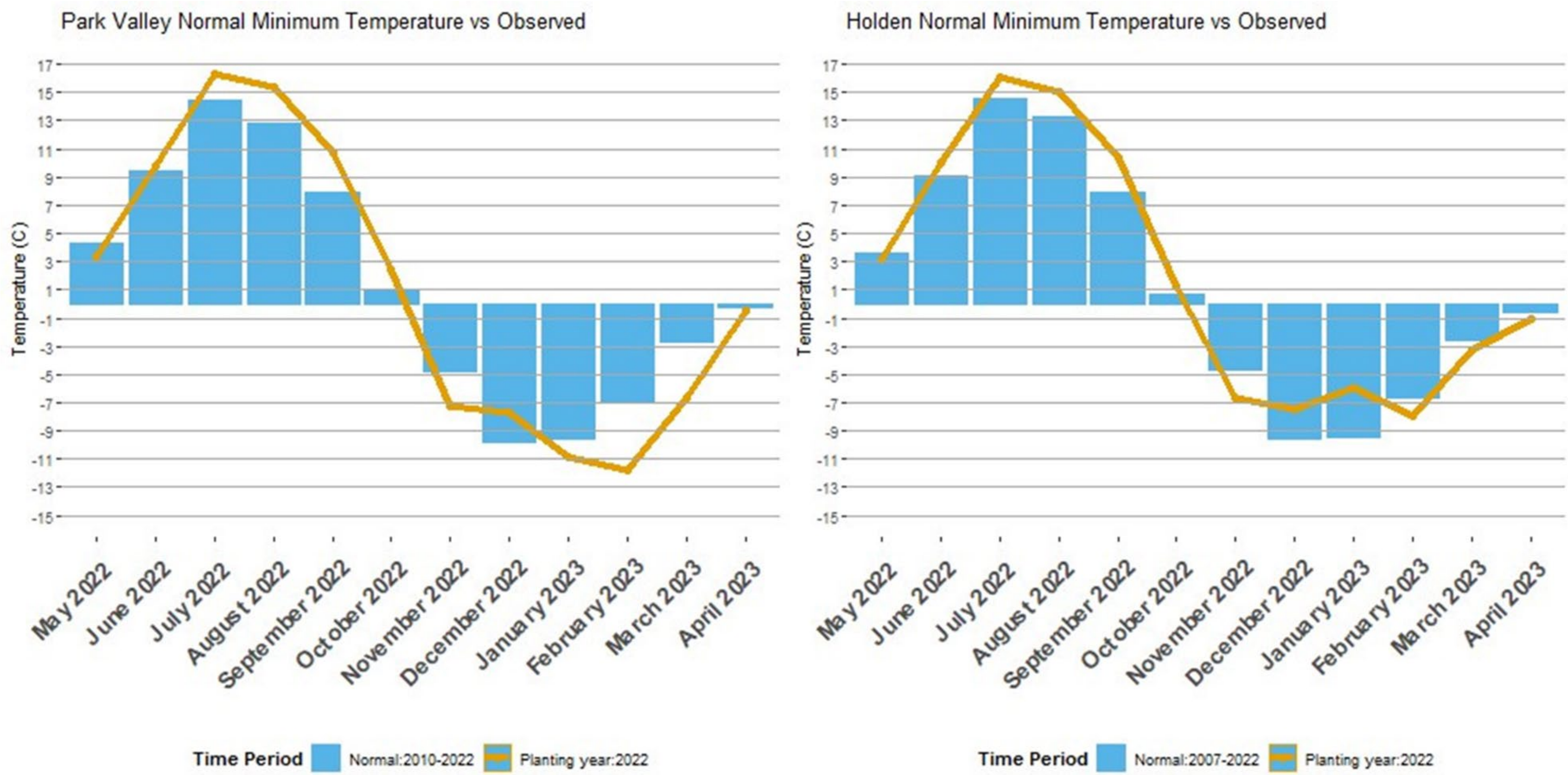


Figure 11: Minimum temperature
Figure 11a: Park valley average minimum temperature vs observed for cohort 1
Figure 11b: Holden average minimum temperature vs observed for cohort 1

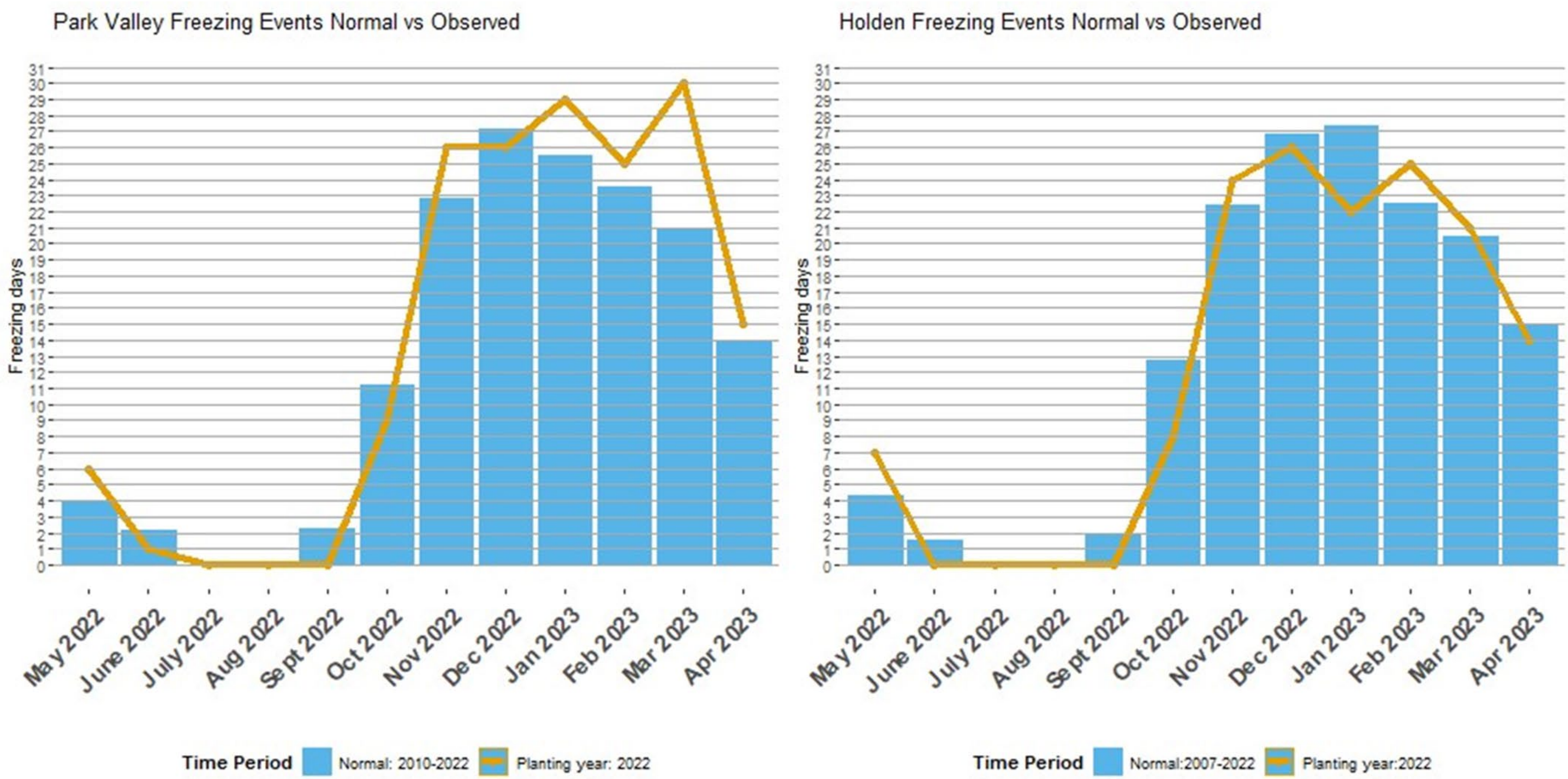
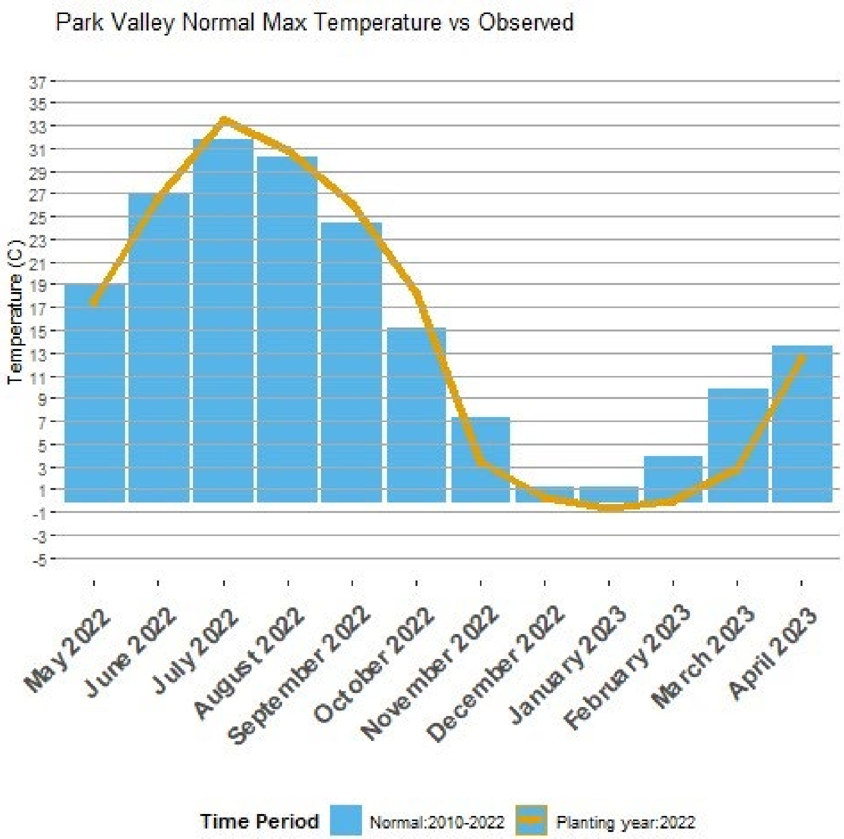


Figure 12: Freezing events
Figure 12a: Freezing events for park valley cohort 1 ‘
Figure 12b: Freezing events for Holden cohort 1



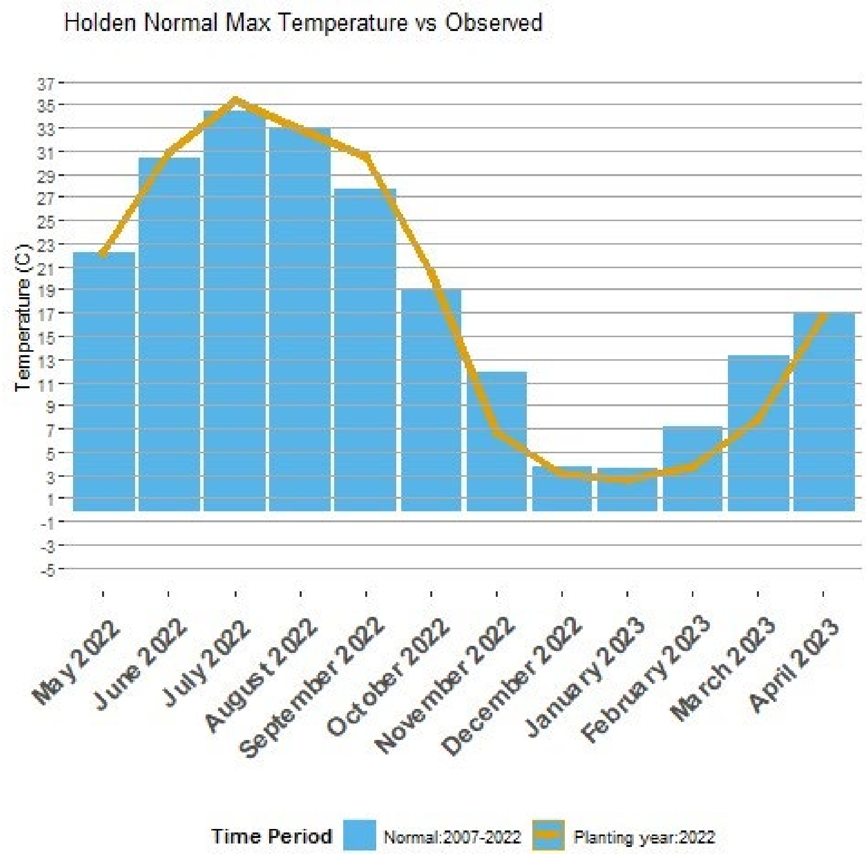


Figure 13: Max temperature
Figure 13a : Max temperature for Park valley cohort 1
Figure 13b: Max temperature for Holden cohort 1.

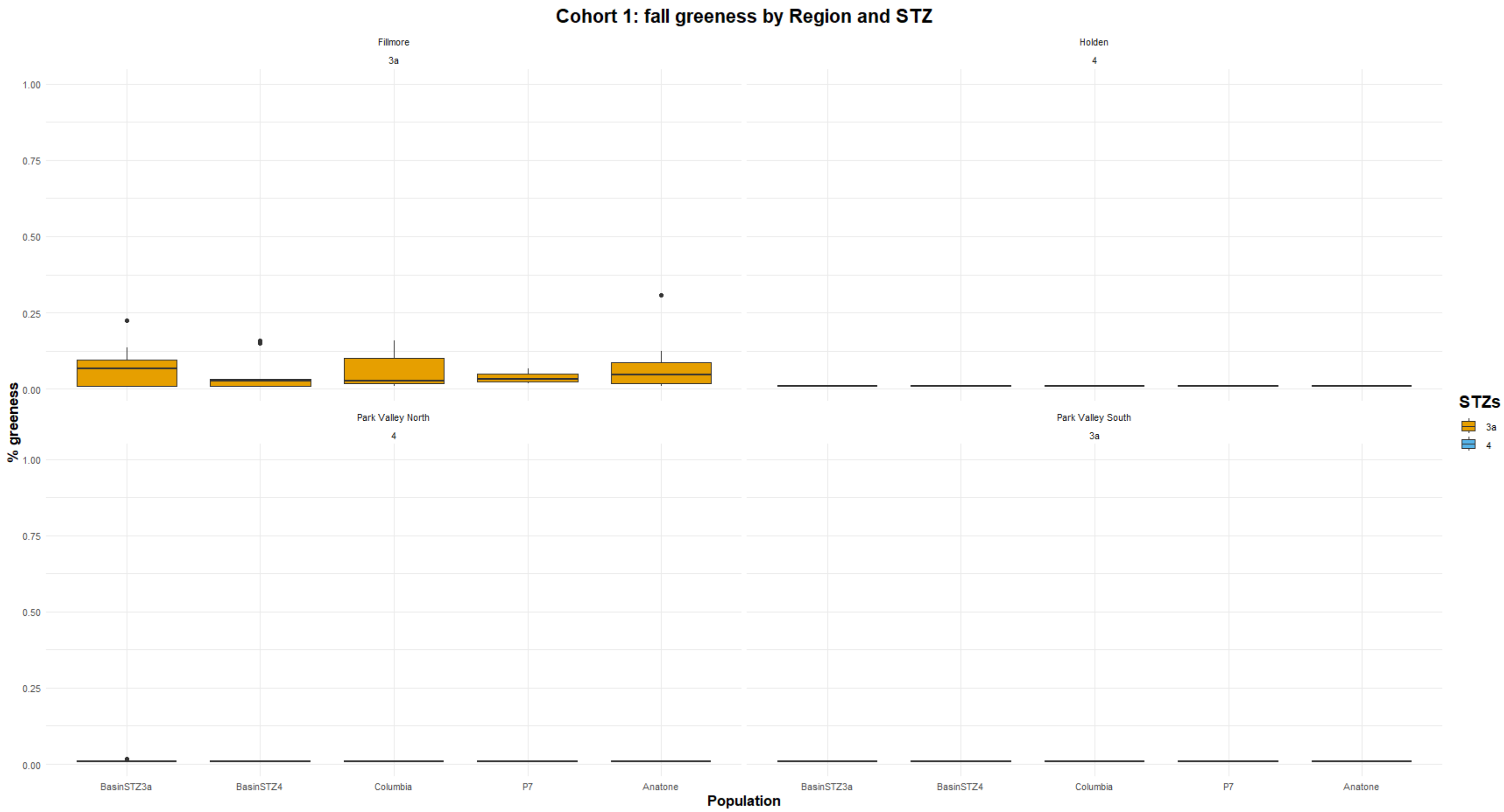


Figure 14: Cohort 1 6-month greenness.

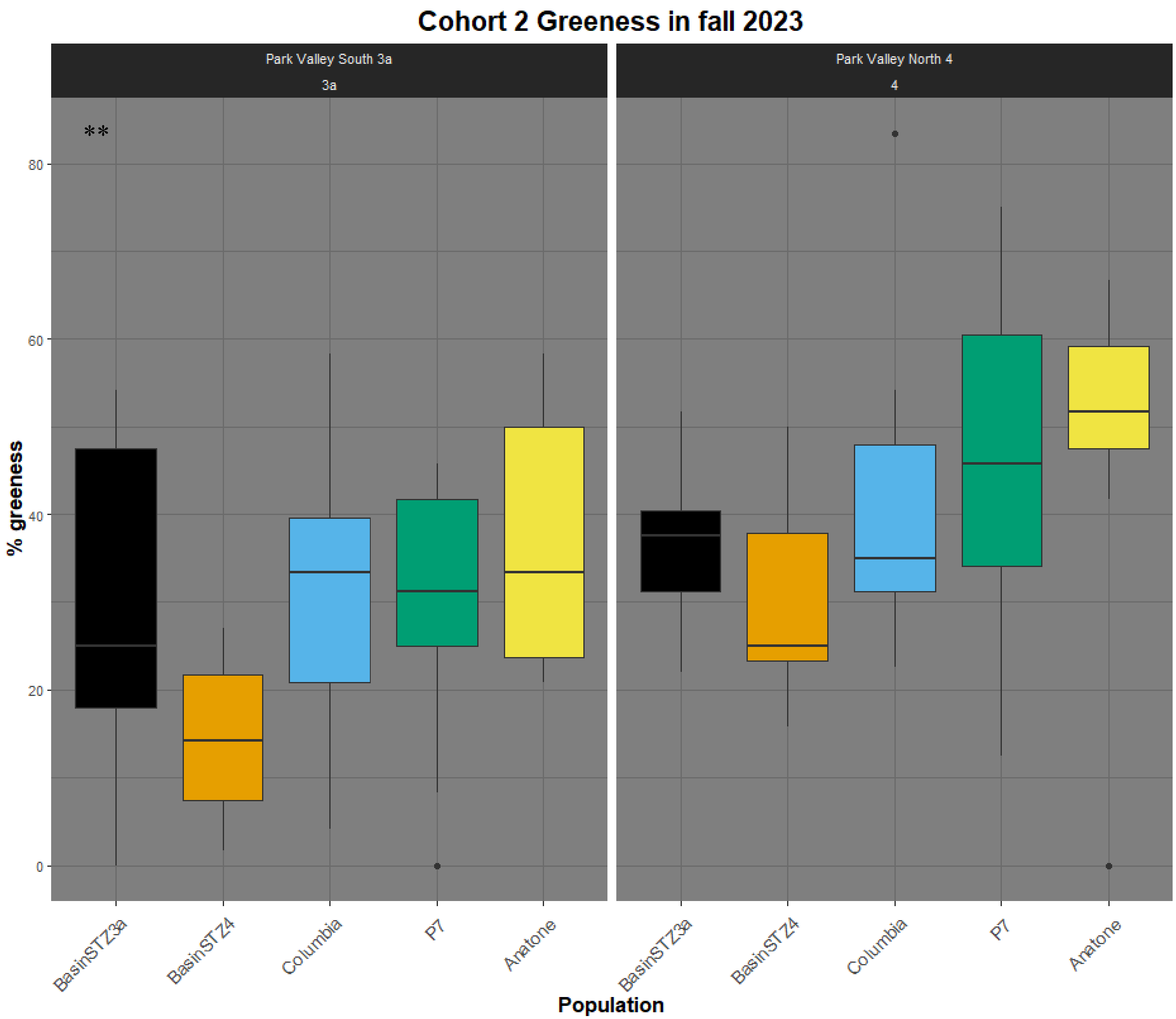


Figure 15: Fall 2023 greenness cohort 2

CHAPTER 4

CONCLUSION

Novel ecosystems are ecosystems that do not have a precedent and may have new combinations of species and different functioning due to human activity (Hobbs et al., 2006). Much of the Intermountain West's (IW) rangelands have undergone disturbances leading to changes in ecosystems. Historical disturbances in the IW, such as overgrazing by livestock, droughts, wildfires, and invasive species, have caused widespread ecosystem changes by reducing vegetation and increasing the presence of non-native species. (Svejcar et al., 2017). Land managers are working to address these changes by suppressing non-native plants and reintroducing native species.

Seedlings in these restoration projects face many challenges for successful establishment and persistence. These include drought, competition from invasive annuals plants, and herbivory. There is a demand for native plant materials with improved traits to help them to overcome these obstacles. Plant functional traits are important for restoration practitioners as they select plant materials, as measuring functional traits offers insights into how plants may fare in a natural setting.

To contribute to plant materials development, I sought to explore functional trait variation of two experimental plant materials for use in the Central Basin and Range (BR) region within the Great Basin. My main goal was to understand functional trait variability and wildland performance between the two BR materials, BasinSTZ3a and BasinSTZ4, and three previously released materials from outside of the BR (Anatone, Columbia and P7).

In comparing functional traits, I found variation in specific leaf area (SLA) and tillering dynamics between the BR populations. BasinSTZ4 had traits related to smaller size and greater investment into leaf structure. BasinSTZ4 had lowest tiller numbers, highest tiller mass, and lowest SLA, indicating greater expression of a conservation growth strategy. This may provide advantages at dry BR sites with temperature extremes (hot summers and cold winters).

Meanwhile, BasinSTZ3a had some traits in common with the commonly-seeded Anatone. These traits were related to more productive growth and a less extreme conservation growth strategy, and included high baseline tiller numbers, high regrowth tiller numbers, and lowest tiller mass. These traits suggest a less conservation growth strategy from BasinSTZ3a, than from BasinSTZ4. These traits may confer advantages to BasinSTZ3a at warm, dry BR climates at sites invaded by invasive annual grasses.

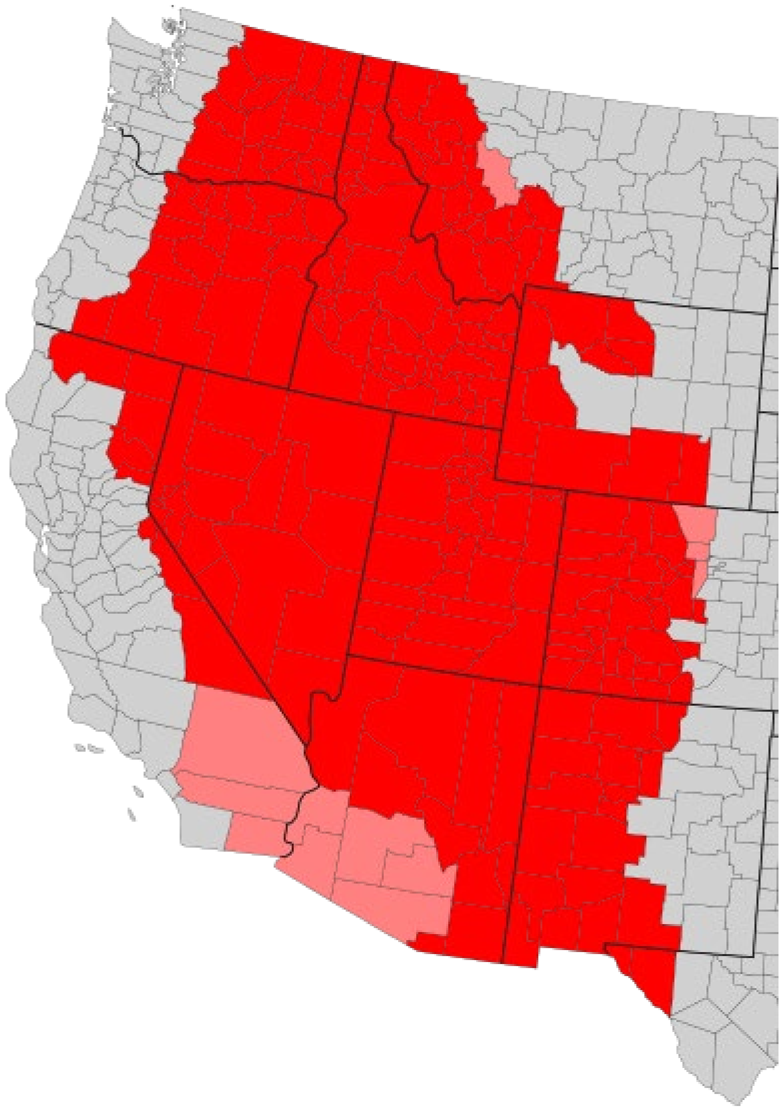
Both BasinSTZ3a and Basin4 had the highest leaf area compensation, suggesting faster renewal of photosynthetic leaf area, a trait commonly associated with grazing-tolerant plants. But, these materials also showed comparatively lower baseline leaf area and dry mass. This suggests that the seedlings may have been shorter in height, which may indicate greater expression of a conservation strategy. The combination of higher leaf area compensation and lower baseline leaf area suggests a possible trade-off between fast growth traits and grazing tolerance, as has been observed in previous studies on bluebunch wheatgrass. However, further research is needed to fully understand this connection.

In the outplanting study, I found low survival in year one. I planted in the spring of 2022, which was followed by a hot and dry summer. When I measured one-year survival, no plants had persisted. I also planted the following year, in Spring of 2023. This year had an extremely wet summer, and when I visited plants again in the fall to measure 6-month greenness, two of the four sites had living, reproductive plants, with much higher greenness ratings than I had recorded for the previous year's planting. At one of those sites, located in STZ 3a, BasinSTZ4 performed the poorest, and all other materials had similarly higher greenness. Though I haven't yet collected one-year survival data for this planting, based on the fall greenness values, I expect to see more survivors. These findings highlight the difficulty of restoring vegetation in the Intermountain West, where there is extreme interannual variability in temperature and precipitation.

In conclusion, this study provides valuable insights into functional trait variation of bluebunch wheatgrass from the Central Basin and Range region. The differences we observed in traits between BasinSTZ3a and BasinSTZ4 suggest their suitability for different site types. Moving forward, further research is needed to better understand how these traits impact wildland performance. This understanding will contribute to developing effective strategies to restore and conserve bluebunch wheatgrass populations in the Intermountain West.

Appendices

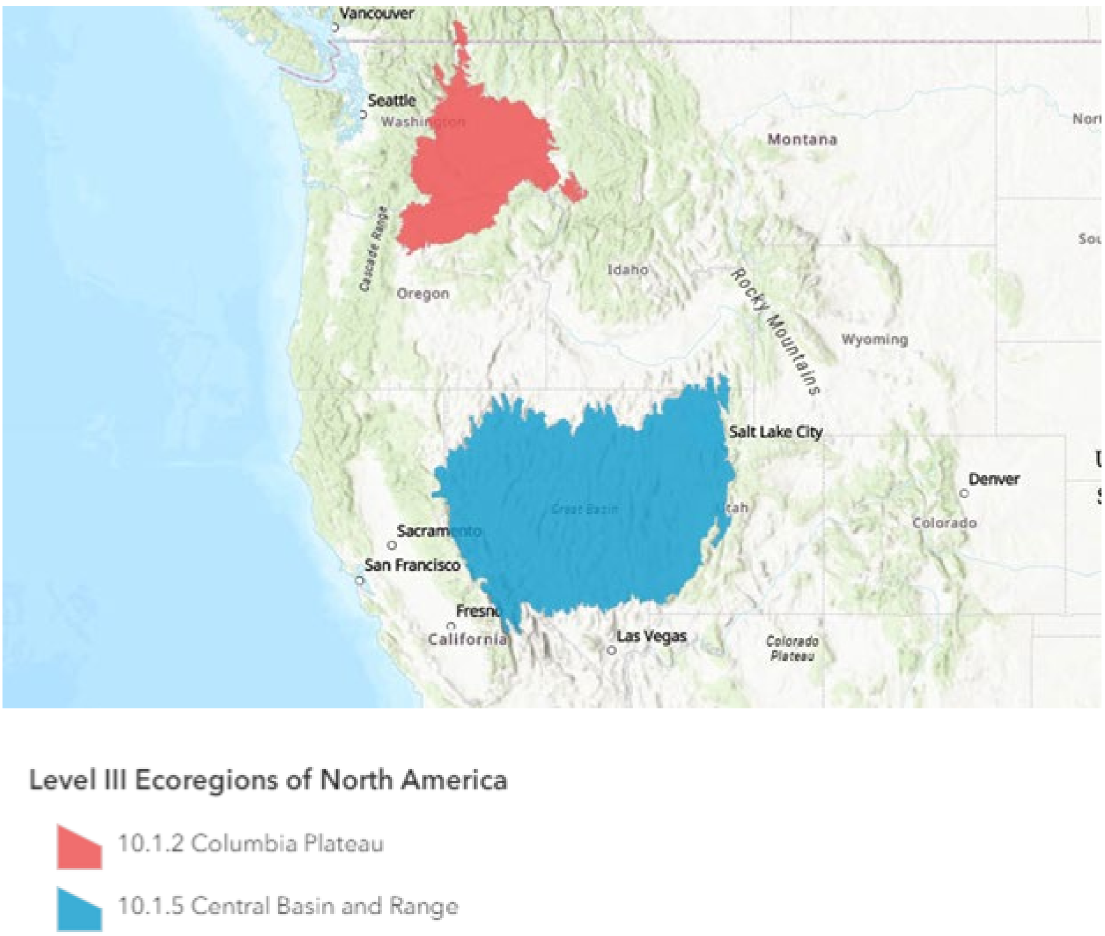
Appendix A: Map of the Intermountain West, United States.



The counties in red are always included, while counties in pink are only sometimes included. Howpper, 2017 [CC BYSA 4.0] from Wikimedia Commons. Available at: upload.wikimedia.org/wikipedia/ commons/thumb/d/d9/Intermountain_West.svg/386px-Intermountain_West.svg.png. Accessed 18 March 2024.

Appendix B: USA Environmental Protection Agency Level III ecoregions of North America.

Map built from ArcGIS Online: Esri. "Topographic" [basemap]. Scale Not Given. "World Topographic Map". March 12, 2024. http://www.arcgis.com/home/item.html?id=30e5fe3149c34df1ba922e6f5bbf808f.



Appendix C: Regrowth Pairwise comparisons.

Dry mass regrowth and leaf area regrowth omitted, as the main effects were not significant.

	Regrowth tiller mass		Regrowth tiller count		Regrowth SLA		Shoot moisture content	
Pairwise comparison	P-value (more conservative model)	Estimate of difference (on the response scale)	P-value	Estimate of difference (on the log scale)	P-value (more conservative model)	Estimate of difference (on the response scaele)	P-value (of regrowth) – more conservative model used	Estimate of difference
BasinSTZ3a - BasinSTZ4	<.0001***	-0.01208	<.0001***	0.7036	0.0057*	21.564	0.9389	-0.017783
BasinSTZ3a - Columbia	0.4417	-0.00189	0.9886	0.0192	0.7491	-5.509	0.1295	-0.063221
BasinSTZ3a - P7	0.0704	-0.0329	<.0001***	0.1834	0.9994	0.977	0.1038	-0.066687
BasinSTZ3a - Anatone	0.9693	0.00066	0.0608	-0.1008	0.0014*	-26.191	1.000	0.000223
BasinSTZ4 - Columbia	<.0001***	0.01019	<.0001***	-0.6844	0.0011*	-27.073	0.3673	-0.045437

BasinSTZ4 - P7	0.0001**	0.00878	<.0001***	-0.5202	0.0078*	-20.587	.3047	-0.048904
BasinSTZ4 - Anatone	<.0001***	0.01274	<.0001***	-0.8044	<.0001***	-47.755	0.9363	0.018007
Columbia - P7	0.6940	-0.00140	0.0003**	0.1642	0.6305	6.487	0.999	-0.003467
Columbia - Anatone	0.1980	0.00255	0.0158*	-0.1200	0.0076*	-20.682	0.1277	0.1277
P7 - Anatone	0.0271*	0.00395	<.0001	-0.2842	0.0010*	-27.168	0.1024	0.1024

Appendix D.: Response to defoliation pairwise comparisons:

Dry mass compensation omitted, main effect not significant

	Leaf area compensation		Relative change in tillers	
Pairwise comparison	P-value	Estimate of difference	P-value	Estimate of difference
BasinSTZ3a - BasinSTZ4	0.9980	0.0250	0.1632	12.965
BasinSTZ3a - Columbia	0.0443**	0.2827	0.2506	-11.424
BasinSTZ3a - P7	0.2129	0.1954	0.2373	-11.613
BasinSTZ3a - Anatone	0.7075	0.1076	.1718	-12.814
BasinSTZ4 - Columbia	0.0701	0.2578	0.0057	-24.389
BasinSTZ4 - P7	0.3183	0.1704	0.0054	-24.578
BasinSTZ4 - Anatone	0.8562	0.0826	0.0038	-25.780
Columbia - P7	0.8309	-0.0874	1.000	-0.188
Columbia - Anatone	0.2963	-0.1751	0.9986	-1.390
P7 - Anatone	0.8288	-0.0878	0.9992	-1.202

Appendix E: Main Effects in analysis of Functional Trait differences among 5 different plant materials.

Trait	Test	P-value	Other summary statistic
Shoot Dry Mass	Baseline mass ~ plant material	3.06e-08 ***	Chi squared value (df = 4): *40.73*
	Regrowth mass ~ plant material	0.051	Sum of squares: 0.00097428
Leaf Area	Baseline area ~ plant material	3.444e-10 ***	Chi squared value (df = 4): *50.098*
	Regrowth area ~ plant material	0.06986	Chi squared value (df = 4): *8.6713*
Tiller Number	Baseline number ~ plant material	2.2e-16 ***	Chi squared value (df = 4): *229.72*
	Regrowth number ~ plant material	2.2e-16 ***	Chi squared value (df = 4): *137.01*
Tiller Mass	Baseline mass ~ plant material	6.684e-15***	Chi squared value (df = 4): *72.514*
	Regrowth mass ~ plant material	< 2.2e-16 ***	Chi squared value (df = 4): *137.01*
SLA	Baseline area ~ plant material	2.2e-16 ***	Chi squared value (df = 4): *81.288*

	Regrowth area ~ plant material	2.11e-05	Sum of squares:3496.5
Shoot Moisture Content	Grams of water ~ plant material	0.03595*	Sum of squares: 0.0132329
Relative change in tillers	Relative change ~ plant material	1.656e-07	Chi squared value (df = 4): *37.177*
Shoot dry mass compensation	Mass compensation ~ plant material	0.05149	Sum of squares: 0.00097428
Leaf area compensation	Area compensation ~ plant material	0.003256	Chi squared value (df = 4): *15.83*

Appendix F: Baseline measurement pairwise comparisons

	Baseline dry mass		Baseline leaf area		Baseline tiller mass		Baseline tiller count		Baseline SLA	
Pairwise comparison	P-value	Estimate of difference (on response scale)	P-value	Estimate of difference (on response scale)	P-value	Estimate of difference (on the log scale)	P-value (more conservative model	Estimate of difference (on the response scale)	P-value	Estimate of difference (on the log scale)
BasinSTZ3a - BasinSTZ4	0.5055	-0.01307	0.9753	-0.909	0.0002**	-0.8753	<.0001***	3.651	0.0927	0.1055
BasinSTZ3a - Columbia	0.0032**	-0.04076	0.0009**	-9.471	0.0017**	-0.6790	0.4713	0.723	0.0139**	-0.1504
BasinSTZ3a - P7	0.0162 *	-0.03197	0.0144*	-6.443	0.0007**	-0.7583	0.0124*	1.777	0.3191	-0.0741

BasinSTZ3a - Anatone	0.4077	-0.01460	0.0550	-5.077	0.2098	-0.2841	0.9922	-0.179	.0039**	-0.1814
BasinSTZ4 - Columbia	0.0372 *	-0.02770	0.0020**	-8.562	0.5190	0.1963	0.0003*	-0.5334	0.0003**	-0.2558
BasinSTZ4 - P7	.1983	-0.01891	0.0350**	-5.534	0.8645	0.1170	0.0088**	-0.3710	0.0043**	-0.1796
BasinSTZ4 - Anatone	.9996	-0.00153	0.1330	-4.168	0.0046**	0.5912	<.0001***	-0.6524	0.0001**	-0.2869
Columbia - P7	.8005	0.00879	0.3635	3.028	0.9620	-0.0793	0.1686	0.1624	0.2947	0.0763
Columbia - Anatone	0.0502	0.02617	0.1072	4.394	0.0531	0.3949	.2796	-0.1190	0.9081	-0.0311**
P7 - Anatone	0.2600	0.01738	0.9019	1.366	0.0193*	0.4742	0.0066	-0.2814	0.0857	-0.1074

Appendix G: Information on climate and soils for each of the four field study sites

(Soil Survey Staff, 2022)

STZ 4: Holden: 39.12780, -112.24279	Semidesert loam (Wyoming big sagebrush) Elevation: 1609 m Mean annual precipitation: 203 mm – 304 mm 8 to 12 inches Mean annual air temperature: 48 to 52 degrees F
STZ 3a: Fillmore: 38.91652, -112.36367	Upland stony loam (Wyoming big sagebrush) Elevation: 1549 m Mean annual precipitation: 304 mm – 355 mm 12 to 14 inches Mean annual air temperature: 46 to 52 degrees F
STZ 4: Park Valley North; 41.82071, -113.27085	Upland stony loam (Black sagebrush) Elevation: 1668 m 304 mm – 406 mm 12-16 inches annual precipitation Mean annual air temperature: 45 to 48 degrees F
STZ 3a: Park Valley South: 41.78651, -113.32484	Semi desert loam (Wyoming Big Sagebrush) Elevation 1601 m 203 mm to 304 mm 8-12 inches annual precipitation Mean annual air temperature 46 to 54 degrees F.

Appendix H: Holden baseline plant cover

Holden			
Code	% cover	Full name	functional group
Total bare ground	80.32	Soil , litter, rock, dung	
%SOIL	64.99	Soil	
%LITTER	15.33	Organic Litter	
%BRTE	7.42	Bromus tectorum	Introduced annual grass
%ALAL	4.49	Alyssum alyssoides	Introduced annual forb
%COAR	3.83	Convolvulus arvensis	Introduced perennial forb
%ERCI	1.36	Erodium cicutarium	Introduced annual forb
%POBU	1.12	Poa bulbosa	Introduced perennial grass
%PSSP	1.02	Pseudoroegneria spicata	Transplanted seedlings

Appendix I: Fillmore baseline plant cover

Fillmore			
Code	% cover	Full name	functional group
Total bare ground	72.18	Soil , litter, rock, dung	
%LITTER	43.95	Organic Litter	
%SOIL	27.39	Soil	
%THIN	11.71	Thinopyrum intermedium	Introduced perennial grass
%BRTE	9.02	Bromus tectorum	Introduced annual grass
%POBU	5.04	Poa bulbosa	Introduced perennial grass
%PSSP	1.04	Pseudoroegneria spicata	Transplanted seedlings
%ROCK	0.85	Rocks/pebbles/stones	
%ERCI	0.68	Erodium cicutarium	Introduced annual forb

Appendix J: Park Valley North baseline plant cover

Park Valley North (STZ4)			
Code	% cover	Full name	functional group
Total bare ground	87.82	Soil , litter, rock, dung	
%Soil	43.12	Soil	
%Litter	23.81	Organic Litter	
%Rock	20.64	Rocks/pebbles/stones	
%Agcr	9.70	Agropyron cristatum	Introduced perennial grass
%Pssp	1.45	Pseudoroegneria spicata	Transplanted seedlings
%Grb	0.39	Chrysothamnus viscidiflorus	Native shrub
%Dung	0.26		
%Pose	0.21	Poa secunda	Native perennial grass
%Save	0.03	Sarcobatus vermiculatus	Native Shrub
%Trdu	0.02	Tragapogon dubius	Introduced annual forb
%Aspu	0.01	Astragalus purshii	Native perennial forb
%PSJU	0.01	Psathyrostachys juncea	Introduced perennial grass

Appendix K: Park Valley South (STZ3a) Baseline plant cover

Park Valley South (STZ3a)			
Code	% cover	Full name	functional group
Total bare ground	92.02	Soil , litter, rock, dung	
%Soil	65.98	Soil	
%Litter	19.18	Organic litter	
%Rock	5.84	Rocks/pebbles/stones	
%Kopr	4.83	Bassia prostrata	Introduced perennial subshrub
%Agcr	1.52	Agropyron cristatum	Introduced perennial grass
%Pssp	1.47	Pseudoroegneria spicata	Transplanted seedlings
%Dung	1.02		
%Agfr	0.19	Agropyron fragilis	Introduced perennial grass
%Brte	0.01	Bromus tectorum	Invasive annual grass

Appendix L: Outline of study factors

Factors	Number	Attributes
Site	2	STZs 3a and 4 are replicated in northern and central Utah. Loamy, 20-40 cm (8-16 inches) precipitation, *Artemisia tridentata* ssp. w*yomingensis/bonnevillensis. Pseudoroegnaria spicata* – ecological sites.
Plot	11 or 12	4.8 m x 4.8 m (12’ x 12’) replicate exclosures.
Plant Materials	5	2 Great Basin sources from STZ 3a and 4 in Utah (Basin and Basin:STZ3a, respectively) and 3 from previously released varieties from the Columbia (P7, Anatone, and Columbia).
Subplot	6	Plant material replicates per plot
TOTAL	720	Plants per experiment.

Appendix M: Other statistics in outplanting study

Test	P-value	Chi-squared value
Cohort 1 6-month survival at Fillmore(STZ3a)	0.1174	7.3746, df = 4
Cohort 1 one-year survival	Not analyzed	
Cohort 2 6-month survival at Park Valley North(STZ4)	0.06308	8.9221, df = 4
Cohort 2 6-month survival at Park Valley South(STZ3a)	0.0003906	20.54, df = 4

Supplement 14:

Pairwise comparisons for cohort 2 6th month survival

	P-value	Estimate of the difference
BasinSTZ3a - BasinSTZ4	0.0214**	0.8360
BasinSTZ3a - Columbia	0.9745	-.1494
BasinSTZ3a - P7	1.000	0.0067
BasinSTZ3a - Anatone	0.5840	-0.3632
BasinSTZ4 - Columbia	0.0031*	-0.9854
BasinSTZ4 - P7	0.0238*	-0.8292
BasinSTZ4 - Anatone	0.0001**	-0.1992
Columbia - P7	0.9708	0.1562
Columbia - Anatone	0.9071	-0.2138
P7 - Anatone	0.5701	-0.3699