Great Basin Native Plant Project Report 2018-2021

2018-2021 Great Basin Native Plant Project Rocky Mountain Research Station Reports

The Great Basin floristic region spans three Level III ecoregions (Central Basin and Range, Northern Basin and Range, and Snake River Plain) and makes up a large portion of the Intermountain West of the United States. Over 75% of the Great Basin is publicly owned and managed by the US federal land management agencies, with state agencies also managing large areas. The primary human uses of public lands are grazing and recreation, with mining and oil and gas development found in some locations. The Great Basin has a range of mostly arid and semi-arid ecosystems from salt desert through coniferous montane forests, generally distributed along elevational gradients in this topographically complex region. Sagebrush steppe is cold desert shrubland dominated by sagebrush (*Artemesia tridentata*). It is the most common ecosystem in the Great Basin, but also of the most conservation concern due to the loss of nearly half of its area to land conversion and human-mediated and natural disturbances. The Great Basin, particularly the sagebrush steppe ecosystem, is a critical habitat for many wildlife species of conservation concern, including the greater sage-grouse, pygmy rabbits, and a number of pollinator species.

Fire return intervals in most places in Great Basin sagebrush steppe have decreased from 100+ years to less than 30 years, due to ongoing annual grass invasions and increasing drought severity and length. Many plant species, sagebrush in particular, are fire-intolerant, unlike plants in arid shrub ecosystems in other parts of the western US, such as the California chaparral. Natural plant community recovery can take many years, often longer than current fire return intervals. As such, post-fire restoration and revegetation, mostly through seeding, is a critical component of conserving the Great Basin sagebrush steppe.

The Great Basin Native Plant Project (GBNPP) is a partnership between the USDA Forest Service Rocky Mountain Research Station (RMRS), the USDI Bureau of Land Management (BLM), and the USDI Fish and Wildlife Service to research and develop native plant materials for use in conservation and restoration of Great Basin ecosystems. The GBNPP was started in 2001 and produces science through support of RMRS researchers and of cooperators at other federal science agencies, universities, and NGOs. GBNPP supported research is focused on the full plant material development and restoration cycle, as outlined by the BLM Plant Conservation Program. In recent years, the GBNPP has concentrated research effort into parts of the plant material development cycle where bottlenecks occur or information is lacking. These goals include: 1) development of species selection and seed collection prioritization tools; 2) seed transfer guideline development based on native plant adaptive variation and genetics; 3) cultivation practice for species that don't fit typical agronomic frameworks; and, 4) seed mix design for long-term restoration of biodiverse Great Basin ecosystems.

Scientists at RMRS focus primarily on goals 1, 3, and 4 and work with cooperators to support research into goal 2, as well as all other aspects of the plant materials development cycle. In addition to leading in-house projects and supporting GBNPP funded cooperator agreements, RMRS scientists also work in collaborations that are aligned with GBNPP goals, where GBNPP funding is often limited and through in-kind support rather than through direct agreements. These aligned projects tend to focus on synthesizing plant material development knowledge at regional, national, and international levels. In this section of the report, projects that are led by RMRS scientists and aligned projects that have occurred or been ongoing between 2018 and 2021 are presented. Cooperator projects that were ongoing in 2021 are presented in the second section of the report. Cooperator reports for all projects that occurred or were ongoing between 2018 and 2020 will be provided as a zip file along with this report.

Francis Kilkenny
USDA Forest Service Rocky Mountain Research Station, Boise ID
December 2021

Workhorse Native Forb Adaptative Genetics and Plant Materials Development for Postfire Restoration of Critical Wildlife Habitat

Science partners:
USDA Forest Service, Rocky Mountain Research Station: Francis Kilkenny (overall project lead), Sarah Barga (overall common garden lead), Jessica Irwin (seed collection lead), Kimberly Rizkowsky (Boise crew lead), Bryce Richardson (genetics co-lead), and Kas Dumroese (plant grow-out lead).
USDI Geological Survey, Flagstaff Campus: Rob Massatti (genetics co-lead), Daniel Winkler, John Bradford
University of Nevada Reno: Beth Leger (Reno and Plymouth common garden lead) and Tessa Bartz
Utah State University: Andrew Kulmatiski (Twin Creeks common garden lead) and Julia Aaronson
The Nature Conservancy: Owen Baughman (Glass Butte common garden lead)

Institutional partners:
Great Basin Native Plant Project (GBNPP); USDI Bureau of Land Management, Great Basin Ecoregion; USDA Forest Service Rocky Mountain Research Station, Lucky Peak Nursery, National Forest System Intermountain Region (Region 4) and Northern Region (Region 1), Western Center for Native Plant Conservation and Restoration; USDI Fish and Wildlife Service, Nevada Office; USDI Geological Survey, Flagstaff Campus; University of Nevada, Reno; Utah State University; The Nature Conservancy; Chicago Botanic Garden; Great Basin Institute; Seeds of Success

Introduction – Restoration efforts that emphasize native germplasm adapted to local environments may be the best approach to ensure long-term ecosystem sustainability. Developing seed transfer zones, using common garden and genomic approaches, is a way to ensure that plant materials used for revegetation are adapted to a given area and that diverse genetic resources are used to enhance ecological stability and resilience. Yet there are no established seed transfer zones for most Great Basin plant species and remarkably little germplasm is conserved for future revegetation needs. At the same time land managers in the Great Basin are faced with changing fire regimes due to annual grass invasions and increasing droughts due to climate change, making sustainable restoration even more challenging into the future. As a result, extensive germplasm collection and seed transfer zone research are urgently needed to abate the erosion of native ecosystems and to improve adaptation of plant materials used for revegetation.

The majority of seed transfer guidelines developed for the Great Basin are for grass and shrub species that play important structural and functional roles in maintaining ecosystem health. However, there are over 5000 plant taxa in the sagebrush biome. Many of these are forb species that are necessary components of the diet of sage-grouse and other wildlife. Additionally, diverse forb communities are the basis of threatened pollinator habitat. Evidence is also building that current revegetation practice using conventional seed mixes containing non-native bunchgrasses, while effective for some revegetation goals, are detrimental to the restoration of biodiverse plant communities and the recovery of sage-grouse and pollinator habitat. Whereas, native-only seed mixes support biodiversity and are promising in moving plant communities toward desired ecological conditions. For native plant restoration to be successful, species will need to be chosen that can be established through seeding, can support wildlife and pollinators across the growing season, and can be cultivated for seed production. A set of forb species that fit these criteria have been identified by the BLM and USFS, and a prioritized species list for seed transfer and plant material development will continue to be curated using ecological and cultivation considerations.

As gaps in existing seed transfer knowledge are defined, common garden work and genomic analyses for identified priority species are being implemented by GBNPP partners and cooperators. Priority restoration species are being tested through a common garden array, where research responsibility is shared with teams across the Great Basin to rapidly assess plant adaptation. The benefit of a common garden array is that it will generate an economy of scale for future seed transfer work, cover a large geographic area effectively, and produce extremely high-quality trait and environmental data. Additionally, these species are being tested using next generation genomics techniques which allows for a complete synthesis of information for each species. The scale of this data collection effort is unprecedented in the Great Basin and is among the highest quality data set of its type in the world. In a time of global change these data will be critical in restoring ecosystems in the face of mounting disturbances.

Seed and tissue collection – From 2017 to 2021 the RMRS Boise lab coordinated the collection of forb seed and tissue for research purposes from across the Columbia, Snake and Great Basins. During this time, a total of 587 wildland collections were made, representing 11 species. Among these collections, 165 were delivered by BLM partners coordinated by the Great Basin Ecoregional program, 19 from the US Fish and Wildlife Service, 45 from partners in the National Forest System, and 358 by RMRS Boise personnel. The majority of these collections were made in coordination with the BLM's Seeds of Success program and with the help of Great Basin Institute interns and Conservation Land Management interns through the Chicago Botanic Garden. These research collections have significantly improved our understanding of the geography and distribution of target species by identifying a total 447 previously undocumented populations not otherwise associated with herbarium records. A variety of factors were considered in selecting species for collection which include their value as sage-grouse and pollinator forage, wide geographic distribution, commonness across their range, ease of field identification, and potential for commercial seed production. The species collected include *Agastache urticifolia*, *Balsamorhiza sagittata*, *Chaenactis douglasii*, *Crepis acuminata*, *Erigeron pumilus*, *Erigeron speciosus*, *Machaeranthera* (*Dieteria*) *canescens*, *Phacelia hastata*, *Sphaeralcea grossulariifolia*, *Sphaeralcea munroana*, and *Sphaeralcea parvifolia*.

Table 1. Number of research seed collections by species and agency from 2017-2021. AGUR = *Agastache urticifolia*, BASA3 = *Balsamorhiza sagittata*, CHDO = *Chaenactis douglasii*, CRAC2 = *Crepis acuminata*, ERPU2 = *Erigeron pumilus*, ERSP4 = *Erigeron speciosus*, MACA = *Machaeranthera (Dieteria) canescens*, SPHAE = *Sphaeralcea* species, used collectively for *S. munroana*, *S. grossulariifolia* and *S. parvifolia*. RMRS = US Forest Service, Rocky Mountain Research Station, BLM = Bureau of Land Management, NFS = US Forest Service, National Forest System, FWS = US Fish and Wildlife Service.

	AGUR	BASA3	CHDO	CRAC2	ERPU2	ERSP4	MACA	PHHA	SPHAE	Total
RMRS	17	42	60	36	34	29	73	54	13	358
BLM	0	6	48	31	5	0	53	4	18	165
NFS	0	5	2	11	1	0	5	20	1	45
FWS	0	11	4	2	0	0	1	0	1	19
Total	17	64	114	80	40	29	132	78	33	587

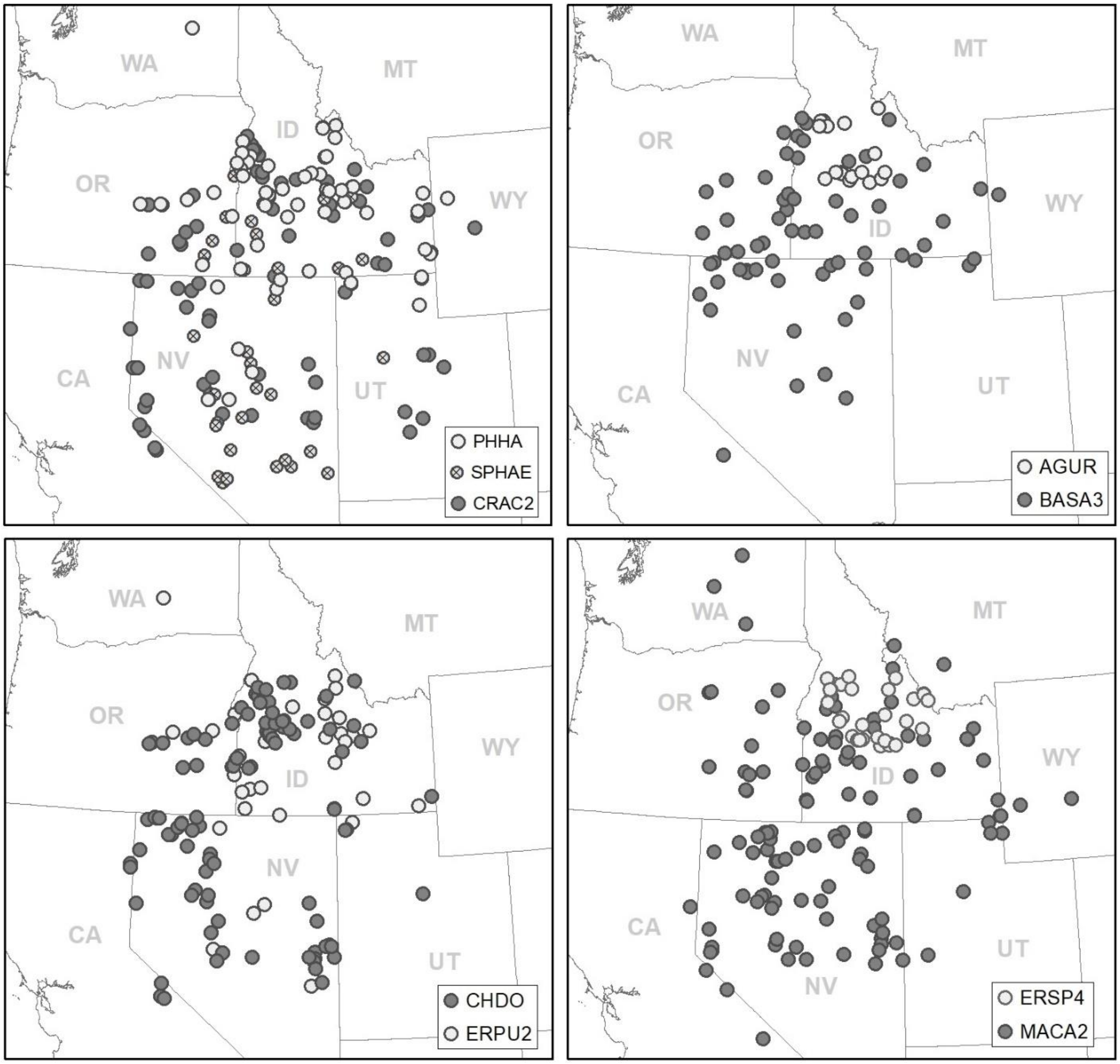

Figure 1. Distribution of research collections from 2017-2021, by species. AGUR = *Agastache urticifolia*, BASA3 = *Balsamorhiza sagittata*, CHDO = *Chaenactis douglasii*, CRAC2 = *Crepis acuminata*, ERPU2 = *Erigeron pumilus*, ERSP4 = *Erigeron speciosus*, MACA = *Machaeranthera (Dieteria) canescens*, SPHAE = *Sphaeralcea* species, used collectively for *S. munroana*, *S. grossulariifolia* and *S. parvifolia*.

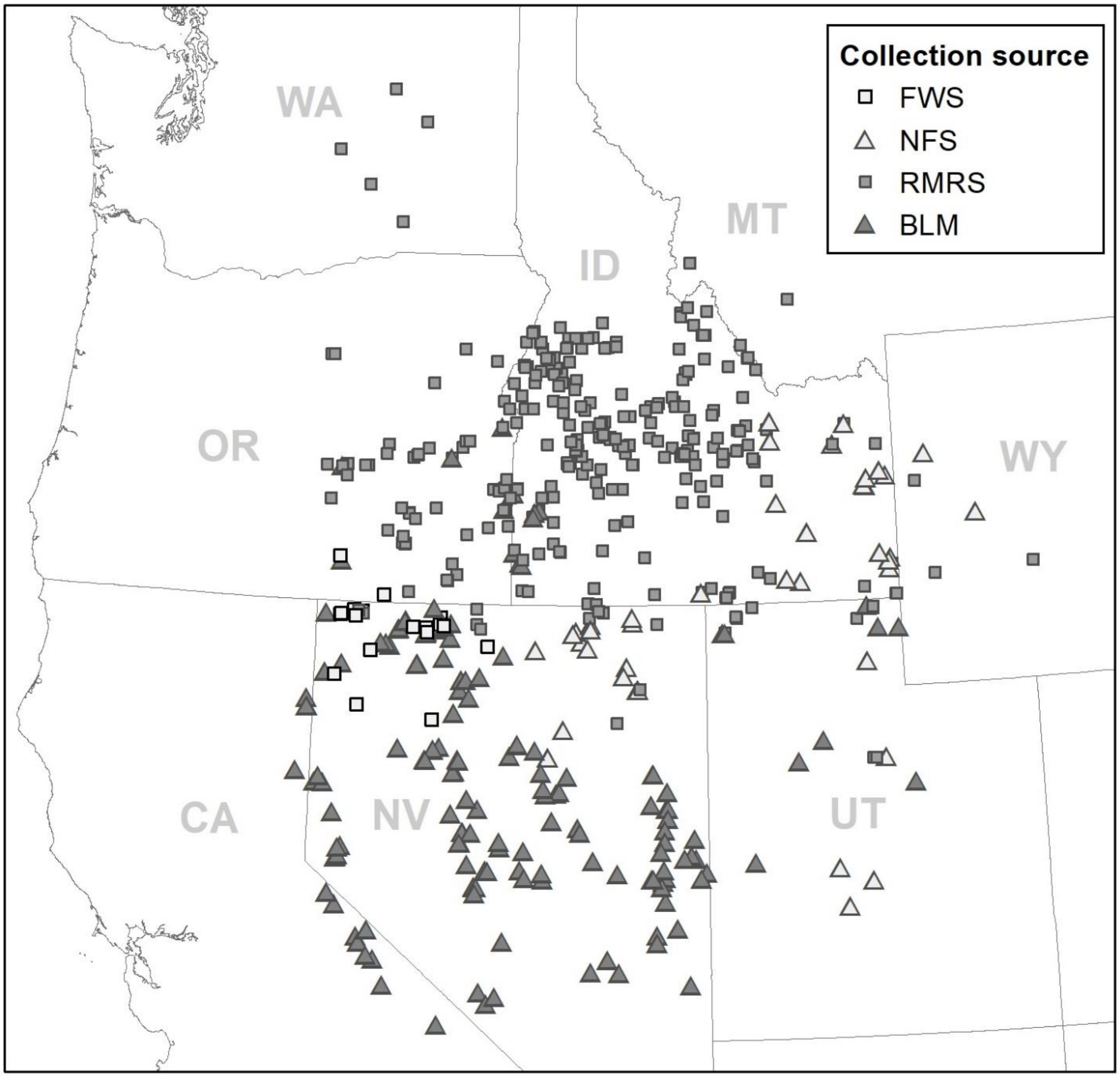

Figure 2. Distribution of research collections from 2017-2021, by contributing agency. RMRS = US Forest Service, Rocky Mountain Research Station, BLM = Bureau of Land Management, NFS = US Forest Service, National Forest System, FWS = US Fish and Wildlife Service.

Forb Common Garden Array: This is a large-scale effort being carried out by the GBNPP with the goal of examining how flexible populations of native forb species are to being moved into novel locations. To test this, and large common garden array has been installed and is being monitored throughout the growing season. This research will provide tools and information to help practitioners successfully incorporate forbs into their restoration seed mixes. With the hope of increasing the availability of genetically appropriate native plant materials and improving success in restoring native plant communities across the Great Basin.

Focal plants - Species in the common garden array include: (installed 2019) *Machaeranthera* (*Dieteria*) *canescens, Chaenactis douglasii,* and *Crepis intermedia,* (installed 2020) *Erigeron pumilus,* (installed 2021) *Phacelia hastata,* (being installed 2022) *Balsamorhiza sagittata.* Experimental plants were grown at the RMRS Moscow greenhouses. Due to extenuating circumstances, the 2019 planting of *Crepis acuminata* was unsuccessful; we are partnering with Beth Leger (UNR) to attempt a second grow-out and installation in 2022. The priority species *Erigeron specious* was not included as part of the GBNPP forb common garden array, but a common garden study was performed through an internal USFS partnership between Region 1 and Region 4. Preliminary seed zones are available from that study.

Table 2. Plant species and populations grown for installation into the common gardens

Species	Number of Populations
Machaeranthera canescens	112
Chaenactis douglasii	89
Erigeron pumilus	39
Phacelia hastata	37
Balsamorhiza sagittata	~69
Crepis acuminata	~71

Common garden locations - As of fall 2021, we have installed 7 forb common gardens in 4 states across the Great Basin (Figure 1). We specifically selected sites that represent a gradient in environmental characteristics related to moisture and temperature.

Data Collection - We selected a suite of relevant traits to measure for the garden plants that include fitness measures, like seed weight and inflorescence number, a variety of pheno-phase measures, and morphological characteristics related to local adaptation, fitness, and general performance. Genetic analyses are also being performed to determine whether ploidy may be a consideration for these species and to enable us to incorporate lineage data into our models. Trait data and plant samples (e.g. leaf, flower, seed) have been collected for *M. canescens, C. douglasii*, and *E. pumilus* and are currently being managed using an Access relational database. Sample processing was delayed due to the COVID-19 pandemic, but has been occurring at a consistent pace starting in 2021. Data analysis is scheduled to begin in 2022.

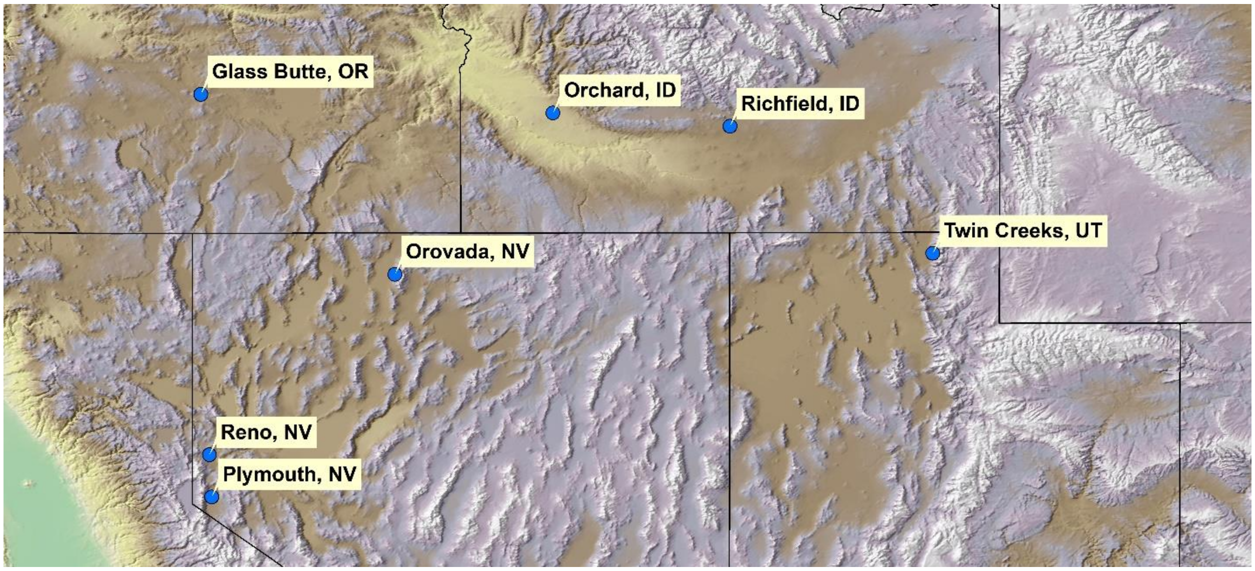

Figure 3. Map of forb common garden locations that are included in this study as of 2021. Additional gardens may be installed for *B. sagittata* in 2022.

Monitoring Partners and Additional Complementary Research – We have partnered with multiple organizations to help with monitoring the common gardens in different locations, including: The Nature Conservancy (Glass Butte, OR), Utah State University (Twin Creeks, UT), and University of Nevada, Reno (Plymouth and Reno, NV). The RMRS Boise lab and our academic project collaborators are also conducting complementary research to add value to the forb common garden study (Table 2).

Table 2. Complementary research conducted by RMRS and academic partners

Researcher	Projects looking at population-level variation
RMRS Boise Lab	Seed dormancy and germination
Utah State University – Logan, UT Julia Aaronson, Andrew Kulmatiski	• Effects of climate, soil chemistry, and soil biology on plant performance • Influence of site-specific soil microbial communities and identifying beneficial microbes
University of Nevada, Reno Tessa Bartz, Beth Leger	• Seedling emergence and survival in response to water availability • Competition with cheatgrass

Seed Germination Study – We have conducted germination studies for both *C. douglasii* and *M. canescens*. We stratified our selection of 19-20 populations to include areas representing a variety of environmental conditions. We applied 4 cold stratification treatments (0, 2, 6, and 10 weeks at 2C) and monitored the seeds for germination for 14 weeks.

Preliminary results indicate that:

- *C. douglasii*: while seeds generally germinated in warm conditions after periods of cold, seeds from some populations germinated under cold conditions

- *M. canescens*: while dormancy is generally low and seeds do not require cold stratification, seeds from some populations germinated under cold conditions

The ability to germinate under cold conditions may be beneficial for populations coming from areas with shorter growing seasons. So, even if it is easy to germinate the seeds in an agronomic setting (e.g. seed increase), there may be population-level variation in adaptations that allow for early germination, under winter conditions. This may be linked to seed source climate and may be important to consider when selecting a population for restoration.

Forb Genetics Study – Beginning in 2017 personnel at RMRS and USGS have collaborated on a study of genetic variation in the focal forb species. The project work flow for each focal species begins by extracting DNA extraction from collected tissue. DNA is then put through flow cytometry to determine ploidy level variation between source populations, and through genomic sequencing to determine genotypes for each source population. Analyses are performed on the sequencing data to understand how genotypes vary within and between source populations and across geographic regions. The populations in this study are selected to match the populations used in the common garden array and 10 individuals are sampled per population. Journal Articles are planned for each species. This data will also be used in analyses of the common garden array data.

Table 3. Progress in genetic marker work for forb species from 2017-2021.

Species	DNA Extraction	Flow Cytometry	Sequencing	Analysis	Journal Article
Erigeron speciosus	Completed	Completed	Completed	Completed	In Revision
Machaeranthera canescens	Completed	Not Needed	Completed	Completed	In Prep
Chaenactis douglasii	Completed	Completed	Completed	In Progress	Not Started
Erigeron pumilus	Completed	In Progress	Completed	In Progress	Not Started
Crepis acuminata	Completed	In Progress	In Progress	Not Started	Not Started
Sphaeralcea spp.	Completed	In Progress	In Progress	Not Started	Not Started
Phacelia hastata	In Progress	In Progress	Not Started	Not Started	Not Started
Balsamorhiza sagittata	In Progress	In Progress	Not Started	Not Started	Not Started

An understanding of genetic variation will help to:

- Inform seed transfer zones by replacing simple division along ecoregional lines with division based on related genetic clusters.
- Avoid outbreeding depression by using sources that are within the same genetic clusters as local populations and with the same ploidy levels.
- Avoid inbreeding depression by selecting sources that have reasonable levels of genetic variation.
- Provide genetic resilience to climate change and other disturbances by using sources with enough genetic variation to allow for continued natural selection.
- Identify unique populations that could be of conservation interest.
- Determine whether genomic signatures of adaptation are sufficient to develop seed transfer zones without the need for common garden studies.

Preliminary results indicate that *Erigeron speciosus* has large northern and southern genetic clusters that are divided by the snake river plain; that *Machaeranthera canescens* is divided into five genetic clusters that generally correspond to level III ecoregions in the western United States; and, that *Chaenactis douglasii* has significant ploidy variation among populations.

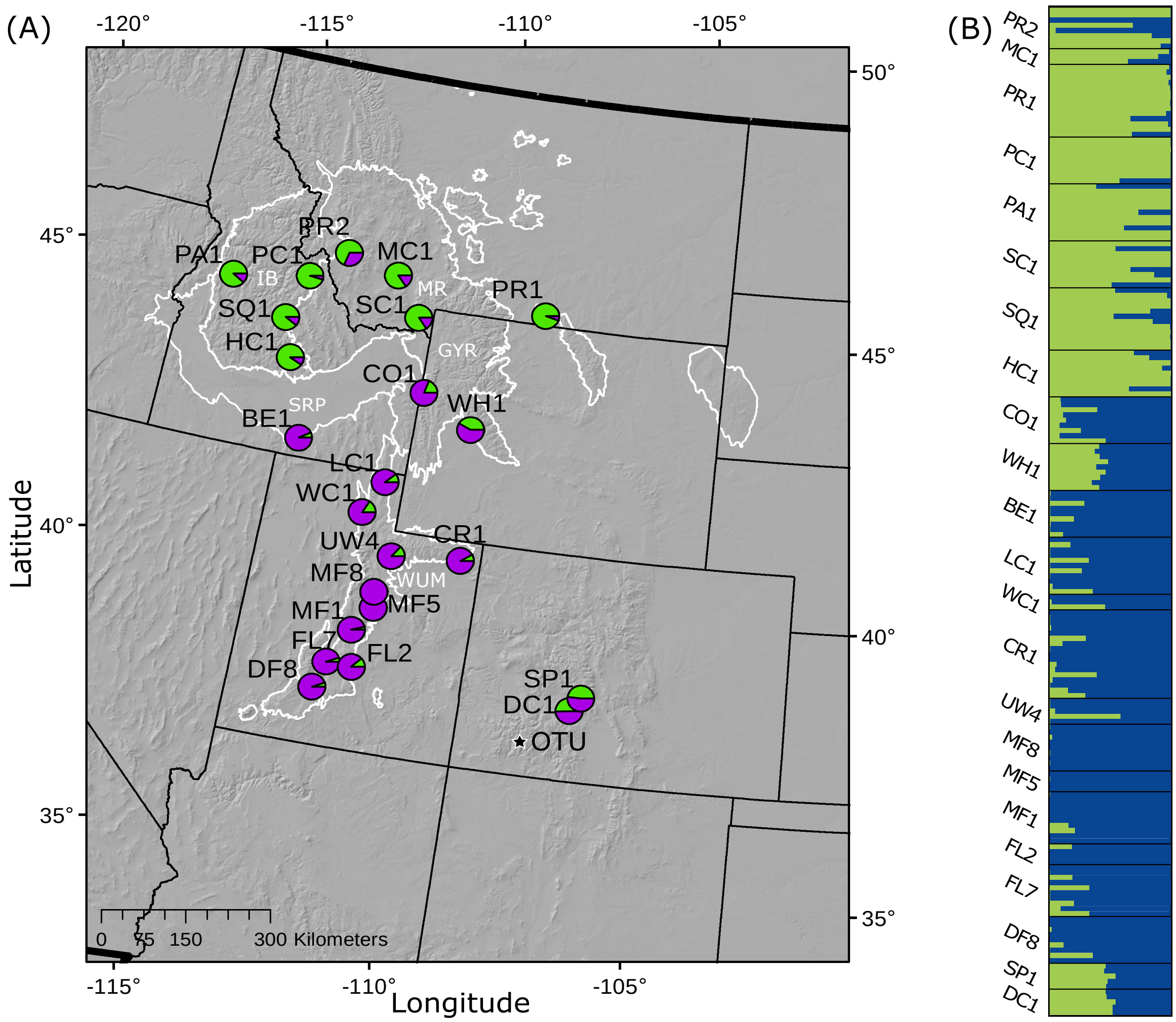

Figure 4. Genetic structure of *Erigeron speciosus* populations. Colors depict distinct clusters. Pie charts with multiple colors indicate likely admixture between clusters.

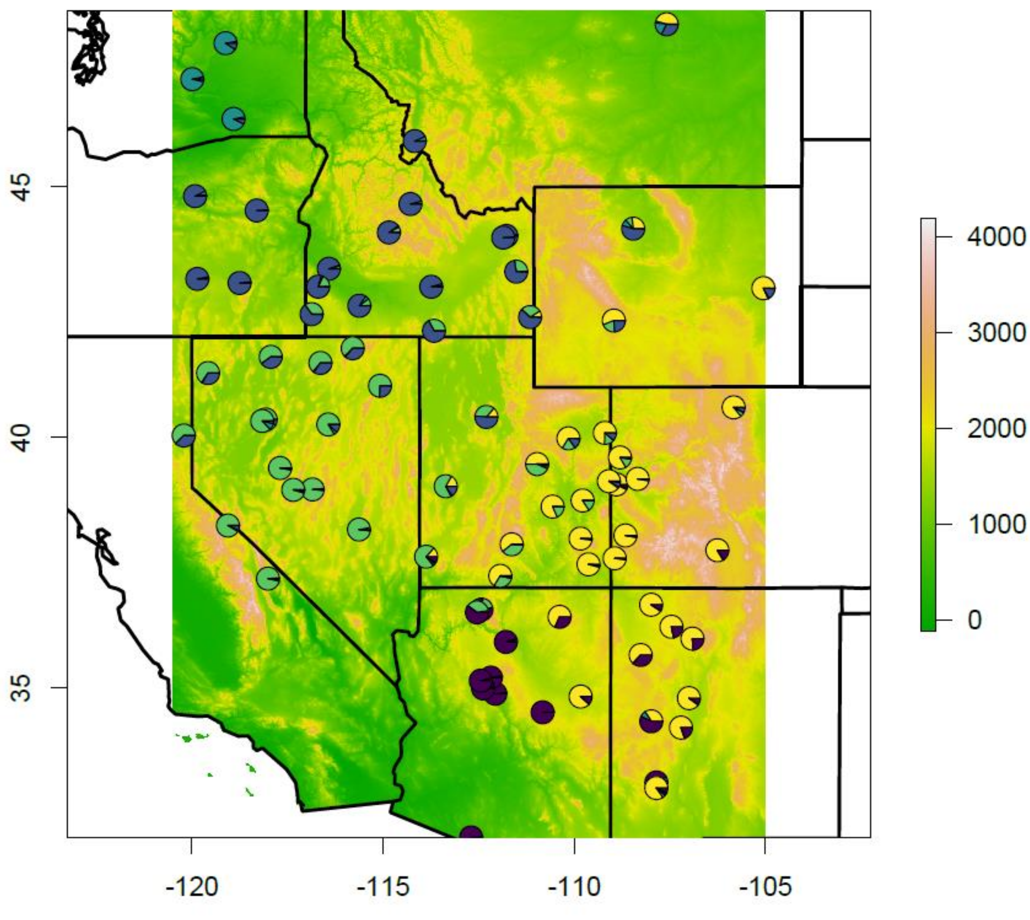

Figure 5. Genetic structure of *Machaeranthera canescens* populations. Colors depict distinct clusters. Pie charts with multiple colors indicate likely admixture between clusters.

Overall, this project will result in the following products:

- Establishment of a common garden array
- General Technical Report: common garden design and installation
- Seed accessions representing many populations of native species used in arid land restoration
- Identification of ploidy and genetic clusters for focal native plant species
- Geospatial seed transfer guidance for use in online tools
- Several publications describing population-level variation in:
  seed germination, soil interactions, drought tolerance, and competition with invasive species

The Bureau of Land Management, US Fish and Wildlife Service, and US Forest Service Intermountain Region are concurrently increasing these focal forb species through IDIQs. This means that once seed zones are established there will already be some seed that corresponds to the established seed zones. So, we are developing seed transfer zones that will be used immediately by placing these seeds into a more accurate adaptive framework.

Vegetation succession in post-fire seeding treatments

Science Partners:
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Utah Division of Wildlife Resources: Danny Summers
Utah Department of Natural Resources: Tyler Thompson
Brigham Young University: Steve Petersen

The objective of this research is to evaluate long-term (15+ years) outcomes of post-fire seeding with conventional and native seed mixes in sagebrush and pinyon-juniper ecosystems of the Great Basin, using a pre-established seeding experiment in Tintic Valley, Utah as a case study. The experiment had been set up following a wildfire that burned across the valley 1999. Four different seed mixes, plus a non-seeded control, had been applied in landscape-scale replicates. Two 'conventional' mixes contained commonly seeded non-native (Eurasian) species such as crested wheatgrass, and the other two mixes were comprised of native species at two levels of diversity and seeding rate. We sought to extend the original three-year evaluation of this experiment (reported by Thompson et al. 2006[1]) to a longer timeframe by re-measuring vegetation attributes 15-17 years later. We have used these data to document successional trends and assess the degree to which restoration objectives are being met on the longer term.

Research was initiated in 2015 through a grant from the Joint Fire Sciences Program, which provided three years of funding, and follow-up work has continued through the Great Basin Native Plant Project. Data were collected in 2015 in partnership with the Utah Division of Wildlife Resources and 2016-2017 in partnership with Brigham Young University. Data have subsequently been analyzed and results have been presented in multiple formats, including nine formal presentations at scientific conferences or other meetings[2], two webinars[3], a final report to the Joint Fire Science Program[4], and a paper published in the journal *Rangeland Ecology and Management* in 2019[5]. The latter paper integrated 2015 data with earlier data from 2002 to reveal the degree of vegetation change and persistence of seed mixes during the intervening period (Figure 1). This paper has been cited by 12 subsequent papers to date (Google Scholar) and has been downloaded 456 times according to USFS records[6]. Another paper, just published in the journal *Applied Vegetation Science*[7], incorporates data from all years to examine successional trajectories of the post-fire seeding treatments and their similarity to reference state communities (Figure 2). A third paper, soon to be submitted to *Restoration Ecology* or a similar journal, takes a closer look at treatment cost-effectiveness and the way in which individual seeded species contributed to restoration objectives. Findings of these studies are relevant to land management in the Great Basin given the continuing application of post-fire seeding with increasing use of native plants.

[1]Thompson TW, Roundy BA, McArthur ED, Jessop BD, Waldron B, Davis JN. 2006. Fire rehabilitation using native and introduced species: A landscape trial. Rangeland Ecology & Management 59:237–248.

[2]Presentations at Conferences/Meetings:

- Ott JE. 2020. Long-term outcomes of a post-fire seeding experiment in Tintic Valley. Invited oral presentation, Utah Society for Range Management 2020 Annual Meeting (virtual), Nov 10-12, 2020.
- Ott JE, Kilkenny FF, Summers DD, Thompson TW. 2020. Vegetation succession following post-fire seeding with conventional and native seed mixes in the Great Basin. Invited oral presentation, 47th Natural Areas Conference (virtual), Oct 14, 2020.

Ott JE. 2019. Outcomes of post-fire seeding experiments using native seed mixes in the Great Basin. Invited oral presentation, Eighth Western Native Plant Conference, Olympia, WA, Nov 12-14, 2019.

Ott JE. 2019. Post-fire seeding experiments with native seed mixes. Invited oral presentation, Intermountain Native Plant Summit IX, Boise, ID, Nov 5-6, 2019.

Ott JE. 2019. Long-term evaluation of seed mixes for post-fire seeding. Invited oral presentation, Utah Partners for Conservation and Development-Watershed Restoration Initiative Regional Partnership Workshop, Richfield, UT, May 29, 2019.

Ott JE, Shaw NL, Kilkenny FF. 2018. Post-fire seeding as a restoration tool: competitiveness and resilience of native seedings in shrub steppe ecosystems of the Great Basin. Invited oral presentation, Society for Ecological Restoration Joint Regional Conference, Spokane, WA, Oct 15-18, 2018.

Ott JE, Kilkenny FF, Summers DD, Thompson TW. 2018. Effects of seeding treatments on vegetation succession following fire in semiarid ecosystems of the Great Basin. Poster presentation, International Association for Vegetation Science 61st Annual Symposium, Bozeman, MT, Jul 22-27, 2018.

Ott JE, Kilkenny FF, Summers DD, Thompson TW. 2018. Long-term effects of post-fire seed mixes: revisiting a large-scale seeding experiment in Tintic Valley, Utah. Oral presentation, 71st Society for Range Management Annual Meeting, Sparks, NV, Jan 28-Feb 2, 2018.

Ott JE. 2017. Strategies and equipment for large-scale, multi-species native seedings in North American drylands. Invited oral presentation, VII World Conference on Ecological Restoration, Foz do Iguaçu, Brazil, Aug 27-Sep 1, 2017.

[3]Webinars:

Kilkenny FF, Ott JE. 2020. Evaluating native seed mixes for post-fire seeding in the Great Basin. Webinar hosted by Natural Areas Association, Jul 28, 2020.

Kilkenny FF, Ott JE. 2020. Seed mix matters! Webinar hosted by USDA Forest Service, Rocky Mountain Research Station, May 20, 2020.

[4]Kilkenny FF, Ott JE, Summers DD, Thompson TW. 2018 Vegetation Succession in Post-fire Seeding Treatments: Final Report to the Joint Fire Science Program.

[5]Ott JE, Kilkenny FF, Summers DD, Thompson TW. 2019. Long-term vegetation recovery and invasive annual suppression in native and introduced postfire seeding treatments. Rangeland Ecology & Management 72:640-653.

[6]U.S. Forest Service Research & Development, publication specific page views: https://www.fs.fed.us/research/ga-service/rd_national_site_view_publication.php?breadcrumb_id=3&rits_prd_id=94082

[7]Ott JE, Kilkenny FF, Summers DD, Thompson TW, Petersen SL. Post-fire succession of seeding treatments in relation to reference communities in the Great Basin. Applied Vegetation Science, 25:e12633.

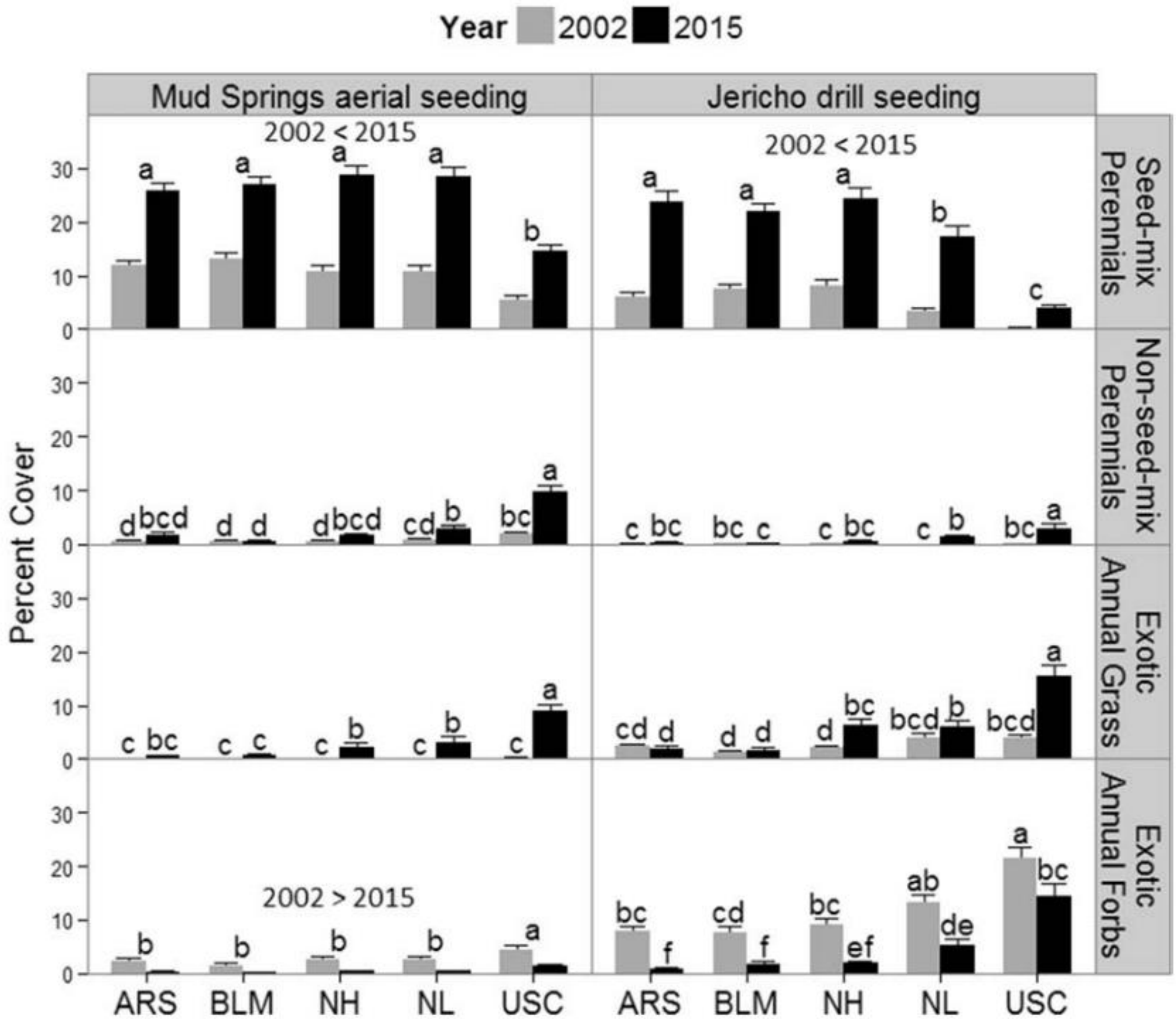

Figure 1. Cover by species group at sites in Tintic Valley, Utah, as recorded 3 yr (2002) and 16 yr (2015) following fire and seeding. Bars indicate means, error bars, standard errors. Within each cell (species groups within sites), means with the same letter are not significantly different ($P < 0.05$). If the treatment × year interaction was not significant, significance is shown separately for treatment (letters spanning both yr) and year (text in cell). Treatments: ARS indicates Agricultural Research Service mix; BLM, Bureau of Land Management mix; NH, Native high diversity mix; NL, Native low diversity mix; USC, unseeded control.[4]

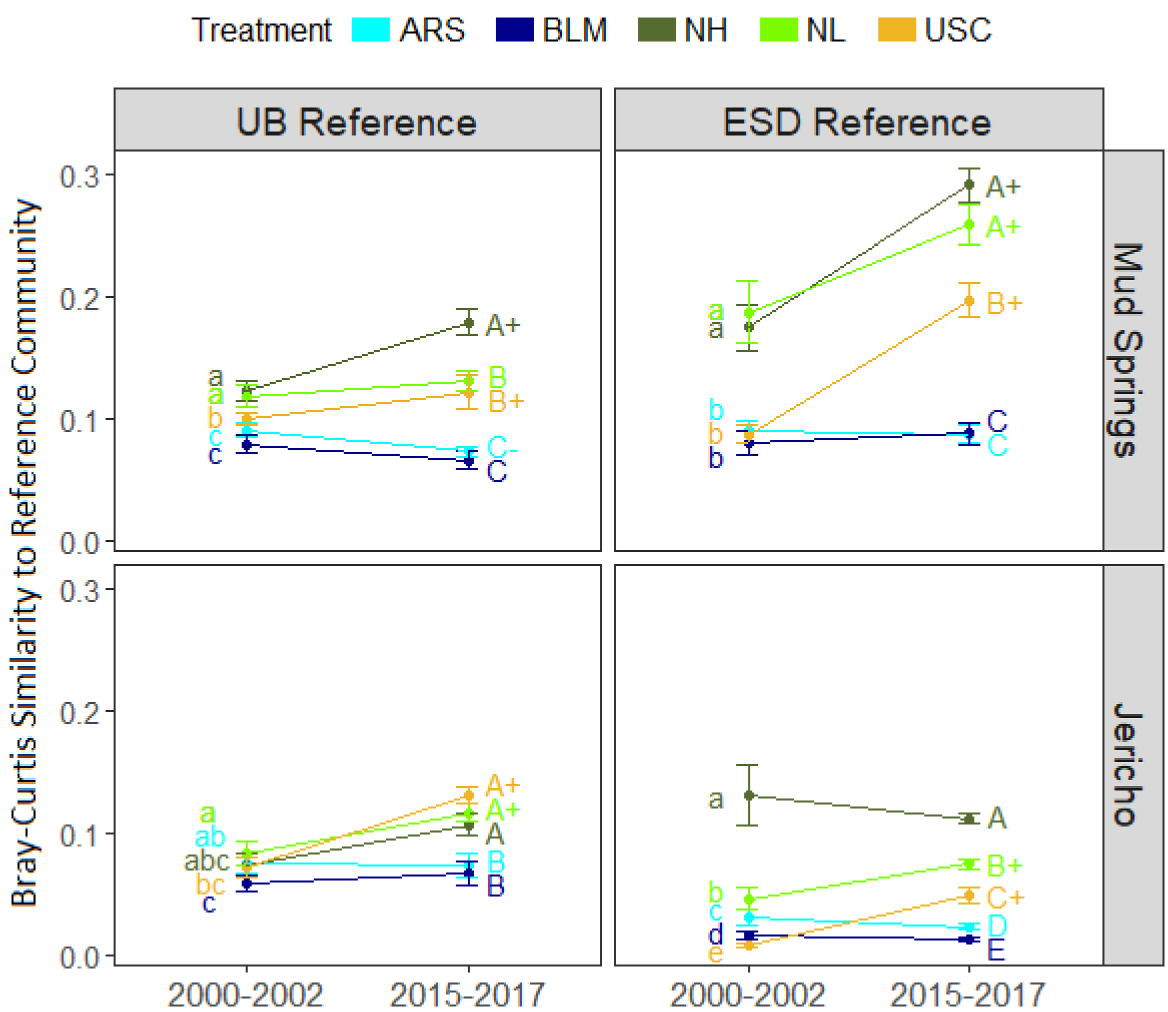

Figure 2. Bray–Curtis similarity of post-fire treatments in relation to reference communities based on local unburned areas (UB) and ecological site descriptions (ESD), during two time periods at two sites (Mud Springs and Jericho) in Tintic Valley, UT, USA. Points and error bars are means and standard errors for each combination of treatment (colors), period (*X*-axes), site (horizontal panels) and reference community (vertical panels); higher values along *Y*-axes indicate greater similarity of treatments to indicated reference communities. In each panel, different letters indicate significant ($p < 0.05$) differences between treatments within periods, i.e., lowercase letters for Period 1 (2000–2002, corresponding to post-fire years 1–3) and uppercase letters for Period 2 (2015–2017, or post-fire years 16–18). Significant ($p < 0.05$) increases and decreases from Period 1 to 2 are shown by + and − symbols, respectively. ARS, Agricultural Research Service mix; BLM, Bureau of Land Management mix; NH, native high-diversity mix; NL, native low-diversity mix; USC, unseeded control.[7]

Adaptive genetic variation and seed transfer guidelines for bottlebrush squirreltail (*Elymus elymoides*)

Science partners:
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USDA Forest Service, Pacific Nothwest Research Station: Bradley St. Clair
University of Nevada Reno: Beth Leger
USDA Agricultural Research Service: Richard C. Johnson
USDA Forest Service, Pacific Northwest Region: Matthew E. Horning

Introduction – Empirical seed transfer zones are tools that can help land managers make informed decisions on seed sourcing and have commonly been modeled using transfer functions, regressions of trait × environment relationships. While transfer functions are relatively straightforward to model, delineating actual seed transfer zone boundaries requires additional methods and depends on both model outputs and management goals that are not always made explicit. Managers may benefit from flexible zone delineations that can be adjusted to encompass broader or narrower transfer criteria, thereby accommodating various transfer risk levels and practical constraints. An under-utilized method for developing seed transfer zones involves multivariate regression trees. Regression tree modeling offers a data-driven approach for determining zone boundaries and an intuitive decision-tree framework for understanding the relative importance of different predictor variables and their interactions. Because of the hierarchical structure of regression tree models, the number of zones is not fixed but can vary depending on stopping criteria, which can be quantitative or practical. Another advantage of regression trees is their ability to directly incorporate categorical variables when developing seed transfer zones.

Intraspecific taxonomic diversity is common in plants and can have important conservation implications, including the complexity it can add to seed sourcing efforts in important restoration species. For species with recognized subspecies, varieties, or phylogenetic structure a central question is whether these intraspecific taxa have different trait × environment relationships and ought to be treated individually when developing seed transfer zones. The regression tree approach provides a way to deal with this issue when developing seed transfer zones, by treating taxonomic or phylogenetic identity as a categorical variable, as we demonstrate for a grass species with multiple intraspecific taxa.

In this study, we characterize adaptive genetic variation in bottlebrush squirreltail (*Elymus elymoides*), a short-lived, self-pollinating, perennial bunchgrass of restoration importance, found in semi-arid regions of western North America, across a core section of its range in the northern Great Basin and Intermountain West region. We present seed transfer zones developed through a regression tree modeling. We use these models and accompanying map projections to assess the relative importance and interacting effects of tested predictor variables, and to highlight a hierarchical interpretation of seed transfer zones that allows flexibility in practical application.

Population Sampling and Experimental Design – Seed was collected between 2007 and 2010 from 110 wildland Elymus populations located in the Intermountain region, western USA, across an area spanning five states and ten EPA Level III Ecoregions. In 2011 seedlings were germinated in growth chambers, then grown in a greenhouse at Oregon State University. Plants were transplanted to common gardens in fall 2011 at three test sites: Central Ferry, Washington; Powell Butte, Oregon; and Reno, Nevada.

Trait Measurements and Analyses – Traits were measured at common gardens in 2013 after a year of growth and acclimatization. Measured traits assessed plant growth, reproduction, leaf morphology, and phenology. Plants were identified to subspecies, based on morphological characteristics, where possible. Relationships between trait variation and climates of population source locations were modeled using a multivariate regression tree (MRT) approach to define seed transfer zones. the MRT algorithm partitions response variables by identifying, at each node in the tree, the predictor variable that minimizes sums variation within groups while maximizing it between groups. Two models were developed, one was 'climate-only' that ignored taxonomy and one 'climate-taxonomy' that used subspecies as a predictor. MRT models were projected geographically onto climate spatial layers. The mapping area consisted of the EPA Level III Ecoregions where one or more populations had been collected plus the climatically-similar Columbia Plateau ecoregion where the Central Ferry common garden site is located.

Taxonomic and Trait Variation – Four subspecies of *E. elymoides* were identified among the populations grown in common gardens: ssp. *elymoides* (57 populations), ssp. *brevifolius* (16 populations), ssp. *californicus* (9 populations), and ssp. *hordeoides* (8 populations). Site and population were major contributors to variation for most measured traits. When broken into components, much of the explained trait variation was due to site effects, ranging from 23% (leaf ratio) to 72% (plant height). Percent variation explained by population ranged from 3% (leaf length) to 24% (heading date).

Zonation Based on Climate Only – MRT modeling resulted in a tree model with 13 terminal nodes, each represented by 5-13 populations (Figure 1). When projected onto the mapping region (Figure 2), this 'climate-only' model defined seed transfer zones that we grouped at three levels of the tree hierarchy. The first level contained three primary zones (A, B, C) differentiated by temperature differential and mean warmest month temperature at the first two partitions of the tree. Each primary zone was further partitioned into 2-3 secondary zones (1, 2, 3) and up to three tertiary zones (a, b, c) differentiated by a variety of precipitation, temperature, degree-day, frost and other climate variables. Zones of the climate-only model occupied distinct geographic areas and elevational bands within the mapping region (Figure 2). The A zones were located primarily on the western side of the region in the vicinity of the Sierra Nevada, Cascades, and western flank of the Blue Mountains. Within this area, A1 occupied higher-elevation, colder/wetter climates than A2. The B2 zones were also concentrated on the western side of the region in areas with relatively low temperature differential and relatively high minimum winter temperatures, though not as high as nearby A2. Farther east, zones B1, B3, C1, and C2 were positioned sequentially along a gradient from colder, snowier climates at higher elevations of interior mountain ranges to warmer, more arid climates at lower elevations. Further subdivisions of the B and C zones were aligned with elevation and/or other geographic features associated with contrasting temperature, or radiation intensity.

Zonation Based on Climate and Taxonomy – When subspecies was included as a predictor variable in the 'climate-taxonomy' MRT model, it was the first variable identified for partitioning (Figure 3). Populations belonging to *E. elymoides* ssp. *brevifolius* and ssp. *californicus*, plus three populations not identified to subspecies, came together in one side of the primary partition, labeled 'bc', opposite the group 'eh' comprised of ssp. *elymoides*, ssp. *hordeoides*, and seven other unidentified populations. Subsequent partitions were determined by climate variables, especially variables measuring temperature or degree-days. The bc group was subdivided into 4 secondary groups, one of which was further partitioned into two tertiary groups, leading to 5 terminal nodes. Separate zone maps were developed for the bc and eh groups by projecting their respective secondary and tertiary subdivisions onto the mapping region (Figure 3). These maps showed that zonation patterns based on trait-climate relationships were similar for both groups of subspecies even though zone boundaries were not identical. These seed transfer zones were also similar to the 'climate-only' seed transfer zones, with the more complex 'climate-only' model appearing to be similar to what a combination of the two 'climate-taxonomy' seed transfer zones would be if intersected.

Traits in Relation to Zones, Climate and Taxonomy – Mean trait values of populations originating in each zone provided a basis for interpreting trait-climate and trait-taxonomy relationships. Patterns of trait expression differed at each common garden site, but in general, plants from cooler/wetter climates were larger, produced more inflorescences, and had wider leaves and later phenology than those from warmer/drier climates. The pattern of large size, high inflorescence number, wide leaves and late phenology was more prominent in the bc subspecies group than eh. Leaves were widest in populations from higher elevation areas in the western and central part of the region, and narrowest in lower elevation areas. Biomass, plant height, inflorescence number, heading date and maturation date were highest/latest in populations from western locations with moderately high precipitation and low temperature differential. The earliest heading and maturation dates were observed in populations from the lowest/driest areas. Biomass, plant height and inflorescence number also tended to be lowest in populations from relatively arid areas, except at Reno where plants from some of the most arid zones were relatively large and produced more inflorescences than plants from elsewhere.

Conclusions –

- Squirreltail trait × environment relationships follow similar patterns to other Great Basin and Intermountain West bunchgrasses, indicating similar spatial patterns of natural selection across these taxa.
- Squireltail has highly plastic trait expression, which might allow for seed sources to establish and persist in areas where they are somewhat maladapted. More research is needed on this.
- Subspecies groupings were an important variable in developing seed transfer zones, although similarities between zonation suggest that the 'climate-only' model may be sufficient for management seeding decisions.
- Multivariate regression trees successfully provided a logical hierarchical structure that will allow managers to combine seed transfer zones for practical purposes.

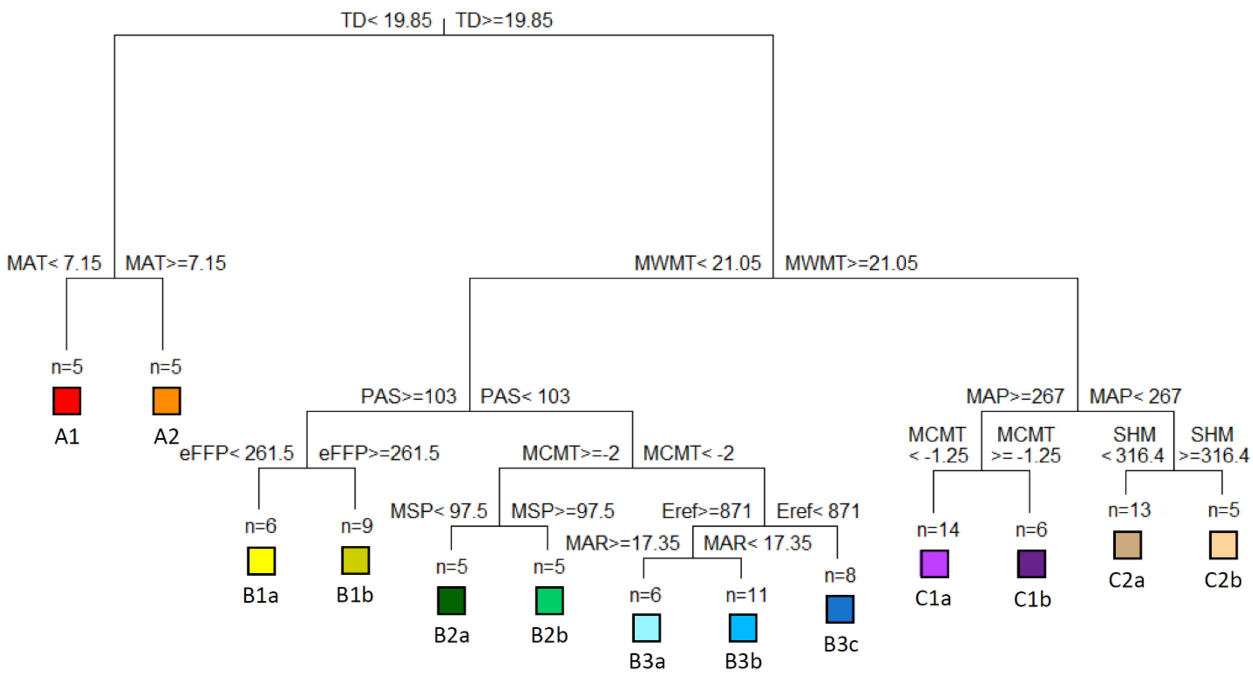

Figure 1. Multivariate regression tree model identifying seed transfer zones based on relationships between climate variables of source locations and traits of 98 *Elymus elymoides* populations measured at three common garden sites. Each partition separates populations differing by principal component (PC) axes of trait values by site at the indicated threshold value of the most strongly correlated predictor variable. Bars show relative values of PC axes 1-18 from left to right, and n indicates the number of populations represented in each terminal leaf. Colors and code names at terminal leaves correspond to seed transfer zones mapped in Figure 2

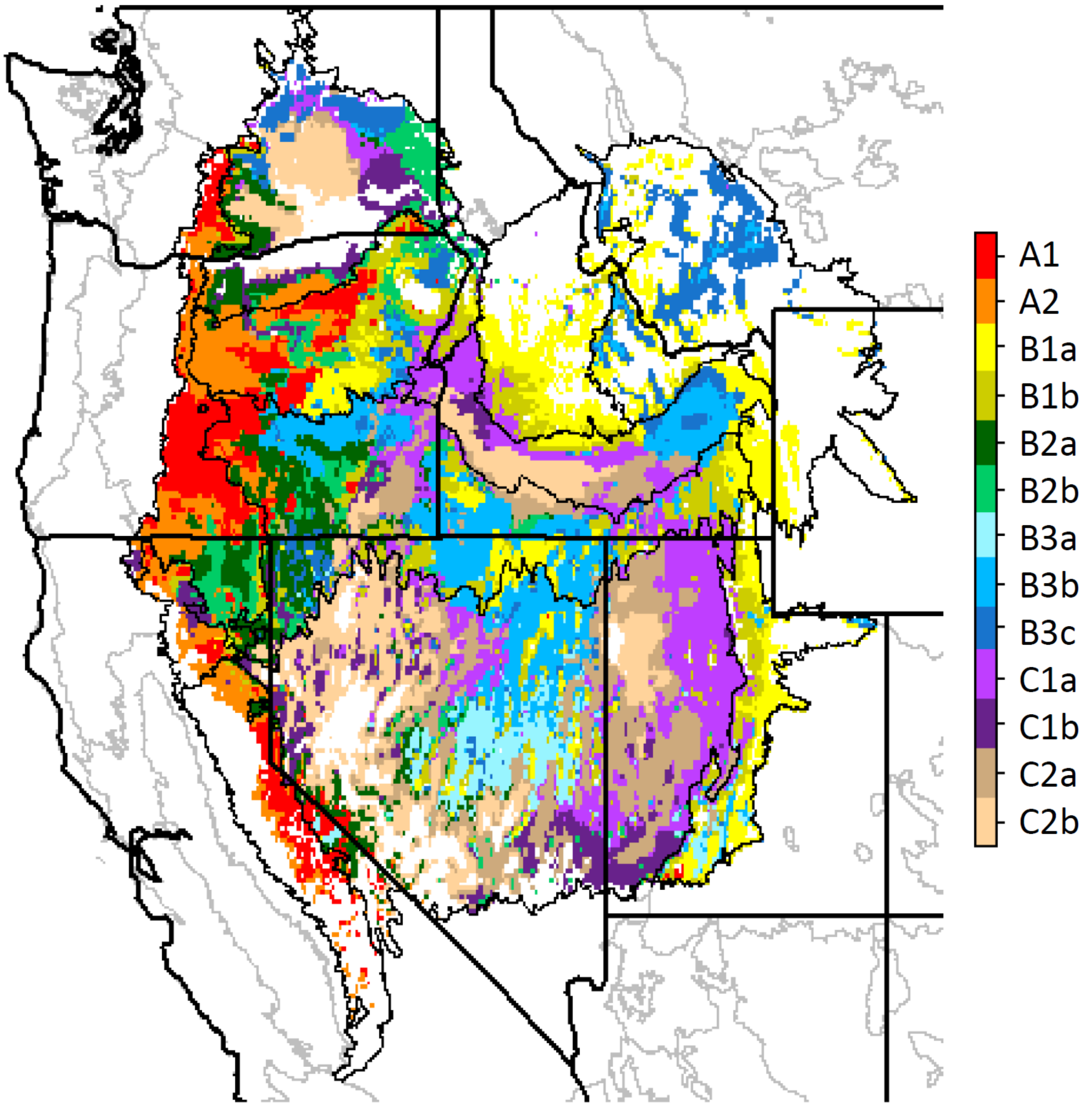

Figure 2. Seed transfer zone map for *Elymus elymoides* based on multivariate regression tree modeling of trait × climate relationships. Zone colors and code names correspond to terminal leaves of model shown in Figure 1. Mapped area is defined by EPA Level III Ecoregions, omitting areas beyond 1 standard deviation of sampled range of climate variables in the study.

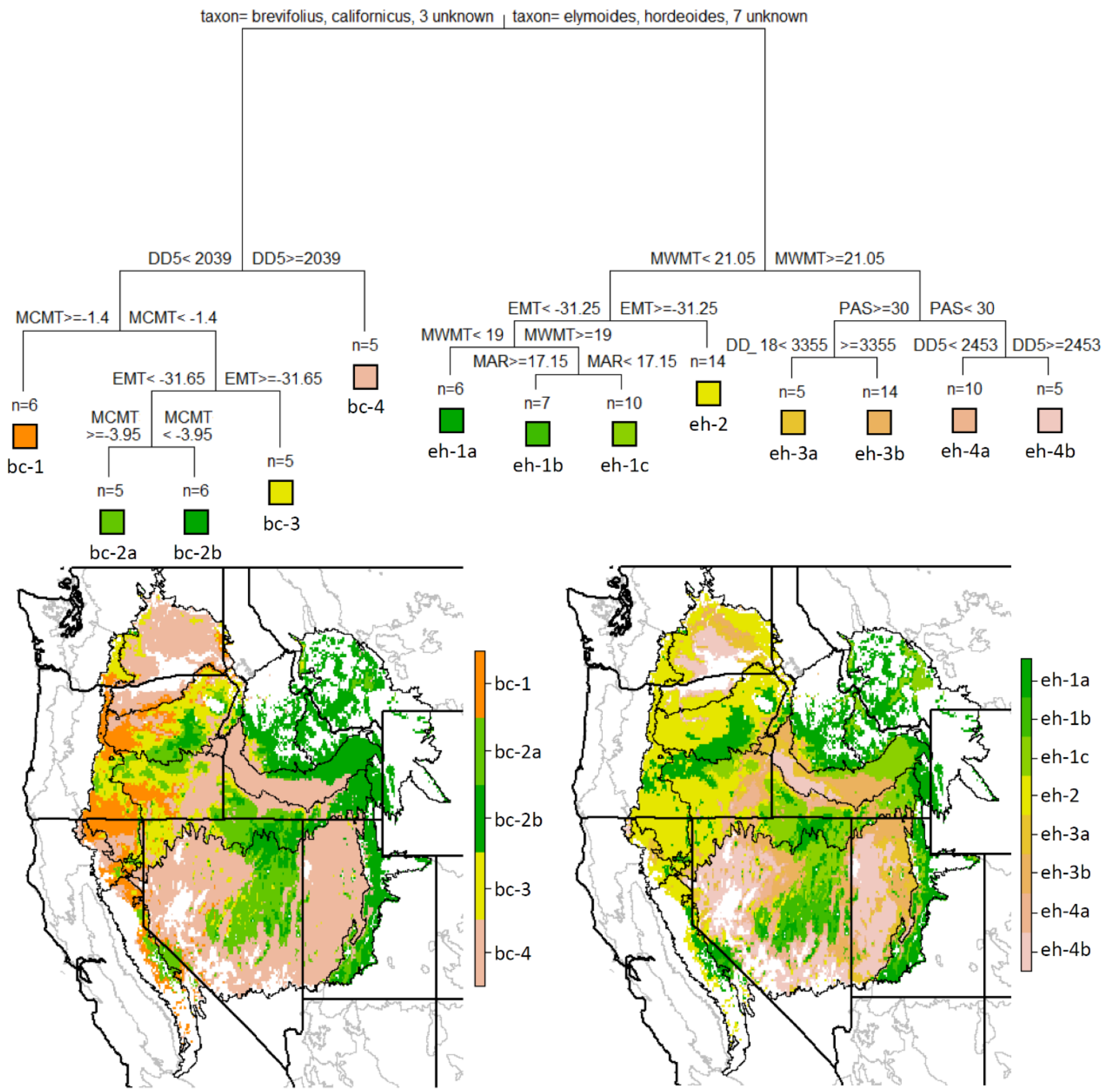

Figure 3. Seed transfer zones for *Elymus elymoides* based on a multivariate regression tree model with taxonomic and climate variables as predictors of trait variables (upper graph), projected onto in the Intermountain region of western North America (lower graphs). Colors at terminal leaves of regression tree correspond to mapped zones. The model shows relationships between predictors (subspecies identity and source-location climate) and traits measured at three common garden sites for 98 populations. Each partition separates populations differing by principal component (PC) axes of trait values by site at the indicated threshold value of the most strongly correlated predictor variable. Bars show relative values of PC axes 1-18 from left to right, and n indicates the number of populations represented in each terminal leaf. Colors and code names at terminal leaves correspond to seed transfer zones mapped in lower graphs. Left map shows zones for branch containing ssp. *brevifolius* and ssp. *californicus* (abbreviated bc); right map, ssp. *elymoides* and ssp. *hordeoides* (abbreviated eh). Mapped area is defined by EPA Level III Ecoregions, omitting areas beyond 1 standard deviation of sampled range, for each subspecies branch, of each climate variables in this study.

Testing the Efficacy of Seed Zones for Re-establishment and Adaptation of Bluebunch Wheatgrass (*Pseudoroegneria spicata*)

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Introduction – Land managers face numerous challenges when maintaining and restoring healthy ecosystems that are resilient to climate change. One challenge is determining and procuring the appropriate plant material to restore an area. In the western United States, plant species span a wide range of climates from arid desert to temperate rainforest to alpine areas. Climatic extremes can lead to natural selection on populations leading to improved survival and reproduction in local environments, i.e. local adaptation. To ensure success in restoration activities and healthy ecosystems, federal agencies have directives to use genetically appropriate materials that are locally adapted. Seed transfer zones have been developed to guide the movement of appropriate plant materials in restoration and reforestation. Seed transfer zones separate areas by plant performance and/or climatic conditions important to plant growth, survival, and reproduction. Provisional seed zones combine minimum winter temperature with aridity (mean annual temperature/mean annual precipitation) to delineate areas of similar climate. Empirical seed zones are delineated for a particular species and incorporate climatic data related to genetically differentiated traits important to survival and reproduction. Empirical seed zones have been developed for over 20 species in the western United States. However, the efficacy of seed transfer zones in predicting the climatic match of seed sources to restoration sites has not been tested in non-tree species.

Focal plant – Bluebunch wheatgrass (*Pseudoroegneria spicata*) is an ecologically important restoration species that provides structure to plant communities and forage for wildlife. Bluebunch wheatgrass is a cool-season, long-lived perennial bunchgrass native to the western United States. This grass thrives in a variety of habitats that range in mean annual temperature (3-11°C), annual precipitation (21-187 cm), and elevation (230-2460 m). Populations of bluebunch wheatgrass differ in several traits important for adaptation to precipitation and temperature (St.Clair et al. 2013). Seed zones for bluebunch wheatgrass demarcate areas with similar climate and populations with climate-associated genetic variation. To examine if populations are locally or regionally adapted requires comparing performance of local and non-local populations in a reciprocal manner. We are using a reciprocal transplant experiment to investigate the efficacy of bluebunch wheatgrass seed zones. Results from this experiment will determine if seed zones for bluebunch wheatgrass are delineated appropriately or if they need modification. We investigate the effectiveness of empirical seed zones for bluebunch wheatgrass by comparing establishment, survival, and reproduction of populations from local seed zones to non-local seed zones.

Objectives –

1. Evaluate adaptation of bluebunch wheatgrass populations from local seed zones relative to non-local seed zones.

2. Model adaptation as a function of source location and common garden climates.
3. Characterize traits and climatic factors important for adaptation.
4. Model effects of climate change on native populations and consequences of assisted migration for responding to climate change.

Methods – We hypothesize that in the long-term, populations from local seed zones will better establish, survive, and reproduce. To test this hypothesis, we established a reciprocal transplant study at 16 sites located in two broad regions (i.e. transects) (Figure 1). The two broad regions include (1) a transect through hot, dry climates of the lower Snake River Plain to the cool, relatively wet climates of the higher elevations of the Great Basin Ranges to the cold, dry climates of the upper Snake River Plain, and (2) a transect from hot, dry climates of the Columbia Plateau to cool, wet climates of the Blue Mountains to the cold, dry climates east of the Blue Mountains. Within each transect, we reciprocally planted native populations from eight seed zones into eight sites that represent the climate of each seed zone (Figure 1). In addition, we planted two propagated lines at all sites and one propagated line at sites at the two extremes of the seed zones.

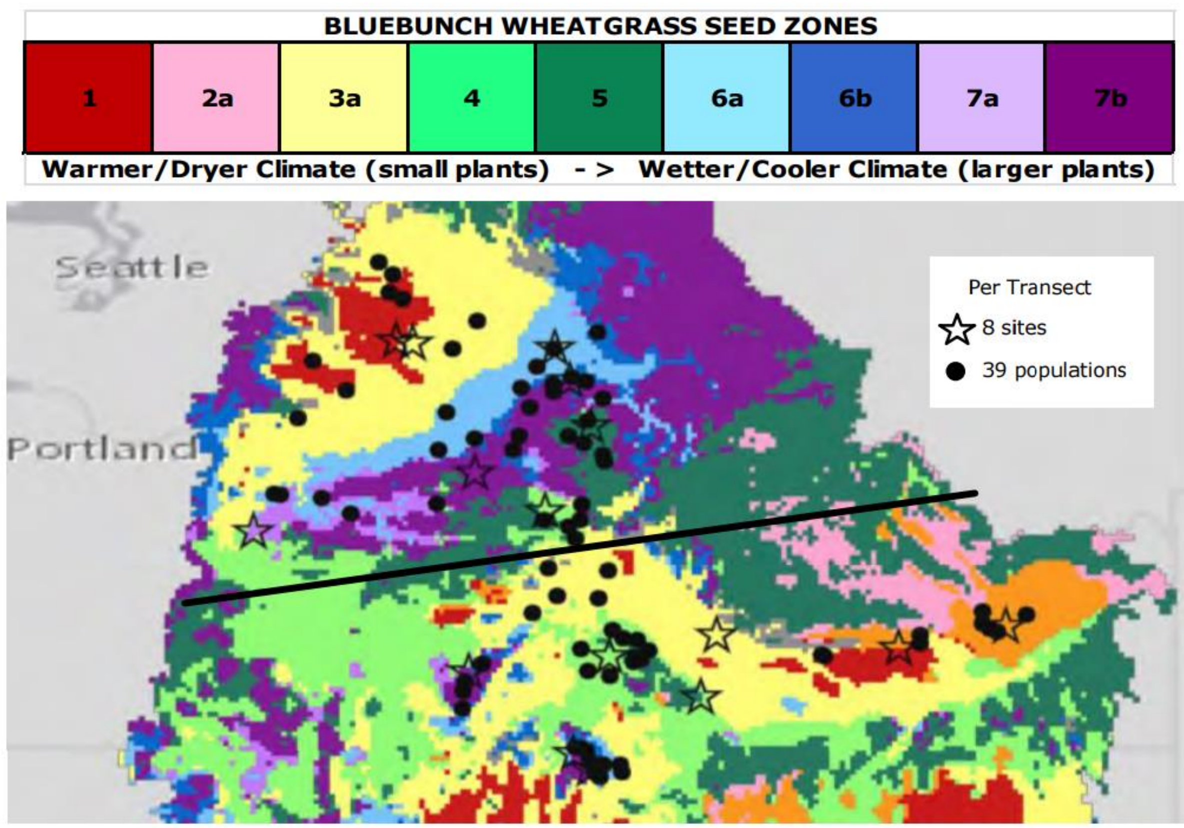

Figure 1. Map of bluebunch wheatgrass populations (black circles) used in eight common gardens (stars) in each transect (delineated) in the northwest US.

Seed was collected from 78 populations throughout the two broad regions in 2013. The RMRS Moscow lab produced containerized planting stock for the study in 2014. Federal, state and private collaborators provided land for common garden sites. The 16 common gardens were identified, prepared and established in 2014. Several common gardens were fenced to protect plants from herbivory. Each common garden was planted with 39 populations and two propagated lines (Anatone and Goldar) for a total of 820 plants surrounded by two rows of buffer plants. A few common garden sites had an additional treatment included: (1) a seed zone from the other transect, or (2) an extra propagated line (Wahluke) for a total of 1000 plants per common garden.

The experimental design at each common garden is a split-plot design with populations planted by seed zone into subplots and all seed zones present within a plot. Each site includes four replicate plots. Each subplot has 25 plants: five replicates of five populations or all one propagated line. However, only four populations were available from the hottest, driest seed zone within each transect and had additional replicates to reach 25 plants per subplot. Spacing is 0.6 m x 0.8 m between plants. Each site was visited in spring and summer of 2015, 2016, and 2017 to record various traits including: leaf length, width and roll; survival; reproductive phenology; herbivory; root crown diameter; and number of reproductive stalks. In 2015 and 2016 reproductive stalks were collected from a subset of individuals per population at each site to estimate seed production. In the summer 2017 after reproductive stalk production, biomass was harvested from a subset of individuals per populations at each site. Weather data loggers were installed at plant height and at 2 m at each site to record local temperature for comparison with regional weather stations and climate interpolation models. Common gardens are managed to control weeds to ensure establishment and to reduce the effect of competing vegetation to allow better evaluation of differences in plant growth, survival, and reproduction associated with local climates.

Site visits to the gardens were also made from 2019-2021 to maintain them in as weed free state as possible. Additional data on plant size, survival and reproduction were taken in most years and at most gardens. Several sites have been retired due to low survival rates or the ending of agreements with local land owners. Currently, 12 sites are still active across the study (6 on each transect).

Preliminary results and discussion – Across 16 common garden sites survival was high (>80%) through the first growing season. One site was completely lost with the majority of plants dying a month after transplant due to an extreme weather event. This site was re-planted, but subsequently lost due to repeated vandalism. Of the 15 sites that remained through 2017, plant survival declined over time to 54% on average. Plants varied in survival, leaf morphology, reproductive phenology and reproduction among common garden sites and among seed sources.

Two analyses are presented here. The first analysis (Figure 2, Table 1), asks how local populations perform relative to the average performance across all non-local populations. In other words, “does local do best?” Populations are defined as local in two ways: 1) populations that are from the same seed transfer zone as the common garden are considered “local”; 2) populations that are from source environments within one standard deviation from the garden location in multidimensional climate space are considered “derived local”. This analysis shows mixed evidence for local adaptation. The average local population performed slightly better than the average non-local population, but this result was only significant in one case (Figure 2, Table 1). Across all measures local was best in 36.7% of cases, the same in 37% of cases, and local was not best in 26.3% of cases. Relative performance depended on how local was defined. Defining local populations by seed transfer zone predicted the presence local adaptation more consistently than predictions based on the “derived local” definition. In particular, across all measure for “local”, local was best in 40% of cases, the same in 31.1% of cases, and local was not best in 28.9% of cases. For “defined local” local was best in 33.3% of cases, the same in 42.3% of cases, and local was not best in 23.8% of cases. “Derived local” also showed significant variation between trait measurements. Taken together this indicates that the use of seed transfer zones in bluebunch wheatgrass seeding will increase the chance of establishment or be neutral in the majority of cases and only be detrimental in some cases.

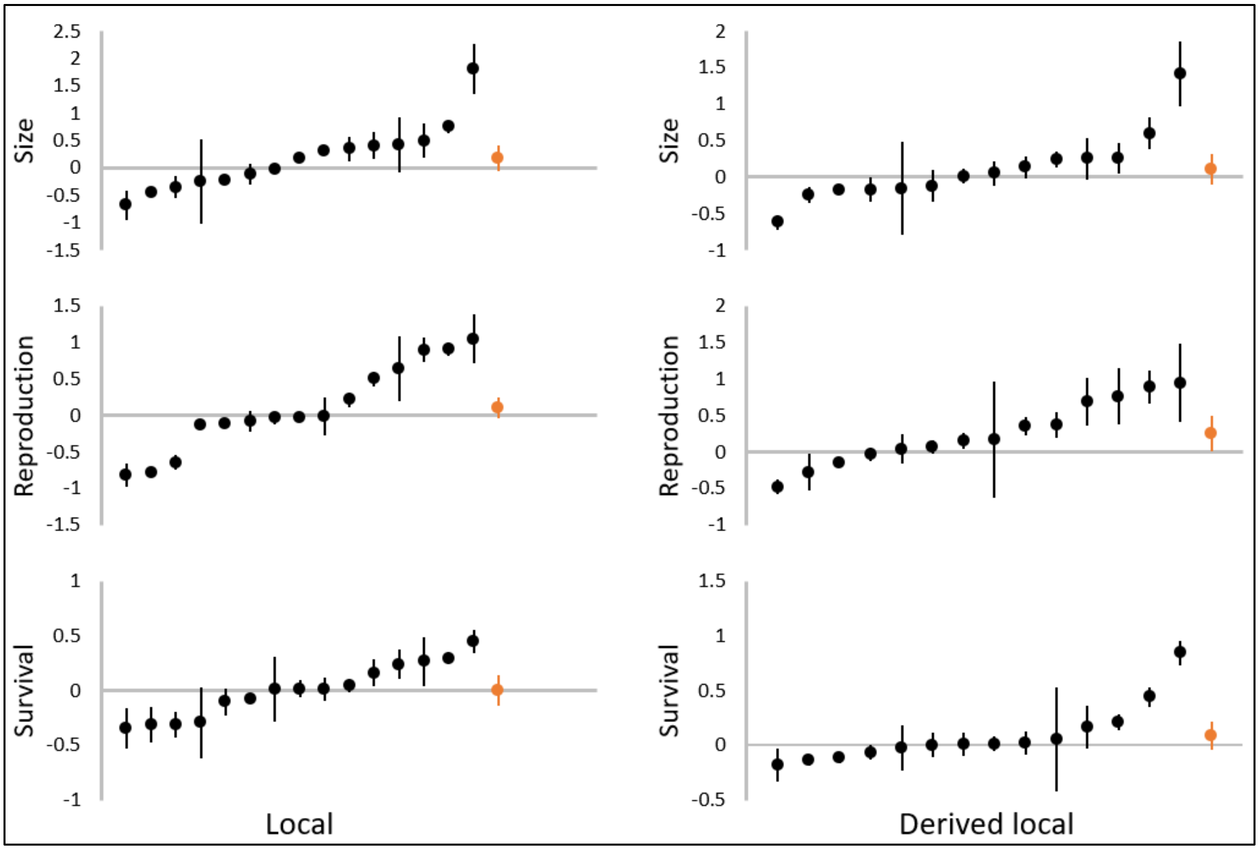

Figure 2. Evaluation of the performance of local populations relative to the average performance of all non-local populations for size, reproduction, and survival. Two definitions for local are used: 1) populations that are from the same seed transfer zone as the common garden are considered “local”; 2) populations that are from source environments within one standard deviation from the garden location in multidimensional climate space are considered “derived local”. Black circles represent the relative performance of the local population at a garden site, gray lines represent the average performance of non-local populations, and orange circles represent the average performance of local populations across all garden sites.

Table 1. Summery table for the analysis presented in Figure 1. Percentages show the proportion of gardens where the relative performance of local populations (see definition in Figure 1) was better, the same as, or worse than the average non-local population for size, reproduction, and survival. Green colors represent higher percentages and red colors represent lower percentages. The "Average Local Best?" column indicates whether the average relative performance of all local populations across the study is greater than the average performance of all non-local populations, and the "Average Significant" column indicates whether this difference is significant.

		Local Best	Local Same	Local Not Best	Average Local Best?	Average Significant?
Local	**Size**	46.7%	26.7%	26.7%	yes	no
	Reproduction	40.0%	26.7%	33.3%	yes	no
	Survival	33.3%	40.0%	26.7%	yes	no
Derived Local	**Size**	28.6%	50.0%	21.4%	yes	no
	Reproduction	50.0%	28.6%	21.4%	yes	yes
	Survival	21.4%	50.0%	28.6%	no	no

For the second analysis we developed models of transfer distance (Figure 2). This shows the change in trait values we might expect based on the difference in climate conditions between the source environment and a garden site. For example, on the Northern transect we would expect that at a given garden, or restoration site, plants from cooler and wetter environments would be larger and plants from warmer and dryer environments would be smaller. The patterns in this analysis show that climatic adaptation is complex and depends on the trait being studied, the locations of the gardens, and the populations used. Patterns of climatic adaptation were similar between common garden transects, but these patterns were much stronger in the Northern transect. This makes sense given that there was also a larger range of variation in common garden environments in the Northern transect. As mentioned above, plants from cooler and wetter areas tended to be larger and plants from warmer and dryer areas tended to be smaller. This corresponds well with known trends in dryland species, where water use can be correlated with size. Reproduction showed the clearest pattern of local adaptation, with plants from the local environment having the highest reproduction at a garden site, and plants from different environments having less reproduction the farther away in climatic space they were sourced from. Survival followed as similar pattern to size, with plants from cooler and wetter areas having higher survival than plants from warmer and dryer areas. This may also be related to life history adaptation to arid environments. Populations from warmer and dryer areas may exhibit a live-fast-die-young strategy where reproduction, rather than long term survival, has the highest contribution to fitness. Transfer distance functions can be used to build and modify seed transfer zones. They also provide managers with the tools to make decisions about how much movement is acceptable, and therefore dictate the size of seed transfer zones. The appropriate size of seed transfer zones is difficult to estimate from studies with few common gardens and is therefore subject to error. In this study, the Northern transect had 8 gardens with usable data, and the Southern transect had 7 gardens with usable data. This number appears to be sufficient to develop robust transfer distance functions. Research into other species will be helped by using common garden arrays of comparable size, since this will allow for robust estimates of transfer distance with a single study.

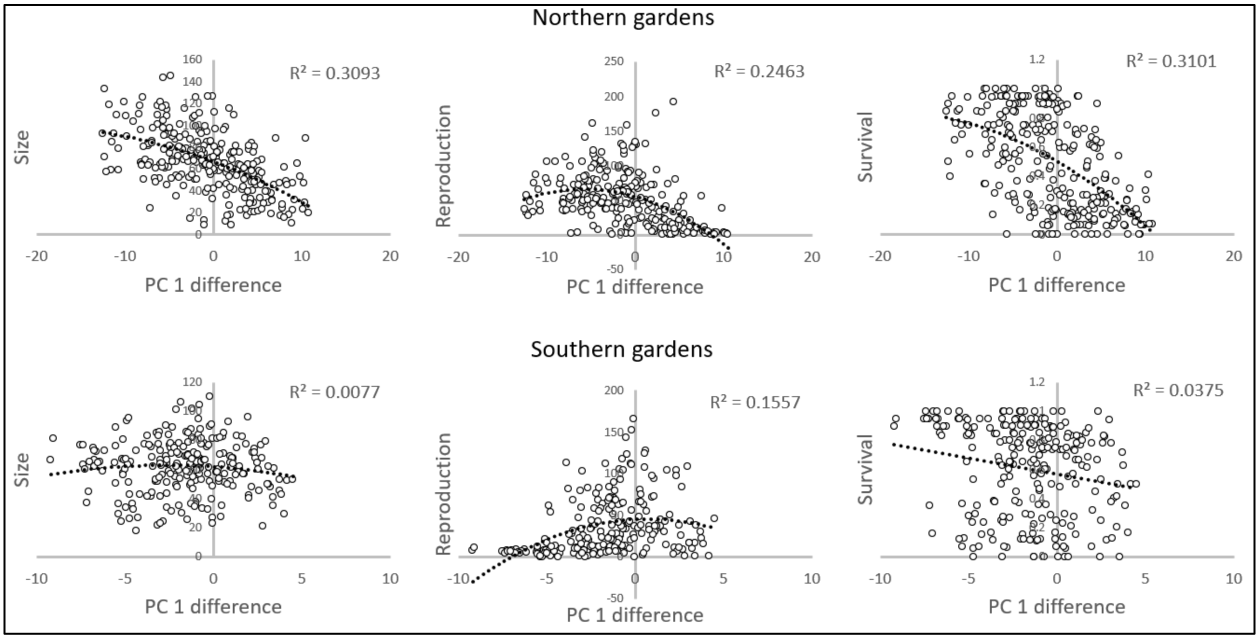

Figure 3. Transfer distance functions for *Psuedoroegneria spicata* Northern and Southern common garden transects for size, reproduction, and survival. PC 1 difference axis indicates expected trait values of a source population at a restoration site given the difference between the climate at the restoration site and the climate at the source location. Negative values on the PC 1 difference axis represent source locations that are cooler and wetter than garden sites, and positive values represent source locations that are warmer and wetter than garden sites.

Management Applications – Since bluebunch wheatgrass is an ecologically important restoration species, it is essential to have effective seed-transfer zones to improve restoration success. This study is examining the efficacy of seed zones for bluebunch wheatgrass to ensure successful establishment and allow for long-term adaptation by maintaining genetic diversity. The results of this study will help land managers to determine sources of bluebunch wheatgrass populations for post-fire restoration of an area. Furthermore, this study will explore the consequences of changing climates for adaptation by substituting space for time to evaluate various populations in different climates. Results from this portion of the study will aid land managers in maintaining and restoring areas considering future climate changes. Long-term productivity and adaptation will be modeled to allow evaluation of trade-offs between different management options for current and future climates. Plant material movement guidelines and associated seed zones can be adjusted based on results from this study and management objectives.

References- St. Clair JB, Kilkenny FF, Johnson RC, Shaw NL, Weaver, G. 2013. Genetic variation in adaptive traits and seed transfer zones for Pseudoroegneria spicata (bluebunch wheatgrass) in the northwestern United States. Evolutionary Applications, 6: 933-948.

Completed bluebunch wheatgrass sub-projects:

Phenological Variation in Bluebunch Wheatgrass (Pseudoroegneria spicata): Implications for Seed Sourcing, Harvest, and Restoration

Science partners:
The Understory Initiative, Oregon: Kathryn Prive
USDA Forest Service, Pacific Northwest Research Station: Holly Prendeville
Oregon State University: Matt Orr and Ron Reuter
USDA Forest Service, Rocky Mountain Research Station: Francis Kilkenny

Abstract- (From publication) To reduce maladaptation in cultivated seed lots, seed transfer zones (STZs) have been developed for grasslands and other habitats using morphological traits and phenological measurements that only capture the first day of events such as flowering and seed ripening. Phenology is closely linked to plant fitness and may affect genetic loss during harvests of seed raised for ecological restoration. Here, we measured the detailed phenologies of 27 populations from six STZs of bluebunch wheatgrass (*Pseudoroegneria spicata*) (Pursh) Á. Löve (Poaceae) raised in a common garden to test whether existing STZs created using a combination of plant morphology and "first-day" phenological measurements adequately capture population-level variation in season-long, detailed phenologies. We also used detailed phenologies to test whether genetic losses may occur during single-pass harvests of commercial seed. Mixed and random effect models revealed differences in detailed reproductive phenology among populations within two of six STZs. The number of individual plants within an STZ not producing harvestable seed during peak harvest levels indicated that 10–27% of individuals from a seed lot could be excluded from a single-pass harvest. Although our findings generally support current STZ delineations for P. spicata, they point to the possible precautionary importance of sourcing from multiple populations and harvesting with multiple passes when resources permit
Table 3. Percentage of individuals lost after hypothetical single-pass harvest at peak ripening. Percent lost indicates the overall percentage of plants that were not in the ripening stage at the time of hypothetical harvest (Table 2).

Table 2. Percentage of individuals lost after hypothetical single-pass harvest at peak ripening. Percent lost indicates the overall percentage of plants that were not in the ripening stage at the time of hypothetical harvest.

Zone	n Plants	Harvest Date	n Ripe	n Lost	% Lost
3a	43	18-Jul	35	8	19
4	61	10-Jul	51	10	16
6a	66	10-Jul	56	10	15
6b	78	10-Jul	57	21	27
7a	78	10-Jul	70	8	10
7b	65	18-Jul	48	17	26
All Zones	391	10-Jul	296	95	24

Population history provides foundational knowledge for utilizing and developing native plant restoration materials

Science partners:
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USDA Forest Service, Rocky Mountain Research Station: Bryce Richardson, Francis Kilkenny
USDA Forest Service, Pacific Northwest Research Station: Holly Prendeville
USDA Agricultural Research Service: Steve Larson and Blair Waldron

Abstract- (From publication) A species' population structure and history are critical pieces of information that can help guide the use of available native plant materials in restoration treatments and decide what new native plant materials should be developed to meet future restoration needs. In the western United States, *Pseudoroegneria spicata* (bluebunch wheatgrass; Poaceae) is an important component of grassland and shrubland plant communities and commonly used for restoration due to its drought resistance and competitiveness with exotic weeds. We used next-generation sequencing data to investigate the processes that shaped P. spicata's geographic pattern of genetic variation across the Intermountain West. *Pseudoroegneria spicata's* genetic diversity is partitioned into populations that likely differentiated since the Last Glacial Maximum. Adjacent populations display varying magnitudes of historical gene flow, with migration rates ranging from multiple migrants per generation to multiple generations per migrant. When considering the commercial germplasm sources available for restoration, genetic identities remain representative of the wildland localities from which germplasm sources were originally developed, and they maintain high levels of heterozygosity and nucleotide diversity. However, the commercial germplasm sources represent a small fraction of the overall genetic diversity of *P. spicata* in the Intermountain West. Given the low migration rates and long divergence times between some pairs of P. spicata populations, using commercial germplasm sources could facilitate undesirable restoration outcomes when used in certain geographic areas, even if the environment in which the commercial materials thrive is similar to that of the restoration site. As such, population structure and history can be used to provide guidance on what geographic areas may need additional native plant materials so that restoration efforts support species and community resilience and improve outcomes.

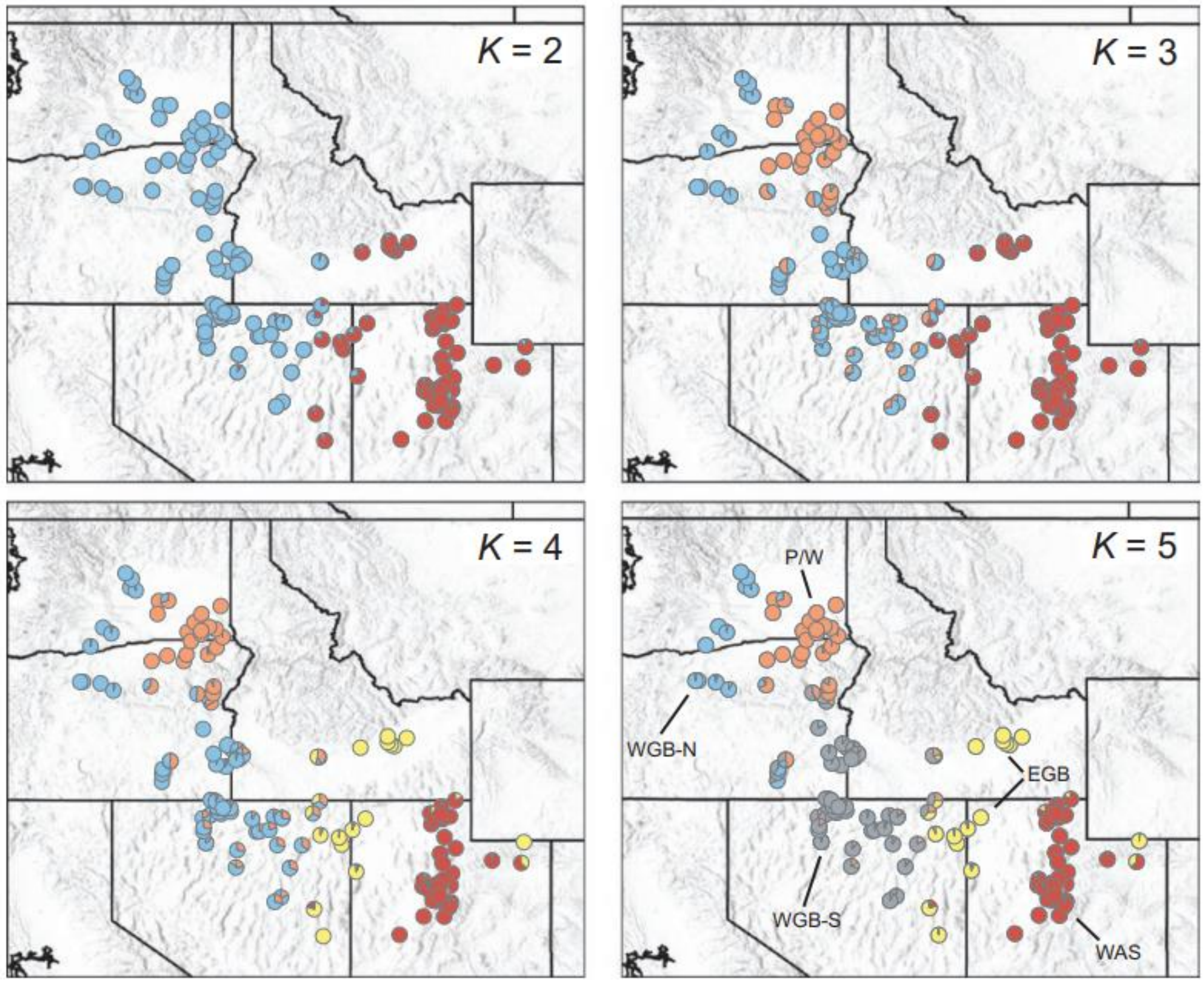

Figure 4. Results of structure analyses for K = 2 to K = 5. The posterior probabilities of individual assignments are averaged within sampling localities and represented by pie charts. Populations discussed in the text are noted for K = 5 and include Palouse/Wallowa (P/W); Western Great Basin (WGB-North and WGB-South); Eastern Great Basin (EGB); and Wasatch (WAS)

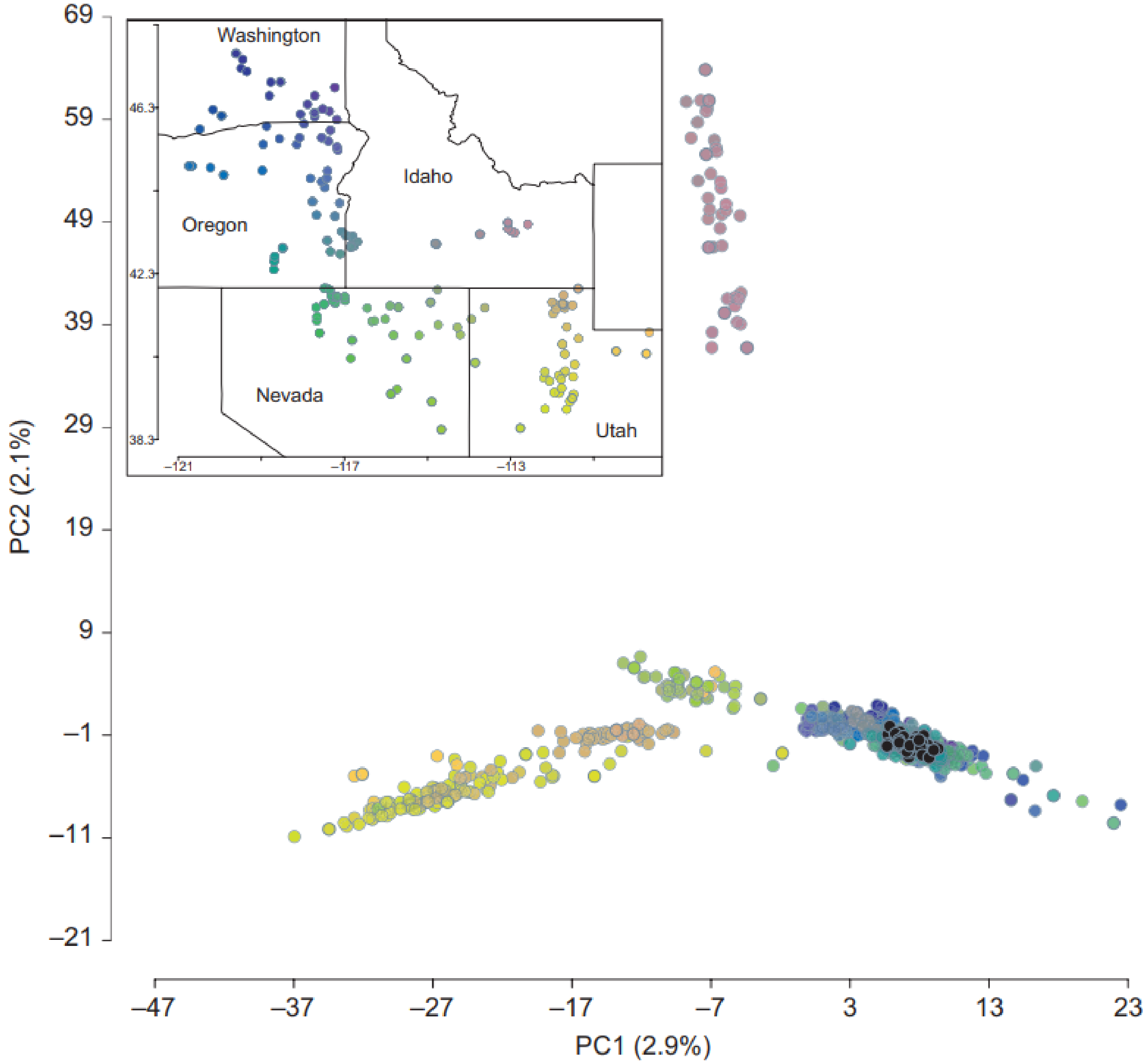

Figure 5. Distribution of diploid *Pseudoroegneria spicata* individuals along principal component 1 and principal component 2 axes of genetic variation. Variation explained by the axes is given in parentheses. Individuals in PC space are colored by their sampling locations, which are displayed in the inset. Individuals colored black represent the commercial germplasm sources. Note that commercial germplasm sources represent only a small fraction of the total genetic diversity of *P. spicata* in this region. Colors in this figure do not correlate to colors that describe populations in Figure 4.

Bluebunch wheatgrass publications:

Massatti R, Prendeville HR, Larson S, Richardson BA, Waldron B, Kilkenny FF. 2018. Population history provides foundational knowledge for utilizing and developing native plant restoration materials. Evolutionary Applications, 11: 2025-2039.

Cooke B, Richardson B, Kilkenny F, St. Clair B, Finch D, Prendeville H. 2019. Science You Can Use (SYCU) - Getting climate-smart with seeds: How a new software tool helps prepare landscapes for expected future conditions. Science You Can Use Bulletin, Issue 35. Fort Collins, CO: Rocky Mountain Research Station. 11 p.

Prive K, Orr MR, Kilkenny FF, Reuter RJ, Prendeville, HR. 2021. Phenological Variation in Bluebunch Wheatgrass (*Pseudoroegneria spicata*): Implications for Seed Sourcing, Harvest, and Restoration. Land, 10: 1064.

GBNPP and RMRS aligned projects

Strong patterns of intraspecific variation and local adaptation in Great Basin plants revealed through a review of 75 years of experiments

Science Partners:
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USDA Forest Service, Rocky Mountain Research Station: Francis Kilkenny and Jeff Ott
USDA Agricultural Research Service: Erin Espeland, RC Johnson
USDA Forest Service, Region 6: Matt Horning
Institute for Applied Ecology: Rob Fiegener and Thom Kaye
USDA Forest Service, Pacific Northwest Research Station: Brad St. Clair

Abstract – (From publication) Variation in natural selection across heterogeneous landscapes often produces (a) among-population differences in phenotypic traits, (b) trait-by-environment associa- tions, and (c) higher fitness of local populations. Using a broad literature review of common garden studies published between 1941 and 2017, we documented the commonness of these three signatures in plants native to North America's Great Basin, an area of extensive restoration and revegetation efforts, and asked which traits and environmental variables were involved. We also asked, independent of geographic distance, whether populations from more similar environments had more similar traits. From 327 experiments testing 121 taxa in 170 studies, we found 95.1% of 305 experiments reported among-population differences, and 81.4% of 161 ex- periments reported trait-by-environment associations. Locals showed greater sur- vival in 67% of 24 reciprocal experiments that reported survival, and higher fitness in 90% of 10 reciprocal experiments that reported reproductive output. A meta-analy- sis on a subset of studies found that variation in eight commonly measured traits was associated with mean annual precipitation and mean annual temperature at the source location, with notably strong relationships for flowering phenology, leaf size, and survival, among others. Although the Great Basin is sometimes perceived as a region of homogeneous ecosystems, our results demonstrate widespread habitat-re- lated population differentiation and local adaptation. Locally sourced plants likely harbor adaptations at rates and magnitudes that are immediately relevant to restora- tion success, and our results suggest that certain key traits and environmental varia- bles should be prioritized in future assessments of plants in this region.

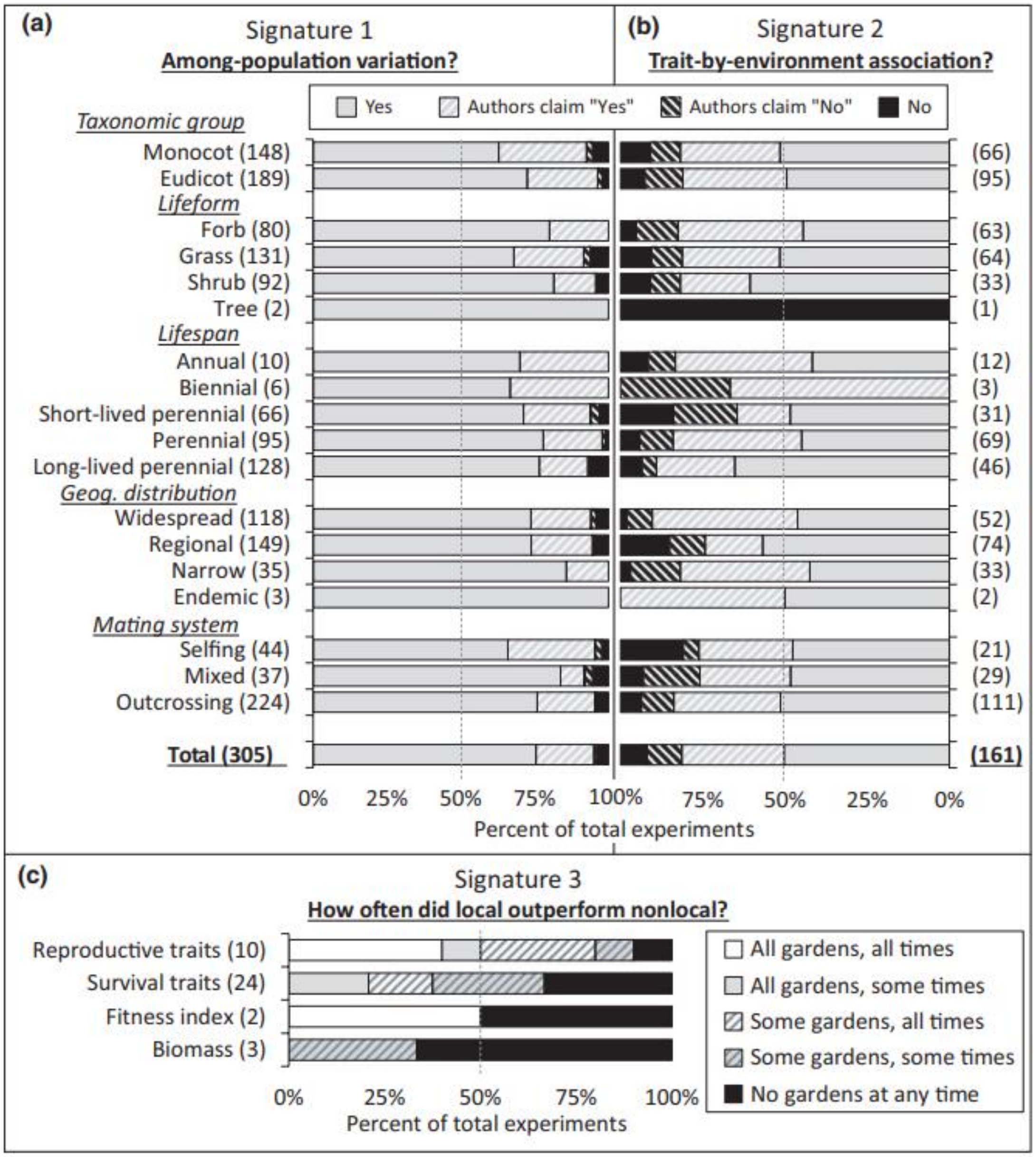


Figure 1. Summary of among- population variation (a, signature 1) and trait-by-environment associations (b, signature 2) for any measured trait, grouped by five life history traits. Summary of local advantage (c, signature 3) for reproductive traits, survival traits, fitness indices, or biomass. Data compiled from 327 experiments from 170 published studies on Great Basin plants (see Appendix S2 and available datasets in electronic supplementary material). For signatures 1 and 2, “Yes” and “No” represent statistical comparisons, while “Authors claim ‘Yes’” and “Authors claim ‘No’” represent textual, claim-based results where supporting statistics were not reported (common in older studies). For signature 3, most experiments had multiple gardens, and many evaluated performance at multiple sampling dates, leading to 5 different scores. These scores, from “All gardens, all times” to “No gardens at any time,” represent a gradient of incidence and frequency of this signature (see Methods). For all panels, numbers in parentheses, (x), indicate the number of experiments scored in a given category, and the dashed gray lines indicate 50%.

Publications – *Baughman OW, Agneray AC, Forister ML, Kilkenny FF, Espeland EK, Fiegener R, Horning ME, Johnson RC, Kaye, TN, Ott J, St. Clair JB*. 2019. Strong patterns of intraspecific variation and local adaptation in Great Basin plants revealed through a review of 75 years of experiments. Ecology and Evolution, 9:6259–6275.

Application of National Seed Strategy Concepts

Science Partners:
Bureau of Land Management: Fred Edwards
Fish and Wildlife Service: Sarah Kulpa
USDA Forest Service, Rocky Mountain Research Station: Francis Kilkenny

Summary – (Lightly edited excerpt from publication) Native plant species are the foundation of sagebrush ecosystems and provide essential habitat for wildlife species, such as Greater sage-grouse (Centrocercus urophasianus; hereafter, GRSG). The National Seed Strategy for Rehabilitation and Restoration (hereafter, Seed Strategy) (PCA 2015) strives to provide all land managers - Federal, tribal, State, county, private, and nongovernmental organization - the tools they need to address ecological restoration across the United States. The Seed Strategy provides a coordinated approach to improving the use of native seed, building Federal and private capacity, and increasing the supply of genetically appropriate native seed. The Seed Strategy recognizes the value of existing native plants and soil seedbanks and acknowledges that not all disturbances or management treatments require active seeding to restore habitat. The Seed Strategy also recognizes that although many nonnative species have been seeded successfully and economically to provide forage and soil stabilization, their ability to support diversity and provide functioning ecosystems to meet multiple use and sustained yield mandates is limited. Successful rehabilitation and restoration must always take into consideration compatibility of species in a seed mix, planting season, and appropriate seeding rates, techniques, technologies, and practices.

Genetically appropriate native plant materials have been historically underdeveloped within the sagebrush biome. This section document focuses on the logistics, challenges, opportunities, and considerations for procuring and using native seed in sagebrush ecosystems at broad (sagebrush biome), mid- (level III ecoregions), and local (project to site) scales. It also discusses local scale tradeoffs that should be considered when managers decide to use nonnative seeds with in the sagebrush biome. It does not address restoration practices and techniques.

Balancing locally adapted seed sources, cultivars, and nonnative species against the realities of implementing a project in the field is a series of tradeoffs. Every project is unique and a one-size-fits-all approach will not work. Sometimes seeding is used as a way to mitigate management risk or simply as insurance. Regardless of why and what is being seeded, the judicious use of seed will not only save money, but also minimize the risk of unintended ecological consequences to naturally recovering native plant communities. As part of any decision to seed, potential tradeoffs should be carefully weighed against the potential future economic and ecosystem costs. Seeding should not always be the first choice. For example, where prescriptive treatments are desired to minimize erosion risks to infrastructure, one-time physical barriers (such as straw wattles and straw mulch) may be more desirable and cost-effective where sufficient native perennial plants exist to promote recovery.

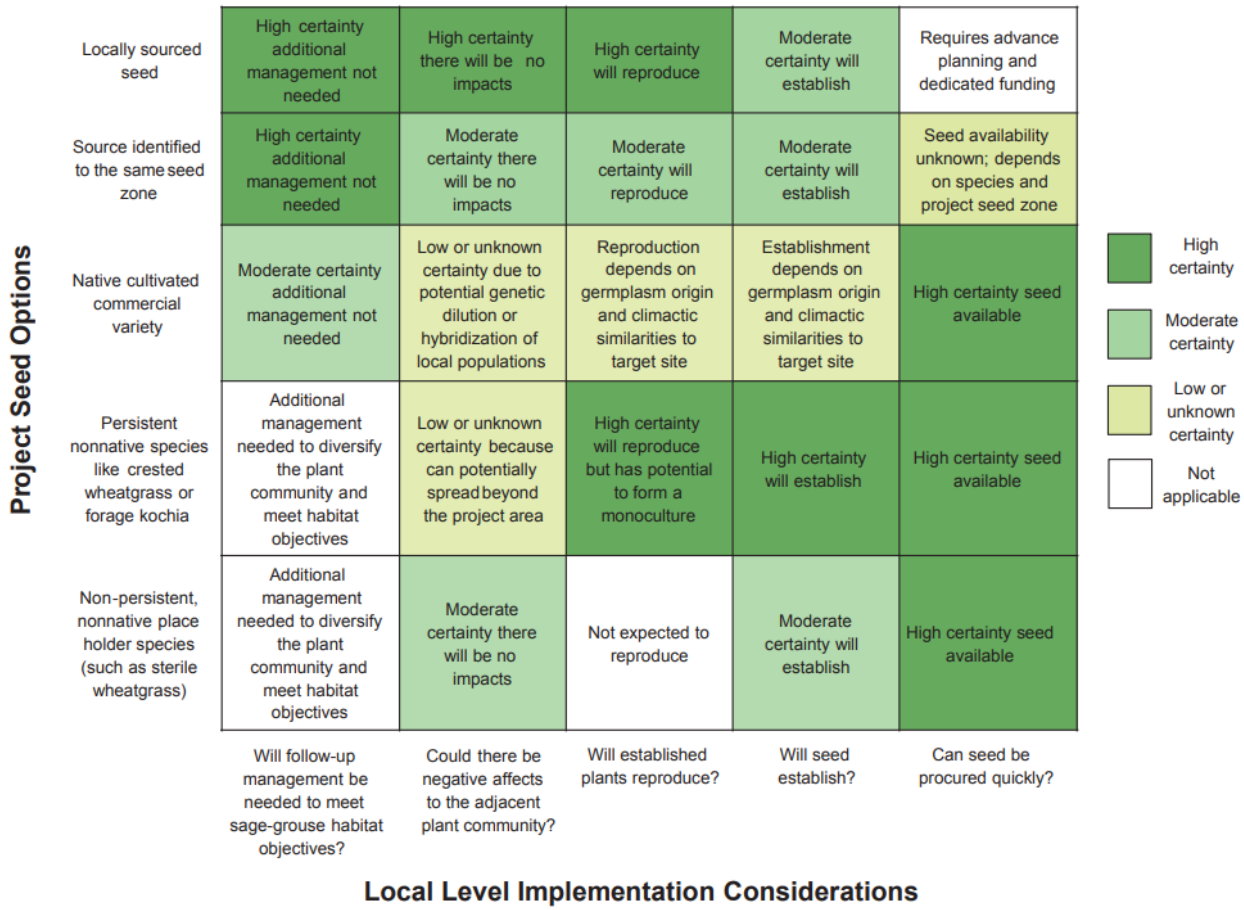


Figure 1. Seed source and local-level considerations for selecting seed sources and types.

Publications – Edwards F, Kulpa SM, Kilkenny FF. 2019. [Application of National Seed Strategy concepts.]() In: Crist MR; Chambers JC; Phillips SL; Prentice KL; Wiechman LA, eds. Science framework for conservation and restoration of the sagebrush biome: Linking the Department of the Interior's Integrated Rangeland Fire Management Strategy to long-term strategic conservation actions. Part 2. Management applications. Gen. Tech. Rep. RMRS-GTR-389. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. p. 113-130.

Priority Actions to Improve Provenance Decision-Making

Science Partners:
University of Adelaide, Australia: Martin Breed, Nick Gellie, and Andrew Lowe
University of Avignon, France: Armin Bischoff
Instituto Forestal Nacional (INFONA), Paraguay: Paula Durruty
Division at Parks Canada, British Columbia: Emily Gonzales
University of Tasmania, Australia: Peter Harrison
Chicago Botanic Garden: Kayri Havens and Pati Vitt
Forest Stewardship Council, Germany: Marion Karmann
Kings Park and Botanic Garden, Australia: Siegy Krauss
USDA Forest Service, Rocky Mountain Research Station: Francis Kilkenny
Big Hole Watershed Committee, Montana: Pedro Marques
Curtin University, Australia: Paul Nevill
Albert Ludwigs University, Germany: Anna Bucharova

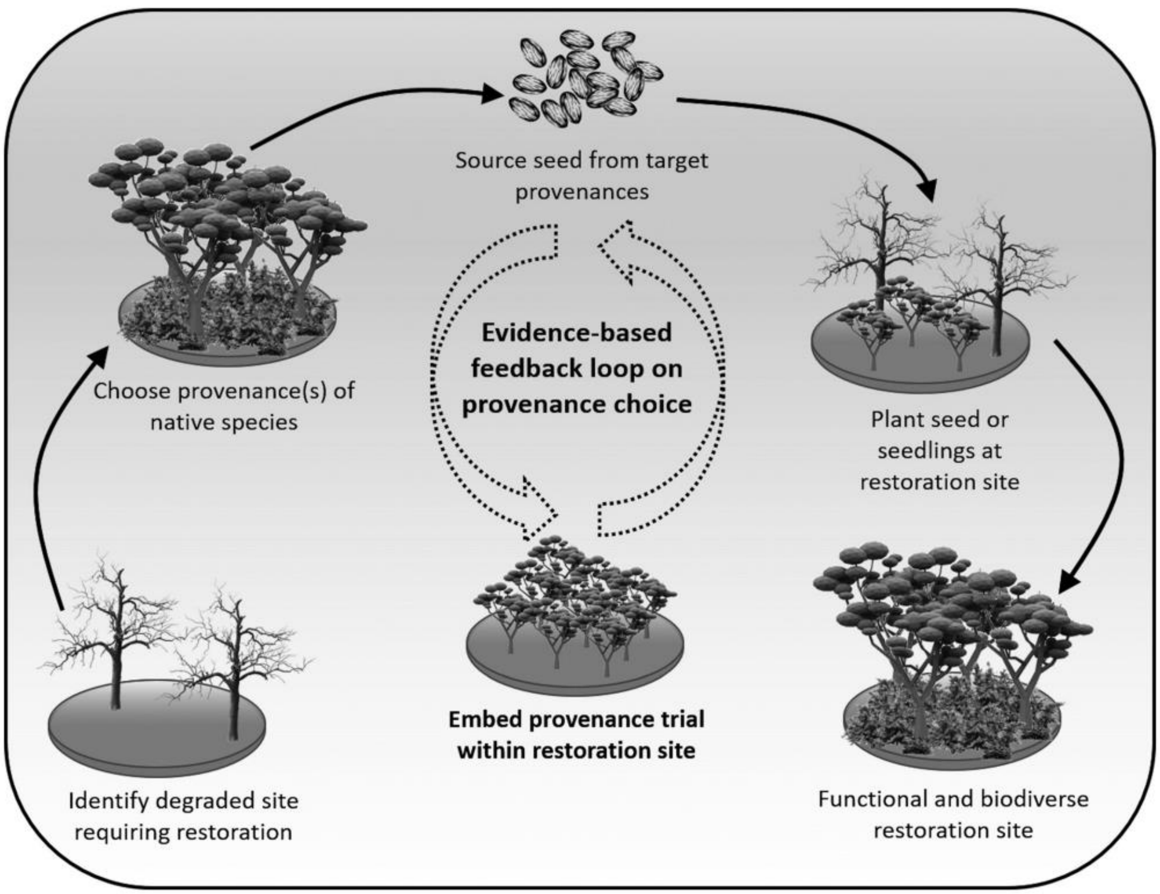


Figure 1. A schematic representation of the evidence-based feedback loop that embedded provenance trials within restoration plantings can have on improving provenance decision-making

Abstract – (From publication) Selecting the geographic origin - the provenance - of seed is a key decision in restoration. The last decade has seen a vigorous debate on whether to use local or nonlocal seed. The use of local seed has been the preferred approach because it is expected to maintain local adaptation

and avoid deleterious population effects (e.g., maladaptation and outbreeding depression). However, the impacts of habitat fragmentation and climate change on plant populations have driven the debate on whether the local-is-best standard needs changing. This debate has largely been theoretical in nature, which hampers provenance decision-making. Here, we detail cross-sector priority actions to improve provenance decision-making, including embedding provenance trials into restoration projects; developing dynamic, evidence-based provenance policies; and establishing stronger research–practitioner collaborations to facilitate the adoption of research outcomes. We discuss how to tackle these priority actions in order to help satisfy the restoration sector's requirement for appropriately provenanced seed.

Publications – Breed MF, Harrison PA, Bischoff A, Durruty P, Gellie NJC, Gonzales EK, Havens K, Karmann M, Kilkenny FF, Krauss SL, Lowe AJ, Marques P, Nevill PG, Vitt PL, Bucharova A. 2018. Priority actions to improve provenance decision-making. BioScience, 68: 510-516.

2021 Great Basin Native Plant Project Cooperator Reports

The following reports are those produced by GBNPP cooperators for RMRS agreement fulfillment purposes. All reports are for joint venture or interagency agreements funded by GBNPP and administered by RMRS. The reports are for 2021 and cover all GBNPP funded agreements that were ongoing or finishing in that year.

The first three reports (pages 42-44) are reports from cooperators working on the RMRS led "Workhorse Native Forb Adaptative Genetics and Plant Materials Development for Postfire Restoration of Critical Wildlife Habitat" project described above (pages 4-12). The rest of the reports cover a range of topics including:

- Development of web-based seed-sourcing tools (pages 45-46).
- Effects of seed mix diversity and soil amendments on soil microbiota and plant community succession (pages 47-48).
- Effects of seed-source on plant-pollinator interactions (pages 49-50).
- GBNPP website updates and science delivery (page 51).
- Synthesis of knowledge on Great Basin Forbs (page 52).
- Effects of precipitation on native plant establishment (page 53).
- Data management support for the Seeds of Success program (page 54).
- Evolutionary implications of seed production (pages 54-58).
- Implications of functional trait differentiation in mixed source populations (pages 59-61).
- Seed increase and cultivation practice of Great Basin forbs (pages 62-65).

These research projects are adding to the knowledge on the development and use of native plants in Great Basin restoration and continuing the cooperative tradition of the GBNPP into the future.

Project Title: Studying patterns of local adaptation in Great Basin Forbs with Common Garden Experiment
Reporting Period: 10/1/2020-9/30/2021
Cooperators: Tessa Bartz, Graduate Student, University of Nevada, Reno; Elizabeth Leger Professor, University of Nevada, Reno

Data collection and maintenance of common gardens in the Reno and Plymouth study sites
First-year data have been collected and reported for the Reno and Plymouth common gardens for *Erigeron pumilus*. Second-year data for *Machaeranthera canescens* have also been reported for the Reno common garden, but are limited due to low re-emergence. *Chaenactis douglasii* did not emerge at all during its second year. Additionally, a set of four temperature loggers were installed at the Reno common garden according to the PI's specifications to record soil and air temperatures within and outside of the common garden. These data have been reported through September and data collection is ongoing.

Minor maintenance has been performed at the Reno common garden due to shrinking fabric and wind blowing up small to medium-sized sections of landscape fabric. These sections did not include any study plants, so damage was minimal. However, some sections of the landscape fabric have shrunk enough that there are small strips of exposed ground between fabric sheets. This will require additional weeding, but the total area is small.

Additional experiments using the same seed sources as the common gardens.
We supervised a germination experiment led by an undergraduate student as part of an honors thesis, which examined how seed weight affected germination of *Chaenactis douglasii* between two generations. The preliminary results for this experiment were presented as a talk at a virtual conference for the Great Basin chapter of Society for Ecological Restoration in May 2021 and at a session for UNR undergraduate research projects in May 2021. These results included that most populations decreased their seed weight after being grown out in the Reno common garden and that seed weight affected whether or not *Chaenactis douglasii* germinated, but did not affect germination speed. Plans are in place to continue this experiment for a second year using the ungerminated seeds from the previous year.
Analysis of a direct-seeded experiment examining a subset of *Chaenactis douglasii* and *Machaeranthera canescens* populations from the common garden is in progress. This experiment looked at how climate-of-origin and water availability affected seedling emergence and survival. Preliminary results were presented as a poster at the virtual ESA conference in August 2021. We found that populations originating from locations with lower annual precipitation had higher emergence and greater early survival relative to populations originating from sites with higher annual precipitation. Furthermore, populations from dry environments responded more readily to increased water treatments, emerging more often than those from wetter environments as water availability increased.

A greenhouse competition study with cheatgrass looking at the same subset of populations from the previous experiment has also been completed. Data from this experiment includes frequency of plants identified by functional group grown from the soil seed bank collected at the population's origin, seedling emergence and survival with and without cheatgrass, and above- and below-ground biomass for target plants and cheatgrass. Analysis of this experiment is in early stages and ongoing.

Project Title: Seed transfer development for important restoration forb species
Reporting Period: 1/1/2021 – 10/05/2021
Cooperators: Owen Baughman, The Nature Conservancy

For the 2021 season, which is now wrapping up, all was on track and no major issues arose. TNC hired one seasonal technician to lead the project. Data were collected regularly, the new fence installed in 2020 resulted in no observed large-mammal grazing of study plants in 2021, and communication with project PI's was made regularly. In addition to being responsible for regularly collecting field data and maintaining the site infrastructure, TNC's lead technician on this project has also been able to assist with lab work associated with this project, helping in Boise at the main RMRS lab to process samples from all of the forb gardens.

The current agreement was funded for three seasons, and we have over half of the funds left after two seasons. This puts us on track to be fully covered for the third season by existing funds.

Below, after each expected outcome (in italics), more details are provided.

1. TNC is to be the primary party responsible for data collection on the Glass Butte study site
    a. TNC was wholly responsible for site data collection and collected this data at appropriate intervals and on time.
    b. To follow data collection and data entry protocols and training provided by Great Basin Native Plant Project staff
    c. Data collection and entry protocols were followed closely, and questions were quickly addressed via direct communication with USDA FS staff. Visits to the site were made weekly or biweekly as appropriate and needed from late May through October.

2. To do basic site maintenance if/when needed on the Glass Butte site
    a. Just as in 2020, there were high winds in spring 2021 that dislodged some of the weed fabric and scrambled the tags for many of the CHDO plants. TNC staff resecured the fabric temporarily, and RMRS staff made a visit to reinforce and fully secure the fabric.
    b. Aside from above, no major incidents occurred that required site maintenance.
    c. Each week, the herbivore fence was inspected for breaks or weakness. None were found.
    d. The main wire gate to the enclosure fell into disrepair and a new post was installed to prevent entry from large mammals.

3. To provide collected data to Great Basin Native Plant Project staff in a timely manner agreed upon in advance
    a. Collected data were prepared and emailed to USDA FS within a week of collection, and physical plant samples were mailed at least every two weeks.

4. To potentially engage in other agreed-on restoration science activities between USFS and TNC, including the opportunity to utilize one of more of the Forb Garden sites for TNC's seed technology restoration science, so long as space is available, and the proposed activities do not hinder other current or planned activities
    a. There were no TNC seed technology science needs for this period in relation to use of one or more USDA FS forb common garden sites.

Project Title: Identifying Mechanisms of Native Plant Success to Improve Restoration Efforts
Reporting Period: 10/1/2020 to 9/30/2021
Cooperators: Andrew Kulmatiski Associate Professor, Utah State University

After vigorous growth of most plants in the 2020 season, most plants in the common garden died between the 2020 and 2021 season. There was very little growth to monitor this season. We tried to get new accessions to plant this season either in the summer or fall (2021), but there were no seeds or seedlings available for us to plant. We hope new seeds or seedlings will become available this fall (2021) for spring 2022 planting.

We collected and established our field experiment Fall 2020. This involved selecting accessions to work with, collecting their seed, collecting and sterilizing soils from three sites and establishing the potted plants in the field. Unfortunately, we had very little germination of the planted seeds. To try to get some growth, we moved the experiment to a greenhouse but still had insufficient germination for replication across treatments. We are now collecting new soil and seeds to restart the experiment in the greenhouse this winter. For this iteration, we will plant germinated seeds into pots. We expect to have initial results from this (now greenhouse) experiment in March 2022.

Project Title: Climate-Smart Restoration Tool
Reporting Period: 10/1/2020-9/30/2021
Cooperators: Brad St. Clair, Research Geneticist, US Forest Service Pacific Northwest Research Station; Nikolas Stevenson-Molnar, Software Engineer, Conservation Biology Institute; Brendan C. Ward, Chief Software Engineer, Conservation Biology Institute; Francis Kilkenny, Research Biologist, US Forest Service Rocky Mountain Research Station; Bryce Richardson, Research Geneticist, US Forest Service Rocky Mountain Research Station

The Climate-Smart Restoration Tool (CSRT) is an interactive web-based application that allows users to match current seed sources with future climate conditions. Natural resource managers must match the climatic adaptability of their seed sources to the climatic conditions of their restoration sites in order to better ensure successful long-term restoration outcomes. There is an urgent need to adopt climate-smart approaches to ecosystem management, but progress has been slow because landowners and natural resource managers lack readily available, site-specific information on which to act. Existing scientific information has been unavailable at the scale needed by natural resource managers and restoration practitioners. The CSRT is designed to (1) easily incorporate new scientific information (e.g., climate projections, physiological thresholds) and (2) allow users to select parameters of interest (e.g., climate change scenarios, and time periods). Climates associated with existing ecoregions and seed zones are displayed so that natural resource managers can choose the appropriate seed source for their restoration site, or decide where seed from a particular source can be planted in the future. Managers ware also able to view spatial maps of current and future climates, seed zones, ecoregions, and other contextual map layers. The CSRT provides the ability to download outputs of the tool to PowerPoint slides, PDF documents, and GeoTIFF files in order to share those results with others and perform additional analysis within desktop data processing environments.

The CSRT delivers information on current and future climate and seed zones. Information on species distributions (i.e., potential future habitats) can be incorporated in this tool in the future. Climate associated with existing ecoregions and seed zones are displayed so that natural resource managers can choose the appropriate seed source for their restoration site, or decide where seed from a particular source can be planted in the future. Because of the uncertainty in climate change projections, the tool is primarily intended as a planning and educational tool. It can be used to explore alternative future conditions, assess risk, and plan potential responses. The tool allows the user to control many input parameters so the results are appropriate for the management practices, climate change assumptions, and risk tolerance of the user.

The CSRT is being implemented following the same method used to develop and publish the Seedlot Selection Tool (SST; https://seedlotselectiontool.org/sst/; Figure 1). A fully functional version of the CSRT is available using the latest open-source software and incorporating mechanisms for its long-term maintenance. We will continue to work collaboratively with key stakeholders to ensure that the application is effective in meeting their needs, using a variety of mechanisms including webinars and targeted outreach.

Bluebunch wheatgrass and mountain big sagebrush genecological functions and species distribution models were added to the CSRT (Figure 1). Progress was also made on the Seedlot Selection Tool including the ability to compare multiple seedlots. This has not yet been implemented into the CSRT.

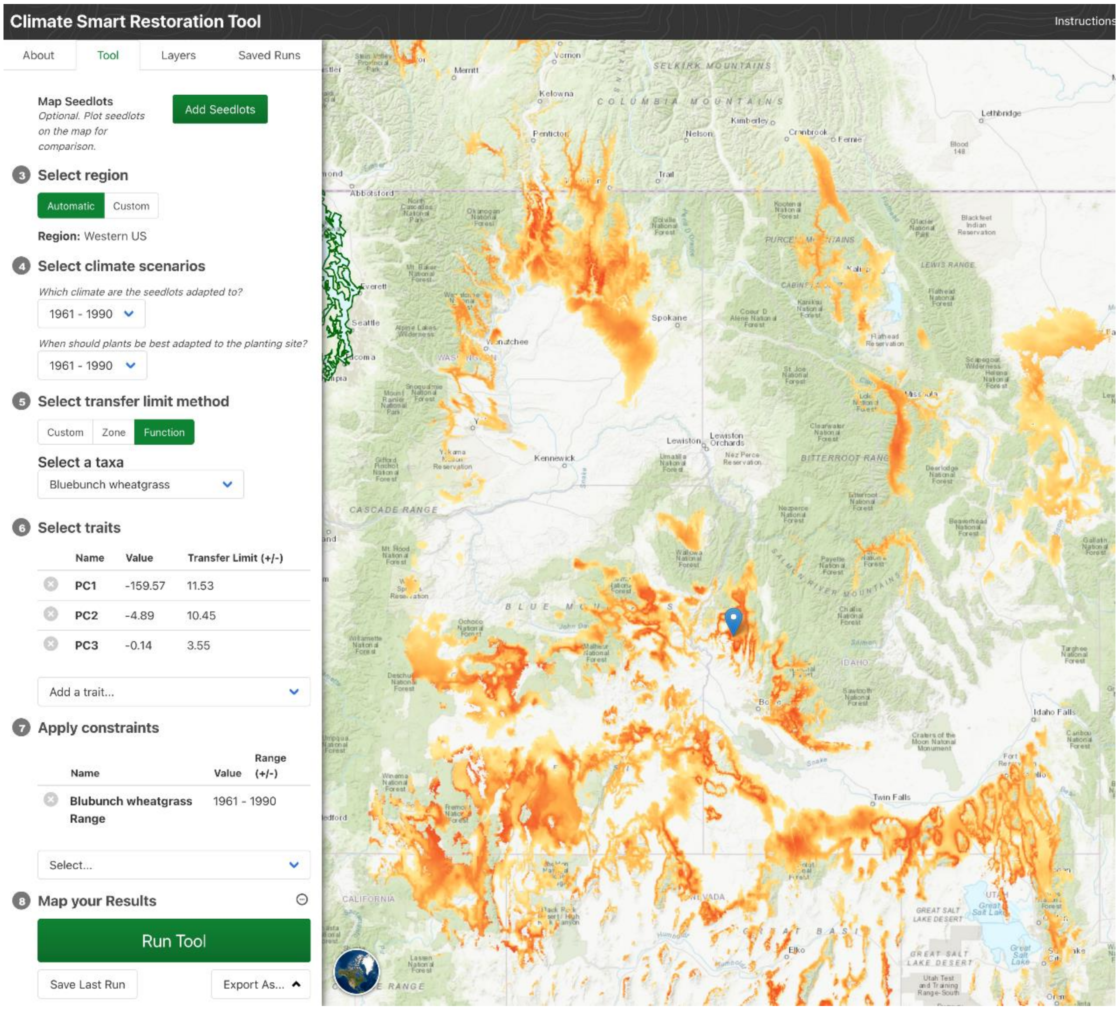


Figure. 1. An example of the seed transfer for bluebunch wheatgrass under contemporary climates. Yellow to red polygons indicate were seed could be acquired for the selected restoration site (blue pin).

CSRT: https://climaterestorationtool.org/csrt/

Publication:
St Clair, John; Richardson, Bryce; Stevenson-Molnar, Nikolas; Howe, Glenn; Bower, Andrew; Erickson, Vicky; Ward, Brendan; Bachelet, Dominique; Kilkenny, Francis; Wang, Tongli. Seedlot Selection Tool and Climate-Smart Restoration Tool: web-based tools for sourcing seed adapted to future climates. In review for Ecosphere

Project Title: Effects of plant species diversity and soil biochar amendments on reestablishment and performance of native sage steppe species

Reporting Period: 8/2020 to 9/2021

Cooperators: Marie-Anne de Graaff, Professor, Boise State University; Luci Wilson, Graduate Student, Boise State University; Francis Kilkenny, USFS Rocky Mountain Research Station

Statement of Progress: A narrative summary of project progress, a comparison of actual accomplishments with proposed goals for the period, any current problems, or unusual developments or delays.

We have made significant progress on this project in 2019, 2020 and 2021. First, we located an experimental field site to conduct our study. On August 6th of 2019, a fire (the "Pothole" fire) burned approximately 70.000 acres 14 miles south of Hammett ID. This fire provided an excellent opportunity to initiate our experiment, which focuses on evaluating how native biodiversity and biochar treatments affect soil properties and feedbacks to native plant communities following a fire.

Experimental design: During the months of August and September of 2019 we located an appropriate site within the burned area to set up our experiment (3 acres in size). The main treatment in the experimental design is 'plant diversity', and subplot treatments consist of 'biochar addition' (3tons/ acre). There are three plant diversity treatments composed of two, four or eight species. Each treatment is composed of 50% forb and 50% grass. Diversity treatments are replicated by 'diversity treatment' where species are randomly selected from the following native grass and forb species:

Grasses	**Forbs**
POSE = *Poa secunda*	PEHU = *Penstamon humilus*
ELEL = *Elymus elymoides*	ERLA = *Eriophylum lanatum*
STCO = *Stipa columbiana*	ERPU = *Erigeron pumilus*
KOMA = *Koelaria macrantha*	ACMI = *Achillea millefolium*
PSSP = *Pseudoreognaria spicata*	CHDO = *Chaenactis douglassii*
SPCR = *Sporobolus cryptandrus*	SPGR = *Sphaeralcea grossularifolia*

Individual plots are 9m*11m, and diversity treatments are replicated 12 times. Alleys between all plots are 3m on all sides, and 10m among blocks. Four replicate field plots for each treatment are situated randomly across three fixed blocks. We also established control plots (no seeding), and *Agropyron cristatum* (crested wheatgrass) plots. These plots were replicated six times (two replicate plots in each block). Subplots (2m*2m) were established across all high diversity (i.e. 8 species) plots and in control and crested wheatgrass plots. Seeding occurred with a reduced-till seeder on October 22nd 2019, but failed due to a particular seed (CHDO) clogging the seeder. To improve on our seeding protocol we cleaned all seeds, and reseeded on November 15, 2019.

Sampling and sample analysis: We collected soils from all experimental field plots (48 plots * 4 cores [2.5cm diameter; depth 0-10cm]) on October 22nd 2019, and in June of 2020. We returned to the site during the first week of July 2021 for vegetation monitoring and collection of soil. We established four permanent vegetation monitoring sites (1mX1m) within each plot to assess plant survival and growth annually. Two of these frames were located at random, whereas the other two frames were strategically placed to capture the diversity treatment. For instance, in a two-species treatment, we determined the location of the permanent monitoring site by ascertaining that the site was comprised of a grass and a forb, thus accurately representing our diversity treatment. Finally, the plots treated with biochar had an additional permanent monitoring frame installed in the top (South- East) corner of each plot. Data collected for vegetation monitoring included species identity, richness, density and percent cover using the Daubenmire cover class method and the class midpoint in data calculation. These data allowed us to determine Shannon-Weaver Diversity index (H'), and using this approach in both 2020 and 2021, we confirmed that our diversity treatments (i.e. 2, 4 and 8 species plots) were successfully established. Species richness and diversity vary significantly among these plots, thus providing us with the foundation from which we can evaluate how species diversity of native plants impacts soil properties and the success of restoration projects; the primary goal of this project.

To evaluate the impact of our treatments on soil properties, soils were collected (2.5cm diameter cores; depth 0-10cm) from each one of the experimental field plots, and stored at -20^{o}C until further processing. The soils collected in 2019 and 2020 have been analyzed for nitrogen mineralization, and those collected in 2021 are being processed for similar analyses. Data indicate that there are no significant differences in total mineralizable nitrogen among seeded diversity treatments, indicating that increasing diversity does not affect soil N availability to plants. However, soil N availability was significantly higher in plots that were not planted than in those that were planted, rendering them more susceptible to invasion by non-native grasses. Additionally, plots planted with crested wheatgrass had higher mineral N concentrations than native species plots. This suggests that crested wheatgrass impacts soil processes differently than native grasses. This difference is likely to lead to vastly different successional trajectories owing to higher plant available N concentrations in crested wheatgrass soils, which typically inhibits the ability of native grasses to reestablish. Finally, we have extracted DNA from our soil samples for microbial community analyses, and test samples have been sent to a sequencing facility to complete some test runs that will provide us with information required for further method development.

While we have made considerable progress with this project over the past year, we have also faced significant slowdowns. COVID-19 has forced us to shut-down our laboratory for months over the summer in 2020, and given that this is the time that graduate students typically use to get ahead on their work in the lab, our analyses have proceeded at a significantly slower pace than expected. In addition, while the laboratory re-opened in the Fall of 2020, we were required to operate in a limited capacity, because only three personnel were allowed in the laboratory at a time. This further limited progress. Our laboratory is currently operating at full capacity, which helps increase the speed with which we can analyze our samples, however, we are still faced with frequent personnel loss due to sickness and required quarantine. In summary, we are accomplishing the goals which we set out to accomplish for this project, but at a slower pace than originally anticipated.

Project Title: Plant-Pollinator Interaction Provenance Trials
Reporting Period: 10/1/2020-9/30/2021
Cooperators: Kevin Grady Associate Research Professor, Northern Arizona University

Narrative: We developed two common gardens for evaluating genetic variation in adaptation to climatic and biotic components. Both gardens are located near Carson City, NV at the USFS Plymouth Work Station. Gardens were planted in October, 2019 by project PI and USFS contacts Tim Kellison, Dirk Netz, and fire crew and Carson City USFS staff volunteers. An irrigation system was installed prior to planting including a 4,000 gallon water pillow and a pressure regulator, water pump powered by portable generator. Irrigation delivered to site through a system of 6 hose bibs positioned throughout the gardens. After planting, the area was also fenced with wire-mesh fencing installed to 12" below soil surface and extending about 4' above the soil.

A total of 6,000 plants were planted. 3,000 in an area dominated by cheatgrass where sagebrush had been removed. 3,000 in an area with intact sagebrush. In this manner, it is possible to evaluate restoration effectiveness in multiple ecosystems in a hot environment. The rationale for not planting in the originally proposed high elevation site was that the maintenance requirements and fencing requirements proved to be untenable logistically. A 6' fenced area would have been needed to block deer. Also, watering the area would have required double the capacity as for one site – another water pillow, another generator, another system of hoses – we calculated that the effort was too much and site too remote to create a well-maintained site. We subsequently decided on the dual garden site at Plymouth with easier access to water and maintenance and much easier for technicians to access.

During the course of the past year, we have located an additional site that does have adequate management to allow for development of a common garden. This site is near Beatty, Nevada on the 7J ranch maintained by the Nature Conservancy. Our group is already working there on riparian restoration. This site is ideal for investigating local adaptation of the western edge of Nevada plant populations. We suggest developing a site at this location in the future – can evaluate Great Basin and Mojave Desert populations – maybe some of the most drought tolerant genotypes in the world.

With left over plugs, we also develop a series of school gardens in the Flagstaff area at three schools. This community project – EcoKids -has great potential to get local people to participate in the development of plant materials for restoration. The idea is that seeds developed from school common gardens will be used to develop common gardens in student's back yards. At the end of the year, seeds will be banked and we can use for regional restoration work. We have developed a series of online activities for COVID inspired home schooling – that links kids to these new planting efforts. We would love to expand this concept to other communities in the southwest.

Accomplishments: Development of common gardens and planting of 5,000 plants that were propagated at NAU greenhouse that included 10 species and 6 populations of each species. We would like to expand this effort to include one more common garden at Beatty NV and expand seed collection efforts to include western NV populations. There is a historic irrigation set up fed by a natural spring that could also be used to support a seed production field. We would like to test the promise for such a system by getting the irrigation online and planting 2-acres of cultivar seeds.

We monitored phenology, growth rate, and survivorship on all planted seedlings throughout 2020 and in summer 2021. Results of these measurements are currently being analyzed and will be included in a publication in 2022.

Problems: in 2019, we experienced problems related to COVID-19. We filed a COVID plan with USFS on April 1, 2019. We were forced to change plans in terms of supporting a MS student. We had a MS student on board but they dropped out of school in February 2020 due to emotional issues. We hired a professional field technician – Scott Massed – to conduct measurements in 2020 and in summer 2021, PI Grady conducted survival and growth measurements.

Unusual Developments: Same as above.

Future Directions: With an additional $70k, we could collect seeds from Mojave Desert to Carson City Great Basin, grow at NAU, and plant at both Carson City and Beatty, NV. We could also plant a 2-acre field to troubleshoot the development of a seed production field (we have similar tests planned near Moab and Farmington).

Project Title: Science Delivery – Great Basin Native Plant Project
Reporting Period: 3/2021 to 9/30/2021
Cooperators: Corey Gucker Coordinator, Writer

Most of the FY2021 project focus has been on updating the GBNPP logo and building a new GBNPP website. The new website has been developed using the Word Press platform. It includes a redesign of the main menu options as well as a search feature for GBNPP documents and webinars. Although all content from the old website has been migrated to the new site, the content must be updated manually to utilize the new search feature. To date, nearly all GBNPP annual reports and journal articles published 2012-2019 have been updated on the new website. We are in the final stages of the GBNPP logo redesign.

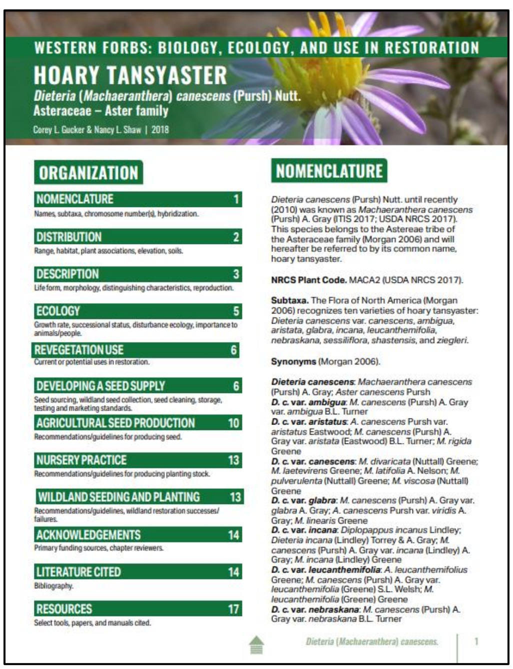
Project Title: Forb Seed Manual
Reporting Period: 10/1/2020 to 9/30/2021
Cooperators: Corey Gucker Communications Specialist, Great Basin Fire Science Exchange; Nancy Shaw, Research Botanist Emeritus, USFS Rocky Mountain Research Station

Finalized or completed draft reviews for seven species: orange agoseris (*Agoseris aurantiaca*), annual agoseris (*A. heterophylla*), shaggy fleabane (*Erigeron pumilus*), common woolly sunflower (*Eriophyllum lanatum*), barestem biscuitroot (*L. nudicaule*), nineleaf biscuitroot (*L. triternatum*), and silverleaf phacelia (*Phacelia hastata*).

This process includes handling all phases of researching, writing, soliciting outside reviews, and developing final layout pdf documents that are ADA (American's with Disabilities Act) compliant.

Completed chapters can be viewed at https://greatbasinfirescience.org/western-forbs-restoration/

Sample pages:



WESTERN FORBS: BIOLOGY, ECOLOGY, AND USE IN RESTORATION

HOARY TANSYASTER

Dieteria (*Machaeranthera*) *canescens* (Pursh) Nutt.
Asteraceae – Aster family

Corey L. Gucker & Nancy L. Shaw | 2018

ORGANIZATION

NOMENCLATURE 1
Names, subtaxa, chromosome number(s), hybridization.

DISTRIBUTION 2
Range, habitat, plant associations, elevation, soils.

DESCRIPTION 3
Life form, morphology, distinguishing characteristics, reproduction.

ECOLOGY 5
Growth rate, successional status, disturbance ecology, importance to animals/people.

REVEGETATION USE 6
Current or potential uses in restoration.

DEVELOPING A SEED SUPPLY 6
Seed sourcing, wildland seed collection, seed cleaning, storage, testing and marketing standards.

AGRICULTURAL SEED PRODUCTION 10
Recommendations/guidelines for producing seed.

NURSERY PRACTICE 13
Recommendations/guidelines for producing planting stock.

WILDLAND SEEDING AND PLANTING 13
Recommendations/guidelines, wildland restoration successes/failures.

ACKNOWLEDGEMENTS 14
Primary funding sources, chapter reviewers.

LITERATURE CITED 14
Bibliography.

RESOURCES 17
Select tools, papers, and manuals cited.

NOMENCLATURE

Dieteria canescens (Pursh) Nutt. until recently (2010) was known as *Machaeranthera canescens* (Pursh) A. Gray (ITIS 2017; USDA NRCS 2017). This species belongs to the Astereae tribe of the Asteraceae family (Morgan 2006) and will hereafter be referred to by its common name, hoary tansyaster.

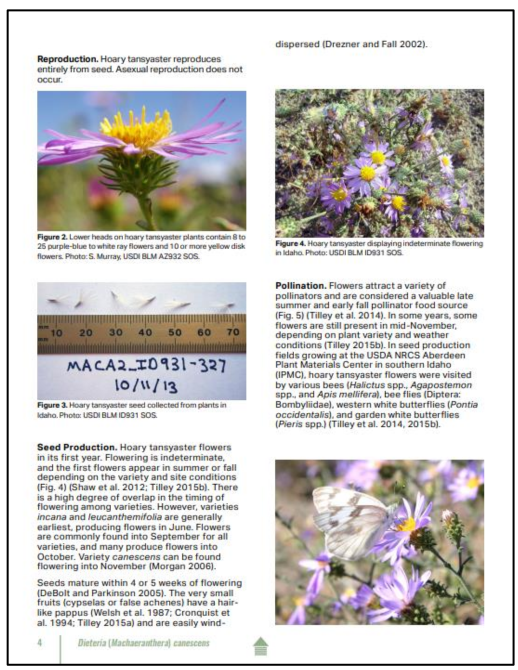
NRCS Plant Code. MACA2 (USDA NRCS 2017).

Subtaxa. The Flora of North America (Morgan 2006) recognizes ten varieties of hoary tansyaster: *Dieteria canescens* var. *canescens*, *ambigua*, *aristata*, *glabra*, *incana*, *leucanthemifolia*, *nebraskana*, *sessiliflora*, *shastensis*, and *ziegleri*.

Synonyms (Morgan 2006).

Dieteria canescens: *Machaeranthera canescens* (Pursh) A. Gray; *Aster canescens* Pursh
D. c.* var. *ambigua: *M. canescens* (Pursh) A. Gray var. *ambigua* B.L. Turner
D. c.* var. *aristatus: *A. canescens* Pursh var. *aristatus* Eastwood; *M. canescens* (Pursh) A. Gray var. *aristata* (Eastwood) B.L. Turner; *M. rigida* Greene
D. c.* var. *canescens: *M. divaricata* (Nuttall) Greene; *M. laetevirens* Greene; *M. latifolia* A. Nelson; *M. pulverulenta* (Nuttall) Greene; *M. viscosa* (Nuttall) Greene
D. c.* var. *glabra: *M. canescens* (Pursh) A. Gray var. *glabra* A. Gray; *A. canescens* Pursh var. *viridis* A. Gray; *M. linearis* Greene
D. c.* var. *incana: *Diplopappus incanus* Lindley; *Dieteria incana* (Lindley) Torrey & A. Gray; *M. canescens* (Pursh) A. Gray var. *incana* (Lindley) A. Gray; *M. incana* (Lindley) Greene
D. c.* var. *leucanthemifolia: *A. leucanthemifolius* Greene; *M. canescens* (Pursh) A. Gray var. *leucanthemifolia* (Greene) S.L. Welsh; *M. leucanthemifolia* (Greene) Greene
D. c.* var. *nebraskana: *M. canescens* (Pursh) A. Gray var. *nebraskana* B.L. Turner

Dieteria (*Machaeranthera*) *canescens*. 1



Reproduction. Hoary tansyaster reproduces entirely from seed. Asexual reproduction does not occur.

Figure 2. Lower heads on hoary tansyaster plants contain 8 to 25 purple-blue to white ray flowers and 10 or more yellow disk flowers. Photo: S. Murray, USDI BLM AZ932 SOS.

MACA2_ID931-327
10/11/13

Figure 3. Hoary tansyaster seed collected from plants in Idaho. Photo: USDI BLM ID931 SOS.

Seed Production. Hoary tansyaster flowers in its first year. Flowering is indeterminate, and the first flowers appear in summer or fall depending on the variety and site conditions (Fig. 4) (Shaw et al. 2012; Tilley 2015b). There is a high degree of overlap in the timing of flowering among varieties. However, varieties *incana* and *leucanthemifolia* are generally earliest, producing flowers in June. Flowers are commonly found into September for all varieties, and many produce flowers into October. Variety *canescens* can be found flowering into November (Morgan 2006).

Seeds mature within 4 or 5 weeks of flowering (DeBolt and Parkinson 2005). The very small fruits (cypselas or false achenes) have a hair-like pappus (Welsh et al. 1987; Cronquist et al. 1994; Tilley 2015a) and are easily wind-dispersed (Drezner and Fall 2002).

Figure 4. Hoary tansyaster displaying indeterminate flowering in Idaho. Photo: USDI BLM ID931 SOS.

Pollination. Flowers attract a variety of pollinators and are considered a valuable late summer and early fall pollinator food source (Fig. 5) (Tilley et al. 2014). In some years, some flowers are still present in mid-November, depending on plant variety and weather conditions (Tilley 2015b). In seed production fields growing at the USDA NRCS Aberdeen Plant Materials Center in southern Idaho (IPMC), hoary tansyaster flowers were visited by various bees (*Halictus* spp., *Agapostemon* spp., and *Apis mellifera*), bee flies (Diptera: Bombyliidae), western white butterflies (*Pontia occidentalis*), and garden white butterflies (*Pieris* spp.) (Tilley et al. 2014, 2015b).

4 *Dieteria* (*Machaeranthera*) *canescens*

Project Title: Precipitation effects on native species establishment in post-fire restoration
Reporting Period: 6-15-20 to 10-29-21
Cooperators: Beth Newingham Research Ecologist, Agricultural Research Service

We have completed analyses and drafted a manuscript for the previous agreement related to this agreement, which is planned to be submitted within six months. In addition, we have modified our Automated Rainfall Manipulation System (ARMS) to improve both rainfall reduction and addition. To test the efficiency of the redesigned ARMS, we used a rainfall simulator to deliver rainfall events varying from 0.5-4.0 inches per hour. We are currently analyzing the rainfall simulator data and will prepare a methods manuscript. We built 12 new ARMS and are currently revisiting the experimental design and determining possible field sites for deployment.

Project Title: Research related to the management and use of native plant materials for restoration
Reporting Period: Oct. 1, 2020 to Sept. 30, 2021
Cooperators: Kayri Havens, Sr. Director, Ecology and Conservation, Chicago Botanic Garden; Andrea Kramer, Director of Restoration Ecology, Chicago Botanic Garden; Jacob Zeldin, Research Ecologist, Chicago Botanic Garden

Below we report on 2 projects: "Seeds of Success data management" and "Production of mixed source seed lots for *Penstemon pachyphyllus*." We also provide information on a related project, "Understanding functional trait variation within and between populations of three restoration-priority forb species (*Machaeranthera canescens*, *Machaeranthera tanacetifolia*, *Heterotheca villosa*), and implications for mixing populations in a restoration setting," not funded by this agreement but related to Great Basin restoration which may be of interest to you.

Project 1: Seeds of Success Data Management
Kayri Havens Sr. Director, Ecology and Conservation, Chicago Botanic Garden

Project Accomplishments:
Seeds of Success (SOS) has had a productive collecting season in 2021, despite COVID-19 and severe drought across much of the western US. Year to date, 32 teams have entered 212 accessions into BG-BASE for the 2021 collecting season. These collections represent 135 unique taxa from 38 plant families across 15 ecoregions and 9 states. More collections are expected to be entered throughout the fall as late-season species ripen and fall precipitation makes its way across the southwest.

Many teams focused on collecting species listed on the Seed Increase contracts. SOS teams have made 67 collections of 27 different IDIQ forb and grass species in 2021. As with past years, most collections (around 63 percent) were made on BLM land in 2021, with a small handful of collections also made on U.S. Forest Service and state lands, and an even smaller percentage originating on private land.

Work has been completed to facilitate the upload of data into BG-BASE from Seeds of Success sites who are using Survey 123, a data collection application from ArcGIS that is used among some of the collection teams. This will eliminate the current duplicity of teams logging data in the field into Survey 123 and then later re-inputting the same data into the SOS data portal. Automated daily, weekly, and monthly backups continue to be archived. Quarterly database maintenance tasks (re-indexing and data verification) were completed. The Seeds of Success Data Portal continues to be hosted and maintained by BG-BASE, Inc.

Project 2: Production of mixed-source seed lots of *Penstemon pachyphyllus* for restoration use in the Great Basin as a model for understanding how neutral and potentially adaptive genetic diversity changes during the production process:

Andrea Kramer, Director of Restoration Ecology, Chicago Botanic Garden; Jacob Zeldin, Research Ecologist, Chicago Botanic Garden

Project progress - BACKGROUND
Research has shown that the production process can alter the genetic diversity of produced seed in unintended ways (Basey et al. 2015; Espeland et al. 2017), and guidance has been developed to minimize the loss or change of genetic diversity throughout this process. However, very little research is

available to guide specific practices, particularly when producing pooled-source seed on a large scale for widespread use.

A mixed-source seed lot of *Penstemon pachyphyllus* is being produced by the USFS for restoration use in Provisional Seed Zone (PSZs) 15-20/3-6 in the Central Basin and Range ecoregion, where the species has the largest number of records in the USGS's BISON database. To produce these pooled sources, Scott Jensen (USFS, Provo) and Kevin Gunnell (UDWR, Ephraim) collected seeds from 18 natural populations, produced seeds in production beds at Fountain Green (Ephraim), maintaining each population in separate rows, and then bulked seed and distributed it to Quarter Circle J Seeds in Vale, OR, where it was sown in Fall 2016. Seeds were also sown into an experimental restoration site at Hamblin Valley, presenting an excellent opportunity to follow genetic diversity through the production and use process.

Additionally, previous research (Kramer 2009) identified outbreeding depression in crosses made between populations in the Great Basin and Colorado Plateau. Research by Broderick et al. (2011) showed that there may be variation in chromosome size within this species, which may explain the outbreeding depression Kramer detected. Because it is possible that seeds produced for the Great Basin mixed-source seed lots may be used for restoration efforts in the Colorado Plateau, it is important to understand the extent to which outbreeding depression may result from crosses between a range of different populations across these regions.

Project Goals

Our objectives were to assess how neutral and potentially adaptive genetic variation may be impacted by this production process and restoration use, and to pinpoint any potential bottlenecks so future work can mitigate steps in the production process that reduce desired genetic diversity. We also worked to understand how chromosome size varies among populations of *P. pachyphyllus* across the Great Basin used in the mixed-source seed lot relative to populations from the Colorado Plateau, and to assess whether measured differences in chromosome size contribute to outbreeding depression when crosses are made between populations.

Project Methods

Two graduate students (Katie Kucera and Rafael Urbina-Casanova) in the Plant Biology and Conservation graduate program, offered jointly by Northwestern University and Chicago Botanic Garden, were engaged in collecting and synthesizing data for this research. Both were advised by Dr. Andrea Kramer.

Neutral genetic markers (Kramer et al. 2011) and assays of potentially adaptive variation in seed germination requirements were used to investigate how genetic diversity and source population representation shift following use of a mixed-source seed lot to establish a seed production field and three restoration sites. Our mixed-source seed lot contained nineteen source populations of the perennial forb, *Penstemon pachyphyllus*, from six mountain ranges in the Great Basin, USA.

Plants sourced from Great Basin populations used in the mixed-source seed lot, as well as from the Colorado Plateau, were grown by Scott Jensen and delivered to Chicago Botanic Garden in fall 2020. In May 2021, when plants were flowering, crosses were performed within and among populations of for all population pairs, with both parents tracked for each cross. Seed production was recorded and all fruit produced were harvested in late summer 2021. We are now in the process of cleaning and counting seeds, and will be x-raying each seed once our new x-ray machine arrives in January 2022.

Additionally, fresh leaf material for each individual included in our crosses (n = 110, representing 20 populations total) was shipped to Bryce Richardson in Boise, ID for flow cytometry to determine chromosome size.

Project Accomplishments

In this reporting period, we achieved our goal of publishing the results of our genetics research on the mixed-source seed lot. In this manuscript, we report results of our work illustrating that, while populations from all six mountain ranges used in the mixed-source seed lot were present in production and restoration sites, representation of each source mountain range shifted unpredictably. Populations from one mountain range were particularly overrepresented at production and restoration sites relative to their composition in the original seed mix. We also found that, despite using the same multi-source seed lot for production and restoration sites, resulting source population composition varied, suggesting that local conditions favored some populations over others. Significant among-population variation in seed germination requirements may, in part, explain shifts in source population representation in the production and restoration sites.

We also achieved our goal of conducting crosses within and between populations of all populations provided by Scott Jensen from the Great Basin and Colorado Plateau, and understanding differences in chromosome sizes among these populations, as flow cytometry results confirmed a clear difference in chromosome size between populations in the Colorado Plateau and Great Basin. We are still in the process of quantifying the results of the crosses within and between populations to determine whether crosses between the Great Basin and Colorado Plateau led to outbreeding depression.

Project Applications for Management

• When increasing seeds from a multi-source seed lot in an irrigated production field, unexpected and unpredictable shifts in the representation of source populations may occur that may change the representation of source populations in seeds produced for restoration efforts.
• When a multi-source seed lot is used at different restoration sites, the representation of source populations may shift even in the first generation, potentially in response to selection for the best-adapted genotypes for the site.
• If source populations have different seed germination requirements, these differences may influence which populations are able to establish at sites with varying climatic conditions.
• Variation in chromosome size between populations in the Colorado Plateau and Great Basin may lead to outbreeding depression, so until further research is conducted, seeds sourced from populations in one region should not be used for restoration in the other.

Project Publications

Kucera KF, Fant JB, Jensen S, Landeen M, Orr E, Kramer AT. (2021). "Genetic variation and structure change when producing and using mixed-source seed lots for restoration." Restoration Ecology. Online early: e13521

References

Basey, A. C.; Fant, J. B.; Kramer, A. T. 2015. Producing native plant materials for restoration: ten rules to collect and maintain genetic diversity. Native Plants Journal, 16:37-53.

Broderick, S., Stevens, M., Geary, B., Love, S., Jellen, E., Dockter, R. Daley, S., Lindgren, D. (2011). A survey of Penstemon's genome size. Genome 54, 160-173.

Espeland, E. K.; Emery, N. C.; Mercer, K. L.; Woolbright, S. A.; Kettenring, K. M.; Gepts, P.; Etterson, J. R. 2017. Evolution of plant materials for ecological restoration: insights from the applied and basic literature. Journal of Applied Ecology, 54:102-115.

Kramer, A.T. 2009. Inbreeding and Outbreeding depression in crosses spanning geographic and genetic distances In: in Ecological genetics of Penstemon in the Great Basin, U.S.A. PhD in Biological Sciences, Ecology and Evolution. University of Illinois at Chicago.

Kramer, A. T.; Fant, J. B.; Ashley, M. V. 2011. Influences of landscape and pollinators on population genetic structure: Examples from three Penstemon (Plantaginaceae) species in the Great Basin. American Journal of Botany, 98:109-121.

Project 3: Understanding functional trait variation within and between populations of three restoration-priority forb species (*Machaeranthera canescens*, *Machaeranthera tanacetifolia*, *Heterotheca villosa*), and implications for mixing populations in a restoration setting*

Andrea Kramer, Director of Restoration Ecology, Chicago Botanic Garden; Jacob Zeldin, Research Ecologist, Chicago Botanic Garden

*Though not supported by funds from this agreement, information about the following project may be relevant to US Forest Service interests and has therefore been included for your information.

Highlights

Four studies using at least one of the priority species each have been undertaken to 1) quantify the extent of functional trait variation within and among populations; 2) assess plasticity in functional traits in response to different stressors; 3) understand how genotypes within populations perform when grown with other individuals of the same genotype, with genotypes from the same population, or with genotypes from other populations; and 4) understand how populations perform when grown with individuals from the same population relative to other species.

Project Description

Introduction (largely from Zeldin et al. 2020, Journal of Applied Ecology)

Plant functional traits strongly influence plant community assembly, ecosystem processes, and invasion dynamics (Roscher et al., 2012). As such, plant functional trait research holds great promise to inform ecological restoration practices (Werden et al., 2018). However, a historical focus on among-species rather than within-species variation in functional traits means this promise has yet to be fully realized (but see Herrera, Medrano, & Bazaga, 2015). This gap makes identifying appropriate populations for mixing to produce germplasm with desired genetic diversity and trait representation for restorations challenging.

Filling this gap requires a more mechanistic understanding of the impact of functional trait variation on ecological dynamics, but insufficient data to understand fine-scale structure of functional trait variation within and among populations inhibit these efforts. Additionally, implicit in recommendations to mix populations for restoration is the assumption that heritable, adaptive trait variation is greater between populations than within. However, because functional trait research provides limited understanding of how variation is structured within and among populations (and even then, only for highly clonal species; Breza, Souza, Sanders, & Classen, 2012), this may not be the best or only approach. For example, in species with extensive trait variation within individual populations, mixing multiple populations may be less necessary to achieve high genetic or trait diversity, as long as the full extent of diversity in the population is captured in germplasm produced for restoration. As such, understanding intraspecific functional structure is necessary to effectively produce diverse plant material for restoration. It is also important to understand how plastic functional trait variation is, particularly in stressful environments, and what it means for how individual plants, populations, and species interact with one another to support diverse plant communities.

Objectives

The objective of this research is to assess how functional trait diversity is structured within and between populations of priority restoration species, to understand how plastic this variation may be under stressful conditions, and to investigate how this variation may influence the outcomes of competitive interactions when individuals from different populations and species are planted together.

Methods

Our first experiment used micropropagation techniques to generate clones of four populations collected as part of the Seeds of Success program for three restoration-relevant forb species (*Machaeranthera canescens*, *Machaeranthera tanacetifolia*, *Heterotheca villosa*) to determine the structure of intraspecific functional trait variation. We measured functional traits in a common environment, assessing 1) how functional trait variation is structured within and among populations while controlling for variation within genotypes, and 2) whether populations differ in individual and multivariate functional traits while accounting for trait variation within and among genotypes. This research was led by Jacob Zeldin and Andrea Kramer, with support from two other graduate students in our program (Alicia Foxx, PhD; Taran Lichtenberger, MS; both graduated in 2020).

Our second experiment again utilized micropropagation techniques to generate clones of two populations of *Machaeranthera canescens* (the same populations and clones as in experiment one). These clones were subjected to four different growing environments imposed in the nursery at Chicago Botanic Garden (drought stress, light stress, and drought x light stress, as well as a control) and plasticity in functional traits was measured. This work was led by Jacob Zeldin, Andrea Kramer, and Taran Lichtenberger, as well as an undergraduate student (Jennet Chang) participating in our summer Research Experiences for Undergraduates program.

Our third experiment again utilized micropropagation techniques to generate clones of two populations of *Machaeranthera canescens* (the same populations and clones as in experiment one and two) grown in a nursery at Chicago Botanic Garden to assess the impact of growing clones in monoculture, mixed with other clones from the same population, and mixed with clones from a different population. This experiment measured competitive outcomes and overall productivity above and below ground, and asked whether variation in productivity could be predicted by the identity of the clones in each planting. This work was led by Taran Lichtenberger, and supported by Andrea Kramer (her MS thesis advisor) and Jacob Zeldin (her MS committee member).

Our fourth experiment utilized seeds from three populations of each of two species (*Machaeranthera canescens* and *Heterotheca villosa*) also utilized in experiment one. Seeds were used to produce seedlings that were integrated into a competition trial in the Chicago Botanic Garden greenhouses that tested how each population performed when planted with other populations of the same species and other populations of a different species. Plants were harvested and above- and belowground trait and biomass measures were made on all plants. This allowed us to assess whether different combinations of plantings displayed competitive or facilitative interactions. This work was led by Alicia Foxx (PhD; defended in 2020), and supported by Andrea Kramer (her PhD advisor).

Expected Results and Discussion

In experiment one, we found substantial variation both within and among populations of our study species for all functional traits measured. In some cases, variation within populations was as great as that between populations. These results were recently published in Journal of Applied Ecology (Zeldin et al. 2020)

In experiment two, we found significant variation in plastic responses in some traits and populations in response to different stressors. These results are currently being written up for publication.

In experiment three, we found evidence that the genotypes that performed most poorly in monoculture performed better when planted in mixture with other genotypes from the same and different populations. These results are currently being written up for publication – this is the MS thesis research of Taran Lichtenberger, who successfully defended her research on 4/1/20. These results are currently being written up for publication.

In experiment four, we found that populations responded differently when planted singly versus in mixture with other populations from the same species as well as from different species. These results are currently being written up for publication – this is the PhD thesis research of Alicia Foxx, who successfully defended her dissertation on 1/28/20. These results are currently being written up for publication.

Management Applications and/or Seed Production Guidelines
In experiments one and two, we showed variation in important functional traits both within and among populations of each study species, which may influence seed sourcing decisions. In experiment three we showed that genotypes that may perform poorly when planted alone can do better when planted in a mixture, which may make it more likely than expected that more genotypes will be able to survive and reproduce when populations are mixed. However, experiment four also showed that not all populations perform similarly when planted together. This suggests that genetically diverse mixed-source seed lots produced for restoration may vary widely in their performance in the field, depending on which populations are included in the mix.

Presentations
None in 2020 (public dissertation and thesis defense presentations planned for March and April 2020 were canceled due to COVID).

Publications
Zeldin, J., T. Lichtenberger, A. Foxx, E. R. Williams, and A. T. Kramer. (2020). Intraspecific functional trait structure of restoration-relevant species: implications for restoration seed sourcing. Journal of Applied Ecology.

Three additional papers planned for submission to peer-reviewed journals in 2021 or 2022 (one per chapter).

Additional Products
We continue to work on manuscripts for projects 2, 3, and 4. At least one manuscript (for project 4) will be submitted by the end of 2021 (with authors A. Foxx, F. Fort, and A. Kramer).

References
Breza, L. C.; Souza, L.; Sanders, N. J.; & Classen, A. T. 2012. Within and between population variation in plant traits predicts ecosystem functions associated with a dominant plant species. Ecology and Evolution 2:1151–1161
Herrera, C. M.; Medrano, M.; Bazaga, P. 2015. Continuous within-plant variation as a source of intraspecific functional diversity: Patterns, magnitude, and genetic correlates of leaf variability in Helleborus foetidus (Ranunculaceae). American Journal of Botany 102:225-232.
Roscher, C.; Schumacher, J.; Gubsch, M.; Lipowsky, A.; Weigelt, A.; Buchmann, N.; Schmid, B.; Schulze, E.D. 2012. Using plant functional traits to explain diversity–productivity relationships. PLoS ONE 7: e36760.

Werden, L.K.; Alvarado, J.P.; Zarges, S.; Calderón, M.E.; Schilling, E.M.; Gutiérrez, L.M.;, Powers, J.S. 2018. Using soil amendments and plant functional traits to select native tropical dry forest species for the restoration of degraded Vertisols. Journal of Applied Ecology 55:1019-1028.

Project Title: Great Basin Research Center Seed Increase
Reporting Period: 10/01/2020 to 09/30/2021
Cooperators: Melissa Landeen GBRC Project Leader, Utah Division of Wildlife Resources; Kevin Gunnell, GBRC Coordinator, Utah Division of Wildlife Resources; Scott Jensen, Botanist, USFS Rocky Mountain Research Station

Highlights
Continued small-scale production grow-out for seed increase of 13 species of native Great Basin forbs.
Harvested seed from 11 native Great Basin forb species (Table 1).
Established 23 new seed-increase beds with perennial or biennial plants in spring 2021 at our Fountain Green Farm.

Project Description
The ongoing native seed increase project at the Utah Division of Wildlife Resources (UDWR) Great Basin Research Center (GBRC) uses wildland-collected seed to establish small-scale production plots. We then use seed harvested from the production plots to supply seed for commercial-scale seed production and to provide material for further research. We also use the seed increase plots to conduct research (both formal and informal) on propagation protocols, including techniques for increasing seed production. This work is being carried out by the GBRC in conjunction with the USFS Rocky Mountain Research Station (RMRS) Provo Shrub Sciences Lab wildland seed collection project.

Introduction
One of the greatest limitations to native vegetation restoration efforts is a lack of seed material to meet the demands of large-scale restoration and ongoing research. The research at the GBRC emphasizes native forbs, which serve many purposes in wildland communities. Forbs are not only an essential component of sage-grouse (*Centrocercus urophasianus* and *C. minimus*) habitat and diet, but many are important for sustaining native pollinator populations (Crawford et al. 2004, Dumroese et al. 2016). In order for successful restoration of native vegetation in rangeland environments, land managers require seed in sufficient quantities at reasonable prices and a knowledge of the best practices for successful germination and establishment of the seed. The native plant research at the GBRC helps meet these needs through the increase of small-scale wildland collections of native species for release to larger-scale commercial production, or to researchers for further testing.

Objectives
- Increase seed from wildland collections to support further research and increase distribution of native seed to commercial growers.
- Keep detailed records of practices, plant growth, and production to assess methods to increase seed production of native species in agronomic settings.

Methods
In conjunction with the USFS Rocky Mountain Research Station (RMRS), we selected priority species and accessions of wildland collected seed to be grown for seed production on the UDWR farm in Fountain Green, UT or the Snow College farm in Ephraim, UT. For each species we began by planting a single accession and evaluated it for production potential. For species that had been previously evaluated and were suited for agricultural seed increase, we acquired and increased multiple genetic sources from the same provisional seed zone. Each source was grown and studied independently. Our farm at Fountain Green contains 156 production beds (80 ft x 5 ft), each equipped with subsurface drip irrigation. Our

farm at Snow College in Ephraim currently houses 2 production fields (0.5 acres) equipped with surface sprinkler irrigation.

For each species we evaluated and assessed factors such as peak bloom date, seed production capacity under irrigation, potential for mechanical harvest, and length of productive life span.
We maintained and harvested the production fields to maximize seed production of native species. When opportunities arose, we developed propagation protocols and conducted research on ways to increased establishment and production. We kept detailed records on growth traits and production techniques to further assess best practices for seed production.

Results and Discussion
In spring 2021 we transplanted new Great Basin source materials into 23 beds at our Fountain Green farm. We harvested seed from 43 beds in the fall of 2021, with at least an additional 27 perennial or biennial beds that will mature and produce seed in 2022. We intend to seed or transplant additional Great Basin materials (number and species yet to be determined) in Fall 2021 or Spring 2022.

In fall 2021 we successfully harvested seed from one germplasm ("NBR1") of basalt milkvetch (*Astragalus filipes*), one accession of Aspen fleabane (*Erigeron speciosus*), three accessions of redroot buckwheat (*Eriogonum racemosum*), one accession of sulfer-flower buckwheat (*Eriogonum umbellatum*), five accessions of Nevada showy goldeneye (*Heliomeris multiflora nevadensis*), two accessions of Lewis flax (*Linum lewisii*), one accession of smooth-stem blazing star (*Mentzelia laevicaulis*), one accession of ragwort (*Packera sp.*), four accessions of firecracker penstemon (*Penstemon eatonii*), two accessions of palmer penstemon (*Penstemon palmeri*), 15 accessions of thick leaf penstemon (*Penstemon pachyphyllus*), and one accession of desert prince's plume (*Stanleya pinnata*). Harvest information is noted in Table 1.

Where multiple accessions of the same species are being grown and harvested in the same year, those sources were collected from the same provisional seed zone (either 15-20 Deg. F/3-6 or 15-20 Deg. F/6-12) for the Great Basin. All sources were grown, harvested, and cleaned separately, but are pooled by seed zone to incorporate genetic material from all sources before being sent to a commercial grower. Harvested material is being stored at the GBRC seed warehouse until a willing qualified commercial grower can be found.

Table 1. Harvest information for species grown for increase by the GBRC in 2019.

Species/Collection	Peak Bloom Date	Harvest Date(s)	Cleaned Seed (g)
Astragalus filipes			
NBR1	--	1-Jul	Pending
Erigeron speciosus			
Region 4 – JP collection	30-Jun	29-Jul—5-Aug	Pending
Eriogonum racemosum			
Highway 40 Daniel	21-Jul	12-Aug—25-Aug	Pending
Pigeon Water	29-Jul	25-Aug—2-Sep	Pending
Elkhorn	11-Aug	2-Sep—13-Sep	Pending
Eriogonum umbellatum			
Region 4 – JP pooled collection	9-Jun	6-Jul—5-Aug	Pending

Table 1. Harvest information for species grown for increase by the GBRC in 2019.

Species/Collection	Peak Bloom Date	Harvest Date(s)	Cleaned Seed (g)
Heliomeris multiflora nevadensis			
Burnt Peak	30-Jun	22-July—28-Sep	Pending
Jackrabbit Mine 2	30-Jun	22-July—28-Sep	Pending
Mendha	30-Jun	22-July—28-Sep	Pending
Pioche	30-Jun	22-July—28-Sep	Pending
Basin of Silver King Mtn	30-Jun	29-July—13-Sep	Pending
Linum lewisii			
Crestline Road	20-May	30 Jun—13-Sep	Pending
Mendha	20-May	30 Jun—13-Sep	Pending
Mentzelia laevicaulis			
Cherry Creek Road	4-Aug	28-Sep	Pending
Packera sp.			
Charleston Deeth	2-Jun	30-Jun—6-Jul	Pending
Penstemon eatonii			
Hamilton	9-Jun	9-Aug	Pending
Mount Wilson	9-Jun	9-Aug	Pending
Pine Grove	9-Jun	9-Aug	Pending
Shoal Creek	9-Jun	9-Aug	Pending
Penstemon pachyphyllus			
Antelope Mountain	26-May	22-Jul	1088.62
Cherry Creek East	26-May	28-Jul	1474.17
Cleve Creek	26-May	28-Jul	453.59
Coyote Gallows	26-May	28-Jul	1564.89
Lexington Creek	26-May	28-Jul	Pending
Lexington Creek East	26-May	22-Jul	Pending
Miller Basin Road	26-May	22-Jul	453.59
NF Road 578	26-May	21-Jul	Pending
North Canyon Pass (A)	26-May	22-Jul	Pending
North Canyon Pass (B)	26-May	28-Jul	Pending
Pine Grove Reservoir	26-May	22-Jul	4082.33
Pleasant Valley	26-May	22-Jul	126.08
Powerline	26-May	28-Jul	317.52
Sacramento Pass	26-May	28-Jul	793.79
Schellbourne	26-May	28-Jul	1927.77
Skunk Springs	26-May	28-Jul	Pending
Penstemon palmeri			
Old Iron Town	30-Jun	28-Sep	Pending
Pass Road	30-Jun	28-Sep	Pending
Stanleya pinnata			
Mills	--	13-Sep	Pending

In 2022 we will continue monitoring and harvesting seed from established plots of *A. filipes*, *E. speciosus*, *E. racemosum*, *E. umbellatum*, *H. multiflora nevadensis*, *Ipomopsis aggregate*, *L. lewisii*, *Packera sp.*, *P. eatonii*, *P. pachyphyllus*, and *P. palmeri*.

Management Applications

The objective of this project is to provide native plant materials to researchers and commercial growers in workable quantities, with the intent that materials will be increased to restoration scales.

References

Crawford, John.A.; Olson, R.A.; West, N.E.; Mosley, J.C.; Schroeder, M.A.; Whitson, T.D.; Miller, R.F.; Gregg, M.A.; Boyd, C.S. 2004. Ecology and management of sage-grouse and sage-grouse habitat. Journal of Range Management 57:2-19.

Dumroese, R. Kasten; Luna, T.; Pinto, J.R.; Landis, T.D. 2016. Forbs: foundation for restoration of Monarch butterflies, other pollinators, and greater sage-grouse in the Western United States. Natural Areas Journal 36:499-501.