Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 2022 Report)

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Photo credit: Shannon Lencioni, U.S. Geological Survey. Public domain.

Photo caption: Small-leaf globemallow (*Sphaeralcea parvifolia*, Malvaceae) in a dryland plant community near Bluff, Utah.

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Introduction

A primary focus of the Colorado Plateau Native Plant Program (CPNPP) is to identify and develop appropriate native plant materials (NPMs) for current and future restoration projects. Multiple efforts have characterized the myriad challenges inherent in providing appropriate seed resources to enable effective, widespread restoration and have identified a broad suite of research activities to provide the information necessary to overcome those challenges (e.g., Plant Conservation Alliance, 2015; Breed et al., 2018; Winkler et al., 2018; NASEM, 2023). Many of the most complex information needs relate to identifying the appropriate sources of plant species that can successfully establish in dryland environments, like the Colorado Plateau, where low and highly variable precipitation is standard. Providing this information requires synergistic research efforts in which results from earlier investigations inform the design of subsequent investigations. The U.S. Geological Survey (USGS) Southwest Biological Science Center's (SBSC's) research activities to support CPNPP in Fiscal Year 2022 (FY22) followed the FY22 Statement of Work to support a research framework that is continually adapting based on the needs of the restoration community and results from previous investigations; the long-term research framework is outlined in the 2019-2023 5-Year Research Strategy (hereafter referred to as the 5-year plan). This research framework provides support for the National Seed Strategy for Rehabilitation and Restoration (Plant Conservation Alliance, 2015), Biden-Harris Administration Executive Order 14008 (*Tackling the Climate Crisis at Home and Abroad*), and Department of Interior Priority #4 (*Working to conserve at least 30% each of our lands and waters by the year 2030*).

Research activities in FY22 centered on landscape genomics, monitoring common garden and seeding experiments near Vernal and Moab, UT, conducting experimental treatments using the GRID (Germination for Restoration Information and Decision-making) framework, and continuing newer genetics projects to investigate the impact of productions techniques on plant materials and restoration treatments on native plant communities. These activities were supported by two biological science technicians. The effects of the SARS-CoV-2 pandemic were less visible in FY22, allowing SBSC to catch up on backlogged laboratory work. The overall progress of SBSC's research remains on track with respect to the 5-year plan. While Dr. Rob Massatti was the only scientist supported by the SBSC-CPNPP agreement in FY22, other scientists, including Drs. John Bradford, Seth Munson, Mike Duniway, Sasha Reed, and Daniel Winkler, spent a considerable amount of time providing expertise and support for individual projects. Work activities performed in support of each 5-year plan goal are discussed in turn.

Goal 1. Resolve patterns and drivers of genetic diversity, structure, and adaptation (i.e., landscape genetics)

Genetic diversity is recognized as an important component of healthy ecosystem functioning (Hughes et al., 2008) and a unit of conservation concern (Hoban et al., 2020), but the consideration of diversity is often not incorporated into the development and use of native plant materials (NPMs) for restoration purposes. However, it is highly likely that consideration of genetic diversity would increase the success of restoration outcomes (e.g., Broadhurst et al., 2008). For example, NPMs with too little genetic diversity may have reduced success due to inbreeding depression, while NPMs that are too genetically different from a local population may reduce restoration success due to outbreeding depression (Hufford et al., 2012). Numerous historical and contemporary processes affect a plant's genetic structure and variation. The application of molecular genetic techniques is valuable for assessing these processes, which in turn can inform the development and deployment of NPMs, a species' genetic diversity and differentiation, taxonomic issues, and adaptation to environmental gradients. Genetic analyses are especially informative when applied to species for which there is little prior knowledge, for example, by generating data that can help structure field-based experimental frameworks, thereby ensuring that experiments will provide informative results. For most of the important Colorado Plateau restoration species, knowledge of adaptive differentiation, genetic diversity, and spatial variation in standing genetic diversity is lacking (Wood et al., 2015).

FY22 Results and Discussion

Data generation and the estimation of genetically informed seed transfer zones is complete for eight priority restoration species (see Table 1). Shapefiles are publicly available on the Western Wildland Environmental Threat Assessment Center's website (https://www.fs.fed.us/wwetac/threat-map/TRMSeedZoneData.php). Analyses are ongoing for three species, *Achnatherum hymenoides, Sporobolus cryptandrus,* and *Elymus elymoides*, to determine how genetic data may best be applied to inform the management of self-fertilizing species with complicated genetic patterns and taxonomic uncertainty. In addition, data analysis will start in FY23 to develop seed transfer zones for *Heterotheca villosa*, which is a collaboration with New Mexico Bureau of Land Management (BLM). These zones will likely be available in FY24. A manuscript is in preparation detailing seed zones and methodology for seven forb species, including *Cleome serrulata, Cleome lutea, Heliomeris multiflora, Sphaeralcea parvifolia, Machaeranthera canescens, Astragalus lonchocarpus,* and *Crepis acuminata*; the *Machaeranthera* and *Crepis* datasets are collaborations with Great Basin Native Plant Project (GBNPP). All data will be made publicly available as official data releases that have gone through internal review at USGS to ensure that they meet Fundamental Science Practices guidelines.

To support the development of species-specific seed transfer guidelines that consider both inferred patterns of adaptation and patterns of genetic differentiation, Dr. Massatti developed an R package (POPMAPS, or Population Management through Ancestry Probability Surfaces; Massatti, 2022) to estimate species' genetic patterns across landscapes. This package provides functions to spatially interpolate patterns of genetic differentiation across a species' distribution based on an empirical genetic dataset and is described in Massatti and Winkler (2022). In addition, POPMAPS utilizes patterns within empirical genetic data to assign a probability to every geographic location regarding the genetic identity of individuals of that species; uncertainty is built into this framework due to the lack of complete sampling across the landscape. This method was used in the development of genetically informed seed transfer zones. The POPMAPS package is available on the USGS gitlab website (https://code.usgs.gov/GWRC/popmaps/).

Dr. Massatti contributed to genomic projects adjacent to CPNPP in FY22. He provided analytical expertise to GBNPP for their ongoing landscape genomic research on *Machaeranthera canescens, Erigeron speciosus* (Richardson et al., 2022)*, Sphaeralcea* species*,* and *Crepis acuminata*; resulting data will support seed transfer and native plant materials development guidance to managers across the Great Basin. Similarly, Dr. Massatti interacted with the Institute for Applied Ecology and New Mexico BLM to inform 1) field-based leaf sampling protocols for future landscape genomic studies across New Mexico, and 2) a project designed to characterize the effects of agricultural production on native plant material development. Interactions with adjacent programs helps ensure that research efforts coincide where possible and may facilitate future, overlapping CPNPP research goals (e.g., genetic effects of production).

Table 1. Species for which molecular data are being gathered and analyzed, and the timeframe for the release of data and reports. Due to field seasons being near the end of fiscal years, the majority of lab work and DNA sequencing occurs in the fiscal year following the year in which work for a species is initiated. According to the 5-year plan, the goal is to release reports and data within one year after DNA sequencing is completed. Green cells = work complete; yellow cells = work in progress; red cells = work not yet initiated.

Species	FY17	FY18	FY19	FY20	FY21	FY22	FY23
Pleuraphis jamesii	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release				
Sporobolus cryptandrus	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release				
Sphaeralcea parvifolia	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release				
Achnatherum hymenoides		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
Cleome lutea		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
Machaeranthera canescens		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
Heliomeris multiflora			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release		
Astragalus lonchocarpus			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release		
Cleome serrulata				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
Elymus elymoides				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
Heterotheca villosa					Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release

Goal 2. Determine adaptive phenotypic variation in natural populations (i.e., common gardens and plant traits)

The ability of land managers to achieve restoration goals is often hindered by a lack of scientifically sound information regarding how to use plant materials across a heterogeneous landscape. To mitigate this knowledge gap, SBSC proposed to establish common gardens at environmentally stratified sites on the Colorado Plateau. Including multiple sources of a species in a common environment (and replicated across environments) enables researchers to tease apart local adaptation (genotype-by-environment interactions), phenotypic plasticity, and the ability of successive generations to respond to novel environmental conditions (Hufford and Mazer, 2003; de Villemereuil et al., 2016). Therefore, common garden experiments allow restoration ecologists to identify seed sources of plants locally adapted to specific climate variables, which regularly occurs across the Intermountain West (Baughman et al., 2019). In addition, common gardens offer exceptional educational, training, and information-sharing opportunities, as they are locations where scientists, growers, and managers can visit together to look at plants and their responses to known conditions.

In addition to common gardens, SBSC researchers will assess plant trait variability across the Colorado Plateau. Understanding variation in plant traits within and among species can help researchers understand how they are able to persist in their current environments, how they may respond to climate variability and land management actions, and how they affect ecological services valued by society. Determining the structural and physiological characteristics of plant populations that allow them to survive under a set of environmental conditions can allow growers to select for these traits in new seed lines and plant materials development. Measuring plant traits of wild populations is also important when collecting seeds for restoration or evolutionary experiments (Li et al., 1998; Cornelissen et al., 2003; Swenson and Enquist, 2007; Makkonen et al., 2012; Frenne et al., 2013). Traits can explain differential performance of populations in experimental and/or common garden environments by serving as a baseline for population phenotypes (Primack et al., 1989; Oleksyn et al., 1998; Vogel et al., 2005; Martin et al., 2007; Vitasse et al., 2009; Hancock et al., 2013; de Villemereuil et al., 2016). Knowledge about which combination of plant traits lead to enhanced performance in a specific environment can inform which species are likely to be successful without having to grow out of all of them. Furthermore, linking the traits of seed sources and plant materials to ecological services, such as soil erosion control or wildlife habitat, can allow land managers to meet intended goals to bolster the health of a managed ecosystem.

FY22 Results and Discussion

Work in FY22 to support field-based experiments centered on collecting seeds for priority species and monitoring the Vernal common garden and seed trialing experiments. Collection efforts resulted in 34 seed collections made for 12 species at 19 sampling locations (Figure 1). Seeds are being cleaned and stored at SBSC in Flagstaff. The common garden established at the beginning of FY21 contained three species (*Machaeranthera canescens, Sphaeralcea parvifolia,* and *Sporobolus cryptandrus*), each represented by twenty seed sources collected across their environmental distributions on the Colorado Plateau (Figure 2). Fifty plants per source were grown in the Northern Arizona University (NAU) research greenhouses starting in April 2020 (3000 plants total). Seedlings were planted into approximately 50 2 x 2-meter plots using a randomized design replicating the planting strategy used to establish the Santa Fe common garden (see below). Seedlings were watered at the time of planting and for several subsequent weeks using fire equipment provided by the Vernal Field Office; post-establishment watering efforts were supported by a Seeds of Success intern working out of the Vernal Field Office. Mortality data were collected for the seedlings in November 2020, and the site was seeded with sterile barley to reduce weed establishment in 2021. Mortality data were collected by USGS technicians in 2021 and 2022. Fewer than 20 individuals survived by July 2021, likely resulting from winter/spring drought conditions; detailed photosynthesis measurements were taken to glean insights from survivors.

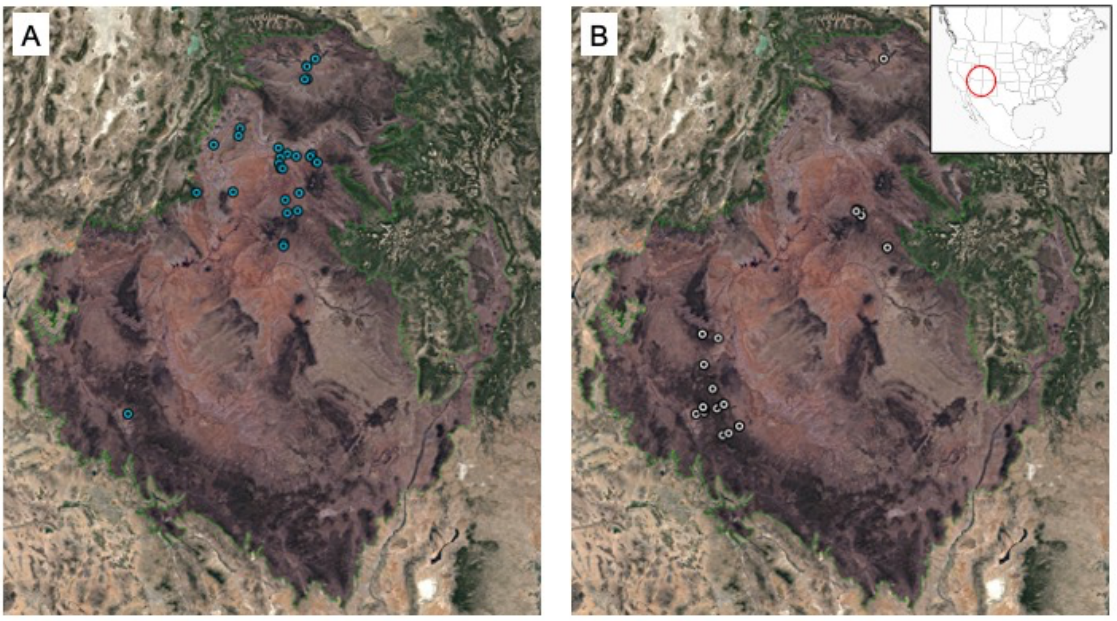

Figure 1. (A) Tissue collection sites to support genomic research projects (see Goals 4 and 5). (B) Seed collection sites to facilitate field-based experiments investigating adaptive phenotypic variation (see Goal 2).

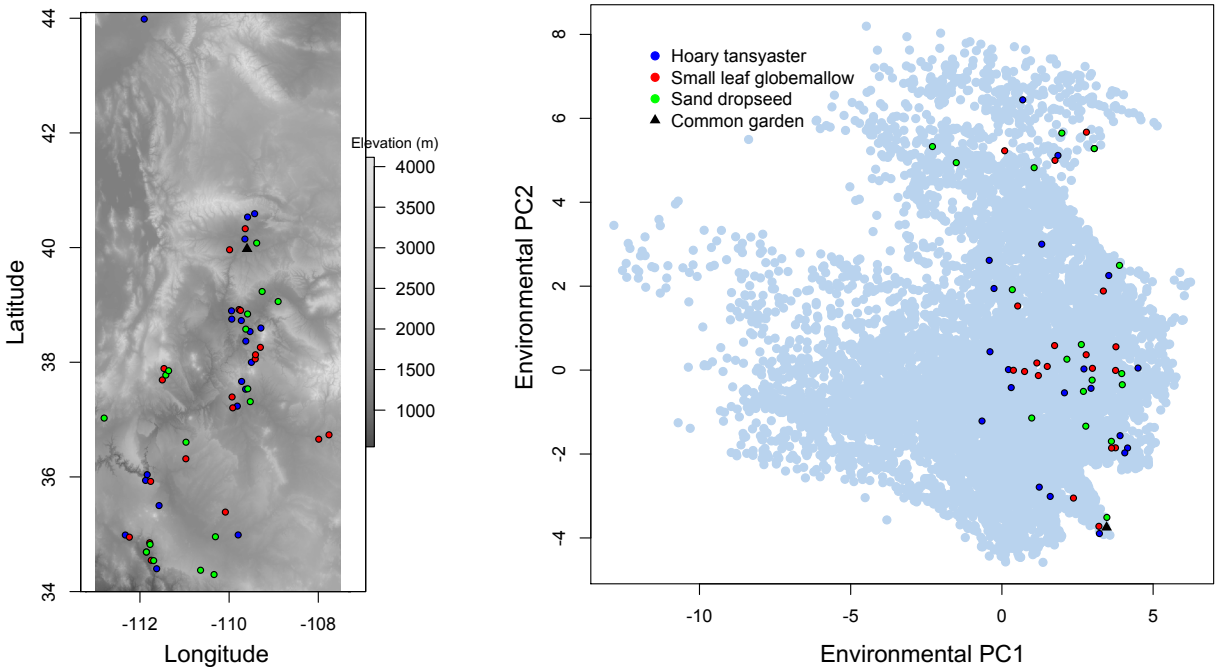

Figure 2. Geographic (left) and environmental (right) sampling of common garden seed sources for *Machaeranthera canescens* (hoary tansyaster – blue), *Sphaeralcea parvifolia* (small-leaf globemallow – red), and *Sporobolus cryptandrus* (sand dropseed – green). The light gray circles in the environmental PCA (right) represent the overall environmental variation of the geographic area (left), with lower values on the environmental PC1 generally representing higher elevations where the focal species are not present.

Similar field-based experimental failures were seen across the region (e.g., personal communication with Mike Duniway and Becky Mann, USGS). By summer 2022, eleven survivors (all *Sporobolus*) were present in the garden.

We leveraged unused space at the common garden, as well as space available at Canyonlands Research Center south of Moab, UT, to initiate a seeding experiment that was planned at the end of FY21 and implemented at the beginning of FY22. The main goal of the seeding experiment was to provide quantitative data regarding the performance of multiple germplasms of three graminoids commonly used in restoration versus climate distance from where the germplasm originated (Table 2). We predict that performance will be negatively correlated with increasing climate distance based on abundant scientific literature supporting local adaptation within plants. Furthermore, for two germplasms (*Achnatherum hymenoides* 'Nezpar*'* and *Bouteloua gracilis* 'Alma'), we acquired seed from agricultural increase fields located in different environmental spaces. Including multiple sources of a germplasm will allow us to quantify effects of the maternal plants' environments on the performance of seed (i.e., maternal effects). We predict that seed produced in increase fields with a more similar climate to the experimental sites will perform better than seed produced in a climate that is more different from the experimental sites. Finally, we will investigate how performance is associated with species diversity, as each germplasm is sown both as a monoculture and with a standard mix of forbs (Table 2).

The seeding experiment in Vernal was monitored in May and July 2022. Counts of living plants illustrate that all species and sources (graminoids and forbs) had difficulty germinating and establishing across plots. *Achnatherum hymenoides* germinated in eight out of twenty plots, with individuals persisting to July in seven. *Elymus elymoides* germinated and established in only one out of sixteen plots. *Cleome serrulata* was the only forb with germination and establishment and was recorded in 13 out of 28 plots. Data do not yet support significant patterns, but it does not look like germination and establishment of *A. hymenoides* is linked to germination and establishment of *C. serrulata.* The Vernal seeding experiment will be monitored in 2023 to understand long-term patterns of survival across plants established in 2022, as well as whether a second winter stratification increases germination of seeds remaining in the plots.

The seeding experiment at Canyonlands Research Center was monitored in May, August, and September 2022. Across all plots (including and excluding forbs), graminoid seed did not germinate. However, all plots seeded with forbs had germination and establishment of most or all forb species. The seeding experiment will be monitored in early 2023 and we are interested to see if a second winter of stratification increases graminoid germination, and if germination is correlated with having forbs present in the plot the previous year. We plan to expand this design and implement similar seeding experiments in 2023; we are in discussion with colleagues to determine a network of sites crossing environmental gradients (e.g., RestoreNet) where we may be able to install this experiment.

Research at the common garden in Santa Fe, NM funded by New Mexico BLM continued in FY22. This garden includes Colorado Plateau seed sources for two CPNPP priority species: *Heterotheca villosa* and *Sporobolus cryptandrus*. Initial data collection is complete and data analysis is underway by a graduate student at NAU, demonstrating how CPNPP benefits, at no cost, from the connections within and among researchers at SBSC and NAU. Mortality data were collected at the Santa Fe common garden in June and August 2022. Data will continue to be collected in FY23 to track long-term survival trends. A manuscript reporting analyses from the initial data collection efforts will be submitted in FY23, and data resulting from this effort will be available from a public data repository following publication.

Dr. Winkler is spearheading data analysis and preparation of a manuscript that details patterns in specific leaf area and stable isotope ratios for fifteen species across the Colorado Plateau, including: *Achnatherum hymenoides, Bouteloua gracilis, Cleome lutea, Cleome serrulata, Elymus elymoides, Heliomeris multiflora, Heterotheca villosa, Machaeranthera canescens, Oenothera pallida, Phacelia crenulata, Pleuraphis jamesii, Plantago patagonica, Sporobolus cryptandrus, Sphaeralcea parvifolia,* and *Stanleya pinnata*. This contribution by an SBSC researcher that is not funded by CPNPP represents a prime example of how CPNPP funds are extended because of the partnership with USGS. The data generated from these activities will be available as data releases and as manuscripts are published; data will be available from the publicly available USGS ScienceBase website.

Goal 3. Quantify seed survival and establishment in the context of growing aridity (i.e., GRID experiments)

Although restoring native plant communities is a key management need for the Colorado Plateau (Copeland et al., 2019), restoration success is often hampered by a lack of understanding of the basic processes that facilitate or impede native plant regeneration (Call and Roundy, 1991). The establishment of plants from seed is highly sensitive to environmental variability and is expected to be dramatically influenced by changing conditions in coming decades. However, our understanding of the drivers and consequences of plant regeneration is surprisingly rudimentary compared to other demographic processes, particularly in drylands. Increased aridity and enhanced weather variability may dramatically impact regeneration in drylands, although the potential consequences (positive or negative) for regeneration have received less attention than mortality or growth. Regeneration of many long-lived dryland plants is notoriously episodic, responding to a relatively rare combination of soil moisture and temperature conditions (Brown and Wu, 2005; Coop and Givnish, 2008; Kolb and Robberecht, 1996; Petrie et al., 2016; Puhlick, et al., 2012; Savage et al., 2013; Schlaepfer et al., 2014). While the details of the conditions that facilitate regeneration remain unclear for many species and locations, the recognized importance of adequate soil moisture underscores the potential negative impacts of rising aridity in coming decades (Feddema et al., 2013; Petrie et al, 2017; Schlaepfer et al., 2015). Indeed, regeneration failures have already been observed, and are expected to continue, across western North America (Allen et al., 2010; Breshears et al., 2009; Stevens-Rumann et al., 2017; Williams et al., 2013).

Table 2. Species and germplasms included in FY22 seeding experiments. Graminoid species were seeded as monocultures and with a standard forb mix. The standard forb mix included all listed forbs and did not vary based on plot or site. Seeds were sown at recommended rates and depths. Source abbreviations include: Great Basin Research Center (GBRC); Aberdeen Plant Materials Center (Aberdeen); Los Lunas Plant Materials Center (Los Lunas); Upper Colorado Environmental Plant Center (UCEPC); BBB Seed (BBB); Western Native Seed (WN).

Species	Germplasm	Source
Achnatherum hymenoides	Nezpar	GBRC
Achnatherum hymenoides	Nezpar	Aberdeen
Achnatherum hymenoides	Paloma	Los Lunas
Achnatherum hymenoides	Chipeta	UCEPC
Achnatherum hymenoides	Ouray	UCEPC
Bouteloua gracilis	Bad River	BBB
Bouteloua gracilis	Alma	GBRC
Bouteloua gracilis	Alma	Los Lunas
Bouteloua gracilis	Bird's Eye	GBRC
Bouteloua gracilis	Hachita	GBRC
Elymus elymoides	Pueblo	GBRC
Elymus elymoides	Massadonna	UCEPC
Elymus elymoides	Little Sahara	GBRC
Elymus elymoides	Turkey Lake	GBRC
Cleome serrulata	Source id'd	WN
Grindelia squarrosa	In development	Aberdeen
Penstemon eatonii	Richfield	Aberdeen
Machaeranthera canescens	Amethyst	Aberdeen

In the context of both increasing environmental stress and the rising prevalence of disturbances, the long-term persistence of many dryland ecosystems and the maintenance of the ecosystem services that they

provide may depend on regeneration of the dominant species that characterize these communities. SBSC researchers are addressing plant establishment questions for priority restoration species using the Germination for Restoration Information and Decision-making (GRID) experimental framework. The goal of this research is to determine if some seed sources (for example, those from more arid locations) can better survive the establishment phase than others, especially under the drier conditions expected to be prevalent across the Colorado Plateau in the coming decades. These results can immediately inform native plant materials development by helping managers understand which seed sources for a species may be able to cope better with increasingly arid environmental conditions.

FY22 Results and Discussion

GRID infrastructure adjacent to the USGS campus in Flagstaff, AZ was utilized in FY22 to conduct two rounds of establishment experiments. Experiments commenced in June and lasted through September. The first trial focused on *Heterotheca villosa* and the second trial utilized *Elymus elymoides*. Watering treatments were designed to mimic the range of precipitation conditions seed sources naturally receive (as determined by analyzing climatological data from their geographic sources), and a technician applied the watering treatments to plots on a regular schedule (Figure 3). Established plants that survived their respective watering treatments were harvested biweekly in a randomized fashion, and the remainder were harvested at the end of the study; collected data included germination date, height measurements, phenology, leaf counts, notes on herbivory, and dry weight of above and below ground biomass, stems, and any flowers/buds present. Future GRID experiments (FY23 and beyond) will be postponed until data from FY19-22 can be analyzed and reported. Data will be available as a publicly-available USGS ScienceBase data release coinciding with the publication of a manuscript.

A winter storm in early 2023 resulted in damage to hoop house #2 in Flagstaff. The metal frame of the hoop house is unsalvageable, and the plastic on all hoop houses was removed to prevent further structural damage. Fortunately, USGS may provide funding for the labor and materials necessary to repair all three hoop houses. While repairs to the metal frame are expected to commence in spring 2023, we will leave the hoop houses uncovered until a new precipitation manipulation experiment is designed (or GRID is continued). In the meantime, plots will experience ambient weather conditions and be utilized for other experiments, for example a seeding trial similar to what was installed at Canyonlands Research Center in 2022 (see above).

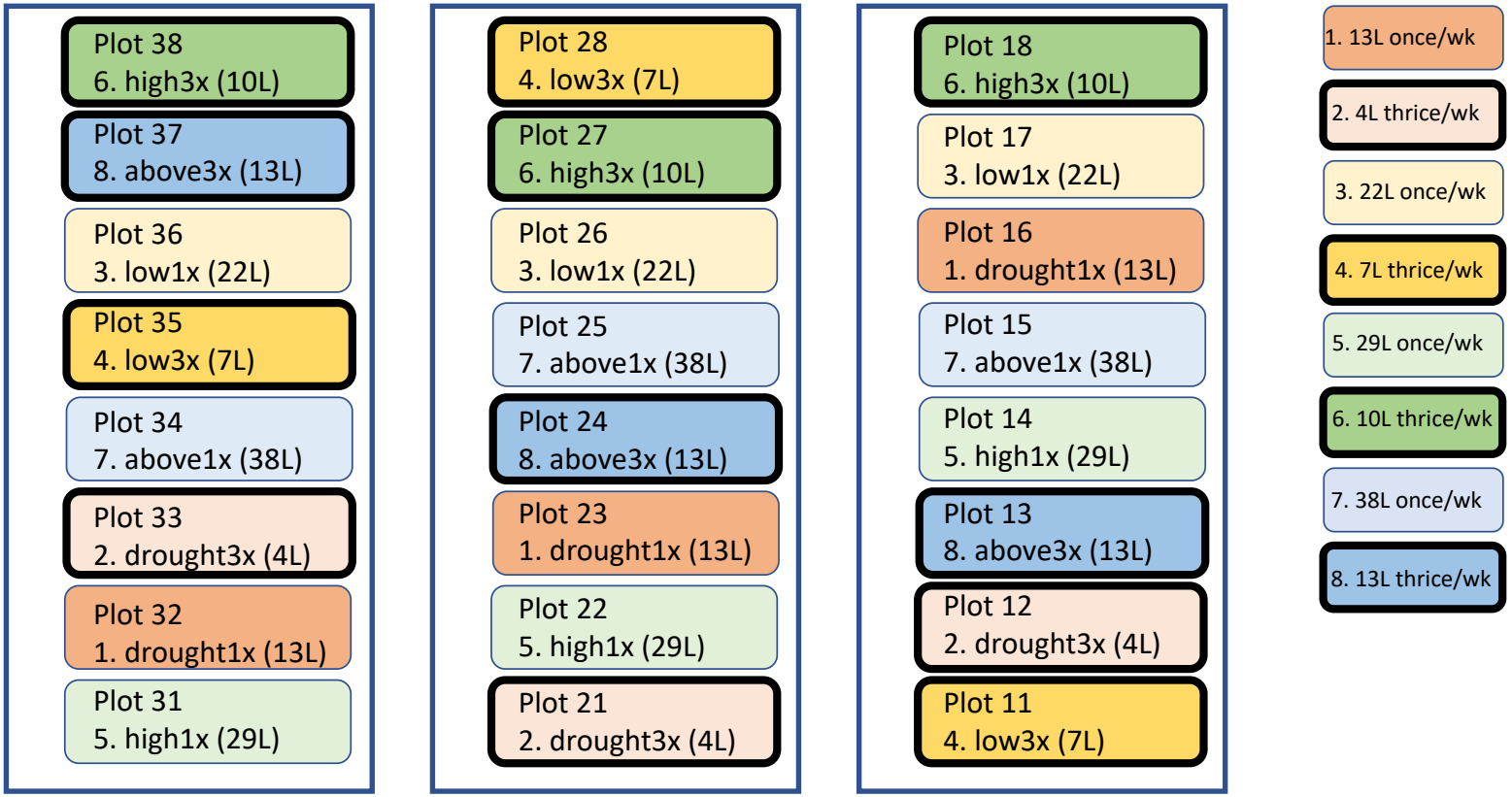

Figure 3. Watering protocol example for an experiment utilizing the GRID infrastructure. Each long rectangle represents a hoop house at the Flagstaff garden (three total), and each hoop house contains eight plots represented by the colored boxes. Plots with thick black borders receive water three times per week, while those without thick black borders receive water once per week (watering legend on the right side of the diagram).

Goal 4. Investigate the impact of seed increase on the genetic identity of restoration materials

As demand grows for genetically appropriate NPMs (Plant Conservation Alliance, 2015; NASEM, 2023), a significant challenge remains the timely development of seed sources for areas that have significant restoration needs. To this end, composite methods are drawing attention because they can utilize small seed accessions collected from wildland sources to develop restoration materials on a relatively short time scale (Bucharova et al., 2019). This process is attractive because: 1) many small seed collections already exist because of Seeds of Success collecting efforts, 2) funding or conducive weather patterns for large-scale seed collection may not be available, and 3) the time necessary to identify and collect an operational collection directly from a wildland population decreases the total number of collections that can be made across the landscape. Despite the potential benefits, the process of developing a native plant restoration material from small seed accessions has unknown impacts on the resulting restoration material (see the 5-year plan for more information). Research aligned with Goal 4 will investigate the genetic impacts of this production method. Our questions include: 1) How does genetic identity/diversity shift across the steps of this production process?; 2) How genetically representative is seed available for restoration compared to the wildland population(s) used to generate the seed?; and 3) How can growing practices be improved/modified such that the seeds available for restoration are more representative of wildland populations?

FY22 Results and Discussion

The production genetics research project includes seed sources for priority restoration species that were selected with the BLM Coordinator in November 2020 and sent for increase at BFI Native Seeds in Moses Lake, WA, and Great Basin Research Center (GBRC) in Ephraim, UT. BFI is increasing five pooled source plant materials representing four species, including: *Sporobolus cryptandrus* (10 seed sources pooled)*, Achnatherum hymenoides* (8 sources), *Sphaeralcea parvifolia* (10 sources), *Plantago patagonica* material 1 (5 sources), and *P. patagonica* material 2 (7 sources). GBRC will increase ten single-source seed accessions representing nine species, including: *Sporobolus airoides, Aristida purpurea, Astragalus lonchocarpus, Bouteloua gracilis, Pleuraphis jamesii* (2 sources)*, Cymopterus bulbosus, Phacelia crenulata, Heterotheca villosa,* and *Cleome lutea.* We will request increased seed from these partners in FY23, which we will germinate at the NAU research greenhouses to obtain leaf tissues for genetic analyses. 550 leaf tissues representing the wildland plant populations are in hand and being processed for next-generation sequencing; when data from agriculturally grown seeds are available, they will be compared to data from the wildland populations so that we can understand the impact of production and increase on genetic diversity.

Dr. Massatti also participated in developing a grant proposal that is funded beginning FY22 by the U.S. Department of Agriculture's Agriculture and Food Research Initiative program titled "Testing Native Plant Materials Resulting from Emerging Techniques: Functional Stability, Genetic Diversity, and Performance in the Context of Ecosystem Services in America's Rangelands." Like CPNPP-funded production research, the goal of the proposed research is to investigate pooled source materials in terms of their performance, focusing on native plant materials that are being increased by Bamert Seed Company in partnership with Southwest Seed Partnership (SWSP) and New Mexico BLM. Research will be led by Dr. Winkler, with assistance from Drs. Faist (Montana State University), Fuentes-Soriano (New Mexico State University), Reed (USGS), Jones (Agricultural Research Service), and Ashlee Wolfe (SWSP), and will directly augment CPNPP research interests.

Goal 5. Investigate the long-term impacts of restoration materials on the genetic identity of plants in their natural communities

Restoration materials have been used to mitigate the impacts of ecosystem disturbances for decades across the Colorado Plateau (Winkler et al., 2018). The use of restoration materials provides clear benefits

to disturbed areas, including soil stabilization, providing food and habitat for wildlife, rejuvenating ecosystem function, and improving the delivery of ecosystem services (Hughes, 2008). However, the study of the impacts of restoration materials on pre-existing communities has received less attention. It has been established that restoration materials can persist at restoration sites for prolonged periods of time (Gustafson et al., 2002; Poehlman et al., 2018), and that the use of non-local restoration materials may have impacts on other organisms in the pre-existing communities (Vandegehuchte et al., 2012; Bucharova et al., 2022). In addition, concerns have been raised regarding "genetic pollution," or the spread of non-local genotypes from restoration materials into natural populations surrounding the restoration site through cross-pollination (Templeton, 1986), and similarly, the spread of invasive genotypes into natural populations (Saltonstall, 2002). In other words, restoration materials may substantially influence local population genetic structure with implications for the survival and reproduction of the population (further discussed in McKay et al., 2005). Given these processes, we designed research to ask: 1) Can we identify admixture resulting from cross-pollination between individuals of the pre-existing population and individuals representing the restoration material; 2) Is there evidence that restoration materials are establishing outside of the restoration area, or that individuals from the pre-existing population are establishing within the restoration treatment?; 3) Do seeds from individuals exhibit signals of cross-pollination; and 4) Do admixed seeds have lower viability and/or display lower seedling vigor compared to non-admixed seeds from both the pre-existing population and the restoration materials?

FY22 Results and Discussion

We used information gathered from BLM field offices, the Utah Division of Wildlife Range Trends monitoring program (hereafter range trends dataset; Utah Division of Wildlife Resources, 2017), and restoration-oriented management actions in projects associated with the Watershed Restoration Initiative (WRI; Watershed Restoration Initiative, 2018) to identify restoration sites and focal species to include in our research design. We narrowed down the list of focal species by determining those that have been used most frequently within restoration treatments. Furthermore, we narrowed down sites by selecting those that contained the focal species pre-disturbance/treatment. After removing species and sites with insufficient sample sizes to conduct analyses, we identified 1,596 leaf tissues for 11 species at 10 restoration sites (Table 3) that allow us to investigate research questions. Leaf tissues were sampled at multiple distances within and outside of restoration treatment boundaries, and sampled plants were monumented to support future seed collections. In FY22, 384 tissues from four species and five restoration sites were sequenced. Data processing and analysis will commence in FY23; all data will be publicly-available on the USGS ScienceBase website following publication.

Table 3. Species, restoration sites, and tissue collections that support the restoration genetics research project. Sites with bolded species names were included in preliminary sequencing efforts; these sites were chosen to represent a range of species' life history characteristics.

Species	Site	Latitude	Longitude	# Collections
Achillea millefolium	Hatch Bench	37.604858	-112.4044	29
Achillea millefolium	Horse Valley	37.807988	-112.63495	108
Achnatherum hymenoides	Circle Cliffs	37.988389	-111.20568	55
Achnatherum hymenoides	Hatch Bench	37.60583	-112.40819	110
Achnatherum hymenoides	Horse Canyon	39.434055	-110.43163	107
Achnatherum hymenoides	McCook Ridge	39.640281	-109.2673	34
Achnatherum hymenoides	Pack Creek	38.443454	-109.37887	113
Achnatherum hymenoides	Strawberry	39.091282	-114.25456	100
Achnatherum hymenoides	Yellow Jacket	37.138425	-112.60179	112
Artemisia tridentata ssp. *wyomingensis*	Hatch Bench	37.607086	-112.40437	40
Artemisia tridentata ssp. *wyomingensis*	Strawberry	39.089549	-114.25644	42
Atriplex canescens	Beef Basin	37.957589	-109.92421	19
Atriplex canescens	Circle Cliffs	37.985117	-111.16237	50
Elymus elymoides	Horse Canyon	39.428484	-110.41138	38
Elymus elymoides	McCook Ridge	39.644222	-109.26463	28
Elymus elymoides	Strawberry	39.05964	-114.2452	100
Leymus cinereus	Circle Cliffs	37.979366	-11.20767	24
Pleuraphis jamesii	Horse Canyon	39.434319	-110.40732	105
Poa secunda	Pack Creek	38.439425	-109.38252	114
Sphaeralcea parvifolia	Circle Cliffs	37.973821	-111.21125	31
Sporobolus contractus	Dugout Flat	38.074432	-110.90116	100
Sporobolus contractus	McCook Ridge	39.651371	-109.26523	106
Sporobolus cryptandrus	Beef Basin	37.955524	-109.92484	19
Sporobolus cryptandrus	Circle Cliffs	37.98063	-111.21091	12

Additional activities

Beyond research and products aligning with Goals 1-5 in the 5-year plan, SBSC researchers participated in an array of additional activities supporting CPNPP. Dr. Massatti and colleagues reported fieldwork accomplishments (e.g., yearly reports for state BLM offices and Navajo Nation on collecting activities), composed internal and public versions of Colorado Plateau Native Plant Program progress reports (e.g., Massatti et al., 2022), and provided an FY23 Statement of Work to the BLM. In addition, Dr. Massatti spent time providing guidance to technicians and researchers working on the Colorado Plateau and ensuring that communication across research groups was maintained so that research is synergistic when possible. SBSC researchers also disseminated research and interacted with other Colorado Plateau researchers at scientific and stakeholder meetings. CPNPP-related research was presented at: the Biennial Conference of Science and Management (September 2022), the Botany Conference (July 2022), the Society for Ecological Restoration Rocky Mountain Chapter Conference (April 2022), in an invited presentation at the California Botanic Garden (April 2022), and at Ecological Society of America (June 2022) (see Appendix 1). Finally, Dr. Massatti participated in Southwest Seed Partnership research committee meetings and National Seed Strategy Federal Implementation Working Group meetings, as

well as in a working group to develop a genetic management plan for the Nevada Seed Strategy in conjunction with Great Basin Native Plant Project and collaborators.

SBSC researchers and technicians supported a broad array of other activities aligned with CPNPP in FY22. Dr. Massatti updated and maintained a public-facing webpage on the SBSC website (https://www.usgs.gov/sbsc/native-plants) that describes SBSC research efforts with respect to CPNPP and the National Seed Strategy. This website serves as a point where all data releases, papers, and software (e.g., the Native Plant Seed Mapping Toolkit: https://rconnect.usgs.gov/seed-toolkit/) that have been reviewed in accordance with USGS Fundamental Science Practices can be easily accessed. In addition, researchers and technicians continued to develop the Plant Materials Project. The goal of this project is to help managers understand how available native plant materials may best be used across the Colorado Plateau and to highlight gaps where native plant material development may be prioritized, and a manuscript will be submitted in FY23 (Winkler et al., *in prep.*). Finally, Dr. Massatti and technicians supported the maintenance and use of CPNPP seed collections in freezers located at the USGS campus in Flagstaff, AZ. Seed collections were newly cataloged (e.g., collections made by Seeds of Success crews) and distributed to researchers. These types of activities will continue in FY23 in support of the CPNPP mission. Products resulting from FY22 research activities are reported in Appendix 1.

Conclusion

As a result of FY22 work, progress has been made to inform restoration efforts across the Colorado Plateau. In particular, genetic studies provide a wide range of information pertinent to native plant materials development and their use in restoration projects, and they will continue to be a central focus of CPNPP-related research by USGS over the next two years (FY23-FY24). With a well-designed research plan, the data gathered from initial genetic studies will inform subsequent experiments such that restoration-related outcomes are maximized. As a result of the USGS-BLM partnership, restoration efforts across the Colorado Plateau and plant materials development for regional use are scientifically informed, and there is strong momentum for continuing to provide knowledge that will improve restoration outcomes.

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Appendix 1. Products resulting from FY22 research activities.

Presentations

2022 Biennial Conference of Science and Management, Flagstaff, AZ. Talk title: 'Addressing redundancy and representation in rare species recovery frameworks using genetic and distributional data.'

2022 Biennial Conference of Science and Management, Flagstaff, AZ. Talk title 'Developing and using native plant materials for restoration across the Intermountain West.'

2022 Botany Conference, Anchorage, AK. Talk title: 'Informing management goals using ancestry probability surfaces.'

2022 Society for Ecological Restoration, Fort Collins, CO. Talk title: 'Knowing your seeds: the importance of within-species variability for promoting restoration success.'

2022 California Botanic Garden, Claremont, CA. Talk title: 'Population genetic data and management's 3 R's: resiliency, redundancy, and representation.'

2022 Federal Implementation Working Group, virtual. Talk title: 'Native Plant Seed Mapping Toolkit: strategies to support native plant material use and development.'

2022 Ecological Society of America, Montreal, Quebec, Canada. Talk title: 'Scaling from site-specific challenges to federal policy in determining restoration outcomes: realistic restoration goals based on seed needs and limited supplies.'

Papers

Massatti R, Winkler DE, Reed S, Duniway M, Munson S, Bradford JB (2022) Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 2022 Report). Report submitted to the U.S. Department of Interior Bureau of Land Management on May 5th, 2022. 19pp.

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Data

Massatti R (2022) Genetically informed seed transfer zones for *Astragalus lonchocarpus*, *Cleome serrulata*, and *Heliomeris multiflora* across the Colorado Plateau and adjacent regions: U.S. Geological Survey data release, DOI: 10.5066/P9H9M79K

Massatti R (2022) *Carex specuicola* data for the southern Colorado Plateau Desert: U.S. Geological Survey data release, DOI: 10.5066/P9LLZ1XD

Software

Massatti R (2022) POPMAPS: An R package to estimate ancestry probability surfaces: U.S. Geological Survey Software Release, https://doi.org/10.5066/P96VLOA5.

Andrews CA, Massatti R (2022) Native Plant Seed Mapping Toolkit software to support decision-making in restoration: U.S. Geological Survey Software Release, *In review*.

Outreach activities by Massatti

USGS website (www.usgs.gov/sbsc/native-plants)

Native Plant Seed Mapping Toolkit (https://rconnect.usgs.gov/seed-toolkit/)

National Native Seed Conference Planning Committee (2023)

Flagstaff Festival of Science (https://scifest.org/2022-festival), “Putting the Right Seed in the Right Place at the Right Time” - research garden tours on the SBSC campus
Federal Implementation Working Group Research Subcommittee
Research Working Group: Developing a genetic management plan for the Nevada Seed Strategy