Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 2021 Report)

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Submitted to Bureau of Land Management May 5th, 2022

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Photo caption: Pinyon-juniper woodland near Moab, Utah.

Suggested citation: 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 2021 Report). Report submitted to the U.S. Department of Interior Bureau of Land Management on May 5th, 2022. 19pp.

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; McCormick et al. 2021). 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 FY21 followed the FY21 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 FY21 centered on landscape genomics, implementing and monitoring a common garden experiment near Vernal, UT, conducting experimental treatments using the GRID (Germination for Restoration Information and Decision-making) framework, and initiating new genetics projects to investigate the impact of production techniques on plant materials and restoration treatments on native plant communities. These activities were supported by four biological science technicians. The SARS-CoV-2 pandemic delayed some aspects of the FY21 workplan, especially for outside laboratory services. However, goals were largely met, and the overall progress of 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 FY21, 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. Products resulting from FY21 research activities are reported in Appendix 1.

Research 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 lack of fertility, vigor, and/or fitness resulting from inbreeding, while NPMs that are too genetically different from a local population may reduce restoration success due to a reduction of fitness resulting from 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, because they generate 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 on adaptive differentiation, genetic diversity, and spatial variation in standing genetic diversity is lacking (Wood et al. 2015).

FY21 Results and Discussion

Data generation and the estimation of genetically informed seed transfer zones are ongoing for four priority restoration species (see Table 1). Delays in reporting seed zones for *Astragalus lonchocarpus* and *Heliomeris multiflora* in FY21 result from genomic sequencing services being delayed due to 1) the SARS-CoV-2 pandemic and 2) an equipment upgrade at the University of Oregon Genomics & Cell Characterization Core Facility (GC3F). GC3F changed their sequencing platform from an Illumina HiSeq 2500 to an Illumina NovaSeq 6000, which produces more data at reduced costs; this upgrade will result in lower long-term sequencing costs for SBSC and CPNPP. However, the new platform interacted differently with SBSC next-generation sequencing libraries, requiring SBSC and GC3F staff to troubleshoot quality issues. The problem was rectified by developing custom primers for our sequencing libraries. We expect to finish seed transfer zones for *A. lonchocarpus* and *H. multiflora* in spring 2022 and for *Cleome s*errulata and *Elymus elymoides* by the end of FY22, bringing us back on schedule according to Table 1. Shapefiles will be publicly available on the Western Wildland Environmental Threat Assessment Center's website (https://www.fs.fed.us/wwetac/threat-map/TRMSeedZoneData.php). Ongoing genetics projects initiated in FY21 include: 1) working with Dr. Bryce Richardson (Rocky Mountain Research Station) to demonstrate best methods for generating seed transfer zones on a joint CPNPP-Great Basin Native Plant Project (GBNPP) *Machaeranthera canescens* dataset; 2) a landscape genomics study of *Sphaeralcea parvifolia*, which is being led by a Masters-level SBSC technician funded by USGS Ecosystem Mission Area; and 3) a landscape genomics study on *Achnatherum hymenoides* and *Sporobolus cryptandrus*, two self-fertilizing, priority restoration species across the Intermountain West. Note that the schedule in Table 1 reflects the 5-year plan, in which focus will shift away from landscape genomics toward other genetically oriented restoration and native plant material production questions from FY21-FY23 (see Goals 4 and 5 below). 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 take into account both inferred patterns of adaptation and patterns of genetic differentiation, Dr. Massatti and Dr. Winkler developed an R package (POPMAPS, or Population Management through Ancestry Probability Surfaces) to extrapolate 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. 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, and a publication is currently being finalized (Massatti and Winkler, *accepted*). POPMAPS and associated data will be available on the USGS github website.

Dr. Massatti contributed to genomic projects adjacent to CPNPP in FY21. He provided analytical expertise to GBNPP for their ongoing landscape genomic research on *Machaeranthera canescens, Erigeron speciosus* (Richardson et al., *in review*), *Sphaeralcea* species, and *Crepis acuminata*; resulting data will support seed transfer and native plant material 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.

Species	FY17	FY18	FY19	FY20	FY21	FY22
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

Research 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 can be common 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 using field-collected samples. 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 material 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 of 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 conduct grow-outs of

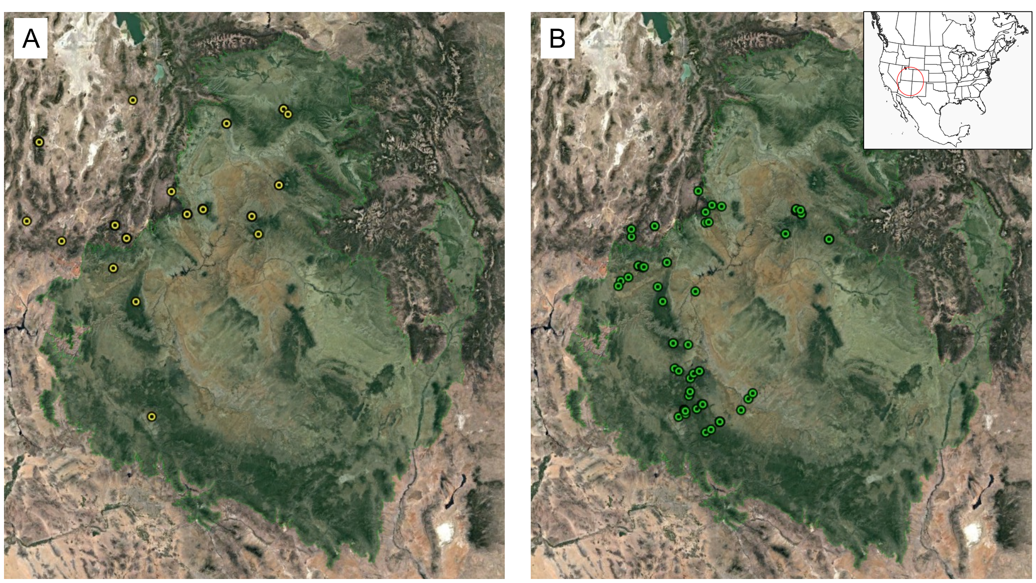

Figure 1. (A) Colorado Plateau tissues collection sites that will facilitate quantifying the impact of restoration materials on natural communities. (B) Seed collection sites to facilitate field-based experiments investigating adaptive phenotypic variation.

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 for land managers to meet intended goals to bolster the health of a managed ecosystem.

FY21 Results and Discussion

Work in FY21 to support field-based experiments centered on collecting seeds for priority species and establishing a common garden experiment on a decommissioned well pad south of Vernal, UT. Collection efforts resulted in 65 seed collections made for 16 species at 35 sampling locations (Fig. 1). Seeds are being cleaned and stored at SBSC in Flagstaff. Common garden infrastructure was established at the beginning of FY21 in October 2020. For each species included in the experimental design (i.e., *Machaeranthera canescens, Sphaeralcea parvifolia,* and *Sporobolus cryptandrus*) (Fig. 2), twenty seed sources representing their environmental distributions across the Colorado Plateau were selected; fifty plants per source were grown in Northern Arizona University (NAU) research greenhouses starting in April 2020 (3000 plants total). The garden site was selected by USGS researchers in Moab and colleagues at the BLM's Vernal Field Office. Site preparation was completed by local contractors and included amending the soil with sulfur fertilizer and organic humus, leveling the site, and erecting a barbed wire perimeter fence to exclude cattle. Seedlings were planted into approximately 50 2 x 2-meter plots in early October using a randomized design replicating the planting strategy used to establish the Santa Fe common garden. 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. An additional chicken wire fence was constructed around plots due to evidence of rabbits in the vicinity of the garden. Mortality data were collected for the seedlings in November 2020, and the site was seeded with sterile barley to reduce weed

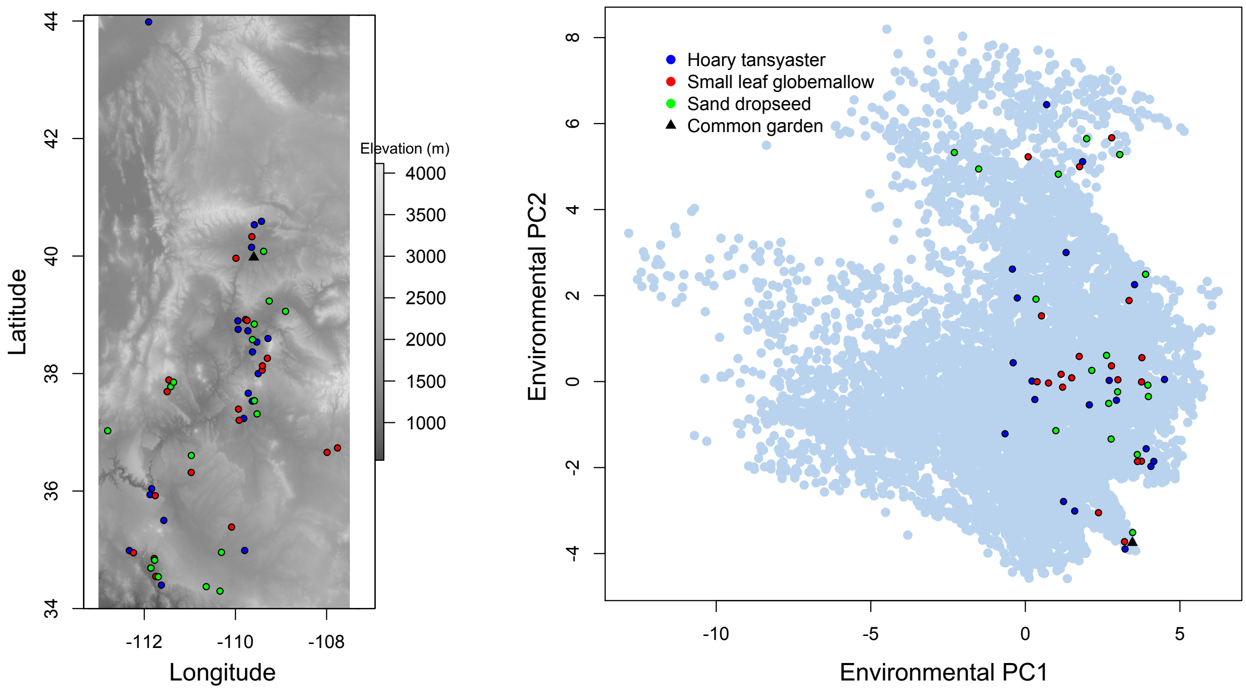

Figure 2. Geographic (left) and environmental (right) sampling of common garden seed sources for *Machaeranthera canescens* (hoary tansyaster - blue), *Sphaeralcea parvifolia* (small-leaf globemallow - red), and *Sporobolus cryptandrus* (sand dropseed - green). The light gray circles in the environmental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

establishment in 2021. Mortality data were collected by USGS technicians in May 2021 and by Dr. Winkler in July 2021. Likely resulting from winter/spring drought conditions, fewer than 20 individuals (all *Sporobolus cryptandrus*) survived by July 2021 – detailed photosynthesis measurements were taken to glean insights from survivors. Similar field-based experimental failures were seen across the region (e.g., personal communication with Mike Duniway and Becky Mann, USGS). A final mortality survey was carried out by Dr. Winkler in October 2021.

Unused space at the common garden, as well as space available at Canyonlands Research Center south of Moab, UT, was leveraged 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 is 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 scientific literature supporting local adaptation within plants. Furthermore, for two germplasms (*Achnatherum hymenoides* 'Nezpar' and *Bouteloua gracilis* 'Alma'), we acquired two sources per germplasm that were grown in agricultural fields that have different climates. 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 smaller climate distance to the experimental sites will perform better compared to seed representing a larger climate distance to the experimental sites. Finally, our experiments include a component where 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). While experiments established in 2021 are designed to provide statistically significant results, we expect to learn from initial trials, modify our experimental design, and implement the experiment across a broader environmental gradient as resources and interest permit.

Research at the common garden in Santa Fe, NM funded by New Mexico BLM continued in FY21. 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 Ella Samuel, a graduate student at Northern Arizona University under the supervision of Dr. Rachel Mitchell, 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 at the end of April 2021 and throughout the summer by a graduate student in Dr. Rachel Mitchell's lab along with seeds that may be useful for future experiments. Data will continue to be collected in FY22 and beyond. A manuscript reporting analyses from the initial data collection efforts will be submitted in FY22, 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 an example of how CPNPP funds are extended as a result of the partnership with USGS. The data generated from these activities will be available as data releases and as manuscripts are published in FY22; data will be publicly available from the USGS ScienceBase catalog.

Table 2. Species and germplasms included in 2021 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

Research 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 are 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). 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 ultimate goal of this research is to determine if some seed sources (for example, those from more arid locations) are able to better survive the establishment phase than others, especially under drier conditions expected to be prevalent across the Colorado Plateau in the coming decades. These results can immediately inform native plant material development by helping managers understand which seed sources for a species may better be able to cope with increasingly arid environmental conditions.

FY21 Results and Discussion

GRID infrastructure adjacent to the USGS campus in Flagstaff, AZ was utilized in FY21 to conduct two rounds of establishment experiments (Fig. 3). Experiments commenced in June and lasted through September. The first trial focused on *Heliomeris multiflora*, while the second trial utilized *Bouteloua gracilis*. 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 (Fig. 4). Established plants that survived in 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. Data analysis efforts are being led by Dr. Winkler and will inform any modifications needed to the protocols before the FY22 experiments. Insufficient FY21 funding required that we perform GRID experiments only at the Flagstaff garden, and further experimental modifications may be necessary to ensure that the collected data are meaningful in the absence of data from the paired garden. Data will be available as a ScienceBase data release coinciding with the publication of a manuscript.

Figure 3. Experimental drought garden (i.e., GRID garden) in Flagstaff, AZ. (A) Week 6 of the *Heliomeris multiflora* experiment. (B) Harvested *Bouteloua gracilis* individual. (C) Aboveground biomass was separated from belowground biomass – biomass components were weighed and dried to investigate source specific drought treatment responses.

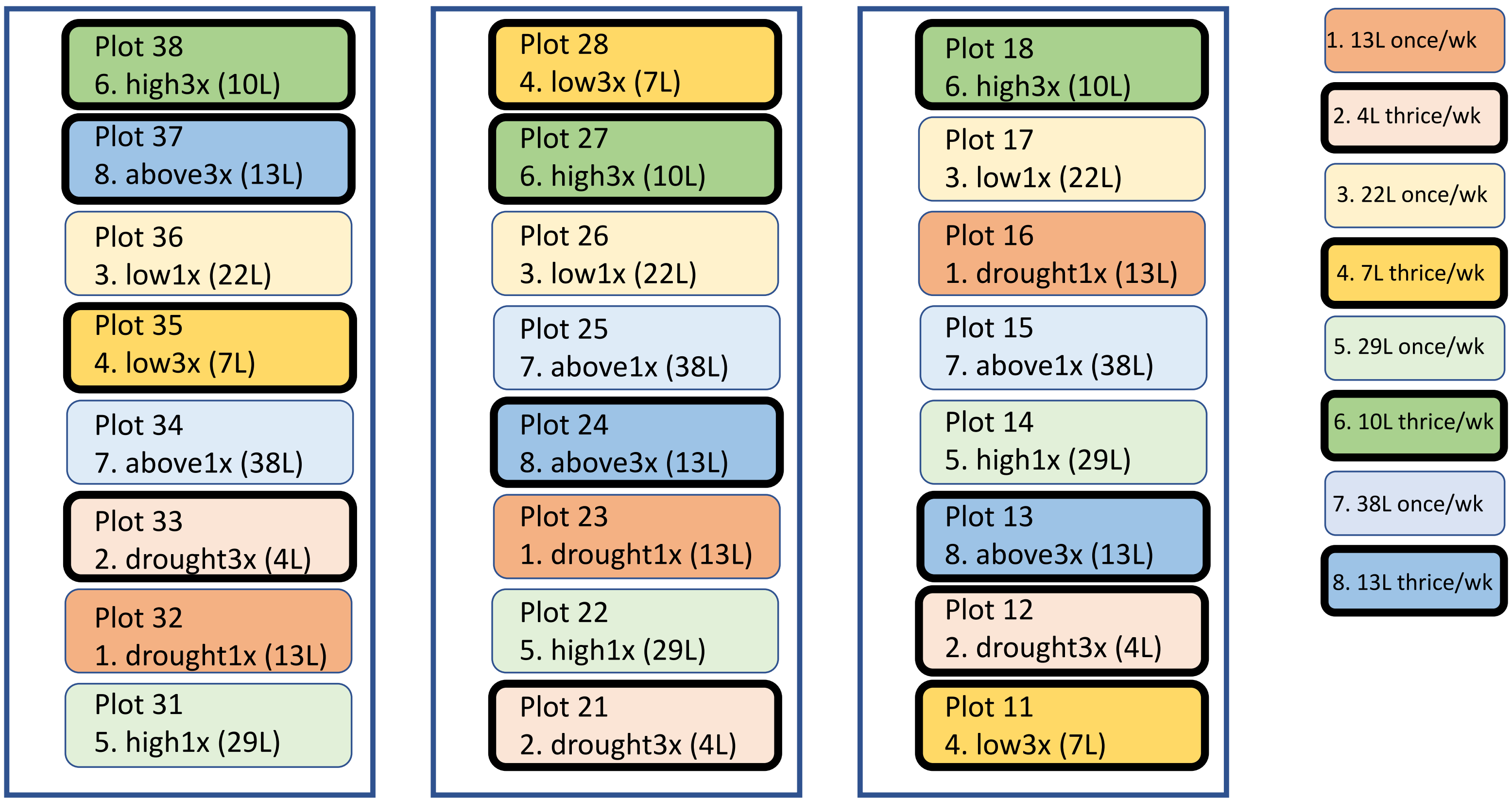

Figure 4. Example watering protocol for an experiment utilizing the GRID infrastructure. Each vertical rectangle represents a hoop house at the Flagstaff garden (three total), and each hoop house contains eight plots represented by the colored boxes. Plots with thick black borders receive water three times per week, while those without thick black borders receive water once per week (watering legend on the right side of the diagram).

Research 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), 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 as a result 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?

FY21 Results and Discussion

Seed sources for priority restoration species were selected with the BLM Coordinator in November 2020 and sent for agricultural production 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.* SBSC technicians will request increased seed from these partners in summer/fall 2022, which we will germinate at the Northern Arizona University research greenhouses to obtain leaf tissues for genetic analyses. In addition, SBSC technicians will germinate seed from the original seed sources (underway at the NAU greenhouses), as well as collect tissue samples (when needed) from the wildland plant populations. When sequencing data are in hand (planned FY23), SBSC will investigate how genetic diversity changes from wildland sources to production fields to increased seed, which will inform native plant material production practices.

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." Similar to CPNPP-funded production research, the goal of 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 and New Mexico BLM. Research will be led by Dr. Winkler, with assistance from Drs. Faist (NMSU), Fuentes-Soriano (NMSU), Reed (USGS), Jones (ARS), and Ashlee Wolfe (SWSP), and will directly augment CPNPP research interests.

Research 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), and that the use of non-local restoration materials may have impacts on other organisms in pre-existing communities (Vandegehuchte et al. 2012). 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 (in terms of individuals) 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?

FY21 Results and Discussion

We are using 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) to identify restoration sites and focal species to include in our research design. We are narrowing down the list of focal species by determining those that have been used most frequently within restoration treatments. Furthermore, we are narrowing down sites by selecting those that contained the focal species pre-disturbance/treatment. During 2021 fieldwork, SBSC technicians collected 1,912 leaf tissues for 26 species at 18 restoration sites (Fig. 1). Leaf tissue sampling was facilitated using geospatial layers representing restoration treatments, and focal species were collected at multiple distances within and outside of restoration treatment boundaries. Sampled plants were geolocated to support future seed collections. Leaf tissue samples are being stored at SBSC in Flagstaff, Arizona. All data will be stored and made available according to details on the 5-year plan. These data will illuminate the risk of using germplasms in restoration that are not genetically appropriate and may provide quantitative support for using the right seed in the right place at the right time.

Additional activities

Beyond research and products aligning with Research Goals 1-5, SBSC researchers participated in an array of additional activities supporting CPNPP. Dr. Massatti and colleagues reported fieldwork accomplishments (yearly reports for state BLM offices and Navajo Nation on collecting activities), composed internal and public versions of the BLM End of Year 20 report (Massatti et al. 2020), and provided an FY22 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. A common venue for disseminating research and interacting with other Colorado Plateau researchers was at scientific and stakeholder meetings. CPNPP-related research was presented at the Natural Areas Conference (October 2020), the Botany Conference (July 2021), and the Society for Ecological Restoration (June 2021). Finally, Dr. Massatti participated in Southwest Seed Partnership research committee meetings and National Seed Strategy Federal Implementation Working Group meetings, and participated in a perspective journal article discussing the benefits of the native plant material development process as implemented in the United States (McCormick et al., 2021).

A broad array of other activities aligned with CPNPP were supported by researchers and technicians in FY21. 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 will serve as a point where all data releases, papers, and programs (e.g., the Native Plant Seed Mapping Toolkit: https://rconnect.usgs.gov/seed-toolkit/) that have been assessed in accordance with the 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; a manuscript will be submitted in FY22. Finally, Dr. Massatti and technicians supported the maintenance and use of CPNPP seed collections in freezers located at the USGS campus in Flagstaff, Arizona. Seed collections were both newly cataloged (e.g., collections made by Seeds of Success crews) as well as distributed to researchers. These types of activities will continue in FY22 in support of the CPNPP mission.

Conclusion

As a result of FY21 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 material 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 (FY22-FY23). 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 material development for regional use are more informed, and there is strong momentum for continuing to provide knowledge that will improve restoration outcomes.

Literature cited

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Appendix 1. List of FY2021 products and presentations relevant to the SBSC-CPNPP partnership.

Papers:

- Massatti R (2021) Genetically-informed seed transfer zones for *Cleome lutea* and *Machaeranthera canescens* across the Colorado Plateau and adjacent regions. Report deliverable to the Bureau of Land Management.
- Massatti R, Winkler DE, Reed SC, Duniway M, Munson S, Bradford JB (2021) Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 2020 Report). Report deliverable to the Bureau of Land Management.
- Winkler DE, Massatti R, Reed SC (2020). Forward-looking dryland restoration in an age of change. Native Plants Journal, 21(3) 268-274.
- McCormick M, Carr A, Massatti R, Winkler DE, DeAngelis P, Olwell M, Native seed development process: How to increase the supply of native seeds to improve restoration success. Restoration Ecology, 29(8) e13499.
- Jones MR, Winkler DE, Massatti R (2021) Demographic modeling informs functional connectivity and management interventions in Graham's beardtongue. Conservation Genetics, 22(6): 993-1003.
- Jones MR, Winkler DE, Massatti R (2021) The demographic and ecological factors shaping diversification among rare *Astragalus* species. Diversity and Distributions, 27(8): 1407-1421.
- Hodel RGJ, Massatti R, Bishop SGD, Knowles LL (2021) Testing which axes of species differentiation underlie covariance of phylogeographic similarity among montane sedge species. Evolution, 75(2) 349-364.

Presentations

- Natural Areas Association presentation: Massatti R, Jones MR, Kilkenny FF, Winkler DE, Using species; patterns of genetic differentiation as the foundation for seed transfer guidelines. October 2020.
- Botany Meeting presentation: Massatti R, Jones MR, Winkler DE, Informing conservation and restoration management using spatially-explicit estimations of genetic diversity. July 2021.
- Society for Ecological Restoration: Winkler DE, Jones MR, Massatti R, Guiding restoration decision-making with modern, robust, and usable genetic information. June 2021.