

Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 2020 Report)

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Cover photograph:

James' galleta grass (*Pleuraphis jamesii*, foreground) in a shrub dominated plant community typical of lower elevations on the Colorado Plateau. Photograph by Shannon Lencioni, USGS, public domain.

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). 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 precipitation is generally low and highly variable. Providing this information requires synergistic research efforts that build upon insight gained from earlier investigations. The U.S. Geological Survey (USGS) Southwest Biological Science Center's (SBSC's) research activities that supported CPNPP in FY20 followed the FY20 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), Department of Interior Secretarial Order #3347 (*Conservation Stewardship and Outdoor Recreation*), and Bureau of Land Management Leadership Priority #1 (*Create a conservation stewardship legacy second only to Teddy Roosevelt*).

Research activities in FY20 centered on landscape genomics, implementing a common garden experiment near Vernal, UT, conducting experimental treatments using the GRID (Germination for Restoration Information and Decision-making) framework, and collecting seeds and leaf tissues in the field in preparation for future experiments. These activities were supported by five biological science technicians. The SARS-CoV-2 pandemic delayed some aspects of the FY20 workplan, especially for contract laboratory services and the construction of the GRID garden in Flagstaff, AZ. 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 FY20, other scientists, including Drs. John Bradford, Seth Munson, Mike Duniway, Sasha Reed, Matt Jones, and Daniel Winkler, spent a considerable amount of time providing expertise and support for individual projects. Work activities performed in support of each goal are discussed in turn.

Resolving patterns and drivers of genetic diversity, structure, and adaptation using landscape genetic approaches

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 genetic 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 genetic structure and variation of plant populations. 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 and spatial variation in standing genetic diversity is lacking (Wood et al., 2015).

FY20 Results and Discussion

During 2020 field work, SBSC collected leaf tissues for CPNPP priority species at environmentally stratified sites across the Colorado Plateau. Because of extensive collecting from 2017 to 2019, few tissue collections were required in 2020, and those that were made were highly targeted. **In total, biological technicians visited 31 sites and made 33 collections of 7 species. These efforts resulted in 306 total tissues (Fig. 1).** Leaf tissue samples are being stored at SBSC in Flagstaff, Arizona. In addition, we requested and sampled herbarium loans from regional herbaria so that genetic variation can be represented across species' distributions.

Data generation is complete or ongoing for ten priority restoration species (see Table 1). After data are available, they are analyzed using the USGS high performance computing cluster. Progress on genetic projects, which include tissue collection, laboratory work and genetic sequencing, and data processing and analysis, is on time (Table 1). Genetically informed seed transfer zones **have been published for *Pleuraphis jamesii*, *Sphaeralcea parvifolia*, and *Sporobolus cryptandrus*, *Machaeranthera canescens*, and *Cleome lutea*** (Massatti 2019a; Massatti 2019b; Massatti 2020a; Massatti 2020b), and shapefiles are publicly available on the Western Wildland Environmental Threat Assessment Center's website (<https://www.fs.fed.us/wwetac/threat-map/TRMSeedZoneData.php>). Important to note, **seed transfer zones reported for these species reflect both patterns of inferred adaptation (e.g., the species' larger inferred response to latitudinal environmental gradients compared to elevational environmental gradients) AND patterns of genetic differentiation that may affect restoration outcomes if not properly accounted for** (see more below). These are the first seed transfer zones available that provide a synthesis of these types of data. Data analysis supporting genetically informed seed transfer zones was also initiated for *Achnatherum hymenoides*; because preliminary genetic results supported the already established seed zones for this species (Johnson et al. 2012), we chose to not provide redundant zones that would confuse the available guidance. Data analysis is ongoing for these six species, as there are additional results that may influence restoration considerations. Upcoming genetics projects 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 will be led by 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 materials production questions from FY21-FY23. Data will be released according to the schedule in Table 1 and will include molecular data and genetically informed maps of seed transfer zones. All data will be made publicly available as official data releases that have gone through internal review at USGS to ensure that they meet the Fundamental Science Practices guidelines. Publication and data releases finalized in FY20 and that were reported in detail in the End of Year FY19 report (Massatti et al. 2020b) include: Massatti (2020c); Massatti and Knowles (2020); Massatti and Shriver (2020); Massatti and Winkler (2020); Massatti et al. (2020a); and Winkler and Massatti (2020).

To support the development of species-specific seed transfer guidelines that take into account inferred patterns of adaptive and neutral genetic differentiation, Dr. Massatti and colleagues have developed an R package (POPMAPS, or Population Management through Ancestry Probability Surfaces) to extrapolate species' genetic patterns across landscapes. First, this package tests whether geographic distance or environmental factors best explain a species' spatial patterns of genetic variation. Next, POPMAPS utilizes empirical genetic data to generate a probability map of genetic identity across the entire species' range, accounting for uncertainty 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 being finalized. POPMAPS and associated data will be available on the USGS github website.

Dr. Massatti had small contributions to genomic projects adjacent to CPNPP in FY20. He provided analytical expertise to GBNPP for their ongoing landscape genomic research on *Machaeranthera canescens*, *Erigeron speciosus*, 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).

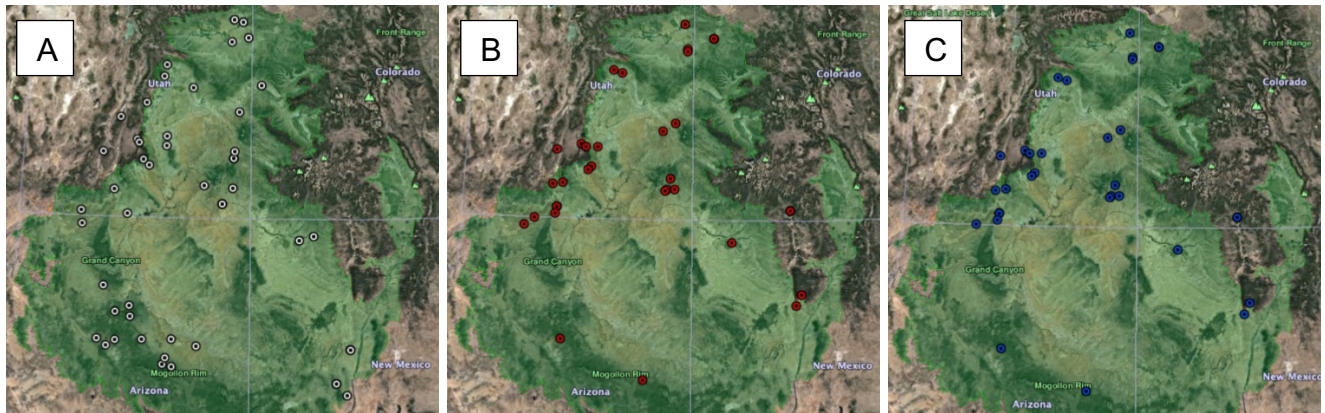


Figure 1. Colorado Plateau collection sites for seeds (A), tissues for genetic analyses (B), and tissues for trait analyses (C) during 2020.

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
<i>Pleuraphis jamesii</i>	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
<i>Sporobolus cryptandrus</i>	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
<i>Sphaeralcea parvifolia</i>	Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release			
<i>Achnatherum hymenoides</i>		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release		
<i>Cleome lutea</i>		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release		
<i>Machaeranthera canescens</i>		Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release		
<i>Heliomeris multiflora</i>			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
<i>Astragalus lonchocarpus</i>			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
<i>Cleome serrulata</i>				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release
<i>Elymus elymoides</i>				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release

Determining adaptive phenotypic variation in natural populations using 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 population sources of a species in a common environment (and replicated across environments) enables researchers to tease apart local adaptation, phenotypic plasticity (genotype-by-environment interactions), 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 able to thrive under specific climate conditions, 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. 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 performances of populations in experimental and/or common garden environments (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 carry out labor-intensive grow-outs. 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.

FY20 Results and Discussion

During 2020 field work, SBSC technicians finished collecting plant trait data at environmentally stratified sites across the Colorado Plateau to fill in gaps for specific geographic locations and species. **Specifically, technicians collected 296 tissues for 6 species across 30 sampling locations (Fig. 1).** Tissue samples are being stored at SBSC in Moab, UT, and raw data are being generated by a biological technician. This work is supported by laboratory space made available by Dr. Sasha Reed. Furthermore, Dr. Winkler is spear-heading data analysis and preparation of a manuscript that details patterns in leaf area and isotope ratios for fifteen species, 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*. Nearly all data are in hand – however, data from a final batch of isotope samples have not yet been received due to closures of laboratory facilities due to the SARS-CoV-2 pandemic. **These contributions by USGS researchers are not funded by CPNPP and represent a prime 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 when manuscripts are published; data will be available from the ScienceBase catalog. A draft manuscript detailing trait patterns for these species will likely be completed in FY21.

Work in FY20 to support common gardens centered on collecting seeds for priority species and establishing common garden experiment on a decommissioned well pad south of Vernal, UT. **Collection efforts resulted in 49 seed collections made for 9 species at 45 sampling locations (Fig. 1).** Seeds are being cleaned and stored at SBSC in Flagstaff. Common garden infrastructure was established in September and October 2020 (Fig. 2). For each species included in the experimental design (i.e., *Machaeranthera canescens*, *Sphaeralcea parvifolia*, and *Sporobolus cryptandrus*), twenty seed sources representing their environmental distributions across the Colorado Plateau were selected; fifty plants per source were grown in the 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, leveling the site, and erecting a barbed wire perimeter fence to exclude cattle. Seedlings were planted into approximately fifty 2 x 2-meter plots in early October 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. An



Figure 2. Common garden experiment established on a decommissioned well pad south of Vernal, UT. Photo credit: Morgan Andrews, USGS, public domain.

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 establishment in 2021.

Additional space is available at the common garden to support future plantings or additional experiments; one experiment discussed by USGS researchers involves seeding trials utilizing available native plant restoration materials for priority restoration species. Funding for additional experiments has yet to be obtained.

Research at the common garden in Santa Fe, NM funded by New Mexico BLM

continued in FY20. 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 Northern Arizona University under the supervision of Dr. Rachel Mitchell (NAU) and Dr. Winkler (USGS), again displaying how CPNPP benefits, at no cost, from the connections among researchers at SBSC and NAU. Data will continue to be collected in FY21 and beyond by a new graduate student working with Dr. Mitchell. In addition, this student will implement a rainfall manipulation experiment in the Santa Fe garden during FY21 utilizing *Plantago patagonica* SOS and USGS seed collections. A manuscript reporting analyses from the initial data collection efforts will be submitted in FY21, and data resulting from this effort will be available at a public data repository following publication.

Quantifying seed survival and establishment in the context of growing aridity using the GRID experimental framework

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 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 (Feddesma, 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 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 in 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 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 better be able to cope with increasingly arid environmental conditions.

FY20 Results and Discussion

GRID infrastructure adjacent to the USGS campus in Flagstaff, AZ was constructed and utilized in FY20 (Fig. 3). Specifically, three hoop houses were erected to exclude natural precipitation to 24 plots, which are each hydrologically isolated using plastic barriers buried to a maximum depth of one meter. Pre-existing soils were amended with sand to more closely mimic soil conditions common across lower elevations of the Colorado Plateau, and probes were buried in each plot to measure soil moisture at various depths. This experimental design mirrors the infrastructure at Canyonlands Research Center (CRC) south of Moab, UT such that parallel experiments at CRC and Flagstaff result in data that can be jointly analyzed. Due to USGS and CRC closures in early spring/summer 2020 resulting from the SARS-CoV-2 pandemic, infrastructure was not completed until early August, causing a delay in the initiation of GRID experiments.

GRID trials at CRC and Flagstaff commenced in August 2020 with *Machaeranthera canescens* seed sources. Watering treatments were designed to mimic the range of precipitation conditions that the seeds 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). The watering protocols were modified compared to the 2019 GRID trial at CRC to ensure a period of moisture availability supporting germination was present before imposing watering treatments representative of the species' source populations. Seedlings were harvested throughout the experimental treatments and at the end of the study; collected data included germination date, root measurements, and fresh/dry weight. Data analysis efforts are being led by Dr. Winkler and will inform any modifications needed to protocols before the FY21 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.

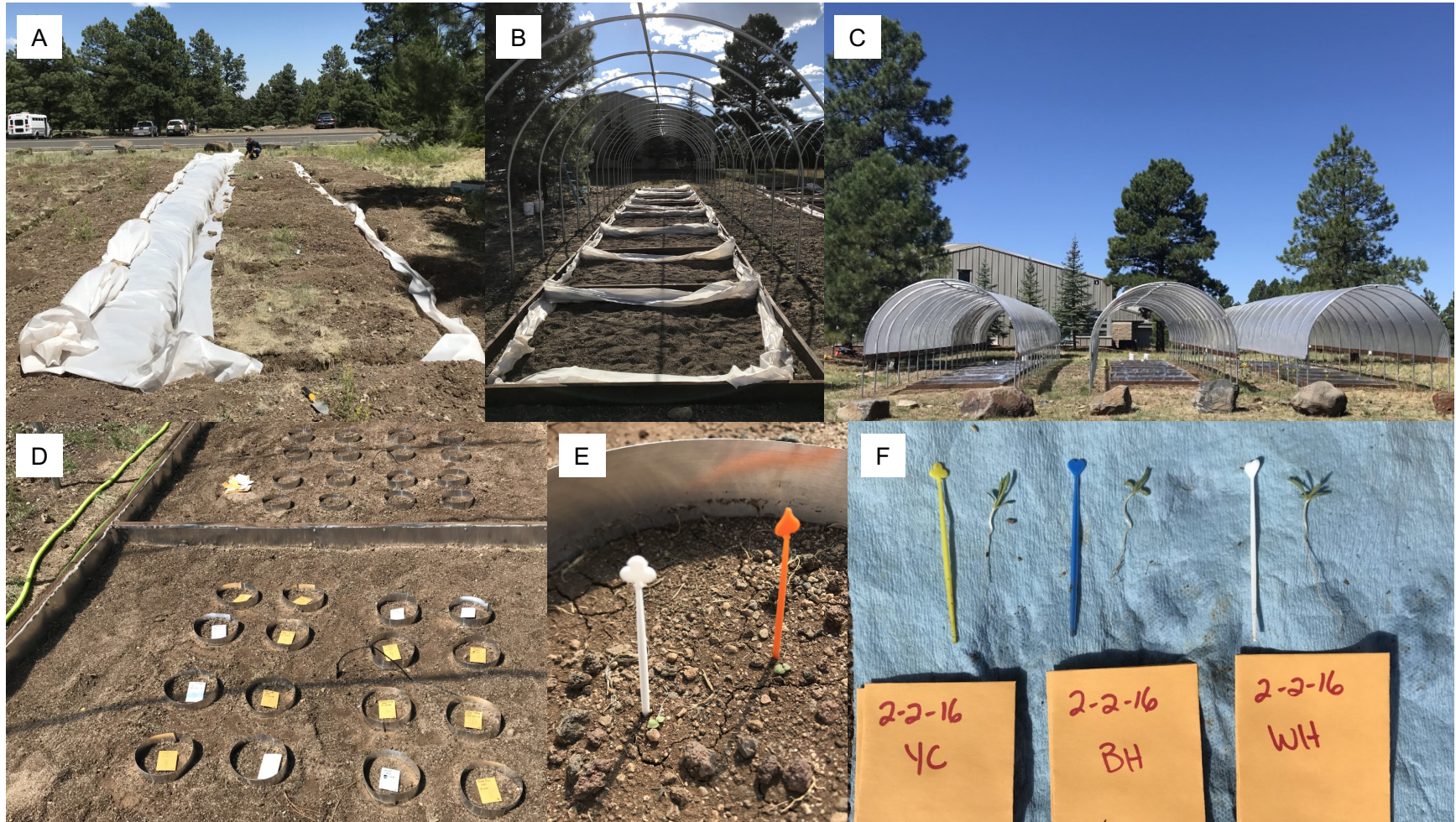


Figure 3. Experimental drought garden (i.e., GRID garden) established in Flagstaff, AZ in 2020. After site selection, plots were trenched and hydrologically isolated with a plastic barrier (A), followed by hoop house and plot frame construction (B). Experiments commenced once the infrastructure was finalized (C). Seed sources were sown into metal collars (D) before watering treatments began. Individual germinants were tracked (E) and harvested (F) at multiple points during the experiment. Photo credits: Morgan Andrews (A, B, D, E), Rob Massatti (C), and Sarah Sterner (F), USGS, public domain.

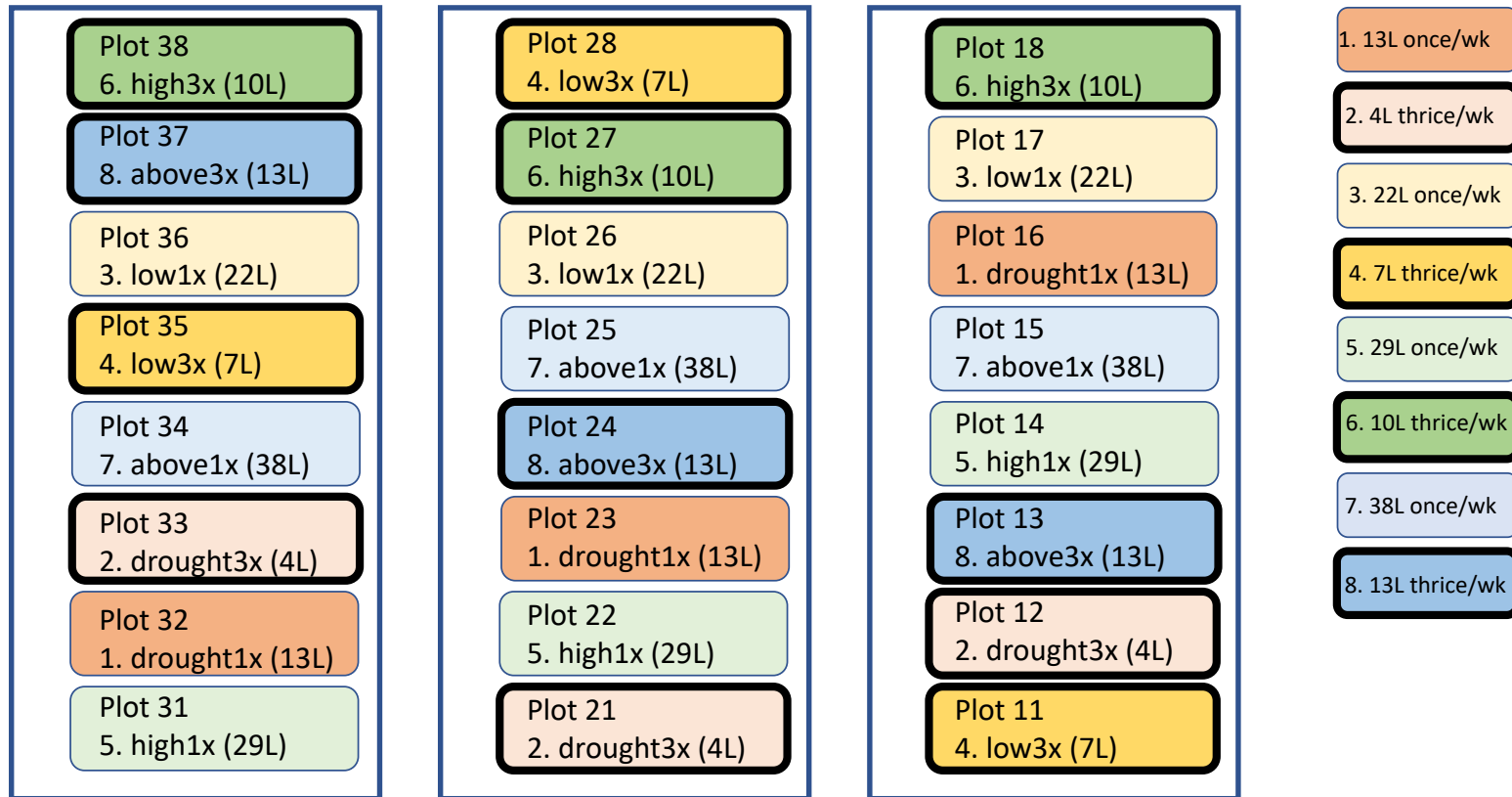


Figure 4. Example watering protocol for an experiment utilizing the GRID infrastructure. Each long rectangle represents a hoop house at the Flagstaff garden (three total), and each hoop house contains eight plots represented by the colored boxes. Plots with thick black borders receive water three times per week, while those without thick black borders receive water once per week (watering legend on the right side of the diagram). Within each plot, 16 metal collars are sown with seeds representing eight seed sources (see Fig. 3D; each seed source is replicated one time in each plot). Given this design and assuming twenty seeds are sown into each collar, each seed source is sown in 48 metal collars (960 seeds total) and subjected to 8 different watering treatments.

Additional activities

Beyond research and products described above, 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 the Navajo Nation on collecting activities), composed internal and public versions of the End of Year 19 report (Massatti et al. 2020b), and provided an FY21 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. Dr. Massatti presented information on the genetic considerations for restoration at the Society for Ecological Restoration – Southwest Chapter (11/9/19), the Intermountain Plant Conference (11/6/19), the Colorado Plateau Native Plant annual meeting (2/25/20), the Natural Areas Conference (10/14/20), the U.S. Fish and Wildlife Service Conservation Science webinar series (8/5/20), and the USGS Ecosystem Mission Area Friday's Findings webinar series (9/4/20). Finally, Dr. Massatti participated in Southwest Seed Partnership steering and research committee meetings and National Seed Strategy Federal Implementation Working Group meetings.

A broad array of other activities aligned with CPNPP were supported by researchers and technicians in FY20. Dr. Massatti updated and maintained a public-facing webpage on the SBSC website (<https://www.usgs.gov/sbnc/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 Climate Partitioning Tool and Seed Selection Tool resulting from Doherty et al. 2017) 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 (see Goal 1 in the 5-year plan). 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. 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. Dr. Massatti also assisted seed source selection for species that will be tested or increased in farm settings at Great Basin Research Center and BFI Native Seeds. Technicians are growing these seed sources at the NAU research greenhouses to collect tissue samples for future genetic analyses. These types of activities will continue in FY21 in support of the CPNPP mission.

Conclusion

As a result of FY20 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 three years (FY21-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 materials development for regional use are more informed, and there is strong momentum for continuing to provide knowledge that will improve restoration outcomes.

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