

Supporting the development and use of native plant materials for restoration on the Colorado Plateau (Fiscal Year 18 Report)

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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 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). Many of the most complex information needs relate to identifying the appropriate plant species and populations 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. U.S. Geological Survey, Southwest Biological Science Center (USGS) research activities in FY18 followed the FY18 Statement of Work (SOW) (“Research supporting native plant materials development for the Colorado Plateau Native Plant Program, FY18”) 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 now outlined in the 2019-2023 5-Year Research Strategy (discussed below; hereafter referred to as the 5-Year Research Strategy). 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*).

The overall focus of activities in FY18 centered on landscape genetics and planning for common garden and other research projects. These activities were supported by two biological technicians that were hired and trained by Dr. Rob Massatti and Dr. Daniel Winkler. Many of the field-related activities, including plant trait measurement and seed/tissue collecting, were assigned to these technicians, which freed Dr. Massatti to work on other research objectives, including the time-consuming activities of processing and analyzing genetic data. A major challenge to field work in FY18 was the drought conditions that pervaded the Plateau during the spring field season. Due to low winter and spring precipitation, many plant communities did not green up in the spring, which prevented the biological technicians from collecting plant trait data, tissues samples, and seeds in many areas. To cope with the dry conditions, the technicians searched sites across a wider range of environmental space and considered a broader suite of species from which to collect data (i.e., species that may be included in research projects in future years). Monsoonal precipitation starting in July supported a late summer/fall field season and allowed the technicians to follow a more normal work plan. While Dr. Massatti was the only scientist supported by the USGS-CPNPP agreement in FY18, other scientists, including Drs. John Bradford, Seth Munson, Mike Duniway, Sasha Reed, Daniel Winkler, and Jayne Belnap, spent a considerable amount of time discussing individual projects and the newly developed 5-Year Research Strategy. Some of these discussions resulted in a publication by Dr. Winkler, in addition to a large group of researchers, practitioners, and others who work on the Colorado Plateau, concerning the restoration challenges facing the Plateau into the future and the types of efforts that may support successful restoration (Winkler et al., 2018). Due to the funding available in FY18, we were not able to establish common garden infrastructure as proposed in Objective 3. However, goals outlined in Objectives 1 and 2 were achieved, as were seed



collections and planning for common garden activities pertaining to Objective 3. Work activities performed in support of each Objective are discussed in turn.

Objective 1. Informing native plant materials development across the Colorado Plateau 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., 2013), 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, because they generate data that can help structure field-based experimental frameworks, thereby ensuring that experiments will provide the 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).

FY18 Results and Discussion

During 2018 field work, USGS collected leaf tissues for CPNPP priority species at environmentally-stratified sites across the Colorado Plateau. Data collection at some sites and for some species was hindered by dry spring growing conditions, as noted above. **Despite difficult spring collecting conditions, biological technicians visited 127 sites and made 243 collections for 23 species, resulting in 2,479 total tissues (Fig. 1).** Leaf tissue samples are being stored at USGS in Flagstaff, AZ. In addition, commercial germplasm sources for priority species were obtained and grown at Northern Arizona University's research greenhouses in Flagstaff, AZ to include in analyses, and herbarium loans from five regional herbaria were requested and sampled so that genetic variation can be represented across species' distributions.

Molecular data have been (or are being) produced for six species, including *Pleuraphis jamesii*, *Achnatherum hymenoides*, *Sporobolus cryptandrus*, *Machaeranthera canescens*, *Sphaeralcea parvifolia*, and *Cleome lutea*. After data are generated, Dr. Massatti uses the USGS high performance computing cluster (i.e., the Yeti supercomputer) to process and analyze data using a custom analytical pipeline (see the 5-Year Research Strategy for details). After the substantial time investment of establishing contracts with a sequencing facility and molecular lab in FY17, work is essentially on time (Table 1). In fact, data analysis is already occurring for *A. hymenoides*, one fiscal year ahead of schedule. In addition, because Dr. Massatti and the biological technicians have been collecting tissue samples for a broad suite of priority species during FY17 and FY18 field work, a portion of the FY19 and FY20 tissue collecting activities are already complete, depending upon which species are chosen for molecular analysis.



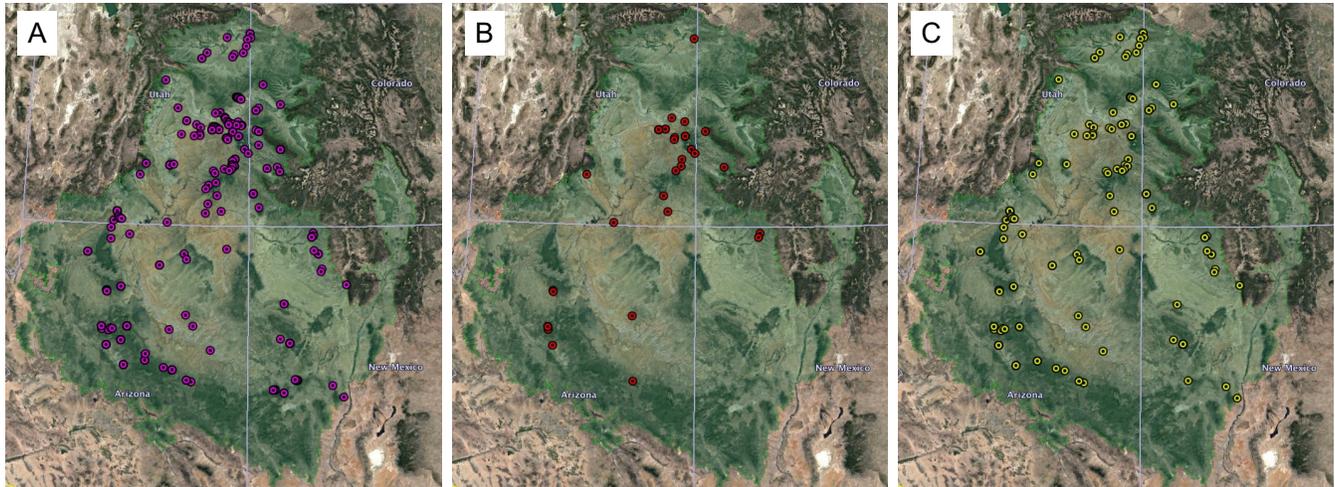


Figure 1. Colorado Plateau collection sites for plant tissues (A), seeds (B), and traits (C) during 2018.

Also note that the schedule in Table 1 reflects the 5-Year Research Strategy, in which focus will shift away from landscape genomics toward other genetically-oriented restoration and 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 will have gone through internal review at USGS to ensure that they meet the Fundamental Science Practices guidelines (<https://www2.usgs.gov/fsp/procedures.asp>). In FY19, we will collect leaf tissues for the priority species decided upon by USGS researchers and the CPNPP Coordinator and continue to work according to the schedule in Table 1.

Several other molecular-based research projects were supported in FY18 by Dr. Massatti. In order to bridge the gap between field-based and lab-based experiments, Drs. Massatti, Duniway, and Dave Hoover (USDA-ARS) initiated a collaboration to generate molecular data for *Pleuraphis jamesii* individuals that were included in the C4 Monsoon experimental garden (a project separately funded by CPNPP), where the goal was to test adaptive versus plastic responses of *P. jamesii* to the monsoonal precipitation gradient. Genetic data have been generated and analyses are underway. Dr. Massatti also finished a collaborative project with Great Basin Native Plant Project working on genetic patterns in *Pseudoroegneria spicata* across the Intermountain West. This project was initiated in FY17 before any CPNPP-related molecular data were available and provided Dr. Massatti the opportunity to develop and test the analytical pipeline that is now applied to CPNPP species. This collaboration resulted in a publication that supports the importance of applying molecular techniques to species of restoration interest so that appropriate knowledge is used to make well-informed decisions regarding the development and use of native plant materials (Massatti et al., 2018a). Finally, Dr. Massatti worked with Dr. Gery Allan (Northern Arizona University) to finish a project initiated by Dr. Troy Wood in FY15 (Massatti et al., 2018b). While this research aligns more with conservation genetics than restoration, it developed crucial information for the preservation of a rare plant (*Astragalus cremonophylax* var. *cremonophylax*) and adds information about patterns of genetic diversity across Colorado Plateau plant species.

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 Research Strategy, 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		
Priority species 1			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
Priority species 2			Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release	
Priority species 3				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release
Priority species 4				Tissue collection	Laboratory work; DNA sequencing	Data analysis; report writing, data release

Objective 2. Trait characterization of plant populations across their Colorado Plateau distributions

Understanding variation in plant traits within and among species can help researchers understand how species 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. Specifically, 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 of which combination of plant traits lead to enhanced performance in specific environments can inform managers and practitioners with respect to which plant materials may promote better restoration success. Furthermore, linking plant traits to ecological services, such as soil erosion control or wildlife habitat, can allow land managers to meet intended goals pertaining to bolstering ecosystem health. As such, it is imperative to assess plant traits in natural populations along the environmental gradients across which they persist.

FY18 Results and Discussion

During 2018 field work, USGS collected plant trait data including plant height, specific leaf area (SLA), and leaf tissues to quantify carbon and nitrogen levels at environmentally-stratified sites across the Colorado Plateau (Fig. 2). Data collection at some sites and for some species was hindered by dry spring growing conditions, as noted above. As such, instead of focusing on generating complete datasets for the FY17 and FY18 species included in genetics analyses (as explained in the FY18 SOW), our strategy was to collect data for priority species as they were encountered. The result of this protocol modification is that it will take at least one more field season to collect a full plant trait dataset for the original set of species (i.e., the species undergoing genetic analyses in FY17 and FY18), but the overall time that it will take to collect data on a larger set of species will remain the same (i.e., some future work is already complete, while some proposed work is incomplete). **Plant trait data were collected for 17 species across 88 sampling locations; specifically, we collected 815 leaf samples to characterize SLA and 1,242 tissues samples for**

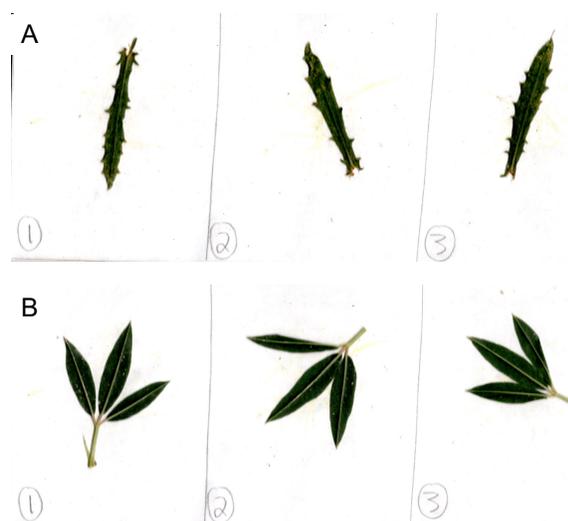


Figure 2. Leaf scans for (A) *Machaeranthera canescens* and (B) *Cleome serrulata* that will be used to calculate specific leaf area. Images taken by a USGS technician.

carbon and nitrogen analyses (Fig. 1). Trait-related tissue samples are being stored, and raw data are being generated (i.e., for tissue analyses), at USGS in Moab, UT by a biological technician. This work is supported by laboratory space made available by Dr. Sasha Reed. In FY19, we will continue to collect the trait data for the species initiated in FY18, as well as those that are slated for inclusion in common or experimental gardens. The data generated from these activities will be publicly available as similar data are generated from common gardens or experimental gardens. However, if these data can support the projects of other researchers, we will expedite their availability. This research effort was realigned in the 5-Year Research Strategy to fall directly within the common garden research objective, as these data will be used in conjunction with similar data collected from common gardens to understand the genotypic and phenotypic basis of plant traits.

Objective 3. Assessing genetically-controlled population performance using common gardens

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, USGS proposed to establish common gardens at environmentally stratified sites on the Colorado Plateau. Including multiple populations 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. 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.

FY18 Results and Discussion

Work in FY18 to support common gardens centered on collecting seeds for priority species that may be used to establish common gardens in future years. **Maternal line seed collections and bulk seed collections were both made for 13 species at 30 sampling locations. In total, 35 bulk and 364 maternal line collections were completed (Fig. 1).** Seeds are being stored at USGS in Flagstaff, AZ. While funding was not available to establish common gardens in FY18, we supported several other opportunities that will facilitate the inclusion of CPNPP priority species into common gardens in FY19. First, a common garden effort is progressing at Northern Arizona University (NAU) with Drs. Kevin Grady, Clare Aslan, and Karen Haubensak, which will incorporate multiple CPNPP priority forb species, including (at least): *Machaeranthera canescens*, *Heterotheca villosa*, *Sphaeralcea parvifolia*, and *Oenothera pallida*. They plan to establish gardens in 2019 in the Sierra Ancha (east of Phoenix, AZ), Flagstaff, AZ, near Carson City, NV, and others. The NAU research group agreed to collaborate with USGS, and especially Dr. Winkler, who has plant physiological experience that they lack. As such, portions (approximately 500 seeds) of some seed collections obtained by USGS biological technicians in 2018 were shared with the NAU research group to assist them in garden establishment according to the 5-Year Research Strategy, including *M. canescens* (2 collections), *H. villosa* (8



collections), *S. parvifolia* (3 collections), and *O. pallida* (3 collections). In addition, the NAU research group allowed USGS to have space in their gardens for two species not incorporated in their research design, including *Helioomeris multiflora* and *Sporobolus cryptandrus*, for which USGS provided 2018 seed collections. While USGS has minimal input on the experimental design, planning, and execution of these gardens, we are hopeful that our collaboration will generate useful data on CPNPP priority species. We note that it is imperative to have additional gardens representing the climate spaces not covered by the NAU efforts and that can include species that are high priority for CPNPP. The power for inferring traits important to plant survival on the Colorado Plateau will greatly increase as the common garden network grows.

A separate effort to establish a common garden is proceeding near Santa Fe, New Mexico under the guidance of New Mexico Bureau of Land Management (BLM) (Zoe Davidson), Institute for Applied Ecology (Melanie Gisler), NAU (Rachel Mitchell), and USGS (Dr. Massatti and Dr. Winkler). New Mexico BLM initiated a 5-year Inter-Agency Agreement with USGS in FY18 to support plant materials research and development for New Mexico. Funding will support a graduate student at NAU and the development of a common garden at the Petchesky Conservation Center near Santa Fe. This garden will be established in spring 2019 and will likely include two CPNPP priority species: *Heterotheca villosa* and *Sporobolus cryptandrus*. Seeds will be sown in NAU's research greenhouses in March 2019 and planted in June 2019. While the focus of populations to include in the common garden will center on the Arizona/New Mexico Plateau Ecoregion (and more specifically within New Mexico; seed provided by the Southwest Seed Partnership and New Mexico BLM), the garden will be large enough to include other populations. As such, Dr. Massatti and Dr. Winkler are working to select other Colorado Plateau populations to establish in the garden, with as much overlap with the NAU gardens (discussed above) as possible.

Activities in FY19 will encompass many of the activities detailed above. Biological technicians will collect seeds for CPNPP priority species during the 2019 field season, specifically with respect to species that may be established in gardens in the near future. We will continue to support the NAU and NM BLM common gardens, although until data collection is required, our involvement will be limited. In addition, much of the collaborative work (i.e., data collection and analyses) provided by USGS to these groups will be through Dr. Winkler, who is not supported by this Agreement. Should funding become available in FY19 to support common garden establishment, we will work to secure the site, build infrastructure, and ensure that we have the seed materials and experimental plan on hand so that plugs can be planted in the garden in either fall 2019 or spring 2020 (as explained in the FY18 SOW).

Additional activities

Beyond the activities detailed above, USGS researchers participated in an array of activities in support of CPNPP that were not outlined in the FY18 SOW. **A large accomplishment initiated in FY18 and completed in early FY19 was the development of the 5-year Research Strategy that will guide USGS research activities during the period of performance for the new USGS – BLM agreement.** This Research Strategy includes the following goals: 1) Provide scientific support to CPNPP; 2) Resolve patterns and drivers of genetic diversity, structure, and adaptation; 3) Determine adaptive phenotypic variation in natural populations; 4) Quantify seed survival and establishment in the context of growing aridity; 5) Investigate the impact of seed increase on the genetic identity of restoration materials; and 6) Investigate the long-term impacts of restoration materials on the genetic identity of plants in their natural communities. Outside of



drafting the Research Strategy and reporting on fieldwork accomplishments (e.g., creating yearly reports for state BLM offices and Navajo Nation on collecting activities), USGS scientists spent time providing guidance to researchers working on the Colorado Plateau, coordinating research efforts, and ensuring that communications across research groups are maintained such that efforts are not duplicated and that research efforts are synergistic whenever possible. A common venue for disseminating research and interacting with other Colorado Plateau researchers was at scientific and stakeholder meetings. For example, Dr. Massatti presented information on the genetic considerations for restoration at the 2018 Southwest Seed Partnership stakeholder meeting, the CPNPP annual meeting, and the Society for Ecological Restoration Southwest Chapter meeting. Information was also provided to managers, practitioners, and professionals in the form of tools and data layers. Specifically, the Climate Partitioning Tool and Seed Selection Tool (Doherty et al. 2017), which may guide habitat restoration and plant materials development, are hosted on the USGS website (https://www.usgs.gov/centers/sbsc/science/restoration-assessment-monitoring-program-southwest-ramps-0?qt-science_center_objects=4#qt-science_center_objects). In addition, species-specific provisional seed transfer zones (Doherty et al. 2017) were made publicly available on the Western Wildland Environmental Threat Assessment Center's website (<https://www.fs.fed.us/wwetac/threat-map/TRMSeedZoneData.php>) to help stratify seed collecting for ten priority restoration species on the Colorado Plateau. A final FY18 activity accomplished by Dr. Massatti was the reorganization of the CPNPP Seeds of Success seed accessions housed at USGS in Flagstaff, AZ. Due to limited capacity, two new freezers were purchased in FY18 and all of the seed accessions were reorganized so that responding to future seed requests will take less time. These types of “additional” activities will continue in FY19 in support of the CPNPP mission. For example, Dr. Massatti (and others) will organize a symposium at the 15th Biennial Conference of Science and Management on the Colorado Plateau & Southwest Region at Northern Arizona University focusing on research in support of CPNPP, as well as present results of genetic analyses at the Botany Conference in Tucson, AZ. The goal of these activities is to bring researchers with common interests together and to provide a forum to discuss restoration on the Colorado Plateau so that research continues in a collaborative and synergistic manner.

Conclusion

As a result of FY18 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 five years (FY19-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 in the notoriously difficult environments of the Southwest.



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