

Washington Rare Plant Care and Conservation
Rare Care *Ex Situ* Seed Storage

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Re: Final Report
BLM Work Order No. L09PX01310

Summary

Washington Rare Plant Care and Conservation (Rare Care) completed seven seed collections of sensitive plant species for the Rare Care *Ex Situ* Seed Storage project. The collections included one each of *Cryptantha leucophaea*, *Cryptantha spiculifera*, *Erigeron piperianus*, *Hackelia hispida* var. *disjuncta*, *Minuartia nuttallii* ssp. *fragilis*, *Penstemon eriantherus* var. *whitedii*, and *Petrophyton cinerascens*. In addition, an attempt was made to collect seeds of a second *Cryptantha leucophaea* population; however, the population could not be relocated. All seeds collected under this contract are stored in the Miller Seed Vault at the University of Washington Botanic Gardens. Including these collections, Rare Care now holds seeds of seventeen sensitive plant species from populations on Bureau of Land Management land in the seed bank. Rare Care also conducted germination tests of BLM collections held in the seed bank. Germination tests were completed for six collections held in the Miller Seed Vault and tests were initiated for six additional collections.

Introduction

Washington Rare Plant Care and Conservation (Rare Care) was contracted by the Bureau of Land Management (BLM) to collect and conserve seeds of species listed under the Endangered Species Act, those considered Species of Concern by the US Fish & Wildlife Service, and BLM sensitive species. The collections were made for *ex situ* conservation of germplasm to preserve the genetic diversity of individual populations. Seeds collected under this project were added to Rare Care's seed bank in the Miller Seed Vault. They will be held until the seeds are used or no longer viable.

Seeds collected under this contract are available for use by BLM for reintroductions and augmentation projects at ecologically appropriate sites. In addition, the seeds may be used for research or for reintroductions at other appropriate sites with the permission of BLM. For collections with more than 200 seeds, a small sample of seeds will be used for germination testing

to document seed viability and develop propagation protocols for the species. The seeds will not be used for other purposes without the approval of BLM.

Methods

Rare Care's seed-banking program is conducted in coordination with other Center for Plant Conservation institutions and follows the protocols presented in the Center for Plant Conservation's 2001 Guidelines of *Ex Situ* Conservation Collection Management (Menges 2004). Activities conducted under this project included seed collection, seed storage, and germination testing.

Species and populations targeted for collection in 2010 were selected based on discussions between Rare Care and BLM botanists. For each species, several potential collection sites were identified and final selection was made based on population size, seed availability, and access issues. The selected collection sites consisted of an entire element occurrence, as defined by NatureServe and tracked by the Washington Natural Heritage Program, or the portion thereof that occur on BLM lands and were accessible at the time of collection.

Activities conducted to collect and prepare seeds for storage included reconnaissance surveys, seed collecting, accessioning, and cleaning. Reconnaissance surveys were completed by the Rare Care program manager and volunteers trained in seed collection. The primary purpose of reconnaissance surveys was to determine the extent and size of the reproductive populations and to establish a timeline for seed maturation. Reconnaissance surveys were typically conducted when the species was in bloom. Populations were subsequently visited four to six weeks later to check seed maturation rates and to collect seeds.

All collections followed standard protocols conforming to the guidelines established by the Center for Plant Conservation (CPC). These protocols were designed to capture the genetic diversity represented in a population while minimizing the size of the collection in order to mitigate any potential negative impacts to the wild population. For most species, the target size of the collection was 20 to 30 seeds from 50 plants, for a total of 1,000 to 1,500 seeds. In accordance with CPC guidelines, the size of the collections did not exceed 10% of the seed production of the population, and no more 30% of the seeds from a single maternal plant were collected. Therefore, the actual size of the collection was dependent on the size of the population and availability of ripe seeds and in some cases was smaller than the target collection size.

In order to estimate the seed production of the wild populations, fruit from five to ten plants were sampled from each population to determine an average number of seeds per fruit and whether any seeds had been lost to seed herbivores, mold, or other circumstances. Seeds from the sampled fruit were saved for storage and the remainder were scattered on the ground in the vicinity of the maternal plant. The average number of fruit per plant was also estimated in order to determine the total number of ripe seeds available in the population based on the average number of seeds per reproductive plant.

Seed collections occurred between June and October, depending on when ripe seeds were available for each species. Collections were made by Rare Care staff and volunteers. All volunteers assisting in seed collections were required to attend a day of training on rare plant

monitoring. All seeds were collected and stored along maternal lines. Paper coin envelopes were used to hold the seeds from one maternal plant. The seeds were placed in the Miller Seed Vault storage room as soon as possible after the collection, usually within 48 hours. Ambient conditions in the storage room were 22% relative humidity and 15° C, which is optimal for drying orthodox seeds (Walters 2004). The seeds were dried for at least one month in order to achieve a moisture content of approximately 6 to 8%. Seeds collected in 2010 are currently being cleaned and counted by trained volunteers. Cleaned seeds will be packaged in aluminum foil packets, heat-sealed, and placed in the freezer where they will be held at -18° C.

Rare Care typically conducts germination testing on most collections held in the vault to document seed viability and track changes of viability over time. Testing is done for all collections that have a sufficient number of seeds and for species that can be propagated using standard horticultural techniques. Orchid seeds are not tested due to the specialized techniques required to propagate them.

Testing was conducted in accordance with Rare Care's protocol and AOSA Rules for seed testing. Move-along experiments (*sensu* Baskin and Baskin, 2004) were used to initially test species for which germination protocols are not already established. Under this method, seeds were moved through a sequence of temperature regimes that simulated seasonal temperature changes in their natural habitat. For Washington species, we used three temperature steps as follows: 5/2° C for winter, 15/8° C for fall/spring, and 24/14° C for summer. The experiments included four treatments - two controls and two move-along sequences, as follows:

- Treatment A: 5/2° C constant
- Treatment B: 24/14° C constant
- Treatment C: 5/2° C (8 weeks) → 15/6° C (4 weeks) → 24/14° C (8 weeks) → 15/6° C (4 weeks) → 5/2° C (8 weeks) → 15/6° C (4 weeks) → 24/14° C (8 weeks)
- Treatment D: 24/14° C (8 weeks) → 15/8° C (4 weeks) → 5/2° C (8 weeks) → 15/6° C (4 weeks) → 24/14° C (8 weeks) → 15/6° C (4 weeks) → 5/2° C (8 weeks)

Each treatment consisted of one Petri dish with 5 to 10 seeds each, for a total sample size of 20 to 40 seeds (depending on availability). Seeds were placed on moist filter paper and exposed to light and dark conditions simulating diurnal light cycles. They were checked weekly for germination. Non-germinated seeds were subsequently tested for viability by seed cutting and making a visual assessment of the condition of the embryo in accordance with AOSA Rules. Results of germination testing were recorded in a database and available to researchers and governmental agencies.

2010 Seed Collections and Accessions

In 2010, Rare Care completed seed collections from seven populations representing five listed and sensitive plant species on BLM land and attempted one additional collection. A summary of these collections follows. Information on the number of seed accessions resulting from these collections is presented in Attachment A.

Completed Collections

1. *Cryptantha leucophaea*

US Fish and Wildlife Service: Species of Concern

BLM Sensitive

Heritage Rank and State Status: G2G3, Sensitive

Seeds were collected on July 28, 2010 from one population of *Cryptantha leucophaea* located on sand dunes near Winchester Wasteway in Grant County (element occurrence [EO] number 62). The population consisted of 58 individuals scattered along the crest and behind one of the smaller dunes. The collection was very late this year because of the unusually wet June. Most of the inflorescences that appeared in May had withered without fruit development, but new inflorescences formed in late June that produced a smaller seed crop in July. This combined with the small size of the population resulted in a seed collection smaller than the target size.

2. *Cryptantha spiculifera*

BLM Sensitive

Heritage Rank and State Status: G4, Sensitive

Seeds were collected on June 24, 2010 from one population of *Cryptantha spiculifera* at Sentinel Mountain in Grant County (EO number 3). Although the population extends for more than five miles along the ridge top, most of the population is on private lands. Therefore, seeds were collected from the western portion of the population accessible from public lands. The population appeared to be healthy, with approximately 1,000 individuals observed. The collection resulted in the target size of 1,000 seeds.

3. *Erigeron piperianus*

BLM Sensitive

Heritage Rank and State Status: G3, Sensitive

Seeds were collected on June 24, 2010 from one population of *Erigeron piperianus* located along Keene Road in Benton County near Kennewick (EO number 71). The population of approximately 800 plants occurs in shrub-steppe habitat in residential area, and a portion of the population was destroyed by a housing development. The area appears to receive heavy foot and bicycle use. The remaining plants appear to be in good condition, and ample seed was available; therefore the collection resulted in the target size of 1,000 seeds.

4. *Hackelia hispida* var. *disjuncta*

BLM Sensitive

Heritage Rank and State Status: G4T2T3, Sensitive

Seeds were collected on June 23, 2010 from one population of *Hackelia hispida* var. *disjuncta*, located at Three Devils Coulee in Douglas County (EO number 17). The population is located on a talus slope where they are not disturbed by grazing, vehicles, or other land use activities. Plants appeared to be healthy and approximately 50% were reproductive. The number of seeds available per plant was somewhat limited; therefore the collection size was slightly lower than the target of 1,000 seeds.

5. *Minuartia nuttallii* ssp. *fragilis*

BLM Sensitive

Heritage Rank and State Status: G5T4, Threatened

Seeds were collected on July 14, 2010 from one population of *Minuartia nuttallii* ssp. *fragilis* located on Sentinel Mountain in Grant County (EO number 1). Although the population is patchily distributed on the bluffs over several miles, only the western portion on the ridge top was sampled for the seed collection because other areas could not be accessed across private lands. This species presents somewhat of a challenge for seed collection because the seeds were very difficult to see, and each flower remnant had to be inspected to determine whether there were any seeds. The plants occur on very steep slopes with very shallow soils over bedrock, making footing difficult. Many of the flowers (more than 80% on most plants) did not appear produce any seeds, and the remainder mostly contained only one seed. In addition, the collection may have occurred later than optimal and many of the seeds may have already dispersed. Therefore, the number of seeds per plant at the time of collection was very low, limiting the size of the collection.

6. *Penstemon eriantherus* var. *whitedii*

BLM Sensitive

Heritage Rank and State Status: G4T2, Sensitive

Seeds were collected on August 10, 2010 from one population of *Penstemon eriantherus* var. *whitedii* at Sentinel Mountain in Grant County (EO number 14). Although the population extends for more than five miles along the ridge top, most of the population is on private lands. Therefore, seeds were collected from the western portion of the population accessible from public lands. The population appeared to be healthy, with approximately 1,000 individuals observed. The collection resulted in the target size of 1,000 seeds.

7. *Petrophyton cinerascens*

US Fish and Wildlife Service: Species of Concern

BLM Sensitive

Heritage Rank and State Status: G1, Endangered

A seed collection was made of *Petrophyton cinerascens* at Earthquake Point in Chelan County (EO number 4) on October 13, 2010. The population of approximately 1,400 plants occurs on basaltic cliffs along the Columbia River. Seeds were also collected from this population in 2009, but the collection was limited to broken inflorescences found at the base of the cliffs. Therefore, the collection was repeated in 2010 in order to build the *ex situ* collection of this rare endemic plant. In 2010, the collection was also made from inflorescence fragments. More inflorescence fragments were found at the base of the cliff this year; therefore, the collection resulted in the target size of 1,000 seeds.

Attempted Collection

1. *Cryptantha leucophaea*

US Fish and Wildlife Service: Species of Concern

BLM Sensitive

Heritage Rank and State Status: G2G3, Sensitive

A reconnaissance site visit was made on May 19, 2010 to a population of *Cryptantha leucophaea* on the south slope of Saddle Mountain in the northeast quarter of Section 24 Township 15 North, Range 23 East (EO number 42). The site is located on a sand deposit in a narrow draw. The sand deposit is moderately vegetated and many of the associated species listed in the original sighting report were present. However, no *C. leucophaea* plants could be located despite a thorough search. Rare Care will add this site to the 2011 monitoring priorities list to have volunteer monitors visit the site to continue searching for this occurrence.

Seed Processing

All seeds have been dried and accessioned, and are currently being cleaned, counted and packaged for long-term storage. Once cleaned, the seeds will be stored at -18° C and become a part of the Rare Care seed bank collection. Copies of the seed collection reports will be sent to BLM once the seed cleaning is completed. An extra copy will be retained in Rare Care’s files.

Germination Testing

Germination testing was completed for six collections in 2010 and is currently underway for six additional collections. Germination tests take approximately 44 weeks to complete. Germination testing of seeds collected in 2010 will commence in 2011 once the seeds are cleaned and processed.

Twenty seeds for *Lomatium tuberosum* and 40 seed for each collection of *Oxytropis campestris* var. *wanapum*, *Polemonium pectinatum* and *Silene spaldingii* were tested in 2010. Each species was tested using four treatments: two move-along treatments and two controls. Results of the germination tests are shown in Table 1.

Table 1. Germination results for tests completed in 2010.

Species	Accession No.	Treatment			
		A	B	C	D
		5/2° C control	24/14° C control	Move-along (5/2° C start)	Move-along (24/14° C start)
<i>Lomatium tuberosum</i>	SV08C02.016-074	0%	0%	60%	60%
<i>Oxytropis campestris</i> var. <i>wanapum</i> ¹	SV08C05.187-234	90%	90%	80%	70%
<i>Polemonium pectinatum</i>	SV08C03.075-136	100%	100%	70%	70%
<i>Polemonium pectinatum</i>	SV08C04.137-186	90%	80%	90%	90%
<i>Silene spaldingii</i>	SV08C23.890-938	80%	50%	100%	70%
<i>Silene spaldingii</i>	SV08C24.939-963	80%	80%	70%	90%

¹Seeds scarified at start of test.

Germination rates for *Lomatium tuberosum* were 60% under the two experimental treatments that provided eight weeks of cold stratification followed by warm stratification. No germination was observed in the cold stratification control or the warm stratification control, indicating that the species requires cold stratification to break dormancy and that cold temperatures hinder germination after dormancy is broken. After cold stratification, germination started under spring conditions (15/6° C).

Good germination was achieved for *Oxytropis campestris* var. *wanapum* under all treatment conditions after scarification. This species does not appear to have physiological dormancy, and can germinate at all temperatures tested.

Good germination was achieved for *Polemonium pectinatum* under all treatment conditions. It does not appear to have physiological dormancy. Germination was delayed by cold temperatures, but the seeds did germinate in the cold treatment. Mold growth occurred on seeds from both collections tested, but was more severe on the collection represented by accessions SV08C04.137-186 and resulted in some seedling mortality. Although the seeds were not soaked in a bleach solution prior to the start of the tests, this would be recommended as part of the propagation protocols for this species.

Germination rates for *Silene spaldingii* ranged between 70 and 100% for treatments that provided cold stratification; however, germination was also observed in the summer control treatment but at a much lower rate. Germination occurred at all treatment temperatures. While 8 weeks of cold stratification was sufficient to achieve approximately 60% germination, higher germination rates were reached after approximately 12 to 16 weeks of cold stratification. These germination rates were comparable to rates achieved by other researchers (USFWS 2007). Similar to *P. pectinatum*, mold growth occurred on the seeds and caused seedling mortality; therefore, seeds should be soaked in a bleach solution before germination.

Conclusion

Washington Rare Plant Care and Conservation completed seven seed collections of seven sensitive plant species during the 2010 season. Collections made of *Cryptantha leucophaea*, *Erigeron piperianus*, *Hackelia hispida* var. *disjuncta*, *Penstemon eriantherus* var. *whitedii*, and *Petrophyton cinerascens* ranged between 1,000 and 1,500 seeds and provide adequate seeds for *ex situ* conservation of these populations. The collection for *Cryptantha leucophaea* resulted in approximately 400 seeds and was limited by the small number of plants in the population as well as the number of seeds available per plant. Finally the *Cryptantha spiculifera* and *Minuartia nuttallii* ssp. *fragilis* seed collections were smaller than the target size due to the limited number of seeds per plant. These collections should be repeated within the next five to ten years to augment the *ex situ* collection. One additional collection of *C. leucophaea* was attempted in 2010 but not completed because the population could not be located.

Good germination success was achieved for all species for which testing was completed in 2010. Cold stratification was required or significantly enhanced germination for two species, *Lomatium tuberosum*, and *Silene spaldingii*, but was not required for *Polemonium pectinatum* or *Oxytropis campestris* var. *wanapum*. Scarification was required for *O. campestris* var. *wanapum*. Mold

growth on the seeds was a problem for *P. pectinatum* and *S. spaldingii*, and the seeds of these species should be soaked in a bleach solution prior to germination.

References

Baskin, C.C. and J.M Baskin. 2004 Germinating Seeds of Wildflowers, an Ecological Perspective. HortTechnology 14(4): 467-473.

Menges, E.S., E.O. Guerrant, and S. Hamzé. 2004. Effects of Seed Collection on the Extinction Risk of Perennial Plants. pp. 305-324 in: Guerrant, E.O., K. Havens, and M. Maunder, eds. *Ex Situ Conservation: Supporting Species Survival in the Wild*. Island Press, Washington.

Walters, C. 2004. Principles for Preserving Germplasm in Gene Banks. pp. 113-138 in: Guerrant, E.O., K. Havens, and M. Maunder, eds. *Ex Situ Conservation: Supporting Species Survival in the Wild*. Island Press, Washington.

Attachment A

Table A-1. Summary of seed accessions of sensitive and listed plant species on BLM land held in the Miller Seed Vault.

Species	WNHP EO #	Accession Numbers ¹	Collection Year	No. of Seeds
<i>Astragalus sinuatus</i>	7	SV06.01-50	2003	5,169
<i>Cryptantha leucophaea</i>	none	SV03.133	2003	919
<i>Cryptantha leucophaea</i>	62	SV10C05.183-218	2010	385
<i>Cryptantha spiculifera</i>	3	SV10C03.093-142	2010	800 ²
<i>Erigeron piperianus</i>	71	SV10C02.043-092	2010	1,000 ²
<i>Erigeron basalticus</i>	7 (formerly 15)	SV03 135-186	2002	2,282
<i>Hackelia hispida</i> var. <i>disjuncta</i>	17	SV10C01.001-042	2010	1,066
<i>Lomatium tuberosum</i>	21	SV08C01.001-015	2008	92
<i>Lomatium tuberosum</i>	38	SV08C02.016-074	2008	425
<i>Minuartia nuttallii</i> ssp. <i>fragilis</i>	1	SV10C04.143-182	2010	300 ²
<i>Oxytropis campestris</i> var. <i>wanapum</i>	1	SV08C05.187-234	2008	1,345
<i>Penstemon barrettiae</i>	13	SV09C04.137-186	2009	1,795
<i>Penstemon eriantherus</i> var. <i>whitedii</i>	14	SV08C06.235	2008	17
<i>Penstemon eriantherus</i> var. <i>whitedii</i>	14	SV10C07.270-319	2010	1,000 ²
<i>Petrophyton cinerascens</i>	4	SV09C19.734-735	2009	894
<i>Petrophyton cinerascens</i>	4	SV10C18.372-713	2010	1,500 ²
<i>Phacelia lenta</i>	4	SV08C08.299-350	2008	6,575
<i>Phacelia lenta</i>	6	SV09C03.101-136	2009	1,437
<i>Polemonium pectinatum</i>	33	SV08C03.075-136	2008	5,221
<i>Polemonium pectinatum</i>	41	SV08C04.137-186	2008	4,001
<i>Silene spaldingii</i>	32	SV08C19.711-734	2008	728
<i>Silene spaldingii</i>	32	SV09C09.369-397	2009	1,169
<i>Silene spaldingii</i>	52	SV08C24.939-963	2008	1,338
<i>Silene spaldingii</i>	83	SV08C18.707-710	2008	89
<i>Silene spaldingii</i>	85	SV08C23.890-938	2008	2,200
<i>Silene spaldingii</i>	85	SV09C08.328-368	2009	1,771
<i>Spiranthes diluvialis</i>	4	SV08C26.966-980	2008	<.020 g
<i>Tauschia hooveri</i>	27	SV09C01.001-050	2009	459
<i>Tauschia hooveri</i>	28	SV09C02.051-100	2009	171

¹Each accession number represents one maternal plant except for accession SV03.133 of *C. leucophaea*.

²Number of seeds estimated based on field notes made by seed collector at the time of collection. An actual count will be available once the seeds are cleaned.