WASHINGTON NATURAL HERITAGE PROGRAM

Seedling Studies of *Eriogonum codium* (Umtanum wild buckwheat)

Prepared for
USFWS, Region 1

Prepared by
Florence Caplow

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Doug Sutherland - Commissioner of Public Lands

Seedling Studies of *Eriogonum codium* (Umtanum wild buckwheat): seedling dynamics, soil seed bank, seedling experiments, and propagation techniques

November 2005

Prepared by
Florence Caplow
Washington Natural Heritage Program
Department of Natural Resources
PO Box 47014
Olympia, WA 98504-7014

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Executive Summary

Erogonum codium (Umtanum wild buckwheat) is a recently described species from Benton County, Washington. *Eriogonum codium* is Endangered in Washington and a Candidate for federal listing. There were 5228 adult plants in the only known population in 1997, and 4418 adult plants in the population in 2005 (a 15% decline in the population in 8 years).

Eriogonum codium has been the subject of an intensive demographic monitoring project since 1997. Mortality has exceeded recruitment in the nine years since monitoring began. Seedling establishment appears to be the limiting factor: only one seedling is known to have survived to flowering. This report summarizes the results of a series of seedling-related studies: 1) the seedling portion of the monitoring study, 2) the seed bank studies from 2002-2005, 3) the cheatgrass and supplemental watering experiments (which were not successful), and 4) propagation technique development.

Seedling production varies between years. The highest year for seedling production was 1999 (125 seedlings). The lowest years for seedling production were 2002 and 2005 (0 seedlings). Total seedling production for the nine years of the study was 278 seedlings. Seedling mortality has been 100% from one year to the next, with the exception of four assumed 1996 seedlings that lived 1 to 3 years, one 1997 seedling that 1 year, one 1998 seedling that was still alive in 2005, and one 2004 seedling not found until 2005. I analyzed seasonal climate conditions (temperature and precipitation in winter and spring seasons of all years) and saw no clear correlations with seedling production or seedling survival. The 1998 seedling first flowered five years after germination.

The seed bank study utilized the services of Ransom Seed Laboratory to analyze the viability and degree of dormancy of both fresh seed and buried seed. Seed was buried in 2002 and samples were retrieved in 2003, 2004, and 2005. The results show a striking loss of viability after the first year of burial. Total viability was between 70% and 78% for the two control samples (including one kept cool and dry for a year), while total viability does not exceed 7% for any buried sample, and is generally below 5%. Viability remains at a low level during all years of burial, and did not significantly decrease with time of burial. The easily germinated fraction of the seed bank does not survive beyond the first year of burial.

The cheatgrass and supplemental watering studies were not successful, due to poor germination of *E. codium* in the studies. Greenhouse propagation in 2003 was successful, and flowering of garden-grown plants occurred within 18 months.

Acknowledgments

Many people and organizations have made this research possible. The Washington Field Office of the Nature Conservancy, the US Department of Energy, the Hanford Reach National Monument, the US Fish and Wildlife Service, and the Washington Native Plant Society have supported the monitoring work over the last 9 years. Peter Dunwiddie of the Nature Conservancy and Katy Beck of Beck Botanical Consulting were both central to the monitoring design, earlier data analysis, and fieldwork. Mark Darrach has volunteered his time for monitoring over the last four years, and led a high school science class at West Sound Academy in an achene counting project. Heidi Newsome of the Hanford Reach National Monument has helped with the monitoring, as have many other staff members from the Monument, the Washington Natural Heritage Program, and the Washington Field Office of the Nature Conservancy. Doug Reynolds of Rain Shadow Nursery did tremendous work developing propagation protocols. Nancy Vivrette of Ransom Seed Lab ran the seed germination trials and provided helpful guidance for the seed burial study, as did Ed Guerrant of the Berry Botanic Gardens.

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1. Introduction

Erogonum codium (Umtanum wild buckwheat) is a recently described species (Reveal et al. 1995). It is a woody, mat-forming perennial. The only known population occurs sporadically in a 2 km strip along the top edge of Umtanum Ridge in Benton County, Washington, in a pumice-like basalt flow top. *Eriogonum codium* is Endangered in Washington (WNHP 2005) and a Candidate for federal listing (USFWS 2005). There were 5228 adult plants in the population in 1997 (Dunwiddie et al. 2001), and 4418 adult plants in the population in 2005 (a 15% decline in the population in 8 years).

Eriogonum codium has been the subject of an intensive demographic monitoring project since 1997. Initial findings from 1997 through 1999 were reported in 2000 (Dunwiddie *et al.* 2000). In 2000 we concluded that *E. codium* is a long lived species (at least 100 years) with high flower production, low germination rates, high seedling mortality, and high variability of growth between individuals and between years. Annual adult mortality ranged from 0% to 4%. Mortality has exceeded recruitment in the nine years since monitoring began. The preliminary analyses suggested a declining population, which was confirmed by the 2005 census, showing a 15% decline.

Seedling establishment appears to be the limiting factor: in nine years of monitoring, only one seedling within the study area is known to have survived to flowering. Seed bank studies can help us understand the source of each year's seedling cohort, and what buffers there may be for poor seed production years. The causes for poor seedling survival are not known, although cheatgrass is present in the population and is known to successfully compete for scarce water resources (Melgoza et al. 1990). Prior to this study, *Eriogonum codium* had never been successfully grown in the greenhouse, and ex situ propagation can play an important role in conservation of rare species (Guerrant et al. 2005). This report summarizes the results of the seedling portion of the monitoring study, the seed bank studies from 2002-2005, the cheatgrass and supplemental watering experiments (which were not successful), and propagation technique development.

2. Seedling dynamics, 1997-2005

<u>Methods</u>

In 1997 a series of 24 1m X 2m permanent plots were randomly selected along three 50 meter belt transects within the largest subpopulation of *Eriogonum codium*. More than 100 individually tagged adult plants have been followed annually since 1997, and we have collected data on length and width of plants, number of inflorescences, and "percent dead" within each adult. Seedlings are mapped within the plots and are counted in May and again in July. We omitted the May seedling search in 1998, 2002, and 2003, since we were generally able to find the year's living and dead seedlings in July. For a detailed discussion of monitoring methods, see Dunwiddie *et al.* 2000.

Mortality between May and July has varied from 67% to 91% (Table 1). In general, we have been successful at re-finding May seedlings during the July survey, whether living or dead. This suggests that most of the year's seedlings were found in the 1998, 2002, and 2003 July survey.

Results and Discussion

Seedling production varies between years (Figure 1). The highest year for seedling production was 1999 (125 seedlings). The lowest year for seedling production was 2002 and 2005 (0 seedlings). The last four years (2001-2005) combined have produced only 10 seedlings, compared to 1998-2001, which produced 201 seedlings.

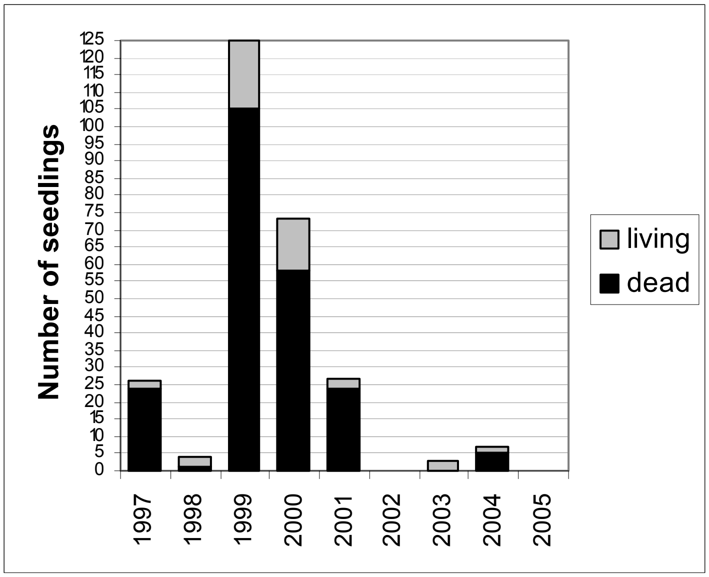

Figure 1. Number of living and dead seedlings of *Eriogonum codium* in July, 1997-2005

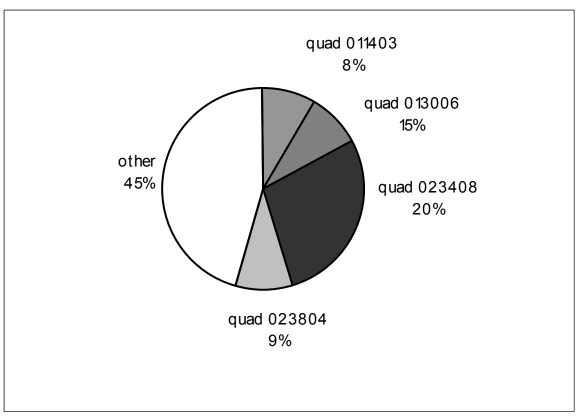

Figure 2. Percentage of seedlings produced by various plots, 1997-2005.

Seedling production also varies widely between plots (quads): four plots out of a total of 24 plots have produced 55% of the total number of seedlings counted since the study began (Figure 2), and two plots have produced no seedlings at all. No plots have produced seedlings in every year, and only two plots have produced seedlings in at least half the years.

Seedling mortality has been 100% from one year to the next, with the exception of four assumed 1996 seedlings that lived, respectively, one year (2) two years (1) and three years (1); one 1997 seedling that lived until 1998; one 1998 seedling not found until 1999 and still alive in 2005; and one 2004 seedling not found until 2005. The 1998 seedling first bloomed five years after germination. In several cases the seedlings that survived were not found during the July survey of the previous year, so they may have germinated later in the season. Seed germination trials conducted by Ransom Seed Laboratory in 1999 found that an average of 55% of the seed germinated, and nearly 5% of the seed was not dormant and germinated in 21 days with moisture and light. This suggests that a fraction of the seed would not require stratification to germinate and could potentially germinate during summer or fall. This is further suggested by the 1997 and 1999 data, in which more seedlings were found in July than in May.

Climate conditions are generally expected to play a role in seedling germination and survival. I analyzed seasonal climate conditions (temperature and precipitation in winter and spring seasons of all years) and saw no clear correlations with seedling production (Table 2). However, when climate data are summarized over all years between 1997 and 2005, precipitation has been below normal during all seasons, and summer temperatures have been above normal, compared to the period 1950-1995 (NOAA-CIRES Climate Diagnostics Center 2005).

Table 1. Total seedling production 1997-2005

	1997 June	1997 July	1998 July	1999 May	1999 July	2000 May	2000 July	2001 May	2001 July	2002 July	2003 July	2004 May	2004 July	2005 May	2005 July
living	23	2	2	121	20	55	15	26	3	0	3	6	2	0	0
dead	1	24	1	1	105	18	58	1	24	0	0	0	5	0	0
total	**24**	**26**	**3**	**122**	**125**	**73**	**73**	**27**	**27**	**0**	**3**	**6**	**7**	**0**	**0**

Table 2. Precipitation and snowfall 1997-2005

			Precip (in)				
Year	# seedlings	Snowfall (in)	Dec-Feb	Mar-May	Jun-Aug	Oct-Feb % normal	March-August % normal
1997	**26**	40.5	5.45	1.36	0.71	132.1	121.0
1998	**4**	8.1	2.7	1.09	0.86	110.9	92.5
1999	**125**	0.9	2.03	0.4	0.95	99.0	82.8
2000	**73**	9.3	2.28	2.28	0.71	91.2	82.8
2001	**27**	14.4	1.38	1.58	1.4	60.7	91.0
2002	**0**	10.7	1.89	0.64	0.82	94.6	70.7
2003	**3**	1.3	5.05	2.57	0.46	103.3	75.4
2004	**7**	28.4	5	1.46	1.8	102.3	113.8
2005	**0**	12.2	1.93	2.92	0.55	66.5	95.1

3. Seed bank studies

Methods

The seed bank study used an indirect method of evaluating the probable soil seed bank by collecting, burying on site, retrieving, and assessing viability of seed samples over time. The material used to store the seeds in the ground is water and gas permeable, and was believed to adequately mimic natural conditions in the soil.

On August 2, 2002, we collected inflorescences from plants from the two largest subpopulations: “powerline” (113 inflorescences) and “main” (84 inflorescences). We collected no more than 2 inflorescences per plant distributed throughout the subpopulations. We also made fallen flower detritus collections from the base of the plants in the powerline subpopulation to see if more seeds were there. Many plants had two kinds of inflorescences – some done flowering and others with fresh blooms.

On August 20, 2002, we cleaned the seed using a fine screen and a light with magnification. The flowers were hand rubbed (because the screens abraded seed) and then the seed was picked out with tweezers, put under a scope, checked (bad ones removed), and counted. The “main” subpopulation of 84 inflorescences produced 158 seeds or 1.88 seeds/inflorescence. The “powerline” subpopulation of 113 inflorescences produced 388 seeds or 3.4 seeds/infl. The fallen flower detritus collections produced 200 seeds. More careful counts of the same cohort by Mark Darrach and a high school science class found 10.68 achenes per inflorescence in the main subpopulation and 11.35 achenes per inflorescence in the powerline population (n = 37 for each population) (report on file with WNHP).

On November 1, 2002, 12 samples of 40 seeds each (subpopulations mixed) were divided into 3 groups of 4 samples each. A 13th sample was set aside for germination testing. Each sample was placed into a 8 by 15 cm packet made of 120µm Nitex nylon, which was then hand-sewn, and the edges sealed with iron-on hemming tape (the iron did not contact seeds and was set on lowest setting). Nitex is a gas and water permeable material used widely in biological sampling and filtration. Each group of packets was placed in a wooden frame divided into 4 quarters divided by cross-pieces. The frames were 50 cm X 50 cm. One packet was placed in each quarter of the frame, approximately 2 cm below the surface of the substrate, which was estimated to be the depth of seeds likely to germinate. The frames were placed along the edge of the main subpopulation, approximately 50 m apart. All frames were fixed to the ground with irrigation stakes. Frame #1 was also secured with rebar. Frames #2 and #3 have rebar nearby (uphill). A GPS unit was used to identify the location of all three frames.

On November 19, 2002, Ransom Seed Laboratory received a sample of 40 seeds for germination and viability testing. They subjected the seeds to their standard germination and viability protocol, which is the following.

1. Seed physically examined.
2. All seeds left for 21 days on top of blotter in light at 20^{o} C. Germinants counted.
3. Remainder cut through cotyledons, 400 ppm GA3 added to the medium and tested an additional 3 days on blotter in light at 20^{o} C.
4. Remainder tested with tetrazolium chloride.

I collected a random packet of seeds from each of the wooden frames on July 11, 2003, July 1, 2004, and July 6, 2005. They were sent to Ransom Seed Laboratory and subjected to the same protocol described above. In addition, I was concerned about the results of the 2003 sampling, and on October 15, 2003 I sent another sample of 40 seeds from the original collection that had been stored for a year.

Results

The results (Figures 3 and 4 and Table 2) show a striking loss of viability after the first year of burial. Total viability was between 70% and 78% for the two control samples (including one kept cool and dry for a year), while total viability does not exceed 7% for any buried sample, and is generally below 5%. Viability remains at a low level during all years of burial, and did not significantly decrease with time of burial. The easily germinated fraction of the seed bank does not survive beyond the first year of burial.

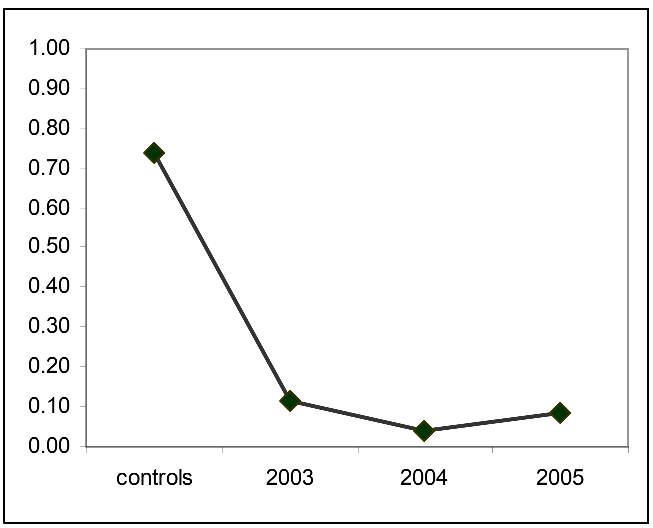

Figure 3. Mean percent seed viability in all samples, control and 2003-2005.

Discussion

Seed bank dynamics are complex, and artificial seed burial studies have drawbacks, among them a tendency to over-estimate the longevity of seeds in the soil, due to, among other factors, protection from seed predators (Thompson 1997). Researchers have also found longer survival of seeds at deeper soil depths (Csontos and Tamas 2003), and this study buried seeds at a very shallow depth (approx 2 cm) , which may have affected the results. In other words, these results cannot be said with certainty to mirror the soil seed bank dynamics. Nonetheless, the results do show that the percent viability of seeds of *E. codium*, when buried at 2 cm, drops sharply after one year and remains low, in comparison with seeds from the same cohort that were stored in a cool and dry location. Based on the soil seed bank classification of Thompson and Grime (1979) *E. codium* would be considered to have a transitory to short term persistent seed bank. This is consistent with some other woody, long-lived species (Csontos and Tamas 2003). Certainly, emergence of buried seeds that are more than one year old is likely to be a rare event.

The cause of seed mortality in the soil is not apparent. Most observations described non-viable seed from the stored samples as "empty". A few were described as "not intact and empty (i.e. these could have germinated in the packet)". Others were described as "intact and empty". Still others were described as "dead (pregerminated)", "dead (fungal bodies on seed coat)", or "abnormal". Clearly, some seed died as a result of fungal attack, and some seed may have attempted to germinate in the packet, but most of the seed died without obvious cause, and may simply not be long-lived.

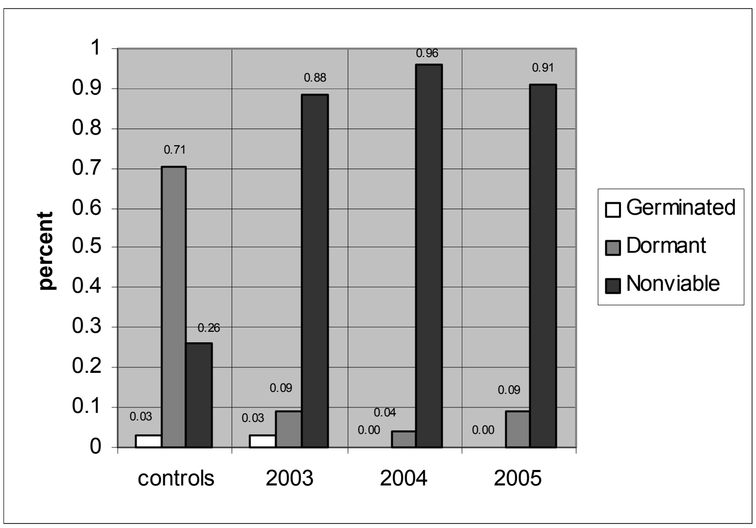

Figure 4. Percent seed that a) germinated, b) was dormant, or c) was non-viable, by year.

Table 3. Results of seed viability study. Numbers represent numbers of seed in each category.

	Germinated	Dormant-germinated with GA3	Dormant-viability assessed with TZ	Dead	Empty	Other Non-viable	Total Viable	Total Non-viable	Total
Control (11/2002)	2	26	0	9	0	3	**28**	**12**	40
Control (10/2003)	1	24	14	3	7	1	**39**	**11**	50
2003									
sample A	1	2	0	5	32	0	**3**	**37**	40
sample B	1	3	0	6	30	0	**4**	**36**	40
sample C	1	6	0	1	32	0	**7**	**33**	40
2004									
sample A	0	0	0	0	20	20	**0**	**40**	40
sample B	0	3	0	2	8	27	**3**	**37**	40
sample C	0	2	0	2	8	28	**2**	**38**	40
2005									
sample A	0	6	0	2	32	0	**6**	**34**	40
sample B	0	1	0	0	39	0	**1**	**39**	40
sample C	0	3	0	1	36	0	**3**	**37**	40

4. Cheatgrass competition and supplemental watering experiments

The intent of the cheatgrass experiment was to evaluate the effects of cheatgrass on seedling survival in a greenhouse environment (study design in Appendix A). Despite successful propagation of *Eriogonum codium* by Rainshadow Nursery in 2002, the seeds stratified in 2003 using the same methods and at the same nursery did not germinate, so the greenhouse experiment was aborted.

The intent of the supplemental watering field experiment was to evaluate the effects of supplemental watering on seed survival in situ. We used seed collected in 2002 and stored in a cool, dry location for 18 months. The seed was stratified on moist paper towels at Rainshadow Nursery beginning on 1/24/2004, and taken out of stratification on 3/9/2004. At that time many seeds had germinated and the radicles were visible. The seed was sorted into 6 samples of 33 seeds each. Six 25 cm by 25 cm plots were established: two in the vicinity of each of the seed burial plots. Each one was in an open area without other perennial vegetation. All plots were weeded of cheatgrass. The soil was watered thoroughly before planting, and 33 seeds per plot were placed with tweezers just below the surface of the soil. One of each set of plots was watered thoroughly on 4/21/04, 5/14/04, and 7/1/04. All plots were checked for seedlings at each visit and on all subsequent visits in 2004 and 2005. No seedlings were seen in any plot in either 2004 or 2005.

We know the viability of the 2002 seed both immediately after collection and more than a year later (see Section 4 of this report). Total viability was quite high for both samples (mean of 71%). Most seeds were dormant to some degree, but many of the outplanted seeds had clearly germinated as a result of stratification. Precipitation was normal during this period (NOAA-CIRES Climate Diagnostics Center 2005). It may be that some unknown factor is needed for germinant survival in the natural population (seed depth or other factors).

In summary, the complete lack of germinants for the cheatgrass study and germinant survival for the supplemental watering study cannot be easily explained, and given the rarity of this species, we do not recommend further seed experiments.

5. Propagation techniques

Rain Shadow Nursery in Kittitas, Washington (approximately 40 miles to the northwest of the population) developed propagation techniques for *Eriogonum codium* in 2002 and 2003.

Seeds were cold moist stratified for up to 60 days in late winter and early spring of the year after collection. Germination occurred over an extended period of time and some germination occurred without stratification (which is corroborated by the results of the seed testing, that showed some seeds germinating without special treatment).

Germination and growth were done in both soil-less #5 Sunshine mix and native soils from the site, although germination was more successful in 2002, when the soil-less mix was used. The seedlings were grown in 10 cu. in. tubes and were fertilized every 2-4 wks with ½ strength Petersen's soluble fertilizer.

Tubelings transplanted easily and grew very well in a 50/50 coarse sand/topsoil mix outside with little supplemental watering in the Kittitas Valley. A 2002 cohort transplant flowered in 2003, 18 months after germination.

One tubeling transplanted to western Washington survived the summer but did not survive its first winter. This species is unlikely to be suited to western Washington conditions, even in a garden environment.

6. Summary

These studies help us to understand more of the biology of this vulnerable species. The results of these studies are summarized below.

1. Only one seedling has survived to flowering since the study began. Time between germination and flowering was five years.
2. Most seedlings die in the first year.

3. In some years, no seedlings were produced in the study area.
4. There is no clear correlation between temperature and/or precipitation and seedling production or survival.
5. Seed viability is nearly 70% in fresh or stored seed, but drops sharply after the first year of burial.
6. Some seed can germinate without special treatment, but only in the first year of burial.
7. Plants can successfully be grown from seed, but germination is unpredictable.
8. Germination is most successful with long stratification (up to 2 months).
9. In an eastern Washington garden environment, flowering can occur as early as 18 months after germination.

The ability to grow plants from seed in the greenhouse is significant for long-term conservation of the species, although no transplanting back to the site has been attempted. The combination of the lack of a seed bank, wide variability in seedling germination, and very poor seedling survival all confirm the vulnerability of this species to decline and/or extirpation. These results will be relevant for transition matrix modeling, which is planned for 2006-2007.

7. Citations

Csontos, P. and J. Tamas. 2003. Comparison of soil seed bank classification systems. Seed Science Research 13: 101-111.

Dunwiddie, P.W., K.A. Beck and F.E. Caplow. Demographic studies of *Eriogonum codium* Reveal, Caplow and Beck (Polygonaceae) in Washington. In: Reichard *et al.* editors. *Conservation of Washington's native plants and ecosystems*. Washington Native Plant Society, Seattle, Washington.

Guerrant, E. O. Jr., K. Havens. And M. Maunder, eds. 2005. *Ex Situ Plant Conservation*. Island Press, Washington, D.C.
NOAA-CIRES Climate Diagnostics Center. 2005. Website accessed November 21, 2005. http://www.cdc.noaa.gov/.

Melgoza, G., R. S. Nowak and R. J. Tausch. 1990. Soil water exploitation after fire: competition between *Bromus tectorum* (cheatgrass) and two native species. Oecologia 83(1): 7-13

Reveal, J.L., F.E. Caplow and K.A. Beck. 1996. *Eriogonum codium*, a new species from southcentral Washington. Rhodora 97, No. 891: 350-356.

Thompson, K. 1997. *The Soil Seed banks of Western Europe*. Cambridge University Press. Cambridge, UK.

Thompson, K. and J.P. Grime. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67: 893-921.

US Fish and Wildlife Service. 2005. Species assessment and listing priority form: *Eriogonum codium*. Accessed November 21, 2005. http://ecos.fws.gov/docs/candforms_pdf/r1/Q3HN_P01.pdf

Washington Natural Heritage Program. 2005. List of plants tracked by the Washington Natural Heritage Program. Website accessed November 3, 2005. http://www.dnr.wa.gov/nhp/refdesk/lists/plantrnk.html

Appendix A

Study design for 2003 cheatgrass study

Pilot Study, 2003

Eriogonum codium seedling response to *Bromus tectorum*

Question: When *Eriogonum codium* seedlings are grown with varying ratios of *Bromus tectorum*, does performance (days of survival or biomass) of *Eriogonum codium* seedlings differ significantly from *Eriogonum codium* seedlings grown without *Bromus tectorum*?

Hypothesis A: There is a significant difference (power of 90%, $\alpha = .10$) between days of survival of *Eriogonum codium* seedlings grown with varying ratios of *Bromus tectorum* and those grown without *Bromus tectorum*.

Hypothesis B: There is a significant difference (power of 90%, $\alpha = .10$) between biomass after 90 days of *Eriogonum codium* seedlings grown with varying ratios of *Bromus tectorum* and those grown without *Bromus tectorum.*

Methods:

Four treatments, each in 634 cm^3 pots: (2 ERCO = 1 BRTE)

A. Control	2 ERCO	
B. 1:1	2 ERCO	1 BRTE
C. 1:2	2 ERCO	2 BRTE
D. 1:4	2 ERCO	4 BRTE

Arranged in the following manner: (* = ERCO, B = BRTE)

A. [*   *]

B. [*   B   *]

C. [* B B *]

D. [* BBBB *]

Each treatment with five replicates.

Eriogonum codium will be germinated, then pricked out at 2-4 days after germination and placed in the pots with seeds of *Bromus tectorum*. All pots are filled with substrate from the Umtanum Ridge site where *Eriogonum codium* occurs.

The pots will be watered to saturation, then placed outside and subject to normal precipitation but no additional watering.

The number of days to mortality for *Eriogonum codium* will be recorded, and all seedlings alive after 90 days will be dried and weighed to record biomass.

A report will be prepared that describes all methods, results of study, and discussion of the implications for the full study in 2004.

Full study, 2004

Methods will be adjusted based on the results of the 2003 pilot study, but the intent is to conduct the same experiment with 10 replicates per sample.

Appendix B
Photographs of seedlings of Eriogonum codium

Looking for seedlings, 2005

Seedling, 2004

Second year juvenile, 2005

Probably second or third year
juvenile (outside plots) 2005

It's perfect!

July 11, 2003

Announcing the first flowering of E. codium #112, 1998 germinant and the only seedling tough enough to make it to adulthood! Yahoo!