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NATIVE PLANT CONSERVATION PROGRAM

Malheur wirelettuce (*Stephanomeria malheurensis*) reintroduction and seed bulking: 2011 recovery efforts

Prepared by
Jordan Brown, Rebecca Currin and Robert Meinke

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CONTACT INFORMATION

Jordan Brown
Native Plant Conservation Program, Oregon Department of Agriculture
Department of Botany and Plant Pathology, Oregon State University
Cordley 2082
Corvallis, Oregon 97331
(541) 737-2346
brownj@science.oregonstate.edu

Rebecca Currin
Native Plant Conservation Program, Oregon Department of Agriculture
Department of Botany and Plant Pathology, Oregon State University
Cordley 2082
Corvallis, Oregon 97331
(541) 737-4135
currinr@science.oregonstate.edu

Dr. Robert Meinke
Native Plant Conservation Program, Oregon Department of Agriculture
Department of Botany and Plant Pathology, Oregon State University
Cordley 2082
Corvallis, Oregon 97331
(541) 737-2317
meinker@science.oregonstate.edu

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Introduction

Background

Stephanomeria malheurensis (Figure 1) is an extremely rare member of the Asteraceae family, known historically from only one location in Harney County, Oregon. The species was first discovered by Dr. Leslie Gottlieb on Bureau of Land Management (BLM) land in July of 1966, and subsequently described in 1978 (Gottlieb 1978). The U.S. Fish and Wildlife Service listed this species as endangered in 1982 (USFWS 1982). By 1985 no *S. malheurensis* plants were observed at the original site of discovery, and the species was considered extinct in the wild. Disappearance of *S. malheurensis* was correlated with a large increase in cheatgrass (*Bromus tectorum*), a non-native invasive annual grass which had appeared at the site after a fire in 1972 (Brauner 1988, Gottlieb 1991).

Figure 1. *Stephanomeria malheurensis* flowering in the wild.

Fortunately, Dr. Gottlieb had collected *S. malheurensis* seed and sent some to Berry Botanic Garden (BBG) for storage at their seed bank facilities in Portland, Oregon. In 1987, the first attempt at reintroducing the species back into this site was initiated. One thousand seedlings were grown at BBG and transplanted in the Narrows site in late April of that year. In 1989, an additional 80 seedlings were outplanted at the site. Plants persisted at Narrows in annually decreasing numbers (see Appendix 1 for a summary of *Stephanomeria* plant counts at the Narrows site), and by 2004 the species had once again disappeared from the site and was considered extinct in the wild.

Currently, the species "persists" primarily in seed storage facilities at Berry Botanic Garden in Portland. There is some concern that long-term storage of the thin-walled seeds (Figure 2) may be problematic. As the viability of stored seeds potentially dwindles, the likelihood of absolute extinction of this species increases. In order to maintain an adequate safeguard against extinction and provide an additional source of propagules for outplanting and recovery work, it is imperative to both re-establish the *S. malheurensis* population at the Narrows site, as well as increase and replace the stored seeds of this species on a regular basis. In addition, long-term survival of this species depends upon the establishment of new populations on administratively protected sites. Consequently, the goals of this project are to (1) re-establish a population of *S. malheurensis* at the original type location at Narrows, (2) establish a new population of *S. malheurensis* at least one more administratively protected location, and (3) increase the number of stored seeds of this critically endangered species.

Figure 2. Thin-walled *Stephanomeria malheurensis* achenes.

Species description

Stephanomeria malheurensis is an annual member of the Asteraceae family. Seeds of *S. malheurensis* germinate in the early spring (usually starting around the first week of April; Dr. Leslie Gottlieb, professor emeritus, University of California Davis, personal communication), and subsequently form glabrous-leaved basal rosettes of up to 15 cm in diameter (Figure 3). This species typically bolts in late May-June, forming a wiry network of branches generally less than 30 cm long (Figure 4). Flower heads are numerous and clustered or single on short peduncles, with 5-6 (rarely up to 11) florets per head (USFWS 1990). Flower heads contain 5-6 ligules approximately 8.2-9.4 mm in length and 3.2-3.6 mm wide starting in late June. Flowers are white, light to dark pink, or salmon colored (Figure 4). Fruits mature from July through October. Each head produces 5-sided achenes averaging 3.3-3.8 mm long and bearing 9-12 pappus bristles (Gottlieb 1978a; Figure 5).

Figure 3. *Stephanomeria malheurensis* rosette.

Figure 3. *Stephanomeria malheurensis* flowers. Flower color can range from white to pale pink to dark pink to salmon.

Figure 5. *Stephanomeria malheurensis* achenes.

Stephanomeria malheurensis is thought to have recently evolved from *S. exigua* ssp. *coronaria* (Figure 6), and both species were originally present at the type location for *S. malheurensis*. This example of sympatric speciation is believed to have been initiated with the change in a single gene governing self-compatibility. *S. exigua* ssp. *coronaria* is an obligate outcrosser, while *S. malheurensis* is highly self pollinating (Gottlieb 1973). The two species are very similar in appearance, and it is difficult to differentiate between them in the field. Fruits seem to be the most reliable method; those of *S. malheurensis* are larger, heavier (*S. malheurensis* seeds are about 1/3 longer and two times heavier than those of *S. exigua* ssp. *coronaria*), and have pappus bristles that frequently split from a thickened base (Gottlieb 1991).

Figure 6. *Stephanomeria malheurensis* (left, red circle) compared to *S. exigua* ssp. *coronaria* (right, yellow circle).

Habitat

The single known naturally occurring population of *Stephanomeria malheurensis* was located at the Narrows Area of Critical Environmental Concern (ACEC), approximately 25 miles south of Burns in Harney County, Oregon, on a hillside of volcanic tuff layered with thin crusts of limestone (Figure 7; USFWS 1990, Guerrant 1996). This differs from surrounding soils, which are derived from basalt (Gottlieb 1973). The altitude at the site is approximately 1500 meters (Gottlieb 1977), and the shrub-steppe vegetation is dominated by big sagebrush (*Artemisia tridentata*), rabbitbrush (*Ericameria nauseosa* and *E. viscidiflora*), and cheatgrass (*Bromus tectorum*) (Gottlieb 1973, Gottlieb 1991, Guerrant 1996). For more information regarding associated species at the Narrows site, see Currin and Meinke 2007.

Figure 7. The original *Staphanomeria malheurensis* type location at the Narrows.

Threats

Stephanomeria malheurensis has a very restricted range (known historically from only one site), and even the highest counts of this population were quite low. Consequently, it is extremely vulnerable to any disturbance at that site (USFWS 1990). In fact, the decline and subsequent disappearance of *S. malheurensis* from Narrows over the past 30 years demonstrates just how critically endangered this species continues to be. Before the Narrows fire in 1972, *S. malheurensis* inhabited the open areas between shrubs and bunchgrasses (Guerrant 1996). After the fire, cheatgrass invaded the site and at times formed an almost complete groundcover in the open areas. Higher levels of cheatgrass corresponded with a decline in *S. malheurensis* (Brauner 1988). In addition to the threat of cheatgrass and other invading exotics, *S. malheurensis* is also vulnerable to future fires. The Narrows site is administratively protected by the BLM, but any change in the administrative status of the site exposes *S. malheurensis* to the potential threat of grazing or mining.

Methods

Seed acquisition

In 2007, ODA obtained the seed used for transplant growing and seed bulking from Natasha Sherman, a graduate student in Dr. John Burke's lab at University of Georgia. The Burke lab seed was acquired from both Dr. Gottlieb and the Berry Botanic Garden (which had also originally received its seed from Dr. Gottlieb), and was subsequently germinated and grown for the purpose of seed bulking. The Gottlieb seed was bulked by Ms. Sherman in 2004, and the Berry seed was bulked by Dr. Burke a few years earlier (Dr. John Burke, professor, University of Georgia, personal communication).

The Burke lab provided 82 maternal lines of seed. Forty-eight were bulked directly from lines provided by Dr. Gottlieb. The other 34 lines were the result of hand-pollination (with plants either selfed or crossed) conducted by the Burke lab. ODA added to the available seed by bulking as many of the lines as possible each year from 2007-2011 (see Currin et al. 2007, Currin and Meinke 2008, Currin and Meinke 2009, and Currin and Meinke 2010). In 2007-2011, seed was collected and immediately stored at Oregon State University (OSU) in paper coin envelopes kept in a sealed plastic container and surrounded by silica gel beads. The plastic container was kept in a refrigerator set at 4°C. Based on a recommendation from Dr. Gottlieb, in 2010 and 2011 we allowed the seeds to dry in their coin envelopes for approximately four weeks before placing the envelopes in the refrigerator.

In 2011, ODA germinated seed from each of the available sources: that which had been received from the Burke lab and that which had been bulked by ODA in 2007, 2008, 2009, or 2010.

Seed germination

ODA selected 4509 seeds representing 77 of the originally available 82 maternal lines, as defined by separate lineage/accession numbers utilized by the Burke lab, for germination in 2011. Seed from the five maternal lines not represented in the 2011 recovery efforts had all been used in the previous years. For maternal lines that had seed bulked by ODA from 2007-2010, either ODA seed or a combination of ODA and Burke seed was used.

During germination, maternal lines remained separated. Seeds were placed in Petri dishes on thick filter paper (up to 50 seeds per dish) and moistened with distilled water (Figure 8). Petri dishes were placed in a dark refrigerator set at 13°C in Dr. Ken Johnson's lab at OSU on February 11, 2011. Petri dishes were checked daily, and remoistened as needed. Seeds were defined as germinated when the radicle emerged through the seed coat. Seeds that had not germinated by February 22, 2011 (11 days later) were considered unviable.

Figure 8. Germinated *S. malheurensis* seeds, labeled by source, and ready to be potted.

Seedling cultivation

ODA potted seedlings once the hypocotyl reached at least four millimeters in length. Seedlings were planted in flats of 3” square pots filled with Supersoil® (a commercial potting mix) during the period of February 14-22, 2011.

Flats were immediately placed outside in the OSU nursery yard (Figure 9). The plants were watered overhead, as needed, using a watering wand. A clear plastic rain shield was attached to 3/4”-diameter PVC hoops to prevent overwatering of the seedlings due to natural rainfall. Seedlings were fertilized weekly with Dyna-gro® (a commercially available form of Hoagland’s solution) at the manufacturer’s recommended application strength.

Figure 9. *Stephanomeria malheurensis* growing in the OSU nursery yard under plastic to protect plants from over-watering due to natural rainfall.

Seedlings were sorted into two groups: those destined for outplanting and those reserved for seed bulking in the greenhouse (Figure 10). Seedlings selected for outplanting were transported to eastern Oregon sites on April 11, 2011.

Seedlings used for the seed bulking efforts remained in the greenhouse yard until they bolted and started producing flowers. On June 13, 2011, plants were transported into the greenhouse to facilitate seed collection. See "Seed bulking" section for more information.

Figure 10. Seedlings were watered and fertilized while under cultivation (left), and eventually sorted to reserve specific maternal lines for seed bulking (right).

Site selection

The first site, Narrows, is the type locality for the species, and is located approximately 25 miles south of Burns, Oregon on the Bureau of Land Management's Narrows Area of Critical Environmental Concern (ACEC). (See Narrows site description in Habitat section above.) This site was chosen because it is the only known site to have supported *S. malheurensis* in the past. In 2007 and 2008, *Stephanomeria malheurensis* rosettes were outplanted in the area of the ACEC known to historically support this species. In 2009, a second site (Narrows 2) containing what appeared to be appropriate *S. malheurensis* habitat was selected for outplanting within the ACEC. In 2010 seemingly suitable habitat just east of Narrows 2 was selected as a third outplanting area within the ACEC, designated Narrows 3 (Figure 11). Due to limited suitable habitat, in 2011 the space once separating Narrows 2 and 3 sites was planted, thereby joining the two planting areas.

Figure 11. Location of four *Stephanomeria malheurensis* outplanting sites: Narrows 1, Narrows 2, Narrows 3 and Dunes (indicated by yellow triangles).

The Dunes site was proposed for *S. malheurensis* introduction after the discovery of a *S. exigua* ssp. *coronaria* population there during a pipeline survey conducted by a BLM botanist. The Dunes site is located approximately 10 miles to the southwest of the Narrows site. The site is on BLM land, and therefore administratively protected. (For more information regarding the Dunes habitat, see Currin and Meinke 2007). During the first two years of this study, transplants were reintroduced at the original Narrows site, and introduced at the Dunes site. However, in 2009 (the third year of the study), a decision was made to focus outplanting efforts at the Narrows site. The Dunes site was monitored for recruitment from previous outplantings, but no additional transplants were introduced at this site in 2009. No monitoring of this site occurred in either 2010 or 2011.

Study design

All introduction sites are surrounded by a cattle exclosure fence. The entire Narrows ACEC had been fenced to exclude cattle when it was designated as an ACEC in 1974. Burns BLM staff oversaw the installation of cattle exclosure fencing at the Dunes site (approximately one acre was fenced) in April of 2007.

In 2007, eight macroplots were established at each of two sites (Narrows 1 and Dunes). Because herbivory had been a problem during 1987 reintroduction attempt, in 2007 each site had four of the macroplots located within rabbit exclosure fencing, and four macroplots without rabbit exclosure fencing (for more information see Currin and Meinke 2007). However, 2007 results showed no significant difference between the survival and reproduction of *S. malheurensis* transplants located within the rabbit exclosures and the survival and reproduction of those located outside of the exclosures. Consequently, in subsequent years new macroplots were installed without rabbit exclosure fencing around them. In 2008, six additional macroplots were installed at each of the two sites (Narrows 1 and Dunes). (See Currin and Meinke 2008 for more information about 2007 and 2008 outplanting configurations.) In 2009, four additional macroplots were established at each Narrows site and in 2010 an additional three macroplots were added as well.

In 2011, three new macroplots were established at each of the Narrows sites (1, 2 and 3), for a total of nine newly planted plots (Figure 12). Each of the macroplot corners were marked with a 2 foot long piece of rebar tied with brightly-colored flagging. A 2-foot-long wooden stake with the macroplot number was also placed in the northwest corner of each plot.

Figure 12. New macroplots were installed at the Narrows 1 site after carefully selecting the most apparently suitable habitat.

Either seventeen or eighteen one-meter-square microplots were established in each macroplot in 2011. Microplots were marked with a central 2-foot-long rebar stake. A metal tag with the plot number was attached to the rebar stake with wire (Figure 13). Each microplot contained eight plants. Plants were placed around the central stake along the outer edge of the meter square plot, with one transplant at each corner, and one transplant halfway between each corner (Figure 14). Plants of different ages and sizes were evenly distributed amongst sites, macroplots and microplots.

Figure 13. *Stephanomeria malheurensis* microplots were marked with 2-ft-long rebar stakes (left). Each microplot contained eight *S. malheurensis* transplants arranged in a one-meter-square plot around the rebar stake (right).

Outplanting

On April 11, 2011, the *S. malheurensis* plants were transported to the introduction sites in the back of pickup trucks, on racks made of wooden 2x4s and metal fencing (Figure 14). During the following three days, seedlings were outplanted at the three Narrows sites. A total of 415 seedlings were outplanted at the Narrows 1 site, 416 seedlings were planted at the Narrows 2 site, and 408 seedlings were planted at the Narrows 3 site (Figure 15).

Figure 14. Plants were transported from the Willamette Valley to the Narrows sites on custom built racks that were loaded in the back of a pickup truck.

Figure 15. Macroplots and plants were arranged before planting began in earnest.

At the time of transplanting, seedlings had been in their pots for 49-57 days. Although seedling rosette diameters were not measured at the time of outplanting in 2011, a brief assessment had estimated diameters ranging from 3-10 cm (Figure 15).

Transplants were thoroughly watered at the time of outplanting, with care taken to apply water slowly and gently so that the water did not run off from the planting area before it could soak in, and so that plants were not buried in the soil due to the flooding action of the water. Water was stored at each site in portable wildland firefighting water tanks provided by the BLM's Burns District office and transported to the plots in garden watering cans (Figure 16). During the eight weeks following outplanting, BLM and ODA staff watered the transplants weekly (unless heavy rains had occurred) (Figure 17).

Figure 16. Relative size of *Stephanomeria malheurensis* seedlings upon planting (left). Watering transplants was made possible by BLM water tanks (right).

Figure 17. Watering was completely necessary, even with the light levels of precipitation that fell during planting, because the hydrophobic soils at the site were relatively dry and didn't absorb or hold water well.

Direct sowing seed plots

With the lack of funding, direct sown seed plots were not installed this season. However, with the copious amounts of seed that was bulked in 2011, there are sufficient supplies to both bank seed for *ex situ* conservation as well as provide for direct sown seed plots in the future. For more information regarding previous direct sown seed plots refer to Currin and Meinke 2007 and Currin and Meinke 2010.

Monitoring

2011 transplant survival: On June 8, 2011, all *S. malheurensis* transplants were monitored to determine transplant survival rates and the number of bolting (reproductive) individuals (Figure 18). At that time all other macroplots (those established from 2007-2010) were monitored for the presence of seedlings as well.

2011 transplant reproduction: ODA staff did make several informal monitoring trips to the site later in the summer to confirm that most of transplants did flower and set seed. However, due to the reduction in funding for the project in 2011, ODA was not able to conduct the intensive reproductive monitoring (involving the counting of all flowers and fruits produced by both the 2011 transplants and recruited individuals from the previous years' outplanting) that usually occurred in late July or early August. Therefore, the ultimate reproductive output of *S. malheurensis* transplanted in 2011 was estimated using reproductive data from the previous years' reports. For more information regarding past reproductive monitoring refer to Currin and Meinke 2010.

Figure 18. Revisiting *Stephanomaria malheuresis* transplant plots for survival monitoring after approximately 8 weeks.

Recruitment from 2007, 2008, 2009, and 2010 transplants and directly-sown seed: Each year there is the potential for new *S. malheurensis* plants to grow from seed either produced by past years' transplants or seed previously hand-sown. Although monitoring of the Narrows sites for any such recruitment of *S. malheurensis* individuals was not completed in 2011 due to a lack of funding, the three seed plots installed in 2010 at the Narrows site were checked for recruitment in 2011 by BLM biologist Caryn Meinicke. (For more information regarding past recruitment monitoring refer to Currin and Meinke 2010.)

Seed bulking

One of the primary focuses of the 2011 work was to increase the amount of bulked *S. malheurensis* seed available for future recovery efforts. As a result, the number of plants grown for seed bulking purposes was increased in 2011. ODA cultivated 674 plants grown from seed from 65 of the Burke lab accessions for seed collection. Seventeen Burke lab maternal lines were not used for seed bulking in 2011 due to one the following reasons: 1) no more seed was available for that line, 2) sufficient seed for that line had been bulked in previous years, or 3) all the cultivated plants for that line died before producing seed in 2011. Plants not sent to Harney County for outplanting remained in the greenhouse yard until they bolted and began to produce flowers. They were transported inside to a greenhouse room on June 13, 2011. At this point, plants from the same maternal line/accession were grouped in plastic trays so that seed could be sorted and collected separately. Plants were watered as needed (after collecting seed, since the spray could knock achenes off of their receptacles, making collection more difficult) and fertilized weekly with Dyna-Gro®, a complete liquid plant fertilizer, according to manufacturer instructions.

ODA staff collected seeds three to four times per week starting in mid-June 2011 and continuing through October of that same year. Seeds that dropped into the parent plant's pot or into a tray were attributed to that tray's maternal line. Collected seed was separated by maternal line/accession and placed in coin envelopes. After allowing the seeds to further dry in the envelopes for approximately four weeks, they were stored in a refrigerator set at 4° C with the seed originally obtained from the Burke lab (see "Seed acquisition" section above).

The method used for estimating the number of collected seeds involved sub-sampling by weight. Samples of 50 achenes, collected from nine different bulked maternal line sources, were used to formulate an average weight of achenes. ODA staff then tested this method by counting all of the achenes collected for each of several of the maternal lines, weighing the achenes, estimating the count based on the weight, and comparing the estimated count with the actual count. These trials showed that estimating the achene counts worked well for lines that produced large amounts of achenes, but that the percent error was often too large when estimating smaller quantities of achenes. Subsequently, the smaller samples (those with less than 100 achenes) were counted for better accuracy, whereas the number of achenes in each of the larger samples was estimated using an average weight of 0.056 g/50 achenes.

Figure 19. Fruiting and flowering *Stephanomeria malheurensis* plants kept in the greenhouse for seed bulking; (inset) mature achenes ready for collection.

Results

Seed germination

Stephanomeria malheurensis seeds began germinating within three days of being moistened and placed in the refrigerator. Within 11 days, 70% of the seeds had germinated (Table 1; Figure 20). ODA staff discontinued seed germination after 11 days. Germination rates varied between Burke accession numbers and year bulked, ranging from 0-100%.

Table 1. *Stephanomeria malheurensis* seed germination rates from 2007 - 2011.

	2007	2008	2009	2010	2011
# Seeds germinated	1090 (86%)	1679 (80%)	1799 (72%)	2400 (65%)	3175 (70%)
# Seeds not germinated*	179 (14%)	430 (20%)	712 (28%)	1296 (35%)	1334 (30%)
Total # seeds used	1269	2109	2511	3696	4509

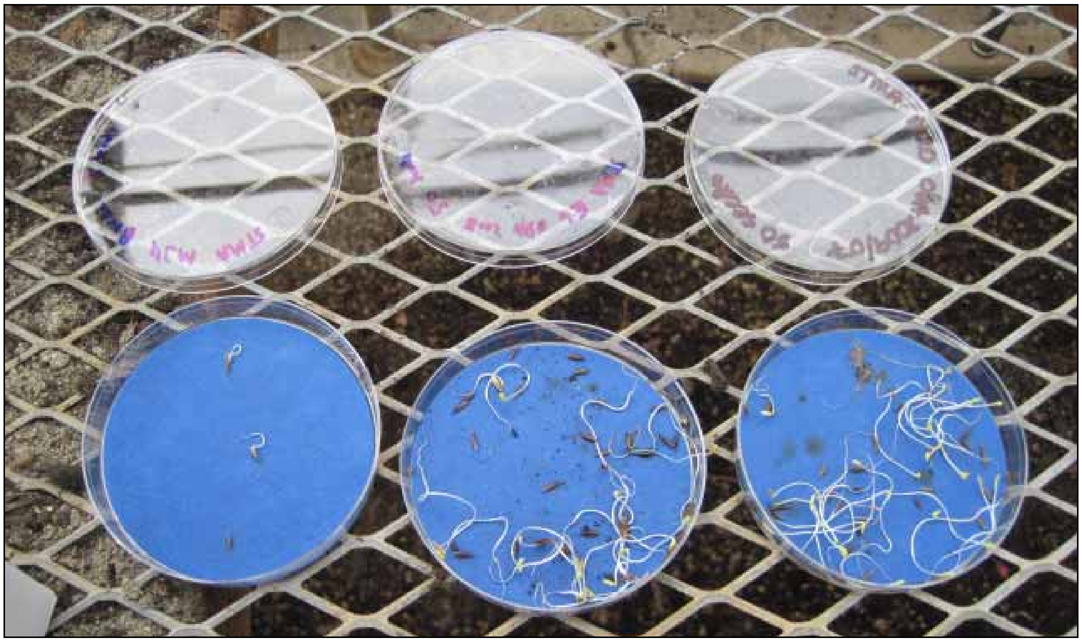

Figure 20. Overall germination rates remained relatively high (although highly variable by maternal line) during the largest scale *Stephanomeria malheurensis* cultivation effort to date.

Seedling cultivation

Of the 3175 planted seedlings, 1239 (39.0%) were transported to the reintroduction sites for outplanting, 674 (21.2%) were kept at OSU for seed bulking, and 1262 (39.8%) died during cultivation (Table 2). Mortality of seedlings during cultivation varied between groups of plants grown from seeds of different Burke accessions, ranging from no mortality at all (0%) to 100% mortality (Figure 21).

Table 2. *Stephanomeria malheurensis* seedling fates in 2011.

	# of Seedlings	% of Seedlings
Potted after germination	3175	100%
Outplanted in Harney County	1239	39.0%
Used for seed bulking	674	21.2%
Died during cultivation*	1262	39.8%

* Plants that either died before outplanting in Harney County, or did not ever produce achenes during seed bulking efforts.

Figure 21. The condition of young seedlings (left), along with the weather and several other factors, can greatly affect the survival of the tiny vulnerable plants after they have been potted (center) and moved outside to be cultivated under rain barriers (right).

Transplant survival

A total of 1239 *S. malheurensis* seedlings were outplanted at the Narrows sites in mid-April of 2011. Eight weeks after being outplanted, approximately three quarters (75%) of the transplants (931 seedlings) were alive, having survived the initial shock of transplantation (Table 3). At that point, 80% (333 plants) of the 2011 Narrows 1 transplants were alive, which is the highest survival rate by site that was observed this year. Comparatively, Narrows 2 showed 74% (308 plants) survival and Narrows 3 had 71% (290 plants) survival as of June 8, 2011 (Table 3; Figures 22 and 23). For more information and trends of survival and reproductive output from previous years, refer to Currin and Meinke 2010.

Table 3. 2011 *Stephanomeria malheurensis* transplant survival after 8 weeks at each of the three Narrows sites.

Date	Status	Narrows 1	Narrows 2	Narrows 3	Total (All Sites)
4/13/11	**Outplanted**	415	416	408	1239
6/8/11 (8 weeks)	**Alive**	333 (80%)	308 (74%)	290 (71%)	931 (75%)
	Dead	82 (20%)	108 (26%)	118 (29%)	308 (25%)

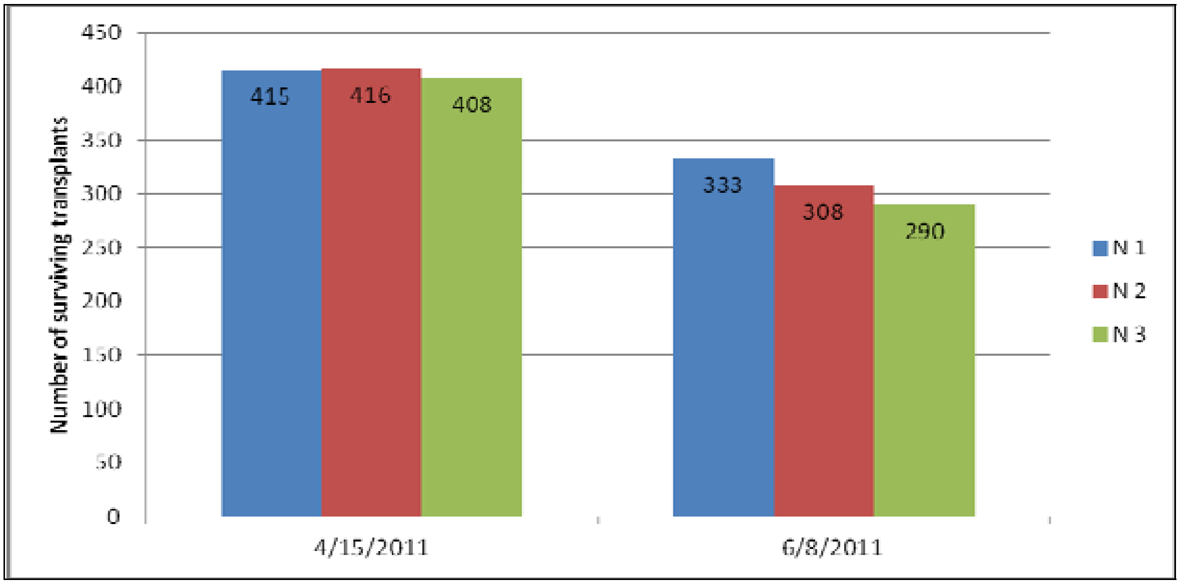

Figure 22. 2011 *Stephanomeria malheurensis* transplant survival after 8 weeks at each of the three Narrows sites (N1 = Narrows 1 [plots 35, 36, & 37], N2 = Narrows 2 [plots 38, 39, & 40], N3 = Narrows 3 [plots 41, 42, & 43]).

Figure 23. The vast majority of *Stephanomeria malheurensis* individuals outplanted in 2011 were olting, or producing flowering stalks, by the time survival monitoring took place. *S. malheurensis* transplants are in the yellow circles.

Herbivory was once again observed in many plots. Flowering stalks were browsed, and in some cases, plants were dug up by animals (Figure 24).

Figure 24. Healthy *S. malheurensis* transplants (left) are subject to the same threats as wild plants, such as above ground herbivory (center) leading to the loss of the flowering stems, and below ground herbivory (right) resulting in a damaged root system and often mortality.

Transplant reproduction

Although ODA was unable to count reproductive output of transplants using the same methodology used in previous years, we did estimate the number of seeds produced by the 2011 transplants using a weighted average number of 45.3 achenes/plant from the previous year's data (Currin and Meinke 2010). As discussed in the previous year's report, this number was most likely an underestimate of the achenes produced. There were 931 surviving (and bolting) transplants in early June of 2011. The estimated number of achenes produced by 2011 transplants was 42,174 achenes.

Recruitment from 2010 directly-sown seed

Only three of the approximately 40 plots were monitored for recruitment this season due to a lack of funding and personnel resources. A total of 56 new recruits were found within the three directly-sown seed plots installed in 2010 (plots 32, 33, and 34) when they were visited by Caryn Meinicke (BLM) on August 25, 2011 (Table 4). Plot 32, in the Narrows 1 site,

Table 4. Number of *Stephanomeria malheurensis* recruits found within/near macroplots at Narrows 1, 2, and 3 sites. Only direct-seeded plots installed in 2010 were monitored during 2011; the total number of recruits reported in 2011 may be artificially low as a result of incomplete monitoring.

Site	Year installed	Macroplot	# Recruits in 2008	# Recruits in 2009	# Recruits in 2010	# Recruits in 2011
N1	2007	1	3	0	0	n/a
		2	0	0	9	n/a
		3	0	0	5	n/a
		4	1	5	15	n/a
		5	0	0	0	n/a
		6	0	0	0	n/a
		7	0	0	4	n/a
		8	5	1	7	n/a
		Subtotal	**9**	**6**	**40**	**n/a**
	2007	**Seed plots**	**0**	**5**	**2**	**n/a**
	2008	9	n/a	2	13	n/a
		10	n/a	21	98	n/a
		11	n/a	8	63	n/a
		12	n/a	9	200	n/a
		13	n/a	3	43	n/a
		14	n/a	0	19	n/a
		Subtotal	**n/a**	**43**	**436**	**n/a**
	2009	15	n/a	n/a	0	n/a
		16	n/a	n/a	13	n/a
		17	n/a	n/a	13	n/a
		18	n/a	n/a	4	n/a
		Subtotal	**n/a**	**n/a**	**30**	**n/a**
	2010	23, 24, 25	n/a	n/a	n/a	n/a
	2010	**Seed plot 32**	**n/a**	**n/a**	**n/a**	**44**
N2	2009	19	n/a	n/a	95	n/a
		20	n/a	n/a	23	n/a
		21	n/a	n/a	39	n/a
		22	n/a	n/a	19	n/a
		Subtotal	**n/a**	**n/a**	**176**	**n/a**
	2010	26, 27, 28	n/a	n/a	n/a	n/a
	2010	**Seed plot 33**	**n/a**	**n/a**	**n/a**	**8**
N3	2010	29, 30, 31	n/a	n/a	n/a	n/a
	2010	**Seed plot 34**	**n/a**	**n/a**	**n/a**	**4**
		Total	**9**	**54**	**684**	**56**

held a total of 44 plants. The directly-sown seed plot in the Narrows 2 site, plot 33, had only eight observed plants at the time of monitoring. Four plants observed in plot 34, in the

Narrows 3 site. (For information on more thorough recruitment monitoring of previous years, refer to Currin and Meinke 2010.)

Seed bulking

Seed bulking continued to be very successful during the fifth year of this project. A total of 674 plants from 88 maternal lines were grown for achene production at OSU. There was a wide range in the average number of achenes produced/plant for each maternal line (0 - 967 achenes/plant). However, on average each plant produced approximately 67 achenes for a total of approximately 45,184 achenes produced in 2011 (Table 5).

Table 5. Number of *Stephanomeria malheurensis* seeds produced by plants cultivated in the Oregon State University greenhouse.

	2007	**2008**	**2009**	**2010**	**2011**
# Maternal lines used	26	66	51	63	88
# Plants producing achenes	89	198	155	471	674
Total # achenes collected	16,520	23,028	18,276	71,850	45,184
Average # achenes/plant	186	116	116	150	67

Discussion

The Malheur wirelettuce (*Stephanomeria malheurensis*) is one of the rarest plants in the United States, having gone extinct in the wild over two decades ago. Recent recovery efforts were only made possible by the collection and *ex situ* storage of this species' seed by Dr. Leslie Gottlieb and staff from the Berry Botanic Garden (BBG). This species' future survival depends upon the establishment of multiple self-sustaining populations in administratively protected areas. The recovery efforts begun by ODA, USFWS, the BLM, BBG and others over the last five years show promise, and are a good start. However, as is common when attempting to recover critically endangered species, our ultimate results will depend on the ongoing support and continuing efforts of each of the partners that have contributed to conserving this rare plant.

During the fifth year of this *Stephanomeria malheurensis* recovery work, the protocols and methods developed for this project helped us accomplish goals without encountering major obstacles. Stored seeds continued to germinate at fairly high rates, and although these rates had declined slightly from 2007-2010, during 2011 there was a slight increase (from 65% in 2010 to 70% germination). Decreased germination rates over time may be due in part to the increased age of the seeds. It is also possible that seeds that have been bulked for successive generations in the greenhouse experience a slight decrease of fitness. In order to minimize this possibility, we have used the "closest to wild-collected" seed (that which was obtained from the Burke lab) for seed bulking purposes whenever possible. Germination rates continued to vary greatly between maternal lines, with some lines having no germination at all, while others had 100% of their seeds germinate. However, because of the potentially limited diversity of available seed stock (all seed comes from the original Gottlieb collections in the early 1970s), we will continue to utilize as many of the lines as possible (rather than only using those that germinate at high rates) in an effort to maximize the genetic diversity of the plants used for either reintroduction or seed bulking.

In 2011, seedling mortality during cultivation increased slightly, to about 40%, from the approximate 30% mortality observed the past two years. Once again, this was probably due to the particularly cold, wet spring experience in the Willamette Valley (where the seedlings were cultivated). While efforts were made to shield seedlings from excessive rain and improve drainage, some of them developed a common nursery fungal disease (*Botrytis* sp.) and perished. This year many of the late germinating seedlings, which for the most part appeared to be less vigorous or sickly, were potted up in an attempt to save every germinated seedling. Unfortunately, many of these damaged or sickly seedlings died. These additional losses contributed to the high seedling mortality observed this year.

The ultimate reproductive fate of the *Stephanomeria malheurensis* individuals that were transplanted in 2011 remains unknown. Reproductive monitoring was not completed due to a lack of funding and personnel resources. On June 8, 2011, during the final watering of the transplants after eight weeks on site, survival monitoring was conducted. The 75% survival observed after eight weeks is very close to the 76% survival observed six weeks after transplanting in 2010. Sixteen weeks after their outplanting, 56% of the cohort of 2010 transplants survived to reproduce. With the similarity between the 2010 and 2011 survival rates at the time of last watering, we may predict a similar percentage of transplants survived to reproduce this season as well. These rates remain well within the range expected for similar projects involving rare species introduction (Allen 1995, Drayton and Primack 2000, Currin and Meinke 2004, Kohn and Lusby 2004, Guerrant and Kaye 2007).

Although recruitment from seeds produced by 2007, 2008, 2009, and 2010 transplants was not monitored in 2011, monitoring of the directly-sown seed plots established in 2010 was conducted thanks to the generosity of BLM botanist Caryn Meinicke. The seed plot located within the Narrows 1 site exhibited the highest levels of recruitment, with 44 plants observed. The directly-sown seed plots installed in Narrows 2 and 3 held fewer plants, with 8 and 4 plants respectively. The majority of these recruits were found in areas with little competing vegetation where they would often occupy bare ground with apparently healthy soil crusts. The most successful directly-sown seed plot (within Narrows 1) had very little encroachment by either native grasses or cheatgrass, and had an apparently healthy soil crust component, which are likely contributing factors for recruitment. The Narrows 2 directly-sown seed plots had a significant presence of cheatgrass (mostly on the west side of the plot) as well as natural litter/debris which might help explain the lower recruitment here. The least successful directly-sown seed plot, in Narrows 3, also appeared to be the most infested with cheatgrass, and have the most litter present, which decreased the amount of open area potentially available for seedling recruitment. Although there did appear to be a strong soil crust component at both the Narrows 2 and 3 plots, perhaps the amount of competing vegetation and litter more strongly influenced the lower levels of recruitment in these areas.

These hypothesized implications of the site conditions mentioned above were also mirrored in the transplant survival monitoring data. The Narrows 1 site appeared to be the most hospitable and also had the highest survival rate, whereas survival decreased slightly with the apparently degraded conditions of Narrows 2 and 3. The variable survival and recruitment observed across sites could also be the result of unforeseen environmental factors inherent to each site or even annual variation between sites.

Relative to the results of the 2007 directly-sown seed plots, the recruitment observed this year is very encouraging. Originally in 2007 only 1,000 seeds were used per site, equally portioned between five one-meter-square plots, and no recruitment was observed during the first year after transplanting (Currin and Meinke 2007, Currin and Meinke 2008). During the second and third year following seeding, there was recruitment in the seed plots, albeit at very low levels. The three directly-seeded plots installed in 2010 were created on a larger scale with the hopes of higher recruitment. Single 10 x 10 meters-squared plots were installed in each of the three Narrows sites and stocked with approximately 15,000 seeds. It is very encouraging to already see recruitment after the first season (which wasn't the case for the 2007 seed plots) and as a result of the increases in sown seed, we hope to see much higher levels of recruitment in the upcoming years.

It has been observed that *S. malheurensis* achenes can survive in a seed bank for at least one year, and likely more, which is encouraging in regards to the directly-sown seed plot results. The fluctuations in establishment and recruitment rates observed during the past several years of recovery efforts have been attributed to variability in sites and conditions as well. Therefore, monitoring of these seed plots over the next several years is necessary for understanding the contributions made to the reproductive capacity of the *S. malheurensis* population, which will likely be staggered over time. More information about the success of direct seed-sowing as a reintroduction strategy will become available as monitoring of the three large-scale seed plots installed in 2010 continue over the next few years.

In 2011, on-the-ground recovery efforts continued, although monitoring was curtailed due to a reduction in funding and available resources. The reproductive and recruitment monitoring were not completed because of this. This absence of data comes at a most inopportune time, leaving us unable to confirm whether the positive trends noticed last year are continuing. The 2010 reproductive and recruitment monitoring results were very encouraging for *S. malheurensis* recovery efforts; the total number of reproductive *S. malheurensis* individuals, the percent of reproductive transplants, and the number of recruits had reached the highest levels since recovery efforts have resumed. The approximately 71,000 achenes bulked in 2010 was the largest yield so far, and the estimated over 60,000 achenes produced onsite is the second largest estimated reproductive output.

In light of these very positive results from 2010, and with the significant contributions of 2011, we are approaching a turning point along the road to recovery for this species. Now, more than ever, it is critical to maintain our commitment to recovery efforts for the continued bolstering of the fragile *S. malheurensis* populations to the point they can sustain themselves. With the highly successful results of 2010, and promising recruitment already observed in the directly-sown seed plots, there is new hope for this extremely rare species. At this phase monitoring is very important in order to confirm our suspicions of progress and not falsely assuming success. Recruitment monitoring will be very important for evaluating the effectiveness of the directly-sown seed plots, which may prove to be a viable and economical method for establishing reproductive plants on site in the future. With continued support and cooperation from all of the *S. malheurensis* partners, Malheur wire-lettuce will be one step closer to recovery in 2012.

Management recommendations

- Continue reintroducing *Stephanomeria malheurensis* seedlings annually at multiple sites within the Narrows ACEC in order to re-establish the only known (and currently presumed extinct) population of this species (Recovery Plan Objectives 1 and 5)

- Continue monitoring Narrows reintroduction sites for transplant reproduction and new recruitment (Recovery Plan Objective 56)

- Monitor Dunes introduction site for recruitment at least one more time (Recovery Plan Objective 56)

- Continue utilizing survival, reproduction, and recruitment data to refine our understanding of *S. malheurensis* habitat requirements and integrate this knowledge into future micro- and macro-site selection

- Identify at least one site on the Malheur National Wildlife Refuge for establishment of new *Stephanomeria malheurensis* populations (Recovery Plan Objective 52)

- Identify additional sites located on administratively protected land for establishment of new *Stephanomeria malheurensis* populations (Recovery Plan Objective 52)

- Establish and monitor additional new populations of *Stephanomeria malheurensis* on administratively protected lands (Recovery Plan Objectives 5 and 56)

- In order to maximize genetic diversity within newly created populations of *S. malheurensis,* continue to include as many of the available maternal lines as possible in both reintroduction and seed bulking efforts

- Continue bulking *Stephanomeria malheurensis* seeds to use in future recovery efforts, as well as to increase and replace the number of stored seeds of this endangered species in order to maintain an adequate safeguard against extinction (Recovery Plan Objective 51)

- Collect seed from transplants at each outplanting site in order to replenish seed stock with fresh seed from individuals that have successfully reproduced in the wild

- Continue exploration of direct seed sowing as an alternative method for new population establishment

- Monitor directly-sown seed plots established in 2010 for at least three years to compare effectiveness of reintroduction by seed vs. transplants

- Investigate methods for controlling cheatgrass (*Bromus tectorum*), which continues to be a threat at the Narrows (especially Narrows 1) site (Recovery Plan Objectives 1 and 12)

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Appendix

Stephanomeria counts at Narrows

Italicized numbers are estimates by Dr. L.D. Gottlieb. Bolded text indicates greenhouse-grown transplants that were reintroduced to the site. N1 = original Narrows site, N2 = second Narrows outplanting site (established in 2009), N3 = third Narrows outplanting site (established in 2010). The total number of recruits in 2011 may be artificially low as a result of incomplete monitoring (only directly-sown seed plots installed in 2010 were monitored in 2011).

Year	# *Stephanomeria*	# *S. malheurensis*	Source
1968	*100*	*3*	Gottlieb 1974
1969	*5,000*	*150*	Gottlieb 1974
1970	*5,000*	*150*	Gottlieb 1974
1971	*25,000*	*750*	Gottlieb 1974
1972	*<500*	*<15*	Gottlieb 1974
1973	No count	No count	
1974	*12,000*	*228*	Gottlieb 1977
1975	*35,000*	*1050*	Gottlieb 1977
1976	No count	No count	
1977	No count	No count	
1978	375	*20+*	USFWS 1990
1979	24	*0*	USFWS 1990
1980	No count	No count	
1981	No count	50+	USFWS 1990
1982	12	9+	USFWS 1990
1983	No count	No count	
1984	No count	No count	
1985	No count	0	Brauner 1988
1986	No count	**34 transplants**	Davidson 1986
1987	12	**1000 transplants**	Brauner 1988
1988	No count	31	Raven 2001
1989	No count	939 (**80 transplants**)	Raven 2001
1990	No count	0 (**200 transplants**)	Raven 2001
1991	No count	387	Raven 2001
1992	No count	105	Raven 2001
1993	No count	280	Raven 2001
1994	No count	36	Raven 2001
1995	No count	413	Raven 2001
1996	No count	24	Raven 2001
1997	No count	0	Raven 2001
1998	No count	52	BLM 1999
1999	No count	0	BLM 1999
2000	210 (*S. exigua* ssp. *coronaria*)	113	BLM 2000
2001	No count	28	BLM 2003
2002	No count	17	BLM 2003
2003	No count	5	BLM 2003
2004	0	0	Hall 2006
2005	5	0	BLM 2005
2006	0	0	Hall 2006

Stephanomeria counts at Narrows (continued)

Year	# *Stephanomeria*	# *S. malheurensis*	Source
2007	0	**428 transplants**	Currin et al. 2007
2008	0	**608 transplants +** 9 recruits	Currin & Meinke 2008
2009	0	**520 transplants** + 54 recruits (N1) + **576 transplants** (N2)	Currin & Meinke 2009
2010	0	**1224 transplants** (N1, N2, N3) + 684 recruits	Currin & Meinke 2010
2011	0	**1239 transplants** (N1, N2, N3) + 56 recruits	Currin & Meinke 2011