Establishment of Two Experimental Populations of Wolf's Evening Primrose (*Oenothera wolfii*)

Final Report for 2004

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Abstract

Oenothera wolfii (Wolf's evening primrose) is a biennial to short-lived perennial endemic to beach habitats in northwestern California and southwestern Oregon. *O. wolfii* faces several imminent threats, including habitat loss and alteration due to coastal development and roadside maintenance, competition with exotic species, and hybridization with the common garden escapee *O. glazioviana*. Initial results from the experimental reintroduction of *O. wolfii* at two study sites are promising, with high transplant survival and reproduction rates. Future monitoring is needed to assess the long-term success of these experimental populations.

Introduction

The showy biennial to short-lived perennial *Oenothera wolfii* (Munz) Raven, Dietrich & Stubbe (Wolf's evening primrose) occurs in only a small number of isolated populations. This taxon is surprisingly rare, considering that it can almost behave like a "weedy" species, and establishes fairly large populations in moderately disturbed areas (Carlson et al. 2001, Imper 1997). Its current precarious status results from having a limited geographical range and being faced with several pressing threats, including habitat loss and hybridization with an escaped garden cultivar, *O. glazioviana*. Currently, Wolf's evening primrose is listed as "Rare and Endangered Throughout Its Range" by the California Native Plant Society (list 1B) and the Oregon Natural Heritage Program (list 1), as "Threatened" by the State of Oregon, and as a "Species of Concern" by the U.S. Fish and Wildlife Service (ORNHIC 2004).

The purpose of this study is to develop a protocol for the establishment of a new *O. wolfii* population. Two locations have been selected as pilot sites for introduction efforts. Originally, both sites were to be located within the New River ACEC; however, due to potential conflicts with another rare plant (*Phacelia argentea*) at one of the sites, the second introduction site was relocated to Oregon Department of Transportation (ODOT) land just south of Gold Beach, Oregon. This study evaluates the survival and reproductive success of transplanted rosettes of various sizes, as well as comparing transplant establishment in weeded and unweeded plots.

Species description

Oenothera wolfii grows from 50 to 200 cm in height, forming a basal rosette of elliptical leaves from which rises a branched flowering stalk, with increasingly smaller leaves arranged along the stem (Figure 1). The pale yellow to yellow flowers are usually less than 40 mm in diameter, with separate petals and stigmas generally placed lower than anthers (Figure 2). Stems, sepals and fruits are often red-tinged and fairly pubescent, often with glandular hairs (Carlson et al. 2001). In spite of these easily identifiable characteristics, the taxonomy and identification of this species' subsection (*Euoenothera*) is considered difficult, due to the high level of interfertility within the group (Imper 1997).

Figure 1. *Oenothera wolfii*.

Figure 2. *Oenothera wolfii* flower.

Geographic range

Currently there are seven known populations of *O. wolfii* in Oregon: Port Orford, Hubbard Creek, Humbug Mountain, Sister's Rock, Otter Point, Pistol River and Zwagg Island (Gisler and Meinke 1997, Figure 3). Visits to all of these populations (with the exception of Humbug Mountain) in September 2004 showed that all populations are present, with the number of individuals in each population ranging from about 40 to several thousand plants. The Humbug Mountain population was visited by Carlson fairly recently, and is also assumed to be extant (Carlson et al. 2001). There are an additional nine populations in California, with locations ranging from Crescent City down to Cape Mendocino (Gisler and Meinke 1997, Imper 1997).

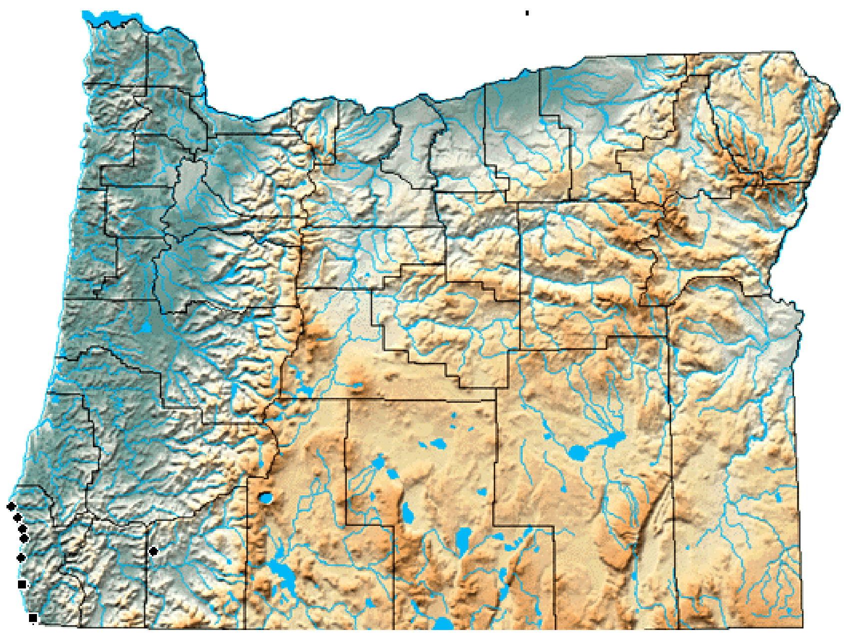

Figure 3. Map of extant *Oenothera wolfii* populations in Oregon. Map provided courtesy of the Oregon Flora Project.

Habitat description

Oenothera wolfii grows in well-drained soil or sand, on or adjacent to coastal beaches. Like other rare species of *Oenothera*, the specific substrate characteristics do not appear to be critical (Pavlik and Manning 1993). The species seems to prefer some disturbance, and is able to move opportunistically into recently disturbed areas (Tom Kaye, Institute for Applied

Ecology, personal communication). The Port Orford population is located on the beach itself, taking advantage of gaps in the ever-present *Ammophila arenaria* created by the dumping of sand on the beach while dredging the bay. Several other populations reside on the partially stabilized beach dunes, where other vegetation provides some protection but frequent disturbance still occurs. *Oenothera wolfii* is also found on the bluffs immediately above the beaches. The vegetation cover on the bluffs ranges from almost complete cover (Hubbard Creek, Pistol River) to areas where bare soil and rock are exposed (Sister's Rock, Otter Point). Once again, *O. wolfii* appears to prefer some disturbance, since the populations on less stabilized substrate were much larger than those in completely vegetated habitat.

Associated species include *Abronia latifolia, Abronia umbellata* ssp. *breviflora*, *Achillea millefolium*, *Ammophila arenaria*, *Anaphalis margaritacea*, *Baccharis pilularis*, *Bromus* sp., *Cytisus scoparius, Daucus carota*, *Elymus mollis*, *Equisetum arvense*, *Eriogonum* sp., *Fragaria chiloensis*, *Garrya elliptica*, *Gaultheria shallon*, *Lonicera involucrata*, *Lotus corniculatus*, *Lupinus* sp., *Mimulus guttatus*, *Morella californica*, *Petasites palmatus*, *Phacelia argentea*, *Picea sitchensis*, *Plantago* sp., *Polygonum paronychia*, *Pteridium aquilinum*, *Rubus spectabilis*, and *Salix hookeriana* (ORNHIC 2003, personal observation).

Current threats

Oenothera wolfii is faced with several imminent threats. The first concern, habitat loss and alteration, is a common one for many rare and endangered plants. Coastal development, and the dune stabilization efforts that often accompany it, has negatively impacted *O. wolfii* habitat. Roadside maintenance is another cause of disturbance. Several *O. wolfii* populations grow adjacent to Highway 101, and activities such as road expansion, culvert maintenance and herbicide spraying may potentially harm these populations. *Ammophila arenaria*, or European beach grass, was introduced during highway stabilization projects in the 1930s, and has proceeded to spread to almost every beach in Oregon. This exotic plant's habit of stabilizing dunes while establishing almost a monoculture has further reduced available habitat for *O. wolfii* (Gisler & Meinke 1997, Imper 1997).

Additionally, *O. wolfii* is able to hybridize with the common garden escapee *O. glazioviana*. Morphological studies indicate that there is widespread hybridization throughout the California populations (Carlson et al. 2001). As many of the Oregon populations are near major roadsides, making them at risk of future hybridization with *O. glazioviana* as well. To effectively conserve the species, it is imperative that new *O. wolfii* populations be established in protected areas, away from highways, using seed from uncompromised *O. wolfii* populations while they still exist.

Literature Review

The current rate of species extinction is of increasing concern to scientists, policy-makers, and members of the general population that appreciate the diversity found in nature. This unprecedented loss of species diversity can be largely attributed to exponential growth of the human race and the subsequent impacts of this increased population on the natural world (Falk and Olwell 1992). Conservation biologists and restoration ecologists are utilizing a variety of tools to stem the tide of species extinction.

Rare and endangered plant conservation goals often include a reintroduction component, where an attempt is made to establish new populations in natural settings. Pavlik et al. (1993) list experimental creation of a population within the historic range of a rare plant as the first phase of recovery for that plant. Often these reintroduction efforts try to establish new populations on administratively protected sites, where future protection and monitoring are more likely to occur. Planning, reintroduction, and monitoring of dune restoration projects are in their infancy (Pickart and Sawyer 1998), but as the knowledge about how to conduct a reintroduction project grows, success stories become more common (Allen 1994, Bowles et al. 1993, Kaye 1995).

As more rare plant reintroduction studies take place, a growing body of literature provides guidance for those attempting such projects in the future. Rare plant reintroduction projects should include the following steps: selection of a reintroduction site, acquisition of propagules, preliminary ex situ studies, experiment design and installation, demographic monitoring and evaluation (Pavlik et al. 1993). Overall success is defined as the creation of a new, self-sustaining population within the historic range of the plant (Pavlik 1997). However, both short-term and longer ranged goals should be developed. Short-term goals might include the completion of the life cycle (in situ) of the plant being reintroduced. Long-term objectives might be met by achieving a pre-determined minimum viable population size through natural recruitment of second generation cohorts (Pavlik 1996).

Methods

Seed Germination

Seeds collected from the Port Orford wild population of *Oenothera wolfii* in September of 2002 were used to propagate plants for establishment of the experimental populations. Seeds were stored in a dry, dark location in paper bags, at room temperature, during the time between collection and germination. The seeds were germinated in petri dishes (50 seeds/dish) lined with germination paper (Figures 4 and 5) in August and September of 2003. Germination took place in a Oregon State University greenhouse, where temperatures were maintained at 70°/65° C and lights were on twelve hours/day. Germination paper was sprayed with distilled water as needed.

Figure 1. *O. wolfii* seed germination

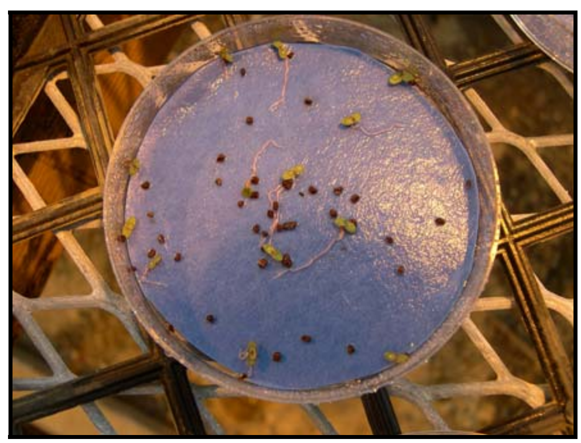

Figure 2. *O. wolfii* seeds after 1 week

Previous studies provided little information about germination techniques. In order to determine whether or not it was necessary to treat the seeds to prevent fungal growth on the seeds, two germination trials were conducted. In each trial, half of the seeds were rinsed with a 5% bleach solution prior to placement on germination paper.

Due to the ease with which *O. wolfii* seeds were germinated in the initial trials, and additional data provided on past *O. wolfii* seed germination trials (Tom Kaye, Institute for Applied Ecology, personal communication), additional germination trials were unnecessary. However, seeds of reproductive plants in both experimental populations were germinated, along with seeds of several comparable wild populations, in order to assess seed viability of the individuals in the experimental populations (See “Seed Viability” section below).

Seedling Cultivation

Germinated seeds were planted in 2” x 2” x 2 1/2” deep cells filled with a 2/3 sand, 1/3 peat moss planting mixture (Figures 6 and 7). Seedlings were watered as needed, and fertilized every three weeks with approximately 1/8 teaspoon 20-30-20 water soluble all-purpose fertilizer.

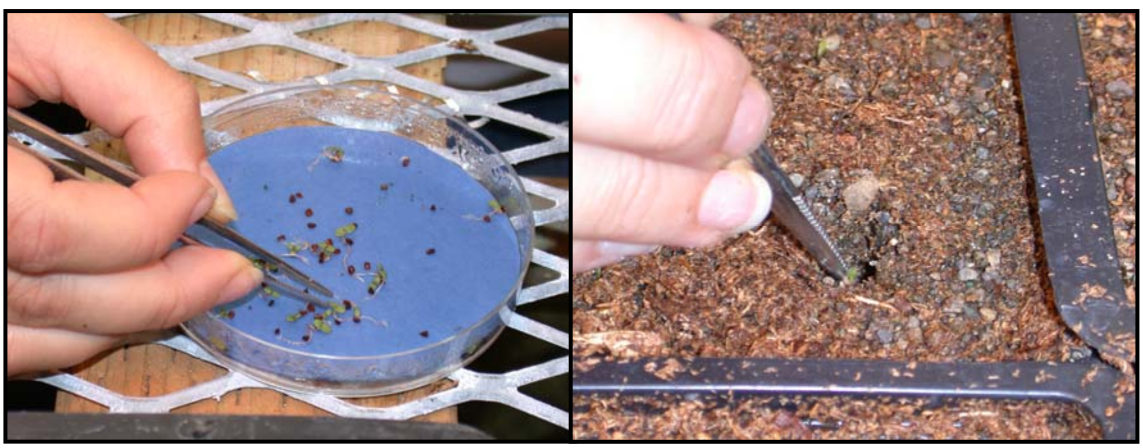

Figure 6. Germinated seeds being planted in peat moss/sand planting medium.

Seedlings were transplanted into 4” x 4” x 6” deep pots after 34 days (Figures 7 and 8). At the time of planting, large rosettes were approximately 30 cm in diameter and small rosettes were approximately 14 cm in diameter. After plants had been growing for six weeks, white flies infested the larger plants in the greenhouse, and were treated by the greenhouse staff with the insecticide Duraplex TR. The white fly infestation did not appear to cause visible harm to the *O. wolfii* plants.

Figure 7. 14 day old *O. wolfii* seedlings.

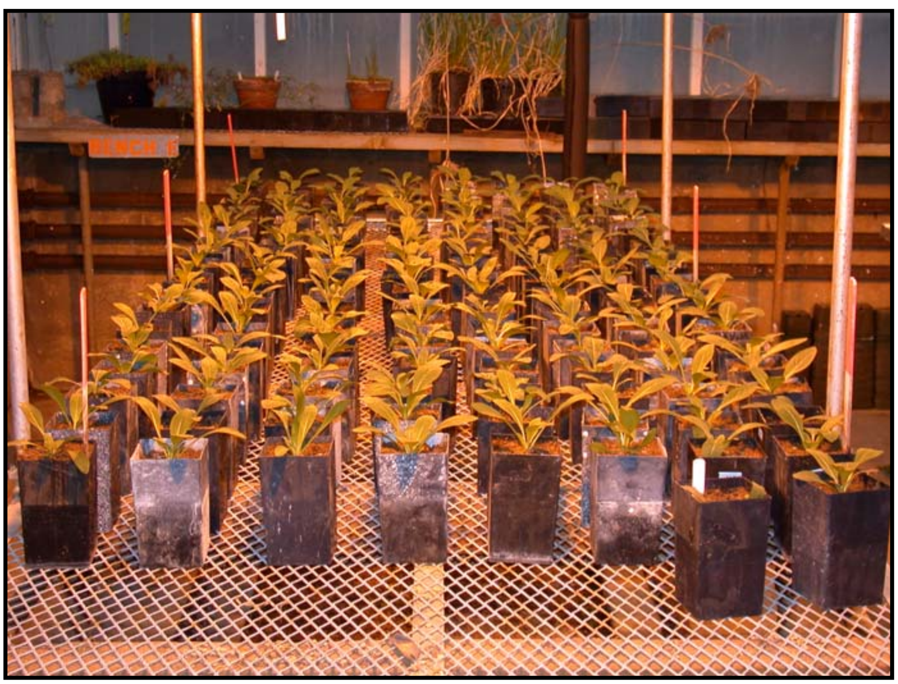

Figure 8. 34 day old *O. wolfii* seedings transplanted into larger pots.

New Population Site Selection

Several criteria were used for selecting sites for introduction of the experimental populations. Sites needed to be fairly close to or within current range of *O. wolfii*. Ideally, they would be isolated from roads and potential hybridization threat. The habitat needed to be similar to that of natural populations for the best chance of success. Finally, for long-term monitoring and protection, the sites needed to be on land that was already being managed by a public agency.

The first site, Lost Lake (Figure 9), is part of the New River Area of Critical Environmental Concern (ACEC), within the Bureau of Land Management's Coos Bay District. Covering 72 acres, the Lost Lake area is located roughly five miles south of Bandon. About a mile from the ocean, it abuts a larger area of State Park land, and consists of inland dunes and shore pine woods. Accessible by a dirt road and located several miles from Highway 101, it is less likely to be exposed to the threat of hybridization. The habitat appears to be compatible with *O. wolfii* needs. Lost Lake is located slightly north of *O. wolfii*'s current range.

Figure 9. *Oenothera wolfii* plots at Lost Lake.

Figure 10. Meyers Creek site, viewed from across Highway 101

The second site, Meyers Creek, is located on the hillside just above Highway 101, about nine miles south of Gold Beach (Figure 10). The land belongs to the Oregon Department of Transportation, and the site is about half of a mile from the Pistol River wild population of *O. wolfii*. The habitat is almost identical to that of the existing population, and ODOT is supportive of the project. The one concern is the proximity to Highway 101, which increases the chance of hybridization and disturbance from ODOT workers and highway travelers.

Experimental Population Outplanting: Lost Lake

Forty plants of each of two ages (39 and 73 days old) were planted at the Lost Lake site in late October. In order to facilitate transportation of the plants to the Lost Lake site (a short hike into the site was required to reach the plots), the larger, older rosettes were removed from their pots, excess planting medium was shaken from their roots, and they were placed in ziplock bags with a damp paper towel. Bagged plants were transported to Lost Lake in large coolers with ice (Figure 11). Since the effect of bagging the plants was unknown, an additional ten large plants were transplanted to the site while still in their pots, serving as controls in case the bagging had a negative effect on the survival of the other large plants. The type of propagule (large bagged rosette, large rosette in pot, small rosette) planted in each square meter plot was randomly determined.

Figure 11. Large rosettes were removed from pots and transported in ziplock bags in coolers.

In addition to evaluating the impact of rosette size on survival and reproduction, the study also looked at the impact of competing vegetation on the establishment of the new plants. . Due to the fact that much of the Lost Lake site had little or no ground cover, and because BLM staff was concerned with the impact of ground cover removal on the site, plots were selected so that there was a range of percent ground cover within the plots, rather than actually removing the ground cover from half of the plots (as was done at Meyers Creek). Fifty percent of the plots had no ground cover in them, and fifty percent of the plots were selected to span the following categories of percent ground cover: 1-25%, 26-50%, 51-75%, and 76-100% (Figures 12-15).

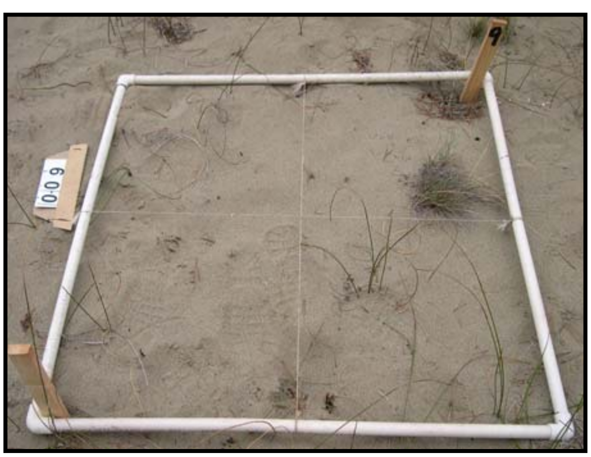

Figure 12. Example of a Lost Lake plot with 0-25% ground cover

Figure 13. Example of a Lost Lake plot with 26-50% ground cover

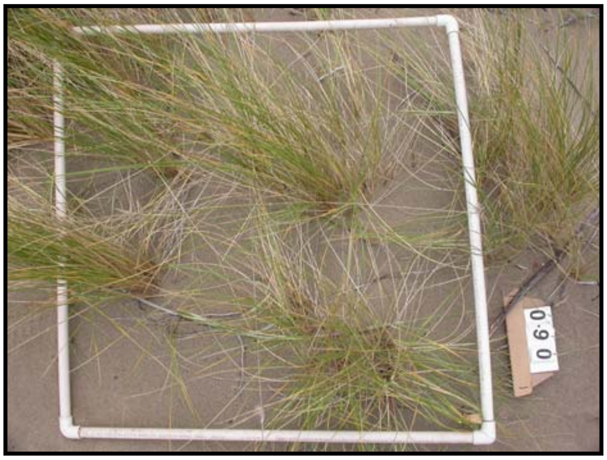

Figure 14. Example of a Lost Lake plot with 51-75% ground cover

Figure 15. Example of a Lost Lake plot with 76-100% ground cover

At the time of outplanting, the fall rains had not yet begun. Each plot's propagules (large rosette, small rosette, and seed) were provided with one liter of water at the time of planting (Figure 16). An additional liter of water was provided to each of the plots three weeks later.

Figure 16. Large rosette at Lost Lake receiving one liter of water at time of outplanting.

In addition to the 90 transplant plug plots established at Lost Lake, ten seed plots were also created, with 200 seeds sown in each plot. In five of the seed plots, the seeds were buried ¼" below the sand (Figure 17), while in five of the plots the seeds were scattered on the surface. A liter of water was sprinkled over the seeds at each plot at the time of sowing.

Figure 17. Lost Lake seed plot, with seeds buried ¼".

Experimental Population Outplanting: Meyers Creek

Rosette age and size: Due to logistical delays, plants at the Meyers Creek site were older at the time of outplanting, although the difference in age between the two treatment groups was the same. The planting at Meyers Creek occurred in mid-November (three weeks after the Lost Lake planting), and the older plants were 94 days old, while the younger plants were 60 days old. The type of rosette (younger vs. older) was randomly assigned to each plot.

Ground cover removal: Unlike Lost Lake, the Meyers Creek site is completely covered with shrubs, forbs and graminoids. Fifty percent of the half meter^2 plots were randomly chosen to have their ground cover removed at the time of outplanting (Figure 18). Ground cover was removed by hand. Randomization was also used to assign 50 percent each of the large and small rosettes to plots with vegetation removed, and to assign 50 percent of each propagule type to plots with existing vegetation untouched. A liter of water was given to each of the plants at the time of planting.

Figure 18. Meyers Creek half meter^2 plot with small rosette and ground cover removal.

Seed plots: Ten seed plots were also established at Meyers Creek, with 200 seeds sown per plot. Five of the plots had their vegetation removed, and five did not. The vegetation removal treatment was assigned to plots randomly, and seed plots were sprinkled with a liter of water at the time of sowing.

Environmental Factors

In order to determine if there was a relationship between plant survival and reproductive success and the environment, the following environmental factors were measured at each plot or site: ground moisture levels, slope, aspect and pH. At Lost Lake, the percentage of vegetation cover is also being treated as an environmental factor, rather than a treatment.

Ground moisture: In order to determine if there was any relationship between ground moisture levels and the survival and reproductive success of individuals in the experimental populations, volumetric water content measurements were taken at each plot in March, June and September of 2004, using a Hydrosense© water meter (Campbell Scientific, Inc. 2001). On each date, three measurements were obtained for each plot. These three measurements were then averaged to obtain the ground moisture measurement used for analysis.

Aspect: The aspect of each plot was determined with a compass. The overall aspect of each site was also noted.

Slope: The slope of each plot was estimated using a half full rectangular bottle of water. A line was drawn on the side of the bottle when it was laying sideways on a level surface, giving a baseline slope. The bottle was then placed on each microplot, and angle between the drawn line and the line of the water surface was measured with a protractor.

Heat Load: Because of the difficulty of utilizing aspect numbers in analysis (an aspect of one degree and an aspect of 359 degrees, while only two degrees apart, would show up as completely different in the analysis), aspect, slope and latitude were combined into one environmental factor, heat load. Heat load was calculated using an equation (adjusted R^2 = 0.983) developed by McCune and Keon (2002).

Soil pH: Because individual plots at each site were located fairly close together, soil pH was measured for the overall site, rather than at each individual plot (John Hart, Department of Crop and Soil Science, Oregon State University, personal communication). Soil samples were taken from three different locations at each site, and submitted to the Oregon State University Department of Crop and Soil Science's Central Analytical Laboratory for pH analysis.

Monitoring

Both sites were visited monthly for the year and a half following outplanting. Relevant data, such as herbivory and other evidence of disturbance were recorded. Photographs were taken of each plant throughout the monitoring period, and ground water measurements were taken quarterly.

By early September of 2005, reproductive plants had bolted, flowered, and set fruit. Fruits on the bottom of the flowering stalk were already mature and beginning to dehisce. All measurements with regards to plant size and reproduction were taken September 8-12, 2005. If the plant had reproduced, plant size was determined by measuring the height of the tallest branch and the number of branches. If the plant had not reproduced, plant size was determined by measuring the diameter of the rosette at its widest point. If there was more than one rosette, the diameter spanned the two rosettes, taken together, at their widest point combined. The number of fruits was counted for each reproducing plant. One fruit was randomly selected from each of three areas (bottom, middle, top) of each reproductive plant, for a total of three fruits per plant. Seeds from the three fruits were combined, counted and divided by three, in order to obtain an estimated average number of seeds per fruit for each plant. Due to the small size of *Oenothera wolfii* seeds, seeds were weighed as a group in order to determine the average weight of the seed.

Seed Viability

In order to estimate the percentage of viable seed for each reproducing individual in the experimental populations, fifty seeds from each plant were germinated in the greenhouse, following seed germination protocol established earlier (See "Seed Germination" section above). In order to compare seed viability of experimental and wild populations, seed collected at three comparable wild populations (Port Orford, Hubbard Creek and Pistol River) in September 2004 were also germinated. Finally, in order to compare seed germination rates between fresh (collected September 2004) seed, one-year-old seed (collected September 2003), and two-year-old seed (collected September 2002), seed collected from the Port Orford and Hubbard creek wild populations in the fall of 2002 and 2003 were also germinated. These germination trials were conducted in the Oregon State University greenhouses in February 2005.

Analysis

Statistical analysis of the data was conducted utilizing the program S-PLUS®, version 6.2. Factors impacting whether or not individuals in the experimental populations survived or reproduced were analyzed using logistic regression. Other response variables (plant size, number of fruits and seeds, seed weight and germination rates, etc.) were analyzed using linear regression and ANOVA.

Because Lost Lake and Meyers Creek were planted at different times (Meyers Creek plants were transplanted three weeks later than those of Lost Lake), resulting in transplants of different ages (Meyers Creek plants were three weeks older), and because the environmental factors at the two sites were very different, Meyers Creek and Lost Lake populations were analyzed separately, as two different experiments, rather than aggregating the data.

Results

Seed germination

Seeds started germinating within five days of being placed in the dishes. Seed germination was not difficult; germination rates ranged from 30 percent to 59 percent (Table 1). Bleaching the seeds to reduce fungal growth did not improve germination rates. In one trial, there was no significant difference between the germination rates of the bleached and unbleached seeds (2-sided p-value = 0.076), and in the other trial, the unbleached seeds actually germinated at a significantly higher rate than those which were not bleached (2-sided p-value = 0.006) (Table 1). Overall, there was little fungal growth on any of the seeds, regardless of the treatment.

Table 1: Results of 2003 seed germination trials: bleached vs. unbleached.

	Trial 1		**Trial 2**	
Treatment	**Bleached**	**Unbleached**	**Bleached**	**Unbleached**
Mean # seeds germinated (out of 50)	20.4 (40.8%)	15.1 (30.2%)	27.6 (55.2%)	29.8 (59%)
Standard Error	1.30	1.25	0.80	0.87
n (# Petri dishes)	19	19	20	20
T-Statistic	2.94		-1.82	
2-sided p-value	0.006		0.076	

Cultivation

There was no difficulty in cultivating *Oenothera wolfii* plants. Almost 100 percent of the transplanted germinated seeds survived. It is interesting to note that while mature *O. wolfii* plants in wild populations have thick taproots, plants in the greenhouse did not. Their roots were fine, filamentous, and were evenly dispersed throughout the planting medium at the time of transplant.

Transplant Survival

Propagule size: Overall, transplant survival was high at both experimental population sites. At Lost Lake, 80 (89 percent) of the transplants (small, big bagged, big pots) survived. Because big plants which were bagged for transportation survived, the ten big plants in pots (controls in the event that bagged plants all died) were not included in statistical analysis. At Meyers Creek, only one small plant died after transplanting, giving an overall survival rate of 99 percent (Table 2). There was a significant difference in survival rates between sites (t-statistic = 2.17, 2-sided p-value = 0.03).

At Lost Lake, 33 small transplants (83 percent) and 37 of the large transplants (93 percent) survived (Table 2, Figure 19). However, this difference in survival rates was not statistically significant (t-statistic = 1.323, 2-sided p-value = 0.187). At Meyers Creek, only one transplant (a small plant) did not survive, giving an overall survival rate of 99 percent. Once again, there was no statistical difference between the survival rate of the two propagule sizes (Table 2, Figure 19).

Ground cover: Ground cover presence also did not affect plant survival rates. At Lost Lake, 39 transplants (87 percent) located in bare plots with no groundcover survived, and 41 transplants (91 percent) located in plots with groundcover survived. There was no statistical difference in survival rates of transplants in plots different ground cover classes (t-statistic = 0.673, 2-sided p-value = 0.441). At Meyers Creek, the one plant which did not survive was in a plot where the ground cover was not removed, and the survival rates based on ground cover status were not statistically significant (Table 3, Figure 20).

Table 2. Transplant survival of different sized propagules at Lost Lake and Meyers Creek.

	LOST LAKE				MEYERS CREEK		
	Big (Bagged)	Small	Big (Pots)	Total	Big	Small	Total
Survived	37 (93%)	33 (83%)	10 (100%)	80 (89%)	40 (100%)	39 (98%)	79 (99%)
Died	3 (7%)	7 (17%)	0 (0%)	10 (11%)	0 (0%)	1 (2%)	1 (1%)
Total Planted	40	40	10	90	40	40	80

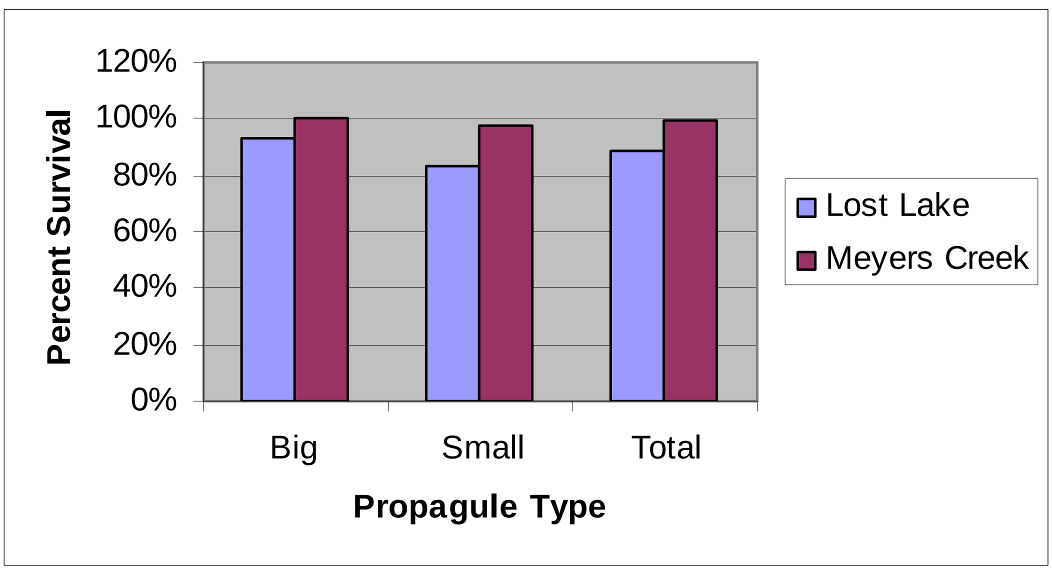

Figure 19: Transplant survival for big and small plants at Lost Lake and Meyers Creek.

Table 3: Transplant survival of propagules in plots with different ground cover status at Lost Lake and Meyers Creek.

		Survived	Died	Total
Lost Lake	**Total planted**	80 (89%)	10 (11%)	90
	Total with no groundcover	39 (87%)	6 (13%)	45
	Total with groundcover	41 (91%)	4 (9%)	45
	1-25% groundcover	10 (91%)	1 (9%)	11
	26-50% groundcover	11 (92%)	1 (8%)	12
	51-75% groundcover	14 (88%)	2 (12%)	16
	76-100% groundcover	6 (100%)	0 (0%)	6
Meyers Creek	**Total planted**	79 (98%)	1 (2%)	80
	Groundcover removed	40 (100%)	0 (0%)	40
	Groundcover left	39 (98%)	1 (2%)	40

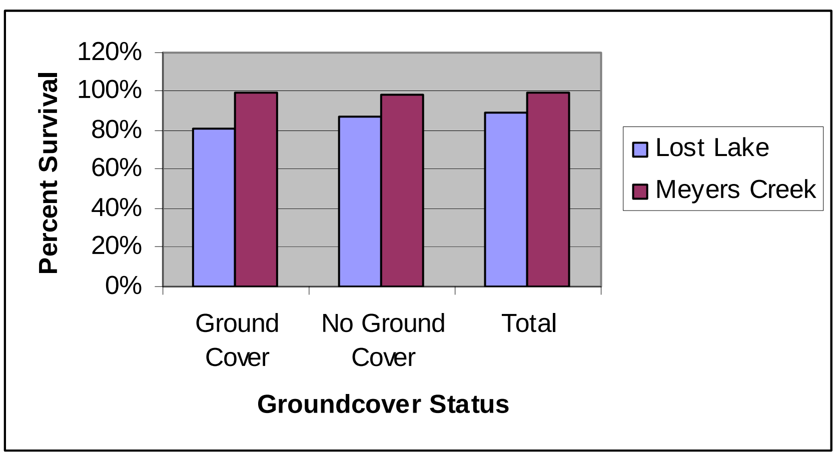

Figure 20: Transplant survival for plots with and without ground cover.

Environmental factors: Finally, there was no evidence that environmental factors (ground moisture levels, slope and heat load) were significantly associated with the survival of transplants at either Lost Lake or Meyers Creek (all 2-sided p-values > 0.05).

Transplant Reproduction

Propagule size: At Lost Lake, 14 plants (18 percent) reproduced in the first growing season after transplanting. All of the reproducing plants were large transplants; 11 large bagged plants (30 percent) and three large potted plants (30 percent) produced flowering stalks and set fruit (Table 4, Figure 21).

At Meyers Creek, propagule size at time of transplanting significantly impacted whether or not the plant reproduced in the first growing season (t-statistic = -3.404, 2-sided p-value = 0.006). A total of 46 (58 percent) plants reproduced by September 2004 (Table 4, Figure 21). Thirty-one of these were large transplants (78% of the large plants) and 15 were small (38 percent). The odds of a large propagule reproducing were 2.3 times the odds of a small propagule reproducing (95% confidence interval 1.4-3.8).

Table 4: Reproduction of large and small transplants at Lost Lake and Meyers Creek.

	LOST LAKE				**MEYERS CREEK**		
	Big (Bagged)	**Small**	**Big (Pots)**	**Total**	**Big**	**Small**	**Total**
Reproduced	11 (30%)	0 (0%)	3 (30%)	14 (18%)	31 (78%)	15 (38%)	46 (58%)
Didn't Reproduce	26 (70%)	33 (100%)	7 (70%)	66 (82%)	9 (22%)	24 (62%)	33 (42%)
Total (Survived)	37	33	10	80	40	39	79

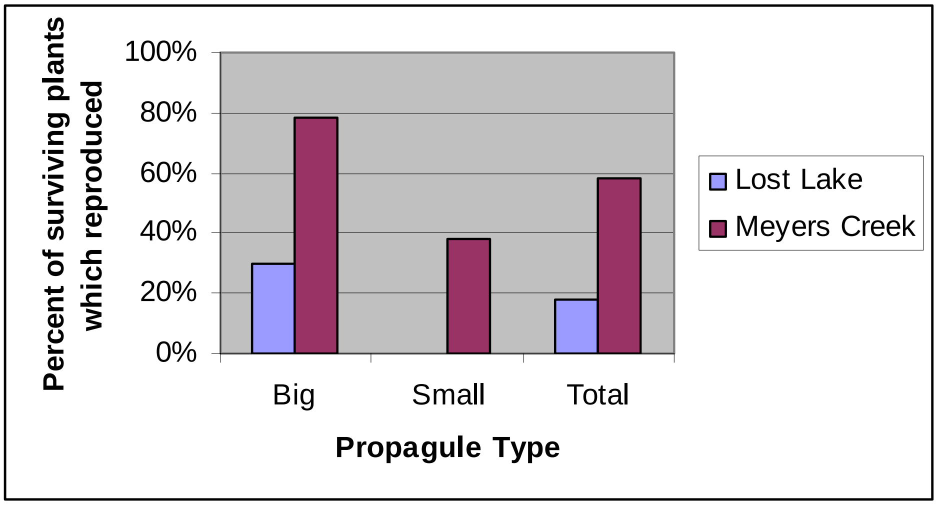

Figure 21: Reproduction of large and small transplants at Lost Lake and Meyers Creek.

Ground cover: Ground cover presence did not significantly impact whether or not plants reproduced at the Lost Lake site (t-statistic = 0.225, 2-sided p-value = 0.810). In plots with no ground cover, six (15 percent) of the transplants reproduced in the first year. In plots with ground cover, eight transplants (20 percent) reproduced in the first year (Figure 22). These eight plants were spread throughout ground cover classes, although none of the plants located within plots with 100 percent ground cover reproduced (Table 5).

At Meyers Creek plots where the ground cover was removed, 31 of the transplants (78 percent) reproduced in the first year (Table 5, Figure 22). In plots where ground cover remained (at 100 percent), only 15 plants (38 percent) reproduced in the first year. This difference was statistically significant (t-statistic = -3.230, 2-sided p-value = 0.001). The odds of transplants reproducing in the first year were 2.2 times greater if ground cover was removed (95% confidence interval 1.4-3.6).

Table 5: Reproduction of transplants with different ground cover percentages at Lost Lake, and reproduction of Meyers Creek transplants in plots with ground cover removed vs. not removed.

		Reproduced	Didn't Reproduce	Total surviving plants
Lost Lake	**Total**	14 (18%)	66 (82%)	80
	Total with no groundcover	6 (15%)	33 (85%)	39
	Total with groundcover	8 (20%)	33 (80%)	41
	1-25% groundcover	2 (20%)	8 (80%)	10
	26-50% groundcover	3 (27%)	8 (73%)	11
	51-75% groundcover	3 (21%)	11 (79%)	14
	76-100% groundcover	0 (0%)	6 (100%)	6
Meyers Creek	**Total**	46 (58%)	33 (42%)	79
	Groundcover removed	31 (78%)	9 (22%)	40
	Groundcover left	15 (38%)	24 (62%)	39

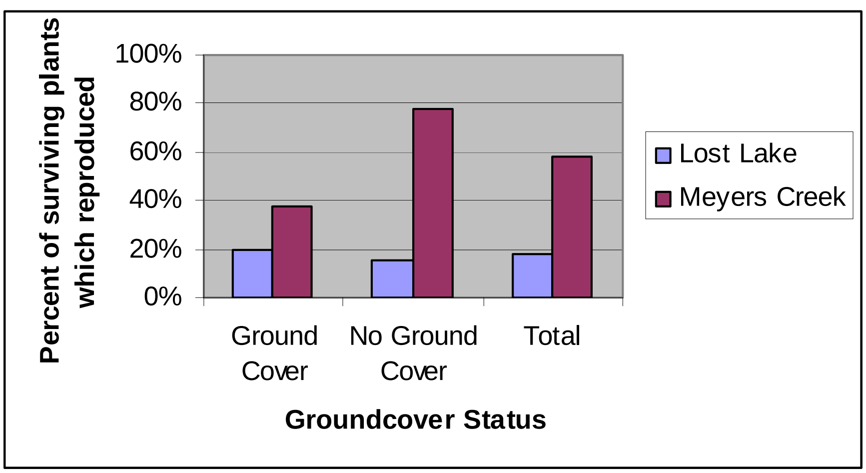

Figure 22: Reproduction of transplants in plots with ground cover vs. those in plots with no ground cover.

Environmental factors: At Lost Lake, there is evidence that several of the environmental factors (June ground moisture percentage, slope and heat load) are associated with whether or not transplants reproduced in the first year (slope t-statistic = 2.986, 2-sided p-value = 0.0028; June moisture t-statistic = -2.620, 2-sided p-value = 0.0088; heat load t-statistic = 2.418, 2-sided p-value = 0.018).   At Meyers Creek, the environmental factor data are not as clear. Both March and June ground moisture levels appear to be associated with plant reproduction, but the association is reversed, with March ground moisture levels positively related to reproduction (t-statistic = 2.763, 2-sided p-value = 0.0058) and September ground moisture levels negatively associated with reproduction (t-statistic = -2.229, 2-sided p-value = 0.022). The rest of the environmental factors are not significantly associated with whether or not transplants reproduced in the first year.

Transplant Reproductive Vigor

In addition to measuring whether or not plants survived or reproduced, a variety of reproductive vigor measurements (plant height, number of branches, number of fruits, number of seeds per fruit, average seed weight and germination rates) were recorded for each reproducing plant.

Propagule size: Because no small plants reproduced at Lost Lake, no analysis of transplant size impacts on these variables could be performed. However, out of the 14 large plants which reproduced, three of those remained in their pots during transportation, rather than being bagged. Although the numbers of individuals are too small to draw statistical conclusions, the three potted large plants performed better in all reproductive vigor categories (Table 6).

At Meyers Creek, large transplants had more branches (average of 3.6 vs.1.1), more fruits (average of 76.5 vs. 45.7), heavier seeds (0.348 mg vs. 0.318 mg), and higher rates of seed germination (46.8% vs. 34.0%). The size of the transplant significantly impacted the number of fruits, with large propagules producing, on average, 25 more fruits than small propagules (2-sided p-value = 0.0028, 95% confidence interval: 11-39). Propagule size was also significant for plant height (2-sided p-value = 0.005); large transplants were roughly nine centimeters taller than small transplants (95% confidence interval: 3cm - 14cm). Small propagules had slightly more seeds per fruit (287.8 vs. 277.1), but this difference was not significant. Among the plants which did not reproduce, small plants had a larger diameter at the end of the growing season (average of 29.9 cm vs. 24.1 cm); however, this difference was not statistically significant either.

Table 6: Comparison of plant size and reproductive success between different propagule sizes and sites. Numbers in parentheses are standard errors.

		LOST LAKE				**MEYERS CREEK**		
		Big (Bag)	**Small**	**Big (Pot)**	**Total**	**Big**	**Small**	**Total**
Reproducing plants:	**Average # Branches/ Plant**	1.3 (0.2)	n/a	2.0 (1.0)	1.4 (0.3)	3.6 (0.5)	1.1 (0.1)	2.8 (0.4)
	Average # Fruits/Plant	10.4 (1.3)	n/a	16.7 (3.8)	11.7 (1.4)	76.5 (10.6)	45.7 (7.1)	66.5 (7.8)
	Average # Seeds/Fruit	174.3 (17.5)	n/a	223.6 (26.7)	185.7 (15.5)	277.1 (11.8)	287.8 (21.3)	280.6 (10.4)
	Average Weight/ Seed (mg)	.300 (.012)	n/a	.366 (.018)	.308 (.011)	.348 (.008)	.318 (.022)	.338 (.009)
	Average % Seed Germination	44.8 (6.1)	n/a	42.0 (13.0)	44.1 (5.3)	46.8 (2.6)	34.0 (5.6)	42.7 (2.7)
	Average Height (cm)	32.7 (2.5)	n/a	38.0 (5.4)	33.9 (2.3)	65.1 (3.0)	66.3 (4.1)	65.5 (2.4)
Non-reproducing plants:	**Average diameter (cm)**	14.8 (0.6)	9.2 (0.6)	15.6 (1.3)	12.1 (0.6)	24.1 (2.0)	29.9 (2.2)	28.3 (1.7)

Ground cover status: The impacts of ground cover status on reproductive vigor are summarized in Table 7. At Lost Lake, ground cover presence was significantly and positively related to the number of seeds per fruit (2-sided p-value = 0.046). Transplants in plots with ground cover produced roughly 73 more seeds per fruit than those in plots without ground cover (95% confidence interval: 22-125). Ground cover presence was not associated with any other reproductive vigor measurements.

At Meyers Creek, ground cover removal significantly impacted the number of fruits produced (2-sided p-value = 0.001). Plants in plots where the ground cover was removed produced 26 fewer fruits (95% confidence interval: 12-40). Removal of ground cover also significantly impacted the height of the plant (2-sided p-value = 0.001), with plants in plots with ground cover removed an estimated 10 cm taller than those in plots where the ground cover was not removed (95% confidence interval: 4-15). Ground cover did not significantly affect other reproduction vigor measurements.

Table 7: Comparison of reproductive success for plots with different ground cover status. Fifty percent of Meyers Creek plots had ground cover removed as a treatment, while 50% of Lost Lake plots were located in areas with no ground cover present. Numbers in parentheses are standard errors.

		LOST LAKE			**MEYERS CREEK**		
		No Ground Cover	**With Ground Cover**	**Total**	**Ground Cover Left**	**Ground Cover Removed**	**Total**
Reproducing plants:	**Average # Branches/ Plant**	1.5 (0.3)	1.4 (0.4)	1.4 (0.3)	2.2 (0.4)	3.1 (0.5)	2.8 (0.4)
	Average # Fruits/Plant	12.2 (2.7)	11.4 (1.6)	11.7 (1.4)	41.3 (6.6)	78.7 (10.5)	66.5 (7.8)
	Average # Seeds/Fruit	148.3 (16.0)	217.7 (18.3)	185.7 (15.5)	290.2 (17.3)	276.0 (13.1)	280.6 (10.4)
	Average Weight/ Seed (mg)	.324 (.020)	0.295 (.010)	.308 (.011)	.330 (.015)	.342 (.012)	.338 (.009)
	Average % Seed Germination	43.3 (7.8)	44.9 (7.7)	44.2 (5.3)	48.0 (5.2)	40.1 (3.0)	42.7 (2.7)
	Average Height (cm)	32.2 (2.5)	35.2 (2.4)	33.9 (2.3)	61.3 (4.1)	67.5 (2.9)	65.5 (2.4)
Non-reproducing plants:	**Average diameter (cm)**	12.0 (0.8)	12.2 (0.7)	12.1 (0.6)	28.2 (1.9)	28.5 (3.8)	28.3 (1.7)

Additional environmental factors: For the most part, there was little evidence that environmental factors were associated with the reproductive vigor of the transplants at Lost Lake. However, there were several exceptions to this generalization. Slope and June ground moisture were significantly associated with reproductive plant height (2-sided p-values =

0.0008, 0.0138, respectively). There was evidence that the interaction between slope and heat load was also significant for reproductive plant height (2-sided p-value = 0.0002).

At Meyers Creek, environmental factor results were inconclusive, as well. Heat load was significantly and positively associated with the number of fruits (2-sided p-value = 0.013). March ground moisture levels were positively associated with average seed weight (2-sided p-value = 0.035). September ground moisture levels were negatively associated with plant height, while June ground moisture levels were positively associated with plant height (2-sided p-values = 0.001 and 0.014, respectively).

Discussion

New population introduction of rare plants within their historical range, as well as the augmentation of existing populations, is a valuable tool used to improve the demographic dynamics of these species. There are several advantages to establishing multiple populations. First, the risk of extirpation due to catastrophic events can be spread out over discrete populations, with surviving populations able to serve as seed sources to re-establish new populations (Menges 1991). Secondly, multiple populations can offset genetic drift in limited, isolated populations, providing more opportunities for the species to evolve in response to various selective pressures (Huenneke 1991, Templeton 1991). The combined threats of habitat loss and hybridization make *Oenothera wolfii* a prime candidate for new population establishment.

Oenothera wolfii seeds germinate easily with no vernalization or scarification treatment. There appears to be no real benefit to treating the seeds with bleach prior to germinating them – fungal growth was not a problem with either the bleached or unbleached seeds, and germination rates for the two treatments were not significantly different . Cultivation of *O. wolfii* in the greenhouse was similarly lacking in obstacles – almost all of the germinated seedlings survived transplantation into pots, and plants grew quickly and healthily in the greenhouse. One interesting observation was the difference in root development between greenhouse-grown plants, which had thin, filamentous roots, and individuals in natural populations, which develop thick taproots. This difference is most likely due to the availability of water and nutrients in the greenhouse setting. However, transplant survival did not appear to be greatly impacted by this morphological difference.

Transplant survival was high at both sites – Meyers Creek only lost one plant (1%), and ten Lost Lake transplants (11%) did not survive. Although there was no statistically difference between survival of different propagule sizes, overall more small plants perished (8) than their larger counterparts (3). It is possible that with larger sample sizes, this difference might become significant. Also, all of the three large plants which died at Lost Lake were plants which had been removed from their pots and bagged for transportation. Once again, although statistically this difference was not significant, it is recommended that plants be transplanted in their pots when at all possible during future introduction efforts. Ground cover status (Lost Lake) and removal (Meyers Creek) did not significantly impact survival rates of transplants, either.

When it came to reproduction, however, propagule size did matter. At Meyers Creek, 31 large transplants reproduced (as opposed to 15 small transplants), and large transplants were taller, and had more branches, more fruits, heavier seeds and higher germination rates than their small counterparts. At Lost Lake, no small transplants reproduced the first year, although many of the plants overwintered a second year as rosettes, and further monitoring is necessary to determine if they will reproduce in the upcoming growing season.

Ground cover removal also positively impacted reproduction rates at Meyers Creek, with plants in plots where the ground cover was removed being more likely to reproduce in the first growing season (31 plants vs. 15 reproductive plants in plots where ground cover was not removed). Lost Lake results were less clear; ground cover removal was not possible, and ground cover percentages were treated as an environmental factor, rather than a treatment. Ground cover percentages were not significantly associated with reproduction; however, plots with some ground cover produced roughly 70 more seeds per fruit than those in plots without ground cover. This may be attributed to the conditions at Lost Lake, where plots with no ground cover were located on open dune habitat with large amounts of moving sand and less ground moisture retention, which might have caused more stress to the plants.

Overall, the relationship between measured environmental factors and transplant survival and reproduction was difficult to establish. Although some factors did appear to be associated with reproductive success, there were no consistent trends which would allow predictions to be made about the appropriateness of future sites. Field observations highlighted the fact that plants performed better at Meyers Creek, where they were close to the ocean (as opposed to Lost Lake, where plants were almost a mile inland). Meyers Creek substrate retained moisture better, due to the humus in the soil and the ground cover. When identifying future reintroduction sites, it is recommended that they be located directly on or above the ocean beach when possible, since this is where natural populations are located, and where the experimental population thrived.

Initial results suggest that reintroduction of *Oenothera wolfii* into suitable sites has the potential for success. Because *O. wolfii* is primarily a biennial, further monitoring is needed to determine whether or not these introduced populations are self-sustaining. Many of the transplanted propagules survived but did not reproduce during the first growing season after transplantation. Also, it is difficult to determine the success of a reintroduction project without evaluating recruitment of new individuals resulting from the naturally sown seed of the reproducing transplants. Monitoring during the upcoming growing season will determine whether or not seedlings are present, but ideally monitoring would continue through at least the fall of 2006, in order to determine if those seedlings established and reproduced as well.

Recommendations

• Continue monitoring of all transplant sites to determine the ultimate feasibility of reintroduction/augmentation projects for *Oenothera wolfii*. Because this taxon is biennial, several more years of data are required to confidently evaluate the ability of reintroduced populations to become self-sustaining and contribute to recovery.

• Utilize information from these initial reintroduction efforts to develop specific protocols to promote success of future reintroduction projects. Information on site suitability and preparation, propagule selection, and environmental factors should be incorporated into protocols for reintroduction of this species.

• Implement reintroduction and augmentation projects using protocols developed with data from the current study.

• Site selection is crucial to success of new populations establishment. Future reintroduction projects should be limited to sites which are close to or within the current range of the plant, adjacent to or directly on the beach (either on bluffs above beach or on beach sand close to the bluffs, where some sand stabilization has occurred). Sites should be exposed to moderate disturbance but have some ground cover established.

• In order to facilitate reproduction during the first growing season, larger propagules should be used for transplanting. If a large percentage of the selected site's substrate is covered with vegetation, removal of ground cover is recommended as well.

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