

Acknowledgements

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Abstract

Oenothera wolfii (Wolf's evening primrose) is a rare and showy, yellow-flowered biennial to short-lived perennial endemic to the beach and coastal bluff habitats along the southern Oregon and northern California coast. Known from only a few populations, this beautiful plant faces many of the same threats that other coastal species must battle, including habitat loss and alteration due to coastal development and roadside maintenance and competition with exotic species. In addition, *O. wolfii* hybridizes with the common garden escapee *O. glazioviana*, with genetic dilution and hybrid swarm populations resulting. In order to promote the conservation of *O. wolfii* and assess the feasibility of introducing new populations within the historic range of this species, plants were cultivated in the greenhouse and transplanted to two experimental field sites on the southern Oregon coast in 2003. Directly-sown seed plots were installed at a third site in 2007, and in 2010 and 2011 rosettes were outplanted at this site as well. Based on initial results, the introduction and establishment of new populations of *O. wolfii* appears to be possible. However, further monitoring shows that selecting introduction sites containing appropriate habitat is critical to the longer-term success of new populations. Due to the difficulty of determining the crucial elements of appropriate habitat before attempting an introduction, it is recommended that multiple sites be established initially. Larger-scale population creation efforts can then be conducted at those sites which have the best initial success.

Introduction

Oenothera wolfii (Munz) Raven, Dietrich & Stubbe (Wolf's evening primrose) is a showy biennial to short-lived perennial that occurs in only a small number of isolated populations located along the coast in southwestern Oregon and northwestern California. In the right circumstances, this species can establish fairly large populations in moderately disturbed areas (Imper 1997, Carlson et al. 2001). Unfortunately, these favorable circumstances are hard to find, and currently Wolf's evening primrose is quite rare. Its precarious status is due to 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, as a "Species of Concern" by the U.S. Fish and Wildlife Service (ORNHC 2004), and as a "Bureau Sensitive" species by the Bureau of Land Management (personal communication, Tim Rodenkirk, Botanist, Bureau of Land Management, Coos Bay).

The purpose of this ongoing study is twofold: 1) to develop a protocol for the establishment of new *O. wolfii* populations and 2) to establish several new populations of this rare species. Two initial locations were selected as pilot sites for introduction efforts in 2003. A third introduction site was added to the study in 2007, and a potential fourth site was identified in 2009. Initial monitoring focused on assessing transplant survival and reproductive success. Because this species is a biennial, monitoring in later years shifted to evaluating longer-term seedling recruitment, survival and reproductive success at each new population.

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,



Figure 1. *Oenothera wolfii* plants in flower and fruit at Port Orford.

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 2. *Oenothera wolfii* flower.

Although considered a biennial, *O. wolfii* can behave as a facultative perennial. Typically seeds will germinate during the first year and produce small rosettes (Figure 3). The rosettes will then bolt in the spring of the second growing season and produce flowers by May or June, although some flowering plants can be seen well into the fall. Seed set occurs in August and September, followed by senescence (Imper 1997). However, when subjected to unusual stress (i.e. drought), individuals of *O. wolfii* may wait several years before flowering, and when conditions are good, an individual may produce new rosettes on the side or base of the senesced flowering stalk (Figure 4), and repeat the cycle in subsequent years (personal observation).



Figure 3. *Oenothera wolfii* first year rosette.



Figure 4. New rosette on base of previous year's flowering stalk.

The light colored flowers, long floral tube, and evening opening of the flowers suggests hawkmoths (Sphingidae) as likely pollinators of *O. wolfii*. However, researchers studying this species have observed few pollinators (Carlson et al. 2001). On one occasion two solitary bees (*Halictus* sp.) were observed collecting pollen and drinking nectar at *O. wolfii* plants in Oregon, and bumblebees (*Bombus* spp.) were seen visiting the flowers of one California population of *O. wolfii*. This species is self-compatible, and self-pollination frequently occurs, although flowers covered with pollinator exclusion bags set slightly less seed than those which were not covered (Carlson et al. 2001).

Seed set is generally high in *O. wolfii*, with an individual plant producing an average of over 100 fruit capsules, each of which typically contains over 250 seeds (Carlson et al. 2001). Other species of *Oenothera* have seeds which remain viable for many years, allowing them to develop long-lived seed banks (Pavlik 1987, Baskin and Baskin 1994). Although no seed bank studies have been conducted using *O. wolfii* seeds, the hard seed coat suggests that the seeds of this species could also remain viable for long periods of time, providing they are not exposed to ideal germination conditions.

Geographic range

Currently there are seven known natural 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 5). 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).

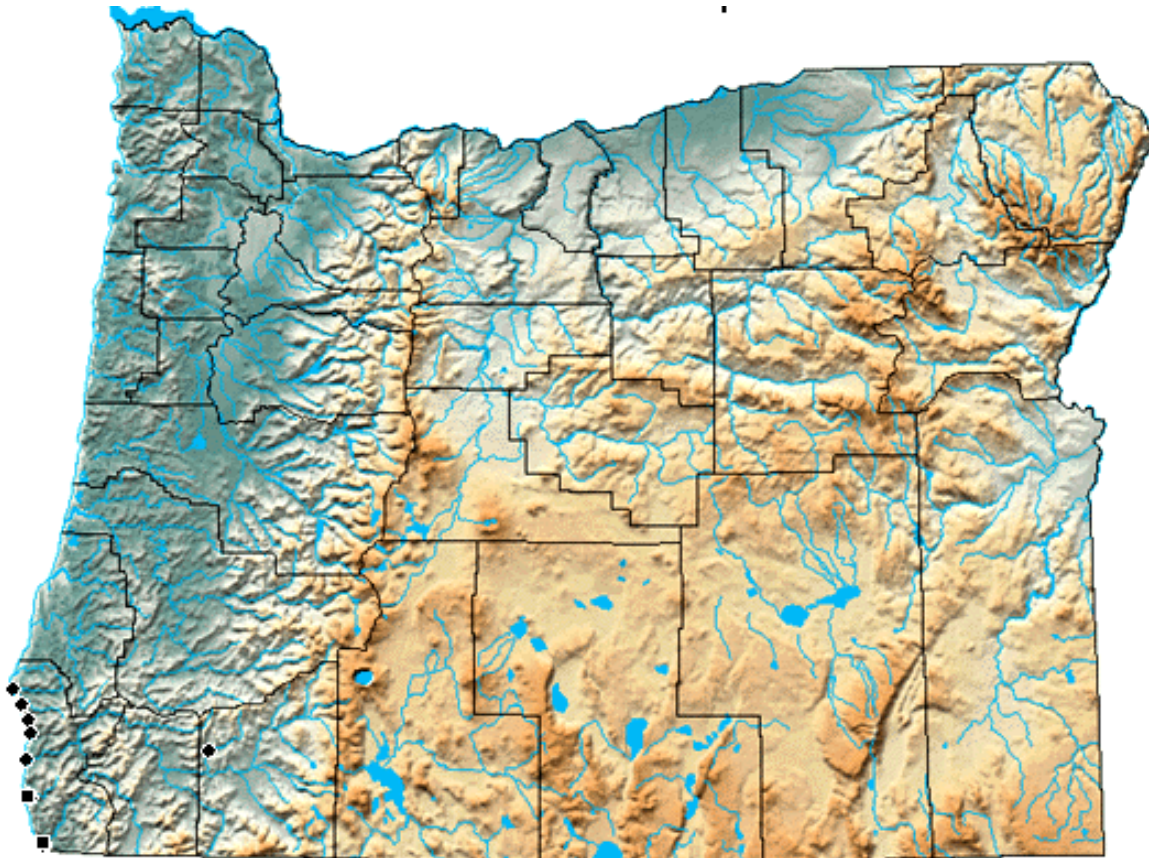


Figure 5. Map of extant *Oenothera wolfii* populations in Oregon. The inland dot that is located in Jackson County represents an unvouchered observation in the Oregon Flora Project database. Oregon Department of Agriculture botanists visiting this site were unable to locate *O. wolfii*, and it is thought that the species was misidentified in this observation (K. Amsberry, personal communication, November 5, 2005). 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. Moisture (obtained from precipitation and sea spray) is needed through much of the first year to sustain young plants until their long taproot develops (Imper 1997). 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 on March 13, 2004). The Port Orford population is located on the foredune itself, taking advantage of gaps in the ever-present *Ammophila arenaria* created by

the dumping of sand on the beach while dredging the bay (Figure 6). Several other populations reside on the partially stabilized beach dunes, where other vegetation provides some protection but frequent disturbance still occurs.



Figure 6. *Oenothera wolfii* in fruit on the foredune at Port Orford, Oregon.

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, Figure 7 and Pistol River, Figure 8) to areas where bare soil and rock are exposed (Sister's Rock, Otter Point; Figures 9 and 10). 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.



Figure 7. Hubbard Creek *Oenothera wolfii* habitat.



Figure 8. *Oenothera wolfii* plant growing with competing vegetation at Pistol River. Population located on the hillside immediately above Highway 101.



Figure 9. *Oenothera wolfii* plants growing on rocky beach at Sister's Rock.



Figure 10. *Oenothera wolfii* habitat at Otter Point. A few plants are found scattered on the beach where there are gaps in the European beachgrass, but most of the individuals are located on the slope above the beach.

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., *Menophila arnaria*, *Mimulus guttatus*, *Myrica 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 wolffii 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. wolffii* habitat. Roadside maintenance is another cause of disturbance. Several *O. wolffii* 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. wolffii* (Gisler and Meinke 1997, Imper 1997; Figures 11 and 12).



Figure 11. European beach grass has become the dominant species along the beach at Otter Point.



Figure 12. European beachgrass (*Ammophila arenaria*) invasion on the foredune at Port Orford.

Additionally, *O. wolfii* is able to hybridize with the common garden escapee *O. glazioviana* (Figure 13). Morphological studies indicate that there is widespread hybridization throughout the California populations (Carlson et al. 2001). More recent genetic studies further support the assertion that *O. wolfii* readily hybridizes with other *Oenothera* species, and that this hybridization is widespread (DeWoody and Hipkins 2005). In fact, the work of DeWoody and Hipkins indicates that several of the Oregon populations may already contain hybridized individuals. In addition, many of the Oregon populations which are currently considered to be “pure” *O. wolfii* are located near major roadsides, making them at risk of future hybridization with *O. glazioviana*. 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.



Figure 13. Flower of the non-native garden escapee, *Oenothera glazioviana*. Note the large, overlapping petals typical of this species.

Literature review

If observed over a long enough period of time, every population of plants faces local extinction (Silvertown and Charlesworth 2001). Consequently, conservationists are not necessarily concerned with extinction itself, but rather its rates and causes. History has even seen periods where large numbers of species became extinct. According to fossil records, the last round of large-scale extinctions started during the late Pleistocene (roughly 30,000 years ago), following a long period of steadily growing biodiversity, and continued until about 1000 years ago (Myers 1993). At no previous time in history, however, have periods of mass extinction occurred over such a limited time frame (Lande 1988). It is this unprecedented loss of large numbers of species within a very short period of time that scientists find so disturbing. The mid 1900s heralded the beginning of this most recent era of mass extinctions, and they continue to the present day (Myers 1993). It is estimated that up to

25% or more of the plant and animal species that are currently recognized could disappear over the next 50 years (Wilson 1992, Myers 1993, Schemske et al. 1994). In the U.S. alone, over 40% of the 17,000 native vascular plant taxa appear on one or more rare plant list, and the Center for Plant Conservation estimates that almost 800 of these will be vulnerable to extinction within the next decade (Morse 1996). The U.S. Fish and Wildlife Service, which tracks the most imperiled species in our country, currently lists 747 native plant taxa as threatened or endangered, indicating the need for immediate action to prevent extinction (U.S. Fish and Wildlife Service 2006).

This unprecedented loss of species diversity over a relatively short period of time 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, Myers 1993). Habitat loss and degradation, pollution, over-harvesting, climate change, and the human-facilitated spread of invasive species and plant pathogens are some of the primary ways that our species has negatively affected plant populations (Cane and Tepedino 2001).

The current trend of increasing numbers of species extinctions (and degradation of the ecosystems in which they reside) is a disturbing one, but scientists, policy-makers, and members of the general public who value the earth's biodiversity are not giving up without a fight. The relatively new fields of conservation biology and restoration ecology have arisen out of this crisis, and conservation biologists and restoration ecologists are utilizing a variety of tools to stem the tide of species extinction.

Two general approaches are advocated by those interested in conserving rare plants: protect those populations still in existence, and increase the number of wild plants by introducing, reintroducing or augmenting populations within appropriate habitat in their historical range. These two approaches are complimentary, but not equal in priority. The first and most important strategy is the preservation of existing undisturbed populations and the habitat in which they reside. Only secondarily should introduction be relied upon as a strategy for insuring species survival (Falk et al. 1996, Drayton and Primack 2000).

However, there are cases in which rare plant introduction is a valuable tool in the fight against widespread loss of biological diversity. Conservation biologists and land managers are frequently faced with the need to intervene in order to save species from extinction (Falk et al. 1996). Setting aside protected sites is often not sufficient, and even on administratively protected lands, sites can be negatively impacted by erosion, logging, grazing, invasion of exotic species, pollution, or wide scale climate change (Falk and Olwell 1992). For some species, it is too late to preserve most of their original habitat. For others, population sizes have fallen below a minimum viable population size, and they will not recover without intervention. When destruction of some natural populations has already occurred, and remaining populations are threatened with extirpation as well, sometimes the only remaining option is attempting to create new populations (Falk 1987, Guerrant and Pavlik 1997).

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 (Bowles et al. 1993, Allen 1994, Bowles and McBride 1996, Cully 1996, McDonald 1996, Walck et al. 2002, Lofflin and Kephart 2005, Rimer and McCue 2005).

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 source and storage

Seeds collected from the Port Orford population of *Oenothera wolfii* in September of 2002 were used to propagate plants for establishment of the experimental populations in 2003. For a complete description of seed germination methodology and results, refer to “Establishment of an Experimental Population of Wolf’s Evening Primrose (*Oenothera wolfii*) at the New River Area of Critical Environmental Concern (ACEC)” (Currin 2005). The 2002 Port Orford seeds were also used to establish a new population of *O. wolfii* at the third experimental site (Four-mile Road) in 2007. From 2002-2007, the clean, dry seeds were stored in brown paper bags, in the dark and at room temperature.

In September of 2009, approximately 50,000 additional seeds were collected from the Port Orford, Pistol River, and Sisters Rock *O. wolfii* populations. These seeds were cleaned and stored in the same manner as those collected in 2002. In September of 2010, seed was collected from approximately 25 parent plants in the Port Orford population, and in September 2011, seed was collected from 34 parent plants at the same location. For both years, collected seed was cleaned and stored using the previously described method.

Seed germination

On October 4, 2011, using seed collected that same year from the Port Orford population, seeds were scattered on wet filter paper in Petri dishes and placed on a bench in an Oregon State University greenhouse. Temperatures were maintained at 70°/65° C and lights were on twelve hours/day (Figure 14). Seeds were kept damp with distilled water as needed. Approximately 50 seeds were placed in each Petri dish. (For a detailed description of previous *Oenothera wolfii* seed germination trials, see Currin 2005.)



Figure 14. *Oenothera wolfii* seeds beginning to germinate in Petri dishes.

Seedling cultivation

Oenothera wolfii transplants used for introduction at both the initial two sites (Pistol River and Lost Lake) in 2003, as well as those outplanted at Four-mile Road in 2010, were cultivated in a greenhouse at Oregon State University. For a complete description of 2003 cultivation methods and results, see “Establishment of an Experimental Population of Wolf’s Evening Primrose (*Oenothera wolfii*) at the New River Area of Critical Environmental Concern (ACEC)” (Currin 2005).

On October 18, 2011, germinated seedlings were planted in 3” plastic pots (18 pots/flat) in Supersoil potting soil. A total of 360 plants (20 flats) were cultivated this year for outplanting. Plants were fertilized weekly and watered as needed.

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. A total of three experimental *O. wolfii* introduction sites have been established as part of this study – two in 2003 and one in 2007 (see Appendix 1 for site maps).

Lost Lake

The first site, Lost Lake (Figure 15), 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. Potential threats to any *O. wolfii* population established at this site include disturbance caused by illegal use of the site for off-road vehicles and hunting, and invasion of the non-native European beachgrass.



Figure 15. *Oenothera wolfii* transplant plots at Lost Lake.

Meyers Creek

The second site, Meyers Creek, is located on the hillside just above Highway 101, about nine miles south of Gold Beach (Figure 16). The land belongs to the Oregon Department of Transportation, and the site is about half of a mile from the Pistol River natural population of *O. wolfii*. The habitat at Meyers Creek is almost identical to that of the Pistol River 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.



Figure 16. Meyers Creek introduction site. *Oenothera wolfii* was outplanted on the slope above Highway 101.

Four-mile Road

Four-mile Road was selected as a third introduction site in 2007. Also part of the New River ACEC, this site is located approximately one mile to the south of the Lost Lake site. This small patch of slightly inland dune habitat is within site of the ocean, situated roughly a quarter mile from the water. In general, the substrate is more stabilized than that of Lost Lake, although there are several patches with bare sand (Figure 17). The dominant vegetation includes *Poa macrantha* and *Carex obnupta*. This site also supports a very small population of another state-listed species, *Phacelia argentea* (silvery phacelia). It was thought that this site's close proximity to the ocean (and subsequent access to the additional moisture and nutrients associated with ocean spray), its partially stabilized substrate (which still is subject to some disturbance), and its administratively protected status made this site an ideal candidate for introduction of *O. wolfii*. The only concern at this site is potential disturbance caused by recreational site users (i.e. hikers or birders) who do not remain on the walking trails passing through the site.



Figure 17. Partially stabilized dune habitat at the Four-mile Road site.

Floras Lake

A fourth *O. wolfii* introduction site was tentatively selected in the fall of 2009. Floras Lake is owned by the BLM, and currently hosts a small population of *Phacelia argentea* (silvery phacelia), another rare coastal species (Figure 18). Although the site looks promising, there is a fair amount of European beachgrass present. The BLM has initiated beachgrass removal efforts, and it is hoped that the site will be ready for the introduction of both *O. wolfii* seeds and additional *Phacelia argentea* plants in the spring or fall of 2012.



Figure 18. Potential *Oenothera wolfii* introduction site at Floras Lake.

Experimental population establishment

The number of *O. wolfii* transplants and/or seeds used for introduction at all three sites is summarized below in Table 1. For a complete description of outplanting methodology and study design for the 2003 introduction sites (Lost Lake and Meyers Creek), refer to “Establishment of an Experimental Population of Wolf’s Evening Primrose (*Oenothera wolfii*) at the New River Area of Critical Environmental Concern (ACEC)” (Currin 2005). See Appendices 2-4 for maps of plot locations at each site, and Appendix 5 for GPS coordinates for each plot.

Table 1. Summary of number of transplants and/or seeds used in the introduction of *Oenothera wolffii* at three sites.

Site	Outplanting date	# Small transplants	# Large transplants	# Seeds
Lost Lake	10/24/03	40	50*	2,000
Meyers Creek	11/13/03	40	40	2,000
Four-mile Road	10/20/07	n/a	n/a	50,000
	1/30/10	n/a	n/a	20,000
	10/16/10	143	n/a	n/a
	Planned for December 2011	300	n/a	n/a

* Because large transplants were removed from pots and transported in plastic bags at Lost Lake, an additional 10 large transplants were transported in pots and outplanted as controls.

On October 20, 2007, 10 seed plots were established at the Fourmile. Each seed plot contained approximately 5,000 seeds, for a total of 50,000 seeds sown at this site.

Due to the prohibitive amount of time it would take to individually count 50,000 *O. wolffii* seeds, seed counts were approximated using seed weight. To estimate seed weight, 40 packets of previously counted seeds (ranging from 200-1000 seeds per packet) were weighed on an electronic scale (accurate to 0.0001 g). The average weight per seed was calculated for each packet, and an overall average weight per seed was then calculated for all of the packets combined. This number was multiplied by 5,000, giving an average weight per 5,000 seeds (in grams). The 2002 Port Orford seed was then grouped in coin envelopes, with each packet weighing the estimated average weight per 5,000 seeds (Figure 19).



Figure 19. Envelope containing approximately 5,000 *Oenothera wolfii* seeds.

The 10 seed plots were three meters long and one meter wide. Each plot was marked at each corner with a 2' wooden stake. Plot numbers (S1-S10) were written on each stake in thick permanent ink (Figure 18). The 5,000 seeds were sprinkled evenly over the surface of the plot. Because the rainy season had already begun, no supplemental water was provided to the seeds at the time of sowing.

Due to concerns about the viability of the five-year-old seed used to establish the seed plots at Four-mile Road in 2007, an additional 2,000 newly collected (in September 2009) seeds were sown in each of the 10 Four-mile Road seed plots in early 2010. Once again, the seeds were sprinkled evenly over the surface of each of the plots (Figure 20).



Figure 20. *Oenothera wolfii* seed plot established at Four-mile Road. The 3m x 1m plots are marked in each corner with 2' wooden stakes.



Figure 21. Sowing *Oenothera wolfii* seeds in plots at Four-mile Road.

In October 2010, 18 transplant plots were installed at the Four-mile Road site. At the time of outplanting, *O. wolfii* rosettes were 38 days old. Each plot was one meter² in size, with the center marked by a 2' wooden stake with the plot number clearly written in indelible ink. Each plot contained eight plants (one plot only had seven plants) evenly placed along the outside edge of the square (Figure 22). Sandy soil allowed for hand installation of the plants (Figure 23). The rosettes were carefully watered in at the time of outplanting (~ one liter of water/transplant; Figure 24) and a GPS point was taken for each plot.



Figure 22. *Oenothera wolfii* transplant plot at Four-mile Road in October 2010.



Figure 23. Rebecca Currin demonstrates planting methodology while BLM biologist Tim Rodenkirk and volunteers Ann Jaster, Thea Cook, Henry Cook, Jeff Cook, and Hal Darst observe.



Figure 24. Bureau of Land Management biologist Tim Rodenkirk waters newly transplanted *Oenothera wolfii* rosettes while Rebecca Currin and volunteer Thea Cook document plot locations with a Garmin GPS.

Monitoring

Both of the sites that were established in 2003 (Lost Lake and Meyers Creek) were visited monthly for the year and a half following outplanting. Transplant survival and reproduction data were recorded at each site. Additional relevant data, such as herbivory and other evidence of disturbance, were recorded as well. Photographs were taken of each plant throughout the monitoring period. All measurements with regards to plant size and reproduction (and subsequently used in the statistical analysis of these variables) were taken September 8-12, 2004. For a complete description of the September 2004 monitoring methodology and results, refer to “Establishment of an Experimental Population of Wolf’s Evening Primrose (*Oenothera wolffii*) at the New River Area of Critical Environmental Concern (ACEC)” (Currin 2005).

From 2005 - 2006, no funding was available for monitoring the Lost Lake and Meyers Creek sites. Consequently, these sites were only monitored twice (in September of 2005 and October of 2006) during this time period.

From 2007 - 2011, the two initial introduction sites were monitored three-four times/year. By the beginning of 2007, many of the wooden stakes at Lost Lake had rotted, and some of the plot numbers were difficult to read. Consequently, monitoring at this site was conducted by searching the general area where the original plots were located and recording the total number and status (reproductive or vegetative) of any *O. wolffii* plants found. At Meyers Creek, transplant survival and reproductive status data were recorded. Seedling recruitment, including recruited seedling reproductive status, was also noted (Figure 25).

The Four-mile Road site was installed in October of 2007. This site was monitored quarterly in 2008 and 2009, and three times each in 2010 and 2011 (one more monitoring trip is planned for 2011). During each monitoring visit, all seed plots and their surrounding area were thoroughly searched for *O. wolffii* seedlings.



Figure 25. Field assistant Hal Darst (with help from budding young botanist Terrwyn Darst-Currin) monitoring *Oenothera wolfii* at Four-Mile Road.

Results

Lost Lake

Initial survival of transplants at Lost Lake was quite high (89%). In this first year, only 14 of the transplants reproduced (flowered and set seed). Fifty-one of the original 90 transplants (57%) were still alive two years after being transplanted. By the third year, only seven of the original transplants were still alive. By the fall of 2007, only three plants could be located at the Lost Lake site, and by the fall of 2008, only one of those plants remained. This sole survivor was still present in the fall of 2009, although it was still vegetative, and had never reproduced (Figure 26). We were unable to locate any *Oenothera wolfii* plants at the Lost Lake site in 2010 or 2011. Sample sizes were too small to determine if transplant size, ground cover or other environmental factors significantly influenced transplant survival over the longer period of time. Despite repeated searches for *O. wolfii* seedlings in the areas

containing both the transplant and directly-sown seed plots, there was no indication of seedling recruitment at this site. See Table 2 for a summary of transplant survival and reproduction at Lost Lake.

Table 2. Summary of *Oenothera wolfii* transplant survival, reproduction and seedling recruitment at Lost Lake.

	Year							
	2004	2005	2006	2007	2008	2009	2010	2011
Transplants								
# originally planted (in Fall 2003)	90	90	90	90	90	90	90	90
# surviving	80	51	7	3	1	1	0	0
# reproducing	14	0	0	0	0	0	0	0
# seedlings recruited	0	0	0	0	0	0	0	0
Seed plots								
# originally sown	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2000
# rosettes	0	0	0	0	0	0	0	0
# reproductive plants	0	0	0	0	0	0	0	0
Total # plants at site	80	51	7	3	1	1	0	0

Meyers Creek

A summary of transplant survival and reproduction at Meyers Creek can be found in Table 3. Initial survival at Meyers Creek was almost 100% (79 out of the 80 transplants were alive after one year). Forty-six transplants reproduced (produced flowers and seeds) in 2004, and the directly sown seed produced seven rosettes in the first year. Forty-six transplants were still alive the following year (2005), with 23 of them reproducing. The seed plots contained eight rosettes in 2005. By the fall of 2006, 17 of the original transplants were still alive, and nine of them reproduced. In addition, there were eight new rosettes in the transplant plots, indicating that recruitment had occurred. The seed plots contained seven individuals this same year, four of which had reproduced. By 2007, it was no longer possible to differentiate between transplanted rosettes (that had never reproduced) and new rosettes (that were the

result of recruitment). As a result, the number of individuals present and their reproductive status were recorded during subsequent monitoring visits. There were nine rosettes and one reproductive individual located in the transplant plots, and two rosettes located in the seed plots in the fall of 2007. By the fall of 2008, this number had decreased to two reproductive plants and two vegetative individuals. In 2009, there were four vegetative individuals present – one in a seed plot, two in transplant plots, and one approximately two meters outside of the transplant plots, presumably the result of recruitment from seed produced by a previously reproductive transplant. In 2010, two of the 2009 vegetative plants had produced flowering stalks, and an additional three vegetative plants were present at the site. In 2011, two reproductive plants were found. Although no vegetative plants were located this year, thick two-to-three-foot-high vegetation made the search very difficult, and it is quite possible that some vegetative plants were present, but missed (Figure 26).



Figure 26: Competing vegetation at Meyers Creek made location of recruited vegetative *Oenothera wolfii* plants difficult.

Table 3. Summary of *Oenothera wolfii* transplant survival, reproduction and seedling recruitment at Meyers Creek.

	Year							
	2004	2005	2006	2007	2008	2009	2010	2011
Transplants								
# originally planted (in Fall 2003)	80	80	80	80	80	80	80	80
# survived*	79	46	17	n/a	n/a	n/a	n/a	n/a
# reproduced	46	23	9	n/a	n/a	n/a	n/a	n/a
# seedlings recruited	0	0	8	n/a	n/a	n/a	n/a	n/a
Total # rosettes	n/a	n/a	n/a	9**	1**	3**	1**	0***
Total # reproductive plants	n/a	n/a	n/a	1**	1**	0**	2**	1
# original transplants that had reproduced by each year	46 (58%)	62 (78%)	68 (85%)	n/a	n/a	n/a	n/a	n/a
Seed plots								
# originally sown (in Fall 2003)	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
# rosettes	7	8	3	2	1	1	2	0***
# reproductive plants	0	0	4	0	1	0	0	1
Total # plants	86	54	32	12	4	4	5	2

* # survived = original transplants still alive at the time of monitoring

** By 2007, it was difficult to determine whether plants found in the plots were original transplants or recruited individuals from seed dropped by reproductive transplants in previous years.

*** Dense vegetation made the search for rosettes very difficult.

Overall, 68 (85%) of the transplants reproduced within three years of being outplanted (Table 4). Nine of these individuals produced flowering stalks in two different years during the period from 2004 to 2007. One individual produced flowering stalks in three separate years during that same time period. See Currin 2005 for more information about effects of transplant size and removal of ground cover on initial transplant survival and reproduction.

Table 4. Summary of *Oenothera wolfii* transplant reproduction at Meyers Creek as of October 2007.

Type of transplant	# Transplants
Total # transplants installed in 2003	80
Total # transplants that reproduced by 2007	68 (85%)
Total # transplants that produced flowering stalk(s) in one year from 2004-2007	58 (73%)
Total # transplants that produced flowering stalks in two years from 2004-2007	9 (11%)
Total # transplants that produced flowering stalks in three years from 2004-2007	1 (1%)

Four-mile Road

Seed plots

So far, no *O. wolfii* plants have been located in or around the 10 seed plots at Four-mile Road. At the time of sowing in 2007, the seeds used were five years old (collected in the fall of 2002). Previous seed germination tests showed similar germination rates for fresh (48%), one-year-old (54%), and two-year-old (48%) seed (Currin, unpublished data). However, it has not been determined how long seeds kept in cool, dry storage will remain viable. In order to investigate the viability of the seed collected in 2002, additional seed germination trials were conducted in 2008-2009.

Because of the lack of seed germination and/or seedling establishment at the Four-mile site during the first year after sowing, in the fall of 2008 it was decided that additional plots should be installed and sown with freshly collected seed. Results from this second direct-sowing attempt would hopefully indicate if lack of seed germination/seedling establishment at the site was due to poor quality seed or inappropriate seedling establishment habitat.

In order to collect fresh seed from the nearest natural population of *O. wolfii*, the Port Orford population was visited in September of 2008. Unfortunately, a thorough survey of the site showed a marked decrease in the size of the population there. While the Port Orford population contained hundreds of *O. wolfii* individuals when it was censused in 2004 (Currin, unpublished data), only 30-40 plants were observed during the 2008 survey. In addition,

only four small reproductive plants were found, so no seed was collected. As a result, additional seed plots were not installed at the Four-mile Road site in 2008.

In September of 2009, the Port Orford population was revisited, and a sufficient number of reproductive plants were located to allow for new seed to be collected. In addition, fresh seed was collected from the Pistol River and Sister's Rock populations. Each of the Four-mile Road seed plots was re-sown with an additional 2,000 seeds in January of 2010. However, no recruitment has been observed in the seed plots at Four-mile Road during 2010 and 2011 monitoring trips (Table 5).

Transplant Plots

Of the 143 *O. wolfii* transplants outplanted in October of 2010, 99% of the plants (141 plants) survived the initial transplant shock and were still alive as of September 2011 (Table 5). Fourteen (10%) of the transplants bolted and produced fruits and seed during the year following being outplanted (Figure 27).



Figure 27. Bolting *Oenothera wolfii* transplant at Four-mile Road. Plant developed side branches after being browsed.

Table 5. Summary of *Oenothera wolfii* monitoring results at Four-mile Road (transplant survival, reproduction and seedling recruitment).

	Year				
	2007	2008	2009	2010	2011
Transplants					
# originally planted (in Fall 2010)	n/a	n/a	n/a	143	143
# surviving	n/a	n/a	n/a	142	141
# reproducing	n/a	n/a	n/a	0	14
# seedlings recruited	n/a	n/a	n/a	0	0
Seed plots					
# originally sown (2007)	50,000	50,000	50,000	50,000	50,000
# originally sown (2010)	n/a	n/a	20,000	20,000	20,000
# rosettes	0	0	0	0	0
# reproductive plants	0	0	0	0	0
Total # plants at site	0	0	0	142	141

Discussion

The goal of rare plants introductions is to increase the number and/or size of their populations, and thereby decrease their risk of extinction. By increasing the number of populations of threatened and endangered plants, we spread out the risk of extirpation due to catastrophic events over discrete populations. These self-sustaining populations can also go on to serve as seed sources to re-establish new populations (Menges 1991). In addition, 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).

Preservation of existing natural populations should be the primary goal of plant conservationists. Maintaining focus on the protection of these populations, which we already know have the ability to survive and reproduce in their current locations, is more

likely to be effective than attempting introduction of new populations in unproven sites. However, preservation of existing sites is not always possible. In fact, it is already too late for some populations of at-risk species; many have succumbed to threats such as competition from non-native invasive species and habitat destruction and degradation. Most of the remaining populations face increasing threats to their long-term survival as well. In light of this situation, the creation of new populations of rare plants within their historical range, as well as the augmentation of existing populations, is a valuable and necessary tool to be used in the battle against rare plant species extinction.

Oenothera wolfii is an excellent example of a rare plant taxon that faces just these sorts of pressures. Limited to a handful of populations in a narrow geographical and ecological range, and faced with the combined threats of habitat loss, competition from non-native species, and hybridization, this species is a prime candidate for new population establishment.

Oenothera wolfii is relatively easy to cultivate *ex situ*, and initial monitoring showed that transplants of this species can have high survival rates – after one year Meyers Creek only lost one plant (98% survival), 89% of Lost Lake transplants survived as well (Currin 2005), and 100% of the Four-mile Road transplants have weathered the initial transplant shock and are still alive several months after being outplanted. Since seed germination, seedling cultivation and initial survival of transplants are some of the primary hurdles that must be overcome in order to successfully establish a new population, this is encouraging news for this species.

Furthermore, many of the surviving transplants at the first two sites went on to reproduce, producing thousands of seeds and initiating the establishment of a seed bank at each of the introduction sites. In fact, although *O. wolfii* is a biennial, and expected to senesce after two growing seasons, many of the original transplants were still alive two to three years after outplanting. Some of these longer-lived “biennials” produced flowering stalks in multiple years. Amazingly, a few of the original transplants were still alive in 2007, four years after

being outplanted at the introduction sites. See Currin 2005 for a more complete description and analysis of first year transplant survival and reproduction.

Overall, the differences in habitat and transplant performance between the initial two introduction sites highlight the need for appropriate site selection when planning rare plant introductions. Furthermore, when the species in question is a biennial, seedling recruitment is imperative to the success of the endeavor. As of the fall of 2009 (six years after outplanting), no seedlings were found in or near the transplant or seed plots at Lost Lake. Although recruitment can be limited by both the availability of seed and suitable microsites (Eriksson and Ehrlén 1992), the abundant seed production of *O. wolfii* suggests that a lack of suitable habitat is the issue at this site.

Meyers Creek, in contrast, saw some seedling recruitment in the years following outplanting, both within and near the transplant and seed plots. However, recruitment has been limited, and by 2011 the Meyers Creek population contained only two plants. Although removal of ground cover before outplanting positively impacted whether or not transplants reproduced in the first year at Meyers Creek (Currin 2005), surrounding vegetation quickly recolonized the plots where ground cover was removed, and within a few years it was difficult to determine which plots had their ground cover removed initially. Seed germination occurred only in the seed plots which had ground cover removed before the seed was sown, and all but one of the transplant plots exhibiting seedling recruitment had ground cover removed at the time of outplanting. This suggests that competing vegetation can negatively impact *O. wolfii* seedling recruitment, and that control of competing vegetation may be necessary (at least initially) for successful establishment of new populations of *O. wolfii*. Other studies have shown that herbaceous competitors can have minimal effect on established individuals and yet easily impair the survival of emerging seedlings (Winn 1985, Guerrant and Pavlik 1997). It is very probable that the lack of additional recruitment and the overall decline in the number of individuals at the newly created Meyers Creek population is at least in part due to the inability of the *O. wolfii* seedlings to compete with the heavy vegetation present at the site. In introduction sites where natural disturbances do not remove competing vegetation

periodically, it might be necessary to provide some form of ongoing vegetation management to remove competition and encourage recruitment of new *O. wolfii* seedlings.

Seedling recruitment is also an issue at the Four-mile Road site. As of the fall of 2011, there was no sign of establishment of seedlings in or around the seed plots at this site, in spite of tens of thousands of seeds being sown on two different occasions. The first batch of 50,000 seeds used in 2007 was five years old, and subsequent seed germination testing of this seed showed very low germination rates. However, the second batch of 20,000 seeds, sown in spring of 2010, had been collected the previous year (in the summer of 2009). This seed was also used to grow the transplants put out at Four-mile in 2010, and it germinated readily (~40% germination) in the fall of 2010. Therefore, it is unlikely that poor seed viability was the cause of the lack of seedling recruitment from the more recently sown seed. This suggests that the Four-mile site may not contain appropriate habitat for seedling recruitment. However, before abandoning this site altogether, one more approach is in the process of being tried – the outplanting of rosettes. First-year rosettes were installed at the Four-mile Road site in the fall of 2010, and another round of outplanting is scheduled for the fall of 2011. Future monitoring of the performance of these transplants (survival, reproduction and subsequent recruitment of seedlings) will determine once and for all the appropriateness of the site for this species.

Finally, there appears to be a strong possibility that *O. wolfii* can be established at Floras Lake. The fact that *Phacelia argentea*, another rare coastal species, is already present and the current efforts to remove European beachgrass from the site are moving forward make this site attractive for future outplanting efforts. There have been some initial delays in European beach grass removal treatments at this site, but it is anticipated that it will be ready for introduction of *O. wolfii* plants and/or seeds sometime in 2012.

Eight years after the original installation of transplants at two initial sites, results suggest that the introduction of *Oenothera wolfii* into suitable sites has the potential for success. Although poor long term transplant performance at Lost Lake indicates that this site is unsuitable for the introduction of *O. wolfii*, the Meyers Creek population shows moderate

success after eight years. The Four-mile site is still being evaluated, although initial results suggest that although transplants appear to be doing well at the site, there may not be suitable habitat for seedling recruitment and establishment. Because *O. wolffii* is primarily a biennial, continued monitoring is needed at Meyers Creek to determine whether or not a seed bank has been established and the introduced population is self-sustaining. Continued monitoring is needed at Four-mile Road as well. Floras Lake should be targeted for introduction as soon as beachgrass removal is completed. Finally, a search for new introduction sites should be continued. It is only through the selection of multiple new sites for new population creation that we can refine the site selection criteria. Although some sites will ultimately prove to be inappropriate, it is hoped that enough locations will successfully support new populations, enabling us to eventually recover this beautiful and rare species.

Recommendations

To be completed in final report.

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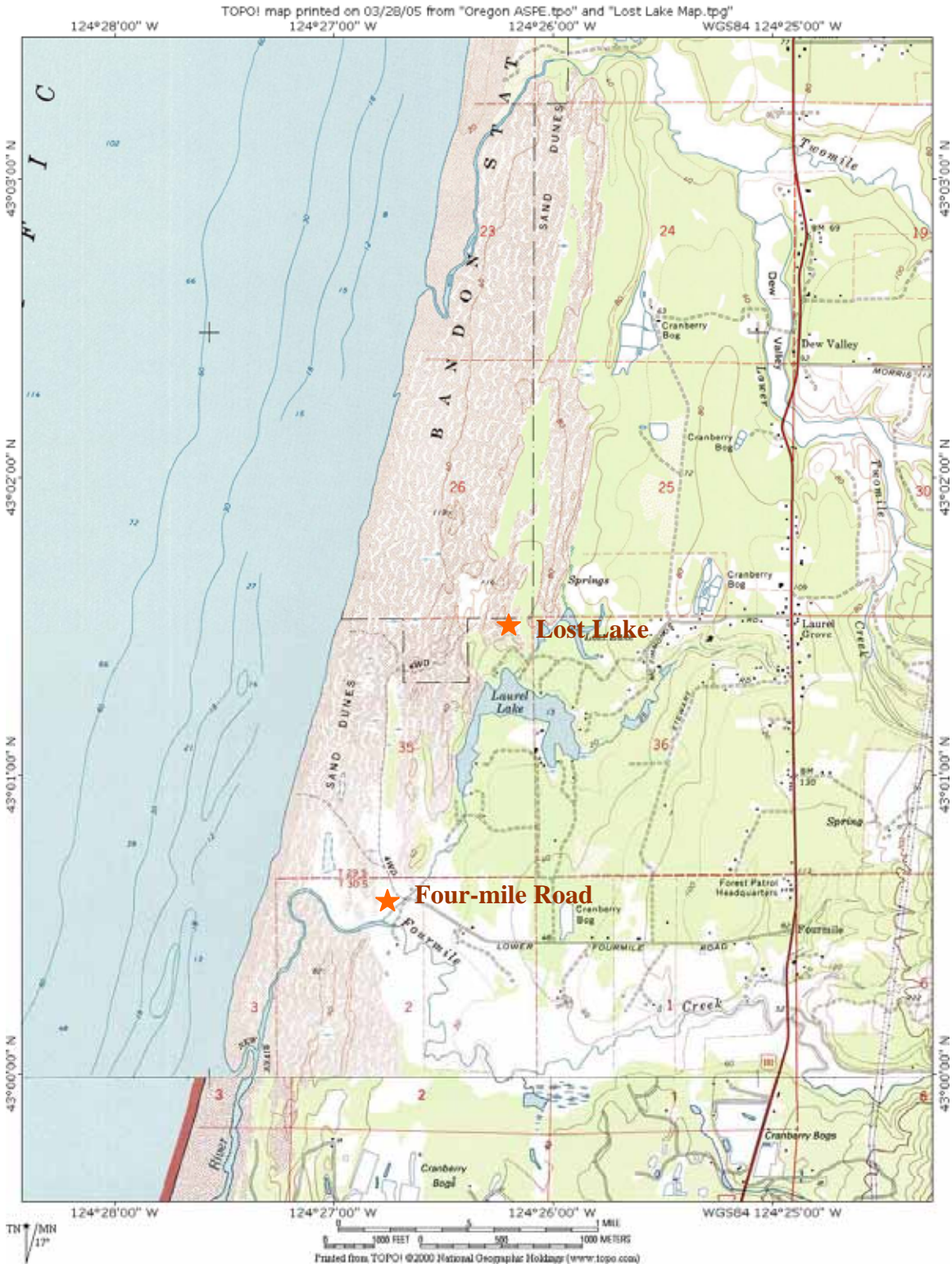
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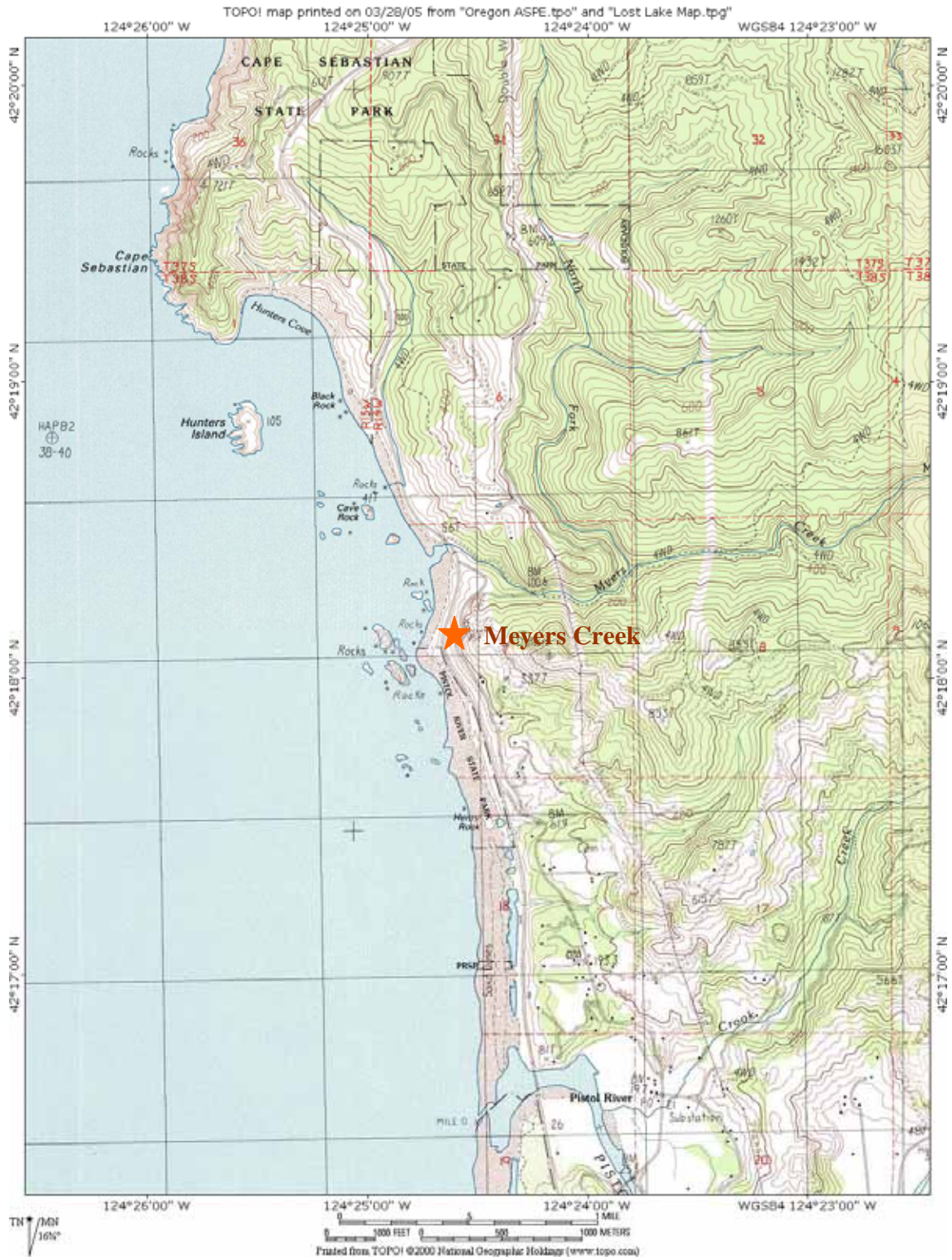
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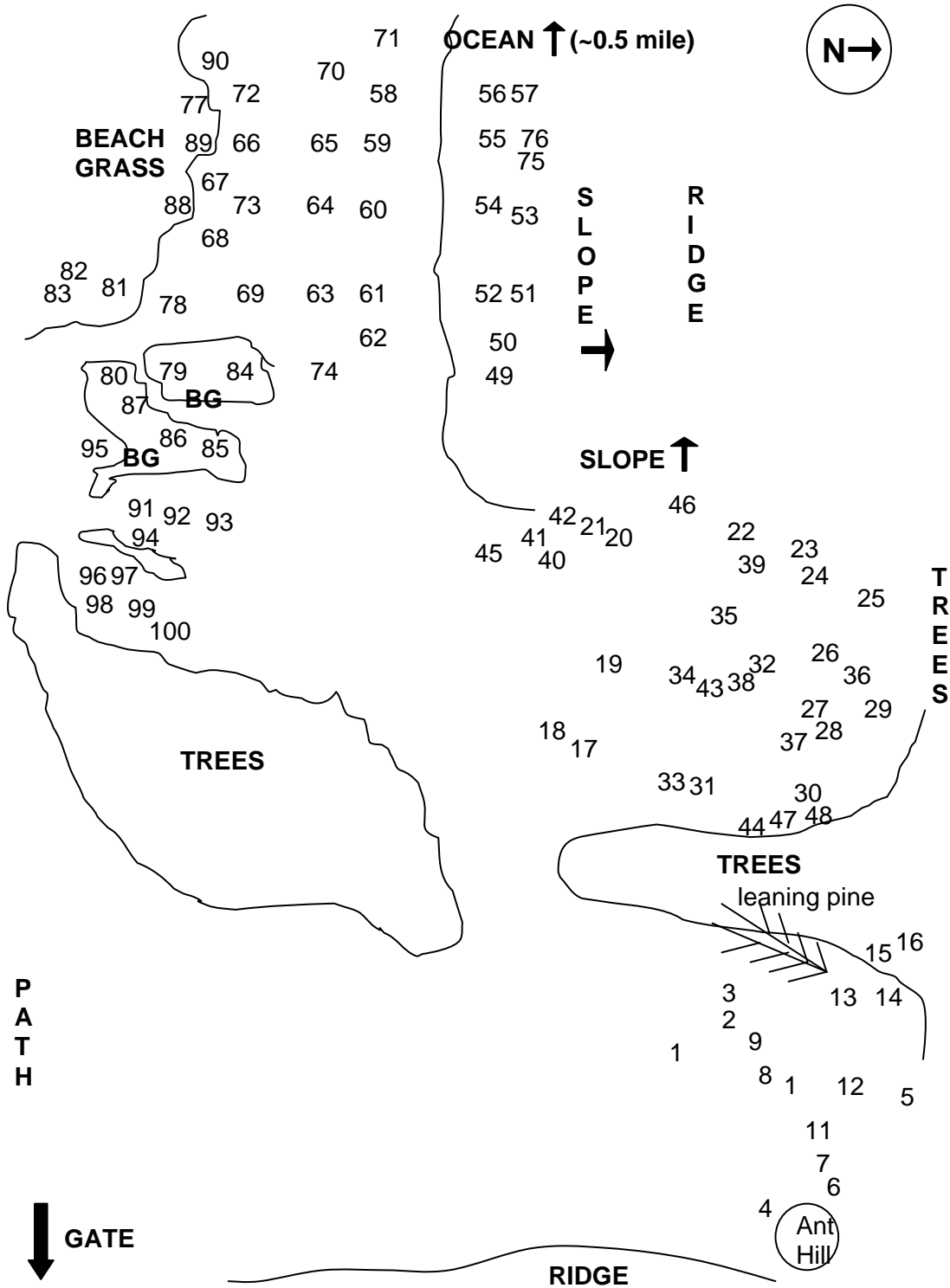
Appendices

Appendix 1: Site maps

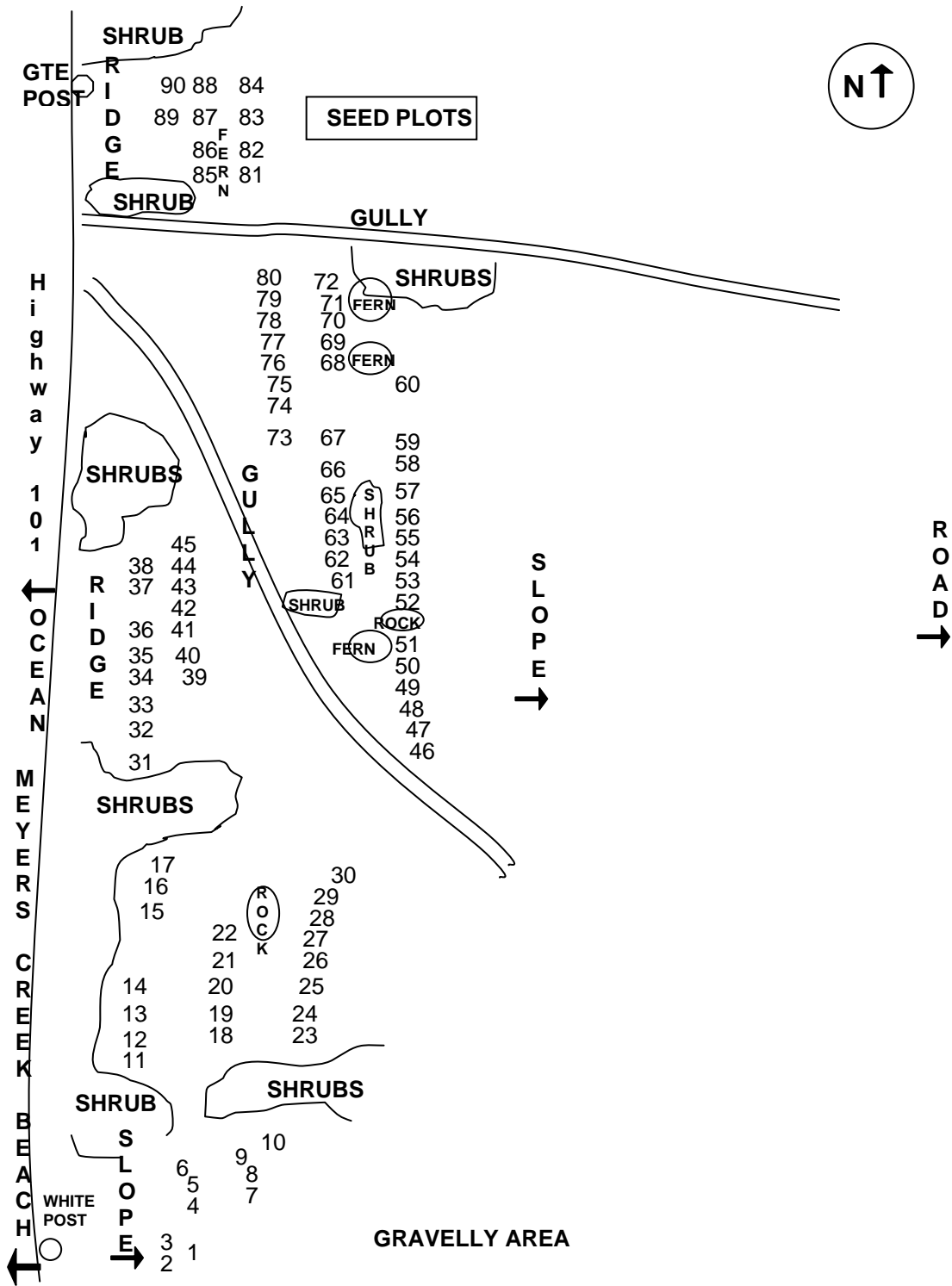




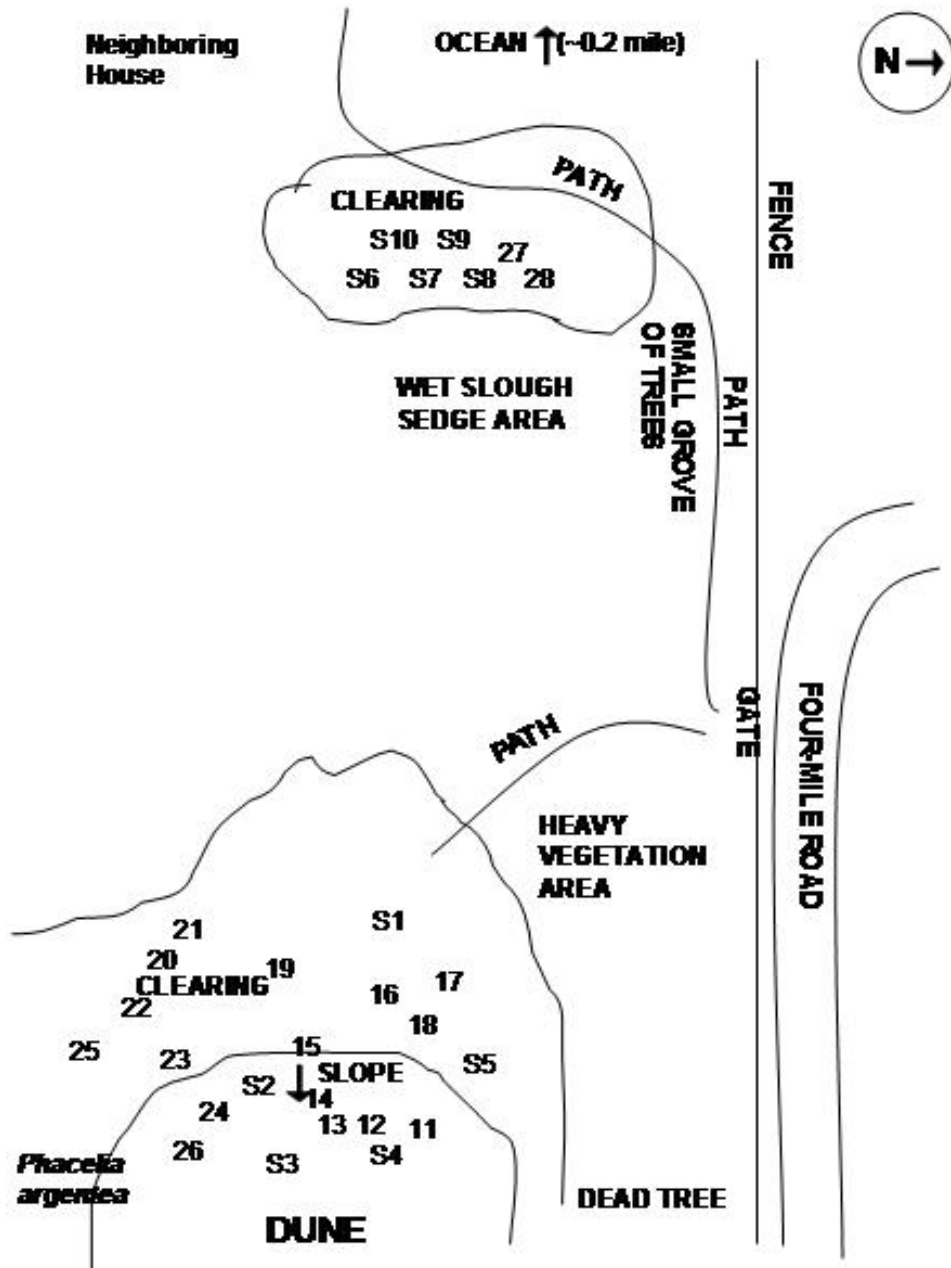
Appendix 2: Lost Lake planting map



Appendix 3: Meyers Creek planting map



Appendix 4: Four-mile Road seed plot planting map



Appendix 5: Plot GPS coordinates

Lost Lake

Plot	GPS	Plot	GPS
1	N43°1.506', W124°26.175'	51	N43°1.513', W124°26.241'
2	N43°1.508', W124°26.177'	52	N43°1.512', W124°26.242'
3	N43°1.508', W124°26.178'	53	N43°1.513', W124°26.242'
4	N43°1.507', W124°26.173'	54	N43°1.511', W124°26.243'
5	N43°1.514', W124°26.173'	55	N43°1.511', W124°26.245'
6	N43°1.511', W124°26.171'	56	N43°1.511', W124°26.247'
7	N43°1.512', W124°26.172'	57	N43°1.512', W124°26.247'
8	N43°1.508', W124°26.177'	58	N43°1.510', W124°26.246'
9	N43°1.509', W124°26.177'	59	N43°1.511', W124°26.245'
10	N43°1.509', W124°26.176'	60	N43°1.510', W124°26.243'
11	N43°1.512', W124°26.174'	61	N43°1.510', W124°26.241'
12	N43°1.513', W124°26.175'	62	N43°1.511', W124°26.240'
13	N43°1.513', W124°26.176'	63	N43°1.508', W124°26.242'
14	N43°1.513', W124°26.174'	64	N43°1.508', W124°26.244'
15	N43°1.514', W124°26.179'	65	N43°1.509', W124°26.245'
16	N43°1.514', W124°26.179'	66	N43°1.508', W124°26.245'
17	N43°1.509', W124°26.208'	67	N43°1.507', W124°26.244'
18	N43°1.509', W124°26.210'	68	N43°1.507', W124°26.243'
19	N43°1.509', W124°26.211'	69	N43°1.508', W124°26.242'
20	N43°1.509', W124°26.211'	70	N43°1.509', W124°26.247'
21	N43°1.510', W124°26.214'	71	N43°1.510', W124°26.248'
22	N43°1.511', W124°26.213'	72	N43°1.509', W124°26.247'
23	N43°1.511', W124°26.212'	73	N43°1.508', W124°26.244'
24	N43°1.512', W124°26.212'	74	N43°1.510', W124°26.239'
25	N43°1.514', W124°26.211'	75	N43°1.513', W124°26.243'
26	N43°1.514', W124°26.210'	76	N43°1.513', W124°26.243'
27	N43°1.512', W124°26.207'	77	N43°1.507', W124°26.247'
28	N43°1.513', W124°26.208'	78	N43°1.506', W124°26.242'
29	N43°1.514', W124°26.207'	79	N43°1.507', W124°26.239'
30	N43°1.512', W124°26.205'	80	N43°1.505', W124°26.239'
31	N43°1.508', W124°26.207'	81	N43°1.503', W124°26.241'
32	N43°1.512', W124°26.210'	82	N43°1.503', W124°26.242'
33	N43°1.509', W124°26.209'	83	N43°1.503', W124°26.241'
34	N43°1.510', W124°26.209'	84	N43°1.507', W124°26.241'
35	N43°1.511', W124°26.211'	85	N43°1.507', W124°26.238'
36	N43°1.514', W124°26.209'	86	N43°1.507', W124°26.237'
37	N43°1.512', W124°26.208'	87	N43°1.506', W124°26.238'
38	N43°1.513', W124°26.209'	88	N43°1.505', W124°26.244'
39	N43°1.511', W124°26.211'	89	N43°1.507', W124°26.246'
40	N43°1.509', W124°26.213'	90	N43°1.508', W124°26.248'
41	N43°1.509', W124°26.213'	91	N43°1.502', W124°26.234'
42	N43°1.508', W124°26.218'	92	N43°1.503', W124°26.233'
43	N43°1.512', W124°26.210'	93	N43°1.504', W124°26.232'
44	N43°1.510', W124°26.205'	94	N43°1.502', W124°26.232'
45	N43°1.508', W124°26.213'	95	N43°1.502', W124°26.236'
46	N43°1.511', W124°26.216'	96	N43°1.502', W124°26.228'
47	N43°1.512', W124°26.205'	97	N43°1.502', W124°26.230'
48	N43°1.513', W124°26.202'	98	N43°1.504', W124°26.228'
49	N43°1.511', W124°26.238'	99	N43°1.505', W124°26.228'
50	N43°1.511', W124°26.240'	100	N43°1.504', W124°26.226'

Meyers Creek

Plot	GPS	Plot	GPS
699 (1)	N42°18.127' W124°24.654'	646	N42°18.146' W124°24.644'
700 (2)	N42°18.126' W124°24.654'	647	N42°18.146' W124°24.645'
603	N42°18.127' W124°24.655'	648	N42°18.146' W124°24.645'
604	N42°18.127' W124°24.655'	649	N42°18.147' W124°24.645'
605	N42°18.128' W124°24.655'	650	N42°18.147' W124°24.645'
606	N42°18.129' W124°24.655'	651	N42°18.148' W124°24.644'
607	N42°18.129' W124°24.651'	652	N42°18.150' W124°24.648'
608	N42°18.128' W124°24.651'	653	N42°18.150' W124°24.648'
609	N42°18.128' W124°24.652'	654	N42°18.150' W124°24.648'
610	N42°18.129' W124°24.651'	655	N42°18.150' W124°24.647'
611	N42°18.136' W124°24.654'	656	N42°18.150' W124°24.647'
612	N42°18.137' W124°24.654'	657	N42°18.150' W124°24.647'
613	N42°18.138' W124°24.654'	658	N42°18.151' W124°24.644'
614	N42°18.138' W124°24.654'	659	N42°18.151' W124°24.644'
615	N42°18.140' W124°24.654'	660	N42°18.152' W124°24.646'
616	N42°18.141' W124°24.654'	661	N42°18.149' W124°24.646'
617	N42°18.142' W124°24.653'	662	N42°18.149' W124°24.646'
618	N42°18.137' W124°24.651'	663	N42°18.149' W124°24.646'
619	N42°18.137' W124°24.651'	664	N42°18.150' W124°24.645'
620	N42°18.138' W124°24.651'	665	N42°18.150' W124°24.645'
621	N42°18.138' W124°24.651'	666	N42°18.151' W124°24.645'
622	N42°18.139' W124°24.651'	667	N42°18.152' W124°24.645'
623	N42°18.137' W124°24.649'	668	N42°18.153' W124°24.645'
624	N42°18.137' W124°24.649'	669	N42°18.153' W124°24.645'
625	N42°18.137' W124°24.649'	670	N42°18.153' W124°24.647'
626	N42°18.138' W124°24.648'	671	N42°18.153' W124°24.647'
627	N42°18.138' W124°24.648'	672	N42°18.154' W124°24.647'
628	N42°18.138' W124°24.648'	673	N42°18.152' W124°24.648'
629	N42°18.139' W124°24.649'	674	N42°18.153' W124°24.647'
630	N42°18.141' W124°24.650'	675	N42°18.153' W124°24.647'
631	N42°18.145' W124°24.652'	676	N42°18.153' W124°24.648'
632	N42°18.145' W124°24.652'	677	N42°18.152' W124°24.647'
633	N42°18.145' W124°24.652'	678	N42°18.153' W124°24.645'
634	N42°18.146' W124°24.653'	679	N42°18.153' W124°24.645'
635	N42°18.146' W124°24.653'	680	N42°18.154' W124°24.646'
636	N42°18.147' W124°24.652'	681	N42°18.157' W124°24.649'
637	N42°18.148' W124°24.653'	682	N42°18.157' W124°24.649'
638	N42°18.148' W124°24.654'	683	N42°18.157' W124°24.649'
639	N42°18.147' W124°24.650'	684	N42°18.158' W124°24.648'
640	N42°18.147' W124°24.651'	685	N42°18.157' W124°24.650'
641	N42°18.147' W124°24.651'	686	N42°18.157' W124°24.650'
642	N42°18.147' W124°24.653'	687	N42°18.157' W124°24.650'
643	N42°18.147' W124°24.653'	688	N42°18.158' W124°24.649'
644	N42°18.147' W124°24.653'	689	N42°18.157' W124°24.650'
645	N42°18.148' W124°24.653'	690	N42°18.158' W124°24.651'

Four-mile Road

Plot Number	GPS Coordinates
S1*	43°00.637'N, 124°26.870'W
S2*	43°00.632'N, 124°26.861'W
S3*	43°00.629'N, 124°26.858'W
S4*	43°00.632'N, 124°26.853'W
S5*	43°00.640'N, 124°26.855'W
S6*	43°00.624'N, 124°26.926'W
S7*	43°00.625'N, 124°26.925'W
S8*	43°00.628'N, 124°26.924'W
S9*	43°00.626'N, 124°26.927'W
S10*	43°00.624'N, 124°26.930'W
11**	43°00.637'N, 124°26.858'W
12**	43°00.635'N, 124°26.850'W
13**	43°00.634'N, 124°26.853'W
14**	43°00.636'N, 124°26.856'W
15**	43°00.635'N, 124°26.857'W
16**	43°00.637'N, 124°26.860'W
17**	43°00.640'N, 124°26.860'W
18**	43°00.638'N, 124°26.859'W
19**	43°00.635'N, 124°26.861'W
20**	43°00.633'N, 124°26.862'W
21**	43°00.635'N, 124°26.861'W
22**	43°00.631'N, 124°26.860'W
23**	43°00.631'N, 124°26.857'W
24**	43°00.631'N, 124°26.856'W
25**	43°00.629'N, 124°26.860'W
26**	43°00.630'N, 124°26.853'W
27**	43°00.634'N, 124°26.870'W
28**	43°00.632'N, 124°26.875'W

* Seed plots (S1-S10)

** Transplant plots (11-28)