AN ABSTRACT OF THE THESIS OF

Rebecca E. Currin for the degree of Master of Science in Botany and Plant Pathology presented on March 6, 2007.
Title: Conservation of *Oenothera wolfii* (Onagraceae): Introducing a Threatened Plant into Two Protected Locations in Oregon

Abstract approved

Robert J. Meinke

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 on the southern Oregon and northern California coast. This rare species is currently listed as Threatened in Oregon, and is considered a Species of Concern by the U.S. Fish and Wildlife Service. Its status results from having a limited geographical and ecological range while facing several threats, including habitat loss and hybridization with an escaped garden cultivar, *O. glazioviana*. 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 the course of this study, seed germination, cultivation and transplanting protocols, as well as site selection criteria, were developed or refined. Additionally, the survival and reproductive success of transplanted rosettes of various sizes were evaluated and transplant success in weeded versus unweeded plots was compared. While rosette size did not affect transplant survival, larger transplants were more likely to reproduce in the first year after transplanting and to have larger numbers of flowering stalks, flowers and seeds than smaller transplants. Transplants were more reproductively successful in plots from which ground cover was removed at the time of transplanting. Overall, transplants were more successful at the southernmost site, which was located on the bluff immediately above the ocean beach on relatively stable

substrate, as opposed to the northern site, which was located approximately one kilometer inland on open, moving sand dunes. Based on initial results, the introduction and establishment of new populations of *O. wolfii* appears to be possible. The knowledge regarding the successful cultivation and transplantation of this species reported in this thesis will be useful for future introduction projects.

Conservation of *Oenothera wolfii* (Onagraceae): Introducing a Threatened Plant into Two Protected Locations in Oregon

by
Rebecca E. Currin

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Rebecca E. Currin, Author

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TABLE OF CONTENTS

Page

Chapter 1: Introduction 1

THE CURRENT PROBLEM: PLANET-WIDE LOSS OF BIODIVERSITY 1

CAUSES OF EXTINCTION 2

IMPORTANCE OF BIODIVERSITY 6

RECENT RESPONSES TO THE LOSS OF BIODIVERSITY 8

PLANT CONSERVATION AND INTRODUCTION 10

LITERATURE CITED 17

Chapter 2: Establishment of Two Experimental Populations of the Threatened *Oenothera wolfii* (Onagraceae) on the Southern Oregon Coast 25

INTRODUCTION 25

Research overview/goals 25

Study system 25

Study species 27

Hypotheses and rationale 47

METHODS 51

Seed source selection 51

Seed collection and storage 52

Seed germination 53

Seedling cultivation 56

Development of site selection criteria 59

Site descriptions 60

Experimental population outplanting: Lost Lake 63

Experimental population outplanting: Meyers Creek 68

Environmental factors 70

Monitoring 72

Analysis 73

RESULTS 74

Seed germination 74

Cultivation 74

Environmental factors 75

Transplant survival 76

Transplant reproduction 79

Transplant reproductive vigor 85

Seedling recruitment 89

Seed viability 89

DISCUSSION 91

LITERATURE CITED 97

TABLE OF CONTENTS (continued)

Page

Chapter 3: Conclusions and Management Recommendations 105

GENERAL CONCLUSIONS 105

RECOMMENDATIONS 110

Bibliography 112

Appendices 125

APPENDIX 1: LOST LAKE MAP 126

APPENDIX 2: MEYERS CREEK MAP 127

APPENDIX 3: LOST LAKE SITE MAP 128

APPENDIX 4: MEYERS CREEK SITE MAP 129

APPENDIX 5: RESEARCH PLOT GPS LOCATIONS 130

APPENDIX 6: PROTOCOL FOR ESTABLISHMENT OF NEW *OENOTHERA WOLFII* POPULATIONS 132

LIST OF FIGURES

Figure Page

1. *Oenothera wolfii* reproductive plant………………………………………. 29

2. *Oenothera wolfii* flower.……………………………….……………….. 29

3. *Oenothera wolfii* first year rosette..……………………………………... 30

4. New rosettes on the base of the previous year's flowering stalk………….. 31

5. Map of extant *Oenothera wolfii* populations in Oregon…………………... 33

6. Map of *Oenothera wolfii*'s range in northern California…………………...34

7. *Oenothera wolfii* population on the foredune at Port Orford, Oregon……...35

8. *Oenothera wolfii* plant growing with competing vegetation at Hubbard Creek………………………………………………………………. 37

9. Pistol River population of *Oenothera wolfii*, located on the hillside immediately above Highway 101…………………………………….…... 38

10. *Oenothera wolfii* plants growing on rocky beach at Sister's Rock………... 39

11. *Oenothera wolfii* habitat at Otter Point…………………………………… 40

12. European beachgrass (*Ammophila arenaria*) on foredune at Port Orford… 42

13. Flower of the non-native garden escapee, *Oenothera glazioviana*.………… 45

14. Putative *Oenothera wolfii* x *O. glazioviana* population in California....….. 46

15. Collecting *Oenothera wolfii* seeds from the Port Orford population..…….. 53

16. *Oenothera wolfii* seed germination in Petri dishes in an OSU greenhouse... 54

17. *Oenothera wolfii* seeds after one week on moistened filter paper…………55

18. Removing germinated *Oenothera wolfii* seeds from Petri dish…………….56

19. Germinated *Oenothera wolfii* seeds being planted in a peat moss/sand planting medium..…………………………………………………………... 57

LIST OF FIGURES (Continued)

Figure Page

20. *Oenothera wolfii* seedlings 14 days after being planted in 5 cm cells..…....57

21. 34-day-old *Oenothera wolfii* seedlings transplanted into 10 cm x 10 cm x 15 cm pots…………………………………………………………………...58

22. *Oenothera wolfii* plots at Lost Lake site……………………………………61

23. Meyers Creek site, viewed from across Highway 101…………………….. 62

24. Large rosettes being removed from pots for transportation to outplanting site……………………………………………………………….. 64

25. Large rosettes were removed from pots and transported to Lost Lake in plastic bags placed in coolers…………………………………………… 64

26. Example of Lost Lake plot with 0-25% ground cover…………………….. 65

27. Example of Lost Lake plot with 26-50% ground cover…………………… 65

28. Example of Lost Lake plot with 51-75% ground cover…………………… 66

29. Example of Lost Lake plot with 76-100% ground cover………………….. 66

30. Lost Lake transplants receiving one liter of water at the time of outplanting………………………………………………………………. 67

31. Lost Lake seed plot, with seeds buried 0.5 cm..…………………………… 68

32. Meyers Creek half-meter^2 plot with small rosette and ground cover removed…………………………………………………………..…. 69

33. Hydrosense© ground moisture meter with 12” sensors…………………... 71

34. Estimating slope for a plot at Meyers Creek………………………………. 71

35. Transplant survival of big and small transplants at Lost Lake and Meyers Creek after one year………………………………………………. 77

LIST OF FIGURES (Continued)

Figure | Page

36. Transplant survival for plots with and without ground cover after one year.. ……………….……………….…………………………………………79

37. Reproduction of big (excluding those transported in pots) and small transplants at Lost Lake and Meyers Creek after one year..………………..80

38. Reproduction of plants in plots with ground cover (of any class, from 1-100%) vs. those in plots with no (0%) ground cover....…………………. 83

LIST OF TABLES

Table Page

1. Results of 2003 seed germination trials: bleached vs. unbleached seeds..… 74

2. Range of values for environmental factor data at Lost Lake and Meyers Creek……………………………………………………………………..75

3. Transplant survival of different sized rosettes at Lost Lake and Meyers Creek after one year ……………………………………………….76

4. Transplant survival in plots with different ground cover status at Lost Lake and Meyers Creek after one year……………..……………………..... 78

5. Reproduction of big and small transplants at Lost Lake and Meyers Creek after one year……………………………………………..………..80

6. Reproduction of transplants in plots with different ground cover percentages at Lost Lake, and reproduction of Meyers Creek transplants in plots with ground cover removed vs. not removed...……………………82

7. Environmental variables significantly associated with transplant reproductive vigor variables……………………………………………….. 84

8. Comparison of plant size and reproductive success between different transplant sizes and sites…………………………………………………… 86

9. Reproductive success of transplants in plots with different ground cover status……………..………………………………………………………… 89

LIST OF APPENDICES

Appendix Page

1. Lost Lake map…………………………………………………...………… 126

2. Meyers Creek map…………………………………………………………. 127

3. Lost Lake site map……………………………………………………….... 128

4. Meyers Creek site map...………………………………………….……….. 129

5. Research plot GPS locations……….………………………………………. 130

6. Protocol for establishment of new *Oenothera wolfii* populations.………… 132

CONSERVATION OF *OENOTHERA WOLFII* (ONAGRACEAE): INTRODUCING A THREATENED PLANT INTO TWO PROTECTED LOCATIONS IN OREGON

Chapter 1: Introduction

"*Only within the moment of time represented by the present century has one species – man – acquired significant power to alter the nature of his world.*"

Rachel Carson, 1962

"*How difficult to imagine this place without a human presence…*"

Edward Abbey, 1968

THE CURRENT PROBLEM: PLANET-WIDE LOSS OF BIODIVERSITY

Given a long enough time period, every population of plants faces local extinction (Silvertown and Charlesworth 2001). Therefore it is not extinction itself, but rather its rates and causes which concern conservationists. It is not even numbers of extinctions which are necessarily alarming - periods where large numbers of species became extinct have occurred throughout history. 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. This current era of mass extinctions started in the middle of the 1900s, and continues to the present day (Myers 1993), which means a process that in the past took place over thousands of years is now being condensed into a fraction of that time. Since little information is available on contemporary natural extinctions, it is not clear whether extinctions caused by humans occur in the same manner as do natural extinctions (Kruckeberg and Rabinowitz 1985), and the ramifications of this accelerated loss of species are not yet fully known.

Roughly 1.7 million species have been identified worldwide. However, scientists believe that there are huge numbers of organisms yet to be described, and a conservative estimate of the actual number of species on the planet is probably closer to ten million (Myers 1993). About 200 species of vascular plants are already presumed to have become extinct in the United States, with over half of those extinctions occurring in Hawaii (Morse 1996). 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). Since the number of extant species is very likely underestimated, it is quite probable that the number of extinctions is underestimated as well, and many of these species could be lost before they are even discovered and described. 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 rarest of the rare, 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 2006a). Lastly, it is not simply the total number of species that should be used as the standard measurement of biodiversity or its loss; intra-specific diversity is important too. On average, each species consists of several hundred genetically distinct populations (Myers 1993). The loss of distinct populations diminishes the gene pool that comprises a species and its populations, and ultimately results in the loss of the underlying building blocks of biodiversity.

CAUSES OF EXTINCTION

Although many factors have contributed to the relatively high rates of extinction seen over the past 50 years or so, the vast majority of lost biodiversity is due to anthropogenic causes (Myers 1993). The fast-paced growth of the human population has had far-reaching impacts on the natural world. 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). Of these factors, the destruction and fragmentation of habitat has undeniably caused the greatest impact (Pantone et al. 1995, Huxel and Hastings 1999). Of the twenty identified causes of plant rarity in a study conducted in Germany, the greatest threat to species persistence was habitat elimination, with causal agents including agriculture, alteration in land use, strip mining and land abandonment (Sukopp and Trautmann 1981). In the Swiss Jura Mountains, a study of more than 150 calcareous grassland species monitored over 35 years pointed at habitat fragmentation and consequent isolation as the major cause of extinction (Fischer and Stocklin 1997). In the United States, the Center for Plant Conservation cites habitat loss and degradation as a threat for the vast majority of the plant species listed as endangered in their National Collection of Endangered Plants (Center for Plant Conservation 2006). Closer to home, a status summary of 165 rare vascular plant taxa in Oregon listed habitat loss and/or degradation due to urban and agricultural development, mining, grazing or recreational use as a threat to over 95% of the species reviewed (Meinke 1980).

Widespread habitat loss results in remnant native plant populations that are spread across the landscape in small, fragmented patches. Habitat fragmentation, and the resulting isolation and smaller size of populations, creates a host of additional threats to plant populations. A growing body of research links population size to population fitness (Widén 1993, Fischer and Matthias 1998, Groom 1998, Reed 2005). Small populations can suffer a decrease in fitness in the form of reduced survival and fecundity. These effects can be attributed to both environmental and genetic causes.

One result of small, fragmented populations is increased vulnerability to evolutionary hazards such as inbreeding depression and genetic drift (Ellstrand 1992). Normally, genetic variation is distributed among populations and individuals within populations through the mechanics of four evolutionary forces (mutation, natural selection, migration, and genetic drift). The genetic structure of small, isolated populations tends to be dominated by the influence of genetic drift (Barrett and Kohn 1991), and the subsequent loss of variation can make these types of populations less able to adapt to changing environments (Schemske et al. 1994). Additionally, reduced genetic variation can increase susceptibility to pests and diseases (Barrett and Kohn 1991), and therefore increase the risk of local extinction. When comparing diversity of isozyme loci in 11 pairs of congeneric species, Karron (1987) documented reduced genetic diversity in rare species, as compared to their more common congeners. These results were attributed in part to genetic bottlenecking, or the genetic drift associated with populations reduced in size due to fluctuations in abundance. In small artificial populations of *Lolium multiflorum*, monitoring of the change in allele frequencies and levels of heterozygosity at three isozyme loci demonstrated that smaller populations experienced a much greater loss in genetic variation than those populations with more individuals (Polans and Allard 1989). One of the goals of conservation is to maintain existing levels of genetic variation in species that are rare or threatened (Barrett and Kohn 1991), so that populations retain their ability to adapt to future environmental changes.

In addition to the potential genetic consequences resulting from population isolation, there may be ecological ramifications as well. Rare species may have less efficient means of dispersing to new sites than their more common congeners and might have to traverse larger distances between sites due to specialized habitat requirements (Primack 1996); these challenges are further exacerbated by human-caused habitat fragmentation. Vertebrate seed disperser populations have been reduced or eliminated by hunting and habitat destruction, and in some cases, physical barriers such as fences, roads, farmlands, and human habitations prevent or retard the dispersal of seeds (Peters and Darling 1985). Another negative consequence of populations being separated by large distances is the interruption of pollinator movement and function. For obligatory outcrossing species, reproductive failure can occur when small, isolated patches are unable to attract the needed pollinators. Population size can be the critical factor in these cases, since larger patches are often able to attract pollinators regardless of their degree of isolation (Groom 1998). Lack of pollinators can lead to reduced seed set (Jennersten 1988, Lamont et al. 1993, Agren 1996, Morgan 1999, Moody-Weis and Heywood 2001), which ultimately may reduce the fitness of the population. Finally, small fragmented populations are particularly vulnerable to stochastic events, such as environmental catastrophes, which can lead to extirpation (Schemske et al. 1994, Amsberry 2001).

Even when habitats have not been destroyed or fragmented, many remaining areas of relatively intact habitat are still beset with problems. Many ecosystems are overrun with non-native invasive species, and this increased competition places additional pressure on rare native plant taxa (Seabloom et al. 2003). Increased numbers of exotic species in native habitats may increase the risk of hybridization between rare species and introduced non-natives (Levin et al. 1996, Allendorf et al. 2001). Habitat disturbance can also bring previously allopatric natives into contact, potentially effecting hybridization among species that occupied distinct habitats prior to human interference (Gisler 2003).

In addition to the direct damage caused by destruction of habitat, over-exploitation, and increased competition from introduced plant or animal species, habitats are indirectly impacted by human activity. Chronic pressure has been applied to many species due to a combination of human factors that have altered environments in ways that inhibit or interrupt reproduction, dispersal, and colonization of new sites (White 1996, Drayton and Primack 2000). For example, the production and dispersal of ground-level ozone or acid precipitation can change the biological and physical characteristics of a location, with the effects accumulating dramatically over time. Global climate shifts, in particular the increased levels of carbon dioxide in the atmosphere and subsequent global warming, changes in temperature and precipitation regimes world-wide. In turn, this can potentially have a negative environmental impact on all or a portion of the geographic ranges of many species (Drayton and Primack 2000). When these changes occur against the backdrop of a natural landscape, fragmented by human disturbance, the result can be extirpation for those populations unable to emigrate from the altered and now unsuitable habitat.

Finally, many of these threats do not respect the boundaries of sites designated as refuges for remaining in situ populations of rare species. Air pollution, invasive exotic species, and the wider effects of human alterations of natural processes impact habitats on a landscape scale. It is very difficult, if not impossible, to prevent these types of threats from causing widespread harm, even in protected areas. Legal protection cannot guarantee survival of genes, species or ecosystems (White 1996).

IMPORTANCE OF BIODIVERSITY

The loss of species has been well documented. When preservation of biodiversity comes into conflict with other land uses, whether they are economic (such as extraction of resources) or social (i.e., recreational use), the important question is why do we care? What value does biodiversity represent?

There are many ways in which plants are essential to the survival and well-being of humans. Plants provide our food, either directly or through the sustenance of animals that we eat. Every agricultural crop currently in production was derived from wild plants, and remaining wild relatives of these crops are continuously explored for unutilized genes imparting disease resistance, cold hardiness, and other beneficial traits. These traits have the potential to diversify our currently genetically homogenous agricultural crops (Affolter 1997). In the field of medicine, many currently used pharmaceuticals were originally derived from plants, although most are now synthesized in laboratories. Examples include senna (*Cassia* spp.), which was used as a laxative as far back as 1500 B.C.; foxglove (*Digitalis* spp.), the original source for the heart medication digitalis: and willow (*Salix* spp.) bark, used for its aspirin-like properties for hundreds of years (Duffin 1999). Plants provide anti-infective agents such as emetine, quinine, and berberine, which are all still being used today in the fight against microbial infections (Iwu et al. 1999). Ornamental cultivars and varieties, the development of which contribute to the growth of Oregon's substantial nursery industry, provide aesthetic beauty. Finally, the new and growing field of bioremediation, or the use of plants to clean up pollution in the environment, dramatically demonstrates that there are many untapped uses for plant species and the genes that they carry (Krämer and Chardonnens 2001, U.S. Environmental Protection Agency 2001). Once plant species become extinct, not only do we lose the direct benefits the plants provide, but any undiscovered and unutilized beneficial genes they may contain are permanently lost as well.

In addition to the obvious and direct benefits that individual plant species provide to human beings, intact ecosystems also provide many services. Healthy plant communities provide soil erosion control and storm water retention. Native plants retain nutrients released into the soil by decomposition, holding nutrients on-site and ultimately maintaining the productivity of the ecosystem in question (Risser 1998). Plants moderate temperatures in environments such as riparian areas by providing shade. Native plants provide habitat and food for many organisms, both large and small. Additionally, native plants often have unique associations with insects (Kaye 2001). When rare plant species deliver vital community services, such as acting as host plants for pollinators, the impacts of species extinction are greater than the direct effects. Losing these plants can cause a cascading series of extinctions. The subsequent loss of pollinators can in turn harm other species that require insect-facilitated pollination in order to reproduce. Even if pollinators are not essential to the survival of a plant species, they can enhance the fruit set and size, seed production and viability, seedling vigor, and genetic diversity of plant populations (Cane and Tepedino 2001), and their loss can undermine the long-term evolutionary potential of the species. The contribution each member of a functioning ecosystem makes toward the delivery of these services is not generally known, and it is risky to dispense with any of the individual components of the system before obtaining this knowledge.

Ecosystem functions are based upon dynamic interactive systems, where synergistic interactions are significant. Any given system member's tolerance of one stress tends to be lower when other stresses are in operation (Myers 1993). Currently most ecosystems are under pressure from multiple stressors, making many members more vulnerable to the threat of extinction. This is especially true for types of environments, such as tropical forests, wetlands, coral reefs and estuaries (Myers 1993). When mass extinctions occur, evolution is disrupted and the speciation process is forced to work with a greatly reduced pool of species. As more and more species are lost from these interdependent systems, the interactive nature of the system breaks down. It is hard to say when this ecosystem degradation becomes irreversible, and function is lost.

There are many examples of why the protection of plant biodiversity is valuable on a practical human-benefit basis. However, the current trend of biodiversity loss raises additional ethical and philosophical questions. As the dominant species on this planet, do human beings consider all forms of life (human or otherwise) intrinsically valuable? Do we want to not only allow, but cause, the wholesale destruction and loss of other species? Fortunately, many people do value biodiversity for its own same, and support the idea that non-human life has intrinsic value, and that plants and animals should be conserved regardless of their benefits to humans (Naess 1986).

RECENT RESPONSES TO THE LOSS OF BIODIVERSITY

Although news of increasing numbers of species extinctions and ecosystem degradation is daunting, all is not without hope. There are a growing number of scientists, policy-makers, and members of the general public who are looking for ways to reverse the conservation trends of the last century. Responses are both legal and programmatic, with new laws and fields of research being created to address our current need for change.

The field of conservation biology has arisen out of crisis – species and ecosystems are being destroyed, and immediate action is needed to stem the tide of loss. Conservation biology is a multi-disciplinary field, combining the knowledge of population biologists, taxonomists, ecologists and geneticists. It combines science and intuition; when action must occur before all the needed information is available, intuition is drawn upon as well (Soulé 1985). The goals of conservation biology practitioners are to provide principles and tools for preserving biological diversity; understand the effects of human activities on species, communities, and ecosystems; and develop practical approaches to preventing the extinctions of species and reintegrate endangered species into functioning ecosystems (Soulé 1985, Primack 2004).

In the United States, the federal Endangered Species Act (ESA) provides the strongest legal protection for biodiversity, and is one of the best tools available to limit anthropogenically caused extinctions of fish, wildlife and plants. Passed by the Senate and House of the 93rd Congress in mid December of 1973, and signed into law by former President Nixon eight days later, this law grants government agencies the authority to prevent additional loss and start the process of restoring that which has been damaged (Bartel 1987). The ESA focuses on the protection of individual species (or, in the case of animals, individual populations as well), classifying those most vulnerable to extinction as either threatened or endangered (with those species classified as endangered being considered the most vulnerable to extinction). Of the plant species (not including subspecies or varieties) formally listed under the ESA by the early 1990s, 80% had a median population size of fewer than 120 individuals (Wilcove et al. 1993). The ESA requires that a recovery plan be provided for every listed taxon, which clearly describes the research and management actions necessary to support the recovery of that species (Schemske et al. 1994). There is evidence that this strategy can work; the earlier a declining species is listed, the better are its chances for successful recovery (Wilcove et al. 1993). Roughly 30% of the species for which a recovery plan was in place by 1990 are now increasing in abundance (Schultz and Gerber 2002).

PLANT CONSERVATION AND INTRODUCTION

There are two general approaches to the conservation of 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. (Note: In this thesis, introduction is used as the general term encompassing introduction of a species into a site within its historical range, whether or not the species was known to exist at that exact site in the past, and whether or not an existing population of the species already resides at the site.) 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).

Preservation of existing rare plant populations and the relatively intact ecosystems that host them should play the more important role in rare plant conservation for a variety of reasons. Once protected, these systems can serve as reference points as attempts are made to restore additional areas of habitat (Falk et al. 1996). The myriad of factors necessary for a species' survival and reproduction are likely to exist in most places where natural populations occur. In contrast, selecting sites for rare plant introduction can be a game of chance, with the odds of the new site containing all of the necessary variables for the species' survival (i.e. soil types, hydrology, precipitation levels, heat and light exposure, mycorrhizal associations, and pollinators) being low, at best.

In addition, turning too quickly to introduction, reintroduction and augmentation increases the risk that these relatively unproven techniques will be used to undermine the primary goal, which should be retention of original habitats. We have already seen this with wetland mitigation, where the destruction of existing wetlands is frequently permitted as long as mitigation is implemented. Construction of new “wetlands” is one form that this mitigation takes; however, there is little evidence that these newly created sites function similarly to natural wetland sites (Magee et al. 1999, Gutrich and Hitzhusen 2004, Brooks et al. 2005). Additionally, not all restoration is initiated with conservation as the primary motivation. Developers and public agencies finding a sensitive species in the way of construction or resource extraction projects often propose relocation of populations as a way of mitigating the negative impacts of the proposed project. California alone has received hundreds of applications by private developers wanting to relocate populations of rare species to more convenient locations (Falk et al. 1996).

In spite of these cautions, there are cases in which introduction will be a valuable tool in the fight against widespread loss of biological diversity. Conservation practitioners, advocates, land managers and regulators are increasingly faced with the need to intervene in order to save species from extinction (Falk et al. 1996). Setting aside protected sites is necessary, but often not sufficient. 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 circumstances are such that the destruction of some natural populations has already occurred, and remaining populations are threatened with extirpation as well, the only remaining option may very well be using all available knowledge of the species and its habitat to attempt to create new populations (Falk 1987, Guerrant and Pavlik 1998).

Since the passing of the Endangered Species Act in the United States, new focus has been placed on those species most in danger of becoming extinct, and most efforts to prevent the loss of biodiversity are directed at individual species (Primack 2004). Introduction is being used increasingly by federal, state, and private conservation agencies, and reintroduction is included in the recovery prescription for nearly one-fourth of the plant species federally listed under the ESA (Falk and Olwell 1992, Falk et al. 1996). Since the outright loss of a species is preceded by the incremental loss of its populations, reversing this trend through the introduction and establishment of new populations is considered necessary for many of these species. The hope is that enough self-sustaining populations will be established that these precarious species may eventually become delisted (Bowles et al. 1993, Pavlick et al. 1993b).

In general, there are a limited number of situations that warrant using translocation or introduction of endangered plants as a recovery tool (Gordon 1994). When rare species are located on unprotected land that is slated for a ground-disturbing activity (such as development) that will destroy the plants and/or their habitat, rescuing these plants and translocating them to a protected site with suitable habitat may be the only option. Species with limited numbers of small or severely declining populations, poorly protected natural populations, dispersal problems, and habitat fragmentation issues are also good candidates for introduction (Falk et al. 1996). When land managers are protecting or restoring native habitat, rare plant introduction can be a valuable part of the restoration efforts. Not only does the successful establishment of new populations increase the species diversity of the restored ecosystem, but careful location of these sites within a network of populations can strengthen the connectivity of existing populations and improve metapopulation functions such as pollinator services or colonization of extirpated patches or other unoccupied habitat (Huxel and Hastings 1999). Finally, when introduction experiments are carefully planned, executed, and monitored, the results of the research can provide valuable knowledge about both the species itself and the field of conservation in general.

As the body of knowledge about how to conduct an introduction project grows, success stories are becoming 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). Although rare plant introductions are still in their infancy (Pickart and Sawyer 1998), and many of the efforts documented in the literature had been monitored for only a few years, some case results have shown at least preliminary success. For example, *Hymenoxys acaulis* var. *glabra* has been successfully introduced onto two nature preserves in Illinois (DeMauro 1994). *Amsinckia grandiflora* was introduced successfully into an unoccupied site within its historic range (Pavlik 1996). Two years after *Echinacea laevigata* was introduced into two sites in Georgia, 75-94% of the transplants were still surviving (Alley and Affolter 2004). Greenhouse-grown individuals of *Abronia umbellata* var. *breviflora* were successfully introduced into several sites on the Oregon coast (Kaye et al. 1998, McGlaughlin et al. 2002). While these introductions can be considered complete only when the species are safely reestablished in their ecological and evolutionary context (Allen 1994), this tool provides some hope for the preservation of biodiversity for the future.

At the same time, however, we cannot rely on introduction as the answer to all of our extinction woes. Many of the introductions attempted over the last twenty years have had mixed results, and not all projects have been successful (Brumback and Fyler 1996, Pantone et al. 1995). In Great Britain, a 1991 British Nature Conservancy Council study revealed that only 22% of the 144 plant introduction attempts reviewed were considered successes (Allen 1994). Sometimes the failures result from insufficient biological or ecological knowledge of the taxon (Falk and Olwell 1992, Falk et al. 1996, Kohn and Lusby 2004) and its habitat. Often researchers lack understanding of the ecosystem-level interactions and processes that are critical to species recovery (Ehrenfeld 2000). Frequently, unsuccessful recovery attempts reflect an inability to isolate and assess the most important factors that contribute to the taxon’s rarity. These factors might be extrinsic to the life history of the species (i.e., habitat destruction, exotic competitors and predators, lack of pollinators) or they might reflect intrinsic biological attributes that limit distribution and abundance (i.e., low fecundity, lack of genetic variability, short-range dispersal mechanisms) (Pantone et al. 1995).

Logistical challenges can also cause introduction project failure. Sharing characteristics with the flora in general, rare plants may produce few propagules to begin with (Weekley and Race 2001), and their propagules may have specialized germination requirements (Baskin et al. 1997, Stewart et al. 2003). Rare plants may be particularly difficult to cultivate in a greenhouse setting (Reinartz 1995), and some rare plants require mycorrhizal colonization to reach a transplantable size (Barroetavena et al. 1998). Once plants are ready for transplantation, inappropriate introduction site selection can also result in project failure (Fiedler and Laven 1996). Rare species can have specific site requirements, although the nature of these requirements is often largely unknown (Drayton and Primack 2000, Kohn and Lusby 2004). Soil type, texture and moisture retention (Fielder and Laven 1996), lack of pollinators (Karron 1987) or necessary mycorrhizal associates (Barroetavena et al. 1998), and presence of herbivores (Alley and Affolter 2004) are all factors that can critically affect the appropriateness of an introduction site.

Finally, perhaps the greatest roadblock to evaluating project success is the lack of clear performance measures, documentation, and monitoring (Pavlik 1996, Sutter 1996). Many of these attempts are conducted opportunistically or under tight time schedules with limited budgets (Falk et al. 1996). Without criteria for success clearly delineated, it is difficult to say whether or not an introduction attempt has been successful. Even if performance measures are identified, resources are often not available for monitoring. In fact, one of the strongest criticisms of introduction experiments, aside from the lack of consistency in experimental design and execution, is the lack of consistent monitoring (Hall 1987, Mehrhoff 1996, Guerrant and Pavlik 1998). Even when studies do have a monitoring component, data are usually collected for only a year or two after the initial transplanting, and the collected data are often not compiled, analyzed or published (Amsberry 2001).

These examples of introduction success and failure have led to an increased body of literature providing guidance for those attempting such projects in the future. As our knowledge of what factors improve the chance of introduction success increases, so will our ability to wield this tool more effectively. In order to determine whether or not a project is successful, however, we must first decide what constitutes success. Pavlik (1997) defines overall success of an introduction project as the creation of a new, self-sustaining population within the historic range of the plant. However, when planning an introduction project, it is important to further break this down into a series of measurable goals for both the short- and long-term. To facilitate this, Pavlik (1996) laid out four general categories by which introduction goals and success can be assessed: abundance (establishment, vegetative growth, fecundity, population size), extent (dispersal, number of populations, distribution of populations), resilience (genetic variation, resistance to perturbation, dormancy), and persistence (self-sustainability, microhabitat variation, community "membership"). The first two, abundance and extent, can be measured in a shorter time frame (0-10 years). Researchers might set short-term goals such as completion of the life cycle (in situ) of the plant being introduced. Resilience and persistence, however, can only be tested over long periods of time. Long-term objectives might be met by achieving a pre-determined minimum viable population size through natural recruitment of second generation cohorts. Ultimately, the hoped-for result of introduction efforts is to reestablish the natural role of multiple, semi-independent, self-sustaining populations in order to lessen extinction risk and maintain genetic variation among populations (Guerrant 1996, White 1996). Introduced populations should be as capable as their natural equivalents of integrating fully into their ecosystem and its functions, and meeting the challenges of the changing environment either through evolution or migration (Pavlik 1996).

When writing about designing new populations of rare plants, Guerrant (1996) states that the single most effective design feature for reducing overall risk of failure is to introduce multiple populations and manage them as metapopulations. This inclusion of spatial analyses into the site location decision-making process (i.e., the relationship of newly created sites to existing natural populations of the target species) may improve a species' chance of recovery (Bowles and McBride 1996, Huxel and Hastings 1999, Drayton and Primack 2000). Introducing the species into appropriate habitat close to already occupied patches increases the efficacy of the recovery effort. A number of patches must be restored before a positive effect on population levels is seen. The most important parameter of metapopulation function may be the population turnover rate (colonization vs. extinction rates) (Schemske et al. 1994). In effect, restoration efforts increase this rate by allowing biologists to facilitate seed dispersal and establishment when a species appears to be unable to disperse on its own (Menges 1991, Primack 1996). Finally, the establishment of multiple introduction sites also allows for researchers to pose more complex hypotheses involving larger numbers of variables, and to more rigorously test these hypotheses statistically.

Ultimately, most introduction attempts remain unique events, limited in geographic scope (Falk et al. 1996). With efforts to establish new populations of rare plants ending in failure more often as not, it is important to ensure that rare plant material obtained for propagation and introduction (whether it be seeds, bulbs, or plants themselves) is not used in vain. Each introduction attempt should be planned and implemented as an experiment that provides valuable knowledge not only about the population, species, or site in question, but also about the larger framework of landscapes, ecosystems, and conservation in general. Every introduction project is a chance to observe processes and their consequences (i.e., founder effects) first hand (Lewin 1989). Every project that proceeds without gathering baseline data is a lost opportunity for learning (Falk et al. 1996). However, every introduction project that has been carefully planned and monitored, even if its new populations fail, can still be considered a success by contributing to our knowledge of rare and endangered plants (Pavlik 1996).

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Chapter 2: Establishment of Two Experimental Populations of the Threatened *Oenothera wolfii* (Onagraceae) on the Southern Oregon Coast

INTRODUCTION

Research overview/goals

The purpose of this study is twofold. The first goal is to develop a protocol for the establishment of new *Oenothera wolfii* populations. The second goal is to create two experimental populations of this species. Two locations were selected as pilot sites for introduction efforts. Originally, both sites were to be located within the New River Area of Critical Environmental Concern (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, compares transplant establishment in weeded and unweeded plots, and analyzes the effect of various environmental variables on transplant survival and reproduction. In addition, seed germination rates were assessed for seeds from different natural populations and of different ages.

Study system

The Oregon coastline is a wild and beautiful state resource. Spanning roughly 350 miles (563 km), the coast has provided many Oregonians with a place to live, work, and engage in recreational activities such as fishing, camping, walking on the beach and whale watching. The Oregon Coast also provides critical habitat to many plants, seabirds, fish, sea lions and gray whales. In 1973, Governor Tom McCall declared the beaches of Oregon “owned by the people” (Oregon State Public Research Group 2006), and Oregonians continue to value this ownership.

This study took place on the southern part of the Oregon coastline, a unique area where a variety of environmental transitions occur. South of Cape Blanco, in Curry County, the trend of the coast is south-southeast, with most of the shoreline being rockier and much less regular in detail than the north (Cooper 1958). High bluffs and steep slopes adjacent to the ocean predominate (Franklin and Dyrness 1973). However, sand dunes comprise roughly 45% of Oregon’s total coastline (Wiedemann 1978), and there are many sandy beaches along the southern coast as well. In this corner of Oregon, the Klamath Mountains merge topographically with the Coast Range, providing substrate materials which are older and more complex in structure than those found to the north (Cooper 1958).

The coastline experiences heavy winter precipitation with dry summers. The greatest moisture deficiency occurs in July and August (Cooper 1958), although fog can mitigate the dry summer conditions (Franklin and Dyrness 1973). The climate along the southern coast tends to be warmer and drier than that of the northern shoreline (Franklin and Dyrness 1973). Cooper (1958) found wind to be the single most important climatic element affecting the ecology of sand dunes, with summer being the greatest season of activity due to the more frequent north to northwest winds. Winter winds tend to arrive from the south to southwest, and are less frequent and of higher velocity than the winds of summer.

The shift in climate, geology and topography is reflected in the vegetation as well. Cooper (1958) described the dominant vegetation of the Oregon coast as part of the Pacific Coastal Forest Complex, and Kumler (1969) divided the coastal vegetation into nine communities, ranging from sparsely dispersed pioneer herbaceous plants on relatively barren shifting sand to near-climax dune forest. Overall, however, the southern Oregon Coast is characterized more by herbaceous and shrubby communities than forest. Ocean-front plant communities tend to be made up of sand-colonizers and sand-stabilizers, both of which must be able to withstand the extreme conditions encountered next to the ocean. Harsh conditions and microenvironmental variability caused by wind and wave action make seed germination, emergence of seedlings, and seedling establishment difficult (Maun 1994). Once plants survive beyond the seedling stage, they must tolerate heavy salt spray, low nutrient levels in the sandy substrate, and strong winds which cause desiccation and abrade the plants with sand.

In addition to the harsh conditions and variable microclimates that make seedling establishment challenging, rare species that grow in dune and coastal environments are subject to a variety of external threats to their existence. The fragile nature of these ecosystems is particularly vulnerable to the types of recreational usage found in these areas; foot and off-road vehicle traffic can easily destroy early successional communities (Cooper 1958, Bowles et al. 1993). In some areas of the coast, timber harvesting and grazing can negatively impact vegetation (Cooper 1958). Dune stabilization efforts, especially those using *Ammophila arenaria*, a non-native grass introduced in the late 1800s, have also completely changed the dune ecosystems (Wiedemann et al. 1969). Finally, coastal development, whether it be roads, recreational facilities, or commercial or residential structures, has had a negative impact on the native vegetation.

Study species

Listing Status

Oenothera wolfii (Munz) Raven, Dietrich & Stubbe (Wolf's evening primrose) is a biennial to short-lived perennial with only a small number of isolated populations. This taxon is surprisingly rare, considering that it can behave like a "weedy" species, and establishes fairly large populations in moderately disturbed areas (Imper 1997, Carlson et al. 2001). Its current precarious status is due to a limited geographical range and several pressing threats, including habitat loss and hybridization with an escaped garden cultivar, *O. glazioviana*. In California, Wolf's evening primrose is listed as "Rare and Endangered Throughout Its Range" by the California Native Plant Society (List 1B; California Native Plant Society 2006). The Oregon Natural Heritage

Information Center (2004) also considers *O. wolfii* “Threatened or Endangered Throughout Its Range” (List 1). The U.S. Fish and Wildlife Service describes *O. wolfii* as a “Species of Concern” (Oregon Natural Heritage Information Center 2004), and the State of Oregon lists this species as “Threatened” (Oregon Department of Agriculture 2006).

Species Description

General 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 the anthers (Figure 2). Stems, sepals, and fruits are typically red-tinged and pubescent, often with glandular hairs (Carlson et al. 2001). In spite of these easily determined characteristics, identification of *O. wolfii* and other species within subsection *Euoenothera* is considered difficult, due to the high level of interfertility within the group (Imper 1997).

Technical description: Wagner and Raven (1993) provide the following technical description of *O. wolfii* in the Jepson Manual:

> Plant biennial, rosetted, densely minutely strigose; many hairs also with red, blister-like bases, some glandular. Flowering stems are erect and 5-10 dm in height. Leaves are cauline, ranging from 5-18 cm in length, narrowly lanceolate to elliptic, and wavy-dentate. The inflorescence a spike. The hypanthium is 30-46 mm across. Sepals are 17-28 mm in length, with free tips in bud erect and 1-3 mm in length. Petals are 13-23 mm long, yellow fading reddish orange. Fruits are 30-48 mm long and 5-8 mm wide, narrowly lanceolate, and ± straight. Seed is 1-2 mm across, angled and irregularly pitted. 2n=14.

Life history/breeding system: Although considered a biennial, *O. wolfii* can behave as a facultative perennial. Under normal conditions, seeds will germinate during the first

Figure 1. *Oenothera wolfii* reproductive plant.

Figure 2. *Oenothera wolfii* flower.

year and produce small rosettes (Figure 3). The rosettes typically 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 (personal observation).

Figure 3. *Oenothera wolfii* first year rosette.

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

The long floral tube, light colored flowers, 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

(Pavlik 1987, Baskin and Baskin 1993), allowing them to develop long-lived seed banks. 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 16 known populations of *O. wolfii* scattered along 260 km (160 miles) of the western United States coastline between Port Orford, Oregon and Cape Mendocino, California. Seven of these populations are in Oregon: Port Orford, Hubbard Creek, Humbug Mountain, Sister's Rock, Otter Point, Pistol River and Zwagg Island (Gisler and Meinke 1997; Figure 5). All Oregon populations were extant in September of 2004. The number of individuals in each population ranging from approximately 40 to several thousand plants. (Note: The Humbug Mountain population was not visited in 2004; however, this site was visited by Carlson et al. [2001] three years earlier, and is also assumed to be extant.) There are an additional nine populations in California, between Crescent City and Cape Mendocino (Gisler and Meinke 1997, Imper 1997; Figure 6).

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* (Pavlik and Manning 1993), the specific substrate characteristics (as long as it is well-drained) do not appear to be critical to the establishment of *O. wolfii*. *Oenothera wolfii* populations tend to be situated south of a headland or promontory, or near the mouth of a river, in locations that are somewhat protected from northwesterly exposure (Center for Plant Conservation 2004). The species seems to require some disturbance, and is able to move opportunistically into recently disturbed areas (T. Kaye, personal communication, March 3, 2005). The Port Orford population is located on the foredune itself (Figure 7). Here *O. wolfii* plants

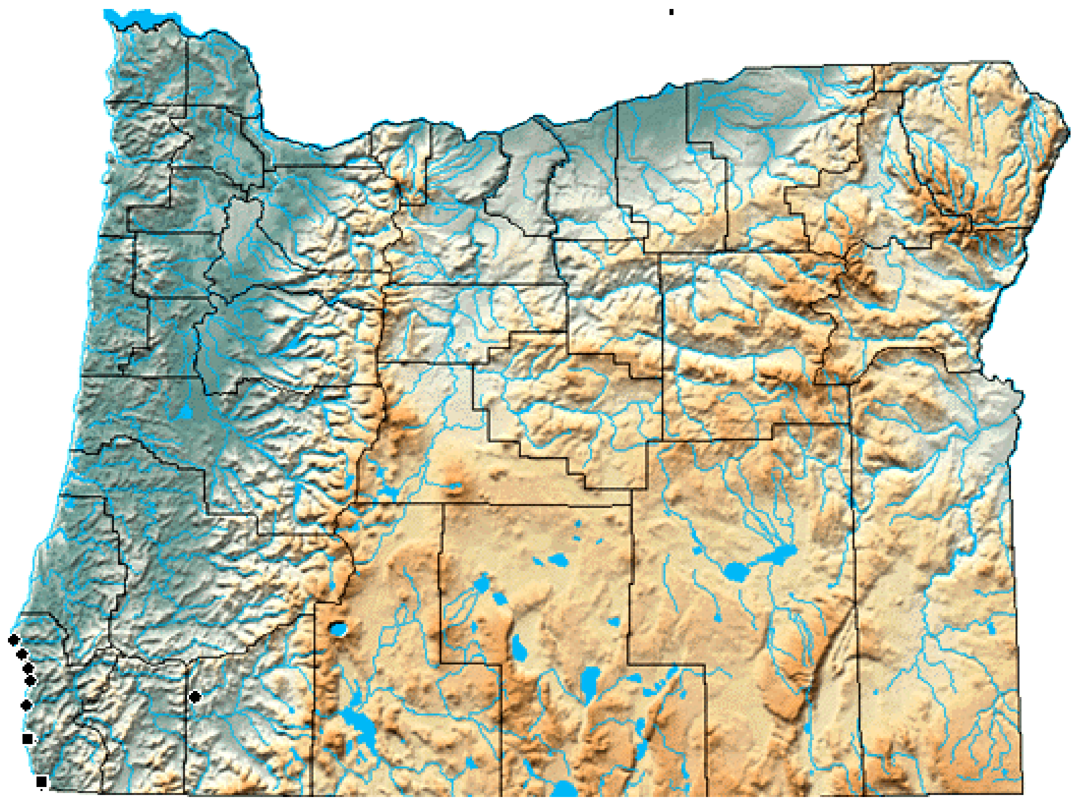

Figure 5. Map of extant *Oenothera wolfii* populations in Oregon. The inland dot that is located in Jackson County represents an unvouchered observation in the Oregon Flora Project database. Oregon Department of Agriculture botanists visiting this site were unable to locate *O. wolfii*, and it is thought that the species was misidentified in this observation (K. Amsberry, personal communication, November 5, 2005). (Map provided courtesy of the Oregon Flora Project.)

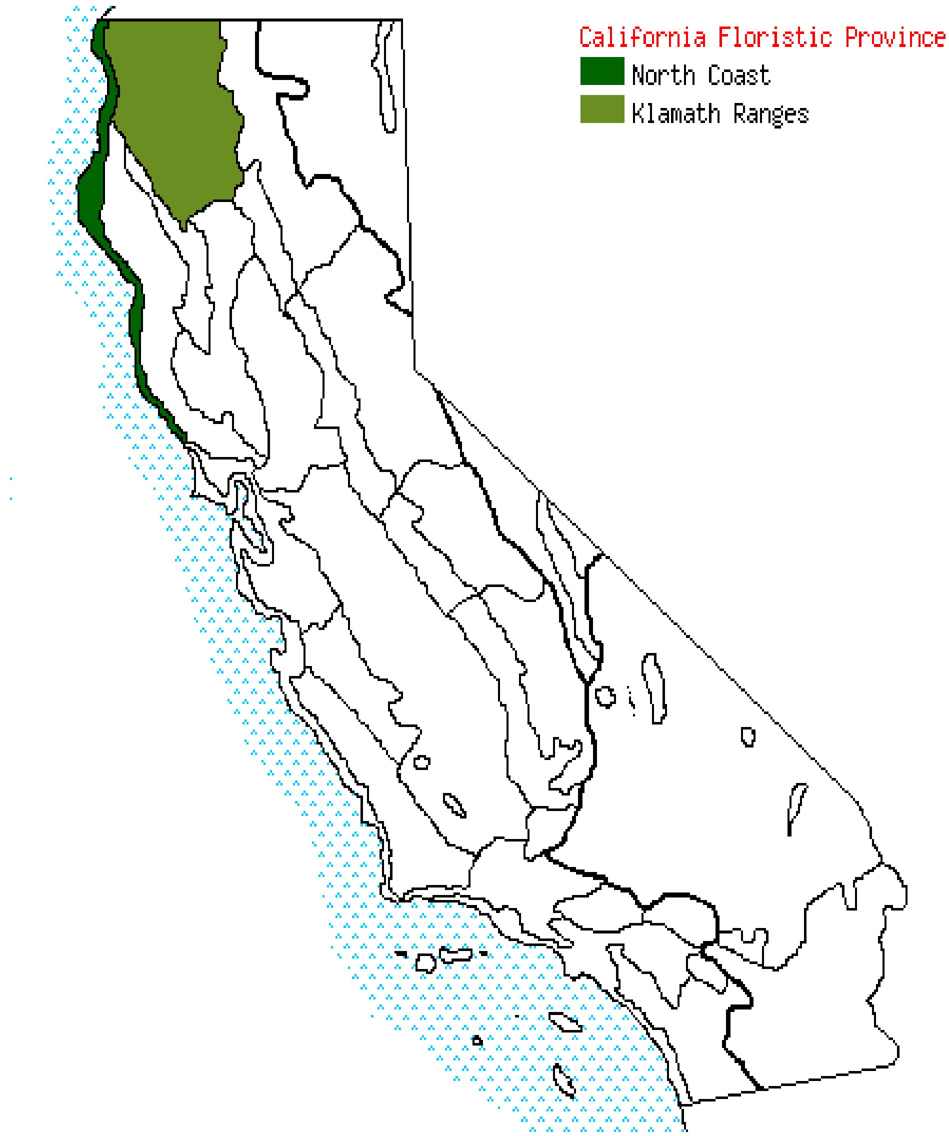

Figure 6. Map of *Oenothera wolfii*'s range in northern California. The California populations are located within the North Coast floristic province (dark green). (Map provided courtesy of the Jepson Flora Project.)

Figure 7. *Oenothera wolfii* population on the foredune at Port Orford, Oregon.

take advantage of gaps in the *Ammophila arenaria* swards that have followed the dumping of sand on the beach following bay dredging. Several other populations reside on partially stabilized beach dunes, where other vegetation provides some protection but frequent disturbance still occurs. At one population in California, a few individuals can be found growing in cracks in a beachside parking lot (Imper 1997).

Oenothera wolfii is also found on bluffs immediately above the beach. The vegetation cover on these bluffs ranges from almost complete (Hubbard Creek, Pistol River; Figures 8 and 9) to sparse in areas where bare soil and rock are exposed (Sister's Rock, Otter Point; Figures 10 and 11). Once again, *O. wolfii* appears to prefer some disturbance, and minimal competition; populations on less stabilized substrate are 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*, *Fragaria chiloensis*, *Garrya elliptica*, *Gaultheria shallon*, *Lonicera involucrata*, *Lotus corniculatus*, *Lupinus* sp., *Mimulus guttatus*, *Myrica californica*, *Phacelia argentea*, *Picea sitchensis*, *Plantago* sp., *Polygonum paronychia*, *Pteridium aquilinum*, *Rubus spectabilis*, and *Salix hookeriana* (Oregon Natural Heritage Information Center 2003, personal observation).

Figure 8. *Oenothera wolfii* plant growing with competing vegetation at Hubbard Creek.

Figure 9. Pistol River population of *Oenothera wolfii*, located on the hillside immediately above Highway 101. This population forms the basis for an Oregon Department of Transportation Special Management Area (SMA).

Figure 10. *Oenothera wolfii* plants growing on rocky beach at Sister's Rock.

Figure 11. *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.

Current threats

Oenothera wolfii faces several imminent threats. Habitat has been lost due to development and road construction and maintenance, and *O. wolfii*'s habitat has been invaded by exotic species. Additionally, *O. wolfii* is capable of hybridizing with an escaped garden ornamental, *O. glazioviana*, potentially destroying the genetic integrity of the rare species (Imper 1997).

Habitat loss: Habitat loss and alteration is a common threat for many rare and endangered plants, and *O. wolfii* is no exception. Coastal development, and the dune stabilization efforts that often accompany it, have negatively impacted this rare species' habitat. Near Crescent City the recent expansion of both commercial and residential development has eliminated or altered *O. wolfii* habitat (Imper 1997). The large population at the Luffenholz site in California was almost completely destroyed by the construction of a beach access parking lot in 1962 (Imper 1997). Roadside maintenance also impacts *O. wolfii*. Several populations grow adjacent to Highway 101, and activities such as road expansion, bridge work, culvert maintenance, and herbicide spraying may harm these populations.

***Non-native invasive species*:** Non-native invasive plants are a threat to the biodiversity and function of every major type of ecosystem in the world, and those along the western Unites States coast are no exception (Daehler 2003, Seabloom et al. 2003). Human activities that increase resource availability and alter disturbance regimes often differentially increase the performance of non-native invasives over that of native species (Daehler 2003). When non-natives are able to tolerate human disturbance and disperse in association with human activities, their ranges can rapidly expand. At the same time, rare native species with limited or hindered dispersal and an inability to adapt to changes caused by human disturbances often experience range contractions (Primack 1996). The non-native invaders displace native plant species and reduce habitat suitability for native animals (Colton and Alpert 1998). Conservation of rare species is hindered by the establishment of invasives in rare plant environments, with the increased competition reducing the ability of rare species to establish, survive, grow, and reproduce (Walck et al. 1999, Kaye 2001).

Oenothera wolfii's preferred habitat is not exempt from the threat of non-native invasive species. Many non-natives have found their way to the west coast, but one species, *Ammophila arenaria* (European beachgrass; Figure 12), stands out as having the most impact on this environment. Native to sandy coasts of the North, Baltic, Mediterranean, and Black Seas, *A. arenaria* was introduced along the northwest Pacific coast during highway stabilization projects in the 1930s. Since then it has spread rapidly, both naturally and by planting, along the entire northern Pacific coastline from Canada to Mexico (Wiedemann 1987). The result has been the development of a massive foredune system in the coastal dune areas that acts as a barrier to the inland movement of sand. This decreasing sand activity encourages the establishment of pioneer vegetation and eventually leads to an increase in the area covered by the regional climax forest (Wiedemann 1978). *Ammophila arenaria* outcompetes native vegetation in several ways. In addition to its extraordinary tolerance to sand burial, this species is also able to allocate a greater percentage of nitrogen to live blades (and less to tillers and roots) than many plant species, enabling it take up more of the available above ground space (Pavlik 1983). This exotic plant's habit of stabilizing dunes while establishing almost a monoculture has further reduced available habitat for *O. wolfii* (Gisler and Meinke 1997, Imper 1997).

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

Hybridization: The combination of human-caused habitat modifications and additional vectors for organism translocation has resulted in dramatic increases in the rates of plant hybridization and introgression worldwide. Once ecological barriers are disrupted by human activities and closely related species are brought into closer proximity, factors such as unspecialized pollinators and weak crossing barriers can promote hybridization (Levin et al. 1996). Whether or not introgression is also present, increased hybridization has directly and indirectly contributed to the extinction of populations and species of many plant and animal taxa (Sanders 1987, Liston et al. 1990, Salas-Pascual et al. 1993, Rhymer and Simberloff 1996, Allendorf et al. 2001). Direct effects include demographic swamping and genetic assimilation, as well as hybrid offspring competition with parents for resources and establishment microsites, and an increase in herbivore and pathogen pressures.

Allendorf et al. (2001) identify three types of hybridization. All three can either occur naturally or be anthropogenic in origin. It is the latter situation which is of most concern to conservationists. The first of type, hybridization without introgression, primarily produces first generation offspring (F1s) that are often sterile. In the cases where one of the parents is rare or threatened, the threat is not so much from genetic mixing, but rather the wasted reproductive effort on the part of that rare parent (as hybrid seed is produced at the expense of conspecific seed), which could eventually pose a demographic risk. In addition, some sterile hybrids spread through asexual reproduction, filling up microsites previously available to the parents (Levin et al. 1996). The second type of human-caused hybridization occurs when F1s are not sterile and backcross with one or both of the parent species. Widespread introgression may follow, creating the possibility of the third hybridization scenario: complete admixture of the species, essentially destroying parental populations. This genetic swamping of rare taxa may result in the functional extinction of “pure” populations of rare species (Levin et al. 1996). This last type of hybridization is difficult to stop, especially if the hybrids are fertile and mate with other hybrids, as well as parental individuals.

Hybridization may prove to be the most pressing threat to *O. wolfii* (Imper 1997). This rare species hybridizes with the common garden escapee *O. glazioviana* (Figure 13), and greenhouse experiments indicate that *O. wolfii* can also hybridize with other members of the genus (Wasmund and Stubbe 1986). *Oenothera glazioviana* originated in Europe, apparently as a stabilized hybrid between two North American species brought to Europe for ornamental purposes. This hybrid subsequently spread around the globe as a garden plant and weed, becoming naturalized on every continent except Antarctica. This weedy species is able to accept pollen from *O. wolfii* and produce viable offspring. Field surveys conducted by Imper (1997) noted that *O. wolfii* x *O. glazioviana* hybrids typically occurred near *O. glazioviana* populations, while *O. wolfii* populations tended to not co-occur with the other two species. This suggests that *O. wolfii* is less receptive to *O. glazioviana* pollen than *O. glazioviana* is receptive to *O. wolfii* pollen. However, *O. wolfii* is able to accept pollen from the *O. wolfii* x *O. glazioviana* hybrids, making it susceptible to introgression (Imper 1997).

In many cases, the detection of hybridization can be difficult, since not all hybrid individuals will display phenotypes which are intermediate to the parental individuals (Smith 1992, Schwarzbach et al. 2001). While some hybrids may have intermediate phenotypes, other hybrid morphologies may be very similar to those of one of the parent species. Morphological characters are not always reliable, and even when hybridization is certain, it is not always clear if the hybrid is a first generation (F1) individual, or the result of a backcross or later generation hybrid (Allendorf et al. 2001). However, more recent technologies provide additional tools for determining the hybrid status of individuals. Given sufficient variation in neutral, bi-parentally inherited genetic markers, statistical methods provide a way to determine whether a plant is a first generation hybrid, a second generation hybrid, or an introgressed individual resulting from backcrosses with either parental species (DeWoody and Hipkins 2004).

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

Although identifying hybrid offspring can be difficult, *O. wolfii* x *O. glazioviana* plants often display intermediate phenotypes (Figure 14). Morphological studies suggest that widespread hybridization with *O. glazioviana* has occurred throughout the California populations of *O. wolfii* (Carlson et al. 2001), and the genetic integrity of many of these populations is questionable (Imper 1997). These findings were later confirmed through the molecular work of DeWoody and Hipkins (2004). Currently, only one California population has avoided the residential development which has brought this species in contact with the garden escapee (Imper 1997). Although most of the Oregon populations are not threatened by residential development at this time, many of them are near major roadsides, placing them at risk of hybridization with *O. glazioviana* in the future.

Figure 14. Putative *Oenothera wolfii* x *O. glazioviana* population in California. (Photo courtesy of Oregon Department of Agriculture)

The mating system of these two species makes it likely that *O. wolfii* will be susceptible to genetic swamping by *O. glazioviana*. Cytogenetic studies show that approximately half of the mature pollen grains produced by *O. wolfii* are sterile, due to its structurally heterozygous genome maintained by balanced lethals (Wasmund and Stubbe 1986). Pollen exclusion experiments demonstrate that *O. wolfii* is self-compatible, producing the majority of seed by self-pollination (Carlson et al. 2001). In contrast, *O. glazioviana* is primarily an outcrosser (Imper 1997). This difference in available pollen and the resulting asymmetric pollen flow creates conditions favorable to hybridization where the two species occur sympatrically (DeWoody and Hipkins 2004).

Often the F1 offspring of interspecific hybridization events are highly vigorous, with enhanced pollen production and dispersal capabilities threatening the genetic integrity of the native plant populations (Anttila et al. 2000). *Oenothera glazioviana* x *O. wolfii* hybrids potentially fit this scenario, as they are very fertile, vigorous, and appear to be more aggressive than either parent (Imper 1997). However, the hybrids have slightly different habitat tolerances than *O. wolfii*. They are able to thrive in gravelly roadside soils, whereas *O. wolfii* prefers native, sandier soils. The hybrids are also less tolerant of salt (Imper 1997).

In general, conservation strategies for rare or threatened taxa should account for any hybridization potentially resulting from human actions (DeWoody and Hipkins 2004). In the case of *O. wolfii*, the effective conservation of this species will probably require that new *O. wolfii* populations be established in protected areas, away from highways and residential areas, using seed from uncompromised *O. wolfii* populations while they still exist.

Hypotheses and rationale

This study was designed to meet two related goals: develop a protocol for establishing new populations of *O. wolfii*, and subsequently establish two experimental populations of this species. Drayton and Primack (2000) recommend utilizing many sites, numerous transplants, and various methods in order to increase the rates of successful introduction for the species in question. Due to logistical constraints, only two sites were selected for this study. At each of the two transplant sites, the effects of rosette size, competing vegetation, and selected environmental variables were evaluated.

Creating new populations of rare species by direct seeding allows for the inclusion of a greater number of genotypes in new population with less expense and effort than required when cultivating and transplanting plants (Primack 1996). Direct seeding also fosters the selection of genotypes suited for the site at the seedling stage (Bowles et al. 1993). However, the vast majority of the literature supports using plants instead of seeds for introduction attempts (Mauder 1992, Bowles et al. 1993, Primack 1996, Drayton and Primack 2000, Lofflin and Kephart 2005). Germinating seeds and seedlings experience high vulnerability and high mortality (Guerrant 1996). When sowing seeds in a new site, frequently only small percentages of the seeds germinate and survive as seedlings (Mauder 1992). Because rare plant material is at a premium, harvesting the large numbers of seed necessary for creating a new population via direct sowing can harm the source population (Lofflin and Kephart 2005). Collecting a small amount of seed, propagating plants ex situ, then transplanting plants into the target site after they are past the vulnerable seedling stage makes better use of the rare plant material. The chances of the new population surviving, flowering, producing and dispersing seeds, and creating a second generation of plants are enhanced with the use of transplants (Primack 1996). Because cultivating multiple generations of greenhouse-grown plants may lead to artificial selection for traits favored in the greenhouse, first generation greenhouse plants should be used for introduction efforts (Alley and Affolter 2004).

The following six hypotheses are divided into two groups. The first group (Hypotheses 1-3) involves the effects of treatments and environmental factors on transplant survival and reproduction. The second group (Hypotheses 4-6) addresses questions regarding seed germination and the impacts of seed source and age on viability.

Hypothesis 1: The size of the transplant will affect its survival and reproduction.

Research has shown that typically, larger founder individuals are more successful, ultimately yielding the largest populations (Guerrant 1996) of newly established plants. In order to test the hypothesis that the age of the transplant will affect its survival and reproduction, two age classes of *O. wolfii* were transplanted at each site. Due to logistical issues, the ages of the transplants were slightly different at each site, although the difference between the two age classes within each site was the same. A small amount of seed was also sown at each site.

Hypothesis 2: The presence of ground cover within the transplant plot will affect the transplant's survival and reproduction.

Many rare species require specific microhabitats for seed germination and seedling establishment to occur (Primack 1996). However, Kruckeberg and Rabinowitz (1985) point out that species with the ability to survive in unusual habitats are not necessarily intolerant of more common environments; in some cases they are simply less able to compete with the increased levels of vegetation found in more hospitable conditions. Environments with high levels of disturbance, such as those found along the coast, may favor some plant species by limiting the competition for light and nutrients (Primack 1996). *Oenothera wolfii* may be one of these species.

This study evaluated the effect of competition on transplant success. Competition often negatively impacts establishment of introduced populations (Carlsen et al. 2000). Removal of vegetative competition increases seedling emergence, juvenile survival, and overall survival and reproduction (Morgan 1997), and the presence of neighboring vegetation can significantly reduce growth and reproduction of *Helianthus paradoxus* (Bush and Van Auken 1997). Failing to consider the effect of competing vegetation on transplant survival can reduce the efficiency of introduction projects (Mehrhoff 1996). Initially, removal of competing vegetation was to be a treatment at both study sites. However, because management conflicts prevented ground cover removal at Lost Lake, the presence of competing vegetation was evaluated as an environmental variable instead of an experimental treatment at this site.

Hypothesis 3: Environmental variables such as ground moisture, slope and heat load will affect the survival and reproduction of transplants.

Of all the criteria used in the selection of introduction sites, suitable habitat is the most critical to the success of newly created populations (Falk et al. 1996). Because many rare species have specialized habitat requirements (Rabinowitz et al. 1986), transplant success depends on appropriate microsite selection (Kohn and Lusby 2004, Maschinski et al. 2004). Mortality and reproductive failure are more likely to occur when plants are introduced into atypical habitat (Pavlik et al. 1993b). Observing occupied microsites helps to identify specific factors characteristic of suitable habitat. Soil texture and chemical composition, water regime, slope, exposure, community associates, availability of pollinators, habitat size, and degree of disturbance are important parameters to consider when selecting introduction sites (Pavlik et al. 1993b). In this study, several environmental variables (ground moisture, slope, aspect, heat load, and soil pH) were chosen to assess which microsite elements might affect transplant survival and reproduction.

Hypothesis 4: Treating seeds with a diluted bleach solution before germination will affect the germination rates.

Previous studies indicate that *O. wolfii* seed germinates easily. Reported germination rates range from 12% to nearly 100%, with higher rates of germination occurring when seeds were exposed to alternating temperature regimes (Imper 1997; Carlson et al. 2001; T. Kaye, unpublished data). According to accounts of previous germination efforts, no pretreatment of seed is necessary (Imper 1997). To test the hypothesis that fungal growth on the seeds might reduce germination in the greenhouse, half of the germinated seeds were rinsed with a diluted bleach solution prior to placement on germination paper.

Hypothesis 5: Source population will affect seed germination rates.

It is often useful to obtain information on life-history and demographic characteristics of naturally functioning populations so that a baseline of what is “normal” may be established (Sutter 1996). In order to determine whether there are differences in seed viability and germination rates between source populations, and to compare seed viability of experimental and wild populations, seeds collected at three comparable natural populations (Port Orford, Hubbard Creek and Pistol River) were germinated.

Hypothesis 6: Age of seeds will affect seed germination rates.

Little is known about the length of time during which *O. wolfii* seeds remain viable. In order to compare seed germination rates between seeds of different ages, seeds collected from the Port Orford natural population in the fall of 2002, 2003 and 2004 were germinated together in early 2005.

METHODS

Seed source selection

The chances of successful population establishment can be improved with careful selection of the founding plant material. Ideally, a well-designed founding population will survive and rapidly expand, eventually developing a complex and genetically diverse demographic structure of its own. The origin of the source material used to establish founding populations has become a topic of debate in the world of plant conservation (Kaye 2001). In general, however, many conservationists agree that several criteria should be considered when selecting a source population. Material for introduction should be obtained from a source population located as geographically close to the outplanting site as possible. In addition, the source population habitat should be ecologically similar to that of the outplanting site. These two factors may increase the chance of the sites sharing similar selective pressures (Guerrant 1996, Hufford and Mazer 2003), which may in turn give the introduced plants more of a “home site advantage” (Montalvo and Ellstrand 2000). Use of locally adapted seed reduces the risk of outbreeding depression from crosses between restored populations and neighboring natural populations, and lowers the threat of hybridization and introgression between ecotypes or subspecies (Kaye 2001). In order to increase the initial genetic diversity of the founding population, plant material used to create the founding population should be collected from relatively large populations, since they often have greater genetic diversity than smaller populations (Helenurm 1998). Finally, the source population should be large enough that removal of seeds or plants needed for introduction efforts do not reduce its viability.

The Port Orford *O. wolfii* population was selected as the source for seeds used to propagate plants for transplantation in this study. Port Orford is the farthest north of the known *O. wolfii* populations, and it is the closest, geographically, to this study's northern introduction site. Ecologically, the Port Orford habitat is fairly similar to that of the northern outplanting site, with open dunes colonized by European beach grass (*Ammophila arenaria*). Port Orford seeds were used for both introduction sites. Although a natural population of *O. wolfii* (Pistol River) is located within a mile of the southern introduction site, this population was determined to be too small to use as a source of introduction material.

Seed collection and storage

Oenothera wolfii seeds were collected from the Port Orford population in September of 2002, when capsules were dry and starting to dehisce (Figure 15). When the seeds used for cultivating transplants for new population establishment come from only a few sources, there is a risk of a founder effect (limited genetic variability in the founding population, which potentially decreases the population's ability to adapt to environmental changes in the future). Genetic drift, caused by small population size and fluctuations in abundance, can result in genetic bottlenecks, and the impacts can be severely negative (Friar et al. 2000, Hufford and Mazer 2003). In order to ensure that the genetic diversity of the Port Orford population was well represented, and reduce the risk of genetic bottlenecks, seed was collected from 75 randomly chosen parental plants located throughout the population. Seeds were stored in a dry, dark location in paper bags, at room temperature, during the time between collection and germination.

Figure 15. Collecting *Oenothera wolfii* seed from the Port Orford, Oregon population. (Photo by S. Gisler)

Seed germination

Germination protocol: In order to have rosettes of two different age classes for outplanting, seeds were germinated in two batches. The first batch was started on July 27, 2003, and the second batch was started on September 9, 2003. Seeds were placed on moistened germination paper in Petri dishes, 50 seeds to a dish (Figure 16). A total

Figure 16. *Oenothera wolfii* seed germination in Petri dishes in an OSU greenhouse. The blue filter paper (left) holds the unbleached seeds and the pink filter paper (right) holds the bleached seeds.

of 40 dishes (2000 seeds) were used in each batch. Seeds were germinated in a greenhouse located at Oregon State University (OSU), where they were exposed to 12 hours of light each day and subjected to an alternating night/day temperature regime (18.3/21.1°C). Seeds were sprayed with distilled water to prevent desiccation as needed, and they began to germinate within a week of placement in the Petri dishes. Once most of the germinated seeds had formed cotyledons (after approximately one week, Figure 17), they were planted in soil (see "Seedling Cultivation," below).

Bleach treatment: In each of the two seed germination batches (see "Germination protocol" section, above), half of the seeds were rinsed with a 5% bleach solution prior to placement on germination paper.

Seed viability tests: In order to estimate the percentage of viable seed for each reproducing transplant, fifty seeds from each plant were germinated in the greenhouse,

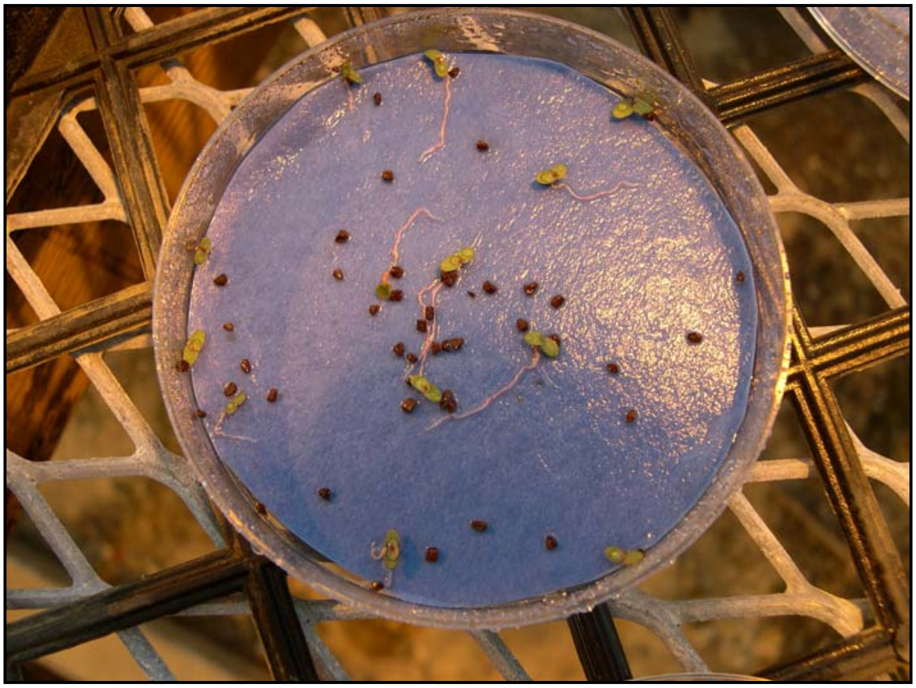

Figure 17. *Oenothera wolfii* seeds after one week on moistened filter paper.

following the seed germination protocol (without bleaching) established earlier (see "Germination protocol" section above). In addition, seeds 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.

Later in this study, seeds collected from mature plants in both introduced populations were germinated, along with seeds of several comparable natural populations, in order to assess seed viability of the individuals in the newly created populations (See "Seed Viability" section below). Germination methods were identical to those described above, although no bleach treatment was used.

Seedling cultivation

The newly emerged cotyledons and radicals of the germinated seeds are extremely fragile, so each germinated seed was carefully transported from its Petri dish by gently grasping the tip of one of the cotyledons with tweezers. These recently sprouted "seedlings" were planted in 5 cm x 5 cm x 6.25 cm deep cells filled with a 2/3 sand, 1/3 peat moss planting mixture (Figures 18 - 20). Cells were then placed under high pressure sodium lights in an OSU greenhouse facility and exposed to 12 hours of light/day. Daytime temperature was set at 21.1°C, and the temperature at night was set at 18.3°C. Seedlings were watered as needed, and fertilized every three weeks with diluted MiracleGro®.

Figure 18. Removing germinated *Oenothera wolfii* seeds from Petri dish.

Figure 19. Germinated *Oenothera wolfii* seeds being planted in peat moss/sand planting medium.

Figure 20. *Oenothera wolfii* seedlings 14 days after being planted in 5 cm cells.

Seedlings were transplanted into 10 cm x 10 cm x 15 cm deep pots after 34 days, using the same sand/peat moss/potting soil mixture as was previously (Figure 21). After plants had been growing for six weeks, white flies infested the larger plants in the greenhouse. Greenhouse staff treated the infestation with the insecticide Duraplex TR. The white fly infestation did not cause visible harm to the *O. wolfii* plants.

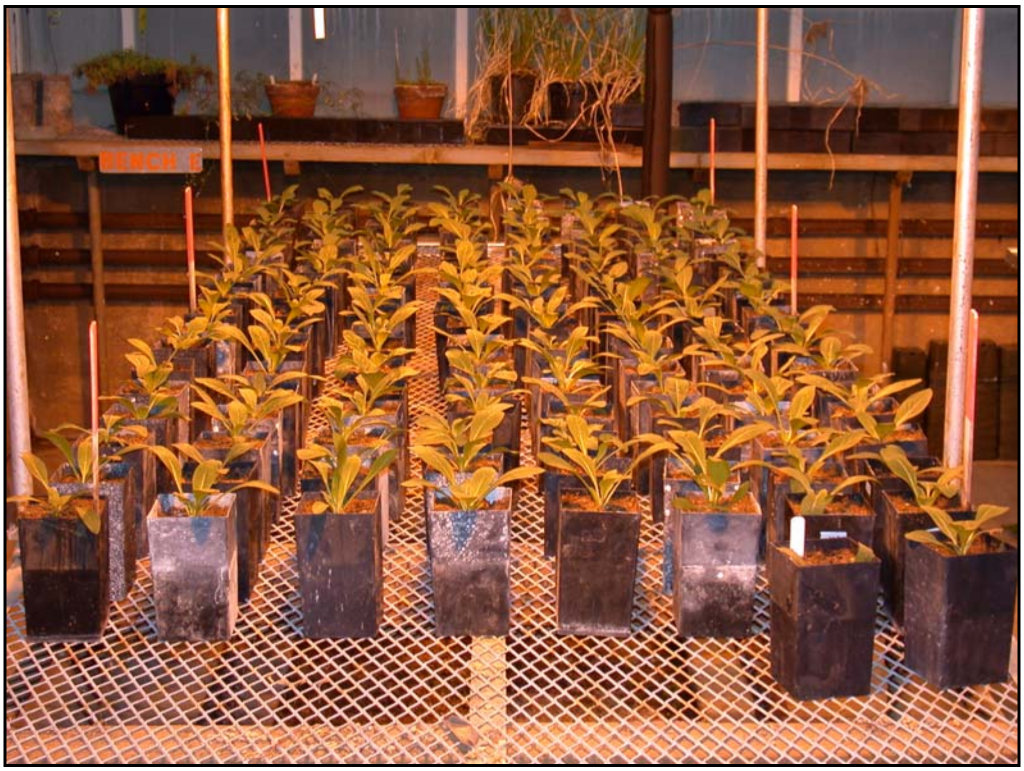

Figure 21. 34-day-old *Oenothera wolfii* seedlings transplanted into 10 cm x 10 cm x 15 cm pots.

When plants are moved from the greenhouse to the outdoors, there is a risk of transplant shock due to the abrupt change in environment. The transplant stress can cause increased mortality rates, especially if the transplants encounter additional stressors (such as unexpected drought) once they are transplanted outside (Drayton and Primack 2000). In order to reduce the environmental change experienced by transplanted *O. wolfii* rosettes, the plants were "hardened off" by turning off the heat in the greenhouse two weeks before transplanting them to sites at the coast.

Development of site selection criteria

Both logistic and biological criteria must be considered when selecting sites for the introduction of rare plants (Fiedler and Laven 1996). Logistically, newly created populations of rare plants have the most permanent conservation value when they are located on land that is administratively protected and managed for the benefit of the introduced species and its habitat (Falk and Olwell 1992). Introduction sites must be accessible to researchers transporting plant material and equipment to the location and monitoring the populations over time. At the same time, ideal sites are not subject to foot or vehicular traffic, and they are not located adjacent to commercial, residential, or agricultural development (Pavlik et al. 1993b). Choosing introduction sites that are removed from human impacts helps prevent human-caused damage to the plants and their habitat. This isolation also serves to separate the newly created population from potentially cross-compatible congeners that associate with disturbance, thereby avoiding undesired genetic exchange (Levin et al. 1996).

Because it is difficult to identify the specific environmental factors that either allow or prevent the establishment of new populations, probability of success is increased when multiple sites are selected for experimental introduction (Drayton and Primack 2000). Creating multiple new populations of rare species, and managing these populations as a metapopulation, provides a buffer against extirpation caused by random catastrophic events (Guerrant 1996). Surviving populations can serve as seed sources to reestablish new colonies in vacated sites (Menges 1991, Bowles and McBride 1996). A larger number of populations potentially leads to more genetic differentiation among sites, increasing the overall heritable diversity of the species and providing greater opportunities for evolution in response to varying selective pressures (Huenneke 1991). In addition, if multiple sites are planned for introduction attempts, Kutner and Morse (1996) suggest that several sites be located slightly outside of the species' current or historical distribution. Experimental locations located a little more poleward or at slightly higher elevations than the historical range of the plant provide an extra margin for survival for taxa which are potentially vulnerable to climate change.

Oenothera wolfii has been transplanted successfully in several locations. In 1989, plants located near Trinidad in Humboldt County, California were transplanted to mitigate a beach access improvement project's negative impacts to that population (Imper 1997). All 13 of the transplanted individuals survived initially, and roughly 150 seedlings resulted from directly sown seed. After seven years of monitoring, the newly created population remained extant, although at very low numbers. In another mitigation situation, individuals of the Port Orford *O. wolfii* population were transplanted from one area of the foredune to another during habitat restoration efforts for *Abronia umbellata* ssp. *breviflora*. Many of these transplants survived and reproduced after transplantation (T. Kaye, unpublished data).

All of these recommendations were considered when developing site selection criteria for the introduction of *O. wolfii* in this study. Locations should be close to or within the current range of *O. wolfii*. Ideally, they would be isolated from roads in order to reduce foot or vehicular traffic and minimize the potential threat of hybridization with *O. glazioviana*. The habitat should be ecologically similar to that of natural populations. Finally, for long-term monitoring and protection, the sites should be located on land managed by a public agency.

Site descriptions

In order to assess transplant survival over a broad range of environmental variables, and ultimately to increase the number of and connectivity between populations of *O. wolfii*, two sites were selected for introduction. The northern site, Lost Lake (Figure 22), is part of the New River Area of Critical Environmental Concern (ACEC), within the Bureau of Land Management's Coos Bay District. Covering 0.29 km^2, the Lost Lake area is located approximately eight kilometers south of Bandon (see Appendix 1 for map). Situated about 1.5 km inland, it abuts a larger area of State Park land. Habitat consists of inland dunes and *Pinus contorta* woods. The property is rarely visited; there are no signs advertising its presence, and the site is accessed by either a private gravel road or a dirt hiking trail. Because the site does not have paved road access and is located several kilometers from Highway 101, it is unlikely to be exposed to the threat of hybridization. The habitat appears to be compatible with *O. wolfii* needs. Open dunes are partially colonized by native vegetation, as well as European beachgrass. The substrate is primarily sand, and the areas where transplants are located receive full sun throughout the day. Lost Lake is located approximately 32 km north of *O. wolfii*'s current range.

Figure 22. *Oenothera wolfii* plots at Lost Lake site.

The southern site, Meyers Creek, is located on a hillside directly above Highway 101, approximately 13.5 km south of Gold Beach (Figure 23). Managed by the Oregon

Department of Transportation (ODOT), the site is about one kilometer from the Pistol River natural population of *O. wolfii* (see Appendix 2 for map). The habitat at this site is almost identical to that of the existing population at Pistol River; the steepness of the slope varies but primarily faces westward, the site is close enough to the ocean to be subjected to winds and spray, the vegetation cover is almost 100%, and the substrate contains more organic matter than that of the Lost Lake site (and that of the seed source site, Port Orford, as well). ODOT currently manages the right-of-way for weeds and vegetation which might block traffic views, and is supportive of the project. The transplant plots are located above the area typically impacted by roadside maintenance. Because this site is in close proximity to Highway 101, transplants could potentially be affected by disturbance from ODOT workers and highway travelers, as well as having an increased risk of hybridization exposure.

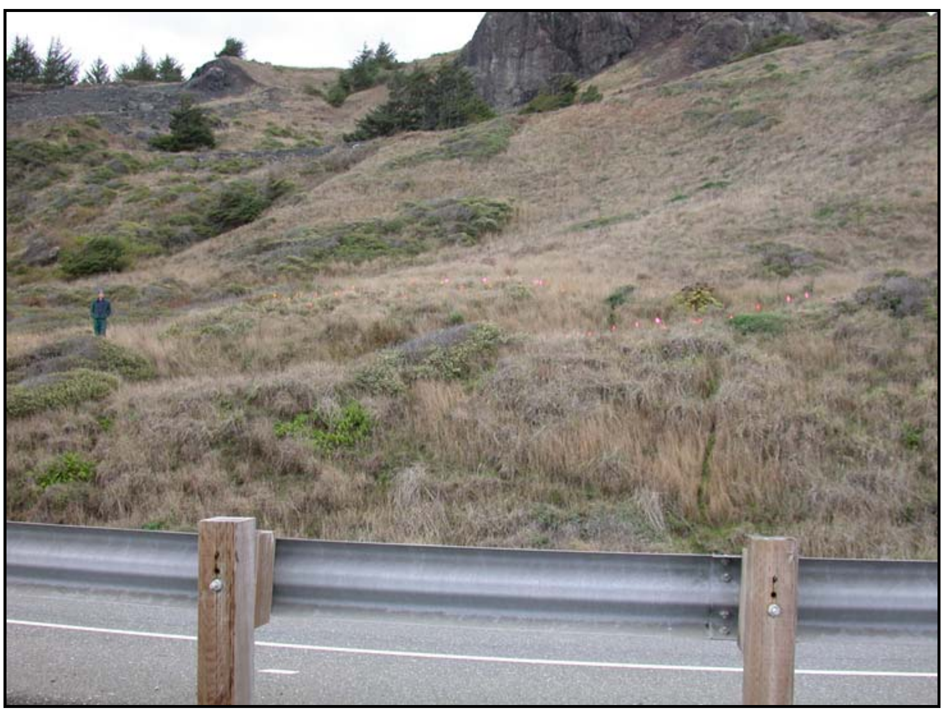

Figure 23. Meyers Creek site, viewed from across Highway 101. Pink flags indicated location of research plots.

Experimental population outplanting: Lost Lake

Forty plants 39 days old and 40 plants 73 days old were planted at the Lost Lake site in late October, 2003 (see Appendix 3 for research plot map). At the time of planting, older rosettes were approximately 30 cm in diameter and small rosettes were approximately 14 cm in diameter. 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 the roots, and plants were placed in plastic bags with a damp paper towel. Bagged plants were transported to Lost Lake in large coolers with ice (Figures 24 and 25). To evaluate potential effects of bagging the plants, an additional ten large plants were transplanted to the site while still in their pots. Upon arrival at the site, rosette types were randomly assigned to treatment plots, with one plant located in each plot. Plots were marked with 60 cm wooden stakes at two corners (Figure 26).

In addition to evaluating the impact of rosette size on survival and reproduction, we also analyzed the impact of competing vegetation on the establishment of the new plants. The Lost Lake site had little or no ground cover in the areas chosen for introduction of *O. wolfii*. Because BLM staff were concerned about the possible effects of ground cover removal, this treatment was not included at the Lost Lake site. Instead plots were selected to include a range of percent cover classes. Fifty percent of the plots (45 plots) had no ground cover in them, and 50% of the plots were selected to span the following categories of percent ground cover: 11 plots with 1-25%, 12 plots with 26-50%, 16 plots with 51-75%, and six plots with 76-100% (Figures 26-29). (Note: It was very difficult to find plots in the 76-100% ground cover category, which resulted in an uneven distribution of ground cover classes.)

Figure 24. Large rosettes being removed from pots for transportation to outplanting site.

Figure 25. Large rosettes were removed from pots and transported to Lost Lake in plastic bags placed in coolers.

Figure 26. Example of Lost Lake plot with 1-25% ground cover.

Figure 27. Example of Lost Lake plot with 26-50% ground cover.

Figure 28. Example of Lost Lake plot with 51-75% ground cover.

Figure 29. Example of Lost Lake plot with 76-100% ground cover.

At the time of outplanting, the fall rains had not yet begun. Introduction efforts involving another coastal dune species, *Abronia umbellata* ssp. *breviflora*, showed that transplant success was affected by the amount of moisture available to plants at the time of transplanting (Kaye 2003). To improve the chances of survival for *O. wolfii* transplants, each rosette received one liter of water (either tap or bottled) at the time of outplanting (Figure 30). An additional liter of water was provided to each of the plots three weeks later.

Figure 30. Lost Lake transplants receiving one liter of water at the time of outplanting.

In addition to the 90 transplant plug plots established at Lost Lake, 10 seed plots were also created, with 200 seeds sown in each plot. In five of the seed plots, the seeds were buried approximately 0.5 cm below the sand (Figure 31). In the other five 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, and again three weeks later. Seed plots contained no vegetative cover.

Figure 31. Lost Lake seed plot, with seeds buried 0.5 cm.

Experimental population outplanting: Meyers Creek

Due to logistical delays, plants at the Meyers Creek site were older than those at Lost Lake 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, 2003 (three weeks after the Lost Lake planting). Older plants were 94 days old, and younger plants were 60 days old. The type of rosette (younger vs. older) was randomly assigned to each plot.

Unlike the site at Lost Lake, the Meyers Creek site is essentially completely covered with shrubs, forbs and graminoids. Fifty percent of the half-meter-squared plots were randomly assigned the ground cover removal treatment at the time of outplanting (Figure 32). This treatment was implemented by removing vegetation by hand with a polaski. Fifty percent each of the large and small rosettes to plots were randomly assigned to plots with vegetation removed, and 50% of each rosette size were planted in plots with existing vegetation untouched. A liter of water was given to each of the rosettes at the time of planting.

Figure 32. Meyers Creek half-meter^2 plot with small rosette and ground cover removed.

Ten seed plots were also established at Meyers Creek, with 200 seeds sown per plot. Five of the plots were randomly chosen for vegetation removal. Seed plots were sprinkled with a liter of water at the time of sowing.

Environmental factors

To determine the 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 was treated as an environmental factor, rather than a treatment.

***Ground moisture*:** Soil moisture can be an important factor in transplant survival (Morgan 1997) and seedling establishment (Maschinski et al. 2004). To determine the relationship between soil moisture levels and the survival and reproductive success of individuals in the experimental populations of *O. wolfii*, volumetric water content was measured to 30 cm deep for each plot in March, June, and September of 2004, using a Hydrosense© water meter (Campbell Scientific, Inc. 2001; Figure 33). On each date, three measurements were obtained for each plot. These three numbers were 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 (Figure 34). 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).

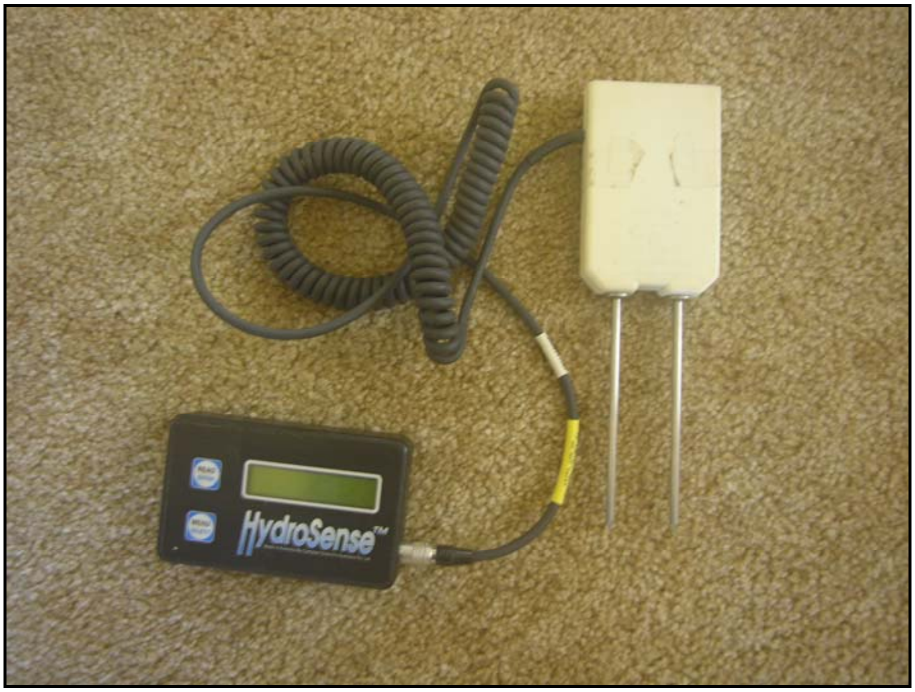

Figure 33. Hydrosense© ground moisture meter with 12" sensors.

Figure 34. Estimating slope for a plot at Meyers Creek.

***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 June 11, 2004). Soil samples were collected at three locations at each site (each sample consisting of one trowel scoop of soil taken from a depth of approximately 10 cm), and submitted to the Oregon State University Department of Crop and Soil Science's Central Analytical Laboratory for pH analysis.

Monitoring

Monitoring is essential for any introduction project. It is only by determining the abundance, resilience, and persistence of the introduced population that success can be evaluated (Sutter 1996). Both *O. wolfii* introduction sites were visited monthly for 18 months following outplanting. Individual plants were monitored for survival, size, fecundity, and seedling recruitment. Although the majority of seeds were expected to fall within close vicinity of their parents (Howe and Smallwood 1982), the entire introduction area was surveyed for seedling recruitment each visit. Additional observations, such as herbivory and other evidence of disturbance, were recorded. Photographs were taken of each plant throughout the monitoring period, and ground moisture was measured quarterly for the first year.

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 plant size and reproduction data were recorded (at both sites) September 8-12, 2005. For reproducing individuals, plant size was determined by measuring the height of the tallest branch and the number of branches. For vegetative individuals, plant size was determined by measuring the diameter of the rosette at its widest point. When more than one rosette was present, 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) on each reproductive plant, for a total of three fruits per plant. Seeds from these three fruits were combined, counted, and divided by three to obtain an estimated average number of seeds per fruit for each plant. Due to the small size of *O. wolfii* seeds, seeds were weighed as a group in order to determine the average weight of each seed.

Analysis

Statistical analysis of the data was conducted utilizing the program S-PLUS®, version 6.2. The effects of the treatments (transplant size and ground cover removal) and environmental variables (ground moisture, slope, heat load and ground cover status) on transplant survival and reproduction were analyzed using logistic regression. (Note: Because no small transplants reproduced at Lost Lake, we were unable to assess the effect of transplant size on reproduction at this site using logistic regression. Instead, a chi-squared test was used for this analysis.) The effects of the treatments and environmental variables on transplant plant size and reproductive vigor response variables (plant height and diameter, number of fruits and seeds, seed weight and germination rates, etc.) were analyzed using linear regression. The effect of bleaching seeds before germination was analyzed using a two-sampled t-test. Differences in seed germination rates between the three natural populations (Port Orford, Hubbard Creek, and Pistol River) were analyzed with a series of two-sampled t-tests. Linear regression was used to assess the effect of seed age on germination rates.

Transplants at Lost Lake and Meyers Creek were planted at different times, resulting in transplants of different ages (Meyers Creek plants were three weeks older). Additionally, the environmental factors at the two sites were different. Consequently, data from Meyers Creek and Lost Lake were analyzed separately, as two 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% to 59% (Table 1). Bleaching the seeds to reduce fungal growth had mixed results. In the first trial, bleached seeds germinated at a significantly higher rate than those that were not bleached (Table 1). However, in the second trial there was no statistically significant difference between the bleached and unbleached seed germination rates (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 seeds. In Trial 1, there was a statistically significant difference between the germination rates of bleached and unbleached seeds. Trial 2 did not show a statistically significant difference between the two treatments.

	Trial 1		**Trial 2**	
Treatment	**Bleached**	**Unbleached**	**Bleached**	**Unbleached**
Mean # seeds germinated (out of 50 per Petri dish)	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

Oenothera wolfii plants were not difficult to cultivate in the greenhouse. Almost 100% of the transplanted seedlings 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.

Environmental factors

Soil pH: At Lost Lake, pH values ranged from 5.6 to 5.8 (Table 2), while at Meyers Creek the range of pH was slightly higher (5.8-6.2).

Ground moisture: Overall, the soil at Lost Lake consistently held less water than the soil at Meyers Creek (Table 2). In March, ground moisture levels ranged from 0-5% at Lost Lake, while they ranged from 5-68% at Meyers Creek. In June, Lost Lake's ground moisture levels remained about the same (3-5%), and Meyers Creek ground moisture levels ranged from 5-89%. In September, the ground moisture levels at Lost Lake were the same as those in June (3-5%), and Meyers Creek ground moisture levels ranged from 6-43%.

Slope: The slope of the plots located at the Lost Lake site ranged from completely flat (0°) to 20° (Table 2). Meyers Creek had a broader range of slope (0°-33°).

Aspect: Due to the uneven terrain at both sites, plot slopes could face any direction. The aspect (with slope and latitude) was used to calculate a heat load value (Table 2).

Table 2. Range of values for environmental factor data for Lost Lake and Meyers Creek.

Environmental Factor	Site	Range
pH	Lost Lake	5.6-5.8
	Meyers Creek	5.8-6.2
March ground water percentage	Lost Lake	0-5%
	Meyers Creek	4-68%
June ground water percentage	Lost Lake	3-5%
	Meyers Creek	5-89%
September ground water percentage	Lost Lake	3-5%
	Meyers Creek	6-43%
Slope	Lost Lake	0-20°
	Meyers Creek	0-33°
Heat load	Lost Lake	0.742-1.008
	Meyers Creek	0.726-1.024

Transplant survival

Transplant size: Overall, transplant survival one year after outplanting was high at both experimental population sites. At Lost Lake, 80 (89%) of the transplants (small, big bagged, big pots) survived. Because the big plants that were bagged for transporting survived, the ten big plants that were transported in pots (controls in the event that bagged plants all died) were not included in most of the statistical analyses. At Meyers Creek, only one small plant died after transplanting, giving an overall survival rate of 99% (Table 3). Overall survival rates differed significantly between sites (logistic regression, 2-sided p-value = 0.03).

At Lost Lake, 33 small transplants (83%) and 37 of the large transplants (93%) survived (Table 3, Figure 35). However, rosette size at the time of outplanting did not significantly affect transplant survival rates (logistic regression, 2-sided p-value = 0.187). At Meyers Creek, all 40 of the large transplants survived (100%) and 39 of the small transplants survived (98%). Once again, there was no statistical difference between the survival rate of the two transplant sizes (Table 3, Figure 35).

Table 3. Transplant survival of different sized rosettes at Lost Lake and Meyers Creek after one year. The difference in transplant survival between big and small transplants was not statistically significant (see text).

	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

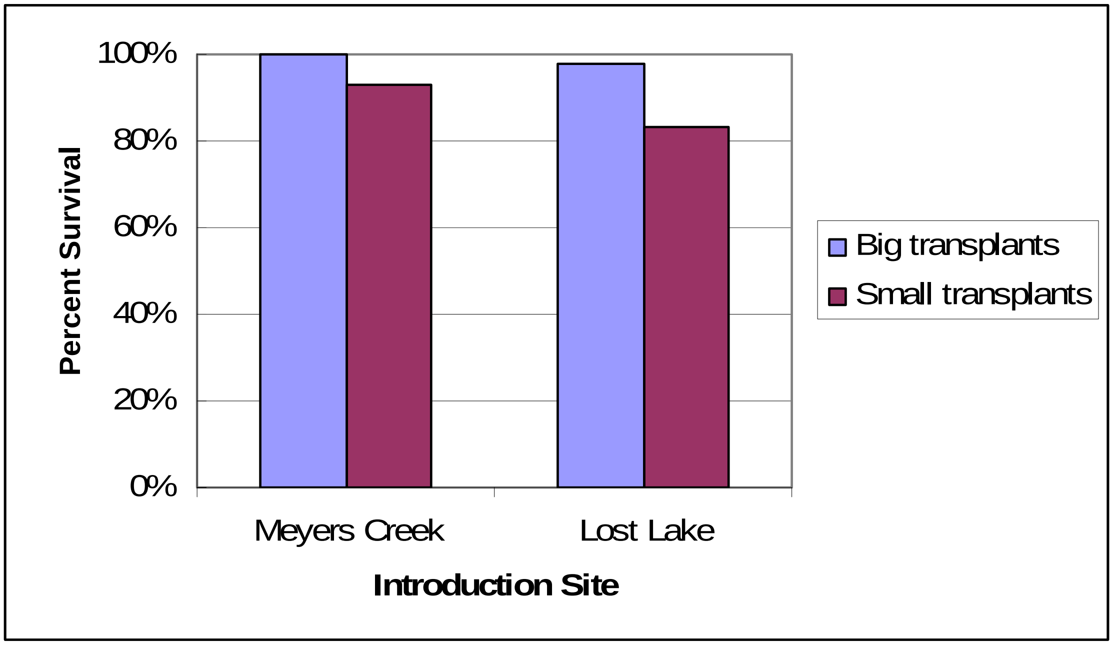

Figure 35. Transplant survival of big and small plants at Lost Lake and Meyers Creek after one year. Differences were not statistically significant (see text).

Ground cover: Ground cover presence did not affect plant survival rates either. At Lost Lake, 39 transplants (87%) located in bare plots with no ground cover survived, and 41 transplants (91%) located in plots with groundcover survived. There was no statistical difference in survival rates of transplants in plots of different ground cover classes (logistic regression, 2-sided p-value = 0.441). At Meyers Creek, the one plant that did not survive was in a plot where the ground cover was not removed, and the survival rates based on ground cover status did not differ significantly (Table 4, Figure 36).

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 (logistic regression, all 2-sided p-values > 0.05; Table 7).

Table 4. Transplant survival in plots with different ground cover status at Lost Lake and Meyers Creek after one year. The differences in transplant survival between ground cover classes were not statistically significant (see text).

		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

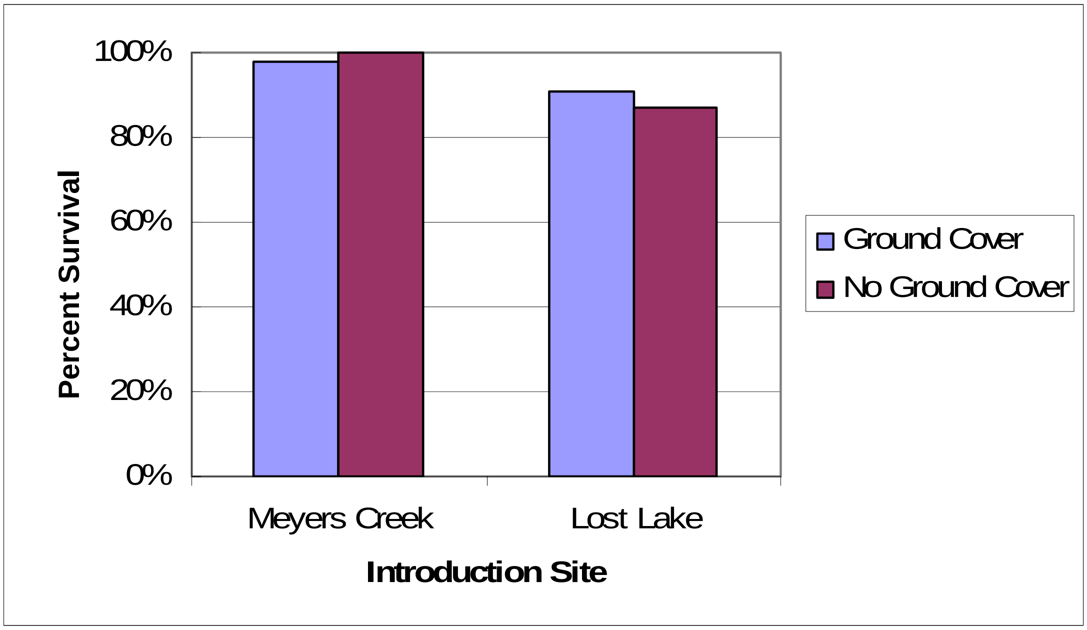

Figure 36. Transplant survival for plots with and without ground cover after one year. The differences in transplant survival between ground cover classes were not statistically significant (see text).

Transplant reproduction

Transplant size: At Lost Lake, transplant size at time of transplanting significantly impacted plant reproduction in the first growing season (chi-squared test, 2-sided p-value < 0.0005). Fourteen plants (18% of surviving plants) reproduced in the first growing season after transplanting at this site. All of the reproducing plants were large transplants; 11 large bagged plants (30% of surviving large bagged plants) and three large potted plants (30% of surviving large potted plants) produced flowering stalks and set fruit (Table 5, Figure 37).

At Meyers Creek, transplant size at time of transplanting significantly impacted plant reproduction in the first growing season (logistic regression, 2-sided p-value = 0.006). A total of 46 plants (58% of surviving plants) reproduced by September 2004 (Table 5, Figure 37). Thirty-one of these were large transplants (78% of the surviving large plants) and 15 were small (38% of the surviving small plants). The odds of a large transplant reproducing were 2.3 times the odds of a small transplant reproducing (95% confidence interval: 1.4-3.8).

Table 5. Reproduction of big and small transplants at Lost Lake and Meyers Creek after one year. The difference in reproduction was statistically significant for both sites (see text).

	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

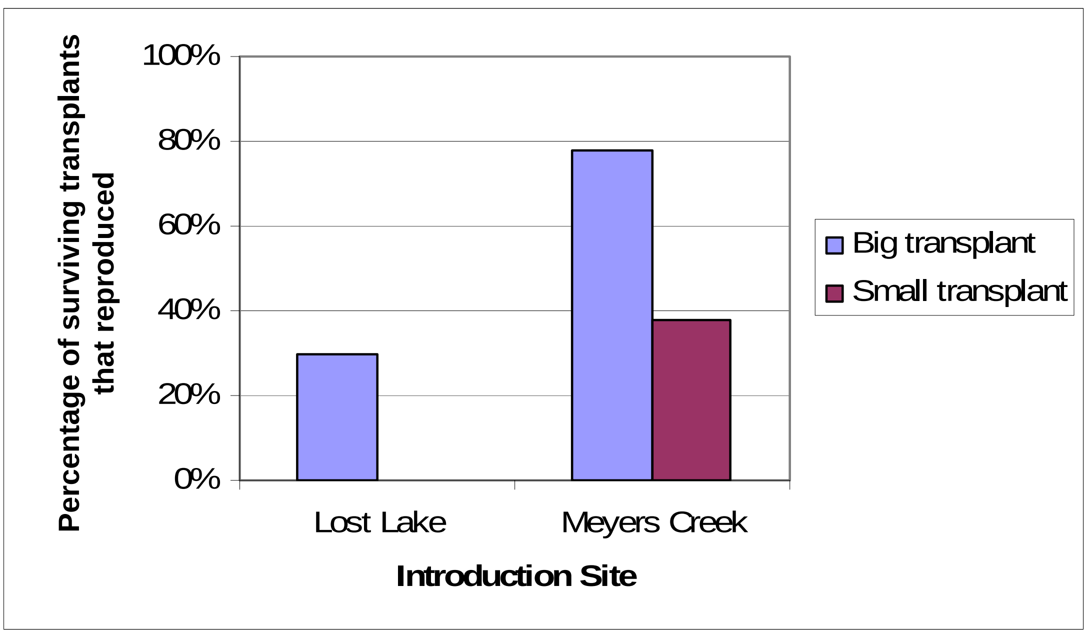

Figure 37. Reproduction of big (excluding those transported in pots) and small transplants at Lost Lake and Meyers Creek after one year. These differences were statistically significant (see text).

Ground cover: Ground cover presence did not significantly impact plant reproduction at the Lost Lake site (logistic regression, 2-sided p-value = 0.810). In plots with no ground cover, six (15% of the surviving plants) of the transplants reproduced in the first year. In plots with ground cover, eight transplants (20% of surviving plants) reproduced in the first year (Table 6, Figure 38). These eight plants were in plots located in three of the four ground cover classes (1-25%, 26-50%, 51-75%). No plants in plots with 76-100% ground cover reproduced in the first year.

In Meyers Creek plots with ground cover removed, 31 of the transplants (78% of surviving plants) reproduced in the first year (Table 6, Figure 38). In plots where ground cover remained (at essentially 100%), only 15 plants (38% of surviving plants) reproduced in the first year. This difference was statistically significant (logistic regression, 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).

Environmental factors: At Lost Lake, several of the environmental factors assessed (June ground moisture percentage, slope, and heat load) were associated with transplant reproduction in the first year (linear regression; slope 2-sided p-value = 0.0028, June moisture 2-sided p-value = 0.0088, heat load 2-sided p-value = 0.018; Table 7). At Meyers Creek, evaluation of the environmental influences did not produce as clear a picture. Both March and September ground moisture levels appeared to be associated with plant reproduction, but the association was reversed, with March ground moisture levels positively related to reproduction (logistic regression, 2-sided p-value = 0.0058) and September ground moisture levels negatively associated with reproduction (logistic regression, 2-sided p-value = 0.022). The rest of the environmental factors measured were not significantly associated with transplant reproduction in the first year (Table 7).

Table 6. Reproduction of transplants in plots with different ground cover percentages at Lost Lake, and reproduction of Meyers Creek transplants in plots with ground cover removed vs. not removed. Differences were not statistically significant at Lost Lake. *The difference in reproduction between plants in plots with and without ground cover was statistically significant at Meyers Creek (2-sided p-value = 0.001, see text).

		Reproduced	**Did not 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

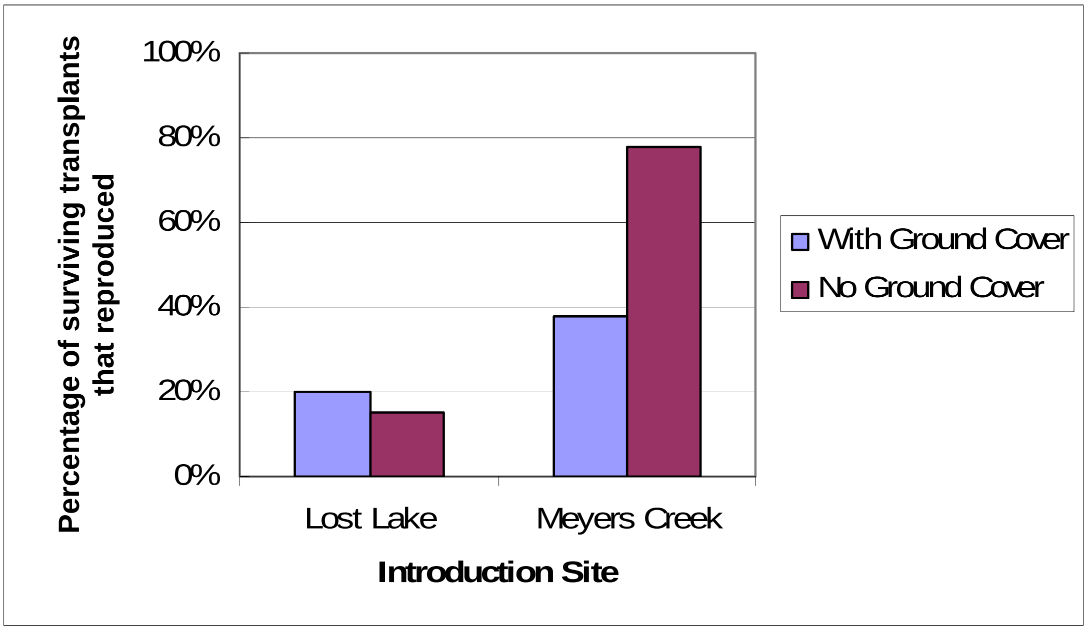

Figure 38. Reproduction of transplants in plots with ground cover (of any class, from 1-100%) vs. those in plots with no (0%) ground cover. Differences were statistically significant at Meyers Creek, but not at Lost Lake (see text).

Table 7. Environmental variables significantly associated with transplant reproductive vigor variables. Shaded areas indicate statistical significance. Signs (+/-) indicate the direction of the association.

		Sept H_2O	March H_2O	June H_2O	Slope	Heat Load
Reproduction	**Lost Lake**			-	+	+
	Meyers Creek	-	+			
Fruits/Plant	**Lost Lake**					
	Meyers Creek					+
Seed Weight	**Lost Lake**				+	
	Meyers Creek		+			
Height	**Lost Lake**			-	-	
	Meyers Creek		-			+
Seed Germination	**Lost Lake**					
	Meyers Creek		-			

Transplant reproductive vigor

In addition to collecting data on plant survival and reproduction, a variety of reproductive vigor measurements (flowering stalk height, number of branches, number of fruits, number of seeds per fruit, average seed weight, and germination rates) were recorded for each reproducing plant.

Transplant size: The impacts of transplant size on reproductive vigor are summarized in Table 6. Because no small plants reproduced at Lost Lake, no analysis of transplant size impacts on these variables could be performed. However, three out of the 14 large plants that reproduced were transplanted from pots, and the remaining nine had been bagged when transported. Although the numbers of individuals are too small to draw statistical conclusions, the three potted large plants tended to perform better in almost all reproductive vigor categories (seed germination rates were slightly lower; Table 8).

At Meyers Creek, the number of fruits produced by a transplant in the first year was significantly and positively impacted by the size of the transplant. Large reproductive transplants produced an average of 76.5 fruits, and small reproductive transplants produced an average of 45.7 fruits (linear regression, 2-sided p-value = 0.003, 95% confidence interval of difference in number of fruits: 11-39). Overall, a trend of larger transplants being more reproductively vigorous was observed; however, these differences were not statistically significant (see Table 8).

Ground cover status: At Lost Lake, ground cover presence was significantly and positively related to the number of seeds per fruit (2-sided p-value = 0.046; Table 9). Transplants in plots with ground cover produced an average of 69 more seeds per fruit than those in plots without ground cover (95% confidence interval: 18-120). Ground cover presence did not affect any other reproductive vigor measurements at this site.

Table 8. Comparison of plant size and reproductive success among different transplant sizes and sites. Numbers in parentheses are standard errors. Numbers which are bold with an asterisk indicate statistically significant differences.

		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)

Table 9. Reproductive success of transplants in plots with different ground cover status. 50% of Meyers Creek plots had ground cover removed as a treatment, and 50% of the plots were left with ground cover intact (at essentially 100%). At Lost Lake, 50% of the plots were located in areas with no ground cover and 50% were located in areas with a range of ground cover classes (1-100%). Numbers in parentheses are standard errors. Bold numbers with asterisks indicate statistically significant differences.

		LOST LAKE			**MEYERS CREEK**		
		With Ground Cover	**Without Ground Cover**	**Total**	**Ground Cover Left**	**Ground Cover Removed**	**Total**
Reproducing plants:	**Average # Branches/ Plant**	1.4 (0.4)	1.5 (0.3)	1.4 (0.3)	2.2 (0.4)	3.1 (0.5)	2.8 (0.4)
	Average # Fruits/Plant	11.4 (1.6)	12.2 (2.7)	11.7 (1.4)	**41.3* (6.6)**	**78.7* (10.5)**	66.5 (7.8)
	Average # Seeds/Fruit	**217.7* (18.3)**	**148.3* (16.0)**	185.7 (15.5)	290.2 (17.3)	276.0 (13.1)	280.6 (10.4)
	Average Weight/ Seed (mg)	0.295 (0.010)	0.324 (0.020)	0.308 (0.011)	0.330 (0.015)	0.342 (0.012)	0.338 (0.009)
	Average % Seed Germination	44.9 (7.7)	43.3 (7.8)	44.2 (5.3)	48.0 (5.2)	40.1 (3.0)	42.7 (2.7)
	Average Height (cm)	35.2 (2.4)	32.2 (2.5)	33.9 (2.3)	61.3 (4.1)	67.5 (2.9)	65.5 (2.4)
Non-reproducing plants:	**Average diameter (cm)**	12.2 (0.7)	12.0 (0.8)	12.1 (0.6)	28.2 (1.9)	28.5 (3.8)	28.3 (1.7)

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, on average, 37 more fruits (95% confidence interval: 23-51). Interestingly, we observed a trend of plants in plots which did not have ground cover removed producing slightly more seeds per fruit (an average of 290 seeds/fruit, as opposed to 276 in plots where ground cover was removed), and these seeds germinating at slightly higher rates (48% vs. 40%) than the seeds from plants in plots with ground cover removed. However, these differences were not statistically significant. Ground cover did not affect any other reproductive vigor measurements at Meyers Creek (see Table 9).

Additional environmental factors: For the most part, environmental variables were not associated in a statistically significant manner with transplant performance variables at Lost Lake. However, there were several exceptions to this generalization (Table 7). (Note: all p-values result from linear regression analysis, unless otherwise stated.) Slope was significantly and positively associated with transplant reproduction (logistic regression, 2-sided p-value = 0.003), seed weight (2-sided p-value = 0.039), and negatively associated with reproductive plant height (2-sided p-value < 0.001). Heat load was significantly and positively associated with reproduction (logistic regression, 2-sided p-value = 0.018). June ground moisture levels were significantly and negatively associated with reproduction (logistic regression, 2-sided p-value = 0.009) and reproductive plant height (2-sided p-value = 0.014). Finally, the interaction between slope and heat load was significantly associated with reproductive plant height (2-sided p-value < 0.001).

At Meyers Creek, the influences of environmental factors on reproductive attributes were generally not apparent as well. Once again, there were several exceptions (Table 7). Heat load was significantly and positively associated with the number of fruits/plant (2-sided p-value = 0.013) and negatively associated with reproductive plant height (2-sided p-value = 0.003). March ground moisture levels were positively associated with reproduction (logistic regression, 2-sided p-value <0.001) and average seed weight (2-sided p-value = 0.035), and negatively associated with reproductive plant height (2-sided p-value = 0.005) and seed germination rates (2-sided p-value = 0.004). September ground moisture levels were negatively associated with reproduction (logistic regression, 2-sided p-value = 0.001).

Seedling recruitment

In order for an introduced population of a rare plant to be considered viable, especially when the species is annual or biennial, transplants must survive and reproduce, and the recruitment of new individuals from the seeds produced by transplants must occur (Kaye 2003). Eventually the population must become self-sustaining, with new recruitment balancing any mortality that occurs. Unfortunately, the duration of this study did not allow for a thorough assessment of seedling recruitment. As of September of 2005 (two years after outplanting, one year after some transplants had flowered and set seed), no seedlings were found in or near the transplant plots.

Seed viability

Seed source: On average, 48.3% of the seeds collected from Port Orford *O. wolfii* plants germinated, as opposed to average germination rates of 23.5% for Hubbard Creek seeds and 46.9% for Pistol River seeds (Figure 39). The difference between average seed germination rates for the Port Orford and Pistol River populations was not statistically significant (two-sample t-test, 2-sided p-value = 0.841). There was a statistically significant difference between the Hubbard Creek population's average seed germination rate and the rates for the two other sites (two-sample t-test, Port Orford 2-sided p-value < 0.001, Pistol River 2-sided p-value < 0.001).

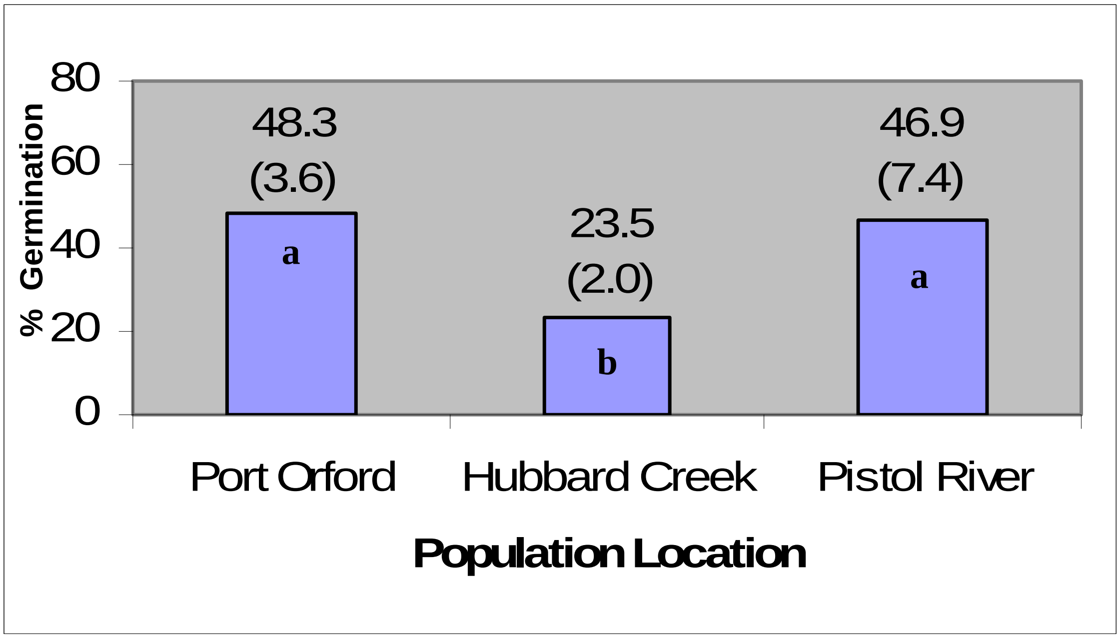

Figure 39. Comparison of germination rates for *Oenothera wolfii* seeds collected from the Port Orford, Hubbard Creek, and Pistol River populations. The difference between Port Orford and Pistol River seed germination rates was not statistically significant (a), but Hubbard Creek seeds (b) germinated at a statistically significant lower rate than those of the other two populations.

Seed age: An average of 48.5% of the 3-year-old (collected from the Port Orford population in 2002) *Oenothera wolfii* seed germinated in 2005 (Figure 40). Two-year-old seed (collected in 2003) germinated at an average rate of 54.9%, and on average, 48.3% of the seed stored for one year (collected in 2004) germinated in 2005. These differences were not statistically significant (linear regression, 2-sided p-value = 0.922).

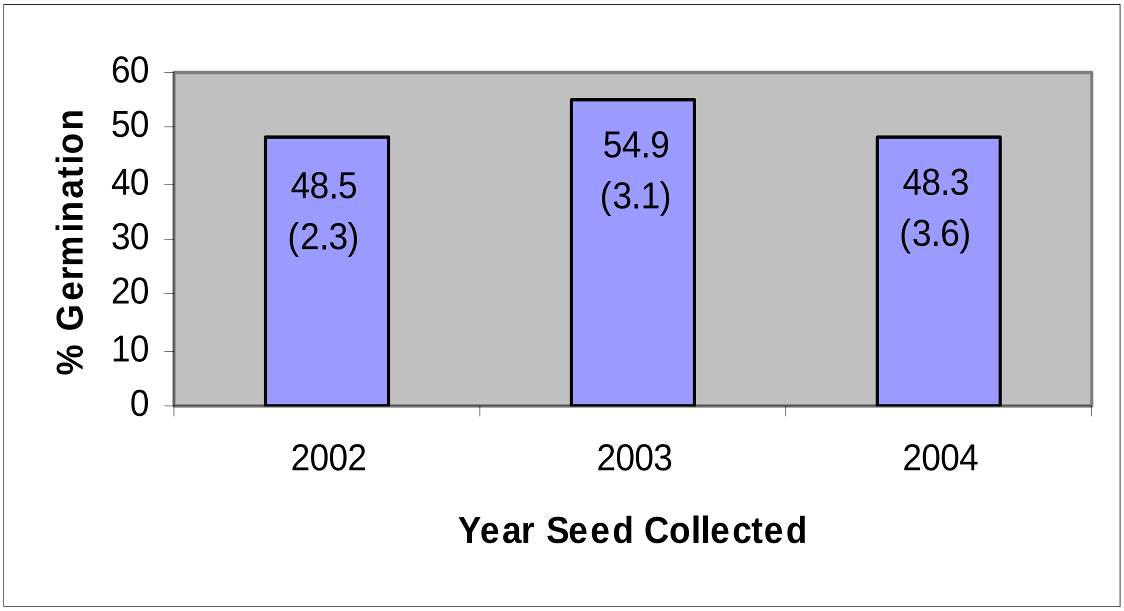

Figure 40. Comparison of germination rates of *Oenothera wolfii* seeds collected from the Port Orford population in 2002, 2003, and 2004. Differences were not statistically significant.

DISCUSSION

Oenothera wolfii seeds germinated easily with no vernalization or scarification treatment. There appeared to be no real benefit to treating the seeds with bleach prior to germinating them – while one trial did result in a statistically significant difference in germination rates (with a greater number of bleached seeds germinating), there was no statistically significant difference between the germination rates of bleached and unbleached seeds in the second trial. In either case, fungal growth was not a problem with either the bleached or the unbleached seeds.

Seed source did affect the rates of germination. Seeds from Hubbard Creek's *O. wolfii* population germinated at a statistically significant lower rate than seeds from either the Port Orford or the Pistol River populations. It is possible that these results indicate lower levels of fitness in plants at Hubbard Creek, which might be expected, given the small size of this population (approximately 40 individuals, with only 16 of them reproductive). However, seeds from the Pistol River population, which contains roughly the same number of individuals as the Hubbard Creek population (and in 2004 had only seven reproductive plants), had germination rates which were, on average, almost double those for seeds collected from Hubbard Creek. Further study is needed to determine the causes of this difference in seed viability, as well as to investigate whether or not these differences occur over multiple years, and between additional *O. wolfii* populations.

Seed age, at least initially, did not affect germination rates of seeds from the Port Orford *O. wolfii* population. Seeds collected in 2002, 2003, and 2004, and subsequently germinated in the greenhouse in 2005, all germinated at rates of approximately 50%. Differences in germination rates were not statistically significant. This is not entirely surprising, since *O. wolfii* seeds have a hard seed coat, which serves to protect them from the harsh coastal environment and presumably allows them to remain viable until they reach a microsite favorable for germination and seedling establishment. Once again, future germination trials are needed to determine how long *O. wolfii* seed can remain viable.

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 well in the greenhouse. Greenhouse-grown plants developed thin, filamentous roots in their pots, whereas individuals in natural populations develop thick taproots. This difference could simply be a function of age (greenhouse-grown plants were young rosettes at the time of transplantation, whereas plants examined in natural populations were larger and presumably older). The lack of a taproot in greenhouse-grown plants might also be the result of access to water and nutrients in the greenhouse setting. However, transplant survival rates were very high in spite of this morphological difference, and once transplanted, greenhouse-grown rosettes did form taproots at the introduction sites.

Transplant survival after one year 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 significant difference between survival of different transplant sizes, overall more small plants perished (eight) than their larger counterparts (three). It is possible that with larger sample sizes, this difference might be significant. Also, the three large plants that died at Lost Lake were all plants that 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 directly from their pots during future introduction efforts. Neither the removal of ground cover at Meyers Creek nor the percent of ground cover in plots at Lost Lake had a significant impact on transplant survival.

There are instances where transplant survival is not impacted by outplanting treatments or environmental conditions, but transplant vigor and reproductive output are affected by these external factors (Rimer and McCue 2005). Plants that survive in suboptimal conditions often don't have enough resources available to allow growth and reproduction. In the case of *O. wolfii*, transplant size affected reproduction. At Meyers Creek, 31 large transplants reproduced (as opposed to 15 small transplants). Large transplants 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. Transplant size does not always determine the success of the transplanted individual (Kohn and Lusby 2004). However, one study using stochastic modeling to analyze data from multiple introduction studies found that individuals of the smallest size class were at the greatest risk for extinction, and in general, the largest founder individuals yielded the largest populations (Guerrant 1996).

Although ground cover removal did not have a significant impact on transplant survival at Meyers Creek, the removal of vegetative competition did have a positive impact on transplant reproduction. Transplants in plots with ground cover removed were more likely to reproduce in the first growing season (31 plants vs. 15 reproductive plants in plots where ground cover was not removed). This corroborates the results of other studies, which found vegetative competition had a negative impact on the performance of transplants (Bush and Van Auken 1997, Morgan 1997, Carlsen et al. 2000). 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 survival or reproduction. However, plots with some ground cover produced roughly 70 more seeds per fruit than those in plots without ground cover. Lost Lake plots with no ground cover were located on open dune habitat with large amounts of moving sand and low ground moisture retention. It is likely that this increased stress reduced seed set.

As of the summer of 2005 (almost two years after outplanting), no seedlings were found in or near the transplant plots. Although recruitment can be limited by both the availability of seed and suitable microsites (Eriksson and Ehrlen 1992), the abundant seed production of *O. wolfii* suggests that a lack of suitable habitat is the issue at this site. Many of the plots at Lost Lake were located on open sand dunes, in habitat characterized by shifting sand and little ground cover. These factors decreased the available moisture at Lost Lake, and this lack of moisture combined with the other harsh environmental factors made this site relatively inhospitable for seedling establishment. Low availability of moisture and the resulting desiccation of seedlings is often a leading cause for emergent seedling mortality (Larcher 1995). Although the Port Orford population is located on dunes as well (in this case, the foredune), and at first glance this population might appear to occupy similar habitat to that at Lost Lake, the plants at Port Orford tend to be found in protected gaps in the European beachgrass, in sites where the sand is partially stabilized and plants are somewhat protected from the wind.

In contrast, the substrate at Meyers Creek was almost completely covered with vegetation. Even plots from which vegetation was removed at the time of outplanting only had a half meter^2 gap created. While this was enough to remove competition for the founding individual, the flowering stalk height and the ocean winds make it likely that many of the seeds fell outside of the cleared area. 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 1998).

Finally, it is important to remember that these sites were only monitored for one year after reproductive transplants set seed. It can often take more than two years to see seedling recruitment, especially if seeds need a particular set of environmental or climatic circumstances in order to germinate. In one study, no recruitment was observed for two years after sowing *Hypericum gentianoides* seeds, then in the third year of monitoring 38 plants appeared. Monitoring of another southern Oregon coastal dune species, *Abronia umbellata* ssp. *breviflora,* also showed that populations could be recolonized from an existing seed bank. Even when no plants were observed one year, new plants appeared the following growing season (Kaye 2003).

Overall, the relationships between measured environmental factors and transplant survival and reproduction were difficult to detect. Although some factors were associated with reproductive success, there were no consistent trends which would allow predictions to be made about the appropriateness of future sites. This may be due to the similar microhabitats of plots within each site. Environmental factors such as soil pH and moisture levels did not vary much between plots, and were not related to differences in transplant performance. 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 roughly 1.5 km inland). Meyers Creek substrate retained moisture better than that of Lost Lake, due to the humus in the soil and the higher levels of ground cover. Although there are natural populations of *O. wolfii* found on open, moving sand, these populations are located right on the foredune, where the lack of moisture in the substrate is perhaps offset by ocean spray and fog. Future introduction sites should be located directly above the ocean beach, since this is where natural populations are located, and where the Meyers Creek population thrived.

Initial results suggest that introduction 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 will recruit new individuals and ultimately become self-sustaining. Many of the transplanted rosettes survived but did not reproduce during the first growing season after transplantation. However, it is difficult to determine the success of an introduction project without evaluating recruitment of new individuals resulting from the naturally sown seed of the reproducing transplants. Monitoring over the next several years will determine whether or not seedlings are present, and whether or not recruited individuals are able to survive, flower, and set seed themselves. Long-term monitoring is important, since there are many cases of introduction projects appearing successful initially, but eventually failing due to a variety of factors (Allen 1994, Brumback and Fyler 1996, Raven 2001, U.S. Fish and Wildlife Service 2006a).

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Chapter 3: Conclusions and Management Recommendations

"*If we squander the capital represented by living nature around us, we threaten life itself*."

E.F. Schumacher, 1973

GENERAL CONCLUSIONS

Oenothera wolfii's status is increasingly precarious, as multiple pressures are brought to bear on this beautiful coastal species. Due to *O. wolfii*'s preference for habitat situated immediately next to the ocean, this plant is competing with roads, recreational areas, and commercial and residential development for space. Existing populations have been negatively impacted by roadside maintenance, expansion of beach access facilities, and recreational use along coastlines. In its favor, this species produces large numbers of seed and expands into favorable habitat where there is little competition (Imper 1997). However, increased competition with dune stabilizing species, especially the non-native *Ammophila arenaria*, has restricted *O. wolfii*'s ability to expand into otherwise favorable habitat, and has contributed to the decline of this species. Finally, the escape of the cross-compatible garden cultivar *O. glazioviana* is a pressing concern for *O. wolfii*. Many of the California populations are thought to contain some hybrids (Imper 1997, Carlson et al. 2001, DeWoody and Hipkins 2004), and most of the Oregon populations are located near major roadsides, exposing them to the risk of hybridization at some point in the future. Given these factors, recovery of this species requires not only the conservation of existing populations, but the creation of new, genetically intact populations in locations which are both administratively protected and ecologically appropriate.

This research contributes to the conservation and recovery of *O. wolfii* by providing additional information regarding seed germination methodology, transplant cultivation and outplanting protocols, and introduction site selection. Our seed germination studies confirm that germinating *O. wolfii* seed is a straightforward process, with no after-ripening, scarification, bleaching, or other pre-germination treatments required. Our results also indicate that *O. wolfii* transplants can be cultivated in standard greenhouse conditions with few difficulties. Our transplanting protocol provides valuable knowledge for future introduction efforts. This study showed that an individual of *O. wolfii* produces hundreds of seeds, and that a large percentage of this seed is viable and germinates readily. This suggests that this species, similar to other rare and common species of *Oenothera* (Pavlik et al. 1993a), is not reproductively limited. In addition, results from our research have further clarified the ecological requirements of *O. wolfii*, and provide some guidelines for future site selection. This increased body of knowledge instills confidence in the potential for successful introduction efforts in the future.

New information regarding protocols for establishing populations of *O. wolfii* has emerged from this study. However, additional questions remain, and considerably more work is needed before this rare species is fully recovered. We now have a better idea of what types of habitat are needed (and what types of habitat are not as suitable) for the successful introduction of *O. wolfii*. In particular, results indicate that sites should be located either on the foredune or the bluffs immediately above the beach. Although some neighboring vegetation may be helpful for sand stabilization and nutrient accumulation in the soil, it appears that *O. wolfii* performed better in areas with limited competition and few immediately associated species. Beyond that, specific microsite criteria have yet to be identified, and future research looking at factors such as vegetative gap size, associated species, and soil structure will assist in narrowing down the environmental characteristics of ideal introduction sites.

Long-term viability of the new *O. wolfii* populations is another area needing further study. Ultimately, the objective of rare plant introduction is the creation of self-sustaining populations capable of persisting in the face of future evolutionary and environmental changes (Pavlik 1996, Kaye 2003). Although this study provides ample evidence that it is possible to successfully transplant *O. wolfii* individuals on the southern Oregon coast, successful population persistence and viability will be reflected by consistent seedling recruitment. In spite of *O. wolfii*'s prolific seed production, seedling recruitment, whether from seed that was experimentally sown or from the seed produced by transplants, has been extremely limited during the short span of this study. At the Lost Lake site, this is likely due to the inhospitable and probably unsuitable habitat. Dense vegetative cover and litter (which reduce opportunities for seed to make contact with the soil and germinate) could explain lack of seedling recruitment at Meyers Creek. There are several courses of action which might improve opportunities for seedling recruitment, and therefore increase chances of successful population establishment. One possibility is to manipulate the habitat in ways which encourage seedling recruitment, such as removing neighboring vegetation or built-up litter (Lawrence 2005). For introduction sites with large amounts of non-native vegetation (such as European beachgrass), creation of semi-protected gaps in vegetation may increase success (Pavlik and Manning 1993; Tom Kaye, personal communication on March 3, 2005). Another possible method for improving the long-term persistence and expansion of the new population is to increase the number of transplants used and the number of years in which transplants are installed at the introduction site. Larger populations have a better chance of reproducing the genetic structure of natural populations (McGlaughlin et al. 2002), and introduction efforts using as many transplants as possible (Primack 1996), over as many years as possible (Drayton and Primack 2000), increase the chances of transplant survival and seedling recruitment.

A third issue that needs to be addressed is that of the hybridization between *O. wolfii* and *O. glazioviana*. Although field observations of plant morphology show that Oregon populations of *O. wolfii* have yet to be infiltrated with *O. glazioviana*, preliminary genetic work (DeWoody and Hipkins 2004) suggests that some Oregon populations may have already experienced hybridization. Close tracking of the spread of *O. glazioviana* and suspected hybrid populations is needed in order to determine which *O. wolfii* populations might best serve as “pure” sources of seed for future introduction projects. In addition, further protection of non-hybridized *O. wolfii* populations might include working with agencies (i.e., Oregon Department of Transportation and county maintenance departments) to eradicate all roadside populations of *O. glazioviana*.

This study encountered challenges which can help direct future work. Both study sites are located on public lands – one federally owned and one managed by the State of Oregon. The Meyers Creek site belongs to the Oregon Department of Transportation (ODOT) and the Lost Lake site is managed by the Bureau of Land Management (BLM). Each of these agencies had their own concerns about the study. The Oregon Department of Transportation does not have a conservation focus, although the agency has been proactive in managing the populations of listed species found on their lands. Staff at ODOT were concerned that a new population of *O. wolfii* on the highway right-of-way would interfere with roadside maintenance activities. This concern was addressed by situating the experimental population high enough on the bank above the highway so as to not be impacted by mowing and spraying, but close enough to the road to still be within the habitat preferred by the species. The BLM was already managing the Lost Lake site as part of the New River Area of Critical Environmental Concern (ACEC), and as such did have a conservation focus to their management plan. Originally, both of the introduction sites were to be located on the ACEC. However, a small population of another state listed species (which is also a federal species of concern), silvery phacelia (*Phacelia argentea*), was discovered at the second proposed site, and BLM staff was concerned that the *O. wolfii* study would have a negative impact on this other species. In light of this concern, we identified an alternative location for our second introduction site, and did not use the area where the silvery phacelia grew. In addition, the BLM was concerned about the effect of ground cover removal (one of the study treatments) at the ACEC. We worked with BLM botanists and land managers to adjust the study plan to take this concern into account. Future introduction efforts will most likely take place on public land, and recovery work will have to be coordinated with the agencies that manage this land. Due to the nature of *O. wolfii* habitat preferences, likely candidates for future partnering include ODOT, the BLM, and the Oregon Parks and Recreation Department (OPRD). In order to minimize conflicts and increase the odds of project success, it is imperative that site visits and land manager review of the proposed study design happen early in the planning process.

The goal of species recovery will only be achieved by multiple partners, both public and private, working together. No one entity has the resources to successfully accomplish all of the tasks necessary for the protection and recovery of most threatened and endangered species. This study is no exception, and without the assistance of many partners, the project would never have moved forward. In addition to the cooperation and support of the introduction site land managers (ODOT and the BLM), financial support from the Oregon Department of Agriculture, the U.S. Fish and Wildlife Service, the BLM, and the Native Plant Society of Oregon contributed to the success of this project. We were also able to use the many resources at Oregon State University, including greenhouse facilities and the technical expertise of faculty in the Departments of Botany and Plant Pathology and Crop and Soil Science. This study provides an excellent example of how those engaging in conservation efforts must identify partners with common goals and leverage resources from multiple sources in order to achieve recovery objectives.

Many studies examining the causes of species rarity and extinction have concluded that threats to these species are primarily extrinsic, caused by human disturbance and destruction of habitat (Pavlik 1987, Pavlik et al. 1993a, Fiedler and Laven 1996). Ideally, recovery efforts should focus on prevention and reversal of the detrimental effects of human activity on habitat quality and quantity (Pantone et al. 1995). However, for some of our most endangered species, including *O. wolfii*, protection of existing populations is not enough (Falk et al. 1996), and the creation of new populations will be necessary to achieve recovery. Because current plant protection laws primarily protect threatened or endangered species on public lands, it is on these lands (combined with privately owned properties with permanent conservation status) that our recovery efforts must be focused. The public agencies and private conservation groups charged with managing rare species on their lands are looking for practical and applicable recommendations for both conserving existing populations and establishing new populations (Gordon 1994). This study has sought to assist land managers in these efforts. By increasing our knowledge of how to establish new populations of *O. wolfii*, or augment existing ones, we are one step closer to being able to create secure, self-sustaining populations of this species in protected areas.

RECOMMENDATIONS

(Note: See Appendix 6 for overall protocol for seed germination, transplant cultivation and new population establishment of *Oenothera wolfii*.)

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

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

3. Site selection is crucial to the success of new population establishment. Future introduction projects should be limited to sites that are close to or within the current range of the plant, adjacent to or directly on the beach (either on

bluffs above the 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.

4. In order to facilitate reproduction during the first growing season, larger rosettes should be used for transplanting.

5. Because larger populations have the potential for greater genetic variability and less risk of extirpation, it is recommended that future introduction projects involve as many transplants as logistically feasible.

6. Ideally, efforts to establish new populations of *O. wolfii* should involve multiple years of outplanting transplants.

7. If a large percentage of the selected site's substrate is covered with vegetation, removal of ground cover (in order to reduce competition and create gaps for seedling recruitment) is recommended.

8. Implement introduction and augmentation projects using protocols developed with data from the current study.

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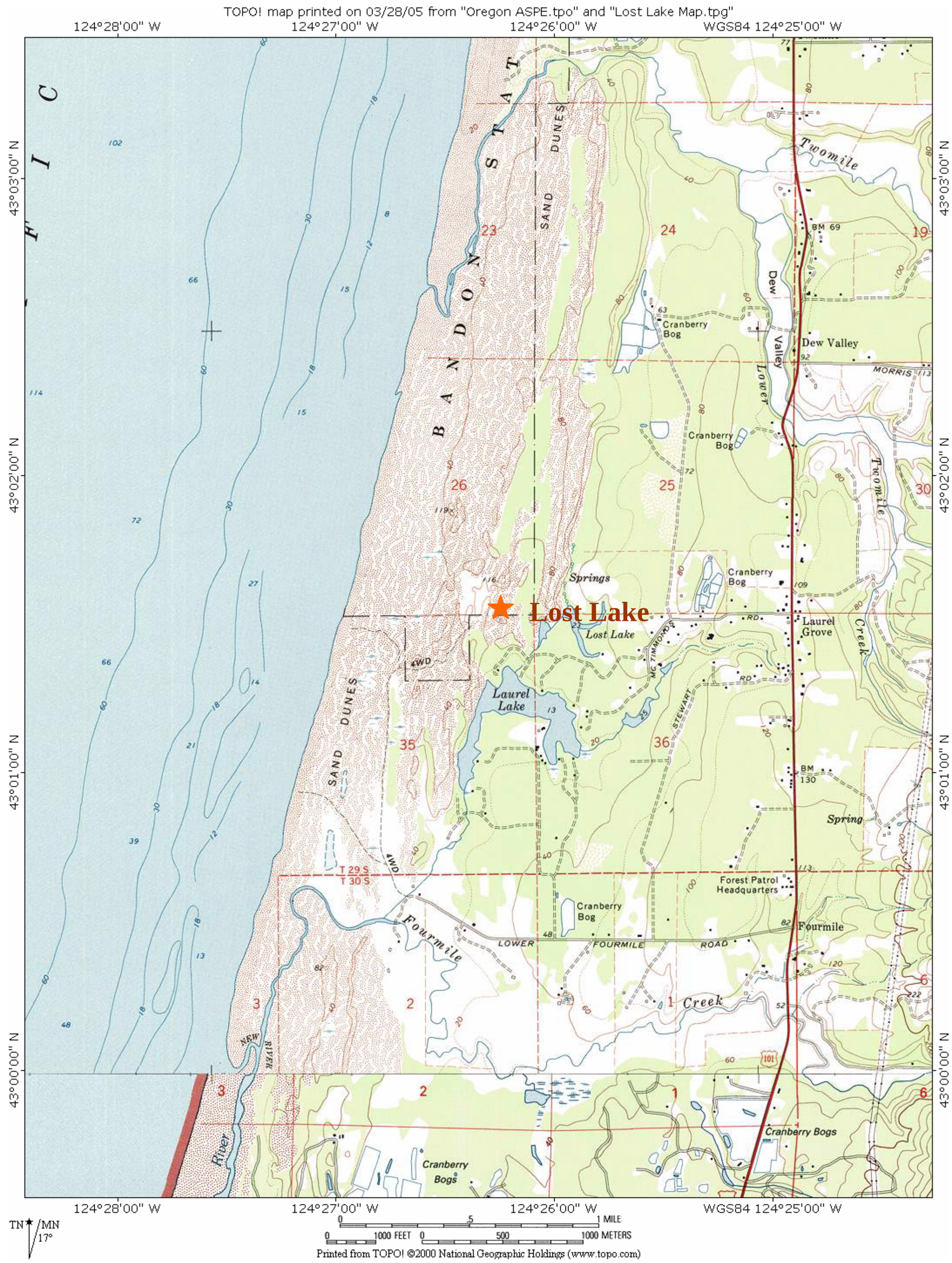
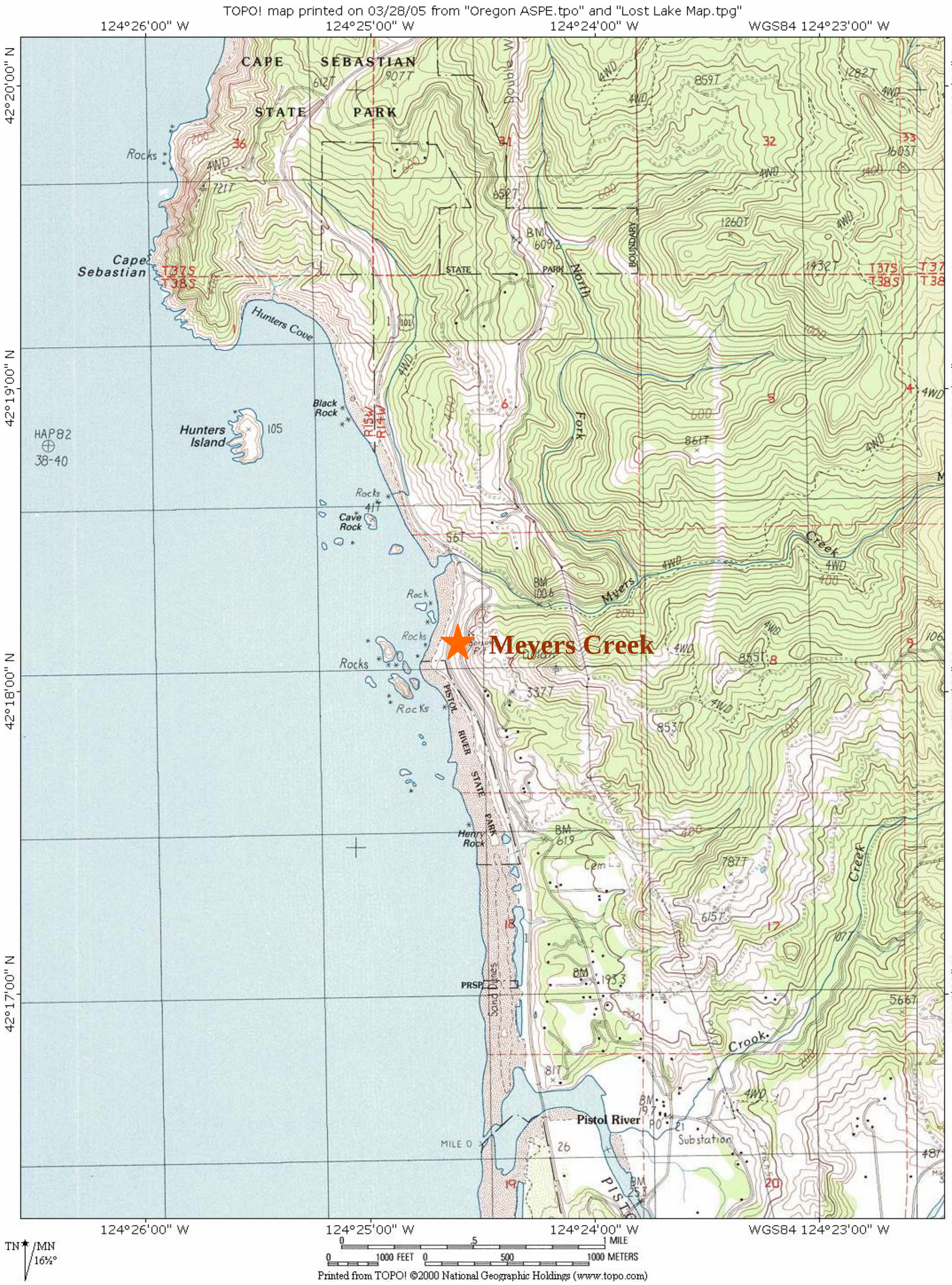
Appendices

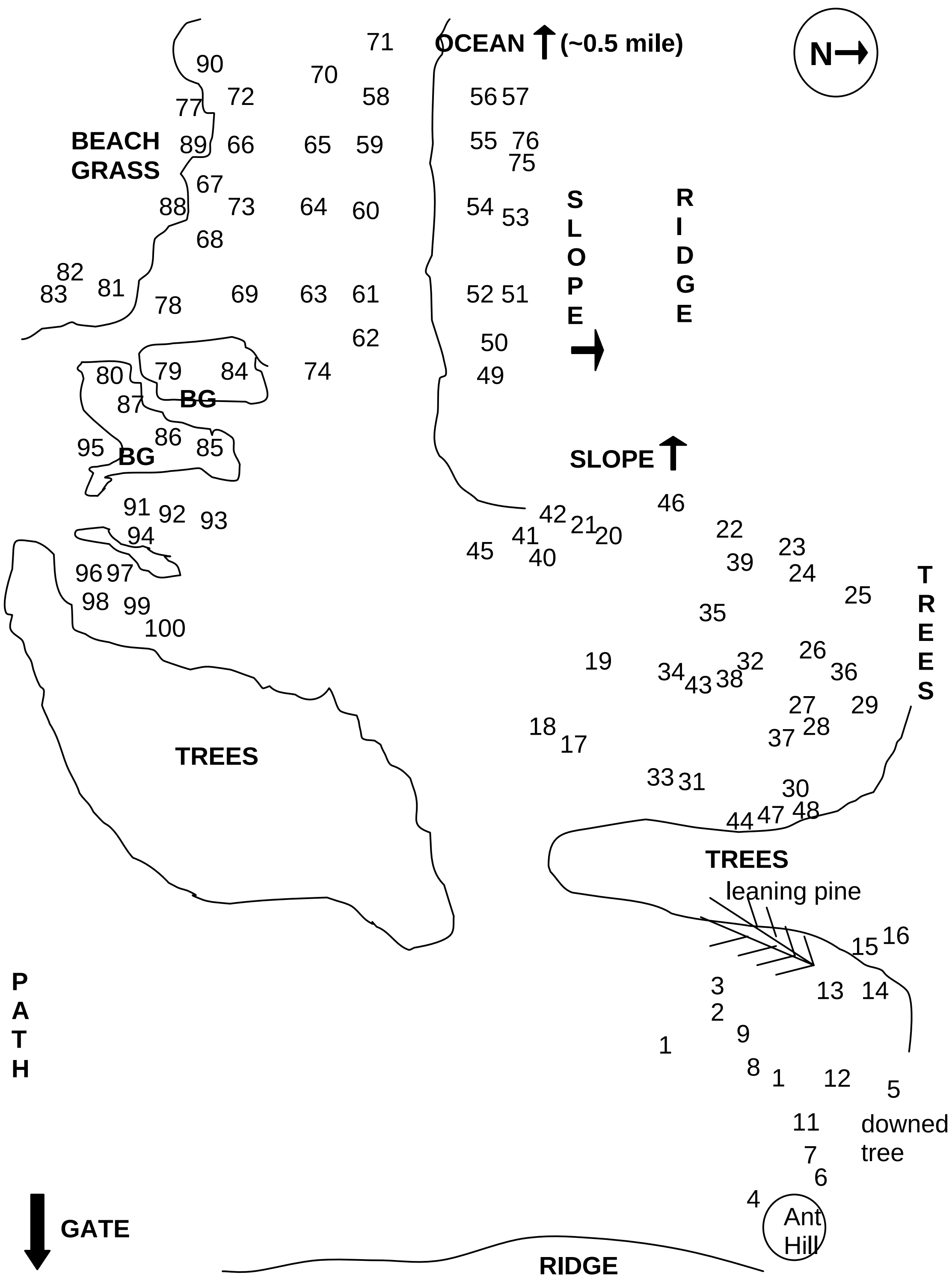
APPENDIX 1: LOST LAKE MAP



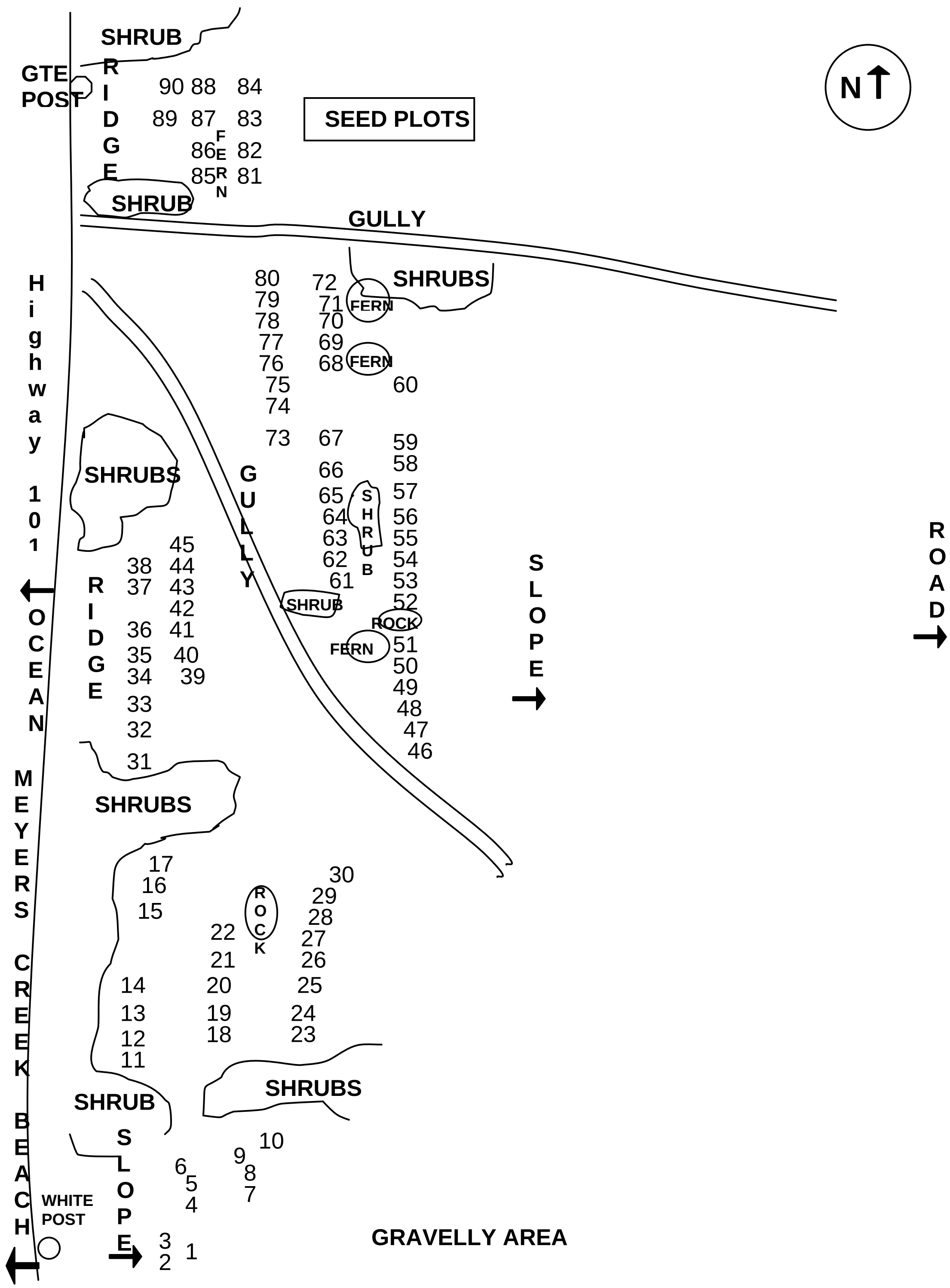
APPENDIX 2: MEYERS CREEK MAP



APPENDIX 3: LOST LAKE SITE MAP



APPENDIX 4: MEYERS CREEK SITE MAP



APPENDIX 5: RESEARCH PLOT GPS LOCATIONS

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’

APPENDIX 5: RESEARCH PLOT GPS LOCATIONS (continued)

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’	566	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’

APPENDIX 6: PROTOCOL FOR ESTABLISHMENT OF NEW *OENOTHERA WOLFII* POPULATIONS

Task	Protocol
Seed source population selection	Select seed source sites that: • are geographically close to introduction site • have similar environmental characteristics • support populations of at least 100 individuals (with at least 25 reproductive individuals)
Seed collection	• Collect no more than 10% of seed from any individual • Collect seeds from at least 50 individuals randomly selected from throughout the population
Seed storage (if necessary)	• Store seeds in paper bags or coin envelopes in a dry, dark location at room temperature • Seeds may be stored for at least three years in this manner
Seed germination	• Germinate seeds under greenhouse lights set at 12 hours of light/day • Germinate seeds at alternating temperatures (can expect ~50% germination within 2 weeks at alternating 18.3/21.1°C; most seeds germinate in the first week) • Germinate on moistened filter paper in Petri dishes, mist with tap or bottled water as necessary
Seedling cultivation	• Carefully transplant seedlings from Petri dish to pots by either gently grasping cotyledon (rather than radical) with tweezers, or lifting seedling with a probe/toothpick • Grasp seedling by cotyledon, rather than radical • Plant seedlings in 5 cm cells filled with pre-moistened 2:1 sand/peat moss potting mixture • Place potted seedlings in greenhouse under high pressure sodium lights set at a 12 hour day length, with daytime temperatures set at 21.1°C and nighttime temperatures set at 18.3/21.1°C • Water as necessary • Fertilize every 2-3 weeks with ½ strength all-purpose plant fertilizer, such as MiracleGro® • When rosettes are 3-4 weeks old, transplant them into 10 cm x 10 cm x 15 cm pots filled with 2:1 sand/peat moss potting mixture • Turn off heat in greenhouse on and taper off watering seedlings 1-2 weeks before outplanting

APPENDIX 6: PROTOCOL FOR ESTABLISHMENT OF NEW *OENOTHERA WOLFII* POPULATIONS (continued)

Task	Protocol
Introduction site selection	Select introduction sites that are: • within the current range of *O. wolfii* • administratively protected (i.e., public land or private land with permanent conservation easement) • not adjacent to commercial, residential, or agricultural development • isolated from human impacts (i.e., recreational use) Select as many introduction sites as possible
Microsite selection	Select outplanting microsites that: • are located on the foredune or the banks/bluffs immediately above the foredune • are subject to a moderate amount of disturbance (i.e., the occasional landslide, or some sand movement) • are not completely dominated by other vegetation, especially non-native weeds
Introduction site preparation	If site contains high levels (>75% ground cover) of competing vegetation, clear any vegetation within ½ meter of the transplants
Transplant transportation	• Transport seedlings in their pots, if at all possible • If unable to transport seedlings in pots, remove them from their pots and shake off excess soil (being careful to not remove any of the roots), place plants in large sealable plastic bags with a moistened paper towel, and place bags in coolers • Do not store plants in coolers for more than one day
Number of transplants/number of years of transplanting	• Plant at least 500 transplants at each site (the more, the better) • Outplant every year for three to five years in order to build the *O. wolfii* seed bank at the introduction site
Transplanting	• Plant seedlings in the fall, once the fall rains have begun • Plant seedlings so that the top of the potting mixture is level with the ground • Water the seedlings thoroughly immediately after planting • Label plants or plots for future monitoring • Map and GPS plot/plant locations
Site maintenance	If there is little or no bare ground (which provides more favorable seed germination and seedling establishment microsites) at the introduction site, periodic (every three to five years) control of competing vegetation may be needed

APPENDIX 6: PROTOCOL FOR ESTABLISHMENT OF NEW *OENOTHERA WOLFII* POPULATIONS (continued)

Task	Protocol
Monitoring	• Census new population annually for at least five years, counting reproductive and vegetative plants • After five years, census (or sample) population every three years