AN ABSTRACT OF THE THESIS OF

Matthew G. Groberg for the degree of Master of Science in Botany and Plant Pathology presented on December 16, 2013

Title: An Experimental Reintroduction of *Pleuropogon oregonus*, a Rare Wetland Grass Native to Oregon.

Abstract approved:

Robert J. Meinke

Pleuropogon oregonus Chase is a rare wetland grass endemic to eastern Oregon. The species is composed of two widely separated populations, one in Lake County and one in Union County. In order to reduce the risk of extinction, the Oregon Department of Agriculture Native Plant Conservation Program initiated several reintroduction projects for the species in the early 2000s. Because there were some morphological distinctions (implying genetic differences) between the two population groups, plants from the different counties were considered to represent separate conservation units in reintroduction projects. The Union County ecotype established well at a site in Grant County (Logan Valley), but reintroduction attempts with the Lake County ecotype failed to produce self-perpetuating populations.

This brings up the question of whether successful establishment is related to habitat quality at the reintroduction sites, the ecotype used, or some combination of both. In addition, the small natural patch sizes, mostly clonal growth, and low caryopsis production suggests that Oregon semaphore grass might suffer from low genetic diversity

and inbreeding depression. Therefore, the question of whether separate ecotypes should be mixed in reintroduction projects merits consideration. On the one hand, planting allopatric ecotypes together runs the risk of disrupting co-adapted gene complexes. Yet if material used in reintroductions exhibits inbreeding depression, mixing disparate populations may be advantageous. Because environmental conditions often fluctuate in wetlands, vegetative community characteristics may be useful in measuring suitability.

Ecotypes were reintroduced in separate areas and mixed in one area at the same reintroduction site, i.e., End Creek, in Union County. Although an effort was made to reintroduce ecotypes into areas that were similar, in terms of environmental conditions and vegetative communities, there were landscape-wide differences in the outplanting areas that were not measured in this study, such as changes in hydrology. Because the area in which the mixed ecotypes were reintroduced did not have any surviving plants, the question of whether mixing ecotypes promoted caryopsis production could not be answered. The areas with the most survival were in full sun, completely saturated soil and had vegetative communities similar to the source sites. Although more plants at End Creek survived in the Union County area than the Lake County area, this difference was attributed to the inadvertent selection of presumably better quality habitat in this area.

High quality habitat appears to be the most significant factor affecting reintroduction success. Although reintroduction areas may appear to be suitable on a small scale, the effect of the surrounding landscape may affect establishment in ways that are not predictable on a small scale. This was most apparent in the mixed plot area which seemed appropriate on the small scale, but contained no surviving plants upon monitoring.

AN EXPERIMENTAL REINTRODUCTION OF *PLEUROPOGON OREGONUS*, A RARE WETLAND GRASS NATIVE TO OREGON

by

Matthew G. Groberg

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 16, 2013
Commencement June 2014

Master of Science thesis of Matthew G. Groberg presented on
December 16 2013

APPROVED

Major Professor, representing Botany and Plant Pathology

Head of the Department of Botany and Plant Pathology

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.

Matthew G. Groberg, Author

ACKNOWLEDGEMENTS

I am very grateful for the assistance of Dr. Karen Antell for helping us secure a location for this reintroduction project in Union County. In addition, she helped collect plants at the Union County site as well as assisted in outplanting at End Creek and gave insight about the history of End Creek. I would like to thank Jordan Brown, Amy Golub-Tse, and Kelly Amsberry for their help collecting plants, planting at End Creek, and collecting baseline environmental data. Thanks to my dad Greg Groberg and brother Kaylen Groberg for their help setting up plots and collecting baseline environmental data. Thanks also to ShyAnne Woods and Dr. Robert Meinke for their help monitoring the population at End Creek.

For funding, I thank the Oregon Department of Agriculture Native Plant Conservation Program and the Portland Garden Club. In addition I received much support from the Botany and Plant Pathology department through Graduate Teaching Assistantships, which I am also very grateful for.

I would also like to express my gratitude to everyone in the Native Plant Conservation Program for giving me the wonderful opportunity to work with them as well as their encouragement for me to continue my education in graduate school.

Lastly, I would like to acknowledge those who reviewed and helped edit this thesis, including my committee members: Dr. Robert J. Meinke (major professor), Dr. Stephen C. Meyers, Dr. Frank Andy Jones and Dr. Jonathan Kaplan (graduate representative). Kelly Amsberry and Rebecca Currin were of great assistance in reviewing earlier drafts of this thesis, and I am very grateful to them as well.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

AN EXPERIMENTAL REINTRODUCTION OF *PLEUROPOGON OREGONUS*, A RARE WETLAND GRASS NATIVE TO OREGON

CHAPTER 1: INTRODUCTION

Morphology and Life History

Pleuropogon oregonus Chase is a perennial, wetland obligate grass endemic to eastern Oregon (Chase 1938). The erect racemes of this species are made up of six or seven spikelets spreading to one side of the rachis, giving the appearance of semaphores (signaling flags or railway signals) and the genus the common name of semaphore grass (But et al. 1985; Figure 1). Each of these spikelets usually has seven to fourteen florets. When the plant is not producing inflorescences, it can be distinguished from many other grasses by the prominent mid-vein on the leaf blade, the prow-shaped leaf apex, and the soft, glabrous leaf blade, although these characters are not unique to the species (Figure 2). Culms grow from an elongated rhizome and can develop into large clonal patches.

Pleuropogon oregonus has an unusual pattern of floral development. The upper florets contain only pistils, while the lower florets are hermaphroditic (Figure 3). The species produces flowers from early June to late July and fruits until the middle of August. The upper pistillate florets mature first, making the spikelets, as a whole, protogynous. Within the hermaphroditic lower florets, the anthers mature first, making them protoandrous. This pattern of development has also been observed in *Pleuropogon californicus*, but is uncommon in general in the Poaceae (But et al. 1985). Separation of the sexes onto separate flowers, which develop at different times, is usually an adaptation to encourage outcrossing (Crawley 1997). Outcrossing is a common strategy for long-lived perennial plants that can afford to be selective with their pollen source, in comparison with annual plants which must produce seed each year.

Figure 1. Infloresence of *Pleuropogon oregonus*. The spikelets spread to one side of the rachis giving them the appearance of semaphores.

Figure 2. *Pleuropogon oregonus* can be distinguished morphologically when not producing inflorescences from most grasses by the prominent mid-vein.

Figure 3. Each spikelet of *Pleuropogon oregonus* is divided into pistilate florets (A) which mature first and bisexual florets (B) which mature after the pistilate florets have senesced.

In grasses, the seed coat becomes fused with the ovary wall forming a fruit-seed complex called a caryopsis. Caryopsis production in *P. oregonus* is fairly limited (Figure 4). Although the majority of the pollen is viable, only about 10 percent of the florets produce caryopses in nature (But et al. 1985). This low caryopsis production may be related to genetic load associated with small population sizes affecting embryonic development, such as in *Lupinus sulphureus* ssp. *kincaidii* (Severns et al. 2011) or *Dedeckera eurekensis* (Weins et al. 1988). However, in contrast to the lupine, *P.*

oregonus evidently does not have a long lasting seed bank, in which outcrossing between generations increases seed production, since dormancy is easily broken with only an application of water in *P. oregonus* (But et al. 1985; Gisler and Meinke 2003).

Figure 4. Only about 10 percent of the ovaries in *Pleuropogon oregonus* produce viable caryopses (left). The majority are aborted before developing (right).

Although caryopsis production is low in natural populations, vegetative reproduction through the species' well developed rhizomes is common (Gisler and Meinke 2001; Figure 5). In fact, vegetative reproduction was thought to be the only means of reproduction in *P. oregonus* before a more detailed study of caryopsis production was performed (But et al. 1985). The extensive asexual reproduction, low sexual reproduction, and the small population sizes suggest that the species may suffer from low genetic diversity and inbreeding depression (a reduction of fitness due to increased genetic drift in small populations; Barrett and Kohn 1991) (Gisler and Meinke 2003).

Pleuropogon oregonus exhibits several wetland-specific adaptations. Plants have rhizomes containing porous aerenchyma (Figure 5), a tissue commonly found in wetland obligate plants. Aerenchyma facilitates gas exchange for cellular respiration in inundated areas by creating additional pore space for gasses within the tissue (Colmer 2002). In addition to the aerenchyma in the rhizomes, the roots of *P. oregonus* contain starch for energy storage. This storage ability allows the plant to lie dormant when conditions are not optimal for above ground growth, such as during floods.

Although plants of *P. oregonus* are well-adapted to wetland conditions, adaptations for growth in water make the species less adapted to dry conditions. A study on aerenchyma production in rice (*Oryza sativa*) demonstrated that the tissues of cultivars grown in areas that experienced periodic droughts contained less pore space than cultivars grown in permanently inundated soil (Colmer 2002). However, unlike rice, *P. oregonus* likely may not have enough genetic variation to adapt to different habitats.

Figure 5. Clonal reproduction is common. Aerenchyma tissue (left cross section insert) in the rhizome of *Pleuropogon oregonus* allows it to carry out gas exchange in soil saturated with water. Starch is stored in the roots (right cross section insert).

History, Distribution, and Legal Status

Pleuropogon oregonus has a history of being rare, or at least overlooked. Although *P. oregonus* was first collected from a site in Union County in 1886 by W. C. Cusick, it was not formally described as a species until almost 50 years later, in the late 1930s (Chase 1938). The type locality was referred to by its local name, Hog Valley, but the actual location was unclear. This site was finally identified as being in Union County in 1901, when it was relocated by A. B. Leckenby. Then, in 1936, the species was found by Morton E. Peck at second location, growing in swampy ground west of Adel in Lake County, Oregon. This collection was significant in that it was about 200 miles away from previous collections in Union County. However, because the species was not collected again for over forty years, by 1975 it was thought to be extinct (But et al. 1985). The species was relocated in 1979 at what was assumed to be the same sites in Union and Lake counties (Figure 6).

The genus *Pleuropogon* consists of four species restricted to the west coast of North America, and one species broadly distributed in the arctic (But 1977; Barkworth et al. 2007). Since the center of diversity for the species is located in northern California, this area is thought to be the location where the most recent common ancestor for the genus first appeared (But 1977). As members of the genus moved northward, *P. oregonus* is speculated to have diverged from *P. californicus* and staying east of the Cascade Mountains. Both *P. oregonus* and *P. refractus* (restricted to the west of the cascades in Oregon) occupy wetland habitats in Oregon (But 1977).

Figure 6. Watershed map of Oregon indicating the locations of the two naturally-occurring populations of *Pleuropogon oregonus* in Lake County and Union County.

There are two possible explanations for this widely disjunct distribution that have been considered. Caryopses from plants occupying the Lake County sites may have been spread to suitable habitat in Union County (or vice versa) by migrating waterfowl through long distance dispersal (see Nathan et al. 2008). Alternatively, the two populations may originally have been part of a series of meta-populations connecting the two extant sites. Modification of land (by livestock grazing or farming by settlers) may have caused the extirpation of intermediate populations, resulting in the disjunct distribution that we see today.

Livestock grazing is one of the most extensive uses of land in the inland Pacific Northwest (Kauffman and Krueger 1984). Cattle often congregate in wetland areas which provide a source of water, shade, and large amounts of vegetation. This impacts wetland species by removing above ground vegetation, increasing soil compaction and erosion, and altering nutrient cycling (Fleischner 1994). Cattle in the Blue Mountains area of northeastern Oregon sometimes remove 75 to 85 percent of stream bank vegetation when browsing a riparian area (Gillen et al. 1985). The sparse historical record for *P. oregonus* makes determining the pre-settlement range difficult, so it is not certain if and how grazing affected the current distribution of the species.

Pleuropogon oregonus is listed as Threatened by the Oregon Department of Agriculture (ODA) (see ORBIC 2010 for listing status). Despite its rarity, this species is not currently listed by the U.S. Fish and Wildlife Service (USFWS). It is, however, a federal Species of Concern. The Oregon Biodiversity Information Center (ORBIC) considers *P. oregonus* to be “critically imperiled because of extreme rarity or because it is somehow especially vulnerable to extinction or extirpation, typically with 5 or fewer occurrences” on both a global and state level (ORBIC 2012), giving it a Heritage Rank of G1/S1 and placing it on ORBIC’s List 1.

Previous Reintroduction Projects

Species known to occur in only a few locations are vulnerable to extinction due to random environmental variation (Menges 1992). Strategies conservationists use to reduce this risk include creating new populations or augmenting the existing populations to mitigate for local population extirpations (Lande 1998). This may be a viable option for *P. oregonus*.

A morphometric study comparing the two populations of *P. oregonus* in a common garden environment determined that individuals from the two populations were morphologically dissimilar (Gisler and Meinke 2003). If these differences are a result of natural selection (and not genetic drift), then mixing the two putative ecotypes in a reintroduction project could potentially result in a reduction in fitness due to the break-up of co-adapted genes— a phenomenon known as outbreeding depression (Hufford and Mazer 2003). To prevent this possibility, outplanting prior to the current study included only plants grown from stock collected near the destination site (Amsberry and Meinke 2004).

The Lake County ecotype was introduced in close proximity to the source site at Camas Creek (a USFWS site) and Parsnip Creek (a Bureau of Land Management site). Unfortunately, there was very low survival of *P. oregonus* plants at these sites (Amsberry and Meinke 2004). This low survival was initially attributed to grazing and competition with exotic weeds. However, when vegetation was removed and grazing exclosures were set up at the Parsnip Creek site, plants still did not survive. However, there was an unpredicted change in hydrology and invasive weeds established in the planting areas after competing vegetation was removed from the plots, which were likely the reasons for poor establishment at the Parsnip Creek site (Brown et al 2012).

The Union County ecotype was planted in 2002 at a site in Grant County (Logan Valley), since there were no suitable sites available for outplanting on publicly owned land near the extant Union County population at the time. Logan Valley is located on Burns Paiute tribal land and is managed for wildlife habitat. Plants established exceptionally well in these sites, with thousands of plants observed between 2005 and 2010 (Brown et al. 2012).

Project Objectives

The main objective of this study has been to develop methods for creating viable, self-perpetuating populations of *P. oregonus*. The second objective was to establish a new population of *P. oregonus* on protected land, while minimally impacting the source populations used as stock sources. The main approach to meeting these objectives was an experimental outplanting. These two objectives include aspects of both “project success” and “biological success”, respectively (Pavlik 1996). Project success is evaluated based on the amount of information gained through the project. Whereas biological success is evaluated based on how much reintroduction assists in the conservation of the species. This work was attempted in partnership with a conservation group (the Blue Mountains Conservancy) that expressed a willingness to assist in monitoring the created population in the future to help determine if it is self-perpetuating.

Questions, Hypotheses, and Predictions

Can outplanting success be predicted by source ecotype and environmental characteristics of the destination site? The following hypotheses will be tested to answer this question: 1) there is difference in the ability of each ecotype to survive, grow, and reproduce in outplantings (regardless of habitat quality), 2) there is a difference in the optimal habitat requirements between each ecotype, and 3) the optimal habitat requirements are the same for both ecotypes, making habitat quality the limiting factor in outplantings. The first hypothesis will be supported if there is a difference in the intercept of multiple linear regressions with survival, growth, and reproduction as dependent variables and soil moisture, light intensity, or pH between ecotypes. The second hypothesis will be supported if there is a difference in the effect of some of these measured environmental characters on survival, growth, and reproduction between ecotypes (different slopes in a multiple linear regression). If the last hypothesis is supported each ecotype will have the same intercept and slope for the effect of these measured environmental characters.

Should the ecotypes be mixed or kept separate in reintroductions? The hypothesis tested to answer this question is: mixing ecotypes during a reintroduction will result in increased caryopsis production as opposed to keeping ecotypes separate, since increasing genetic diversity can ameliorate the effects of inbreeding depression. This would be supported if there is increased caryopsis production by plants present in plots with both ecotypes compared to plots having single ecotypes. Although determining if the progeny of these crossed ecotypes have increased rates of survival, growth, and reproduction would more completely answer this question, this cannot be answered in the time frame of the current project, but may be answered through subsequent monitoring.

Can the suitability of an outplanting site be predicted based on its similarity to the habitat at the source site? The hypothesis that will be tested is: the characteristics of the vegetative communities at the source sites indicate the hydrological regime that *P. oregonus* can grow in. This would be supported if similarity between habitats of the reintroduction plots and source sites, as measured by percent cover of wetland species, natives, perennials, or graminoids, is correlated with in increased rates of survival, growth, and reproduction of reintroduced *P. oregonus* plants.

CHAPTER 2: LITERATURE REVIEW

Reintroduction and Rare Plant Conservation

Value of Reintroduction

We live in an era of global change in which the current rate of species extinction is 100 to 1000 times higher than it was during pre-human dates (Pimm et al. 1995). This loss of biodiversity is projected to increase as our population grows.

Maintaining biodiversity has many measureable benefits. Biodiversity increases net primary productivity in an ecosystem through resource partitioning (Costanza et al. 2007). In contrast to a monoculture, where the same nutrient requirements are present in each individual, diverse communities may use different resources at the same time or the same resources at different times. Loss of biodiversity can lead to widespread epidemics as well as the emergence of new diseases due to coevolving pathogens (Altizer et al. 2003). Diverse ecosystems, compared to non-diverse ecosystems, are, in general, less susceptible to invasions by exotic species (Hooper et al., 2005). In addition, many ecosystem functions are dependent on interactions between multiple species (Chapin III et al. 1998; Duffy 2009).

In addition to increasing biodiversity, reintroduction projects of rare species can have other beneficial effects on the communities from which they were extirpated. Reintroductions of keystone species like the gray wolf can have a large impact on the community, although they only represent a small fraction of it, through regulating herbivore populations (Beazley and Cardinal 2004). Restoration efforts for Kincaid's lupine (*Lupinus sulphureus ssp. kincaidii*), which is the larval host plant for the endangered Fender's blue butterfly, has assisted in the conservation of both species since they have such a close relationship (Wilson et al. 2003).

Reintroductions bolster existing populations of rare species, assisting in their conservation. The creation of meta-populations can reduce the impact of stochastic events on local populations by allowing propogules to recolonize an area after extinction events (Lande 1998). Reintroduction projects involving cultivation can result in higher adult survival than projects which only use seeds, since watering and fertilizing seedlings provides abundant resources and the natural enemies (herbivores and pathogens) may be controlled more efficiently in cultivation (Albrecht and Maschinski 2012). In addition, reintroductions using multiple sources can restore genetic diversity lost through habitat modification, destruction, or fragmentation (Gustafson et al. 2002).

Global climate change presents unique challenges to conservation (Schlesinger et al. 2001). Evidence in the fossil record suggests that during previous eras of global climate change, many species survived by migrating to new locations. However, much of the available habitat to which species could migrate is now destroyed, severely degraded, or fragmented due to recent human activities (Schlesinger et al. 2001), making it unlikely that narrowly endemic species could reach more favorable habitat if migration were necessary. Although global climate change may alter the natural habitats of endemic plant species, many land management agencies lack a concrete policy for introducing endangered plant species into new areas (McLachlan et al. 2006).

Reintroduction ecology, the combined field of restoration genetics and ecology, is relatively new, and consequently there are still many unanswered questions about how reintroductions should be performed (Maschinski and Haskins 2012). Compared to vertebrate reintroductions, there are very few published results for rare vascular plant species reintroductions (Noël et al. 2010). In addition, many reintroduced populations are monitored for less than four years (Godefroid et al. 2011), making it difficult to determine if self-perpetuating populations are being created.

Although some ecologists suggest that the process of reintroduction creates a genetic bottleneck (Monks et al. 2012) in new populations (see “Genetic Threats” below), others have shown that newly created populations can have levels of genetic diversity that are similar to the source populations (Lloyd et al. 2012). The contradictory results of studies such as these reflect the need for well-designed reintroduction experiments that address the effects of genetic diversity on the stability of created populations (Guerrant Jr. 1996; Pavlik 1996; Kennedy et al. 2012).

Threats to Rare Species

Habitat Loss

The main direct threat to rare plants in Oregon is the modification of land for human uses (Kaye et al. 1997). In Oregon, grazing, timber harvest, recreation, urbanization, agriculture, and mining all impact natural habitats and potentially affect native plant populations. The majority of the rare and endangered plants in Oregon are negatively affected by grazing and timber harvest, compared to the other land uses listed above (Kaye et al. 1997). Livestock grazing modifies habitat by removing above ground vegetation, increasing soil compaction and erosion, and altering nutrient cycling and the biological communities (Fleischner 1994).

Areas conserved as relatively unaltered habitat can still be affected by ecosystem fragmentation, and many species may be unable to disperse between “islands” of suitable habitat (Saunders et al. 1991). In addition, fragmentation affects larger scale ecosystem functions such as nutrient cycling, hydrology, wind and light intensity on the edges of each patch. Habitat fragmentation can also effectively lower the interbreeding population size (see “Genetic Threats” below).

Climate change can also result in habitat loss for a target species (Parmesan 2006). Recent changes in the climate associated with greenhouse gas emissions have caused a change in the distribution and phenology of plants across multiple ecosystems. Species which have a narrow range are the least resilient to this. Although some populations can adapt to local climate change, there is little evidence that new adaptations can spread rapidly enough at the species level to counter the negative impacts of climate change. As the climate changes faster than species can adapt, their current ranges may not be able to support them in the future (Dalrymple et al. 2012; Haskins and Keel 2012). Some ecologists have suggested assisting narrowly endemic species in their migration through outplanting to areas predicted to be suitable as the climate changes (Kutner and Morse 1996; Maschinski et al. 2012).

As humans have increased their ability to travel long distances, the introduction of exotic species has become more common and is now considered one of the largest threats to biodiversity (Gurevitch and Padilla 2004). Exotic species often colonize an area after it has been disturbed (Hobbs and Huenneke 1992). Accordingly, the decline of many rare plant species is related to both habitat modification and the proliferation of invasive weeds (Gurevitch and Padilla 2004).

Genetic Threats

Inbreeding depression, a reduction in fitness due to genetic drift increasing the frequency of deleterious alleles in a population, can be a major threat to rare species (Barrett and Kohn 1991; Hedrick and Kalinowski 2000). Genetic drift refers to the random change in allele frequencies from generation to generation in a sexually reproducing population, in contrast to natural selection which favors increased fitness (Crawley 1997). As the population size decreases, the amount of genetic drift increases, because it is more likely that a few random individuals will contribute a disproportionate amount of genes to the next generation. Therefore small populations are more likely to have deleterious alleles rise in frequency and become fixed in their gene pools, preventing natural selection from favoring other alleles.

Figure 7 shows a simulation (developed by the author using R statistical software and Microsoft Excel) of five selectively neutral genes in a population of 10, 100 and 1,000 randomly mating, annual, diploid individuals. Each gene has two alleles which started at a 50/50 ratio in the population. Individuals were randomly selected, with replacement, to contribute to the next generation (assuming the population size is stable). This simulation illustrates that there is increased genetic drift in small populations.

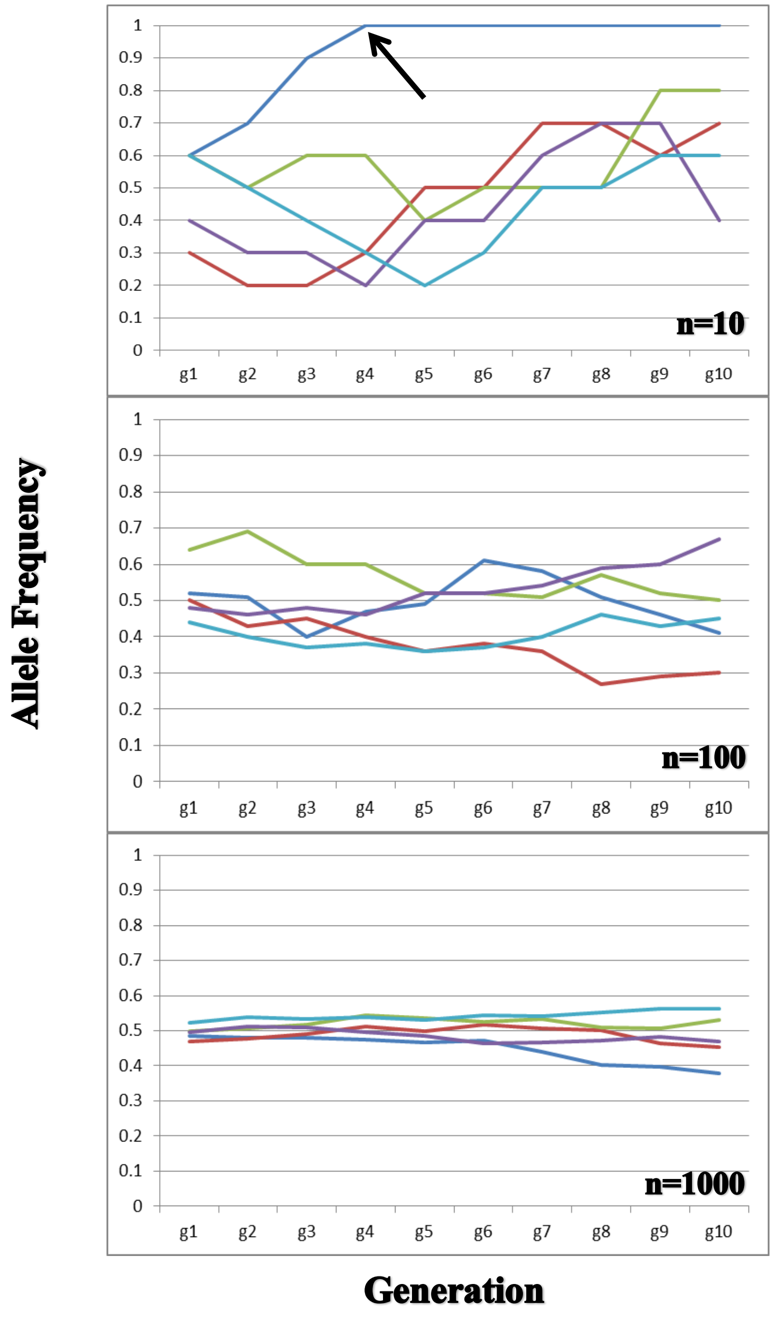

Figure 7. A computer simulation showing genetic drift in a population of 10 (top), 100 (middle) and 1,000 (bottom) randomly mating diploid individuals. The Y axes represent the proportions of the population containing a particular allele. The X axes represent the generations since the ratio of each allele was 50/50. The line indicated with an arrow shows an allele which became fixed in the fourth generation of the population through genetic drift alone.

Inbreeding depression has several important effects on rare species. The decreased genetic diversity through randomly fixed alleles prevents the population from adapting in response to changing environmental pressures, and can limit range expansion by restricting the ability to adapt to new habitats (Huenneke 1991). In addition, inbreeding depression can lead to an "extinction vortex" in rare species, a negative feedback loop in which the reduction in population size reduces fitness and the reduction in fitness further reduces the population size (Solé and Mills 1998).

The threat of inbreeding depression in rare plant reintroductions is two-fold. First, rare plants are often composed of small population sizes. Second, only a fraction of the number of individuals in these populations is selected to form the genetic structure of the new population (Monks et al. 2012). Although a created population may increase in numbers to a level which is similar to a natural population, the effective population size will still be low due to the limited genetic diversity of the population founders (Crawley 1997). It is important to consider the concept of effective population size, since the goal of a reintroduction is typically to create adaptive populations.

Rare ecotypes or small populations with unique gene pools that are in close proximity to more abundant common ecotypes are subject to genetic swamping (McKay et al. 2005). This is because there is more pollen migrating from the large population to the small population than vice versa. For this reason, augmenting a small extant population with a large number of individuals should be done with care, since unique alleles present in the smaller extant population could be lost through genetic swamping.

Current Issues in Reintroduction

Definitions and Goals of Reintroduction

Reintroduction has been defined as “the release and management of a plant into an area which it formally occurred, but in which it is now extinct or believed to be extinct” (Godefroid et al. 2011). Occasionally, outplanting projects for narrowly endemic species are performed outside the taxons’ known range (Morse 1996). However, reintroducing into the historic range of the species is ideal, as this process is likely to better match the species with the environment in which it evolved (McDonald 1996).

Although reintroducing a species within its historic range is preferable, it may not be practical for several reasons. Historic records for rare plant ranges are often inaccurate or incomplete, especially for those species experiencing a significant range reduction prior to the initiation of rare plant surveys. There may also be a lack of suitable habitat remaining within the known historic range of a species. For example, the historic range of a species may consist of privately owned land, and land owners may be uninterested in conservation. In addition, as the climate changes, some projects have included climate change models for assisted migration to new areas which are projected to have suitable habitat (Maschinski et al. 2012). Since the ideal goal of reintroducing a species back into its historic range is often not possible, selection of the best available habitat may be necessary.

The success of a reintroduction project can be measured in two ways: “biological success” and “project success” (Pavlik 1996). The “biological success” of a reintroduction can be evaluated by monitoring for survival, growth, and reproduction. “Project success” can be evaluated based on the amount of information gained about the species, or reintroductions in general, through the work. Although a project may be extremely successful biologically, if it is not set up in a way that allows for the reasons of success to be determined (baseline data collection, randomized set-up, a diversity of environmental conditions, etc), then it may have low project success. This is because it is difficult to make generalizations which can be applied to future projects. Therefore, when possible, both “biological success” and “project success” should be considered as goals in creating a new population.

Selecting Reintroduction Sites

Selecting appropriate reintroduction sites is a challenge due to the biological, physical, logistical, and historical factors (Fiedler and Laven 1996). The presence of appropriate pollinators and fruit dispersal agents may be critical to success for some species, as may the presence of appropriate mycorrhizae in the soil (Moora et al. 2004). Physical characteristics to consider include soil quality, climate, hydrology, light intensity, and elevation. Land ownership, accessibility, and requirement that the land owner or manager commit to multiple years of monitoring may also influence reintroduction site selection. The location of the reintroduction site in relation to the historic range of the species (if known), and the previous land uses at the site may also be important.

However, geographic affinity is not necessarily a reliable predictor of reintroduction success. Instead, the ecological similarity of the source and reintroduction sites may be a more appropriate predictor (Lawrence and Kaye 2011). Critics of the “local is best” paradigm suggest that keeping sources too local may maintain fragmentation and inbreeding within disjunct populations, resulting in less evolutionary adaptability (Sgro et al. 2010). In addition, due to changes in natural environments, the current habitat may not reflect the conditions in which the alleles were selected. Although using local sources for reintroduction plant material may prevent potential issues such as genetic swamping of a local ecotype, the sites closest to the source population may not be the most optimal for reintroduction (McKay et al. 2005). Because the current genetic make-up of locally adapted gene pools can be based on stressors present in the past, in a changing environment there are likely to be “time lags” resulting in species that are adapted to conditions no longer present in their current location (Dawkins 1982; Sgro et al. 2010).

Selecting Population Founders

One question that arises when creating populations of rare plants is whether genetic diversity should be maximized by reintroducing propagules from multiple sources in a single site, or should outplanting stock be kept separate to preserve locally adapted genetics (Angermeier and Karr 1994)? Ecotypes are typically kept separate to conserve locally adapted genetics and prevent potential outbreeding depression, a loss in fitness due to a break up of co-adapted genes or introduction of maladaptive intermediates

(Hufford and Mazer 2003). However, populations created with multiple sources can exhibit increased average heterozygosity and diversity of allele combinations, with no negative effect on fitness, and increased environmental adaptability (Gustafson et al. 2002).

Including new allele combinations in reintroductions allows the environment to select against deleterious alleles that have become fixed through genetic drift (Hedrick and Kalinowski 2000). Mixing ecotypes in reintroductions may result in increased heterozygote fitness and adaptive variability due to the introduction of new allele combinations that are available for selection. However, potential increases or decreases in fitness due to mixing ecotypes should be evaluated on a case by case basis depending on the scale of differentiation between ecotypes. A genetic study done on *Silene vulgaris*, a widespread introduced species in North America, indicated that inbreeding reduced fitness and crosses between regions had mixed results (some regions showing increased fitness, and some showing decreased fitness; Bailey and McCauley 2006). This illustrates that even within a single species the potential increase or decrease in fitness as a result of crossing ecotypes is context dependent and should be determined through experimentation.

Ultimately we must determine whether “biological integrity” or “biological diversity” is the main goal of a reintroduction. Creating populations with multiple sources may increase genetic diversity, but decrease genetic integrity, since the gene combinations in each donor population are related to the specific conditions in which they evolved (Angermeier and Karr 1994). The logic behind the idea that integrity should be preserved over diversity is based on the assumption that humans should not alter the current distribution of ecotypes. However, if human activity is the principle reason for ecotype differences, it could be argued that mixing ecotypes can increase genetic diversity to a level similar before fragmentation occurred (Gustafson et al. 2002).

Some species, such as the Torrey pine (*Pinus torreyana*) have little or no genetic variation within populations, but exhibit variation between populations (Millar and Libby 1991). In cases such as this, it may be better to mix ecotypes in reintroductions since the differences between ecotypes are most likely the result of genetic drift associated with a founder effect rather than natural selection. However, this is a complex issue, and experimental reintroductions in which ecotypes are introduced both separately and together may be the best way to determine the effect of mixing different genetic sources on fitness (Guerrant Jr. 1996).

Collection and Cultivation

Determining the appropriate life history stage to be used as stock for a reintroduction project is critical for its success. For example, seeds, rhizomes, bulbs, and cultivated juvenile or adult plants can all be used. In general, cultivation to adult plants before outplanting increases the chance of survival, since the plants can be fertilized and the natural enemies (herbivores and pathogens) can be controlled in the early, most vulnerable, stages of development (Albrecht and Maschinski 2012). Because removing a large number of seeds or adult plants from a source population could negatively impact the populations further, care should be taken to prevent disturbance of the existing source plants. However, in cases where the source population cannot be conserved (i.e., it is on private land slated for development), it may be better to collect as much as possible for reintroduction on protected land.

Clonally propagating plants to produce stock for outplanting produces large numbers of plants for species that reproduce poorly by seed, such as *Fritillaria gentneri* (Amsberry and Meinke 2005). A large population created from clones effectively functions like a small population due to the low genetic diversity. However, for species that do not produce much seed, collection and propagation of clones is a good way to reduce the impact to the source populations. Although many clonal plant species have been very successful in nature (Cook 1983), the lack of genetic recombination can cause DNA mutations to be accumulated in the somatic meristem cells (Weins et al. 1988) and can lead to a "mutational meltdown," an accelerating reduction in fitness known as Muller's ratchet (Gabriel et al. 1993). This phenomenon was pointed out by the geneticist Muller in 1964 when studying small asexual populations. The basic idea is that because there is no meiotic genetic recombination or sex in clonal populations, each new generation of clones will have no less mutations than the parents of the clones. In a sexually reproducing population, these mutations can be lost in some gametes through genetic recombination in meiosis, or masked through a non-mutated allele in one of the parents. Therefore, when outplanting clonally propagated stock in which seed production is limited by genetic diversity, it may be best to include multiple sources to allow genetic recombination to occur during seed production.

<u>Special Considerations for Reintroducing Wetland Plant Species</u>

Properties and Definitions of Wetlands

Types of Wetlands

Although there are many different definitions of wetlands, the United States Environmental Protection Agency (EPA 2013) states that:

> “Wetlands are areas where water covers the soil, or is present either at or near the surface of the soil all year or for varying periods of time during the year, including during the growing season. Water saturation largely determines how the soil develops and the types of plant and animal communities living in and on the soil. Wetlands may support both aquatic and terrestrial species. The prolonged presence of water creates conditions that favor the growth of specially adapted plants and promote the development of characteristic wetlands soils."

However, this encompasses a wide diversity of habitat types and can be subdivided into several ecologically unique groups, such as marshes, swamps, bogs, and fens. These wetland types are diverse in terms of their physical properties and include very different species (EPA 2013).

Physical Characteristics and Nutrient Cycling

As mentioned above, wetlands include permanently and temporarily wet areas that may contain flowing or static water. This includes fresh, brackish, and salt water.

When wetlands are drained for other land uses, such as to prevent flooding, restoring the original hydrology is often complex and not easily accomplished (Zedler 2000).

The dominant vegetation type in a wetland is also an important factor that may affect the hydrology through changes in transpiration and stream flow (Huxman et al. 2005). According to the EPA definitions, marshlands are dominated by grasses, whereas swamps are dominated by trees or shrubs. Bogs and fens are dominated by *Sphagnum* moss and differ primarily in their acidity (bogs being more acidic; EPA 2013). Because wetlands are diverse in their physical characteristics, the plant communities adapted to them are similarly varied (i.e., species that can only live in ephemeral wetlands may be annuals and put out a seed bank to survive during the off season; whereas a perennial species adapted to being submerged year round might be dependent on the water for structural support).

Wetlands have unique soils and nutrient cycling dynamics that can be complex and sensitive to changing land use (Verhoeven et al. 2006). Fluctuations in the saturation of the soil can result in the presence of differing communities of microorganisms due to the presence or absence of high levels of oxygen. Changes in wetland use can result in nutrients becoming unavailable to plants or other organisms, since the microbial community often plays a large part in nutrient cycling. In addition, changes in wetland use can result in inefficient nitrification and denitrification, increasing the production of N_2O, a greenhouse gas that also contributes to stratospheric ozone depletion (Morse and Bernhardt 2013).

Using Wetland Indicator Species

Wetland plants vary greatly in their habit and form. They may be rooted in saturated soil, completely submerged by water, possess floating leaves, be emergent from water, or free floating. Wetland plants often have unique adaptations that allow them to survive in inundated conditions. One example of this is the production of arenchyma tissue (Evans 2003). This is a special porous tissue that facilitates gas exchange in the underground portion of plants in soil inundated by water. Other adaptations include reduction of the cuticle, linear divisions of the leaves, and extensive vegetative reproduction (Cronk and Fennessy 2001).

Because wetland hydrology is variable, wetland indicator species are often used in order to delineate wetland boundaries (Tiner 1991). A plant species can be given a wetland indicator rank based on the frequency it is found in a wetland or upland area. There are five main rankings: obligate wetland species, facultative wetland species, facultative species, facultative upland species, or obligate upland species. This ranking system has been effectively used to delineate wetland boundaries and is often a good predictor of the hydrological regime. However, since both obligate wetland plants and facultative wetland plants can occur in the same area, weighting the percent cover of each wetland indicator category, and calculating the average weighted wetland indicator number, can give a more precise estimate of whether an area will support wetland species in a reintroduction (Amsberry 2001).

Ecological and Economic Importance of Wetland Plants

Wetland plant species have many important ecological functions. They form the basis of many aquatic food webs, and also provide habitat for insect larvae, fish, amphibians, and migrating waterfowl (Tiner Jr. 1984). In addition, maintaining wetlands in agricultural areas increases regional species diversity (Thiere et al. 2009). Wetland vegetative communities provide shading and bank stability, increasing the amount of dissolved oxygen and reducing the amount of particulate matter in streams, both key factors affecting the health of fish and filter feeding arthropods (Osborne and Kovacic 1993). Since wetland plants can survive in regularly inundated areas, they provide an important source of biodiversity and habitat complexity in these inundated areas. Vegetative diversity increases the diversity of microbial organisms responsible for important nutrient cycling processes (Zak et al. 2003).

Wetland plant communities provide a variety of important ecosystem services. They help remove excess ammonium from surface waters as well as filter toxins from drinking water (Verhoeven et al. 2006). Wetland vegetation slows flood water, providing an economic advantage during large storms that otherwise might cause damage to crops or property (Mitsch and Gosselink 2000). In addition, wetlands provide habitat for economically important fish and waterfowl. Shallow water wetlands are estimated to contribute to about 40 percent ($33 trillion per year) of global ecosystem services, even though they only make up about 1.5 percent of the Earth's surface (Costanza et al. 1997).

In addition to the ecological and economic importance of conserving wetland plant species, wetlands have intrinsic worth, such as aesthetic and educational values. It can also be argued that we have a moral responsibility to prevent extinction of any species, as a result of our actions. The values that people place on wetlands and wetland species are difficult to measure, but related to factors such as gross domestic product as well as population density in areas surrounding the wetlands (Brander et al. 2006).

Wetland Restoration and Rare Wetland Species Reintroductions

Many of Oregon’s native wetlands have been modified for agriculture, rangeland, and urban development. For example, urban growth in Portland, Oregon between 1982 and 1992 resulted in the degradation and loss of 40 percent of surrounding wetlands (Holland et al. 1995). Cattle graze in riparian corridors frequently in the Blue Mountains area of northeastern Oregon, sometimes removing 75 to 85 percent of stream bank vegetation (Gillen et al. 1985). Other human influences in Oregon that degrade or fragment habitats for wetland species include mining, logging, road construction, water diversion for irrigation, fertilizer seepage, and dam construction (Wissmar 2004).

Although wetland restoration is similar to upland restoration in its goals of restoring biodiversity and ecosystem function, wetlands are often much more complicated to restore (Zedler 2000). The historical flooding and disturbance regimes are often lost when wetlands are drained and converted to other land uses. In addition, the surrounding landscape in which a wetland is restored can affect the functioning of the wetland. For example, poorly placed restored wetlands can result in large amounts of sediment from runoff water in the surrounding area. For all of these reasons it is often difficult to predict the success of restoring a wetland (Zedler 2000).

Wetlands which are restored from agricultural lands often have a loss of microbial mediated nutrient cycling (Morse and Bernhardt 2013). Large inputs of fertilizer often negatively affect microorganisms involved in the nitrogen cycle (Matson et al. 1997). Once the input of fertilizer stops, the soil becomes nutrient deficient until the microbial community can be restored. This is problematic since many wetlands are converted to agriculture due to the proximity to a water source for irrigation (Verhoeven et al. 2006).

A meta-analysis of the reintroduction of 25 rare wetland plants on restored wetlands suggests that the most important predictor of success is ecological similarity to the source sites (Noël et al. 2010). Ecological similarity in this meta-analysis was calculated based on ecological indicator traits of the species present at each site. The authors used a multivariate ordination method to scale ecological similarity, and then calculated the distance in multivariate space between the source sites and the restored wetlands across all 25 species. Since it is difficult to predict how similar a restored wetland will be compared to the source wetlands, comparing ecological communities at the source and restored wetland may be a good approach for restoring wetland species.

Although comparing vegetative communities in terms of their species cover is important information to have, using traits of the species to compare communities is also commonly done (McCune et al. 2002). Comparing traits of species, rather than species themselves, can be useful for making generalizations about community similarity, since some species may not occur in the sites that are compared. In addition, comparing species traits allows for interpretations that can be linked back to environmental traits in the communities being compared.

CHAPTER 3: MATERIALS AND METHODS

Objectives and Questions

The main objective of this study is to research methods for creating new populations of *Pleuropogon oregonus* which are self-perpetuating. This would provide information about the species and can be considered a “project success” criterion (Pavlik 1996). The three main questions being asked for this are: 1) Can outplanting success be predicted by source ecotype and environmental characteristics of the destination site? 2) Should the ecotypes be mixed or kept separate in reintroductions? 3) Can the suitability of an outplanting site be predicted based on its similarity to the habitat at the source site?

A second objective is to create an additional population on protected land to assist in the conservation of the species. Since this objective is not related to gaining information about reintroducing the species, but directly assisting its conservation, it is considered a “biological success” criterion (Pavlik 1996).

These objectives will be evaluated through an experimental outplanting in which both ecotypes are reintroduced at the same site. They will be placed across a gradient of conditions in an area with both ecotypes and areas with each one separate.

Collection and Cultivation

In the spring of 2012, a total of approximately 150 tillers of *P. oregonus* were collected, as clumps of rhizomes with about 30 to 50 tillers attached, from one source population in Union County and one in Lake County (Figure 8). Tillers were defined as aboveground shoots growing from a rhizome which could become physiologically independent clones if separated from the rest of the plant (refer back to Figure 5). A clump is a group of tillers which may or may not be connected to each other physiologically. Small numbers of plants were collected in order to minimally disturb the natural populations. Although plants were growing vegetatively, they had not yet developed reproductive stalks. Each clump was collected with the soil surrounding the roots intact and stored in a clear plastic bag. Bag tops were left open and kept cool during transportation to prevent desiccation.

Figure 8. Union County site collection, spring 2012. Clumps were placed in gallon sized zip-lock bags with soil surrounding roots left intact.

To prepare the plants for cultivation, each clump of *P. oregonus* was divided into smaller groups of about 10 tillers and evenly distributed in small plastic swimming pools filled with wet potting mix (Figure 9). Ecotypes were kept in separate pools throughout the process. Plants were cultivated in the Oregon State University greenhouse yard in Corvallis, Oregon during the summer of 2012. To promote growth, plants were regularly weeded and watered until inundated, and were fertilized weekly using a mixture of 7.5 L tap water and 2 ml DynaGro (N:P:K = 7:9:5) until one month before outplanting.

Figure 9. *Pleuropogon oregonus* growing in swimming pools during the summer of 2012 at the Oregon State University greenhouse yard. Plants reproduced clonally until the pools were filled.

Before the outplanting in September 2012, plants in these pools were divided into clumps of about 20 tillers each and the soil was gently removed from the rhizomes in a bucket of water (Figure 10). Each of these clumps were placed in an individual bag and kept cool during transportation to the reintroduction site.

Figure 10. Soil being removed from *Pleuropogon oregonus* rhizomes in preparation for outplanting.

Site Selection

End Creek is a restored wetland near La Grande in Union County (Figure 11). In the mid-1800s through early 1900s, End Creek, along with many of the other wetlands in the Grande Ronde valley, was drained (Antell 2010). Up until the 1950s the site was used as a dairy farm. After that End Creek was farmed for wheat until it became a part of the Federal Wetlands Reserve Program in 2005. At this time, natural flooding cycles were returned and native vegetation reemerged. Additionally, native species were planted to provide a wetland habitat corridor for salmon. End Creek is currently managed by the Blue Mountains Conservancy. Students and researchers use the area as an ecological preserve and a research site (Antell 2010).

Figure 11. End Creek is a protected wetland managed by the Blue Mountains Conservancy. It is used as an ecological preserve and habitat corridor for salmon. In addition, it is used as a study site for students at Eastern Oregon University. It was drained sometime between the mid-1800s and early 1900s and only recently restored as a wetland (Antell 2010).

Although this area is a restored wetland that may be ecologically different from the source sites, it was the best available habitat on protected land known to us in Union County. By comparing the vegetative communities at End Creek to the source sites, this will help answer the question of whether restored wetlands provide a similar ecological community to the source wetland. Because wetland draining, as described above, results in habitat destruction for *P. oregonus*, determining whether a restored wetland will be able to support *P. oregonus* may be critical for the future survival of the species.

Plot Set-Up, Baseline Data Collection, and Outplanting

To determine if *P. oregonus* ecotypes should be mixed or kept separate in future reintroductions, three outplanting areas (Figure 12) were selected at End Creek for this study (one for mixed ecotypes, one for Lake County plants and one for Union County plants). To determine if environmental characteristics of the reintroduction sites could predict establishment success, each area was subdivided into four habitat types: wet and shady, wet and open, dry and shady, and dry and open (Figure 13). In each of these habitat types, a linear transect with 5 $1m^2$ plots was established, using PVC pipe and pin flags to mark the corners of each plot, for a total of 20 plots in each area (Figure 14). Although plots were set up in this fashion to create a similar gradient of conditions in each outplanting area, habitat heterogeneity between areas and within habitat types made it necessary to measure vegetation and environmental characteristics for each plot quantitatively.

Figure 12. Three outplanting areas were selected in order to evaluate the difference between mixing ecotypes and keeping ecotypes separate in reintroductions. The red dots represent the ends of each transect.

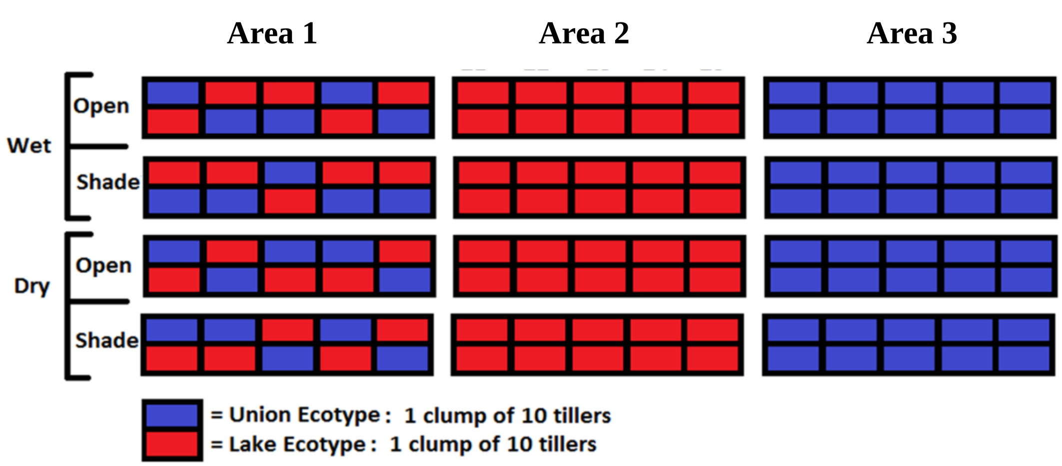

Figure 13. Each area was subdivided into four different habitats to create a gradient of environmental conditions. Due to habitat heterogeneity between areas and within habitat types, baseline environmental and vegetative cover was measured in each plot.

Figure 14. *Pleuropogon oregonus* clumps being watered after planting.

Before the outplanting in mid-September 2012, baseline data on plant species cover and environmental conditions in each plot were measured. The vascular plant species in each plot were identified and the percent cover of each was visually estimated. Light intensity data at 1 m height was measured using an Extech™ light meter. Volumetric soil moisture was measured from the surface soil using a Hydrosense™ moisture probe. Soil acidity was also estimated using a Kelway® direct reading pH tester. If the soil was too dry for an accurate reading, water was added to a sample of the soil to create a slurry.

Four hundred tillers total were planted in each of the areas at End Creek. In Area 1, all plots contained both ecotypes randomly assigned to one side of the plot or the other. In Area 2 only the Lake County ecotype was planted and in Area 3 only the Union County ecotype was planted. Each plot had two clumps of rhizomes with 10 tillers each, placed about 20 cm apart from each other. After the clumps were planted, they were each watered with about 0.5L of water to reduce transplant shock. This was the only external habitat modification performed during the reintroduction.

In addition to Areas 1-3, seven bulk plots were set up along a stream bed in between the planting areas to assist in the biological success of the reintroduction at End Creek. Because *P. oregonus* is wind-pollinated, these large plots were needed in order to allow sufficient pollen movement to promote caryopsis production. Two bulk plots were planted with the Lake County ecotype alone, two plots with the Union County ecotype alone and three plots with both ecotypes. All bulk plots received approximately 125 tillers broken up into clumps of about ten.

Monitoring

The outplanted population at End Creek was monitored on July 25th 2013 for survival, growth, and reproduction. Although we had planned to install pollinator exclusion bags in order to collect caryopses later in the year, none of the plants flowered in 2013, and bags were not necessary.

Within each plot, emergent tillers were counted. In addition, the number of leaves produced by tiller was counted and each leaf blade length was measured. After measuring the widths of several leaves, it was assumed that all leaves were very close to one centimeter in width, since there was very little variation between plants. Because rhizomes may put out multiple tillers or focus on leaf production in a few tillers, a vegetative robustness metric of total leaf area per plot was used, assuming each leaf was 1cm wide.

Data Analysis

Although fitness is ultimately measured as reproductive success, vegetative robustness can be an indicator for increased seed production in future years (Hutchings 1997). In addition, plants that survive are more fit than plants which did not survive, since they have the ability to reproduce in the future. Although plants did not produce inflorescences this year, survival (emergence of any tillers in a plot) and vegetative robustness (total leaf area in a plot) were analyzed to estimate fitness in future years.

R Statistical Software version 2.13.1 was used for data analysis. I used two different measures to estimate fitness: survival, and total leaf area per plot. Since survival is a binomial response variable, I used a general linearized model with a binomial logistic distribution to estimate parameter effects and error (Ramsey and Schafer 2002). Since there was non-constant variance in the leaf area graph, I performed a log(x+1) transformation so linear regressions could be performed. With both the survival and leaf area regressions, I started with a fully saturated model with the predictors of: ecotype (a binomial predictor), soil pH, light intensity, volumetric soil moisture, and all interactions between these. I then did a backwards elimination of predictors with the least significance and compared the Akaike Information Criterion (AIC) values to determine the simplest adequate model (Ramsey and Schafer 2002). Since the interaction between soil moisture and light intensity was significant, this term was labeled "habitat suitability" and was rated on a scale of 0 to 10 (0 = full shade and/or no moisture, 10 = open sun and fully saturated soil).

A percent cover matrix of vascular plant species in fifteen 1 m^2 plots that were occupied by *P. oregonus* in the Lake and Union County sites (from Gisler and Meinke 2003) was added to the percent cover matrix of the End Creek plots. This matrix was relativized by total cover per plot, so that the total cover in each plot equaled 100 percent. For each species in the matrix, the following traits were attributed based on the USDA Plants database (USDA 2012): wetland indicator status (wetland obligate, wetland facultative, facultative, and upland facultative), native status (native or exotic), and life history (perennial or annual). In addition, a growth habit trait of graminoid (Poaceae, Cyperaceae, and Juncacae) or non-graminoid was used to separate the species into functional groups. A matrix of percent cover of each trait per plot was calculated by multiplying the percent species cover matrix by the species trait matrix. A weighted average wetland indicator status was calculated by assigning a value of 1-4 for each wetland indicator category, multiplying the percent cover by this and then adding the total weighted cover.

For the community analysis I used the "vegan: community ecology" package (Oksanen et al. 2011) available in R. I created a distance matrix of the percent cover of community traits using a "city block" distance measure. This was done to approximate dissimilarities in the community traits of each plot. Because the pairwise comparisons of community traits showed non-linear relationships (as is common with community data; McCune et al. 2002), I used Kruskal's Non-metric Multidimensional Scaling (NMS). I used a maximum of 500 iterations, a convergence tolerance of 0.00001 and 100 random starting configurations. These values represent a slow and thorough search for the most representative ordination, with the lowest stress value. Stress is a measure of departure from monotonicity between ordination distances and the ranked dissimilarities of the distance matrix. A high stress value means that the ordination is a poor fit for the data (see McCune et al. 2002 for a summary on NMS and community analysis). To determine if survival in each plot was related to similarities to the source sites I calculated the shortest distance of each point on the ordination from the centroid of the source sites (average X and Y coordinates). I then used a binary logistic regression with community distance as an independent variable and survival as the dependent variable.

Average total monthly precipitation data in La Grande were taken from the Western Regional Climate Center website (WRCC 2013) and total monthly precipitation data from the year after outplanting were taken from the Weather Underground website (Wunderground 2013). Although plots in the three areas appeared to be similar on the m^2 level, lack of precipitation may have resulted in a shift in the water table. Because the mixed ecotype plots were in a different area than the separate ecotype plots, which appeared to be more recently farmed, there were landscape-wide differences not measured in the study. As a result of this, the mixed plots were excluded from the statistical analysis.

CHAPTER 4: RESULTS

Summary of Outplanting Success

Survival of *Pleuropogon oregonus* tillers was relatively low at the monitoring visit on July 2013 (Table 1). The exceptionally low amount of precipitation received in the La Grande area during the year following the outplanting at End Creek probably reduced survival (Figure 15). Rainfall was less than half of the normal amount in most months while the plants were at End Creek with no rainfall at all during November and December. This lack of precipitation following the outplanting may have caused some of the plants to desiccate before they became established.

Table 1. Summary of the July 2013 monitoring after out-planting on September 2012.

Area	Number of plots with re-emergence	Average tillers per plot from survivors in m^2 plots	Total number of tillers in bulk plots	Average number of leaves per tiller	Average leaf length of survivors
Mixed	0/20 plots 0/3 bulk plots	0	0,0,0	0	0
Lake	4/20 plots 2/2 bulk plots	3.33	4, 10	3.36	14.51
Union	10/20 plots 2/2 bulk plots	2.67	9, 4	3.92	19.61

Because no tillers emerged in the mixed plots, my initial question of whether mixing ecotypes increased caryopsis production could not be answered. Although planting Area 1 initially appeared to be similar in terms of soil moisture, light intensity, and percent cover of wetland indicator species to the other two areas, the stream was narrower and the water current appeared to be faster here (Figure 16). This area appears to be more recently farmed (refer back to Figure 12), and although the environmental conditions and plant communities were similar on the m^2 plot level, there were landscape-wide differences (such as water flow) not detected on the plot level. The low amount of precipitation, therefore, may have affected Area 1 more significantly by further narrowing the stream and exposing the plants to the dry banks. Because Area 1 appeared to be different than the other two areas in ways that were not measured in this study (i.e. landscape-wide differences), it was not included in the statistical analysis.

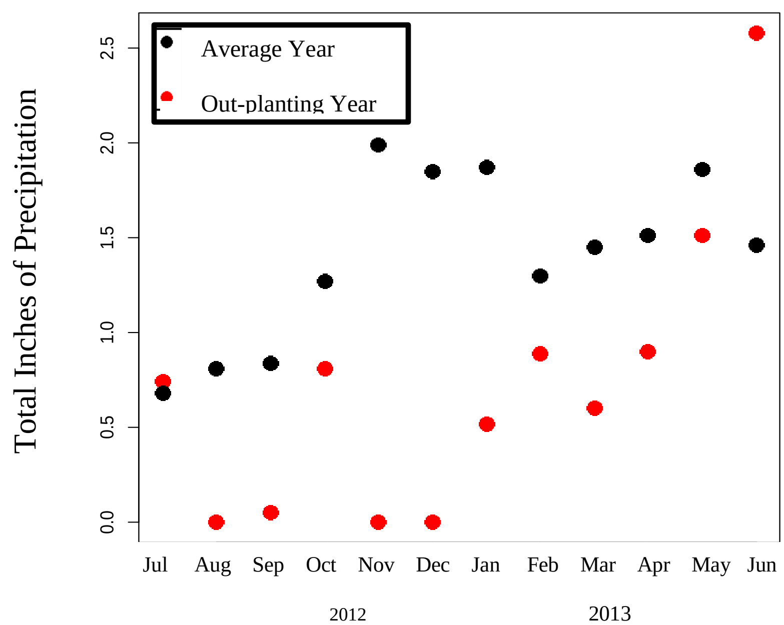

Figure 15. The abnormally low precipitation during the year after outplanting likely caused many plants to dry out before becoming established. Average climate data were obtained through the Western Regional Climate Center (WRCC 2013) webpage and precipitation in the year of outplanting were obtained from the Weather Underground webpage (Wunderground 2013).

Figure 16. Although plots in all three areas were initially exposed to similar environmental conditions, including percent cover of wetland indicator species, landscape-wide differences had a significant effect on survival. The low amount of precipitation after outplanting may have altered the water table more significantly in planting Area 1 (left) compared to the other two planting areas (right).

None of the introduced plants at End Creek produced inflorescences after outplanting, and so fitness was estimated using survival and total leaf area per plot rather than fecundity. Survival is a binary variable where 1 = plots with surviving plants and 0 = plots without surviving plants.

<u>Habitat Requirements</u>

The majority of the plants which survived were in plots in direct light (about 1,000 lux) and mostly saturated soil (about 90% of the pore space) (Figure 17). When the soil moisture level is multiplied by the light intensity, this value best predicts the odds of survival (since there are both dry and sunny plots and wet and shady plots in which plants did not survive). This interaction term, "habitat suitability," is scaled from 0-10 (0 = no light and/or soil moisture, 10 = full sun and completely saturated soil). The simplest adequate model for predicting survival included only two significant linear predictors: an interaction term between light intensity, and soil moisture and an intercept (Table 2).

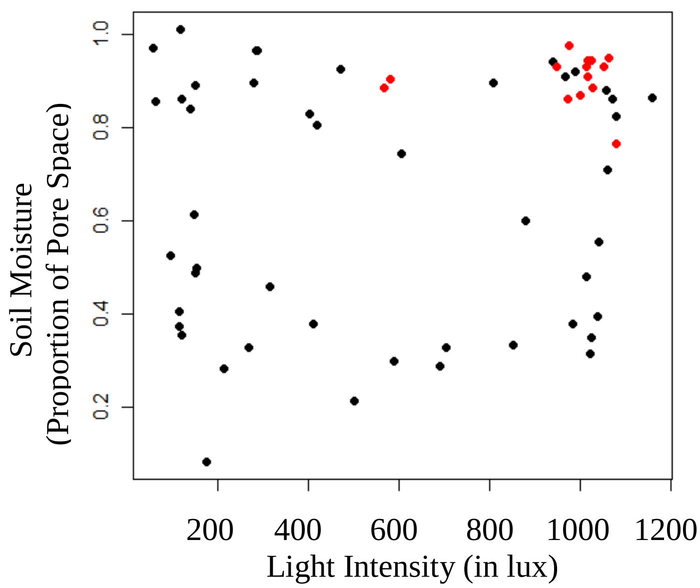

Figure 17. A scatterplot showing the interaction between light intensity, soil moisture, and survival of *Pleuropogon oregonus*. The red points indicate plots which contained surviving plants.

The coefficients and standard errors in a logistic equation are expressed in log odds and can be interpreted by exponentiation (Ramsey and Schafer 2002). The intercept's coefficient is -4.917, meaning that the odds of *P. oregonus* surviving in completely dry soil and/or full shade is nearly zero, or about a 7 in 1,000 chance. The light x moisture interaction had a significant positive coefficient of 0.7225, meaning that the odds of survival increase by about 106 percent for each unit increase in the light x moisture interaction term. When habitat suitability reaches about five, the odds of survival increase dramatically (Figure 18). There is a slight tapering in the odds of survival near the end of the graph due to the few plots in the upper range which did not contain surviving plants.

Table 2. Summary of the simplest adequate model describing the effect of the interaction of volumetric soil moisture and light intensity on the odds of survival of *Pleuropogon oregonus*. AIC= 26.3.

Coefficient	Estimate in log odds	Standard Error in log odds	P-value
Intercept	-4.9175	1.4797	0.000890
Habitat Suitability (Moisture x Light)	0.7225	0.1974	0.000253

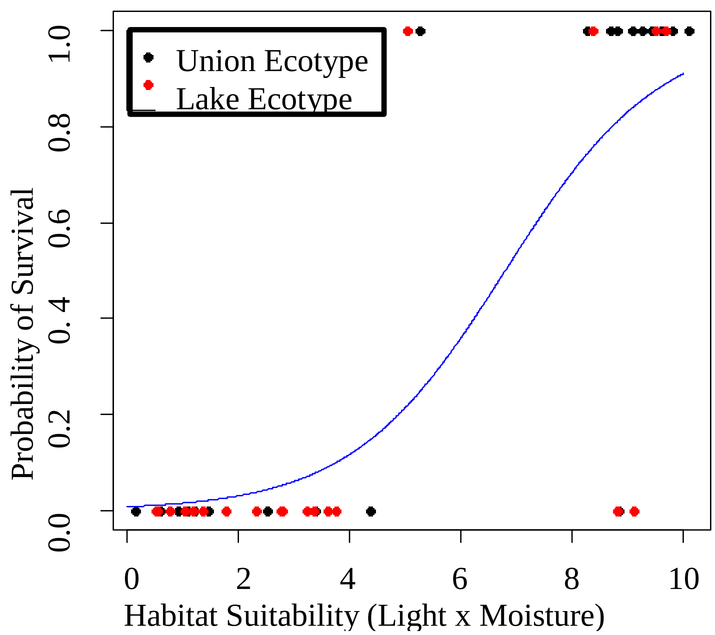

Figure 18. Logistic regression of the effect of habitat suitability (soil moisture x light intensity) on survival. The blue line represents the smoothed fitted model of the logistic regression.

The main difference between the logistic regression on survival and the multiple linear regression for log(leaf area per plot) was the detection of a difference between the Union County and Lake County ecotypes (Table 3). The interaction term between light intensity and soil moisture was very significant ($p<0.0000$) in this model (as it was in the logistic regression). This effect was the same with both ecotypes, but there was also some evidence that there was an overall difference in total leaf area per plot ($p= 0.0862$).

Table 3. Summary of the simplest adequate multiple linear regression model predicting the effects ecotype and habitat suitability on log(leaf area per plot). AIC = 144.7.

Coefficient	Estimate	Standard Error	P-value
Lake Intercept	-0.95304	0.39941	0.0223
Union Intercept	-0.15619	0.45203	0.0862
Habitat Suitability (Moisture x Light)	0.79685	0.06314	<0.0000

However, since there are many plots with no survival in the lower range of the graph, the assumptions of constant variance and normally distributed data are not met with all of the data points (Figure 19). Since zero is the lowest possible value, there is no variance in these plots, making interpretation of the standard error and coefficient estimates misleading. Another problem in interpreting the zero values using linear regression is the set minimum value for leaf area. Because zero is the lowest possible value, an area which is very unsuitable has the same fitness score as an area which is only slightly unsuitable. For this reason the logistic regression on survival data is probably a better model for estimating differences in fitness between each ecotype.

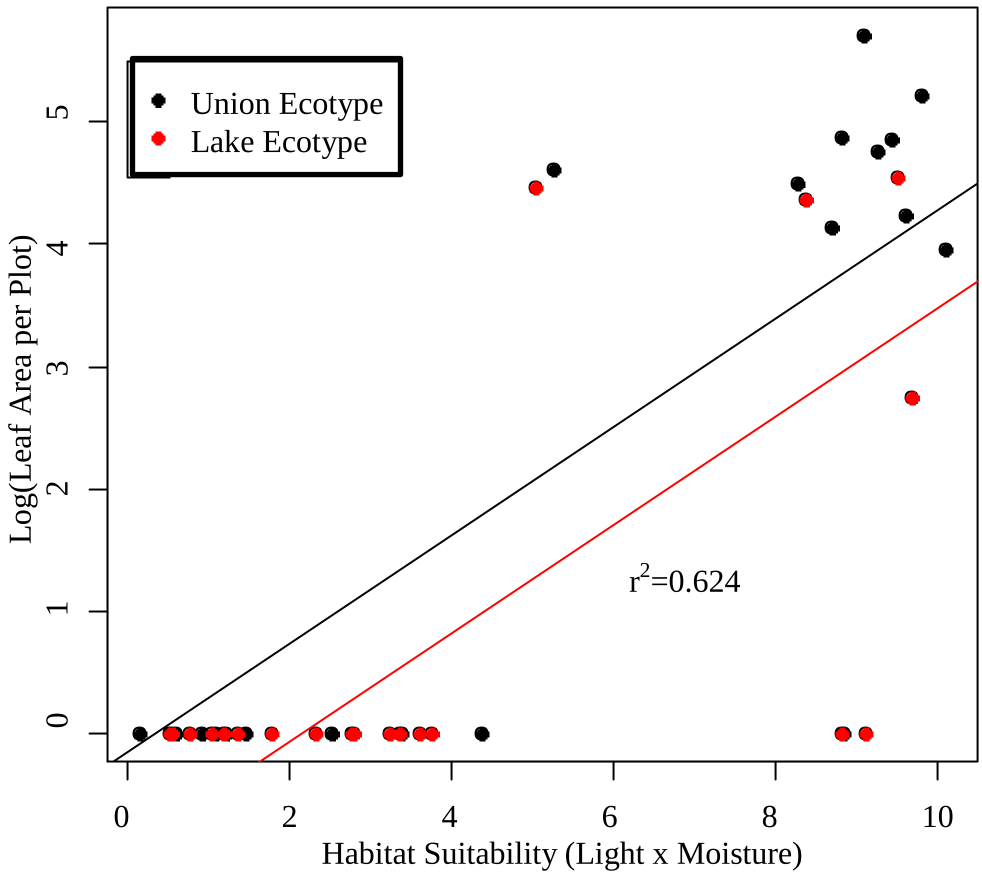

Figure 19. The effect of habitat suitability (soil moisture x light intensity) on log(leaf area per plot). Although the effect of light and moisture was the same for both ecotypes, there was less *Pleuropogon oregonus* leaf area overall in plots with the Lake County ecotype.

Community Analysis

The non-metric scaling ordination had a final stress score of 5.265057, indicating that it is a "good ordination with no real risk of drawing false inferences" (McCune et al. 2002). Values from the source sites in Union and Lake counties form a cluster of points in the ordination and most of the plots containing surviving plants are close to this cluster (Figure 20).

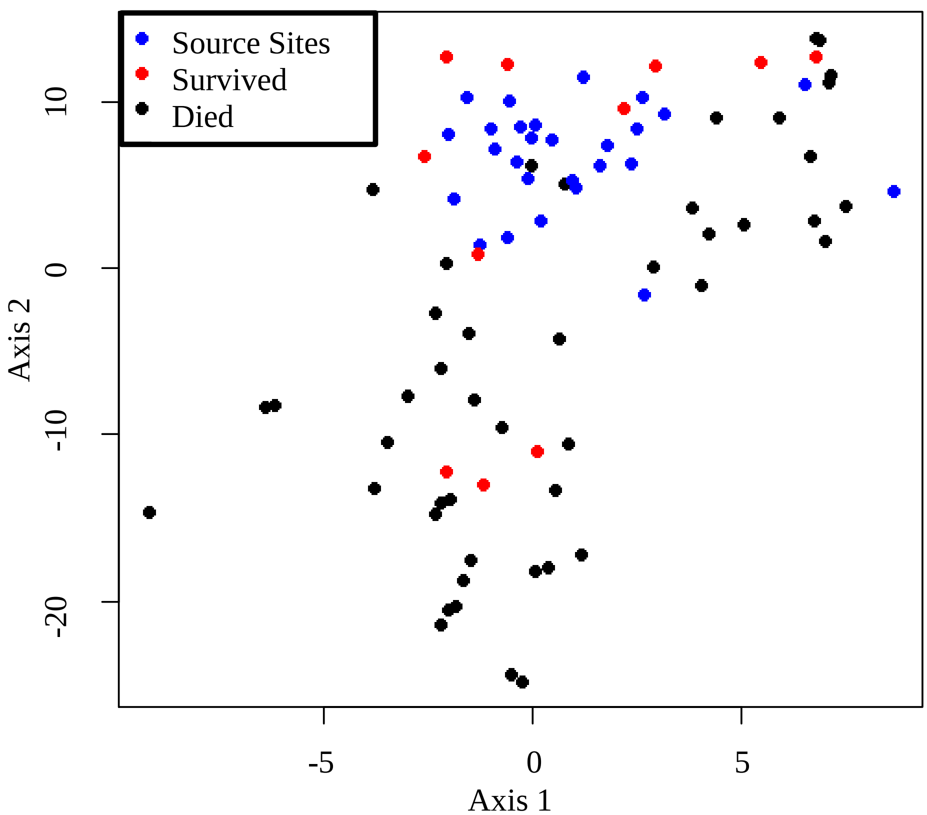

Figure 20. A Non-metric Multidimensional Scaling ordination with a final stress of 5.265057. Distances represent dissimilarities in community structure in each m^2 plot.

The Euclidean distance from the centroid of the source site on the ordination has a significant negative effect on the odds of survival (Table 4; Figure 21). The coefficient of the intercept (1.64173) suggests that if the plots were exactly on the centroid of the source site, the *P. oregonus* plants would have about a 5 to 1 chance of surviving. The coefficient for the effect of community distance (-0.17576) indicates that odds of survival decrease by about 16 percent for each unit of distance away from the centroid.

Table 4. Summary of the relationship of dissimilarities between the community structure at the source sites and survival of *Pleuropogon oregonus* indicated with a logistic regression.

Coefficient	Estimate in log odds	Standard Error in log odds	P-value
Intercept	1.64173	0.76229	0.03126
Community Distance	-0.17576	0.06098	0.00395

Since the distances of plots on the NMS ordination represent non-linear relationships with the community characteristics, these distances must be compared to the original predictors to interpret the meaning of distance from the source community (Figure 22). Plots that are similar to the source community have a higher percent cover of native species and a lower wetland indicator number (indicating a larger percentage of wetland obligate species). In addition, although there does not appear to be a linear relationship between the percent cover of perennial species and distance from the source community, all of the plots with surviving plants were also composed of only perennial species.

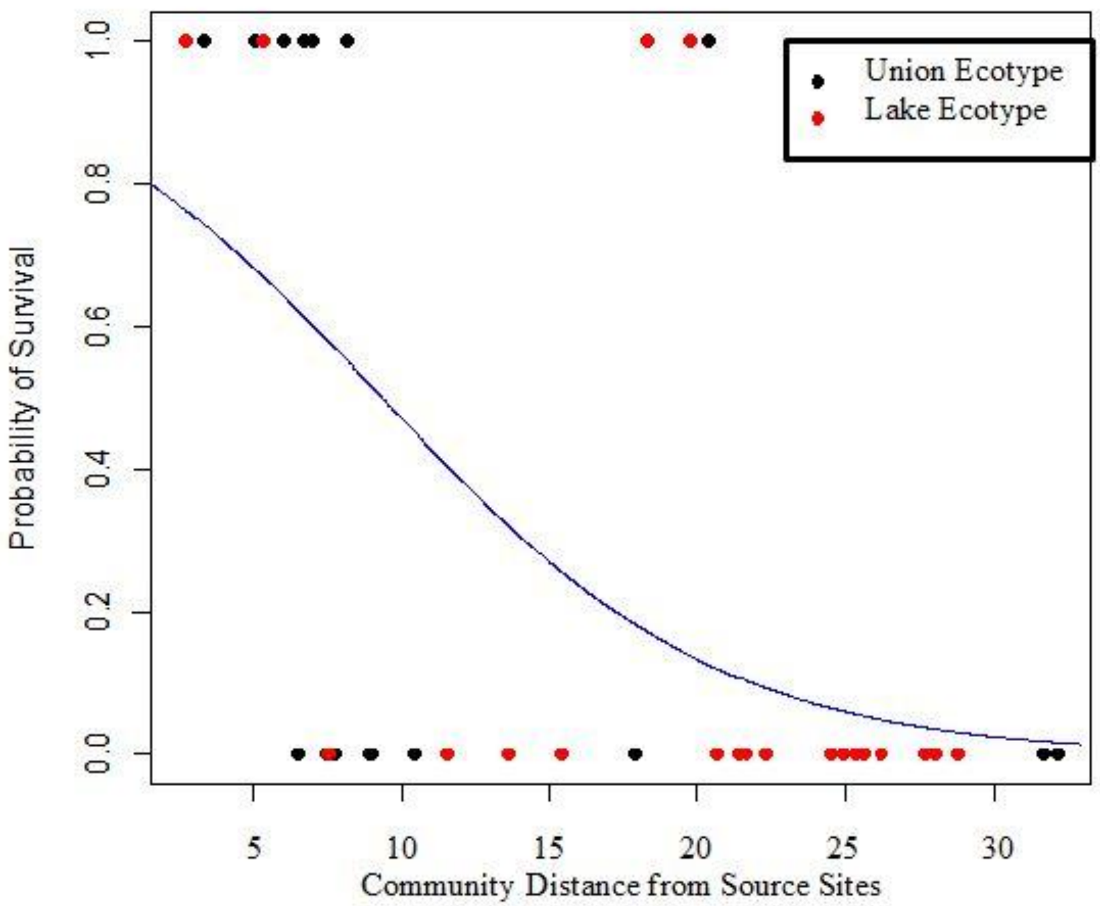

Figure 21. Logistic regression showing the effect of dissimilarity to the source community and the probability of survival. The blue line represents the fitted model of the logistic regression. Plants in plots that were similar to the source community had the highest probability of survival, whereas plots which had different plant communities were less likely to support *Pleuropogon oregonus*.

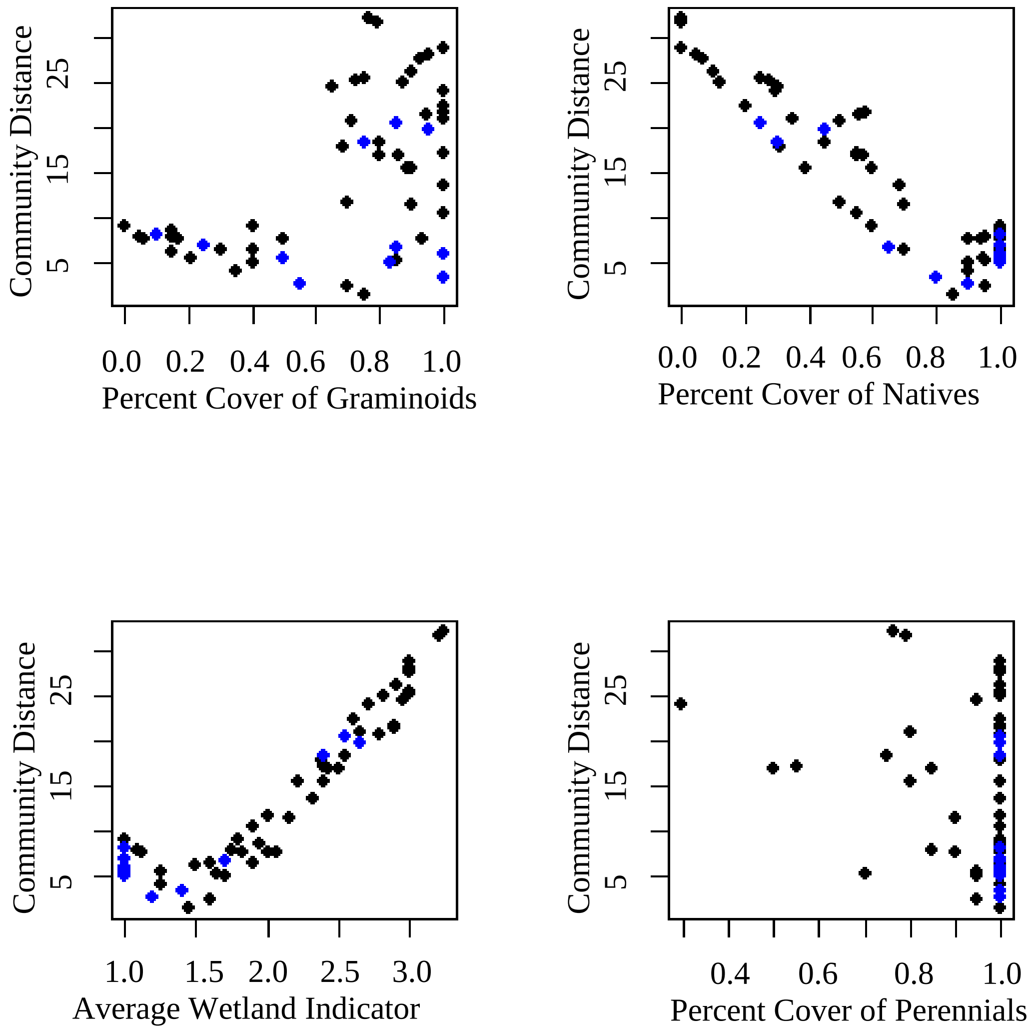

Figure 22. Community distance is related mostly to a lower percent cover of native species and a higher average wetland indicator number (indicating a larger proportion of upland plants). In addition, the plots with surviving plants (shown in blue) are all in areas dominated by perennial species.

Synthesis

Combining the results of the community analysis and the environmental analysis produces a better picture of factors affecting survival (Figure 23). Although there were more Union County plots than Lake County plots with surviving plants, these plots were presumably in a more optimal location. Most of the Union County plots with surviving plants had completely saturated soil (with a stream running through them) and were in full sun (Figure 24). They likely had a year-round water source since they were dominated by native, perennial, wetland species such as *Veronica americana* and *Glyceria grandis*.

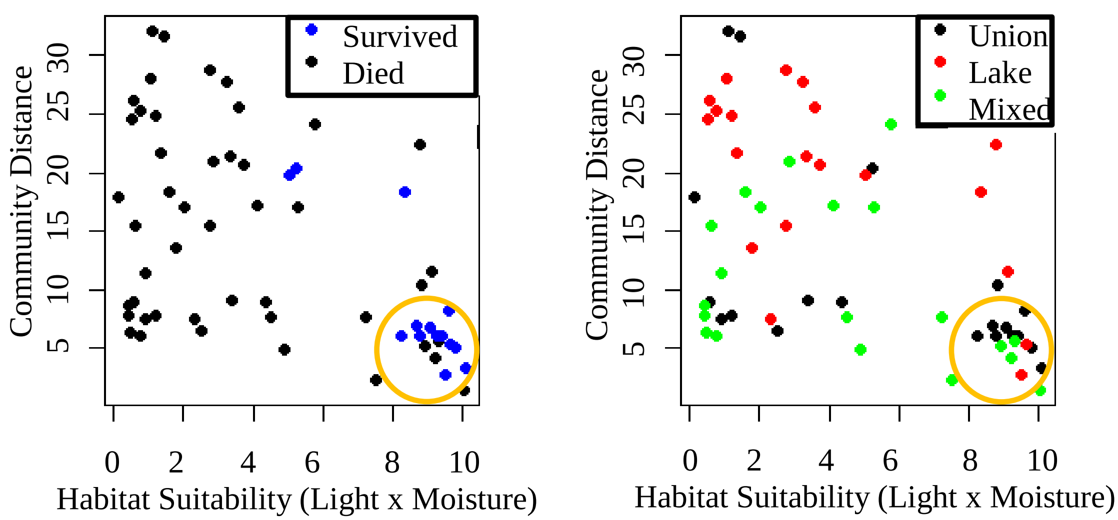

Figure 23. Survival of plants is more likely in an environment that has fully saturated soil, full sun, and a similar vegetative community to the source sites (mostly native, wetland obligate, perennial species).

None of the plants flowered this year. Poor growth conditions may have prevented reproduction, or plants may need more time to develop before reproducing. The mixed Union and Lake County bulk plots did not contain any surviving plants. This may be due to a lower than average water table due to limited precipitation. However, plants in the bulk plots with only Lake or Union County plants survived in areas that were open, moist, and contained native perennial wetland species.

Figure 24. The most optimal conditions for the *Pleuropogon oregonus* survival were areas that were dominated by native perennial wetland species and in were open to full sun. A vegetative *P. oregonus* tiller is indicated with a red circle.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

Selecting Outplanting Areas for *Pleuropogon oregonus*

Since there is a strong interaction between light intensity and soil moisture, the best areas for establishment of *Pleuropogon oregonus* during this study were open meadows in areas saturated with water year round. Because of the presence of aerenchyma tissue, these inundated conditions should not significantly reduce the potential for gas exchange in the underground portions of the plant (Colmer 2002). In addition, the species establishes better in full sun, so choosing reintroduction areas that are sunny, as well as wet annually, would result in the greatest likelihood of success.

The changes in water table (from pre-disturbance levels) in restored wetlands are often difficult to predict (Zedler 2000). Identifying perennial wetland obligate species in the plots should help indicate which areas are likely to have annually-saturated conditions (Tiner 1991). Many of the plots at End Creek had a high proportion of wetland obligate plants, indicating that they were normally saturated year long. However, chance events, such as a drought, may alter the water table in a given year, negatively impacting establishment of outplanted *P. oregonus* plants.

Under future climate change scenarios, wetlands are expected to be significantly impacted as a result of changing hydrology (Erwin 2009). Wetland restoration projects will have to account for this by analyzing the habitat on multiple scales, and determining how they will be affected by the projected altered hydrology.

The conditions for survival appear to be similar for both ecotypes (Tables 2 and 4, Figures 18 and 20). Because the availability of quality habitat is often the factor limiting reintroduction success, placing plants in high quality sites is more important than ecotype selection. Identifying suitable habitat should be a priority in future reintroduction effort for *P. oregonus*.

Although the majority of publications describing rare plant reintroduction projects report successful establishment, there is also a need to report on projects with little establishment (Godefroid et al. 2011). Even though this reintroduction project did not have much biological success, information was gathered that could be applied to other reintroductions for this species, suggesting project success (Pavlik 1996). The survival of some of the outplanted individuals indicates that this site can potentially support *P. oregonus*, but the fact that no surviving plants could be found in Area 1 suggests that apparently suitable habitat may not always result in a self-perpetuating population. There were plots in this area that were very similar to the source community and had a significant amount of soil moisture and light intensity (Figure 23). More effort should be made in future projects on quantifying these landscape-wide differences affecting hydrology.

Differences Between Ecotypes

The difference in survival between the two ecotypes after accounting for the variation in microhabitat characteristics was not significant. Although survival was lower in the plots planted with the Lake County ecotype, this difference can potentially be attributed to the inadvertent selection of drier and/or shadier conditions for these plots.

However, even when accounting for the differences in microhabitat, the Lake County plants were less vegetatively robust than those from Union County. Contrastingly, the Lake County ecotype was actually more vegetatively robust under greenhouse cultivation conditions in an earlier study (Gisler and Meinke 2003). That said, there were several important differences between these studies. The earlier study used reproductive plants that were genetically distinct (since they were cultivated from caryopses collected from the field). In contrast, this study compared clones outplanted into the field. The difference in total leaf area produced could result in a difference in ability to produce inflorescences, but this will have to be determined through future monitoring.

In addition to leaf area not being a perfect estimate of fitness, lack of survival reduced the sample size for average leaf length to only ten for Union County and four for Lake County. Because of this, the logistic model was likely a better fit for estimating fitness of the ecotypes planted at End Creek. Since the simplest adequate model did not include an overall difference in survival between the ecotypes, or a different slope for each ecotype, habitat quality appears to be more of a significant factor in determining outplanting success than genetic source. Since vegetative community characteristics at both source sites clustered together in the NMS ordination, both ecotypes may have functionally similar habitats, even though they are in separate counties.

Limitation of the Study

The lack of survival of either ecotype in many plots suggests that *P. oregonus* requires very specific conditions in order to survive. The year after outplanting also may have just been a poor growth year due to the limited precipitation. Although an effort was made to locate plots in three areas that were similar in their gradient of environmental conditions, yet separate from each other to prevent cross pollination, the mixed plots were inadvertently set up in an area which exhibited differences not measured in this study. This area appears to have been farmed more recently (Figure 12) than the other two areas, and therefore may have soils which differed. Although the soil moisture and light intensity were similar here, the surrounding landscape likely had an effect on the water table that was different than the other two areas. The low levels of precipitation during the winter immediately after planting may have affected these plots more severely, since the hydrology appears to have been different in this area.

If suitable sites could be located for outplanting in Lake and Union counties, reciprocal transplants of both ecotypes would be a good follow-up to this work. This would allow for a more confident conclusion as to whether there actually is local adaptation in each ecotype.

The low survival of *P. oregonus* observed in this study may be related to the use of plants produced asexually from limited collections of rhizomes. Although clumps of plants were collected from different parts of the source populations, these collections may not have included sufficient genetic diversity to allow plants to survive under a wide range of conditions.

Since plants in the mixed plots did not survive their first year, my study was unable to determine the effect of ecotype mixing (notably the potential increase in genetic diversity) on caryopsis production. In addition, successfully crossing the wind pollinated florets manually is very difficult. The question of increased fitness in outcrossed offspring may be answered more confidently by allowing the ecotypes to cross through wind pollination, then determining the paternal source through DNA sequencing. Evaluating the amount of genetic diversity present in each population would also help assess the effect of inbreeding depression on each ecotype (Neale 2012).

Why was the Previous Reintroduction in Logan Valley so Successful?

Logan Valley is located in Grant County and is managed to promote wildlife diversity by the Burns Paiute tribe. Compared to the outplanting sites at End Creek, Logan Valley has more areas with slow moving, wide streams. It is not surrounded by agricultural land and the hydrology has not been significantly altered by humans, although beaver have colonized the area. The natural hydrology, native vegetation, and lack of heavy grazing probably provide an ideal habitat for *P. oregonus* establishment (Brown et al. 2012).

However, since the population is still being augmented with additional cultivated plants, and analysis of growth, survival, and reproduction for that project is not done on a per plot basis, it is difficult to determine to what degree the population is self-perpetuating. In fact, population numbers in Logan Valley were much lower in 2013 compared to the previous year, and several areas that supported hundreds of tillers in previous years were dry and only had a handful of surviving plants (personal observation). Continued monitoring of this site once planting ceases will help determine if the created population here can persist without continued human intervention.

Future Directions in the Conservation of *Pleuropogon oregonus*

The Logan Valley site is administratively protected and provides suitable habitat for a viable introduced population of the Union County ecotype. However, if the two *P. oregonus* ecotypes are to be kept separate in the future, a suitable, protected site should also be secured for the Lake County ecotype. Several new outplanting areas in annually saturated sites in Lake County are being evaluated in 2013. Monitoring of the pilot plantings being used for evaluation will provide data valuable to selecting future sites for large scale outplantings.

Since Logan Valley currently supports the only successful outplanting for *P. oregonus*, more cultivated plants will be planted in this site in areas that are likely to be wet all year. Since mixing the ecotypes did not produce analyzable data in my study, we are no closer to determining the benefits of keeping ecotypes separate verses mixing them. It appears that both ecotypes require similar conditions for survival. If, at some point, we do determine that mixing the ecotypes increases caryopsis production or offspring fitness, then further plantings should be mixed.

Conclusions

The two main objectives in this thesis were to research methods for creating a self-perpetuating population of *Pleuropogon oregonus*, and to contribute to the recovery of the species by creating an additional population on protected land. Thus both “project success” and “biological success” were considered (Pavlik 1996). Although after the first year of monitoring there was little establishment, low tiller production, and no flowering plants, further monitoring would help determine the long term biological status of the new population at End Creek. This project was informative in terms of habitat selection protocol, but due to the loss of plants in the mixed plots, one of the initial questions could not be answered.

This project suggests that reintroductions of *P. oregonus* are most limited by habitat quality, rather than ecotype used. The most significant predictor of survival and total leaf area was the interaction between light intensity and soil moisture. The Lake County plants were less vegetatively robust than the Union County plants, but issues associated with interpreting many values of zero in a linear regression made the logistic analysis better suited for describing the survival data. Although more of the Union County ecotype survived, this difference could be explained by the inadvertent selection of plots that have presumably better quality habitat. If more sites become available in both counties for outplanting, future researchers could perform reciprocal transplants in each county to more confidently determine if the differences between ecotypes are related to natural selection.

Plants in the mixed plots did not survive in Area 1, so assessing the effect of mixing ecotypes on caryopsis production and offspring fitness could not be done. Although there was no biological success in this area, it still provided information about habitat selection. Even though an area might appear to be suitable on a small scale, both ecotypes could not establish in any of the plots in this area, which appeared to be more recently farmed. It may be that the low precipitation during the year following outplanting (2012-2013) affected this area differently than the other two areas. Because artificial pollination is very difficult to achieve for this wind-pollinated species, future researchers could answer the question of whether crossing the ecotypes will increase fitness by mixing the two ecotypes in cultivation, keeping track of the maternal lines, and using DNA sequencing to determine the paternal source of the offspring.

Similarity of plant cover in each study plot to the vegetative communities at the source sites is related to a higher probability of survival. The clustering of both source areas in the ordination (Figure 20) suggests that both ecotypes have functionally similar vegetative communities. Although this was not as accurate of a predictor of survival as measurements of environmental data, selecting plots that have a high proportion of wetland obligate, native, and perennial species would increase the probability of survival.

In conclusion, there are still many unanswered questions about the best methods for creating new populations of *P. oregonus*, and this study has shown the difficulty of restoring populations of this particular narrowly endemic species. However, as we learn more about reintroducing species such as this, there may be a day when *P. oregonus* will no longer be threatened by extinction.

LITERATURE CITED

Albrecht, M.A. and J. Maschinski 2012. Influence of founder population size, propagule size, propagule stages, and life history on the survival of reintroduced plant populations. Pages 171-188 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Altizer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology and Evolution **18**: 589-596.

Amsberry, K. 2001. Conservation biology of *Plagiobothrys hirtus* (Boraginaceae): evaluation of life history and population enhancement. Thesis to Oregon State University, Department of Botany and Plant Pathology.

Amsberry, K. and R.J. Meinke. 2004. Biological status survey: investigating population establishment of the state endangered and federal species of concern grass species *Pleuropogon oregonus*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, OR.

Amsberry, K. and R.J. Meinke. 2005. Developing cultivation methods and a local facility for the propagation establishment and augmentation of *Fritillaria gentneri* in southwest Oregon. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, OR.

Angermeier, P.L. and J.R. Karr. 1994. Biological integrity verses biological diversity as policy directives. Bioscience **44**: 690-697.

Antell, K. 2010. Blue Mountains Conservancy. End Creek wetlands restoration project history. Accessed on June 2013. http://www.bluemountainsconservancy.org/research/rr_endcreek.html

Bailey, M.F. and D.E. McCauley. 2006. The effects of inbreeding, outbreeding, and long-distance gene flow on survivorship in North American populations of *Silene vulgaris*. Journal of Ecology **94**: 98-109.

Barkworth, M.E., L.K. Anderton, K.M. Capels, S. Long, and M.B. Piep. 2007. Manual of grasses for North America. Utah State University Press, Logan.

Barrett, S.C.H. and J.R. Kohn. 1991. Genetic and evolutionary consequences of small population sizes in plants: implications for conservation. Pages 3-30 in D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Beazley, K. and N. Cardinal. 2004. A systematic approach for selecting focal species for conservation in the forests of Nova Scotia and Maine. Environmental Conservation **31**: 91-101.

Brander, L.M., R.J.G.M. Florax, and J.E. Vermaat. 2006. The empirics of wetland valuation: a comprehensive study and meta-analysis of the literature. Environmental and Resource Economics **33:** 223-250.

Brown, J., K. Amsberry, and R.J. Meinke. 2012. Averting federal listing of Oregon semaphore grass (*Pleuropogon oregonus*) through cultivation and population creation: year 3, 2010. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, OR.

But, P.P.H. 1977. Systematics of *Pleuropogon*. Thesis submitted to the University of California, Berkly.

But, P.P.H., J. Kagan, V.L. Crosby, and J.S. Shelly. 1985. Rediscovery and reproductive biology of *Pleuropogon oregonus*. Madroño **32:** 189-190.

Chapin III, F.S., E.S. Zavaleta, V.T. Eviner, R.L. Naylor, P.M. Vitousek, H.L. Reynolds, D.U. Hooper, S. Lavorel, O.E. Sala, S.E. Hobbie, M.C. Mack, and S. Díaz. 1998. Consequences of changing biodiversity. Nature **405:** 234-242.

Chase, A. 1938. New grasses from Oregon. Journal of the Washington Academy of Sciences **28:** 52-53.

Colmer, T.D. 2002. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy, and deep-water rice (*Oryza sativa* L.). Annals of Botany **91:** 301-309.

Cook, R.E. 1983. Clonal plant populations: a knowledge of clonal structure can affect the interpretation of data in a broad range of ecological and evolutionary studies. American Scientist **71:** 244-253.

Costanza, R., R. d'Arge, R. de Groot, S. Farberk, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Suttonkk, and M. van den Belt. 1997. The value of the world's ecosystem services and natural capitol. Nature **87**: 253-260.

Costanza, R., B. Fisher, K. Mulder, S. Liu, and T. Christopher. 2007. Biodiversity and ecosystem services: a multi-scale empirical study of the relationship between species richness and net primary production. Ecological Economics **61:** 478-491.

Crawley, M.J. 1997. Sex. Pages 157-213 in M.J. Crawley, editor. Plant ecology, second edition. Blackwell Science Ltd, Oxford.

Cronk, J.K. and M.S. Fennessy. 2001. Wetland plants: biology and ecology. CRC Press, Boca Raton, Florida.

Dalrymple, S.E., E. Banks, G.B. Stewart, and A.S. Pullin 2012. A meta-analysis of threatened plant reintroductions from across the globe. Pages 31-50 188 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Dawkins, R. 1982. Constraints on perfection. Pages 30-54 in The extended phenotype. Oxford University Press, New York.

Duffy, J.E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. Frontiers in Ecology and the Environment **7:** 437-444.

EPA. 2013. Wetland types. Environmental Protection Agency website. Accessed October 2013. http://water.epa.gov/type/wetlands/types_index.cfm

Erwin, K.L. 2009. Wetlands and global climate change: the role of wetland restoration in a changing world. Wetlands Ecology and Management **17:** 71-84.

Fiedler, P.L. and R.D. Laven. 1996. Selecting reintroduction sites. Pages 157-169 in D.A. Falk, C.I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Fleischner, T.L. 1994. Ecological costs of livestock grazing in western North America. Conservation Biology **8:** 629-644.

Gabriel, W., M. Lynch, and R. Burger. 1993. Muller's ratchet and mutational meltdowns. Evolution **47:** 1744-1757.

Gillen, R.L., W.C. Krueger, and R.F. Miller. 1985. Cattle use of riparian meadows in the Blue Mountains of Northeastern Oregon. Journal of Range Management **38:** 205-209.

Gisler, S.D. and R.J. Meinke. 2001. Biological status survey: seed production, seed germination requirements, and cultivation protocols for the state endangered species, *Pleuropogon oregonus*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, OR.

Gisler, S.D. and R.J. Meinke. 2003. Developing biogeographically based population introduction protocols for the Species of Concern grass, *Pleuropogon oregonus*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, OR.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A. Stevens, R. Aguraiuja, C. Cowell, C.W. Weekley, G. Vogg, J.M. Iriondo, I. Johnson, R. Dixon, D. Gordon, S. Magnanon, B. Valentin, K. Bjureke, R. Koopman, M. Vicens, M. Virevaire, and T. Vanderborght. 2011. How successful are plant species reintroductions. Biological Conservation **144:** 672-682.

Gurevitch, J. and D.K. Padilla. 2004. Are invasive species a major cause of extinctions? Trends in Ecology and Evolution **19:** 470-474.

Guerrant, Jr., E.O. 1996. Designing populations: demographic, genetic, and horticultural dimensions. Pages 171-207 in D.A. Falk, C.I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Gustafson, D.J., D.J. Gibson, and D.L. Nickrent. 2002. Genetic diversity and competitive abilities of *Dalea purpurea* (Fabaceae) from remnant and restored grasslands. International Journal of Plant Science **163:** 979-990.

Haskins, K.E. and B.G. Keel. 2012. Managed relocation: panacea or pandemonium. Pages 229-242 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Hedrick, P.W. and S.T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics **31:** 139-162.

Hobbs, R.J. and L.F. Huenneke. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology **6:** 324-337.

Holland, C.C., J.E. Honea, S.E. Gwin, and M.E. Kentula. 1995. Wetland degradation and loss in the rapidly urbanizing area of Portland, Oregon. Wetlands **15:** 336-345.

Hooper,D.U., F.S. Chapin III, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A.J. Symstad, J. Vandermeer, and D.A. Wardle. 2005. Effects of biodiversity on ecosystem functioning. Ecological Monographs **75:** 3-35.

Huenneke, L.F. 1991. Ecological implications of genetic variation in plant populations. Pages 31-44 in D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Hufford, K.M. and S.J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution **18:** 147-155.

Hutchings, M.J. 1997. Other aspects of performance. Pages 340-342 in M.J. Crawley, editor. Plant ecology, second edition. Blackwell Science Ltd, Oxford.

Huxman, T.E., B.P. Wilcox, D.D. Breshears, R.L. Scott, K.A. Snyder, E.E. Small, K. Hultine, W.T. Pockman, and R.B. Jackson. 2005. Ecohydrological implications of woody plant encroachment. Ecology **86:** 308-319.

Kauffman, J.B. and W.C. Krueger. 1984. Livestock impacts on riparian ecosystems and streamside management implications. Journal of Range Management **37:** 430-38.

Kaye, T.N., R.J. Meinke, J. Kagan, S. Vrilakas, K.L. Chambers, P.F. Zika and J.K. Nelson, editors. 1997. Patterns of rarity in the Oregon flora: implications for conservation and management. Pages 1-10 in Conservation and management of native plants and fungi. Native Plant Society of Oregon, Corvallis.

Kennedy, K., M.A. Albrecht, E.O. Guerrant Jr, S.E. Darymple, J. Maschinski, and K.E. Haskins 2012. Synthesis and future directions. Pages 265-276 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Kutner, L.S. and L.E. Morse 1996. Reintroduction in a changing climate. Pages 23-48 in D.A. Falk, C.I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Lande, R. 1998. Anthropogenic, ecological, and genetic factors in extinction and conservation. Researches on Population Ecology **40:** 259-269.

Lawrence, B.A. and T.N. Kaye. 2011. Reintroduction of *Castilleja levisecta*: effects of ecological similarity, source population genetics, and habitat quality. Restoration Ecology **19:** 166-176.

Lloyd, M.W., R.K. Burnett, K.A. Engelhardt, and M.C. Neel. 2012. Does genetic diversity of restored sites differ from natural sites? A comparison of *Vallisneria americana* (Hydrocharitaceae) populations within the Chesapeake Bay. Conservation Genetics **16:** 753-765.

Maschinski, J.E., D.A Falk, S.J. Wright, J. Possley, J. Roncal, and K.S. Wendelberger. 2012. Optimal locations for plant reintroductions in a changing world. Pages 109-130 in J. Maschinski and K.E Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J. and K.E. Haskins 2012. Introduction. Pages 1-7 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Matson, P.A., W.J. Parton, A.G. Power, and M.J. Swift. 1997. Agricultural intensification and ecosystem properties. Science **277:** 504-509.

McCune, B., J.B. Grace, and D.L. Urban. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach.

McDonald, C.B. 1996. The regulatory and policy context. Pages 87-101 in D.A Falk, C. I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

McKay, J.K., C.E. Christian, S. Harrison, and K.J. Rice. 2005. How local is local? A review of practical and conceptual issues in the genetics of restoration. Restoration Ecology **13:** 432-440.

McLachlan, J.S., J.J. Hellmann, and M.W. Schwartz. 2006. A framework for debate of assisted migration in an era of climate change. Concervation and Policy **21:** 297-302.

Menges, E.S. 1992. Stochastic modeling of extinction in plant populations. Pages 253-275 in P.L. Fiedler and S.K. Jain, editors. Conservation biology: the theory and practice of nature conservation preservation and management. Chapman and Hall, London.

Millar, C.I. and W.J. Libby. 1991. Strategies for conserving clinal, ecotype, and disjunct population diversity in widespread species. Pages 149-170 in D.A Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York.

Mitsch, W.J. and J.G. Gosselink. 2000. The value of wetlands: importance of scale and landscape. Ecological Economics **35:** 25-30.

Monks, L., D. Coates, T. Bell, and M.L. Bowles 2012. Determining success criteria for reintroductions of threatened long-lived plants. Pages 189-208 in J. Maschinski and K.E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Moora, M., M. Öpik, R. Sen, M. Zobel. 2004. Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species. Functional Ecology **18:** 554-562.

Morse, L.E. 1996. Plant rarity and endangerment in North America. Pages 7-22 in D.A. Falk, C.I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Morse, J.L. and E.S. Bernhardt. 2013. Using ^{15}N tracers to estimate N_2O and N_2 emissions from nitrification and denitrification in coastal plain wetlands under contrasting land uses. Soil Biology and Biochemistry **57:** 635-643.

Nathan, R., F.M. Schurr, O. Spiegel, O. Steinitz, A. Trakhtenbrot, and A. Tsoar. 2008. Mechanisms of long-distance seed dispersal. Trends in Ecology and Evolution **23**: 638-647.

Noël, F., D. Prati, M. van Kleunen, A. Gygax, D. Moser, and M. Fischer. 2010. Establishment success of 25 rare wetland species introduced into restored habitats is best predicted by ecological distance to source habitats. Biological Conservation **144:** 602-609.

ORBIC. 2010. Rare, threatened, and endangered vascular plant list. Oregon Biodiversity Information Center. Accessed on June 2012. http://orbic.pdx.edu/rte-species.html

ORBIC. 2012. Rare species ranking definitions. Oregon Biodiversity Information Center. Accessed on June 2012. http://orbic.pdx.edu/rte-defs.html

Osborne, L.L. and D.A. Kovacic. 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. Freshwater Biology **29:** 243-258.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. The Annual Review of Ecology, Evolution, and Systematics **37:** 637-669.

Pavlik, B.M. 1996. Defining and measuring success. Pages 127-156 in D.A Falk, C.I. Millar, M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC

Pimm, S.L., G.L. Russell, J.L. Gittleman, and T.M. Brooks. 1995. The future of biodiversity. Science **269:** 347-350.

Ramsey, F. and D. Schafer. 2002. The statistical sleuth: a course in methods of data analysis. Brooks/Cole, Belmont.

Saunders, D.A., R.J. Hobbs, and C.R. Margules. 1991. Biological consequences of ecosystem fragmentation: a review. Conservation Biology **5:** 18-24.

Schlesinger, W.H., J.S. Clark, J.E. Mohan, and C.D. Reid. 2001. Global environmental change: effects on biodiversity. Pages 175-223 in M.E. Soulé and G.H. Orians, editors. Conservation biology: research priorities for the next decade. Island Press, Washington, DC.

Severns, P.M., A. Liston, and M.V. Wilson. 2011. Habitat fragmentation, genetic diversity, and inbreeding depression in a threatened grassland legume: is genetic rescue necessary? Conservation Genetics **12:** 881-893.

Sgro, C.M., A.J. Lowe, and A.A. Hoffmann. 2010. Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary Applications **4:** 326-337.

Solé, M.E. and L.S. Mills. 1998. No need to isolate genetics. Science **282:** 1658-1659.

Thiere, G., S. Milenkovski, P.E. Lindgren, G. Sahlén, O. Berglund, and S.E. Weisner. 2009. Wetland creation in agricultural landscapes: Biodiversity benefits on local and regional scales. Biological Conservation **142:** 964-973.

Tiner Jr, R.W. 1984. Wetlands of the United States: current status and recent trends. United States Fish and Wildlife Service, Newton, Massachusetts.

Tiner, R.W. 1991. The concept of a hydrophyte for wetland identification. Bioscience **41:** 236-237.

USDA. 2012. United States Department of Agriculture Plant Database. Accessed on June 2012. http://plants.usda.gov

Verhoeven, J.T.A., B. Arheimer, C. Yin, and M.M. Hefting. 2006. Regional and global concerns over wetlands and water quality. Trends in Ecology and Evolution **21:** 96-103.

Weins, D., C.I. Davern, and C.L. Calvin. 1988. Exceptionally low seed set in *Dedeckera eurekensis*: is there a genetic component of extinction? Pages 19:20 in H.C. Doyle-Jones V., editor. Plant biology of eastern California. University of California Press, Los Angeles.

Wilson, M.V., T. Erhart, P.C. Hammond, T.N. Kaye, K. Kuykendall, A. Liston, A.F. Robinson Jr, C.B. Schultz, and P.M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus ssp. kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal **23:** 72-83.

Wissmar, R.C. 2004. Riparian corridors of Eastern Oregon and Washington: functions and sustainability along lowland-arid to mountain gradients. Aquatic Sciences **66:** 373-387.

WRCC. 2013. Western Regional Climate Center. La Grande, Oregon. Accessed on July 2013. http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?orlagr

Wunderground. 2013. Weather History for KORLAGRA5. Accessed on July 2013. http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KORLAGRA5

Zak, D.R., W.E. Holmes, D.C. White, A.D. Peacock, and D. Tilman. 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology **84:** 2042-2050.

Zedler, J.B. 2000. Progress in wetland restoration ecology. Trends in Ecology and Evolution **15:** 402-407.