OREGON DEPARTMENT OF AGRICULTURE

NATIVE PLANT CONSERVATION PROGRAM

Population Creation as a Recovery Tool for the Federal Candidate *Artemisia campestris* var. *wormskioldii*

Phase Two: Large Scale Outplanting

Years 1 and 2

Addendum

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Factors influencing reintroduction success

Many authors have suggested that the establishment of new populations of threatened and endangered species is essential for their long-term survival (Maunder 1992; Allen 1994; Falk et al. 1996; Jusaitis et al. 2004; Albrecht & McCue 2010). Reintroduction, the placement of plant material into a site within the historic range of a species, is a conservation tool that can reduce extinction potential by increasing population numbers and size (Maunder 1992; Walck et al. 2002; Brumback et al. 2003). Reintroduction projects often contain an experimental component, which provides researchers with the opportunity to gain biological and ecological information on selected species (Guerrant & Kaye 2007). In addition to furthering the recovery of the species, these projects can help conservationists develop a set of overarching reintroduction principles (Menges 2008). Although reintroduction, a component of conservation biology, is still a new field with some shortcomings (Godefroid et al. 2011; Drayton & Primack 2012), there are limited alternatives available to those attempting to recover species. Plant reintroduction is an active management strategy that promotes the retention of biodiversity, and carefully planned reintroduction projects are an essential recovery tool for land managers addressing species decline in our changing world.

There are several key factors that can influence reintroduction success: type and number of transplants introduced (Godefroid et al. 2011), number of reintroduction attempts (Kaye 2008; Guerrant 2012), genetic diversity of source material (Neale 2012), habitat similarity of reintroduced site to wild sites (Noel et al. 2011), and site preparation and management (Drayton & Primack 2012). To accurately document the success of these factors, long term monitoring is essential (Guerrant 2012). By understanding which factors aid in success, reintroduction practitioners can strategically plan projects and determine priorities in terms of time and budget.

The type and quantity of propagules or transplants can have a significant impact on the success of the reintroduction (Falk et al. 1996; Godefroid et al. 2011). Seeds, seedlings, or more mature plants represent the most common option, but rhizomes or vegetative cuttings are often used as well. There are advantages and disadvantages to using each type of plant material, and determining the appropriate type can be difficult. For example, Reckinger et al. (2010) found that transplants of *Scorzonera humilis*, a rare member of the Asteraceae found in Britain, performed better, in terms of size, flowering, fruiting time, and survival, than seeds in a reintroduced population after four years. In a reintroduction of *Brachycome muelleri*, another rare Asteraceae member, Jusaitis et al. (2004) also found that reintroduced seedlings had higher rates of survival and fitness than seeds. These results are consistent with other recommendations of using transplants over seeds (Guerrant 1996a; Albrecht & Maschinski 2012). However, there is a cost for this success: the use of seedlings or adult plants in a reintroduction can be prohibitively expensive. The cultivation, transportation, and outplanting of transplants is much more labor intensive than the direct sowing of seed.

On the other hand, seeds are a practical choice for reintroduction for several reasons. Seeds can be transported easily to sites and for some species, seed production is abundant, and seeds can be acquired in prolific numbers. If transplant production costs are high, seeds may be the suitable option, especially when available in large quantities (Kaye & Cramer 2003). Researchers can also determine the suitability of sites for germination in the wild by sowing seeds directly into a reintroduction site. However, collecting seeds may decrease the opportunity for recruitment in the parent population (Bottin et al. 2007). The use of seeds may also require more reintroduction site preparation, such as reducing competing vegetation to encourage seedling establishment (Jõgar & Moora 2008).

To evaluate which type of plant material would best promote establishment success, a study comparing several of the most promising options would be the best approach (Kaye 2009). If such a study is not an option, consideration of other factors such as the species' life history strategy, may guide the decision. For example, seeds have been successfully used most often with annual species (Albrecht & Maschinski 2012), whereas perennial species may establish faster with transplants (Alley & Affolter 2004). The amount of available material may influence the decision, as many rare species are known from only a few populations and seeds could be difficult to acquire. Finally, seed losses are often greater in the wild than in a controlled setting (Albrecht & Maschinski 2012), so propagating transplants from seeds in a greenhouse may reduce seed losses at this stage.

Reviews have shown that, regardless of the type of plant material used for reintroduction, the number of propagules or transplants introduced can affect project success (Bottin et al. 2007; Godefroid et al. 2011; Albrecht & Maschinski 2012; Guerrant 2012). The likelihood of creating a viable population is greater with increasing amounts of seeds or transplants. A large founding population can provide insurance against stochastic events (Guerrant 1996a), and transplant mortality in the first year after outplanting (Monks et al. 2012).

Multiple outplanting events are often used to create a large founding population at reintroduction sites (Guerrant 2012). This approach, while ideal, is usually dependent on time and funding. In addition, to promote establishment, several reintroduction attempts at multiple sites may be conducted to counteract many unknown factors in a reintroduction (Maschinski & Duquesnel 2006; Kaye 2008). For example, Bottin et al. (2007) created six populations at three sites to aid in the recovery of *Arenaria grandiflora*, an endangered plant found on cliffs and calcareous rocks in the forests near Paris. Population sizes significantly decreased at two of the sites due to herbivory and invasive species. However, the populations at the third site, although decreasing slightly after initially thriving, did successfully establish (Bottin et al. 2007). This demonstrates how multiple reintroductions can serve as a bet-hedging strategy.

The creation of multiple populations can also aid in metapopulation structure (Guerrant 1996a), an important consideration for some species. Metapopulation structure consists of a network of populations in various stages of colonization and succession (Primack 1996), and implies connectivity and gene flow throughout population patches (Maschinski 2006). This is an important concept for rare species recovery, especially in cases where habitats have become degraded and fragmented (Maschinski & Haskins 2012). Reintroductions can create stepping stones between natural populations and created ones, therefore improving metapopulation structure and the overall status of the species.

A large founding population should contain as much genetic diversity as possible, to maximize resiliency to environmental change (Guerrant 1996a). The use of single versus multiple plant material sources for reintroduction is grounds for a debate (Kaye 2001; Maschinski et al. 2013). Use of a single source may be optimal depending on availability of material, or a desire to maintain local ecotypes (Montalvo & Ellstrand 2000) and reduce the opportunity for outbreeding depression (Hufford & Mazer 2003). However, the use of plant material from multiple sources has been shown to increase germination rates (Albrecht & McCue 2010), survivorship, flowering, and fruiting (Reckinger et al. 2010). The use of multiple sources can also protect against threats that are the result of low genetic diversity such as inbreeding depression (Kephart 2004) and reduced seed set (Severns 2003). Up to this point, the "local is best paradigm" has been the prevalent hypothesis for restoration projects (Kaye 2001; McKay et al. 2005), but this view is shifting with increased research (Maschinski et al. 2013), showing the benefits of using plant material from multiple source populations.

Source populations located in habitat that is ecologically similar to that of the outplanting site may provide plant material most genetically suitable for a successful reintroduction (Dalrymple & Broome 2010). Measurement of characteristics such as soil type (Maschinski et al. 2004), vegetation composition and abundance (Noel et al. 2011), or hydrology (Rimer & McCue 2005) may determine the ecological similarity between source site and reintroduction site. Lawrence & Kaye (2009) found that transplants of

Castelleja levisecta grown from seed collected from ecologically similar sources performed better than transplants grown from seed collected from geographically close sources. They measured ecological similarity by vegetation composition and soil characteristics. Understanding this relationship between source population and reintroduction site habitats may guide the choice of which sources to use for plant material.

Selecting sites for reintroduction should be guided by ecological similarity to sites supporting natural populations as well. On the broad end, sites for reintroduction should be within the historic range of the species (Kaye 2008), but teasing apart the fine details of site appropriateness can be difficult. This is especially so if the species has been absent from an area for a long period of time (Lawrence & Kaye 2006). Assessing factors such as those previously mentioned for ecologically similar source material, i.e. soil characteristics, and vegetation composition and abundance, may determine which sites are ecologically appropriate. However, site selection can be complicated. Even if two planting sites appear similar based on one trait, differences in another trait could be the determinant of establishment. For example, Rimer & McCue (2005) planted seedlings of *Helenium virginicum* at two sites with similar hydrology to determine the effects of site on survivorship and growth. Both planting sites were on the edge of a seasonal sinkhole pond, the habitat of natural populations of *H. virginicum*, but plants at one site performed better than those at the other. They attributed this to differences in vegetation abundance, a factor not previously considered in site selection (Rimer & McCue 2005). The site that performed better had less competing established vegetation for the sun-loving *H. virginicum*, a trend that also followed their weed exclusion treatment (Rimer & McCue 2005). Finally, proximity to natural populations may be a poor indicator of appropriate sites, as other extrinsic factors (such as herbivory) may be the determinant of project success (Holl & Hayes 2006). Because of a myriad of interacting habitat factors, as well as the often limited number of examples of natural populations, reintroductions should be designed to include multiple microsites to determine the best habitat (Burmeier & Jensen 2009; Maschinski et al. 2012).

Preparation or post-planting management may be necessary to encourage and promote successful establishment of a newly created population once sites have been selected. Invasive and weedy species are known to negatively impact native vegetation (Jusaitis et al. 2004; Jusaitis 2005). The reduction of invasive species at reintroduction sites to acceptable levels is often essential (Kaye 2008). For example, Jusaitis (2005) found that transplant survival and growth was higher in plots when weeds were removed when compared to transplant survival and growth in weedy plots. To reduce the negative effects of herbivores on newly planted populations, fencing or caging may be necessary as well (Maschinski et al. 2004; Jusaitis 2005; Holl &Hayes 2006). Jõgar & Moora (2008) tested various management techniques for reintroducing *Gladiolus imbricatus*, a rare meadow species, including mulching, mowing, and traditional cutting by scythe and removal of hay. They found that mulching was the best technique for encouraging establishment and growth of this seed limited species, and suggested that this technique be applied in future meadow restoration practices (Jõgar & Moora 2008). Most reintroduction sites will need some type of site preparation before planting or management after introduction, both of which may be guided by the characteristics of the site or the species.

Follow-up monitoring is crucial for assessing the success of a project once the planting stage is complete. Monitoring plans should be identified in the planning stages of the project, to ensure that this important step in any reintroduction work is not overlooked. Monitoring should be as long-term as possible, to capture changes in the population over multiple years and detect any patterns of demographic variation. Drayton & Primack (2012) retracted earlier statements of success, reported in 2000, of their reintroduction of eight species. Initially, they had monitored their sites for the first three years after reintroduction, but visits to sites 15 years later revealed that all but two populations had disappeared. This report underscores the benefits of long-term monitoring to avoid premature assumptions of success. Monitoring time may depend on the species (e.g. annual vs. long-lived perennial) and the data collected. Monitoring may be more intense in the first years of the project and taper off as the population becomes stable (Guerrant 1996a). Although the details may vary by project and species, long-term monitoring is essential for any reintroduction.

While all of these factors may influence the success of a reintroduction project, it is important to understand what is meant by success and to define it in terms of goals. A successful project could mean establishing a new population, testing germination and planting techniques, or determining evidence of recruitment. It is important for restorationists to provide measures of success that are meaningful and adequate given the amount of effort that goes into reintroduction (Monks et al. 2012). Several recent reviews suggest that a reintroduction project is successful when the new population has recruited a second and sometimes third generation (Godefroid et al. 2011; Monks et al. 2012). The persistence of the population through the natural creation of satellite populations would indicate great success (Primack & Drayton 1997). While this can be a tall order, it is a vital element to strive for in recovery plans for endangered species.

It is with the consideration of these factors that may influence reintroduction success that the plan to experimentally reintroduce northern wormwood (*Artemisia campestris* var. *wormskioldii*) was designed.

Study species

Northern wormwood is a tap-rooted, low-growing perennial with leaves that are 2.5-10 cm long, two to three times divided into mostly linear divisions with fine silky hairs (Amsberry & Meinke 2011). Inflorescences extend above leaves and have outer pistillate, fertile flowers with inner sterile disk flowers (Figure 1.1). Flowering occurs in April and May, with fruit set a month later. Achenes are very small (5-10 mm) and glabrous.

Northern wormwood is restricted to dynamic cobble bar environments on the Columbia River, and is known from only two natural populations. Both are in Washington, one located at Miller Island and the second at Beverly, 300 river km apart (Arnett 2010). The habitat at these two native sites differs considerably. Miller Island plants are rooted in basalt covered with a thick layer of surface sand, and plants at Beverly are found on a peninsula with various sizes of pebbles and cobbles, and low, sparse vegetation (Figure 1.2). Both of these populations are declining, presumably due to a combination of drifting sand, flooding, and weed infestations (Arnett 2010; Amsberry & Meinke 2011). Other native plant species associated with northern wormwood include *Camissonia contorta, Chrysothamnus nauseasus* var. *albicaulis, Eriogonum compositum*, *Phacelia hastata,* and *Salix exigua.*

Figure 1.1 Close-up of northern wormwood inflorescence. Photo by A. Brickner.

The Oregon Department of Agriculture's (ODA) Native Plant Conservation Program (NPCP) has been working on recovery efforts for this species since 2005 (Amsberry et al. 2007; Amsberry & Meinke 2011). NPCP staff surveyed for existing populations and appropriate habitat in 2005 and 2006, and found several sites suitable for a pilot outplanting (Amsberry et al. 2007). One of these, Squally Point, which is located approximately eight km west of The Dalles, OR, on the Columbia River, was selected for reintroduction. Three outplanting events were completed at this site. In spring 2008, 359 greenhouse grown transplants were planted at Squally Point. Two augmentations of 232 and 303 transplants were added in fall 2008 and spring 2010, respectively (Amsberry & Meinke 2011). This newly created population has been declining since 2010 due to unusually high and fluctuating water flow and unauthorized recreational use, but valuable information has been gathered from this pilot project. Seed germination and transplant production methods were developed and microsite selection techniques were used to determine the most appropriate planting locations. This work provided baseline data and methodology used in the current study.

Figure 1.2. Habitat at the natural population of northern wormwood, Beverly, in Grant County, Washington. Photo by A. Brickner.

Status

Northern wormwood is a candidate for Federal listing and is considered a "Spotlight Species" by the U.S. Fish and Wildlife Service (USFWS 2011). Spotlight Species are "Threatened, Endangered, and Candidate taxa selected by USFWS to receive additional agency focus in regard to recovery efforts" (Amsberry & Meinke 2011). While not listed federally, northern wormwood is listed as critically imperiled by the Oregon Biodiversity Information Center (ORBIC 2010), Endangered by the Oregon Department of Agriculture (ODA 2012), and Endangered by the Washington Natural Heritage Program (Washington Department of Resources 1997).

Threats

Populations of northern wormwood are declining, and this can be attributed to several definite and potential causes. First, construction of multiple dams on the Columbia River has directly resulted in loss of individuals and habitat (Gamon 1989; Carlson 1998). Much of the riverbanks on the Columbia River are covered in large, stabilizing rocks, an inappropriate substrate for northern wormwood (Gamon 1989; Amsberry & Meinke 2011). Without appropriate habitat, northern wormwood has been unable to colonize new sites. Second, multiple dams on the river have changed the seasonal flows of the river through reduction in spring floods and increased inundation (National Research Council 2004; USFWS 2011). This change in hydrology may affect the growth and reproduction of northern wormwood plants (USFWS 2011). Loss of plants by changing water levels has been observed at the created site, Squally Point (personal observation) (Figure 1.3). Finally, the occurrence of invasive species along the Columbia River and in northern wormwood populations represents a great threat. Invasive species reduce habitat, compete for resources, and are often cited as the cause of decline of native plant populations (Kaye 2009).

Figure 1.3. Northern wormwood plant inundated in June at the first reintroduced Oregon population (Squally Point). Photo by A. Brickner.

Literature Cited

Albrecht, M., and K. McCue. 2010. Changes in demographic processes over long time scales reveal the challenge of restoring an endangered plant. Restoration Ecology **18:**235-243.

Albrecht, M. A., and J. Maschinski. 2012. Influence of founder population 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.

Allen, W.H. 1994. Reintroduction of endangered plants. Bioscience **44:**65-68.

Alley, H., and J. Affolter. 2004. Experimental comparison of reintroduction methods for the endangered *Echinacea laevigata* (Boynton and Beadle) Blake. Natural Areas Journal **24:**345-350.

Amsberry, K., R. Currin, and R.J. Meinke. 2007. Reintroducing *Artemisia campestris* var. *wormskioldii* to Oregon: site selection, cultivation, and pilot outplanting. Report prepared for U.S. Fish and Wildlife Service, Portland Office. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Amsberry, K., and R. J. Meinke. 2011. Population creation as a recovery tool for the Federal candidate species *Artemisia campestris* var. *wormskioldii*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Arnett, J. 2010. Wormskiold's northern wormwood (*Artemisia borealis* var. *wormskioldii*): Miller Island Conservation Plan. Report prepared for U.S. Fish and Wildlife Service. Washington Department of Natural Resources, Olympia, Washington.

Bottin, L., S. Le Cadre, A. Quilichini, P. Bardin, J. Moret, and N. Machon. 2007. Re-establishment trials in endangered plants: a review and the example of *Arenaria grandiflora*, a species on the brink of extinction in the Parisian region (France). Ecoscience **14:**410-419.

Brumback, W. E., D. M. Weihrauch, and K. D. Kimball. 2003. Propagation and transplanting of an endangered alpine species Robbins' cinquefoil. Native Plants Journal **Spring**.

Burmeier, S., and K. Jensen. 2009. Experimental ecology and habitat specificity of the endangered plant *Apium repens* (Jacq.) Lag. at the northern edge of its range. Plant Ecology & Diversity **2:**65-75.

Carlson, M.L. 1998. Status report for *Artemisia campestris* L. ssp. *borealis* var. *wormskioldii* [Bess.] Cronq. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Dalrymple, S. E., and A. Broome. 2010. The importance of donor population identity and habitat type when creating new populations of small cow-wheat *Melampyrum sylvaticum* from seed in Perthshire, Scotland. Conservation Evidence 1-8.

Drayton, B., and R. Primack. 2000. Rates of success in the reintroduction by four methods of several perennial plant species in eastern Massachusetts. Rhodora **102:**299-321.

Drayton, B., and R. Primack. 2012. Success rates for reintroductions of eight perennial plant species after 15 years. Restoration Ecology **20:**299-303.

Falk, D. A., C. I. Millar, and M. Olwell. 1996. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington D.C.

Gamon, J. 1989. Report on the status of *Artemisia campestris* L. var. *wormskioldii* [Bess.] Cronquist. Washington Natural Heritage Program, Olympia, Washington.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A. Stevens, R. Aguraiuja, C. Cowell, C. Weekley, G. Vogg, J. Iriondo, I. Johnson, B. 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.

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

Guerrant, E. O. 2012. Characterizing two decades of rare plant reintroduction. Pages 9-29 in J. Maschinski and K. E. Haskins, editors. Reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Guerrant, E. O., and T. N. Kaye. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany **55:**362-370.

Holl, K., and G. Hayes. 2006. Challenges to introducing and managing disturbance regimes for *Holocarpha macradenia*, an endangered annual grassland forb. Conservation Biology **20:**1121-1131.

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

Jõgar, U., and M. Moora. 2008. Reintroduction of a rare plant (*Gladiolus imbricatus*) population to a river floodplain - How important is meadow management? Restoration Ecology **16:**382-385.

Jusaitis, M. 2005. Translocation trials confirm specific factors affecting the establishment of three endangered plant species. Ecological Management & Restoration **6:**61-67.

Jusaitis, M., L. Polomka, and B. Sorensen. 2004. Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). Biological Conservation **116:**251-266.

Kaye, T. N. 2001. Common ground and controversy in native plant restoration: the SOMS debate, source distance, plant selections, and a restoration-oriented definition of native. Native plant propagation and restoration strategies. Corvallis (OR): Nursery Technology Cooperative and Western Forestry and Conservation Association. p**:**5-12.

Kaye, T. N. 2008. Vital steps toward success of endangered plant reintroductions. Native Plants Journal **9:**313-322.

Kaye, T. N. 2009. Toward successful reintroductions: the combined importance of species traits, site quality, and restoration technique. Pages 99-106. California Native Plant Society Conservation Conference.

Kaye, T. N., and J. R. Cramer. 2003. Direct seeding or transplanting: the cost of restoring populations of Kincaid's lupine. Ecological Restoration **21:**224-225.

Kephart, S. 2004. Inbreeding and reintroduction: progeny success in rare *Silene* populations of varied density. Conservation Genetics **5:**49-61.

Lawrence, B., and T. Kaye. 2006. Habitat variation throughout the historic range of golden paintbrush, a Pacific Northwest prairie endemic: implications for reintroduction. Northwest Science **80:**140-152.

Lawrence, B., and T. Kaye. 2009. Reintroduction of *Castilleja levisecta*: effects of ecological similarity, source population genetics, and habitat quality. Restoration Ecology **19:**166-176.

Maschinski, J. 2006. Implications of population dynamic and metapopulation theory for restoration. Pages 59-87 in D. Falk, M. Palmer, and J. Zedler, editors. Foundations of restoration ecology. Island Press, Washington, DC.

Maschinski, J., J. Baggs, and C. Sacchi. 2004. Seedling recruitment and survival of an endangered limestone endemic in its natural habitat and experimental reintroduction sites. American Journal of Botany **91:**689-698.

Maschinski, J., and J. Duquesnel. 2006. Successful reintroductions of the endangered long-lived Sargent's cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. Biological Conservation **134:**122-129.

Maschinski, J., 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 109-129 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. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J., S.J. Wright, S. Koptur, and E.C. Pinto-Torres. 2013. When is local the best paradigm? Breeding history influences conservation reintroduction survival and population trajectories in times of extreme climate events. Biological Conservation **159:**277-284.

Maunder, M. 1992. Plant reintroduction: an overview. Biodiversity and Conservation **1:**51-61.

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 genetics of restoration. Restoration Ecology **13:**432-440.

Menges, E. S. 2008. Restoration demography and genetics of plants: when is a translocation successful? Australian Journal of Botany **56:**187-196.

Monks, L., D. Coates, T. Bell, and M. 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.

Montalvo, A., and N. Ellstrand. 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. Conservation Biology **14:**1034-1045.

National Research Council. 2004. Managing the Columbia River: instream flows, water withdrawals, and salmon survival. The National Academies Press. Washington, DC.

Neale, J. R. 2012. Genetic considerations in rare plant reintroduction: practical applications (or how are we doing?). Pages 71-88 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Noel, F., D. Prati, M. Van Kleunen, A. Gygax, D. Moser, and M. Fischer. 2011. 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.

Oregon Biodiversity Information Center (ORBIC). 2010. Rare, threatened and endangered plants and animals of Oregon. Oregon Biodiversity Information Center, Portland Oregon. Accessed on October 22, 2012. http://orbic.pdx.edu/documents/2010-rte-book.pdf.

Oregon Department of Agriculture (ODA), Native Plant Conservation Program. 2012. Oregon listed plants. Oregon Department of Agriculture, Salem, Oregon. Accessed on October 22, 2012. http://www.oregon.gov/ODA/PLANT/CONSERVATION/Pages/statelist.aspx.

Pavlik, B. M. 1996. Defining and measuring success. Pages 127-155 in D. A. Falk, C. Millar, and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered species. Island Press, Washington, DC.

Primack, R. 1996. Lessons from ecological theory: dispersal, establishment, and population structure. Pages 209-233 in D. Falk, C. Millar, and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Primack, R.B., and B. Drayton. 1997. The experimental ecology of reintroduction. Plant Talk **October:** 26-28.

Reckinger, C., G. Colling, and D. Matthies. 2010. Restoring populations of the endangered plant *Scorzonera humilis*: influence of site conditions, seed source, and plant stage. Restoration Ecology **18:**904-913.

Rimer, R., and K. Mccue. 2005. Restoration of *Helenium virginicum* Blake, a threatened plant of the Ozark Highlands. Natural Areas Journal **25:**86-90.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation **110:**221-229.

U.S. Fish and Wildlife Service. 2011. Species assessment and listing priority assignment form for Northern Wormwood. U.S. Fish and Wildlife Service, Lacey, Washington. Accessed on March 28, 2013. http://www.fs.fed.us/r6/sfpnw/issssp/documents/planning-docs/cp-fws-candidate-va-artemisia-borealis-2011-05.pdf

Walck, J. L., T. E. Hemmerly, and S. N. Hidayati. 2002. The endangered Tennessee purple coneflower *Echinacea tennesseensis* (Asteraceae): its ecology and conservation. Native Plants Journal **3:**54-64.

Washington Department of Natural Resources. 1997. *Artemisia campestris* L. ssp. *borealis* Hall & Clem.var. *wormskioldii* (Bess.) Cronquist species profile. Washington Department of Natural Resources, Olympia, Washington. Accessed on October 22, 2012. http://www1.dnr.wa.gov/nhp/refdesk/fguide/pdf/arca.pdf.

Chapter 2

SOURCE MATERIAL AND TRANSPLANT PRODUCTION FOR THE PREPARATION OF A LARGE-SCALE REINTRODUCTION

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Abstract

Creating a large, genetically diverse founding population is an essential component of a successful reintroduction. Studies show that genetic diversity can positively influence germination, survivorship, flowering, and seed set, and should be considered in the initial stages of a reintroduction project. In combination with genetic diversity, large founding populations can encourage establishment as well as provide a buffer against initial transplant mortality due to factors such as herbivory and disturbance. Once source material is collected, knowledge of horticultural practices is important to produce transplants in large quantity. In this study, we assessed using genetically diverse plant material (in this case, seeds from the two remaining natural populations) on seed germination and transplant cultivation of the rare plant, northern wormwood (*Artemisia campestris* var. *wormskioldii* [Besser ex Hook.] Cronquist [Asteraceae]). Seed sources used for this study were defined as all seeds collected from a single population, regardless of the year collected or whether seeds were wild collected or greenhouse grown. Each population source included seeds from several accessions, defined by year. Seeds were collected from the two remaining natural populations, Miller Island and Beverly, both in Washington and set 300 river km apart. Our results indicated that germination rates varied greatly by seed source and year, with germination rates ranging from 93.0% to 7.5%. Variation in seed germination within sites may be attributed to inbreeding depression at Miller Island and environmental conditions at Beverly. The use of multiple sources and accessions was critical in obtaining enough viable seeds to reach our goal of 2,000 transplants. Overall, 62.4% of the seeds tested

germinated. Approximately 70% of these seedlings (2,400) survived to grow to transplant size. The results of this study contributed to development of germination and propagation protocols for future recovery efforts of northern wormwood.

Introduction

Genetically diverse source material and the introduction of a large founding population can positively affect a reintroduction project (Godefroid et al. 2011). A reintroduced population that retains the genetic resources necessary to be resilient to environmental change is a common goal for reintroduction practitioners (Guerrant 1996a). Genetic diversity also increases the odds that at least some individuals in the founding population will be adapted to the reintroduction site environment. Using a large number of founders can aid in achieving this goal, and decrease the likelihood of the created population succumbing to problems such as inbreeding depression (Severns 2003). Selection of founding population plant material sources requires careful assessment, and choosing which type of plant material to reintroduce (e.g. seeds, whole plants, rhizomes, etc.) may control the success of the project (Maschinski et al. 2012).

Among restoration ecologists there is an ongoing debate about whether to use single or multiple sources for plant material in reintroductions (Kaye 2001; Neale 2012; Maschinski et al. 2013). Studies have shown that genetic diversity of restored populations can be equal to that of native populations when founders come from multiple parent sources (Gustafson et al. 2002; Lloyd et al. 2012). A diverse mix of source material can also improve measures of performance such as germination (Albrecht & McCue 2010), survivorship, flowering, and seed set (Sheridan & Karowe 2000;

Reckinger et al. 2010). Diverse source material representing different genotypes will affect the expression of these traits (Falk et al. 2002). However, trying to maintain local ecotypes to reduce the potential for outbreeding depression may be a goal of the project (Montalvo & Ellstrand 2000; Kaye 2001). Therefore, a single source may be preferred when matching seed source to reintroduction site. Maintaining a single ecotype at a given site can increase the fitness of a reintroduced population when favorable traits have evolved in a particular population, making individuals well-adapted to that site (Montalvo & Ellstrand 2000). Although outbreeding depression is less common than inbreeding depression, it has been exhibited in a few plant species (Ferdy et al. 2001; Sletvold et al. 2012) and is a reasonable concern for conservation biologists. Finally, in certain situations, with rare plant reintroductions, only one source of material may be available (Guerrant 1996b; Albrecht & Penagos 2012), making the decision simple.

Although single source reintroductions occasionally have merit, maximizing the genetic diversity of a founding population through collection from multiple sources is a more common approach (Godefroid et al. 2011). A genetically diverse founding population may increase the ability of the species to establish in a new environment, and respond to future changes in that environment (Maschinski et al. 2013). Variables such as available sources or project goals may help restoration ecologists to choose between using single or multiple sources. A reintroduction may be designed as an experiment to determine which sources perform best for future recovery work (Lawrence & Kaye 2006).

Once the number of parent plant populations has been decided upon, the type of plant material (i.e. seeds or transplants) must be determined. This choice will depend upon many factors, including the quantity of material, funding, project goals, and species biology (Guerrant 1996a; Guerrant & Kaye 2007; Albrecht & Maschinski 2012). Seeds and transplants are the most common choices, but there are other options such as rhizomes or bulbs. Despite the strengths and weaknesses of each choice, recent reviews point to transplants, i.e. whole plants, having higher rates of survival and reproduction in reintroductions (Godefroid et al. 2011; Guerrant 2012). Using larger transplants can accelerate population establishment (Alley & Affolter 2004), and initial characteristics of success, such as flowering and fruiting, can be seen earlier in the project (Jusaitis et al. 2004). However, there are some risks and costs associated with using transplants. Transplants grown in the greenhouse for multiple generations may be adapted to the greenhouse environment and have reduced performance at a reintroduction site. In addition, the cultivation of transplants is usually expensive (especially when compared to sowing seeds directly). Seeds are often selected because they can be collected in large amounts, are more cost-effective (Kaye & Cramer 2003), and are relatively undemanding to distribute on site. Potential low germination (Holl and Hayes 2006) and increased time to reproductive size, which can slow population growth (Albrecht & McCue 2012), are often the drawbacks to using seeds.

The quantity of plant material used to create the founding population will be determined by the type of material selected. In general, reintroductions that use seeds will have a larger founding size to make up for potentially low germination rates in the wild. Creating a large founding population can be more complicated with transplants, as more time and effort goes into growing and placing plants on site. In a review of 238 reintroductions, Godefroid et al. (2011) found that researchers used an average of 1,551 seeds or 400 transplants in each reintroduction attempt. While there are no specific guidelines for determining the founding size of a population, reviews of reintroduction studies show that the larger the founder population is, the greater the chance for success (Godefroid et al. 2011, Albrecht & Maschinski 2012). Available material and other practical constraints may guide the decision (Guerrant 2012). Minimum viable population requirements (Shaffer 1981) described in recovery plans for endangered species may also assist in determining founder population size.

For the viability of a reintroduction project, it is critical to have knowledge of horticultural practices and logistics. An understanding of seed germination and seedling care is essential to obtaining the appropriate quantity of transplants with minimal losses (Maschinski et al. 2012). Pilot cultivation studies may be conducted to test protocol before beginning large-scale propagation. These can also aid in the development of a timeline for the project to determine the expected outplanting date and then backtrack to determine start dates for cultivation. Understanding the timeframe from seed germination to transplant enables the development of a logistical plan incorporating elements such as transportation of material to the site and designating a number of days for planting. Trial studies can also inform researchers of the need for additional techniques to increase seed germination. For example, Brumback et al. (2003) treated *Potentilla robbinsiana* seeds with gibberelic acid (a plant hormone used to stimulate germination) before sowing in the spring. This treatment was not needed when seeds were sown in the fall, since they were subjected to natural stratification between winter freezing and thawing conditions. Both methods resulted in increased germination compared to the sowing of untreated seeds in the spring.

In this study, we looked at seed germination rates in relation to multiple population sources, as well as transplant production in anticipation of a large-scale reintroduction of the rare plant, northern wormwood (*Artemisia campestris* var. *wormskioldii* [Besser ex Hook.] Cronquist [Asteraceae]).

Northern wormwood is a tap-rooted perennial restricted to dynamic cobble bar environments on the Columbia River, and is known from only two natural populations in Washington, Miller Island and Beverly, which are set 300 river km apart (Arnett 2010). Northern wormwood is listed as Endangered in both Oregon and Washington (ODA 2012; Washington Department of Natural Resources 1997). This species is also a Candidate for listing and designated a “Spotlight Species” by the U.S. Fish and Wildlife Service (USFWS 2011).

Northern wormwood is an excellent candidate for a large-scale reintroduction for several reasons. Previous work completed by the Oregon Department of Agriculture (ODA) has shown that seeds of this species germinate readily in the greenhouse and plants are easy to care for (Amsberry et al. 2007). Several pilot outplantings completed by ODA have shown that northern wormwood plants can establish in selected sites, and that these transplants also produce a large amount of seed (Amsberry & Meinke 2011). This seed has been collected and stored *ex situ*, and is available for further recovery efforts. This paper will address the off-site preparation needed to support the outplanting of northern wormwood, focusing on germination rates of different seed sources and transplant production. The objective was to grow 2,000 plants from seed in the greenhouse at Oregon State University (OSU). To achieve this, we measured germination rates from multiple population seed sources and developed a germination and transplant production protocol suitable for large-scale outplantings.

Methods

Seed sources

One objective of this project was to maximize the diversity of the founding population. Seeds were selected from the two remaining native populations, Miller Island and Beverly. The Beverly source included seed collected from plants cultivated in the greenhouse at OSU from seed originally collected at this native site. Each population source included seeds collected from at least four separate years, creating nine accessions (Table 2.1). Seeds collected in different years may reflect the different environmental conditions under which they were produced. This provided spatiotemporal variation that would contribute to the diversity of the founding population.

The Miller Island population is on the east end of the island in an area of drifting sand, with plants rooted in basalt bedrock. This population is declining most likely due to burial by sand (Arnett 2010). Seeds from Miller Island were collected in June of 2011 from seven plants that had been bagged earlier in the spring. Of the approximately 1,900 seeds collected, 390 were used for this project. The rest were stored at OSU to be used for future recovery efforts. Five hundred seeds from Miller Island were also contributed by the former Berry Botanic Garden (BBG), now the Rae Selling Berry Seed Bank and Plant Conservation Program, in Portland, Oregon. In cooperation with the U.S. Forest Service, Oregon Department of Agriculture, and Washington Department of Natural Resources, BBG staff collected northern wormwood seeds at Miller Island in three different years: 1991, 2004, and 2006. Seeds were subsequently stored at BBG's seed bank. The seeds from 1991 were part of a bulk collection and seeds from 2004 and 2006 were from specific accessions, representing 10 and 7 parent plants, respectively. However, from this point forward, collections from 2004 and 2006 will be referred to as accessions in terms of the year collected, not parent plant. Miller Island is approximately 10 km downriver from Rufus Island, the reintroduction site, and represents a geographically similar source.

The second source, Beverly, is found further north on the Columbia River in Washington, approximately 300 km upstream from our reintroduction site (Rufus Island). This population occurs on a cobble-sand peninsula that experiences changing water levels as a result of the nearby Wanapum Dam (USFWS 2011). Seeds are collected here annually by the Grant County Public Utility District, some of which were sent to ODA to be stored at OSU (Figure 2.1). Seeds for this project were collected from this site in 2008-2010. Seeds were not separated by maternal line. Flooding of the population prevented seed collection in 2011 and 2012 (Mark Woodward, pers. comm.). A total of 1,231 seeds collected over three years were used for this project. The habitat at Beverly is similar to some of that found on Rufus Island, so this source represents an ecologically similar source for some planting locations at this reintroduction site.

Table 2.1. Number of seeds by year from each population source. Beverly Cultivated seeds were collected from large adult plants started from Beverly seeds, but grown in the greenhouse yard at OSU. Miller Island BBG seeds were collected in the wild and stored at the Rae Selling Berry Seed Bank at the former Berry Botanic Garden in Portland, Oregon. The Berry Botanic Garden is now the Rae Selling Berry Seed Bank and Plant Conservation Program, housed at Portland State University.

Source	Number of seeds used	Accession	Location
Beverly Cultivated	1230	2008	Corvallis, OR
Beverly Cultivated	40	2011	Corvallis, OR
Beverly	310	2008	Beverly, WA
Beverly	305	2009	Beverly, WA
Beverly	616	2010	Beverly, WA
Miller Island	390	2011	Klicktat CO, WA
Miller Island: BBG	250	1991	Klicktat CO, WA
Miller Island: BBG	219	2004	Klicktat CO, WA
Miller Island: BBG	31	2006	Klicktat CO, WA
Total	3,391	9	

Beverly seeds were also collected from cultivated plants at OSU. ODA has maintained 30 northern wormwood plants in their nursery yard to be used for seed collection. These plants were started in 2005 from seed collected from the Beverly population. They are grown in five gallon pots, watered regularly, and experience a considerably more stable environment (Figure 2.2) when compared to that of natural populations located on the Columbia River. At the time of this project, over 10,000 northern wormwood seeds collected from these plants were stored at OSU. For this project, 1,230 seeds from the 2008 collection were used, as well as 40 seeds collected from these plants in 2011. This source will be referred to as Beverly Cultivated throughout the rest of this paper. The total number of seeds used in this study collected from two population sources across seven separate years, was 3,391.

Figure 2.1. Bagged plant for seed collection at the native population, Beverly in May 2011. Seeds were collected by the Grant County Public Utility District and sent to ODA for storage. Photo by K. Amsberry.

Seed germination

Transplants grown from seeds were selected for this project based on the methods developed for the pilot outplanting conducted by ODA (Amsberry et al. 2007). Plants were easily grown in the greenhouse and transplants showed initial establishment (Amsberry & Meinke 2011). Germination was carried out at the OSU greenhouse in June, 2011. Prior work (Amsberry et al. 2007; Amsberry & Meinke 2011) indicates that healthy northern wormwood seeds can be determined by size and color: dark brown or grey and plump seeds indicate potentially more viable seeds, while green or small seeds can indicate unripe or unviable seed. To determine how many seeds to use for germination, a pilot study was conducted two months prior to the actual start date of the project. For this pilot study, ten seeds from Beverly 2008 and 2010 and Beverly Cultivated 2008 were germinated in Petri dishes on filter paper wetted with distilled water and covered with the lid. These dishes were set in the greenhouse at OSU, subjected to the natural light, and monitored for germination. Rates calculated from this pilot study were used to estimate the number of seeds to use for each seed source to reach our goal. Seeds from Miller Island were not used in the pilot study as they had not yet been acquired.

Figure 2.2. Plants in the greenhouse yard at OSU grown for seed collection. These plants were started from Beverly seed in 2005 and represent different environmental conditions under which seeds were developed. Seeds collected from these plants in 2008 and 2011 were used for this project. Photo by K. Amsberry.

For the germination study, seeds from each accession were placed in Petri dishes on filter paper wetted with distilled water. Each dish received ten to 15 seeds (Figure 2.3), except for some from Miller Island BBG dishes, where seeds were separated by accession and some accessions had less than ten seeds available. Temperature in the greenhouse ranged from 18-29 C°, and filter papers were moistened as needed (almost daily). After one week, seeds from some accessions did not show any germination, so a second group of seeds were prepared for germination to ensure that we reached our goal of 2,000 plants. The method for both sets of germination was based on previous germination of northern wormwood seeds by developed by ODA (Amsberry et al. 2007; Amsberry & Meinke 2011), as well as information collected during the pilot seed study conducted two months prior. No seed germination pre-treatments were used.

Germination rates were determined by observing seeds for 17 days. Germination was defined as the breaking of the seed coat and the emergence of a 1 mm-long radicle. Each day, the number of new seeds that had germinated in each dish was recorded, as well as the presence of mold and greenhouse temperature. After 17 days most seeds had germinated and mold was becoming widespread in Petri dishes. Mean germination rates were analyzed with a one-way ANOVA with significance set at $p \leq 0.05$. Tukey's honest significant difference (HSD) test was used to determine which means differed. Analyses were performed in R, an integrated suite of software facilities for data manipulation and calculation (R Core Team 2012, version 2.11.1).

Figure 2.3. Fifteen seeds from the Beverly 2010 accession completely germinated in a Petri dish. Photo by A. Brickner.

Transplant production

Seedlings were planted one week after germination began (Figure 2.4). Seedlings were 1-3 cm long at this stage, and were planted in 10.16 cm square pots filled with Sunshine Professional Growing Mix® (Canadian sphagnum peat moss, horticultural grade perlite, pumice, and dolomite limestone) and watered at the time of planting. Plants were separated by seed source, labeled, and cultivated in the greenhouse for eight weeks (Figure 2.5). Plants were watered daily, and fertilized weekly with Dyna-gro® (7-9-5) liquid plant food. Fungus gnats hindered growth of plants for the pilot outplanting

(Amsberry & Meinke 2011), so a layer of quartz was placed around plants. Losses were minimal at this stage, although a few seedlings did develop past cotyledon stage. After eight weeks, plants were placed outside to be conditioned to the outdoor environment (Figure 2.6). Fertilization was discontinued at this stage as plants were well developed and were beginning to become potbound.

Figure 2.4. Planting seedlings one week after germination. Photo by A. Brickner.

Figure 2.5. Transplants growing in the greenhouse at OSU. Photo by A. Brickner.

Figure 2.6. Over 2,400 northern wormwood plants being acclimatized at OSU. Photo by A. Brickner.

Results

Seed germination

Germination rates varied across all sources (Figure 2.7). The accessions with the highest germination rates were Beverly 2010 (93.0%), both Beverly Cultivated years (91.3% and 87.5% for 2008 and 2011, respectively), and Beverly 2008 (77.4%). Most surprisingly, the seeds from Miller Island 1991 germinated at a rate of 78.7%. Seeds from Miller Island that were collected in 2011 had the lowest germination rate (7.5%) during the observation period. However, many more seeds from this source germinated after observations ceased, adding to the diversity of the founding population.

Climate and dam discharge data were gathered to evaluate the potential influence of these factors on germination rates for seeds collected at the Beverly site. Temperature and precipitation data were available from the Priest Rapids Dam Weather Station (Western Regional Climate Center 2013), located approximately 12 km from the Beverly population. Wanapum Dam discharge data were available from the Fish Passage Center (Fish Passage Center 2013). Monthly temperatures did not differ among the three seed collections years (Figure 2.8). However, precipitation during May and June (when seeds are maturing) was much higher in 2010 (Figure 2.9). In 2008, although precipitation was low, high levels of water from dam discharge may have provided adequate moisture for seed maturation (Figure 2.10). In 2009, both precipitation and discharge were low, resulting in inadequate moisture for seed maturation and poor germination rates. Demography data depict a dramatic reduction in population size at Miller Island (J.

Arnett pers. comm. May 9, 2011; Figure 2.11). This decline mimics the reduction in seed germination rates for seeds collected over a similar time period (Figure 2.7).

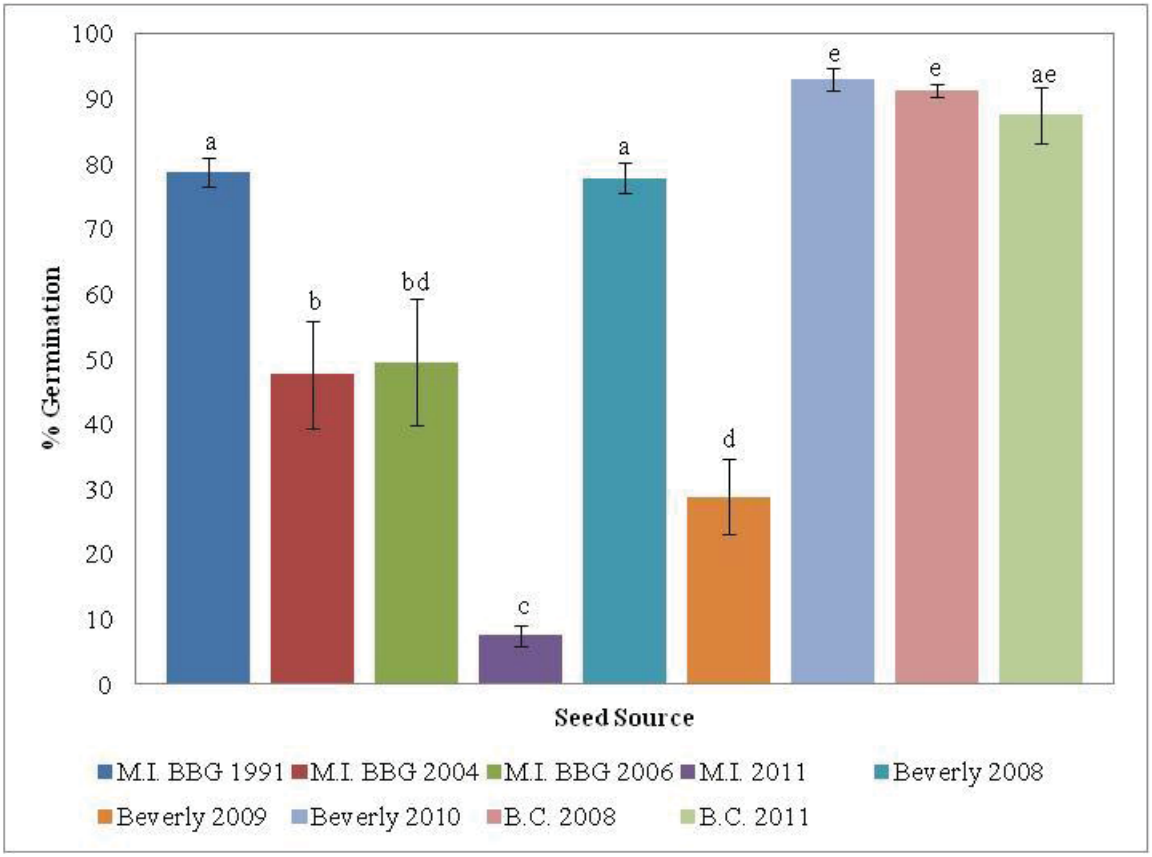

Figure 2.7. Mean percent germination by seed source after 17 days. Seeds were germinated in Petri dishes in the greenhouse at OSU. M.I. BBG = Miller Island seed from the Berry Botanic Garden, M.I. = Miller Island, B.C. = Beverly Cultivated. Error bars represent standard error (n = 23 for M.I. 1991, n = 23 for M.I. 2004, n = 7 for M.I. 2006, n = 24 for M.I. 2011, n = 21 for Beverly 2008, n = 20 for Beverly 2009, n = 41 for Beverly 2010, n = 82 for B.G. 2008, and n = 4 for B.G. 2011). Columns with different letters differ significantly from each other ($p < 0.05$).

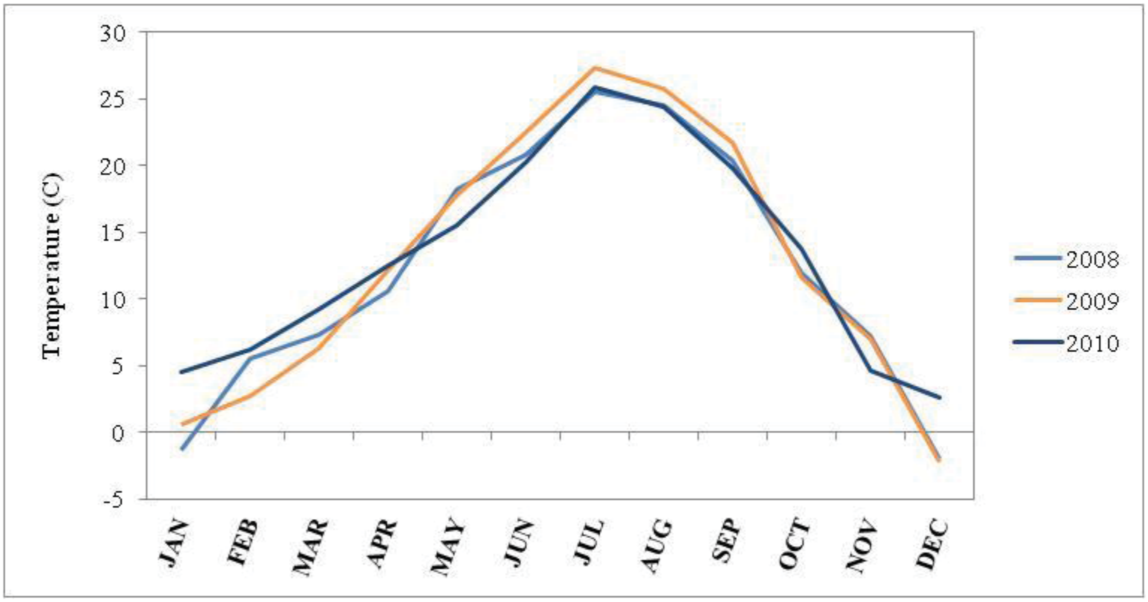

Figure 2.8. Average monthly temperature (°C) from the Priest Rapids Dam Weather Station (Western Regional Climate Center 2013).

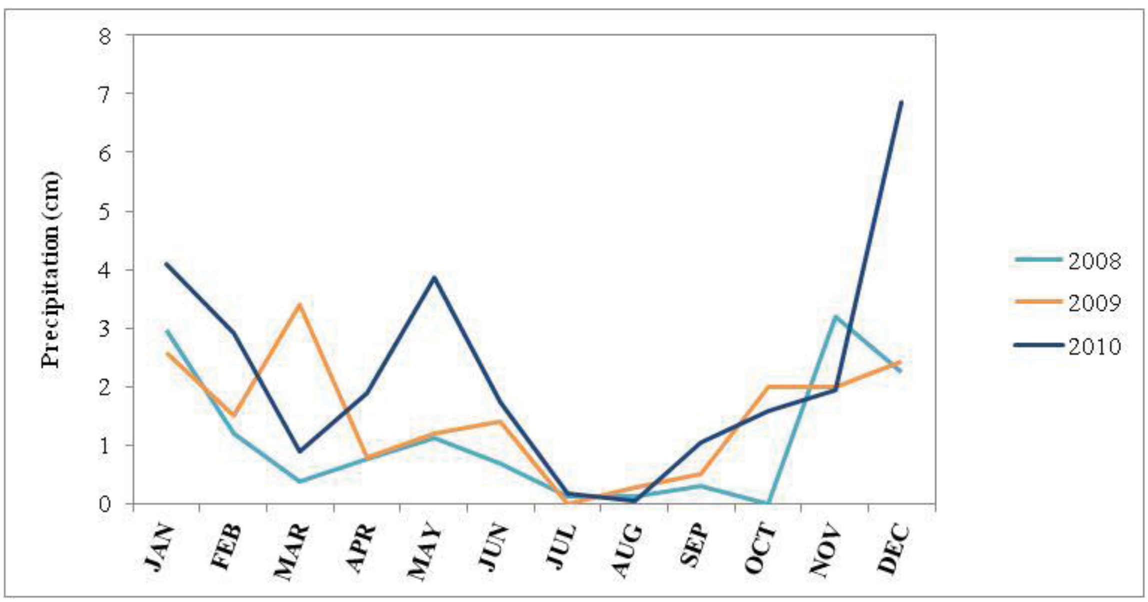

Figure 2.9. Average monthly precipitation (cm) from the Priest Rapids Dam Weather Station (Western Regional Climate Center 2013).

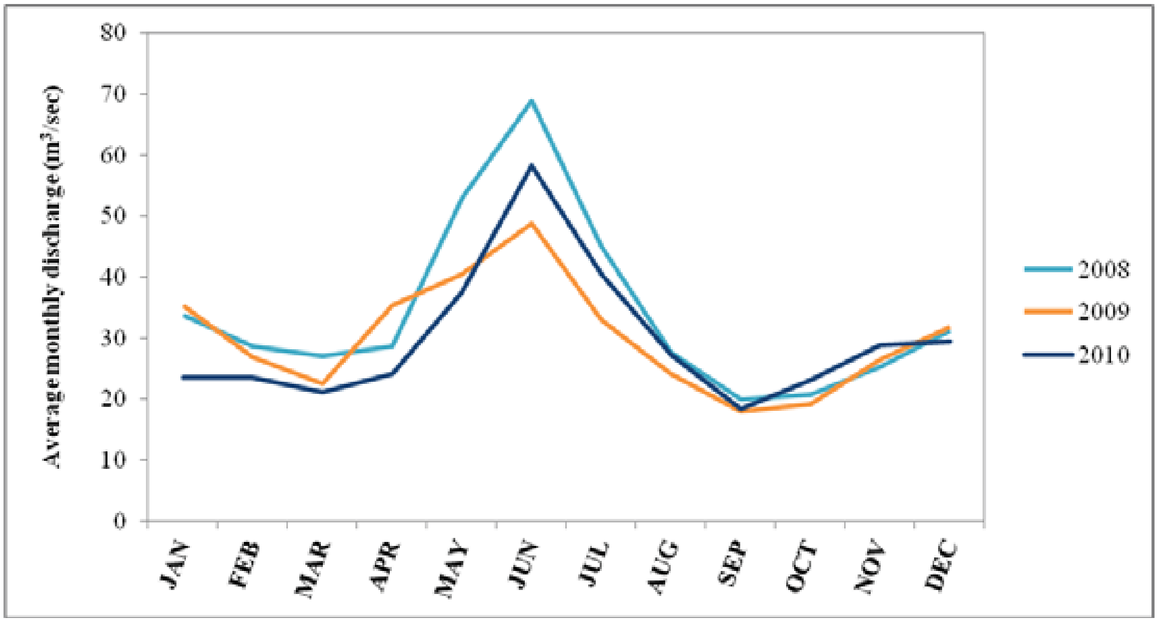

Figure 2.10. Average monthly discharge (m^3/sec) from the Wanapum Dam located upriver from the Beverly population (Fish Passage Center 2013).

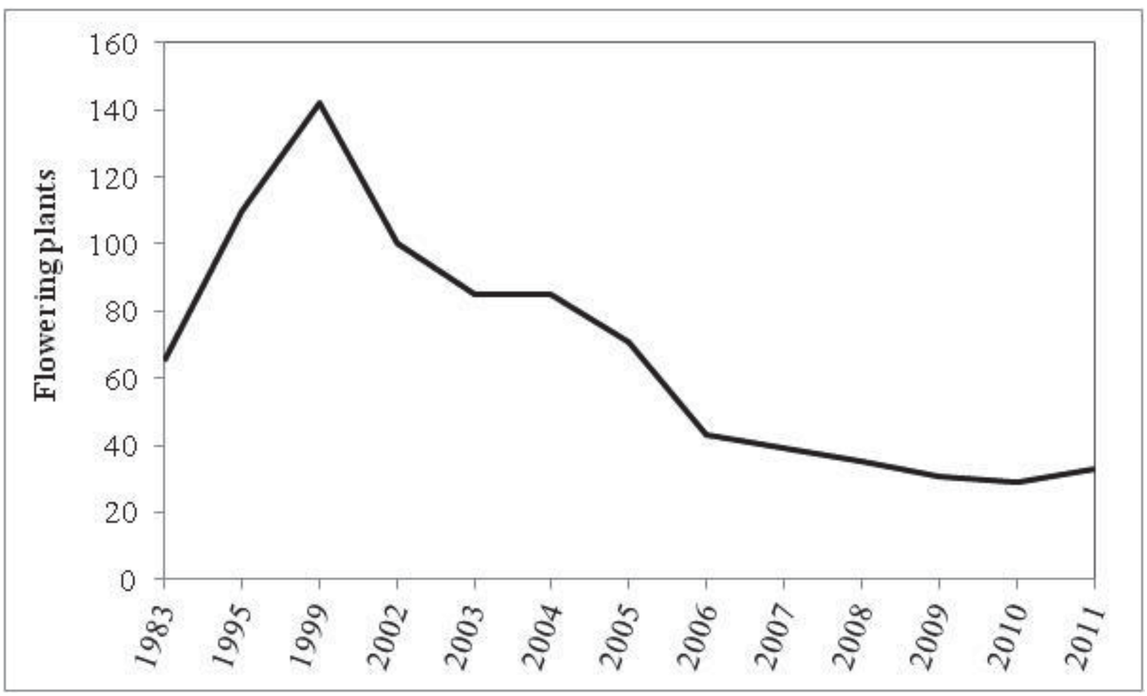

Figure 2.11. Results from demography data collected from 1983 to 2011 at Miller Island (J. Arnett, pers. comm. May 9, 2011).

Discussion

Acquiring propagules for rare plants can be challenging as minimal material may be available or may come at a high cost to the parent population (Guerrant 1996b; Guerrant & Kaye 2007). Despite the rarity of northern wormwood, we were able to utilize nine accessions of seeds from two native sources, spanning a 20 year collection period. This diverse compilation of seeds produced a cohort of transplants with a range of genetic traits. Greater diversity of the founding population contributes to a better probability of population establishment (Guerrant 1996a). Maximizing germination is important for rare plant species as seed availability can be limited (Guerrant & Kaye 2007) and every seed that does not germinate could be considered a loss to the species. The use of an *ex situ* source can reduce the impact of seed collection from the wild populations by managing for the viability of these populations (Cochrane et al. 2007).

Variation in seed germination is often attributed to genetic and environmental factors (Gutterman 2000), but determining the germination characteristics of a species can take multiple years and experimental treatments (Andersson & Milberg 1998). Results from our study show variation in germination rates between seeds collected from the two native populations and between years within each of these populations. These results suggest that genetic factors and environmental conditions may be influencing germination rates of northern wormwood seed.

One such genetic factor that may reduce plant fitness is inbreeding depression (Silvertown & Charlesowrth 2001). Reduced seed set and seed viability, in combination with small population size, are symptoms of inbreeding depression. (Sheridan & Karowe

2000; Silvertown & Charlesworth 2001; Severns 2003; Charlesworth & Willis 2009; Angeloni et al. 2011). The germination rates of four Miller Island seed collections spread across 20 years, paired with 28 years of demography data (Figure 2.11), suggest that inbreeding depression is increasing in this population. Seeds collected in 1991 had significantly greater germination rates than those collected in subsequent years. Demographic data collected between 1983 and 1999 indicates that the population grew from 65 to 142 northern wormwood plants (Joe Arnett, pers. comm. May 9, 2011; Figure 2.11). As the Miller Island population declined between 2002 and 2011, so did the germination rates of seeds collected from 2004, 2006, and 2011. In 2004, the Miller Island population consisted of 85 flowering plants, a 60% decrease from 1999. By 2006, there were 43 plants. In 2011, only 33 flowering northern wormwood plants were located. The lack of recruitment in this population may also be evidence of reduced seed viability. Inbreeding depression is a complex phenomenon (Angeloni et al. 2011). Our results corroborate with results of other studies that have shown a correlation between small population size, reduced seed viability, and potential inbreeding depression (Sheridan & Karowe 2000; Thorpe & Kaye 2011). Therefore, it is possible that inbreeding depression is the cause of reduced germination rates for seeds in this study.

Several germination studies have shown inter-annual variation in seed viability similar to that found in seeds collected from the wild Beverly population (Beckstead et al. 1996; Andersson & Milberg 1998; Herranz et al. 2010). Environmental conditions, as opposed to genetics, are often credited with differences in seed viability between years (Galloway 2001; Luzuriaga et al. 2006). Unpredictable environments may create more variation (Beckstead et al. 1996) and allow for greater germination in years with more favorable environmental conditions.

The Beverly population experiences arid to semi-arid conditions characteristic of the Columbia Basin (Gamon 1989; Carlson 1998), along with fluctuating water levels provided by the dams. The differences in germination rates of the three wild collected accessions from Beverly (2008, 2009, and 2010) may be attributed to this hot, dry, and dynamic environment. Seeds collected in all three years germinated at significantly different rates. Climate data from the Priest Rapids Dam Weather Station (Western Regional Climate Center 2013) indicates that there was no difference in temperature during the growing season during these three years. However, precipitation data (Western Regional Climate Center 2013) and discharge data from the Wanapum Dam (Fish Passage Center 2013), found upstream from the population, may explain the variation. Northern wormwood plants flower in April and May, and set seeds by late May to early June. Precipitation in May was significantly greater in 2010 than in 2009. Beverly 2010 and Beverly Cultivated 2008 and 2011 seed accessions exhibited similar germination rates. The cultivated plants received regular watering in the spring and the amount of water available to northern wormwood plants during flowering may influence seed viability. Discharge from the Wanapum Dam was lowest in May 2010 and plants were probably not inundated during this time, another factor that may contribute to viable seed production. The drier flowering season of 2009 may have caused northern wormwood plants to allocate resources to factors associated with survival and growth, not seed viability. While 2008 was also fairly dry when compared to 2010, the discharge from the dam was higher in that year, and may have provided northern wormwood access to groundwater that mitigated the effects of lower rainfall.

Variation in population size can explain differences in plant fitness traits between sites (Fischer & Matthies 1998; Morgan 1998; Reed 2005; Leimu et al. 2006; Thorpe & Kaye 2011). Studies that have found a correlation between population size and germinability are rare (Morgan 1998; Jacquemyn et al. 2001; but see Sheridan & Karowe 2000), but some correlation has been shown between factors that may affect germinability such as seed set (Morgan 1998) and seed mass (Jacquemyn et al. 2001). Inbreeding depression in the Miller Island population may account for the lower germination of seeds from this site in comparison to the larger Beverly site. While the Beverly population had some inter-annual variation in seed viability, the average seed germination rate across five years was significantly greater than the average germination rate of seed from Miller Island ($p < 0.0001$, Figure 2.7). Many factors determine the viability of seeds, such as maternal and paternal environments (Galloway 2001), population size (Thorpe & Kaye 2011), and genetic factors (Beckstead et al. 1996). These factors may contribute to the variation in viability of northern wormwood seeds.

The primary goal of this study was to determine germination rates in terms of seed source and year, with the intent of supporting the paradigm of multiple sources for plant material for reintroduction (Maschinski et al. 2013). This study supports the use of multiple sources and provides new information on the germination ecology of the rare northern wormwood. Additional information on a rare species can contribute to understanding the causes of rarity (Schemske et al. 1994), as well as help guide management decisions.

Literature Cited

Albrecht, M., and K. McCue. 2010. Changes in demographic processes over long time scales reveal the challenge of restoring an endangered plant. Restoration Ecology **18:**235-243.

Albrecht, M., and J. Penagos. 2012. Seed germination ecology of three imperiled plants of rock outcrops in the southeastern United States. Journal of the Torrey Botanical Society **139:**86-95.

Albrecht, M. A., and J. Maschinski. 2012. Influence of founder population 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.

Alley, H., and J. Affolter. 2004. Experimental comparison of reintroduction methods for the endangered *Echinacea laevigata* (Boynton and Beadle) Blake. Natural Areas Journal **24:**345-350.

Amberry, K., R. Currin, and R.J. Meinke. 2007. Reintroducing *Artemisia campestris* var. *wormskioldii* to Oregon: site selection, cultivation, and pilot outplanting. Report prepared for U.S. Fish and Wildlife Service, Portland Office. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Amsberry, K., and R. J. Meinke. 2011. Population creation as a recovery tool for the Federal candidate species *Artemisia campestris* var. *wormskioldii*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Andersson, L.,and P. Milberg. 1998. Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Science Research **8:**29-38.

Angeloni, F., N.J. Ouborg, and R. Leimu. 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. Biological Conservation **144:**35-43.

Arnett, J. 2010. Wormskiold’s Northern wormwood (*Artemisia borealis* var. *wormskioldii*): Miller Island Conservation Plan. Report prepared for U.S. Fish and Wildlife Service. Washington Department of Natural Resources, Olympia, Washington.

Beckstead, J., S. Meyer, and P. Allen. 1996. *Bromus tectorum* seed germination: between-population and between-year variation. Canadian Journal of Botany-Revue Canadienne De Botanique **74:**875-882.

Bottin, L., S. Le Cadre, A. Quilichini, P. Bardin, J. Moret, and N. Machon. 2007. Re-establishment trials in endangered plants: a review and the example of *Arenaria grandiflora*, a species on the brink of extinction in the Parisian region (France). Ecoscience **14:**410-419.

Brumback, W. E., D. M. Weihrauch, and K. D. Kimball. 2003. Propagation and transplanting of an endangered alpine species Robbins' cinquefoil. Native Plants **Spring**.

Carlson, M.L. 1998. Status report for *Artemisia campestris* L. ssp. *borealis* var. *wormskioldii* [Bess.] Cronq. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Charlesworth, S., and J.H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics **10**:783-796.

Cochrane, J., A. Crawford, and L. Monks. 2007. The significance of ex situ seed conservation to reintroduction of threatened plants. Australian Journal of Botany **55:**356-361.

Falk, D.A., E.E. Knapp, and E.O. Guerrant. 2002. An introduction to restoration genetics. Prepared by the Society for Ecological Restoration for Plant Conservation Alliance, Bureau of Land Management, U.S. Department of Interior.

Ferdy, J., S. Loriot, M. Sandmeier, M. Lefranc, and C. Raquin. 2001. Inbreeding depression in a rare deceptive orchid. Canadian Journal of Botany-Revue Canadienne De Botanique **79:**1181-1188.

Fischer, M., and D. Matthies. 1998. Effects of population size on performance in the rare plant *Gentianella germanica*. Journal of Ecology **86:**195-204.

Fish Passage Center. 2013. River data: Wanapum dam. Fish Passage Center, Portland, Oregon. Accessed on March 28, 2013. http://www.fpc.org/river_home.html.

Galloway, L.F. 2001. The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). American Journal of Botany **88(5):**832-840.

Gamon, J. 1989. Report on the status of *Artemisia campestris* L. var. *wormskioldii* [Bess.] Cronquist. Washington Natural Heritage Program, Olympia, Washington.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A. Stevens, R. Aguraiuja, C. Cowell, C. Weekley, G. Vogg, J. Iriondo, I. Johnson, B. 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.

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

Guerrant, E. O. 1996b. Reintroduction of *Stephanomeria malheurensis*, a case study. Pages 399-402 in D. Falk, C. Millar, and P. Olwell, editors. Restoring diversity: strategies for reintroductions of endangered plants. Island Press, Washington, DC.

Guerrant, E. O. 2012. Characterizing two decades of rare plant reintroduction. Pages 9-29 in J. Maschinski and K. E. Haskins, editors. Reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Guerrant, E. O., and T. N. Kaye. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany **55:**362-370.

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

Gustafson, D., D. Gibson, and D. Nickrent. 2004. Competitive relationships of *Andropogon gerardii (*Big Bluestem) from remnant and restored native populations and select cultivated varieties. Functional Ecology **18:**451-457.

Gutterman, Y. 2000. Maternal effects on seeds during development. Pages 59-84 in M. Fenner, editor. Seeds: The ecology of regeneration in plant communities, 2nd ed. CAB International Publishing. Wallingford, UK.

Herranz, J.M., P. Ferrandis, and E. Martinez-Duro. 2010. Seed germination ecology of the threatened endemic Iberian *Delpinium fissum* subsp. *sordidum* (Ranunculaceae). Plant Ecology **211:**89-106.

Holl, K., and G. Hayes. 2006. Challenges to introducing and managing disturbance regimes for *Holocarpha macradenia*, an endangered annual grassland forb. Conservation Biology **20:**1121-1131.

Huang, Z., Y. Gutterman, and Z. Hu. 2000. Structure and function of mucilaginous achenes of *Artemisia monosperma* inhabiting the Negev Desert of Israel. Israel Journal of Plant Sciences **48:**255-266.

Jacquemyn, H., R. Brys, and M. Hermy. 2001. Within and between plant variation in seed number, seed mass and germinability of *Primula elatior*: effect of population size. Plant Biology **3:**561-568.

Jusaitis, M., L. Polomka, and B. Sorensen. 2004. Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). Biological Conservation **116:**251-266.

Kaye, T. N. 2001. Common ground and controversy in native plant restoration: the SOMS debate, source distance, plant selections, and a restoration-oriented definition of native. Native plant propagation and restoration strategies. Corvallis (OR): Nursery Technology Cooperative and Western Forestry and Conservation Association. p**:**5-12.

Kaye, T. N. 2008. Vital steps toward success of endangered plant reintroductions. Native Plants **9:**313-322.

Kaye, T. N., and J. R. Cramer. 2003. Direct seeding or transplanting: the cost of restoring populations of Kincaid's lupine. Ecological Restoration **21:**224-225.

Lawrence, B., and T. Kaye. 2009. Reintroduction of *Castilleja levisecta*: effects of ecological similarity, source population genetics, and habitat quality. Restoration Ecology **19:**166-176.

Leimu, R., P.M. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology **94:**942-952.

Lloyd, M., R. Burnett, K. Engelhardt, and M. 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 **13:**753-765.

Luzuriaga, A.L., A. Escudero, and F. Perez-Garcia. 2006. Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). Weed Research **46:**163-174.

Maschinski, J., M. A. Albrecht, L. Monks, and K. E. Haskins. 2012. Center for Plant Conservation best reintroduction practice guidelines. Pages 277-306 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J., S.J. Wright, S. Koptur, and E.C. Pinto-Torres. 2013. When is local the best paradigm? Breeding history influences conservation reintroduction survival and population trajectories in times of extreme climate events. Biological Conservation **159:**277-284.

Menges, E.S. 1991. Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biology **5:**158-164.

Montalvo, A., and N. Ellstrand. 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. Conservation Biology **14:**1034-1045.

Morgan, J.W. 1998. Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology **13:**266-273.

Neale, J. R. 2012. Genetic considerations in rare plant reintroduction: practical applications (or how are we doing?). Pages 71-88 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Oregon Department of Agriculture (ODA), Native Plant Conservation Program. 2012. Oregon listed plants. Oregon Department of Agriculture, Salem, Oregon. Accessed on October 22, 2012.
http://www.oregon.gov/ODA/PLANT/CONSERVATION/Pages/statelist.aspx.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Reckinger, C., G. Colling, and D. Matthies. 2010. Restoring populations of the endangered plant *Scorzonera humilis*: influence of site conditions, seed source, and plant stage. Restoration Ecology **18:**904-913.

Reed, D.H. 2005. Relationship between population size and fitness. Conservation Biology **19:**563-568.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation **110:**221-229.

Schemske, D.W., B.C. Husband, M.H. Ruckelshaus, C. Goodwillie, I.M. Parker, and J.G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology **75(3):**584-606.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. BioScience **31**:131-134.

Sheridan, P., and D. Karowe. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia. American Journal of Botany **87:**1628-1633.

Silvertown, J., and D. Charlesworth. 2001. Introduction to plant population biology, 4th ed. Blackwell Publishing. Malden, MA.

Sletvold, N., J. Grindeland, P. Zu, and J. Agren. 2012. Strong inbreeding depression and local outbreeding depression in the rewarding orchid *Gymnadenia conopsea*. Conservation Genetics **13:**1305-1315.

Sletvold, N., J. Grindeland, P. Zu, and J. Agren. 2012. Strong inbreeding depression and local outbreeding depression in the rewarding orchid *Gymnadenia conopsea*. Conservation Genetics **13:**1305-1315.

Thorpe, A.S., and T.N. Kaye. 2011. Conservation and reintroduction of the endangered Willamette daisy: effects of population size on seed viability and the influence of local adaptation. Native Plants Journal **12(3):**289-298.

U.S. Fish and Wildlife Service. 2010. Spotlight Species Action Plan for Northern Wormwood. U.S. Fish and Wildlife Service, Lacey, Washington. Accessed on December 6, 2010. http://ecos.fws.gov/docs/action_plans/doc3088.pdf

Washington Department of Natural Resources. 1997. *Artemisia campestris* L. ssp. *borealis* Hall & Clem.var. *wormskioldii* (Bess.) Cronquist species profile. Washington Department of Natural Resources, Olympia, Washington. Accessed on October 22, 2012. http://www1.dnr.wa.gov/nhp/refdesk/fguide/pdf/arca.pdf.

Western Regional Climate Center. 2013. Cooperative Climatological Data Summaries: Washington, Priest Rapids Dam. Accessed on April 15, 2013. http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?wa6747

Yang, X., M. Dong, and Z. Huang. 2010. Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. Plant Physiology and Biochemistry **48:**131-135.

Chapter 3

ECOLOGICAL FACTORS INFLUENCING TRANSPLANT SUCCESS IN THE EXPERIMENTAL REINTRODUCTION OF NORTHERN WORMWOOD

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Abstract

Northern wormwood (*Artemisia campestris* var. *wormskioldii*) is a rare plant found on the banks of the Columbia River and may benefit from reintroduction work. Although threatened with extinction, virtually nothing is known about the species' biology, and little is known about wormwood habitat or recruitment requirements. Accordingly, an experiment was designed to investigate the effects of three ecological factors on transplant survival. A secondary goal of the project was to create a population in support of on-going recovery efforts. In October, 2011, we planted 2,100 greenhouse grown transplants on Rufus Island, Sherman County, Oregon, which is located within the historic range of the species. We transplanted 1,450 of these plants in experimental plots to examine the impacts of: (1) substrate type; (2) distance from the water line; and (3) presence or absence of the invasive shrub, false indigo (*Amorpha fruticosa*). We found that the sand substrate resulted in the greatest survival of transplants while compacted rock and loose rock substrates exhibited higher mortality. Based on our data and specifically for this site, a minimum planting distance of nine meters from the water line in October, 2011, is recommended for transplanting to curb losses from fluctuating water levels. There was no significant difference in transplant survival between treated and control false indigo plots. However, this invasive plant should not be ruled out as a threat to northern wormwood, given the short timeframe of this study. Data collected on reproductive output implied that our created population has the potential to be self-sustaining. This was strengthened in September of 2012 with the discovery of 11 wormwood seedlings, but continued monitoring will be necessary to understand the

reproductive capabilities of this population. Results from this reintroduction, in terms of habitat requirements and reintroduction protocol, can be extrapolated for use in further recovery efforts.

Introduction

Active conservation efforts are essential for ensuring the maintenance of plant diversity around the world. Reintroduction is a conservation tool that can create new populations of declining species as part of our efforts to preserve biodiversity (Maunder 1992; Maschinski & Haskins 2012). Reintroduction practitioners can reduce extinction potential of particularly vulnerable taxa, through the creation of new populations within the historic range of a species (Walck et al. 2002; Brumback et al. 2003). Whenever feasible, such projects can also include an experimental component to gain information on the ecology and biology of the species (Guerrant & Kaye 2007).

Plants have become endangered due to a variety of anthropogenic threats such as the introduction of invasive species (Jusaitis 2005) or habitat loss (Alley & Affolter 2004). Vulnerability may also be related to natural causes, including dispersal limitations (Walck et al. 2002) or being endemic to specific, limited habitats (Maschinski et al. 2004; Rimer & McCue 2005). Determining the causes of rarity and identifying threats can aid in recovery efforts for listed species. The use of data gathered from experimental reintroductions can be important in the recovery of selected species (Jõgar & Moora 2008; Lawrence & Kaye 2009).

Reintroductions that incorporate an experimental aspect are useful for several reasons, such as obtaining additional information on the ecology of a vulnerable species.

In a study of *Apium repens*, a rare umbellifer found in Germany, Burmeier and Jensen (2009) tested competitive ability and inundation tolerance of seedlings to identify recovery priorities. They found *A. repens* to be a weak competitor but able to tolerate fresh-water inundation, factors that will guide site selection for recovery efforts. Maschinski et al. (2004) tested introduction by sowing seeds in two habitats previously unoccupied by the species *Purshia subintegra*, a rare shrub. They also sowed seeds in its natural habitat, limestone mesas. They observed some seed germination in all habitat types, but found germination in the unoccupied habitats limited by soil moisture. This suggests that reintroductions should be restricted to the typical habitat of choice for the species. Studies such as these imply that baseline data on habitat specificity can be a crucial component of reintroduction planning as they will guide appropriate site selection for future reintroduction efforts.

Experimental reintroductions can also add to our knowledge of reproductive response in some species. One study tested different maternal lines and planted seedlings along a water inundation gradient to determine factors that influence the height and flowering of *Helenium virginicum*, a threatened species of seasonal sinkhole ponds in Virginia and Missouri. Maternal line and water inundation gradient had a significant effect on height and flowering (Rimer & McCue 2005). Maternal lines varied in height from 92.41 to 24.13 cm and in potential reproductive output by 128.67 to 0 flowers, suggesting underlying genetic effects on plant fitness (Rimer & McCue 2005). These results underscore the need for conservationists to consider genetic factors when selecting plant material for reintroduced populations to recover rare and threatened species.

Researchers can test different methods by conducting reintroductions as an experiment, which can help standardize protocols that may have wide applicability (Kaye 2008). Selecting plant material for the founding population is one of the preliminary steps in a reintroduction (Guerrant & Kaye 2007; Maschinski et al. 2012a). Seeds, seedlings, whole plants, rhizomes, and vegetative cuttings are examples of plant material often used, with the common choice being between seeds and whole plants. (Guerrant 1996a). There are advantages and disadvantages to all options (Guerrant & Kaye 2007; Kaye 2009). For example, seeds are relatively easy to sow on site and can be acquired in prolific numbers. However, reviews have shown that seeds result in low germination in the wild (Godefroid et al. 2012; Guerrant 2012). The production cost of transplants can be high, but seedlings or whole plants have shown greater survival rates than seeds in reintroductions (Jusaitis 2004; Reckinger et al. 2010). Many rare species are known from only a few populations, making seeds more difficult to acquire. As a result, the use of transplants may be appropriate to minimize the use of valuable seed that may not germinate when sowed on-site. Ideally, reintroductions are designed to evaluate the relative suitability of the type of plant material, which will aid in future recovery efforts (Alley & Affolter 2004; Jusaitis 2004).

Regardless of whether or not the founding population of a reintroduction consists of seeds or transplants, attention is often given to germination requirements in reintroduction studies. Pre-treating seeds of the federally endangered *Potentilla robbinsiana* with gibberelic acid (a plant hormone used to stimulate germination) increased germination in the greenhouse yard two-fold over those sown without pre-treatment. Previous germination work proved difficult for the alpine species at the greenhouse found close to sea level (Brumback et al. 2003). The discovery of this seed germination pre-treatment method aided in the delisting of the species in 2002 (USFWS 2002), because it helped identify the best method whole plant production of *P. robbinsiana* (Brumback et al. 2003). The realignment of a popular hiking path reduced the potential for anthropogenic habitat disturbance, which also contributed to the recovery of this species. Likewise, Qi et al. (2009) found information on the germination requirements of *Bretschneidera sinensis*, an endangered tree found in China, lacking and subsequently tested multiple methods to improve propagule production for conservation. Without the knowledge of a crucial germination requirement, reintroduction work may be ineffective.

Site preparation and maintenance, through removal of weedy or invasive species, can be used to improve transplant success (Kaye 2008; Maschinski et al. 2012a). Jusaitis (2005) observed improved seed germination and seedling establishment of *Acacia whibleyana* in non-weedy versus weedy sites. In a similar study, Jusaitis et al. (2004) found greater seed germination of *Brachycome muelleri* in weed free plots. Seedlings are often considered to be the most vulnerable stage for a plant (Maschinski et al. 2004). Therefore, removal of weeds before and after reintroduction can increase the likelihood of plant establishment and recruitment of future generations.

Reintroduction is still a relatively new component of conservation biology (Godefroid et al. 2011), and each reintroduction project yields data that can contribute to improvements in the field. Two decades of plant reintroductions with mixed methods and results have brought to attention the need for a set of guidelines to reference (Maschinski et al. 2012a). Reintroduction projects with experimental components will provide evidence for factors that will determine the success of the methods being used. It is important to communicate these successes and failures, as all types of information will produce more efficient efforts (Menges 2008).

Northern wormwood (*Artemisia campestris* var. *wormskioldii* [Besser ex hook.] Cronquist [Asteraceae]) is a tap-rooted biennial to perennial restricted to dynamic cobble bar environments on the Columbia River. It is known from only two natural populations in Washington, Miller Island and Beverly, which are 300 river km apart (Arnett 2010). Both of these populations are declining, presumably due to a combination of drifting sand, flooding, and weed infestations (Arnett 2010; Amsberry & Meinke 2011). Northern wormwood is listed as Endangered in both Oregon and Washington (ODA 2012; Washington Department of Natural Resources 1997). It is also a Candidate for federal listing and designated a “Spotlight Species” by the U.S. Fish and Wildlife Service (USFWS 2011).

Current recovery efforts are being undertaken by the Oregon Department of Agriculture (ODA), Washington Department of Natural Resources, and the U.S. Fish and Wildlife Service. The natural populations are monitored annually for survival and recruitment (Arnett 2010; Amsberry & Meinke 2011), and seed collection is active at these sites when hydrology permits. ODA has been working on recovery efforts for this species since 2005 (Amsberry et al. 2007; Amsberry & Meinke 2011). ODA staff surveyed for existing populations and appropriate habitat in 2005 and 2006, and found several sites suitable for a pilot outplanting (Amsberry et al. 2007). The Squally Point population was planted in the spring of 2008 with two augmentations in the fall of 2008, and the spring of 2010 (Amsberry & Meinke 2011). As of 2010, this newly created population has been declining due to unusually high and fluctuating water flow and unauthorized recreational use, but valuable information has been gathered from this pilot project. Seed germination and transplant production methods were developed and microsite selection techniques were used to determine the most appropriate planting locations. These efforts provided baseline data and methodology used in this study.

The decline of northern wormwood can be attributed to several actual and potential causes. First, construction of multiple dams on the Columbia River has directly resulted in loss of individuals and habitat (Gamon 1989; Carlson 1998). Much of the riverbanks on the Columbia River are covered in large, stabilizing rocks, an inappropriate substrate for northern wormwood (Gamon 1989; Amsberry & Meinke 2011). Without appropriate habitat, northern wormwood has been unable to colonize new sites. Second, multiple dams on the river have changed the seasonal flows of the river through reduction in spring floods and increased inundation (National Research Council 2004; USFWS 2011). This change in hydrology may affect the growth and reproduction of northern wormwood plants (USFWS 2011). Loss of plants swept away by changing water levels have been observed at the created site, Squally Point (personal observation) (Figure 1.3). Finally, the occurrence of invasive species along the Columbia River and in northern wormwood populations represents a great threat. Invasive species reduce habitat, compete for resources, and are often cited as the cause of decline of native plant populations (Kaye 2009).

A large-scale reintroduction of northern wormwood to a new site was the next logical step in the recovery of this species. Previous work established methodology for introducing northern wormwood plants into new sites, but more information on the species' ecology is necessary to promote recovery (Amsberry et al. 2007; Amsberry & Meinke 2011). This paper will describe the experimental reintroduction of northern wormwood, which examined three ecological factors that may affect transplant success.

Methods

Study site

Our experimental reintroduction of northern wormwood took place on Rufus Island, Sherman County, Oregon. This site is located roughly 38.6 km (along I-84) east of The Dalles. Rufus Island is part of a series of cobble bars that were created during construction of the John Day Dam and I-84 in the 1940s-60s (Figure 3.1). Rufus Island is managed by the Army Corps of Engineers (ACOE) as a fish and wildlife habitat enhancement project. ODA conducted habitat surveys in 2005 and 2006 and chose Rufus Island as a strong potential outplanting site for northern wormwood based on ecological components and administrative protection (Amsberry & Meinke 2007). Habitat on Rufus Island is similar to that found at the two known native sites, with low vegetation and a mix of rock and sand substrate (Figure 3.2). A list of the most common associate species can be found in Appendix B.

Figure 3.1. Rufus Island (see yellow circle). is located approximately 5 miles west of the town of Rufus, OR. It is part of a series of cobble bars created during dam construction. Interstate-84 runs adjacent to the island.

Figure 3.2. The shoreline habitat on Rufus Island consists of small to large rocks and low, sparse vegetation. Photo by A. Brickner.

Treatments

It is with the consideration of the aforementioned threats, i.e. loss of habitat, change in hydrology, and invasive species, that our reintroduction of northern wormwood was designed. Three ecological factors, substrate type, distance from the water, and invasive species, were selected for experimental treatments.

Substrate type

Due to the construction of the dams and I-84, northern wormwood populations have been lost, and little is known about this species' specific habitat requirements (Gamon 1989; Carlson 1998). In addition to this, the habitat at the two natural sites differs. Miller Island plants are rooted in basalt bedrock but with a thick layer of surface sand, and plants at Beverly are found on a peninsula with various sizes of cobbles and pebbles, and low, sparse vegetation. Therefore, two substrates comparable to those found at the natural sites were selected to determine which is most conducive to transplant survival. Sand substrate (Figure 3.3) represents the substrate found at Miller Island as well as the created population at Squally Point, which has shown some initial success despite its current decline (Amsberry & Meinke 2011). Compacted rock on Rufus Island (Figure 3.4) is similar to that at Beverly and is thought to be most appropriate due to this similarity. A third substrate, loose rock, was also chosen for reintroduction (Figure 3.5). While not quite representing substrates found at Beverly and Miller Island, the loose rock is associated with the dynamic cobble bar environment of the Columbia River, where northern wormwood populations have historically been located.

Figure 3.3. Sand substrate on the east end point. Photo by A. Brickner.

Figure 3.4. Compacted rock substrate. Photo by A. Brickner.

Figure 3.5. Loose rock substrate. Photo by A. Brickner.

Distance from the water line

The second threat, change in hydrology, was assessed by determining a minimum planting distance from the water line for transplants. Many of the losses at the introduced population, Squally Point, can be attributed to changing water levels. In the summer of 2012, a large fragment of sand dune present on the site was washed away, taking northern wormwood transplants and seedlings with it (personal observation). By determining a minimum planting distance from the water line, we can hope to curb losses due to fluctuating water levels.

False indigo plots

The third threat, invasive species, was assessed through the removal of an invasive shrub, false indigo (*Amorpha fruticosa*). False indigo is considered an invasive, exotic species in Washington (Washington State Department of Ecology 2011). It was listed as a Class B noxious weed in Washington in 1988 (Washington State Noxious Weed Control Board 2010), and in 2013 in Oregon (ODA 2013). False indigo produces a large quantity of seed and spreads quickly, displacing native riparian species (Washington State Department of Ecology 2011). It is found along the Columbia and Snake Rivers in Oregon and Washington, and grows extensively on the Oregon banks of the Columbia River (Amsberry & Meinke 2011). False indigo is of concern for northern wormwood populations as false indigo can colonize the limited appropriate habitat for the rare species.

Transplant propagation

Transplants were propagated at the greenhouse facilities at Oregon State University (OSU), Corvallis, Oregon. Seeds were collected from two sources: the two natural populations, Miller Island and Beverly. Seeds from Beverly were collected in the wild, as well as from plants grown at the OSU greenhouses by ODA. These plants were started from Beverly seed in 2005. Seeds collected from the two native populations consisted of nine accessions defined by year of collection. Using seeds collected in different years maximized the diversity in the founding population for a species with limited sources for seed collection. A more genetically diverse founding population may increase the ability of the species to establish at its new site, as well as respond to environmental change.

In June of 2011, over 3,400 seeds were germinated to acquire our goal of 2,000 transplants. A pilot study was conducted to obtain germination rates in March of 2011. For this pilot study, ten seeds from three accessions were germinated in Petri dishes on filter paper wetted with distilled water. These dishes were set in the greenhouse at OSU, subjected to natural light, and monitored for germination. Rates calculated from this pilot study were used to estimate the number of seeds to use for each seed source to reach our goal of 2,000 plants. Protocol from previous work by ODA (Amsberry et al. 2007; Amsberry & Meinke 2011) was also used as a reference for propagation. Seeds were germinated using the same method as the pilot study. Seedlings were planted one week after germination began. Seedlings were 1-3 cm long at this stage and were planted in 10.16 cm square pots filled with Sunshine Professional Growing Mix® (Canadian sphagnum peat moss, horticultural grade perlite, pumice, and dolomite limestone) and watered at the time of planting. Plants were watered daily and fertilized weekly with Dyna-Gro® (7-9-5). After eight weeks in the greenhouse, transplants were placed outside to be acclimatized. Over 2,400 transplants were propagated during this stage.

Site selection and transplant protocol

Planting sites for ecological factors were chosen carefully based on the treatment. The experimental outplanting took place on Rufus Island, October 11-13, 2011, at various sites along the outer limb of the island (Figure 3.6). Northern wormwood plants were planted with the help of ODA field crew members and volunteers. Transportation to the site was provided by ACOE (Figure 3.7). Each treatment site had a variable quantity of transplants and all plants were watered once after planting with water from the Columbia River.

Substrate type

Of the three substrates, sand is only found in one small area on Rufus Island. Several areas of compacted rock were present on site, and the largest of these was selected for plot placement. Finally, loose rock substrate is found along the banks adjacent to the main river channel. A large area, set away from the two other substrates, was selected to maintain a more or less even distance between substrate plots (Figure 3.6).

Twenty plots were placed along a transect within each substrate type, and the location of plots was identified according to position along a meter tape, and whether the plot was on the north or south side of the tape (e.g., a plot designated as Plot 1, 1-2m, S, would be located between meters 1 and 2 on the south side of the transect line). Transect length differed by substrate, and two shorter transects (as opposed to one longer transect) were used in the sandy area, due to limited space. Start, middle, and end of transect lines were marked with rebar and numbered tags. Attempting to place rebar in the corners of each plot would have presented a time issue as it was quite difficult to get rebar into the compacted rock. To be consistent, the transect-plot lines were used for all three substrates.

Two hundred northern wormwood greenhouse starts were arrayed along transect lines within each of the three substrates being evaluated, with 10 plants placed in each of 20 $1m^2$ plots. Plot locations were laid out selectively based on appearance of substrate. Because some of the sand substrate contained large rocks, the sandiest areas were selected for plot location. To be consistent, plot locations for the compacted and loose rock substrates were selected by appearance, not randomly.

Distance from the water line

A large area on Rufus Island was graded down by ACOE as part of their wildlife habitat enhancement work. We chose this area to evaluate optimal planting distance from the water line, since earlier observations (during the growing season) indicated dramatic changes in water level (Figure 3.8). Four transects, each 16 meters in length, were set up here, perpendicular to the water line. Transects started six meters from the water’s edge and ended at a line of tall vegetation. Some of this vegetation was removed to maximize the length of the transects. Transects were started six meters from the water’s edge because of the ACOE habitat enhancement project. This left a six meter buffer from the water's edge. Results will take this buffer into consideration for recommended planting distances. The water line at time of planting in October was similar to that in March, 2011 (Figure 3.8), and is thought to be the low water line.

Within each of the four transects, three northern wormwood plants were placed every half meter, perpendicular to the transect line and spaced approximately 0.3 m apart (Figure 3.9). In other words, every square meter had six plants for a total of 99 plants per transect and 396 plants for the entire area. Substrate here consisted of large and medium sized rocks with sand underneath. Planting in this area required some pick axing.

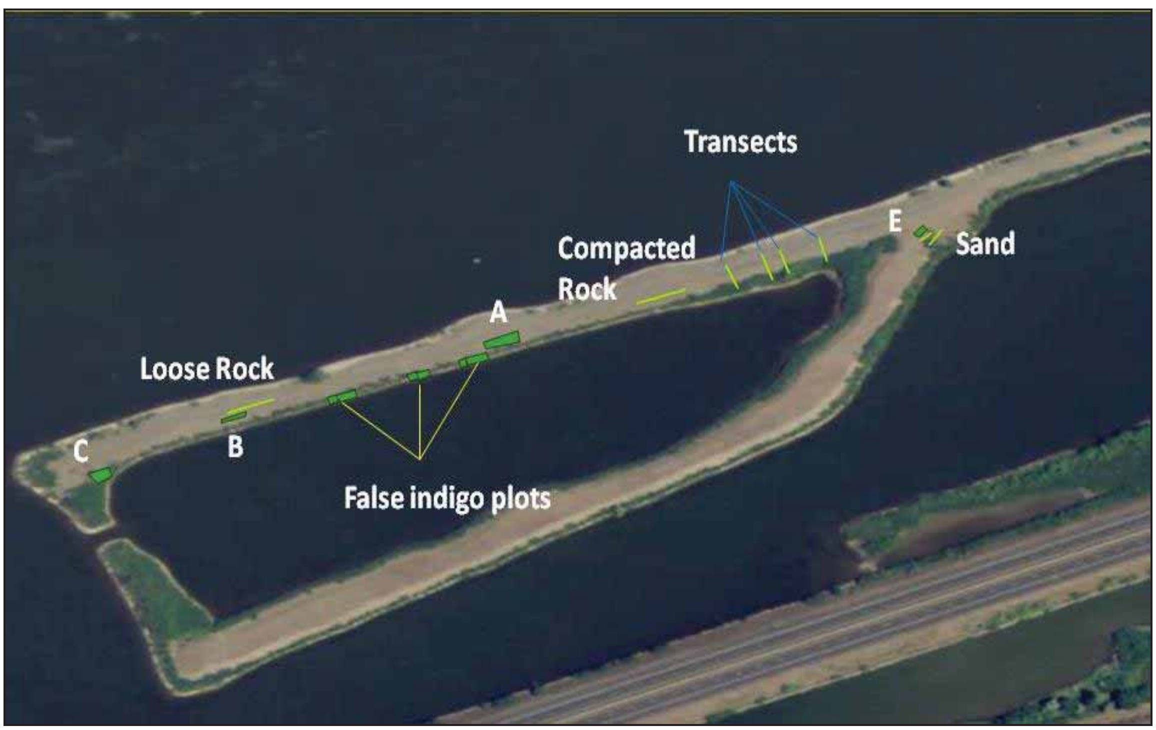

Figure 3.6. Treatment planting locations and surplus plant patches, represented by letters A, B, C, and E.

Figure 3.7. Transportation provided by Army Corps of Engineers was essential to this project. Photo by A. Brickner.

Figure 3.8. Location of graded down area chosen for distance from the water line transects on Rufus Island. Aerial view shows dramatic changes in water level during the growing season of 2011.

Figure 3.9. Row of three transplants in the distance from the water line transects. Photo by A. Brickner.

False indigo

To investigate the potential effects of false indigo on northern wormwood transplants, three replicates of paired plots were established. Three discrete (treated) plots (4 m^2) were created by treating all false indigo plants using a cut-stem method with a 50/50 mix of Element 3A specialty herbicide and nonionic surfactant (Figure 3.10). In the area adjacent to each treated plot, false indigo plants were left untreated. These untreated plots varied in length from 7-14m. The untreated plots were longer than the treated plots, as the goal was to plant northern wormwood within the false indigo, which was harder to accomplish if space was constrained.

Both untreated and treated plots received 75 transplants, or 150 plants per pair. In both types of plots, plants were placed two meters away from the water line in an attempt to prevent inundation by rising water levels. Plants were placed amongst false indigo plants in the control plots (Figure 3.11). A total of 450 plants were planted within all three replicates.

Figure 3.10. Treated false indigo plot. Photo by A. Brickner.

Surplus plant patches

In order to aid in recovery efforts and use additional northern wormwood transplants not planted in experimental treatments, 650 plants were outplanted in four patches of various sizes. Locations were chosen based on similarity to three of the experimental treatments: sand, compacted rock, and removed false indigo plots. This was an attempt to determine appropriate planting locations without the data from the experimental sites, which could provide a more accurate prediction of habitat appropriate for northern wormwood.

Figure 3.11. Northern wormwood transplant amongst false indigo. Photo by A. Brickner.

Monitoring and data collection

Transplanted northern wormwood plants were monitored for survivorship three times in 2012: January 4, May 1, and September 18. This entailed recreating the planting set up (e.g. laying transect lines) and counting each individual plant within a treatment area to record survival.

Data on reproductive output, in the form of number of flowering plants and number of inflorescences, was collected on May 1, 2012. Upon arriving at Rufus Island, we found many plants had been subjected to herbivory. Only the inflorescences, not foliage, had been browsed, mainly in the sand substrate. Browsed plants typically had some evidence of the floral stem remaining and were easily counted (Figure 3.12). Therefore, our measurements of reproductive output were adjusted to account for this. In addition to the original two parameters, we collected data on number of completely browsed plants and number of browsed inflorescences. A “browsed plant” was defined as an inflorescence with no flowers remaining. Any plant that had more than three flowers on at least one inflorescence was considered a “contributing plant”. This meant that it had the potential to produce seed and contribute to the next generation. This data was used to determine potential (before herbivory) and actual (after herbivory) reproductive output.

Figure 3.12. A completely browsed flowering northern wormwood plant, located in the sand substrate. Photo by A. Brickner.

On June 5, 2012, 1.4% of total intact inflorescences from three of the experimental treatments, i.e., sand, compacted rock, and distance from the water, were collected (Figure 3.13). Collected inflorescences were selected based on robustness of plants (in terms of number of inflorescences) in May 2012, and represented 91% of the total intact inflorescences. Inflorescences were brought back to OSU for cleaning and seeds were counted. A subset of these seeds was tested for viability by placing ten seeds from each treatment in Petri dishes on filter paper, wetting with distilled water and setting in the greenhouse at OSU. Within one week, seeds had either germinated or were thought to have been spoiled by mold. The germination rates gathered from this viability test was used to estimate total viable seed.

Figure 3.13. Seed collection in the sand substrate, June, 2012. Photo by J. Brown.

Data analysis

All statistical analyses were performed using R, an integrated suite of software facilities for data manipulation and calculation (R Core Team 2012, version 2.11.1). All graphs were created with Microsoft Excel (Microsoft Office 2007). We compared mean number of plants surviving per plot by substrate type, using a one-way ANOVA with significance set at a $p \leq 0.05$. Tukey's honest significant difference (HSD) test was used to determine which means differed and by how much. Reproductive output measures from the substrates were analyzed in the same way. The intended method of analysis for determining the minimum planting distance from the water line was to have been linear regression. However, herbivory at the end of the transect created a bimodal distribution, and the analysis was revised to quadratic regression to predict survival along the transect. To test the difference in survival and reproductive output between paired false indigo plots, we used a paired t-test with significance set at $p \leq 0.05$. For reproductive output, the total number of flowering plants and inflorescences, without herbivory, were used in to determine the full potential of the site. No statistical analysis was performed on the surplus plant populations but survivorship was recorded during all three monitoring visits.

Results

Transplant survival

A total of 38.36% of the transplants (823 plants) survived after one year. Survival varied from 78% to 23% across all planting sites (Figure 3.14).

Substrate type

There was a significant difference in mean survivorship of plants per plot between substrate types one year after outplanting (one-way ANOVA, $p \leq 0.001$). Results from Tukey's HSD test indicated transplants in the sand substrate type exhibited significantly less mortality than transplants in either rock substrates. Plots located in the sand substrate had 3.9 more surviving transplants than those in compacted rock ($p \leq 0.0001$; 95% CI 2.19 to 5.6), and 4 more survivors than those in loose rock substrate ($p \leq 0.0001$; 95% CI 2.3 to 5.7). There was not a significant difference in number of surviving plants per plot between plots in the compacted and loose rock substrates ($p = 0.9$).

Distance from the water line

Results from a quadratic regression indicate that a minimum planting distance from the waterline in October for transplant survival is nine meters, and that maximum survival (100% with six transplants/m^2) can still be achieved up to 24 m, as long as plants are not subjected to herbivory (R^2=0.87, $p \leq 0.001$) (Figure 3.15). This final planting interval had six meters added at either end to account for the buffer created by the ACOE's habitat enhancement project. Even though our transects were 16 m long, the losses at the far end were due to herbivory, not by rising water levels.

False indigo plots

A paired t-test showed no significant difference in northern wormwood survival between treated and untreated false indigo plots ($p = 0.19$). Mean survival was 22.6 plants in the treated plots and 16.0 plants in the untreated plots. Due to their proximity to the shoreline, rising water levels had negative effects on plants in both types of plots, and flooding in the lower half of plots resulted in lower survival across both treatments (Figures 3.14, 3.16).

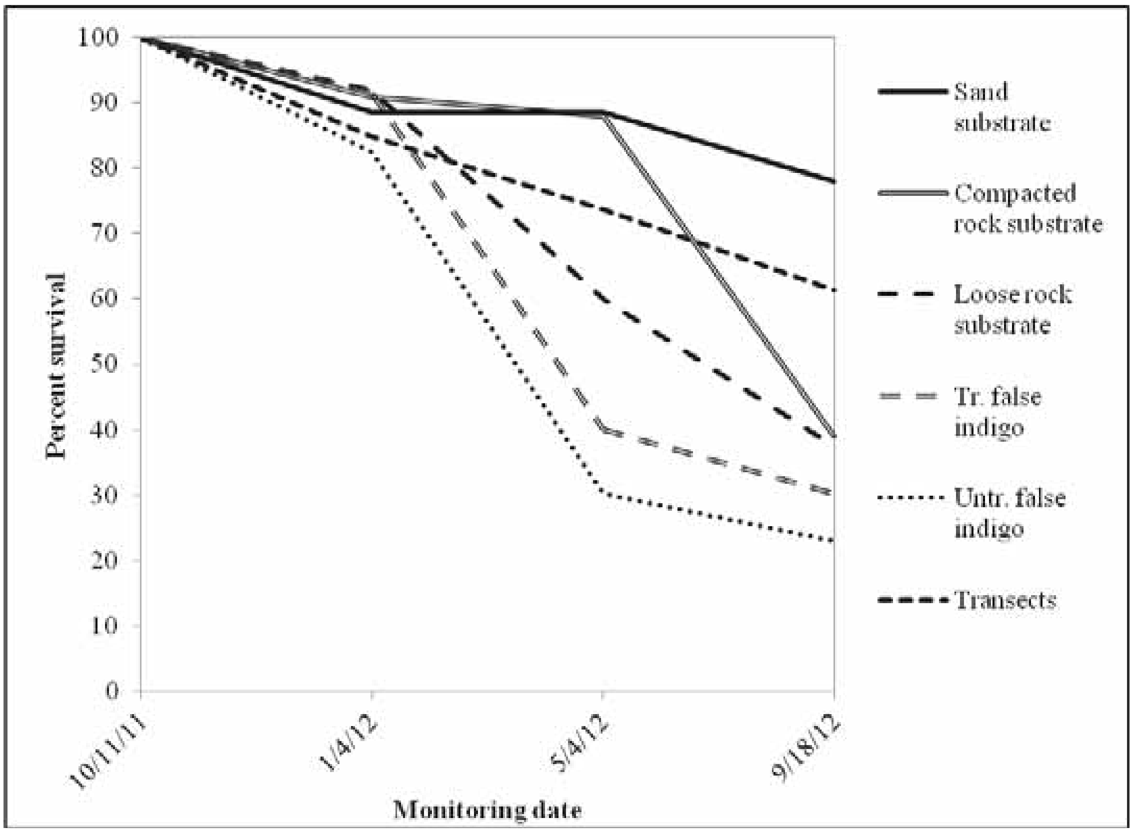

Figure 3.14. Percent of transplant survival over one year at experimental transplanting sites. "Tr." refers to the plots with removed false indigo plants and "Untr." refers to areas with unremoved false indigo plants. "Transects" refers to the transects created to determine minimum planting distance from the water line.

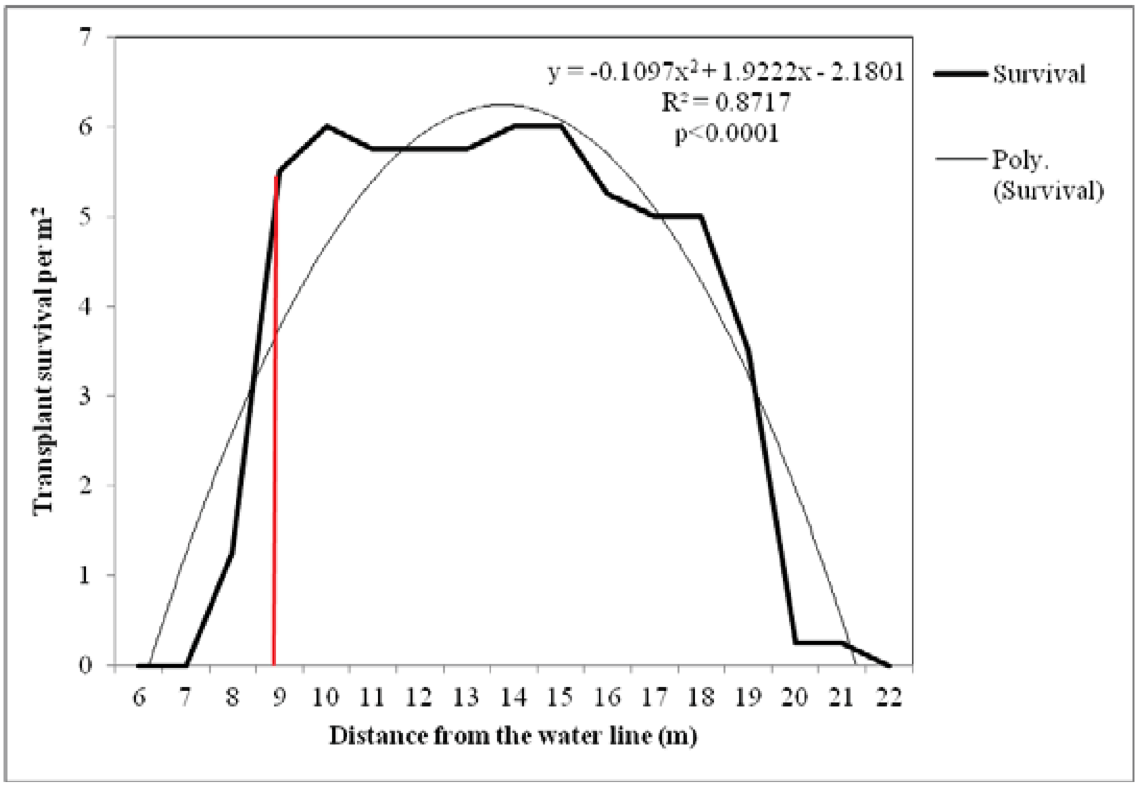

Figure 3.15. Results from quadratic regression indicate a minimum planting distance of nine meters for survival of 5.5 transplants. “Poly. (Survival)” = the regression trendline. Maximum survival was six plants/m^2. Transects started six meters from the edge of the water and were 16 meters long.

Figure 3.16. Plants in the treated false indigo plots were flooded in May of 2012. Photo by A. Brickner.

Surplus plant patches

Transplant survival varied across the four surplus plant patches. Total percent survival was 24% or 155 plants. This survival is much lower than total survival across the experimental locations when the surplus plant data was excluded (44.8%). The patch surplus with the greatest survival rate was set near the sand substrate, the substrate with the greatest survival rate. Two of the larger patches were planted in substrate similar to that of the compacted rock plots, which fared poorly in comparison to the plots in the sand substrate. The fourth patch was located in an area where false indigo plants were removed. This area was flooded. These results emphasize the importance of experimental reintroductions, which can provide more complete data on factors affecting transplant survival.

Reproductive output

Substrate type was significantly correlated with the number of flowering plants and number of inflorescences per plot (one-way ANOVA, $p \leq 0.0001$) (Figures 3.17 and 3.18). Results from Tukey's HSD test indicated that plots in the sand substrate had 4.8 more flowering plants per plot ($p \leq 0.0001$; SE ± 2.05) and 31.85 more inflorescences per plot ($p \leq 0.0001$; 95% CI 16.4 to 47.3) than plots in the loose rock substrate. Plots in the compacted rock substrate supported 4.5 more flowering plants per plot ($p \leq 0.0001$; SE ± 1.93) and 21.5 more inflorescences per plot ($p \leq 0.0001$; 95% CI 6.0 to 37.0) than plots in the loose rock substrate. The sand and compacted rock substrate plots did not differ in the number of flowering plants and inflorescences produced per plot ($p = 0.9$ and 0.2, respectively). The number of flowering plants and inflorescences produced did not differ significantly between the paired false indigo plots ($p = 0.07$ and 0.28, respectively). Data on reproductive output in the transects was not analyzed statistically but is included in the reproductive output table (Table 3.1).

A total of 4,582 seeds were collected from the 37collected inflorescences (1.4% of the total produced). These results were used to calculate the average number of seeds per inflorescence by collection site. Using this average, we calculated an estimate of the number of seeds produced, before and after herbivory, at each collection site. The calculations estimated that there were between 33,264 to 260,752 seeds before herbivory and 24,012 to 243,896 seeds after herbivory, depending on collection site (Table 3.2).

Ten seeds from each collection site were tested for viability as described in Methods. The germination rates varied from 70% to 90%, indicating that seeds produced by our outplanted population had good potential for germination.

In a surprising turn of events, in September 2012 we found 11 seedlings on Rufus Island (Figure 3.19). Seedlings were found between the “distance from the water line” transects, approximately three to six meters from each transect line and nine meters from the water line. The substrate found here is a mixture of sand with large and medium rocks.

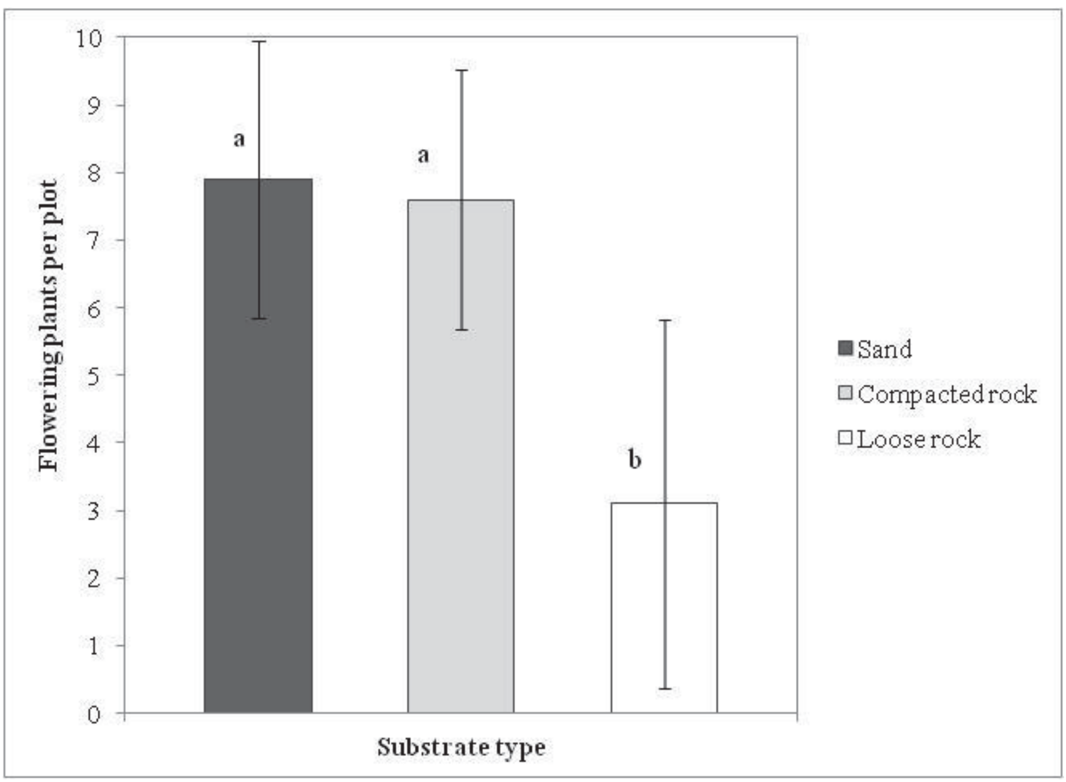

Figure 3.17. The mean number of flowering plants per plot in three substrate types. Each plot has 10 plants. Different letters represent significant differences at the $p < 0.05$ level. Error bars indicate standard error (n=20).

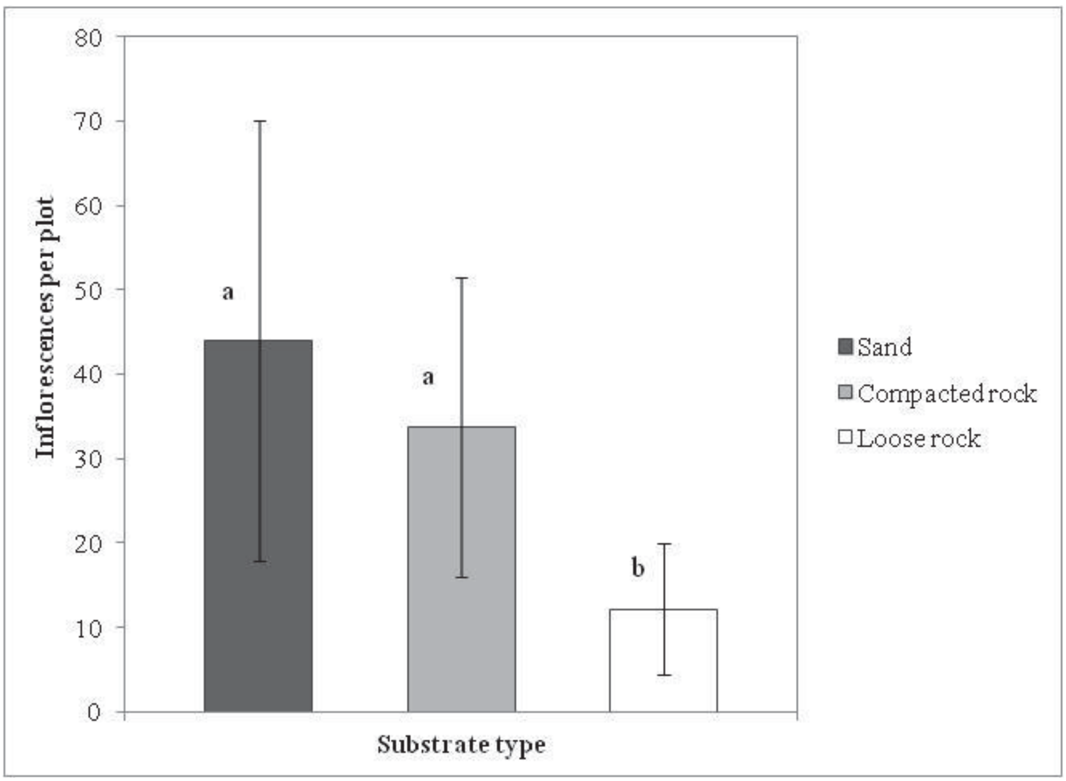

Figure 3.18. The mean number of inflorescences per plot per treatment. Each plot had 10 plants. Different letters represent significant differences at the $p < 0.05$ level. Error bars indicate standard error (n=20).

Table 3.1. Reproductive output, estimated using the number of flowering plants and inflorescences produced, with and without herbivory, at all transplanting sites. "NC" indicates that data was not collected for that parameter at that site. "Treated F.I." = treated false indigo plots. "Untr. F.I." = untreated false indigo plots.

Treatment	Total flowering plants	Flowering plants browsed	Flowering plants contributing (non-browsed)	Total flower stems produced	Flowering stems browsed	Flowering stems intact	Mean flower stems/plant (total)
Sand	158	42	116	880	384	496	5.6 +/-4.8
Compacted rock	152	0	152	674	7	667	4.4 +/-3.9
Loose rock	62	2	60	243	32	211	4.0 +/- 3.5
Treated F.I.	3	0	3	17	4	13	5.6 +/-4.7
Untr. F.I.	9	0	9	64	41	23	7.1 +/-15.3
Distance	256	10	246	1516	98	1418	6.0 +/-1.8
Surplus	46	0	46	NC	NC	NC	NC
Total	686	54	632	3394	566	2828	40.8

Table 3.2. Estimates of total potential and actual seed produced. The total estimated seeds produced (before herbivory) was calculated using the total number of inflorescences without herbivory, multiplied by the average seeds per stem. Actual seed produced (after herbivory) was calculated using the number of intact inflorescences. "Transects" indicates the plants planted in transects created to determine a minimum planting distance from the water line.

Treatment	Total # of inflorescences collected	Total # of seeds collected	Average # of seeds per stem	Total # of stems produced (before herbivory)	Total # of stems produced (after herbivory)	Total estimated seeds (before herbivory)	Actual seeds (after herbivory)
Compacted rock	10	895	90	674	667	60,660	60,030
Sand	7	252	36	924	496	33,264	24,012
Transects	20	3,435	172	1516	1418	260,752	243,896

Figure 3.19. One of 11 seedlings found near plants in the “distance from the water line” transects.

Discussion

Survival of our northern wormwood transplants on Rufus Island varied across all planting locations. Due to the experimental nature of this project, total percent survival may be considered low (39.36%). However, we planted a relatively large founding population (2,091 transplants) and as of September 2012, the population on Rufus Island consisted of 823 plants. This population is currently the second largest of four total northern wormwood populations, both natural and created. By planting as many individuals as was feasible, given time and logistics, we were able to defend against initial decline of newly transplanted populations within the first year (Menges 2008; Monks et al. 2012), and still have a relatively large population remaining. The presence of 11 seedlings indicates that this population has promise for producing subsequent generations, although this cannot be known without further monitoring. Reintroductions that include an experimental component can answer ecological questions (Guerrant & Kaye 2007), especially concerning rare species where few examples of natural populations may be available for observation (Lawrence & Kaye 2009). Our reintroduction examined three ecological factors that may influence transplant success. The results from this study provide more details on the habitat requirements of northern wormwood.

Substrate type

The substrate preference of northern wormwood is difficult to discern as the substrates found at the two natural populations differ considerably. Most plants at Miller Island are rooted in basalt with a top layer of sand, but three plants grow directly out of a basalt outcrop. Plants at the natural Beverly site grow in mixed rock substrate with some sand and little soil development (Gamon 1989; Carlson 1998). This experimental treatment was designed to determine more specific substrate requirements for establishing new northern wormwood populations.

On Rufus Island, transplants performed better, in terms of survival, flowering plants, and inflorescences, in the sand substrate than in the compacted rock and loose rock substrates. Initial survival of transplants in the compacted rock substrate was similar to those in the sand substrate in May 2012, but the plots located in compacted rock suffered many transplant losses over the summer. This could be attributed to a difference in elevation between the sand and compacted rock substrate sites. Both natural populations are found near water level and may benefit from access to groundwater when precipitation is low (USFWS 2011). The compacted rock site had a slightly higher elevation and may have been elevated enough to deter transplant access to groundwater during the summer. The sand substrate site is located closer to water level and had fewer losses over the summer. Further investigation into elevational effects on northern wormwood might clarify this difference.

The loose rock substrate was a poor substrate for northern wormwood transplant survival. This is likely due to the shifting nature of the loose rock and minimal developed soil underneath available for plants to take root. No other vegetation was found within the loose rock substrate site either, while the sand and compacted rock substrate sites supported some sparse vegetation. This may be another indicator that loose rock is a poor substrate for vegetation of any type. Several plots in the loose rock were completely buried by rocks, and some plants were washed out of plots and found several meters away from their original location. The results from this experiment indicate that a combination of sand and rock substrate, found at or near water level, is most appropriate for northern wormwood transplants.

Distance from the water line

Native plants found in riparian areas are often adapted to natural flow regimes and have life-history strategies to complement these environments (Naiman & Décamps 1997; Greet et al. 2012). The pre-dam riparian environment on the Columbia River consisted of more dynamic cobble bars that provided habitat for the early colonizing northern wormwood (Gamon 1989; Carlson 1998; Amsberry & Meinke 2011). However, the creation of dams on the river have altered seasonal flows (National Research Council 2004), reducing cobble bar habitat (Gamon 1989; Carlson 1998) and often inundating northern wormwood plants for much of their growing season (USFWS 2011). This inundation may hinder northern wormwood plants from spreading viable propagules to the small amount of remaining available habitat (Amsberry & Meinke 2011). Our results from the experimental transects set to determine a minimum planting distance from the water line support the theory that the altered water regime has a negative effect on northern wormwood.

In May 2012, transplants in the “distance from the water line” transects that were placed within nine meters of the water line were buried by rocks which had shifted with changing water levels earlier in the spring. Some of these plants did produce inflorescences which were only just visible above the new rock piles. This burial may have resulted in a loss of seeds and potential recruitment, as this site was the only site to produce seedlings. In addition, the altered flow and timing of flooding caused by the dam management may cause seedlings to be more vulnerable to burial or inundation. Since we cannot remove the dams near northern wormwood populations, managers should take into consideration the altered flow regimes for riparian plant conservation (Merritt et al. 2010). To aid in the establishment of future created northern wormwood populations, planting distance from the current water line should be taken into consideration. As each site will be unique in altered flow regime, an observation period, pre-outplanting, may be necessary to determine appropriate distance from the water line for transplants.

False indigo plots

Invasive species have been shown to reduce biodiversity and abundance of native species (Jusaitis 2005; Kaye 2008), and riparian areas may be particularly vulnerable to reduced native plant diversity caused by exotic species (Merrit et al. 2000; Greet et al. 2012). These two factors may have strong implications for rare plant conservation in riparian zones. Although we found no significant difference in survival between treated and untreated plots, we believe that false indigo may still present a threat to northern wormwood populations. Our study was short-term (one year), and effects of false indigo on northern wormwood transplants may take longer to become evident.

For example, other northern wormwood populations, natural and created, are found in full sun (pers. obs.), and the shade created by the larger false indigo plants may negatively affect transplants in subsequent years. Finally, more research on the effects of false indigo on recruitment of northern wormwood should be undertaken to evaluate the threat potential of this invasive species.

The history of false indigo may also provide insight into how it may affect northern wormwood populations. False indigo was originally introduced to the west coast for bank stabilization in the 1920s (Washington Department of Ecology 2011), but is now a noxious weed in Washington and Oregon (Washington Department of Ecology 2011; Oregon Department of Agriculture 2013). After being introduced for cultivation purposes, false indigo also became invasive in riparian areas in Ukraine (Protopopova et al. 2006). A potentially comparable case to the spread of false indigo is that of *Tamarix ramosissma* in the western U.S. Introduced in the 1800s, *T. ramosissima* is now the third most prevalent woody species of riparian areas in the western U.S. (Stromberg et al. 2007). The recent addition of false indigo to the Oregon Noxious Weed List (Oregon Department of Agriculture 2013) indicates its potential to be a great threat to riparian areas in the west (Washington Department of Ecology 2011). Control of false indigo and other invasive species will improve habitat quality, and may aid in establishment of future northern wormwood populations.

Reproductive output

Monitoring of reproductive output is vital to predicting whether or not a reintroduced population will become self-sustaining (Sutter 1996; Monks et al. 2012). Results from our reproductive output data show that the population on Rufus Island has potential to create future populations. All treatment sites showed some reproductive output, in terms of flowering plants, and some sites had transplants that bloomed profusely. Herbivory negatively affected some transplant sites, but the large founding population compensated for this.

The unexpected presence of 11 seedlings went beyond our initial definition of success considering the short timeframe of the study. Recruitment occurred in the “distance from the water line” site and may represent conditions ideal for wild germination of northern wormwood seed. This substrate consisted of sand with a top layer of medium to large sized rocks. The rocks perhaps created a wind barrier so when seeds fell from inflorescences, they fell between large rocks and into sand that provided an appropriate medium for germination. It can take years for reintroduced populations to show signs of recruitment (Drayton & Primack 2012), so this observance was particularly lucky. In contrast to this, reintroduced populations may reproduce in the first years of a project and then taper off (Albrecht & McCue 2010). This illustrates the need to be cautious about overly positive predictions based on initial recruitment at Rufus Island, and highlights the need for long-term monitoring of this population.

Conclusion

The results from each ecological treatment provide a piece of the northern wormwood habitat puzzle. When viewed as a whole, our results reveal information about the habitat requirements of northern wormwood. A combination of rock and sand substrate is the most appropriate substrate for northern wormwood establishment, especially when located at or near water level. Placing plants at least nine meters from the water line may decrease losses due to changing water levels. Finally, removal of invasive and weedy species will reduce competition and improve habitat quality. Rufus

Island is set in a dynamic river environment and the northern wormwood population is vulnerable to disturbances created by this environment such as rising water levels and herbivory, but by using methods recommended in this study, reintroduction can be a successful conservation tool for promoting the recovery of northern wormwood.

Literature Cited

Albrecht, M., and K. McCue. 2010. Changes in demographic processes over long time scales reveal the challenge of restoring an endangered plant. Restoration Ecology **18:**235-243.

Alley, H., and J. Affolter. 2004. Experimental comparison of reintroduction methods for the endangered Echinacea laevigata (Boynton and Beadle) Blake. Natural Areas Journal **24:**345-350.

Amberry, K., R. Currin, and R.J. Meinke. 2007. Reintroducing *Artemisia campestris* var. *wormskioldii* to Oregon: site selection, cultivation, and pilot outplanting. Report prepared for U.S. Fish and Wildlife Service, Portland Office. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Amsberry, K., and R. J. Meinke. 2011. Population creation as a recovery tool for the Federal candidate species *Artemisia campestris* var. *wormskioldii*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Arnett, J. 2010. Wormskiold’s Northern wormwood (*Artemisia borealis* var. *wormskioldii*): Miller Island Conservation Plan. Report prepared for U.S. Fish and Wildlife Service. Washington Department of Natural Resources, Olympia, Washington.

Brumback, W. E., D. M. Weihrauch, and K. D. Kimball. 2003. Propagation and transplanting of an endangered alpine species Robbins' cinquefoil. Native Plants Journal. **Spring**.

Burmeier, S., and K. Jensen. 2009. Experimental ecology and habitat specificity of the endangered plant *Apium repens* (Jacq.) Lag. at the northern edge of its range. Plant Ecology & Diversity **2:**65-75.

Carlson, M.L. 1998. Status report for *Artemisia campestris* L. ssp. *borealis* var. *wormskioldii* [Bess.] Cronq. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Drayton, B., and R. Primack. 2012. Success rates for reintroductions of eight perennial plant species after 15 Years. Restoration Ecology **20:**299-303.

Gamon, J. 1989. Report on the status of *Artemisia campestris* L. var. *wormskioldii* [Bess.] Cronquist. Washington Natural Heritage Program, Olympia, Washington.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A. Stevens, R. Aguraiuja, C. Cowell, C. Weekley, G. Vogg, J. Iriondo, I. Johnson, B. 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.

Greet, J., R.D. Cousens, and J.A. Webb. 2012. More exotic and fewer native plant species: riverine vegetation patterns associated with altered seasonal flow patterns. River Research and Applications.

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

Guerrant, E. O. 2012. Characterizing two decades of rare plant reintroduction. Pages 9-29 in J. Maschinski and K. E. Haskins, editors. Reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Guerrant, E. O., and T. N. Kaye. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany **55:**362-370.

Jõgar, U., and M. Moora. 2008. Reintroduction of a rare plant (*Gladiolus imbricatus*) population to a river floodplain - How important is meadow management? Restoration Ecology **16:**382-385.

Jusaitis, M. 2005. Translocation trials confirm specific factors affecting the establishment of three endangered plant species. Ecological Management & Restoration **6:**61-67.

Jusaitis, M., L. Polomka, and B. Sorensen. 2004. Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). Biological Conservation **116:**251-266.

Kaye, T. N. 2008. Vital steps toward success of endangered plant reintroductions. Native Plants **9:**313-322.

Lande, R. 1988. Genetics and demography in biological conservation. Science. **241:**1455-1460.

Lawrence, B., and T. Kaye. 2009. Reintroduction of *Castilleja levisecta*: effects of ecological similarity, source population genetics, and habitat quality. Restoration Ecology **19:**166-176.

MacDougall, A., and R. Turkington. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology **86:**42-55.

Maschinski, J., M. A. Albrecht, L. Monks, and K. E. Haskins. 2012a. Center for Plant Conservation best reintroduction practice guidelines. Pages 277-306 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J., J. Baggs, and C. Sacchi. 2004. Seedling recruitment and survival of an endangered limestone endemic in its natural habitat and experimental reintroduction sites. American Journal of Botany **91:**689-698.

Maschinski, J., and K.E. Haskins. 2012. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maunder, M. 1992. Plant reintroduction: an overview. Biodiversity and Conservation **1:**51-61.

Menges, E. S. 2008. Restoration demography and genetics of plants: when is a translocation successful? Australian Journal of Botany **56:**187-196.

Merrit, D.M., M.L. Scott, N.L. Poff, G.T. Auble, D.A. Lytle. 2010. Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. Freshwater Biology **55:**206-225.

Microsoft. 2007. Microsoft Excel. Redmond, Washington: Microsoft, 2007. Computer Software.

Monks, L., D. Coates, T. Bell, and M. 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.

Naimen, R.J., and H. Décamps. 1997. The ecology of interfaces: riparian zones. Annual Review of Ecology and Systematics **28:**621-658.

National Research Council. 2004. Managing the Columbia River: instream flows, water withdrawals, and salmon survival. The National Academies Press. Washington, DC.

Olden, J., and T. Rooney. 2006. On defining and quantifying biotic homogenization. Global Ecology and Biogeography **15:**113-120.

Oregon Department of Agriculture (ODA). 2013. Oregon State Noxious Weed List, B List. Accessed on April 29, 2013. http://www.oregon.gov/ODA/PLANT/WEEDS/Pages/statelist2.aspx#B_list

Qi, Q., C. Hong-Feng, X. Fu-Wu, L. Dong-Ming, and H. Xiao-Gai. 2009. Seed germination protocol for the threatened plant species, *Bretschneidera sinensis* Hemsl. Seed Science and Technology **37:**70-78.

Protopopova, V.V., M.V. Shevera, and S.L. Mosyakin. 2006. Deliberate and unintentional introduction of invasive weeds: a case study of the alien flora of Ukraine. Euphytica **148:**17-33.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Reckinger, C., G. Colling, and D. Matthies. 2010. Restoring populations of the endangered plant *Scorzonera humilis*: influence of site conditions, seed source, and plant stage. Restoration Ecology **18:**904-913.

Rimer, R., and K. Mccue. 2005. Restoration of *Helenium virginicum* Blake, a threatened plant of the Ozark Highlands. Natural Areas Journal **25:**86-90.

Sutter, R. D. 1996. Monitoring. Pages 235-264 in D. Falk, C. Millar and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

U. S. Fish and Wildlife Service. 2002. Accessed on September 13, 2012. Robbins' cinquefoil (*Potentilla robbinsiana*) Species Profile. http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=Q20V

U.S. Fish and Wildlife Service. 2011. Species assessment and listing priority assignment form for Northern Wormwood. U.S. Fish and Wildlife Service, Lacey, Washington. Accessed on March 28, 2013. http://www.fs.fed.us/r6/sfpnw/issssp/documents/planning-docs/cp-fws-candidate-va-artemisia-borealis-2011-05.pdf

Walck, J. L., T. E. Hemmerly, and S. N. Hidayati. 2002. The endangered Tennessee purple coneflower *Echinacea tennesseensis* (Asteraceae): its ecology and conservation. Native Plants Journal **3:**54-64.

Washington Department of Ecology. 2011. Non-native invasive freshwater plants: Indigo bush (*Amorpha fruiticosa*). Accessed on February 20, 2013. http://www.ecy.wa.gov/programs/wq/plants/weeds/aqua014.html

Washington Department of Natural Resources. 1997. *Artemisia campestris* L. ssp. *borealis* Hall & Clem.var. *wormskioldii* (Bess.) Cronquist species profile. Washington Department of Natural Resources, Olympia, Washington. Accessed on October 22, 2012. http://www1.dnr.wa.gov/nhp/refdesk/fguide/pdf/arca.pdf.

Washington State Noxious Weed Control Board. 2010. Noxious weeds list, class B. Accessed on February 20, 2013. http://www.nwcb.wa.gov/searchResults.asp?class=B

Chapter 4: Conservation recommendations

Based on the results presented in this report, the following conservation actions are recommended for further recovery efforts of northern wormwood.

1. Continue to monitor the population at Rufus Island to determine demographic patterns and effects of ecological treatments. Ideally, ten or more years of monitoring will determine the level of success for this project and whether a self-sustaining population of northern wormwood has been created.

2. The methods presented are recommended for use as general protocol for reintroduction of northern wormwood including germination, propagule production, and site suitability and management. This protocol can help guide funding and timelines.

3. Transplants are recommended over seeds. Transplants can be grown in a greenhouse in early summer and be large enough for outplanting after 10-12 weeks.

4. A large founding population (>2000 transplants) is recommended to provide a buffer against initial losses due to transplant shock and stochastic events.

5. Planting sites have substrate, elevation, competing vegetation, and distance from the water line requirements. Substrate should consist of some sand with various sized rocks. The elevation of sites should be close to water level. Sites should be free of large, competing vegetation, especially the invasive shrub, false indigo. Other invasive species on site should be monitored for management. Plants

should be planted at least nine meters from the low water line. A period of pre-planting observation may be necessary to understand the changes in flow at a given site.

6. Seed collection from both natural sites, Beverly and Miller Island, should continue to maintain *ex situ* seed collections. This will provide some genetic diversity and flexibility for germinating enough seeds to propagate a large quantity of transplants.

7. Plants grown in the greenhouse yard at Oregon State University should continue to be maintained for seed collection. New plants should be added to this greenhouse stock as necessary to maintain genetic diversity of seeds.

8. A reintroduction that monitors individual transplants grown from different sources may help determine demographic patterns of outplanted populations.

9. Suitable habitat appears to be a limiting factor for northern wormwood. To help alleviate this factor, surveys for future planting sites may need to be expanded beyond the banks of the Columbia River to, for example, the Deschutes or John Day Rivers and their tributaries. Administrative protection will be important for any new site due to the harm sustained at the other reintroduced population, Squally Point, by unauthorized recreation.

10. Recruitment also appears to be a limiting factor. Studies on seed dispersal and germination in the wild may be necessary to ensure the persistence of northern wormwood populations.

Literature Cited

Albrecht, M., and K. McCue. 2010. Changes in demographic processes over long time scales reveal the challenge of restoring an endangered plant. Restoration Ecology **18:**235-243.

Albrecht, M., and J. Penagos. 2012. Seed germination ecology of three imperiled plants of rock outcrops in the southeastern United States. Journal of the Torrey Botanical Society **139:**86-95.

Albrecht, M. A., and J. Maschinski. 2012. Influence of founder population 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.

Allen, W.H. 1994. Reintroduction of endangered plants. Bioscience **44:**65-68.

Alley, H., and J. Affolter. 2004. Experimental comparison of reintroduction methods for the endangered Echinacea laevigata (Boynton and Beadle) Blake. Natural Areas Journal **24:**345-350.

Amberry, K., R. Currin, and R.J. Meinke. 2007. Reintroducing *Artemisia campestris* var. *wormskioldii* to Oregon: site selection, cultivation, and pilot outplanting. Report prepared for U.S. Fish and Wildlife Service, Portland Office. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Amsberry, K. and R. J. Meinke. 2011. Population creation as a recovery tool for the Federal candidate species *Artemisia campestris* var. *wormskioldii*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Andersson, L. and P. Milberg. 1998. Variation in seed dormancy among mother plants, populations and years of seed collection. Seed Science Research **8:**29-38.

Angeloni, F., N.J. Ouborg, and R. Leimu. 2011. Meta-analysis on the association of population size and life history with inbreeding depression in plants. Biological Conservation. **144:**35-43.

Arnett, J. 2010. Wormskiold's Northern wormwood (*Artemisia borealis* var. *wormskioldii*): Miller Island Conservation Plan. Report prepared for U.S. Fish and Wildlife Service. Washington Department of Natural Resources, Olympia, Washington.

Beckstead, J., S. Meyer, and P. Allen. 1996. *Bromus tectorum* seed germination: between-population and between-year variation. Canadian Journal of Botany-Revue Canadienne De Botanique **74:**875-882.

Bottin, L., S. Le Cadre, A. Quilichini, P. Bardin, J. Moret, and N. Machon. 2007. Re-establishment trials in endangered plants: a review and the example of *Arenaria grandiflora*, a species on the brink of extinction in the Parisian region (France). Ecoscience **14:**410-419.

Bowen, B. 2011. Natural areas protection at its best; protecting the Tennessee purple coneflower (*Echinacea tennesseensis*). Natural Areas Journal **31:**326-330.

Brumback, W. E., D. M. Weihrauch, and K. D. Kimball. 2003. Propagation and transplanting of an endangered alpine species Robbins' cinquefoil. Native Plants. **Spring**.

Burmeier, S., and K. Jensen. 2009. Experimental ecology and habitat specificity of the endangered plant *Apium repens* (Jacq.) Lag. at the northern edge of its range. Plant Ecology & Diversity **2:**65-75.

Carlson, M.L. 1998. Status report for *Artemisia campestris* L. ssp. *borealis* var. *wormskioldii* [Bess.] Cronq. Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.

Charlesworth, S., and J.H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics. **10**:783-796.

Cochrane, J., A. Crawford, and L. Monks. 2007. The significance of ex situ seed conservation to reintroduction of threatened plants. Australian Journal of Botany **55:**356-361.

Dalrymple, S. E., and A. Broome. 2010. The importance of donor population identity and habitat type when creating new populations of small cow-wheat *Melampyrum sylvaticum* from seed in Perthshire, Scotland. Conservation Evidence**.** 1-8.

Drayton, B., and R. Primack. 2000. Rates of success in the reintroduction by four methods of several perennial plant species in eastern Massachusetts. Rhodora. **102:**299-321.

Drayton, B., and R. Primack. 2012. Success rates for reintroductions of eight perennial plant species after 15 Years. Restoration Ecology **20:**299-303.

Falk, D. A., C. I. Millar, and M. Olwell. 1996. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington D.C.

Falk, D.A., E.E. Knapp, and E.O. Guerrant. 2002. An introduction to restoration genetics. Prepared by the Society for Ecological Restoration for Plant Conservation Alliance, Bureau of Land Management, U.S. Department of Interior.

Fenster, C., and L. Galloway. 2000. Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). Conservation Biology **14:**1406-1412.

Ferdy, J., S. Loriot, M. Sandmeier, M. Lefranc, and C. Raquin. 2001. Inbreeding depression in a rare deceptive orchid. Canadian Journal of Botany-Revue Canadienne De Botanique **79:**1181-1188.

Fischer, M., and D. Matthies. 1998. Effects of population size on performance in the rare plant *Gentianella germanica*. Journal of Ecology **86:**195-204.

Fish Passage Center. 2013. River data: Wanapum dam. Fish Passage Center, Portland, Oregon. Accessed on March 28, 2013. http://www.fpc.org/river_home.html.

Galloway, L.F. 2001. The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). American Journal of Botany. **88(5):**832-840.

Gamon, J. 1989. Report on the status of *Artemisia campestris* L. var. *wormskioldii* [Bess.] Cronquist. Washington Natural Heritage Program, Olympia, Washington.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A. Stevens, R. Aguraiuja, C. Cowell, C. Weekley, G. Vogg, J. Iriondo, I. Johnson, B. 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.

Greet, J., R.D. Cousens, and J.A. Webb. 2012. More exotic and fewer native plant species: riverine vegetation patterns associated with altered seasonal flow patterns. River Research and Applications.

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

Guerrant, E. O. 1996b. Reintroduction of *Stephanomeria malheurensis*, a case study. Pages 399-402 in D. Falk, C. Millar, and P. Olwell, editors. Restoring diversity: strategies for reintroductions of endangered plants. Island Press, Washington, DC.

Guerrant, E. O. 2012. Characterizing two decades of rare plant reintroduction. Pages 9-29 in J. Maschinski and K. E. Haskins, editors. Reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Guerrant, E. O., and T. N. Kaye. 2007. Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany **55:**362-370.

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

Gustafson, D., D. Gibson, and D. Nickrent. 2004. Competitive relationships of *Andropogon gerardii (*Big Bluestem) from remnant and restored native populations and select cultivated varieties. Functional Ecology **18:**451-457.

Gutterman, Y. 2000. Maternal effects on seeds during development. Pages 59-84 in M. Fenner, editor. Seeds: The ecology of regeneration in plant communities, 2nd ed. CAB International Publishing. Wallingford, UK.

Herranz, J.M., P. Ferrandis, and E. Martinez-Duro. 2010. Seed germination ecology of the threatened endemic Iberian *Delpinium fissum* subsp. *sordidum* (Ranunculaceae). Plant Ecology. **211:**89-106.

Holl, K., and G. Hayes. 2006. Challenges to introducing and managing disturbance regimes for *Holocarpha macradenia*, an endangered annual grassland forb. Conservation Biology **20:**1121-1131.

Huang, Z., Y. Gutterman, and Z. Hu. 2000. Structure and function of mucilaginous achenes of *Artemisia monosperma* inhabiting the Negev Desert of Israel. Israel Journal of Plant Sciences **48:**255-266.

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

Jacquemyn, H., R. Brys, and M. Hermy. 2001. Within and between plant variation in seed number, seed mass and germinability of *Primula elatior*: effect of population size. Plant Biology **3:**561-568.

Jõgar, U., and M. Moora. 2008. Reintroduction of a rare plant (*Gladiolus imbricatus*) population to a river floodplain - How important is meadow management? Restoration Ecology **16:**382-385.

Jusaitis, M. 2005. Translocation trials confirm specific factors affecting the establishment of three endangered plant species. Ecological Management & Restoration **6:**61-67.

Jusaitis, M., L. Polomka, and B. Sorensen. 2004. Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). Biological Conservation **116:**251-266.

Kaye, T. N. 2001. Common ground and controversy in native plant restoration: the SOMS debate, source distance, plant selections, and a restoration-oriented definition of native. Native plant propagation and restoration strategies. Corvallis (OR): Nursery Technology Cooperative and Western Forestry and Conservation Association. p**:**5-12.

Kaye, T. N. 2008. Vital steps toward success of endangered plant reintroductions. Native Plants **9:**313-322.

Kaye, T. N. 2009. Toward successful reintroductions: the combined importance of species traits, site quality, and restoration technique. Pages 99-106. California Native Plant Society Conservation Conference.

Kaye, T. N., and J. R. Cramer. 2003. Direct seeding or transplanting: the cost of restoring populations of Kincaid's lupine. Ecological Restoration **21:**224-225.

Kephart, S. 2004. Inbreeding and reintroduction: progeny success in rare Silene populations of varied density. Conservation Genetics **5:**49-61.

Kirchner, F., A. Robert, and B. Colas 2006. Modelling the dynamics of introduced populations in the narrow-endemic *Centaurea corymbosa*: a demo-genetic integration. Journal of Applied Ecology **43:**1011-1021.

Lande, R. 1988. Genetics and demography in biological conservation. Science. **241:**1455-1460.

Lawrence, B., and T. Kaye. 2006. Habitat variation throughout the historic range of golden paintbrush, a Pacific Northwest prairie endemic: implications for reintroduction. Northwest Science **80:**140-152.

Lawrence, B., and T. Kaye. 2009. Reintroduction of *Castilleja levisecta*: effects of ecological similarity, source population genetics, and habitat quality. Restoration Ecology **19:**166-176.

Leimu, R., P.M. Mutikainen, J. Koricheva, and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology **94:**942-952.

Lloyd, M., R. Burnett, K. Engelhardt, and M. 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 **13:**753-765.

Luzuriaga, A.L., A. Escudero, and F. Perez-Garcia. 2006. Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). Weed Research. **46:**163-174.

MacDougall, A., and R. Turkington. 2005. Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology **86:**42-55.

Maschinski, J. 2006. Implications of population dynamic and metapopulation theory for restoration. Pages 59-87 in D. Falk, M. Palmer, and J. Zedler, editors. Foundations of restoration ecology. Island Press, Washington, DC.

Maschinski, J., M. A. Albrecht, L. Monks, and K. E. Haskins. 2012a. Center for Plant Conservation best reintroduction practice guidelines. Pages 277-306 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J., J. Baggs, and C. Sacchi. 2004. Seedling recruitment and survival of an endangered limestone endemic in its natural habitat and experimental reintroduction sites. American Journal of Botany **91:**689-698.

Maschinski, J., and J. Duquesnel. 2006. Successful reintroductions of the endangered long-lived Sargent's cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. Biological Conservation **134:**122-129.

Maschinski, J., D. A. Falk, S. J. Wright, J. Possley, J. Roncal, and K. S. Wendelberger. 2012b. Optimal locations for plant reintroductions in a changing world. Pages 109-129 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. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Maschinski, J., S.J. Wright, S. Koptur, and E.C. Pinto-Torres. 2013. When is local the best paradigm? Breeding history influences conservation reintroduction survival and population trajectories in times of extreme climate events. Biological Conservation. **159:**277-284.

Maunder, M. 1992. Plant reintroduction: an overview. Biodiversity and Conservation **1:**51-61.

McGlaughlin, M., K. Karoly, and T. Kaye. 2002. Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellata* subsp *breviflora* (Nyctaginaceae). Conservation Genetics **3:**411-420.

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 genetics of restoration. Restoration Ecology. **13:**432-440.

Menges, E.S. 1991. Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biology **5:**158-164.

Menges, E. S. 2008. Restoration demography and genetics of plants: when is a translocation successful? Australian Journal of Botany **56:**187-196.

Merrit, D.M., M.L. Scott, N.L. Poff, G.T. Auble, D.A. Lytle. 2010. Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. Freshwater Biology **55:**206-225.

Microsoft. 2007. Microsoft Excel. Redmond, Washington: Microsoft, 2007. Computer Software.

Monks, L., D. Coates, T. Bell, and M. 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.

Montalvo, A., and N. Ellstrand. 2000. Transplantation of the subshrub *Lotus scoparius*: testing the home-site advantage hypothesis. Conservation Biology **14:**1034-1045.

Morgan, J.W. 1998. Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology **13:**266-273.

National Research Council. 2004. Managing the Columbia River: instream flows, water withdrawals, and salmon survival. The National Academies Press. Washington, DC.

Naimen, R.J., and H. Décamps. 1997. The ecology of interfaces: riparian zones. Annual Review of Ecology and Systematics **28:**621-658.

Neale, J. R. 2012. Genetic considerations in rare plant reintroduction: practical applications (or how are we doing?). Pages 71-88 in J. Maschinski and K. E. Haskins, editors. Plant reintroduction in a changing climate: promises and perils. Island Press, Washington, DC.

Noel, F., D. Prati, M. Van Kleunen, A. Gygax, D. Moser, and M. Fischer. 2011. 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.

Olden, J., and T. Rooney. 2006. On defining and quantifying biotic homogenization. Global Ecology and Biogeography **15:**113-120.

Oregon Biodiversity Information Center (ORBIC). 2010. Rare, threatened and endangered plants and animals of Oregon. Oregon Biodiversity Information Center, Portland Oregon.
Accessed on October 22, 2012. http://orbic.pdx.edu/documents/2010-rte-book.pdf.

Oregon Department of Agriculture (ODA), Native Plant Conservation Program. 2012. Oregon listed plants. Oregon Department of Agriculture, Salem, Oregon. Accessed on October 22, 2012.
http://www.oregon.gov/ODA/PLANT/CONSERVATION/Pages/statelist.aspx.

Oregon Department of Agriculture (ODA). 2013. Oregon State Noxious Weed List, B List. Accessed on April 29, 2013.
http://www.oregon.gov/ODA/PLANT/WEEDS/Pages/statelist2.aspx#B_list

Pavlik, B. 1997. Perspectives, tools, and institutions for conserving rare plants. Southwestern Naturalist **42:**375-383.

Pavlik, B. M. 1996. Defining and measuring success. Pages 127-155 in D. A. Falk, C. Millar, and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered species. Island Press, Washington, DC.

Primack, R. 1996. Lessons from ecological theory: dispersal, establishment, and population structure. Pages 209-233 in D. Falk, C. Millar, and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Primack, R.B., and B. Drayton. 1997. The experimental ecology of reintroduction. Plant Talk **October:**26-28.

Protopopova, V.V., M.V. Shevera, and S.L. Mosyakin. 2006. Deliberate and unintentional introduction of invasive weeds: a case study of the alien flora of Ukraine. Euphytica **148:**17-33.

Qi, Q., C. Hong-Feng, X. Fu-Wu, L. Dong-Ming, and H. Xiao-Gai. 2009. Seed germination protocol for the threatened plant species, *Bretschneidera sinensis* Hemsl. Seed Science and Technology **37:**70-78.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Reckinger, C., G. Colling, and D. Matthies. 2010. Restoring populations of the endangered plant *Scorzonera humilis*: influence of site conditions, seed source, and plant stage. Restoration Ecology **18:**904-913.

Reed, D.H. 2005. Relationship between population size and fitness. Conservation Biology **19:**563-568.

Rich, T., C. Gibson, and M. Marsden. 1999. Re-establishment of the extinct native plant *Filago gallica* L. (Asteraceae), narrow-leaved cudweed, in Britain. Biological Conservation **91:**1-8.

Rimer, R., and K. Mccue. 2005. Restoration of *Helenium virginicum* Blake, a threatened plant of the Ozark Highlands. Natural Areas Journal **25:**86-90.

Severns, P. 2003. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation **110:**221-229.

Schemske, D.W., B.C. Husband, M.H. Ruckelshaus, C. Goodwillie, I.M. Parker, and J.G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology. **75(3):**584-606.

Shaffer, M.L. 1981. Minimum population sizes for species conservation. BioScience. **31**:131-134.

Sheridan, P., and D. Karowe. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia. American Journal of Botany **87:**1628-1633.

Silvernail, I., and R.J. Meinke. 2008. Patterns of ecotypic variation and the germination and cultivation requirements of *Lomatium cookii*. Native Plant Conservation Program, Oregon Department of Agriculture, Salem, Oregon.

Silvertown, J., and D. Charlesworth. 2001. Introduction to plant population biology, 4^{th} ed. Blackwell Publishing. Malden, MA.

Sletvold, N., J. Grindeland, P. Zu, and J. Agren. 2012. Strong inbreeding depression and local outbreeding depression in the rewarding orchid *Gymnadenia conopsea*. Conservation Genetics **13:**1305-1315.

Sutter, R. D. 1996. Monitoring. Pages 235-264 in D. Falk, C. Millar and M. Olwell, editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, DC.

Thorpe, A.S., and T.N. Kaye. 2011. Conservation and reintroduction of the endangered Willamette daisy: effects of population size on seed viability and the influence of local adaptation. Native Plants Journal. **12(3):**289-298.

U. S. Fish and Wildlife Service. 2002. Accessed on September 13, 2012. Robbins' cinquefoil (*Potentilla robbinsiana*) Species Profile. http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=Q20V

U.S. Fish and Wildlife Service. 2010. Spotlight Species Action Plan for Northern Wormwood. U.S. Fish and Wildlife Service, Lacey, Washington. Accessed on December 6, 2010. http://ecos.fws.gov/docs/action_plans/doc3088.pdf

U.S. Fish and Wildlife Service. 2011. Species assessment and listing priority assignment form for Northern Wormwood. U.S. Fish and Wildlife Service, Lacey, Washington. Accessed on March 28, 2013. http://www.fs.fed.us/r6/sfpnw/issssp/documents/planning-docs/cp-fws-candidate-va-artemisia-borealis-2011-05.pdf

U.S. Fish and Wildlife Service. 2011. Species profile: Tennessee purple coneflower. Accessed on October 12, 2012. http://www.fws.gov/ecos/ajax/speciesProfile/profile/speciesProfile.action?spcode=Q1VU

Walck, J. L., T. E. Hemmerly, and S. N. Hidayati. 2002. The endangered Tennessee purple coneflower *Echinacea tennesseensis* (Asteraceae): its ecology and conservation. Native Plants Journal **3:**54-64.

Washington Department of Ecology. 2011. Non-native invasive freshwater plants: Indigo bush (*Amorpha fruiticosa*). Accessed on February 20, 2013. http://www.ecy.wa.gov/programs/wq/plants/weeds/aqua014.html

Washington Department of Natural Resources. 1997. *Artemisia campestris* L. ssp. *borealis* Hall & Clem.var. *wormskioldii* (Bess.) Cronquist species profile. Washington Department of Natural Resources, Olympia, Washington. Accessed on October 22, 2012. http://www1.dnr.wa.gov/nhp/refdesk/fguide/pdf/arca.pdf.

Washington State Noxious Weed Control Board. 2010. Noxious weeds list, class B. Accessed on February 20, 2013. http://www.nwcb.wa.gov/searchResults.asp?class=B

Western Regional Climate Center. 2013. Cooperative Climatological Data Summaries: Washington, Priest Rapids Dam. Accessed on April 15, 2013. http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?wa6747

Yang, X., M. Dong, and Z. Huang. 2010. Role of mucilage in the germination of *Artemisia sphaerocephala* (Asteraceae) achenes exposed to osmotic stress and salinity. Plant Physiology and Biochemistry **48:**131-135.

Appendix A: Protocol for transplant propagation for a large-scale reintroduction of northern wormwood

Task	Protocol
Seed source selection	• Use seeds from as many sources as possible to maximize the diversity of the founding population • Seeds from ecologically similar habitats such as the Beverly population may result in transplants more likely to establish and survive at wild sites
Seed storage	• Seeds should be stored in a dry, cool place in small coin envelopes
Seed germination	• Germinate seeds in Petri dishes with filter paper in a greenhouse • Use new or sanitized Petri dishes to reduce risk of mold • Use filter paper and wet thoroughly with distilled water • Place seeds on wet filter paper, maximum of 15 • Re-wet seeds as necessary • Seeds should be ready to be planted within one week
Seedling cultivation	• Prepare pots by filling with potting soil, drench with water, pat down soil, fill pot to the top with soil and wet top one more time • Using tweezers, carefully pull seedling off of filter paper • Using a pencil, poke a hole in the center of the pot and carefully place seedling in hole • Gently pat soil in and around seedling • Mist seedlings until they are larger and then use regular watering methods • After 6-8 weeks, place plants outdoors to get conditioned to the environment • Suggested transplant size for outplanting will take 10-12 weeks to achieve

Appendix B: Rufus Island species list

Achillea millefolium
Ailanthus altissima
Amorpha fruticosa
Anthriscus scandicina
Asclepias fascicularis
Bromus tectorum
Camissonia contorta
Centaurea diffusa
Chrysopsis villosa
Chrysothamnus nauseosus var. *albicaulis*
Convolvulus arvensis
Descurainnia pinnata
Epilobium sp.
Erodium cicutarium
Eriogonum compositum
Geranium sp.
Glycyrrhiza lepidota
Helenium autumnale var. *grandiflorum*
Hordeum sp.
Hypericum perforatum
Lomatium grayi
Myosotis discolor
Oenothera pallida var. *pallida*
Phacelia hastata
Plantago patagonica
Polygonum majus
Potentilla sp.
Rubus armeniacus
Salix exigua
Solidago sp.
Verbascum thapsus
Verbena bracteata