Restoring Abandoned Agricultural Lands in Puget Lowland Prairies: A New Approach

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A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2013

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Program Authorized to Offer Degree:

Environmental and Forest Sciences

University of Washington

Abstract

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Puget Lowland Prairies are one of the most endangered ecosystems in the United States. Restoration has been occurring for more than thirty years and has focused on extant sites with some native species present. Generally, actions first began with invasive species control, followed by reintroduction of fire, and then seeding and planting. Significant improvements have been made, however existing habitat continues to be converted and additional strategies to restore the ecosystem need to be explored. Developing techniques and methods for restoring abandoned agricultural lands, and other lands with no native species present, to native prairie is a critical information need, as it will likely be an important future strategy. This dissertation explored techniques to restore native prairie habitat on a suite of abandoned agricultural lands over a large spatially and temporally replicated experiment, the Prairie Habitat Restoration Project (PHRP). I first explored techniques and factors affecting the seedling establishment and survival of a rare species, a hemiparasitic plant endemic to the Puget Lowland Prairies,

Castilleja levisecta. I seeded *C. levisecta* in combination with different site preparation and seeding treatments across 4 sites over 2 years. Next, I tested the effect of host plant identity and container size on survival and reproduction of *C. levisecta* in a replicated outplanting study with two potential host species. Finally, I tested a novel approach we call "Staged-Scale Restoration" (SSR) that rigorously explored multiple habitat treatments within an adaptive management framework. I implemented SSR by identifying several promising restoration strategies and testing them in small, replicated experimental plots. Based on the results of these small-scale tests, the most successful treatments were applied to increasingly larger scaled-up areas. My first study demonstrated that direct seeding is an effective strategy for establishing large populations of *C. levisecta.* My second study found that the presence of a host species, and the identity of that host, significantly affected the survival and performance of *C. levisecta*. My final study demonstrated that SSR enables adaptive management to be implemented in restoration projects while minimizing risks, improving scientific rigor, and providing a cost effective approach.

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ACKNOWLEDGEMENTS

First I would like to thank my primary advisor Jon Bakker and Peter Dunwiddie, who served effectively as my co-advisor. You have each provided above and beyond support for my academic journey and I will always be grateful. Also my other committee members Janneke Hille Ris Lambers, Kern Ewing, and Sarah Hamman have all provided helpful feedback and advice.

The U.S. Fish and Wildlife Service provided generous support and the majority of funding for all of the research in this dissertation. I would like to especially thank Ted Thomas for your support of this research. Also thanks to land managers and agencies that have assisted in implementing this research at their respective sites; WDFW, The Nature Conservancy, Pacific Rim Institute, Thurston County, Dave Hays, Lisa Younger, Robert Pelant, Seth Luginbill, Sanders Freed.

Thanks to TNC for giving me lots of flexibility while working full time. Thanks to my first supervisor at TNC, Pat Dunn for your encouragement to begin, and thanks to my current supervisor Dave Rolph for your unwavering support for me to finish. Also to all my colleagues at TNC that have been so supportive. Also to my lab group at UW for all your support and advice over the years.

The numbers of people that have helped implement, maintain, and monitor the research arrays have been enormous, and I would not have been able to do this without incredible generosity from so many people. I have likely forgotten some people who have helped, and ask your pardon. Betsie DeWreede, Mike and Marion Jarisch, and the Tuesday Volunteer Crew were invaluable in collecting seeds and cleaning seeds from the research plots. Thanks to the following for monitoring and other help: Adam Martin, Anita Goodrich, Ashley Smithers, Allison McGrath, Amber Unger, Angela Winter, Briana Abrahms, Betsie DeWreede, Bob W., Becca Reilly, Brian Kapusta, Ben Waldron, Charlotte Ballog, Caitlin Guthrie, Cassie Johnson, Cheryl Lowe, Dan Bransford, David Parker, Dave Wesdowski, Eva Robinson, Rachel Mitchell, Ryan Haugo, Emily Borodkin, Ellen Sherck, Etsuko Reistroffer, Grace Diehl, Gail Trotter, Heron Brae, Heather Van Varen, Joe Bettis, Jared Tarr, Kathryn Hill, Korena Mafune, Kyle Pinjuv, Karen Reagan, Karen Wells, Laney Widener, Laurel Carver, Lindsey Hamilton, Lisa Hintz, Laura Kress, Liza Norment, Leighton Olive, Marcia Anne Rosenquist, Mark Roth, Megan Erickson, Natalie Footen, Otis Bell, Heather Ostle, Paul Griffith, Paul P., Robyn Andrusysvyn, Robyn B., Silvain Amiet, Spencer Alexander, Sarah Clarke, Scott Stavely, Sarah Farr, Valerie Verhei, Veronica Wisniewski, Jessica Da Bell, Will, Dan, Pratt, MCJ, MD, Andy, AG, Marion Jarisch, Shawn Zinewski and the WCC crew. Also thanks to "The Monitors"; you know who you are. Also thanks to Mason McKinley at CNLM and Jim Lynch and the Fire Crew on JBLM for all the pro-bono fire assistance.

A special thanks to Mark Roth from TNC and then CNLM, without whom I could not have even hoped to get the field research implemented. Thank you for the endless hours and the multiple near death experiences. Hopefully, this work will be a suitable offering to the Whidbey gods.

Finally, thank you to my friends and family, to Florence and Hawthorn, who arrived in the middle of this journey, and especially to my wife Rain, who has been incredibly supportive while sacrificing countless weekends and nights for the past five years.

Chapter 1

A General Overview of Puget Lowland Prairies and Current and Future Conservation

Background

Grasslands, prairies, and savannas cover over 40% of the Earth's land surface and are found in every terrestrial region of the world (Curtin and Western 2008). However, worldwide grasslands have become one of the most imperiled ecosystems and are facing increasing threats by multiple anthropogenic activities (Ceballos *et al.* 2010). In North America there have been declines in areas of native prairie ranging as high as 99.9%, while overall it is one of the most impacted ecosystems (Samson and Knopf 1994).

In Western Washington, Puget Lowland Prairies are part of the Willamette Valley-Puget Trough-Georgia Basin Ecoregion (Figure 1.1). They are a relatively small grassland ecosystem and usually don't even get mentioned in assessments of grassland habitat globally or regionally such as by the World Temperate Grasslands Conservation Initiative (Peart 2008). However, they too have declined significantly since settlement by Europeans, from more than 73,000 ha to less than 7,000 ha (Crawford and Hall 1997), and only 2-3% are still dominated with native species, though these estimates are more than a decade old and should be updated (Dunwiddie and Bakker 2011).

The largest remaining remnants of Puget Lowland Prairie are located in South Puget Sound on Joint Base Lewis-McChord (JBLM). There are additional protected prairies in South Puget Sound located off of JBLM, and several smaller protected areas in North Puget Sound (Figure 1.2). There are also a handful of privately owned sites, some of which are used for grazing. Most of the remaining remnants tend to be small and highly fragmented, and have lost the functional connectivity they used to share. Importantly, the prairies that remain are not representative of the breadth of prairie habitat that existed before conversion. Deeper soil and wetter prairies were disproportionately impacted when the first prairies were converted, as they were the most valuable for agriculture (Easterly et al. 2005, Lea 2006). Therefore the remaining examples of Puget Lowland Prairies are highly skewed towards sites on the poorest soils (Dunwiddie and Bakker 2011).

Naturally, the biota associated with these remnants is highly degraded as well, particularly with additional stresses present such as invasive species. For example, in a study of 15 of the largest prairie remnants in South Puget Sound, Dunwiddie et al. (2006) found that 40% of the native plant species that occurred in the past are absent from all the sites today. One of the species of particular concern is *Castilleja levisecta* (Golden paintbrush), a federally threatened hemiparasite endemic to Puget Lowland Prairies. In addition to flora, Puget Lowland Prairies have imperiled mammals (Stinson 2005), birds (Altman 2011), butterflies (Schultz *et al.* 2011), and other invertebrates (Fazzino et al. 2011). Given the level of fragmentation and degradation, the habitat and the species dependent on these prairies will continue to decline without robust protection and restoration.

Ecological Restoration Research in Puget Lowland Prairies

Restoration work in Puget Lowland Prairies has been consistently guided by the use of ecological restoration research to first better understand the system and then to help answer the most pressing questions. For example, in 1996, very early in the restoration of Puget Lowland Prairies, practitioners and researchers collaborated as part of the South Puget Sound Prairie Landscape Working Group conference to collate existing information on habitat, rare species, restoration techniques, and protection efforts (Dunn and Ewing 1997). The resulting volume on the ecology and conservation of system guided restoration techniques and research needs for the next decade. During that time, both the restoration work and research grew in sophistication. Restoration and associated research grew from summarizing information and exploring basic restoration techniques, to striving to understand more complex interactions such as overarching strategies across the ecoregion (Stanley et al. 2011), non-target effects of herbicides on butterflies (LaBar and Schultz 2012), plant community responses to multiple restoration actions (Rook *et al.* 2011), and the effective use of fire as a restoration tool (Hamman *et al.* 2011), to name a few. The evolving restoration work and research culminated in another volume of collected papers from the Cascadia Prairie Oak Partnership conference in 2011 (Dunwiddie and Bakker 2011) that will undoubtedly guide and advance future restoration. A consistent theme of both the work and the research over the last three decades is the urgency for both the protection and restoration of Puget Lowland Prairies, and the understanding of how to do that most effectively.

Past and Present Conservation Efforts

Fortunately, state and federal agencies and non-governmental organizations like The Nature Conservancy, Pacific Rim Institute, and the Center for Natural Lands Management (CNLM) have or are working urgently to protect and restore Puget Lowland Prairies. Generally, restoration in Puget Lowland Prairies focused initially on the removal of biological threats, followed by the restoration of ecological processes, and then planting and seeding to overcome seed limitations of native species. Combined, these three areas of effort have significantly improved habitat of extant lowland prairie sites across the region.

Control of Biological Threats

Controlling biological threats were the first restoration actions undertaken in Puget Lowland Prairies in the early 1990's, and consisted initially of abating structure changing invasive species like Scotch broom (*Cytisus scoparius*) on individual sites (Dunn and Ewing 1997). Following the reduction or control of Scotch broom, other problematic invasive species including a suite of European pasture grasses among others, have been identified as priorities to control. In general, non-native species are targeted for control if they change the structure of the prairies or form monocultures and exclude native species. Many non-native species are not controlled, and some such as *Plantago lanceolata* are even encouraged due to the use of that species by a rare butterfly. Invasive species control is accomplished by mechanical control such as mowing or hand pulling, herbicides, and the use of prescribed fire.

Restoration of Ecological Processes

The restoration of ecological processes has mainly consisted of the return of fire to the system through the use of prescribed fire. Like many ecosystems in the United States, the Puget Lowland Prairies are a fire-adapted system that was anthropogenically maintained for thousands of years by indigenous peoples (Boyd 1999). Fire prevents the invasion of the prairies by tree species such as Douglas fir (*Pseudotsuga menziesii*), and provides areas for new germination by native herbaceous species. Prescribed fire is also effective at controlling many non-native species and restoring low vegetation prairie structure. The first prescribed fires in this system were conducted in the late 1990s; it became a well-developed component of the restoration program by 2005 (McKinley, 2013 personal communication).

Native Species Seed Limitation

The use of native plantings and seeding for restoration in Puget Lowland Prairies is done to address the depleted seed banks and limited seed dispersal of many species. Generally, plantings and seeding are conducted into sites where problematic invasive species have been controlled or reduced. Plantings have historically been small in scale and diversity (< 10 species), however recently plantings of several hundred thousand plants annually have occurred at some sites (Lynch, 2013 personal communication). Seeding is a relatively new restoration method. Larger scale seeding of the predominant native grass, *Festuca roemeri,* began in the early 2000s but was delayed for several years due to seed contamination (Dunwiddie and Delvin 2006). Seeding with more than a few species is beginning to occur on larger scales, but is still limited in scope. For example, the native seeding done over several hectares with more than 26 species as part of this research represented the largest and most diverse seeding conducted in this system to date. However, seed production is being increased by CNLM and larger and more diverse seedings will be an important restoration strategy in the future.

Recovery of Castilleja levisecta

Recovery of *Castilleja levisecta* from the twelve wild populations to the goal of at least twenty has been proceeding methodically since the federal listing of the species in 1997. Initial work included thorough assessments of existing populations and production of recovery and reintroduction plans (Caplow 2004, Chappell and Caplow 2004). Recovery will require both establishing the species at many new sites and ensuring conditions for reproduction, such as the presence of the appropriate density and diversity of host species. Limited research has occurred to determine appropriate host species and the effect of host species identity on *C. levisecta* performance, and there is a need for additional research in this area. Early reintroduction work focused almost exclusively on outplantings, as initial direct seeding efforts proved unreliable (Pearson and Dunwiddie 2006) and using seeds as founders was not recommended in the reintroduction plan (Caplow 2004). However, direct seeding is now being done successfully throughout the species' range, including in Oregon where it had been extirpated (Kaye 2011). Overall the trajectory of the species' recovery has been on a sharp rise over the last several years, mainly due to the use of seeds as founders. In fact, 2013 is the first year that blooming *C. levisecta* plants from introduced populations are projected to be greater than those from the existing wild populations. I believe the recovery of the species is likely within the next decade, assuming funding and consistent efforts continue.

Collaborative Management and Increased Effort

While the remaining Puget Lowland Prairie sites are owned by many different entities, increasingly restoration is approached from an ecosystem perspective (Dunwiddie and Bakker 2011), and sites are managed as a whole with coordination of restoration and research. Restoration funding through U.S. Fish and Wildlife Service (USFWS) and the Department of Defense's Army Compatible Use Buffer Program (ACUB) has been instrumental in helping align multiple agencies and organizations conducting management and restoration. For example, USFWS has contributed more than $1 million (US) in grants over the last 15 years directed to recover *C. levisecta* (Dunwiddie and Bakker 2011), while ACUB has contributed more than $8 million (US) over the last six years towards protection, restoration, and management of lands off of JBLM for four rare species found on the military installation (Anderson, 2013 personal communication).

This additional funding and coordination has resulted in increased effort across all areas of work. An example of the expansion of effort in restoration is evident in the recent prescribed fire history. The number of prescribed fires conducted by all partners in Puget Lowland Prairie restoration has steadily increased from just 2 in 2005 to more than 50 in 2012 and acreage burned in the same period has increased from 55 acres to 2,500 acres (Figure 1.3). This increased restoration effort and coordination has resulted in significant improvement of prairie quality across multiple protected sites complete with reintroduction of some of the species rarest species such as Taylor's checkerspot (*Euphydryas editha taylori*).

Future Conservation Priorities

While the protection, restoration, and associated research that have occurred in Puget Lowland Prairies to date are critical, they are restricted to extant sites and do not address restoration of sites where no native species are present, such as sites with deeper soils like abandoned agricultural lands. I believe that restoration of these kinds of lands to native prairie habitat may be as feasible or even more feasible than restoration of extant degraded sites and most significantly, could add a new suite of lands for prairie conservation in the region.

The process of returning agricultural land to native vegetation has long been of interest to plant ecologists (Marushia and Allen 2011), however research has usually focused on the natural process of succession such as Meiners' et al. (2002) studies on 40 years of old field succession. In many parts of the world however, grasslands that were historically converted to agricultural use are now being restored as the intensity of agricultural land use has declined over the last two decades, particularly in the United States and Europe (Goklany 2002, Prach et al. 2013). In the Midwest for example, grassland restoration of former agricultural lands has been occurring for decades (Zajicek et al. 1986, Polley et al. 2005). Restoring abandoned agricultural lands is less common in the Western United States, though its importance is increasingly recognized (Kulmatiski et al. 2006, Marushia and Allen 2011).

Developing techniques and methods for restoring abandoned agricultural lands to native Puget Lowland Prairie is a critical information need, as it will likely be an important future strategy for the recovery of the ecosystem. As part of my overall research I explored techniques to restore native prairie habitat on a suite of abandoned agricultural lands over a large spatially and temporally replicated Prairie Habitat Restoration Project (PHRP). For this dissertation, I present three separate studies, within the context of the PHRP. I focus two of my studies on *Castilleja levisecta* and the final study on the methodology of my field research. In the first study, I explore techniques for direct seeding of *C. levisecta.* This species has previously had limited success in direct seeding. I determine which treatments and combination of treatments in the PHRP result in the establishment, survival, and reproduction of the species. My second study focuses further on *C. levisecta* in a separate experiment designed to better understand the effect of host, host species, and planting size on survival and performance. I conducted a replicated two-year host plant and pot size experiment with *C. levisecta,* also on abandoned agricultural land. My final study takes a broader view and focuses on an innovative design feature I employed as part of the PHRP, Staged-Scale Restoration (SSR). SSR is a method to apply adaptive management in a restoration context; where promising restoration approaches that can be applied at large scales are identified and tested in small, replicated experimental plots directly on the restoration site. Restoration of the site proceeds in progressive steps by implementing the most successful approaches at increasingly larger scales that build on accumulated experience. I illustrate the SSR approach using the PHRP as a case study, though the technique also has applicability in other ecosystems.

Overall, I believe these studies contribute to the robust body of restoration ecology in the Puget Lowland Prairies and grassland restoration ecology. Each of these studies provides new information that should advance the recovery of one of the rarest species in the region and of Puget Lowland Prairies.

Note to the reader: Chapters 2 – 4 are each intended as a separate manuscript for publication. There is some variation in formatting among these chapters due to differing requirements among the intended publication outlets. There is also some duplication between chapters such as in description of sites or methods.

Chapter 1 Figures

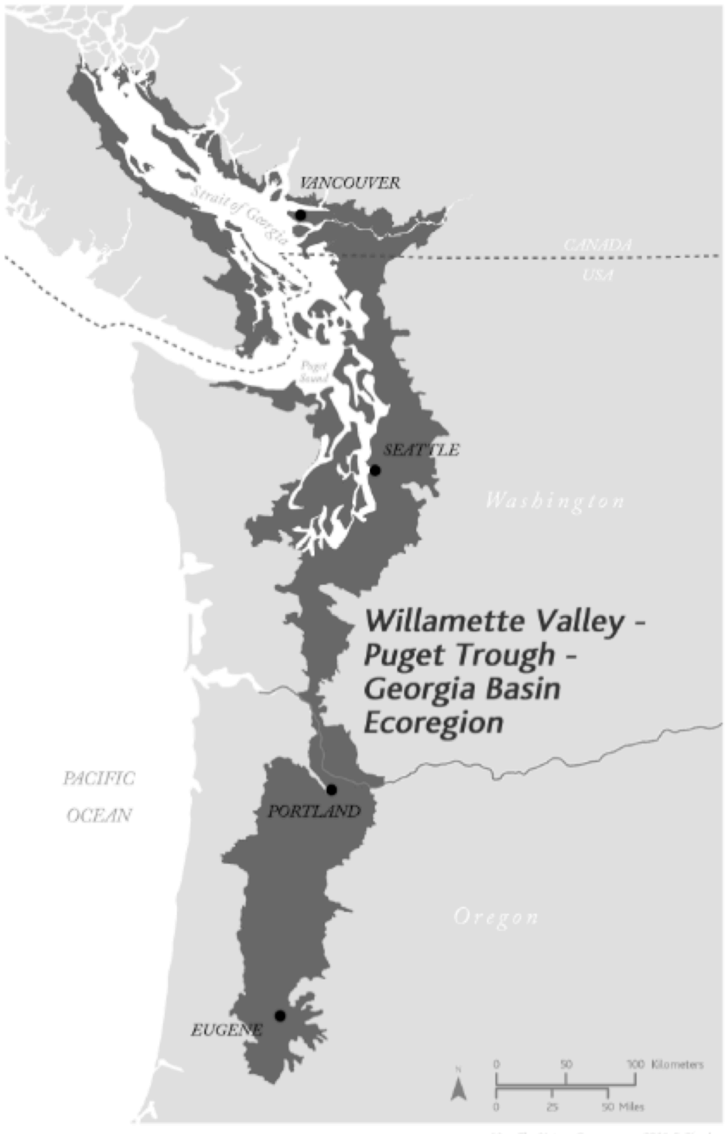

Figure 1.1. The Puget Lowland physiographic province consists of a broad, low-lying region situated between the Cascade Range of Washington to the east and the Olympic Mountains and Willapa Hills to the west. It is the “Puget Trough” of the Willamette Valley-Puget Trough-Georgia Basin Ecoregion. Puget Lowland Prairies refer to all of the grasslands that exist within this geographic area.

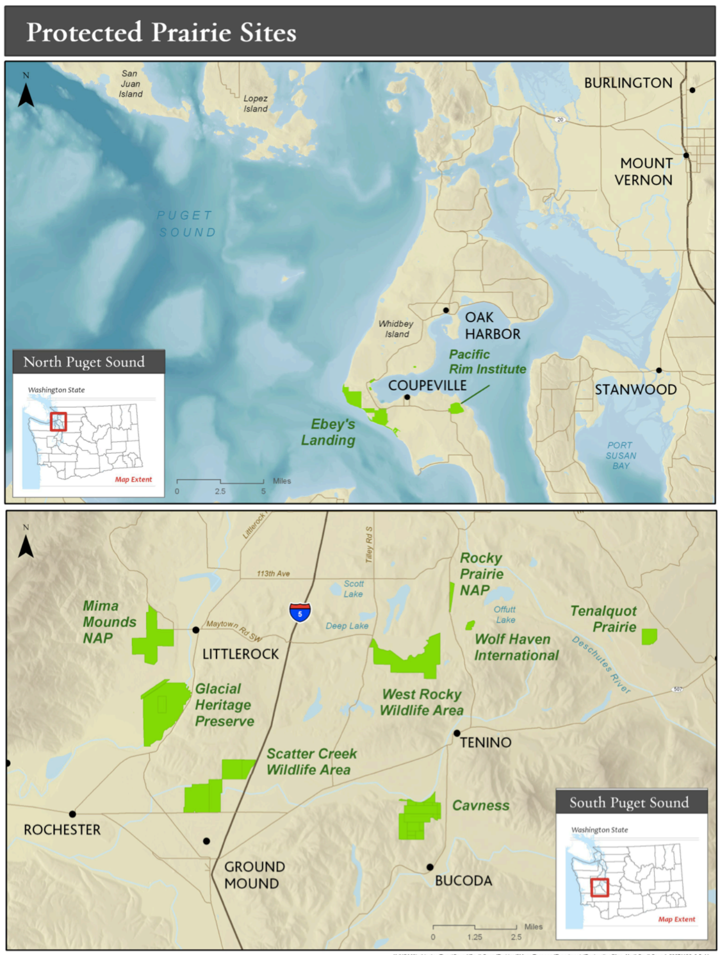

Figure 1.2. Protected Puget Lowland Prairie sites in North and South Sound. (The Nature Conservancy of Washington 2013).

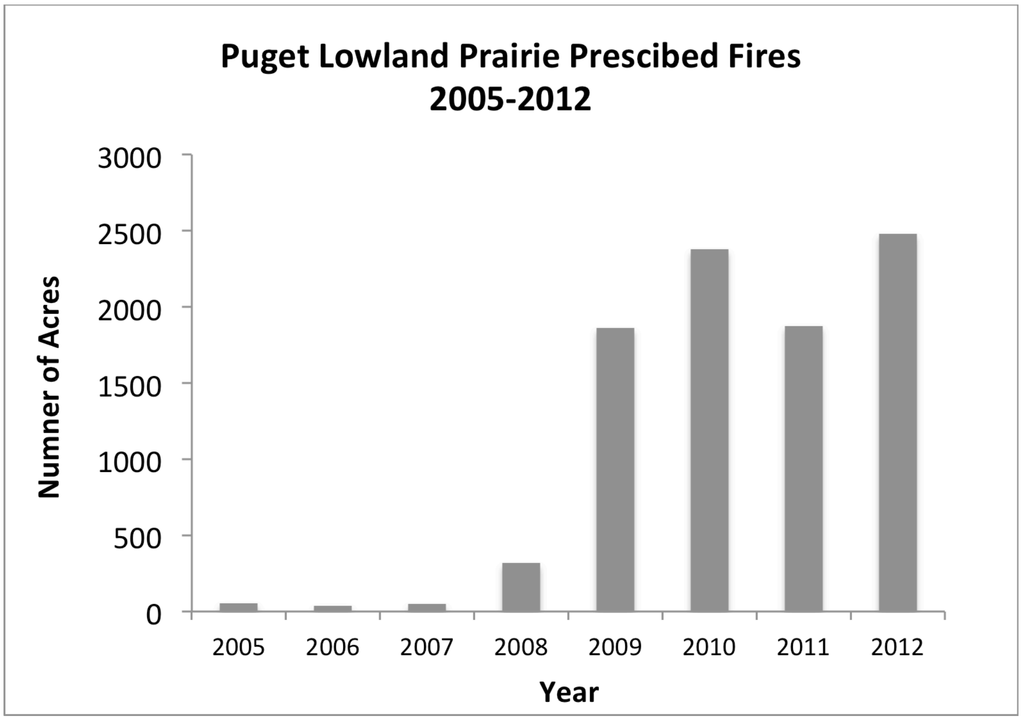

Figure 1.3. Puget Lowland Prairie prescribed fires from 2005 to 2012. In addition to the acreage of fires increasing from 55 to 2,500 in that time frame, the number of fires conducted also increased from 2 in 2005 to more than 50 by 2012 (McKinley 2013, unpublished data).

Chapter 2

Direct Seeding in Abandoned Agricultural Lands to Recover a Rare Species, Golden Paintbrush (*Castilleja levisecta*)

Castilleja levisecta (Golden paintbrush) is a federally threatened hemiparasite associated with one of the most endangered ecosystems, the Puget Lowland prairies of Western Washington. Effective recovery requires understanding how to efficiently establish large numbers of individuals at a site. Recent restoration actions have increased the amount and availability of *C. levisecta* seeds for recovery. I compared several restoration strategies in a rigorous experimental design, which included preparing sites for seeding using broadcast burning, solarization, and herbicide. Three different mixtures of 21 to 26 native forbs and grasses were sown, including *C. levisecta* at densities of 75, 110, and 150 seeds per square meter. Strategies were replicated over three years (2010, 2011, 2012) and four sites to understand when and where they were most effective. All sites were former agricultural lands and contained no native species. Results demonstrate that direct seeding is an effective establishment technique for *C. levisecta,* with establishment at all sites, and suggest that broadcast burning and repeated herbicide application are promising site preparation treatments across all sites. Additionally, this research suggests former agricultural areas and more productive soils should be considered for *C. levisecta* recovery and Puget Lowland prairie restoration. Further research should investigate whether differences in establishment relate to the presence and abundance of host plants as well as the effect of *C. levisecta* presence and density on neighboring prairie community assemblage.

Key Words: *rare species, parasitic plants, agricultural lands restoration, prairie restoration*.

Introduction

Native grasslands are one of the most imperiled ecosystems in the world and continue to face threats from development, invasive species, and woody encroachment (Ceballos et al. 2010, Carter and Blair 2012). However, many grasslands that were historically converted to agricultural use are being restored as the intensity of agricultural land use has declined over the last two decades, particularly in the United States and Europe (Goklany 2002, Prach et al. 2013). For example, in Germany, grassland restoration is the second most implemented conservation mitigation method in the country, with a focus on restoration of former agricultural lands (Conrad and Tischew 2011). In the United States, grassland restoration of former agricultural lands has been occurring for decades (Zajicek et al. 1986, Polley et al. 2005).

After extended periods of agricultural use, restoring diverse grassland plant communities requires large additions of native seed, due to seed dispersal limits, and the fact that desirable target species are usually no longer present in the seed bank (Wagner and Pywell 2011, Carter and Blair 2012). However, grassland restorations generally fail to produce the diversity of plant species found in native remnants (Dickson and Busby 2009). There are multiple reasons for this lack of success, including incorrect seed mixes (Van der Putten et al. 2000, Dickson and Busby 2009), lack of appropriate management (Warren et al. 2002), or not understanding the requirements for germination and establishment (Wagner and Pywell 2011). Additionally, grassland restorations often do not match native remnants because rare or conservative species are excluded from the seed mix (Polley et al. 2005).

In general, rare species are poorly recruited from the seedbank or disperse themselves very poorly (Pegtel 1998). Therefore, it is logical to include them in a seed restoration mix. However, restoration practitioners may be hesistant to include rare species in a seed mix, as broadcasting seeds could be perceived as a waste of seeds (Hedberg and Kotowski 2010). Additionally, knowledge of rare species biology and ecology, which can inform site selection, may be lacking (Pegtel 1998). There is increasing evidence, however, of the importance of including as diverse a seed mix as feasible, including latter-successional species or rarer species, early in restoration efforts to ensure success (Kardol et al. 2008, Dickson and Busby 2009, Prach et al. 2013). It is within this context of restoring abandoned agricultural land, or severely degraded grassland, with a diverse, native mix of species, that I explore techniques to establish *Castilleja levisecta* (Golden paintbrush), a rare hemiparasite endemic to prairies in the Pacific Northwest of the United States.

In this study, as part of the Prairie Habitat Restoration Project (PHRP), I replicated direct seeding and site preparation treatments with a diverse, native grassland seed mix including *C. levisecta* over two years and four sites, to examine three questions regarding its establishment and survival. First, which sites and site preparation treatments were most effective in establishing the species, and in which years? Second, which factors such as site, seeding mix, or site treatment affected the population trend? Finally, which factors affected flowering and therefore reproduction of the species?

Methods

Study species

Castilleja levisecta (Orobanchaceae) is a short-lived (ca. 5-7 years) hemiparasitic perennial. In the wild, mature plants typically have up to a dozen (occasionally more) flowering stems, which bloom from late April to early July. Each stem has 5-10 capsules, which begin to ripen in August, each bearing 100+ minute seeds. The species has been listed as threatened by the U.S. Fish and Wildlife Service (2000), and is restricted to twelve remaining populations in the San Juan Archipelago and the Puget Lowland. Extant populations are largely found on sandy, well drained soils of glacial origin (Chappell and Caplow 2004, Kaye and Lawrence 2008). A recovery goal for the species calls for 20 populations of at least 1,000 flowering individuals by 2018 (U.S. Fish and Wildlife Service 2000). To help achieve this goal, a reintroduction plan has been written that calls for the establishment of new populations within its historical range, and the augmentation of existing populations, many of which are quite small (Caplow 2004). The recovery plan does not recommend using seeds as founders for a recovery population, likely due to the limited seed availability at the time the plan was written.

Study Sites

My study was installed in four Puget Lowland prairies. Glacial Heritage and West Rocky Wildlife Area are sites in South Puget Sound, while Smith Prairie and Ebey's Landing Preserve are on Whidbey Island (Figure 2.1). The 490 ha Black River-Mima Prairie-Glacial Heritage Preserve (Glacial Heritage) is one of the largest protected grasslands in western Washington. Glacial Heritage is owned by Thurston County and Washington Department of Fish and Wildlife (WDFW) and managed by the Center for

Natural Lands Management. Approximately 12 km east of Glacial Heritage, the 135 ha West Rocky Wildlife Area is owned and managed by WDFW. Smith Prairie is the largest protected grasslands (70 ha) on Whidbey Island and is owned and managed by Pacific Rim Institute. Approximately 10 kilometers to the northwest, the 20 ha Ebey's Landing Preserve is owned and managed by The Nature Conservancy.

Although native or semi-native prairie covers portions of each of these sites, the research area within each site is located where cultivation for agriculture occurred for many years and no native species were present or detected when the study began. To prepare each site prior to initiation of the restoration experiments, I applied a non-selective herbicide (glyphosate) to kill all existing vegetation. This began the process of reducing weed abundance throughout each site, and produced a relatively uniform set of conditions in which to install the experimental treatments.

Experimental Design

The PHRP experimental design consisted of seven combinations of site preparation and seed mix (Table 2.1) randomly assigned to 35 treatment plots at each site. Treatments included combinations of 3 site preparation methods and 3 seeding mixes. The plots were approximately 40 meters square in South Sound and 25 meters square in North Sound, with each plot separated by 2-meter aisles and borders. Smaller arrays were established in North Sound sites due to limited seed availability. Cumulatively, each array occupies approximately 0.26 ha in South Sound and 0.19 ha in North Sound.

Plots assigned to the broadcast burn treatment were burned in the summer prior to seeding. The solarization treatment consisted of plowing and roto-tilling the soil and installing a 2mm clear plastic in June, which remained in place until September. Plots with the 2-year herbicide treatment were sprayed as needed (never allowing any species to set seed that germinated) for 2 seasons before seeding, and these plots were seeded one year after the other plots within the respective array.

Seeding occurred in early November each year. Seeding was done with a broadcast seeder. The ground was harrowed before seeding and raked after seeding on all plots. Seed mix treatments each contained 21-26 native forbs and grasses and approximately 700 seeds per square meter. Three grass:forb mixes were tested with ratios (based on number of seeds) of 50:50 (grass rich), 25:75 (mixed), and 2:98 (forb rich). These different ratios were chosen to determine which was most effective in establishing important host and nectar sources for a suite of rare butterfly species in the region. Some studies have shown grass rich mixes effective in suppressing weed and non-native species (Török *et al.* 2010), while others advocate for higher density of forb species for successful restoration (Dickson and Busby 2009). Additionally, I was interested to explore the effect of different seed mix ratios of *C. levisecta* performance. *C. levisecta* was sown at a rate of approximately 75 (grass-rich), 110 (mixed), and 150 (forb-rich) seeds per meter square in each of the seed mixes respectively.

Each of the seven seeding and site-preparation treatments was replicated on five plots within each array. I established arrays at four sites to compare the effectiveness of the same treatments among sites. Finally, I was particularly interested in understanding the degree to which interannual variation affected treatment outcomes as this has been found to be a significant factor in many grassland restoration projects (Young et al. 2005, Sheley and Half 2006). Therefore, I also incorporated temporal replication into the design by repeating the same experimental treatments on separate arrays over three years at each South Sound site, beginning in 2009, and over two years at each North Sound site, beginning in 2010, for a total of ten arrays at the four sites. I omitted the 2009 arrays from this analysis, as I was limited on *C. levisecta* seed in 2009. Therefore I analyzed two arrays at each site in South Sound and two arrays at each site in North Sound for a total of 8 arrays at four sites.

Finally, the PHRP design also included a scaling component that we have termed staged-scale restoration (see Chapter 3). At each site, treatments that are most effective were applied in plots that increased in area by factors of 10 and 100 over the first several years. I did not incorporate these scaled plots into the analysis of establishment, survival, or flowering. However, I calculated the total number of *C. levisecta* plants established at each site in both the arrays and in the scaled plots.

Data Collection

Data were collected each spring for three years from 2010 to 2012. In one meter square permanent quadrats, I measured the density of all *C. levisecta* plants by counting the number of individuals. South Sound plots had 6 quadrats and North Sound plots had four quadrats, which sampled approximately 15% of the plot area. In the spring of 2011 and 2012 I also counted the number of flowering and vegetative plants within each quadrat and the number of flowering stems per plant. In 2012, all of the plots within experimental arrays at all sites were censused for *C. levisecta*. For the censused plots, I recorded the number of flowering plants and the number of flowering stems per plant. I did not count vegetative plants, or plants not flowering in the census.

Statistical Analyses

I used a negative binomial with log link generalized linear model (GLM) to test for effects of treatment, site, and year on the proportion of *C. levisecta* seed that established in year one. To use this GLM, data were transformed by multiplying initial results by 10,000. For analyses of population trend of *C. levisecta* plants by site and treatments after establishment in year one, I used a negative binomial with log link GLM. I used a Poisson loglinear GLM to test for differences in the percentage of plants that flowered and the number of flowering stems per plant. A least significant difference test was used for pairwise multiple comparisons. All analyses were conducted using SPSS Statistics 19 (IBM 2012).

Results

A summary of the effects of site and site preparation and interaction between these terms is found in Table 2.2. A summary of the effects of seeding treatment and site preparation treatments and interaction between these terms if found in Table 2.3

Establishment

For establishment, I was interested to know, in the context of the PHRP, which sites and site preparation treatments (burn, solarize, herbicide) were most effective in establishing the *C. levisecta*. I only looked at the effect of physical treatments and sites and assumed seeding treatments would have no effect in the first year, as all sown species should have an equal chance of establishment regardless of other species in the mix. The establishment results include data from the first year of each array, as I was interested to know treatment effects from the seed I sowed initially. After the first year, I was unable to determine if plants that established were from delayed germination from my initial sowing or from recruitment of established flowering plants.

The percentage of sown *C. levisecta* seed that established, averaged across all four sites, showed a significant difference between all treatments. Burning was most effective with more than 3.0% established, followed by the herbicide treatment (2.0%), and the solarize treatment (1.3%) (Figure 2.2). However, there were strong regional differences in the site preparation effects, which are masked averaging across all sites. Separating the North Sound and South Sound sites shows that burning was the most effective technique for *C. levisecta* at all sites (3.5% and 3% at North and South sound sites). The herbicide treatment was the least effective in North Sound (1.0%), while in South Sound it was similar to the burn treatment (2.8%). Finally, the solarize treatment was as effective as burning in North Sound (3.0%), while in South Sound it was particularly ineffective (<0.01%) (Figure 2.3).

In addition to the strong regional differences, there were strong site differences on *C. levisecta* establishment. Ebey's Landing Preserve in North Sound had the highest establishment (4.5%), followed by Glacial Heritage in South Sound (2.8%). Smith Prairie and West Rocky Preserve had no difference in establishment (1.0%) (Figure 2.4). There was no difference in the proportion of *C. levisecta* seeds that established in the three seed mix treatments of grass-rich, mix, and forb-rich.

Finally, there was significant variability in establishment based on the seeding year. There was more than 4% establishment overall in 2010, followed by less than 1% establishment in 2011 and slighlty more than 2% in 2012 (Figure 2.5). This annual variability was consistent across all sites and treatments. There was no interaction with site preparation treatment, though there was significant interaction with site (data not shown).

Population Trend

For this metric, I was interested to know which factors (site, seed mix treatment, or site treatment) affected the trend of the population, after establishment in the first year. I was not able to conduct an analysis of survival on plants that established in the first year, as I was not tracking individual plants, but rather the averaged number of plants within plots. Therefore I looked at the trend of the population, or the establishment in year three compared to year one, for different treatments, which should include delayed germination of sown seed and recruitment from plants established in the first year that produced seed. As I was looking at the trend from year one to year three, I only analyzed data from the 2010 arrays, where I had data for three years.

In comparing the different sites, the population trend at all sites was positive from year one to year three. At Ebey's Landing Preserve the population decreased between year one and year two, however this was offset by more establishment from years two to three. Similarly establishment across all site treatments also increased with plot age. For example, the percent of sown *C. levisecta* seed that established with the burn treatment increased by 4% from the first year to the third year across all sites. *C. levisecta* survival did decrease between years one and two in the solarize treatment, howeverby year three, establishment was greater than in year one.

As with establishment in year one, there were significant differences in the overall establishment between both site and treatment by year three. Interstingly, the regional differences present in year one, are not apparent in the percent *C. levisecta* seed that has established by year three. The burn treatment remained the most effective (6.0%) at all sites except West Rocky. Herbicide was the most effective treatment at West Rocky and was overall the second most effective treatment (5.0%) at all other sites by year three. The solarize treatment was significantly less effective than the other treatments at all sites (1.3%) (2. 6). In terms of site differences, Glacial Heritage had the highest year three establishment (7.8%), followed by Ebey's Landing (5.0%), Smith Prairie (2.3%) and West Rocky (1.6%) (Figure 2.7). There was no effect of the seed mix treatment on establishment by year three.

Flowering

For flowering, I was interested in which factors affected flower productionas well as the number of flowering stems per plant. In fact, there were no effects of site, seed mix treatment, or site preparation on flowering. The percentage of plants that established and then flowered averaged 66%. There were significant differences in the number of flowering stems per plant, which is an indication of the overall size and vigor of the plant. Glacial Heritage had significantly more flowering stems per plant than the other three sites (Figure 2.8).

Discussion

A primary objective of this study was to determine if direct seeding of *C. levisecta* in severely degraded habitats was an effective technique. I conducted this research with the expectation that I would begin to establish recovery populations of *C. levisecta* at each site. This project implemented the first study of direct seeding *C. levisecta* within a diverse prairie seed mix. It also represented the first large scale (larger than experimental plots) direct seeding of the species. I have found that seeding is an effective technique to establish *C. levisecta* (Figure 2.9). Table 2.4 details the estimated number of plants that established and that were flowering in 2012 at each site. More than 100,000 *C. levisecta* plants have been established at all sites combined over the course of four years. Additionally, more than 75,000 flowering plants were estimated to be present at all sites in 2012. This project has established *C. levisecta* in greater numbers than exist in any of the known wild and introduced populations, and now represents a significant portion of the population. For example, each year the Washington Department of Natural Resources (WDNR) conducts a census of all flowering plants, in which each flowering plant at all of the existing wild and introduced sites is counted. In 2012 the WDNR census found just over 23,000 flowering *C. levisecta* plants across all Washington, Oregon, and British Columbia populations (unpublished data WDNR). The estimated *C. levisecta* flowering plants in the PHRP included more than three times this amount.

The census data that I collected at each experimental array was helpful to ensure that the population and flowering estimations made from scaling the quadrat data do not over estimate the number of *C. levisecta* at any site or within any treatment. By conducting both the quadrat and the census monitoring on the same plots I was able to compare the two and adjust appropriately for estimations of areas that I did not census. The census data were consistently lower by an average of 15-30% than the estimations from the quadrat data, meaning that I always recorded fewer flowering *C. levisecta* plants when I counted each one in a plot compared to when I estimated based on the quadrat data. This difference could be due to a clumped distribution pattern of the plant within the plots. However, given that the quadrat estimation always over-estimated the number of plants and never under estimated, this seems less likely. It may be more likely that plants were simply missed when counting for the census, as the plots are large and it is easy to miss plants or count individuals as one plant. Regardless, I have used a conservative multiplier to the quadrat estimations of 0.7, which accounts for the largest percentage that the census data and quadrat data differed in any plots, to ensure I have not over-estimated.

Site preparation treatments are an important factor in the establishment of *C. levisecta*. Despite the strong regional differences in the first year establishment, overall the burning and repeated herbicide treatments were the most consistently effective site preparation treatments, particularly when considering the net establishment by year three (Figure 2.6). Though the solarization treatment was effective in year one in the North Sound plots, it was not in other years or at other sites. The solarization treatment leaves very little cover on the ground to shelter seedlings emerging in the fall and winter. Winters were generally colder in South Sound, where the sites are further from the influences of Puget Sound. I suspect that this lack of cover contributes to seedling mortality, due to frost heaving and predation during the establishment process. I suspect that this lack of cover contributes to seedling mortality during the establishment process. Both the burning and the herbicide treatments however, leave substantial litter and cover on the ground, which may shelter seedlings in the winter months. In considering the *C. levisecta* establishment in the context of the PHRP and the other 21 to 26 species it was sown with, it was not uncommon for some species to perform consistently better with burn or herbicide treatments while others did better with solarization. It appears that *C. levisecta* does not consistently establish well with the solarization treatment.

The positive population trend of *C. levisecta* from year one to year three across all sites and treatments was surprising. I expected to see a drop in the average number of plants per plot, particularly after year two, due to interspecific competition and seedling mortality. I believe the continued establishment of *C. levisecta* after year one is due to two factors. The first is delayed germination of the seed of *C. levisecta* originally sown. Seed dormancy is a well understood phenomenon in plant ecology (Cohen 1966, Rees 1994) and something I have witnessed with *C. levisecta* when propagating in the greenhouse. My monitoring did not track individual seedlings, but rather the total number of plants within quadrats, which were averaged over each plot. It is likely that some seedlings died, however additional seedlings emerged in year two from seeds sown in year one. The second likely contribution to the increased establishment was recruitment from flowering plants that established in year one. *C. levisecta* flowered and set seed in all treatments where it established, and seeds from these plants likely contributed to additional recruitment. The average number of flowering stems per square meter was between 4 and 12 in year one, depending on the site and treatment. I did not count the number of seed capsules produced per plant, however a conservative estimate would be three per flowering stem, which would result in approximately 1,800 to 5,400 seeds assuming 150 seeds per capsule. This is substantially more seed on a per m^2 basis than I originally sowed.

Surprisingly, the percentage of flowering *C. levisecta* plants or the number of flowering stems per plant did not differ by any of the site preparation or seeding treatments. I expected that by year three, the relative density of different potential host species present in the different seed mixes would manifest with differences in flowering or in the number of flowering stems per plant. Research suggests that parasites like *C. levisecta* that exploit many different hosts do not use them randomly; instead, some hosts may confer significantly greater benefits to the parasite than others (Kelly et al. 1988; Marvier & Smith 1997). I have also documented significant differences in *C. levisecta* survival and seed production based on different hosts (Chapter 3). One of the reasons I likely did not see treatment differences in C. levisecta flowering could be that I used the metric of flowering stems per plant. *C. levisecta* produces flowers on a raceme. By just counting the number of flowering stems instead of counting the number of flowers per raceme or measuring the length of the raceme, I was not able to differentiate between plants that had just a few flowers per raceme versus those with many more. It is also possible that all of the seed mixes had a minimum number of potential host species for the number of *C. levisecta* plants that established and there really were no differences in number of flowering stems per plant. Finally, it could be that not enough time elapsed to observe differences in the effect of host species' presence on *C. levisecta*. There were however, differences in the number of flowering stems per plant based on site, with Glacial Heritage having more than other sites. The high levels of herbivory, particularly by deer browse of flowering stems in the North Sound sites, may explain this significant finding.

Herbivory is one of the factors to consider when using the metric of flowering for *C. levisecta* and perhaps other species. In the North Sound there was significant browsing by both voles and deer, especially at Ebey's Landing. I think the lower percentage of flowering plants at Ebey's is likely due to excessive browse (Table 2.2). Other researchers have noted this issue with *C. levisecta* (Kaye and Lawrence 2008). I documented extensive herbivory by voles in plots with high amounts of cover. Burning and reducing cover is an effective technique for reducing vole browse, as voles generally do not like open ground (Pusenius and Ostfeld 2012). Fencing is currently being used in restoration work in the North Sound to control deer browse.

In addition to flowering, there was also no difference in the proportion of *C. levisecta* seed that established between the three seed mix treatments of grass-rich, mixed, and forb-rich. Approximately 75, 110, and 150 *C. levisecta* seeds were sown in each of these treatments, respectively. An average of just more than one *C. levisecta* plants established in the grass-rich seed mix treatment, compared to slightly less than two in the mixed treatment, and slightly more than three plants in the forb-rich treatment. This suggests that I did not reach seed density saturation and additional *C. levisecta* plants could have been established with a higher density seeding than 150 seeds per square meter, depending on the desired number of plants. In previous seeding studies of *C. levisecta* more than 1,000 seeds per square meter were sown (Dunwiddie, 2012 unpublished data) within an extant prairie site, with lower establishment than I observed in my study. It is interesting that the hemiparasite was able to establish well when seeded simultaneously with its prospective hosts, as opposed to being sown within existing adult host species. The presence of hosts do not appear to be a critical need for seedling establishment, and site suitability may be determined more by edaphic factors and micro-site suitability than presence of host species.

Despite the success of seeding *C. levisecta*, one of the important observations is that the overall establishment rate is generally low, even in the most successful treatments. There has been little research tracking the percent establishment of grassland species in a restoration context, however, an ongoing study in Puget Lowland Prairies has documented extremely low 2nd year establishment for *Festuca roemeri* (1-5%), *Eriophyllum lanatum* (5-15%) *and Potentilla gracilis* (1-3%), three common prairies species frequently used in restoration (unpublished data S. Hamman). Hillhouse and Zedler (2011) recently found that field establishment rates in tallgrass prairie restoration projects were low, with grasses more likely to establish than forbs. The field establishment rate that Hillhouse and Zedler found for 14 forbs and grasses was just over 6%, but was less than 1% for many of the species, despite many of the species having germination rates in the greenhouse over 80% (Hillhouse and Zedler 2011). These results support my findings for establishment rates for *C. levisecta*, which ranged from less than 1% to 4% for some treatments in the first year. The establishment rate for all species sown in the PHRP seed mix was also low, with the average for any year for all sown species below 10%. An important lesson from this research project is to plan for low establishment rates in a field restoration setting, even when a species may have high viability or germination rates.

In addition to low establishment rates, the difference in establishment rate between years was remarkable. In 2010 more than 4% of the *C. levisecta* seeds established compared to less than 1% in 2011. Interannual variation in uncontrolled field conditions is a well known phenomenon in restoration experiments (Vaughn and Young 2010). For example, Bakker et al. (2003) found that the conclusions they made from indentical treatments of grassland restoration techniques made over three different years were contingent on the year of the experimental initiation. Importantly in my case however, the variability was consistent across all the sites and treatments; 2010 was a “better” year for establishment than 2011. It could have also been an issue with seed quality in 2011, though this too would be a manifestation of annual variability. There was interaction between year and site, likely due to the significant drop in effectiveness of solarization at Ebey’s Landing in 2011, compared to 2010. Regardless, interannual variation should be a consideration for sowing rates of *C. levisecta*, and a consistent establishment rate is unlikely and managers should instead plan for a range of establishment rates that will include rates below 1% in some years.

Importance of Site Characteristics

The most significant factor in determining the establishment of *C. levisecta* was the characteristics of the individual sites. In the North Sound, significantly more plants established at Ebey’s Landing compared to Smith Prairie, while in the South Sound, many more plants established at Glacial Heritage compared to West Rocky. Variability of results when working spatially across many sites is a common occurrence in restoration research. For example, in an extensive grassland restoration research project across five European abandoned arable land sites, Van der Putten et. al (2000) also found that local site conditions and how species identities interact with those conditions affected the relationship between the diversity of communities and their susceptibility to invasions. While highly variable results is often an outcome of spatially replicated experiments, it actually highlights the importance of implementing spatial replication; to ensure the scale of results is correctly understood.

One of the common site characteristics of the PHRP is that all of the sites were habitats I refer to as severely degraded, or abandoned agricultural areas. My results highlight the importance of these types of lands for *C. levisecta* recovery, as well as for restoration of other Puget Lowland prairie species. The PHRP is the first attempt in Puget lowland prairie restoration where former agricultural lands were directly restored to native grassland habitat. Previous restoration work in this region has focused only on extant prairie sites. My results demonstrate that the restoration of severely degraded habitats should be considered as part of the recovery strategy for *C. levisecta* and as part of the overall conservation of the Puget Lowland Prairie system.

Edaphic factors may explain the major differences between the PHRP sites and also point to site considerations for future restoration. In comparing the four sites of the PHRP, an interesting result is the relative success of the two sites with more productive agricultural soils. Glacial Heritage and Ebey's Landing had *C. levisecta* establishment rates of 8% and 5% respectively by year three, while Smith Prairie and West Rocky had establishment rates closer to 2% (Figure 2.7). In South Sound, the Glacial Heritage site is characterized by Nisqually loamy fine sand soil type while the West Rocky site is a Spanaway-Nisqually complex soil (NRCS online database). The Spanaway-Nisqually complex soil is one of the most typical soil types for the remaining extant prairies and often includes Mima Mound features. The Nisqually loamy fine sand is uncommon in extant sites that retain native prairie vegetation because the majority of sites with these soils are currently in agricultural production, or were for many decades. Similarly in North Sound, while both sites are classified as a San Juan sandy loam, the Smith Prairie site has substantially more gravel and larger rocks in the upper soil profile and holds less moisture than the Ebey's Landing site. Historic or adjacent land use of the two sites illustrates the relative difference in productivity of the soils. The Smith Prairie site was used for forage and as a game farm for many decades, while the Ebey's Landing site is located in one of the most productive seed growing areas in Washington State, and is surrounded by cultivated fields.

The restoration of formerly productive agricultural sites to native grasslands is a well established practice in both Europe and the midwest United States and lessons learned in these systems can inform restoration of former agricultural lands in Puget Lowland Prairies. One of the overarching lessons learned is that one of the most seriously limiting factors for restoring native grasslands is seed disperal limitation (Donath et al. 2003). High soil fertility is also considered a limiting factor after the cessation of agricultural practices, as the competitiveness of some early successional species in a nitrogen-rich environment can affect plant community succession (McLendon and Redente 1992). However, recent research in grassland restoration suggests that seed addition outweighs soil fertility reduction (Kardol *et al.* 2008) and contributes to reduced exotic species cover (Carter and Blair 2012). My research has demonstrated that these kinds of lands are appropriate for seeding for *C. levisecta*. Based on my results and the experience of others restoring former agricultural areas around the world, I would focus on having a diverse and appropriately dense seed mix to recover *C. levisecta* on these abandoned agricultural lands or lands with more productive soils.

Conclusion

In this study, I replicated direct seeding of *Castilleja levisecta* over 3 years and 4 sites on severely degraded habitat to understand factors affecting its establishment, survival, and flowering. I found that site preparation treatments affected establishment but not flowering rates. Burning and repeated herbicide treatments were the most effective site preparation techniques for *C. levisecta* establishment. I successfully established *C. levisecta* on all sites totaling more than 100,000 plants within 3 years. These results demonstrate that severely degraded habitats such as abandoned agricultural areas are appropriate sites to consider for the recovery of this endangered species.

Establishment rates for Puget Lowland Prairie species sown with *C. levisecta* as part of this experiment were generally quite low (less than 5%). For *C. levisecta*, rates were less than 1% in some treatment combinations and never higher than 5% in the most favorable conditions. Additionally, spatial and temporal variability affected the efficacy of the tested treatments. Land managers should consider sowing high densities of seed mixes and should account for interannual variability at their sites when restoring grassland systems such as in the Puget Lowland Prairies. It may also be possible to alter sowing methods such as with the use of a seed drill to improve seedling establishment.

Future research should seek to understand the effect of the presence and abundance of different host plant species on *C. levisecta* performance. Conversely, understanding the effect of *C. levisecta* on the neighboring community is equally important. Recent research into the use of *Rhinanthus minor*, an annual hemi-parasitic plant, to maintain diversity in European grasslands (Pywell et al. 2004, Westbury and Davies 2006), points to the importance of understanding that relationship. Another potential opportunity to recover *C. levisecta* while achieving other conservation outcomes pertains to interactions with a rare butterfly, Taylor's checkerspot (*Euphydryas editha taylori*), which uses *C. levisecta* as a host. As larger areas of *C. levisecta* are sown, an ideal platform is provided to study interactions with Taylor's checkerspot and explore how the two species might be recovered synergistically. Finally, spatial analysis of the availability and context of severely degraded lands or lands with more productive soils for restoration of *C. levisecta* and other Puget Lowland Prairie species should be conducted to better understand the restoration and conservation opportunities.

Implications for Practice

- Direct seeding of *Castilleja levisecta* on severely degraded habitats is an effective recovery strategy for this endangered species.
- Prescribed fire treatment was consistently the most effective site preparation treatment across all sites and years for *C. levisecta* establishment.
- Establishment rates of *C. levisecta* and other prairie species in sown mixes is generally quite low, and land managers should plan for establishment rates below 5%.
- *C. levisecta* established most successfully on sites with more productive soils.
- Puget Sound lowland prairie restoration practitioners should consider former agricultural lands and lands with more productive soils for the restoration of the *C. levisecta* and Puget Lowland Prairies more generally.

Tables and Figures for Chapter 2

Table 2.1. Site preparation and seeding treatments tested. Plots assigned to the broadcast burn treatment were burned in the summer prior to seeding. The solarization treatment consisted of plowing and roto-tilling the soil and installing a 2mm clear plastic in June, which remained in place until September. Plots with the 2-year herbicide treatment were sprayed as needed for 2 seasons before seeding. Three grass/forb mixes with 21 species in North Sound and 26 species in South Sound were tested with ratios of 50:50 (grass rich), 25:75 (mixed) and 2:98 (forb rich) (See appendix for complete seeding list). Approximately 75, 110, and 150 *C. levisecta* seeds were sown in each mix respectively.

Site Preparation	Seed Mix
B (broadcast burn)	G (grass-rich)
B (broadcast burn)	M (mixed)
B (broadcast burn)	F (forb-rich)
S (solarized)	G (grass-rich)
S (solarized)	M (mixed)
S (solarized)	F (forb-rich)
H (2-year herbicide treatment)	F (forb-rich)

Table 2.2. Effects of Site, Site Preparation, and Site x Site Preparation on *C. levisecta* establishment in year one and in year three. Terms were tested in four experimental arrays and evaluated one or three years after sowing. Significant differences indicated in bold.

Response	Site			Site Prep		Site x Site Prep	
	N	Statistic[a]	*P*-value	Statistic[a]	*P*-value	Statistic[a]	*P*-value
North Sound							
Year 1 Establishment	140	28.60	**.000**	12.66	**.000**	3.12	.209
South Sound							
Year 1 Establishment	140	59.15	**.000**	626.46	**.000**	28.91	**.000**
All Sites							
Year 3 Establishment	140	27.25	**.000**	62.08	**.000**	38.44	**.000**

[a] Test statistic is Wald chi-square for survival (analyzed with negative binomial with log link GLM)

Table 2.3. Effects of Seeding, Site Prep, and Seeding x Site Prep on *C. levisecta* flowering in years two and three. Terms were tested in four experimental arrays and averaged from years two and three years after sowing.

Response	Seeding			Site Prep		Seeding x Site Prep	
	N	Statistic[a]	*P*-value	Statistic[a]	*P*-value	Statistic[a]	*P*-value
Proportion Flowering	123	.234	.890	.695	.706	.259	.878
Number of Flowering Stems Per Plant	123	.073	.964	2.867	.239	.045	.978

[a] Test statistic is Wald chi-square for survival (analyzed with Poisson loglinear GLM)

Table 2.4. *C. levisecta* population and flowering estimates for 2012 at all sites. Estimations were made from data collected in quadrats and then scaled to match area sown at each site. Initial quadrat estimations were compared with plot census data and include a conservative multiplier (0.7) to ensure against over-estimations. The two shaded cells were not measured and are estimated based on observed data from other plots on the sites. Glacial Heritage has significantly more than other sites, as this is the only site where 100x scaled plots were sown with *C. levisecta.*

Site	Array	Plants	Flowering Plants	Percent Flowering	Flowering Stems Per plant
EL	10 x scaled-up plot	788	350	44%	5.9
	2010	1225	726	59%	4.8
	2011	185	34	18%	3.8
EL Total		**2197**	**1110**	**51%**	**4.6**
SP	10 x scaled-up plot	963	663		
	2010	609	442	73%	3.5
	2011	83	34	41%	2.1
SP Total		**1654**	**1139**	**69%**	**3.3**
GH	100 x scaled-up plot	99082	66715		
	10 x scaled-up plot	1400	747	53%	5.4
	2009	668	496	74%	11.8
	2010	4127	3115	75%	7.4
	2011	2163	1271	59%	6.8
GH Total		**107440**	**72342**	**67%**	**7.3**
WR	10 x scaled-up plot	93	93	100%	2.0
	2009	437	361	83%	4.9
	2010	1483	987	67%	5.7
	2011	2013	1442	72%	2.9
WR Total		**2874**	**1744**	**61%**	**4.7**
Grand Total		**114165**	**76335**	**67%**	**5.4**

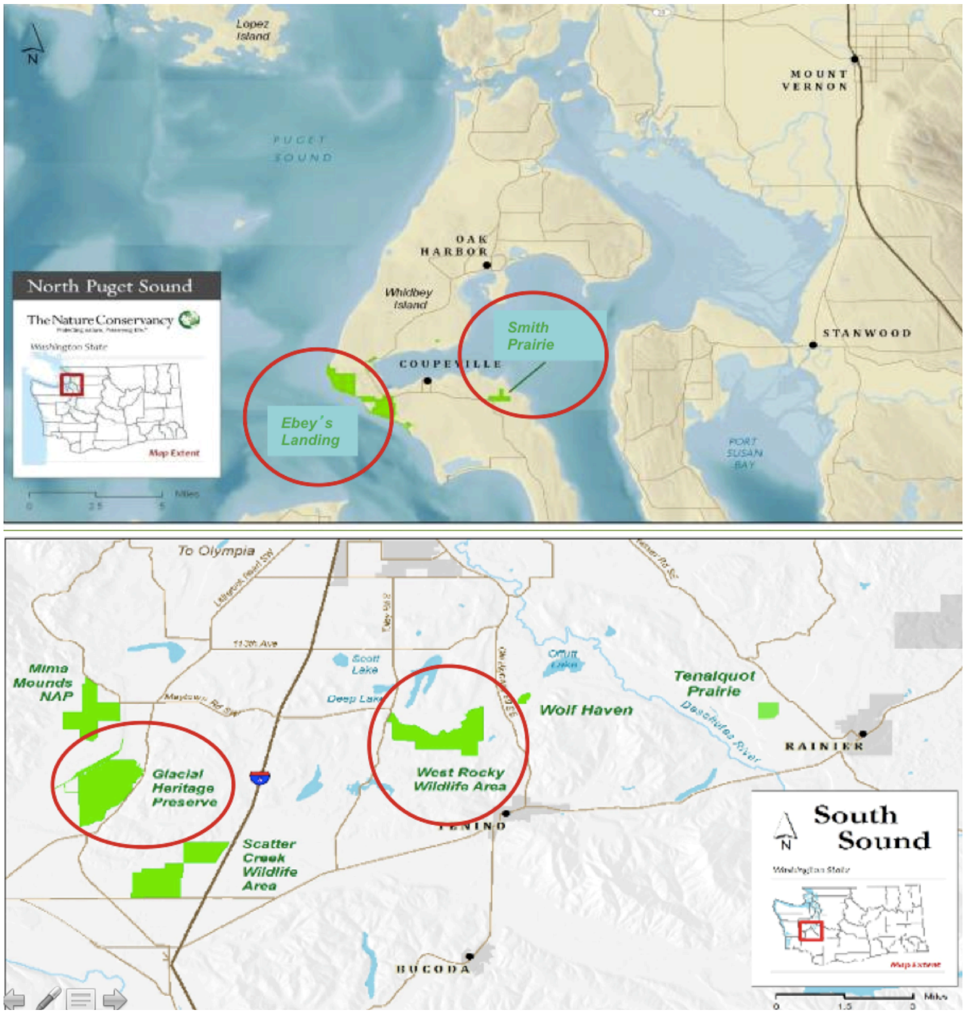

Figure 2.1. Site locations of Prairie Habitat Restoration Project. Ebey's Landing and Smith Prairie are located on Whidbey Island and Glacial Heritage Preserve and West Rocky Wildlife Area are located south of Olympia, WA. The North Sound and South Sound sites are approximately 100 miles apart.

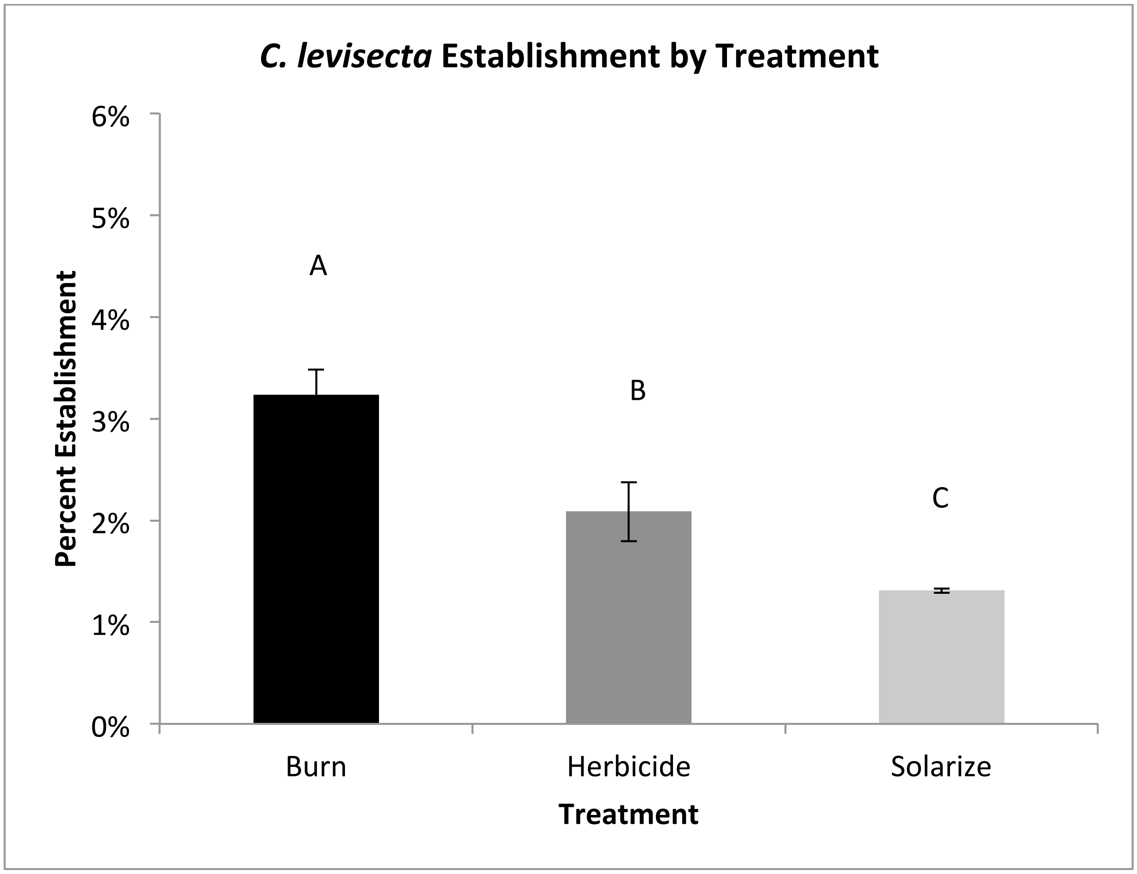

Figure 2.2. Percentage of sown *C. levisecta* that established in year one across all sites by site preparation treatments. Treatments not sharing a common letter are significantly different at P < 0.05 Error bars display one SE.

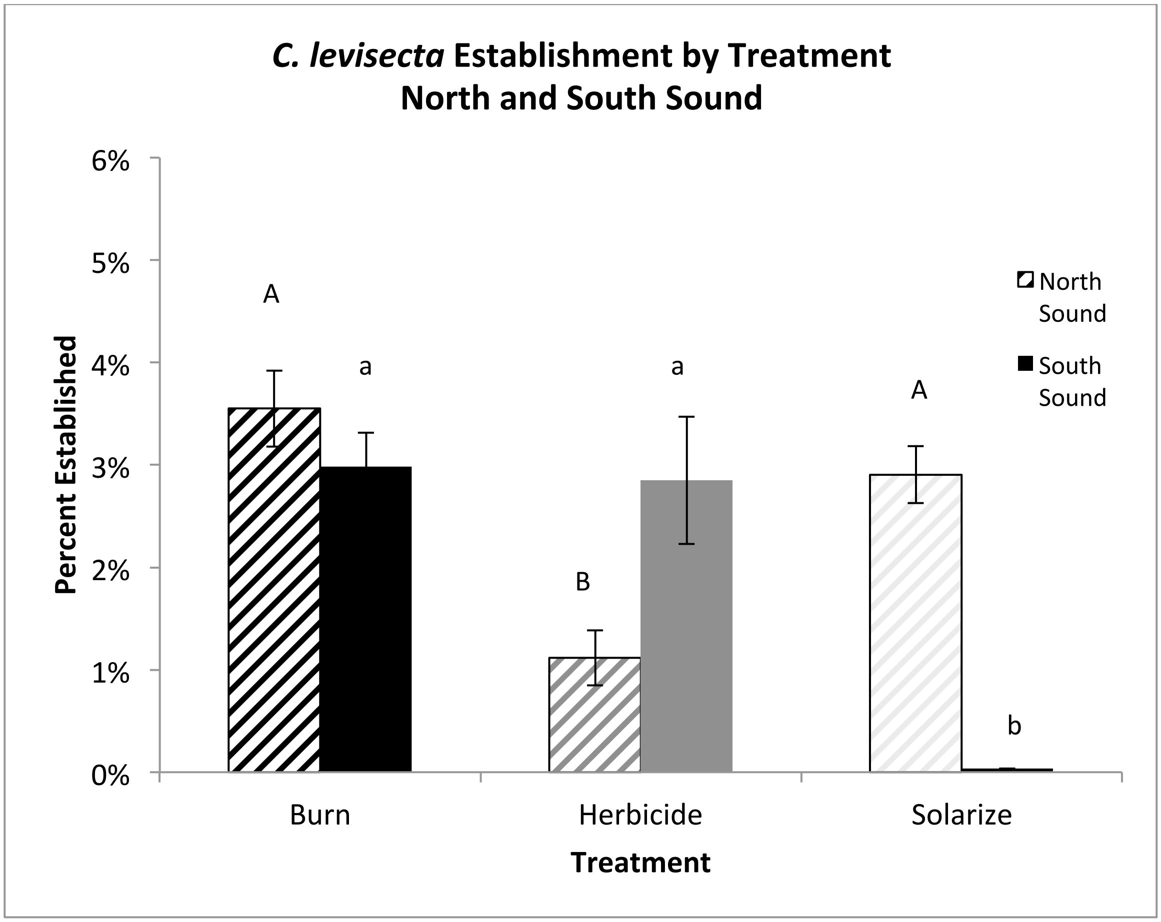

Figure 2.3. Site preparation treatment effects on *C. levisecta* establishment displayed by regions, North Sound and South Sound. Post hoc comparisons made within regions. Capital letters are for North Sound and lower case for South Sound. Treatments not sharing a common letter are significantly different at $P < 0.05$. Error bars display one SE.

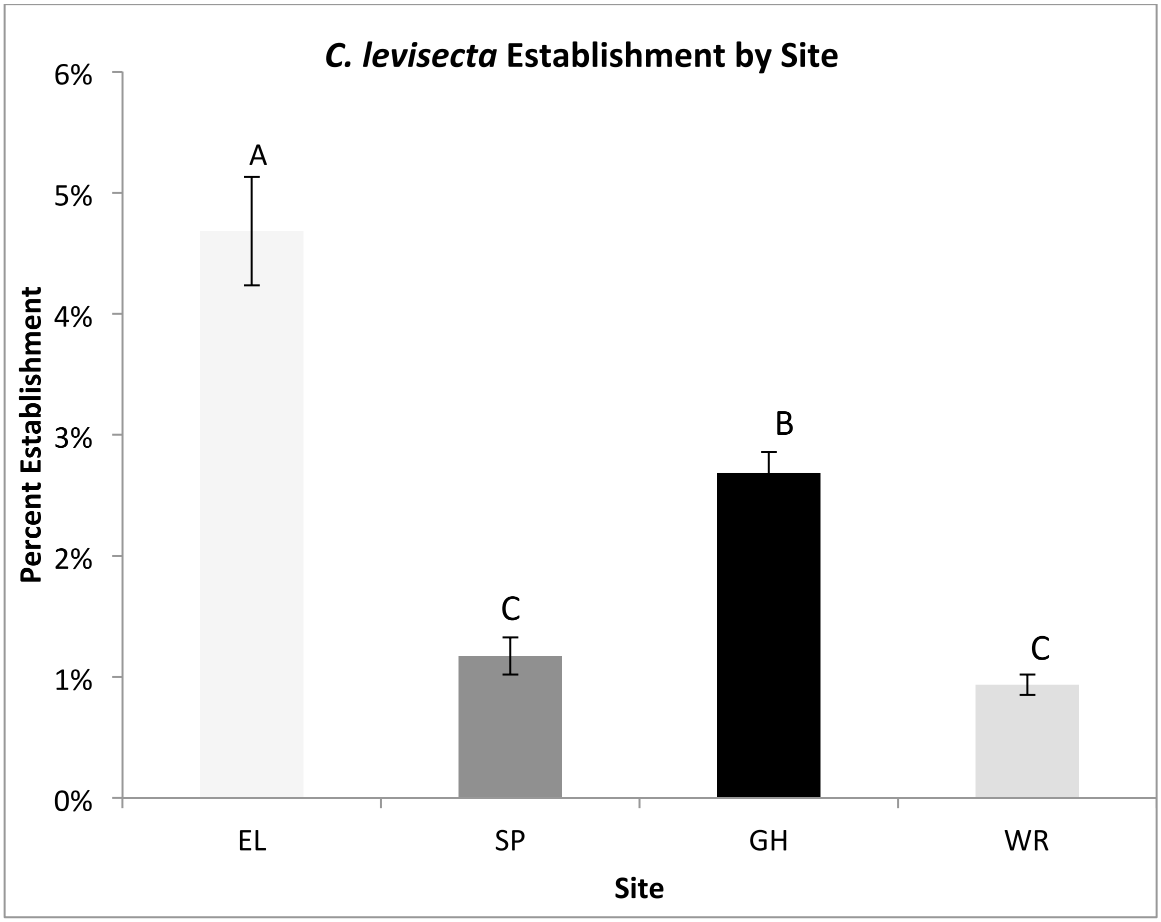

Figure 2.4. *C. levisecta* percentage establishment of sown seeds by site in year 1. Sites not sharing a common letter are significantly different from each other at $P < 0.05$. Error bars display one SE.

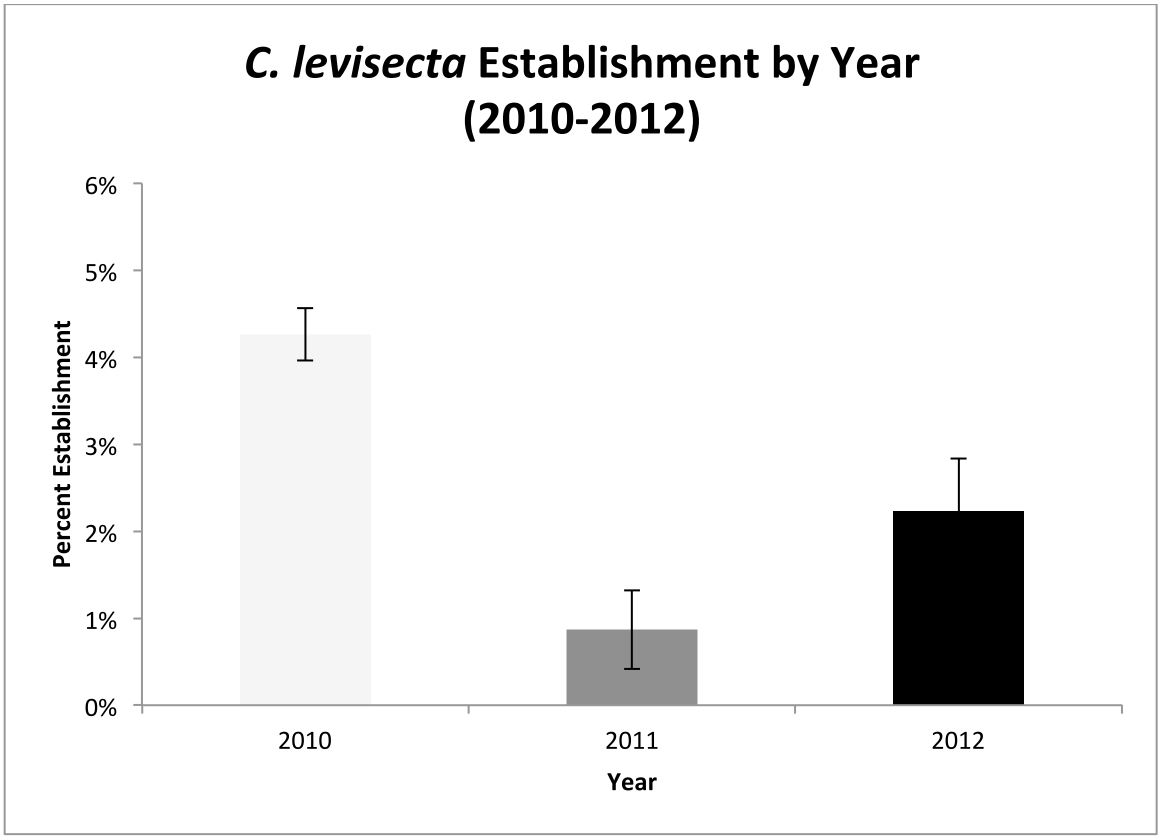

Figure 2.5. Percentage of sown *C. levisecta* that established by year across all sites and treatments. Data from 2012 only includes plots from the herbicide and forb-rich seed treatments. All years are significantly different at P < 0.05 Error bars display one SE.

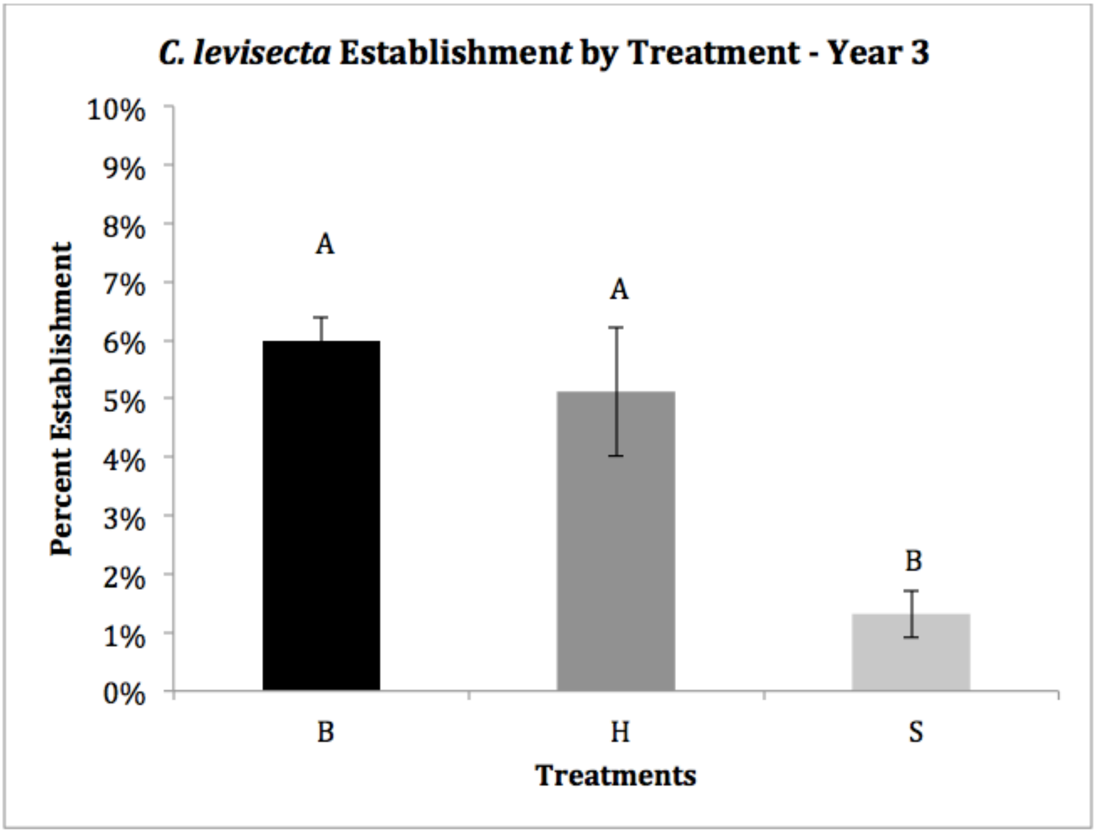

Figure 2.6. Percentage of sown *C. levisecta* that established by year 3 across all sites by site preparation treatments. Treatments not sharing a common letter are significantly different at $P < 0.05$ Error bars display one SE. Note the change in scale on the Y-axis from previous figures.

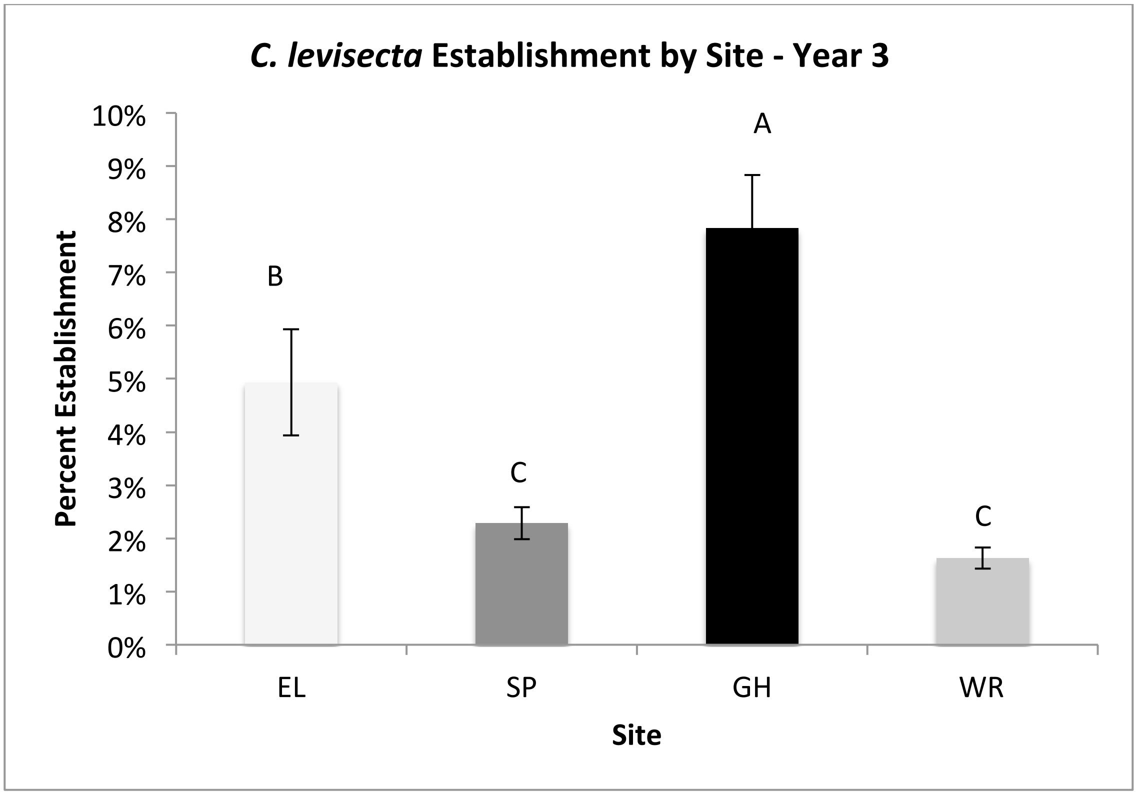

Figure 2.7. *C. levisecta* percentage establishment of sown seeds by site in year 3. Sites not sharing a common letter are significantly different from each other at $P < 0.05$. Error bars display one SE. Note the change in scale on the Y-axis from previous figures.

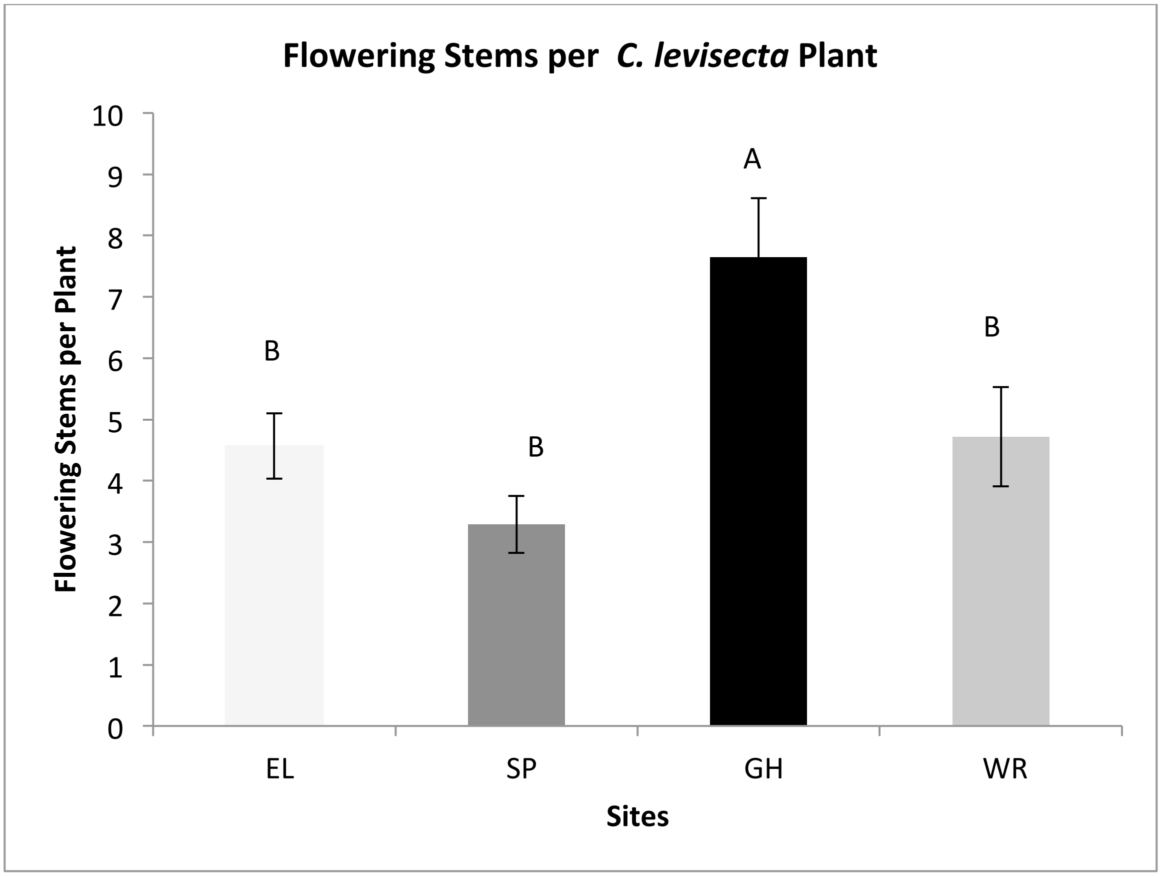

Figure 2.8. Number of flowering stems per *C. levisecta* plants by site in year 3. Sites not sharing a common letter are significantly different from each other at P < 0.05. Error bars display one SE.

Figure 2.9. *C. levisecta* established at Glacial Heritage Preserve by direct seeding with a forb-rich seed mix following prescribed burn site preparation. This plot was sown in the fall of 2009. Image taken in spring of 2013.

Chapter 3

Host Plants and Container Size Affect Success of Outplanted Golden Paintbrush (*Castilleja levisecta*)

Abstract

The establishment of parasitic plants may be limited both by their requirements and by those of their host plants. *Castilleja levisecta* (golden paintbrush) is a federally threatened hemiparasite associated with one of the most endangered ecosystems in the United States, the prairies of western Washington. Recovery of this species requires understanding how sensitive it is to presence and identity of host plants, and whether this sensitivity depends on *C. levisecta* size. I tested the effects of host plant presence and identity (none, *Festuca roemeri*, or *Eriophyllum lanatum*) and initial *C. levisecta* size (grown in 49 or 393 ml pots) on the performance of ~ 600 plants outplanted in a tilled field over two years. Performance was characterized using four metrics: survival, vigor, number of flowering stems, and seed capsule production. *C. levisecta* survival was higher in the presence of a host plant in both planting years. Importantly, host plant identity strongly affected survival and seed production in the second year after planting, with higher survival and seed production of *C. levisecta* plants with *E. lanatum* than with *F. roemeri. C. levisecta* plants grown in larger pots had consistently high survival and produced more seed, but only in year one. These results confirm that some hosts confer significantly greater benefits to the parasite than others. Further research should test which other hosts *C. levisecta* can use, and the mechanisms by which host plant identity might affect *C. levisecta* performance.

Key Words: *Parasitic plants, pot size, rare plants, restoration planting*

Introduction

Reintroductions into suitable habitats are a key strategy in the restoration of self-sustaining populations of threatened and endangered species (Guerrant 1996; Guerrant & Kaye 2007). Various authors have suggested that rare species recovery efforts need to be fundamentally grounded on a demographic (Schemske et al. 1994), genetic (Hamrick *et al*. 1991), or ecological understanding of the species. Recovery of rare, parasitic plants, however, is further complicated by potentially diverse interactions of the species with their hosts. Understanding the availability and utility of host plants is a critical step in the successful reintroduction of parasitic plants (Marvier and Smith 1997). For this study, I explored such host plant relationships with *Castilleja levisecta* (golden paintbrush), a rare hemiparasite endemic to prairies in the Pacific Northwest of the United States.

Most members of the genus *Castilleja* are considered generalist hemiparasites, capable of parasitizing multiple host species (Dobbins and Kuijt 1973). However, research suggests that parasites that exploit many different hosts do not use them randomly; instead, some hosts may confer significantly greater benefits to the parasite than others (Kelly et al. 1988; Marvier & Smith 1997). With *C. levisecta,* there is little information on host requirements or on the biological effects of host species on survival and performance (Wayne 2004, Pearson and Dunwiddie 2006, Kaye and Lawrence 2008), and the results of these studies have been variable and at times contradictory. I surmised that if *C. levisecta* performs better when grown with some host species and not others, recovery efforts for the species might be considerably more successful if they are planted with the most beneficial hosts.

A second focus of my study examined the importance of container size in outplanting success, a common but rarely studied element among restoration practitioners. Propagation and outplanting of greenhouse-grown plugs is costly; plants grown in small containers are cheaper to grow and easier to plant, but may be accompanied by slower growth or increased mortality. I found two studies that evaluated how container size affected outplanting success (Kemery and Dana 2001; Svendsen and Tanino 2006). Both reported that larger container size increased plant growth but did not influence survival of outplanted herbaceous perennials. Having such information for *C. levisecta* could substantially affect both the cost and success of recovery efforts for this species, as outplanted plugs have been widely used at many sites.

In this study, I replicated outplantings in field settings over two years to examine three questions that explore the interrelationships among the variables of host species and container size. First, how does the presence and identity of host species affect survival and performance (flowering and capsule production) of *C. levisecta*? Second, how does greenhouse container size affect these variables, and third, how do host and container size interact?

Methods

Study species

Castilleja levisecta (Orobanchaceae) is a short-lived (ca. 5-7 years) hemiparasitic perennial. In the wild, mature plants typically have up to a dozen (occasionally more) flowering stems, which bloom from late April to early July. Each stem has 5-10 capsules, which begin to ripen in August, each bearing 100+ minute seeds that appear to be primarily wind-dispersed. The species has been listed as threatened by the U.S. Fish and

Wildlife Service (2000), and is restricted to eleven remaining wild populations in the San Juan Archipelago and one in the South Sound of the Puget Lowland. Extant populations are largely found on sandy, well drained soils of glacial origin (Chappell & Caplow 2004b). A recovery goal for the species calls for 20 populations of at least 1,000 flowering individuals by 2018 (U.S. Fish and Wildlife Service 2000). To help achieve this goal, a reintroduction plan has been written that calls for the establishment of new populations within its historical range, and the augmentation of existing populations, many of which are quite small (Caplow 2004).

Study Site

The study was carried out on the 1150 ha Black River-Mima Prairie-Glacial Heritage Preserve (Glacial Heritage), which is one of the largest protected prairies in western Washington (46°87’15”N, 123°05’30”W). Glacial Heritage is owned by Thurston County Parks and Washington Department of Fish and Wildlife (WDFW) and managed by The Center for Natural Lands Management and WDFW. Although native prairie covers most of the site, the research area is located within a 3 ha portion that was cultivated for many years and contained no native species when the study began. Site preparation included application of a non-selective herbicide (glyphosate) to kill all existing vegetation, and tilling prior to planting.

Experimental Design

I compared *C. levisecta* grown with three host treatments: *Eriophyllum lanatum, Festuca roemeri,* and no host. Both species are known to serve as hosts to *C. levisecta* (Kaye and Lawrence 2008). Host and *C. levisecta* plants sown in the fall the year before planting and were grown separately, the *C. levisecta* in two container sizes of 115 ml (small pot) and 393 ml (large pot) (Ray Leach "Cone-tainer" tube and 4 inch diameter pot, respectively), and the hosts in 115 ml containers. All plants were grown outside on benches in sterile potting medium and fertilized with a 10-10-10 (NPK) fertilizer. *C. levisecta* were outplanted with a randomly assigned host in November 2010 in an experimental array and the entire experiment was replicated in a second array in November 2011. A balanced design was not possible due to mortality of some of the greenhouse plants. However, at least 79 replicates of each of the 6 treatments were outplanted in 2010 and at least 97 of each of the replicates were outplanted in 2011 (Table 3.1). *C. levisecta* plants were spaced 1 meter apart; those with hosts were planted with roots touching. To ensure no other potential host species were available, the site was weeded throughout the experiment.

Data Collection

Data were collected in early June 2011 and again in September 2011 for the 2010 array. In June, I recorded *C. levisecta* survival, number of flowering stems, and host species survival. In September, the number of *C. levisecta* stems with capsules and the number of capsules per stem were recorded. Each of these measures was repeated for both arrays in June and September of 2012, resulting in two years of measurement on the 2010 array and one year of measurement on the 2011 array.

Statistical Analyses

Prior to analyses any experimental units where the host died were removed (9 in 2010 and 0 in 2011). I used a binary logistic generalized linear model (GLM) to test for effects of host treatment and container size on *C. levisecta* survival. After analyzing patterns of survival, I restricted subsequent analyses to data only from plants that survived. For analyses of continuous variables (number of flowering stems, number of capsules), I used a Poisson loglinear GLM. A Bonferroni honestly significant difference test was used for pairwise multiple comparisons. All analyses were conducted using SPSS Statistics 19 (IBM 2012).

Results

First year results for the 2010 and 2011 arrays and second year results for the 2010 array are presented below by survival, number of flowering stems, and seed production. A summary of the effects of host identity and container size and interaction between these terms is found in Table 3.2.

Survival

Average first year survival of *C. levisecta* (ignoring container size) was high regardless of host treatment in the 2010 array and lower in the 2011 array. In the 2010 array, 92% survived when growing with *E. lanatum*, 87% with *F. roemeri*, and 82% with the no-host control compared to 80%, 73%, and 63% respectively in the 2011 array. The main effect of container size was significant in both arrays in the first year. However, the small pots had significantly higher survival in the 2010 array (Figure 3.1 i), while the larger pots had higher survival in the 2011 array (Figure 3.1 ii).

By the second year in the 2010 array, the identity of the host had a large effect on survival (Figure 3.2 i). Average survival remained very high (85%) when growing with *E. lanatum*, but had fallen to 60% with *F. roemeri*, and just 33% with the no-host control (all $P < 0.001$). Survival of *C. levisecta* continued to differ significantly between the two container sizes, with total survival remaining higher (67%) in the small pots than in the large pots (53%) ($P < 0.001$). There was no interaction between host and container in any year.

Flowering

During their first year of growth in the field, *C. levisecta* plants produced few flowering stems regardless of the host with which they were grown. The same pattern occurred in both arrays, though more flowering stems were produced in the 2011 array (Figures 3.1 iii, iv). Despite the small number of flowering stems across all treatments, plants with a host produced significantly more flowers than those growing without a host ($P < .05$) in the 2010 array. Plants with *E. lanatum* produced the most flowering stems in each array; however, the no-host treatment outperformed plants with *F. roemeri* in the 2011 array. The average number of flowering stems by container size was significantly higher with the large pots than the small pots in both the 2010 and 2011 array (2010: 1.4 versus 0.8 stems per plant; 2011: 2.4 versus 0.3 stems per plant, all $P < 0.001$).

As with survival, the magnitude of the effect of the host identity on flowering increased in the second year (Figure 3.2 ii). Plants with *E. lanatum* produced on average 5 more flowering stems than plants with *F. roemeri*, and 12 more than *C. levisecta* plants with the no-host treatment. All treatments differed significantly ($P < 0.001$). Unlike in year one, larger pots did not consistently produce more flowering stems. There was no difference in the number of flowering stems by container size alone.

Capsule Production

The overall number of seed capsules produced by *C. levisecta* was slightly higher in the 2011 than the 2010 array (Fig. 3.1 v and vi). In the 2010 array, plants with a host produced significantly more capsules than plants without a host, even though the no-host plants produced more flowering stems (Figure 3.1 iii, v). However, plants with the no-host treatment produced more capsules than those with *F. roemeri* in the 2011 array. As observed with flowering stems, large pots consistently produced more capsules in both arrays. There was a significant interaction between the host and container treatments in both arrays.

Capsule production increased 10-fold from year one to year two in the 2010 array, with much of that production coming from plants with a host, particularly the *E. lanatum* treatment (Figure 3.2 iii). The average number of capsules by host treatment was 116.4 capsules for plants with *E. lanatum*, 44.4 with *F. roemeri*, and 18.1 with no-host control (all $P < 0.001$). Additionally, the number of capsules per flowering stem was also highest with *E. lanatum*, followed by *F. roemeri*, and no host (Figure 3.3). Surprisingly, the larger container size advantage reversed in year two, with small pots producing more capsules on average (140.9) than larger ones (122.7) ($P < 0.001$). Small pots produced more capsules across all treatments in year two with the exception of the no-host treatment, where production was equal and likely contributed to the significant interaction between the host and container treatments.

Discussion

I examined how host species and container size affect two important life history stages that often are critical to recovery: survival from juvenile to adult and seed production. Outplanted plants must not only survive in significant numbers, but also must produce large quantities of seed to overcome the extremely low establishment rates (1% or less) that have been observed in the field. In my study, I explored factors that I hypothesized might affect not only the initial survival of outplantings, but also their vigor, fecundity, and longevity. Over the lifespan of the plants, these factors are likely to affect the total quantity of seed produced. Collectively, these factors play key roles in determining whether recovery plantings successfully establish, whether and how quickly new populations grow, and ultimately whether they thrive to become robust recovery populations.

Survival from Juvenile to Adult

During their first year in the field, most the outplantings survived regardless of host treatment, probably because they were primarily drawing on resources in the potting soil in which they were grown. However, by their second year, the survival of *C. levisecta* plants was significantly greater when growing in association with a host. Others have reported similar results (Matthies 1997), so this enhanced survival was not unexpected. However, the magnitude of this effect was noteworthy by the second year, when more than two and a half times the number of plants grown with hosts survived than those grown without hosts.

It also is clear from my results that the identity of the associated host significantly influenced *C. levisecta* survival in the second year of growth. Although survival was unaffected by host identity in the first year for both the 2010 and 2011 arrays, in the second year plants grown with *E. lanatum* were much more likely to still be alive (85%) compared to *F. roemeri* (60%). Several mechanisms may contribute to these results. *E. lanatum* grows less densely than *F. roemeri* and may provide more growing spaces for the hemiparasite. In the field, I observed *C. levisecta* growing both around and within the canopy of *E. lanatum* plants. In contrast, this was uncommon with *F. roemeri,* where the species usually grew side-by-side but not intermingled (Figure 3.4). Additionally, root morphology could allow for more or higher quality haustorial connections with *E. lanatum*. Finally, *E. lanatum* may actively grow for a longer period than *F. roemeri* allowing for greater resource availability to *C. levisecta*.

In addition to host effects on survival, larger pot sizes also provided high survival in the first year. Despite little evidence in the literature, intuitively I expected that plants grown in the large pots, which contained 4 times the growing space and more nutrients than the small pots, might confer benefits similar to growing with a host plant, and result in high survival. In fact, I did find consistently high survival in larger pots in both years, though small pots had particularly high survival in the 2010 array and outperformed large pots. The higher survival for small containers in the 2010 array was one of the more surprising results. I suggest two potential explanations for these differing results between years. The most likely cause is different environmental conditions for field and greenhouse growth. In the field, conditions for survival were potentially more favorable in the first year due to mild winter conditions after planting and a wet spring the following year. Therefore the advantage that plants in larger pots may have had was mitigated by favorable field conditions for all plants. Given the limited resources that the smaller pots have, it is logical that survival would be more variable depending on environmental conditions in a given year. My survival results are consistent with a 4-year field study across multiple sites in South Puget Sound that show first year survival of *C. levisecta* in the same small pot size between 32% and 92%, with an average of 65% (Dunwiddie 2013, unpublished data). In addition to field conditions, environmental conditions while the plants were being grown out on the outside greenhouse benches could have also more adversely affected larger pots survival results. Svendsen and

Tanino (2006), for example, note how elevated temperature in containers can de-acclimate roots and render them susceptible to low temperature stress. The smaller containers were grown in a contained flat and received less direct solar radiation than the larger containers that were grown separately on benches.

Capsule Production

The number of capsules produced per flowering stem, as well as the number of flowering stems per plant, can vary considerably in *C. levisecta*. In my study, where conditions were optimal, I observed extremely robust plants with more than 20 capsules per stem and over 100 flowering stems, although wild plants are usually much smaller. The number of seeds within each capsule can also vary considerably, but averages 175 seeds per capsule. Availability of resources probably is a major factor contributing to the variability of flowering stems and capsules, and I hypothesize that *C. levisecta* plants growing with different host species may receive substantially different resources through their haustorial connections.

I found that the presence of a host species and the host identity greatly affected flowering and seed capsule production. Capsule production was more than double in the first year of the 2010 array and four times greater in the 2011 array for plants with *E. lanatum* compared to *F. roemeri*. These differences between the hosts become much more pronounced in the second year of the 2010 array. Given the importance of seed production for the maintenance of populations of a short-lived species such as *C. levisecta*, this is a particularly important finding.

To illustrate how the factors of host species and container size combine to influence reproductive capacity in a population, Figure 3.5 projects the number of capsules produced by a hypothetical population of 100 plants starting in year 1, and growing with each of my host and container size treatments. By the second year, plants with *E. lanatum* hosts are projected to produce an average of >34,000 seed capsules compared to just 11,000 for plants with *F. roemeri* (Figure 3.5). The difference in projected seed production between these two hosts is driven by both the lower survival in the second year of plants with *F. roemeri*, but also by lower numbers of capsules per flowering stem. Plants with *E. lanatum* consistently had many more capsules per stem. The average number of capsules per stem was consistently above 12 and as high as 17 across all treatments and years for plants with *E. lanatum*, while the number of capsules per stem for plants with *F. roemeri* averaged 7 and was only as high as 11. If these differential trends in survival and productivity continue, they would result in vastly more seed being produced by plants associated with *E. lanatum* over the lifetime of the founder population.

The greater performance of plants grown with *E. lanatum* could be a function of greater pollination of *C. levisecta* plants when grown with *E. lanatum*, as its showy yellow flowers bloom at the same time as *C. levisecta*. This could attract pollinators to visit *C. levisecta* growing with *E. lanatum* more frequently than those growing with *F. roemeri*. This phenomena has been observed in other *Castilleja* species (Adler et al. 2001). The host identity could also affect the bloom phenology. I observed, for example, plants with *E. lanatum* blooming for longer in the growing season, and the capsules were often still green when I was recording data in September, compared to plants with *F. roemeri,* where capsules were more likely to be senesced. The number and quality of haustorial connections could also be driving this host effect. I attempted to quantify haustorial connections between host species on a subset of two-year-old plants in the 2010 array in collaboration with a graduate student from University of Washington. We found haustorial connections on both, however the removal of soil from the field setting was difficult and it was not clear if all of the haustoria were found (Figure 3.6) (Footen, 2012 unpublished data). Finally, greater seed capsule production in *E. lanatum* could be a function of *E. lanatum* having a longer growing period than *F. roemeri*. This would allow the *C. levisecta* plants to take advantage of the plants active growing stage for a longer period and subsequently produce more seeds.

Larger containers also provided a significant advantage in terms of flowering and capsule production in the first year in both arrays (Table 3.2), a result that is consistent with previous studies on container size effects (Kemery and Dana 2001, Meyer and Cunliffe 2004, Thetford and Miller 2005, Svendsen and Tanino 2006). It is particularly noteworthy, however, that in my study, the positive effect of the larger containers on growth did not continue past the first year, as *C. levisecta* grown in the smaller containers produced more capsules in the second year after producing less in the first year (Figure 3.2). The relative advantage larger container sizes provide in the first year appears to be erased and even reversed by the second year of growth. One explanation for why smaller pot size plants produce more seeds in the second year could be the quantity or quality of their haustorial connections. At the time of planting, for example, there was relatively more of the surface area of the *C. levisecta* roots in small pots adjacent to the host plants, which were in the same size pots, than there was of the larger pots. It is possible that more root connections were made that conferred greater resources in the following growing season. I would not expect this relative advantage of the small pot size to continue, particularly in a more typical field setting where a variety of other hosts beyond the one that I provided are available on all sides of a plant to utilize.

Lessons learned

In addition to my results respective to the research questions, I also have three lessons learned or recommendations, which can be applied to most ecological field studies. My first recommendation is to take care in choosing the right metric to measure results. My results particularly underscore the importance of selecting appropriate metrics. While survival is fairly straightforward to evaluate with most species, authors have used diverse metrics to assess growth, vigor, and fecundity. Height, width, volume, stem and leaf number, and biomass all provide measures of plant size. These, in turn, are often correlated with reproductive output, which may be measured separately using counts of flowering stems, flowers, capsules, fruits, or seeds. Choosing which parameters to monitor involves tradeoffs based on cost, time, season, and biology of the species. In my study, I was specifically interested in the effect of host and container size on the plants' ability to survive, flourish, and produce seed. I initially intended to just use number of flowering stems as a proxy for reproduction. Ultimately, however, I chose to use flowering stems and number of capsules as proxies for reproduction. I added counting the number of seed capsules to have a measure closer to the actual seed production while avoiding the impracticality of counting actual seeds within capsules of each plant. I have already shown that *C. levisecta* plants with hosts produce more flowers and seed capsules and *E. lanatum* produces more of both than *F. roemeri*. I also discovered that plants with a host and particularly those with *E. lanatum* produce more seed capsules for any given number of flowering stems (Figure 3.4). Just counting the number of flowering stems does not give an accurate picture of the species fecundity as host species affects the size of that flowering stem and how many capsules will be produced on any flowering stem. It is also possible that variability in the number, size, and viability of seeds within capsules is also high or affected by host treatments, and may influence the accurate estimation of treatment effects on fecundity.

Secondly, I would advocate for replicating field studies for at least two years to understand the level of annual variability in your system. I replicated this experiment for two years in order to test whether treatment effects were consistent from year to year. The variability that I found in the effect of pot size on survival and slight differences between host treatment effects from year to year is to be expected given all of the other variables affecting these plants in a field setting. While consistent results may be easier to interpret, my variable results are a good reminder to take the results of most short-term field studies cautiously, to not draw conclusions too broadly from limited information, and to replicate experiments temporally if possible. Vaughn and Young (2010) recently made the case for more deliberate investigation of temporal contingency in ecological experimentation in a literature review of more than 500 ecology articles. They found that fewer than 5% of experiments conducted experiments in multiple years while 76% of those that did found significant interaction between treatment and initiation year (Vaughn and Young 2010).

Even with temporal replication, I would strongly recommend continuing monitoring for at least two years to identify any trends. For example, in the first year of the 2010 array there was no significant difference in survival of *C. levisecta* planted with *E.*

lanatum (92%) and *F. roemeri* (87%). This result was also confirmed in the 2011 array with no statistical difference in survival of 80% and 73%, respectively. Based on these results, I might have concluded that host identity makes no or little difference in survival. However, second year results show a much larger and significant difference in the effect of host identity with 85% and 60% survival with *E. lanatum* and *F. roemeri* respectively. I would put more weight on my second year results and the trend that appears to be emerging about the effect of host identity on both survival and seed production, though even these results should not be overstated. I have not replicated this study spatially for example, and site conditions such as presence or lack of herbivores, competing vegetation, edaphic or other factors, may influence the growth and persistence of a particular host species and in turn *C. levisecta* performance. Several recent reviews provide an additional cautionary note about drawing conclusions too quickly. In evaluating the success of plant reintroductions, Godefroid et. al (2011) found that survival rates declined substantially with time in individual experiments. Similarly, Drayton and Primack (2012) revisited their own experiment after 15 years and found that almost all of the eight populations they previously reported as successfully reintroduced had disappeared.

Conclusion

In this study, I replicated outplantings in a field setting over two years to explore how host species and container size affect survival and performance (flowering and fecundity) of *C. levisecta*. I found that both host presence and more importantly host identity significantly affect survival and fecundity. These results demonstrate that *C. levisecta* does not obtain equal fitness from all hosts. Host identity should be an important and perhaps pivotal decision in planning for recovery of the species. Though host requirements and effects are not well understood for *C. levisecta,* I recommend that multiple host species should be examined experimentally in advance for at least two years at a site before taking recovery actions. Restoration practitioners should try to ensure appropriate hosts are available, but also take care in choosing hosts that will be most likely to ensure survival and seed production of *C. levisecta* at a given site. Given that *C. levisecta* recovery is unlikely to occur in a vacuum, but rather as a part of overall recovery at any site, an alternative strategy is to ensure a diverse mix of potential hosts are available in any restoration effort. For example, as part of an active *C. levisecta* recovery project in Oregon, Kaye has found that perennial plant diversity improved *C. levisecta* survival at the microsite scale (2011). Testing hosts in advance in a more controlled environment at a site will provide guidance on this decision.

In addition to host effects, I found that larger pot sizes did result in consistently high survival and significantly more flowering and seed capsule production in year one. Utilizing larger pot sizes may be appropriate when high survival needs to be assured in the first year. However, given that the advantage that larger pot sizes provide appears to be lost after one year, restoration practitioners should weigh the greater expense and difficulty in planting larger container sizes, with the first year goals. For example planting in larger pots may be advisable when seed of a given population is very limited for propagation, and only a small number of plants can be produced for outplanting.

Future research should explore different host species effects on *C. levisecta* survival and performance to better understand some potential mechanisms for host species effects such as haustorial connections to improve the success of future reintroductions of the species.

Implications for Practice

- Choosing the right host for your site can strongly influence initial restoration success of *C. levisecta*. Efforts to identify and include hosts that confer the greatest benefits to the hemiparasites may greatly enhance survival and growth of recovery populations.
- Larger container sizes have consistently high survival and do confer growth and seed capsule production advantages in the first year. Depending on restoration goals, using a larger pot size to ensure survival and performance in year one may be appropriate.
- Temporal replication of outplanting experiments and long-term monitoring (at least two years) may produce results that are highly variable and even contradictory. Conclusions on restoration or treatment effects that are based on short-term results should be viewed with considerable caution.
- Variable selection (flowering stems vs seed capsules) to monitor is an important decision to ensure you can answer the research or restoration questions.
- Treatments that increase the survival of outplantings may not also increase reproduction.

Chapter 3 Tables and Figures

Table 3.1. Number of replicates of each container size and host treatment combination.

Container Size	Host	2010 N	2011 N
Small	*Eriophyllum*	99	98
	Festuca	93	97
	No host	100	100
Large	*Eriophyllum*	93	98
	Festuca	98	97
	No host	79	100

Table 3.2. Effects of host identity and container size on survival, number of flowering stems, and fecundity (number of seed capsules) of *Castilleja levisecta*. Terms were tested in two experimental arrays and evaluated for one or two years after outplanting. Significant effects are in bold type.

Response	Host		Container Size		Host × Container Size	
	Statistic[a]	*P*-value	Statistic[a]	*P*-value	Statistic[a]	*P*-value
Survival						
First Year						
2010 Array	7.5	**0.023**	15.2	**<0.001**	2.0	0.355
2011 Array	10.6	**0.005**	92.6	**<0.001**	1.2	0.549
Second Year						
2010 Array	86.9	**<0.001**	17.4	**<0.001**	2.4	0.303
Flowering Stems						
First Year						
2010 Array	20.3	**<0.001**	46.5	**<0.001**	23.9	**<0.001**
2011 Array	45.2	**<0.001**	130.5	**<0.001**	11.1	**0.004**
Second Year						
2010 Array	512.2	**<0.001**	0.2	.674	26.2	**<0.001**
Fecundity						
First Year						
2010 Array	1762.7	**<0.001**	344.1	**<0.001**	137.4	**<0.001**
2011 Array	441.8	**<0.001**	195.5	**<0.001**	46.2	**<0.001**
Second Year						
2010 Array	9375.2	**<0.001**	115.8	**<0.001**	224.0	**<0.001**

[a] Test statistic is Wald chi-square for survival (analyzed with binary logistic GLM), and Wald chi-square for flowering stems and fecundity (analyzed with poisson loglinear GLM).

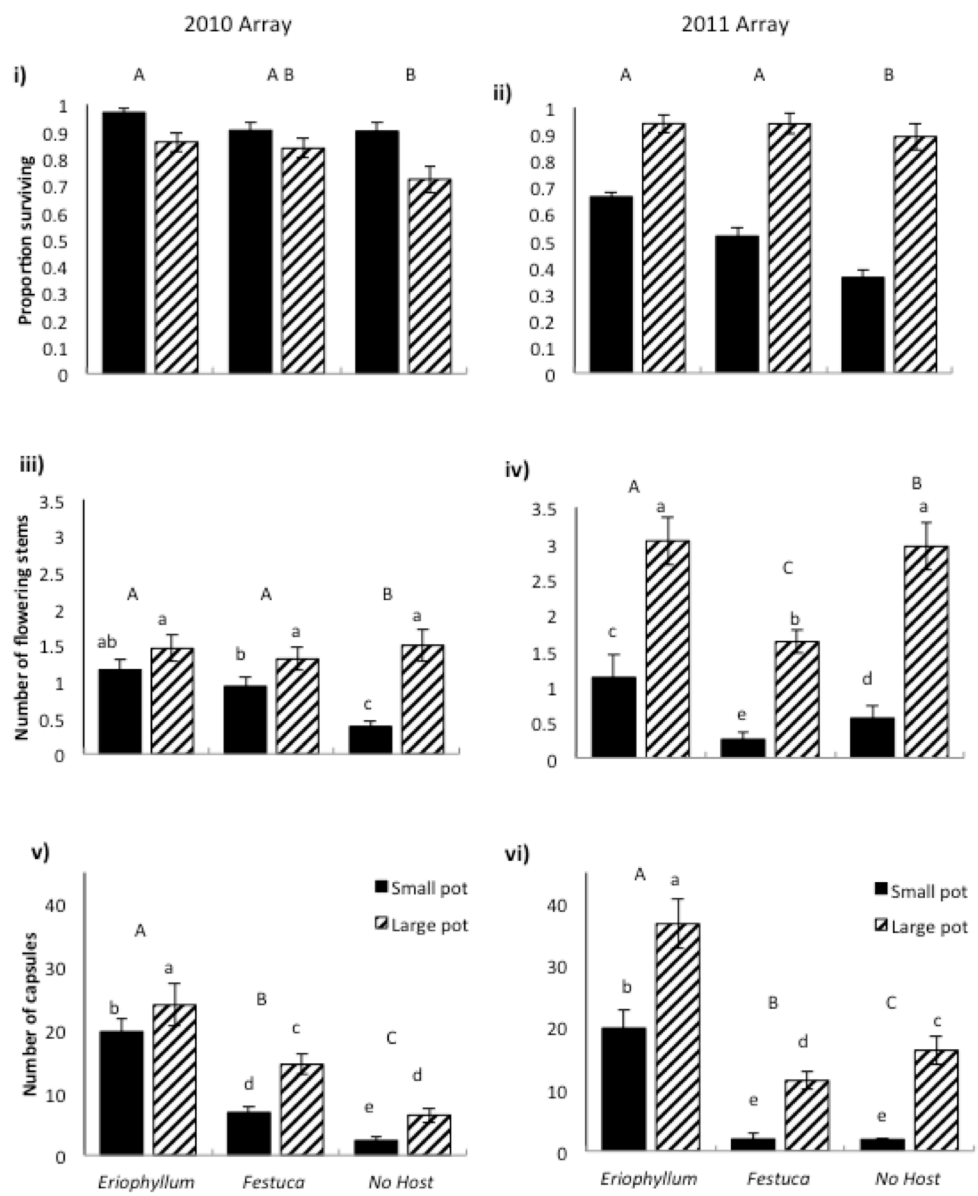

Figure. 3.1. First year results for each replicate. Results are shown with container size separated within each host treatment. i) & ii) *Castilleja levisecta* field survival in 2010 and 2011 arrays. iii) & iv) Average number of *C. levisecta* flowering stems by host treatment in 2010 and 2011 arrays. v) & vi) Average number of *C. levisecta* seed capsules produced in 2010 and 2011 arrays. Host treatments not sharing a common capital letter and host x container combinations not sharing a common lower case letter were significantly different ($P < 0.05$).

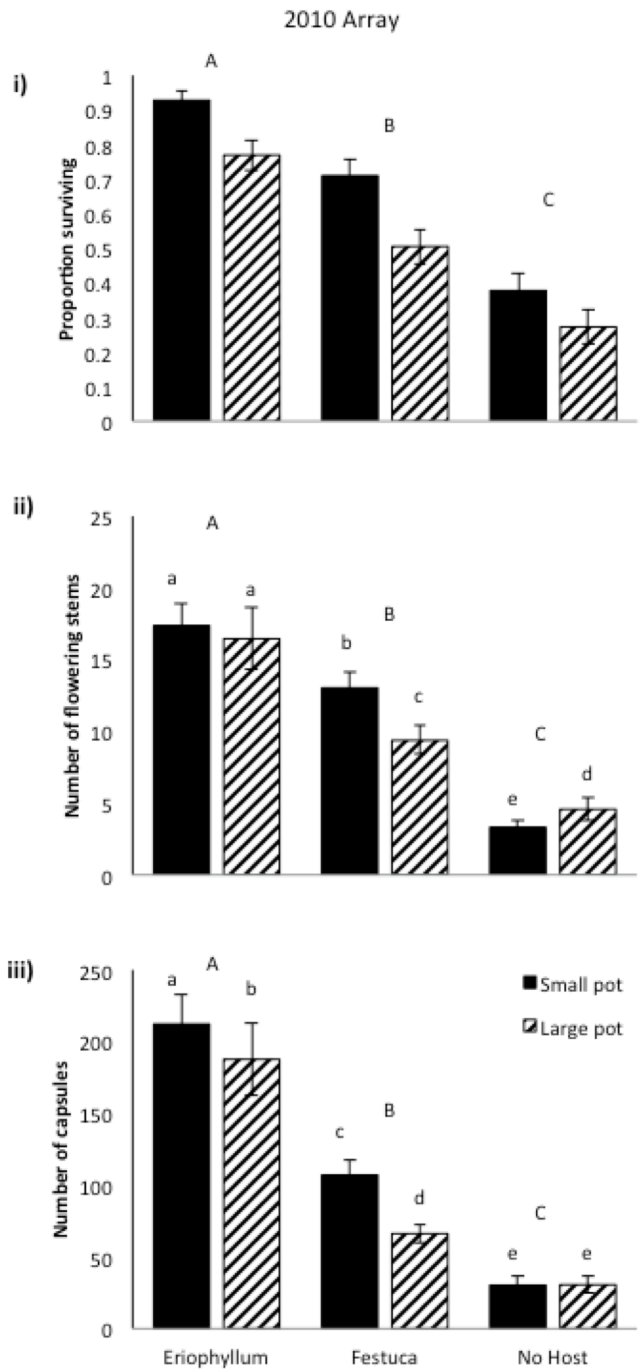

Figure. 3.2. Second year results for 2010 array. Results are shown with container size separated within each host treatment. i) *Castilleja levisecta* field survival. .ii) Average number of *C. levisecta* flowering stems by host treatment. iii) Average number of *C. levisecta* seed capsules produced. Host treatments not sharing a common capital letter and treatment combinations not sharing a common lower case letter were significantly different (P < 0.05).

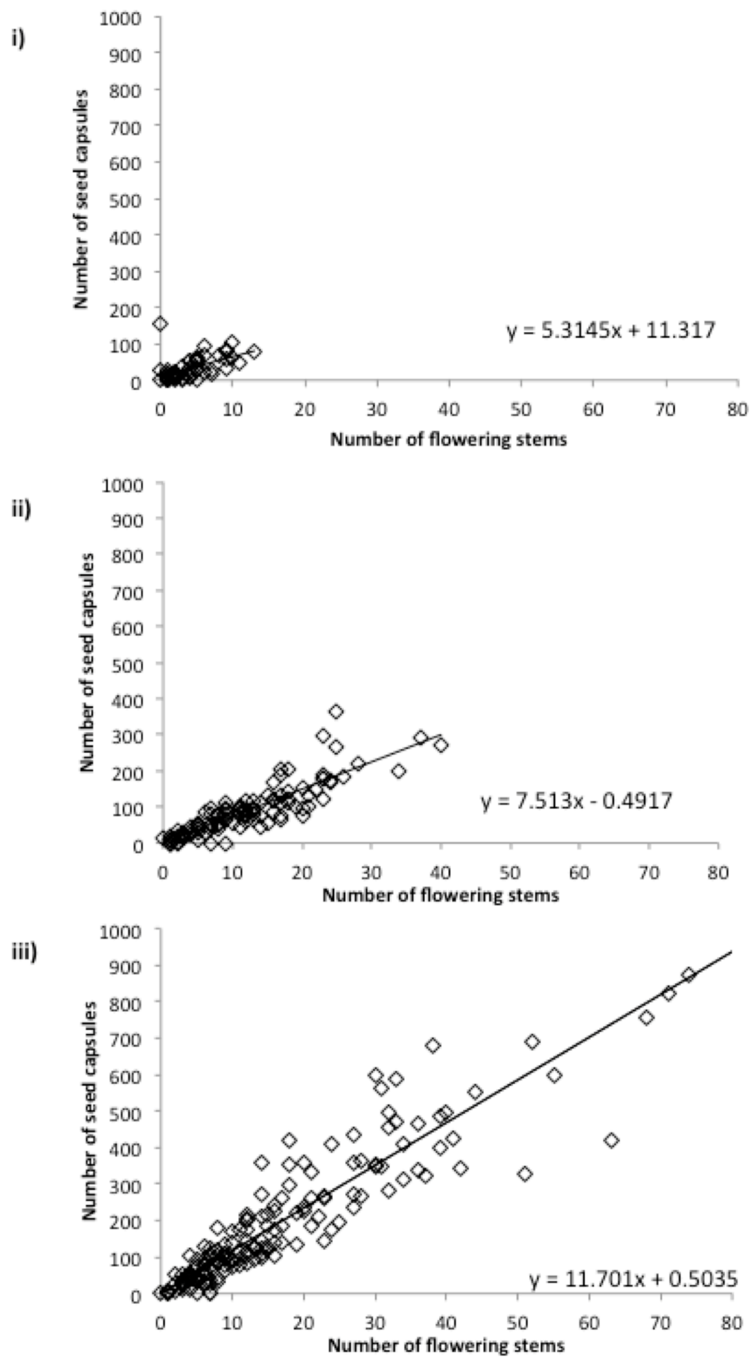

Figure 3.3. Linear relationship between the number of flowering stems and number of seed capsules produced in the second year of the 2010 array with i) no-host, ii) *F. roemeri*, and iii) *E. lanatum* treatments. Analysis of the effect of host on seed capsule production with flowering as a covariate demonstrates a significant interaction between flowering and host as evidenced by the differing slopes of the lines ($P < 0.000$). Flowering stems with *E. lanatum* produce more seed capsules than *F. roemeri* and the no-host treatment.

Figure 3.4. *C. levisecta* growing with *F. roemeri* (top) and *E. lanatum* (bottom).

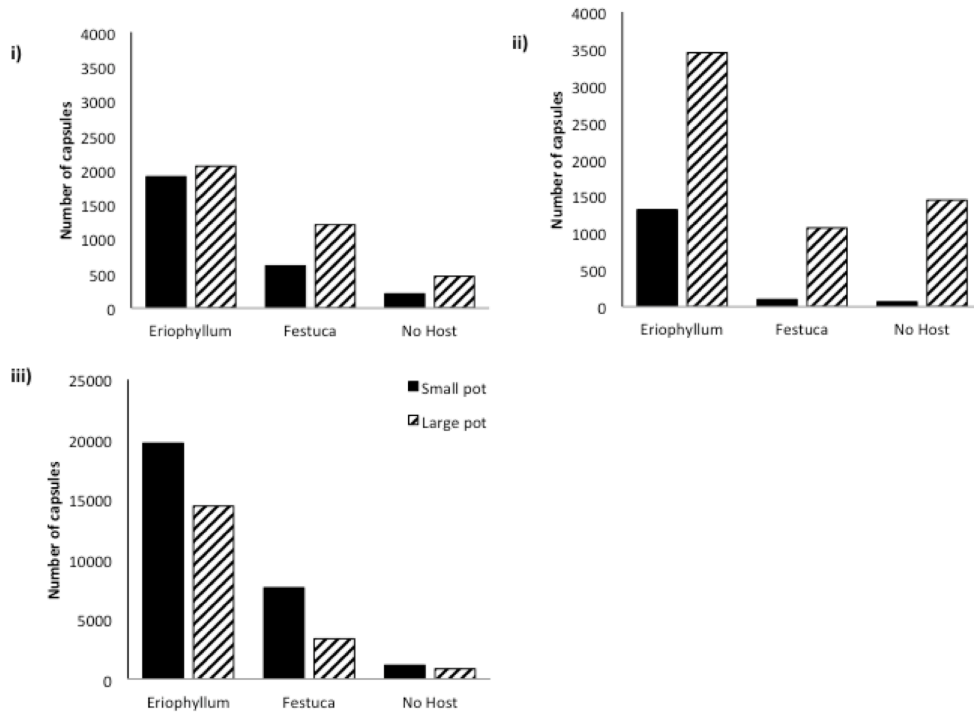

Figure 3.5. Projected seed production from 100 *C. levisecta* plants with associated treatments, calculated by multiplying mean survival by mean number of capsules produced per flowering plant. i) 2010 array year one. ii) 2011 array year one. iii) 2010 array year two (note change in y axis scale from year one arrays to year two array).

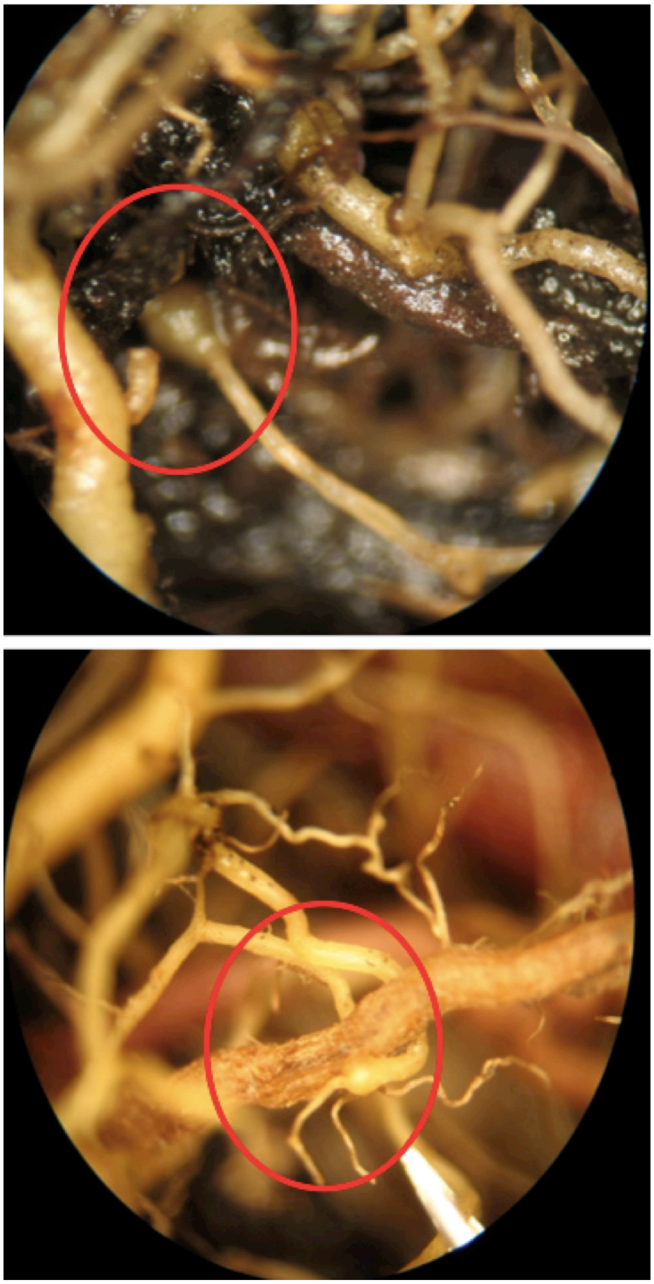

Figure 3.6. Haustorial connections of *C. levisecta* on *F. roemeri* (top) and *E. lanatum* (bottom) from two year old plants in the 2010 array (Footen, 2012).

Chapter 4

Staged-Scale Restoration: A Systematic Adaptive Management Approach for Improving Restoration Effectiveness

Abstract

Over the last several decades, ecological restoration has grown dramatically in both magnitude and sophistication. The use of adaptive management (AM), where rigorous experimental evidence informs large-scale management and restoration efforts, has been greatly discussed and recommended, but rarely implemented. More often, managers implement restoration based largely on anecdotal observations and educated guesses, rather than growing out of rigorous experimental evidence. This can result in costly and time consuming failures. In many prairie ecosystems, the lack of large, intact habitats compels managers to restore communities *de novo,* creating assemblages of native species in abandoned agricultural fields. These efforts often face formidable challenges such as controlling invasive weeds, developing effective methods to establish plant communities, and directing successional processes with appropriate management tools to facilitate ecological restoration. To develop more efficient solutions to these restoration challenges, I tested a "Staged-Scale Restoration" (SSR) strategy in Puget Lowland Prairies that rigorously explores multiple solutions within an adaptive management framework. SSR begins by identifying several promising restoration strategies that can be applied at large scales, and tests them in small, replicated experimental plots. Based on the results of these small-scale tests, the most successful treatments are then applied in increasingly larger scaled-up areas, while incorporating refinements suggested during the small-scale experiments. SSR provides a method for implementing AM in restoration projects that minimizes risks, improves scientific rigor, collaboration among researchers and managers, and provides a cost effective restoration approach. This technique should encourage adoption of AM in restoration projects.

Index terms: Restoration ecology, abandoned agricultural land, prairie restoration

Introduction

Over the last several decades, the restoration of ecologically degraded and destroyed communities has grown dramatically in both magnitude and sophistication (Holl et al. 2003). Early restoration projects tended to be based largely on trial and error, with practitioners implementing and refining treatments based on direct observation of natural systems, personal experience, and informed guesses. The realization of the uncertainty involved in restoration and natural resource management, together with events such as the degradation of the Everglades and collapses in commercial fisheries, led C. S. Holling and associates (Holling 1978) to propose a set of methods that they called "adaptive environmental assessment and management" (AEAM). This concept, soon shortened to adaptive management (AM), is natural resource management conducted in a manner that purposely and explicitly increases knowledge and reduces uncertainty (Holling 1978, Walters and Holling 1990, Rist et al. 2012). AM was greeted with enthusiasm by land and natural resource management agencies and others as it promised an approach to effectively restore and manage resources and reduce uncertainty through learning (Rist et al. 2012).

AM has been widely adopted as an organizing principle by myriad non-governmental organizations and international organizations, and is well established in the natural resource management agency practice in the United States at all levels of government (Ruhl 2008, Rist et al. 2012). With the extensive use of the concept of AM however, it has been simplified and become more of a buzzword than a recognized set of principles (Parma *et al*. 1996). In recent years there have been a number of attempts to clarify the meaning of the term, including distinguishing active from passive AM (Schreiber *et al*. 2004).

Despite these efforts, significant misunderstanding on what constitutes AM exists today from academia to land managers. Often AM is simplified to "learning by doing," and managers who are using a trial-and-error approach to contend with changing resource systems argue that they are already using AM (Rist et al. 2012). The various definitions and understanding for what constitutes AM are also reflected in the literature with opposite positions on the trajectory of AM. On one side are claims that AM is a well-developed theoretical approach (Eberhard *et al*. 2009) that is widely regarded as an effective and powerful framework to support the successful management of natural resources (Mackenzie and Keith 2009). On the other side, detractors point to the rare instances of successful practical applications and high documented failure rates (McLain and Lee 1996, Eberhard et al. 2009, Rist et al. 2012).

I do not resolve the debate on what AM is or should be in this paper. Instead, I first define AM as I understand it, review how AM is currently applied, discuss barriers to its implementation that are commonly cited, and suggest contexts in which it works well. I then propose a new systematic AM approach in a restoration setting, which I have termed "staged-scale restoration," that overcomes some of the barriers and short-comings that have been identified. This approach is then illustrated in a case study involving a five-year restoration study in Puget Lowland prairies, where I demonstrate how this approach can improve effectiveness and efficiency of habitat restoration. Finally, I consider the advantages and limitations of the approach and offer recommendations for where this new approach could prove useful.

Adaptive Management- Background

Clarifying what I mean by ‘adaptive management’ is needed due to the various definitions currently present in the literature. AM has a formal definition and specific operational requirements. However, more than 40 years after the concept was introduced, it means many different things to different people. AM has been used to describe everything from formal natural resource management to encouraging participation in social learning, and many who use the term rarely define what it means (Rist et al. 2012). In a comprehensive review of AM in the literature, only 18% of 187 papers defined AM (Rist et al. 2012). In recent years, there has been a distinction in the literature between “active” and “passive” AM. Generally, active AM is used to describe a process where there is a hypothesis-driven design where different management actions (experiments) are conducted simultaneously, while passive AM involves choosing the best strategy based on existing knowledge and then altering that strategy with new data (Lawler 2009). Walters and Holling (1990) originally introduced the distinction of active and passive adaptation in management. However, they made the distinction to propose AM as an alternative to passive adaptation to management (Rist et al. 2012). Regardless, this distinction is widely used in the literature and textbooks on the subject. The “active” AM term was likely introduced in an attempt to refocus on the experimentation element of the original term (Allen and Gunderson 2011).

For my purposes, I reserve the term AM for restoration that includes a formalized process of learning, ideally with experimentation to test hypotheses, as this was a core concept of AM (Walters 2007). I define active AM as the process by which explicit experimentation testing multiple treatments is implemented as part of the restoration, while passive AM retains a formalized hypothesis-driven learning process but only implements one treatment and does not explicitly experiment with multiple treatments.

Since its introduction and description, AM has been hailed as a solution to endless trial and error approaches to natural resource management and restoration challenges. However, it has failed more often than not (Allen and Gunderson 2011). In fact, it is rare to find examples of successful implementation of AM and it has failed in most large-scale applications. In an insightful review of the design and implementation of AM, Allen and Gunderson (2011) found myriad reasons for why AM failed (Table 4.1) and few examples of success.

Given the rate of failure of an adaptive management approach from examples above and in other situations, it might appear that the approach is flawed and not feasible. However, another explanation for its failing is that it is being applied to inappropriate situations. There are many ways to conduct restoration and manage natural resources and AM is unlikely to be useful in all of them. The broad appeal of the concept of AM, "learning by doing," likely entices its use when not appropriate. Allen and Gunderson (2011) suggest assessing both the risk and the uncertainty when considering natural resource management or restoration. They assert that four approaches are appropriate considering the level of risk and uncertainty of a given issue (Figure 4.1). If the uncertainty is low and the risk is low, then best management practices should be applied, while a nurture and triage approach is appropriate if the risk is higher. Scenario planning is recommended when the perceived risks and uncertainty are so high that planning for a range of outcomes is the best option. Through scenario planning, desired actions can be discovered and diverse interests can potentially reach consensus on how to move forward

(Peterson et al. 2003). AM is considered most appropriate in cases when the uncertainty is high but the risk is relatively low.

Consider, for example, a critically endangered species like the California condor (*Gymnogyps californianus*). When the population was down to 23 individuals in the wild, it was not a good candidate for AM as the risk of experimentation was too great (Allen and Gunderson 2011). Instead, it was appropriately managed with a "Nurture and Triage" approach. All of the known birds were captured and a captive breeding program was instituted. There are now more than 200 birds in the wild and once the population is sufficiently stabilized and the risk is lower, the species can be managed either with best management practices or adaptive management, depending on the uncertainty of the management issue.

On the other hand, habitat restoration is often ideally situated for AM. In a restoration project converting an agricultural field to native grassland, for example, there may be uncertainty on how to establish a plant community to support certain invertebrates. However, the risk to harming the invertebrates or other species with the restoration action is very low. Though the use of AM in habitat restoration appears to be a good fit, there are few specific examples in the literature that illustrate its implementation. Part of the reason for this could be that much restoration work currently falls into a low risk and low uncertainty category and involves the use of best management practices. In other words, people know, or think they know, how to do the restoration and there is no need for AM experimentation to refine techniques. Another explanation is that successful implementations do not generate publishable articles, either because of lack of interest on the part of managers in publishing, or because journal editors do not regard such articles as worthy of publication (Mcfadden et al. 2011). Finally, research shows that ecological management tends to be precautionary in the short term rather than experimental (Hauser and Possingham 2007): actions that have a moderate benefit are preferred over actions with uncertain but marginally larger expected benefits (Rist et al. 2012). Restoration funding is often limited, and the opportunity cost and real cost of conducting an experimental restoration technique on a large scale may be perceived to be too great compared to the benefit of an established technique.

It is within this context of conducting "active" adaptive management in habitat restoration where there is low risk but high uncertainty that I propose a novel approach that adds scientific rigor to restoration adaptive management projects while increasing efficiency, cost effectiveness, and likelihood of restoration success.

Staged-scale Restoration - Design

Staged-scale restoration (SSR) integrates a rigorous, hypothesis driven, experimental design into restoration projects. Fundamental to the concept of SSR is that it fully integrates the experimental arrays used to identify the most effective treatments into the actual restoration of the site. SSR is a multi-year process that begins with small scale experimental plots installed on a site to test plausible alternative treatments, and progressively refines and applies the most promising treatments, based on the experimental results, at increasing scales until the site is restored (Figure 4.2). In stage 1, an experimental array of plots is established that applies a set of restoration treatments to test particular hypotheses. The plots are large enough to use operationally realistic methods, but modest in size such that treatments can be replicated several times within the array without consuming large acreages of the restoration site. As the treatments take effect, the results are monitored and the data are analyzed for stage 2.

The method and intensity of the monitoring in stage 1 will depend on the restoration goals of a project and the level of change or difference that practitioners want to detect between treatments. Rigorous monitoring, such as counting seedlings and recording the cover of all species present within a set of quadrats may be desirable in some situations. However, rigorous monitoring and analysis would not always be required or needed. Under some circumstances, it may be sufficient for practitioners to decide amongst treatments based primarily or solely on visual assessments. The arrangement of the experimental arrays in stage 1 is well suited for managers to walk through and visually compare and contrast different treatments.

In stage 2, the most successful treatment – or a suite of treatments that practitioners would like to continue to compare – is applied to scaled-up larger plots. Continued monitoring of the scaled-up plots, as well as the original experimental array, informs the selection of treatments that are applied to increasingly larger scaled-up areas as restoration continues in subsequent stages.

Many aspects of this process can be customized. The number of treatments tested in the experimental array is up to the practitioners to decide. The scale at which treatments are increased at each stage can be as large or small as desired; in Figure 4.2, they are scaled up by a factor of ten at each stage. The length of time between the stages can vary depending on the timeline, resources, and rate of ecological development of the community. For example, stages could be implemented in consecutive years. However, several years could elapse between each stage so that managers can see how well plant communities establish. Finally, the number of stages in the process can vary depending on the size of the area to be restored and the available resources. The important concept is to use the results from the experimental and smaller scaled areas to gradually and systematically refine and install appropriate restoration treatments that have been tested on the site.

Staged-Scale Restoration - Approach

The SSR approach addresses several of the short-comings and limitations that have plagued managers who attempted to implement AM, and which account for many of the failures of AM described by Allen and Gunderson (2011). Reviewing the reasons for failure in Table 4.1, I have regrouped these under common themes of Risk, Scientific Rigor, Collaboration, and Costs and Value in Table 4.2. SSR can address each of these themes to enable AM to be applied with more confidence and effectiveness by practitioners.

Risk

As customarily practiced, active adaptive management applies experimental treatments directly at operational scales. However, this means that large management areas are committed to treatments with uncertain or even undesirable outcomes, such as when an experimental design includes untreated controls where known problems are left unaddressed. Land managers are understandably less inclined to apply restoration treatments that may not work at a significant scale when known strategies may produce acceptable outcomes, though it is possible that managers may underestimate the level of uncertainty with established practices. SSR design provides an alternative strategy for implementing active adaptive management in a restoration setting that simultaneously reduces the scale of areas dedicated to uncertain experimental treatments. Effectively, SSR expands the instances where AM may be applied in restoration contexts. Consider again Allen and Gunderson's risk and uncertainty matrix. Because SSR can start as small as needed and gradually scale up, practitioners working even in a scenario with very high risk and uncertainty can apply this kind of AM. In fact, I would assert that SSR could replace scenario planning as a strategy for high risk and high uncertainty situations in restoration because practitioners can start small and have minimal impact on the habitat in the initial stages (Figure 4.3). Furthermore, SSR increases confidence that the treatments applied to most of the site will be most successful by incorporating more rigorous experiments and carrying them out directly on that particular site.

Scientific Rigor

A second challenge with AM is a frequent lack of scientific rigor, leaving it open to legitimate criticism as quasi-science (Lee 1999). Replication is one of the powerful components of SSR that distinguishes it from traditional AM, and provides greater scientific rigor, allowing scientists to better understand complex interactions. SSR implicitly contains within-site replication as part of the design. The risk associated with a lack of replication or other experimental design components such as randomization is that managers will make poor decisions thinking they are supported by "science". SSR solves this issue by implementing a robust experimental design, including within-site replication, which can simultaneously test multiple restoration techniques while also making contributions to ecological or restoration theory.

Additional replication including temporal and other spatial replication is easily added to address other common issues when conducting habitat restoration. These additions are not required components of SSR, but rather can be added to increase the robustness of the design, depending on the needs of the practitioner. For example, interannual variation in establishment success, or "year effects", has been a persistent problem for ecological restoration practitioners (Young et al. 2005). SSR can provide insight into this variability by comparing treatments implemented in an experimental array in one year and in scaled up areas in other years. Furthermore, SSR can be expanded to explicitly evaluate the importance of interannual variation by establishing experimental arrays in multiple years. By doing so, SSR can better account for interannual variability and more reliably predict which restoration treatments will work at a site.

In addition to temporal replication, SSR can also accommodate spatial replication by implementing it across sites. If land managers are trying to determine restoration designs for a whole system and not just a single site, or if a site has very heterogeneous characteristics, spatial replication would be important to account for the effect of different site conditions on restoration treatments.

Collaboration

SSR provides a platform for greater collaboration between all parties involved in a restoration project, such as ecological researchers and restoration managers, which has been identified as an area of conflict in other AM applications (Walters 1997, Gregory et al. 2006). The rigorous experimental design can meet fundamental and ancillary research needs of participating researchers while the scaling aspect systematically restores a site and meets a restoration manager’s goals. As with any AM approach, collaboration between researchers, managers, and other stakeholders is critical for success. The design of SSR can enhance this collaboration for several reasons. First, it is able to more fully meet research goals to ensure research agendas don’t hijack management goals. Additionally, the design can test multiple approaches more quickly than traditional AM or a trial and error management approach; therefore, decision makers and policy leads can be more confident in the direction of the restoration. Finally, the design encourages operationally realistic treatments even at the plot level, and this better informs researchers on the available larger scale management options.

Costs and Value

The real costs and opportunity costs of implementing AM are often cited as a reason for not using the approach, particularly when restoration funding is limited. It is difficult to quantify the cost differences between a SSR and a trial and error approach to restoration. The costs of implementing SSR will be greater in the initial stages on a per hectare basis than another approach. However, the primary value of an SSR design is that the level of uncertainty in restoration methods for a site is substantially lower by the time larger areas are being restored. This is likely a substantial cost savings compared to immediately moving forward with untested methods at larger scales, having them fail, and having to restore them a second time. Additionally, the opportunity cost of a delay in getting larger areas restored can be perceived as an impediment. However, with SSR, within a short period of time, large areas can be restored in a systematic and experimental fashion that simultaneously provides robust, scientifically-derived information regarding the relative success of different treatments on a management site. These results can then be scaled up even further if resources allow.

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Staged-Scale Restoration - Case Study in Puget Lowland Prairies

The native Puget Lowland Prairies of western Washington are one of the most endangered ecosystems in the United States and have been nearly extirpated from the region (Noss et al. 1995). It is estimated that <10% of the original 180,000 acres of pre-European settlement prairie remains, and only about 3% is dominated by native prairie species (Crawford and Hall 1997). A suite of rare and endangered species are associated with this habitat, including invertebrates, mammals, birds, amphibians, and plants.

Habitat degradation and loss have been identified in recovery and status documents and by biologists as key factors contributing to the rarity of these plants and animals (USFWS 2000; Stinson 2005) . Restoration is necessary to maintain existing prairie lands and to recover rare and endangered species. To date, prairie restoration efforts in western Washington have focused exclusively on restoring moderately degraded sites that still retain a significant component of native species - the 3% referred to by Crawford and Hall (1997). To reduce fragmentation and enlarge existing prairies that will better sustain rare species, managers must think beyond the remaining prairie parcels to consider the restoration value of cropland or abandoned agricultural lands. This requires that they develop techniques to restore severely degraded areas, or areas without any native species present. Since such restoration has never been attempted in Puget Lowland Prairies, there are many unknowns about how this can be successfully and efficiently accomplished. I therefore sought to develop and test such techniques to restore abandoned agricultural fields to diverse, native prairie habitat that can sustain a suite of rare prairie butterfly species. I implemented SSR with these research restoration treatments to simultaneously restore large areas on each of the sites while conducting the research.

Case Study - Site Description and Design

My study was installed in four Puget Lowland Prairies. Glacial Heritage and West Rocky Preserve are sites in South Puget Sound, while Smith Prairie and Ebey's Landing Preserve are on Whidbey Island. The 490 ha Black River-Mima Prairie-Glacial Heritage Preserve (Glacial Heritage) is one of the largest protected grasslands in western Washington. Glacial Heritage is owned by Thurston County, Washington Department of Fish and Wildlife and managed by The Center for Natural Lands Management. Approximately 12 km east of Glacial Heritage, the 135 ha West Rocky Prairie is owned and managed by the Washington Department of Fish and Wildlife. Smith Prairie is the largest protected grassland (70 ha) on Whidbey Island and is owned and managed by Pacific Rim Institute. Approximately 10 km to northwest, the 20 ha Ebey's Landing Preserve is owned and managed by The Nature Conservancy.

The first stage of SSR involves setting up the experimental array. My experimental design consisted of seven combinations of site preparation methods and seed mixes (Table 4.3) randomly assigned to 35 treatment plots at each site. Treatments included combinations of three site preparation methods and three seeding mixes. Treatments were chosen in collaboration with land managers and were based on the broad goal to create habitat suitable for a suite of rare butterfly species. The plots were approximately 40 meters square in South Sound and 25 meters square in North Sound, with each plot separated by 2-meter aisles and borders. Plots were smaller in North Sound due to more limited seed availability. Cumulatively, each array occupied approximately 0.26 ha in South Sound and 0.19 ha in North Sound, including aisles and borders. Since scalability of treatments is an integral part of the SSR, we selected treatments that could be applied at increasingly larger scales, and used experimental plots sufficiently large to permit the operation of mechanical equipment (Figure 4.4).

The extant vegetation at all sites was dominated by non-native pasture grasses or weedy species; there were no species that I wanted to conserve so the entire array was treated with a non-selective herbicide (glyphosate) using a boom-sprayer prior to application of the experimental treatments. One set of plots was assigned to the broadcast burn treatment, which consisted of burning in the summer prior to seeding. A second set received a solarization treatment, which consisted of plowing and roto-tilling the soil and installing a 2mm clear plastic in June. The plastic remained in place until September, and was intended to kill weeds and seeds by elevating soil temperatures, as has been done in other restoration experiments (Schultz 2002). A third set of plots received a 2-year herbicide treatment; these were sprayed as needed for two seasons before seeding. Because of the longer duration of the preparation treatment, these plots were seeded one year after the other plots within the respective array.

Seeding occurred in early November each year. Seed mix treatments each contained ca. 21-26 native forbs and grasses and ca. 700 seeds per square meter. Three grass:forb mixes were tested with ratios of 50:50 (grass rich), 2:98 (forb rich), and 25:75 (mixed). These different ratios of the number of seeds sown were chosen to determine which was most effective in establishing important host and nectar sources for a suite of rare butterfly species. Some studies have shown grass rich mixes effective in suppressing weed and non native species (Török *et al*. 2010), while others advocate for initially sowing a higher density of forb species, as these species may be difficult to insert later into well-established grass-dominated vegetation (Dickson and Busby 2009).

Each treatment was replicated on five plots within each array. Furthermore, establishing arrays at four sites allowed us to compare the effectiveness of the same treatments among sites that differed in several respects. We were particularly interested in understanding the degree to which interannual variation affected treatment outcomes, so we also incorporated temporal replication into the design. This was accomplished by repeating the same experimental treatments on separate arrays over three years. Results from experimental treatments in Years 1-3 determined which treatment(s) were applied to scaled-up plots in years 2-5 (Figure 4.4).

Case Study - Implementation

Table 4.4 details the scaled plots, treatments, and year of installation for South and North Sound research areas. In the South Sound sites, a total of six 10x and three 100x scaled plots were installed at each site in addition to the three experimental arrays. 10x plots were approximately 400 square meters and 100x plots were approximately 4,000 square meters. In the North Sound, after the three experimental arrays were established, only three 10x scaled plots were installed at each site due to seed limitations, and the fact that we started a year later in the North Sound sites. The total research area including experimental arrays and scaled plots was just over 2 hectares at each South Sound site and 0.3 hectares at each North Sound site.

First year seeding results in South Sound did not show a difference between burning and herbiciding at Glacial Heritage, so both site preparation treatments were scaled by 10x in stage 2. At West Rocky preserve, however, the broadcast burn treatment was ineffective as a site preparation due to the strong seedling response of a non-native weed, *Leucanthemum vulgare*. Therefore, two solarization treatments and one herbicide treatment were scaled by 10x in stage 2. In North Sound in year 3 a similar pattern emerged between sites as in South Sound. Plots at Ebey's Landing showed no difference in physical treatments. However, at Smith Prairie only solarization and herbicide treatments were scaled up in stage 2, as broadcast burning also encouraged significant weed establishment of species such as *Cirsium arvense* and other agriculturally-associated weeds.

By scaling up treatment areas by a factor of ten each year, the majority of the restoration occurred in years 3 and 4. By this time, we had results from the first year of each of the experimental arrays in addition to second and third year results from the first experimental arrays installed. Although our overall establishment data did not show strong differences among the physical treatments in terms of species richness or density established, we began to see trends where the 2-year herbicide treatment was showing the most promising results (Figure 4.5). This was especially true for particular species of concern such as an endangered plant in our seed mix, *Castilleja levisecta*. *C. levisecta* had very poor establishment in solarization treatments. Given that this species was a priority restoration objective, and several of the sites had increased weed establishment associated with the burn treatments, we decided to use the herbicide treatment for most of our stage 2 scaled up site preparation.

Seeding treatments varied by site and generally it was site differences, especially the existing plant community present on each site, which influenced the seeding treatment that we decided to use in the scaled-up plots. As in the physical treatments, we did not see significant differences in the results produced by the three seeding treatments on either the richness or density of seeded species that established at any site in the first year. However, in general we increased the proportion of grass seed in the scaled-up arrays in sites with more broadleaf weed species, to help with weed suppression. At Glacial Heritage for example, where our existing community was predominantly non-native graminoids, we used the forb rich seed mix in scaled-up plots, to maximize the potential nectar and host plant species for butterflies of concern. At West Rocky, however, non-native weed species began to out compete sown species in our first year experimental array, so in consultation with the land manager we chose to scale sowing *Festuca roemeri* only without any additional forb species until non-native weed species could be controlled. In North Sound we used a modified "mix" seed treatment to help suppress weed species, which included the forb-rich seed mix and additional grass seed. Each of the sites received a slightly modified seed mix based on discussions with land managers, species availability, and results in experimental arrays.

The treatments in the scaled up plots (stage 2 and 3) responded as we expected based upon the experimental arrays at each site. Each treatment in scaled plots at a site equaled or outperformed its corresponding treatment combination in the experimental arrays (Figure 4.6). The exception to this was at West Rocky, where we chose to scale a grass treatment with *Festuca roemeri* only while the land manager focused on weed control before other forb species were sown.

Case Study - Summary

Over the course of five years I implemented a SSR design across four sites in severely degraded Puget lowland Prairies. The results included restoration of approximately six hectares and refined restoration techniques for each of the sites. It is important to note that this case study does not represent a basic SSR design, which would be implemented at just one site and with one experimental array. Rather, I wanted to demonstrate the additional components of temporal and spatial replication that could be included in the design. That replication proved to be valuable in this case as I will discuss below with additional thoughts on the SSR design.

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Discussion

My case study implementing a staged-scale restoration design in Puget Lowland Prairies demonstrates that this concept can be an effective adaptive management strategy to restore a site while also conducting a controlled restoration experiment. Restoration of Puget Lowland Prairies provides an ideal environment for implementation of active adaptive management. Many of the sites being restored do not have rare and endangered species present, but habitat restoration is being conducted to support the reintroduction of these species, in addition to *C. levisecta* being included in the seed mix. There is still high uncertainty on how to create appropriate habitat for rare butterfly species, for example, but there is relatively low risk for land managers in trying a suite of restoration techniques. Restoration work is currently not being conducted on severely degraded habitats as with my case study. However, within the extant prairie system, restoration is predominately done with trial and error management and the use of established techniques. I believe AM could be widely adopted in Puget Lowland Prairies overall. One of the reasons it is not currently applied is the unwillingness of land managers to commit large areas to uncertain or undesirable outcomes. In this and other systems, SSR provides land managers with a viable AM framework that does not commit larger areas of a site to restoration until techniques have been tested and refined on that site.

The importance of the “site effect”, or how treatments respond to particular site conditions, was one of the most significant findings in our Puget lowland prairie case study. Despite the fact that within the two regions of North and South Sound, sites were less than 15 kilometers apart, the sequence of scaled up treatments I installed differed at every site, and during the final year of scaling up treatments, the largest scaled up treatments differed at 3 of the 4 sites. The strong site differences that I observed in my case study emphasize the importance of tailoring restoration actions for specific sites. In this respect, SSR is a well-suited adaptive management technique as it can be tailored to the site on which it is applied.

The size of the scaled treatments is also not fixed. I chose to scale first by 10x and then by 100x simply due to the limitation of seed for sowing at large scales in this system. The sowing of the 100x scaled plots at Glacial Heritage in 2011 with 26 native forbs and grasses represented the largest sowing of a diverse prairie seed mix (more than a few species) ever conducted in Puget lowland prairies. Figure 4.7 provides an aerial view of how each of the experimental arrays and scaled plots were implemented on each site.

In addition to sites, the effect of interannual variability on my results is also an important finding. I added the temporal replication to our experimental design as interannual variability is a known issue in restoration design (Vaughn and Young 2010) and I wanted to account for changes in establishment from year to year. The addition of temporal replicates was very useful in determining what treatments to scale at every site. Surprisingly, I had no consistently effective treatment. No treatment worked at all sites in the same year or all years at the same site. For example, the solarization treatment was relatively effective at sites in the South Sound in the first year, but proved ineffective in the experimental arrays from years 2 and 3. If I had not replicated our experimental arrays I may have come to the same conclusion but perhaps only after scaling the treatment on a much larger area. Given the level of interannual variability of weather, establishment, etc., in most ecological systems, I recommend adding this component to SSR.

There are some limitations and considerations to the SSR approach that should be discussed among researchers and land managers. First, the ongoing management of the initial experimental arrays through time should be planned for in advance. Researchers and managers should be on the same page as to how they will be managed. For example, will invasive species be controlled or allowed to remain in order to monitor longer-term effects? Additionally, the research arrays can have significant edge effects that may influence treatments on a plot level differently than at scale. The experimental arrays also leave a “grid” imprint on the site, particularly if larger aisles are maintained between treatments, and weeds must be controlled in the aisles. Finally, the entire approach takes time. While the time could be an advantage, it could also be a limiting factor if restoration funding and timing is time sensitive. One of the advantages of time that I discovered with my case study, however, was that it allowed me to build enough seed resources to continue the scaling up of the restoration, as I was able to collect seed from the 10x scaled plots. Despite the potential limitations with this process, good communication and planning can mitigate most of them, or at least help clarify goals of the restoration.

The costs and benefits of implementing a staged-scale design compared to a trial and error approach or a traditional active adaptive management approach are difficult to determine, but I think the benefits outweigh the extra costs. There are real costs associated with establishing, maintaining, and monitoring the experimental array, so SSR will initially cost more on an area basis than implementing a single alternative. However, the information that the experimental arrays and/or the staged-scaling provide in terms of determining appropriate restoration techniques may be worth the extra costs, particularly if a single treatment was applied to large areas but was ineffective. Seeding, for example, can be a very expensive restoration method as the cost of seeds can be high (Wagner and Pywell 2011). If native seeds are not available commercially, the cost of collecting and propagating seeds can be prohibitive. Consider again West Rocky from my case study example; my first year results from the site looked promising for a couple of the treatments. Only after scaling the first 10x plots did it become evident that the site preparation techniques were not effective. If alternative treatments had been applied on a larger scale immediately, much seed, effort, and funding would have been wasted. SSR reduces the risk of implementing an ineffective treatment over a large area.

Conclusion

Forty years after its introduction, the concept of AM is well established and embraced in the natural resource management and restoration fields. However, there are still relatively few examples of successful application of AM. Many restoration projects provide an ideal platform for AM implementation, yet it is rare to find examples of AM applied in a restoration setting. There are many articulated reasons for AM failure in implementation or adoption, and generally they can be attributed to concern over the risk, scientific rigor, collaboration, and costs and value of the approach. Staged-scale restoration (SSR) provides an alternative strategy for implementing AM in restoration projects that minimizes risks, improves scientific rigor, encourages collaboration among researchers and managers, and provides a cost effective restoration approach. This technique should encourage adoption of AM in restoration projects, while systematically conducting restoration.

Tables and Figures for Chapter 4

Table 4.1. Descriptions of reasons for failure in application of adaptive management (adapted from Allen and Gunderson (2011)).

Author	Reason for AM Failure
Feldman (2008)	Unwillingness of managers to risk experimentation with rare resources
Gregory et al (2006)	Failure of scientists to understand the array of management options
	Lack of attention to building shared understanding with stakeholders
	Scientists overstating their capacity to measure complex interactions
Lee (1999)	Difficulties in translating learning into practice
	The costs and delays in gathering information and learning
	Uncertainty whether the AM approach works
	Valuing action more than learning
Walters (1997)	Research interests hijack management goals
	Bureaucratic and political inaction
Moir and Block (2001)	Timelines too long and perceived costs too high
Walters (2007)	Failure of decision makers to understand the need for AM
	Inadequate funding for increased monitoring need
	Lack of leadership for complex process of AM

Table 4.2. Descriptions of reasons for failure in application of adaptive management (Table 4.1) grouped by common themes.

Risk

- Uncertainty whether the AM approach works
- Unwillingness of managers to risk experimentation with rare resources

Scientific Rigor

- Scientists overstating their capacity to measure complex interactions
- Difficulties in translating learning into practice

Collaboration

- Failure of scientists to understand the array of management options
- Research interests hijack management goals
- Lack of leadership for complex process of AM
- Bureaucratic and political inaction
- Lack of attention to building shared understanding with stakeholders
- Failure of decision makers to understand the need for AM

Costs and Value

- Inadequate funding for increased monitoring need
- The costs and delays in gathering information and learning
- Timelines too long and perceived costs too high
- Valuing action more than learning

Table 4.3. Site preparation and seeding treatments tested. Plots assigned to the broadcast burn treatment were burned in the summer prior to seeding. The solarization treatment consisted of plowing and roto-tilling the soil and installing a 2mm clear plastic in June, which remained in place until September. Plots with the 2-year herbicide treatment were sprayed as needed for 2 seasons before seeding. Three grass/forb mixes with 21 species in North Sound and 26 species in South Sound were tested with ratios of 50:50 (grass rich), 2:98 (forb rich), and 25:75 (mixed).

Site Preparation	Seed Mix
B (broadcast burn)	G (grass-rich)
B (broadcast burn)	M (mixed)
B (broadcast burn)	F (forb-rich)
S (solarized)	G (grass-rich)
S (solarized)	M (mixed)
S (solarized)	F (forb-rich)
H (2-year herbicide treatment)	F (forb-rich)

Table 4.4. Scaled-up treatments at the four restoration sites. Physical treatments are indicated with the first letter and seed mixes with the second letter. The herbicide treatment (H) was the most consistently applied treatment at scale and seeding treatments varied by site. (*Treatment codes: B-Burn, S-Solarization, H-Herbicide, F–Forb, M-Mix, G-Grass*)*100x plots at West Rocky only included *Festuca roemeri* to enable better weed control. The HM treatments in North Sound included "F' treatment densities but included additional grass species to assist with weed control. Overall customization of treatments is an integral part of the SSR design as scaling advances at a particular site.

Region	Site	Scaling	Year 1	Year 2	Year 3	Year 4	Year 5
South Sound	Glacial Heritage	10x		B F	B F	H F	
		10x		S F	H F	H F	
		100x				B F	
		100x				H F	H F
		Array	All	All	All		
	West Rocky	10x		S F	H F	H G	
		10x		S F		H G	
		10x				H G	
		100x				H G*	
		100x				H G*	
		100x				H G*	
		Array	All	All	All		
North Sound	Ebey's Landing	10x			B F		
		10x			S M	H M	
		Array		All	All		
	Smith Prairie	10x			S F	H M*	
		10x				H M*	
		Array		All	All		
Scaled Area by Year (m^2)				1,600	1,950	22,750	6,000
Experimental Area by Year (m^2)			5,200	9,000	9,000		

Total Restored Area 55,000 (m^2)

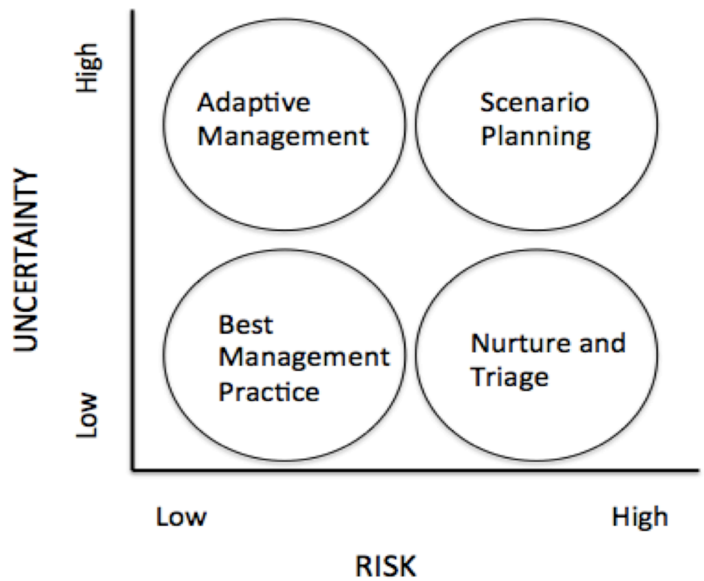

Figure 4.1. Adaptive management (active or passive) is appropriate where the level of risk is relatively low, where it is safe to conduct experiments (adapted from Allen and Gunderson 2011).

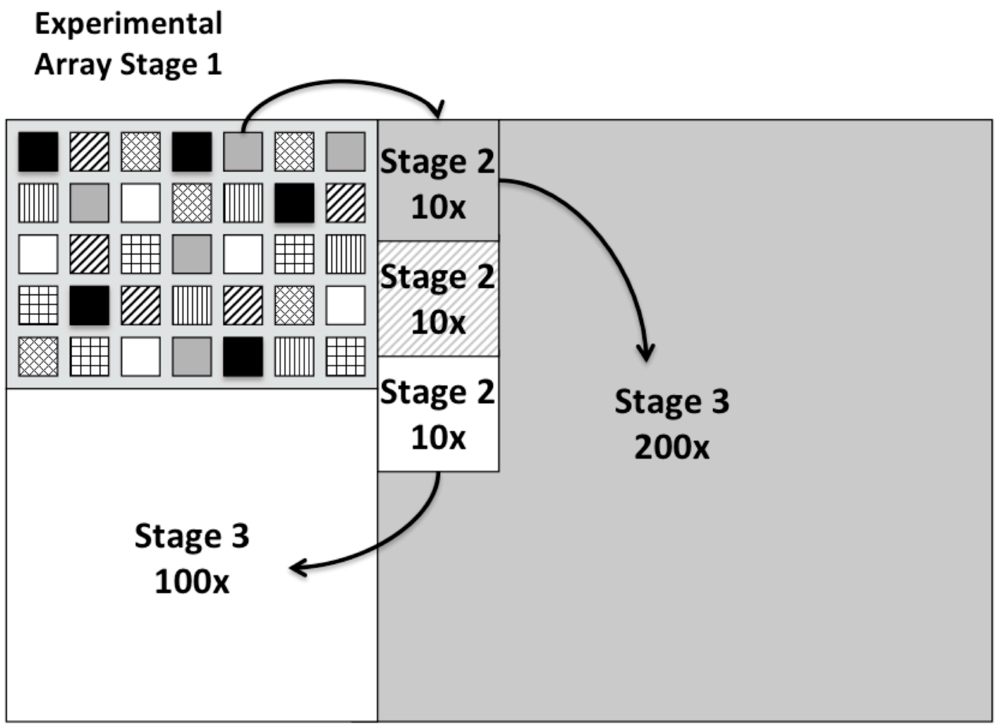

Figure 4.2. Conceptual design of stage-scale restoration where scaled-up areas are 10x as large as experimental plots in stage 2 and 100x as large in stage 3. The different colors and patterns in the experimental array represent different treatments. In this example, in Stage 2, several of the treatments in the experimental array are scaled by 10x. In Stage 3 two treatments are chosen to scale by 100x and 200x. In practice, any number of scaled treatments in stage 2 or 3 can be applied at any scale, and it is possible that scaling of different treatments at even larger and larger scales may be appropriate depending on restoration goals.

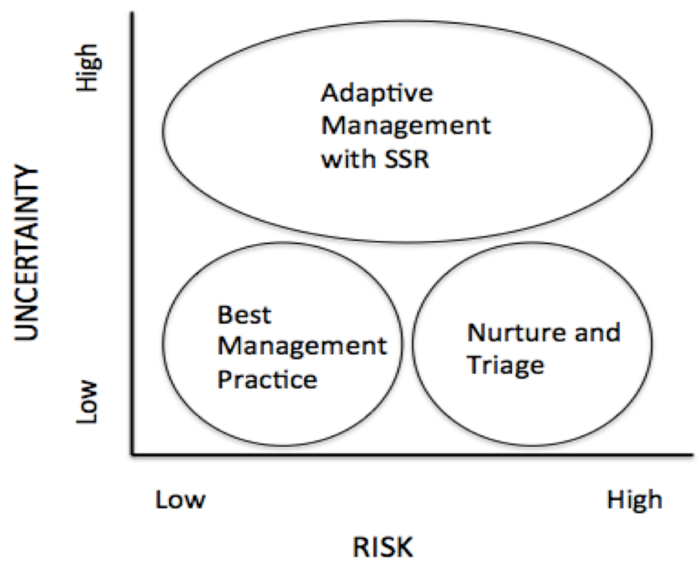

Figure 4.3. SSR expands the instances where AM may be applied in restoration contexts (see Figure 4.1). Since SSR can start as small as needed and gradually scale up, practitioners working even in a scenario with high risk can apply AM using SSR. In figure above SSR replaces scenario planning as a strategy for high risk and high uncertainty situations. It is also appropriate for AM in low risk situations. (Adapted from Allen and Gunderson 2011).

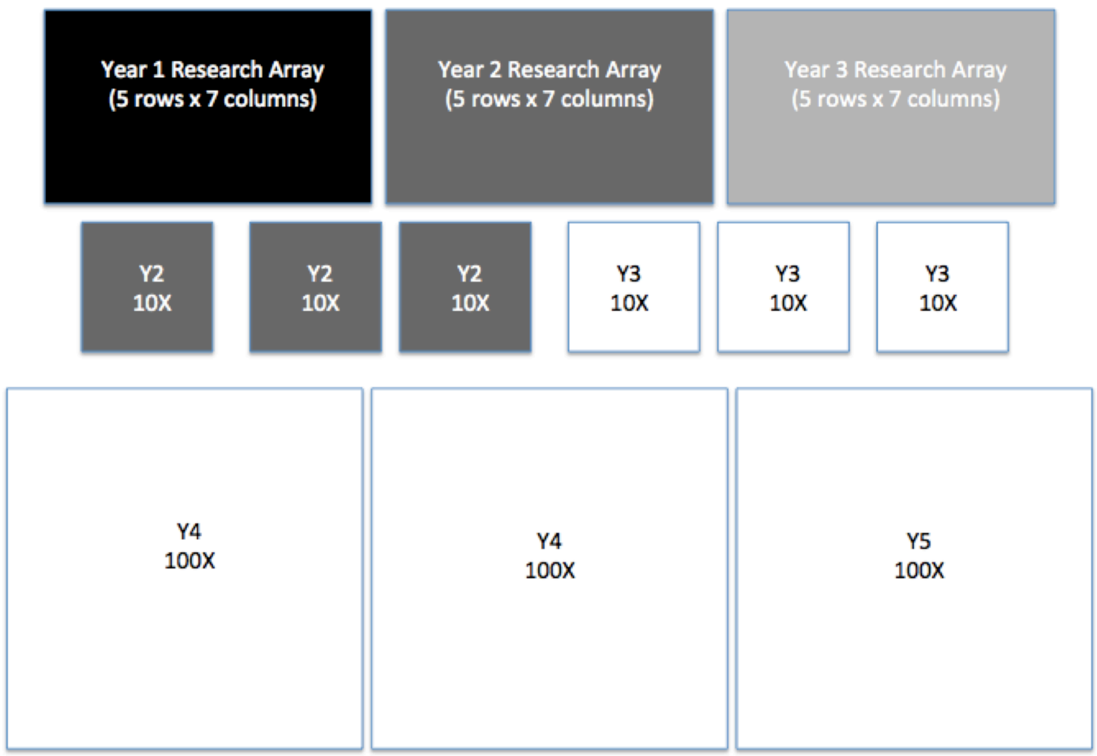

Figure 4.4. Conceptual model of how treatments were implemented in a Staged-Scale Restoration design at a single site in a South Sound Puget Lowland Prairie from Year 1 to Year 5. An array of 35, 40m^2 experimental plots was established in Year 1 to compare 7 treatment combinations ("Year 1 Research Array" – detail of individual plots not shown in figure; black). Spatial replication was accomplished by repeating each treatment on 5 plots within the array (within-site replication), and by establishing arrays at multiple sites (not shown in this figure). Temporal replication was accomplished by repeating the same experimental treatments on 35 plot arrays established in Year 2 (dark grey) and Year 3 (grey). The scaling component of the restoration design began in Year 2, when the two most successful treatments identified on the Year 1 plots were applied to 3 plots that were 10x larger (400m^2) than an experimental plot. This scaled-up treatment was again replicated spatially (multiple sites), and temporally, with the additional 10x plots implemented in Year 3 and 100x plots implemented in Years 4 and 5. (not to scale)

Figure 4.5. First year establishment data for all experimental arrays at all sites. Species richness and density are depicted as a percentage of species seeded. Overall, there were no strong differences in richness or density, though herbicide treatment was more effective, followed by burn and solarize. (Bakker et. al unpublished data)

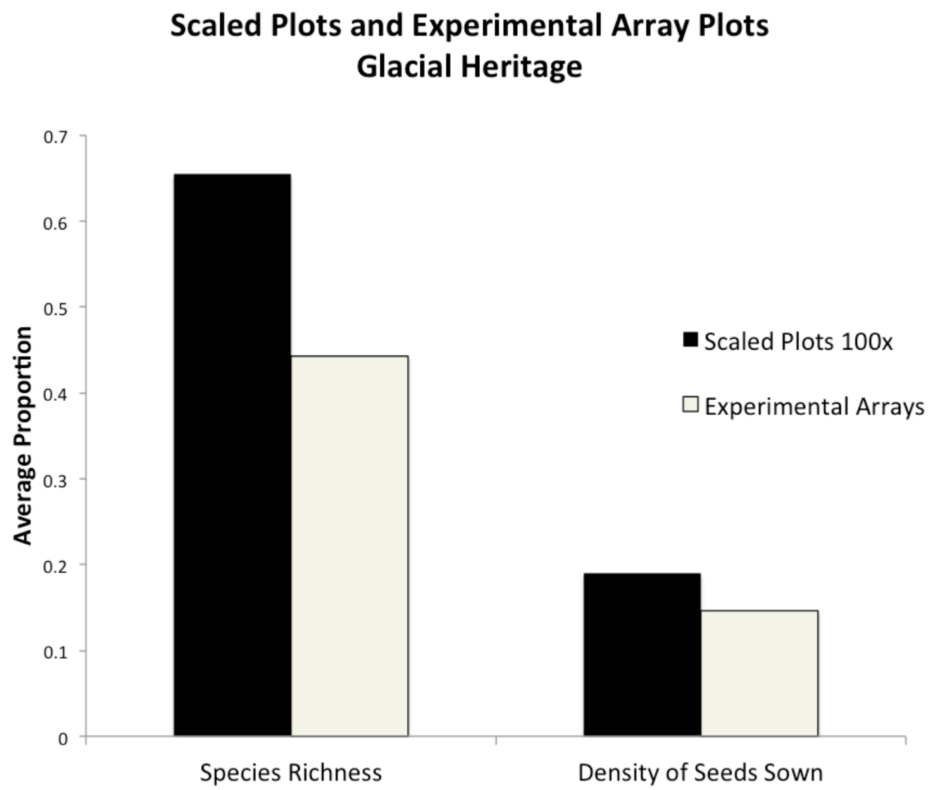

Figure 4.6. First year average proportion of species richness and density of sown species of 100x scaled plots with an Herbicide/Forb treatment from 2012 and corresponding treatment from experimental arrays in 2009, 2010, and 2012.

Figure 4.7. SSR design footprint at each of the four sites, beginning in the top left and working clockwise: Pacific Rim Institute Preserve, Ebey's Landing Preserve, Glacial Heritage Preserve, and West Rocky Preserve. Yellow outlines are the experimental arrays, 10x scaled plots in Blue, and 100x scaled plots in Red. The year represents the year of the first monitoring. Plots were sown the fall before the first monitoring. Some sites have spaces or gaps between arrays or scaled areas. These gaps were left in consultation with the land managers to preserve existing features on the sites or accommodate other research areas. Images are not shown at the same scale.

Chapter 5

Restoring Abandoned Agricultural Lands in Puget Lowland Prairies:

Conclusions and Recommendations for Further Research

The restoration of abandoned agricultural lands to Puget Lowland Prairie will be an important future strategy for the recovery of the ecosystem. There is a growing recognition in the conservation community that existing sites and sites with limited soil and site heterogeneity are not sufficient for the full recovery of the system. In fact, former agricultural lands are currently being considered for purchase in the region to restore to native prairie. Therefore, there is a critical need to develop techniques and methods for restoring abandoned agricultural lands to native habitat. While restoration of former agricultural lands to native grassland is well established in some parts of the world, this project represents the first attempt in this region. In this dissertation I explored techniques to restore native prairie habitat on a suite of abandoned agricultural lands over a large spatially and temporally replicated Prairie Habitat Restoration Project (PHRP).

In my first study (Chapter 2), I focused on one of the region's rarest species, *Castilleja levisecta*, in the context of the PHRP. The PHRP combined three site preparation treatments (burning, solarization, and repeated herbicide application) with three seeding treatments of between 21 to 26 native prairie species. I explored techniques for direct seeding of *C. levisecta*, over 3 years and 4 abandoned agricultural sites, and determined which treatments and combination of treatments resulted in the best establishment, survival, and reproduction of the species. I found that burning and repeated herbicide treatments were the most effective site preparation techniques before seeding. Through the course of this research, I successfully established more than 100,000 *C. levisecta* plants within 3 years on all sites. I also found that sites with more productive soils had better establishment than those with less productive soils. This finding suggests that severely degraded habitats such as abandoned agricultural areas are appropriate sites to consider for the recovery of this endangered species.

My second study (Chapter 3) focused further on *C. levisecta* in a separate experiment, also installed on abandoned agricultural land, designed to better understand the effect of host, host species, and planting size on survival and performance. I replicated outplantings in a field setting over two years to explore how host species and container size affected survival and performance (flowering and fecundity) of *C. levisecta*. I found that both host presence and, more importantly, host identity significantly affected survival and fecundity. Plants grown with a host had higher survival and fecundity than those without, and plants grown with *E. lanatum* significantly out-performed those grown with *F. roemeri*. These results confirm, as with other hemiparasites, that *C. levisecta* does not obtain equal fitness from all hosts. Host identity should be an important and perhaps pivotal decision in planning for recovery of the species. However, as the host requirements and effects are still not well understood for *C. levisecta,* an appropriate strategy is to provide a diverse seed mix in any restoration effort. Kaye, for example, recently found that perennial plant diversity improved *C. levisecta* survival at the microsite scale (Kaye 2011). Until a range of host species is known and the mechanisms driving the relative advantages of host species is better understood, ensuring a suite of potential host species is important, particularly as land managers begin seeding former agricultural lands.

My final study (Chapter 4) took a broader view and focused on an innovative design feature I employed as part of the PHRP, Staged-Scale Restoration (SSR). SSR is a method to apply adaptive management (AM) to a system in a restoration context. Several promising restoration approaches are identified that can be applied at large scales, and then tested in small, replicated experimental plots directly on the restoration site. Restoration of each site proceeded in progressive steps by implementing the most successful approaches at increasingly larger scales. I believe that many restoration projects provide ideal platforms for AM implementation, though it is rare to find examples of AM applied in a restoration setting. My results demonstrate that SSR is a feasible site-specific method for implementing AM in restoration projects that minimizes risks, improves scientific rigor, collaboration among researchers and managers, and provides a cost effective restoration approach. This technique should encourage adoption of AM in restoration projects, while systematically restoring a site.

One of the themes that emerged from each of my studies was that the restoration of former agricultural lands or areas that are devoid of native species is a rational and feasible strategy for Puget Lowland Prairies. In addition to successfully establishing *C. levisecta,* all of the other species in the PHRP mix each established in at least one of the different treatment combinations. Further analyses will explore which species and combinations of species establish best with particular treatments. However, we have established that the restoration trajectory, at least for some sites, can be very quick from completely non-native grassland to native and diverse prairie habitat.

The importance of site in determining the outcome of my different studies was another overarching theme. *C. levisecta* establishment and performance was more affected by the sites than the physical and seeding treatments. Additionally, different treatment combinations were scaled at different sites as part of my Staged-Scale-Restoration (SSR) design. These findings underscore that land mangers should take caution in applying “silver-bullet” treatments, and should ideally use a method such as SSR to tailor restoration treatments to specific sites.

Finally, one of the most striking themes was the role of interannual variability in treatment effects. Whether it was the establishment of seeded species such as *C. levisecta* (Chapter 2), or survival of different size pot treatments (Chapter 3), or the lack of consistent treatment effects in the SSR experiment (Chapter 4), interannual variability was an important driver of the results. The fact that treatments can be replicated, in as close to the same manner as possible, in one year after another and yet have widely varying results definitely limits the ability to predict the outcomes of restoration actions. However, another way to consider this reality is that interannual variability actually ensures heterogeneous communities at a site, assuming the site is restored systematically through time.

Recommendations for Future Research

A comprehensive spatial analysis of potential abandoned agricultural lands that could be restored to native prairie is an important conservation measure that should be conducted. The recently published Prairie Landowner Guide (Carver and Noland 2011) provides a good start with a spatial analysis of “potential” prairie soils, and illustrates the expansion of this habitat type that could occur if lands outside of the extant sites are considered for restoration. However, this analysis needs to be combined with analysis of landscape context (to protected areas for example) and restoration opportunity to develop a list of potential conservation sites. Once restoration occurs on these abandoned agricultural lands sites, researching the management of the sites in comparison with current extant sites is important, as they will likely differ in terms of timing of restoration actions and techniques.

Much of my research focused on recovery of *Castilleja levisecta* and I have several recommendations for further study. First, continued research to understand the effect of the presence and abundance of different host plant species on *C. levisecta* performance should be continued. Conversely, understanding the effect of *C. levisecta* on the neighboring community is equally important. In the future , as more productive sites are considered for restoration, I think practitioners could look to using a species like *C. levisecta* to maintain diversity in sites, just as *Rhinanthus minor*, an annual hemiparasitic plant, is used maintain diversity in European grasslands (Pywell et al. 2004, Westbury and Davies 2006). Lastly, another research need and potential opportunity to recover *C. levisecta* while achieving other conservation outcomes pertains to interactions with a rare butterfly, Taylor's checkerspot (*Euphydryas editha taylori*), which uses *C. levisecta* as a host. As larger areas of *C. levisecta* are sown, an ideal platform is provided to study interactions with Taylor's checkerspot and explore how the two species might be recovered synergistically.

Finally, I recommend applying the Staged-Scale Restoration approach to other Puget Lowland Prairie sites and other ecosystems. It is not necessary to apply either the temporal or spatial replication that I incorporated as part of the PHRP. Further implementation of this adaptive management approach will confirm whether this method of AM will encourage the adoption of AM in restoration projects and ultimately result in more effective restoration on a site by site basis.

Grasslands are likely to remain one of the most imperiled ecosystems on the planet, and future anthropogenic effects such as global climate change are going to require land managers and conservationists to be both adaptive and flexible to protect and restore them. The research that I have detailed here provides alternative strategies for restoring Puget Lowland Prairies and contributes to the robust body of restoration ecology of this system and of grassland restoration ecology overall. It is my hope that this research will specifically help in the recovery of one of our rarest species, *Castilleja levisecta,* and will more broadly provide restoration strategies and ideas to help restore the Puget Lowland Prairie system.

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APPENDIX A

Prairie Habitat Restoration Project Sown Species and Sowing Densities

Species densities are shown by the number of seeds sown per square meter in South Sound and North Sound plots. A zero indicates that the species was not part of the seed mix for that region, as the South Sound and North Sound mixes differed. Densities were estimated based on seed mass and estimated number of seeds per gram. Seed densities varied depending on seed mix, Grass (G), Mix (M), or Forb (F).

		South Sound			North Sound		
Species	Seeds per gram	G	M	F	G	M	F
Achillea millefolium	3129.07	4	6	8	17	18	23
Allium acuminatum	359.28	0	0	0	12	18	23
Allium cernuum	359.28	0	0	0	12	18	23
Aquilegia formosa	324.68	4	6	7	0	0	0
Armeria maritima	359.28	4	6	7	12	18	23
Balsamorhiza deltoidea	166.67	4	6	7	0	0	0
Camassia quamash	333.33	19	28	37	20	30	39
*Castilleja hispida**	8333.33	76	113	148	76	113	148
Castilleja levisecta	8333.33	76	113	148	42	62	81
Cerastium arvense	6382.98	7	11	14	18	27	35
Clarkia amoena	2000.00	0	0	0	0	0	0
Delphinium nuttallii	1041.67	4	6	7	12	18	23
Erigeron speciosus	6666.67	7	11	14	17	26	33
Eriophyllum lanatum	2083.33	7	11	14	53	71	103
Lomatium nudicale	150.00	0	0	0	18	27	35
Lomatium triternatum	246.51	8	11	15	0	0	0
Lomatium utriculatum	546.45	8	12	16	23	27	45
Lupinus albicaulis	25.70	2	3	4	6	9	11
Lupinus bicolor	200.00	0	0	0	35	44	58
Lupinus lepidus	245.50	8	11	15	0	0	0
Lupinus littoralis	200.00	0	0	0	7	10	13
Microseris lanceolata	1612.90	0	0	0	0	0	0
Plagiobothrys figuratus	2000.00	0	0	0	0	0	0
Plectritis congesta	1000.00	11	17	22	47	71	93
Potentilla gracilis	2000.00	18	27	36	0	0	0
Ranunculus occidentalis	431.03	19	28	37	30	13	17
Sericocarpus rigidus	1219.51	19	28	37	0	0	0
Solidago missouriensis	1612.90	8	12	15	0	0	0

Solidago spathulata	1796.40	15	23	29	24	36	46
Viola adunca	1060.07	38	57	74	0	0	0
Sub Total -forbs		**363**	**545**	**712**	**402**	**541**	**727**
Danthonia californica	248.55	167	83	7	53	26	2
Danthonia spicata	1351.35	33	17	1	0	0	0
Festuca roemeri	1421.80	18	9	1	263	131	11
Koeleria macrantha	5000.00	67	33	3	0	0	0
Sub Total - grasses		**285**	**142**	**11**	**315**	**158**	**13**
TOTAL DENSITY		**648**	**687**	**723**	**718**	**698**	**739**

**C. hispida* was sown at Glacial Heritage only in 2009 and at all sites in 2010 and no sites in 2011.

Appendix B

Photographs of Prairie Habitat Restoration Project

Appendix B.1. Installing 2009 array of the Prairie Habitat Restoration Project at Glacial Heritage in the spring of 2008. The entire array was sprayed with an non-selective herbicide (Glyphosate) before installation.

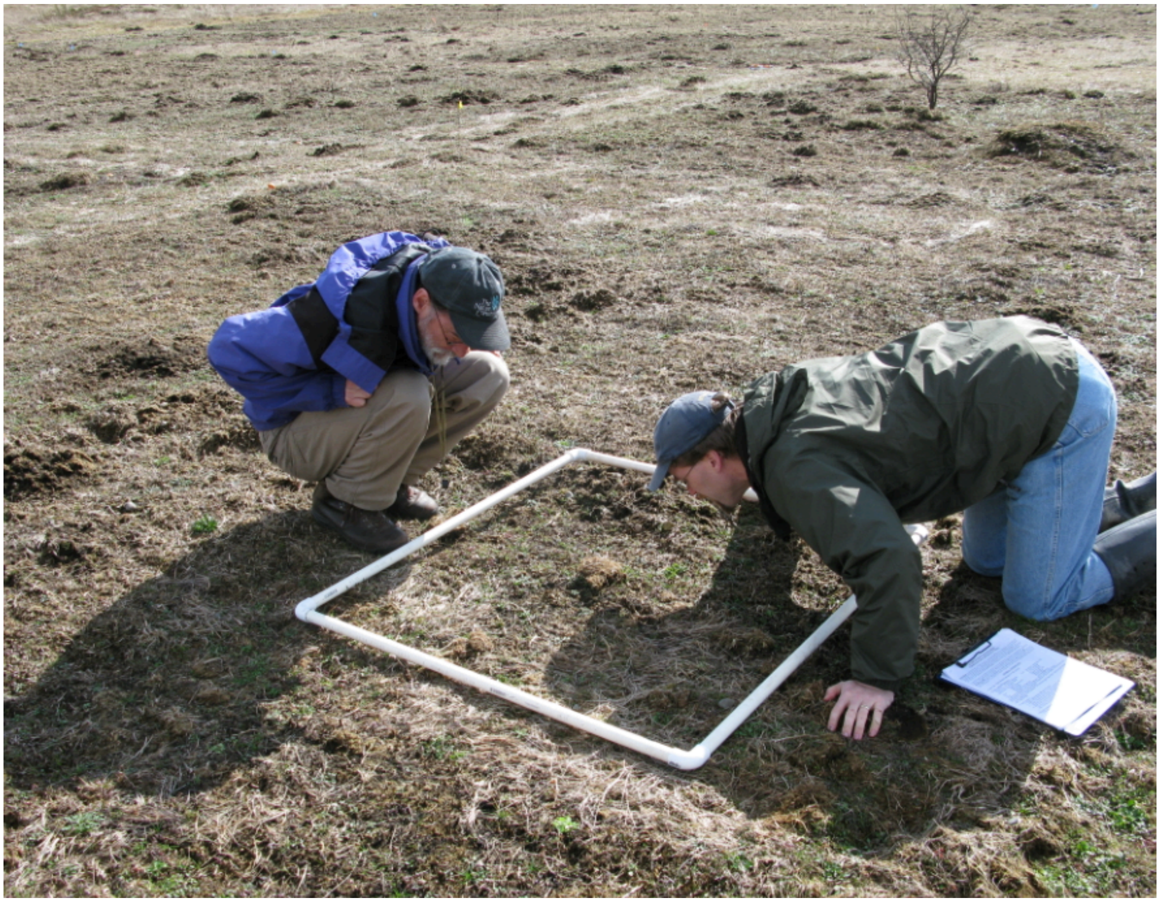
Appendix B.2. Plowing at Smith Prairie at Pacific Rim Institute in preparation for installing solarization plastic for the 2010 array at the site. At all sites solarization plastic was 2mm thick and was installed after plowing followed by roto-tilling.

Appendix B.3. Installing 2mm solarization plastic at Ebey's Landing Preserve site in the summer of 2009. Soil was used to cover the edges and the plastic was pulled as tight as possible before closing final edge. In the North Sound sites, deer proved to be problematic as they walked over the plastic and punctured it on all arrays multiple times.

Appendix B.4. Conducting a prescribed fire at the Prairie Habitat Restoration Project plots at West Rocky Preserve in the summer of 2008. Solarization plastic can be seen on adjacent plots.

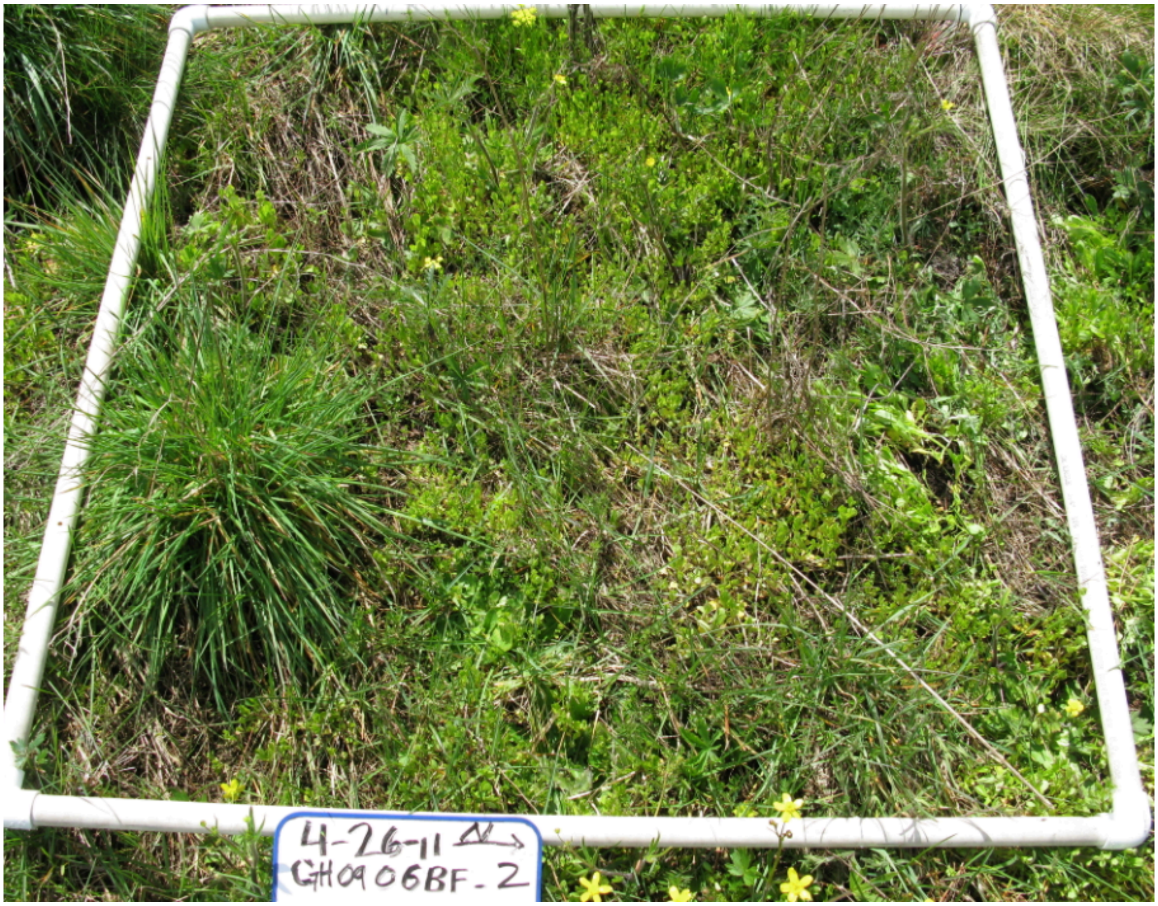
Appendix B.5. Prairie Habitat Restoration Project 2009 experimental array at Glacial Heritage. Image taken in July 2008.

Appendix B.6. Seeding the Prairie Habitat Restoration Project plots at Glacial Heritage in the fall of 2008. Seeding was done with a walk behind drop seeder. Seeds were mixed with wetted medium grade vermiculite to ensure uniform coverage of the plot. The corners of the plots were left unseeded and were covered while seeding as seen above.



Appendix B.7. Monitoring of the Prairie Habitat Restoration Project at West Rocky in the spring of 2009. Monitoring occured in the seeded and unseeded areas of each plot. Monitoring intensity varied with plot size. In the larger (40 m^2) South Sound plots, the seeded area was sampled with 6 quadrats, each 1 x 1 m, and the unseeded area with 2 quadrats, each 0.5 x 0.5 m. In the smaller (25 m^2) North Sound plots, the seeded area was sampled with 4 quadrats, each 1 x 1 m, and the unseeded area with 2 quadrats, each 0.5 x 0.5 m. All quadrats were permanently marked. Density of all sown species was recorded in the first year and cover of all species sown and not sown was recorded in all years. Additionally, the number of *C. levisecta* in flower and the number of flowering stems per plant were also recorded beginning in 2010 through 2013.

Appendix B.8. In addition to vegetation monitoring, a photo of plots and a subset of quadrats were recorded. An example of a quadrat photo monitoring from the Glacial Heritage 2009 array is shown above. Pictured is the “Burn/Forb” treatment from the first spring in 2009 (above), in 2011 (pageX), and in 2013 (pageX). Photo monitoring was conducted annually on all plots at all arrays.



Appendix B.8. Continued. “Burn/Forb” treatment quadrat photo monitoring from 2011.

Appendix B.8. Continued. “Burn/Forb” treatment quadrat photo monitoring from 2013.

Appendix B.9. Overview images of the Prairie Habitat Restoration Project arrays from 2009 at West Rocky (top) and Glacial Heritage (bottom). Site differences are evident from these images where the dominant non-native species, *Leucanthemum vulgare*, at West Rocky significantly impacted native species establishment compared to Glacial Heritage, where the native, *Plectritis congesta,* is most evident in the image above.

Appendix B.10. Overview of plots from the 2010 array at Glacial Heritage Preserve of the Prairie Habitat Restoration Project demonstrating the difference in establishment of *C. levisecta* in burn treatments (left) and solarization treatments (right), both plots received a forb rich seed mix.

Appendix B.11. Overview of 10x scaled plot at Glacial Heritage Preserve in spring 2013.

Appendix B.12. Taylor's Checkerspot (*Euphydryas editha taylori*) larvae feeding on *Castilleja levisecta* plant in plots of the Prairie Habitat Restoration Project in the spring of 2013. Several hundred caterpillars were released as part of a pilot project to study the potential of using *C. levisecta* as a host plant for reintroduction of the butterfly in the future.

Appendix C

Photographs of *Castilleja levisecta* companion planting project

Appendix C.1. *C. levisecta* 4” pots and tube containers before outplanting in the field. Companion plants of *F. roemeri* and *E. lanatum* are pictured in the background in tube containers.

Appendix C.2. Outplanting second companion planting array in fall of 2011 at Glacial Heritage Preserve. The entire array was plowed and tilled before planting..

Appendix C.3. Outplanting of second *C. levisecta* array in fall of 2011 at Glacial Heritage Preserve. The first array, planted in the fall of 2010 is pictured in the background.

Appendix C.4. Example of *C. levisecta* planted with host species, *F. roemeri* (top)*, and E. lanatum* (bottom) in outplanting from 2011 at Glacial Heritage Preserve. *C. levisecta* and host species were grown separately and outplanted together as shown above in the field. As can be seen in this image, the size of the *C. levisecta* varied based on pot size. *C. levisecta* is from a 4" pot in top image and from a tube container in bottom image. Images taken in fall of 2011.

Appendix C.5. Monitoring 2011 companion planting array in the spring of 2012. Survival and the vigor of host and *C. levisecta* plants were recorded as well as the number of flowering stems. In the late summer plants were monitored again to record the number of stems and seed capsules and total number of seed capsules per plant.

Appendix C.6. Above is an example of seed capsules of *C. levisecta* in the 2011 companion planting array. Image taken in the fall of 2012.

Appendix C.7. Above is an example of a two-year-old *C. levisecta* plant growing with *E. lanatum* in the 2010 array. Image taken in the spring of 2012. Many *C. levisecta* plants had more than 50 flowering stems in the second year of growth.

Appendix C.8. Aerial image of 2010 and 2011 *C. levisecta* companion planting arrays. Blooming *E. lanatum* are evident in image and more obvious in 2010 array where plants are larger. The Prairie Habitat Restoration Project experimental plots are visible in the bottom left of the above image, adjacent to the companion planting arrays. Aerial image from July 2012.

VITA

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