AN ABSTRACT OF THE THESIS OF

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Title: Factors Affecting Establishment and Germination of Upland Prairie Species of Conservation Concern in the Willamette Valley, Oregon

Abstract approved:

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Thomas N. Kaye

Identifying mechanisms that determine who lives and dies is the first step in developing successful restoration techniques for rare species and endangered habitats. We studied interactions that affect establishment of native plant forbs of conservation concern at the seedling stage to support the theoretical basis for restoration activities in Pacific Northwest prairies. Specifically, we tested the hypothesis that seedling establishment is controlled by 1) competition with or 2) facilitation by existing vegetation and that the interaction is mediated in part by environmental stress.

We direct-seeded or planted vegetative plugs of *Lupinus oreganus*, *Castilleja levisecta, Erigeron decumbens, Iris tenax* and *Sidalcea malviflora* ssp. *virgata* into 20 plots with a range of community compositions in high-stress upland prairies at each of three sites. We counted seedlings and estimated cover of plant functional groups as well as litter, bare soil and disturbance then used linear regression to test for effects of these factors on seedling establishment.

We found evidence of indirect facilitation of grass on seedling establishment in the first year: higher accumulations of leaf litter increased seedling numbers at two sites. In the second year, there was evidence of facilitation by live vegetation and

litter on seedlings at one site, but no net effect of either competition or facilitation at the other two sites.

Overall, we found more evidence for positive interactions than we did for competition. In particular, litter appeared to have a positive effect on seedling establishment of *L. oreganus* and *S. malviflora* ssp. *virgata*. This is contrary to the common perception that litter inhibits plant establishment but supports the theory that facilitation is more common in high stress sites; practitioners should consider seeding into leaf litter at some sites.

To support a robust approach to conservation and reintroduction of species with dormant seed, we characterized dormancy types and developed germination protocols for *S. malviflora* ssp. *virgata* and *I. tenax. S. malviflora* ssp. *virgata* has physical dormancy and may have physiological dormancy. Scarification followed by four weeks of cold moist stratification was effective in initiating germination. *I. tenax* has morphophysiological dormancy which is overcome by four weeks of warm moist stratification followed by 6-12 weeks of cold stratification. We also conducted a meta-analysis of experiments that tested pre-sowing seed scarification of *L. oreganus* and conclude that breaking physical dormancy prior to direct seeding does not support higher establishment relative to unscarified seeds in this species.

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Factors Affecting Establishment and Germination of Upland Prairie Species of Conservation Concern in the Willamette Valley, Oregon

by
Katherine D. Jones

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

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Katherine D. Jones, Author

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Chapter 1

Restoration of species of conservation concern in an ecosystem context

General Introduction

Ecosystem science is a holistic discipline encompassing many interacting biotic and abiotic components. Ecosystem restoration therefore must also be holistic in its scope. By developing a sound rationale for restoration activities, identifying and capitalizing on processes that drive ecosystem structure (Morse 1996, Primack 1996, Zedler 2005), employing novel approaches and continually adapting their strategy (Seddon et al. 2007, Armstrong and Seddon 2008), managers working toward conservation of rare species and endangered ecosystems can save imperiled places and species for future generations.

The research we present in this thesis focuses on ecosystem processes and species of conservation concern in the Willamette Valley, Oregon. We emphasize integrating individual species biology and the unique environmental conditions of a restoration site. Habitat restoration of upland prairies that focuses on resources for two rare butterflies may help shift from goals from species conservation to ecosystem restoration in this region.

Study System

Prairies of the Willamette Valley are a prime example of a critically endangered ecosystem (Noss et al. 1995). Prior to European settlement, the Willamette Valley of western Oregon was a mosaic of coniferous forests, oak savannas and grassland prairies with high plant and animal diversity. Habitat loss, caused by conversion to agriculture, urbanization and natural succession to shrubland and forest due to loss of disturbance regime has reduced native habitats to a fraction of their pre-settlement extent (Alverson 2005). Before 1850, prairies likely covered 30% (409,000 hectares) of the valley floor (Altman et al. 2001). Upland prairies accounted for approximately 277,000 hectares, two-thirds of Willamette Valley prairies (U.S. Fish and Wildlife Service 2000). Today, less than 0.5% remains (Wilson et al. 2003). With 99.5% habitat loss, the upland prairie ecosystem of western Oregon is one of the most endangered ecosystems in the United States (Noss et al. 1995).

The Willamette Valley is a region with a high concentration of rare species (Kaye et al. 1997) and the greatest concentration of human populations in the state.

Because of urbanization, agriculture and succession, existing patches of intact upland prairie ecosystems have become "biological islands," widely separated from one another. Natural processes alone are no longer able to restore ecosystem function. Due to severe fragmentation, remaining populations of rare species are frequently too small with too little genetic diversity to be sustainable (Kaye 2001, Severns 2003a, Thorpe and Kaye 2011). Active management, including vegetation manipulation, native species reintroduction and native population augmentation is necessary to restore and protect these habitats and species (Wilson et al. 1992, Wilson 1996, Wilson and Clark 2000).

Umbrella Species

The process of ecosystem restoration can be difficult to quantify and difficult to monitor because of the many individual species and related processes to consider. Use of an umbrella species, a single species whose conservation would confer protection to a suite of co-occurring species, is a technique often employed by conservation biologists to protect an ecosystem (Roberge and Angelstam 2004). Wilson *et al* (1997) suggest that the Fender's blue butterfly (*Icaricia icarioides fenderi*) may be an appropriate umbrella species for protecting the upland prairie ecosystem in the Willamette Valley. Another potential umbrella species may be the Taylor's checkerspot butterfly (*Euphydryas editha taylor*).

Fender's blue butterfly

The Fender's blue butterfly (*Icaricia icarioides fenderi*) is a small butterfly, with about a 1-inch wingspan, from the family Lycaenidae, sub-family Polyommatinae (Schultz et al. 2003). This is a federally listed endangered species (U.S. Fish and Wildlife Service 2000) with only 17 known populations and only 2,000-6,000 individuals (Schultz et al. 2011). Most individual populations are located on small patches (<1 ha) of prairie habitat and are separated by distances greater than the an individual butterfly is assumed capable of travelling in its lifetime (Schultz 1998, Schultz et al. 2003).

The life-cycle of the Fender's blue butterfly has several implications for habitat restoration and rare plant conservation. In its' larval stage, Fender's blue butterfly relies on the threatened Kincaid's lupine (*Lupinus oreganus*). It feeds almost exclusively on this species and will utilize other lupines, including spur lupine (*L. arbustus*) and sickle-keeled lupine (*L.* albicaulis), only if Kincaid's lupine is also present(Wilson et al. 1997). As an adult, the butterfly requires suitable nectar sources, primarily obtained from native species including tapertip onion (*Allium acuminatum*), narrow-leaved onion (*A. amplectens*), Tolmie star-tulip (*Calochortus tolmiei*), small camas (*Camassia quamash*), clearwater cryptantha (*Cryptantha intermedia*), wooly sunflower (*Eriophyllum lanatum*), Oregon geranium (*Geranium oreganum*), Roughleaf Iris (*Iris tenax*), pale flax (*Linum angustifolium*), blue flax (*L. perenne*), meadow checker-mallow (*Sidalcea campestris*), rose checkermallow (*S. virgata*), and likely other native wildflowers (Schultz 2001, Schultz et al. 2003, U.S. Fish and Wildlife Service 2010). Canopy cover caused by encroachment of woody species into upland prairies and by the presence of exotic tall grass and shrub species such as false brome (*Brachypodium* sylvaticum), tall oatgrass (*Arrhenantherum elatius*) Scots broom (*Cytisus scoparius*) and Himalayan blackberry (*Rubus discolor*), restricts activity of both larval and adult butterflies, and inhibits growth of Kincaid's lupine (Schultz et al. 2003, Severns 2008a).

It is unlikely that Fender's blue butterflies are capable of traveling more than 2 km in their lifetime, and disperse an average distance of 1 km from their host plant (Schultz 1998). To support viable numbers of individuals and allow for interbreeding between distinct populations of butterflies there must be a network of suitable habitats available within close proximity to known populations. The general rule 'large patches are better than small; near patches are better than far' holds true for the Fender's blue butterfly (Schultz and Crone 2005).

Taylor's checkerspot butterfly

Taylor's checkerspot butterfly (*Euphydryas editha taylori*) is a candidate for Federal endangered species listing. It is a member of the family Nymphalidae. This

species was once widespread throughout western Oregon, Washington and British Columbia but today is reduced to 9-13 sites: 6-10 in Washington, one in British Columbia and two sites in Oregon, in the Willamette Valley (Schultz et al. 2011). In western Oregon, population size is estimated to be fewer than 1000 individuals, ranging from the low to high hundreds depending on the year (Severns 2008b).

In the Willamette Valley, Taylor's checkerspot butterfly exclusively utilizes the non-native English plantain (*Plantago lanceolata*) as its host plant. The native host plant species is not known but Washington populations utilize *Castilleja* sp., *Collinsia* sp. and *Plectritis congesta* (J. Pelham pers. comm. and A. Potter pers. comm. *in* Severns and Warren 2008). The fact that this butterfly depends on an exotic host plant indicates that the historic host is no longer available. Severns and Warren (2008) suggest golden paintbrush (*Castilleja levisecta*) or blue-eyed Mary (*Collinsia* sp) may be the historic host for Taylor's checkerspot in the Willamette Valley. The primary nectar resource for this species is wild strawberry (*Fragaria viginiana*).

Both the Fender's blue butterfly and Taylor's checkerspot are limited by quantity and quality of available habitat. Tall invasive grasses such as tall oat grass (*Arrhenatherum elatius*) and tall fescue (*Festuca arundinacea*) reduce habitat for both butterflies by restricting access to reproductive and nectar resources (Severns 2008b). Restoration of habitat for these species entails 1) invasive species removal, 2) native plant reintroduction and 3) development of multiple patches of restoration sites.

Research Approach

These investigations build on previous studies of invasive species removal techniques to explore the restoration of target plant species by seed. We focus on plant regeneration across a variety of conditions using resource requirements of the Fender's blue butterfly and Taylor's checkerspot butterfly as a framework for ideal habitat conditions. Butterflies were not directly studied; we investigated methods of reintroducing plant species used or required by these butterflies.

Though many species are involved in a fully functioning upland prairie ecosystem, we chose five species that, due to their conservation status or importance

to endangered butterflies, are likely targets for restoration projects: Kincaid's lupine (*Lupinus oreganus* A. Heller), Willamette daisy (*Erigeron decumbens* Nutt.), golden paintbrush (*Castilleja levisecta* Greenm.), roughleaf iris (*Iris tenax* Douglas ex Lindl.), and rose checkermallow (*Sidalcea malviflora* (DC.) A. Gray ex Benth. ssp. *virgata* (Howell) C.L. Hitchc.). Plant nomenclature follows Cook and Sundberg (2011b). All species are perennial forbs native to upland prairies in the Willamette Valley, Oregon.

We investigated establishment and germination for these species, and present results of those investigations here in three chapters. In chapter 2, we present results of a field study that investigated the role of plant community functional groups on establishment of all five of our focal plant species. We utilized a previous research project that manipulated community composition through a series of treatments to reduce invasive grass cover (Stanley et al. 2008), adding seed or vegetative plugs into plots at three sites and following establishment and survival of individuals for two years. In chapter 3, we report on a series of laboratory experiments that were aimed at identifying germination requirements for *S.malviflora* ssp. *virgata* and *I. tenax*, two species hypothesized to have seed dormancy and which have sometimes had low establishment rates in previous restoration efforts. Finally, in chapter 4, we assess the effectiveness of pre-sowing seed scarification of *L. oreganus*, a species with demonstrated physical dormancy. We conducted a meta-analysis of thirteen field experiments that planted both scarified and unscarified seeds and discuss the applicability of this technique (which is necessary for greenhouse propagation) to direct seed for restoration purposes.

The unifying theory is that effective restoration and reintroduction strategies must be multifaceted. Managers should account for unique ecosystem processes of a restoration site, the specific biology of the species they are working with and make use of lessons learned in other restoration projects.

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Chapter 2

Ecological Drivers of Seedling Establishment and Survival

Katherine D. Jones and Thomas N. Kaye.

Abstract

The stress gradient hypothesis states that plant community interactions shift from competition in low stress environments to facilitation in high stress environments. We tested for completion and facilitation in a high stress environment in the Willamette Valley, OR and hypothesized that positive interactions, would be the primary driver of establishment at our sites but that interactions likely differed between plant functional groups and between life history stages.

We direct seeded or planted five species of native perennial forbs of conservation concern, *Lupinus oreganus*, *Castilleja levisecta*, *Erigeron decumbens, Iris tenax* and *Sidalcea malviflora* ssp. *virgata,* into 20 plots at each of three sites. Seedling establishment and survival was tracked over two growing seasons. We used linear regression to test for effects of cover by various plant functional groups, litter, bare soil and disturbance on seedling success.

There was evidence of indirect facilitation of *L. oreganus, S. malviflora* ssp. *virgata* and *C. levisecta* by grass in the form of leaf litter. Facilitation by litter is contrary to the common perception that litter inhibits plant establishment but supports the theory that facilitation is more common in high stress sites. This suggests a phased approach to restoration of degraded habitats. In high stress environments, land managers should consider seeding target forb species prior to eradicating invasive grasses or after successfully establishing native grasses.

Introduction

Habitat loss and competition from non-native species are leading causes of declines in diversity (Morse 1996, Czech 1997, Wilcove et al. 1998). The list of threatened and endangered species is growing at an increasing rate (Pimm and Raven 2000); in some cases, whole ecosystems are at risk of being lost (Noss et al. 1995). Restoring this diversity is now a major challenge facing ecologists and land managers (Dobson 1997, Hobbs and Harris 2001) and there is a clear call to answer these challenges through the direct application of ecological theory (Palmer et al. 1997, Miller and Hobbs 2007).

In applied ecology, primary ecological concepts provide managers with a theoretical framework from which to develop restoration protocols based on the biology of individual species (Sarrazin and Barbault 1996, Palmer et al. 1997, Zedler 2005). For example, in plant community ecology, the stress-gradient hypothesis proposes that competitive community interactions between plant species have greater importance in low or moderate stress environments and that as stress conditions increase, facilitative interactions become more prevalent (Bertness and Callaway 1994, Tielbörger and Kadmon 2000). Within a particular habitat type, effective restoration techniques will differ in locations with differing stress conditions (Padilla and Pugnaire 2006) and it is likely that interactions also will differ among life history stages (Brown and Van Staden 1997, McPeek and Peckarsky 1998, Maestre et al. 2005). Even under a particular stress environment, interspecific plant interactions differ between functional groups because resource use varies among functional groups (Hooper 1997). In prairie systems, grasses, especially non-native invasive species, tend to be taller and faster growing than many native forbs (Blossey and Notzold 1995, Wilson 1998, Wilson and Clark 2001) and therefore frequently suppress native forbs regardless of stress conditions. Also, plant-plant interactions may be completely superseded by interactions between trophic levels. It is well known that disturbance, especially herbivory, can have significant effects on plant community structure and may obscure effects of either competition or facilitation between plant species (Hambäck and Beckerman 2003, Brooker et al. 2006). Plant establishment can be affected by associated vegetation through competition or facilitation, but disturbance could disrupt these processes. Successful conservation efforts, especially reintroduction of species with important ecosystem functions or species of particular conservation concern (Srivastava and Vellend 2005, Isbell et al. 2011) will be achieved through identifying and applying ecological theories that drive community structure and ecosystem dynamics at a particular location (Thorpe and Stanley 2011).

Conservation of species *interactions* is essential to successful ecosystem restoration (Soulé et al. 2003); individual species reintroduction still needs to be

conducted within an ecosystem context. Predator-prey, plant-pollinator and the role of ecosystem engineers are examples of essential relationships to consider in ecosystem restoration planning (Nabhan and Fleming 1993, Jones et al. 1994, Krearns et al. 1998, Soulé et al. 2003, 2005, Daleo and Iribarne 2009). Groups of species involved in mutualistic or supportive interactions are especially important to consider for maintaining ecosystem function and complexity (Menz et al. 2011). For example, whitebark pines are dependent on the Clark's nutcracker for seed dispersal and reproduction (Hutchins and Lanner 1982), the endangered lesser long-nosed bat is a key pollinator of cardon and organ pipe cacti (Fleming et al. 2001), and the feeding behavior of endangered sea otters exerts strong control on the structure of costal kelp forests (Soulé et al. 2003). Plant-insect mutualisms are some of the most essential interactions such that a threat to one of the interacting species can endanger the persistence of the other (Bronstein et al. 2006). Ecology has many stories about obligate interactions such as yucca moths, fig wasps, orchid bees (Kiester et al. 1984) and numerous examples of butterflies that have specific host plants (Bronstein et al. 2006, 2009) indicative of co-evolution and often interdependent life cycles (Dennis et al. 2004).

Endangered plant species conservation requires protecting existing populations but also often requires reintroduction to augment existing populations or to establish new ones (Morse 1996, Guerrant and Kaye 2007, Kaye 2009). Species reintroduction comes with its own set of challenges; in addition to theoretical considerations are issues associated with practical application. One of the many challenges in a species reintroduction project is choosing propagule type (Guerrant and Kaye 2007). Despite lower establishment rates, for many species, if seed availability is high, it is generally more economical to use seed for reintroductions than to use transplants (Kaye and Cramer 2003, Guerrant and Kaye 2007). However, many seeds, though viable and capable of germination, will not do so if they are dormant (Baskin and Baskin 1998) and may require pre-treatment of seeds. Fabaceous species in particular are commonly hard seeded and may require scarification (Singh et al. 1991, Russell

2011). In upland prairies of western Oregon, *Lupinus oreganus* (Fabaceae, Kincaid's lupine) is a hard-seeded species often targeted for conservation projects. Successful reintroduction of this species may require both an understanding of community interactions that affect this plant once growing but also pre-sowing treatments to ensure seed germination.

This research aims to identify interactions, positive and negative, that drive establishment of native species of conservation concern in an endangered grassland ecosystem, upland prairies of the Willamette Valley, Oregon. We focus on five native plant species that are threatened or endangered and provide essential ecosystem functions for two endangered butterflies, the Fender's blue and Taylor's checkerspot. We asked two questions: 1) Which plant community interactions determine establishment and survival of these forbs? And 2) Does pre-sowing scarification enhance establishment of a hard seeded native perennial? We use ecological theories on plant community interactions to suggest novel techniques for seed preparation to enhance reintroduction strategies.

Based on the stress-gradient hypothesis (Bertness and Callaway 1994, Callaway and Walker 1997) and the seed biology of our species we tested several hypotheses:

H1: Plant community interactions in this region are dominated by facilitation at the seedling stage with existing plants protecting vulnerable seedlings. Competition is likely more prevalent at later life history stages as forbs mature and develop similar resource requirements to the established plant community.

H2: Plant community interactions differ between functional groups; grasses are more likely than established forbs to exert a competitive effect on establishment of planted forb species.

H3: Disturbance by herbivores and burrowing rodents will have a negative effect on establishment and will obscure plant-plant interactions if incidence is high.

H4: Pre-treating seeds to overcome physical dormancy will stimulate higher germination resulting in greater establishment.

We planted treated and untreated seeds and vegetative plugs into manipulated prairie communities to address these hypotheses.

Methods

Study System

This research was conducted in upland prairies in the Willamette Valley of western Oregon, USA. The temperate latitudes have experienced some of the greatest losses of biodiversity due to intense development and exploitation by humans (Noss et al. 1995). Grasslands and savannas (prairies) in particular are among the most endangered ecosystems in the United States based on their decline, current extent, imminence of threat, and number of associated threatened and endangered species (Noss and Peters 1995, Noss 2000). The Willamette Valley is no exception; with significant loss of native ecosystems. Prior to European settlement, the Willamette Valley was a mosaic of coniferous forests, oak savannas and grassland prairies with high plant and animal diversity. Habitat loss, caused by conversion to agriculture, urbanization and natural succession to shrubland and forest due to loss of disturbance regime has reduced native habitats to a fraction of their pre-settlement extent (Alverson 2005). Before 1850, prairies likely covered 30% (409,000 hectares) of the valley floor (Altman et al. 2001). Upland prairies accounted for approximately 277,000 hectares, two-thirds of Willamette Valley prairies (U.S. Fish and Wildlife Service 2000). Today, less than 0.5% remains (Wilson *et al.* 2003). With 99.5% habitat loss, the upland prairie ecosystem of western Oregon is one of the most endangered ecosystems in the United States (Noss et al. 1995).

Our five study species are, Kincaid's lupine (*Lupinus oreganus* A. Heller), Willamette daisy (*Erigeron decumbens* Nutt.), golden paintbrush (*Castilleja levisecta* Greenm.), roughleaf iris (*Iris tenax* Douglas ex Lindl.), and rose checkermallow (*Sidalcea malviflora* (DC.) A. Gray ex Benth. ssp. *virgata* (Howell) C.L. Hitchc.). *L.*

oreganus, *E. decumbens* and *C. levisecta* are all threatened or endangered species in the Willamette Valley. *I. tenax* and *S. malviflora* ssp. *virgata* were also included because of seed availability and value as nectar resources for endangered butterflies (Schultz and Dlugosch 1999, Schultz 2001) (see Table 1 for details). Nomenclature follows the Oregon Flora Project (Cook and Sundberg (eds.) 2011).

Our study sites are located at the southern end of the Willamette Valley/Puget Trough/Georgia Basin (WPG) Ecoregion. This region spans almost 600 km from north to south. Soil moisture content, fertility and organic matter increase with latitude. The northern portions of the region therefore have more productive, low stress environments while the southern end is characterized as having less productive, higher stress environments (Richardson et al, in press).

Table 1 Plant species used in experimental seeding and planting

Species Name	**Common Name**	**Family**	**Conservation Status**	**Geographic Range**	**Butterfly resource**	**Plant Material Used**	**# Planted**
Lupinus oreganus	Kincaid's Lupine	Fabaceae	Threatened	Western WA and OR	Host for FBB	Seed	100/split plot
Erigeron decumbens	Willamette Daisy	Asteraceae	Endangered	Western OR	None known	Seed	~1000
Castilleja levisecta	Golden Paintbrush	Orobanchaceae	Endangered	Western WA and BC	Potential host for TCB	Vegetative Plugs	5 at Bellfountain only
Sidalcea malviflora ssp. *virgata*	Rose Checkermallow	Malvaceae	Common	Western WA and OR	Nectar	Seed	~100
Iris tenax	Oregon Iris	Iridaceae	Common	OR, WA, CA	Nectar	Seed and Vegetative Plugs	~100 seeds & 5 plugs

From 2005-2010, the Institute for Applied Ecology (IAE), in coordination with The Nature Conservancy (TNC) conducted a long-term manipulative experiment with the goal of identifying effective techniques for controlling invasive perennial grass species and promoting native plant assemblages in grassland communities throughout the WPG Ecoregion. The IAE/TNC project employed a multi-site, multi-variable design to test the effectiveness of a variety of management treatments. Treatments included the application of a grass-specific herbicide, spring or fall mowing and burning plus application of a post-burn broad-spectrum herbicide on 5 x 5 m experimental units, treatments were followed by seeding native grasses and forbs (Stanley et al., 2008). The treatment units represent a range of community composition from high perennial forb cover with low litter abundance to high invasive grass and litter cover (Richardson et al. in press, Stanley et al. 2008, 2011a, 2011b).

We utilized three of the IAE/TNC study sites (Figure 1), two at William L. Finley National Wildlife Refuge, Pigeon Butte (44°23.9' N, 123°19.2 W) and Bellfountain (44°24.2' N, 123°20.9' W), and one at Fort Hoskins Historic Park (44°40.8' N, 123°27.8' W) administered by Benton County, Oregon. Elevation at study sites ranged from 112-138 m above mean sea level. Soils are all moderately deep to very deep and well-drained. They are formed from colluvium and residuum derived from basalt, igneous bedrock or sedimentary rocks. All sites have a Mediterranean climate, characterized by mild wet winters and dry summers with an average annual precipitation of 171 cm. The two years we made our observations, 2010 and 2011, experienced spring temperatures that were cooler than average with above average precipitation in this region.

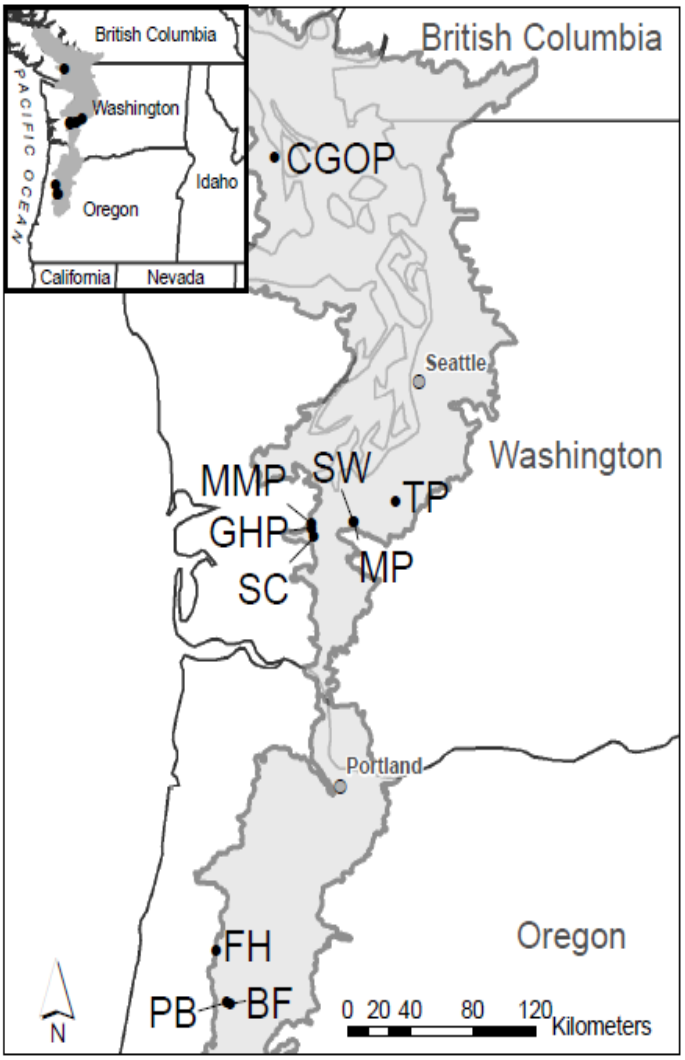

Figure 1 IAE/TNC research sites, ours are the southernmost sites, Pigeon Butte (PB), Bellfountain (BF) and Ft. Hoskins (FH)

Experimental Design

To test for effects of associated vegetation on plant establishment, we seeded or planted plugs of our target species into the 20 manipulated communities at the three Oregon sites. We established 0.5 x 2 meter plots oriented within the IAE/TNC project plots in a quadrant of the plot seeded in 2007. We broadcast seeds of *L. oreganus, S. virgata, I. tenax* and *E.decumbens* into each plot in November of 2009. The number of seeds sown varied with species. Estimates of seed viability from tetrazolium chloride

(TZ) tests were available for *S. virgata* and *I. tenax*, so seed numbers were adjusted to sow approximately 100 viable seeds. Specifically, viability of *S. malviflora* ssp. *virgata* seed was estimated at 84% (commercially reported live seed) and seed number was increased to 119 seeds per plot to achieve an average sowing rate of 100 seeds. Seeds of *I. tenax* came from two commercial sources (Silver Falls Seed Company and Heritage Seedlings) with viabilities of 69% and 77%, and corresponding seeding rates of 129 and 145 seeds to sow an estimated 100 seeds per plot. Previous studies show that *E. decumbens* has low establishment (<1%, Kaye and Brandt 2005) and typically low viability (0-39%, Clark et al. 1997, Thorpe and Kaye 2011). Although viability estimates were not available for this species, we compensated for the poor expected establishment rate by sowing 1000 seeds in each plot, estimated by weight; seven sets of 1000 seeds were hand counted and weighed to estimate the average weight, which was then used to measure the remaining seeds.

For *L. oreganus*, each plot was divided in half to create two 0.5 x 1 meter split-plots. Each side of the split-plot was planted randomly with either 100 scarified or 100 un-scarified lupine seeds. We scarified seeds by hand using a razor blade to break the hard seed coat. Seeds were sown in November of 2009. In addition, 50 *L. oreganus* seeds were broadcast in one 0.5 x 0.5 m plot in each treatment unit in November 2006. We planted five plugs each of *C. levisecta* and *I. tenax* in June and November of 2010, respectively. Plugs of *C. levisecta* were planted at Bellfountain only due to limited availability of this species, while *I. tenax* was planted at all three sites. In both cases, plugs were planted on 50 cm spacing along one edge of the 0.5 x 2 m plots, with *I. tenax* and *C. levisecta* on opposite sides at Bellfountain. Though the range of current conditions in these plots is the result of previous treatments (Richardson et al. *In press*), we characterized the community composition of each plot individually rather than grouped by treatment history. With our study design, we cannot separate effects of community components from treatment history.

Data Collection

Cover estimates

We conducted ocular estimates of cover to the nearest 1% of grasses, forbs, moss, litter, bare soil and disturbance (see Appendix A for examples). Estimates were made for each 0.5 x 0.5 m in the plot. Litter depth was measured at five points in each 1 x 0.5 m plot. Estimates were made for each growing season. In 2011 we centered a 0.5 x 0.5 m plot over lupine planted in 2006 and estimated percent cover of grasses, forbs, moss, litter, bare soil, and disturbance.

Establishment by seed

Throughout this paper, we refer to the proportion of seeds that *establish* rather than *germinate*; and we define establishment as the proportion of seeds that germinated, emerged and produced photosynthetic cotyledons and/or true leaves that were present at the time we visited each plot in 2010 (definition adapted from Harper 1977). This count excludes seeds that may have emerged and died before our survey of the plots as well as seeds that never germinated.

For two consecutive years, 2010 and 2011, we conducted demographic surveys of all experimental plots in April and May of 2010 then again in May 2011. We mapped the location of each individual that established from seed by measuring coordinates to the nearest centimeter within a 1 x 0.5 m plot frame and mapping them on a paper datasheet to scale (Appendix A). Using the map we developed, we relocated individuals the following year. For each individual we counted number of leaves at each survey to get a relative estimate of growth.

In 2011, we measured *L. oreganus* seeds sown in 2006 by counting the number of lupine leaves present. After 5 years of growth, many individuals had grown together and single individuals may have had multiple stems coming from the ground therefore it was often difficult to distinguish one individual from another. We used the number of *L. oreganus* leaves as a measure of overall success of the 50 seeds sown in 2006.

Transplants

For *I. tenax*, we noted if the individual survived, counted the number of leaves and measured the length of the longest leaf blade. *C. levisecta* produces multiple shoots from the ground, we measured the length of each shoot an individual produced and noted whether or not they produced flowers.

Statistical Analysis

Data were analyzed using R statistical software, version 2.14 (2011). Our response variables are the proportion of seeds that establish and proportion of established seeds or vegetative transplants that survive in our experimental plots. Our explanatory variables are percent cover of existing vegetation, grouped by functional group and average litter depth, measured in centimeters.

Cover estimates were averaged across the 0.5 x 1 m split-plot for *L .oreganus* and across the whole 0.5 x 2 m plot for all other species. We used Analysis of Variance and simple descriptive statistics on the community components to verify that our manipulated communities represented a wide range of community conditions. We used Analysis of Variance to test for differences among the three sites and, although establishment did not always significantly differ by site, site was a blocking factor and therefore was accounted for in all Multiple Regression models. We used Simple Linear Regression to test for community interactions with planted *C. levisecta* plugs, and we used Multiple Linear Regression to test for the effect of surrounding vegetation on initial establishment and survival of seeds planted in 2009 and *I. tenax* plugs planted in 2010.

We conducted t-tests to determine if scarification of *L. oreganus* affected establishment rates in 2010. All additional analysis was conducted separately for each seed treatment. Although we determined that there was no density dependence for this species, in 2011 survival was analyzed for each split plot to retain the 0.5 m resolution of cover estimates for all of the seeds with a shared treatment history. Establishment and survival for this species was analyzed for each pre-treatment group separately.

Our threshold for statistical significance was p=0.05 but because our primary interest is identifying any *potential* interactions between our study species and the existing plant community, we report results to p=0.1 as being suggestive or equivocal and worthy of note.

Results

Community Components

Grass cover in our plots ranged from 0 to 80% and differed by site (f=5.77, df=2, p=0.03, ANOVA F-test). Cover by forbs ranged from 19% to 93% and did not differ significantly by site (f=5.77, df=2, p=0.34). Total cover of vascular plants, the sum of grass and forb estimates, ranged from 42 to 121% and did not differ by site (f=0.32, df=2, p=0.72). Litter depth ranged from 0.25 to 5.10 centimeters and was closely correlated with grass cover (p<0.0001; Figure 2). Disturbed area of plots caused primarily by moles ranged from 0 to 50% and differed by site (f=11.35, df=2, p=0.0001).

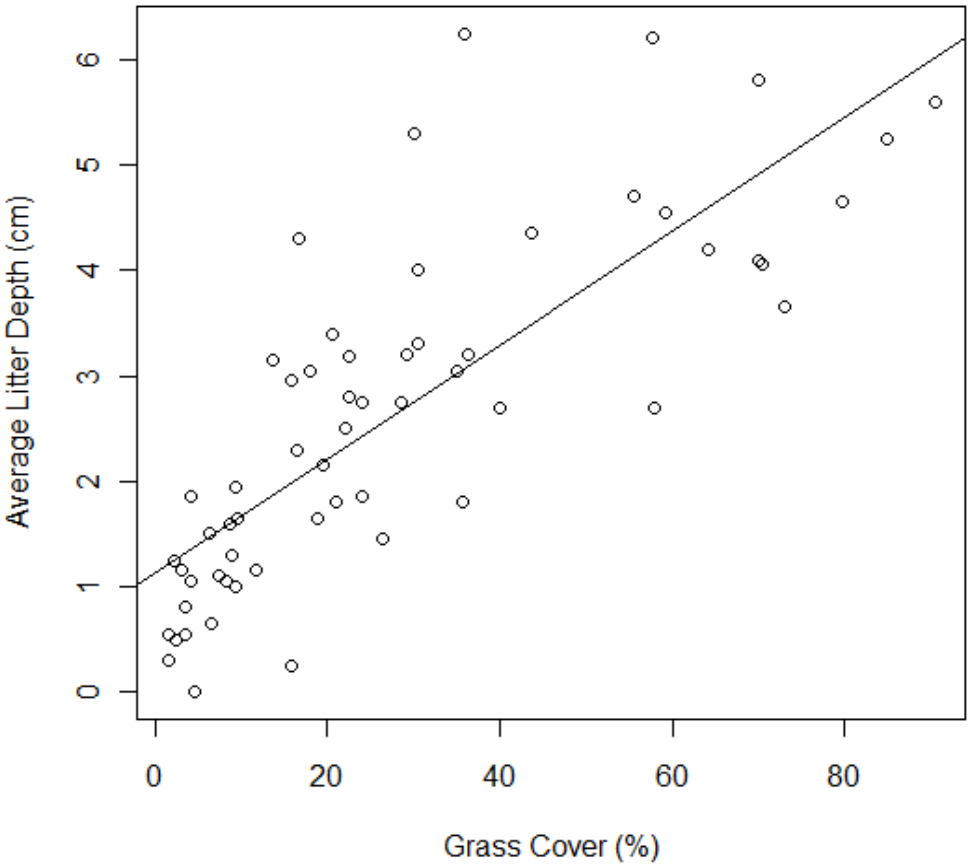

Figure 2 Litter depth compared with grass cover ($p<0.0001$, $R2=0.33$).

Table 2. Summary of all regression models with site as a blocking factor. Bold values are significant at $p \leq 0.1$.

			Establishment				Survival			
		Factor	**Slope**	**Intercept**	**R^2**	***p***	**Slope**	**Intercept**	**R^2**	***p***
Lupinus oreganus	Scarified Seeds	Grass	-0.007	12.1	0.009	0.92	-0.15	38.9	0.23	0.3
		Forb	0.09	7.13	0.03	0.67	0.1	28.4	0.22	0.5
		Moss	0.013	11.68	0.009	0.92	0.09	30.5	0.22	0.6
		Bare	-0.278	12.09	0.03	0.59	0.26	31.6	0.21	0.8
		Disturbed	-0.165	12.1	0.3	0.75	-0.25	34.6	0.22	0.5
		Litter Depth	1.688	8.84	0.03	0.61	-0.25	32.9	0.21	0.9
		Total Veg	0.156	-1.83	0.05	0.37	-0.11	41.1	0.22	0.6
	Non-Scarified Seeds	Grass	0.088	25.5	0.04	0.33	0.1	32.6	0.25	0.55
		Forb	-0.045	30.8	-0.04	0.66	0.07	29.6	0.26	0.27
		Moss	-0.142	29.99	0.04	0.33	0.1	31.8	0.26	0.39
		Bare	-0.021	28.38	0.03	0.66	-1	36.1	0.27	0.16
		Disturbed	-0.251	29.17	0.05	0.28	**-0.7**	**40.3**	**0.39**	**0.0005**
		Litter Depth	**3.394**	**21.36**	**0.1123**	**0.03**	**3**	**24.6**	**0.3**	**0.05**
		Total Veg	0.154	14.81	0.05	0.4	**0.29**	**10.7**	**0.3**	**0.029**
Sidalcea malviflora	Seeds	Grass	-0.01	15.60	0.21	0.78	0.18	29.6	0.09	0.12
		Forb	-0.01	15.71	0.2	0.85	-0.08	40.5	0.06	0.52
		Moss	0.07	14.39	0.21	0.3	0.19	33.9	0.08	0.2
		Bare	0.15	15.03	0.21	0.47	**-1.54**	**40.3**	**0.1**	**0.07**
		Disturbed	-0.15	15.74	0.22	0.2	**-0.78**	**45**	**0.17**	**0.007**
		Litter Depth	-0.3	15.71	0.2	0.76	**5**	**20.2**	**0.19**	**0.004**
		Total Veg	-0.07	21.66	0.2	0.4	0.2	18.14	0.08	0.17
Iris tenax	Transplants	Grass					-0.13	80.8	0.14	0.45
		Forb					0.19	68.1	0.15	0.33
		Moss					-0.3	81.3	0.17	0.13
		Bare					0.9	73.3	0.14	0.48
		Disturbed					0.2	73.1	0.13	0.66
		Litter Depth					-2.5	83.5	0.14	0.35
		Total Veg					0.07	69.1	0.13	0.8
Castilleja levisecta	Transplants	Grass	-0.16	98.6	0.13	0.12	**0.37**	**65**	**0.17**	**0.07**
		Forb	0.16	84.6	0.09	0.21	-0.3	92.5	0.07	0.25
		Moss	0.13	91.4	0.01	0.7	0.76	67.6	0.08	0.22
		Bare	1.32	91.5	0.02	0.59	**-9.6**	**93.4**	**0.33**	**0.008**
		Disturbed	0.39	91.6	0.06	0.3	**-1.4**	**88.6**	**0.24**	**0.03**
		Litter Depth	-1.2	95.3	0.02	0.58	**8.9**	**55.4**	**0.3**	**0.01**
		Total Veg	-0.29	118.7	0.07	0.26	**1.01**	**-6.2**	**0.21**	**0.04**

Lupine

Establishment

L. oreganus establishment did not differ among sites (p=0.78). After accounting for site, there is convincing evidence that litter depth was positively correlated with establishment of non-scarified seeds after accounting for site (p=0.03, R^2=0.11, see Table 2 for all regression models). The correlation with litter depth was demonstrated at Pigeon Butte (p=0.06) and Ft. Hoskins (p=0.069) but not at Bellfountain (p=0.29, Figure 3).

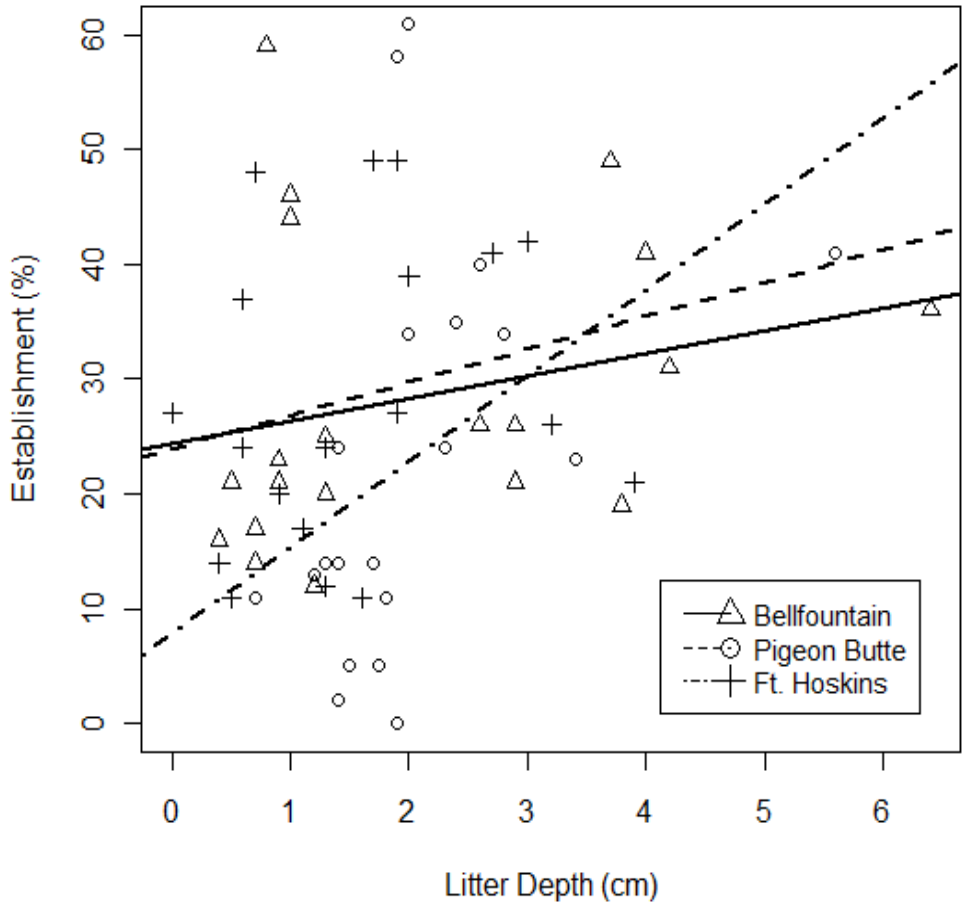

Figure 3. Establishment of *L. oreganus* relative to average litter depth at three sites (p=0.03, R^2=0.11).

Seed scarification significantly reduced seedling establishment of *L. oreganus* across all sites ($p<0.0001$, paired t-test, $df=59$). Split plots seeded with scarified seeds had an average 14% lower lupine establishment than split plots with non-scarified seeds after accounting for site (95% CI 8.7 to 18.3%; Figure 4).

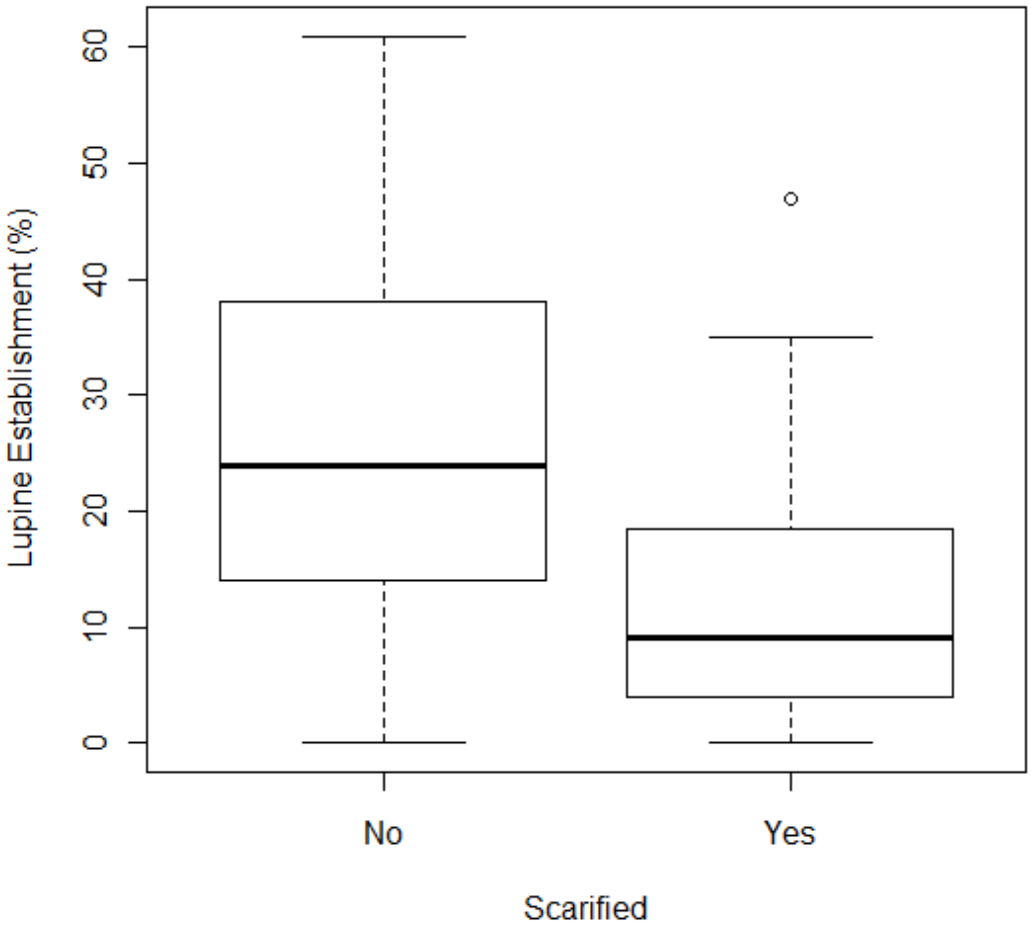

Figure 4 Seedling establishment of *L. oreganus* with and without scarification in the first year after sowing, across all sites ($p=0.0001$).

Seedling survival

Survival in 2011 of plants that established in 2010 differed by site (f=14.97, df=2, p<0.0001). Survival however did not depend on scarification (f=2.25, df=1, p=0.33), nor on the number of plants that established in 2010 (f=0.29, df=1, p=0.48 ANOVA F-test).

Survival of scarified seeds was not correlated with any of the community variables. Non-scarified seed survival was, however, was positively correlated with litter depth after accounting for site (p=0.05, R^2=0.30; Figure 5A). Disturbance by moles was strongly negatively correlated with survival after accounting for site (p=0.0005, R^2=0.39, Figure 5B).

Only half of the plots at Bellfountain and Ft. Hoskins that were planted with *L. oreganus* in 2006 still had lupine present in 2011. Plots without lupine present had about 26% higher grass cover than plots with lupine (p=0.001, 95%CI 11%-41%). Plots with flowering lupine had a higher mean number of leaves (f=23.4, df=1, p=0.0001 ANOVA F-test; Figure 6). Of the plots with lupine, median leaf number was positively correlated with litter depth (p=0.07) after accounting for site. All other community factors appeared to have a neutral effect on median leaf number (Table 3).

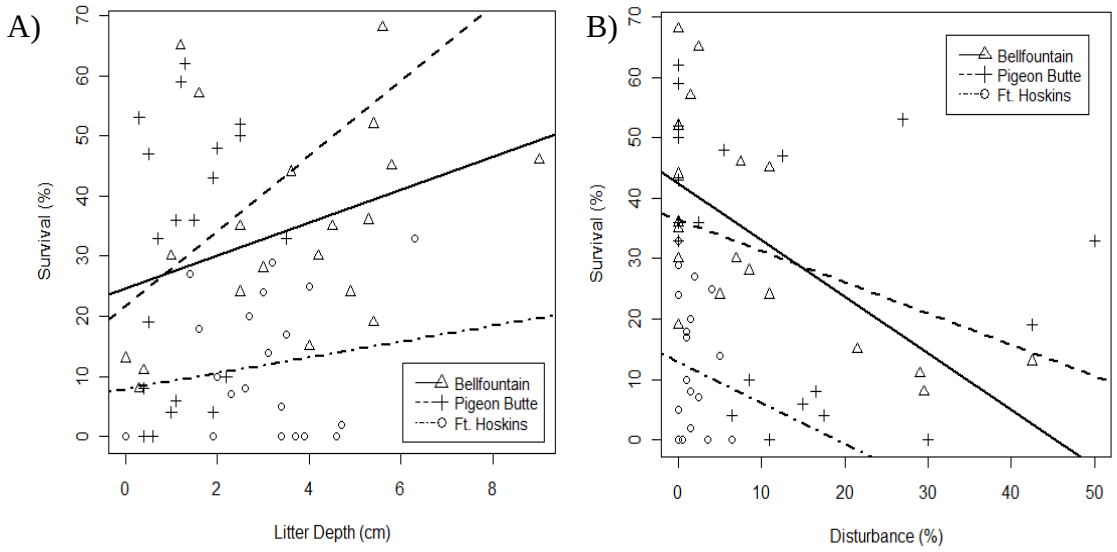

Figure 5. Survival of non-scarified seeds compared to A) litter depth (p= 0.05) and B)soil disturbance from moles (p=0.0005).

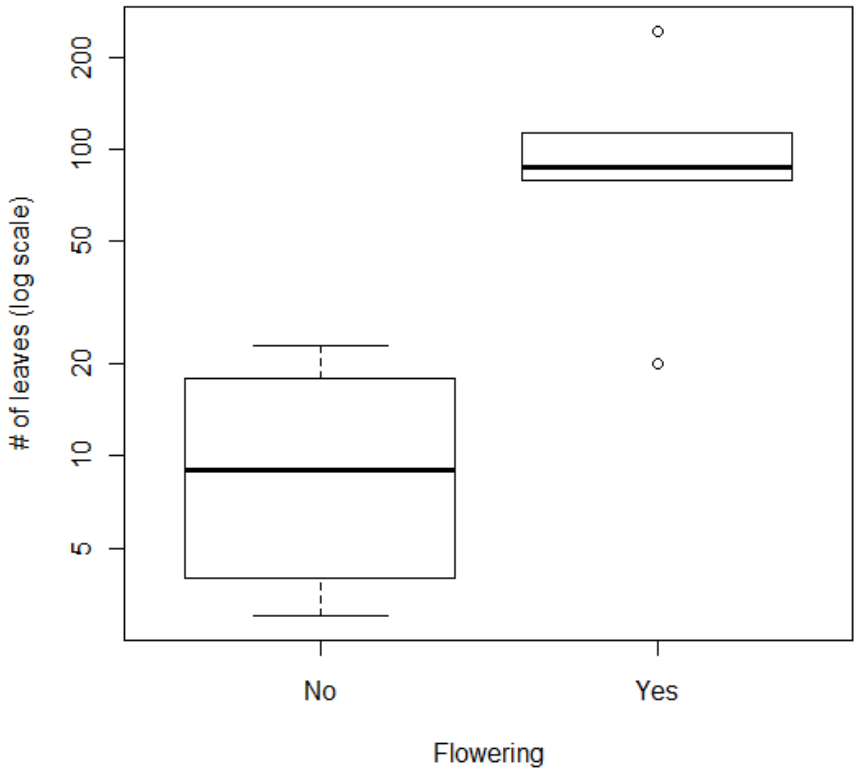

Figure 6 Boxplot of number of *L. oreganus* leaves (log scale) in plots with and without lupine flowers

Table 3 Summary of regression models for median leaf number for surviving *L. oreganus* from seeds planted in 2006. Site is a blocking factor. Bold values are significant at p≤0.1. Note, regressions run on log transformation of leaf number.

Factor	**Slope**	**Intercept**	**R^2**	***p***
Grass	-0.027	4	0.12	0.16
Forb	0.01	2.6	0.05	0.39
Moss	0.007	2.76	0.025	0.58
Bare	-0.04	3.08	0.54	0.37
Disturbed	-0.04	3.21	0.06	0.34
Litter Depth	**0.33**	**1.4**	**0.19**	**0.07**
Total veg	-0.004	3.2	0.009	0.83

Sidalcea

Establishment

Initial establishment of *S. malviflora* ssp. *virgata* differed by site (f=7.49, df=2, p=0.002 ANOVA F-test). After accounting for site, none of the community components we measured were correlated with initial establishment in our multiple regression models.

Seedling survival

Survival of seedlings that established in 2010 did not differ by site (f=1.59, df=2, p=0.21 ANOVA F-test). After accounting for site, litter depth was positively correlated (p=0.004) with seedling survival (Figure 7) and there was equivocal evidence that bare ground (p=0.07) and convincing evidence that incidence of disturbance by moles (p=0.007), were negatively correlated with survival.

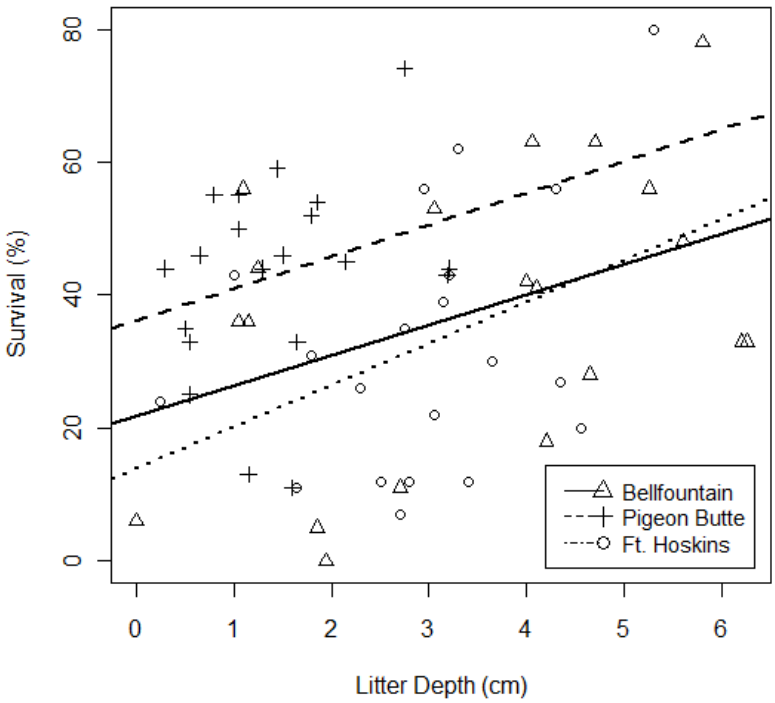

Figure 7 Survival of *S. malviflora* compared to litter depth at three study sites (p=0.004).

Castilleja

Establishment of *C. levisecta* planted in 2010 was not correlated with cover of any of the functional groups we measured, but survival to 2011 was positively correlated with grass (p=0.07, Figure 8A), total cover of grasses and forbs (p=0.04, Figure 8B), and litter depth (p=0.01 Figure 8C). Persistence to 2011 was negatively correlated with bare ground (p=0.008) and disturbance by moles (p=0.03).

Iris

In 2010, we were unable find any *I. tenax* seedlings at any site and therefore could not compare seedling establishment to community characteristics. Our 2011 survey found a total of 29 individuals in only 5 plots that established from seed, most of them at Ft. Hoskins but this was insufficient for statistical analysis.

Survival of transplants

There was an average of 76% survival of transplanted *Iris*. Survival did differ by site (f=4.26, df=2, p=0.019, ANOVA F-test), this effect was driven by the nearly 100% survival at Pigeon Butte (Figure 9). Due to the generally high survival rate overall, we were unable to detect a significant positive or negative affect from any of community component we measured.

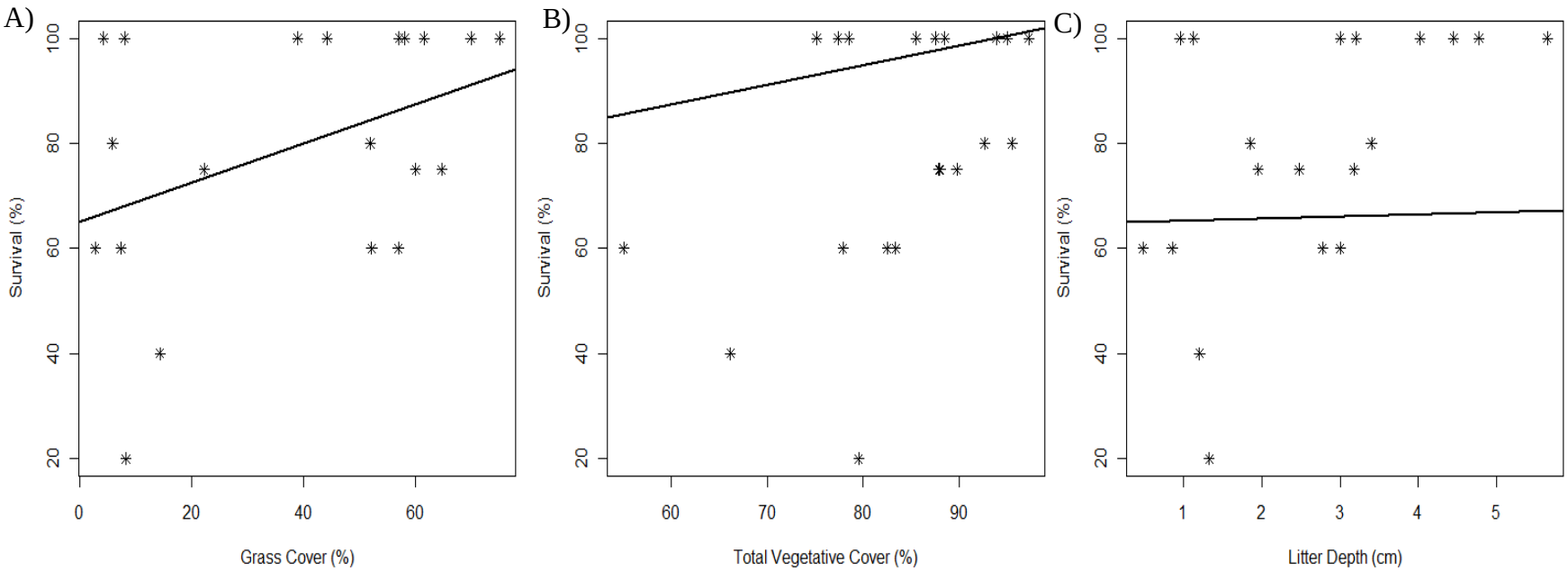

Figure 8. Survival of planted *Castilleja levisecta* as a function of cover of A) grass (p=0.07), B) total cover of vascular plants (p=0.04) and C) litter depth (p=0.01).

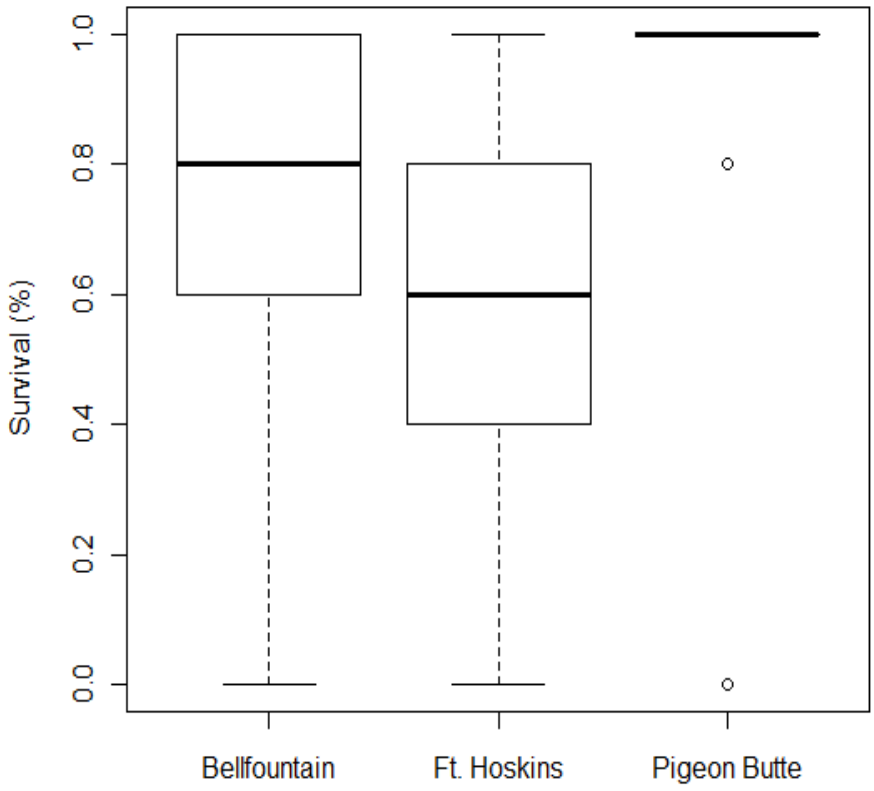

Figure 9 Boxplot of *Iris tenax* survival by site (p=0.019)

Erigeron

We only found a handful of *Erigeron* seedlings in either 2010 or 201l, our sample size was insufficient for statistical analysis. Only nine individuals in two plots were found at Ft. Hoskins, four individuals established in two plots at Pigeon Butte. None of the individuals found in 2010 were relocated in 2011.

Discussion

Facilitation and Competition

Though we did not test a range of stress conditions, our results are consistent with the stress-gradient hypothesis that suggests that stressful environments tend toward facilitative interactions (Richardson et al. *In press*, Bertness and Callaway

1994, Callaway and Walker 1997). Plant interactions in our stressful habitat do tend toward facilitation. Because litter in these prairies is primarily the result of grass, the effects of litter on establishment are an indirect effect of grass. The indirect effect of grass through litter accumulation was the strongest potential driver of seedling establishment of *Lupinus oreganus* and seedling survival of *Sidalcea malviflora* ssp. *virgata* at our sites. Litter accumulation, resulting from abundant native and non-native grasses, was positively correlated with establishment of non-scarified seeds of *L. oreganus,* and *S .malviflora*. Litter depth was also correlated with survival in the second year for *L. oreganus, S. malviflora* ssp. *virgata*, and *Castilleja levisecta*. We saw potentially positive interactions with grass (as a direct effect), moss and total vegetative cover (Table 2) for *C. levisecta* at Bellfountain and *L. oreganus* and *S. malviflora* ssp. *virgata* at individual sites. Most of our planted species performed better with more neighbors. Clark and Wilson (2003) also found that seedling mortality was high in gaps between established plants and attributed this effect to the variable abiotic conditions common to this region.

Litter is generally considered to have an inhibitory effect on seed germination and establishment though this trend is weaker in grasslands than in forest or forb dominated environments (Ryser 1993, Xiong and Nilsson 1999). Both the physical and chemical environments are affected by the presence of plant litter (Facelli and Pickett 1991). Litter can intercept light, regulate temperature and help conserve soil moisture (Amatangelo et al. 2008).

In Willamette Valley prairies, litter has been shown to have both positive and negative effects on seed establishment (Clark and Wilson 2000). For example, Maret and Wilson (2005) found that litter suppressed establishment of broadcast seeds, in Western Oregon grasslands. Their study species all had relatively small seeds compared to *L. oreganus* and *S. malviflora* ssp. *virgata*. Perhaps the negative effects in this case were the result of litter preventing soil-to-seed contact. Jensen and Guteknust (2003) found that in the presence of litter, seedling establishment was positively correlated with seed size. Our larger smooth un-barbed and un-appendaged

seeds may have been more successful at passing through the litter layer to achieve soil contact. In another study, Wilson and Clark (2001) recommend mowing tall invasive grasses without removal of cut material to promote native species already represented in the community which supports our findings that litter was also associated with increased survival of established individual.

Differences in interaction by functional group

We found no indication that grass inhibited establishment in the first two years of any of the species we seeded in our plots. In fact we found the opposite; litter depth, an indirect effect of grass was positively correlated with increased establishment and survival of *L. oreganus*, survival of *S. malviflora* ssp. *virgata* and second year survival of *C. levisecta*.

All of the negative effects we measured for establishment and survival of seedlings in their first two years were correlated with the abiotic factors, disturbance and bare ground. This indicates that the *lack* of neighbors to interact with or burial and removal by burrowing rodents may be negatively affecting the ability to establish and persist. Direct interactions with biotic community components, grass, forbs and moss appear to be primarily neutral in the early stages of establishment but may tend towards competition at later life history stages.

Competition and Facilitation at different life history stages

In our study, the number of leaves was a reasonable proxy for overall performance of *L. oreganus* as it was correlated with flowering and thereby reproductive potential. With respect to leaf number, we saw a potential shift from neutrality to competition with grass cover in five-year old lupine compared to seedlings, but litter depth still was positively correlated with overall performance. Wolkovich et al (2009) demonstrated how litter alters the biotic and abiotic environment and enhances growth of adult *Artemisia californica,* though non-native grasses likely interacted competitively with young *A. californica*. We may see something similar where litter in our study system is interacting positively with our

species at both the seedling and adult stages but that the net balance of facilitative or competitive interaction shifts at an intermediate life history stage (Figure 10A).

We did not have mature individuals for any of the other species we tested in which to compare this trend but we expect that transitions from facilitation or neutrality to a competitive relationships (Figure 10B), especially with grass, may occur for other species as well. Though facilitation is occurring, the shift from facilitative to competitive interactions over the life on an individual may correspond with an overall negative effect on the population over time (Williams and Crone 2006).

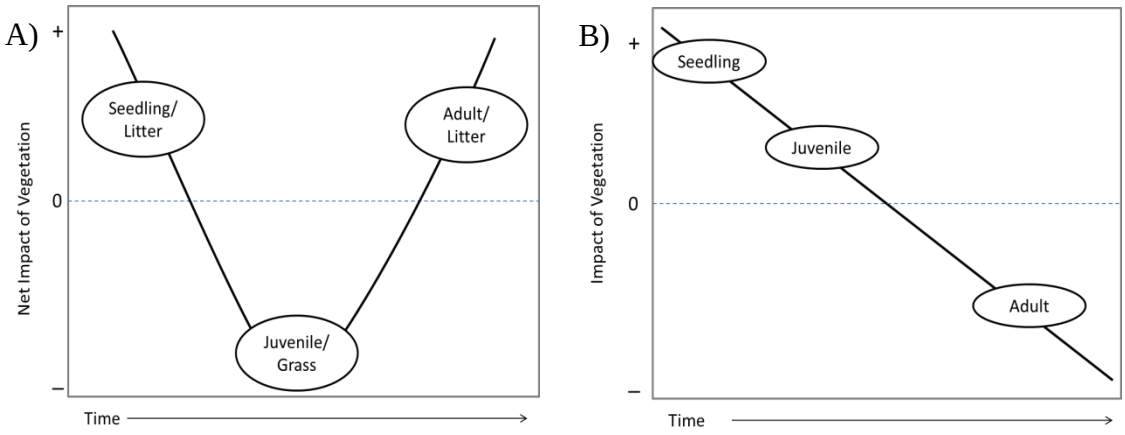

Figure 10. Two conceptual models of the shift between facilitative to competitive interactions with existing community over successive life history stages of an individual A) The net or driving interaction may differs by functional group for different life history stages. B) The interaction with a single functional groups shifts in one direction with successive life history stages.

Seed Pre-treatment

Contrary to our prediction, scarified *L. oreganus* seeds had lower establishment than non-scarified seeds in our study. Severns (2003b) also found that, though scarification is necessary to initiate germination for greenhouse propagation, it does not appear to improve establishment in the field, he surmised, that physical dormancy was overcome by natural means. The breaking of physical dormancy by natural means is a mechanism for ensuring that seeds germinate under conditions and

during seasons most favorable for establishment of the species. Physical dormancy of non-scarified seeds was apparently overcome in the field by natural processes such as (Baskin and Baskin 1998) freeze-thaw dynamics during winter months, the increase in relative humidity during winter months, interactions with soil microbes or a combination of these factors (Baskin and Baskin 1998).

Reduced establishment of scarified seeds may have been the result of early mortality. Scarified seeds germinated earlier in the year than non-scarified seeds in both our study plots and in nursery flats of seeds subject to outdoor conditions (Appendix C). Many of these early germinants may have died because of freezing temperatures, increased pressure by herbivores, increased incidence of pathogens during the wetter part of the winter, or a combination of all these factors. Maret and Wilson (2000) observed high mortality in seeds of grasses and annual forbs that germinated in either the fall or winter.

Iris and Erigeron

Once established, *Iris tenax* had a high survival rate such that it was not possible to correlate iris success with any community component. Like many irises, *I. tenax* is a clonal species (Wilson 2001) and the seeds appear to have dormancy (see chapter 3). For restoration purposes, seed may not be the most efficient way to establish *Iris* (Volis et al. 2007)

Previous studies have shown that establishment of *Erigeron decumbens* seed viability is often very low, especially among seeds from small populations (Thorpe and Kaye 2011) and establishment by seed is also very low, sometimes <1% (Kaye and Brandt 2005). Even so, actual establishment rates in our study sites may have been higher than we observed; seedlings and juvenile *E. decumbens* are very small and may easily be confused with young grasses or young *Plantago lanceolata*, both of which were abundant in our plots (Appendix A). However, our results are consistent with what others have found; this does not seem to be a species that is well suited to direct seeding. Transplants are the best option for reintroduction of this species (Thorpe 2009).

Biological relevance

Although our results are suggestive statistically of positive or negative interactions with community components, some of the effects we observed were weak. In our regression analysis, our strongest positive signals came from the indirect interaction of target species with grass in the form of litter depth. Other community factors such as cover by grasses, forbs and total cover had minor slopes (see for example Figure 8C). Though these slopes appear to be nearly flat in some cases, they still provide evidence that suggests a facilitative interaction between these community factors and the target species. The winters of 2010 and 2011 were mild; they were wetter and cooler than the average for the Willamette Valley. If, as Callaway (1997) posits, facilitative effects increase as abiotic stress increases, we'd expect the positive interactions we observed to be even stronger in warmer, drier years.

Implications for Management

Unfortunately, there is no panacea or universal prescription for ecosystem restoration. Effective restoration strategies must be goal driven (Zedler 2005), therefore strategies must differ depending on the scale (species or community level) at which managers are working. In the case of target species augmentation or reintroduction, the management strategy needs to be tailored to the individual species. In stressful sites, for medium to large-seeded perennial forbs that we tested, *Lupinus* and *Sidalcea* seeding should occur prior to removal of undesirable grass species or after successful establishment of native grasses. Since litter from grasses appears to enhance establishment of these species, we recommend leaving existing litter in place until a couple years after seeding. Mowing to control tall invasive grasses may be implemented if cut material is left on the ground. Targeted removal of invasive grasses should wait until target restoration species are established unless litter addition is included in the management activities. Though removal of invasive grasses may be postponed, it appears that it is a necessary step at some point to establish sustainable populations.

Although scarification is an effective method for overcoming physical dormancy in *Lupinus oreganus* and does enhance germinability of this species in a greenhouse environment, we do not recommend scarification as a pre-treatment for field sowing. It appears that natural processes are sufficient to overcome dormancy of seeds sown in the fall. If seeding occurs in the spring with the goal of immediate establishment, scarification may still be a useful tool though further research is necessary to demonstrate this.

Seeding may not be the ideal method for introducing *Iris tenax*; managers should consider greenhouse propagation and transplanting of this species. Disturbance by moles which dig up, eat, or bury seedlings was the strongest single factor that limited establishment of *Lupinus* and *Sidalcea*. We recommend managers survey restoration sites for burrowing rodents prior to reintroduction. If rodent populations are high, it would be wise to take steps to mitigate the detrimental effects of herbivory and soil disruption that these mammals have on establishing seedlings; rodent exclosures, removal of rodents, or preferentially selecting sites with lower abundance of moles may increase the chances of success.

Plant reintroduction will be more successful by taking the time to characterize the stress environment of restoration sites and developing reintroduction strategies that work with the unique conditions of the site and the individual requirements of the species to capitalize on interactions between plant functional groups.

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Chapter 3

Sprouting seeds to save butterflies: Characterizing dormancy of two perennial plant species of conservation concern

Katherine D. Jones and Thomas N. Kaye

Abstract

Habitat restoration for the endangered *Icaricia icarioides fenderi* (Fender's blue butterfly) requires establishment of host plants as well as nectar resources to support all life stages of the butterfly. *Sidalcea malviflora* ssp. *virgata* (Rose checkermallow) and *Iris tenax* (Roughleaf iris) are two native forbs in the Willamette Valley, OR recognized as providing high value nectar supply for the butterfly, but which have demonstrated poor establishment in past direct seeding indicating that there may be some dormancy in seeds of these species. We characterized the dormancy types of each species and developed germination protocols for greenhouse propagation of plant materials. *S. malviflora* ssp. *virgata* has physical dormancy and may have some physiological dormancy. Optimum germination (55%) was achieved by scarification, followed by four weeks or more of cold moist stratification at 5°C. *I. tenax* has morphophysiological dormancy which was most effectively overcome (63%) by four weeks warm moist stratification at 20/30°C followed by 6-12 weeks cold moist stratification at 5°C. Application of dormancy breaking techniques prior to direct seeding did not increase field establishment of *I. tenax.*

Introduction

Habitat restoration for endangered arthropods is essential to save many species from extinction (Kim 1993, Panzer and Schwartz 1998, Assmann and Janssen 1999, Black et al. 2001). In the Willamette Valley, Fender's blue butterfly (*Icaricia icarioides fenderi* Macy) relies on a suite of plants to support both juvenile and adult individuals (Schultz and Dlugosch 1999). To increase habitat for the Fender's blue butterfly, land managers are focused on invasive species removal and restoration of native plant communities that will support all life stages (Schultz et al. 2011). Reintroduction of resource plants, in addition to larval host plants is necessary to meet habitat needs for this species (Schultz 2001).

Two important nectar resources that are likely candidates for butterfly habitat restoration efforts in the Willamette Valley are *Sidalcea malviflora* (DC.) A. Gray ex Benth. ssp. *virgata* (Howell) C.L. Hitchc.) (Rose checkermallow) and *Iris tenax*

Douglas ex Lindl (Toughleaf iris) (plant nomenclature follows Oregon Flora Project Checklist, Cook and Sundberg (eds.) 2011a, 2011b). In past reintroduction efforts, both species exhibited poor establishment (Kaye, pers. obs.) from seed and both Malvaceae and Iridaceae frequently have some type of dormancy (Winter 1960, Arditti and Pray 1969, Blumenthal et al. 1986, Halse and Mishaga 1988, Baskin and Baskin 1998). Dormancy may be defined as the condition in which viable seeds fail to germinate even when subjected to favorable environmental conditions (Bewley 1997, Baskin and Baskin 2004, Finch-Savage and Leubner-Metzger 2006). Dormancy is caused either by characteristics of the embryo (endogenous dormancy) or by characteristics of seed structures other than the embryo (exogenous dormancy). Exogenous factors may involve hormones that chemically inhibit germination, or physical barriers that may prevent the absorption of water into the seed or block elongation and emergence of the radicle or shoot (Baskin and Baskin 1998).

Sidalcea malviflora ssp. *virgata* is a Malvaceae. Species in this family often have exogenous physical dormancy caused by an impermeable seed coat (Rolston 1978, Baskin and Baskin 1998). The chalaza region of the seed appears to be the location where, in nature, the seed coat is weakened and dormancy is most often overcome (Rolston 1978). The chalaza plug is a structure in the seed, located opposite the micropyle that, under the correct conditions, dislodges and allows water to enter the seed and initiate germination (Winter 1960, Baskin et al. 2000). Physical dormancy is overcome in manipulative experiments (and often in nature) through scarification, breaking, of the seed coat.

Germination of *Iris tenax* has not been previously investigated. The genus *Iris* is known to have strong dormancy in many species (Tillich 2003) and has been described as having physical dormancy (Blumenthal et al. 1986), physiological dormancy (Arditti and Pray 1969, Morgan 1990), morphological and morphophysiological dormancy (Grime et al. 1981, Shipley and Parent 1991, Coops and van der Veld 1995). However, most studies have been conducted on wetland or ornamental species of *Iris*, and it is unknown how well these observations extend to

upland species. Physiological dormancy is the most common type of dormancy (Baskin and Baskin 2004). This type of dormancy is found in all seed types and in nearly all plant families. Physiological dormancy is often overcome through stratification, the process of subjecting seeds to either warm or cold conditions to simulate seasonal temperature fluctuations. Stratification may be applied to either moist or dry seeds.

To support the development of plant materials for restoration projects using these species, we conducted a series of laboratory and field experiments to determine the specific type of dormancy in each species and develop dormancy breaking protocols. Because physiological dormancy is so common, we elected to include stratification in our experimental treatments for both species in addition to testing experimental treatments based on descriptions of dormancy in related species.

Methods

Our experimental design was based on methods described in Baskin and Baskin (1998) for the dormancy types described in the literature for related species. All laboratory experiments were conducted at the Oregon State University Seed Laboratory. We tested the effects of scarification and moist stratification on germination of *Sidalcea malviflora* and *Iris tenax*. Temperature controlled rooms at the Seed Laboratory were used to stratify seeds (Appendix B). Following treatments, seeds were placed in a germination chamber with alternating 15/25°C temperatures and 8/16 hour photoperiods, (8 hours of warm light, 16 hours of cold dark).

We employed a factorial design at each stage of germination trials to determine optimal techniques for breaking seed dormancy for these species. We determined a seed to have germinated if the radicle emerged at least 2 mm beyond the seed coat.

Sidalcea malviflora

We tested scarification in combination with cold moist stratification as methods for breaking dormancy in *Sidalcea malviflora* in a 2 x 7 factorial design. Each of the 14 treatment combinations included 4 replicates of 50 seeds (Table 1).

Scarification was applied to half of the seeds with the other half remaining un-scarified as controls. Scarification of *Sidalcea maliviflora* ssp. *virgata* seeds was achieved by nicking off a piece of the seed coat of each seed with a razor blade; each seed was scarified by hand to ensure the seed coat was broken. Cold stratification (5 ˚C) was applied to both scarification units in 7 treatments ranging from 0-12 weeks of cold stratification (Table 1). Following treatment application, seeds were placed in a germination chamber (15/25 °C) and growth was monitored and recorded after a two-week germination period.

Table 1 Experimental design for germination tests of Sidalcea malviflora ssp virgata.

	# of weeks at 5° C						
	0	2	4	6	8	10	12
Unscarified	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50
Scarified	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50	4 x 50

4x50 = 4 replicates of 50 seeds each. All seeds came from the same commercial source.

Iris tenax

All *I. tenax* seeds for the initial germination tests were provided by Heritage Seedlings Inc. and were from mixed-accession production beds developed from Willamette Valley populations. For the final round of trials, we tested seeds from six different sources from around the Willamette Valley to determine if dormancy breaking methods were consistent throughout the range of this species.

Wet stratification

We conducted several rounds of germination trials on *Iris tenax* to determine optimal germination conditions and characterize the type of dormancy in this species. We tested combinations of warm and cold stratification in a 3 x 7 factorial design. We tested a total of 21 treatment combinations; each treatment combination was replicated four times using 50 seeds for each replicate (Table 2). Warm stratification (20˚/30˚C) was applied in three treatments, 0, 2 and 4 week periods of warm conditions. Cold

stratification (5˚C) followed warm stratification and was tested in seven treatments ranging from 0-12 weeks of cold stratification. Following treatment application, seeds were placed in a germination chamber (15˚/25˚C). Growth was recorded following a two-week germination period. Both the 20˚/30˚C and the 15˚/25˚C chambers have 8/16hr photoperiods, (8hrs of light, 16hrs of dark).

Based on initial results in 2010, we followed up in 2011 by testing the effects of short intervals of high heat applied to dry seeds, followed by cold moist stratification , the effects of giberellic acid on germination compared to warm/wet stratification and wet stratification only (control).

Dry stratification –

We tested a total of nine treatment combinations in a 3 x 3 factorial design (Table 2). Dry stratification was applied in three treatments, 40°C for 24 and 48 hours and 50°C for 25 hours followed by cold (5°C) moist stratification for 0, 4 or 8 weeks. Each treatment combination was replicated 4 times using 50 seeds for each replicate. Growth was recorded following a two-week germination period.

Gibberellic acid-

We tested whether gibberellic acid (GA3) would break dormancy without manipulating temperature. We soaked *Iris* seeds in a 500 ppm GA3 aqueous solution for 25 hours then recorded germination after 2, 4 and 6 week germination periods in 15°/25°C germination chamber.

Field experiment –

Concurrent with the 2011 round of lab tests, we tested warm stratification treatments as pre-treatment techniques for field sowing. One hundred pre-treated seeds were sowed into 70 randomly assigned 1 x 0.5 m plots laid out in in a 7.5 x 27 m grid in an upland prairie at Bald Hill Natural Area in Corvallis, Oregon. The treatments tested were: control (no stratification); 4 weeks of 20/30°C wet stratification; 24 hours at 40°C dry stratification and 24 hours at 50° dry stratification. All seeds were mixed

with vermiculite and broadcast across the plots in fall 2010. Plots were sampled June 2011.

Scarification

The first two rounds of tests indicated that there may be a substance in the seeds that inhibits germination. To test whether the inhibitor was located in the seed coat, we removed the seed coat and compared germination with an un-manipulated control. Seeds were placed in a pneumatic seed scarifier for 5 minutes at 35psi and we visually confirmed that most of the seed coat had indeed been removed by this treatment. We tested 100 scarified and 100 non-scarified seeds, with and without a one week cold stratification period at 5°C (Table 2). The technique used to scarify *Iris* seeds differed from techniques used to scarify *Sidalcea* because we wanted to remove the entire outer covering of the seed rather than cause a break in the seed coat. The pneumatic scarifier was fast and efficient for exposing the endosperm.

Source population

To determine if dormancy is consistent across the range of this species, we tested the germination protocols we developed in the first couple rounds of trials on seeds from throughout the Willamette Valley. Seeds were collected from native populations and from commercial production beds. Our sources represent a range of seeds that are likely to be used in restoration projects in this region (Table 3 and Figure 1). Because in the first three rounds of tests we only observed germination after some combination of warm wet stratification and cold stratification, we elected to use only these treatments in this experiment and vary the lengths of time of both treatments. We tested the effects of warm and cold stratification in a 2x2 factorial design replicated across six seed sources. Warm stratification (20/30°C) was applied for either 1 or 4 weeks followed by cold stratification (5°C) for 4 or 8 weeks.

Statistical Analysis

We used two-way analysis of variance (ANOVA) to test for the effects of scarification and stratification on mean germination of *Sidalcea malviflora* and *Iris*

tenax. We also conducted pairwise comparisons between each treatment group using Tukey HSD test. All analyses for both species were conducted using R statistical software, version 2.14 (2011).

Table 2 Experimental design for germination tests on *Iris tenax*. 4x50x1 = 4 replicates of 50 seeds from 1 seed source

	Treatment	**Cold Stratification**							
		0 wk 5°	1 wk 5°	2 wk 5°	4 wk 5°	6 wk 5°	8 wk 5°	10 wk 5°	12 wk 5°
Wet Stratification	Control	4x50x1	-	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1
	2 wk 20/30° (wet)	4x50x1	-	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1
	4 wk 20/30° (wet)	4x50x1	-	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1	4x50x1
Dry/Wet Stratification & GA3	Control	4x50x1	-	-	4x50x1	-	4x50x1	-	-
	4 wks- 20/30°C (wet)	4x50x1	-	-	4x50x1	-	4x50x1	-	-
	24 hrs - 40°C (dry)	4x50x1	-	-	4x50x1	-	4x50x1	-	-
	48 hrs - 40°C (dry)	4x50x1	-	-	4x50x1	-	4x50x1	-	-
	24 hrs - 50°C (dry)	4x50x1	-	-	4x50x1	-	4x50x1	-	-
	24 hrs - GA3 500ppm	4x50x1	-	-	-	-	-	-	-
	24 hrs - GA3 500ppm, 4 wk germination	4x50x1	-	-	-	-	-	-	-
	24 hrs - GA3 500ppm, 6 week germination	4x50x1	-	-	-	-	-	-	-
Scarification	Control	1x100x1	1x100x1	-	-	-	-	-	-
	Scarified, 5min @ 35psi	1x100x1	1x100x1	-	-	-	-	-	-
Seed Source	1 wk 20/30°	-	-	-	4x50x6	-	4x50x6	-	-
	4 wk 20/30°	-	-	-	4x50x6	-	4x50x6	-	-

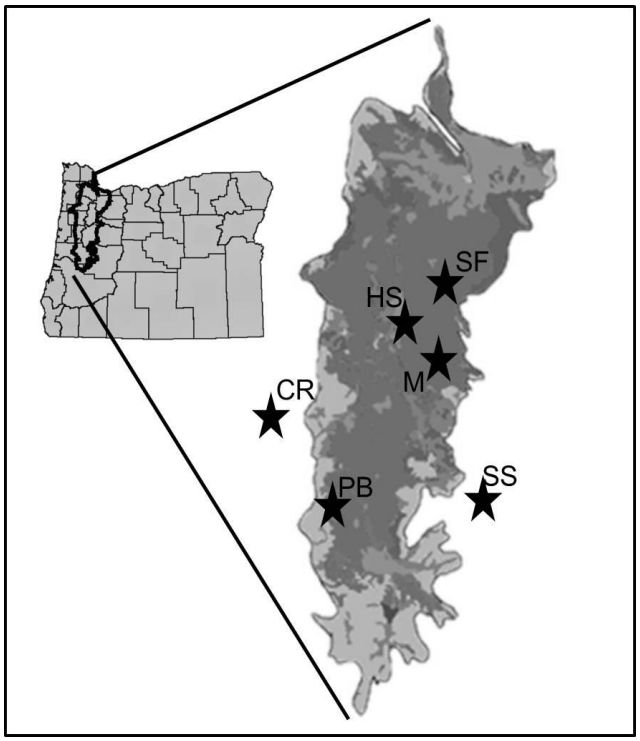

Figure 1 *I. tenax* source locations around the Willamette Valley, Oregon

Table 3 Seed sources of *I. tenax* compared for the final round of germination trials

Source Name	Type	Elevation (meters)	Estimated live seed (%)
Coast Range (CR)	Native Population	1067	60
Heritage Seedlings (HS)	Commercial/ mixed accession; Marion, Polk and Benton Co.	146	66
Mehema (M)	Native Population	155	62
Pigeon Butte (PB)	Native Population	144	40
South Santiam (SS)	Native Population	260	45.5
Silver Falls Seed (SF)	Commercial/ mixed accession; Marion Co.	135	41

Results

Sidalcea malviflora ssp. virgata

Both scarification and stratification were strongly correlated with germination but there was not a statistical interaction between treatments (Table 4). Mean germination of non-scarified seeds ranged from 3% (+/- SE 0.5%) for seeds that did not receive any stratification to a maximum germination of 35% (+/- SE 4%) for seeds that received 12 weeks of cold stratification. Scarified seeds had highest germination after four or more weeks of cold stratification. (Figure 2).

Table 4 Two-way Analysis of Variance for effects of scarification and cold stratification of *S. malviflora* ssp. *virgata*

	df	SS	MS	F-ratio	P
Scarification	1	0.63858	0.63858	47.4639	< 0.0001
Cold Stratification	1	0.43226	0.43226	32.1286	< 0.0001
Scarification x Cold Stratification	1	0.00258	0.00258	0.1917	0.6634
Residuals	52	0.69961	0.013458		

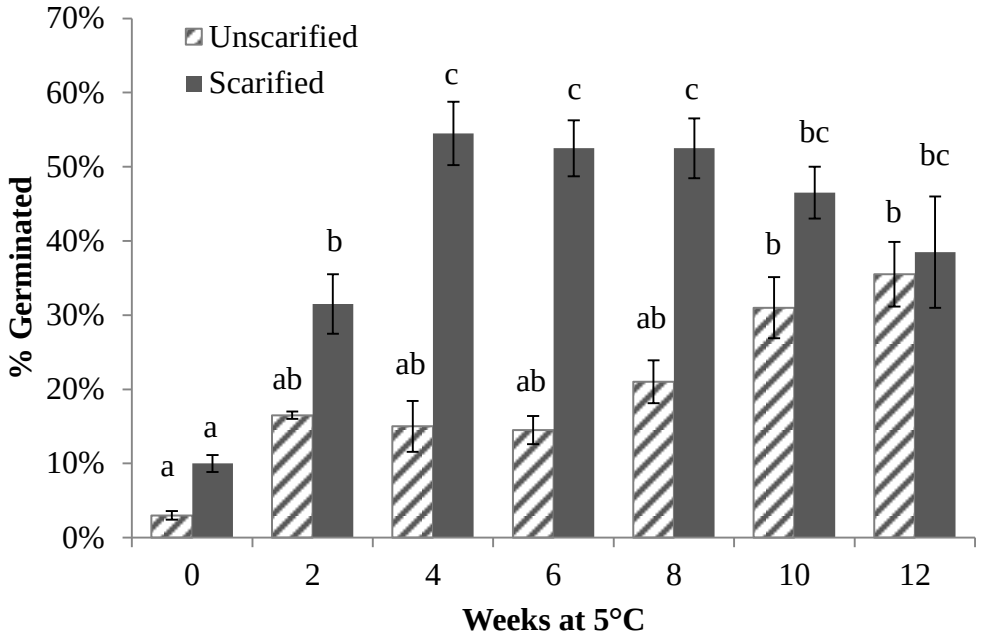

Figure 2 Germination of un-scarified and scarified *S. malviflora*, error bars equal one standard error for the treatment group. Different letters represent statistically different germination response to the different treatments ($p \leq 0.05$).

Iris tenax

Wet Stratification

Two-way analysis of variance indicated that both warm and cold moist stratification were important for germination and that there is a statistically significant interaction between the two treatments (Table 5). Mean germination was greatest when both warm and cold stratification were applied with the greatest mean germination occurring after 4 weeks warm stratification at alternating 20/30°C followed by 8 to 10 weeks cold stratification at a constant 5°C (Figure 3, Table 6).

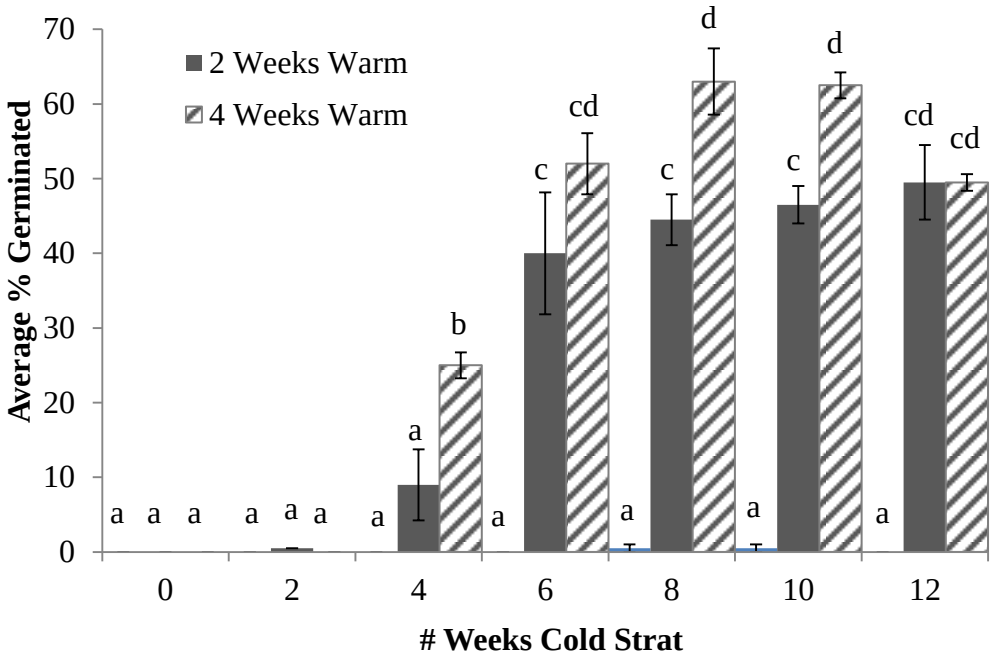

Figure 3 Germination results for *Iris tenax* with 2 and 4 week warm stratification. Mean germination for treatment groups that recieved no warm stratification was 0 and therefore is not shown on here. Bars with the same letter represent means that did not differ significantly ($p \leq 0.05$) based on Tukey HSD.

Table 5 Analysis of variance for effects of cold and warm stratification on the percentage of germination of *I. tenax*

	df	SS	MS	F-ratio	P
Cold	1	16576.2	16576.2	124.227	<0.0001
Warm	1	18000.3	18000.3	134.899	<0.0001
Cold x Warm	1	6864.3	6864.3	51.443	<0.0001
Residuals	80	10674.8	133.4		

Table 6 Mean germination (%) of *Iris tenax*, numbers in parentheses represent standard error of the mean

	Treatment	**Cold Stratification**							
		0 wk 5°	1 wk 5°	2 wk 5°	4 wk 5°	6 wk 5°	8 wk 5°	10 wk 5°	12 wk 5°
Wet Stratification	Control	0(0)	-	0(0)	0(0)	0(0)	1(0.5)	1(0.5)	0(0)
	2 wk 20/30° (wet)	0(0)	-	0.5(0)	9(2.4)	40(4.1)	45(1.7)	47(1.5)	50(2.5)
	4 wk 20/30° (wet)	0(0)	-	0(0)	25(1.7)	52(4.1)	63(4.4)	63(3.5)	50(1.1)
Dry/Wet Stratification & GA3	Control	0(0)	-	-	0(0)	-	0(0)	-	-
	4 wks- 20/30°C (wet)	0(0)	-	-	34(5.5)	-	67(3.9)	-	-
	24 hrs - 40°C (dry)	0(0)	-	-	0(0)	-	0(0)	-	-
	48 hrs - 40°C (dry)	0(0)	-	-	0(0)	-	0(0)	-	-
	24 hrs - 50°C (dry)	0(0)	-	-	0(0)	-	0(0)	-	-
	24 hrs - GA3 500ppm 2 wk germination	0(0)	-	-	-	-	-	-	-
	24 hrs - GA3 500ppm, 4 wk germination	0(0)	-	-	-	-	-	-	-
	24 hrs - GA3 500ppm, 6 week germination	0(0)	-	-	-	-	-	-	-
Scarification	Control	0	0	-	-	-	-	-	-
	Scarified, 5min @ 35psi	0	0	-	-	-	-	-	-
Seed Source (all)	1 wk 20/30°	-	-	-	1.2(0.9)	-	8.4(3.8)	-	-
	4 wk 20/30°	-	-	-	1.6(1)	-	39.3(14.1)	-	-

Dry Stratification

Dry stratification did not initiate germination in any of the treatment combination we tested (Table 6).

Gibberellic acid

None of the seeds that were treated with GA3 germinated after six weeks in the germination chamber.

Field experiment

Sampling efforts only positively identified five *I. tenax* individuals among the 70 plots that were seeded. All seedlings that we found were in either the 20/30°C pre-treatment group or the control group. This low establishment was insufficient for statistical analysis.

Scarification

Scarification did not release seeds from dormancy. There was no germination in either treatment group.

Source Population

With only one exception, all of the source populations we tested had the greatest germination with 4 weeks of warm moist stratification at 20/30° C followed by 8 weeks of cold moist stratification at 5° C (Figure 4).

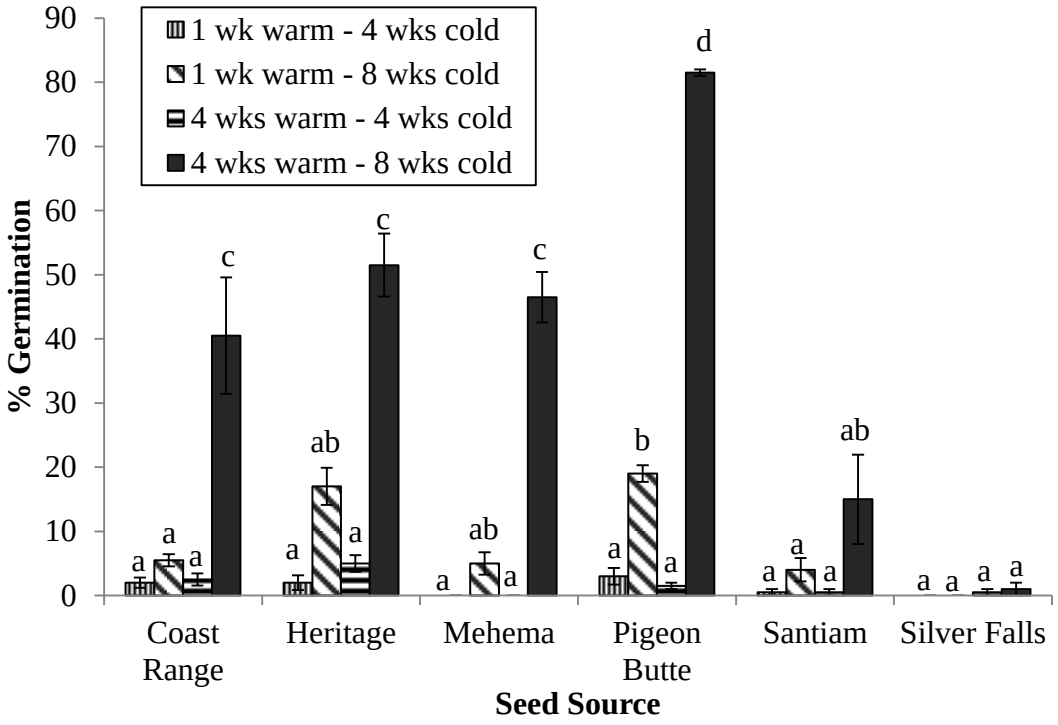

Figure 4 mean percent germination versus treatment combination for six different source populations of *Iris tenax*. Error bars represent one standard error of the mean, bars with the same letter represent means that did not differ significantly ($p \leq 0.05$) based on Tukey HSD.

Discussion

Sidalcea malviflora

S. malviflora has a hard seed coat that appeared to be a primary barrier to imbibition and germination. Its seeds also appear to possess some physiological dormancy, so that germination was highest when scarification and cold stratification were combined. Hard seededness has also been documented in a related wetland species from the Willamette Valley, *Sidalcea nelsoniana*, which had increased germination after scarification of the seed coat with a needle (Halse and Mishaga (1988). Physiological dormancy in *S. malviflora* ssp. *virgata* may be caused by germination inhibiting compounds in the seed, which was overcome by cold

stratification. Another explanation for the effect of cold stratification is simply that the seed coat is only mildly impermeable; the extended period of moist conditions softened the external lipid layers sufficiently to dislodge the chalazal plug, allowing water into the seed. Russel (2011) also found that cold stratification increased germination of *Sidalcea campestris* but concluded that this effect may be a result of slow degradation of the seed coat of a physically dormant species rather than evidence of physiological dormancy.

Under naturally occurring environmental conditions, the chalazal plug of *S. malviflora* may be dislodged by extreme temperatures, such as drying or a freeze-thaw cycle. In the Willamette Valley, seeds sown in the fall successfully overcome dormancy when they are subject to normal winter conditions in the soil (see chapter 2) with few seeds remaining dormant for two winters (personal observation). However, for the purposes of greenhouse propagation, when germination must occur quickly and consistently for all viable seeds we recommend scarification followed by four weeks of cold wet stratification.

Iris tenax

Iris tenax appears to have either deep simple or deep simple epicotyl morphophysiological dormancy as defined by Baskin and Baskin (2004). Highest germination of *I. tenax* was achieved by applying 4 weeks of warm wet stratification followed by 6-12 weeks of cold wet stratification.

The embryo of this species is very small and linear (Appendix C), requiring a period of maturation and growth inside the seed in warm moist conditions (simple morphological dormancy). Germination inhibitors in the seed, likely in the endosperm (Lenz 1955, Arditti and Pray 1969) or embryo must be overcome before radicle emergence occurs (physiological dormancy). Giberellic acid was ineffective at overcoming dormancy, suggesting that the species expresses deep dormancy. Baskin and Baskin (1998) report that morphophysiological dormancy is often overcome through a combination of warm and cold stratification treatments. They state that during the warm period, the embryo elongates and cells differentiate in preparation for

germination; then, during cold wet stratification, germination inhibitors are leached out of the seed. Morgan (1990) reported that a combination of both warm and cold stratification was most effective for germination of *Iris virginiana*. He applied treatments in the reverse order from our tests, cold stratification followed by warm. To overcome both morphological and physiological dormancy both warm and cold stratification were found to be necessary, though the order of treatments may not be important. We did not test the reverse order for *I. tenax*.

The observed requirements for breaking dormancy in the laboratory may be similar to the natural conditions that regulate germination in the field. This species blooms in late spring with seed pod maturation typically occurring in June. Following dispersal, seeds would be subjected to the warm summer temperatures. Though summer is a dry period in the Willamette Valley, residual soil moisture coupled with high temperatures may be sufficient to stimulate development of the embryo within the seed. Germination inhibitors protect the seed from germinating early during the fall months and becoming susceptible to freezing winter temperatures. The slow leaching of inhibitors throughout the cool wet winter, followed by warming temperatures in early spring, initiates emergence of the vulnerable radicle and shoot under favorable spring conditions during the year following seed dispersal.

Our scarification trials support Arditti and Pray's (1969) assertion that germination inhibitors in the genus *Iris* are located in the endosperm (or embryo) rather than in the seed coat. Blumenthal et al. (1986) reported that for two species of *Iris, I. lorteti* and *I. atropurpurea*, the seed coat mechanically restricted germination of the seeds in the micropylar region. If this were also true for *I. tenax* we would have expected some germination among the scarified treatment group. Because of our short trial period, the embryos may not have had adequate time to mature therefore these results may be partially confounded by the fact that seeds also have morphological dormancy.

Our attempts to use dormancy breaking treatments to pre-treat seeds for field sowing were generally unsuccessful. With the exception of the 20/30°C wet

stratification, most of the pre-treatments we tested in the field were also unsuccessful in the lab. The size of *I. tenax* seedlings and their similar appearance to several grass species that dominated the vegetation in our plots made them difficult to identify in a prairie setting and we discovered during sampling that we unintentionally seeded *I. tenax* into plots that already had a large population of *Sisyrinchium* sp., another member of the Iris family, that was difficult to differentiate from juvenile *I. tenax* based on leaf morphology (Appendix B). We cannot be sure of the accuracy of our survey efforts; actual germination and establishment may have been higher or lower than our counts. Nonetheless, based on our results, we recommend transplanting greenhouse grown plants rather than pre-treating seeds and field sowing. Once germinated, seeds of *I. tenax* are easy to transplant and grow readily in greenhouse conditions. In our experience, greenhouse seedlings have a high success rate in outplantings into field conditions (see chapter 2).

Seed dormancy may be under genetic control (Holdsworth 1999, Koornneef et al. 2002); it is possible that, for a given species dormancy may differ with different genotypes and with varied environmental conditions. This does not appear to be the case for *I. tenax*; dormancy appears to be consistent throughout the geographic range in the Willamette Valley. With the exception of Silver Falls, germination was highest for seeds that received 4 weeks of warm moist stratification followed by 8 weeks of cold moist stratification. The Silver Falls seed had been stored for several years and appeared to be low quality. Staining of the embryo and endosperm during the TZ evaluation was often mottled and irregular; the 'low quality' assessment was supported by the growth of mold that greatly exceeded growth of mold on other seed sources.

Habitat restoration for Fender's blue butterfly requires establishing populations of both the host plant *Lupinus oreganus* (Kincaid' lupine), the primary food source for butterfly larvae, and nectar species to support adult individuals. *Sidalcea malviflora* and *Iris tenax* are hardy species that provide reliably high levels of nectar (Schultz and Dlugosch 1999), and are species that are likely to be included in restoration efforts for

the butterfly. By following the dormancy breaking techniques described here, land managers can produce ample plant materials of these species to support habitat restoration for this endangered arthropod.

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Chapter 4

Do seed enhancements promote establishment of direct seeded native species? A meta-analysis on a physically dormant threatened forb.

Katherine D. Jones and Thomas N. Kaye

Abstract

Seed enhancements are widely used in high-value agricultural and horticultural crop production to promote rapid, uniform germination and improve seedling growth. If applied in habitat restoration projects that require plant species reintroductions, such enhancements could prove a valuable tool to increase success of direct seeding efforts. *Lupinus oreganus* (Kincaid's lupine) is a threatened perennial forb that is the host plant for the endangered *Icaricia icarioides fenderi* (Fender's blue butterfly). This species has physical dormancy caused by a seed coat that is impermeable to water. Propagation of this species requires scarification of the seed to initiate germination. We conducted a meta-analysis of 13 studies that tested scarification of *L. oreganus* seeds prior to direct seeding in controlled experiments. We found that, though scarification is necessary for greenhouse propagation, it does not enhance field establishment. For direct seeding of *L. oreganus*, allowing natural controls of dormancy (e.g. temperature, moisture and soil interactions) to determine germination timing is more effective than pretreating seeds to overcome physical dormancy. Scarification may still be an effective treatment for spring planting of *L. oreganus,* but additional field studies are necessary to test this hypothesis. Seed enhancement techniques are novel in the context of habitat restoration and if effective could dramatically change how land managers plant reintroduction but small scale field experiments are necessary before wide spread application of such techniques.

Introduction

Reintroduction of rare plants in habitat restoration projects may be achieved by either direct seeding or transplanting greenhouse grown plants. Although direct seeding generally has a lower success rate than transplants, if enough seed is available, it may be preferred because of lower costs associated with this method (Kaye and Cramer 2003, Guerrant and Kaye 2007). If seeds of the species being planted exhibit some kind of dormancy, the success rate may be even lower than expected.

Dormancy is a key issue for agricultural and horticultural practitioners and much of the research on dormancy comes from these fields (Bewley 1997, Koornneef et al. 2002, Halmer 2004, Sanchez and Mella 2004, Kucera et al. 2005), though increasingly more research on dormancy is coming from ecological perspectives (Vleeshouwers et al. 1995, Kaye 1997, Baskin and Baskin 1998, Finch-Savage and Leubner-Metzger 2006). Dormancy is caused either by characteristics of the embryo (endogenous dormancy) or by characteristics of seed structures other than the embryo (exogenous dormancy). Exogenous factors may involve hormones that chemically inhibit germination, or physical barriers that may prevent the absorption of water into the seed or block elongation and emergence of the radicle or shoot (Baskin and Baskin 1998).

Physical dormancy is an exogenous type of dormancy that is typically caused by a seed coat that is impermeable to water (Rolston 1978, Baskin and Baskin 2004). In a laboratory setting, germination does not occur without first modifying the impermeable seed coat layer so that water is able to enter the seed. Artificial means of breaking physical dormancy include: mechanical scarification, chemical scarification, immersion in boiling water, wet and dry heat, and low temperatures (Baskin and Baskin 1998). Mechanical scarification is a common and reliable method of weakening the seed coat and making it permeable to water (Baskin and Baskin 2004).

Many species in the Fabaceae family have physical dormancy (Baskin and Baskin 1998, Baskin et al. 2000, Baskin 2003, Finch-Savage and Leubner-Metzger 2006, Funes and Venier 2006). The genus *Lupinus* in particular is known for having many species with an impermeable seed coat that must first be softened or broken (usually through scarification) before germination occurs (Burns 1959, Mackay et al. 2001, 1995, 1996, Kaye 1997, Kaye and Kuykendall 2001a, Dehgan et al. 2003, Trindle and Flessner 2003, Karaguzel et al. 2004, Medina-Sánchez and Lindig-Cisneros 2005, Pavlovic and Grundel 2009, Gutiérrez Nava et al. 2010, Jones et al. 2010, Russell 2011, Elliott et al. 2011). We know how to reliably germinate lupines *in*

the laboratory however few studies have been published that discuss the efficacy of scarification on lupine germination *in the field*.

In the horticultural industry, lessons from the lab guide the development of pre-sowing seed treatments, also called seed enhancements, for commercial production of some high value plant and seed materials. One of the primary goals of seed enhancements is to improve germination rates and consistency (Parera and Cantliffe 1994). Seed enhancements include a broad set of techniques that address conditioning, processing, protection, physiological enhancements and seed coating (Taylor et al. 1998, Halmer 2004, 2006).

Here we ask the question, does seed scarification as a pre-sowing treatment improve plant establishment of a hard-seeded species? To address this question, we conducted a meta-analysis of published and unpublished field experiments that included both scarified and un-scarified seeds of a threatened legume with physically dormant seeds to determine if scarification is a useful tool for land managers utilizing a direct seeding approach for restoration and rare plant reintroduction.

Methods

The study species for this meta-analysis is *Lupinus oreganus* A. Heller (Kincaid's lupine), a threatened, long-lived fabaceous perennial endemic to the Willamette Valley of western Oregon. It is the host plant for the endangered Fender's blue butterfly (*Icaricia icarioides fenderi* Macy) (Wilson et al. 1997, 2003, Schultz and Dlugosch 1999, Schultz et al. 2003). Both the lupine and the butterfly are protected by the endangered species act (U.S. Fish and Wildlife Service 2000) and conservation of the butterfly is not possible without also conserving the lupine. For this reason, *L. oreganus* is often targeted for restoration projects in upland prairies of this region. To support restoration efforts for the lupine and the butterfly, an understanding of the germination requirements of the lupine is essential.

Like many lupine species, seeds of *L. oreganus* are physically dormant; to stimulate germination, seeds must first be scarified (Kaye and Kuykendall 2001a, 2001b). Using either mechanical or chemical scarification techniques, managers can

reliably germinate seeds to develop plant materials for transplanting lupine in restoration projects. Since recognizing the physical dormancy of this species, direct seeding reintroductions have frequently utilized scarified seeds.

The 13 field experiments included in this meta-analysis occurred over a 12-year period from 1998 to 2010 and come from one published study (Green Oaks, Severns 2003), Chapter 2 of this thesis (Bellfountain, Ft. Hoskins and Pigeon Butte 2010), two unpublished reports (Turtle Swale and Stark), and five previously unpublished data sets (EE Wilson, Fitton Green, Isabelle, Philomath Prairie, Pigeon Butte 2003 and Raindannce Ranch) provided by the Institute for Applied Ecology (Table 1). All study sites were located in remnant or restored upland prairies in the Willamette Valley, Oregon. Seeds used in each were collected from five different native populations also within the Willamette Valley: Clarks, Fir Butte, Green Oaks, LaBarre, Lupine Meadows, Pearcy and West Hills. Each study tested scarified and un-scarified seeds. Seeds were planted in either fall or winter and seedlings were counted the following spring. We had access to complete datasets for each study rather than summary results only so we could format data to achieve consistency in the types of data we included in the meta-analysis (dataset available in Appendix C). If multiple counts were taken over the growing season, we used the highest count as a measure of total establishment. We used the mean proportion of seeds that established per plot (# of seedlings / # of seeds planted) in each study as a standardized measure to compare between studies. The number of seeds tested in each plot ranged from 25-250 between studies.

Throughout this paper, we refer to the seedling *establishment* rather than *germination*. This is due to the fact that, in field studies, it is often difficult to know with certainty how many seeds germinate; some seeds may germinate and die before a researcher is able to count them. By counting seedling *establishment*, we acknowledge this fact and are only considering seeds that germinate and survive long enough to be counted.

Statistical exploration of data began by conducting two sided t-tests on establishment of seedlings from scarified vs. non-scarified seed. This provided an overview of what each study found and whether they found a statistically significant difference between treatment and control groups. We were unable to conduct any further analysis on the results of our t-tests due to statistical issues inherent with this type of 'vote counting' (see Hedges and Olkin 1980, Gurevitch and Hedges 1999 for more information on this topic). We could describe the difference in means and the error around those estimates, but essentially this approach only indicates the direction of the effect. We calculated another statistic, *d*, to characterize the magnitude of the effect.

Our meta-analysis of effect sizes followed the methods described by Gurevich and Hedges (2001). We began by calculating a standardized mean effect, *d* (also called Hedge's *d*)

$$d = \frac{\bar{x}_i^s - \bar{x}_i^u}{s_i} J$$

where $\bar{x}_i^s$ is the mean proportion of scarified seeds that established for a given study *i*, and $\bar{x}_i^u$ is the mean proportion of un-scarified seeds that established for the same study. The difference in means was divided by pooled standard deviation of the two treatment groups;

$$s_i = \sqrt{\frac{(n_i^s - 1)(s_i^s)^2 + (n_i^u - 1)(s_i^u)^2}{n_i^s + n_i^u - 2}}$$

where n_i^s and n_i^u represent samples size of scarified and un-scarified treatments respectively. *J* is a correction term to account for differing sample sizes between the studies calculated as:

$$J = 1 - \frac{3}{4(n_i^s + n_i^u - 2) - 1}$$

The effect size, *d*, is interpreted as the difference between the two treatment groups expressed in units of standard deviation. A positive value for *d* indicates that the proportion of scarified seeds that established was greater than un-scarified seeds and a

negative value for *d* indicates that un-scarified seeds had greater establishment than scarified seeds. In order to compare effect sizes between studies, we calculated the variance, *v*, in each effect:

$$v_i = \frac{n_i^s + n_i^u}{n_i^s n_i^u} + \frac{d^2}{2(n_i^s + n_i^u)}$$

The inverse of the variance, *w = 1/v* is used to weight the estimate of the cumulative effect size $\bar{d}$,

$$\bar{d} = \frac{\sum w_i d_i}{\sum w_i}$$

and its variance, $s_{\bar{d}}^2$,

$$s_{\bar{d}}^2 = \frac{1}{\sum w}$$

The 95% confidence interval, *CI* for $\bar{d}$ is:

$$CI = \bar{d} \pm Z_{\alpha/2} s_{\bar{d}}^2$$

Q_w is the measure of homogeneity within the group of studies and is similar to a χ^2 statistic with *n*-1 degrees of freedom. Because our studies are not separated into distinct classes, we only considered the within class homogeneity as described by Gurevitch and Hedges (Gurevitch and Hedges 2001)

$$Q_w = \sum w_i \, d_i^2 - \frac{(\sum w_i d_i)^2}{\sum w_i}$$

Table 1 Studies included in meta-analysis of *Lupinus oreganus* seed scarification trials

Study site	Year of Study	# of seeds/plot	# Plots Scarified : Unscarified	Seed Source	Citation
Bellfountain	2010	100	20 : 20	Pearcy	Jones and Kaye (Chapter 2)
EE Wilson	2009	25	19 : 21	Clarks	IAE unpublished data
Fitton Green	2010	250	10 : 10	Lupine Meadows and Clarks	IAE unpublished data
Ft. Hoskins	2010	100	20 : 20	Pearcy	Jones and Kaye (Chapter 2)
Green Oaks	1998	30	11 : 7	Green Oaks	Severns 2003
Isabelle	2000	50	10 : 10	Fir Butte	Kaye and Brandt, 2005
Philomath Praire	2010	250	10 : 10	Lupine Meadows and Clarks	IAE unpublished data
Pigeon Butte	2003	50	5 : 5		IAE unpublished data
Pigeon Butte	2010	100	20 : 20	Pearcy	Jones and Kaye (Chapter 2)
Raindance Ranch	2010	250	10 : 10	Lupine Meadows and Clarks	IAE unpublished data
Starck	2003	25	16 : 14	LaBarre and West Hills	Kaye et al. 2005
Turtle Swale	2003	50	10 : 10	Fir Butte	Kaye and Brandt 2005
Turtle Swale	2002	50	20 : 20	Fir Butte	Kaye and Brandt 2005

Results

Scarification usually decreased establishment of *L. oreganus* seeds in field experiments. T-tests indicated that scarification had a statistically significant ($p<0.05$) negative effect on establishment in seven of the studies, no effect in six data sets, and a positive effect in one study (Table 2). On average, 8% fewer scarified seeds established than did un-scarified seeds after accounting for site differences ($p<0.001$, $R^2=0.36$, 95% confidence interval -2 to -16%).

The cumulative effect size for scarification ($\bar{d}$) was -0.65 standard deviations ($p<0.05$, 95% confidence interval, -0.68 to -0.62 standard deviations). Individual studies had effect sizes ranging from -3.63 to 0.61 (Table 3). Nine of the thirteen studies we analyzed had a negative effect size. Though the difference in mean proportion established roughly followed effect size, it did not take into account variance around the mean or sample size (Figure 1). Our studies did not all exhibit the same effect (Q =66.82, df=12, $p<0.0001$).

Table 2 Results of t-tests for each of the study sites included in this meta-analysis. P-values <0.05 are considered significant for effects of seed scarification on seedling establishment of *Lupinus oreganus*, and symbols indicate whether effects were positive (+), negative (-) or neutral (0).

Study Site	Paired	T	df	p-value	Effect of Scarification
Bellfountain	Yes	-6.12	19	<0.0001	-
EE Wilson	No	-0.129	34.48	0.25	0
Fitton Green	No	-8.47	10.33	<0.0001	-
Ft. Hoskins	Yes	-3.44	19	0.003	-
Green Oaks	No	1.04	14.46	0.32	0
Isabelle	Yes	0.33	9	0.75	0
Philomath Prairie	No	-8.14	10.47	<0.0001	-
Pigeon Butte 2003	No	-1.4	7.98	0.2	0
Pigeon Butte 2010	Yes	-7.44	19	<0.0001	-
Raindance Ranch	No	-8.19	9.203	<0.0001	-
Stark	No	1.43	27.99	0.17	0
Turtle Swale 2002	Yes	2.18	19	0.042	+
Turtle Swale 2003	Yes	-2.397	9	0.04	-

Table 3 Parameter estimates used in the meta-analysis and summary statistics of combined effects of seed scarification on seedling establishment in *Lupinus oreganus*.

Study Site	code	n_{s_i}	n_{u_i}	$\bar{x}_i^s$	$\bar{x}_i^u$	$s_{s_i}^2$	s_u^2	J	d	v	w
Bellfountain	BF	20	20	0.12	0.28	0.13	0.13	0.98	-1.24	0.12	8.39
EE Wilson	EE	19	21	0.07	0.11	0.14	0.11	0.98	-0.37	0.10	9.81
Fitton Green	FG	10	10	0.05	0.28	0.02	0.08	0.96	-3.63	0.53	1.89
Ft. Hoskins	FH	20	20	0.14	0.23	0.11	0.18	0.98	-0.58	0.10	9.59
Green Oaks	GO	11	7	0.43	0.35	0.17	0.15	0.95	0.46	0.24	4.17
Isabelle	IS	10	10	0.26	0.25	0.14	0.13	0.96	0.04	0.20	5.00
Philomath Prairie	PP	10	10	0.10	0.34	0.03	0.09	0.96	-3.49	0.50	1.98
Pigeon Butte	PB03	5	5	0.10	0.33	0.10	0.10	0.90	-0.78	0.43	2.32
Pigeon Butte	PB10	20	20	0.25	0.28	0.09	0.13	0.98	-1.32	0.12	8.20
Raindance Ranch	R10	10	10	0.13	0.16	0.10	0.06	0.96	-1.69	0.27	3.68
Starck	S03	16	14	0.08	0.03	0.09	0.08	0.97	0.19	0.13	7.33
Turtle Swale	TS02	20	20	0.08	0.05	0.09	0.05	0.98	0.32	0.10	9.87
Turtle Swale	TS03	10	10	0.10	0.21	0.12	0.08	0.96	-1.01	0.23	4.44
Combined effects								$\bar{d}$	$s_{\bar{d}}^2$	$CI \pm$	Q_w
								-0.65	0.013	0.028	66.82

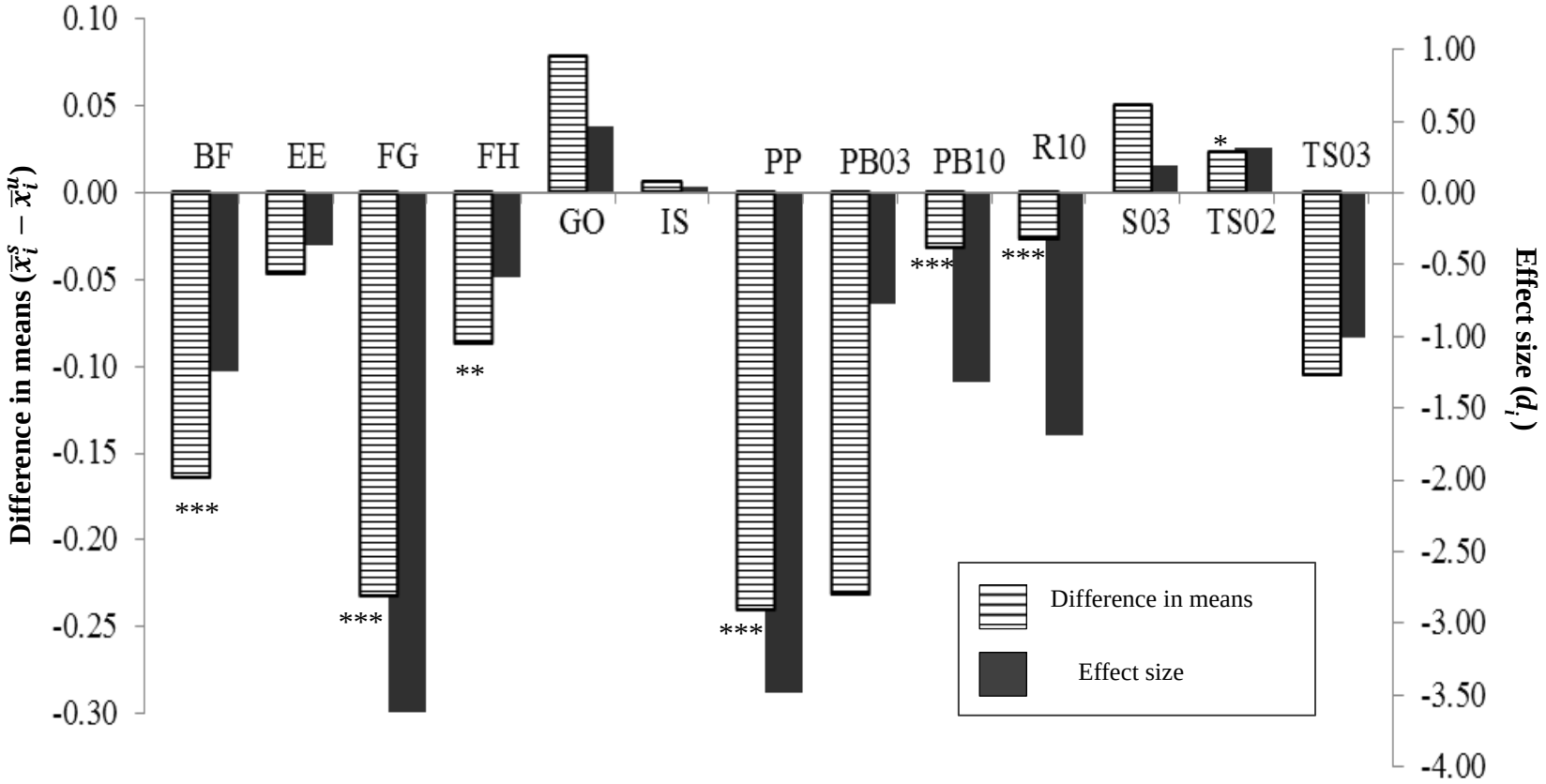

Figure 1 Comparison of differences in means between scarified and unscarified seeds and effect size for each study site. Asterisks indicated statistically significant differences between establishment of scarified and unscarified seeds as follows: *p≤0.05, ** p ≤0.01, ***p ≤ 0.001. See Table 3 for explanation of site codes.

Discussion

Though scarification is necessary to germinate seeds of *Lupinus oreganus* in the greenhouse, treating seeds with this method prior to sowing does not appear to improve seedling establishment of this species at field sites. According to Cohen's (1965) generalized interpretation of effect size magnitude, nearly half of the studies we considered had a "very large" (d >1 standard deviation) negative effect for scarification. Not all of the effect sizes were equal; most were negative though some were positive and the magnitude of the effect size was highly variable. This may be a result of the month that seeding or sampling occurred or may be an artifact of different environmental conditions between years or at different sites. The cumulative effect however is clearly negative.

Our findings with *Lupinus oreganus* are consistent with results of tests with other species used in restorations. For example, seed enhancements, such as scarification and pelleting, decreased emergence relative to untreated seeds of the native perennial *Lupinus argenteus* in eastern Oregon (Shock et al. 2010). Seed priming prior to sowing also had a negative effect on establishment of Wyoming big sagebrush (Booth and Bai 2000). The reasons for these negative effects are unclear but may be related to germination date of treated seeds. Seed dormancy evolved in many species in response to particular environmental conditions; germination occurs naturally at certain times of the year most favorable for growth of that species (Silvertown 1981, Baskin and Baskin 1998). It may be that by breaking seed dormancy prior to field sowing, treated seeds were disadvantaged in their native habitats. In the seven studies in this analysis that demonstrated lower establishment of treated seeds, scarification may have caused seeds to germinate earlier than control seeds the seedlings of which then succumbed to extreme temperatures or herbivory. Alternatively, seeds may have rotted because they imbibed water before temperatures were appropriate for germination and growth. We suspect that, for *L. oreganus*, pressure from herbivores is a primary factor in seedling mortality. In our study (Chapter 2), scarified seeds emerged earlier than unscarified seeds and earlier than

many other prairie species; we have witnessed slugs eating lupine cotyledons and seen evidence that many insects eat them as well (Appendix C).

Horticulture generally deals with species that have been removed from their native environment and therefore require enhancements to stimulate uniform rapid growth and meet industry demands. Some commercial techniques may be inappropriate for direct seeding of native species of conservation concern as the goals of conservation differ from horticulture.

Seed enhancements may still prove useful for direct seeding of some native plants. Coating seeds in material to increase weight and add nutrients or pesticides, seed pelleting or coating, is already in use for direct seeding over large areas in habitat restoration efforts in the Brazilian cerrado (Anese et al. 2010) and may be an option for broad scale seeding in the western rangelands of the United States (Madsen and Lawrence 2010). Based on laboratory characterizations of seed dormancy, other researchers have suggested that application of dormancy breaking pre-treatments may enhance reintroduction efforts (Hardegree 1994, Staden et al. 2000, Raimondi and Kermode 2004, Deering and Young 2006, Brancalion et al. 2010, Wagner et al. 2011). We caution however that seed treatments designed to break dormancy and stimulate early germination may place emergence of seedlings out of synch with their native environment.

It is likely that winter moisture and temperature conditions are responsible for breaking physical dormancy in *L. oreganus* seeds. If seeding occurred in the spring rather than fall or winter, it is possibly that scarification would enhance initial establishment; un-scarified seeds would likely remain dormant and not germinate until the following year though this has not yet been tested. We recommend employing an experimental approach that relies on field trials when considering seed enhancements to improve field establishment of native plants used for habitat restoration.

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Chapter 5

General Conclusion

We found evidence that indirect or direct facilitation from, rather than direct competition with established grasses drove initial establishment and survival of *Lupinus oreganus*, *Sidalcea malviflora*, and *Castilleja levisecta.* This suggests that managers working to establish new populations or augment existing populations of these species may benefit from postponing invasive grass removal until a year or two after direct seeding or transplanting these species. Alternatively, introducing native forbs may be postponed until after re-establishing native grasses at a site.

Restoration of degraded habitats does not occur as a single event. It is an iterative process. Sites must be monitored, strategies may change, and often continual management of restored sites is necessary to maintain restoration (Pastorok et al. 1997, Kaye 2001). Developing a stepwise procedure for native species establishment and invasive species removal fits naturally into this existing restoration framework. To develop transplants of *S. malviflora* or *Iris tenax* for restoration projects, it is necessary to overcome the natural seed dormancy in these species. Through germination trials, we demonstrated that seeds of *S. malviflora* ssp. *virgata* should be scarified followed by 4 weeks of cold stratification to achieve maximum germination in the shortest period of time. *I. tenax* has morphophysiological dormancy; seeds must first be warm stratified for four weeks to initiate growth of the embryo and then exposed to eight weeks of cold moist stratification to leach germination inhibitors for maximum germination. However, seed treatments that promote germination in the lab do not necessarily support establishment in the field. Pre-treatment of *I. tenax* did not increase establishment of direct seeded plots and according to our meta-analysis pre-sowing scarification of *L. oreganus* seeds generally had a neutral or negative affect on establishment relative to untreated controls.

Future research directions

In a recent review on the state of facilitation theory Brooker et al (2008) made many suggestions for future research to better understand the role of positive interactions in ecosystem function. Many of our recommendations for future research mirror theirs. Our research identified facilitation as a potentially important process

related to establishment of some species of conservation concern but our experimental design did not allow us to identify mechanisms of this effect. The increased stress conditions at our sites are determined by a range of factors (Richardson et al. *In press.*). By manipulating individual sources of environmental stress such as temperature, water availability and soil fertility, restoration ecologists may shed light on which resources are limited in this stressful environment and are in turn made available to seedlings by neighboring established plants.

Of greater importance to restoration in this region may be identifying how interactions shift over time and over successive life history stages. Our study only followed individuals for two years; a longer period of observation could address this question. A longer observation period of individuals may also make it possible to determine the impact of plant-plant interactions on plant fitness. Though we included leaf count in our sampling protocol, there was not enough variation to evaluate individual performance; with continued observations and additional measures of fitness including flowering, seed set and above ground biomass, variation between plots may become apparent. A final key question is whether facilitation or competition with functional groups differs by species provenance. Does litter from native grasses facilitate establishment of *L. oreganus* equally to that of non-native species? Do seedlings of non-native species also benefit from interactions with established neighbors?

Pre-treating seeds to enhance direct seeding efforts is already in practice in agriculture (Halmer 2004) and it is likely that some techniques may be applicable to plant species reintroduction. Though our attempts to enhance establishment through pre-treating seeds did not improve restoration outcomes, continued research may still identify alternative options. In particular, seed pre-treatments such as scarification and priming may enhance seeding efforts during suboptimal seasons opening up more of the year for reintroduction activities.

Even with pre-treatments, some species still will not establish at high enough rates to meet restoration goals. In that case, transplants may be the best option.

Characterizing seed dormancy and developing reliable germination protocols for 'difficult' seeds will support propagation efforts and may inform further research on seed pre-treatments.

In 1845, the explorer James Clyman described the Willamette Valley as "prairie all luxuriantly clothed in a rich and heavy coat of vegetation and literally clothed in flowers" (quoted in Alverson 2005). Today the expanses he witnessed have become fragmented and reduced to a fraction of their former extent. However, permanent loss of species and ecosystems in this region can be averted. Restoration strategies that address conditions at both the individual and ecosystem scales offer holistic solutions to these complex issues. Ecological theories of species interactions and environmental controls of seed dormancy provide a framework for developing restoration strategies. In the Willamette Valley, determining drivers of establishment and survival of species of conservation concern will give managers the tools to conserve the biodiversity of this region.

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APPENDICES

Appendix A

Supplemental Material for Chapter 2

Plot Layout

Experimental plots were situated within Collin's Project ("IAE/TNC" project in text, see Stanley et al. 2008 for project details) centered along the edge of the quarter plot seeded only in 2007 along the border with the control, unseeded quarter plot (Figure 1). Seeding in the Collin's project was randomly assigned so the position of our plots differs between plots.

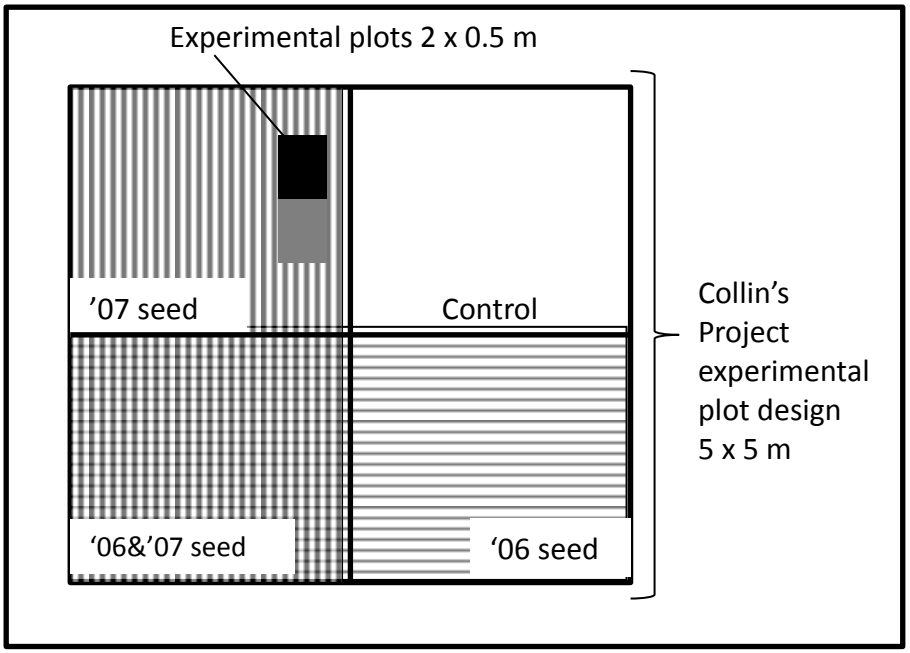


Figure 1 Plot orientation within existing experimental design

Plots were marked with three nails with washers spray painted yellow; nails were oriented so that the top of the "triangle" indicates the top of the plot (Figure 2). Seeding rates were : 100 *Sidalcea,* 100 *Iris,* 1000 *Erigeron* broadcast across the whole plot, 100 scarified and unscarified *Lupinus* in either side of the plot. Assignment of scarified side was random. Five plugs of *Iris tenax* were planted at 0.05 m intervals across the top of the plot, except at Bellfountain where 5 plugs of *Castilleja levisecta* were planted along the top and *Iris* were planted with the same spacing along the bottom edge of the plot (Figure 3-4).

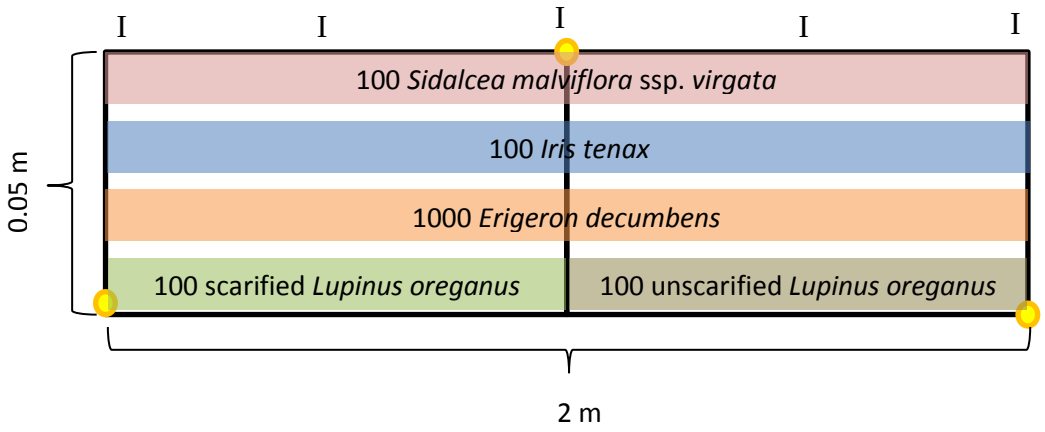


Figure 2 Generalized seeding design and plot layout (differs slightly at Bellfountain)

Figure 3 Iris plugs germinated using protocols discussed in Chapter 3

Figure 4 Iris planting at Bellfountain

Sampling

Plots were sampled using demographic techniques, individuals were mapped so that they could be relocated (Figure 5). Other plot characteristics including disturbance and debris were also mapped.

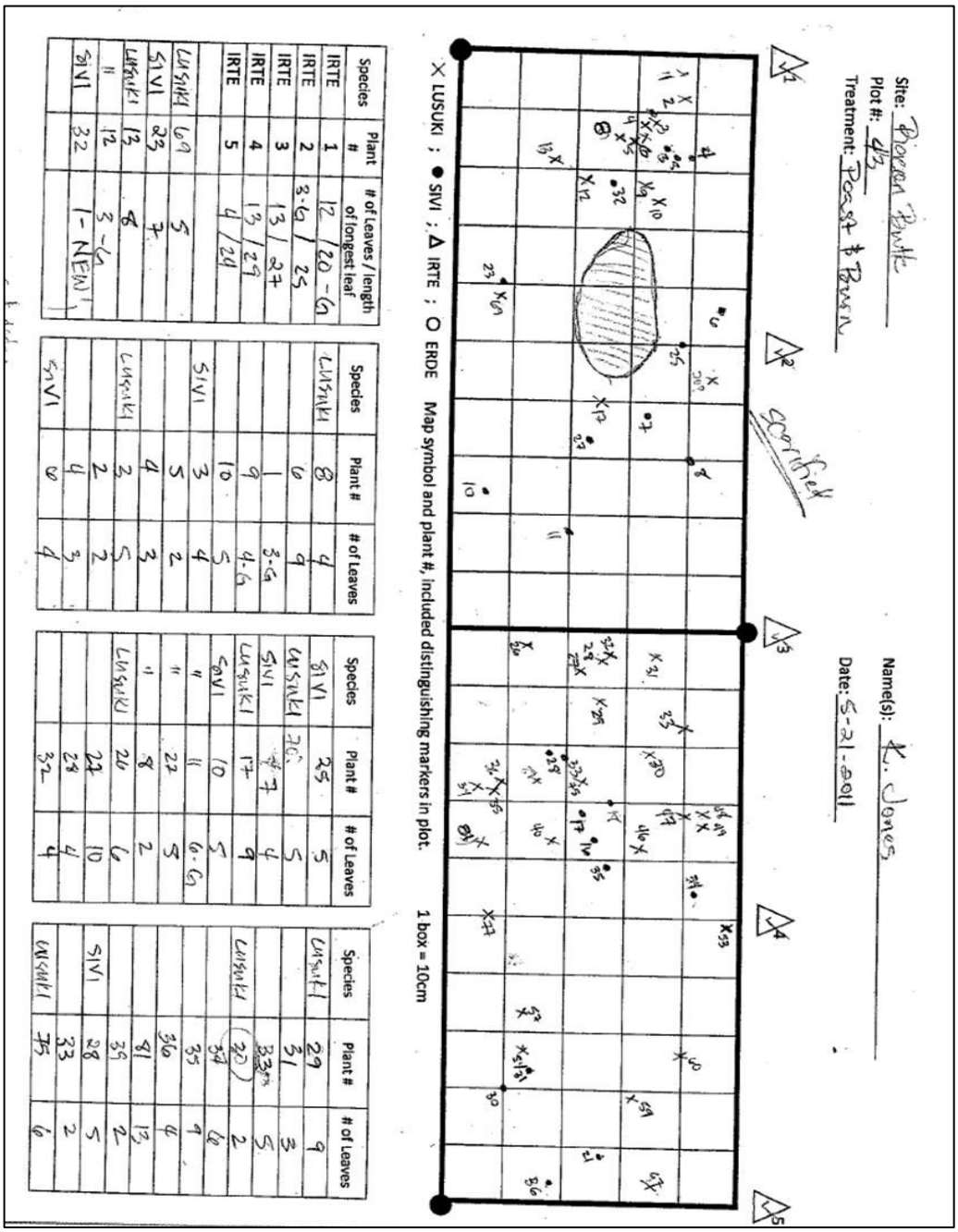


Figure 5 Sample data sheet from 2011

Cover estimates

We made ocular estimates of percent cover of each plant functional group and disturbance on 0.25 m^2 sections of each plot (Figures 6-14)

Figure 6 Grass-dominated plot

Figure 7 Lupine in Grassy plot

Figure 8 Forb-dominated plot

Figure 9 Lupine in a forb dominated plot

Figure 10 Moss-dominated plot

Figure 11 Sidalcea in a mossy plot

Figure 12 Grass derived litter

Figure 13 Sidalcea (cotyledons only) in deep litter

Figure 14 Disturbance by moles

Cryptic Seedlings

We were unable to conduct a statistical analysis on establishment and survival of *Erigeron decumbens* and *Iris tenax* seeded in our plots. Other studies have demonstrated low seed viability and low establishment rates in *E. decumbens* and we demonstrated that *I. tenax* seeds likely have dormancy. Even so, both species may be difficult to find and identify in field conditions. *E. decumbens* is easily confused with seedlings of *Plantago lanceolata* which is abundant at all three sites (Figures 15 &16) and *I. tenax* seedlings have very narrow blade-like leaves easily mistaken for grass .

Figure 15 *Erigeron decumbens* seedling

Figure 16 *Plantago lanceolata* seedling

Figure 15 planted *Iris tenax* seedling

Though we put considerable time into searching each plot and making sure that all of the field technicians were familiar with the characteristics of all the species that we seeded, it is possible that actual establishment rates of both of these species was higher than we observed.

Results

Table 1 Summary of split-plot data for *Lupinus oreganus* in 2010

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
BF	3	L	no	50	37	87	4.5	0.5	0	2.6	26	2.5
BF	6	R	no	35	45.5	80.5	15	0	7.5	1	46	2.52
BF	8	L	no	72.5	20.5	93	8.5	0.5	1	2.9	21	1.67
BF	10	L	no	50.5	42	92.5	6.5	4	2.5	1	44	2.48
BF	12	R	no	29.5	66.5	96	4.5	0	0	4.2	31	2.65
BF	13	R	no	11.5	77.5	89	18	0.5	0	0.7	17	2.94
BF	15	L	no	3.5	81.5	85	7	2.5	12	0.4	16	2.81
BF	17	R	no	60.5	29	89.5	10	2.5	0.5	4	41	2.34
BF	18	L	no	4	70	74	29	4	0	0.5	21	2.05
BF	20	R	no	1.5	79.5	81	20	3	2.5	0.8	59	2.14
BF	23	L	no	0.5	73.5	74	8.5	3.5	22	0.9	23	2.7
BF	24	L	no	6.5	86	92.5	16	4	0	1.2	12	2.67
BF	26	L	no	46.5	26.5	73	5.5	0	17.5	0.9	21	2.14
BF	27	L	no	48.5	49	97.5	8.5	0	0	1.3	25	2.88
BF	33	R	no	58.5	22	80.5	31.5	0	0	6.4	36	2.39
BF	34	R	no	62.5	30.5	93	10.5	0	0	2.9	26	3
BF	35	R	no	12.5	81.5	94	6	0	0	3.7	49	2.8
BF	37	L	no	18	77.5	95.5	1.5	3.5	0	1.3	20	2.65
BF	40	L	no	75.5	28	103.5	5	0	0	3.8	19	2.21

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
BF	44	L	no	1.5	91	92.5	14.5	0	0	0.7	14	2.29
BF	3	R	yes	67	26	93	4	0	0	2.8	1	2
BF	6	L	yes	33.5	43.5	77	16.5	0.5	5	1.7	19	3
BF	8	R	yes	52	55.5	107.5	22.5	2.5	0	1.6	47	2.09
BF	10	R	yes	30	60	90	5	4	2	1.7	15	0
BF	12	L	yes	59	42	101	10	2	0	3.7	2	2.17
BF	13	L	yes	41.5	51.5	93	15.5	1	0.5	1	6	2.91
BF	15	R	yes	11	79	90	9	1.5	1.5	1	3	2.21
BF	17	L	yes	57.5	31.5	89	6.5	0.5	7.5	0.6	17	2.54
BF	18	R	yes	5.5	57.5	63	40.5	0	0.5	0.6	5	1.8
BF	20	L	yes	4.5	66	70.5	13.5	1	16.5	0.3	31	3.26
BF	23	R	yes	1.5	51	52.5	4	2.5	41	1	3	3.02
BF	24	R	yes	9	76	85	20.5	0.5	0	1.2	3	3.22
BF	26	R	yes	52.5	23.5	76	23.5	0	0	0.7	3	3
BF	27	R	yes	46.5	49	95.5	0.5	0.5	0	2.7	12	3.23
BF	33	L	yes	46.5	43.5	90	22.5	0	0	3.7	26	2.93
BF	34	L	yes	73	28	101	5	0	0	3.8	2	3.02
BF	35	L	yes	6.5	89	95.5	4.5	0	0	1.2	31	2.46
BF	37	R	yes	20	69	89	9	0	0	1.8	2	3.03
BF	40	R	yes	85	14	99	1.5	0	0	3.7	8	3.35
BF	44	R	yes	0.5	94	94.5	12	1.5	0	0.9	1	3.07

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
FH	2	R	no	12.5	36	48.5	96.5	0	1	1.4	2	3.3
FH	6	R	no	20.5	72.5	93	5	4	0	1.5	5	2.81
FH	11	R	no	6	82	88	20.5	0	0	1.9	0	3.27
FH	16	L	no	75	20.5	95.5	0	1.5	4.5	2.4	35	2.93
FH	20	R	no	3	95	98	20	3	0	0.7	11	3.48
FH	21	L	no	33.5	61.5	95	1.5	3.5	0	1.4	14	2.64
FH	25	L	no	13.5	64	77.5	1	0	20	1.4	24	2.75
FH	30	L	no	30	66	96	0	0	2.5	1.75	5	3.76
FH	32	L	no	15	77.5	92.5	1.5	5	0	2.8	34	3.05
FH	33	L	no	36	60	96	0	4.5	1	1.9	58	3
FH	37	R	no	37	59	96	24	0	3	3.4	23	3.04
FH	38	L	no	62.5	35.5	98	1.5	3	0	2.3	24	3.18
FH	39	R	no	22.5	73	95.5	8.5	0	1.5	1.2	13	3.2
FH	40	L	no	23.5	72.5	96	0.5	0	1.5	1.7	14	3.04
FH	42	R	no	8.5	82	90.5	21.5	0	0	2	61	3.31
FH	43	L	no	71	54	125	0	1	0	5.6	41	2.48
FH	44	L	no	12	67.5	79.5	21.5	2	0	2.6	40	2.82
FH	49	L	no	27	55.5	82.5	2	7	2	2	34	2.27
FH	50	L	no	14	67.5	81.5	0	2	14.5	1.3	14	2.83
FH	2	L	yes	9	47	56	89	1.5	1	1.9	0	3
FH	6	L	yes	42	42	84	14.5	3.5	0	1.8	3	2.47

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
FH	9	R	yes	62.5	32.5	95	0	0.5	0	5	5	2.45
FH	11	L	yes	8	78.5	86.5	15	6	0.5	2.2	1	2.4
FH	16	R	yes	77.5	16.5	94	0	0	5.5	1.8	7	2
FH	21	R	yes	17	70	87	4	3	0	2.1	11	3
FH	25	R	yes	30	22.5	52.5	3	39.5	5	1.6	8	2.41
FH	30	R	yes	30.5	66	96.5	0	2.5	3	2.3	11	1.8
FH	32	R	yes	28.5	70	98.5	1.5	1	0	2.2	5	2.23
FH	33	R	yes	48.5	48.5	97	2	2.5	0	2.4	33	2
FH	37	L	yes	48	45	93	25.5	1.5	2.5	2.5	15	3
FH	38	R	yes	67.5	29	96.5	12	0.5	1	1.9	17	1.67
FH	39	L	yes	12.5	77.5	90	14	0	0	1.8	6	2.17
FH	40	R	yes	52	43.5	95.5	2	2.5	1.5	1.3	15	2.54
FH	42	L	yes	11	76.5	87.5	37.5	0	0	1.8	34	2
FH	43	R	yes	46.5	38.5	85	1	3	0	3.6	35	2.61
FH	44	R	yes	13.5	65.5	79	62.5	0	0	3	27	2.5
FH	49	R	yes	13.5	80.5	94	0	4	0	1.3	19	2.75
FH	50	R	yes	17	71	88	3	2.5	0	2.6	21	3
PB	2	R	no	3.5	63.5	67	0	31	0	0	27	0
PB	3	L	no	15	63	78	5.5	8.5	1.5	3	42	3.33
PB	5	R	no	15.5	75	90.5	2	3	0	2.7	41	2.6
PB	6	R	no	1.5	67.5	69	0	7	18.5	0.5	11	1

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
PB	9	R	no	44	51.5	95.5	1.5	6	0	1.9	27	2.33
PB	12	L	no			0					21	2.6
PB	17	R	no	11	53.5	64.5	0	1	32.5	0.4	14	2.36
PB	18	R	no	8.5	77.5	86	0	0	20.5	1.3	12	2.5
PB	24	L	no	6.5	82	88.5	0	9.5	0	0.6	37	2.18
PB	26	L	no	62.5	27.5	90	0	2	0	3.9	21	2.8
PB	27	L	no	5	55	60	0	0	42.5	1.1	17	3.24
PB	28	L	no	7	72.5	79.5	1.5	6	11.5	0.6	24	3.43
PB	32	R	no	10.5	61	71.5	3	0	22.5	2	39	3.13
PB	35	R	no	8	74	82	7	13	0	0.9	20	2.33
PB	39	L	no	2	73.5	75.5	2	3.5	13.5	1.9	49	2.2
PB	42	L	no	43.5	46.5	90	0	2	7.5	3.2	26	2.94
PB	43	R	no	2.5	77.5	80	0	0	17.5	0.7	48	2.66
PB	44	R	no	45.5	41	86.5	1.5	2.5	4	1.7	49	2.81
PB	45	R	no	24.5	63.5	88	0	11.5	2.5	1.6	11	3.42
PB	46	R	no	28.5	57	85.5	7	7	5	1.3	24	4.38
PB	2	L	yes	2.5	78.5	81	0	19	0	0.5	3	3
PB	3	R	yes	9.5	71	80.5	1	7	12.5	1.8	9	3.22
PB	5	L	yes	12	82.5	94.5	0	1.5	0	3.8	34	3.91
PB	6	L	yes	3.5	80.5	84	0	2	13.5	0.5	8	3.38
PB	9	L	yes	42	53	95	0	0.5	0	4.3	9	3.22

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
PB	12	R	yes			0					7	3.29
PB	17	L	yes	38	40	78	0	0	20.5	1.6	15	2.93
PB	18	L	yes	11	69.5	80.5	0	2	20	1.1	5	4
PB	24	R	yes	14	70	84	0	8.5	0	1	14	3.29
PB	26	R	yes	42.5	36.5	79	0	0	11.5	2.1	18	3.17
PB	27	R	yes	6.5	52.5	59	0	1	39	0	1	2
PB	28	R	yes	7	79.5	86.5	6	1.5	10	1.1	10	2.5
PB	35	L	yes	10	64.5	74.5	6	15.5	0	0.5	5	3.8
PB	39	R	yes	11	75	86	1.5	2	3.5	1.6	30	3.33
PB	42	R	yes	22	62.5	84.5	0	0	15.5	1.4	4	3
PB	43	L	yes	3	70.5	73.5	0	11	14	1.3	30	3.9
PB	44	L	yes	34	50	84	6	4.5	10	2.2	18	2.83
PB	45	L	yes	22.5	44.5	67	0	17.5	12.5	0.6	4	2.75
PB	46	L	yes	27	47.5	74.5	0	24	0	1.2	4	2.75

Table 2 Summary of split plot data for *Lupinus oreganus* 2011 survey

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	% Survival	Av leaf #
BF	3	L	no	50	36.5	86.5	20	0	0	5.6	68	5.4
BF	6	R	no	74	5	79	28.5	0	21.5	4	15	2.9
BF	8	L	no	74	23.5	97.5	25	0	5	4.9	24	3
BF	10	L	no	62	9.5	71.5	9	2	0	4.2	30	4.2
BF	12	R	no	72.5	30	102.5	55	0	0	5.3	36	3.5
BF	13	R	no	18.5	82.5	101	6	0	0	2.5	35	3.7
BF	15	L	no	4.5	53	57.5	20	5	29	0.4	11	3
BF	17	R	no	70.5	21	91.5	17.5	0	0	3.6	44	3.9
BF	18	L	no	26.5	46	72.5	10	0	11	2.5	24	2.2
BF	20	R	no	6	80	86	4.5	6.5	7	1	30	3.4
BF	23	L	no	6	39	45	12.5	8	42.5	0	13	1.7
BF	24	L	no	5.5	66.5	72	12.5	0	29.5	0.3	8	5
BF	26	L	no	89.5	9	98.5	9	6	0	5.4	19	2.8
BF	27	L	no	22.5	26	48.5	14.5	0	8.5	3	28	4
BF	33	R	no	38	22.5	60.5	10	0	7.5	9	46	3.4
BF	34	R	no	53	45	98	65	0	0	5.4	52	7.2
BF	35	R	no	3	91.5	94.5	16	2	1.5	1.6	57	6.4
BF	37	L	no	82.5	14.5	97	10	0	0	4.5	35	3.4
BF	40	L	no	59.5	23.5	83	10	2.5	11	5.8	45	4.8
BF	44	L	no	6.5	54	60.5	67.5	6	2.5	1.2	65	8.1
BF	3	R	yes	65.5	22	87.5	17.5	0	2	6.8	0	0

Table 2 LUOR 2011 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	% Survival	Av leaf #
BF	6	L	yes	85.5	8	93.5	15	2	12.5	5.3	21	4
BF	10	R	yes	66.5	13	79.5	2.5	0	0	4.2	25	0
BF	12	L	yes	67.5	22	89.5	22.5	0	0	2.9	50	2.9
BF	13	L	yes	17.5	58.5	76	11	2	10	3.6	33	4.5
BF	15	R	yes	14	71.5	85.5	11	4.5	8.5	3.5	0	0
BF	17	L	yes	70.5	11.5	82	7.5	0	17.5	4.5	18	6.6
BF	18	R	yes	21.5	33.5	55	8.5	1.5	32	1.2	0	6
BF	20	L	yes	17.5	56.5	74	9	7	11.5	1.3	28	0
BF	23	R	yes	3	45.5	48.5	6	5.5	39	0	0	6.8
BF	24	R	yes	11	81.5	92.5	23.5	0	3.5	1.8	67	7.3
BF	26	R	yes	91.5	11	102.5	6	0	0	5.8	33	0
BF	27	R	yes	38.5	29.5	68	17.5	0	13.5	5	17	13
BF	33	L	yes	34	35.5	69.5	20.5	0	15.5	3.5	49	0
BF	34	L	yes	58	30	88	65	0	0	4	50	5
BF	35	L	yes	1.5	85	86.5	9.5	1.5	10	0.9	70	6
BF	37	R	yes	87.5	13	100.5	6.5	2	0	6	0	4.5
BF	40	R	yes	80.5	9.5	90	20	0	5	5.8	67	4.6
BF	44	R	yes	8.5	58	66.5	55	3.5	1.5	1	100	3
FH	2	R	no	14	28	42	92.5	0	3.5	3.9	0	5.6
FH	6	R	no	45	28.5	73.5	1.5	6.5	1.5	2.7	20	3.2
FH	9	L	no	10.5	52.5	63	7.5	6.5	2	1.4	27	6.1
FH	11	R	no	20	87.5	107.5	23	0	0	0	0	4.1

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
FH	16	L	no	22.5	56.5	79	0	3	0	3	24	3.9
FH	20	R	no	8.5	88.5	97	6.5	6	1	1.6	18	3.5
FH	21	L	no	26	67.5	93.5	5	9.5	6.5	1.9	0	0
FH	25	L	no	47.5	39	86.5	0	5	0	3.2	29	2
FH	30	L	no	42.5	65	107.5	0	1	1	3.5	17	5.2
FH	32	L	no	42.5	42.5	85	5.5	4	0	3.4	0	2.5
FH	33	L	no	25	44	69	0	12.5	2.5	2.3	7	4
FH	37	R	no	23.5	63.5	87	12	0	0	6.3	33	2
FH	38	L	no	26	41	67	0.5	3.5	0.5	4.6	0	4.7
FH	39	R	no	19	66.5	85.5	4.5	4	1.5	2.6	8	9.3
FH	40	L	no	22	70	92	0	6.5	0	3.7	0	3.6
FH	42	R	no	76	16.5	92.5	13	4	1.5	4.7	2	4.4
FH	43	L	no	51	40	91	1	0.5	0	3.4	5	5.6
FH	44	L	no	15	42.5	57.5	46.5	2.5	1	2	10	4
FH	49	L	no	35	54.5	89.5	0	2.5	4	4	25	3.4
FH	50	L	no	47	40	87	0	14	5	3.1	14	4
FH	2	L	yes	13.5	37.5	51	94	1	0	2.4	0	0
FH	6	L	yes	15	27	42	2	9.5	5.5	7.9	67	3.8
FH	9	R	yes	21	53.5	74.5	5.5	4.5	1.5	4.5	20	6.7
FH	11	L	yes	11.5	87.5	99	10	0	0	0.5	0	5.5
FH	16	R	yes	22.5	40	62.5	0	2.5	1	2.6	14	3

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
FH	20	L	yes	10	86.5	96.5	9	10	9	0.4	20	3
FH	21	R	yes	11.5	46.5	58	1.5	21	6.5	1.4	0	0
FH	25	R	yes	32.5	42	74.5	2	12	1.5	2.2	50	2.7
FH	30	R	yes	27.5	63	90.5	0	0.5	0.5	2.6	9	0
FH	32	R	yes	18.5	51	69.5	1.5	4.5	0	3.2	0	2.8
FH	33	R	yes	19	47.5	66.5	1	12.5	0	2.7	6	0
FH	37	L	yes	10	62.5	72.5	24.5	2.5	0	2.3	13	3.5
FH	38	R	yes	15	61.5	76.5	9.5	8.5	5	2.2	12	4
FH	39	L	yes	14	91	105	1.5	2	0	2	0	3.5
FH	40	R	yes	36.5	52.5	89	0	7	0	2.7	7	3.8
FH	42	L	yes	70	22.5	92.5	15	5	3.5	2.6	0	3
FH	43	R	yes	36.5	60	96.5	0	1.5	0	5.3	9	7.8
FH	44	R	yes	27	32	59	52.5	2.5	2	1.6	0	0
FH	49	R	yes	13	62	75	1	6.5	4.5	1.5	32	3.5
FH	50	R	yes	71.5	21	92.5	3	0	0	6	14	7
PB	2	R	no	1.5	67.5	69	0	5.5	27	0.3	53	0
PB	3	L	no	8	68.5	76.5	13	3	0	1.5	36	6.5
PB	5	R	no	3	87	90	7.5	0	0	2.5	50	14
PB	6	R	no	1.5	59.5	61	2	8.5	11	0.6	0	0
PB	9	R	no	42.5	37.5	80	6.5	0	0	3.5	33	2
PB	12	L	no	11	82.5	93.5	21	2	2.5	1.1	36	3.7

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
PB	17	R	no	2	61	63	0	4	30	0.4	0	0
PB	18	R	no	3.5	73.5	77	2	6	16.5	0.4	8	4
PB	24	L	no	4	95	99	0	2.5	0	1.2	59	12
PB	26	L	no	14	71.5	85.5	4.5	1.5	8.5	2.2	10	0
PB	27	L	no	3.5	76.5	80	2.5	4	15	1.1	6	6
PB	28	L	no	3	84	87	2	11	6.5	1	4	6.5
PB	32	R	no	19	75	94	16.5	1	0	2.5	52	5.5
PB	35	R	no	4.5	56	60.5	0	1.5	42.5	0.5	19	0
PB	39	L	no	38.5	82.5	121	6	5.5	0	1.3	62	3
PB	42	L	no	7.5	77.5	85	6	5	5.5	2	48	0
PB	43	R	no	2.5	79	81.5	2	6	12.5	0.5	47	3.3
PB	44	R	no	52.5	23.5	76	6	0	0	1.9	43	0
PB	45	R	no	5.5	42.5	48	0	1	50	0.7	33	4.3
PB	46	R	no	21.5	62.5	84	2.5	3.5	17.5	1.9	4	3
PB	2	L	yes	1.5	87.5	89	3	7.5	7.5	0.3	0	0
PB	3	R	yes	9.5	81	90.5	3.5	3	7.5	1.7	56	4
PB	5	L	yes	5.5	95	100.5	2	0	0	1.2	63	9.6
PB	6	L	yes	3.5	75.5	79	1.5	1	23	0.4	63	5
PB	9	L	yes	30	28.5	58.5	12	4	14	2.9	56	2.6
PB	12	R	yes	7	81	88	72.5	2.5	0.5	1.5	25	2
PB	17	L	yes	1	79	80	1.5	6.5	13	0.7	7	3

Table 1 LUOR 2010 cont.

Site	trt unit	Side	Scarified	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	# Plants	Av. Leaf #
PB	18	L	yes	5	92	97	4	4	5.5	1.7	20	6
PB	24	R	yes	8.5	86	94.5	0	3	0.5	1.8	47	6.9
PB	26	R	yes	31	65	96	0	1	0	4.167	17	3
PB	27	R	yes	5	68	73	0	5.5	22	1	100	2
PB	28	R	yes	3	82	85	10	4	10	1.3	17	2.5
PB	32	L	yes	20	66	86	13	1	11.5	1.8	38	4.6
PB	35	L	yes	8.5	65	73.5	1.5	5	18	0.8	60	13.7
PB	39	R	yes	14.5	87.5	102	5.5	3.5	0	1.6	67	3.3
PB	42	R	yes	11.5	74	85.5	6.5	6	0	1.3	60	4
PB	43	L	yes	4.5	76.5	81	0	7.5	15	1.1	43	5
PB	44	L	yes	19	66	85	8	3.5	0	1.7	39	3.9
PB	45	L	yes	1.5	69	70.5	1	9.5	22.5	0.4	60	3.3
PB	46	L	yes	35.5	31	66.5	0	2.5	1	3.6	25	3

Table 3 Summary of whole plot data for *S. malviflora* ssp. *virgata* (SIMA) and *C. levisecta* (CALE) in 2010

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	SIMA % establishment	CALE % survival 2010
BF	3	58.5	31.5	90	4.25	0.25	0	2.7	9	60
BF	6	34.25	44.5	78.75	15.75	0.25	6.25	1.35	25	100
BF	8	62.25	38	100.25	15.5	1.5	0.5	2.25	9	80
BF	10	40.25	51	91.25	5.75	4	2.25	1.35	28	100
BF	12	44.25	54.25	98.5	7.25	1	0	3.95	17	100
BF	13	26.5	64.5	91	16.75	0.75	0.25	0.85	15	80
BF	15	7.25	80.25	87.5	8	2	6.75	0.7	6	100
BF	17	59	30.25	89.25	8.25	1.5	4	2.3	16	80
BF	18	4.75	63.75	68.5	34.75	2	0.25	0.55	20	100
BF	20	3	72.75	75.75	16.75	2	9.5	0.55	25	100
BF	23	1	62.25	63.25	6.25	3	31.5	0.95	16	100
BF	24	7.75	81	88.75	18.25	2.25	0	1.2	11	80
BF	26	49.5	25	74.5	14.5	0	8.75	0.8	21	100
BF	27	47.5	49	96.5	4.5	0.25	0	2	12	100
BF	33	52.5	32.75	85.25	27	0	0	5.05	21	100
BF	34	67.75	29.25	97	7.75	0	0	3.35	8	80
BF	35	9.5	85.25	94.75	5.25	0	0	2.45	9	100
BF	37	19	73.25	92.25	5.25	1.75	0	1.55	18	100
BF	40	80.25	21	101.25	3.25	0	0	3.75	9	100
BF	44	1	92.5	93.5	13.25	0.75	0	0.8	9	100
FH	2	10.75	41.5	52.25	92.75	0.75	1	1.65	23	N/A
FH	6	31.25	57.25	88.5	9.75	3.75	0	1.65	10	

Table 3 SIMA & CALE 2010 (cont.)

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	SIMA % establishment	CALE % survival 2010
FH	9	42.75	47	89.75	3.5	0.75	0	3.4	18	
FH	16	76.25	18.5	94.75	0	0.75	5	2.1	25	
FH	20	2.25	86	88.25	23.5	4	1	1	23	
FH	21	25.25	65.75	91	2.75	3.25	0	1.75	9	
FH	25	21.75	43.25	65	2	19.75	12.5	1.5	14	
FH	30	30.25	66	96.25	0	1.25	2.75	2.025	27	
FH	32	21.75	73.75	95.5	1.5	3	0	2.5	21	
FH	33	42.25	54.25	96.5	1	3.5	0.5	2.15	25	
FH	37	42.5	52	94.5	24.75	0.75	2.75	2.95	9	
FH	38	65	32.25	97.25	6.75	1.75	0.5	2.1	17	
FH	39	17.5	75.25	92.75	11.25	0	0.75	1.5	19	
FH	40	37.75	58	95.75	1.25	1.25	1.5	1.5	14	N./A
FH	42	9.75	79.25	89	29.5	0	0	1.9	23	
FH	43	58.75	46.25	105	0.5	2	0	4.6	11	
FH	44	12.75	66.5	79.25	42	1	0	2.8	13	
FH	49	20.25	68	88.25	1	5.5	1	1.65	17	
FH	50	15.5	69.25	84.75	1.5	2.25	7.25	1.95	15	
PB	2	3	71	74	0	25	0	0.25	25	
PB	3	12.25	67	79.25	3.25	7.75	7	2.4	18	
PB	5	13.75	78.75	92.5	1	2.25	0	3.25	26	
PB	6	2.5	74	76.5	0	4.5	16	0.5	17	
PB	9	43	52.25	95.25	0.75	3.25	0	3.1	27	
PB	12	0		0					25	

Table 3 SIMA & CALE 2010 (cont.)

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	SIMA % establishment	CALE % survival 2010
PB	17	24.5	46.75	71.25	0	0.5	26.5	1	12	
PB	18	9.75	73.5	83.25	0	1	20.25	1.2	22	
PB	24	10.25	76	86.25	0	9	0	0.8	24	
PB	27	5.75	53.75	59.5	0	0.5	40.75	0.55	12	
PB	28	7	76	83	3.75	3.75	10.75	0.85	8	
PB	32	10.25	64.25	74.5	1.5	1.75	14.25	2.3	38	
PB	35	9	69.25	78.25	6.5	14.25	0	0.7	35	
PB	39	6.5	74.25	80.75	1.75	2.75	8.5	1.75	17	N/A
PB	42	32.75	54.5	87.25	0	1	11.5	2.3	27	
PB	43	2.75	74	76.75	0	5.5	15.75	1	31	
PB	44	39.75	45.5	85.25	3.75	3.5	7	1.95	29	
PB	45	23.5	54	77.5	0	14.5	7.5	1.1	24	
PB	46	27.75	52.25	80	3.5	15.5	2.5	1.25	23	

Table 4 Summary of whole plot data for *S. malviflora* (SIMA), *I. tenax* (IRTE) and *C. levisecta* (CALE) in 2011

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	% SIMA survival	% IRTE Survival	% CALE persistence 2010-2010
BF	3	57.75	29.25	87	18.75	0	1	6.2	33	40	100
BF	6	79.75	6.5	86.25	21.75	1	17	4.65	28	100	60
BF	8	58	17.5	75.5	12.5	2.25	15.75	2.7	11	0	75
BF	10	64.25	11.25	75.5	5.75	1	0	4.2	18	60	60
BF	12	70	26	96	38.75	0	0	4.1	41	100	100
BF	13	18	70.5	88.5	8.5	1	5	3.05	53	60	75
BF	15	9.25	62.25	71.5	15.5	4.75	18.75	1.95	0	80	20
BF	17	70.5	16.25	86.75	12.5	0	8.75	4.05	63	100	75
BF	18	24	39.75	63.75	9.25	0.75	21.5	1.85	5	100	40
BF	20	11.75	68.25	80	6.75	6.75	9.25	1.15	36	80	60
BF	23	4.5	42.25	46.75	9.25	6.75	40.75	0	6	60	60
BF	24	8.25	74	82.25	18	0	16.5	1.05	36	100	100
BF	26	90.5	10	100.5	7.5	3	0	5.6	48	100	100
BF	27	30.5	27.75	58.25	16	0	11	4	42	80	100
BF	33	36	29	65	15.25	0	11.5	6.25	33	40	100
BF	34	55.5	37.5	93	65	0	0	4.7	63	60	100
BF	35	2.25	88.25	90.5	12.75	1.75	5.75	1.25	44	80	80
BF	37	85	13.75	98.75	8.25	1	0	5.25	56	80	80
BF	40	70	16.5	86.5	15	1.25	8	5.8	78	80	100
BF	44	7.5	56	63.5	61.25	4.75	2	1.1	56	100	100

Table 4 2011 Whole plot summary for SIMA, IRTE and CALE

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	% SIMA survival	% IRTE Survival	% CALE persistence 2010-2010
FH	2	13.75	32.75	46.5	93.25	0.5	1.75	3.15	39	40	
FH	9	15.75	53	68.75	6.5	5.5	1.75	2.95	56	100	
FH	16	22.5	48.25	70.75	0	2.75	0.5	2.8	12	80	
FH	20	9.25	87.5	96.75	7.75	8	5	1	43	60	
FH	21	18.75	57	75.75	3.25	15.25	6.5	1.65	11	40	
FH	25	40	40.5	80.5	1	8.5	0.75	2.7	7	40	
FH	30	35	64	99	0	0.75	0.75	3.05	22	100	
FH	32	30.5	46.75	77.25	3.5	4.25	0	3.3	62	40	
FH	33	22	45.75	67.75	0.5	12.5	1.25	2.5	12	80	
FH	37	16.75	63	79.75	18.25	1.25	0	4.3	56	60	
FH	38	20.5	51.25	71.75	5	6	2.75	3.4	12	80	
FH	39	16.5	78.75	95.25	3	3	0.75	2.3	26	100	N/A
FH	40	29.25	61.25	90.5	0	6.75	0	3.2	43	80	
FH	42	73	19.5	92.5	14	4.5	2.5	3.65	30	60	
FH	43	43.75	50	93.75	0.5	1	0	4.35	27	0	
FH	44	21	37.25	58.25	49.5	2.5	1.5	1.8	31	40	
FH	49	24	58.25	82.25	0.5	4.5	4.25	2.75	35	60	
FH	50	59.25	30.5	89.75	1.5	7	2.5	4.55	20	40	
PB	2	1.5	77.5	79	1.5	6.5	17.25	0.3	44	100	
PB	3	8.75	74.75	83.5	8.25	3	3.75	1.6	11	100	
PB	5	4.25	91	95.25	4.75	0	0	1.85	54	80	
PB	6	2.5	67.5	70	1.75	4.75	17	0.5	35	100	

Table 4 2011 Whole plot summary for SIMA, IRTE and CALE

Site	trt unit	% Grass	% Forb	Total Veg	% Moss	% Bare	% Disturbed	Litter Depth (cm)	% SIMA survival	% IRTE Survival	% CALE persistence 2010-2010
PB	9	36.25	33	69.25	9.25	2	7	3.2	44	100	
PB	17	1.5	70	71.5	0.75	5.25	21.5	0.55	33	100	
PB	18	4.25	82.75	87	3	5	11	1.05	55	100	
PB	26	22.5	68.25	90.75	2.25	1.25	4.25	3.18	43	100	
PB	27	4.25	72.25	76.5	1.25	4.75	18.5	1.05	50	100	
PB	28	3	83	86	6	7.5	8.25	1.15	13	100	
PB	32	19.5	70.5	90	14.75	1	5.75	2.15	45	100	
PB	35	6.5	60.5	67	0.75	3.25	30.25	0.65	46	100	
PB	39	26.5	85	111.5	5.75	4.5	0	1.45	59	100	N/A
PB	42	9.5	75.75	85.25	6.25	5.5	2.75	1.65	33	100	
PB	43	3.5	77.75	81.25	1	6.75	13.75	0.8	55	100	
PB	44	35.75	44.75	80.5	7	1.75	0	1.8	52	100	
PB	45	3.5	55.75	59.25	0.5	5.25	36.25	0.55	25	100	
PB	46	28.5	46.75	75.25	1.25	3	9.25	2.75	74	0	

Appendix B

Supplemental Material to Chapter 3

Iris tenax seed coat

Iris tenax has a tacky coating on the seed coat (Figure 1). During germination trials, we did not address the seed coat texture as being involved in dormancy. Some species of *Iris* are hypothesized to be myrmecochorous, seeds are dispersed by ants. Though there is no true elaiosome on *Iris tenax* seeds, this coating may have something to do with dispersal. Our scarification trials were intended to determine if there was a chemical that was inhibiting germination. However we also tested whether the coating somehow inhibited imbibition or the rate of imbibition of water into the seed.

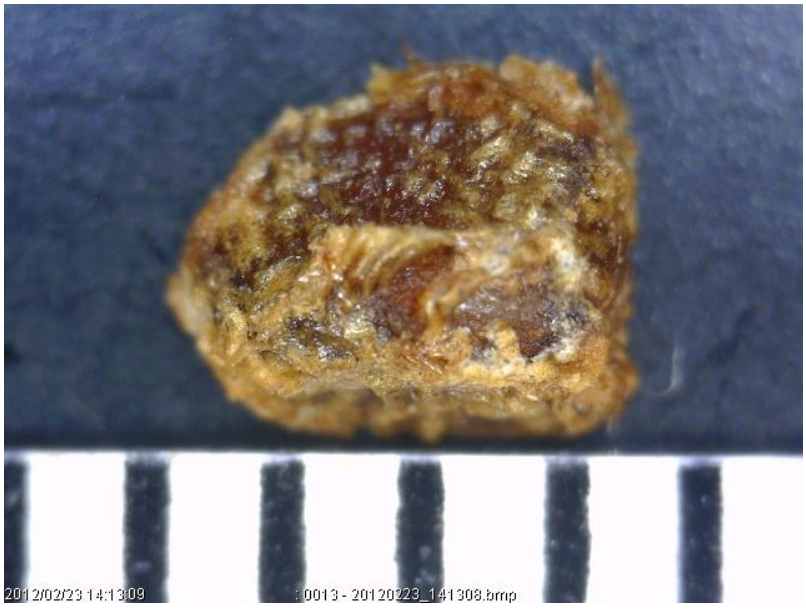


Figure 1 Seed coat of a mature dry seed of *I. tenax*

We counted 100 seeds of *I. tenax* seeds that had been scarified (whole seed coat removed) in the pneumatic scarifier for 5 minutes at 35 psi and 100 seeds that had not been scarified (control). Seeds were weighed then placed on moistened blotter paper for and re-weighed seeds every 15 minutes for an hour, then again after 24 hours.

Interestingly, unscarified seeds imbibed water at a faster rate than scarified seeds and had a greater total increase in weight (Table 1). The total weight of unscarified seeds increased by about 51% after 24 hours whereas scarified seeds only increased by about 45% (we only had a single replicate and therefore cannot estimate error).

We must be careful about extrapolating too far from limited results but it is tempting to consider the role of this seed coat with relation to germination. It is difficult to differentiate between water that is absorbed into the seed or may be absorbed by seed coat of unscarified seeds (except by comparison to scarified seeds). If water is being absorbed by the seed coat, which appears likely, this may be an adaptation to prevent desiccation of the seed during the development of the embryo prior to germination.

Table 1 Increase in weight of 100 scarified vs. 100 unscarified seeds of *I. tenax* following imbibition

Time following immersion in Water (min)	**Weight of 100 Unscarified Seeds (g)**	**Weight of 100 Scarified Seeds (g)**
0 –dry seed	1.16	1.04
15	1.35	1.099
30	1.39	1.11
45	1.42	1.13
60	1.44	1.15
Overnight	1.75	1.51

Germination trials

All seeds were placed in square plastic boxes lined with moistened sterile filter paper then placed in either the warm or cold chambers at the OSU seed lab (Figure 2). Following the experimental period, germinated seeds were planted in conetainers and were donated to restoration projects in the region (Figure 3).

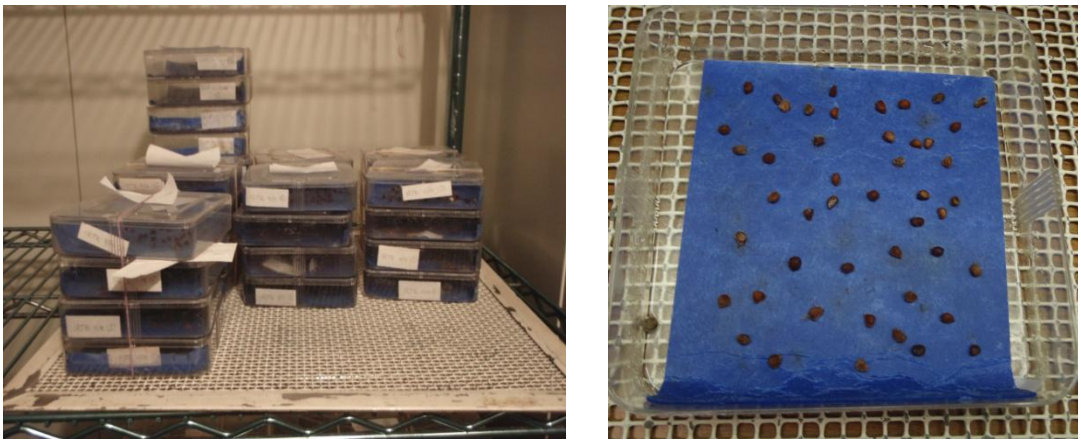


Figure 2 Seed boxes in cold stratification (left), *Iris* seeds on filter pater (right)

Figure 3 Germinated *Sidalcea* seeds (left) and *Sidalcea* planted in conetainers for restoration projects

A note about mold

There was significant development of mold on seeds of both species. Some of the lower germination rates we recorded for *Sidalcea* at the end of the experimental period was likely due to seed or germinant mortality caused by seed. It was not always easy to distinguish between a moldy seed that had germinated and one that had not (see for example Figure 4). Mold even appeared on seeds that were rinsed in a bleach solution prior to stratification treatments suggesting that spores are carried within the seed. Interestingly, seeds from different populations carried different varieties of mold (Figure 5).

Some seeds of both *Sidalcea* and *Iris* still germinated despite the presence of mold though high incidence of mold did seem to be associated with seeds with lower viability. We hypothesize that negative effects of mold on germinated seeds may be neutralized by interactions with soils.

Figure 4 Mold on *S. malviflora* ssp. *virgata* seeds and germinants

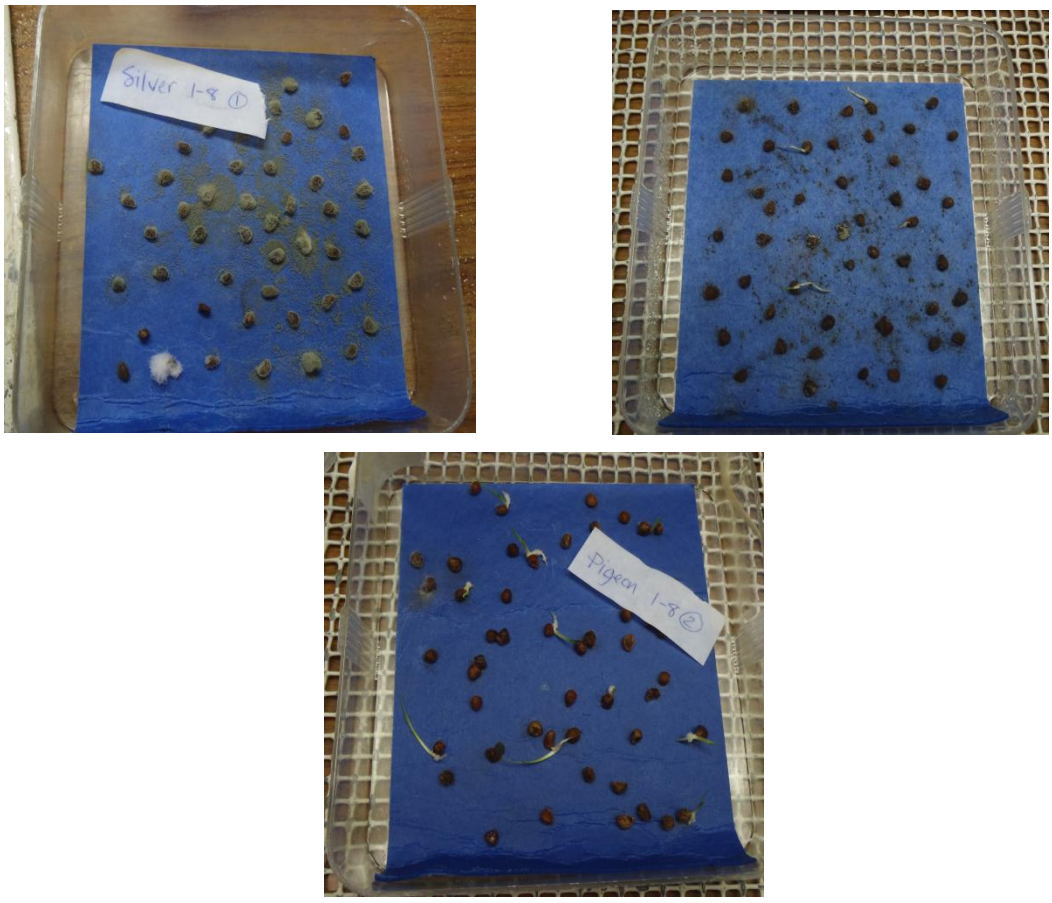


Figure 5 Seeds of *I. tenax* from the Silver Falls commercial production with whitish mold (top left), seeds from Mehema (top right) produce a black mold while seeds from Pigeon Butte have hardly any mold at all (center).

Morphological dormancy in *Iris tenax*

The embryo of a mature seed of *I. tenax* are only about 1 mm long, ¼ the length of the whole seed (Figure 6). The embryo nearly quadruples in size be for germination occurs supporting our hypothesis that further development and likely cellular differentiation of the embryo within the seed is necessary prior to radicle emergence.

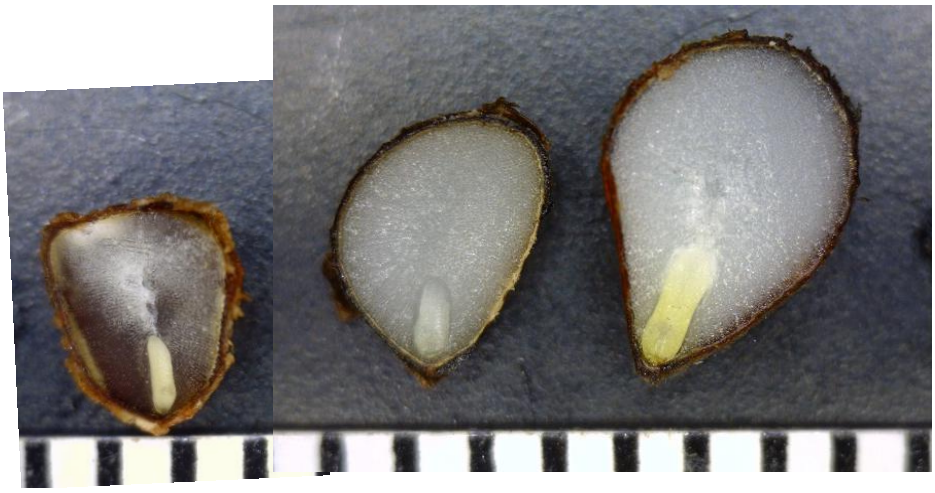
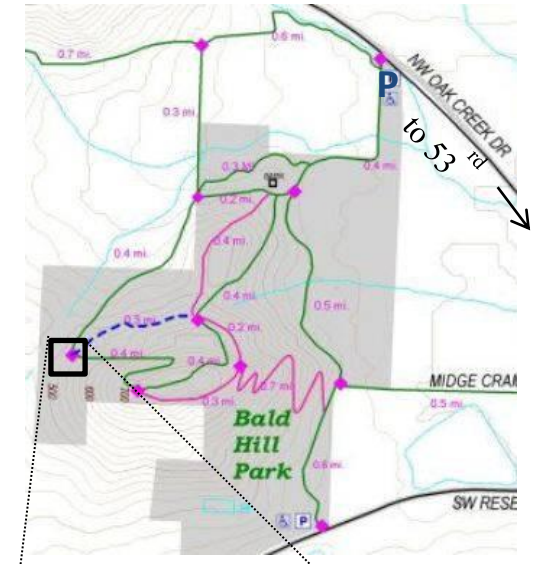


Figure 6 Embryos of *Iris tenax* dry seed (left), after 3 weeks warm stratification (middle) and just before emergence ~12 weeks total stratification (right). One hatch mark = 1 mm.

***Iris tenax* field trials**

Seed pretreatment experiments were conducted in an upland prairie at Bald Hill Natural Area in Corvallis, Oregon, USA (see site map Figure 4). The site was 27 x 6 m; plots were laid out along each of 5-27 m tape measures at 2 m intervals beginning at 0 m. Seed treatments included: Control, 4 wks @ 20/30°C, 24 hrs @ 40°C and 24 hrs @ 50°C. Assignment of treated seeds to plots was randomized across the whole site (Table 2).

The north corner has a very dense patch of another Willamette Valley Iridaceae, *Sisyrinchium* sp., smaller patches are present throughout the site (Figure 8). If any *Iris* did establish at this site, they may not be recognizable until they flower in a couple years.



Park at Oak creek parking area and follow the bike trail, turn right on the barn trail and stay on lower trails through prairies. IRTE plot is north of the trail just past the junction with the forest path. Northern corner is marked with a 4ft. White post, other corners are marked with orange construction markers covering rebar posts. First plot is at origin and is to the west of the transect, additional plots positioned every other meter.

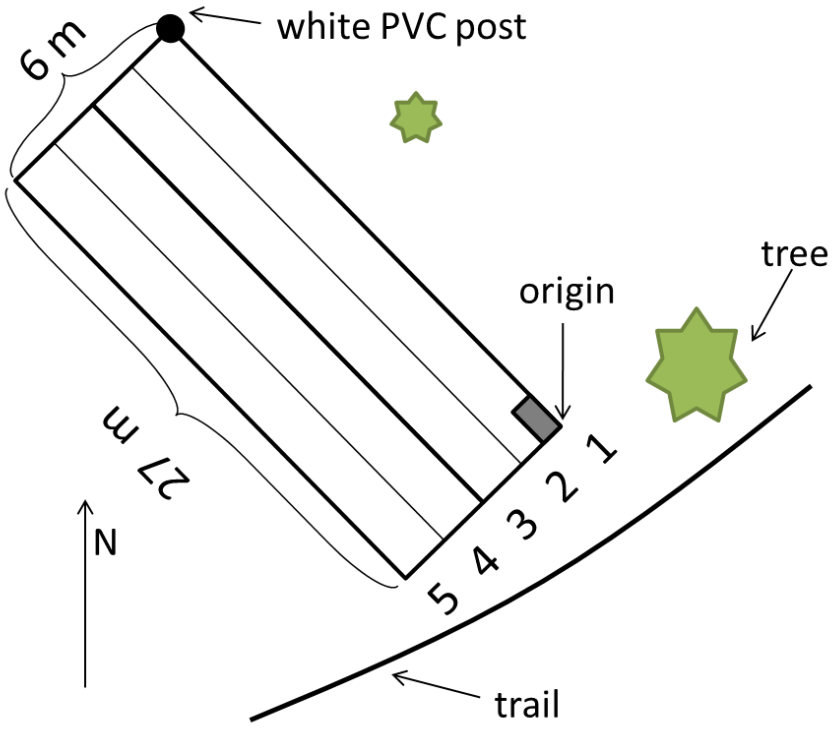


Figure 7 Site map and layout for *Iris* seed pretreatment experiment at Bald Hill Natural Area, Corvallis, OR.

Table 2 Seed pretreatment plot assignments

Row 1	**0 m**	**Row 2**	**1.5 m**	**Row 3**	**3 m**	**Row 4**	**4.5 m**	**Row 5**	**6 m**
0	20/30	0	20/30	0	control	0	40	0	50
2	control	2	20/30	2	50	2	control	2	20/30
4	20/30	4	control	4	50	4	20/30	4	20/30
6	40	6	40	6	20/30	6	40	6	40
8	40	8	50	8	40	8	20/30	8	20/30
10	50	10	40	10	control	10	control	10	50
12	20/30	12	50	12	20/30	12	40	12	40
14	control	14	50	14	40	14	50	14	40
16	40	16	40	16	control	16	50	16	50
18	50	18	40	18	40	18	40	18	50
20	20/30	20	20/30	20	control	20	control	20	50
22	50	22	50	22	20/30	22	40	22	20/30
24	40	24	20/30	24	50	24	50	24	50
26	20/30	26	50	26	20/30	26	20/30	26	40

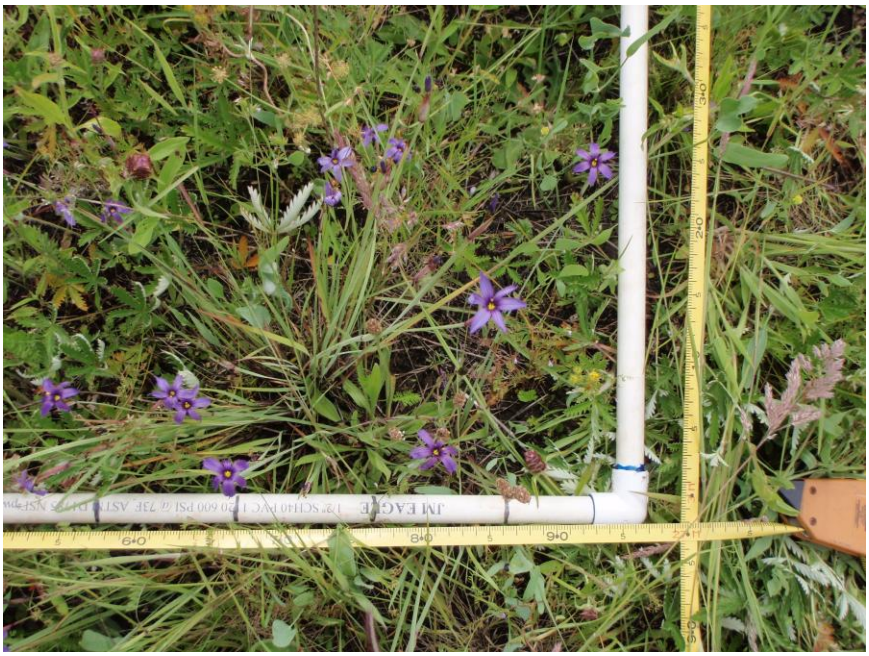


Figure 8 Native population of *Sisyrinchium* sp. growing a plot seeded with *Iris tenax* at Bald Hill Natural Area.

Appendix C

Supplemental Material to Chapter 4

Seedling mortality

To support field experiments for Chapter 2, we planted nursery trays filled with bark and potting soil with each of our study species then left them untended outside to germinate as the seasons dictated. The purpose of this was to get a sense of the timing and percentage of emergence and to help identify each species at various stages of development. The results of our lupine 'reference flats' may help explain some of the mechanism behind equal or lower field establishment of scarified *Lupinus oreganus* seeds compared to unscarified seeds (Figure 1).

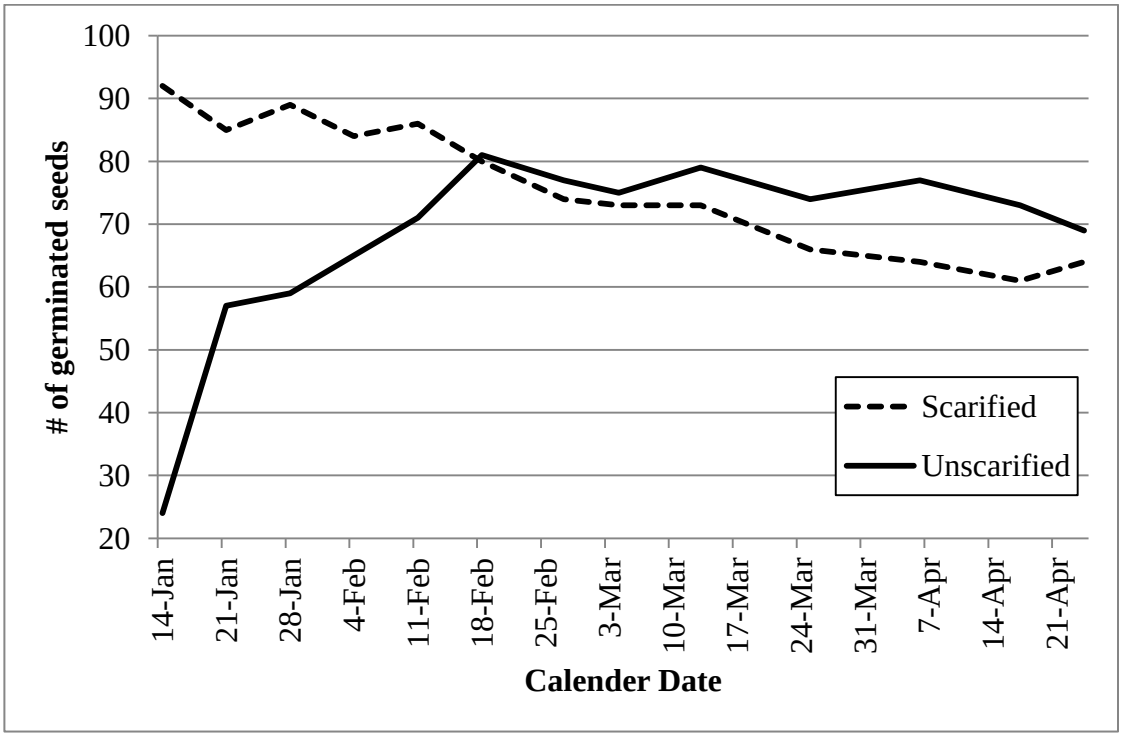


Figure 1 Germinated seeds of scarified and unscarified seeds by date in reference flats.

We planted 100 seeds each of scarified and unscarified seeds. Though we did not catch the date of first germination it is clear that scarified seeds germinated earlier than unscarified seeds. Perhaps most importantly, it appears that unscarified seeds all germinated at once, these same flats several weeks earlier had no germination.

The timing as well as the rate of germination may be key to overall establishment. Unscarified seeds germinate over a longer period of time with some individuals geminating early and others later. If, as we posited in Chapter 4, scarified seeds germinate early and then succumb to herbivory and/or extreme temperatures and, as we saw in our reference flats, maximum germination occurs all at once, then there are no intact seeds remaining to replace individuals that die. We expect that more sources of mortality on germinated seeds in the field than in our protected nursery flats (Figure 2).

Figure 2 Scarified seeds of *L. oreganus* emerged before surrounding vegetation (top) and were noticed by herbivores (bottom) at Bellfountain field site. Photo taken February 3, 2010.

Meta-analysis raw data

Much of the data used for our meta-analysis of scarification pre-treatments of *Lupinus oreganus* is from unpublished sources. All previously unpublished data is presented here with the expressed approval of the researchers responsible for the data (Table 1).

Table 1 Raw data used for germination meta-analysis.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
BF	3	no	100	26	Nov	yes	2010
BF	3	yes	100	1	Nov	yes	2010
BF	6	no	100	46	Nov	yes	2010
BF	6	yes	100	19	Nov	yes	2010
BF	8	no	100	21	Nov	yes	2010
BF	8	yes	100	47	Nov	yes	2010
BF	10	no	100	44	Nov	yes	2010
BF	10	yes	100	15	Nov	yes	2010
BF	12	no	100	31	Nov	yes	2010
BF	12	yes	100	2	Nov	yes	2010
BF	13	no	100	17	Nov	yes	2010
BF	13	yes	100	6	Nov	yes	2010
BF	15	no	100	16	Nov	yes	2010
BF	15	yes	100	3	Nov	yes	2010
BF	17	no	100	41	Nov	yes	2010
BF	17	yes	100	17	Nov	yes	2010
BF	18	no	100	21	Nov	yes	2010
BF	18	yes	100	5	Nov	yes	2010
BF	20	no	100	59	Nov	yes	2010
BF	20	yes	100	31	Nov	yes	2010
BF	23	no	100	23	Nov	yes	2010
BF	23	yes	100	3	Nov	yes	2010
BF	24	no	100	12	Nov	yes	2010
BF	24	yes	100	3	Nov	yes	2010
BF	26	no	100	21	Nov	yes	2010
BF	26	yes	100	3	Nov	yes	2010
BF	27	no	100	25	Nov	yes	2010
BF	27	yes	100	12	Nov	yes	2010

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
BF	33	no	100	36	Nov	yes	2010
BF	33	yes	100	26	Nov	yes	2010
BF	34	no	100	26	Nov	yes	2010
BF	34	yes	100	2	Nov	yes	2010
BF	35	no	100	49	Nov	yes	2010
BF	35	yes	100	31	Nov	yes	2010
BF	37	no	100	20	Nov	yes	2010
BF	37	yes	100	2	Nov	yes	2010
BF	40	no	100	19	Nov	yes	2010
BF	40	yes	100	8	Nov	yes	2010
BF	44	no	100	14	Nov	yes	2010
BF	44	yes	100	1	Nov	yes	2010
TS02	10E	no	50	3	January	yes	2002
TS02	10W	yes	50	2	January	yes	2002
TS02	11E	yes	50	1	January	yes	2002
TS02	11W	no	50	0	January	yes	2002
TS02	12E	yes	50	1	January	yes	2002
TS02	12W	no	50	2	January	yes	2002
TS02	13E	no	50	1	January	yes	2002
TS02	13W	yes	50	1	January	yes	2002
TS02	14E	yes	50	3	January	yes	2002
TS02	14W	no	50	3	January	yes	2002
TS02	15E	no	50	5	January	yes	2002
TS02	15W	yes	50	9	January	yes	2002
TS02	16E	no	50	10	January	yes	2002
TS02	16W	yes	50	9	January	yes	2002
TS02	17E	yes	50	8	January	yes	2002
TS02	17W	no	50	5	January	yes	2002
TS02	18E	no	50	2	January	yes	2002
TS02	18W	yes	50	15	January	yes	2002
TS02	19E	yes	50	1	January	yes	2002
TS02	19W	no	50	3	January	yes	2002
TS02	1E	yes	50	1	January	yes	2002
TS02	1W	no	50	3	January	yes	2002
TS02	20E	no	50	1	January	yes	2002
TS02	20W	yes	50	13	January	yes	2002

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
TS02	2E	no	50	3	January	yes	2002
TS02	2W	yes	50	6	January	yes	2002
TS02	3E	yes	50	3	January	yes	2002
TS02	3W	no	50	0	January	yes	2002
TS02	4E	yes	50	4	January	yes	2002
TS02	4W	no	50	2	January	yes	2002
TS02	5E	no	50	0	January	yes	2002
TS02	5W	yes	50	1	January	yes	2002
TS02	6E	yes	50	1	January	yes	2002
TS02	6W	no	50	2	January	yes	2002
TS02	7E	no	50	1	January	yes	2002
TS02	7W	yes	50	1	January	yes	2002
TS02	8E	no	50	6	January	yes	2002
TS02	8W	yes	50	21	January	yes	2002
TS02	9E	yes	50	0	January	yes	2002
TS02	9W	no	50	0	January	yes	2002
TS03	51E	no	50	7	January	yes	2003
TS03	51W	yes	50	4	January	yes	2003
TS03	52E	yes	50	5	January	yes	2003
TS03	52W	no	50	20	January	yes	2003
TS03	53E	no	50	9	January	yes	2003
TS03	53W	yes	50	12	January	yes	2003
TS03	54E	no	50	9	January	yes	2003
TS03	54W	yes	50	6	January	yes	2003
TS03	55E	no	50	8	January	yes	2003
TS03	55W	yes	50	0	January	yes	2003
TS03	56E	yes	50	3	January	yes	2003
TS03	56W	no	50	9	January	yes	2003
TS03	57E	no	50	14	January	yes	2003
TS03	57W	yes	50	1	January	yes	2003
TS03	58E	no	50	8	January	yes	2003
TS03	58W	yes	50	16	January	yes	2003
TS03	59E	no	50	12	January	yes	2003
TS03	59W	yes	50	2	January	yes	2003
TS03	60E	yes	50	1	January	yes	2003
TS03	60W	no	50	7	January	yes	2003

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
EE	1	yes	25	1	Nov	yes	2008
EE	2	yes	25	4	Nov	yes	2008
EE	3	yes	25	0	Nov	yes	2008
EE	4	yes	25	0	Nov	yes	2008
EE	5	yes	25	0	Nov	yes	2008
EE	6	no	25	3	Nov	yes	2008
EE	7	no	25	7	Nov	yes	2008
EE	8	yes	25	2	Nov	yes	2008
EE	9	yes	25	0	Nov	yes	2008
EE	10	no	25	2	Nov	yes	2008
EE	11	yes	25	0	Nov	yes	2008
EE	12	yes	25	1	Nov	yes	2008
EE	13	no	25	5	Nov	yes	2008
EE	14	yes	25	0	Nov	yes	2008
EE	15	yes	25	0	Nov	yes	2008
EE	16	no	25	7	Nov	yes	2008
EE	17	yes	25	0	Nov	yes	2008
EE	18	no	25	2	Nov	yes	2008
EE	19	no	25	1	Nov	yes	2008
EE	20	no	25	1	Nov	yes	2008
EE	21	no	25	10	Nov	yes	2008
EE	22	no	25	0	Nov	yes	2008
EE	23	no	25	5	Nov	yes	2008
EE	24	no	25	6	Nov	yes	2008
EE	25	yes	25	0	Nov	yes	2008
EE	26	no	25	0	Nov	yes	2008
EE	27	no	25	1	Nov	yes	2008
EE	28	yes	25	0	Nov	yes	2008
EE	29	no	25	3	Nov	yes	2008
EE	30	no	25	1	Nov	yes	2008
EE	31	no	25	2	Nov	yes	2008
EE	32	yes	25	1	Nov	yes	2008
EE	33	no	25	0	Nov	yes	2008
EE	34	yes	25	9	Nov	yes	2008
EE	35	yes	25	13	Nov	yes	2008
EE	36	no	25	0	Nov	yes	2008

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
EE	37	no	25	3	Nov	yes	2008
EE	38	no	25	1	Nov	yes	2008
EE	39	yes	25	1	Nov	yes	2008
EE	40	yes	25	0	Nov	yes	2008
FG	1	yes	250	23	fall	no	2010
FG	2	no	250	76	fall	no	2010
FG	3	no	250	46	fall	no	2010
FG	4	no	250	50	fall	no	2010
FG	5	no	250	69	fall	no	2010
FG	6	yes	250	10	fall	no	2010
FG	7	no	250	76	fall	no	2010
FG	8	no	250	122	fall	no	2010
FG	9	yes	250	16	fall	no	2010
FG	10	no	250	60	fall	no	2010
FG	11	yes	250	4	fall	no	2010
FG	12	yes	250	10	fall	no	2010
FG	13	no	250	75	fall	no	2010
FG	14	yes	250	12	fall	no	2010
FG	15	yes	250	19	fall	no	2010
FG	16	yes	250	9	fall	no	2010
FG	17	yes	250	7	fall	no	2010
FG	18	yes	250	13	fall	no	2010
FG	19	no	250	62	fall	no	2010
FG	20	no	250	68	fall	no	2010
FH	2	no	100	2	Nov	yes	2010
FH	2	yes	100	0	Nov	yes	2010
FH	6	no	100	5	Nov	yes	2010
FH	6	yes	100	3	Nov	yes	2010
FH	9	no	100	11	Nov	yes	2010
FH	9	yes	100	5	Nov	yes	2010
FH	11	no	100	0	Nov	yes	2010
FH	11	yes	100	1	Nov	yes	2010
FH	16	no	100	35	Nov	yes	2010
FH	16	yes	100	7	Nov	yes	2010
FH	20	no	100	11	Nov	yes	2010
FH	20	yes	100	15	Nov	yes	2010

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
FH	21	no	100	14	Nov	yes	2010
FH	21	yes	100	11	Nov	yes	2010
FH	25	no	100	24	Nov	yes	2010
FH	25	yes	100	8	Nov	yes	2010
FH	30	no	100	5	Nov	yes	2010
FH	30	yes	100	11	Nov	yes	2010
FH	32	no	100	34	Nov	yes	2010
FH	32	yes	100	5	Nov	yes	2010
FH	33	no	100	58	Nov	yes	2010
FH	33	yes	100	33	Nov	yes	2010
FH	37	no	100	23	Nov	yes	2010
FH	37	yes	100	15	Nov	yes	2010
FH	38	no	100	24	Nov	yes	2010
FH	38	yes	100	17	Nov	yes	2010
FH	39	no	100	13	Nov	yes	2010
FH	39	yes	100	6	Nov	yes	2010
FH	40	no	100	14	Nov	yes	2010
FH	40	yes	100	15	Nov	yes	2010
FH	42	no	100	61	Nov	yes	2010
FH	42	yes	100	34	Nov	yes	2010
FH	43	no	100	41	Nov	yes	2010
FH	43	yes	100	35	Nov	yes	2010
FH	44	no	100	40	Nov	yes	2010
FH	44	yes	100	27	Nov	yes	2010
FH	49	no	100	34	Nov	yes	2010
FH	49	yes	100	19	Nov	yes	2010
FH	50	no	100	14	Nov	yes	2010
FH	50	yes	100	21	Nov	yes	2010
GO	2	yes	30	13	fall	no	1998
GO	4	yes	30	24	fall	no	1998
GO	5	yes	30	13	fall	no	1998
GO	7	yes	30	13	fall	no	1998
GO	9	yes	30	15	fall	no	1998
GO	10	yes	30	19	fall	no	1998
GO	11	yes	30	12	fall	no	1998
GO	12	yes	30	10	fall	no	1998

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
GO	13	yes	30	8	fall	no	1998
GO	17	yes	30	9	fall	no	1998
GO	18	yes	30	6	fall	no	1998
GO	20	no	30	4	fall	no	1998
GO	21	no	30	13	fall	no	1998
GO	24	no	30	8	fall	no	1998
GO	25	no	30	9	fall	no	1998
GO	28	no	30	9	fall	no	1998
GO	29	no	30	17	fall	no	1998
GO	30	no	30	14	fall	no	1998
IS	1	no	50	4	Nov	yes	2000
IS	1	yes	50	3	Nov	yes	2000
IS	2	no	50	20	Nov	yes	2000
IS	2	yes	50	16	Nov	yes	2000
IS	3	no	50	1	Nov	yes	2000
IS	3	yes	50	1	Nov	yes	2000
IS	4	no	50	16	Nov	yes	2000
IS	4	yes	50	18	Nov	yes	2000
IS	5	no	50	10	Nov	yes	2000
IS	5	yes	50	9	Nov	yes	2000
IS	6	no	50	19	Nov	yes	2000
IS	6	yes	50	23	Nov	yes	2000
IS	7	no	50	12	Nov	yes	2000
IS	7	yes	50	10	Nov	yes	2000
IS	8	no	50	13	Nov	yes	2000
IS	8	yes	50	18	Nov	yes	2000
IS	9	no	50	20	Nov	yes	2000
IS	9	yes	50	18	Nov	yes	2000
IS	10	no	50	12	Nov	yes	2000
IS	10	yes	50	14	Nov	yes	2000
PP	1	no	250	50	fall	no	2010
PP	2	no	250	100	fall	no	2010
PP	3	no	250	60	fall	no	2010
PP	4	yes	250	25	fall	no	2010
PP	5	no	250	78	fall	no	2010
PP	6	no	250	106	fall	no	2010

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
PP	7	no	250	84	fall	no	2010
PP	8	yes	250	19	fall	no	2010
PP	9	no	250	63	fall	no	2010
PP	10	no	250	96	fall	no	2010
PP	11	no	250	95	fall	no	2010
PP	12	no	250	121	fall	no	2010
PP	13	yes	250	37	fall	no	2010
PP	14	yes	250	20	fall	no	2010
PP	15	yes	250	34	fall	no	2010
PP	16	yes	250	27	fall	no	2010
PP	17	yes	250	20	fall	no	2010
PP	18	yes	250	20	fall	no	2010
PP	19	yes	250	28	fall	no	2010
PP	20	yes	250	20	fall	no	2010
PB03	11	yes	50	16		no	2003
PB03	12	no	50	24		no	2003
PB03	13	no	50	11		no	2003
PB03	14	no	50	14		no	2003
PB03	15	yes	50	14		no	2003
PB03	16	yes	50	17		no	2003
PB03	17	yes	50	7		no	2003
PB03	18	yes	50	8		no	2003
PB03	19	no	50	18		no	2003
PB03	20	no	50	16		no	2003
PB10	2	no	100	27	Nov	yes	2010
PB10	2	yes	100	3	Nov	yes	2010
PB10	3	no	100	42	Nov	yes	2010
PB10	3	yes	100	9	Nov	yes	2010
PB10	5	no	100	41	Nov	yes	2010
PB10	5	yes	100	34	Nov	yes	2010
PB10	6	no	100	11	Nov	yes	2010
PB10	6	yes	100	8	Nov	yes	2010
PB10	9	no	100	27	Nov	yes	2010
PB10	9	yes	100	9	Nov	yes	2010
PB10	12	no	100	21	Nov	yes	2010
PB10	12	yes	100	7	Nov	yes	2010

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
PB10	17	no	100	14	Nov	yes	2010
PB10	17	yes	100	15	Nov	yes	2010
PB10	18	no	100	12	Nov	yes	2010
PB10	18	yes	100	5	Nov	yes	2010
PB10	24	no	100	37	Nov	yes	2010
PB10	24	yes	100	14	Nov	yes	2010
PB10	26	no	100	21	Nov	yes	2010
PB10	26	yes	100	18	Nov	yes	2010
PB10	27	no	100	17	Nov	yes	2010
PB10	27	yes	100	1	Nov	yes	2010
PB10	28	no	100	24	Nov	yes	2010
PB10	28	yes	100	10	Nov	yes	2010
PB10	32	no	100	39	Nov	yes	2010
PB10	32	yes	100	28	Nov	yes	2010
PB10	35	no	100	20	Nov	yes	2010
PB10	35	yes	100	5	Nov	yes	2010
PB10	39	no	100	49	Nov	yes	2010
PB10	39	yes	100	30	Nov	yes	2010
PB10	42	no	100	26	Nov	yes	2010
PB10	42	yes	100	4	Nov	yes	2010
PB10	43	no	100	48	Nov	yes	2010
PB10	43	yes	100	30	Nov	yes	2010
PB10	44	no	100	49	Nov	yes	2010
PB10	44	yes	100	18	Nov	yes	2010
PB10	45	no	100	11	Nov	yes	2010
PB10	45	yes	100	4	Nov	yes	2010
PB10	46	no	100	24	Nov	yes	2010
PB10	46	yes	100	4	Nov	yes	2010
R10	1	yes	250	2	Fall	yes	2010
R10	2	no	250	41	Fall	yes	2010
R10	3	yes	250	2	Fall	yes	2010
R10	4	no	250	29	Fall	yes	2010
R10	5	yes	250	3	Fall	yes	2010
R10	6	yes	250	3	Fall	yes	2010
R10	7	yes	250	2	Fall	yes	2010
R10	8	no	250	39	Fall	yes	2010

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
R10	9	yes	250	1	Fall	yes	2010
R10	10	no	250	21	Fall	yes	2010
R10	11	no	250	24	Fall	yes	2010
R10	12	no	250	52	Fall	yes	2010
R10	13	no	250	59	Fall	yes	2010
R10	14	yes	250	5	Fall	yes	2010
R10	15	no	250	35	Fall	yes	2010
R10	16	yes	250	4	Fall	yes	2010
R10	17	no	250	60	Fall	yes	2010
R10	18	yes	250	1	Fall	yes	2010
R10	19	no	250	29	Fall	yes	2010
R10	20	yes	250	0	Fall	yes	2010
S03	1-1	no	25	0	Dec	yes	2003
S03	1-2	no	25	3	Dec	yes	2003
S03	1-3	no	25	7	Dec	yes	2003
S03	1-4	no	25	0	Dec	yes	2003
S03	1-5	no	25	0	Dec	yes	2003
S03	2-6	no	25	0	Dec	yes	2003
S03	2-8	no	25	1	Dec	yes	2003
S03	2-9	no	25	0	Dec	yes	2003
S03	2-11	no	25	0	Dec	yes	2003
S03	3-12	no	25	0	Dec	yes	2003
S03	3-13	no	25	0	Dec	yes	2003
S03	3-14	no	25	0	Dec	yes	2003
S03	3-15	no	25	0	Dec	yes	2003
S03	3-15	no	25	0	Dec	yes	2003
S03	1-1	yes	25	6	Dec	yes	2003
S03	1-2	yes	25	1	Dec	yes	2003
S03	1-3	yes	25	4	Dec	yes	2003
S03	1-4	yes	25	0	Dec	yes	2003
S03	1-5	yes	25	6	Dec	yes	2003
S03	2-6	yes	25	3	Dec	yes	2003
S03	2-7	yes	25	0	Dec	yes	2003
S03	2-7	yes	25	0	Dec	yes	2003
S03	2-8	yes	25	0	Dec	yes	2003
S03	2-9	yes	25	1	Dec	yes	2003

Table 1 - raw data cont.

Site Code	plot #	Scarified	# Seeded	# Germinants	Month Seeded	Paired Design	Year Sampled
S03	2-10	yes	25	0	Dec	yes	2003
S03	2-10	yes	25	1	Dec	yes	2003
S03	2-11	yes	25	1	Dec	yes	2003
S03	3-12	yes	25	5	Dec	yes	2003
S03	3-13	yes	25	0	Dec	yes	2003
S03	3-14	yes	25	2	Dec	yes	2003