Astragalus peckii Population Establishment: Developing Protocols for Seed Germination, Seedling Cultivation, and Planting

by
Tabitha Pearson

A THESIS

submitted to

Oregon State University

University Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Botany
(Honors Scholar)

Presented November 13, 2015
Commencement June 2016

AN ABSTRACT OF THE THESIS OF

Tabitha Ann Pearson for the degree of Honors Baccalaureate of Science in Botany presented on November 13, 2015. Title: *Astragalus peckii* Population Establishment: Developing Protocols for Seed Germination, Seedling Cultivation, and Planting.

Abstract approved: ____

Richard Halse

Astragalus peckii Piper is a threatened Oregon endemic in the pea family (Fabaceae). This species faces extinction due to loss of habitat. Recovery efforts include augmenting existing populations and creating new ones. The purpose of this study was to determine the best methodology for population augmentation of *Astragalus peckii* by evaluating methodologies for 1) seed germination and seedling cultivation, and 2) transplant survival and seed emergence. Older seeds required manual scarification to induce germination while younger seeds germinated without treatment. Seedlings were placed in three soil treatments with varying ratios of potting mix and native soil. Seedlings experienced high rates of mortality unrelated to treatment. Seeds and seedlings were planted in the fall along four transects of varying disturbance and previous species occupation. Spring survival rates provided little evidence for an increase in population size of *Astragalus peckii* in the four transects after planting.

Key Words: Botany, conservation, Oregon

Corresponding e-mail address: pearsota@onid.oregonstate.edu

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APPROVED:

Richard Halse, Mentor, representing Botany and Plant Pathology

Aaron Liston, Committee Member, representing Botany and Plant Pathology

Kelly Amsberry, Committee Member, representing the Oregon Department of Agriculture

Toni Doolen, Dean, University Honors College

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

Tabitha Ann Pearson, Author

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ASTRAGALUS PECKII POPULATION ESTABLISHMENT: DEVELOPING PROTOCOLS FOR SEED GERMINATION, SEEDLING CULTIVATION, AND PLANTING

Chapter 1

INTRODUCTION

Astragalus peckii Piper, a member of the pea family (Fabaceae), is a perennial milkvetch known by the common name Peck's milkvetch. This species is endemic to Oregon and is threatened by habitat loss from off-road vehicle use, urbanization, and agricultural development (Amsberry and Meinke 2003; Carr et al. 2009). *Astragalus peckii* is listed by the Oregon Department of Agriculture as a threatened species, defined as any native plant species likely to become endangered within the foreseeable future throughout all or a significant portion of its range (Oregon Administrative Rules 2014). Oregon state law requires the conservation of threatened and endangered species on all non-federal public lands (Oregon Revised Statutes 2014).

Conservation efforts are vital for maintaining the balanced ecosystem of native habitats and species (Bradshaw 1993). Long-term monitoring of natural populations identifies species in decline and reintroduction projects can significantly decrease the time needed for a species to recover (Reay and Norton 1999). Projects that incorporate germinating native seeds and cultivating seedlings are essential for the recovery-based creation and augmentation of rare plant populations. Such projects can help to ascertain the validity of mass propagation projects based on survival success rates (Rodrigues et al. 2015). Because propagules of many rare plants can be difficult to obtain due to their scarcity, researchers must be able to have established methodologies available to maximize the number of successfully cultivated seedlings. Developing protocols for identifying the best habitat in which to plant the cultivated seedlings is also essential. Established methodologies can increase the chance of a successful reintroduction and reduce the impact of propagule collection from limited natural populations (Bayraktar et al. 2015).

Biodiversity conservation is especially important due to the current high extinction rate of species. Humans have caused up to a 1,000 percent increase in the background rate of species extinction. The current exceptionally high extinction rate is called the Anthropocene extinction event, the sixth mass extinction event on the planet (Wagler 2011). Plants may go extinct before they ever have the chance to be researched or even identified. Loss of species impacts the ecosystem by eliminating components that may be critical to their continued function. Research is needed in order to document the contributions made by individual species. Humans can value plants based on their usefulness, but usefulness needs research and discovery, something that cannot take place if plants are driven extinct because they are seen as having little practical or intrinsic value. For example, an important cancer drug was extracted from the plant *Taxus brevifolia* in the 1960s. Prior to this discovery, *T. brevifolia* was routinely destroyed in logging operations because it was seen as having little value (Minore et al. 1996).

Citizens may become more actively concerned about environmental issues when they are able to participate in projects within their community. Even well-intentioned ecological projects can fail because of citizen resistance to government initiatives developed without citizen input (Booth and Skelton 2011). My project utilized both volunteers from a local school and citizen scientists residing within the community. Middle-school students helped collect some range and population data for *A. peckii* and volunteers from the citizen's group Friends of the Tumalo Wildlife Corridor (FotTWC) assisted with transplanting and watering the seedlings planted to increase population size. Citizen support is vital to completing environmental projects. In most communities, citizens do not support or understand the conservation of natural areas. The lack of support can result in damage at conservation sites, and even destruction of research plots and loss of data. Full participation from citizens is needed to protect sites and research projects from damage. Damage to sites has been shown to reduce plant taproot formation and drought tolerance (Amsberry and Meinke 2003).

ASTRAGALUS PECKII

Astragalus peckii is a member of the pea family (Fabaceae). The Fabaceae is one of the most important angiosperm families in the world with regard to economics, second only to the grasses. The Fabaceae includes a variety of beans and lentils as well as clovers and alfalfa (Gisler and Meinke 2001). Plants in this family are often ecologically important because some species have root nodules. These nodules provide space for nitrogen fixing bacteria, which turn atmospheric nitrogen into a form usable by plants (Brewin 2010).

Peck's milkvetch is a low, densely leafy plant with a taproot that grows best on open flats or gentle slopes among sagebrush in pumice soil in the Cascade Range (Piper 1924) and is characterized by its unique petioles which persist for one or two years (Barneby 1964). Stems are 1-3 dm long (Meinke and Gisler 2001). Flowers are between 5-7 mm long, hermaphroditic, and produced in creamed-colored inflorescences of 5-9 flowers (Piper 1924; Barneby 1964; Gisler and Meinke 2001). An average of 1.0-1.5

small, black seeds are produced per three-angled fruit. A typical individual produces about 300 seeds in a season (Gisler and Meinke 2001; Amsberry and Meinke 2003). Peck's milkvetch plants seem to rely on insect pollination, as plants bagged before budding have a very low fruit production (Gisler and Meinke 2001). Peck's milkvetch is a component of plant communities that include *Artemesia tridentata* and *Chrysothamnus viscidiflorus* as well as juniper-woodlands. Peck's milkvetch grows best in open habitats (Amsberry and Meinke 2003).

Peck's milkvetch is endemic to Oregon and grows from east of Sisters, OR in Deschutes County, south to Chiloquin, OR in Klamath County. The northern end of the species population range is marked by two historic populations that occur near Sisters, OR. Most populations, about 30, are located next to the Tumalo Dam. Populations can range from hundreds of thousands of plants over several acres to 10-100 individuals. Populations have become fragmented by development. Several populations are scattered near Chemult, OR including a historical population near Odell Lake. The southern end of the population consists of one large and two smaller populations near Chiloquin, OR (Amsberry and Meinke 2003).

Seeds for this study were collected from Bull Flat, near the Tumalo Dam and Sisters, OR, and from Beaver Marsh, near the middle of the population range by Chemult, OR. The site at Bull Flat was used for transplanting the seedlings and planting seeds and has a stable to declining *A. peckii* population (Martin 2010). The winters in Bull Flat are cold and snowy, while the summers are hot and dry. At Bull Flat the 30-year normal for precipitation is 41.4 mm in January and112.7 mm in July with annual mean precipitation of 310.9 mm. The maximum normal temperature is 5.4° C in January and

28.4° C in July (PRISM Climate Group 2015). The soils in this habitat are rich in pumice from the eruption of Mt Mazama 7,700 years ago (Mandeville et al. 2009).

Plants of *A. peckii* provide a pupation and feeding site for the herbivorous moth *Sparganothis tunicana* (Martin 2010). Moths can be used as indicator species to signal a healthy ecological system because they are an integral part of the food chain, and may also indicate other ecological factors such as habitat disturbance (Summerville et al. 2004). Moth herbivory increases the mortality of *A. peckii* at Bull Flat, especially early in the growing season, but this mortality was not correlated with negative population growth (Martin 2010).

GOALS

1. Creation of a methodology for seed germination and transplant cultivation of *A. peckii.*
2. Evaluation of the success of transplanted seedlings and sown seeds in sites with two levels of disturbance and two levels of naturally occurring *A. peckii* population.
3. Collaboration with the local community to increase awareness of conservation and to evaluate the success of restoration projects in areas with routine visitation by citizens.

Chapter 2 begins with a study focusing on the methods for scarifying seeds of *A. peckii* collected from two sites in three years, and discusses the causes of age-related physical seed dormancy. After the seeds have germinated, the study examines survival of seedlings planted in native soil and potting mix. The study discusses possible causes for seedling mortality and suggests steps for future research.

Chapter 3 focuses on the planting of the *A. peckii* seedlings and seeds of varying age and site source, in native habitat. This chapter also discusses the species composition of the plant community in the sites selected for planting. Four habitat types were selected based on the number of *A. peckii* currently present and on the level of disturbance. Habitat locations and the number of *A. peckii* per habitat were recording using a GPS. Survival rates were assessed seven months after the initial fall planting. This chapter also discusses the viability of future reintroductions based on seed emergence and transplant survival as well as the challenges of reintroduction projects.

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Chapter 2

CREATING A METHODOLOGY FOR SEED GERMINATION AND SEEDLING CULTIVATION OF *ASTRAGALUS PECKII*, A THREATENED OREGON ENDEMIC

METHODS

1. Germination Trials

Germination trials were conducted to determine the best methodology for maximizing the germination of *Astragalus peckii* seeds and to create seedlings for cultivation and outplanting. Seeds were collected from native sites of established *A. peckii* populations. Peck's milkvetch populations are scattered from the northern end of their range near Sisters, OR to the south end of their range near Chiloquin, OR. Several populations are located in the middle of this range near Chemult, OR (Fig. 1). Two sites were used to collect seeds. One site is at Bull Flat (44º 09′ 40.35″N, 121º 26′ 05.54″W, 1062-m elevation) and is located near Sisters, Oregon and managed by the Bureau of Land Management (Martin 2010). The other site is south of Bull Flat at Beaver Marsh (43º 06′ 23.348″ N, 121º 48′ 53.372″ W, 1407-m elevation) and is located near Chemult, Oregon and managed by the Oregon Department of Transportation (K. Amsberry, personal communication).

Five lots of seeds were collected over three years and from the two sites: Beaver Marsh 2013 (M13), Beaver Marsh 2014 (M14), Bull Flat 2012 (B12), Bull Flat 2013 (B13), and Bull Flat 2014 (B14). Seeds were collected from mature plants. Seeds were either manually removed from the pods or removed using a wooden block and a screen. When pressure was applied to the pods with the block, the seeds came out and were separated from the pods with the screen. Previous studies noted the difficulty of removing seeds from the pods (Gisler and Meinke 2001). The screen and block method allowed for more seeds to be processed than by manual removal. The seeds were then placed in envelopes and labelled with lot identification and year of collection.

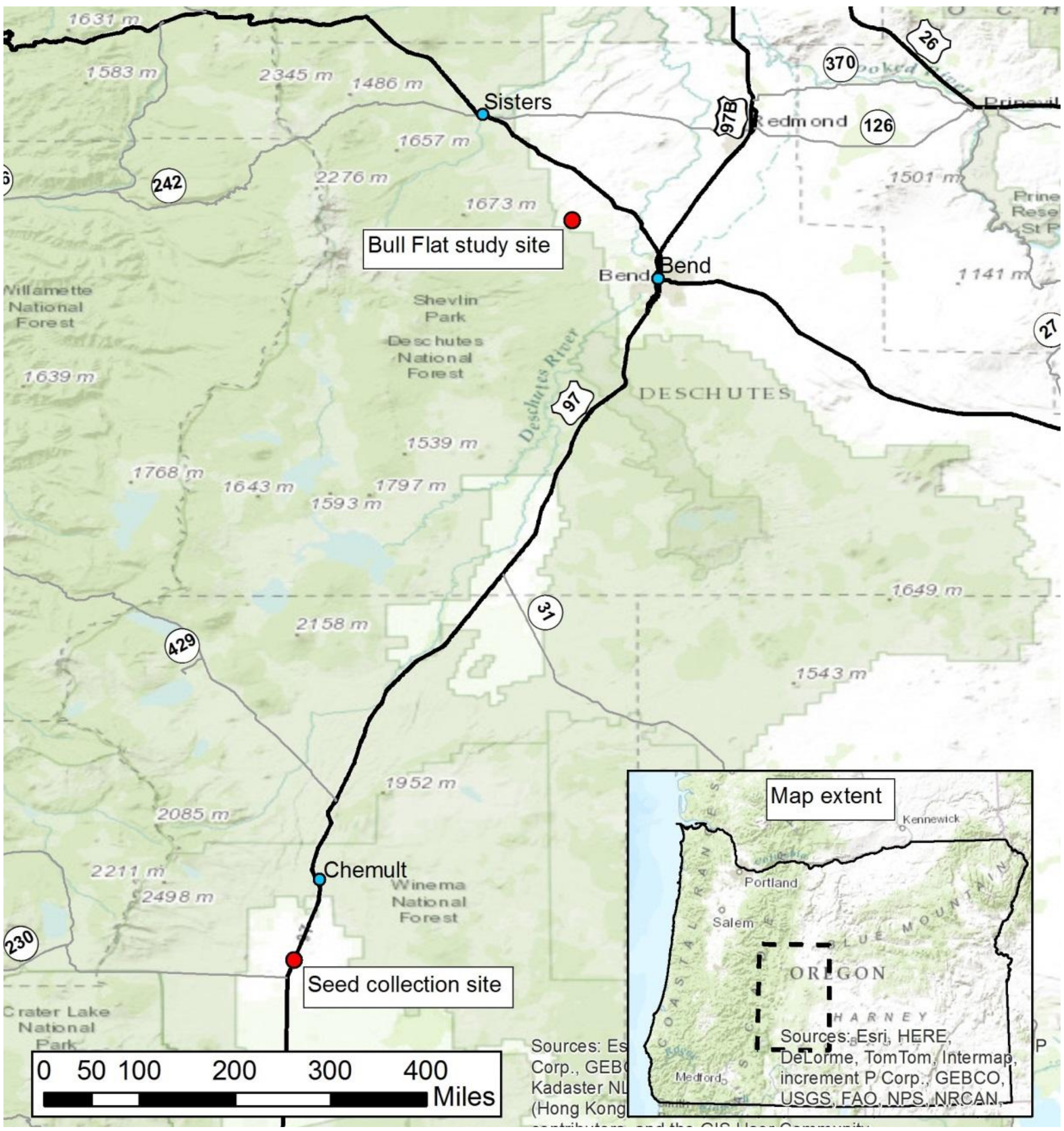


Figure 1. Seeds were collected from Bull Flat near Bend, OR and Beaver Marsh near Chemult, OR over three years. The Bull Flat site was used for the vegetation study and the planting of seeds and seedlings.

One hundred and fifty seeds from each seed lot were selected and 50 seeds in each lot were scarified with a scalpel under a microscope, 50 seeds were scarified with sandpaper under a microscope, and 50 seeds were left as an untreated control (for a total of 750 seeds). Scarified seeds were treated under a microscope to prevent damage to the embryos. Scarification was chosen as a treatment because a previous study showed an 85% germination rate with treatment (Gisler and Meinke 2001). Each treatment was separated into batches of ten seeds for a total of five replications per seed lot and treatment type, and seeds were placed in petri dishes on moistened filter paper using deionized water in the Oregon State University greenhouses under ambient conditions (Fig. 2).

Figure 2. Petri dishes with ten seeds and five replications per site, year, and treatment were placed on a table in the OSU greenhouses.

2. Seedling Cultivation

As seeds germinated, seedlings were planted in 4 inch band pots lined with fiberglass mesh. Planting soils in pots were watered the day before planting and again after the seedlings were added to each pot. Deep band pots were selected to encourage strong taproot formation and to help ensure seedling survival after transplanting in the native site.

The seedlings were placed in mixtures of potting mix (Metro Mix 360) and native soil (collected from Bull Flat): 100 seedlings were planted in pure potting mix, 100seedlings were planted in potting mix and 1 cup of native soil, and 100 seedlings were planted in potting mix and 2 tablespoons of native soil . Previous studies had tried growing the seedlings with inoculations of *Rhizobium*, but hadn't collected soil from the native site that may have had specific strains of bacteria in symbiosis with *A. peckii* (Gisler and Meinke 2001). Each seedling was selected by site (Bull Flat or Beaver Marsh) and year (2012, 2013, 2014) and randomly assigned a pot using a random number generator. The pots were then arranged randomly in groups of 100 per table (Figures 3, 4).

In addition to the plants grown for the study, 125 additional seedlings were grown in potting mix to ensure sufficient numbers for transplanting at the native site. Twenty-five of the additional seedlings were placed outside of the greenhouse. All seedlings were placed outside of the greenhouse eight weeks before planting to allow them to become accustomed to outside conditions.

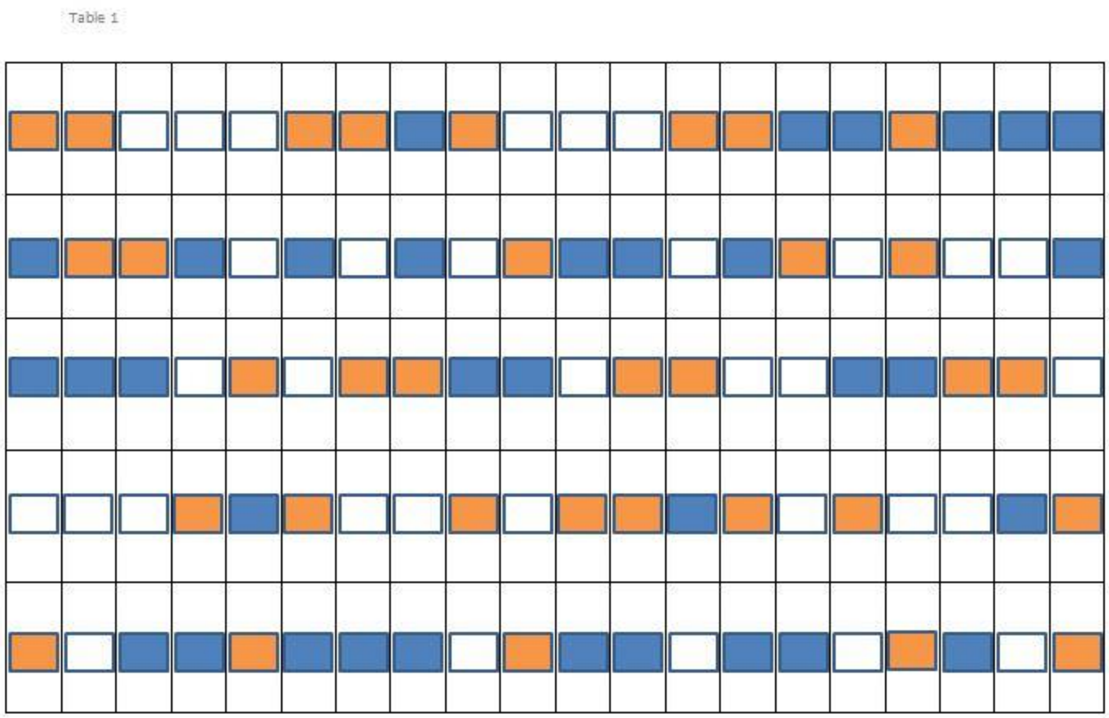


Figure 3. The setup of a table of 100 plans with three treatment types randomly arranged.

Figure 4. Each pot contained a seedling selected from the five seed lots by a random number generator. Orange tabs indicate 1 cup of native soil per pot, white tabs indicate pure potting mix per pot, and blue tabs indicate 2 tablespoons of native soil per pot.

Thirteen days after planting, seedlings experienced a high mortality rate of 40-50% per table and a layer of quartz was added to the top of the soil line (Fig. 5) to prevent damping off. This treatment also mimicked the native habitat of *A. peckii*, which grows in open habitat where young plants are routinely exposed to dry conditions and wind (Amsberry and Meinke 2003).

Figure 5. A 15-day old seedling covered with quartz to mimic the dry soil of Bull Flat and to prevent fungal disease.

RESULTS

1. Germination Trials

The number of germinated seeds in each dish was recorded daily for 15 days after the seeds were placed in petri dishes. Photographs were also taken each day. Seeds from both sites collected in 2014 had significantly better germination rates when untreated than untreated seeds collected in 2012 or 2013 (Fig. 6).

2013 **2014**

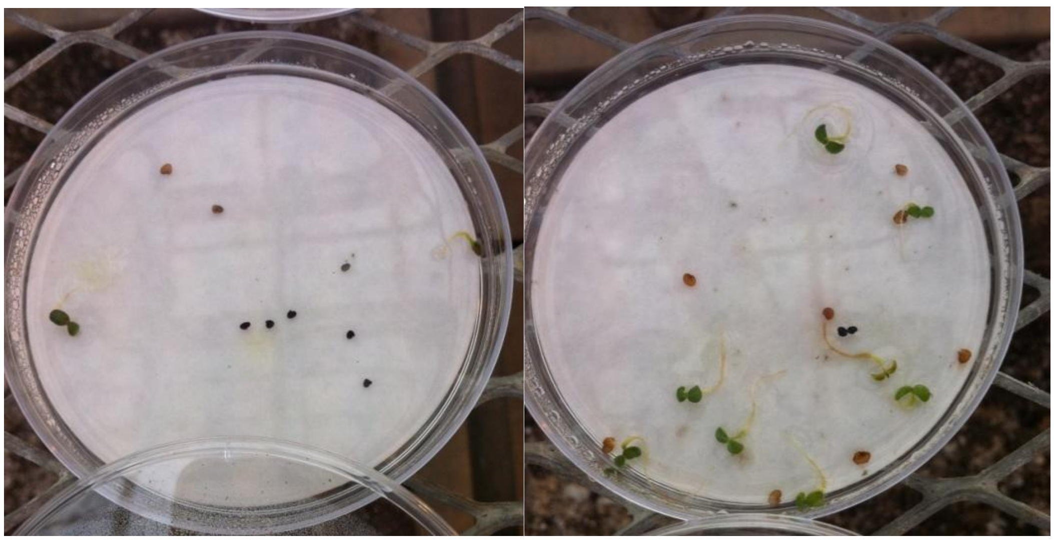


Figure 6. Untreated seeds collected in 2014 germinated at significantly higher rates than untreated seeds collected in 2013.

Seeds scarified with sandpaper germinated at 90% for all years. Seeds scarified with a scalpel germinated at rates of 75% for years 2012 and 2013, but at rates of 55% for 2012. Untreated seeds germinated at 70% for 2014, but were under 30% for 2012 and 2014 (Fig. 7).

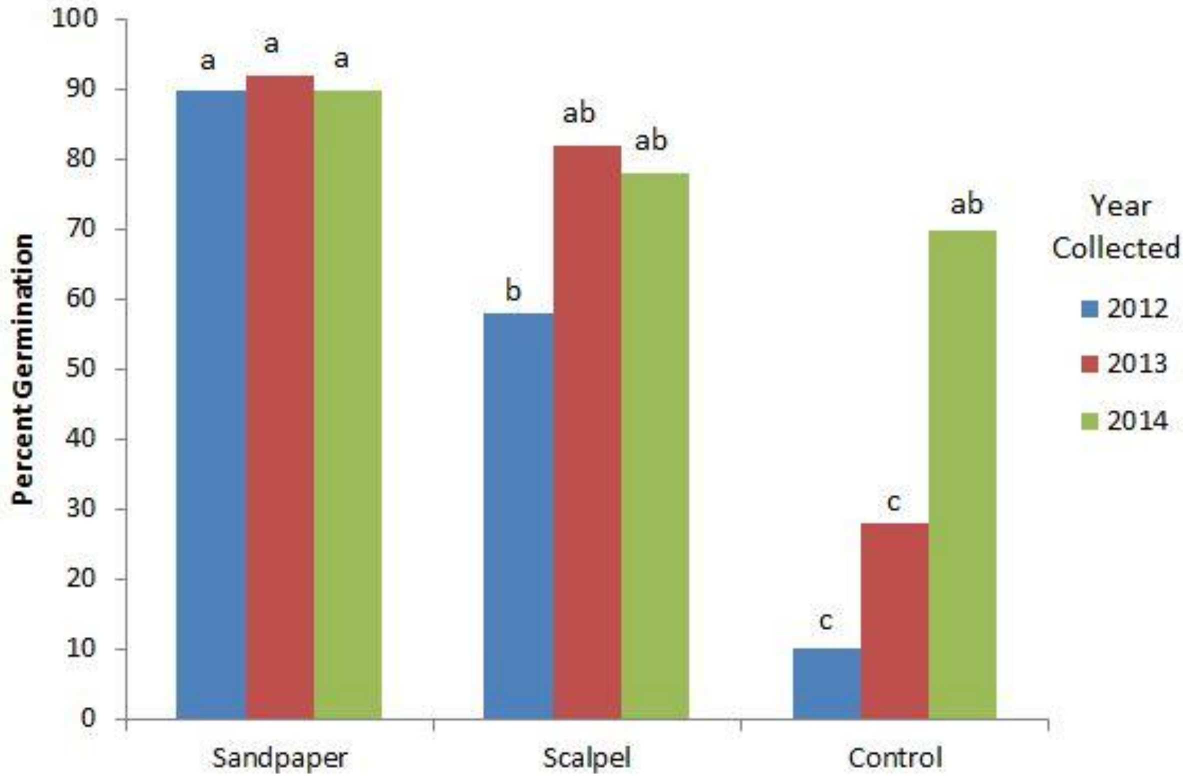


Figure 7. Average percent germination for seeds of three ages (collected in 2012, 2013, and 2014) and three treatments (scarified with sandpaper, scarified with scalpel, and control) after 15 days of germination. Data from seeds from both sides are combined. Bars with the same letter are not significantly different.

Seeds from the year 2013 at Beaver Marsh were unable to achieve high germination rates without scarification, but seeds from the year 2014 achieved high germination rates without treatment (Fig. 8).

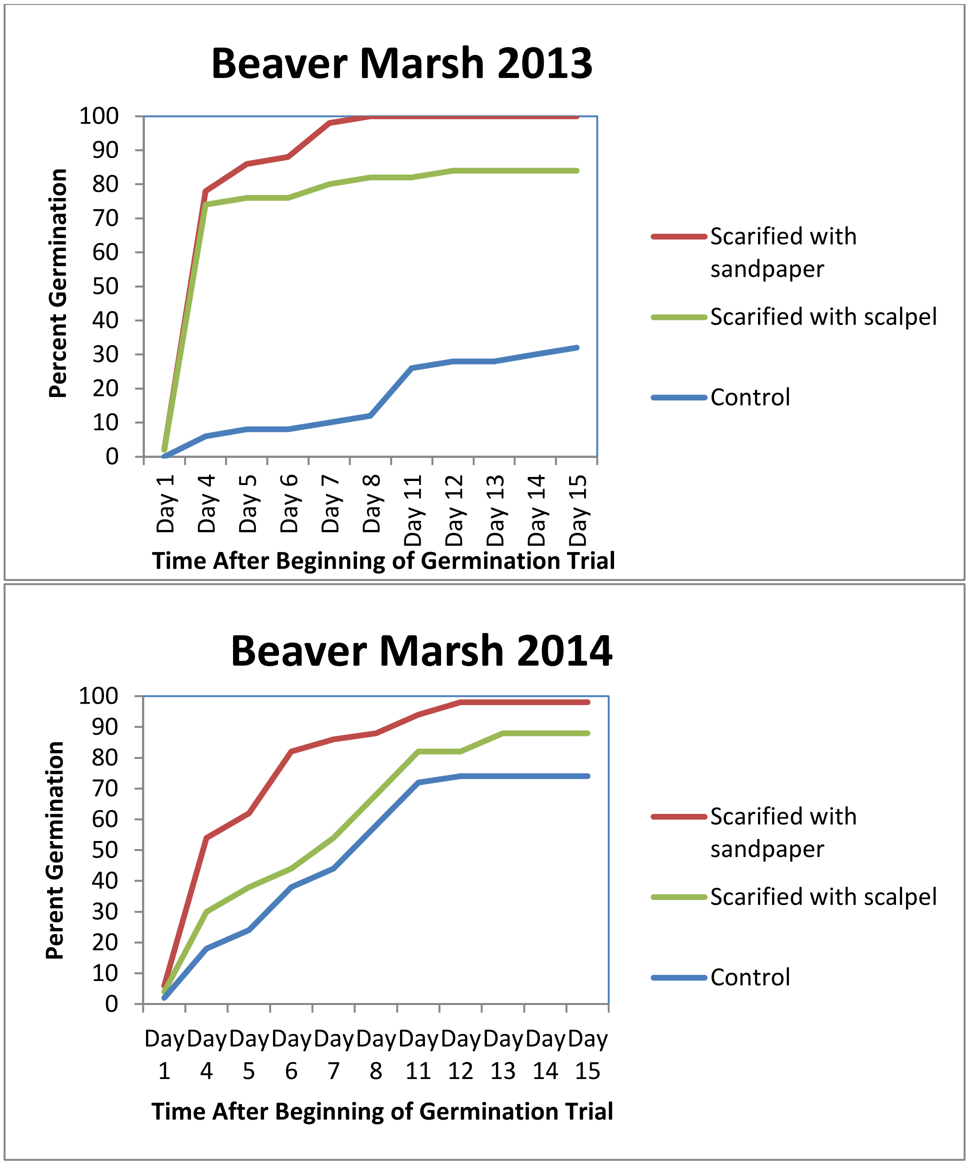
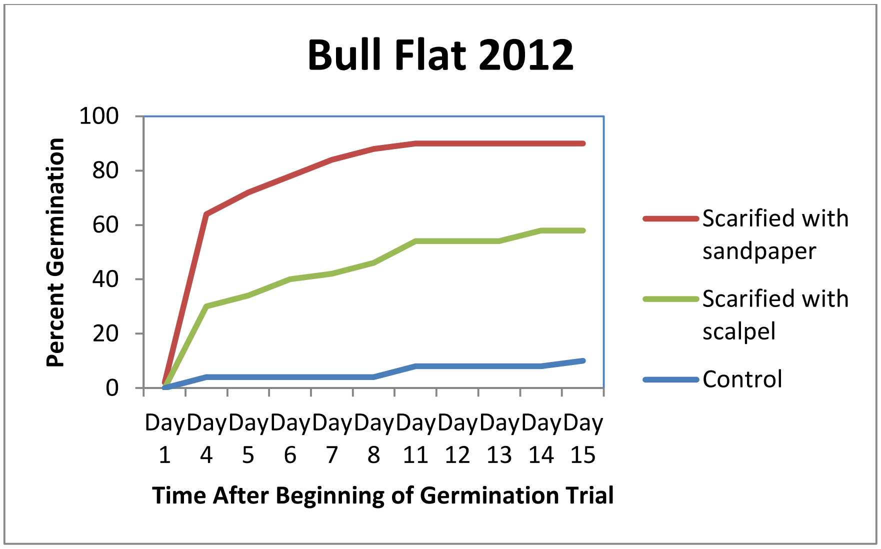
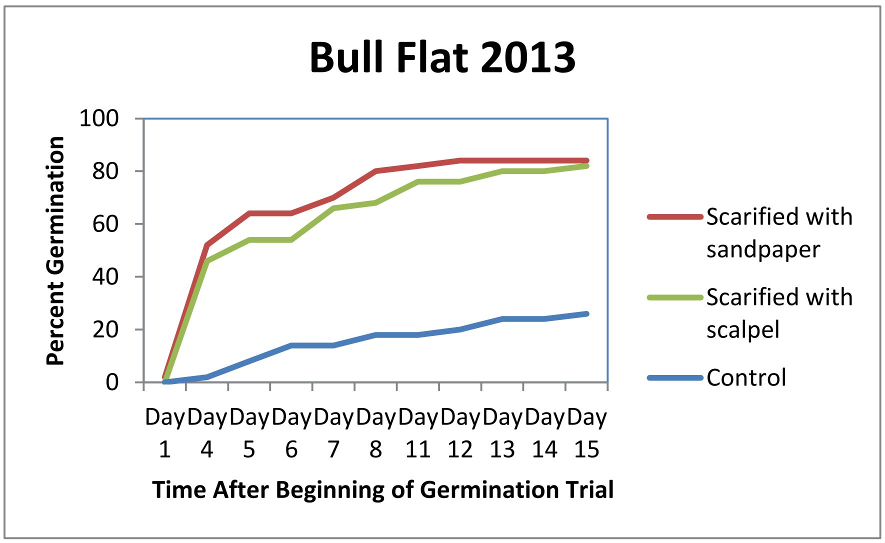


Figure 8. The average percentage of seeds that germinated over 15 days for three treatment groups and two years for collection site Beaver

Seeds collected in 2012 from both sites germinated very poorly without scarification, but seeds collected in 2013 germinated at higher rates, and seeds collected in 2014 germinated at rates not significantly different than the sandpaper and scalpel treatment groups (Fig. 9).





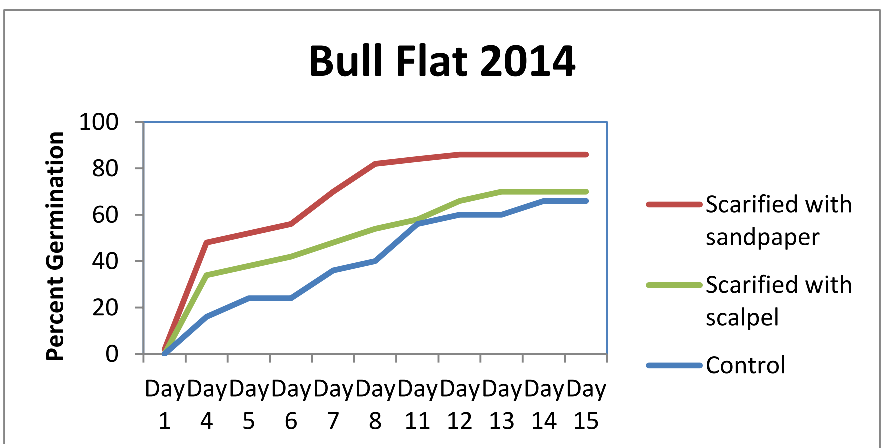


Figure 9. The average percentage of seeds that germinated over 15 days for three treatment groups and three years for collection site Bull Flat.

2. Seedling Cultivation

Seedling mortality did not differ significantly among the three treatments (p= 0.745 at alpha 0.05) (Table 1). Because mortality was high, a sample was sent to the OSU Plant Pathology Diagnostic Laboratory to determine a possible fungal or pest cause for the mortality rate; previous studies had documented "damping off" of cultivated seedlings (Gisler and Meinke 2001). However, the lab results showed no fungal or pest evidence for mortality in the sampled seedlings.

Table 1. Chi-squared test found no significant difference between seedling survival and treatment (p=0.745).

Chi-square (Observed value)	0.588
Chi-square (Critical value)	5.991
DF	2
p-value	0.745
alpha	0.05

While the mortality for the seedlings was high, the surviving seedlings appeared as healthy as plants at the native site. Seedlings grown outside were generally shorter and stouter than those grown in the greenhouse (Fig. 10), though they did not experience a lower rate of mortality. All seedlings were placed outside of the greenhouse in flats eight weeks before the date they were to be transplanted to Bull Flat to allow sufficient time for hardening off to the cold weather. During this time they were treated for aphids after an infestation was discovered, but no seedlings were lost due to aphids or treatment for aphids.

Figure 10. Ten week old seedlings grown outside (top) and in the greenhouse (bottom).

DISCUSSION

1. Germination Trials

The seeds in this study showed increasing physical dormancy with age. The one or two year old seeds collected in 2012 and 2013 from both Beaver Marsh and Bull Flat required manual scarification by sandpaper or scalpel to break the seed coat and to allow the embryo to be imbibe water, while seeds collected in 2014 were able to germinate the same year they were collected. This is useful information for agencies wishing to propagate Peck's milkvetch. If seeds are able to germinate without scarification, it will decrease the hours of work required to manually scarify the small seeds from older years without damage to the embryo.

There was no significant difference between using the scalpel or sandpaper as scarification treatment, though the sandpaper treatment seemed to produce more seedlings quickly if one were to do a large-scale propagation where high yields were necessary. Further research can develop methodologies for creating most seedlings with the least amount of labor, such as acid scarification, which is done on other species of Fabaceae. Some studies have indicated increased vigor in germination of seeds where acid scarification is used over hot water scarification (Fabiao et al. 2014). At the present, the best methodology seems to be to treat the seeds with sandpaper under a microscope if the seeds are over one year old or to germinate seeds collected during the current season.

The increasing physical dormancy of seeds is an adaptive trait that promotes the long-term survival of the Peck's milkvetch population. Seeds displaying physical dormancy are able to increase their germination distribution because seeds of different ages will germinate at different times. “Afterripening” is the mature, parent-separated seed’s process of losing this physical dormancy in response to environmental circumstances (Foley 2001). Seeds receiving more physical abrasion will germinate more quickly than seeds that remain protected by their seed coat (Patanè and Gresta 2006). This is especially useful in an extreme environment such as Bull Flat, where conditions can change quickly and staggering episodes of germination is beneficial. Physical dormancy prevents a mass germination that would kill all seedlings after a single extreme temperature event when the seedlings are still vulnerable.

Physical dormancy is found in 15 angiosperm families, including Fabaceae (Baskin and Baskin 1998). In all of these families, a “specialized structure is associated with water-impermeable layers” (Baskin and Baskin 2000). This layer prevents germination by blocking water from penetrating the seed coat. Germination may resume upon the disruption of these structures (Baskin and Baskin 2000).

The water-impermeable coat of dormant seeds is caused by a layer over the seed of phenolic and suberin compounds in the palisade cells of the seed coat (Smýkal et al. 2014). This impermeable layer is formed after the maturation of the seed, but there is a period of time between maturation and seed-coat dormancy where seeds are able to germinate before reaching their maximum dry weight (Gresta et al. 2011). The establishment of physical dormancy and development of the water-resistant seed coat takes place in the dehydration of the maturation process (Baskin and Baskin 1998). In one study, seeds of *Scorpius subvillosus* L. were able to germinate at rates of 100% before maturation, but germination rates decreased with storage (Cristaudo et al. 2008).

These findings are consistent with those of our study. Seeds of *A. peckii,* were also able to germinate for a certain period before becoming physically dormant.

To break physical seed dormancy in nature, environmental factors are required, such as changes in temperature, fire, or ingestion by animals (Baskin and Baskin 1998) Temperature is often considered the primary factor for changing dormancy states, especially in temperate regions (Baskin and Baskin 2003). Temperature changes may be sufficient to remove or disrupt structures of the seed coat preventing water uptake. Seeds displaying physical dormancy are often able to germinate after the seed coat has been broken under a large range of conditions in relation to both temperature and light (Baskin and Baskin 2000). Seeds in Bull Flat and Beaver Marsh are exposed to temperature extremes that may aid the breaking of physical dormancy.

Seeds of *A. peckii* should be considered physically dormant if seeds older than the immediate season are used. Once their seeds coats have been broken they are able to germinate at rates of up to 90%. Future research could determine the cause of breaking of physical dormancy in nature at *A. peckii* plants' native site, as this could lead to identification of seed treatment methods that could be used on-site to improve seed germination and population numbers.

2. **Seedling Cultivation**

The cultivation of many rare and endangered plants is not well-researched and high mortality rates are common in efforts to produce transplants. Additionally, only a small amount of material should be collected from natural populations to avoid further loss in viability (Bayraktar et al. 2015). The seedlings experienced the same mortality when grown in potting soil as when they were grown in native soil mixtures. Because native soil did not increase seedling viability, collection of soil in natural populations is not warranted. The ability to use potting mix in cultivation projects results in results in a much smaller impact on the environment of native sites. Future research could potentially emulate the environment of Bull Flat in a more realistic way to encourage seedling survival. This may include growing the seedlings in perlite, which emulates the volcanic soil of the native habitat (Gisler and Meinke 2001). Bull Flat is hot and dry in the summer with low humidity, while OSU is located in the Willamette Valley and experiences a much milder season. Seedlings may also normally experience a high mortality rate in their native site because of the harsh conditions, which would make a low seedling percent survival rate normal. Survival rates of individual maternal lines were not tested in our study; it is possible that some lines survive better than others.

The 25 plants grown outside in the small pilot study experienced similar mortality to the plants grown in the greenhouse. However, they were shorter and stouter than their greenhouse-grown compatriots. In other studies, plants grown in greenhouses exhibited different characteristics than plants grown outside, such as variation in growth caused by light spectrum differences (Shirley 1929) or exposure to elements such as low humidity (Preece and Sutter 1991). As native plants at Bull Flat also tend to be short and stout, future seedling cultivation may be best outside if the seedlings are to most closely resemble native plants. However, researchers will also need to consider the risk for additional pests, as aphids were a problem with the seedlings grown outside, and consider contrasts in climate between OSU and the native sites.

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Chapter 3

SITE SELECTION, VEGETATION DATA, AND PLANTING OF THE RARE OREGON ENDEMIC *ASTRAGALUS PECKII* IN FOUR HABITAT TYPES

METHODS

1. Site Selection and Vegetation Data

Four 50 meter long transects were placed in Bull Flat, OR (44º 09′ 40.35″N, 121º 26′ 05.54″W, 1062-m elevation) located in the Tumalo Wildlife Corridor near Sisters, OR east of the Three Sisters. Transects were placed in habitat types classified by disturbance and occupation by current populations of *Astragalus peckii.* One transect was placed in each of four habitat types: undisturbed and under-occupied, undisturbed and occupied, disturbed and under-occupied, and disturbed and occupied (Fig. 11).

Each transect site was marked with a white stake at the beginning and end of 50 meters, then each stake labeled with metal tags as 101, 102, 103, or 104. Twenty quadrats were placed on each of the four transects in each site for a total of 80 quadrats. Quadrat placement was determined by a random number generator and a diagram of the 50 meter transect (Fig. 12). Every meter on the transect was assigned a number from 1-100 on both sides. Twenty random numbers were then drawn from the 100 for placement of each quadrat of one meter squared on the assigned meter of the transect. The placement of the quadrats was repeated for each transect in the four selected sites. In each quadrat, the present number of *A. peckii* was recorded as well as the GPS location. Percent cover of all vegetation by species was also recorded (Fig. 13).

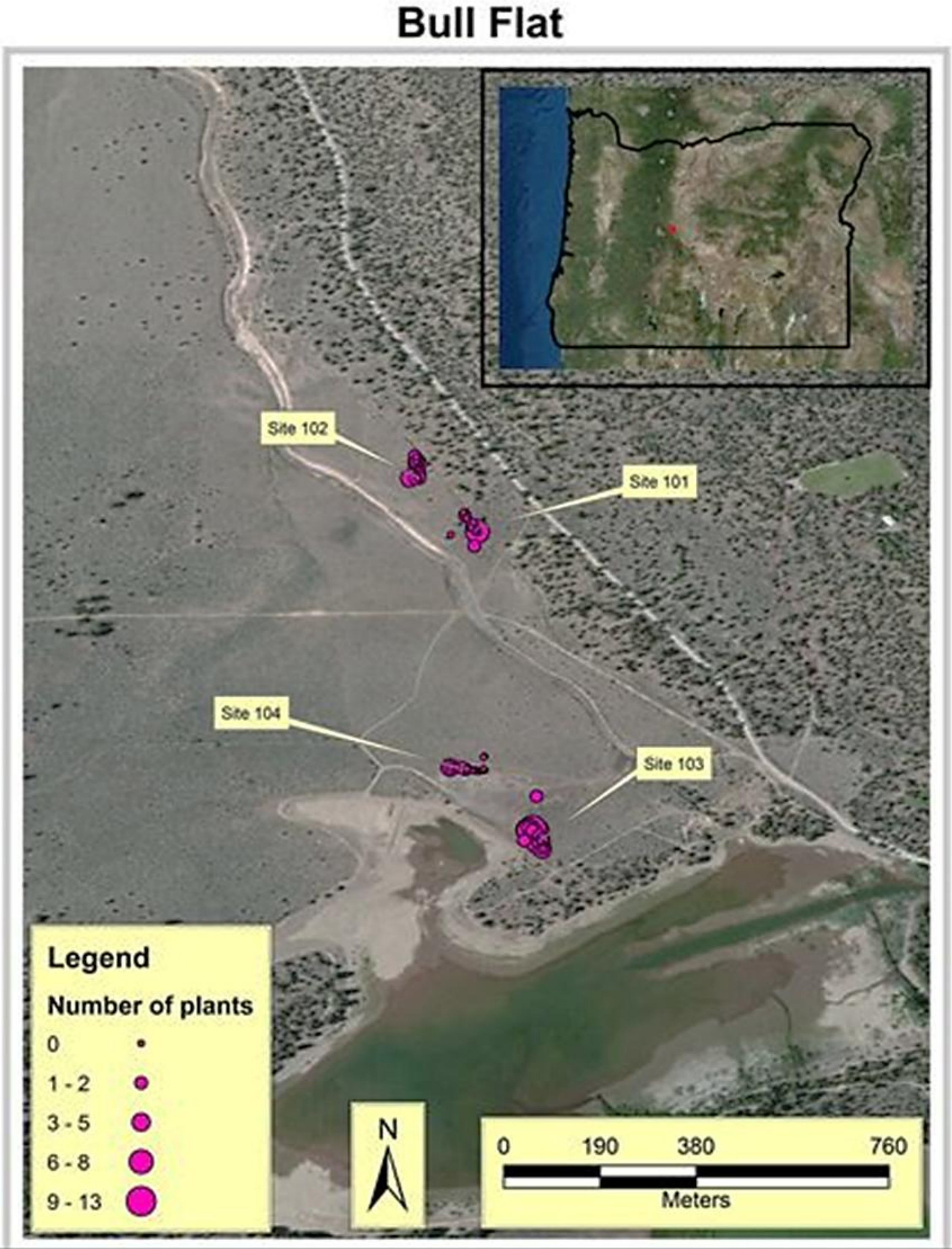

Figure 11. Sites 103 and 104 are in disturbed habitat and Sites 101 and 102 are in undisturbed habitat.

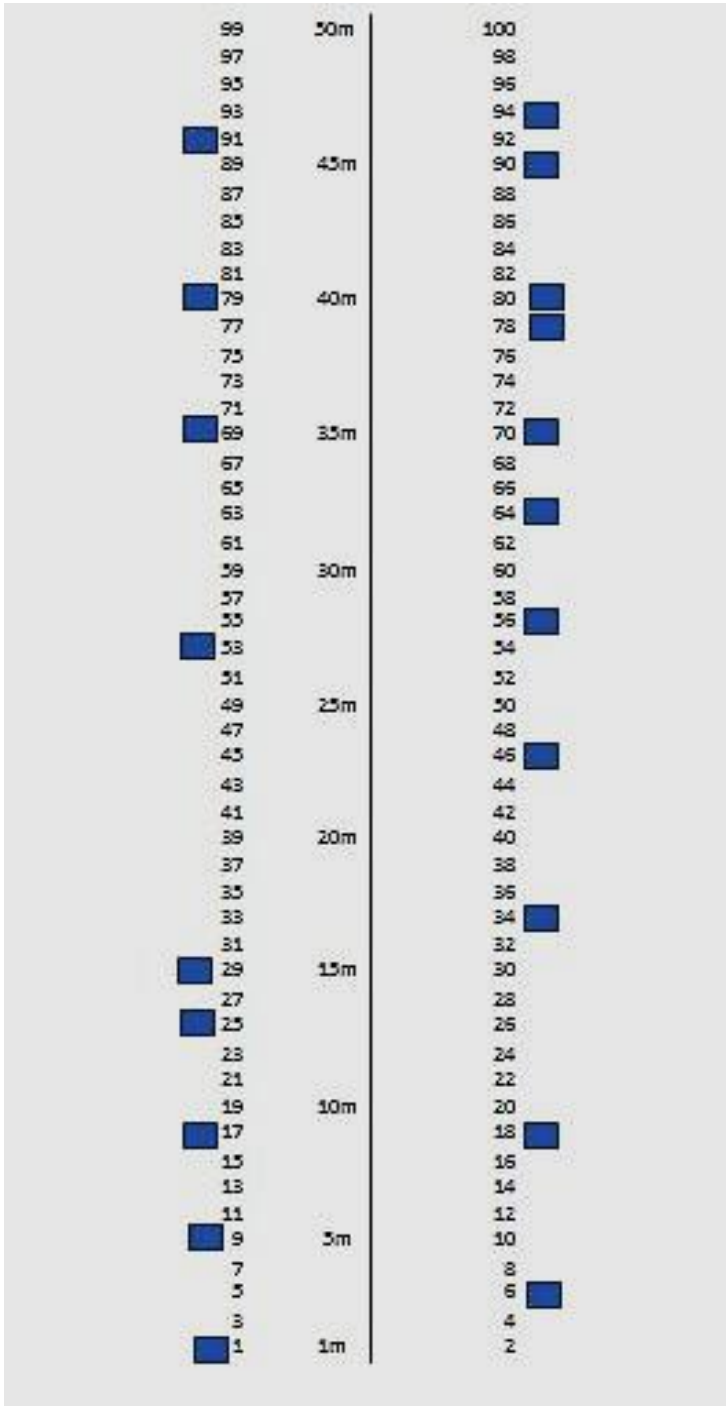

Figure 12. The quadrat setup around each of four 50m long transects was established using a random number generator.

Figure 13. A researcher records GPS and vegetation data on a quadrat at the beginning of a transect in Bull Flat.

Occupied Sites 102 and 103 generally contained a large and established population of Peck's milkvetch. Each of the 40 quadrats placed in these sites contained between 0 and 14 *A. peckii* plants. Under-occupied Sites 101 and 104 were located in habitats that did not contain a large or established population of *A. peckii*. The 40 quadrats in these sites contained between 0 and 5 *A. peckii* plants per quadrat.

Transects were also classified as disturbed or undisturbed (Fig. 14). Disturbed sites generally experienced off-road vehicle usage while undisturbed areas had no vehicle use. Disturbed areas were generally defined by low percent vegetation cover of no more than

20% per quadrat. Sites 103 and 104 were located in disturbed areas. Two quadrats were exceptions to the 20% cover or less guideline in disturbed Site 103 because of a large percent cover by dead vegetation. However, more than 75% of disturbed quadrats contained 5% cover or less.

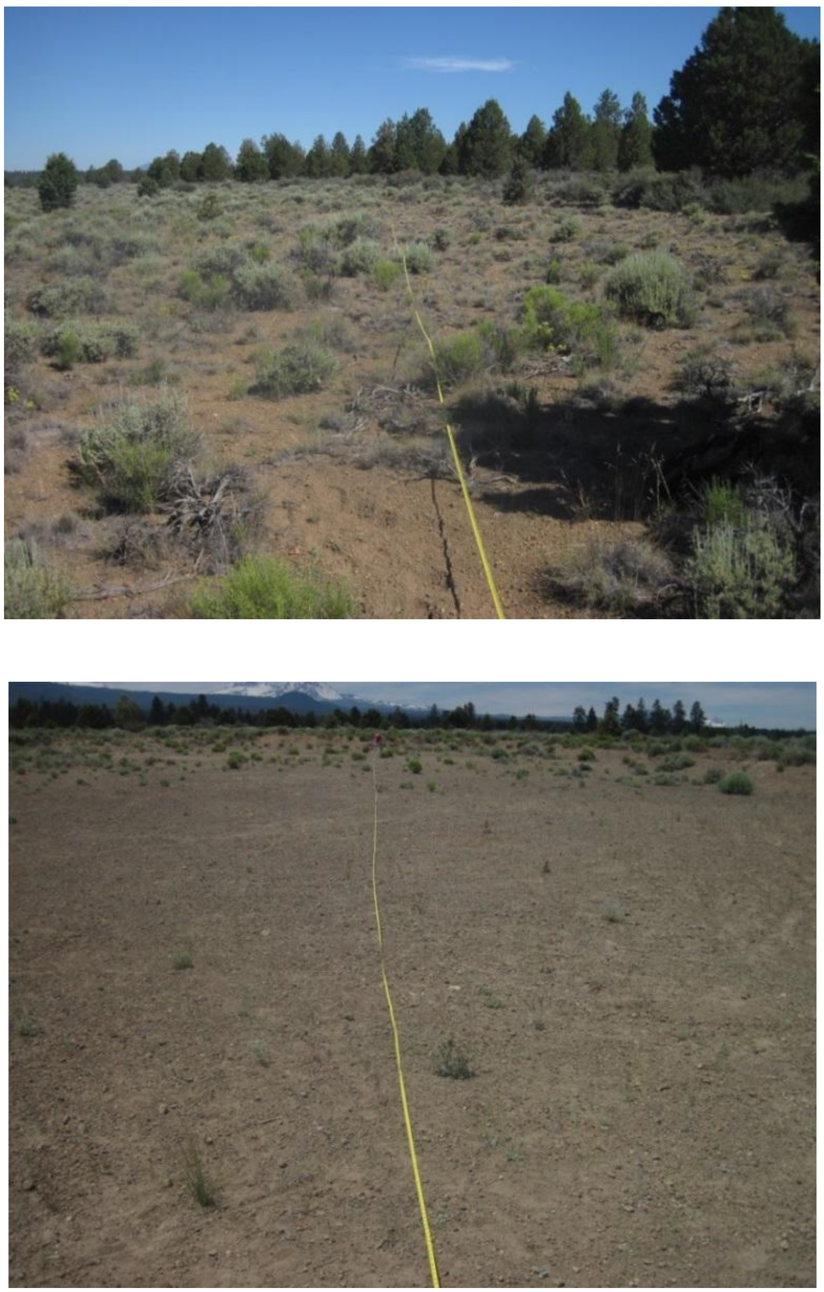

Figure 14. Undisturbed site (above) and disturbed site (below).

Percent cover of all species was recorded in each quadrat (Fig. 15). Plants with their base within the quadrat area were counted as present in the quadrat, including plants with their bases inside the quadrat and other branches or plant cover protruding over and outside the quadrat area. Plants with branches or other cover over or inside the quadrat, but with bases outside of the quadrat, were not counted as a species present in the quadrat. Percent cover was recorded as the area of the quadrat covered by vegetation.

Figure 15. A quadrat in Bull Flat along a 50 meter long transect in undisturbed, occupied habitat (Site102).

2. Planting

Seeds were selected from five seed lots. These lots were collected from Beaver Marsh or Bull Flat over the years 2012, 2013, and 2014. In order to increase genetic variability in each seeded plot, all seeds were mixed together, and divided into packets for sowing.

Six hundred and forty mixed seeds were counted manually and weighed in at 0.7 grams. Another 640 seeds were counted and also weighed 0.7 grams. The remaining 18 packets were made by weighing the seed mixture to yield 0.7 grams, or approximately 640 seeds. A total of 12,800 seeds were placed in 20 packets for planting in the quadrats at the Bull Flat site. The majority of seeds were collected from Bull Flat 2012 at 52% of the total mixed 12,800 seeds, with Bull Flat 2013 and 2014 both contributing 5%. Seeds collected from Beaver Marsh in 2013 made up 24% of the total and seeds collected from Beaver Marsh in 2014 made up 14% of the total.

Quadrats were randomly assigned to be control, seed, or seedling plots using the transplants described in Chapter 2. Of the twenty quadrats per site (80 quadrats total in all four sites), ten were assigned to be seedling plots and five each were assigned to be seed and control plots. Each transect at each site was assigned the same control, seed, and seedling plot arrangement made by the diagram.

Seeds were planted in each assigned quadrat by volunteers from FotTWC. Flagging was placed in each corner of the quadrats for the volunteers, marking each by color as “seed” or “seedling” plots. Each transect had five assigned seed quadrats per site for a total of twenty seed quadrats. Volunteers used pre-made packets of 640 seeds from the variety of locations to plant the seeds in the quadrats. Volunteers were instructed to water the ground and gently brush the soil in each quadrat aside around existing plants to place seeds in the soil bed. Rulers were provided to ensure seeds were all planted two centimeters deep. After placement and covering with soil, the area inside the quadrat was watered again. Unfortunately, volunteers accidentally planted seeds in the plots assigned as controls in Sites 101 and 102 and no seeds were planted in Sites 103 and 104.

Volunteers also assisted with the seedling transplants. There were ten assigned seedling quadrats on each transect per site for a total of 40 quadrats. Each quadrat contained four seedlings for a total of 160 seedlings over all four sites. Seedling quadrats were also watered before transplant of seedlings. Each seedling was removed from the band pot in which it was transported (Fig. 16) and placed in a square formation inside the quadrat. Seedlings were not assigned by their previous treatment in native or potting soil, but they were assigned by their growth location (greenhouse or outside). Plants that were grown outside the greenhouse were generally shorter and stouter than those grown in the greenhouse. For the planting, each seedling plot was assigned three greenhouse plants and one outside plant to ensure maximum survival and to avoid having areas with all greenhouse or outside grown plants. After seedlings were transplanted they were watered. Each seedling was marked with a wooden stake an inch north of the seedling to assist with location of the seedlings in the spring for survival rate.

Seeds and seedlings were planted November 8, 2014. Native seedlings survive their first summer and must also survive the winter conditions. Within a week of planting the seedlings, Bull Flat was hit by exceptionally hard frost and within two weeks seedlings were covered with snow.

Seeds are assumed to germinate and emerge in the spring and summer. Seeds which exhibit physical dormancy, such as those of *A. peckii* plants, typically do not exhibit physiological dormancy requiring treatment (i.e. the need for cold stratification). They are able to germinate at a range of temperatures once dormancy is broken (Baskin and Baskin 1998). Therefore, it is possible that seeds germinate once physical dormancy is broken, possibly in nature by heat or cold, and will therefore germinate in the warmer temperatures of spring and summer.

Figure 16. Volunteer removes *Astragalus peckii* plant from band pot, revealing root structure.

RESULTS

1. Site Selection and Vegetation Data

Species present in at least one of the quadrats over the four sites were *Artemesia tridentata* Nutt., *Chrysothamnus viscidiflorous* (Hook.) Nutt., *Achnatherum lemmonii* (Vasey) Barkworth, *Bromus tectorum* L., *Erigonum umbellatum* Torr., *Chrysothamnus nauseosus* (Pallas) Britton, *Purshia tridentata* (Pursh) DC., *Lupinus aridus* Douglas, *Linum perenne* L., and *Gayophytum humile* Juss (Table 2).

Table 2. Plant key of species found in at least one quadrat of the 80 surveyed.

Species	Code
Artemesia tridentata	ARTR
Chrysothamnus viscidiflorus	CHVI
Achnatherum lemmonii	ACLE
Bromus tectorum	BRTE
Eriogonum umbellatum	ERUM
Chrysothamnus nauseosus	CHNA
Purshia tridentata	PUTR
Lupinus aridus	LUAR
Linum perenne	LIPE
Gayophytum humile	GAHU

In Site 101, which was undisturbed and under-occupied (Table 3), there were no more than three *A. peckii* plants per quadrat (5-60 percent cover). Key species were *A. tridentata* and *C. viscidiflorus,* at least one of which was found in each of the twenty quadrats in Site 101. Thirteen quadrats had *A. tridentata* while eleven had *C. viscidiflorus*. Two quadrats contained *A. lemmonii* and one each contained *C. nauseosus* and *L.perenne* (Table 3)*. Bromus tectorum* (cheatgrass) did not cover the quadrats surveyed in Site 101, although this weed was found in some disturbed quadrats of Site 104.

Table 3. Species composition of quadrats in Site 101.

Quadrat Number	**Number of *A. peckii* present**	**Cover Percent**	**Associated species present**
1	0	5	ARTR, CHVI
6	0	60	ACLE, ARTR
9	0	50	ARTR
17	3	5	CHVI
18	0	10	ARTR
25	0	15	ARTR, CHVI
29	0	15	ARTR, CHVI
34	0	15	CHVI
46	0	50	ARTR, CHVI
53	0	5	CHVI
56	0	5	ARTR , CHNA
64	0	5	ACLE, ARTR, LIPE
69	0	30	ARTR
70	0	40	ARTR
78	1	20	ARTR
79	0	30	CHVI
80	0	15	ARTR
90	0	40	CHVI
91	0	15	CHVI
94	0	10	ARTR, CHVI

Site 102 was undisturbed and occupied (Table 4). There were up to four *A. peckii* plants in each quadrat (1-50 percent cover). This site also did not contain *B. tectorum* in the quadrats surveyed. This occupied site had a different variety of species than the under-occupied site. *A. tridentata* was found in eight quadrats and *C. viscidiflorus* in three quadrats, less than the number in Site 101. Site 102 had thirteen plots with *A. lemmonii* as well as six plots of *E. umbellatum* and one of *L. perenne*. The naturally occurring occupied areas supported fewer *A. tridentata* and *C. viscidiflorus* and a higher amount of grasses such as *A. lemmonii* (Table 4).

Table 4. Species composition of Site 102.

Quadrat Number	**Number of *A. peckii* present**	**Cover Percent**	**Associated species present**
1	2	20	ACLE, ARTR
6	3	10	CHVI, F
9	1	10	ARTR
17	2	30	CHVI
18	0	40	ACLE, ERUM
25	4	30	ACLE, ARTR
29	0	10	ACLE
34	0	30	ACLE, ARTR
46	0	10	ACLE
53	1	30	ARTR, ERUM
56	0	5	ERUM
64	0	5	ERUM
69	4	1	ACLE, ERUM
70	0	5	ACLE
78	2	1	ACLE
79	1	20	ACLE
80	0	30	ARTR
90	2	10	ACLE
91	2	10	ACLE, ARTR, CHVI
94	0	50	ACLE, ARTR, ERUM

Disturbed and occupied Site 103 (Table 5) also contained many quadrats with *Achnatherum lemmonii*, a feature this site has in common with undisturbed occupied Site 103. Each quadrat in Site 103 contained between 0 and 13 *A. peckii* plants (0-13 percent cover). Seventeen quadrats in Site 103 contained *A. lemmonii*, three contained *C. nauseosus* or *A. tridentata*, two contained *P. tridentata*, and one contained *G. humile* or *C. viscidiflorus* (Table 5). No quadrats in Site 103 contained *B.tectorum.*

Table 5. Species composition of Site 103.

Quadrat Number	**Number of *A. peckii* present**	**Cover Percent**	**Associated species present**
1	2	1	ACLE
6	1	10	ACLE
9	7	5	ACLE
17	7	5	ACLE
18	4	5	ACLE, CHNA
25	13	5	ACLE, CHNA
29	12	5	ACLE
34	1	5	ACLE
46	1	5	ACLE, GAHU
53	0	5	ACLE, CHVI
56	2	20	ACLE, PUTR
64	0	1	ACLE
69	1	1	ACLE
70	0	1	ACLE
78	2	10	ACLE
79	2	5	ACLE
80	8	20	ARTR
90	2	1	ACLE, ARTR
91	0	100	PUTR
94	3	95	ARTR

Disturbed and under-occupied Site 104 (Table 6) was notable for its low vegetation percent cover of 1 percent or less, and in the case of three quadrats, no cover at all. There were five or less *A. peckii* in each quadrat in this site (Table 6). The most common species in this area were *E. umbellatum* and *G. humile,* which were present in three quadrats each. Small *A. tridentata* were also found in three quadrats as well as one quadrat with *C. viscidiflorus*, two with *C. nauseosus*, and another two with *L. aridus*. Two quadrats in this site contained *B. tectorum*.

Table 6. Species composition of Site 104.

Quadrat Number	**Number of *A. peckii* present**	**Cover Percent**	**Associated species present**
1	0	0.5	ERUM
6	0	0.5	ERUM
9	0	0.5	ERUM
17	0	0.5	GAHU
18	0	0.5	GAHU
25	0	0	*
29	0	0	*
34	0	1	CHVI
46	0	0	*
53	0	1	LUAR
56	0	1	BRTE
64	1	1	ARTR
69	1	1	ARTR
70	0	1	ARTR , CHNA
78	0	0	*
79	0	1	CHNA
80	0	1	GAHU
90	5	1	BRTE
91	2	1	CHNA
94	0	1	LUAR

The occurrence of three species in the quadrats were compared between occupied and under-occupied sites using independent one-tailed t-tests. There is evidence to suggest that occupied sites have a higher occurrence of *A.lemmonii* (p= 0.02 at alpha 0.05) than do under-occupied sites. There is little evidence to suggest occupied sites have lower levels of *A. tridentata* (p= 0.3) or *C. viscidiflorus* (p= 0.3) at alpha 0.05.

2. Planting

Ten seed and ten seedling quadrats were planted in Site 101, and the number of plants of *A. peckii* present in all quadrats was recorded in Jun. A paired t-test found little evidence for a larger final number of *A. peckii* in the quadrats after planting seeds and seedlings at Site 101 (p-value 0.209 at alpha 0.05) (Table 7).

Table 7. One-tailed paired t-test for Site 101 before and after planting.

	before	**after**
Mean	0.2	0.35
Observations	20	20
df	19	
P(T<=t) one-tail	0.209696	
t Critical one-tail	1.729133	

Undisturbed, occupied Site 102 also had seeds and seedlings in all twenty assigned quadrats, but no data sets were collected. When researchers returned to the area in June, all stakes, tags, and markers for seedlings had been removed from the area and from each quadrat. While GPS data was available showing the approximate location of each plot, the loss of the exact transect area and quadrat location made accurate data collection infeasible.

In June, seedlings were observed in all plots planted with seedlings in Site 103 (seeds were not planted in this site).Unfortunately, the last five seedling quadrats had been destroyed by the dumping of agricultural dirt filled with common garden weeds. In the seedling plots, there is little evidence to suggest a larger number of *A. peckii* in the final quadrats (p= 0.208 at alpha 0.05) or in the control quadrats (p= 0.06 at alpha 0.05) (Table 8).

Table 8. One-tailed paired t-test for Site 102 before and after planting.

	before	**after**
Mean	3	1.625
Observations	8	8
df	7	
P(T<=t) one-tail	0.20814	
t Critical one-tail	1.894579	

Site 104 had ten seedling quadrats (no seed quadrats). There is little evidence to suggest a higher final number of *A. peckii* in the seedling quadrats (p= 0.172 at alpha 0.05) or in the control quadrats (p= 0.39 at alpha 0.05) (Table 9).

Table 9. One-tailed paired t-test for Site 104 before and after planting.

	before	**after**
Mean	0.3	0.4
Observations	10	10
df	9	
P(T<=t) one-tail	0.17172	
t Critical one-tail	1.83311	

Some quadrats that received transplants experienced an overall loss of plants from the original number. Of the 640 seeds planted in Site 101, five emerged, for an emergence rate under 1%.

DISCUSSION

1. Site Selection and Vegetation Data

An interesting finding of the vegetation survey was the lack of *B.tectorum* in undisturbed sites and in the disturbed, occupied site. *Bromus tectorum* (cheatgrass), is an annual, early-successional species introduced in the late 1800s, possibly by livestock, from Mediterranean Europe. In states such as Nevada, cheatgrass is present in almost all sagebrush-bunchgrass communities (Knapp 1996). Because cheatgrass is an early-successional species, it can outcompete native perennials such as Peck's milkvetch. Cheatgrass was found in the disturbed, under-occupied site in Bull Flat, suggesting that as areas are disturbed, cheatgrass is able to grow better the native perennials. As more disturbance occurs in Peck's milkvetch populations due to development, the growth of cheatgrass could be favored over native species. Climate change is predicted to increase the prevalence of invasive species with certain common traits especially favored in fragmented habitats, such as increased nitrogen levels in the soil (Dukes and Mooney 1999). Future ecological research at Bull Flat will need to consider the presence of invasive early-successional species and the effects of changing climate on the prevalence of these invasives.

The vegetation survey also provided a catalog of the species diversity in both occupied and under-occupied sites. Notably, a significantly larger population of *A.lemmonii* was found sites occupied by *A. peckii*. This native perennial bunchgrass provides forage for large animals and seeds for small animals (Darris et al. 2007). Because significantly more *A. lemmonii* is found in sites occupied by *A. peckii*, the presence of this species may indicate sites suitable for transplants. Future projects may consider areas with *A. lemmonii* an ideal habitat for transplantation of *A. peckii* seedlings based on the historical co-occupation by these two plants. Native bunchgrasses are essential for the ecology of the area, and their displacement by annuals such as cheatgrass should be a primary research concern.

Selection of sites suitable for reintroduction or augmentation projects is often a challenge especially when working with species that require habitats that are also rare. Unique habitats are lost with increasing development, such as the loss of wetland habitat due to urban development in Portland (Holland et al. 1995). As habitat around Bull Flat is lost due to development, opportunities to research components of this habitat, such as its ability to support *A. peckii*, will also be reduced.

2. Planting

The results of this study suggest that further research is needed before transplanting and/or sowing seeds can be recommended for large-scale reintroductions of Peck's milkvetch. Transplanting and sowing seeds did not increase the number of *A. peckii* plants present in the quadrats studied. Sites 101 and 104 experienced a slight, though not significant, increase in *A. peckii* plants while Site 102 experienced a decrease in plants. Additionally, while the results were not significant, some quadrats in all sites that received transplanted seedlings or planted seeds experienced an overall loss in the number of *A. peckii* plants. This may suggest transplantation can harm existing populations.

Seeds emerged from one site that could be evaluated at a rate under 1%. This may suggest that removing large numbers of seeds from the population may be harmful if the return in emergence is so low. A factor potentially affecting seed emergence was that over half of the seeds planted were collected from Bull Flat in 2012. The germination study demonstrated increasing physical dormancy with age, so it is possible seeds new seeds will emerge at higher rates. Previous research has suggested a lower percent seed viability the longer seeds are in the seed bank at Bull Flat (Martin 2010), possibly as the physical dormancy of the seeds increases. Therefore, future research on natural mechanisms to break dormancy in *A. peckii* and the induction of seed emergence from the seed bank may be essential. Another possibility suggested by the low seed bank survival and the increased physical dormancy is that the seeds of *A. peckii* typically germinate in the season they are collected, before physical dormancy sets in and before survival in the seed bank diminishes.

Water scarcity could have affected this study, as global changes and drought have caused dramatic changes to plant communities. Climate change has increased the mortality of trees in the forest canopy globally due to increased drought and heat extremes, including areas not normally thought of as drought-prone and in addition to normal fluctuations in tree mortality. The increased die-back of forests will have long-term implications for the global climate, as they are currently an important component for the sequestration of carbon (Allen et al. 2010). The die-back of forests has the potential to accelerate drought and heat increases as feedback mechanisms are lost. As habitats prone to drought receive smaller amounts of precipitation and are exposed to more extreme heat, species may not be able to survive the new conditions, which will impact the ecosystem as a whole.

Precipitation could have affected the study because the transplants were done in a much wetter than average month following a year that was much drier than average. There was 5.8 times less precipitation in November 2013 than in November 2014 (PRISM Climate Group 2015). As more extremely wet or dry years are observed, plant communities may face increased challenges.

While Bull Flat receives very little precipitation in the summer, it is coated with snow in the winter. However, snowpack has also been decreasing in conjunction with increases in the global frequency and duration of drought. Snowpack provides storage of winter precipitation that can revive plants over the dry summers. The snowpack in the West, particularly in the Cascade Mountains and in northern California, has declined precipitously due to climate change even when we take changes in the use of land or other variations in forest canopy into consideration (Mote et al. 2005). Future research and assessment of the viability of restoration projects will have to take into account the effect of increased temperatures and drought as well as decreased winter precipitation and lack of snowpack.

3. Challenges

Reintroduction projects can face unique challenges because the environment for the study conditions can change at any time, a challenge not often faced by in-lab experiments. In addition, community involvement can be essential for the success of ecology projects, as uncooperative community members may destroy projects by removing flags, entering sites marked for research, or causing other damage to restoration sites.

Awareness and involvement of the community in restoration work can help mitigate some of the challenges faced by damage of the environment and study property. The volunteers assisting this study (FotTWC) were essential for promoting the protection of the site. When known trespassers were disrupting the protected areas in the Tumalo Wildlife Corridor, they ensured more signs were placed to spread awareness of projects and to remind trespassers of bans on certain uses of the area. The FotTWC also assisted our work at Bull Flat by locating and sending us plants we had banged for seed collections and helping with the seed and seedling planting.

A challenge to this study was the dumping of agricultural dirt over the last 10 meters of the disturbed and occupied Site 103. Not only did this invalidate data for this transect, several common garden weeds were also in the dirt. These weeds could spread and disrupt the ecology of the entire area. The area was also host to shooting and drinking, as shells and bottles were found, especially in the disturbed site. Horse trailer marks were also observed in the disturbed site; vehicle use in this area is prohibited.

An additional challenge to this study was the loss of the control quadrats in Sites 101 and 102 and the loss of the seed quadrats in Sites 103 and 104. The twenty packets were distributed after the flagging for "seed" and "seedling" quadrats had been placed for volunteers. Instructions were given to place seeds in "seed" flagging areas without conveying that some of the "seed" quadrats were control. This resulted in sites 101 and 102 having each ten seed and seedling plots and sites 103 and 104 having each 10 seedling and 10 control plots. While volunteer help contributes to project success in many ways, good management and planning is also essential to ensure correct scientific procedures are followed.

Another challenge to the study was the unpredicted hard frost and snow that covered the area shortly after the seedlings were planted. Seedlings may have had a better chance for survival if they had been given some time to recover from the transplant and time to acclimate to the weather changes. In the future seedlings could be planted earlier in the season to see if more seedlings survive with additional time before winter.

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