Assessing Local Adaptation in Four Native Grass Species

By

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Prairies in the Pacific Northwest have been reduced to less than 1% of their pre-settlement distribution. Prairie restoration typically requires larger quantities of seeds than can be wild-collected on site, thus site managers often contract with farmers to produce seed. However, if selection pressures in farm fields are radically different than that of sites to be restored, then it is possible that the restoration will fail. We experimentally addressed these concerns with four species of grasses that are being used for restoration in a 956-hectare county park in the S. Willamette Valley. We sought to determine whether the distance between the site of seed production and the site of restoration was within a seed transfer zone by using a reciprocal transplant experiment. Seedlings were assessed for growth and mortality over fifteen-weeks from April-August 2013. We asked whether growing location and seed source affected growth and mortality. We found little evidence for local adaptation. However, we found significant growing location effects on growth and mortality between the two sites; on average plants survived better at Mt. Pisgah, but grew larger at Albany. Adult plants already growing at these sites for these species were also evaluated to determine whether growing location had an effect on damage to the adults and their reproductive output. Location did influence the amount of damage experienced by adults of four species. *Festuca roemeri* had less damage at Mt. Pisgah.

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INTRODUCTION

Human activities in ecosystems throughout the world have ubiquitously altered natural habitats and plant communities. Large-scale restoration projects have become increasingly common as a way to combat habitat degradation and loss of species, and to preserve ecosystem services and ecological interactions among organisms. Understanding restoration genetics is an integral part of a successful restoration project (Falk et al. 2001). The underlying genetics of plant materials used in restoration affect the ability of populations to adapt and thrive long term on a site.

Willamette Valley native prairie restoration has been a focus of both public and private organizations. Examples include the Buford Recreation Area and private large - scale habitat restorations run by The Nature's Conservancy. Restoration has become necessary to respond to human caused disturbances in the fire regime and inundation by invasive non-native species (Clark and Wilson 2001). Historically the fire regime was very different when the Kalapuya people intentionally maintained the native prairie. Prior to European settlement, they maintained a frequent fire regime that kept the grasslands free from woody shrubs and the savannah dominated by Oaks. The arrival of European settlers altered this periodic burning, which had become a part of the natural cycling of the ecosystem (Boyd 1999, Clark and Wilson 2001). Furthermore, inundation by invasive species has altered the strength of competition experienced by native species in the valley, in addition to changing ecosystem structure and function (Cremieux et al 2008). A decrease of regular burning and the introduction of invasive exotics are two significant reasons why many local native plant species, and native plants throughout the world, are under threat (Myerson and Mooney 2007), including the species discussed herein.

The use of native plants has been established as having importance in maintaining historic ecosystem function following human disturbance (Lesica and Allendorf 1999, Bischoff et al. 2006, Myerson and Mooney 2007). Augmentation of existing populations of plants in remaining habitat patches, such as the oak savannah and prairie, involves reseeding using locally collected material at sites like Buford Park Recreation Area, hereafter known by its common name, Mt. Pisgah. The use of local materials is important because it preserves historic genetic composition in the population (Myerson and Mooney 2007, Bischoff et al. 2006) while also protecting against damage caused by swamping of local genotypes with non-local, potentially maladapted individuals (Miller et al. 2010). Furthermore, local material is better able to withstand rare destructive events such as storms and drought that don’t occur annually (Bischoff et al. 2006, Myerson and Mooney 2007). The goals of restoration projects are to preserve an endangered habitat that once covered the majority of the Willamette Valley while also maintaining the genetic integrity of the plant communities. Maintaining genetic integrity is important for insulating species assemblages against the effects of climate change (Falk et al. 2001), because it preserves genomic variation and therefore the capacity to evolve (McKay 2005).

Local Context

Habitat restoration in the Willamette Valley often takes place in isolated patches of preserved habitat such as oak savannah areas at Mt. Pisgah. Restoration projects are a major practical component of the greater discipline of conservation biology. The goals of conservation biology are simple to understand, but often complicated to implement. Conservation biologists seek to understand ecosystems and preserve their function, biodiversity and critical species, in the face of destructive forces created by humans.

One of the major concerns that have arisen in recent years for restoration managers is the importance of local adaptation in the species used for restoration, and the spatial scale over which local adaptation occurs (McKay 2005). Local adaptation refers to a set of genotypes found in a species range that confer greater fitness (survival and reproduction) to that species in a specific habitat. For example, a species may exist in a range that has an elevation gradient; individuals living near sea level are better suited to the conditions near the sea than species living in the foothills and vice versa. Other genotypes in a species that have lower fitness can be considered maladapted for that ecosystem. Local adaptation has been documented in a wide variety of organisms, but for most species, the spatial scale for local adaptation is unknown (McKay et al. 2005, Bischoff et al. 2006). I assessed four native grasses for evidence of local adaptation between two sites over an 85-km distance, in order to make inferences about the spatial scale of adaptation. To examine adaptation, I used a reciprocal transplant with weekly measurements of seedling growth and mortality. A reciprocal transplant is an experimental design using common gardens; these common gardens contain all species from both seed production sites. It is reciprocal because the garden is repeated at all field sites.

Species Descriptions

The four grasses used in this study were all once common to the Willamette Valley and now exist in greatly reduced numbers, primarily due to changes in the fire regime and invasion by other grasses from Eurasia. *Bromus sitchensis* (Trin). is a large perennial grass that grows between 120-180 cm tall. Historically it was common to moist forest edges, riparian corridors, oak woodlands and savanna. *Bromus sitchensis* is bright green and has large, open panicles and broad leaves with long drooping inflorescences (plants.usda.gov). The second species under study was *Danthonia californica* (Bolander), which can grow in a broad range of heights from 30-130 cm. This bunchgrass can produce cleistogamous seeds in the inflorescence stalk. *D. californica* prefers open areas such as prairies, meadows, fields, and savannas, it can be found throughout Oregon, but is more common to western Oregon (plants.usda.gov). Each inflorescence commonly has only 2-4 spikelets. *Elymus glaucus* (Buckley) is the largest of the species under study and can grow to 200 cm tall. Commonly lower leaves exhibit a purplish color, lending to the common name: blue wild rye. This species grows in meadows, thickets and open forests (plants.usda.gov). The final species, *Festuca roemeri* (Pavlick E.B. Alexeev), is a smaller bunchgrass with sage green leaves and pink to purplish inflorescence stalks (plants.usda.gov). None of these species are endangered on their own, but potential special local adaptation to conditions specific to Mt. Pisgah make this analysis important in maintaining the long term ecological balance in the restoration area. It is important to assess these four grass species specifically, because they are widely used for restoration at Mt. Pisgah and other restoration projects throughout the Pacific Northwest and if these species show strong local adaptation, then other species used might as well.

Restoration challenges

A major hurdle that restoration of native prairie needs to overcome is finding adequate sources of local seed for the project in a cost effective way (Miller et al. 2010, Knapp and Rice, 2011). On-site production is often not possible, or not possible for the quantities needed. On-site production of local seed for an individual restoration project can be cost inefficient if seed can be produced at a large scale for many restoration projects within a single seed transfer zone. Seed transfer zones are geographic areas within which individuals can be transferred with no detrimental effects on mean population fitness (Hufford and Mazer 2003). Without in-depth genetic studies of individual species, ecoregions can be approximated as an effective seed transfer zone (Miller et al. 2010). Miller at al. (2010) suggested that out of five native species they studied, four could use the Willamette Valley ecoregion as an appropriate seed transfer zone. However, none of the species they looked at was a grass. Furthermore, their study only used common gardens, which are not as effective as reciprocal transplants in determining local adaptation (Kawecki and Ebert 2004), and local experts disagree with some of their interpretation of the data (Stewardship and Technical Advisory Committee (STAC) for Friends of Mt. Pisgah, personal communication). My work focuses specifically on local adaptation, and the consequences of being farmed off-site for four species from the grass family (Poaceae).

To facilitate seed increase for restoration, wild collected seeds are being farmed (grown in a single species culture) at two sites: the native plant nursery on site at Mt. Pisgah, and off site farms in the Albany area. Plant populations can experience strong inadvertent directional selection during cultivation (Lesica and Allendorf 1999). My experiment attempted to answer two main questions; are the grasses locally adapted to conditions at Mt. Pisgah? And, are the selection pressures (both natural and unintended management selection) experienced by plants grown in Albany enough to cause a genetic shift that would make the population maladapted to the specific conditions at Mt. Pisgah?

These questions are important to site managers at Mt. Pisgah because researchers previously in this local context have not addressed them for this site and these species. To address these questions I used a reciprocal transplant experiment in which seeds produced at Mt. Pisgah and in Albany were planted both back to the site where they were produced, and to the other site. I measured seedling growth and survival. In addition I took advantage of adult plants currently producing seeds at these sites. These plants were measured for damage by pathogens and herbivores and reproduction.

METHODS

Field Sites

The experimental field sites were selected by the site managers at each location, for their availability of use during the spring and summer. The Mt. Pisgah (Buford Park Recreation Area) site was on the eastern side of the park on an east-facing slope in an open oak savannah (latitude 43° 59’ 42” N, longitude 122° 56’ 44” W, elevation 640 ft.). This site is located in on top of Philomath soil, a soil series found on convex hills in the Willamette Valley. This soil is well drained and the soil texture is silty clay, meaning that it is primarily composed of small soil particles. Philomath soils are usually moist, but can dry out and crack in the heat of summer (www.nrcs.usda.gov).

The Albany site is part of the farm belonging to the Kenagy family. It was an upper corner of a field that had been producing *Festuca roemeri*, but which had not been planted this year and was left fallow for the season (latitude 44° 39’ 39” N, longitude 123° 5’ 39” W, elevation 180 ft.). This site was located on Chapman soil. Chapman soils are very deep and well drained, common on low stream terraces and flood plains in the Willamette Valley. Slopes are 0 to 3 percent. Chapman soil texture is loam, a mixture of sand, clay and silt that is regarded as class I agricultural soil (www.nrcs.usda.gov). The clay portion of Chapman loam is made of smectite minerals, which contribute to cracking of the soil in summer.

Sources of seeds and plants

For the reciprocal transplant experiment, seeds of all the species were initially wild collected from Buford Park. These wild collected seeds were then either outplanted to a commercial grass seed farm in Albany (Kenagy Family Farm) or the Native Plant Nursery at Mt. Pisgah. All seeds used in this experiment were harvested from adult plants in 2012.

The adults plants that were measured for fitness and pest damage were planted out at different times, depending on the species. *Bromus sitchensis* adults were all planted in 2008. Seeds from *Danthonia californica* came from adults planted in 2004 (Pisgah) and 2008 (Albany). *Elymus glaucus* adults were planted at Pisgah in 2008 and Albany in 2011, while *Festuca roemeri* adults were planted in 2004 (Pisgah) and 2010 (Albany).

Site Preparation

The two common gardens were planted on March 16, 2013 in 10m by 10m plots using a randomized blocked design. Each block contained all the different treatments (species and seed source) in the experiment. The blocks are thus also replicates. Each field site had six blocks holding 40 individuals, five from all eight populations. Each block contained ten seeds from each species, five from Pisgah and five from Albany. The blocks were planted as a five by eight seed grid with the seeds separated by 20 cm from the other seeds to reduce competition between individuals under study.

Prior to planting, each seed was attached to a colored toothpick that indicated seed source and species, with nontoxic Elmer’s glue. Prior experiments with grasses in the Roy lab (Roy et al. 2011) had established that glue was indeed non-toxic, and the germination adjacent to toothpicks made finding grass seedlings possible at later dates in the field. The toothpicks were then inserted into the soil following the randomized order within each block. Toothpicks were inserted to a depth that allowed the grass seeds to experience the light necessary for germination, where the top of the seed was just below the surface of the soil. Seeds were planted on March 16, given three weeks to germinate, with weekly measurements commencing on April 7. Because of extremely low germination rates, *Danthonia californica* was dropped from the seedling experiment and only included in the adult portions. Therefore, all seedling experiments reflect data collected from *Bromus sitchensis*, *Elymus glaucus* and *Festuca roemeri*.

Measurements

To examine selection on these plants, I measured characteristics associated with growth, survival and reproduction as well as the presence of natural enemies (insects and pathogens). Each week, starting April 7th and ending with August 1st, seedlings were measured for height in centimeters using a ruler to assess growth across time. Height means the height from the base of the seedling, where it meets the soil, to the tip of the longest leaf, stretched to its fullest extent. Seedlings living at Pisgah were planted into a site where they experienced competition from other plants that were present before planting (mostly non-native grasses), whereas at the farm in Albany, there were bare soils and no competition when the plants were small. These differences in planting environment complicated the measurement of survival. Survival is tricky with plants. They can appear to be dead one week and be alive the next. It is hard to know if they are dead or not as long as they maintain some green in the leaves. Further, it was difficult to fully assess when a seedling had died at Pisgah because of other vegetation growing immediately adjacent to the grass seedlings. While conducting the measurement, when I was unable to locate individual seedlings for multiple weeks at the Pisgah location, they were considered “dead” from the week they first went missing. At Albany, seedlings experienced virtually no competition, so it was more obvious when a seedling had died. Because of the occasional difficulty in locating a seedling, missing size data for *F. roemeri* individuals (the smallest species) were inferred by averaging the heights between the weeks when a measurement was available in order to have enough values to perform the statistical analysis.

The adult plants were growing in plots containing many plants at each site. To obtain 20 plants for measurement, I randomly chose distances in meters along the length of the plots of each species. For example At Pisgah, plants were grown in 9 m by 5 m rectangles. To measure 20 plants, the plant closest to each meter marker was measured, along with the plant closest to the 2.5 m marker along the short edges of the plot. At the larger Albany fields, distances were randomly selected along a 30 m transect.

Once the plants were selected, size was measured in centimeters using length and width of the plant base, and height of vegetation. These three measurements were multiplied to yield cubic size, a measurement more representative of the total plant size. Height of reproductive stalks was also measured on the adult plants. The total number of flowering stalks was counted for each adult plant, along with the number of florets on a single flowering stalk. These values were multiplied together to calculate the estimated number of seeds per plant. Damage categories, such as rasping, chew, red spots etc., were assessed as either present or absent on each adult plant. Interaction with pathogens and other natural enemies has been suggestion to contribute to patterns of local adaptation (Cremieux 2008, Kawecki and Ebert 2004). Overall percent damage was assessed visually for each plant.

Statistical Analysis

Time to mortality for the seedlings was analyzed using a fit parametric survival analysis, assuming a Weibull distribution. When plants were still alive at the end of the experiment, they were indicated in the data as being “censored”, so that surviving individuals would not bias the mean death dates. The statistical model included seed source, growing location and the interaction between source and location. Average survival times were calculated based on the week death was recorded.

The weekly growth rate of the seedlings from April 7th to August 1st was tested using repeated-measures MANOVA (multivariate analysis of variance). Repeated measures was used because these data are internally correlated (the size the week before is linked to the size the following week), thus these are not independent data.

Adults were assessed for resistance to damage by pests and for reproduction in the different environments. An ANOVA (Analysis of Variance) was used to test how location and plant size affected reproductive output (estimated seed number). These explanatory variables were also used as predictors of percent damage on adults. Estimated seed number was calculated by multiplying the number of flowering stalks on one plant by the number of florets on one stalk. Seed numbers and plant size were log transformed so the residuals would fit the assumptions of ANOVA. Estimated percent damage was transformed using the arcsine transformation, as this transformation is useful for percentage data (Zar 1996). JMP ProV 9.0.2 (SAS 2010) was used for all data analysis.

RESULTS

Seedling Mortality

To determine whether local adaptation was a significant factor affecting survivorship, the influence of growing location and seed source on mortality were assessed, as well as the interactions between these variables. Local adaptation would be suggested if seeds from a locality had the best survival, growth or reproduction at this locality.

Where plants were grown mattered to survival. An overall model in which all the species were included indicated significantly higher survivorship at Mt. Pisgah (Table 1). To determine whether all the species responded the same, individual species were then analyzed separately. Seeds of the three species *Bromus sitchensis,* and *Elymus glaucus* survived best at Mt. Pisgah (Fig. 1, Table 1). This result occurred regardless of the original source of the seed material. For *Bromus sitchensis* at Mt. Pisgah, 26 survived out of a total of 45 germinating for a 57.8% survivorship, versus Albany, which had 19 survivors out of 48 germinated seeds for 39.6% survival by the end of the study (chi square “growing location”; p = <0.0001. DF 1,3 Table 1). *Elymus glaucus* seedlings living at the Mt. Pisgah site had 17 survivors from 33 germinated seeds for 51.5% survival, whereas at Albany had 12 survivors from 38 germinated for 31.6% survival, (chi square “growing location”; p = <0.0001. DF 1,3 Table 1).

Differences in mortality between the two growing locations for *Festuca roemeri* were not significant but the trends were similar to the other species. *Festuca roemeri* seedlings living at Mt. Pisgah had nine survivors out of 26 seedlings for 34.6% survival at Pisgah versus Albany and only five survived out of 23 for a 21.7% survival rate at Albany. The growing location differences alone were not significant, likely due to a low germination rate to begin with.

For two species there were no significant seed source effects. Mortality results from seed source for *Bromus sitchensis* were significant (p = 0.0517 DF 1,3. Table 1).

Suggesting that seeds produced at Mt. Pisgah may be stronger competitors, and survive better. This may have been possible to determine more definitively had the sample size been larger. For *Festuca roemeri*, there was a significant interaction between seed source and growing location (chi square “growing location * seed source”; p = 0.0231 DF 1,3. Table 1). This indicates that local adaptation may be occurring in *F. roemeri*.

Table 1. Mortality Effect Liklihood Tests

Source	**Nparm**	**DF**	**L-R Chi-square**	**Prob>ChiSq**
Bromus sitchensis				
Seed Source	1	1,3	3.79	0.0517
Growing Location	1	1,3	69.7	**<.0001***
Seed Source*Growing Location	1	1,3	0.815	0.3666
Elymus glaucus				
Seed Source	1	1,3	2.09	0.1480
Growing Location	1	1,3	25.8	**<.0001***
Seed Source*Growing Location	1	1,3	2.62	0.1053
Festuca roemeri				
Seed Source	1	1,3	1.88	0.1701
Growing Location	1	1,3	0.133	0.7154
Seed Source*Growing Location	1	1,3	5.16	**0.0231***
Overall Species Effect Tests				
Seed Source	1	1,3	0.263	0.6077
Growing Location	1	1,3	60.6	**<0.0001***
Seed Source*Growing Location	1	1,3	3.06	0.0803

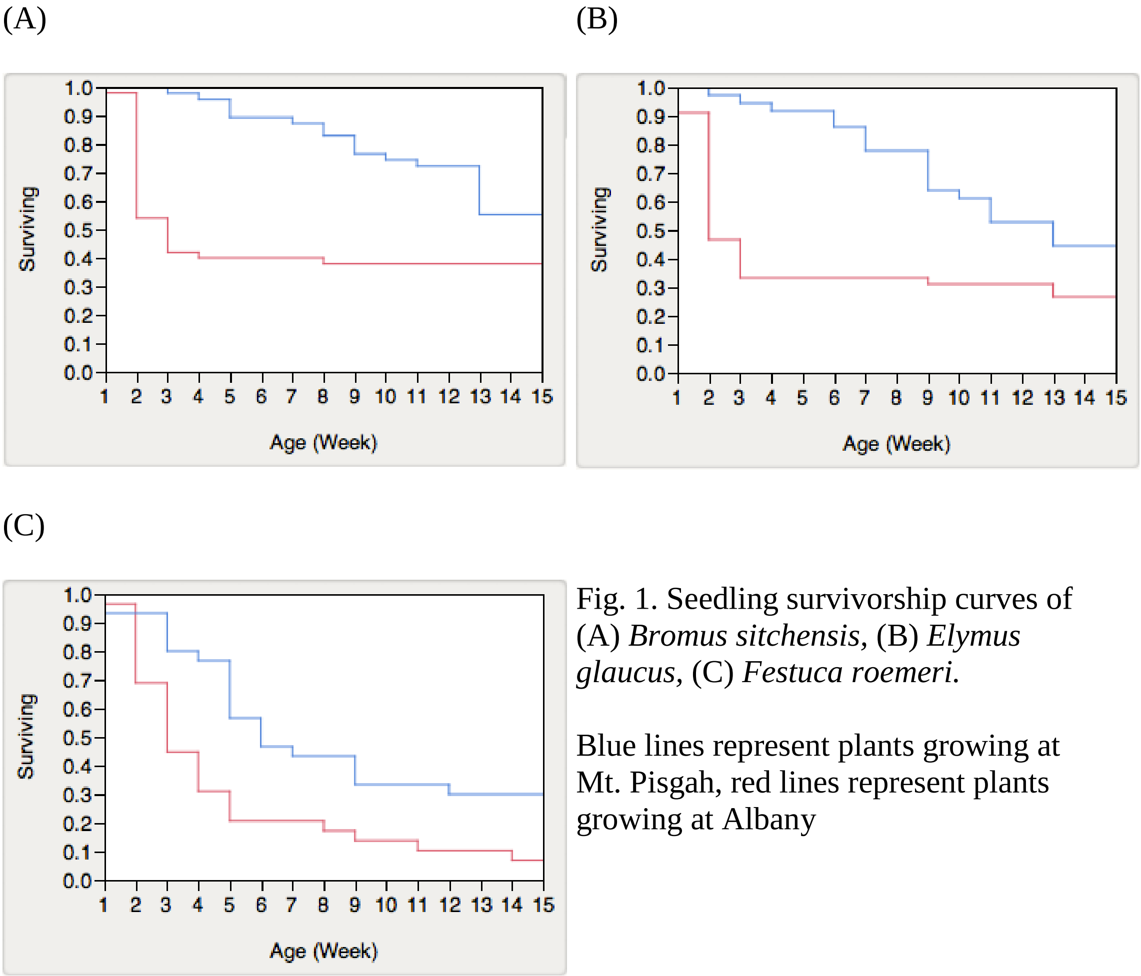

Fig. 1. Seedling survivorship curves of (A) *Bromus sitchensis,* (B) *Elymus glaucus,* (C) *Festuca roemeri.*

Blue lines represent plants growing at Mt. Pisgah, red lines represent plants growing at Albany

Seedling Growth

The locality at which plants were grown tended to affect growth. While the plants survived better at Mt. Pisgah, they tended to grow faster at Albany. When using repeated-measures MANOVA for all fifteen dates of measurement these differences in growth rates were significant only for *E. glaucus* ($F = 61.03_{1,6}$ $p = 0.0002$) and were nearly significant for *B. sitchensis* ($F = 3.85_{1,14}$ $p = 0.0699$). Differences in growth were not significant for *F. roemeri*. For this species towards the final weeks of the study, average heights decreased due to the death of larger individuals at the Albany site.

Seed source did not significantly affect growth rates for any of the species (Table 2). There were also no growing location by seed source interactions for growth rate (Table 2).

Table 2. Growth effect summary for each species individually

Species	Growing Location	Seed Source	Growing location * seed source
Bromus sitchensis	0.0699	0.1547	0.2379
Elymus glaucus	**0.0002**	0.8607	0.4591
Festuca roemeri	0.2930	N/A*	N/A*

*high death rate the Albany site made it impossible to compare seed source over the duration of the experiment for *Festuca roemeri* because not enough data was available (sample size: five individuals at Albany and nine at Pisgah)

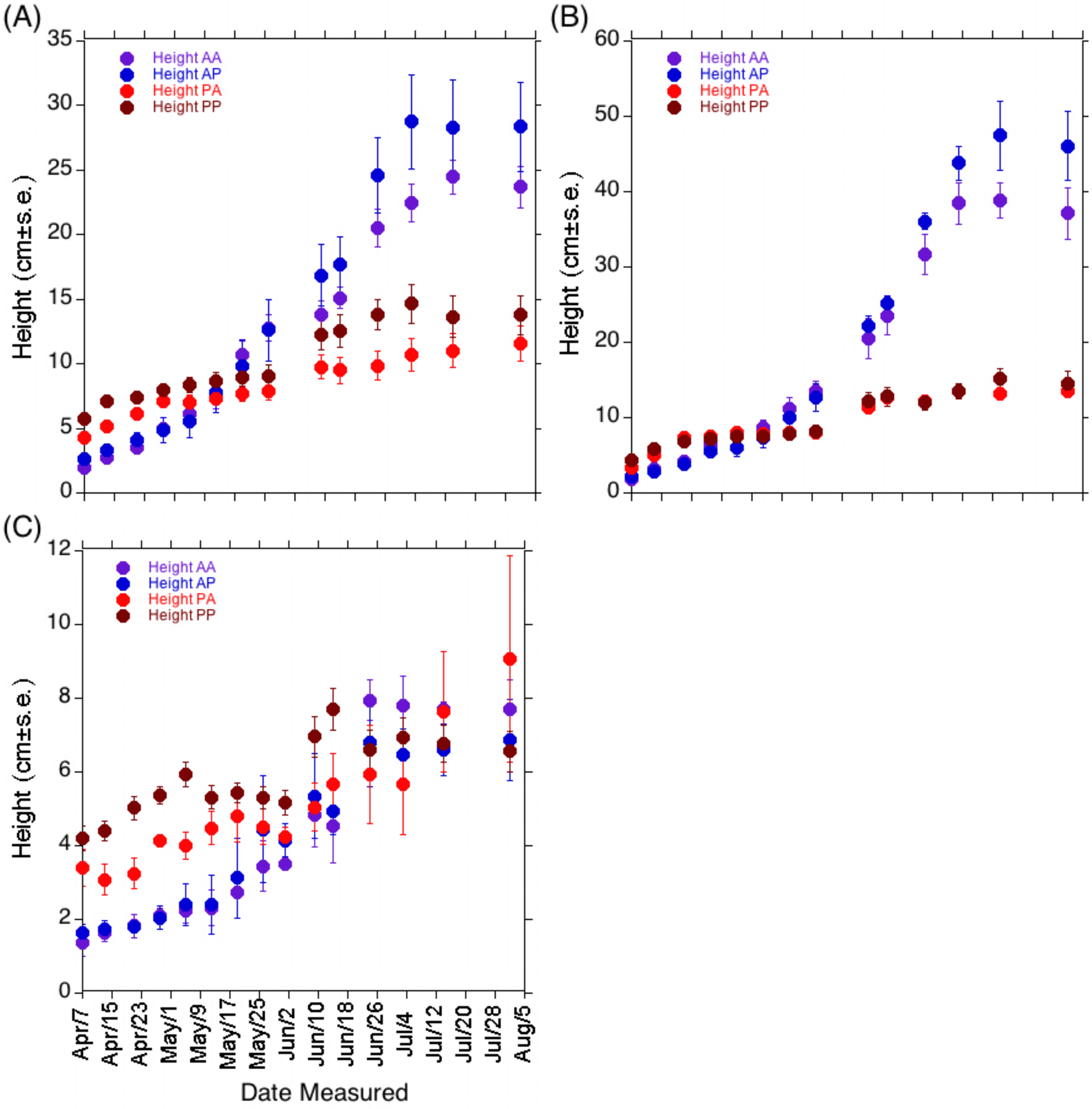

Figure 2. Seedling growth over time for (A) *Bromus sitchensis*, (B) *Elymus glaucus*, (C) *Festuca roemeri*

The first letter represents growing location. The second letter represents seed source. For example, AP is Pisgah seed growing at Albany.

Adult Damage

Adult plants living at both experimental locations, Mt. Pisgah and Albany, experienced relatively similar rates of attack by various pests (herbivores and pathogens) despite the difference in location. Growing location was only a significant explanatory variable for adults in one species, *Festuca roemeri.* Individuals of this species living in Albany experienced a greater degree of damage than individuals living at Mt. Pisgah. The average damage on plants living in Albany for *F. roemeri* was 12.1 ± 2.5% and the average for Pisgah was 3.57 ± 0.74%. Size was also assessed to determine whether larger plants experienced higher rates of attack but this was not a significant explanatory factor for any species. For *Bromus sitchensis,* there was some significant interaction between size and growing location, indicating that size depended on location (Table 3).

Table 3. Adult effect likelihood tests percent damage

Species	**Size**	**Growing Location**	**Size * Location**
Bromus sitchensis	0.6436	0.5844	**0.0254***
Danthonia californica	0.5236	0.1882	0.6987
Elymus glaucus	0.0822	0.9614	0.9579
Festuca roemeri	0.8453	**0.0061***	0.2093

Adults were also assessed to determine whether the amount of pest damage influenced the amount of reproduction, as estimated by the number of seeds. This was significant only for *Elymus glaucus*. Contrary to expectations, for this species, more damage resulted in a greater number of seeds (p = 0.0011). For all other species damage did not appear to affect the number of seeds produced by each plant.

Individual damage types were assessed on all adult plants measured at each site. These types were identified in the data set as either "present" or "absent" on the plant. The types of damage used to assess the adults were the presence of insects or insect traces such as aphids, insect eggs, frass and caterpillars. Herbivory patterns were also monitored, including: chew by insects, linear and non-linear rasping of leaf tissue. Finally discolorations were also monitored, this category included rust, white spots, tan streaks, tan spots, brown spots either <2mm or >2mm, red spots of these size classes, black spots and leaf twist. When comparing numbers of individuals affected with each type of damage in a chi-square test at both sites, none of the individual types of damage were found to be significantly different.

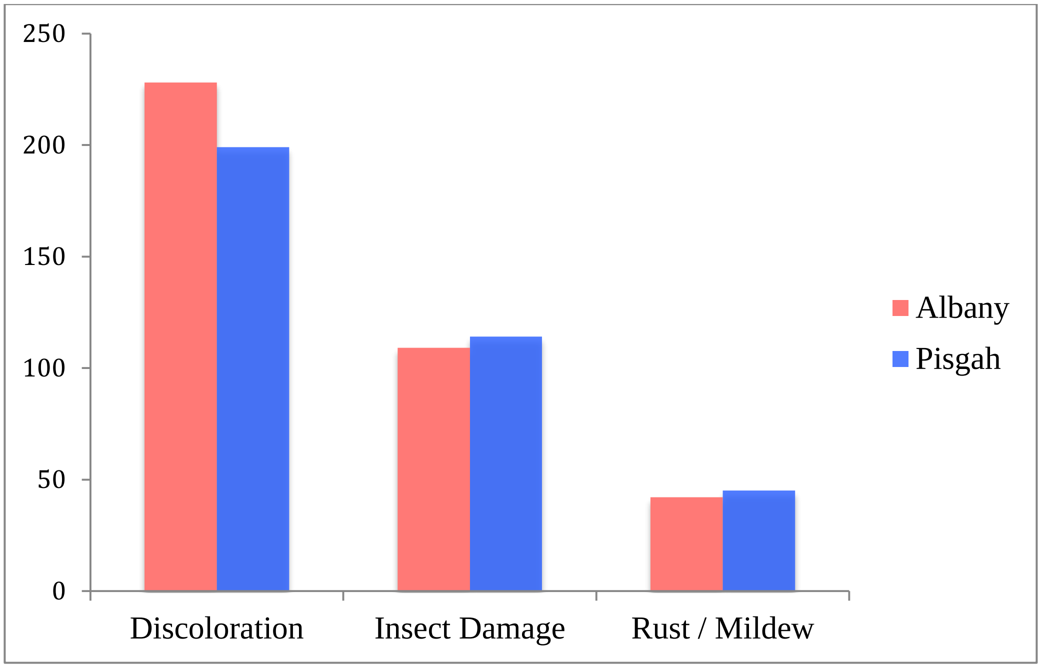

Figure 3. Total number of damages "present" on adult individuals in each feeding guild

These categories were combined for a second analysis to see if feeding guild was significant. The guilds were insects (aphids, eggs, frass, caterpillars, chew, rasping), rusts and mildews (rust, white spots) and fungal spots (tan streaks, tan spots, brown spots, red spots, black spots). Damage by none of the guilds proved to be significantly influenced by location. The extent of damage is influenced by location but the actual pests present are similar between sites.

DISCUSSION

The Buford Park Recreation Area is managed by the Stewardship and Technical Advisory Committee (STAC) on matters concerning the restoration of the historical prairie ecosystem. These managers wanted to know whether the seeds they use in restoration were appropriate, since some were being produced on site and others were being produced on a farm 85 km away. Were the plants producing seeds responding to differential selection pressures present in a commercial grass seed farm, as opposed to a local environment at Mt. Pisgah? In addition to comparing how the seedlings performed, managers also wanted to know whether location was affecting the reproduction of adult grasses, and their ability to resist natural enemies. From a reciprocal transplant experiment, I found that location was the most important factor affecting the survivorship and growth of seedlings. Furthermore, there was no detectable effect of seed source. This meant that seeds from both production areas appeared equally suited to all habitats they were outplanted into when compared against each other. Finally, the interactions *between* growing location and seed source that would suggest local adaptation were minimal, only occurring in one species, *Festuca* *roemeri,* for seedling growth; if the seeds produced had been locally adapted, then a strong homesite advantage would have been present. This means that growth and survivorship would have been highest for plants from a location that were planted back to that same location.

Contrary to expectations, the location affects on mortality and size resulted in an opposite pattern of seedling success. The highest rate of mortality occurred at Albany. However, the seedlings that were able to survive at Albany grew much larger than those growing at Pisgah. *Bromus sitchensis* and *Elymus glaucus* individuals living at Albany were able to grow over double the size of their Mt. Pisgah counterparts. I even observed seed production in four *Elymus glaucus* individuals growing at Albany during the final weeks of measurement. These four individuals were the only seedlings planted that were able to reproduce by the end of the season.

One potential explanation for these results is the differences in the soil type existing at the two locations. The Albany location was a farm field of class I agricultural soil (www.nrcs.usda.gov). The class I designation denotes maximum fertility and the highest possible crop yields (www.nrcs.usda.gov). Given that at Albany, a small number of seedlings planted in March were able to reproduce by August, it is interesting that survivorship was so low. This suggests strong selection forces at Albany that could potentially lead to local adaptation in the future if adult plants were given more generations of outplanting. High mortality increases the potential loss of genetic diversity. This is consistent with a previous study of genetic change during commercial cultivation of a grass. When another perennial grass was studied for genetic changes during seed increase, an 8% loss of genetic diversity was found after just two generations (Ferdinandez et al. 2004).

Additional differences in environmental factors at the two sites, besides soil type, exerted selection pressure on the young grasses. Seedlings died rapidly at Albany, where there was no shade, during an early period of unseasonal heat. Large cracks in the soil appeared within the first few weeks of measurement and could have caused extensive damage to young root structures of the seedlings at Albany. Most of the Albany deaths occurred during the third week of measurement (fig. 1), coinciding with the early hot dry period in the spring, potentially suggesting that bare agricultural lands select for seedlings that can withstand the extreme heat events of that year because sun protection is greatly reduced in open fields.

Differential survivorship between the two locations is an important evolutionary chance for the seedlings. While evidence of local adaptation is likely to become more obvious in years of climactic extremes (Lesica and Allendorf 1999), the mortality effect in particular is important because high death rates at Albany can create a genetic bottleneck for seedlings planted for restoration as a result of these extremes. The differences in seedling success as the result of their growing location suggest the potential for adaptation over a longer period of outplanting for the adults than was tested. This experiment underscores the importance of performing reciprocal transplants when large areas are under restoration because growing location can exert a strong influence on the performance of plant material under cultivation. We agree with Lesica and Allendorf (1999) and Falk et al. (2001) that care must be taken in selecting sources of seed for restoration, and that the underlying genetics of plant materials affects long term project success. Furthermore this experiment contributes to the call by other researchers for more studies in local adaptation on restoration projects (McKay et al. 2005, Kawecki and Ebert 2004).

The results of the experiment might have been more conclusive regarding seed source, and the location and seed source interaction, had the period under study been extended. For most studies of local adaptation, measurements are taken over one season or one year. It is better to measure for an extended period because rare selection events like storms and drought are the primary drivers of local adaptation. Reciprocal transplant studies have been touted as a way for restoration managers to determine the best sources of local seed for restoration projects (Kawecki and Ebert 2004, Bischoff et al. 2006).

One of the unplanned strengths of this experiment was the period of hot dry weather in April that I considered a specific kind of rare selection event. This heat wave can be approximated as a drought for the seedlings over the single season studied. Distance between locations is not always the best proxy for estimating a seed transfer zone, which is why a relatively small distance of 85 km may still have effects on local adaptation. Other research suggests that differences in the abiotic and biotic factors, such as elevation, soil, climate, pathogens and natural enemies are the best for estimating local adaptation (Hufford and Mazer 2003). Increasing the size of the study to have more individuals for the seedling experiment would have generated a larger data set for statistical analysis. However, given the limitations of labor and the necessity of doing measurements at both sites in a single day, the experiment was as big as was feasible.

The adult damage survey revealed that location did not have any measurable effect on reproduction. This measurement was somewhat influenced by the techniques I used to determine seed output. To assess reproduction, I estimated the number of seeds produced. This was done by counting the total number of flowering stalks (culms) on each plant and then assuming that multiplying culms by the number of florets found on a randomly selected stalk would give a reasonable estimate of seed number. We also found that the natural enemies at both locations showed that the general communities of crop pests living at the two locations were similar. However, visual estimates of damage to *Festuca roemeri* adults living at Albany were significantly higher and the damage more extensive on the individual plants than damage estimates of individuals at Mt. Pisgah.

I observed a nearly ubiquitous rust fungus infection on adults of this species living in the Albany farm field. Overall, rust fungus was the single greatest contributor to the higher damage values at Albany. Other studies of perennial grasses using reciprocal transplants have found local adaptation in regards to rust resistance (Cremieux et al. 2008). However the distance between the areas used was much larger. Understanding the influences of natural enemies on local adaptation was important because patterns of pest resistance can be highly differentiated between populations (Cremieux et al. 2008). However, our experiment did not show location dependent interactions with pests, suggesting that the spatial scale is within a range of similar adaptation to natural enemies.

Another potential factor affecting the results of the adult damage experiment was our assessment of types of adult damage. Our methods were somewhat limited because I used the nominal categories of “present” or “absent” for different types of damage. It takes more yes/no data (=nonparametric) to be statistically significant than continuous percent damage data, such as was used for the overall damage assessments on the adults (Zar 1996). Had percent damage measurements been made for each type of damage, more statistical power would have been available to assess site differences of damage type and guilds.

Other studies of local adaptation in perennial grass species have found various patterns of local adaptation. Cremieux et al. 2008 conducted an experiment over multiple locations in Europe, that also used a reciprocal transplant, found opposite patterns of resistance to natural enemies in the two species studied. This suggests that it is difficult to predict species interactions between host plants and their natural enemies, and the effects of those interactions on local adaptation, without doing experiments with the species under consideration (Cremieux et al. 2008). Results like those in Cremieux underlie the importance of assessing patterns of adult damage between Mt. Pisgah and Albany. Local adaptation is a complex process, and comprehensive studies of natural populations using reciprocal transplants are the best way to tease apart the boundaries of a seed transfer zone (Kawecki and Ebert 2004).

Local adaptation in populations should not be assumed without experiments to test for it, because the ability of phenotypic plasticity (changes in morphology in response to the environment) is often sufficient to respond to the various habitats in an environmental gradient (McKay et al. 2005). Phenotypic plasticity is a potential explanation for why we found no homesite advantage from our experiment. Interspecific competition was not directly assessed in this experiment, but in a previous study using many of the genera we used, local adaptation was found to occur over a small spatial scale (170 km) when interspecific competition was accounted for (Rice and Knapp 2008). This suggests that as restored areas become revegetated, local genotypes may become more important to survival and reproduction.

Finally, much of this experiment was motivated to further investigate the results of the experiment done by Miller et al. (2010), where they argue that the entire Willamette Valley ecoregion is an appropriate seed transfer zone. While the common garden of Miller et al. (2010) was not as good at examining local adaptation as reciprocal transplants are, the experiment I conducted lent credence to their conclusion that the Willamette Valley can be considered an appropriate seed transfer zone. Nonetheless, while there was no strong homesite advantage apparent in the overall performance of seedlings in terms of growth and survivorship, the strong location effects suggest the potential of future adaptation given more years of selection in the two locations.

PRACTICAL APPLICATIONS

Because evidence of local adaptation was minimal, economic considerations can be prioritized by the STAC to produce the maximum quantity of seed needed to restore the prairie. With this in mind, site managers at Mt. Pisgah should feel comfortable sourcing seed from farms in Albany if plant materials used for seed increase are refreshed every few years with wild collected seed from the restoration site itself. Refreshing the populations of the seed-producing outplanted adults will insulate against the potential long-term effects of site differences. Because all of the seed was coming from adults that were less then ten years old, local adaptation is not expected to occur over the course of a decade for these species unless a strong selection force arises at either Albany or Mt. Pisgah. One selection force to be considered in the future is climate change. This has a huge potential to alter ecosystems. Therefore, the highest priority for restoration managers must be to preserve genetic diversity and therefore the capacity of a population to adapt to change. If the STAC continues to source seed from Albany, the main consideration should be a wide sampling protocol to gather a diverse population of seed for outplanting at a commercial grass seed farm, because the risk of local adaptation is relatively low. In a prairie ecosystem, grasses are important members of the plant community. Much of the project success hinges on the ability of site mangers to reseed degraded areas with genetically appropriate native species. Studies like this one are relatively simple to implement given sufficient volunteer labor and can be a useful tool for any large restoration project looking to find commercial sources of native seed.

LITERATURE CITED

Bischoff, A., L. Cremieux, M. Smilauerova, C. S. Lawson, S. R. Mortimer, J. Dolezal, V. Lanta, A. R. Edwards, A. J. Brook, M. Macel, J. Leps, T. Steinger, and H. Muller-Scharer. 2006. Detecting local adaptation in widespread grassland species - the importance of scale and local plant community. Journal of Ecology 94:1130-1142.

Boyd, R. Strategies of Indian burning in the Willamette Valley. *in* R. Boyd, editor. Indians, fire and the land in the Pacific Northwest. Oregon State University Press, Corvallis, Oregon.

Clark, D. L. and M. V. Wilson. 2001. Fire, mowing, and hand-removal of woody species in restoring a native wetland prairie in the Willamette Valley of Oregon. Wetlands 21:135-144.

Cremieux, L., A. Bischoff, M. Smilauerova, C. S. Lawson, S. R. Mortimer, J. Dolezal, V. Lanta, A. R. Edwards, A. J. Brook, T. Tscheulin, M. Macel, J. Leps, H. Muller-Scharer, and T. Steinger. 2008. Potential contribution of natural enemies to patterns of local adaptation in plants. New Phytologist 180:524-533.

Falk, Donald A., Eric E. Knapp, Edgar O. Guerrant. An introduction to restoration genetics. Report prepared by the Society for Ecological Restoration for the Plant Conservation Alliance, Bureau of Land Management, US Department of the Interior and the US Environmental Protection Agency. November 2001.

Ferdinandez, Yasas S. N., Bruce E. Coulman, Yong-Bi Fu. Detecting changes over two generations of seed increase in an Awned Slender Wheatgrass population using AFLP markers. Crop Science. Vol. 45 (2005): 1064-1068.

Hamrick, J. L., M. J. W. Godt. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions: Biological Sciences. Vol. 351 No. 1345. (1996): 1291-1298

Hedrick, Phillip W. Conservation genetics: where are we now?. Trends in Ecology and Evolution. Vol. 16 No. 11 (2001): 629-636

Hufford, Kristina M. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution. Vol. 18 No. 3 (2003): 147-155.

Kawecki, Tadeusz J. Dieter Ebert. Conceptual issues in local adaptation. Ecology Letters. Vol. 7 (2004): 1225-1241

Knapp, Eric E. Kevin J. Rice. Effects of competition and temporal variation on the evolutionary potential of two native bunchgrass species. Restoration Ecology. Vol. 19 No. 3 (2011): 407-417

Legendre, P., M. R. T. Dale, M. J. Fortin, P. Casgrain, and J. Gurevitch. 2004. Effects of spatial structures on the results of field experiments. Ecology 85:3202-3214

Lesica, Peter., Fred W. Allendorf. Ecological genetics and the restoration of plant communities: mix or match?. Restoration Ecology. Vol. 7 No. 1 (1999): 42-50.

Little, T. M. and F. J. Hills. Agricultural experimentation: design and analysis. John Wiley and Sons, New York. 1978.

McKay, John K., et al. How local is local – a review of practical and conceptual issues in the genetics of restoration. Restoration Ecology. Vol. 13 No. 3 (2005): 432-440. Print.

Miller, Stephanie A. et al. Can an ecoregion serve as a seed transfer zone? Evidence from a common garden study with five native species. Restoration Ecology. Vol.

Myerson, Laura A., Harold A. Mooney. Invasive alien species in an era of globalization. Frontiers in Ecology and the Environment. Vol. 5 No. 4. (2007): 199-208.

Oregon Department of Fish and Wildlife. Willamette Valley ecoregion. Oregon Conservation Strategy (2006): 234-253.

PLANTS Database. United States Department of Agriculture. November 2013. plants.usda.gov

Rice, Kevin J., Nancy C. Emery. Managing microevolution: restoration in the face of global change. Frontiers in Ecology and the Environment. Vol. 1 No. 9. (2003): 469-478

Rice, Kevin J., Eric E. Knapp. Effects of competition and life history stage on the expression of local adaptation in two native bunchgrasses. Restoration Ecology. Vol. 16 No. 1. (2007): 12-23.

Roach, D. A. and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology and Systematics 18:209-235.

Roy, B. A., T. Coulson, W. Blaser, T. Policha, J. Stewart, K. Blaisdell, and S. Güsewell. Population regulation by enemies of the grass *Brachypodium sylvaticum*: demography in native and invaded ranges Ecology 92 (2011): 665-675.

Scheiner, S. M. and J. Gurevitch, editors. 1993. Design and Analysis of Ecological Experiments. Chapman & Hall, New York.

Soule, Michael E. What is conservation biology? Bioscience, Vol. 35 No. 11, The Biological Diversity Crisis. (1985): 727-734

Stelkens, Rike B., Manuel Pompini, Claus Wedekind. Testing for local adaptation in brown trout using reciprocal transplants. BMC Evolutionary Biology. (2012) 12:247.

Turesson, G. 1922. The plant species in relation to habitat and climate. Hereditas 6:99-152.

Vergeer, Philippine, Esther Sonderen, N. Joop Outborg. Introduction strategies put to the test: local adaptation versus heterosis. Conservation Biology. Vol. 18 No. 3 (2004): 812-821.

Wilson, Barbara L. Richard Bainerd, Nick Otting. Poaceae. Oregon Flora Project. (2013)

Wulff, R. D. 1995. Environmental maternal effects on seed quality and germination.*in* J. Kigel and G. Galili, editors. Seed development and germination. Marcel Dekker, Inc., New York.

Zar, J.H. 1996. Biostatistical Analysis. 3rd Edition. Prentice Hall, Upper Saddle River, New Jersey.