Adaptive Genetic Variation of Broadleaf Lupine (*Lupinus latifolius*) and Implications for Seed Transfer

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Abstract

Broadleaf lupine, (*Lupinus latifolius*) is a common native legume with potential utility for erosion control. The purpose of this study is to gain insight into species germination and growth and to establish seed transfer guidelines by estimating patterns of genetic variation and relating these patterns to environmental gradients. Seed samples from 83 populations of lupine were collected on the Mt. Hood National Forest, sown in the greenhouse, and outplanted into two replicate garden plots at two locations. Physical and phenological data were recorded for each seedling. Overall germination was 74% with 95% survival of outplanted individuals. Seedlings established extensive root systems and average plant height at 4.5 months was 27 cm. We found genetic variation for almost all traits examined and much of this variation is related to seed source, however patterns are complex and neither clinal nor classification models are completely satisfactory in explaining the variability among sources.

Keywords

seed source, provenance, planting zone, seed zone, native legume, erosion control

Introduction

For decades, national forests in Oregon and Washington have used 10 to 12 native conifer species for reforestation projects, but until recently haven't focused on the other 3,000 shrubs, grasses, sedges, and rushes native to the Pacific Northwest. Traditionally we have used domesticated Eurasian grasses and legumes for erosion control and understory planting, largely due to availability, low cost, and ease of establishment. However, many land managers are concerned about the negative consequences of sowing these species in natural landscapes. Thus, interest in using a wide array of native species in site rehabilitation and revegetation projects has increased dramatically.

There is substantial information on conifer planting, so we know how to collect, store, propagate, and deploy native tree seed, but an comparable body of knowledge has not yet been built for native understory species. After the failure of some plantations in which native tree seedlings were planted far from their source environments, investigations began into levels of genetic variation within and among populations of Pacific Northwest conifers. Results showed that some species are broadly adapted (e.g. western white pine, *Pinus monticola*, Campbell and Sugano, 1989), while other species are relatively closely adapted to their seed source origin (e.g. Douglas-fir, *Pseudotsuga menziesii*, Campbell, 1986). From this research seed transfer guidelines emerged, which help us to 1) collect quality seed (while retaining adequate levels of genetic diversity); 2) produce seed efficiently; and 3) increase the likelihood of successful outplanting.

Formulating Seed Transfer Guidelines and Seed Zones

Seed transfer guidelines can be formulated in different ways. Conservative guidelines based on factors we think are important, such as elevation and geographic barriers to pollen and seed movement, have been used in the past. Recently these were modified based on studies with other species or studies in different areas (Forest tree seed zones 1996). While this is inexpensive and rapid, it can lead to collecting and storing more seed lots than are necessary to efficiently produce adapted seed. Patterns of allozyme variation have also been used to limit seed transfer (Westfall and Conkle 1992). These procedures rely on sampling a geographic area, determining amounts and patterns of allozyme variation in the sample, and relating the patterns to geographic variables such as latitude, longitude, and elevation. However, patterns of isozyme variation are difficult to translate into understandable patterns of adaptive variation (Linhart 1995).

Studies of adaptive genetic variation, while expensive and time consuming, offer the best information with which to design seed transfer guidelines, particularly for species which we intend to propagate and use large quantities of seed. Briefly, the procedure involves sampling a designated geographic area by collecting seeds from well-distributed mother plants growing at different source locations. All seed from one mother plant comprise a family. Several families are collected from a subset of the sources to allow estimates of within population variation. Seedling families are grown in environmentally uniform plots and data are recorded on plant size and growth rhythm, or phenological, traits, such as date of budburst, flowering, or senescence. Usually two contrasting environments, such as warm/cold, are used to reveal more variation among families. Since growth environments are uniform, observed differences among sources and families should be due to genetic variation. Regression and classification analyses are then used to relate genetic variation among sources to geographic and topographic variables, such as latitude, longitude, elevation, precipitation, aspect, etc. From these relationships, seed transfer risk is estimated and guidelines are formulated which minimize the risk of planting failure due to maladaptation. (Campbell and Sorensen 1978).

Studies of genetic variation in annual and perennial plants show that differentiation among populations from different seed source locations often occurs along gradients, similar to that found in conifer species (Linhart 1995). Therefore, methods used to formulate seed transfer guidelines in conifer species should be transferable to many of the shrubs, grasses, and forbs used in restoration planting projects.

Common Garden Study of Broadleaf Lupine

Broadleaf lupine (*Lupinus latifolius*) is common and widespread throughout the Cascade Range in Oregon and Washington. It is a good candidate for erosion control seed mixes: it grows well in tough sites, colonizes disturbed areas, has a deep root system for stabilizing soil, and forms associations with nitrogen fixing bacteria. In order to efficiently produce large quantities of seed, information on how many separate seed lots to maintain is needed. This study was undertaken to determine how closely adapted this species is to source environments and how far seed can be safely moved, or how many seed lots need to be maintained. This report focuses on the procedures involved in this study and what we have learned about adaptation in lupine to date.

Materials and Methods

Sampling

Seed pods from 152 mother plants growing at 83 different source locations throughout the Mt Hood National Forest were collected during the summers of 1995 and 1996. One family was collected at each of 21 locations (25.3%), two families at 57 locations (68.7%), and three or more families at each of 5 locations (6.0%). Pods were stored in kraft paper bags and allowed to dry and shatter inside the bags. Seed was then cleaned, sealed in plastic bags, labeled, and stored in a freezer until sowing in the spring of 1997.

Source locations were entered into a GIS system and the following geographic and topographic variables were determined for each source location: latitude and departure (in meters), elevation (feet), average annual precipitation (inches), and aspect (degrees). Aspect was transformed into two variables, EWaspect=sine(aspect) and NSaspect=cosine(aspect).

Greenhouse Culture and Field Outplanting

Seedlings were grown at the Natural Resource Conservation Service Plant Materials Center (PMC) greenhouse in Corvallis Oregon. Prior to sowing a scarification trial was conducted to determine optimum scarification time in a seed tumbler for small seed lots. Seed was scarified and sown into 2" peat pots in early February 1997. Pots were arranged in a replicated design in which family blocks were randomized in two replications. Germination of test seedlings was 74%. By May 1 seedlings had developed extensive root systems and were ready for outplanting.

Seedlings were outplanted to two field sites which had been prepared by tilling, covering the plot area with black plastic mulch fabric for weed control, and installing a drip irrigation system for early plant establishment. One site is located at the PMC and was planted on May 8 and 9. The other site is located at Wind River Nursery (WRN) in Carson Washington and was planted on May 16 and 17. Overall transplanting survival was 95%. Seedlings were planted at 18 in. by 36 in. spacing in a design where family row plots (4 plants from the same family in a row) were randomized in two replications at each location. Experimental plots were surrounded by a two row buffer to minimize edge effects. Plants will be grown and data collected until fall of 1999.

Traits

Generally traits could be grouped into 5 broad categories – greenhouse traits, plant size, morphology, growth rhythm, and pest resistance. Measurements were taken at each site and evaluated independently, for example first year crown height at PMC and first year crown height at WRN are two separate traits. Traits measured in the greenhouse were number of days to emergence (Julian date), and when the first true leaf attained 2.5 cm in diameter. Plant size traits include measurements of crown height (cm), flower stalk height (cm), and crown diameter (cm). From these measurements crown volume, height-diameter ratio, and growth rate were calculated. Plant morphology traits include leaflet width (mm), leaflet length (mm), petiole length (mm), and overall plant form (5 form classes, upright – prostrate). Growth rhythm traits were date of bud-burst (Julian date), date of flower opening (Julian date), senescence (5 classes), secondary crown growth (5 classes), bud-break stage (6 classes), and flower advancement (proportion of flower bud stalks in bloom). Pest resistance was reflected in powdery mildew abundance (4 classes) and grasshopper damage (4 classes). Other traits scored include over-winter survival and overall plant vigor (5 classes). Seed size will be measured on seed collected from test plants in 1999.

Analysis

Analysis generally follows methods outlined in Campbell (1986) and Sorensen and Weber (1994). Briefly, analysis consists of identifying those traits showing large amounts of source genetic variation using analysis of variance and variance component estimation methods. Traits highly correlated with other traits are deleted and factor analysis is used to derive a smaller number of linear combinations (called factors or principal components). Twenty-seven traits were included in the factor analysis for this study and 5 factors were retained. Factor scores of these components are calculated for each source and regressed against site variables (latitude, longitude, elevation, etc) or evaluated in hierarchical models using site classification variables (seed zone, elevation band, eco-class, etc.).

Results and Discussion

Sample Site Distribution

Source locations spanned approximately 38 minutes latitude (47 miles) and 38 minutes longitude (30 miles). Mean elevation was 3582 feet above sea level (range 932-5432) and mean annual precipitation was 24.6 cm per year (range 8.7-41.7). Nearly 27% of the locations were east facing slopes, of the remainder 12,8,10,5,8,14,and 16 percent were on north, northeast, northwest, south, southeast, southwest, and west aspects respectively. Sample distribution was slightly skewed, with more samples coming from the northeast and southwest portions of the Forest (correlation between latitude and longitude = .42, p<.0001). Elevation increased going east (correlation =.40, p<.0002), while precipitation decreased going east (correlation = -.46, p<.0001) and increased going north (correlation =.39, p<.0002).

Site Effects

Plants grew larger and flowered earlier at the Plant Materials Center (PMC) than at the Wind River (WRN) site. At the end of the first growing season, median plant height and diameter at PMC were 33 and 41 cm respectively. At WRN median plant height and diameter were both 22 cm. Average date of flower opening in 1997 at PMC was July 12 and at WRN, July 21. Source level correlations for size traits between the two locations averaged .50.

Traits and Factor Analysis

Over 60 traits have shown significant genetic variation in the study to date. Of these, 27 having the largest amounts of source variation have been selected for factor analysis.

Five factors were retained in the factor analysis which explained 74% of the source variation in the 27 traits. Factor 1 (22% of the source variation) reflected budburst, growth at PMC, flower color, early growth in the greenhouse, and survival at PMC. Sources with large scores for this factor tended to begin growth early in the season, attained large size at Corvallis, had good over-winter survival in Corvallis, and had predominantly white colored flowers. Factor 2 (18%) was heavily influenced by overall plant form at WRN, with some influence from height growth at both locations. Sources with high scores for this factor were generally upright plants, had relatively high height-diameter ratios, and were taller in both environments. Factor 3 (18%) reflected size and growth rate at WRN only, sources with large, rapid growing plants had high scores for this factor. Factor 4 (9%) demonstrated source variation in leaflet width and leaflet length. Factor 5 (7%) showed variation for mildew susceptibility, high scoring sources had greater mildew occurrence at both sites.

Regression and Classification Analyses

Multiple regression equations relating factor scores to geographic and topographic variables were complex, with latitude, longitude, and precipitation being the most important variables (Table 1.).

The equation for factor 1 explained 42% of the source-related variation.

Table 1. Linear correlation coefficients of factor scores with geographic variables and regression model R-square values. (Correlation is significant at p=.05 level if the absolute value of the correlation >.21)

Factor	Elev.	Lat.	Long.	Prec.	N-S Aspect	E-W Aspect	Model R-square
1	-0.06	0.35	0.24	0.05	0.08	0.17	0.42
2	-0.27	0.28	-0.08	0.50	0.08	-0.08	0.23
3	-0.23	0.12	-0.50	0.27	0.06	-0.04	0.61
4	0.27	0.31	0.21	0.17	-0.05	0.02	0.37
5	-0.05	-0.42	-0.31	-0.08	-0.07	-0.16	0.25

Latitude, longitude, and their interaction had the largest effects, with elevation and EWaspect interactions being less important. Scatter plots of factor scores show sources with high scores for this factor occur predominately in the northeast part of the forest and sources with low scores occur in the southern part of the area. Twenty-three percent of the source variation in factor 2 was explained by precipitation and it's quadratic term alone. Scatter plots indicate high scoring sources for this factor occur in the western part of the sampled area, while low scoring sources occur in the eastern part.

The equation for factor 3 had the greatest explanatory power of any, explaining 61% of the variation in this factor. It was also the most complex, retaining 11 variables, and scatter plots do not show any clear or obvious trends. Latitude, elevation, precipitation, and interactions of both aspect variables with precipitation and latitude were important variables, while longitude had weak effects. Thirty-seven percent of the source variation in factor 4 was explained by geographic and topographic variables. Latitude was the most important variable, with lesser amounts of variation explained by longitude, elevation, and NSaspect. The equation for factor 5 explained 25% of the variation. Latitude and EWaspect interactions with precipitation and elevation were important variables. Similar to the situation for factor 3, plots for factors 4 and 5 show no clear geographic grouping.

Seed zone was the only consistent significant effect ($p<.0001$) in classification analyses for all 5 factors. When analyses were conducted with seed zones grouped into a 'westside' zone and an 'eastside' zone, zones were significant for all but factor 3. Effects of sources-within-zones were always significant ($p<.01$). Eco-class, 500 foot elevation bands, and aspect effects were all consistently non-significant.

Based on these results, there seems to be as much, or more, source-related variation in lupine as in Pacific Northwest conifers which have been investigated. Furthermore, this variation is significantly related to physiographic and climatic variables, which suggests that it is adaptive.

Implications for Seed Transfer

Adaptive variation in lupine is complex, neither clinal nor classification models were completely satisfactory in explaining the variability among sources observed in this study to date. However, two trends seem evident: 1) Elevation should not be an important factor for limiting seed transfer. The effects of elevation were small in regression and classification analyses. 2) Latitude, and occasionally longitude, were important in regressions and seed zone was a significant effect in the classifications. Consequently, seed transfers in latitude and longitude should be more limited than transfers in elevation.

Seed transfer risk estimation has not been done yet, and detailed interpretation would be premature. Also, data collection for this study is not yet complete, and relationships could change with the addition of new data.

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