# Using Common Garden Studies to Inform Seed Transfer Zones for Willamette Valley Species 

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## Preface

This report is the result of a cooperative project between the Institute for Applied Ecology (IAE) and public agencies. IAE is a non-profit organization whose mission is conservation of native ecosystems through restoration, research and education. Our aim is to provide a service to public and private agencies and individuals by conduction habitat restoration, developing and communicating information on ecosystems, species, and effective management strategies, and through working with schools and interns to provide educational opportunities.

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## EXECUTIVE SUMMARY

The question of what seed is genetically and ecologically appropriate for restoration projects in the Willamette Valley has been hotly debated for the last two decades. The lack of data regarding variability of native Willamette Valley species has hampered seed production and restoration efforts and has caused costly delays - both biological and financial. To address this lack of data, we utilized common garden studies to measure differences and similarities among populations and to provide informed seed transfer zones recommendations.

## Findings and recommendations:

- In general, populations of the five species we examined showed little geographic structure to their variation in the Willamette Valley, suggesting that a single seed zone may be recommended for this ecoregion, with some exceptions. Movement of seeds between this ecoregion and others is currently not recommended.
- Sidalcea campestris:

We recommend a single seed transfer zone for S. campestris within the Willamette Valley.

- Eriophyllum lanatum var. lanatum:

Most populations within the Willamette Valley appear to represent a single ecotype. A population at Kingston Prairie is somewhat unique and we do not recommend using seed from that site in large scale production efforts for widespread use.

- Epilobium densiflorum:

Most populations appear to be of a single ecoptype, but three populations were clearly morphologically and phenologically similar to one another and divergent from other populations (Kingston and Sublimity Prairies, Coburg Rd, and Mt Richmond). We caution against the use of the four atypical populations outside of similar habitats and geographic areas.

- Potentilla gracilis var. gracilis:

We recommend a single seed transfer zone for P. gracilis within the Willamette Valley. Conservatively, we suggest seed from Yamhill County be omitted from large-scale seed increases for use elsewhere in the Willamette Valley to reduce the chance of including $P$. gracilis var. fastigiata in areas where it is not currently distributed.

- Prunella vulgaris ssp. lanceolata:

We recommend a single seed transfer zone for Prunella vulgaris ssp. lanceolata within the Willamette Valley.

## Key Messages:

- Genetic differences among populations do not appear to follow a geographic pattern based on our measured characteristics.
- Large within population-level variation is common. Some population level differences are detectable among all species
- Most morphological and phenological traits were poorly correlated with geographic and climatic variables. Therefore, populations included in these studies appear to be of a single ecotype.
- This study included populations only below 230 m elevation in the Willamette Valley. Elevation may be an important factor driving ecotypic differentiation in the Willamette Valley, but it does not appear to be significant below 230 m. Our highest Willamette Valley populations were at Kingston (224 m) and Sublimity Prairies ( 213 m ) and these were atypical in some cases. However, this may be due to differences in soil type rather than elevation. Regardless, seeds from these sites should be avoided for widespread mixing with other low elevation sources.
- We do not recommend using seed from outside the Willamette Valley without further investigation.
- The scope of inference of these results is limited to the populations included in the common garden. Sampling from additional populations could alter these findings (for example, some Willamette Valley counties and higher elevations were not represented).
- Additional common garden research should broaden the number of populations examined to include a full range of environmental and climatic conditions in which wild populations occur. Also, future studies should determine the presence and significance of local adaptation.
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#### Abstract

Restoration of prairie habitats in the Willamette Valley is urgently needed but often limited by seed availability. Information on the distribution of genetic variation within and among local populations of native species can help managers understand how this variation is structured on the landscape and use available seed resources most efficiently. We used common garden studies of five species to determine differences among populations and to evaluate seed transfer decisions for natives used in habitat restorations. The primary goal of this project is to identify practical seed transfer zones in the Willamette Valley for movement of native seeds from source locations to restoration sites.


## Methods (Brief)

## Seed Collection and Propagation:

Seeds were collected for each species from multiple (10-30) georeferenced populations across, and in some cases outside, the Willamette Valley. On average, five individuals per population of each of the 5 species were grown in a completely randomized design at the USDA-NRCS Plant Material Center in Corvallis Oregon. Between 15 and 25 phenological and morphological traits were measured for all individuals. Traits were tailored to maximize quantification of variability of important traits among populations. Several botanical experts or species authorities were consulted for recommendations during the trait selection process.

## Data Analysis:

To detect trends and patterns in the measured morphological and phenological variables nonmetric multidimensionalscaling (NMS) was used. NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales. We compared the relative position of each population on the ordination by visually assessing each ordination axis. Due to the degree of difficulty assessing individual sampling units within a population, additional coding subgroup overlays such as county, Level 4 Ecoregion, and 20 mile Eugene buffer we used. These subgroup overlays were based on geographic-administrative or habitat units that are either in practice in the Willamette Valley or logical potential seed transfer zone boundaries. Spatial clusters defined by hierarchical agglomerative cluster analysis were also used as an overlay to provide unbiased spatially defined units. To gain insight into what mechanism may be influencing the observed variation, correlations between ordination axes and the environmental (geographic and climatic) variables were performed.

## Species:

The species used in these common garden studies are native Willamette Valley forbs:
Sidalcea campestris, Eriophyllum lanatum var. lanatum, Epilobium densiflorum, Potentilla gracilis var. gracilis, and Prunella vulgaris ssp. lanceolata. A unique report for each species follows a general introduction.

## Introduction

The use of native seed in restoration, reintroduction, and the creation of new populations is widely employed as a habitat enhancement tool by governmental and private organizations (Reinartz 1995). Maximizing the successful establishment of plant materials is an essential goal in restoration efforts. The diversity of species used, the diversity of populations within a species, and how well adapted the source populations are to the restoration site are all essential factors that can determine the degree of establishment success. While the importance of species-level diversity in restoration is relatively well understood, substantial debate surrounds the issue of which populations within a species should be used in restoration (McKay et al. 2005).

The question of which source populations should be used in restoration is fundamentally a question of the genetics of the source populations and the genetics of the existing native populations in the area of the restoration sites. The genetics of source populations for plant materials can, in fact, have significant impacts on restoration success (see examples in Broadhurst et al. 2008). The impacts can occur through an increase or decrease in the fitness of the plants used in restoration, the plants already present at the site and nearby populations, and alterations in fitness of offspring from the existing and new populations. Additionally, there are potentially broader evolutionary impacts involving selection or drift acting on the frequency of new alleles (genes) introduced at the restoration site, which could result in new evolutionary trajectories for the natural populations.

If the genetics of the source population(s) are a poor match for the local conditions at the restoration site, using sources from non-local (distant) areas may result in poor establishment. Furthermore, if there are native populations present at the site that show strong local adaptation, bringing in divergent genes may threaten the genetic integrity of the local populations and reduce fitness by the introduction of maladaptive genes. Alleles that are foreign to a population may disrupt coadapted gene complexes resulting in a decline in fitness in first and second generation offspring (Lynch and Walsh 1997, Pélabon et al. 2005).

Alternatively, mixing populations from different areas may result in increased vigor of the offspring (i.e., heterosis and positive epistasis, see Fenster and Dudash 1994 and Lynch and Walsh 1997). This "hybrid vigor" is often observed when crossing inbred lines of crops (e.g., Moll 1965), but it is also observed in native plant populations, especially when the populations are small and isolated (Fenster and Dudash 1994). Having a greater number of genotypes at restoration sites also increases the probability that one of those genotypes performs better, either by chance or due to adaptation to similar selective pressures found at the restoration site. Mixing populations in restoration sites may also help re-establish gene flow that has been inhibited by more recent habitat fragmentation. This may be particularly relevant in the Willamette Valley that has just $1 \%$ of the formerly extensive and largely contiguous native prairie habitats persisting (Christy and Alverson 1994, Clark and Wilson 2005).

The ability to predict when restoration success improves by using local seed sources versus a composite of populations from greater distances is not readily apparent (e.g., Lawrence and Kaye 2008). Further, the degree of genetic and geographic distance in which local fitness declines is not predictable. Local adaptation may occur on the scale of hundreds of meters or thousands of kilometers (Fenster and Galloway 2000, Pèlabon et al. 2005). In general, however,
the degree of local adaptation is a function of three components: amount of additive genetic diversity present in the populations, how divergent and strong the selective pressures are among populations, and how much gene flow among populations occurs. The presence of large amounts of additive genetic diversity allows populations to evolve more quickly to selective pressures. Having strong selective pressures that are different for different populations drives the adaptations of each population in alternative directions. Last, if populations have a large amount of gene flow among populations, local adaptations cannot easily develop, even if there is sufficient additive genetic variation and strong divergent selection. It should also be mentioned that local adaptations are more difficult to develop for very small populations that face greater impacts of non-adaptive change due to genetic drift.

While the degree of local adaptation is one side of the source population issue, the degree to which populations are fixed for deleterious alleles is another factor that can have significant influence on the fitness of offspring at the restoration site. Small and isolated populations are particularly prone to harboring alleles that reduce fitness. In some cases, deleterious mutations can increase in frequency and threaten population persistence (i.e., "mutational meltdown" Lynch et al. 1995). When outcrossed, these deleterious alleles can be masked in the heterozygous condition in the offspring. So, the mean fitness of offspring increases when crossing two inbred populations that are fixed for deleterious alleles at different loci. Last, adding more alleles per locus in the population allows an opportunity for selection to remove the deleterious alleles. This will allow greater evolutionary flexibility and in this context will result in higher short-term fitness of the first few generations.

## Seed Source Guidelines

While predicting the consequences to the restoration success and surrounding populations is extremely difficult and few data are present, most recommendations advocate the use of "local" seed (Broadhurst et al. 2008). Using local seed is based on the idea that populations may be locally adapted and seeds from nearby populations will have higher establishment rates and there will be fewer negative consequences to surrounding populations through the introduction of maladaptive genes (Keller et al. 2000; McKay et al. 2005; O’Brien et al. 2007 in Broadhurst et al. 2008). In some cases the guidelines are based on spatial distances. The Nature Conservancy and West Eugene Wetlands Partnership in the Willamette Valley follow the guideline of a 20 mile radius from the restoration location (West Eugene Wetlands Seed Collection Manual 2003). English Nature in the United Kingdom use sources within 5 miles of the restoration site and 15 km is used for the Western Australian Forest Management Plan 2004-2014 (Broadhurst et al. 2008). In other cases, correspondence of environmental variables between source populations and the restoration site is recommended (Mortlock 2000, in Broadhurst et al. 2008). Genetically delineated seed zones have been used by the U.S. Forest Service when they are available, but they may include topographic, climatic, and substrate information when the genetic data is insufficient (Knapp and Dyer 1998 and reference therein, in Broadhurst et al. 2008). Often, however, we lack any information on the spatial or ecological scale in which plant materials should be moved, and most decisions are based on best guesses without an understanding of adaptive and non-adaptive variation (Broadhurst et al. 2008).

## Adaptive and Non-Adaptive Variation

Local adaptation by populations is expected to take on many forms. Populations may have physiological or anatomical adaptations to soil conditions or other environmental factors that are not readily apparent. For example, populations of willows in regions with historic moose populations are more heavily defended with tannins and other defensive compounds than regions lacking historic moose populations (Spalinger and Hobbs 1992) and populations of Mimulus growing on toxic mine tailings look morphologically similar, yet have physiological mechanisms for tolerating heavy metals not found in other populations (Allen and Sheppard 1971). In most cases of populations developing physiological adaptations, it is expected that morphological traits will diverge as well, either through pleiotropy (i.e., one gene causing expression in more than one trait), or because local adaptation requires selection and reduced inter-population gene flow. Without the homogenizing effects of gene flow, morphological or phenological traits of populations are likely to diverge. Local adaptation is commonly expressed directly, however, in the plants' morphology or phenology (e.g., low-growing plants from higher elevations, see Clausen, Keck, and Heisey 1940; more heavily pubescent Arabidopsis in regions with invertebrate herbivores; floral morphologies that best match the local pollinator pool, see Armbruster 1993; flowering time in Syringia across latitude, see Briggs and Walters 1997).

Local adaption is, therefore, expected to result in a pattern of populations looking and behaving differently and in a manner that is consistent with their environment. For example, populations of plants from high elevation populations may all possess a more prostrate growth form than low elevation populations. We should also expect that individuals within a population show a reduction in variation for traits most strongly associated with local adaptation.

Within-species variation in morphology, phenology, and genetics is partitioned at multiple levels, within and among individuals, and among populations. Variation among populations is often the most obvious source observed in vascular plants (see Briggs and Walters 1997) and the component of variation that should correlate most strongly with local adaptation. Differences among populations are due to three potential sources: environmental, genetic, and environmental by genetic interactions. Different climates, soil conditions, ecological interactions (e.g., herbivory or competition), and maternal effects are examples of environmental sources of variation that are commonly observed. The genetic source of variation is due to different alleles in the populations resulting in variation in expression (phenotypes). For example, some populations may have alleles that code for more than one flower color, leaf dissection, etc. Individuals with different genetics (i.e., genotypes) may also produce dissimilar morphological and phenotypic expressions that respond differently to various environmental conditions. For example some genotypes may produce broader and thinner leaves when grown in shaded habitats, but other genotypes may not.

Use of locally adaptive individual reduces the risk that a plant is not adapted to its environment. Yet, genetically "local" is a nebulous concept. However, it can be defined simply as "plant materials that reflect the amount and type of genetic diversity that is typical for a particular plant species in the area under consideration" (Rogers and Montalvo, 2004).

## Approaches to Evaluating Seed Sources

Seed transfer zones outline regions within which plant materials can be transferred with little risk that they will be poorly adapted to a new location. Seed zones attempt to maintain diverse natural genetic structure through use of locally adapted or genetically appropriate native plant populations. Guidelines for seed transfer are established using studies that identify adaptive differences among populations. Common garden studies explore the relative influences of genotype and environment on phenotype (i.e., genes versus environment in producing individuals) (Clausen, Keck, and Heisey 1940, Rogers and Montalvo 2004). Studies using common gardens typically use populations from various geographic sources grown in a uniform environment. In this way, environmental variation is minimized and the remaining differences are genetically based. Traits believed to be related to adaptation are measured and the relationships between traits and environment/climatic variables of the source locations are determined. The more overlap that exists with environmental variables and measured traits suggests a greater chance of successful plant survival, assuming that locally derived seed sources are best adapted to similar climatic variables. It should be noted that locally derived seed sources do not always perform better (Raabová et al. 2007). Seed sources from the more distant populations of the rare Castilleja levisecta performed better at restoration sites than closer populations (Lawrence and Kaye 2008).

Seed transfer guidelines in the form of seed transfer zones were first established by the federal government in the 1930's (McCall 1939). The general ecological construct behind these zones were stimulated by tree provenance tests beginning as early as the $18^{\text {th }}$ century in Europe, which were implemented to increase production of timber products. Seed zones, as defined by the USDA Forest Service Seed Handbook, reflect an area with altitudinal limits within which soil and climate are reasonably uniform, indicating a higher probability of maintaining a species of plant adapted to that particular set of environmental conditions. Seed transfer guidelines and ultimately zones are species specific and in many cases do not extend even into similar families or genera. Common garden studies have increasingly been conducted to evaluate seed transfer decisions for native grasses and forbs used in habitat restoration projects (Gordon and Rice 1998, Knapp and Rice 1998, Montalvo and Ellstrand 2001, Erickson, Mandel and Sorensen. 2004, Doede 2005). However, little information regarding native plants seed zones within the Willamette Valley ecoregion is currently available even though several large scale restoration projects are underway (e.g., West Eugene Wetlands Partnership, U.S. Fish and Wildlife Service, Bureau of Land Management, Natural Resources Conservation Service, The Nature Conservancy, Portland Metro, and the Institute for Applied Ecology).

## Project Goals:

This report summarizes the findings for Sidalcea campestris, Eriophyllum lanatum var. lanatum, Epilobium densiflorum, Potentilla gracilis var. gracilis, and Prunella vulgaris ssp. lanceolata. The primary goal of this study was to delineate seed transfer zone(s) for these species within the Willamette Valley, which will assist in the identification of genetically appropriate sources of this species for restoration and revegetation enhancement projects.

# Distribution of morphologic and phenological variation of Sidalcea campestris in the Willamette Valley: a common garden study to inform seed transfer zones 



## Materials and Methods

## Study species

Sidalcea campestris Greene (Malvaceae) is a native perennial commonly found in upland prairie habitat of the Willamette Valley, Oregon. Its distribution extends from southern Washington to central Oregon, primarily in the Willamette Valley; it is uncommon east of the Cascades, but a few scattered populations extend as far east as Idaho. It is considered "apparently globally secure" and "apparently secure" in Oregon by NatureServe (G4-S4) (2008). Sidalcea campestris has small, pink or white flowers that are contained in dense, hairy calyxes in a terminal raceme (Gisler 2003). It typically flowers from June to August. Sidalcea campestris is gynodiecous, where populations are composed of hermaphrodite and female (male-sterile) individuals. Flowers of hermaphrodites have larger petals and female flowers are substantially smaller and lack anthers. Female individuals are self-incompatible and require pollen transfer between individuals for seed set. Although hermaphrodites are self-compatible, protandry (mechanism whereby anthers dehisce pollen before stigma is receptive) minimizes self pollination (Gisler 2003). As such, insect pollination is generally required for seed production for both sexes.

## Population sampling

Sidalcea campestris seeds were collected from 32 populations distributed throughout the Willamette Valley in August 2006 when the seeds were mature, but had not dehisced (Fig. 1, Table 1). Latitude, longitude, and elevation were recorded for each location. To avoid impacts to natural populations no more than $25 \%$ of available seed was collected from all individual plants at each location. A large variation in natural population sizes were present for this species; population sizes ranged from 5 individuals to greater than 350. Habitats at nearly all the sites were similar prairie remnants along roadsides or small natural areas. These populations had roughly similar substrates and hydrology. For analysis, locations were classified based on individual populations, county, EPA defined level 4 Ecoregion (Griffin and Omernik 2008) (Apendix I), and presence or absence within a Eugene 20 mile buffer zone (West Eugene Wetlands Seed Collection Manual, 2003).

## Experimental design

Seeds were sown on February 12, 2007 into flats of Ray Leach "Cone-tainers" and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center in Corvallis, Oregon. Ten cone-tainers were started for each population ( $\mathrm{N}=320$ ), and were randomly placed within flats. Seeds were lightly scarified by rubbing them on sandpaper prior to sowing. Flats were watered and placed in an unheated greenhouse. Average daytime temperatures ranged between 10 and $15.5^{\circ} \mathrm{C}$ and nighttime temperatures were between 4.4 and $7.2^{\circ} \mathrm{C}$. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After ten weeks in the greenhouse, plants were moved to a shade house and allowed to acclimatize for several weeks to outdoor temperatures.

Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. The study site was then covered with three inches of bark mulch to further aid in weed suppression. Plants 1-10 from each population were chosen replicates; if plants 1-10 did not emerge or survive additional plants were used to bring the total number of plants per population to ten. Some populations did not have ten plants due to mortality thus only 205 S. campestris study plants were transplanted using a completely randomized design in June 2007. An additional row of $S$. campestris was planted on each side of the plot as a border row to buffer against edge effects. Plants were placed 0.7 meters apart within rows and rows were placed one meter apart.

## Plant trait measurement

Traits were chosen based on characteristics described in Hickman (1993) and Hitchcock and Cronquist (2001). Traits thought to have adaptive significance, or associated with reproductive success, taxonomically important traits, and traits with high degrees of variability between local congeners (S. campestris, S. virgata, and S. cusickii) were included (Gisler 2003). While measuring pre-defined traits, additional traits were included based on apparent visual differences among plants. Several botanical experts or species authorities were consulted for
recommendations during the trait selection process. Table 2 shows a list of the traits and how they were measured.

Phenological and morphological traits were measured for all individual hermaphrodite $S$. campestris plants during 2008. All morphological traits were measured in a single day. To reduce measurement error, one person measured traits while a second recorded. Floral traits were recorded three weeks from when an individual first flowered using only newly opened flowers. Morphological traits less than 25 cm in length were measured by dial calipers to 0.01 mm . Traits greater than 25 cm were measured with a meter stick to the nearest centimeter. When measuring a single trait in triplicate on an individual, no measurements were made from the same organ (e.g., average petal length was the mean length of three petals from three separate flowers chosen haphazardly). Infructescence congestion and leaf dissection were calculated as a ratio of multiple traits (Table 2). Emergence date was monitored on a daily basis; when conetainers contained more than one seedling, germination was recorded for the first seedling that emerged in each cone-tainer. Flowering date was monitored on transplanted individuals three times a week until a value was obtained for each study plant. Female plants, misidentified plants, and plants with missing values were removed from the analysis.

## Climatic Data

Climatic conditions at each population location site were characterized using digital maps produced in ArcGIS 9.3 and data generated by PRISM climate models (PRISM group, 2008). PRISM is an analytical model that uses point data and a digital elevation model (DEM) to generate gridded estimates of monthly and annual average daily maximum/minimum climatic parameters (PRISM group, 2008). PRISM incorporates a conceptual framework that addresses the spatial scale and pattern of climate variables that allows for estimation of variables in regions with heterogeneous terrain (PRISM group, 2008). PRISM was parameterized to use 1971-2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for a single location is no better than half the resolution of the cell. Variables were modeled monthly. An annual average was produced by averaging the monthly grids. For this analysis, mean annual temperature (USANNAV) and precipitation (USANNP), mean dates of the first (SPRFRST) and last frost (FLLFRST), and the number of frost free days (FRSTFREE) was gathered for each population based on each population's unique latitude (LAT) and longitude (LONG).

## Data analysis

Ordination analyses were performed on morphological and phenological variables using nonmetric multidimensional scaling (NMS) based on Euclidean distance measures (Kruskal 1964). Analyses were completed using PcORD 5 (McCune and Mefford 1995). NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales (Peterson and McCune 2001). To account for variable collection on different scales (i.e., Julian days and centimeters) data were relativized using the
standard deviates of each column variable. Prior to relativization, emergence and flowering date variables were monotonically log transformed to compress high values and spread low values by expressing values as orders of magnitude. Monotonic transformation of individual variables allows for independent changes to data point values without altering their rank. Individual's greater than two standard deviates from the mean Euclidian distance were defined as outliers and removed from the analysis (CB0909, CB0217, CB0409, RO0422, HR0410, HR1202, RU1015, KI0101, KN0819, NB0704).

Since adaptive variation is important in designating seed transfer zones and is most directly related to variation within and among seed sources, we used individuals as our sampling units instead of mean population estimates. Thus, the main data matrix consisted of individual plants and traits measured at the common garden. To visually clarify the distribution of sampling units in ordination space, a second matrix with additional information (latitude, longitude, county, elevation, and climatic variables) was overlayed or jointly-biplotted.

NMS uses an iterative search for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). To ensure that the ordination was below an acceptable level of stress, we used a random seed with 250 runs of real data. Dimensionality was assessed visually using a scree plot. Monte Carlo simulations using 250 replicate were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used.

We compared the relative position of each population in ordination space by visual assessment. Due to the degree of difficulty assessing individual sampling units within a population, we used additional coding subgroup overlays such as county ( $n=6$ ), Level 4 Ecoregion ( $\mathrm{n}=3$ ), 20 mile Eugene buffer $(\mathrm{n}=2$ ) (Table 1). These subgroup overlays were based on geographic-administrative or habitat units that are either in practice in the Willamette Valley or logical potential seed transfer zone boundaries. To identify spatial clustering based on latitude and longitude we used hierarchical agglomerative clustering patterns calculated using Euclidean distances and nearest neighbor linkages to maximize distinctness of groups. The resulting clustering dendrogram was scaled using a log transformation and information was retained at the 5.1E-02 level (Fig. 2). This allowed us to partition the dataset into more homogenous subsets based exclusively on geographic location. This subset was then used as an additional plot overlay.

We quantitatively compared the relative position of populations in the ordination using Euclidian distances and unblocked Multi-Response Permutation Procedure (MRPP) analysis on weighted groups. Data were relativized for MRPP analysis using the standard deviation of each variable. MRPP is a nonparametric test used to examine whether populations on matrix plots occupy different regions of ordination space.

Correlations between ordination and the environmental variables were calculated using Pearson Correlation Coefficients. The percent of variation in the original ordination was also recorded. A Bonferroni correction was used when multiple comparisons were performed to maintain a low probability of relationships appearing significant when, in fact, they only appear significant by chance.

Univariate calculations were made for each variable by population. Trait means were used to produce pairwise Pearson Correlation Coefficients for each variable with latitude, longitude, and climatic variables using SAS 9.2 (SAS Institute 2008). Ordination axes were individually regressed on geographic and climatic variables in PcORD.

## Results

## Morphological and phenological patterns across populations

The Nonmetric Multidimensional Scaling (NMS) analysis provided a 3-dimensional ordination best solution to the data based on a final stress of 18.39849, a final instability of 0.00001 , and 484 iterations. Using Clarke's cutoff for acceptable instability, values between 10 and 20 represent a usable picture (McCune and Grace 2002). Therefore, values at the upper end, such as our results, suggest a potential to be misleading and thus too much reliance on details of the plots should be discouraged. Together the axes explained $76.2 \%$ of the variance: $24.2 \%$, $37.4 \%$ and $18.8 \%$ for axes 1,2 , and 3, respectively. Axis 1 was strongly influenced by total raceme height, and axis 2 was strongly influenced by basal leaf measurements (mean leaf length, width, lobe A, and lobe dissection). Although some traits show strong correlations to ordination axes, they are inconclusive in explaining the distribution of individuals in ordination space (Fig. 3; Table 3). Since the majority of variation is accounted for within axis 1 and 2 , they are used to visually describe the data.

NMS ordination provided little evidence of discrete clustering of populations in ordination space based on morphological and phenological characteristics. However, post hoc analysis using Multi-Response Permutation Procedure (MRPP) indicated some significant differences between individual populations (Table 4a). This pattern was not immediately apparent in the ordination plots (Fig. 4). With 496 total comparisons only 23 pairs of populations were significantly different representing just $3 \%$ of the population level contrasts. Glaser x Parker, Tupper x Turner, and Mt. Richmond dominated these comparisons. Interestingly, these populations are not significantly different than each other, and they are not geographically close together (Fig. 1). Further, while MRPP suggests statistical significance of populations, many $A$ values were close to zero, indicating little within population similarity (i.e., individuals were not necessarily more similar to other members of their populations than to individuals from other populations). The $A$ statistic is a descriptor of within-group homogeneity compared to random expectation (McCune and Grace 2002). If heterogeneity within groups equal chance expectation then $A=1$; however, if less agreement (heterogeneity) exists within groups than expected by chance, then $A=0$. In such cases where small $A$ values are statistically significant careful consideration of the ecological significance of the results is warranted. $A$ values of less than 0.3 represent substantial heterogeneity (variability) between contrasted groups.

Populations that dominate many of the population level comparisons have the potential to strongly influence subsequent overlays used in analysis. County as a subgroup overlay suggests it is not sufficient in visually defining differences in plant variation based on the traits measured (Fig. 5). Post hoc MRPP analysis, however, suggests Linn County was significantly different
from all other counties in the analysis (Table 4 b ). This difference is largely driven by the inclusion of the Glaser x Parker in the Linn County group of populations. Similarly, no visual clustering was found in the Eugene overlay, which shows no difference between plants within or outside the 20 mile Eugene seed transfer zone (Fig. 6). Individuals from within the 20 mile Eugene seed transfer zone overlap in morphology and phenology with individuals outside of the 20 mile zone. Although a significant difference was detected between populations within and outside the Eugene 20 mile buffer ( $\mathrm{T}=-2.5044066, \mathrm{~A}=0.00312368, p=0.02$ ) based on post hoc MRPP, this does not match individual population differences. In this case, the $A$ value is exceptionally low suggesting no real ecological difference exists.

Ten geographic clusters were defined using Hierarchical Clustering (Fig. 2). At this coarse clustering level, ordination revealed separation of Cluster 5 (Glaser x Parker, Sand Ridge) from all other clusters (Fig. 7). MRPP suggests several differences in clusters, yet they are similar to those suggested at the population level (Table 4c). Cluster 5 is significantly different than all other clusters. Again, these populations are low in sample units and include Glaser x Parker. Cluster 10, which includes the most northern populations, is significantly different than Clusters 2 and 6 . Clusters 2 and 6 are both geographically adjacent to each other and are located in the southern portion of Benton and northern Marion counties. Thus, using unbiased spatially defined groups of populations through Hierarchical Clustering indicates that populations that are closer are often not more similar.

EPA defined Ecoregions (Level 4) within the Willamette Valley did not cluster in the ordination; much overlap of individuals between these zones is apparent (Fig. 8). Post hoc MRPP analysis again indicates Ecoregion level differences are not significant suggesting that this is not an appropriate delineation of seed zones (Table 4d). These results correspond to other overlays investigated in this analysis.

## Correlations of traits with geographic and climatic variables

Nineteen traits were measured or calculated for each individual in each population. Ranges and means ( $\pm 1 \mathrm{SD}$ ) of populations indicate substantial variability both between and within populations (Appendix A). High levels of within population variability suggest nearly all populations are highly polymorphic in the Willamette Valley. Population level variability is apparent in most traits. In particular, phenological measurements, although less important in ordination, show substantial differences in timing among populations. Seed germination began February 21 and continued until May 3, 2007, with a mean date of March 15. Initial flowering began as early as April 20 and as late as June 30. Only 20 individuals, incidentally from Glaser x Parker and Sand Ridge (Linn County) flowered prior to June. Average flowering was June 10. This suggests that although phenology was not statistically important it is still biologically important in differentiating populations, particularly those populations that are consistently different in this ordination.

Regression of ordination axes with climatic and geographic variables identified a few weak associations (Table 3), (Figs. 9-10). Neither date of emergence nor flowering date proved to be correlated with latitude or longitude. It is important to note that correlations with axes are difficult to ascertain since variables are not necessarily linearly correlated or parallel to axes.

Some significant correlations were present between univariate traits and geographic or climatic variables. Longitude was correlated with several plant traits specifically those related to leaf morphology (Table 5). The relationship was positive for the basal leaf mean length from base of sinus to base of leaf and lobe dissection and negative for the mean length from outermost lobule to base of sinus (Table 5). Frost free date was also positively associated with basal leaf mean length from base of sinus to base of leaf and lobe dissection.

## DISCUSSION

Population level differences do not appear to follow a geographical pattern based on the measured characteristics in this study. Three populations (Glaser x Parker, Tupper Turner, and Mt. Richmond) were significantly different than most other populations and similar to one another; however, these populations are separated by between $20-60 \mathrm{~km}$ and numerous populations were found between them (some populations less than 1 km away). Inflorescence length, total plant height, and leaf morphology appear to be strongly correlated to ordination axis, but are highly variable within and among populations. Interestingly, prior morphometric analysis of individuals in situ found plant height as an important characteristic (Lambert 2008) with heights shortest in the Linn County area, which corresponds to the findings in this study. Most other generalizations regarding plant height and geographic area did not correspond to our findings perhaps due to the differences in populations and traits used in the analyses. High degree of variability within and among populations were common in all Pairwise MRPP population comparisons (represented by $A$ values less than 0.3 ), with only Glaser x Parker showing mild signs of reduced variability. This is most likely an artifact of small sample size and potential inbreeding or relatedness of the individuals used in the common garden.

Support for a difference between plants within or outside the 20 mile Eugene seed transfer zone was extremely weak, suggesting no predictable ecological difference exists. In a few cases populations separated by a few kilometers were detectibly different, but generally populations were not different from one another when separated by distances of up to 150 km . Populations grouped by Ecoregion, also did not show any differences. Therefore, the coarse previous habitat types defined by the Ecoregions do not appear to be related to the morphology of $S$. campestris populations. County is an artificial boundary often used by managers, although rarely biologically meaningful. In this study, county proved ineffectual in defining clusters in ordination space. Linn County was found to be significantly different than all other counties; however, large within group variability was present and Linn County contains the distinctive Glaser x Parker population. Grouping populations based in Hierarchical Clustering of geography again separated out the cluster containing the Glaser x Parker population. Using this method, northern populations were significantly different than populations in the southern portion of Benton and northern Marion counties, but no different than the populations in Lane County. Only Cluster 5 (Glaser x Parker and Sand Ridge) proved to have moderate $A$ values in pairwise comparisons. Hierarchical Clustering is a better tool for defining spatially clustered geographic areas though, in this case, they do not correspond to differences in morphological or phenological variability of these measured populations.

Despite the slight difference of the Glaser x Parker population with other population it is important to realize that this population in nature is extremely small (only five individuals). Thus it is not surprising measurements taken on individuals from this population show very little variability; individuals in this population are likely closely related and inbred and therefore more likely to be similar to one another and different from other populations. These differences most likely represent non-adaptive genetic drift, rather than local adaptation. We strongly recommend the addition of other populations and more individuals within Linn County in future seed guideline studies. Too few seed sources and representative samples from populations make accurate inferences this part of the valley slightly more complicated to ascertain.

## Correlations of traits with geographic and climatic variables

Morphological and phenological traits were poorly correlated with geographic and climatic variables. High levels of within population variability, in most traits, suggest high levels of polymorphism in populations in the Willamette Valley. Some significant, although weak, correlations were found between univariate traits and geographic or climatic variables. Basal leaf length and lobe dissection decreases with increasing latitude (and increases with number of frost free days), while it simultaneously decreases for the mean length from outermost lobule to base of sinus. For all past seed zones studies within the Willamette Valley, little to no correlation with geographic and climatic variables was found (Erickson, Mandel, and Sorensen 2004; St. Clair, Mandel, and Vance-Borland 2005). The paucity of strong climatic by trait relationships is anticipated since the Willamette Valley has a very homogenous climate. Additionally, it should be emphasized that the Willamette Valley was a more or less expansive and continuous prairie and savanna that experienced large-scale disturbances such as fire and floods prior to EuroAmerican settlement (Johannessen et al. 1971, ODFW 2005). These factors suggest that strong differential selective pressures in different regions were unlikely and larger contiguous habitats undergoing broad-scale disturbances would facilitate substantial gene flow across the Willamette Valley. Strong local adaptation for various sites within the Willamette Valley is therefore not likely.

The strong overlap of measured characteristics across populations suggests movement of seed among populations with similar environments within the Willamette Valley would result in a high probability of plant establishment. We did not have representation of S. campestris populations above 229 m in elevation and it is possible that populations from higher elevations may show local adaptation. We therefore recommend that plant materials from below 229 m are not mixed with higher elevation plants. In addition, we do not recommend movement of seed from sources outside the Willamette Valley, or for populations inhabiting unusual habitats within the Willamette Valley without further study.

## RECOMMENDATIONS

In summary, our data indicate there is a general lack of morphological and phenological differentiation in populations of $S$. campestris across the Willamette Valley. While a few populations were morphologically different, there were no apparent spatial, climatic, or environmental factors that were related to the populations. One of these populations, at least, is
very small and likely inbred. Glaser x Parker, Tupper x Turner, and Mt. Richmond populations proved to be slightly divergent, particular in terms of phenology (although not significantly in this analysis). We therefore caution against use of these three populations outside of similar habitat and geographic area. Else, we recommend a single seed transfer zone for S. campestris within the Willamette Valley under 229 m and from $45.39^{\circ}$ latitude in the north to $43.92^{\circ}$ in the south. We did not include plants from outside of this area and cannot assume their inclusion in this seed transfer zone. We suggest that using multiple populations in reintroductions will increase restoration success and assist in restoring more historic levels of gene flow. Last, additional studies are recommended to determine the presence and/or scale of local adaptation and the genetic basis for the adaptations if they are found.

Table 1. List of population names with corresponding abbreviations and number of individuals represented per population as sampling units. County: $1=$ Benton, $2=$ Polk, $3=$ Lane, $4=$ Linn, $5=$ Marion, 6 = Yamhill. Eugene: $1=$ within 20 mile buffer, $2=$ not present in buffer. Cluster is based on dendrogram interpretation at the 5.1E-02 level. EPA defined Ecoregion level 4: $1=$ Valley Foothills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Gallery Forest.

| Population | Population Codes |  | N | Latitude | Longitude | County | Eugene | Cluster | Ecoregion |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Airlie x DeArmond | AD | 1 | 2 | 44.743550 | -123.284900 | 1 | 1 | 1 | 2 |
| Alpine Rd. | AL | 2 | 7 | 44.324300 | -123.341500 | 1 | 2 | 3 | 2 |
| Airlie x Saurkraut | AS | 3 | 3 | 44.444541 | -123.192378 | 2 | 1 | 2 | 2 |
| Berry Creek Rd. | BC | 4 | 6 | 44.729000 | -123.361000 | 2 | 1 | 1 | 1 |
| Berthelsdorf | BE | 5 | 7 | 45.156330 | -123.324900 | 6 | 1 | 10 | 1 |
| Bellfountain x 53rd | BL | 6 | 5 | 44.506200 | -123.319200 | 1 | 1 | 4 | 2 |
| Coburg Ridge | CO | 7 | 3 | 44.164000 | -123.099000 | 3 | 2 | 7 | 2 |
| Camas Swale | CS | 8 | 7 | 43.959000 | -123.012000 | 3 | 2 | 8 | 3 |
| Dillard Rd. | DL | 9 | 3 | 43.959000 | -123.027000 | 3 | 2 | 8 | 3 |
| Dykstra Rd. | DY | 10 | 8 | 44.196690 | -123.190880 | 1 | 2 | 7 | 2 |
| E.E. Wilson | EE | 11 | 10 | 44.702100 | -123.203700 | 1 | 1 | 1 | 2 |
| Glaser x Parker | GP | 12 | 6 | 44.527450 | -123.017200 | 4 | 1 | 5 | 2 |
| Helt | HE | 13 | 8 | 43.925590 | -123.051000 | 3 | 2 | 8 | 3 |
| Hill Rd. | HI | 14 | 7 | 45.252000 | -123.230000 | 6 | 1 | 10 | 2 |
| Junction City | JC | 15 | 10 | 44.102373 | -123.113838 | 3 | 2 | 7 | 3 |
| Kingston Prarie | KN | 16 | 8 | 44.775340 | -122.744300 | 4 | 1 | 9 | 1 |
| McFarland Rd. | MC | 17 | 10 | 44.359000 | -123.333800 | 1 | 2 | 3 | 2 |
| Monroe | MN | 18 | 10 | 44.183005 | -123.174685 | 1 | 2 | 7 | 3 |
| Morrows | MO | 19 | 4 | 44.912850 | -123.327300 | 2 | 1 | 6 | 2 |
| Masonville Rd. | MS | 20 | 10 | 44.714870 | -122.847000 | 6 | 1 | 9 | 1 |
| Mt. Richmond | MT | 21 | 2 | 45.180000 | -123.290000 | 6 | 1 | 10 | 1 |
| Maxfield Creek | MX | 22 | 3 | 45.388400 | -123.256400 | 2 | 1 | 10 | 1 |
| Panther Creek | PC | 23 | 6 | 45.262000 | -123.227000 | 6 | 1 | 10 | 2 |
| Rockyford Rd. | RF | 24 | 8 | 45.358000 | -123.253000 | 6 | 1 | 10 | 2 |
| Red Prarie Rd. | RP | 25 | 7 | 45.085000 | -123.415000 | 6 | 1 | 11 | 2 |
| Smithfield Rd. | SM | 26 | 3 | 44.590062 | -123.155075 | 2 | 1 | 1 | 2 |
| SandRidge | SN | 27 | 3 | 44.530300 | -122.967600 | 4 | 1 | 5 | 2 |
| SR 22 | SR | 28 | 4 | 44.933800 | -123.186350 | 2 | 1 | 6 | 1 |
| Story x Maxfield Creek | ST | 29 | 3 | 44.817000 | -122.794000 | 2 | 1 | 9 | 1 |
| Sublimity Prarie | SU | 30 | 4 | 44.841010 | -122.743800 | 5 | 1 | 9 | 1 |
| Tampico Rd | TP | 31 | 10 | 44.694000 | -123.250600 | 1 | 1 | 1 | 2 |
| Tupper x Turner | TU | 32 | 2 | 45.375000 | -123.262000 | 6 | 1 | 10 | 2 |

Table 2. List of phenological and morphological traits measured on S. campestris plants in common garden study.

| Trait | Abbreviation | Measurement Method |
| :---: | :---: | :---: |
| Emergence date | JULEMER | Initial Julian date of cotyledons emergence monitored on a daily basis recorded for the first seedling that emerged in each cone-tainer. |
| Flowering date | JULDATE | Initial Julian date of flowering monitored on transplanted individuals three times a week until a value was obtained for each study plant. |
| Mean basal leaf length | AVELEAFL | Distance (mm) from base leaf to the tip of the middle lobe measured on 3 haphazardly selected basal leaves. |
| Mean basal leaf width | AVELEAFW | Distance (mm) across widest part leaf measured on 3 haphazardly selected basal leaves. |
| Mean basal leaf lobule to sinus length | AVEAL | Mean length from outermost lobule to base of sinus on 3 haphazardly selected basal leaves |
| Mean basal leaf sinus to base length | AVEBL | Mean length from base of sinus to base of leaf on 3 haphazardly selected basal leaves |
| Leaf dissection | LOBEDISS | Calculated as a ratio of multiple traits: length of outermost lobule to base of sinus /(length of base of sinus to base of leaf + length of outermost lobule to base of sinus) |
| Mean lobe width | AVELOBEW | Mean width (mm) between outermost lobule on middle lobe on 3 haphazardly selected basal leaves |
| Height | HT | Total height of plant (cm) base to tip |
| Total number of racemes | TOTRACE | Total number of racemes |
| Mean number of branches | AVEBRANC | Number of branches on a raceme counted for 3 haphazardly selected racemes |
| Flower color | FLWRCLR | Flower color ranked according to appropriate web wheel colors: |
| Mean petal length | AVEPETL | Mean petal length (mm) on 3 haphazardly selected flower heads |
| Mean petal width | AVEPETW | Mean petal width (mm) on 3 haphazardly selected flower heads |
| Mean sepal length | AVESEPL | Mean sepal length (mm) on 3 haphazardly selected flower heads |
| Tallest raceme length | AVEINFLL | Length (cm) from the base of the plant to the tip of the tallest inflorescence |


| Mean inflorescence <br> length | AVEINFLFL | Mean length (cm) from bottom to top of inflorescence (after <br> seed set) measured on 3 haphazardly selected racemes |
| :--- | :--- | :--- |
| Mean number of fruits | AVEFLWR | Mean number of fruits on a measured inflorescence length <br> counted on 3 haphazardly selected racemes |
| Infructescence congestion | RFLOWCM | Calculated as the ratio of two measured traits: Mean number <br> of fruits/ mean infructescence length |

Table 3. Correlation of axes with measured trait variables and secondary geospatial and climatic variables. Kendall' tau is a rank regression estimate of correlation, whereas $\mathrm{R}^{2}$ is the square of the linear correlation coefficient.

| Axis: | 1 |  | 2 |  | $\frac{3}{\mathbf{R}^{2}}$ | Tau |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathbf{R}^{2}$ | tau | $\mathbf{R}^{2}$ | Tau |  |  |
| Latitude | 0.02 | -0.06 | 0.01 | 0.05 | 0.01 | 0.05 |
| Longitude | 0.09 | 0.21 | 0.00 | -0.04 | 0.00 | 0.01 |
| Emergence date | 0.02 | 0.07 | 0.01 | 0.03 | 0.00 | -0.03 |
| Flowering date | 0.20 | -0.27 | 0.00 | 0.04 | 0.05 | -0.07 |
| Mean basal leaf length | 0.05 | 0.15 | 0.80 | -0.73 | 0.00 | 0.00 |
| Mean basal leaf width | 0.09 | 0.21 | 0.75 | -0.69 | 0.01 | -0.04 |
| Mean basal leaf lobule to sinus length | 0.25 | 0.33 | 0.59 | -0.56 | 0.17 | -0.28 |
| Mean basal leaf sinus to base length | 0.18 | -0.27 | 0.00 | -0.06 | 0.40 | 0.48 |
| Leaf dissection | 0.27 | 0.38 | 0.20 | -0.30 | 0.40 | -0.50 |
| Mean lobe width | 0.04 | 0.16 | 0.58 | -0.56 | 0.01 | -0.02 |
| Mean sepal length | 0.00 | -0.02 | 0.01 | -0.23 | 0.05 | 0.25 |
| Mean petal length | 0.03 | -0.11 | 0.15 | -0.25 | 0.04 | 0.16 |
| Mean petal width | 0.00 | -0.03 | 0.08 | -0.20 | 0.15 | 0.32 |
| Total number of racemes | 0.09 | -0.19 | 0.03 | 0.11 | 0.02 | -0.12 |
| Height | 0.44 | -0.46 | 0.05 | -0.14 | 0.11 | -0.25 |
| Tallest raceme length | 0.58 | -0.55 | 0.01 | -0.06 | 0.09 | -0.19 |
| Mean number of branches | 0.18 | -0.29 | 0.00 | 0.00 | 0.27 | -0.31 |
| Mean inflorescence length | 0.02 | -0.13 | 0.06 | -0.20 | 0.00 | -0.03 |
| Mean number of fruits | 0.05 | -0.16 | 0.03 | -0.11 | 0.00 | -0.04 |
| Infructescence congestion | 0.00 | -0.03 | 0.02 | 0.11 | 0.00 | -0.02 |
| First day of fall frost | 0.00 | 0.03 | 0.02 | -0.07 | 0.00 | 0.00 |
| Number of frost free days | 0.02 | 0.07 | 0.02 | -0.07 | 0.00 | 0.04 |
| Last day of spring frost | 0.03 | -0.13 | 0.01 | 0.07 | 0.00 | -0.07 |
| Mean annual temperature | 0.01 | 0.07 | 0.00 | -0.01 | 0.00 | 0.04 |
| Mean annual precipitation | 0.01 | -0.05 | 0.00 | 0.05 | 0.00 | -0.06 |

Table 4. Significantly different MRPP multiple pair-wise comparisons with adjusted Bonferroni correction, test statistic $T$ and agreement statistic $A$ grouped by (a) population ( $p<0.0001$ ) (b) county ( $p<0.003$ ) (c) dendrogram defined cluster ( $p<.005$ ) (d) Ecoregion ( $p<0.008$ ). Comparisons with moderate biological significance are italicized ( $\mathrm{A} \geq 0.17$ ).
(a)

| Populations |  |  | $\boldsymbol{T}$ | $\boldsymbol{A}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 7 | -0.2118503 | 0.0055277 |
| 1 | vs. | 27 | -1.7125852 | 0.1593815 |
| 3 | vs. | 21 | -2.1920574 | 0.1625477 |
| 3 | vs. | 32 | -0.3954577 | 0.0188168 |
| 7 | vs. | 21 | -1.8830267 | 0.132168 |
| 9 | vs. | 32 | -0.3287708 | 0.0188821 |
| 19 | vs. | 21 | -0.7647124 | 0.0595707 |
| 21 | vs. | 26 | -0.5488585 | 0.0427297 |
| 22 | vs. | 32 | -0.430647 | 0.0214264 |
| 26 | vs. | 32 | -0.139224 | 0.0102359 |
| 27 | vs. | 32 | -0.6542673 | 0.0515612 |
| 12 | vs. | 17 | -7.9175569 | 0.1797211 |
| 15 | vs. | 24 | -6.9948547 | 0.1148342 |
| 16 | vs. | 24 | -6.9224756 | 0.1261569 |
| 11 | vs. | 12 | -7.6864755 | 0.1714481 |
| 12 | vs. | 31 | -7.7595996 | $\mathbf{0 . 2 0 1 5 7 6 6}$ |
| 12 | vs. | 15 | -7.741227 | $\mathbf{0 . 2 0 8 0 7 5 9}$ |
| 12 | vs. | 18 | -7.7277856 | $\mathbf{0 . 2 1 9 2 8 0 5}$ |
| 15 | vs. | 25 | -6.1852833 | 0.0957027 |
| 16 | vs. | 20 | -6.2019496 | 0.1074663 |
| 12 | vs. | 24 | -7.3993061 | $\mathbf{0 . 2 4 0 8 9 3 2}$ |
| 16 | vs. | 25 | -5.9172661 | 0.1114811 |
| 10 | vs. | 12 | -6.5008451 | 0.1898459 |

(b)

| County |  |  | $\boldsymbol{T}$ | $\boldsymbol{A}$ |
| :--- | :--- | :--- | ---: | ---: |
| 1 | vs. | 4 | -12.88 | 0.03998 |
| 6 | vs. | 4 | -13.324 | 0.05672 |
| 3 | vs. | 4 | -9.3211 | 0.05328 |
| 2 | vs. | 4 | -8.6951 | 0.0462 |
| 6 | vs. | 3 | -5.8151 | 0.0187 |
| 2 | vs. | 6 | -5.688 | 0.01801 |
| 1 | vs. | 6 | -5.5952 | 0.01235 |
| 1 | vs. | 2 | -4.806 | 0.0126 |

(e)

| Ecoregion |  | $\boldsymbol{T}$ | $\boldsymbol{A}$ | $\boldsymbol{p}$ |
| :---: | :---: | :---: | :---: | :---: |
| 2 | vs. | 1 | 0.0129 | 0.0000 |
| 2 | vs. | 3 | -0.4117 | 0.0006 |
| 1 | vs. | 3 | -1.7505 | 0.0058 |

(c)

Table 5. Univariate Pearson Correlation Coefficients of a subset of measured traits with geographic and climatic variables. Significant correlations are shown in bold.

| Variable | Longitude | Latitude | First Frost Date | Frost Free <br> Days | Last Frost Date | Mean Annual Temperature | Mean Annual Precipitation |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Height <br> p-value | $\begin{array}{r} -0.23422 \\ 0.197 \\ \hline \end{array}$ | 0.10135 <br> 0.581 | $\begin{array}{r} -0.11342 \\ 0.5365 \\ \hline \end{array}$ | $\begin{array}{r} -0.19676 \\ 0.2804 \\ \hline \end{array}$ | $\begin{array}{r} 0.25484 \\ 0.1592 \\ \hline \end{array}$ | $\begin{array}{r} -0.11725 \\ 0.5228 \\ \hline \end{array}$ | $\begin{array}{r} 0.06439 \\ 0.7262 \\ \hline \end{array}$ |
| Tallest Raceme length p-value | $\begin{array}{r} -0.25827 \\ 0.1535 \\ \hline \end{array}$ | $\begin{array}{r} 0.00372 \\ 0.9839 \\ \hline \end{array}$ | 0.00117 <br> 0.9949 | $\begin{array}{r} -0.03385 \\ 0.8541 \\ \hline \end{array}$ | $\begin{array}{r} 0.0674 \\ 0.714 \\ \hline \end{array}$ | $\begin{array}{r} -0.14609 \\ 0.425 \\ \hline \end{array}$ | $\begin{array}{r} 0.15932 \\ 0.3838 \\ \hline \end{array}$ |
| Mean basal leaf length p-value | $\begin{array}{r} 0.12482 \\ 0.4961 \\ \hline \end{array}$ | $\begin{array}{r} -0.1888 \\ 0.3007 \end{array}$ | $\begin{array}{r} 0.15953 \\ 0.3831 \\ \hline \end{array}$ | 0.14132 <br> 0.4404 | $\begin{array}{r} -0.09414 \\ 0.6083 \\ \hline \end{array}$ | 0.01671 $0.9277$ | $\begin{array}{r} 0.03132 \\ 0.8649 \\ \hline \end{array}$ |
| Mean basal leaf width p-value | $\begin{array}{r} 0.13638 \\ 0.4567 \\ \hline \end{array}$ | $\begin{array}{r} -0.2038 \\ 0.2631 \\ \hline \end{array}$ | $\begin{array}{r} 0.19642 \\ 0.2813 \\ \hline \end{array}$ | 0.21171 $0.2447$ | $\begin{array}{r} -0.18949 \\ 0.2989 \\ \hline \end{array}$ | $\begin{array}{r} -0.02721 \\ 0.8825 \\ \hline \end{array}$ | $\begin{array}{r} 0.06414 \\ 0.7273 \\ \hline \end{array}$ |
| Mean basal leaf lobule to sinus length p-value | $\begin{array}{r} 0.37474 \\ 0.0346 \\ \hline \end{array}$ | $\begin{array}{r} -0.4314 \\ 0.0137 \\ \hline \end{array}$ | $\begin{aligned} & 0.2947 \\ & 0.1016 \\ & \hline \end{aligned}$ | 0.31606 0.078 | $\begin{array}{r} -0.28123 \\ 0.1189 \\ \hline \end{array}$ | 0.19291 <br> 0.2901 | $\begin{array}{r} -0.12317 \\ 0.5018 \\ \hline \end{array}$ |
| Mean basal leaf sinus to base length p-value | $\begin{array}{r} -0.41005 \\ 0.0198 \\ \hline \end{array}$ | $\begin{array}{r} 0.30888 \\ 0.0854 \\ \hline \end{array}$ | $\begin{array}{r} -0.17111 \\ 0.3491 \\ \hline \end{array}$ | $\begin{array}{r} -0.24406 \\ 0.1783 \\ \hline \end{array}$ | $\begin{array}{r} 0.28145 \\ 0.1186 \\ \hline \end{array}$ | $\begin{array}{r} -0.18528 \\ 0.31 \\ \hline \end{array}$ | $\begin{aligned} & 0.1112 \\ & 0.5446 \\ & \hline \end{aligned}$ |
| Mean lobe width $p$-value | $\begin{array}{r} -0.16759 \\ 0.3592 \\ \hline \end{array}$ | $\begin{array}{r} -0.0225 \\ 0.9028 \\ \hline \end{array}$ | $\begin{array}{r} 0.05864 \\ 0.7499 \\ \hline \end{array}$ | $\begin{array}{r} 0.01059 \\ 0.9541 \\ \hline \end{array}$ | 0.04612 $0.8021$ | $\begin{array}{r} -0.05242 \\ 0.7757 \\ \hline \end{array}$ | $\begin{array}{r} 0.00675 \\ 0.9707 \\ \hline \end{array}$ |
| Leaf dissection p-value | $\begin{array}{r} 0.45538 \\ 0.0088 \\ \hline \end{array}$ | $\begin{array}{r} -0.4484 \\ 0.0101 \\ \hline \end{array}$ | $\begin{array}{r} 0.28569 \\ 0.1129 \\ \hline \end{array}$ | 0.33695 <br> 0.0593 | $\begin{array}{r} -0.33225 \\ 0.0632 \\ \hline \end{array}$ | $\begin{array}{r} 0.22748 \\ 0.2105 \\ \hline \end{array}$ | $\begin{array}{r} -0.12945 \\ 0.4801 \\ \hline \end{array}$ |

Figure 1. Mapped collection locations (populations) of S. campestris within the Willamette Valley. Circled populations represent clusters defined by hierarchical cluster analysis.


Figure 2. Resulting dendrogram from Hierarchical Cluster Analysis. Populations of the same color are grouped into spatial clusters defined by similar latitude and longitude.


Figure 3. NMS 3-dimensional ordination. Each symbol represents an individual in multivariate space. Symbols that are closer spatially are more similar morphologically and phenologically. Individuals from Population 12 (Glaser x Parker) are circled with a red ellipse.


Figure 4. NMS ordination of individuals within each population with variables (axis 1 vs. 2). Multiple individuals (sample units) are represented by a single color for each population.


Axis 1

Figure 5. NMS ordination with County overlay (axis 1 vs. 2). County: $1=$ Benton, $2=$ Polk, $3=$ Lane, $4=$ Linn, $5=$ Marion, $6=$ Yamhill.


Axis 1

Figure 6. NMS ordination with Eugene overlay (axis 1 vs. 2). Individuals within 20 miles of Eugene are shown in red; those outside of this seed transfer zone are shown in green.


Figure 7. NMS ordination using Clusters defined by Hierarchical Cluster Analysis overlay (axis 1 and 2).


Axis 1

Figure 8. NMS ordination using Ecoregion Level 4 defined by EPA (axis 1 and 2): $1=$ Valley Foot hills, 2 = Prairie Terraces, $3=$ Willamette River and Tributary Galley Forest.


Axis 1

Figure 9. NMS ordination axis 1 vs 2 with climatic variable correlations jointly biplotted with population (a) annual precipitation, (b) annual temperature (c) first day of fall frost (d) last day of spring frost (e) number of frost free days (average growing season length). Red lines indicate regression lines ( r ), while the blue line represents Kendall' tau (a rank regression). The size of each symbol represents the relative magnitude of the climate variables.
(a)

SICA NMS


(b)

(c)

(d)

(e)


Figure 10. NMS ordination axis 1 vs. 2 with geographic variable correlations jointly biplotted with population (a) latitude, (b) longitude. Red lines indicate regression line (r), while the blue line represents Kendall' tau (a rank regression). The size of each symbol represents the relative magnitude of latitude and longitude.
(a)

(b)


# Distribution of morphologic and phenological variation of Eriophyllum lanatum in the Willamette Valley: a common garden study to inform seed transfer zones 



## Materials and Methods

## Study species

Eriophyllum lanatum (Pursh) Forbes (Asteraceae) is a common native annual or perennial found in upland prairie habitat of the Willamette Valley, Oregon. This species is considered globally secure and is not ranked at the subnational level (G5 - SNR; NatureServe 2008). Its distribution extends from British Columbia to California and is found well east of the Cascades. Twelve varieties of E. lanatum are currently recognized; six occur in Oregon: var. achillaeiodes, var. grandiflorum, var. integrifolium, var. lanatum, var. lanceolatum, and var. leucophyllum (USDA 2008). Our analysis was confined to the variety E. lanatum var. lanatum. Eriophyllum lanatum is a perennial that typically has flower heads on single stalks, with 8-13 yellow ray flowers that bloom all spring and summer. This species is an important and distinctive member of Willamette Valley remnant prairies and is widely used in restoration.

## Population sampling

Eriophyllum lanatum var. lanatum seeds were collected in 2006 from 16 populations distributed throughout the Willamette Valley, one population south of the Willamette Valley, and two populations in the state of Washington (Fig. 1, Table 1). Latitude, longitude, and elevation were recorded at each location. At each location, no more than $25 \%$ of available seed was collected from each identified plant. A large variation in natural population sizes were present for this species; population sizes ranged from 10 to greater than 300 individuals. For analysis, locations were classified based on individual populations, county, EPA defined level 4 Ecoregion (Griffin and Omernik 2008), presence or absence within a Eugene 20 mile buffer zone, and presence or absence within the Willamette Valley.

## Experimental design

Seeds were sown on December 15, 2006 into flats of Ray Leach "Cone-tainers" and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center (PMC). The PMC is located in Benton County, Corvallis, Oregon, at 68.5 m elevation. Thirty cone-tainers were started for each population $(\mathrm{N}=224)$, and were randomly placed within flats. Flats were watered and placed in polyethylene bags and moved to a walk in cooler (dark, constant temp of $1.1^{\circ} \mathrm{C}$ ). On February 18, 2007, the cooler temperature was changed to $12.7^{\circ} \mathrm{C}$. On March 21, 2007 flats were moved to an unheated greenhouse. Average daytime temperatures ranged between 15.5 and $23.8^{\circ} \mathrm{C}$ and nighttime temps were between 7.2 and $12.7^{\circ} \mathrm{C}$. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After ten weeks in the greenhouse, plants were moved to a shade house and allowed to acclimatize for several weeks to outdoor temperatures.

Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. The study site was then covered with three inches of bark mulch to further aid in weed suppression. The 224 E. lanatum var. lanatum study plants were transplanted on May 25, 2007 using a completely randomized design. An additional row of E. lanatum was planted on each side of the plot as a border row to buffer against edge effects. Plants were placed 0.6 meters apart within rows and rows were placed one meter apart.

## Trait measurement

Traits were chosen based on characteristics described in Hickman 1993, and Hitchcock and Cronquist 2001. Traits thought to have adaptive significance, or associated with reproductive success, taxonomically important traits, and traits with high degrees of variability between varieties (E. lanatum var. achillaeoides and var. intergrifolium) were included. While measuring pre-defined traits, additional traits were included based on apparent visual differences among plants. Several botanical experts or species authorities were consulted for recommendations during the trait selection process. Table 2 shows a list of the traits and how they were measured.

Phenological and morphological traits were measured for all individual E. lanatum plants during the summer of 2008. Each growth trait was measured in a single day. To reduce measurement error, one person measured traits while a second recorded. Floral traits were
measured using only new flowers. Morphological traits less than 25 cm in length were measured by dial calipers to 0.01 mm . Traits greater than 25 cm were measured with a meter stick to the nearest centimeter. When measuring a single trait in triplicate on an individual, no measurements were made from the same organ (e.g., average petal length was the mean length of three petals from three separate flowers chosen haphazardly). An additional index of flower color was calculated as the difference in petal length of each colored segment of an individual petal (whole flower petal length - inner flower petal color length). Emergence date was monitored on a daily basis; when cone-tainers contained more than one seedling, germination was recorded for the first seedling that emerged in each cone-tainer. Flowering date was monitored on transplanted individuals three times a week until a value was obtained for each study plant. Two plants with missing values were removed from the analysis (LT0711 and RU0921).

## Climatic Data

Climatic conditions at each population location site were characterized using digital maps produced in ArcGIS 9.3 and data generated by PRISM climate models (PRISM group, 2008). PRISM is an analytical model that uses point data and a digital elevation model (DEM) to generate gridded estimates of monthly and annual average daily maximum/minimum climatic parameters (PRISM group, 2008). PRISM incorporates a conceptual framework that addresses the spatial scale and pattern of climate variables that allows for estimation of variables in regions with heterogeneous terrain (PRISM group, 2008). PRISM was parameterized to use 1971-2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for a single location is no better than half the resolution of the cell. Variables were modeled monthly. An annual average was produced by averaging the monthly grids. For this analysis, mean annual temperature (USANNAV) and precipitation (USANNP), mean dates of the first (SPRFRST) and last frost (FLLFRST), and the number of frost free days (FRSTFREE) was gathered for each population based on each population's unique latitude (LAT) and longitude (LONG).

## Data analysis

Ordination analyses were performed on morphological and phenological variables using nonmetric multidimensional scaling (NMS) based on Euclidean distance measures (Kruskal 1964). Analyses were completed using PcORD 5 (McCune and Mefford 1995). NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales (Peterson and McCune 2001). To account for variable collection on different scales (ie. Julian days and centimeters) data were relativized using the standard deviates of each column variable. Prior to relativization, difference in flower color, mean number of heads per inflorescence, and leaf width were monotonically log transformed, and total number of racemes was squareroot transformed to compress high values and spread low values by expressing values as orders of magnitude. Monotonic transformation of individual
variables allows for independent changes to data point values without altering their rank. Individuals greater than 2 standard deviates from the mean Euclidian distance were defined as outliers and removed from the analysis (CB0909, CB0217, CB0409, RO0422, HR0410, HR1202, RU1015, KI0101, KN0819, NB0704).

Since adaptive variation is important in designating seed transfer zones and is most directly related to variation within and among seed sources, we used individuals as our sampling units instead of mean population estimates. Thus, the main data matrix consisted of individual plants and traits measured at the common garden. To visually clarify the distribution of sampling units in ordination space, a second matrix with additional information (latitude, longitude, county, elevation, and climatic variables) was overlayed or jointly-biplotted (Table 1).

NMS uses an iterative search for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). To ensure that the ordination was below an acceptable level of stress, we used a random seed with 250 runs of real data. Dimensionality was assessed visually using a scree plot. Monte Carlo simulations using 250 replicates were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used.

We compared the relative position of each population in ordination space by visual assessment. Due to the degree of difficulty assessing individual sampling units within a population, we used additional coding subgroup overlays such as county ( $\mathrm{n}=7$ ), 20 mile Eugene buffer ( $\mathrm{n}=2$ ), and presence or absence within the Willamette Valley ( $\mathrm{n}=2$ ) were used (Table 1). To identify spatial clustering based on latitude and longitude we used hierarchical agglomerative clustering patterns calculated using Euclidean distances and nearest neighbor linkages to maximize distinctness of groups. The resulting clustering dendrogram was scaled using a log transformation and information was retained at the $75 \%$ and $87.5 \%$ levels (Fig. 2). This allowed us to partition the dataset into more homogenous subsets based exclusively on geographic location. These subsets were then used as additional plot overlays.

We quantitatively compared the relative position of populations in the ordination using Euclidian distances and unblocked Multi-Response Permutation Procedure (MRPP) analysis on weighted groups. MRPP is a nonparametric test used to examine whether populations on matrix plots occupy different regions of ordination space. Correlations between ordination axes and the environmental variables were calculated using Pearson Correlation coefficients. The percent of variation in the original ordination was also recorded. A Bonferroni correction was used when multiple comparisons were performed to maintain a low probability of relationships appearing significant when, in fact, they only appear significant by chance.

Univariate calculations were made for each variable by population. Traits were used to produce pairwise Pearson Correlation Coefficients for each variable with latitude, longitude, elevation, and climatic variables using SAS 9.2 (SAS Institute 2008). Ordination axes were individually regressed on geographic and climatic variables in PcORD.

## Results

## Morphological and phenological patterns across populations

The Nonmetric Multidimensional Scaling (NMS) analysis provided a 3-dimensional ordination best solution to the data based on a final stress of 19.98888, a final instability of 0.00001 and 146 iterations. Using Clarke's cutoff for acceptable instability, values between 10 and 20 represent a usable picture (McCune and Grace, 2002); however, values at the upper end suggest a potential to mislead and thus too much reliance on details of the plots should be discouraged. Together the axes explained $71.7 \%$ of the variance, $19.1 \%, 25.8 \%$ and $26.8 \%$ for axes 1,2 , and 3 respectively. Axis 1 was strongly influenced by flower petal measurements (flower color petal length, mean ray length and width), axis 2 the difference in color, and axis 3 was strongly influenced by the categorically defined flower color. These traits show strong correlations to ordination axes, and explain much of the distribution of individuals in ordination space (Fig. 3; Table 3). Since the majority of variation is accounted for within axis 2 and 3, they are used to visually describe the data.

NMS ordination provided evidence of discrete clustering of populations in ordination space based on morphological and phenological characteristics. Post hoc analysis using MultiResponse Permutation Procedure (MRPP) indicated significant differences between many individual populations (Table 4a). Although much overlap between populations exists, many populations seem to be clustered in ordination space (Fig. 4). With 161 total comparisons 156 pairs of populations were significantly different representing $97 \%$ of the population level contrasts. Further, many $A$ values were close to zero, suggesting little within group dissimilarity. $A$ values close to zero, indicate little within population similarity (i.e., individuals were not necessarily more similar to other members of their populations than to individuals from other populations). The $A$ statistic is a descriptor of within-group homogeneity compared to random expectation (McCune and Grace 2002). If heterogeneity within groups equal chance expectation then $A=1$; however, if less agreement (heterogeneity) exists within groups than expected by chance, then $A=0$. In such cases where small $A$ values are statistically significant careful consideration of the ecological significance of the results is warranted. $A$ values of less than 0.3 represent substantial heterogeneity (variability) between contrasted groups.

Using county as a subgroup overlay indicates that county is sufficient in visually defining some differences in plant variation based on the traits measured (Fig. 5). Individuals from Linn and Douglas Counties, as well as those in the State of Washington, are distinctly clustered away from individuals throughout the remaining Willamette Valley counties. Polk County individuals may also be clustered; however, those within Washington, Yamhill and Lane counties do not appear to be clustered by county and greatly overlap visually. Post hoc MRPP analysis suggests all counties differ significantly from one another, which is expected since it reflects much of the population level contrasts (Table 4b). Populations that dominate many of the population level comparisons have the potential to strongly influence subsequent overlays used in analysis.

Similarly, some clustering of predefined population groups appears in the Eugene overlay. This clustering is greatly influenced by individuals outside the Willamette Valley, as well as the more unusual individuals found in Linn County (Fig. 6). Again, significant differences were detected between populations as being either outside or within the Eugene 20 mile buffer ( $\mathrm{T}=-28.814378, \mathrm{~A}=0.01837289, p<0.0001$ ) based on post hoc MRPP; however
significant difference are not present when the Washington (Olympic Mountains) populations are removed. This pattern matches many of the individual population and county level differences.

The Willamette Valley visual overlay suggests that individuals within the valley cluster based on the traits measured (Fig. 7). Individuals found outside the valley, whether in the State of Washington or in Douglas County, Oregon cluster seemingly surrounding individuals within the valley. Again, this corresponds to significant differences within the population and county overlays further suggesting that, at a minimum, the Willamette Valley as a whole should be considered a distinct seed zone. Individuals within the Valley that appear close to clusters outside the valley are all from Kingston Prairie and Cole School Rd (Linn County), which are both at elevations greater than 219 m . To explore this pattern further, we grouped populations into a high elevation overlay that defined all populations greater than 219 m as high elevation. Here most of the low elevation populations tightly cluster (Fig. 8a). Jointly biplotting the difference in color with elevation suggests that flower petals, of lower elevation populations, are a single color or have a larger inner flower petal color length (Fig. 8b).

Clustering of populations based on Hierachical Clustering at the 9.2E-06 (75\%) and 6.2E08 (87.5\%) levels using Euclidean distances resulted in 3 and 5 geographic clusters respectively (Fig. 9). At the 75\% coarse clustering level, visual inspection of ordination revealed separation of Clusters 2 and 3 from each other but both overlap with Cluster 1. MRPP suggests several differences in clusters comparable to population and county levels (Table 4). Clustering at the $87.5 \%$ level suggests 2,4 and 5 as separate but again this yields the same results (Fig. 10). Thus, hierarchically defined clusters appear ineffectual for grouping our data for use as a meaningful ordination overlay. When populations outside the Willamette are included in the analysis, county appears to be a better geographic representation than clusters defined by hierarchical clustering.

EPA defined ecoregions (level 4) within the Willamette Valley overlapped in the ordination (Fig. 11). Ecoregions not found in the Willamette Valley were concentrated and few individuals overlapped with populations within the Valley further indicating that use of seed from outside the Willamette Valley is not appropriate. Post hoc MRPP analysis again indicates ecoregion level differences are significant but again small A values suggest this difference is not biologically meaningful (Table 4 e). These results correspond to other overlays investigated in this analysis.

## Correlations of traits with geographic and climatic variables

Twenty-two traits were measured or calculated for each individual in each population. Ranges and means ( $\pm 1 \mathrm{SD}$ ) of populations indicate substantial variability both between and within populations (Appendix B, C). High levels of within population variability suggest high levels of polymorphism in populations. Population level variability is apparent in most traits.

Regression of ordination axes with climatic and geographic variables identified a few weak associations (Table 3), (Figs. 12-13). It is important to note that correlations with axes are difficult to ascertain since variables are not necessarily linearly correlated or parallel to axes.

Pearson's Correlation coefficients between univariate traits and geographic and climatic variables identified some significant but weak correlations. Longitude was negatively correlated with mean ray length and width, as well as flower color and flower petal length but these
associations were very weak (Table 5). Elevation was weakly correlated with mean ray width, the difference in flower color and negatively associated with flower color 2 (Table 5). Frost free date was also positively associated with the difference in color yet negatively associated with flower color 2. The first frost free day of spring was positively correlated with mean ray length and width, and the difference in color, while negatively associated with flower color 2. Mean annual temperature and precipitation had the strongest correlations with the difference in color, flower color 2 and emergence date (Table 5). Although significant, no correlation was stronger than 0.46 (diffcolr $\times$ usannp). Using a subset of populations found only within the Willamette Valley, all correlations are further reduced and no correlation is stronger than 0.40 (longitude $\times$ total racemes). Morphological and phenological variables are poorly correlated with geographic and climatic variables especially between populations in the Willamette Valley.

## DISCUSSION

Populations of E. lanatum var. lanatum show some discreet clustering in ordination space based on morphological and phenological characteristics; however, these clusters do not appear to follow a clear geographic pattern within the Willamette Valley. Populations that are closer together geographically do not appear to be more similar morphologically. The traits in the NMS ordination that explained the most variation across populations were flower color, flower color petal length, and the difference in flower color. These traits were moderately correlated with all axes, but are highly variable both within and among populations. That is, individuals within populations displayed a large range in flower color traits, as well as different populations tending to have different colored ray flowers.

Using county as a subgroup overlay suggests that county is marginally sufficient in visually defining differences in plant variation based on the traits measured. Relative to the distribution of this species, however, few counties are represented within this study. Eriophyllum lanatum var. lanatum is clearly distributed to a greater extent within each county, but these populations are not represented within this common garden (Oregon Plant Atlas, December 5, 2008). This substantially limits our ability to make inferences in areas undersampled or absent from the collection. County is an artificial boundary often used by managers and is rarely biologically meaningful. Although county appears to be adequate to define areas, substantial amounts of missing data limit the usefulness of this classification to inform seed zones. We strongly recommend addition of other populations and more individuals within all counties in future seed guideline studies. Too few seed sources and representative samples from populations make accurate inferences in these areas of the valley difficult to ascertain.

Although much overlap between populations exists, many populations seem to be clustered in ordination space. MRPP post hoc analysis suggests little within group dissimilarity between populations. The within group dissimilarity is more than expected by chance. Not surprisingly, Hurricane Ridge, Elwha (elev. $1602 \mathrm{~m}, 248 \mathrm{~m}$ ), and North Bank/Roseburg (elev. 244 m ) appear to cluster away from most other populations, but have some overlap with populations within the Willamette Valley above 200 m in elevation. Regardless of whether the
population was in the State of Washington or in Douglas County, Oregon, those outside the Willamette Valley cluster away from those within. This pattern is further exemplified in all other overlays. Willamette Valley plants tend to have greater variability in floral traits, while those outside the Valley display less differentiation in flower color. In particular, the high Olympic populations had relatively small lengths of inner flower color in relation to the entire petal length creating larger differences in color. This pattern may be due to pollinator-driven selection, non-pollinator driven selection (e.g., see Strauss and Whittall 2006), or non-adaptive factors. High levels of and/or current gene flow in the Willamette Valley is likely responsible for the uniformity across low elevation populations. The differences inside and outside of the Willamette Valley suggests that, at a minimum, seed sources from outside the Willamette Valley are not recommended for use in restoration within the Valley.

Individuals within the Willamette Valley that are most morphologically similar to clusters outside the valley (in the ordination) are from Kingston Prairie and Cole School Rd (Linn County), which are both at elevations greater than 219 m . Populations grouped as low elevation (less than 219 m ) tightly cluster particularly when color differences (DIFFCOLR ) are less pronounced. However, overlap exists along the boundary of these clusters in the ordination, revealing overlap of individuals within Lane and Washington County populations. This corresponds to the many population level MRPP contrasts that indicate a high degree of variability within and between populations. Cole School Rd and Hacker Rd depart from this pattern and were generally less variable within populations. This is likely an artifact of relatedness of individuals within the common garden. Collection records indicate small populations at both locations (three and ten individuals. respectively). Since each population is represented by 20 common garden individuals, there is no question that some level of relatedness exists among individuals. During the original seed collection maternal records were not kept and instead seed was bulked by location. Random selection of seed was used to propagate replicates for each population but for small populations the probability of selecting related replicates is high. Reduced levels of within population variance are expected when individuals within a population are related. Without maternal records indicating relatedness of individuals, within population variance can not be accurately estimated. Removing these populations from the analysis did not change the general patterns observed and instead it clarified the distinctness of populations outside the Willamette Valley (not shown).

## Correlations of traits with geographic and climatic variables

Morphological and phenological traits were poorly correlated with geographic and climatic variables. Pearson's Correlation Coefficients between univariate traits and geographic or climatic variables identified some significant correlations. The strongest positive correlation was represented by the difference in flower color and annual average precipitation. Later emergence dates with lower amounts of annual average precipitation was the only moderate phenological/climatic correlation. Since populations from outside the Willamette Valley are from much different environments, stronger correlations with climatic variables were anticipated. However, these results may indicate that mean values based on these PRISM data may be too coarse. Investigation of finer scale climatic variables to discern slight microclimate difference
could prove useful. Alternatively, little correlation between climate and morphological or phenological variation may exist for this taxon.

## RECOMMENDATIONS

In summary, our findings suggest that populations of E. lanatum var. lanatum, within the Willamette Valley counties included in this study are morphologically and climatically within a single ecotype up to approximately 200 m in elevation. Although elevation did not appear as a strong geographic variable in the correlation matrix, it is a measureable marker that delineates Kingston Prairie from the remaining populations. Kingston Prairie is a TNC preserve which represents one of the best examples of native Willamette Valley Prairie that has retained much of its original vegetation in both wet and dry upland areas. Kingston Prairie also has much different soil structure than most other prairies in the valley where it is characterized by basalt bedrock that underlays shallow soil. Due to its uniqueness in both soil structure and as a population which stands out in this analysis, we do not recommend using seed from Kingston Prairie in large scale production efforts for widespread use at restoration sites in the Willamette Valley. We also do not recommend movement of seed from sources outside the Willamette Valley, or beyond within Valley distributions without further study. Realistically this study has too few seed sources and representative samples to make precise inferences valley wide. Populations from a full range of environmental and climatic conditions in which this species occurs should be included in future seed guideline studies. Additionally, studies are recommended to determine the presence and/or scale of local adaptation and the genetic basis for the adaptations if they are found.

Table 1. List of population names with corresponding abbrevations and number of individuals represented per population as sampling units. County: $1=$ State of Washington, $2=$ Washington (county), $3=$ Yamhill, $4=$ Polk, $5=$ Linn, $6=$ Lane, $7=$ Douglas (outside Willamette Valley). Eugene: $1=$ within 20 mile buffer, $0=$ not present in buffer. Willamette Valley $1=\mathrm{IN}, 2=$ OUT. Within 20 mile Eugene buffer $=1$, outside $=2$. Cluster75 is based on dendrogram interpretation at the $9.2 \mathrm{E}-06$ level and Cluster 87 at the $6.2 \mathrm{E}-08$ level.

| Population | Code | Eugene | Cluster75 | Cluster87 | Ecoregion | County | Willamette <br> Valley | LAT | LONG |  |
| :--- | :---: | :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Coble | 1 | CB | 1 | 1 | 1 | 2 | 6 | 1 | 44.06434 | -123.20040 |
| Cooper Mountain | 2 | CM | 0 | 2 | 3 | 1 | 2 | 1 | 45.44922 | -122.87190 |
| Crowe | 3 | CR | 0 | 2 | 3 | 1 | 4 | 1 | 45.05702 | -123.47150 |
| Cole School Rd | 4 | CS | 0 | 2 | 4 | 1 | 5 | 1 | 44.72621 | -122.79930 |
| Elwha | 5 | ER | 0 | 3 | 5 | 4 | 1 | 2 | 44.14750 | -123.28750 |
| Fisher Butte | 6 | FB | 1 | 1 | 1 | 2 | 6 | 1 | 44.05571 | -123.25270 |
| Greenhill | 7 | GR | 0 | 2 | 3 | 1 | 3 | 1 | 45.16600 | -123.31300 |
| Hacker Rd | 8 | HA | 1 | 1 | 1 | 2 | 6 | 1 | 44.06807 | -123.24900 |
| Hurricane Ridge | 9 | HR | 0 | 3 | 5 | 4 | 1 | 2 | 45.37649 | -123.25870 |
| Hazel Dell | 10 | HZ | 1 | 1 | 1 | 1 | 6 | 1 | 44.02975 | -123.22120 |
| Kirk Pond | 11 | KI | 1 | 1 | 1 | 2 | 6 | 1 | 44.11000 | -123.27000 |
| Kingston Prairie | 12 | KN | 0 | 2 | 4 | 1 | 5 | 1 | 44.77534 | -122.74430 |
| Long Tom ACEC | 13 | LT | 1 | 1 | 1 | 2 | 6 | 1 | 44.14059 | -123.28090 |
| North |  |  |  |  |  |  |  |  |  |  |
| Bank/Roseburg | 14 | NB | 0 | 1 | 2 | 5 | 7 | 2 | 44.13450 | -123.29520 |
| Oxbow East | 15 | OE | 1 | 1 | 1 | 2 | 6 | 1 | 44.05659 | -123.18420 |
| Oxbow West | 16 | OW | 1 | 1 | 1 | 2 | 6 | 1 | 44.05278 | -123.18880 |
| Royal Amazon | 17 | RA | 0 | 2 | 4 | 2 | 5 | 1 | 44.57280 | -122.79900 |
| Rose Prairie | 18 | RO | 1 | 1 | 1 | 2 | 6 | 1 | 44.08100 | -123.23500 |
| Rupers | 19 | RU | 0 | 2 | 3 | 1 | 4 | 1 | 45.03515 | -123.42490 |

Table 2. List of phenological and morphological traits measured on E. lanatum plants in common garden study.

| Trait | Trait | Measurement |
| :---: | :---: | :---: |
| Emergence date | JULEMR | Date of cotelydon emergence (Julian) |
| Flowering date | FLWRJUL | Date of first flower was recorded for each individual. This traits was recorded three times a week until a value was obtained for each study plant (Julian) |
| Mean flower ray length | AVERAYL | Mean flower ray length was measured on three flower heads chosen haphazardly (mm) |
| Mean flower ray width | AVERAYW | Mean flower ray width was measured on three flower heads chosen haphazardly (mm) |
| Mean flower head diameter | AVEFHDIA | Mean flower head diameter (does not include rays) (mm) |
| Total number of racemes | EXTOTRCM | The number of racemes was counted in a 0.125 subsample and was extrapolated to calculate the total number of racemes within an individual plant. |
| Height | HT | Total height from base to tallest flower head (cm) |
| Diameter | DIA | Total plant diameter measured across the top of the plant (cm) |
| Leaf arrangement | LARRGT | Leaf arrangement: <br> 1 Opposite <br> 2 Alternate |
| Leaf edge | LEAFEDG | Leaf edge: <br> 1 Planar <br> 2 Revolute |
| Inner flower color | FLCR1 | Often 2 colors appeared on a single ray. In this case the inner flower color was measured in terms of length on a single ray (mm). |
| Whole flower ray length | FCPTL | On the date inner flower color was measured, a measure of the entire ray length was also measured (mm). |
| Difference in color | DIFFCOLR $\Theta$ | Difference in flower color was calculated by subtracting the inner flower color from the whole ray length. |
| Categorical flower color | FLCL2 | Categorical:   <br> 1 DY Dark Yellow <br> 2 Y Yellow <br> 3 LY Light Yellow <br> 4 DYY Dark Yellow (inner) Yellow (outer) <br> 5 DYLY Dark Yellow (inner) Light Yellow (outer) |
| Mean number of flowers per raceme | AVEHDINFL | Mean number of flowers per raceme was measured on three racemes chosen haphazardly |
| Mean peduncle length | AVEPEDL | Mean peduncle length was measured on three racemes chosen haphazardly (mm) |
| Mean flower ray length | AVERAY | Mean ray length measured on three flower heads chosen haphazardly (mm) |
| Leaf color | LEAFCLR | Categorical: <br> 1 Not silvery <br> 2 Moderately silvery <br> 3 Very silvery |
| Mean basal leaf length | AVELEAFL | Mean basal leaf length measured leaves chosen haphazardly(cm) |


| Mean number of leaf lobes | AVELOBE | Mean number of lobes present on one side of a leaf <br> measured on three leaves chosen haphazardly |
| :--- | :--- | :--- |
| Mean leaf tip | AVELEAFTIP | Mean leaf tip: Pointed or rounded (recorded for 3 <br> haphazardly chosen leaves) |
| Leaf width | LEAFW | Leaf width measured on a single basal leaf chosen <br> haphazardly $(\mathrm{cm})$ |

Table 3. Correlation of axes with measured trait variables and secondary geospatial and climatic variables.

|  | Axis 1 |  | Axis 2 |  | Axis 3 |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathbf{R}^{\mathbf{2}}$ | tau | $\mathbf{R}^{\mathbf{2}}$ | tau | $\mathbf{R}^{\mathbf{2}}$ | tau |
| Elevation | 0.066 | 0.089 | 0.055 | -0.235 | 0.028 | -0.150 |
| Latitude | 0.054 | 0.024 | 0.038 | -0.128 | 0.073 | -0.082 |
| Longitude | 0.281 | -0.364 | 0.023 | -0.016 | 0.004 | 0.032 |
| First date of Fall frost | 0.025 | -0.037 | 0.045 | 0.120 | 0.067 | 0.126 |
| Number of frost free days | 0.100 | -0.101 | 0.029 | 0.062 | 0.081 | 0.136 |
| Last date of spring frost | 0.172 | 0.345 | 0.015 | 0.075 | 0.080 | -0.107 |
| Mean annual temperature | 0.083 | -0.017 | 0.071 | 0.166 | 0.092 | 0.070 |
| Mean annual precipitation | 0.078 | -0.025 | 0.076 | -0.182 | 0.108 | -0.161 |
| Flowering date | 0.006 | -0.040 | 0.004 | -0.035 | 0.000 | 0.035 |
| Mean ray length | 0.484 | $\mathbf{0 . 4 8 7}$ | 0.009 | -0.053 | 0.029 | 0.098 |
| Mean ray width | 0.490 | $\mathbf{0 . 4 7 8}$ | 0.000 | 0.017 | 0.002 | -0.034 |
| Mean flower head diameter | 0.022 | -0.110 | 0.001 | -0.066 | 0.002 | -0.010 |
| Total number of racemes | 0.101 | -0.207 | 0.073 | -0.231 | 0.150 | 0.287 |
| Height | 0.001 | 0.019 | 0.242 | -0.357 | 0.118 | 0.209 |
| Diameter | 0.004 | -0.061 | 0.299 | -0.364 | 0.211 | 0.327 |
| Flower color | 0.087 | 0.242 | 0.250 | 0.340 | $\mathbf{0 . 5 5 1}$ | $\mathbf{0 . 5 5 4}$ |
| Flower color petal length | $\mathbf{0 . 5 0 7}$ | $\mathbf{0 . 5 1 6}$ | 0.057 | -0.159 | 0.070 | 0.176 |
| Difference in color | 0.037 | 0.107 | 0.461 | $\mathbf{- 0 . 5 0 6}$ | 0.332 | -0.403 |
| Mean number of flowers heads per raceme | 0.011 | 0.081 | 0.109 | -0.226 | 0.017 | 0.032 |
| Mean peduncle length | 0.005 | -0.033 | 0.123 | -0.253 | 0.141 | 0.263 |
| Mean ray length | 0.138 | -0.302 | 0.019 | -0.093 | 0.001 | -0.030 |
| Emergence date | 0.005 | 0.011 | 0.124 | 0.239 | 0.025 | 0.123 |
| Mean leaf length | 0.095 | 0.194 | 0.035 | -0.125 | 0.043 | -0.118 |
| Mean number of lobes | 0.064 | -0.178 | 0.103 | -0.236 | 0.019 | -0.097 |
| Basal leaf width | 0.185 | 0.282 | 0.000 | -0.013 | 0.044 | -0.180 |

Table 4. Significantly different MRPP multiple pair-wise comparisons with adjusted Bonferroni correction, test statistic T and agreement statistic A grouped by (a) population ( $p<0.0003$ ) (b) county ( $p<0.003$ ) (c) dendrogram defined cluster 75 ( $p<0.001$ ) (d) dendrogram defined cluster 87.5 ( $p<0.001$ ).
(a)

| Populations | T | A | 10 vs. 18 | -16.99578 | 0.13446 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| vs. 10 | -16.76739 | 0.13763 | 12 vs. 14 | -13.36997 | 0.10395 |
| 2 vs. 4 | -16.09353 | 0.09615 | 12 vs. 16 | -14.05758 | 0.11504 |
| 2 vs. 5 | -15.75794 | 0.09108 | 12 vs. 17 | -13.46725 | 0.08429 |
| 2 vs. 6 | -15.40643 | 0.08986 | 12 vs. 18 | -13.03645 | 0.08799 |
| 2 vs. 8 | -16.54999 | 0.10461 | 12 vs. 19 | -14.70170 | 0.09447 |
| 2 vs. 9 | -15.14626 | 0.09278 | 13 vs. 19 | -12.48738 | 0.08679 |
| 2 vs. 10 | -18.92427 | 0.12424 | 14 vs. 17 | -15.61606 | 0.14794 |
| 2 vs. 13 | -14.19794 | 0.09372 | 1 vs. 4 | -12.98632 | 0.08985 |
| 2 vs. 14 | -15.60339 | 0.11260 | vs. 9 | -13.43063 | 0.09571 |
| 3 vs. 4 | -17.92360 | 0.17564 | 1 vs. 12 | -11.79175 | 0.07863 |
| 3 vs. 12 | -15.22418 | 0.12825 | 1 vs. 14 | -14.04833 | 0.14234 |
| 4 vs. 5 | -18.15656 | 0.11547 | vs. 6 | -14.94918 | 0.12379 |
| 4 vs. 6 | -15.39986 | 0.09749 | 4 vs. 16 | -14.26376 | 0.11484 |
| 4 vs. 8 | -14.60259 | 0.08887 | vs. 13 | -16.69123 | 0.14156 |
| 4 vs. 9 | -17.36063 | 0.11702 | vs. 14 | -11.57045 | 0.07953 |
| 4 vs. 10 | -22.86811 | 0.21045 | vs. 16 | -12.34004 | 0.09373 |
| 4 vs. 11 | -14.18262 | 0.10627 | 5 vs. 18 | -13.86182 | 0.09739 |
| 4 vs. 13 | -16.44888 | 0.12275 | vs. 10 | -15.24368 | 0.14521 |
| 4 vs. 14 | -17.37905 | 0.15458 | vs. 10 | -16.45549 | 0.12831 |
| 4 vs. 17 | -13.57363 | 0.08583 | vs. 12 | -12.52939 | 0.07559 |
| 4 vs. 18 | -13.97110 | 0.09397 | vs. 16 | -14.29642 | 0.12152 |
| 4 vs. 19 | -18.55718 | 0.13403 | vs. 17 | -15.57354 | 0.11798 |
| 5 vs. 6 | -17.52462 | 0.12313 | vs. 16 | -13.75169 | 0.10855 |
| 5 vs. 8 | -16.60671 | 0.10105 | vs. 18 | -14.01815 | 0.10311 |
| 5 vs. 10 | -16.13506 | 0.10948 | 10 vs. 11 | -15.00840 | 0.12987 |
| 5 vs. 12 | -16.53944 | 0.10244 | 12 vs. 13 | -10.81108 | 0.06984 |
| 5 vs. 17 | -15.07620 | 0.10310 | 13 vs. 14 | -14.26551 | 0.14428 |
| 6 vs. 9 | -15.63665 | 0.10060 | 14 vs. 18 | -14.72049 | 0.14469 |
| 6 vs. 10 | -20.51342 | 0.16447 | 1 vs. 5 | -12.83637 | 0.08847 |
| 6 vs. 12 | -12.93374 | 0.07720 | vs. 5 | -15.42277 | 0.13697 |
| 6 vs. 14 | -16.19013 | 0.14279 | vs. 17 | -13.96890 | 0.12190 |
| 6 vs. 19 | -12.36992 | 0.07286 | 5 vs. 11 | -13.70603 | 0.10465 |
| 8 vs. 14 | -16.76758 | 0.14273 | vs. 16 | -12.79725 | 0.10205 |
| 9 vs. 12 | -15.27813 | 0.09767 | 13 vs. 17 | -12.89916 | 0.09768 |
| 9 vs. 13 | -14.65773 | 0.10660 | 13 vs. 18 | -12.99500 | 0.10678 |
| 9 vs. 17 | -15.18180 | 0.10694 | vs. 8 | -14.04526 | 0.11094 |
| 10 vs. 12 | -21.04095 | 0.17448 | 5 vs. 7 | -13.55572 | 0.11345 |
| 10 vs. 13 | -17.35907 | 0.15588 | vs. 8 | -14.12930 | 0.09529 |
| $10 \quad$ vs. 14 | -18.42114 | 0.18890 | 8 vs. 13 | -13.81187 | 0.10639 |
| 10 vs. 15 | -14.23492 | 0.10561 | 14 vs. 16 | -14.04374 | 0.15843 |
| 10 vs. 16 | -15.96552 | 0.13885 | 14 vs. 19 | -13.59980 | 0.12468 |
| 10 vs. 17 | -18.45178 | 0.15246 | 17 vs. 19 | -10.96699 | 0.06871 |


| 2 vs. 7 <br> 4 vs. 15 | -10.23488 | 0.06799 | $\begin{array}{llll}6 & \text { vs. } & 11 \\ 6 & \text { vs. } & 7\end{array}$ | -7.45507 | 0.04664 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 4 vs. 15 | -12.84255 | 0.09327 | 6 vs. 7 | -7.88690 | 0.05562 |
| 11 vs. 14 | -14.18097 | 0.16511 | 11 vs. 18 | -8.37990 | 0.06437 |
| 7 vs. 9 | -12.99978 | 0.10803 | 6 vs. 17 | -7.38050 | 0.04166 |
| 7 vs. 12 | -11.19881 | 0.08372 | 8 vs. 19 | -9.88898 | 0.06835 |
| 11 vs. 12 | -9.84468 | 0.06538 | 15 vs. 19 | -8.08696 | 0.05520 |
| vs. 3 | -12.67012 | 0.11566 | 11 vs. 15 | -7.82060 | 0.06225 |
| vs. 13 | -11.30227 | 0.09095 | 3 vs. 8 | -9.62384 | 0.08276 |
| 2 vs. 16 | -9.97067 | 0.06380 | 10 vs. 19 | -7.64152 | 0.04634 |
| vs. 17 | -9.67232 | 0.05409 | 6 vs. 18 | -6.87493 | 0.04190 |
| vs. 14 | -11.64208 | 0.08930 | 1 vs. 11 | -6.70176 | 0.04967 |
| 3 vs. 18 | -10.91536 | 0.08965 | 7 vs. 11 | -7.36479 | 0.06237 |
| 5 vs. 19 | -12.74474 | 0.08195 | 11 vs. 19 | -6.48338 | 0.04364 |
| 8 vs. 9 | -11.99994 | 0.07572 | 9 vs. 19 | -7.79298 | 0.04980 |
| vs. 18 | -13.07696 | 0.09850 | 1 vs. 6 | -5.53977 | 0.03488 |
| 12 vs. 15 | -12.17118 | 0.09081 | 4 vs. 12 | -5.72101 | 0.03062 |
| 3 vs. 14 | -13.60587 | 0.15785 | 5 vs. 9 | -4.84728 | 0.02511 |
| 9 vs. 11 | -11.95229 | 0.09030 |  |  |  |
| 16 vs. 19 | -10.15806 | 0.07413 |  |  |  |
| 2 vs. 3 | -9.96916 | 0.06489 |  |  |  |
| 11 vs. 13 | -10.18856 | 0.08149 |  |  |  |
| 1 vs. 2 | -9.46554 | 0.05630 |  |  |  |
| 4 vs. 7 | -10.82984 | 0.07981 |  |  |  |
| 2 vs. 12 | -9.47843 | 0.05025 |  |  |  |
| vs. 14 | -13.20211 | 0.16823 |  |  |  |
| 14 vs. 15 | -13.11921 | 0.14379 |  |  |  |
| 13 vs. 16 | -13.50754 | 0.15159 |  |  |  |
| 8 vs. 15 | -11.98787 | 0.08809 |  |  |  |
| 3 vs. 15 | -11.75268 | 0.10441 |  |  |  |
| 9 vs. 10 | -11.09700 | 0.06874 |  |  |  |
| 13 vs. 15 | -11.69256 | 0.10011 |  |  |  |
| 3 vs. 16 | -11.10815 | 0.11030 |  |  |  |
| vs. 19 | -9.01703 | 0.05806 |  |  |  |
| 2 vs. 18 | -9.30388 | 0.05438 |  |  |  |
| 7 vs. 13 | -11.54648 | 0.10945 |  |  |  |
| 2 vs. 15 | -9.79410 | 0.06192 |  |  |  |
| 3 vs. 7 | -11.12973 | 0.10955 |  |  |  |
| 6 vs. 13 | -8.57120 | 0.05255 |  |  |  |
| 3 vs. 13 | -12.47211 | 0.12260 |  |  |  |
| 9 vs. 15 | -10.44495 | 0.07164 |  |  |  |
| 3 vs. 10 | -10.23230 | 0.07831 |  |  |  |
| 11 vs. 16 | -10.68928 | 0.10351 |  |  |  |
| 2 vs. 19 | -8.84978 | 0.04725 |  |  |  |
| 5 vs. 15 | -10.43839 | 0.07067 |  |  |  |
| 6 vs. 15 | -8.45633 | 0.05296 |  |  |  |
| 3 vs. 11 | -11.09340 | 0.10619 |  |  |  |
| 3 vs. 9 | -11.96133 | 0.10548 |  |  |  |
| 7 vs. 19 | -9.06845 | 0.06862 |  |  |  |
| 2 vs. 11 | -8.75575 | 0.05640 |  |  |  |
| 11 vs. 17 | -8.82501 | 0.06368 |  |  |  |
| 8 vs. 11 | -11.28912 | 0.09374 |  |  |  |
| 18 vs. 19 | -8.50294 | 0.05509 |  |  |  |
| 7 vs. 8 | -10.03969 | 0.08044 |  |  |  |


| County |  | T | $\mathbf{A}$ |  |
| :--- | :--- | :--- | :---: | :---: |
| 6 | vs. | 2 | -15.67016 | 0.01857 |
| 6 | vs. | 4 | -21.64843 | 0.02474 |
| 6 | vs. | 5 | -39.67795 | 0.04350 |
| 6 | vs. | 1 | -40.29290 | 0.04454 |
| 6 | vs. | 3 | -27.16104 | 0.03357 |
| 6 | vs. | 7 | -34.42795 | 0.04394 |
| 2 | vs. | 4 | -11.68747 | 0.04803 |
| 2 | vs. | 5 | -16.64525 | 0.05972 |
| 2 | vs. | 1 | -20.20784 | 0.07573 |
| 2 | vs. | 3 | -16.54999 | 0.10461 |
| 2 | vs. | 7 | -15.60339 | 0.11260 |
| 4 | vs. | 5 | -31.99740 | 0.11697 |
| 4 | vs. | 1 | -21.95853 | 0.07749 |
| 4 | vs. | 3 | -13.27774 | 0.06761 |
| 4 | vs. | 7 | -19.28299 | 0.11837 |
| 5 | vs. | 1 | -31.62970 | 0.09294 |
| 5 | vs. | 3 | -17.99896 | 0.06768 |
| 5 | vs. | 7 | -21.55827 | 0.10162 |
| 1 | vs. | 3 | -18.97881 | 0.07291 |
| 1 | vs. | 7 | -15.27939 | 0.06284 |
| 3 | vs. | 7 | -16.76758 | 0.14273 |

(c)

| Cluster $75 \%$ |  |  | T | A |
| :---: | :---: | ---: | :---: | :---: |
| 1 | vs. | 2 | -11.44048 | 0.00833 |
| 1 | vs. | 3 | -27.72797 | 0.02938 |
| 2 | vs. | 3 | -33.94514 | 0.04600 |

(d)

| Cluster87\% |  | T | $\mathbf{A}$ |  |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 3 | -12.73099 | 0.01251 |
| 1 | vs. | 4 | -23.96985 | 0.02538 |
| 1 | vs. | 5 | -32.01758 | 0.03703 |
| 1 | vs. | 2 | -30.09158 | 0.04098 |
| 3 | vs. | 4 | -27.45711 | 0.04957 |
| 3 | vs. | 5 | -29.43585 | 0.06460 |
| 3 | vs. | 2 | -25.07209 | 0.07289 |
| 4 | vs. | 5 | -33.11121 | 0.07725 |
| 4 | vs. | 2 | -23.89580 | 0.07894 |
| 5 | vs. | 2 | -15.27939 | 0.06284 |

(e)

| Ecoregion |  |  | $\mathbf{T}$ |
| :---: | :---: | :---: | :---: |
| 2 | vs. | 1 | -19.777 |
| 2 | vs. | 4 | -39.699 |
| 2 | vs. | 5 | -31.965 |
| 1 | vs. | 4 | -26.851 |
| 1 | vs. | 5 | -23.237 |
| 4 | vs. | 5 | -15.279 |

Table 5. Univariate Pearson Correlation Coefficients of measured traits with geographic and climatic variables. Significant correlations are shown in highlighted bold.

|  | Latitude | Longitude | Elevation | First day of fall frost | Last day of spring frost | Mean annual temperature | Mean annual precipitation |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Emergence date | 0.0567 | 0.0745 | -0.2541 | 0.2573 | -0.2966 | 0.3814 | -0.4102 |
| p-value | 0.3131 | 0.1843 | <. 0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 |
| Flowering date | 0.0435 | 0.0506 | -0.1566 | 0.1807 | -0.2650 | 0.1973 | -0.1951 |
| $p$-value | 0.4389 | 0.3676 | 0.0051 | 0.0012 | <. 0001 | 0.0004 | 0.0005 |
| Mean ray length | -0.0505 | -0.3224 | 0.2083 | -0.1247 | 0.3208 | -0.2332 | 0.2264 |
| $p$-value | 0.3684 | <. 0001 | 0.0002 | 0.0260 | <. 0001 | <. 0001 | <. 0001 |
| Mean ray width | 0.2141 | -0.3672 | 0.3561 | -0.2556 | 0.3811 | -0.3068 | 0.2857 |
| p-value | 0.0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 |
| Mean flower head diameter | -0.0658 | 0.0723 | -0.0378 | $\mathbf{0 . 1 2 0 6}$ | -0.1442 | 0.1463 | -0.1524 |
| $p$-value | 0.2414 | 0.1977 | 0.5008 | 0.0313 | 0.0099 | 0.0089 | 0.0064 |
| Total racemes | 0.0372 | 0.3718 | -0.0964 | -0.0114 | -0.1541 | 0.0292 | -0.0304 |
| $p$-value | 0.5081 | <. 0001 | 0.0856 | 0.8393 | 0.0058 | 0.6033 | 0.5885 |
| Height | 0.1435 | 0.0816 | -0.0352 | 0.0234 | -0.1491 | 0.0882 | -0.1022 |
| $p$-value | 0.0103 | 0.1462 | 0.5312 | 0.6772 | 0.0076 | 0.1160 | 0.0684 |
| Diameter | 0.0396 | 0.1464 | -0.0391 | 0.0137 | -0.1072 | 0.0438 | -0.0505 |
| p-value | 0.4810 | 0.0088 | 0.4866 | 0.8074 | 0.0558 | 0.4352 | 0.3686 |
| Leaf arrangement | 0.0676 | $\mathbf{0 . 2 1 2 5}$ | 0.0661 | -0.0589 | -0.0637 | -0.0399 | 0.0496 |
| p-value | 0.2284 | 0.0001 | 0.2392 | 0.2944 | 0.2569 | 0.4781 | 0.3769 |
| Leaf edge | 0.0349 | -0.0693 | 0.0475 | 0.0093 | 0.0044 | -0.0042 | 0.0050 |
| $p$-value | 0.5342 | 0.2174 | 0.3982 | 0.8682 | 0.9376 | 0.9401 | 0.9298 |
| Flower color | -0.0139 | -0.2087 | -0.2151 | 0.2749 | -0.1712 | 0.2818 | -0.3007 |
| $p$-value | 0.8046 | 0.0002 | 0.0001 | <. 0001 | 0.0021 | <. 0001 | <. 0001 |
| Flower color petal length | 0.0156 | -0.2674 | 0.1859 | -0.1351 | 0.2709 | -0.2239 | 0.2096 |
| $p$-value | 0.7818 | <. 0001 | 0.0008 | 0.0157 | <. 0001 | <. 0001 | 0.0002 |
| Difference in color | 0.0256 | 0.0187 | 0.3555 | -0.3797 | 0.3725 | -0.4514 | 0.4603 |
| $p$-value | 0.6491 | 0.7399 | <. 0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 |
| Categorical flower color | -0.0726 | 0.0541 | -0.3205 | 0.3259 | -0.3313 | 0.4007 | -0.4177 |
| $p$-value | 0.1956 | 0.3357 | <. 0001 | <. 0001 | <. 0001 | <. 0001 | <. 0001 |
| Mean number of flower heads per raceme | 0.1303 | 0.0162 | 0.1417 | -0.0551 | 0.0059 | -0.0965 | 0.0869 |
| p-value | 0.0200 | 0.7729 | 0.0113 | 0.3267 | 0.9165 | 0.0854 | 0.1212 |
| Mean peduncle length | 0.0514 | 0.1312 | -0.0993 | 0.1264 | -0.2470 | $\mathbf{0 . 2 1 1 8}$ | -0.2270 |
| $p$-value | 0.3598 | 0.0191 | 0.0767 | 0.0239 | <. 0001 | 0.0001 | <. 0001 |
| Mean number of rays | -0.0658 | 0.3279 | -0.0647 | 0.0697 | -0.1910 | 0.1251 | -0.1234 |
| $p$-value | 0.2415 | <. 0001 | 0.2491 | 0.2145 | 0.0006 | 0.0255 | 0.0276 |
| Leaf color | -0.1507 | 0.0228 | 0.0422 | 0.1050 | -0.0476 | 0.0368 | -0.0434 |
| p-value | 0.0070 | 0.6853 | 0.4523 | 0.0610 | 0.3969 | 0.5130 | 0.4401 |
| Mean leaf length | -0.1054 | -0.1805 | -0.0013 | 0.0085 | 0.0590 | -0.0193 | 0.0314 |
| $p$-value | 0.0600 | 0.0012 | 0.9820 | 0.8798 | 0.2932 | 0.7316 | 0.5763 |
| Mean number of lobes | 0.1484 | 0.2423 | -0.0091 | -0.0692 | -0.0973 | -0.0310 | 0.0292 |
| $p$-value | 0.0079 | <. 0001 | 0.8720 | 0.2179 | 0.0827 | 0.5816 | 0.6033 |
| Leaf tip | 0.1555 | -0.0438 | $\mathbf{0 . 2 0 2 3}$ | -0.1525 | 0.1238 | -0.1745 | 0.1696 |
| p-value | 0.0054 | 0.4353 | 0.0003 | 0.0064 | 0.0271 | 0.0018 | 0.0024 |
| Leaf width | -0.1433 | -0.1483 | 0.0477 | 0.0272 | 0.0750 | -0.0301 | 0.0281 |
| $p$-value | 0.0104 | 0.0080 | 0.3963 | 0.6283 | 0.1817 | 0.5917 | 0.6165 |

Figure 1. Population distribution of E. lanatum seed collection locations within and outside the Willamette Valley. Circles represent hierarchically defined clusters at the $87.5 \%$ level. Lines connecting clusters represent clusters defined at the $75 \%$ level.


Figure 2. Hierarchical Dendrogram of populations to detect clustering defined by geographical variables Latitude and Longitude (a) 9.2E-06 (75\% information remaining) (b) 6.2E-08 (87.5\% information remaining).
(a)

(b)


Figure 3. NMS 3-dimensional ordination; Population is used as the overlay. Each symbol represents an individual in multivariate space. Symbols that are closer spatially are more similar morphologically and phenologically. Vectors (red lines) indicate variables with significant correlations to axes $\left(\mathrm{R}^{2}(\right.$ or tau $\left.)<0.50\right)$.

## ERLA



Figure 4. NMS ordination of individuals within each population with variables with $>0.50 \mathrm{R}^{2}$ value. Vectors (red lines) indicate variables with significant correlations to axes ( $\mathrm{R}^{2}<0.50$ ). Multiple individuals (sample units) are represented by a single color.

ERLA


Axis 2

Figure 5. NMS ordination with county overlay. County: $1=$ State of Washington, 2 = Washington (county), $3=$ Yamhill, $4=$ Polk, $5=$ Linn, $6=$ Lane, $7=$ Douglas (outside Willamette Valley).


Axis 2

Figure 6. NMS ordination with Eugene overlay. Individuals within 20 miles of Eugene are shown in green, those outside of this seed transfer zone are shown in red.


Figure 7. NMS ordination with populations present or absent in the Willamette Valley: $2=\mathrm{IN}, 1$ = OUT

ERLA


Axis 2

Figure 8. NMS ordination using Elevation as an overlay where $1=$ elevation $<200 \mathrm{~m}$ and $2=$ elevation $>200 \mathrm{~m}$ (a) axis 3 and 2 rotated by categorical flower color (b) diffcolr jointly biplotted with high elevation overlay rotated by categorical flower color.
(a)


Axis 2
(b)


Axis 2

$$
\begin{gathered}
\text { DIFFCOLR } \\
\text { Axis } 2 \\
r=-.823 \text { tau }=-.628 \\
\text { Axis } 3 \\
r=.248 \text { tau }=.165
\end{gathered}
$$



Figure 9. NMS ordination using Cluster overlay defined by hierarchical cluster analysis at 75\% level.


Axis 2

Figure 10. NMS ordination using Cluster overlay defined by hierarchical cluster analysis at $87.5 \%$ level.


Figure 11. NMS ordination using Ecoregion level 4 defined by EPA (axis 3 and 2): $1=$ Valley Foot hills, 2 = Prairie Terraces, $3=$ Willamette River and Tributary Galley Forest.

ERLA


Axis 2

Figure 12. NMS ordination with climatic variable correlations jointly biplotted with population (a) annual precipitation, (b) annual temperature (c) first day of fall frost (d) last day of spring frost (e) number of frost free days (average growing season length). Red lines indicate regression lines (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes.
(a)


## ERLA



Axis 2

Axis 2
$r=-.409$ tau $=-.199$
Axis 3
$r=.053$ tau $=.054$
(b)

(c)

ERLA



| $P O P$ |  |
| :--- | :--- |
| $\Delta$ | 1 |
| $\Delta$ | 2 |
| $\Delta$ | 3 |
| $\Delta$ | 4 |
| $\Delta$ | 5 |
| $\Delta$ | 6 |
| $\Delta$ | 7 |
| $\Delta$ | 8 |
| $\Delta$ | 9 |
| $\Delta$ | 10 |
| $\Delta$ | 11 |
| $\Delta$ | 12 |
| $\Delta$ | 13 |
| $\Delta$ | 14 |
| $\Delta$ | 15 |
| $\Delta$ | 16 |
| $\Delta$ | 17 |
| 18 |  |
| $\Delta$ | 19 |


(d)

(e)


Figure 13. NMS ordination axis 2 vs 3 with geographic variable correlations jointly biplotted with (a) Latitude, (b) Longitude, (c) elevation. Red lines indicate regression line (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes.
(a)


Axis 2

Axis 2
$r=-.319$ tau $=-.109$
Axis 3
$r=.016$ tau $=.053$
(b)

(c)
ERLA


Axis 2
ELEV
Axis 2
$r=-.257$ tau $=-.225$
Axis 3
$r=.111$ tau $=.137$


# Distribution of morphologic and phenological variation of Epilobium densiflorum in the Willamette Valley: a common garden study to inform seed transfer zones 



## Materials and Methods

## Study species

Epilobium densiflorum (Lindl.) Hoch \& Raven (Onagraceae) (synonym: Boisduvalia densiflora), is an annual herb commonly found in wet prairie habitat of the Willamette Valley, Oregon. Its distribution extends from California north to British Columbia, and east of the Cascades to Montana and Nevada. Epilobium densiflorum has small, pink flowers that are clustered in leafy, terminal and lateral spikes (Pojar and Mackinnon, 1994). It typically flowers from June through August and is thought to be predominately selfing (Seavy et al. 1977), although pollination by bees and syrphid flies has previously been reported (Raven 1979). This species is considered globally secure (G5) and is not ranked in Oregon (NatureServe 2008). While common in the Willamette Valley, E. densiflorum is considered endangered in British Columbia due to habitat loss (COSEWIC 2005, NatureServe 2008).

## Population sampling

Epilobium densiflorum seeds were collected in 2006 from 22 populations distributed throughout the Willamette Valley (Fig. 1,Table 1). Latitude, longitude, and elevation were
recorded at each location. At each location, no more than $25 \%$ of available seed was collected from each identified plant. A large variation in natural population sizes were present for this species; population sizes ranged from 50 individuals to greater than 1000. For analysis, locations were classified based on individual populations, county, EPA defined level 4 Ecoregion (Griffin and Omernik 2008), and presence or absence within a Eugene 20 mile buffer zone (West Eugene Wetlands Seed Collection Manual, 2003).

## Experimental design

Seeds were sown in February 22, 2006 into flats of Ray Leach "Cone-tainers" (Stuewe \& Sons, Inc., Corvallis, OR) and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center in Corvallis, Oregon. Thirty cone-tainers were started for each population ( $\mathrm{N}=660$ ), and were randomly placed within flats. The PMC is located in Benton County, Corvallis, Oregon, at 225 ft elevation. Average daytime temperatures ranged between 4.4 and $12.8^{\circ} \mathrm{C}$ and nighttime temperatures were between 1.1 and $4.4^{\circ} \mathrm{C}$. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After ten weeks in the greenhouse, plants were moved to a shade house and allowed to acclimatize for several weeks to outdoor temperatures. Given the high germination rates for all populations, it was decided to reduce the sample size to 15 replicates per population ( $\mathrm{N}=330$ ).

Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. A drip irrigation system was installed on the field. Drip tapes were placed in long rows across the field 3 ft apart. The field was irrigated approximately once every two weeks to help the plants establish during the first summer. The study site was then covered with three inches of bark mulch to further aid in weed suppression. The 330 E. densiflorum study plants were transplanted in June 2007 using a completely randomized design. An additional row of $E$. densiflorum was planted on each side of the plot as a border row to buffer against edge effects. Plants were placed 2 m apart within rows and rows were placed one meter apart.

## Plant trait measurement

Traits were chosen based on characteristics described in Winn and Gross (1993). Traits thought to have adaptive significance, or associated with reproductive success, taxonomically important traits, and traits with high degrees of variability between varieties (E. densiflorum var. densiflora and E. densiflorum var. salina) were included. While measuring pre-defined traits, additional traits were included based on apparent visual differences among plants. Several botanical experts or species authorities were consulted for recommendations during the trait selection process. Table 2 shows a list of the traits and how they were measured.

Phenological and morphological traits were measured for all individual E. densiflorum plants during the summer of 2008. Each growth trait was measured in a single day. To reduce measurement error, one person measured traits while a second recorded. Floral traits were measured using only new flowers. Morphological traits less than 25 cm in length were measured by dial calipers to 0.01 mm . Traits greater than 25 cm were measured with a meter stick to the nearest centimeter. When measuring a single trait in triplicate on an individual, no measurements were made from the same organ (e.g., average petal length was the mean length of
three petals from three separate flowers chosen haphazardly). Emergence date was monitored on a daily basis; when cone-tainers contained more than one seedling, germination was recorded for the first seedling that emerged in each cone-tainer. Flowering and seed set date was monitored on transplanted individuals three times a week until a value was obtained for each study plant. Plants with missing values were removed from the analysis (SR1611, WI0112, WI0316, WI0608).

## Climatic Data

Climatic conditions at each population location site were characterized using digital maps produced in ArcGIS 9.3 and data generated by PRISM climate models (PRISM group, 2008). PRISM is an analytical model that uses point data and a digital elevation model (DEM) to generate gridded estimates of monthly and annual average daily maximum/minimum climatic parameters (PRISM group, 2008). PRISM incorporates a conceptual framework that addresses the spatial scale and pattern of climate variables that allows for estimation of variables in regions with heterogeneous terrain (PRISM group, 2008). PRISM was parameterized to use 1971-2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for a single location is no better than half the resolution of the cell. Variables were modeled monthly. An annual average was produced by averaging the monthly grids. For this analysis, mean annual temperature (USANNAV) and precipitation (USANNP), mean dates of the first (SPRFRST) and last frost (FLLFRST), and the number of frost free days (FRSTFREE) was gathered for each population based on each population's unique latitude (LAT) and longitude (LONG).

## Data analysis

Ordination analyses were performed on morphological and phenological variables using nonmetric multidimensional scaling (NMS) based on Euclidean distance measures (Kruskal 1964). Analyses were completed using PcORD 5 (McCune and Mefford 1995). NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales (Peterson and McCune 2001). To account for variable collection on different scales (i.e., Julian days and centimeters) data were relativized using the standard deviates of each column variable. Prior to relativization, mean flower petal length was monotonically log transformed to compress high values and spread low values by expressing values as orders of magnitude. Monotonic transformation of individual variables allows for independent changes to data point values without altering their rank. Individuals greater than two standard deviates from the mean Euclidian distance were defined as outliers and removed from the analysis (BF0511, CO0308, CO0704, FI0502, KN0412, KN1905, LP0415, MR0911, MS0213, SR0603).

Since adaptive variation is important in designating seed transfer zones and is most directly related to variation within and among seed sources, we used individuals as our sampling units instead of mean population estimates. Thus, the main data matrix consisted of individual
plants and traits measured at the common garden. To visually clarify the distribution of sampling units in ordination space, a second matrix with additional information (latitude, longitude, county, elevation, and climatic variables) was overlayed or jointly-biplotted .

NMS uses an iterative search for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). To ensure that the ordination was below an acceptable level of stress, we used a random seed with 250 runs of real data. Dimensionality was assessed visually using a scree plot. Monte Carlo simulations using 250 replicates were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used.

We compared the relative position of each population in ordination space by visual assessment. Due to the degree of difficulty assessing individual sampling units within a population, additional coding subgroup overlays such as county ( $\mathrm{n}=7$ ), 20 mile Eugene buffer $(\mathrm{n}=2)$, and visually defined clusters $(\mathrm{n}=5)$ were used (Table 1 ). These subgroup overlays were based on geographic-administrative or habitat units that are either in practice in the Willamette Valley or logical potential seed transfer zone boundaries. To identify spatial clustering based on latitude and longitude we used hierarchical agglomerative clustering patterns calculated using Euclidean distances and nearest neighbor linkages to maximize distinctness of groups. The resulting clustering dendrogram was scaled using a log transformation and information was retained at the $75 \%$ and $50 \%$ levels (Fig. 2). This allowed us to partition the dataset into more homogenous subsets based exclusively on geographic location. These subsets were then used as additional plot overlays.

We quantitatively compared the relative position of populations in the ordination using Euclidian distances and unblocked Multi-Response Permutation Procedure (MRPP) analysis on weighted groups. MRPP is a nonparametric test used to examine whether populations on matrix plots occupy different regions of ordination space. Correlations between ordination axes and the environmental variables were calculated using Pearson Correlation coefficients. The percent of variation in the original ordination was also recorded. A Bonferroni correction was used when multiple comparisons were performed to maintain a low probability of relationships appearing significant when, in fact, they only appear significant by chance.

Univariate calculations were made for each variable by population. Traits were used to produce pairwise Pearson Correlation Coefficients for each variable with latitude, longitude, elevation, and climatic variables using SAS 9.2 (SAS Institute 2008). Ordination axes were individually regressed on geographic and climatic variables in PcORD.

## Results

## Morphological and phenological patterns across populations

The Nonmetric Multidimensional Scaling (NMS) analysis provided a 3-dimensional ordination best solution to the data based on a final stress of 15.03680, a final instability of 0.00001 , and 306 iterations. Using Clarke's cutoff for acceptable instability, values between 10 and 20 represent a usable picture (McCune and Grace, 2002); however, values at the upper end suggest a potential to mislead and thus too much reliance on details of the plots should be
discouraged. Together the axes explained $85 \%$ of the variance, $42.7 \%, 18.9 \%$ and $23.3 \%$ axes 1 , 2 , and 3 respectively. Axes 1 and 2 were moderately influenced by flower and seed set date, plant height and diameter, while axis 3 was more strongly influenced by mean leaf length and width. Leaf measurements showed the strongest correlations to ordination axes, explaining some of the distribution of individuals in ordination space (Fig. 3; Table 3). Since axes 1 and 3 explain the majority of the variance in the ordination they are used in all 2-dimensional graphical data representations.

NMS ordination provided evidence of discrete clustering of populations in ordination space based on morphological and phenological characteristics. Although much overlap between populations exists, some populations seem to be clustered in ordination space (Fig. 4). Post hoc analysis using Multi-Response Permutation Procedure (MRPP) indicated significant differences between many individual populations (Table 4a). With 231 total comparisons 171 pairs of populations were significantly different representing $74 \%$ of the population level contrasts. Further, many significant MRPP population level comparisons had $A$ values above 0.40 suggesting a moderate level of within group similarity. The $A$ statistic is a descriptor of withingroup homogeneity compared to random expectation (McCune and Grace 2002). If heterogeneity within groups equal chance expectation then $A=1$; however, if less agreement (heterogeneity) exists within groups than expected by chance, then $A=0$. In such cases where small $A$ values are statistically significant careful consideration of the ecological significance of the results is warranted. $A$ values of less than 0.3 represent substantial heterogeneity (variability) between contrasted groups. The largest within group dissimilarities occur in the Kingston, Sublimity Prairie, Coburg Rd., and Mt. Richmond populations respectively. These populations appear as distinct clusters in ordination space (Fig. 4) particularly in terms of flowering and seed set dates represented by axis 1 .

Populations that dominate many of the population level comparisons have the potential to strongly influence subsequent overlays used in analysis. Using county as a subgroup overlay suggests that county is insufficient in defining differences in plant variation (Fig. 5). Individuals within all counties do not appear to be clustered by county and greatly overlap. Despite the large amount of overlap in ordination space, post hoc MRPP analysis suggests all counties differ significantly from one another, which is expected since this analysis reflects much of the population level contrasts (Table 4b). Despite significant differences in MRPP, $A$ values are exceptionally low. These low values indicate little confidence that these groupings of individuals by county are in fact unique.

Similarly, some clustering appears in the Eugene overlay, which is greatly influenced by individuals found in Kingston, Sublimity Prairie, Coburg Rd, and Mt. Richmond populations (Fig. 6). Significant differences were detected between populations as being either outside or within the Eugene 20 mile buffer ( $\mathrm{T}=-28.814378$, $\mathrm{A}=0.01837289, p<0.0001$ ) based on post hoc MRPP. Again a small $A$ value indicates almost no differences between groups. This pattern matches county level differences, suggesting neither county nor the Eugene buffer represent adequate defining overlays.

Clustering of populations based on Hierachical Clustering at the 6.4E-02 (75\%) and 1.3E$01(50 \%)$ levels using Euclidean distances resulted in eight and five geographic clusters
respectively (Fig. 2). At the $75 \%$ clustering level, visual inspection of ordination revealed separation of clusters 7 (Kingston and Sublimity Prairies) and 8 (Mt. Richmond) from all other clusters but overlap with each other. MRPP suggests several differences in clusters comparable to population level differences (Table 4a,c,d). Clustering at the coarser $50 \%$ level suggests little visual separation of groups yielding slightly better results than those at the county level (Fig. 7). It appears population or perhaps clustering at the $75 \%$ level are better geographic overlay representations than county or clusters at the $50 \%$ level (Fig. 8).

EPA defined Ecoregions (level 4) within the Willamette Valley did not clearly cluster in the ordination; much overlap of individuals exist between these zones (Fig. 9). The concentration the Valley Foothills Ecoregion at the top of the ordination reflects individuals from Kingston and Sublimity Prairies and Mt. Richmond (the most northern population). Individuals from Spores tend to concentrate in the lower half of the ordination, but overlaps with Coburg Rd, Granger Avenue, Lupine Meadows, and others. This pattern suggests that although some clustering is occurring it is not geographically defined. Post hoc MRPP analysis indicates ecoregion level differences are significant however, again $A$ values are very small. These results correspond to other overlays investigated in this analysis.

## Correlations of traits with geographic and climatic variables

Nine traits were measured or calculated for each individual in each population. Ranges and means ( $\pm 1 \mathrm{SD}$ ) of populations indicate substantial variability both between and within populations (Appendix D). High levels of within population variability suggest high levels of genetic polymorphism in populations in the Willamette Valley. Population level variability is apparent in most traits, as well.

Regression of ordination axes with climatic and geographic variables identified a few weak associations (Table 3), (Figs. 10-11). It is important to note that correlations with axes are difficult to ascertain since variables are not necessarily linearly correlated or parallel to axes.

Pearson's Correlation coefficients between univariate traits and geographic and climatic variables identified some significant but weak correlations. Latitude and longitude were negatively correlated with flowering and seed set dates, as well as plant diameter, but these associations were very weak (Table 5). The strongest associations were positive correlations of pubescence and longitude, as well as diameter and annual average temperature; however, these traits did not appear to be strongly correlated with ordination axes (Table 5). Although significant, no correlation was stronger than 0.43 (diameter $\times$ mean annual temperature). Morphological and phenological variables are poorly correlated with geographic and climatic variables especially.

## DISCUSSION

Population level differences do not appear to follow a distinct geographical pattern based on the measured characteristics in this study. Flowering and seed set dates, height and diameter, as well as leaf length and width appear to be strongly correlated to ordination axes. Although much overlap exists between populations, some populations are clustered in ordination space. Kingston, Sublimity Prairie, Coburg Rd, and Mt. Richmond populations appear as a distinct
cluster in ordination space particularly in terms of flowering and seed set dates. At the population level, much between group dissimilarity exists. Clustering of individuals within a population reflects reduced variability. Reduced variability is expected in annual species, and is even more pronounced when the species is predominantly autogamous. With small sample collections and autogamous reproduction, we expect relatedness within populations used in the common garden. However, without data regarding maternal individuals sampled, it is not possible to accurately estimate within population variance.

County is an artificial boundary often used by managers although rarely biologically meaningful. In this study, county proved ineffectual in visually defining clusters in ordination space. Linn County was found to be significantly different than all other counties, however this is largely due to the inclusion of the unusual Kingston and Sublimity Prairie populations in that group. Using county provided no additional insight into defining spatial clusters. Clustering based on Hierarchical Clustering at the $75 \%$ level again separated out the cluster containing the Kingston and Sublimity Prairies, and in a separate cluster Mt. Richmond. EPA defined ecoregions yielded similar results concentrating individuals from Kingston and Sublimity Prairies and Mt. Richmond (the most northern population). Population level differences appear to be the best overlay for visual interpretation; however, Hierarchical Clustering at the $75 \%$ level provided a geographic representation that was moderately better than either county or coarser hierarchical levels.

Since clear population level differences exist based on these data, although geographic patterning is unclear, we strongly recommend adding other populations throughout the Willamette Valley in future seed guideline studies. Too few population sources and representative samples from populations make accurate inferences more difficult to ascertain.

Overlap of measured characteristics between populations suggests movement of seed between populations with similar environments would allow for the survival of plants used in restoration within the Willamette Valley. However, in this study, the populations Mt. Richmond, Kingston and Sublimity Prairies show a high degree of clustering away from other populations, primarily based on early flowering and seed set. If flowering plants are used in restorations where pollinator activities are inappropriately timed, mixing of genetic material can be reduced limiting future generation of plants that require outcorssing. This is less critical on the for autogamous annuals, as in this case; however, the potential effects of using plants with different flowering times on associated pollinators in a restoration are unknown. Until populations from a full range of environmental and climatic conditions in which this species occurs are included in future studies, at a minimum we do not recommend movement of seed from sources outside the Willamette Valley, or beyond the distribution we have sampled. Additionally, we caution against the use of the three "unusual populations" outside of similar habitats and geographic areas.

## Correlations of traits with geographic and climatic variables

Although the ordination was largely explained by phenological characteristics, morphological and phenological traits were poorly correlated with geographic and climatic variables. With little differences in climatic variables within the Willamette Valley based on
coarse PRISM data, no significant correlations were anticipated; finer measures of climatic extremes or microclimate are highly recommended in future analyses of climatic variables for this species due to its habitat requirements. For all past seed zones studies within the Willamette Valley, little to no correlation with geographic and climatic variables was found (Clausen, Keck, and Hiesey 1940; Erickson, Mandel, and Sorensen 2004; St. Clair, Mandel, and Vance-Borland 2005).

Epilobium densiflorum requires wet winters for successful seed germination, yet thrives during summer drought conditions allowing it to exist in areas of high environmental stress. Although this species has a high level of tolerance to both wet and dry extremes, mortality occurs when soil moisture is insufficient to meet the needs of a plant. The specialized, open, ephemerally wet habitats of this species are becoming increasingly fragmented and encroached upon by woody and invasive species. Habitat alterations can substantial change hydrology and reduce water availability for these plants. Loss of habitat coupled with its annual reproductive biology suggests that once a population is lost, there is negligible probability of recolonization. Since this species requires such a particular microclimate for stand success, more precise climatic measurements should be included in future analysis.

## RECOMMENDATIONS

In summary, our data indicate there are recognizable differences in populations across the Willamette Valley for E. densiflorum. Three populations were clearly morphologically and phenologically similar to one another and divergent from other populations. Two of these populations were closely located and shared a similar and unusual habitat. Kingston Prairie is a TNC preserve which represents one of the best examples of native Willamette Valley Prairie that has retained much of its original vegetation in both wet and dry upland areas. Kingston and Sublimity Prairies also have a much different soil structure than most other prairies in the valley where it is characterized by basalt bedrock that underlays shallow soil. The third population, Mt. Richmond, was ca. 100 km to the northwest and occurred in a typical wet meadow in valley foothill habitat (other associated species found at this location include Carex densa, Juncus sp.). A fourth population, Coburg Rd, was also divergent from other populations but was not phenologically or morphologically similar to the other three aberrant populations. No apparent spatial, climatic, or environmental factors were related to population variation. We therefore recommend limiting seed movement to sources from sources within the Willamette Valley, and not beyond the distribution of populations we have sampled. Additionally, we caution against the use of the four "unusual populations" outside of similar habitats and geographic areas. We suggest that using multiple populations in reintroductions will increase restoration success and assist in restoring more historic levels of gene flow. Last, additional studies are recommended to determine the presence and/or scale of local adaptation and the genetic basis for the adaptations if they are found.

Table 1. List of population names with corresponding abbrevations and number of individuals represented per population as sampling units. County: $1=$ Washington, $2=$ Yamhill, $3=$ Marion, $4=$ Linn, $5=$ Benton, $6=$ Lane. Eugene: $1=$ within 20 mile buffer, $0=$ not present in
buffer. Clusters are based on Hierachical Cluster Analysis dendrogram interpretation at the $6.4 \mathrm{E}-02$ and $1.3 \mathrm{E}-01$ levels, retaining $75 \%$ and $50 \%$ of the original information respectively. EPA defined Ecoregion Level 4: $1=$ Valley Foothills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Gallery Forest.

| Population | Code |  | County | Eugene | Cluster7 | Cluster5 | Ecoregion | Elevation | Latitude | Longitude |
| :--- | ---: | :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Ankeny WLR | 1 | AK | 4 | 0 | 1 | 1 | 2 | 62 | 44.8004 | -123.0682 |
| Belts Drive | 2 | BD | 5 | 1 | 3 | 2 | 2 | 137 | 44.3000 | -123.0200 |
| Bald Hill | 3 | BH | 3 | 0 | 2 | 1 | 2 | 148 | 44.5675 | -123.3322 |
| Bellfountain Rd x 53rd St. | 4 | BF | 3 | 0 | 2 | 1 | 2 | 89 | 44.5062 | -123.3192 |
| Baskett Slough | 5 | BS | 2 | 0 | 5 | 4 | 2 | 84 | 44.9855 | -123.2642 |
| Coburg Rd | 6 | CO | 6 | 1 | 3 | 2 | 2 | 115 | 44.1640 | -123.0990 |
| Deer Creek Park | 7 | DC | 1 | 0 | 6 | 4 | 1 | 170 | 45.1700 | -123.3900 |
| Finely | 8 | FI | 3 | 0 | 2 | 1 | 2 | 110 | 44.4178 | -123.3008 |
| Gahr Farm | 9 | GH | 1 | 0 | 6 | 4 | 1 | 150 | 45.1660 | -123.3130 |
| Granger Avenue | 10 | GR | 3 | 0 | 2 | 1 | 2 | 150 | 44.6287 | -123.2323 |
| Jackson Creek | 11 | JC | 3 | 0 | 2 | 1 | 1 | 227 | 44.6141 | -123.2883 |
| Kingston Prairie | 12 | KN | 5 | 0 | 8 | 5 | 1 | 224 | 44.7753 | -122.7443 |
| Lupine Meadow | 13 | LM | 3 | 0 | 2 | 1 | 2 | 93 | 44.5511 | -123.3488 |
| Lakepark Skate Park | 14 | LP | 3 | 0 | 2 | 1 | 2 | 93 | 44.6325 | -123.2418 |
| Mt. Richmond | 15 | MR | 1 | 0 | 7 | 4 | 1 | 87 | 45.3884 | -123.2564 |
| Masonville Rd. | 16 | MS | 1 | 0 | 6 | 4 | 1 | 116 | 45.1800 | -123.2900 |
| Oak Creek | 17 | OA | 5 | 0 | 4 | 3 | 2 | 211 | 44.4953 | -122.8762 |
| ODOT Mitigation Site | 18 | OD | 5 | 0 | 4 | 3 | 2 | 84 | 44.5464 | -123.0031 |
| OSU Horse Center | 19 | OS | 3 | 0 | 2 | 1 | 2 | 98 | 44.5746 | -123.3106 |
| Sublimity Prairie | 20 | SP | 4 | 0 | 8 | 5 | 1 | 213 | 44.8410 | -122.7438 |
| Spores | 21 | SR | 6 | 1 | 3 | 2 | 3 | 127 | 44.0947 | -122.9424 |
| Wintercreek | 22 | WI | 2 | 0 | 2 | 1 | 2 | 110 | 44.7220 | -123.2415 |

Table 2. List of phenological and morphological traits measured on E. densiflorum plants in common garden study.

| Trait | Abbreviation | Description |
| :--- | :--- | :--- |


| Diameter | DIAM | Distance across broadest part of plant (cm) |
| :--- | :--- | :--- |
| Height | HT | Total height of plant (cm) |
| Mean flower petal <br> length | AVEPETL | Length of flower petal measured on 3 haphazardly chosen flowers |
| Emergence date | JULEMER | Date at first sign of cotyledon emergence (Julian) |
| Flowering Date | JULFLWR | Date at first sign of flowering (Julian) |
| Pubescence | PUB | Degree pubescence was recorded (1 low - 4 high) |
| Mean leaf length | AVELEAFL | Upper leaf length were measured on 3 haphazardly chosen flowers <br> $(\mathrm{mm})$ |
| Mean leaf width | AVELEAFW | Upper leaf width were measured on 3 haphazardly chosen flowers <br> $(\mathrm{mm})$. |
| Seed set date | JULSEED | Date at first sign of mature fruit (Julian) |

Table 3. Correlation of axes with measured trait variables and secondary geospatial and climatic variables. Kendall' tau is a rank regression estimate of correlation, whereas $\mathrm{R}^{2}$ is the square of the linear correlation coefficient.

|  | Axis 1 |  | Axis 2 |  | Axis 3 |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathbf{R}^{\mathbf{2}}$ | tau | $\mathbf{R}^{\mathbf{2}}$ | tau | $\mathbf{R}^{\mathbf{2}}$ | tau |
| Latitude | 0.079 | -0.137 | 0.01 | -0.07 | 0.052 | 0.132 |
| Longitude | 0.088 | -0.061 | 0.155 | 0.233 | 0.001 | 0.034 |
| Elevation | 0.058 | -0.083 | 0.047 | 0.124 | 0 | -0.011 |
| Flowering date | $\mathbf{0 . 5 4 3}$ | $\mathbf{0 . 4 9 8}$ | $\mathbf{0 . 4 5 4}$ | $\mathbf{- 0 . 5 1 1}$ | 0.112 | -0.237 |
| Height | $\mathbf{0 . 4 8 2}$ | $\mathbf{0 . 5 1 7}$ | 0.002 | 0.032 | 0.002 | 0.018 |
| Diameter | $\mathbf{0 . 4 8 1}$ | $\mathbf{0 . 5 0 2}$ | 0.019 | 0.085 | 0.001 | 0.021 |
| Emergence date | 0 | -0.015 | 0.01 | -0.044 | 0.004 | -0.042 |
| Mean flower petal length | 0.01 | 0.064 | 0.047 | 0.145 | 0.031 | 0.112 |
| Seed set date | $\mathbf{0 . 4 5 6}$ | $\mathbf{0 . 5 0 9}$ | $\mathbf{0 . 5 3}$ | $\mathbf{- 0 . 5 5 6}$ | 0.149 | -0.251 |
| Mean leaf length | 0.101 | 0.223 | 0.098 | -0.21 | $\mathbf{0 . 5 7 1}$ | $\mathbf{0 . 5 3 6}$ |
| Mean leaf width | 0 | -0.003 | 0.076 | -0.192 | $\mathbf{0 . 6 7 7}$ | $\mathbf{0 . 6 3 6}$ |
| First date fall frost | 0.088 | 0.231 | 0.001 | -0.037 | 0.07 | -0.195 |
| Number of frost free days | 0.013 | 0.167 | 0.003 | -0.06 | 0.057 | -0.189 |
| Last day of spring frost | 0.008 | 0.061 | 0.019 | -0.076 | 0.034 | 0.125 |
| Mean annual temperature | 0.164 | 0.19 | 0.004 | -0.025 | 0.006 | -0.026 |
| Mean annual precipitation | 0.069 | -0.123 | 0.018 | 0.094 | 0.014 | 0.121 |

Table 4. Significantly different MRPP multiple pair-wise comparisons with adjusted Bonferroni correction, test statistic $T$ and agreement statistic $A$ grouped by (a) population ( $p<0.0002$ ) (b) county ( $p<0.003$ ) (c) dendrogram defined cluster at the $75 \%$ level ( $p<0.005$ ) (d) dendrogram defined cluster at the $50 \%$ level ( $p<0.007$ ). Comparisons with biological significance are italicized ( $\mathrm{A} \geq 0.30$ ).
(a)

| Populations |  |  |  | $\boldsymbol{T}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | -18.147 | $\boldsymbol{A}$ |
| 1 | vs. | 3 | -650 |  |
| 1 | vs. | 4 | -7.985 | 0.120 |
| 1 | vs. | 5 | -6.570 | 0.147 |
| 1 | vs. | 6 | -114 |  |
| 1 | vs. | 7 | -6.666 | 0.352 |
| 1 | vs. | 8 | -9.627 | 0.176 |
| 1 | vs. | 9 | -10.038 | 0.202 |
| 1 | vs. | 10 | -12.844 | 0.292 |
| 1 | vs. | 11 | -9.357 | 0.182 |
| 1 | vs. | 12 | -17.319 | 0.470 |
| 1 | vs. | 14 | -9.118 | 0.207 |
| 1 | vs. | 15 | -16.522 | 0.413 |
| 1 | vs. | 16 | -7.763 | 0.141 |
| 1 | vs. | 18 | -15.210 | 0.346 |
| 1 | vs. | 20 | -17.822 | 0.460 |
| 1 | vs. | 21 | -11.256 | 0.277 |
| 1 | vs. | 22 | -13.391 | 0.317 |
| 2 | vs. | 3 | -15.991 | 0.368 |
| 2 | vs. | 4 | -12.915 | 0.268 |
| 2 | vs. | 5 | -18.426 | 0.435 |
| 2 | vs. | 6 | -14.618 | 0.397 |
| 2 | vs. | 7 | -16.369 | 0.395 |
| 2 | vs. | 8 | -13.344 | 0.270 |
| 2 | vs. | 9 | -16.760 | 0.399 |
| 2 | vs. | 10 | -16.963 | 0.413 |
| 2 | vs. | 11 | -12.334 | 0.245 |
| 2 | vs. | 12 | -13.812 | 0.315 |
| 2 | vs. | 13 | -16.691 | 0.356 |
| 2 | vs. | 14 | -15.429 | 0.372 |
| 2 | vs. | 15 | -12.010 | 0.243 |
| 2 | vs. | 16 | -14.642 | 0.321 |
| 2 | vs. | 17 | -14.933 | 0.333 |
| 2 | vs. | 18 | -12.588 | 0.265 |
| 2 | vs. | 19 | -12.135 | 0.264 |
| 2 | vs. | 20 | -13.976 | 0.282 |
| 2 | vs. | 21 | -15.654 | 0.382 |
| 2 | vs. | 22 | -15.488 | 0.390 |
| 3 | vs. | 4 | -6.074 | 0.118 |
| 3 | vs. | 6 | -10.257 | 0.268 |
| 3 | vs. | 10 | -8.065 | 0.178 |
| 3 | vs. | 12 | -15.823 | 0.440 |
| 3 | vs. | 15 | -12.591 | 0.316 |
| 3 | vs. | 18 | -7.356 | 0.160 |
| 3 | vs. | 20 | -15.890 | 0.395 |
| 3 | vs. | 21 | -6.138 | 0.147 |
| 4 | vs. | 5 | -9.387 | 0.163 |
| 4 | vs. | 6 | -13.237 | 0.359 |



| 8 vs. 15 | -11.914 | 0.289 |  | 14 vs. 21 | -8.442 | 0.202 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| vs. 16 | -6.543 | 0.132 |  | 15 vs. 16 | -10.102 | 0.236 |
| 8 vs. 17 | -5.585 | 0.108 |  | 15 vs. 17 | -13.146 | 0.322 |
| 8 vs. 18 | -8.242 | 0.163 |  | 15 vs. 18 | -11.612 | 0.266 |
| 8 vs. 19 | -6.156 | 0.122 |  | 15 vs. 19 | -11.277 | 0.286 |
| 8 vs. 20 | -13.849 | 0.323 |  | 15 vs. 21 | -14.319 | 0.405 |
| 8 vs. 21 | -7.110 | 0.149 |  | 15 vs. 22 | -11.824 | 0.320 |
| 9 vs. 10 | -13.704 | 0.321 |  | 16 vs. 18 | -7.650 | 0.159 |
| 9 vs. 12 | -16.603 | 0.441 |  | 16 vs. 19 | -6.460 | 0.133 |
| 9 vs. 13 | -6.742 | 0.135 |  | 16 vs. 20 | -15.344 | 0.365 |
| 9 vs. 14 | -7.621 | 0.182 |  | 16 vs. 21 | -8.901 | 0.237 |
| 9 vs. 15 | -11.475 | 0.274 |  | 16 vs. 22 | -6.548 | 0.152 |
| 9 vs. 18 | -10.321 | 0.223 |  | 17 vs. 18 | -7.451 | 0.140 |
| 9 vs. 20 | -16.473 | 0.405 |  | 17 vs. 20 | -15.004 | 0.354 |
| 9 vs. 21 | -7.366 | 0.174 |  | 17 vs. 21 | -8.966 | 0.208 |
| 9 vs. 22 | -5.970 | 0.138 |  | 17 vs. 22 | -7.798 | 0.181 |
| 10 vs. 11 | -7.100 | 0.147 |  | 18 vs. 19 | -9.882 | 0.218 |
| 10 vs. 12 | -16.750 | 0.468 |  | 18 vs. 20 | -13.755 | 0.294 |
| 10 vs. 13 | -13.710 | 0.323 |  | 18 vs. 21 | -12.847 | 0.295 |
| 10 vs. 15 | -16.583 | 0.438 |  | 19 vs. 20 | -13.466 | 0.326 |
| 10 vs. 16 | -9.303 | 0.221 |  | 19 vs. 21 | -7.249 | 0.155 |
| 10 vs. 17 | -9.204 | 0.196 |  | 19 vs. 22 | -10.361 | 0.253 |
| 10 vs. 18 | -10.114 | 0.214 |  | 20 vs. 21 | -16.255 | 0.432 |
| 10 vs. 19 | -12.787 | 0.290 |  | 20 vs. 22 | -14.802 | 0.395 |
| 10 vs. 20 | -17.520 | 0.449 |  | 21 vs. 22 | -9.942 | 0.249 |

## (b)

| County |  |  |  | $\boldsymbol{T}$ |
| :--- | :--- | :--- | :--- | :--- |
| 1 | vs. | 2 | -4.9909 | 0.0288 |
| 1 | vs. | 3 | -6.2281 | 0.0233 |
| 1 | vs. | 5 | -9.0588 | 0.0509 |
| 2 | vs. | 5 | -11.2527 | 0.0970 |
| 3 | vs. | 2 | -5.1273 | 0.0254 |


| 3 | vs. | 5 | -13.1855 | 0.0611 |
| :--- | :--- | :--- | :--- | :--- |
| 4 | vs. | 1 | -8.6679 | 0.0554 |
| 4 | vs. | 2 | -13.3443 | 0.1451 |
| 4 | vs. | 3 | -6.7980 | 0.0357 |
| 4 | vs. | 5 | -4.6516 | 0.0453 |
| 4 | vs. | 6 | -10.9973 | 0.0883 |
| 6 | vs. | 1 | -13.0320 | 0.0670 |
| 6 | vs. | 2 | -9.3943 | 0.0715 |
| 6 | vs. | 3 | -9.1658 | 0.0407 |
| 6 | vs. | 5 | -9.6570 | 0.0626 |


| 1 | vs. | 7 | -16.522 | 0.413 |
| :--- | :--- | :--- | :--- | :--- |
| 1 | vs. | 8 | -25.681 | 0.441 |
| 2 | vs. | 4 | -4.820 | 0.018 |
| 2 | vs. | 5 | -4.461 | 0.018 |
| 2 | vs. | 7 | -22.420 | 0.096 |
| 2 | vs. | 8 | -50.900 | 0.207 |
| 3 | vs. | 2 | -14.085 | 0.052 |
| 3 | vs. | 4 | -9.687 | 0.089 |
| 3 | vs. | 5 | -7.501 | 0.091 |
| 3 | vs. | 6 | -13.617 | 0.111 |
| 3 | vs. | 7 | -14.326 | 0.190 |
| 3 | vs. | 8 | -27.565 | 0.273 |
| 5 | vs. | 4 | -7.148 | 0.089 |
| 5 | vs. | 7 | -16.879 | 0.392 |
| 5 | vs. | 8 | -26.626 | 0.446 |
| 6 | vs. | 4 | -6.767 | 0.056 |
| 6 | vs. | 7 | -17.999 | 0.206 |
| 6 | vs. | 8 | -37.551 | 0.377 |
| 7 | vs. | 4 | -16.126 | 0.234 |
| 8 | vs. | 4 | -27.972 | 0.320 |
| 8 | vs. | 7 | -4.654 | 0.060 |

(d)

| Cluster 50\% |  |  |  | $\boldsymbol{T}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | $\boldsymbol{A}$ |  |
| 1 | vs. | 4 | -4.729 | 0.056 |
| 1 | vs. | 5 | -55.216 | 0.012 |
| 1 | vs. | 3 | -6.285 | 0.022 |
| 2 | vs. | 4 | -13.989 | 0.079 |
| 2 | vs. | 5 | -27.565 | 0.273 |
| 2 | vs. | 3 | -9.687 | 0.089 |
| 4 | vs. | 5 | -35.818 | 0.250 |
| 4 | vs. | 3 | -6.523 | 0.037 |
| 5 | vs. | 3 | -27.972 | 0.320 |

(c)

| Cluster 75\% |  |  |  | $\boldsymbol{T}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | -8.524 | 0.035 |
| 1 | vs. | 3 | -10.860 | 0.141 |
| 1 | vs. | 4 | -12.234 | 0.176 |
| 1 | vs. | 5 | -6.570 | 0.114 |
| 1 | vs. | 6 | -8.057 | 0.084 |

Table 5. Univariate Pearson Correlation Coefficients of measured traits with geographic and climatic variables. Significant correlations greater than $( \pm) 0.25$ correlation coefficients are highlighted.

|  | Latitude | Longitude | First <br> date of <br> fall frost | Number <br> of frost <br> free days | Last day <br> of spring <br> frost | Mean <br> annual <br> temperature | Mean <br> annual <br> precipitation |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Flowering date <br> $p$-value | -0.2775 | -0.2620 | 0.3355 | 0.1988 | -0.0353 | 0.3447 | -0.3533 |
| Height | $<.0001$ | $<.0001$ | $<.0001$ | 0.0003 | 0.5277 | $<.0001$ | $<.0001$ |
| $p$-value | -0.2403 | -0.0095 | 0.2359 | 0.1691 | -0.0825 | 0.2505 | -0.1139 |
| Diameter | $<.0001$ | 0.8650 | $<.0001$ | 0.0023 | 0.1389 | $<.0001$ | 0.0408 |
| $p$-value | -0.2746 | -0.0539 | 0.2002 | 0.0872 | 0.0408 | 0.4292 | -0.1521 |
| Pubescence | $<.0001$ | 0.3344 | 0.0003 | 0.1176 | 0.4647 | $<.0001$ | 0.0062 |
| $p$-value | 0.0618 | 0.4102 | -0.0811 | 0.0618 | -0.2080 | -0.0367 | 0.0304 |
| Emergence date | 0.2685 | $<.0001$ | 0.1458 | 0.2678 | 0.0002 | 0.5110 | 0.5867 |
| $p$-value | 0.0124 | -0.0900 | -0.0926 | -0.0662 | 0.0321 | -0.2364 | 0.1453 |
| Mean flower <br> petal length <br> $p$-value | -0.1213 | 0.0294 | 0.0350 | -0.0060 | 0.0489 | 0.1938 | -0.1251 |
| Seed set date <br> $p$-value | 0.0293 | 0.5992 | 0.5306 | 0.9151 | 0.3807 | 0.0005 | 0.0246 |
| Mean leaf length <br> $p$-value | -0.1377 | -0.2713 | 0.1948 | 0.0739 | 0.0613 | 0.2319 | -0.2547 |
| Mean leaf width | 0.0133 | $<.0001$ | 0.0004 | 0.1852 | 0.2720 | $<.0001$ | $<.0001$ |
| $p$-value | 0.4181 | -0.1049 | -0.0219 | -0.0595 | 0.0941 | 0.0340 | -0.0391 |

Figure 1. Population distribution of E. densiflorum seed collection locations within the Willamette Valley (defined by the red outline). Locations within circles represent populations hierarchically clustered at the $75 \%$ level. Circle joined with a line represent populations clustered at the $50 \%$ level.


Figure 2. Hierarchical Dendrogram of populations to detect clustering defined by geographical variables Latitude and Longitude (a) 6.4E-02 (75\% information remaining) (b) 1.3E-01 (50\% information remaining).
(a)

(b)

Epilobium densiflorum


Figure 3. NMS 3-dimensional ordination; Population is used as the overlay. Each symbol represents an individual in multivariate space. Symbols that are closer spatially are more similar morphologically and phenologically. Vectors indicate variables with significant correlations to axes $\left(R^{2}\right.$ (or tau) $<0.50$ ).


Figure 4. NMS ordination of individuals within each population (axis 1 vs 3). Multiple individuals (sample units) are represented by a single color. Kingston (12), Sublimity Prairies (20), and Mt. Richmond (15) are all found within the red ellipse.


Axis 1

Figure 5. NMS ordination with County overlay (axis 1 vs. 3). County: $1=$ Washington, $2=$ Yamhill, $3=$ Marion, $4=$ Linn, $5=$ Benton, $6=$ Lane.


Figure 6. NMS ordination with Eugene overlay (axis 1 vs. 3). Individuals within 20 miles of Eugene are shown in green, those outside of this seed transfer zone are shown in red.


Figure 7. NMS ordination with clustering at the $75 \%$ level (axis 1 vs. 3).


Figure 8. NMS ordination using Cluster overlay defined by hierarchical cluster analysis at 50\% level (axis 3 and 1 ).


Figure 9. EPA defined Ecoregion level 4: $1=$ Valley Foothills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Gallery Forest (axis 1 vs. 3).


Figure 10. NMS ordination jointly biplotted populations with (a) flowering date (b) seed set date. Red lines indicate regression lines ( r ), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes of the variables.
(a)


JULFLR
Axis 1
$r=-.350$ tau $=-.250$
Axis 3
$r=-.733$ tau $=-.494$

(b)


Figure 10. NMS ordination axis 1 vs. 3 with climatic variable correlations jointly biplotted with population (a) annual precipitation, (b) annual temperature (c) first day of fall frost (d) last day of spring frost (e) number of frost free days (average growing season length). Red lines indicate regression lines (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes of the variables.
(a)


Axis 1
UsAMNP

Axis 1
$r=.123$ tau $=.126$
Axis 3
$r=.262$ tau $=.121$
(b)



Axis 1

(c)

(d)

(e)

FRSTFREE
Axis 1
$r=-.240$ tau $=-.193$
Axis 3
$r=-.111$ tau $=-.165$


Figure 11. NMS ordination axis 1 vs. 3 with geographic variable correlations jointly biplotted with ITS seed zone (a) Latitude, (b) Longitude, (c) elevation. Red lines indicate regression line (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes of the variables.
(a)



Axis 1

> LAT
> Axis 1
> $r=.234$ tau $=.135$ Axis 3
> $r=.279$ tau $=.136$

(b)

(c)


Figure 12. Mean flowering and seed set dates for each population.


# Distribution of morphologic and phenological variation of Potentilla gracilis var. gracilis in the Willamette Valley: a common garden study to inform seed transfer zones 



## Materials and Methods

## Study species

Potentilla gracilis Douglas ex Hook. (Rosaceae) is a native perennial commonly found in upland prairie habitat of the Willamette Valley, Oregon. Due to its widespread distribution within this habitat it has been highly recommended for extensive use in restoration projects throughout the Willamette Valley. Four varieties of this species are currently accepted: var. brunnescens (Rydb.) C.L. Hitchc., var. fastigiata (Nutt.) S. Wats., var. flabelliformis (Lehm.) Nutt. ex Torr. \& Gray, and var. gracilis Dougl. ex Hook (USDA 2008). Potentilla gracilis grows in a tufted form with ascending stems with spreading hairs (Hitchcock and Cronquist 2001). Stalks can have few to many flowers that typically flower in late spring to early summer. This species is thought to require outcrossing in perfect flowers; however, the complex to a large extent is apomictic (Clausen, Keck and Hiesey 1940). Potentilla gracilis is the most variable cinquefoil in western North America requiring a technical key to differentiate between the two varieties found within the Willamette Valley (var. gracilis and var. fastigiata); pubscent (var. gracilis) or glabrous (var. fastigiata) under leaves (Turner and Gustafson 2006; Hitchcock and Cronquist 2001). All plant grown in the common garden had, to some degree, pubescent leaves identifying them as Potentilla gracilis var. gracilis.

## Population sampling

Potentilla gracilis var. gracilis seeds were collected in 2005 from 13 populations distributed throughout the Willamette Valley (Fig. 1, Table 1). Latitude, longitude, and elevation were recorded for each location. At each location, no more than $25 \%$ of available seed was collected from each identified plant. A large variation in natural population sizes were present for this species; population sizes ranged from 3 to greater than 1000 individuals. For analysis, locations were classified based on individual populations, county, EPA defined Level 4 Ecoregion, presence or absence within a Eugene 20 miles buffer zone, and visually defining populations into geographic zones based on subjective map interpretation.

## Experimental design

Seeds were sown on February 22, 2006 into flats of Ray Leach "Cone-tainers" and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center in Corvallis, Oregon. Thirty cone-tainers were started for each population ( $\mathrm{N}=390$ ), and were randomly placed within flats. Flats were watered and placed in polyethylene bags and moved to a walk-in cooler. After 90 days of cold-moist stratification, the flats were moved to an unheated greenhouse. Average daytime temperatures ranged between 21.1 and $29.4^{\circ} \mathrm{C}$ and nighttime temperatures were between 7.2 and $10^{\circ} \mathrm{C}$. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After ten weeks in the greenhouse, plants were moved to a shade house and allowed to acclimatize for several weeks to outdoor temperatures. Severe dampening off occurred at the seedling stage, reducing the overall number of plant available for outplanting. In addition, one population, E.E. Wilson, was completely eradicated from the common garden plot after it was identified as Potentilla recta, a noxious Eurasian invasive that is morphologically very similar to $P$. gracilis. Thus, replicates from each population are uneven (between 3 and 30 replicates per population) totaling 179 individuals.

Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. The study site was then covered with three inches of bark mulch to further aid in weed suppression. The 179 P. gracilis study plants were transplanted in June 2006 using a completely randomized design. An additional row of $P$. gracilis was planted on each side of the plot as a border row to buffer against edge effects. Plants were placed 0.6 meters apart within rows and rows were placed one meter apart.

## Trait measurement

Traits were chosen based on characteristics described in Hickman 1993, and Hitchcock and Cronquist 2001. Traits thought to have adaptive significance, or associated with reproductive success, taxonomically important traits, and traits with high degrees of variability between varieties were included. While measuring pre-defined traits, additional traits were included based on apparent visual differences among plants. Several botanical experts or species
authorities were consulted for recommendations during the trait selection process. Table 2 shows a list of the traits measured and how they were measured.

Phenological and morphological traits were measured for all individual $P$. gracilis plants during the summer of 2008. Each morphological trait was measured in a single day. To reduce measurement error, one person measured traits while a second recorded. Morphological traits less than 25 cm in length were measured by dial calipers to 0.01 mm . Traits greater than 25 cm were measured with a meter stick to the nearest centimeter. When measuring a single trait in triplicate on an individual, no measurements were made from the same organ (e.g., average petal length was the mean length of three petals from three separate flowers chosen haphazardly). Emergence date was monitored on all subset of individuals but due to extreme dampening off, several unmonitored individuals from the same population were used as replacements; this trait is not included in the analysis. Flowering date was monitored on transplanted individuals three times a week until a value was obtained for each study plant. Plants with missing values were removed from the analysis.

## Climatic Data

Climatic conditions at each population location site were characterized using digital maps produced in ArcGIS 9.3 and data generated by PRISM climate models (PRISM group, 2008). PRISM is an analytical model that uses point data and a digital elevation model (DEM) to generate gridded estimates of monthly and annual average daily maximum/minimum climatic parameters (PRISM group, 2008). PRISM incorporates a conceptual framework that addresses the spatial scale and pattern of climate variables that allows for estimation of variables in regions with heterogeneous terrain (PRISM group, 2008). PRISM was parameterized to use 1971-2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for a single location is no better than half the resolution of the cell. Variables were modeled monthly. An annual average was produced by averaging the monthly grids. For this analysis, mean annual temperature (USANNAV) and precipitation (USANNP), mean dates of the first (SPRFRST) and last frost (FLLFRST), and the number of frost free days (FRSTFREE) was gathered for each population based on each population's unique latitude (LAT) and longitude (LONG).

## Data analysis

Ordination analyses were performed on morphological and phenological variables using nonmetric multidimensional scaling (NMS) based on Euclidean distance measures (Kruskal 1964). Analyses were completed using PcORD 5 (McCune and Mefford 1995). NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales (Peterson and McCune 2001). To account for variable collection on different scales (Julian days and centimeters) data were relativized using the
standard deviates of each column variable. Prior to relativization, total height, total number of racemes, mean tooth crest to tooth valley, mean tooth valley to midvein, number of racemes per branch, and number of flowers per branch were monotonically log transformed to compress high values and spread low values by expressing values as orders of magnitude. Monotonic transformation of individual variables allows for independent changes to data point values without altering their rank. Individual's greater than two standard deviates from the mean Euclidian distance were defined as outliers and removed from the analysis (SP0510, SR0911, SP1703, OE0905, WC1502, KP0312, FI0612, SP1602, OE1511,SP0903, LU1708, FI1512, BH0306, SP0102, FI1001. SR0911).

Since adaptive variation is important in designating seed transfer zones and is most directly related to variation within and among seed sources, we used individuals as our sampling units instead of mean population estimates. Thus, the main data matrix consisted of individual plants and traits measured at the common garden. To visually clarify the distribution of sampling units in ordination space, a second matrix with additional information (latitude, longitude, county, elevation, and climatic variables) was overlayed or jointly-biplotted.

NMS uses an iterative search for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). To ensure that the ordination was below an acceptable level of stress, we used a random seed with 250 runs of real data. Dimensionality was assessed visually using a scree plot. Monte Carlo simulations using 250 replicate were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used.

We compared the relative position of each population in ordination space by visual assessment. Due to the degree of difficulty assessing individual sampling units within a population, additional coding subgroup overlays such as county ( $n=5$ ), Ecoregion Level 4 ( $n=$ 2), 20 mile Eugene buffer ( $n=2$ ), and visually defined geographic zones $(n=4)$ were used (Table 1). These subgroup overlays were based on geographic-administrative or habitat units that are either in practice in the Willamette Valley or logical potential seed transfer zone boundaries. To identify spatial clustering based on latitude and longitude we used hierarchical agglomerative clustering patterns calculated using Euclidean distances and nearest neighbor linkages to maximize distinctness of groups. The resulting clustering dendrogram was scaled using a $\log$ transformation and information was retained at the 1.4E-06 level (Fig. 2). This allowed us to partition the dataset into more homogenous subsets based exclusively on geographic location. This subset was then used as an additional plot overlay.

We quantitatively compared the relative position of populations in the ordination using Euclidian distances and unblocked Multi-Response Permutation Procedure (MRPP) analysis on weighted groups. Data were relativized for MRPP analysis using the standard deviation of each variable. MRPP is a nonparametric test used to examine whether populations on matrix plots occupy different regions of ordination space.

Correlations between ordination and the environmental variables were calculated using Pearson Correlation Coefficients. The percent of variation in the original ordination was also
recorded. A Bonferroni correction was used when multiple comparisons were performed to maintain a low probability of relationships appearing significant when, in fact, they only appear significant by chance.

Univariate calculations were made for each variable by population. Trait means were used to produce pairwise Pearson Correlation Coefficients for each variable with latitude, longitude, and climatic variables using SAS 9.2 (SAS Institute 2008). Ordination axes were individually regressed on geographic and climatic variables in PcORD.

## Results

## Morphological and phenological patterns across populations

The Nonmetric Multidimensional Scaling (NMS) analysis provided a 3-dimensional ordination best solution to the data based on a final stress of 12.96858, a final instability of 0.00001 and 116 iterations. Using Clarke's cutoff for acceptable instability, values between 10 and 20 represent a usable picture (McCune and Grace 2002); however, values at the upper end suggest a potential to mislead and thus too much reliance on details of the plots should be discouraged. Together the axes explained $88.6 \%$ of the variance, $20.5 \%, 26.4 \%$ and $41.7 \%$ for axes 1,2 , and 3 respectively. Axis 3, which explains the majority of the variability, was strongly influenced by basal leaf and flower petal measurements (mean tooth crest tooth valley, mean tooth valley to midvien, and mean petal width). Axis 2 was weakly correlated $\left(\mathrm{R}^{2}<.50\right)$ with both flowering date and the petal length and width. Axis 1 was strongly influenced by total height and diameter. Leaf tooth traits show the strongest correlations to ordination axes, and adequately explain the distribution of individuals in ordination space (Fig. 3; Table 3). Since the majority of variation is accounted for within axis 2 and 3, they are used to visually describe the data.

NMS ordination provided evidence of discreet clustering of populations in ordination space based on morphological and phenological characteristics (Fig. 4). Post hoc analysis using Multi-Response Permutation Procedure (MRPP) confirms many significant differences between individual populations (Table 4a). More than 58\% of the total population level comparisons resulted significant differences, some with large $A$ values. Fort Hill (FH), the most northern population, dominated the comparisons. These populations were not different than Bald Hill or Hazel Dell. Interestingly, Bald Hill and Hazel Dell are significantly different from each other when analyzed at the population level, but this disappeared when grouped into respective visual zones and clusters.

Using county as a subgroup overlay suggests that county is marginally sufficient in visually defining differences in plant variation based on the traits measured (Fig.4). Not surprisingly, Fort Hill individuals' (Yamhill County) are clustered in the ordination, yet they overlap with individuals represented by both Benton and Lane Counties. Post hoc MRPP analysis suggests all counties are significantly different from one another (Table 4b), although true difference based on $A$ values are only found between Yamhill, Marion, and Linn Counties. Similarly, no clustering was found in the Eugene overlay, which displays no visual difference
between plants within or outside the 20 mile Eugene seed transfer zone. Individuals inside the 20 miles buffer appear to be less variable than those outside (Fig. 5). Significant differences were detected between populations as being either outside or within the Eugene 20 mile buffer ( $\mathrm{T}=-2.5044066, \mathrm{~A}=0.00312368, p=0.02$ ) based on post hoc MRPP, but the extremely low $A$ value suggests little within group dissimilarity and is therefore poor support for a difference between individuals inside and outside of the buffer. Populations that dominate many of the population level comparisons have the potential to strongly influence subsequent overlays used in analysis. These differences could be strongly driven by the influence of the Fort Hill, Sublimity and Kingston Prairie populations.

The visually defined geographic overlay again mimics the results found in the population and county level overlays. The Fort Hill population is distinctly separated from Zones 2 and 4 (Finely, and Kingston/Sublimity Prairies respectively) (Fig. 6). Visual zones 1 and 6 overlap greatly despite being geographically furthest from one another (Fig. 2).

Clustering of populations based on Hierarchical Clustering using Euclidean distances resulted in four geographic clusters (Fig. 2). At this coarse clustering level, visual inspection yields two distinctly different clusters (Clusters 2 and 4). Here the Spores population is clustered with the Linn County populations of Kingston and Sublimity Prairies. MRPP suggests several differences in clusters similar to those suggested at the population, County, and visual zone levels (Table 4a). Hierarchically defined clusters appear better in geographic representation than county but small $A$ values suggest visually defining the clusters may be a more accurate reflection in this example (Fig. 8).

The valley foothill populations of Kingston and Sublimity Prairies appear to have less variability among individuals in the ordination; however, other populations within this ecoregion are quite variable and overlap with most other ecoregions. The Spores population, which represents the only population in the Willamette River Tributary and Gallery Forest ecoregion, is also less variable among individuals in the ordination with some overlap with individuals from other ecoregions. Post hoc MRPP analysis again indicates ecoregion level differences are significant, but low $A$ values suggests questioning the strength of this result (Table 4 d ). These results correspond to all other overlays investigated in this analysis.

## Correlations of traits with geographic and climatic variables

Fourteen traits were measured or calculated for each individual in each population. Ranges and means ( $\pm 1 \mathrm{SD}$ ) of populations indicate substantial variability both between and within populations (Appendices E and F). High levels of within population variability suggest high levels of polymorphism in populations in the Willamette Valley. Population level variability is apparent in most traits.

Regression of ordination axes with climatic and geographic variables identified a few weak associations (Table 3), (Figs. 10-11). Neither date of emergence nor flowering date proved to be correlated with latitude or longitude. It is important to note that correlations with axes are difficult to ascertain since variables are not necessarily linearly correlated or parallel to axes.

Pearson's Correlation Coefficients between univariate traits and geographic or climatic variables identified some significant correlations. Longitude and elevation were correlated with several plant traits, specifically those related to flower petal measurements (Table 5). Both mean tooth crest, tooth valley, and mean tooth crest to mid-vein were negatively correlated with longitude. Mean petal length and width are both positively correlated with the last date of spring frost.

## DISCUSSION

Populations displayed some discrete clustering of populations in ordination space based on morphological and phenological characteristics; however, these clusters do not appear to follow a clear geographic pattern. Basal leaf and flower petal measurements (mean tooth crest, tooth valley, mean tooth valley to midvien, and mean petal width) appear to be strongly correlated to axis 3 but are highly variable within and between populations. Fort Hill (FH), the most northern population, was the only representative of northern Polk County, as well as visually and hierarchically defined clusters based on its proximity from all other populations. This population was characterized by reduced variability in some measurements. Populations of rather small uniform composition in one locality are not unusual for Potentilla gracilis, although nearby populations may be much more variable (Hitchcock 1993). A general measure of basal leaf lobe dissection, which was greater in individuals from this location, appears to set this population apart. Whether this trait is adaptive in this population or if the population is actually var. fastigiata (currently limited to Yamhill Co. within the Willamette Valley based on Oregon Flora Project 2008) warrants further investigation.

Using county as a subgroup overlay indicates that county is marginally sufficient in defining differences in plant variation based on the traits measured based on the very limited number of populations sampled. Potentilla gracilis var. gracilis is clearly distributed extensively within each county but these populations are not represented in this common garden analysis (Oregon Plant Atlas, December 5, 2008). This substantially limits our ability to make inferences in areas under sampled or absent from the collection. Not surprisingly, Fort Hill individuals' (Yamhill County) are clustered in the ordination, yet they overlap with individuals represented by both Benton and Lane Counties. Incidentally these differences have small $A$ values based on post hoc MRPP analysis. Post hoc MRPP analysis suggests Yamhill (FH) is significantly different than both Marion and Linn Counties. However, this again may be biased as each of these counties are represented by only a single population, thus reflecting only population level differences. More populations within all three of these counties should be included for a more accurate depiction of county level variance.

No clear visual clustering was found in the Eugene overlay, which shows no difference between plants within or outside the 20 mile Eugene seed transfer zone. Both groups appear to have high within group dissimilarity and overlap in a large portion of the ordination. Only 33 individuals are represented within Eugene, while 130 are outside. It appears that populations within the buffer are similar in morphological characteristics, however individuals from
populations outside the buffer that overlap with these populations include individuals from Fort Hill, Junction City, Lupine Meadows, and Finely. These results indicate no clear geographically meaningful pattern and little reason to continue using a 20 mile buffer as a boundary for this species. Further investigation of spatial autocorrelation is strongly recommended. Large differences in sample size combined with the high degree of variability within populations may substantially bias this result.

Geographically defined visual and hierarchical cluster yielded similar results as found at the population level. Hierarchical clustering can be useful tool in defining spatial clusters when populations are on continuous scale or when elevation differences are included in the region wished to be defined. County is an artificial boundary often used by managers although rarely biologically meaningful. Although county appears to be adequate to define areas, substantial amounts of missing data limit the usefulness of this classification to inform seed zones. This is also the case in the ecoregion overlay. We strongly recommend the addition of other populations and more individuals within all counties in future seed guideline studies. Too few seed sources and representative samples from populations make accurate inferences to these areas of the Willamette Valley slightly more complicated to ascertain.

## Correlations of traits with geographic and climatic variables

Morphological and phenological traits were poorly correlated with geographic and climatic variables. Pearson's Correlation Coefficients between univariate traits and geographic or climatic variables identified some significant correlations. Little correlation with climatic variables was anticipated since we do not expect to see mean level differences within the Willamette Valley based on coarse PRISM data. These results are similar to other seed zones studies within the Willamette Valley (Erickson, Mandel, and Sorensen 2004; St. Clair, Mandel, and Vance-Borland 2005). Rather than highlighting mean climatic differences within the Willamette Valley, we suggest switching focus to biologically relevant characteristics such as emergence or flowering dates. Despite the absence of statistical significance of phenological traits in this analysis, these traits are likely to be tightly linked to selection and gene flow, related to pollinator activity and invertebrate herbivory. Investigation of less coarse climatic variables to discern slight microclimate difference could prove useful.

Our findings suggest that populations of P. gracilis var. gracilis within the Willamette Valley are morphologically and climatically within a single ecotype up to 227 m in elevation; no collections were from higher elevations. Populations from a full range of environmental and climatic conditions in which each species occurs should be included in future seed guideline studies if a range larger than the Willamette Valley is in question. Thus, we do not recommend movement of seed from sources outside the Willamette Valley, or beyond within Valley distributions without further study. Conservatively, we also do not recommend using seed from Yamhill County so as to reduce the chance of including P. gracilis var. fastigiata in zones where is not currently distributed.

The strong overlap of measured characteristics across populations suggests movement of seed among populations with similar environments within the Willamette Valley would result in
a high probability of plant establishment. Potentilla gracilis is a widespread species ranging in distribution from Alaska to Mexico. Potentilla spp. are able adapt to a variety of environments where they have been documented to alter their leaf morphology in response to environmental changes, particularly drought (Teeri 1978; Loik and Harte 1997). In response to global climate change, where drought and increased temperatures are predicted in the Pacific Northwest, using P. gracilis as a species in seed mixtures for restoration sites could prove essential to restoration success. Within the Willamette Valley, restoration specialists and botanists believe that as a widespread perennial species, Potentilla gracilis provides a foundation for other native plants and pollinators (M. Gisler, personal communication).

## Agricultural production of native plant materials

The goal of acquiring locally adapted seeds is often difficult especially when populations are extirpated from restoration areas and wild collected seed is not abundant or is very expensive. These problems have encouraged agricultural production of many native species. While this aims to solve seed availability it creates equally as difficult considerations. Planting collected seeds in production fields may not capture all the nonrandom mortality of a population. Agricultural production may influence seed dormancy and germination requirements, and survival/death of plants based on production management, essentially selecting for good production plants but perhaps losing diversity in the process. Single year collection does not reflect changes in original population after seed collection (seed from seed bank germination or seeds/pollen arrival from another locations), or environmental/climatic changes (Rogers and Montalvo 2004). In addition, geographically associated pest, hybrids, and alternative genotypes can easily be increased and potentially transferred to new environments. For example, Potentilla recta, a noxious Eurasian invasive with morphology similar to $P$. gracilis can easily be mistaken, as the primary discriminating feature, flower color, is not evident during seed collection. We made this error and the invasive plant was grown in the common garden and a small production field until they flowered, were correctly identified, and destroyed. Collection of only a few noxious seeds can be devastating to a restoration site and not easily noticed in a production field. Identification of individuals within a production field should warrant destruction of the field and vigilance to remove future invasive volunteers. This can result in significant financial losses; however, the financial cost if the invasive species is not detected and seeds are moved to a restoration site will be orders of magnitude greater. Maximizing local adaptation and minimizing ecological and financial risks in agricultural production of native species is a very difficult balance.

## RECOMMENDATIONS

In summary, our data indicate there is some morphological and phenological differentiation in populations of $P$. gracilis var. gracilis across the Willamette Valley. A few populations were clearly morphologically different; however, there were no apparent spatial, climatic, or environmental factors that were related to the populations. Therefore, we recommend a single seed transfer zone for $P$. gracilis var. gracilis within the Willamette Valley
under 227 m and from $45.39^{\circ}$ latitude in the north to $43.92^{\circ}$ in the south. Conservatively, we also do not recommend using seed from Yamhill County to reduce the chance of including $P$. gracilis var. fastigiata in zones where is not currently distributed. We did not include plants from outside of this area and cannot assume their inclusion in this seed transfer zone. We suggest that using multiple populations in reintroductions will increase restoration success and assist in restoring more historic levels of gene flow. Last, additional studies are recommended to determine the presence and/or scale of local adaptation and the genetic basis for the adaptations if they are found.

Table 1. List of population names with corresponding abbrevations and number of individuals represented per population as sampling units. County: $1=$ Benton, $2=$ Polk, $3=$ Lane, $4=$ Linn, $5=$ Marion. Eugene: $1=$ within 20 mile buffer, $2=$ not present in buffer. Zone is based on visually defining geographic population aggregates. Cluster is based on dendrogram interpretation at the $1.4 \mathrm{E}-07$ level. EPA defined Ecoregion Level 4: $1=$ Valley Foothills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Gallery Forest.

| Population | Code | VZone | Eugene | County | Cluster | Ecoregion |  |
| :--- | :--- | :--- | :---: | :---: | :---: | :---: | :---: |
| Bald Hill | BH | 1 | 3 | 2 | 1 | 1 | 2 |
| Fort Hill | FH | 2 | 1 | 2 | 2 | 3 | 2 |
| Finely | FI | 3 | 4 | 2 | 1 | 1 | 2 |
| Hazel Dell | HD | 4 | 6 | 1 | 3 | 2 | 1 |
| Jackson Creek | JC | 5 | 3 | 2 | 1 | 1 | 1 |
| Kingston Prairie | KP | 6 | 2 | 2 | 4 | 4 | 1 |
| Lupine Meadows | LU | 7 | 3 | 2 | 1 | 1 | 2 |
| Oxbow East | OE | 8 | 6 | 1 | 3 | 2 | 2 |
| Philomath Prairie | PP | 9 | 3 | 2 | 1 | 1 | 1 |
| Sublimity Prairie | SP | 10 | 2 | 2 | 5 | 4 | 1 |
| Spores | SR | 11 | 5 | 1 | 3 | 4 | 3 |
| Willow Creek | WC | 12 | 6 | 1 | 3 | 2 | 2 |

Table 2. List of phenological and morphological traits measured on P. gracilis plants in common garden study.

| Trait | Abbreviation | Measurement Methods |
| :---: | :---: | :---: |
| Flowering Date | FLJUL | Flowering date was monitored on transplanted individuals three times a week until a value was obtained for each study plant (Julian) |
| Mean basal leaf height | AVEBASHT | Base of plant to top of basal leaf crown height was measured in 3 haphazardly choosen locations on an individual plant(cm) |
| Total height | TOTHT | Mean total height was calculated from 3 measurements taken from the base to the tip of 3 flowering stalks chosen haphazardly within a plant (cm) |
| Diameter of floral stalk crown | DIAFL | Diameter of flowering stalk crown was measured once at the widest point in the floral crown (cm) |
| Diameter of basal stalk crown | DIABAS | Diameter of basal crown was measured once at the widest point of the basal crown (cm) |
| Mean flower petal length | AVEPETL | Mean flower petal length was calculated from 3 petal length measurements taken from 3 different flowers chosen haphazardly (cm) |
| Mean flower petal width | AVEPETW | Mean flower petal width was calculated from 3 petal width measurements taken from 3 different flowers chosen haphazardly (cm) |
| Total number of racemes | TOTRAAC | Each plant was sectioned into 4 equal quadrats representing $25 \%$ of the plant area. One quadrat was randomly chosen and all racemes counted therein. The total number of racemes was then extrapolated to represent the whole plant. |
| Mean basal leaf length | AVEBASLL | Mean basal leaf length was measured on 3 haphazardly chosen individuals (cm) |
| Mean leaf tooth crown to valley | AVETCTV | Mean basal leaf 3rd tooth crown to tooth valley was measured on 3 haphazardly chosen individuals (cm) |
| Mean leaf valley to midvien | AVETVM | Mean basal leaf valley of 3rd tooth to midvein was measured on 3 haphazardly chosen individuals (cm) |
| Number of branches per raceme | RACEBRAN | Number of branches per raceme based on a single raceme chosen haphazardly |
| Number of flowers per branch | FLBRAN | Number of flowers per branch based on a single branch chosen haphazardly |

Table 3. Correlation of axes with measured trait variables and secondary geospatial and climatic variables.

|  | Axis 1 |  | Axis 2 |  | Axis 3 |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{R}^{2}$ | tau | $\mathrm{R}^{2}$ | tau | $\mathrm{R}^{2}$ | tau |
| Latitude | 0.01 | -0.09 | 0.12 | 0.3 | 0.05 | 0.16 |
| Longitude | 0.03 | 0.14 | 0.25 | 0.33 | 0.06 | 0.1 |
| Elevation | 0 | -0.02 | 0.12 | 0.25 | 0 | 0.02 |
| Flowering Date | 0 | -0.02 | 0.1 | -0.25 | $\mathbf{0 . 5}$ | $\mathbf{- 0 . 5 5}$ |
| Mean basal leaf height | 0.02 | 0.06 | 0.35 | -0.42 | 0.12 | -0.23 |
| Total height | 0.21 | 0.31 | 0.31 | -0.37 | 0.03 | -0.14 |
| Diameter of floral stalk crown | $\mathbf{0 . 5 1}$ | $\mathbf{0 . 5 3}$ | 0.06 | 0.18 | 0.35 | 0.41 |
| Daimeter of basal stalk crown | $\mathbf{0 . 5 6}$ | $\mathbf{0 . 5 5}$ | 0.05 | -0.14 | 0.06 | 0.15 |
| Mean flower petal length | 0.02 | -0.08 | $\mathbf{0 . 5 4}$ | $\mathbf{- 0 . 5 4}$ | 0.35 | -0.43 |
| Mean flower petal width | 0.03 | -0.1 | $\mathbf{0 . 6 4}$ | $\mathbf{- 0 . 6 1}$ | 0.33 | -0.44 |
| Total number of racemes | 0.37 | 0.45 | 0.15 | 0.3 | 0.14 | 0.31 |
| Mean basal leaf length | 0.09 | -0.24 | 0.49 | -0.5 | 0.08 | -0.2 |
| Mean leaf tooth crown to |  |  |  |  |  |  |
| valley | 0.21 | -0.32 | $\mathbf{0 . 6 1}$ | $\mathbf{- 0 . 6 2}$ | 0.15 | -0.25 |
| Mean leaf valley to midvien | 0.23 | -0.34 | $\mathbf{0 . 6 7}$ | $\mathbf{- 0 . 6 4}$ | 0.19 | -0.3 |
| Number of branches per |  |  |  |  |  |  |
| raceme | 0.12 | -0.23 | 0.19 | -0.26 | 0.13 | 0.27 |
| Number of flowers per branch | 0.02 | 0.09 | 0 | 0 | 0.04 | -0.14 |
| First day of fall frost | 0 | 0.05 | 0.15 | -0.2 | 0.04 | -0.06 |
| Number of frost free days | 0 | 0 | 0 | -0.16 | 0.02 | 0.06 |
| Last day of spring frost | 0 | -0.01 | 0.25 | -0.32 | 0.23 | -0.35 |
| Mean annual temperature | 0.01 | 0.1 | 0.13 | -0.18 | 0.08 | -0.11 |
| Mean annual precipitation | 0 | 0.01 | 0.26 | 0.44 | 0.06 | 0.2 |

Table 4. Significantly different MRPP multiple pair-wise comparisons with adjusted Bonferroni correction, test statistic T and agreement statistic A grouped by (a) population ( $p<0.0007$ ) (b) county ( $p<0.005$ ) (c) visual zone ( $p<0.003$ ) ( d ) dendrogram defined cluster ( $p<.005$ ) (e) level 4 ecoregion ( $p<0.003$ ).
(a)

| Populations | $\mathbf{T}$ | $\mathbf{A}$ |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | -12.460734 | 0.2443675 |
| 1 | vs. | 4 | -6.6406762 | 0.1111011 |
| 1 | vs. | 5 | -6.4204746 | 0.0880218 |
| 1 | vs. | 7 | -11.798962 | 0.0749727 |
| 1 | vs. | 8 | -10.677441 | 0.1542717 |
| 1 | vs. | 11 | -10.29278 | 0.1328831 |
| 1 | vs. | 12 | -10.900042 | 0.1608538 |
| 2 | vs. | 3 | -18.09607 | 0.1896577 |
| 2 | vs. | 5 | -5.2686132 | 0.093232 |
| 2 | vs. | 6 | -15.077442 | 0.3021223 |
| 2 | vs. | 7 | -10.712699 | 0.0887577 |
| 2 | vs. | 8 | -6.4275091 | 0.1046239 |
| 2 | vs. | 9 | -5.593547 | 0.249509 |
| 2 | vs. | 10 | -18.041957 | 0.236982 |
| 2 | vs. | 11 | -9.385499 | 0.2250215 |
| 2 | vs. | 12 | -5.2806116 | 0.0857556 |
| 3 | vs. | 4 | -7.826327 | 0.0715135 |
| 3 | vs. | 5 | -8.2208078 | 0.0633894 |
| 3 | vs. | 7 | -15.045056 | 0.0728594 |
| 3 | vs. | 8 | -13.954486 | 0.1169792 |
| 3 | vs. | 10 | -6.6043003 | 0.0308445 |
| 3 | vs. | 11 | -12.47322 | 0.0891159 |
| 3 | vs. | 12 | -13.299915 | 0.1106986 |
| 4 | vs. | 6 | -8.867487 | 0.1452815 |
| 4 | vs. | 10 | -9.8775138 | 0.1109412 |
| 5 | vs. | 6 | -10.457177 | 0.1441864 |
| 5 | vs. | 10 | -11.343213 | 0.1133082 |
| 5 | vs. | 11 | -6.0073018 | 0.112225 |
| 6 | vs. | 7 | -18.604711 | 0.1266606 |
| 6 | vs. | 8 | -13.620833 | 0.2008451 |
| 6 | vs. | 11 | -14.227838 | 0.1764386 |
| 6 | vs. | 12 | -13.270564 | 0.2082119 |
| 7 | vs. | 10 | -23.603027 | 0.1402889 |
| 7 | vs. | 11 | -10.884007 | 0.0805091 |
| 8 | vs. | 10 | -15.59484 | 0.1615738 |
| 8 | vs. $\quad 11$ | -7.4713521 | 0.1474434 |  |


| 10 | vs. | 11 | -13.687406 | 0.1185049 |
| :--- | :--- | :--- | :--- | :--- |
| 10 | vs. | 12 | -15.070673 | 0.1578331 |

(b)

| County |  |  | T | $\mathbf{A}$ |
| :--- | :--- | :--- | ---: | ---: |
| 1 | vs. | 2 | -16.955239 | 0.0585676 |
| 1 | vs. | 3 | -18.304071 | 0.0445266 |
| 1 | vs. | 4 | -10.21416 | 0.0291274 |
| 1 | vs. | 5 | -16.802261 | 0.0446437 |
| 2 | vs. | 3 | -7.9141006 | 0.0642137 |
| 2 | vs. | 4 | -15.077442 | 0.3021223 |
| 2 | vs. | 5 | -18.041957 | 0.236982 |
| 3 | vs. | 4 | -22.893618 | 0.1593029 |
| 3 | vs. | 5 | -25.123367 | 0.1515237 |
| 4 | vs. | 5 | -4.3005621 | 0.025375 |

(c)

| Zone |  | $\mathbf{T}$ | $\mathbf{T}$ |  |
| :--- | :--- | ---: | ---: | ---: |
| 3 | vs. | 1 | -13.090845 | 0.0644084 |
| 3 | vs. | 4 | -7.8759484 | 0.0264395 |
| 3 | vs. | 6 | -12.026719 | 0.044022 |
| 3 | vs. | 2 | -24.770962 | 0.0759022 |
| 3 | vs. | 5 | -11.484749 | 0.0484367 |
| 1 | vs. | 4 | -18.09607 | 0.1896577 |
| 1 | vs. | 6 | -7.4768868 | 0.0644485 |
| 1 | vs. | 2 | -25.090366 | 0.1819491 |
| 1 | vs. | 5 | -9.385499 | 0.2250215 |
| 4 | vs. | 6 | -22.253372 | 0.1474991 |
| 4 | vs. | 2 | -5.2087644 | 0.0180404 |
| 4 | vs. | 5 | -12.47322 | 0.0891159 |
| 6 | vs. | 2 | -32.283423 | 0.180111 |
| 6 | vs. | 5 | -11.036691 | 0.1134262 |
| 2 | vs. | 5 | -18.559656 | 0.1011138 |

(d)

| Cluster |  |  | $\mathbf{T}$ | $\mathbf{A}$ |
| :--- | :--- | :--- | :---: | :---: |
| 1 | vs. | 3 | -16.955239 | 0.0585676 |
| 1 | vs. | 2 | -18.304071 | 0.0445266 |
| 1 | vs. | 4 | -20.615384 | 0.0463652 |
| 3 | vs. | 2 | -7.9141006 | 0.0642137 |
| 3 | vs. | 4 | -25.090366 | 0.1819491 |
| 2 | vs. | 4 | -34.074287 | 0.152637 |

(e)

| Ecoregion |  |  | T | A |
| :---: | :---: | ---: | :---: | :---: |
| 2 | vs. | 1 | -14.513 | 0.030 |
| 2 | vs. | 3 | 9.957 | 0.027 |
| 1 | vs. | 3 | -12.407 | 0.056 |

Table 5. Univariate Pearson Correlation Coefficients of a subset of measured traits with geographic and climatic variables. Significant correlations are shown in italicized.

|  | Latitude | Longitude | Elevation | First day of fall frost | Number of frost free days | Last day of spring frost | Mean <br> annual temp | Mean <br> annual <br> precip |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Flowering Date p-value | $\begin{array}{r} \hline-0.22813 \\ 0.0021 \end{array}$ | $\begin{array}{r} \hline 0.04469 \\ 0.5525 \end{array}$ | $\begin{array}{r} \hline 0.05563 \\ 0.4595 \\ \hline \end{array}$ | $\begin{array}{r} -0.0093 \\ 0.9017 \end{array}$ | $\begin{array}{r} \hline-0.28392 \\ \mathbf{0 . 0 0 0 1} \end{array}$ | $\begin{array}{r} \hline 0.43792 \\ <.0001 \end{array}$ | $\begin{array}{\|r} \hline-0.23672 \\ 0.0014 \end{array}$ | $\begin{array}{r} \hline 0.32814 \\ <.0001 \end{array}$ |
| Mean basal leaf height <br> $p$-value | $\begin{array}{r} -0.15294 \\ 0.041 \end{array}$ | $\begin{array}{r} -0.23111 \\ 0.0019 \end{array}$ | $\begin{array}{r} -0.17727 \\ 0.0176 \end{array}$ | $\begin{array}{r} 0.16333 \\ 0.0289 \end{array}$ | $\begin{array}{r} -0.06705 \\ 0.3725 \end{array}$ | $\begin{array}{r} 0.34796 \\ <.0001 \end{array}$ | $\begin{array}{r} -0.2697 \\ 0.0003 \end{array}$ | $\begin{array}{r} 0.17095 \\ 0.0221 \end{array}$ |
| Total height <br> p-value | $\begin{array}{r} -0.35502 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} \hline-0.24373 \\ 0.001 \\ \hline \end{array}$ | $\begin{array}{r} -0.35283 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} \hline 0.36149 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} \hline 0.22867 \\ 0.0021 \\ \hline \end{array}$ | $\begin{aligned} & \hline 0.1703 \\ & 0.0227 \\ & \hline \end{aligned}$ | $\begin{array}{r} -0.40289 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} \hline 0.29519 \\ <.0001 \\ \hline \end{array}$ |
| Diameter of floral stalk crown p-value | $\begin{array}{r} -0.00086 \\ 0.9909 \\ \hline \end{array}$ | $\begin{array}{r} 0.14922 \\ 0.0462 \\ \hline \end{array}$ | $\begin{array}{r} -0.03608 \\ 0.6316 \\ \hline \end{array}$ | $\begin{array}{r} -0.03215 \\ 0.6692 \\ \hline \end{array}$ | $\begin{array}{r} 0.11672 \\ 0.1197 \\ \hline \end{array}$ | $\begin{array}{r} -0.23317 \\ 0.0017 \\ \hline \end{array}$ | $\begin{array}{r} 0.06237 \\ 0.4069 \\ \hline \end{array}$ | $\begin{array}{r} -0.02072 \\ 0.7831 \\ \hline \end{array}$ |
| Diameter of basal stalk crown p-value | $\begin{array}{r} -0.08495 \\ 0.2582 \\ \hline \end{array}$ | $\begin{array}{r} -0.05325 \\ 0.479 \\ \hline \end{array}$ | $\begin{array}{r} -0.11306 \\ 0.1318 \\ \hline \end{array}$ | $\begin{array}{r} 0.09588 \\ 0.2017 \\ \hline \end{array}$ | $\begin{array}{r} 0.0475 \\ 0.5277 \\ \hline \end{array}$ | $\begin{array}{r} 0.06608 \\ 0.3795 \\ \hline \end{array}$ | $\begin{array}{\|r} -0.07064 \\ 0.3474 \\ \hline \end{array}$ | $\begin{array}{r} 0.08842 \\ 0.2392 \\ \hline \end{array}$ |
| Mean flower petal length p-value | $\begin{array}{r} -0.35466 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.35857 \\ <.0001 \end{array}$ | $\begin{array}{r} -0.24977 \\ 0.0007 \\ \hline \end{array}$ | $\begin{array}{r} 0.33732 \\ <.0001 \end{array}$ | $\begin{array}{r} -0.04506 \\ 0.5492 \\ \hline \end{array}$ | $\begin{array}{r} 0.57 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.41797 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.42691 \\ <.0001 \\ \hline \end{array}$ |
| Mean flower petal width <br> p-value | $\begin{array}{r} -0.44757 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.44067 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.30472 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.44719 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.09867 \\ 0.1888 \\ \hline \end{array}$ | $\begin{aligned} & 0.5037 \\ & <.0001 \\ & \hline \end{aligned}$ | $\begin{array}{r} -0.4924 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.46129 \\ <.0001 \\ \hline \end{array}$ |
| Total number of racemes p-value | $\begin{array}{r} 0.13536 \\ 0.0708 \\ \hline \end{array}$ | $\begin{array}{r} 0.36345 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.18576 \\ 0.0128 \\ \hline \end{array}$ | $\begin{array}{r} -0.24981 \\ 0.0007 \\ \hline \end{array}$ | $\begin{array}{r} -0.13657 \\ 0.0683 \\ \hline \end{array}$ | $\begin{array}{r} -0.15181 \\ 0.0425 \\ \hline \end{array}$ | $\begin{array}{r} 0.24694 \\ 0.0009 \\ \hline \end{array}$ | $\begin{array}{r} -0.10328 \\ 0.1689 \\ \hline \end{array}$ |
| Mean basal leaf length <br> $p$-value | $\begin{array}{r} -0.27045 \\ \mathbf{0 . 0 0 0 3} \end{array}$ | $\begin{array}{r} -0.26621 \\ 0.0003 \end{array}$ | $\begin{array}{r} -0.23912 \\ 0.0013 \\ \hline \end{array}$ | $\begin{array}{r} 0.19733 \\ 0.0081 \\ \hline \end{array}$ | $\begin{array}{r} -0.01525 \\ 0.8395 \\ \hline \end{array}$ | $\begin{array}{r} 0.31578 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.42857 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.28702 \\ <.0001 \\ \hline \end{array}$ |
| Mean leaf tooth crown to valley p-value | $\begin{array}{r} -0.07482 \\ 0.3196 \\ \hline \end{array}$ | $\begin{array}{r} -0.56435 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.19637 \\ 0.0084 \\ \hline \end{array}$ | $\begin{array}{r} 0.27695 \\ 0.0002 \end{array}$ | $\begin{array}{r} -0.04635 \\ 0.5378 \\ \hline \end{array}$ | $\begin{array}{r} 0.48289 \\ <.0001 \\ \hline \end{array}$ | $\begin{array}{r} -0.29398 \\ \quad<.0001 \\ \hline \end{array}$ | $\begin{array}{r} 0.10014 \\ 0.1823 \\ \hline \end{array}$ |
| Mean leaf valley to midvien p-value | $\begin{array}{r} -0.08562 \\ 0.2545 \end{array}$ | $\begin{array}{r} -0.54907 \\ <.0001 \end{array}$ | $\begin{array}{r} -0.23401 \\ 0.0016 \end{array}$ | $\begin{array}{r} 0.28667 \\ 0.0001 \\ \hline \end{array}$ | $\begin{array}{\|r} -0.02513 \\ 0.7384 \end{array}$ | $\begin{aligned} & 0.4635 \\ & <.0001 \end{aligned}$ | $\begin{aligned} & -0.312 \\ & <.0001 \end{aligned}$ | $\begin{array}{r} 0.09777 \\ 0.1929 \\ \hline \end{array}$ |
| Number of branches per raceme p-value | $\begin{array}{r} -0.07892 \\ 0.2937 \end{array}$ | $\begin{array}{r} -0.11682 \\ 0.1194 \end{array}$ | $\begin{array}{r} -0.12929 \\ 0.0846 \\ \hline \end{array}$ | $\begin{array}{r} 0.07899 \\ 0.2933 \end{array}$ | $\begin{array}{r} 0.08812 \\ 0.2408 \end{array}$ | $\begin{array}{r} -0.02348 \\ 0.755 \end{array}$ | $\begin{array}{\|r} \hline-0.16711 \\ 0.0254 \end{array}$ | $\begin{array}{r} 0.04355 \\ 0.5627 \end{array}$ |
| Number of flowers per branch p-value | $\begin{array}{r} -0.0711 \\ 0.3443 \end{array}$ | $\begin{array}{r} -0.16846 \\ 0.0242 \\ \hline \end{array}$ | $\begin{array}{r} -0.02561 \\ 0.7336 \\ \hline \end{array}$ | $\begin{array}{r} 0.16276 \\ 0.0295 \\ \hline \end{array}$ | $\begin{array}{r} 0.14761 \\ 0.0486 \\ \hline \end{array}$ | $\begin{array}{r} 0.00564 \\ 0.9403 \\ \hline \end{array}$ | $\begin{array}{r} -0.01887 \\ 0.8021 \\ \hline \end{array}$ | $\begin{array}{r} 0.01107 \\ 0.8831 \\ \hline \end{array}$ |

Figure 1. Population distribution of $P$. gracilis seed collection locations within the Willamette Valley. Circles represent visually defined clusters. Lines connecting visual clusters represent clusters defined by hierarchically clustering analysis.


Figure 2. Hierarchical Dendrogram of populations to detect clustering defined by geographical variables latitude and longitude.
POGR NMS
Distance (Objective Function)
2.8E-.06

Figure 3. NMS 3-dimensional ordination; Population is used as the overlay. Each symbol represents an individual in multivariate space. Symbols that are closer spatially are more similar morphologically and phenologically.


Figure 4. NMS ordination of individuals within each population (axis 2 vs 3 ). Multiple individuals (sample units) are represented by a single color.


Figure 5. NMS ordination with County overlay (axis 2 vs 3 ). County: $1=$ Benton, $2=\operatorname{Polk}, 3=$ Lane, $4=$ Linn, $5=$ Marion.


Figure 6. NMS ordination with Eugene overlay (axis 2 vs 3). Individuals within 20 miles of Eugene are shown in red, those outside of this seed transfer zone are shown in green.


Figure 7. NMS ordination with Visual Zone overlay (axis 2 vs 3 ).


Figure 8. NMS ordination using Clusters defined by hierarchical cluster analysis overlay (axis 3 and 2).


Figure 9. NMS ordination using Ecoregion level 4 defined by EPA (axis 3 and 2): $1=$ Valley Foot hills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Galley Forest.


Figure 10. NMS ordination axis 2 vs 3 with climatic variable correlations jointly biplotted with ITS seed zone (a) annual precipitation, (b) annual temperature (c) first day of fall frost (d) last day of spring frost (e) number of frost free days (average growing season length). Red lines indicate regression lines (r), while the blue line represents Kendall' tau (a rank regression).
(a)

(b)

(c)

(d)

(e)


Axis 1
FRSTFREE
Axis 1
$r=-.102$ tau $=-.043$
Axis 3
$r=.106$ tau $=.018$


Figure 11. NMS ordination axis 1 vs 3 with geographic variable correlations jointly biplotted with ITS seed zone (a) Latitude, (b) Longitude (c) Elevation. Red lines indicate regression line (r), while the blue line represents Kendall' tau (a rank regression).
(a)

(b)

(c)


# Distribution of morphologic and phenological variation of Prunella vulgaris in the Willamette Valley: a common garden study to inform seed transfer zones 



## Materials and Methods

## Study species

Prunella vulgaris L. ssp. lanceolata (W. Bartram) Hultén (Lamiaceae) is a common native perennial found in wetland habitat of the Willamette Valley, Oregon. Its distribution extends throughout North America and is even considered a weedy species. Another subspecies, Prunella vulgaris ssp. vulgaris, also occurs in Oregon on more disturbed sites and is not native (USDA 2008). The inflorescence of Prunella vulgaris ssp. lanceolata has a terminal dense four angled spike of verticillasters each with six flowers (three flowers per cymule). Flowers are typically purple, but may be white and flower during late spring and early summer. Plants are assumed to predominately outcrossing, however a mixed mating system is likely as many other Prunella species are self-fertile and highly clonal (Hitchcock and Cronquist 1993).

## Population sampling

Prunella vulgaris ssp. lanceolata seeds were collected in 2005 from 10 populations distributed throughout the Willamette Valley (Fig. 1, Table 1). Latitude, longitude, and elevation were recorded for each location. At each location, no more than $25 \%$ of available seed was collected from all individual plants. A large variation in natural population sizes were present fot this species; population sizes ranged from 80 to greater than 2000 individuals. For analysis, locations were classified based on individual populations, county, EPA defined level 4 Ecoregion (Griffin and Omernik 2008), and presence or absence within a Eugene 20 mile buffer zone (West Eugene Wetlands Seed Collection Manual, 2003).

## Experimental design

Seeds were sown in February 22, 2006 into flats of Ray Leach "Cone-tainers" and grown in a greenhouse at the Natural Resources Conservation Service Plant Material Center (PMC). The PMC is located in Benton County, Corvallis, Oregon, at 225 ft elevation. Thirty conetainers were started for each population $(\mathrm{N}=300)$, and were randomly placed within flats. Flats were watered and placed in an unheated greenhouse. Average daytime temperatures ranged between 4.4 and $12.8^{\circ} \mathrm{C}$ and nighttime temps were between 1.1 and $4.4^{\circ} \mathrm{C}$. No supplemental greenhouse lighting was used; seedlings were subjected to typical early spring daylight. After ten weeks in the greenhouse, plants were moved to a shade house and allowed to acclimatize for several weeks to outdoor temperatures.

Prior to transplanting, herbicide was applied to the study site to eliminate any existing weeds. A drip irrigation system was installed on the field. Drip tapes were placed in long rows across the field 3 ft apart. The field was irrigated approximately once every two weeks to help the plants establish during the first summer. The study site was then covered with three inches of bark mulch to further aid in weed suppression. The $232 P$. vulgaris ssp. lanceolata study plants were transplanted in June 2007 using a completely randomized design. Some seedling mortality reduced the overall number of plants available for outplanting. Thus, replicates from each population are uneven (between 12 and 30 replicates per population) totaling 232 individuals. An additional row of $P$. vulgaris ssp. lanceolata was planted on each side of the plot as a border row to buffer against edge effects. Plants were placed 0.6 meters apart within rows and rows were placed one meter apart.

## Trait measurement

Traits were chosen based on characteristics described in Winn and Gross 1993. Traits thought to have adaptive significance, or associated with reproductive success, taxonomically important traits, and traits with high degrees of variability between local conspecifics ( $P$. vulgaris ssp. vulgaris and ssp. lanceolata). While measuring pre-defined traits, additional traits were included based on apparent visual differences among plants. Several botanical experts or species authorities were consulted for recommendations during the trait selection process.

Phenological and morphological traits were measured for all individual $P$. vulgaris var lanceolata plants during the summer of 2007 (Table 2). All morphological traits were measured in a single day. To reduce measurement error, one person measured traits while a second recorded. Floral traits were measured using only new flowers. Morphological traits less than 25 cm in length were measured by dial calipers to 0.01 mm . Traits greater than 25 cm were measured with a meter stick to the nearest centimeter. When measuring a single trait in triplicate on an individual, no measurements were made from the same organ (e.g., average petal length was the mean length of three petals from three separate flowers chosen haphazardly).
Emergence date was monitored on a daily basis; when cone-tainers contained more than one seedling, germination was recorded for the first seedling that emerged in each cone-tainer. Flowering and seed set date was monitored on transplanted individuals three times a week until a value was obtained for each study plant. Plants with missing values were removed from the analysis (SP1401, SP1314, SP1808, SP1705, SP1504, SP1214).

## Climatic Data

Climatic conditions at each population location site were characterized using digital maps produced in ArcGIS 9.3 and data generated by PRISM climate models (PRISM group, 2008). PRISM is an analytical model that uses point data and a digital elevation model (DEM) to generate gridded estimates of monthly and annual average daily maximum/minimum climatic parameters (PRISM group, 2008). PRISM incorporates a conceptual framework that addresses the spatial scale and pattern of climate variables that allows for estimation of variables in regions with heterogeneous terrain (PRISM group, 2008). PRISM was parameterized to use 1971-2000 mean daily maximum/minimum climate variable grids as the predictor grids in the interpolation. The resolution of each cell within the grid is 4 km (averaged within the cell) and therefore the precision of the estimate for a single location is no better than half the resolution of the cell. Variables were modeled monthly. An annual average was produced by averaging the monthly grids. For this analysis, mean annual temperature (USANNAV) and precipitation (USANNP), mean dates of the first (SPRFRST) and last frost (FLLFRST), and the number of frost free days (FRSTFREE) was gathered for each population based on each population's unique latitude (LAT) and longitude (LONG).

## Data analysis

Ordination analyses were performed on morphological and phenological variables using nonmetric multidimensional scaling (NMS) based on Euclidean distance measures (Kruskal 1964). Analyses were completed using PcORD 5 (McCune and Mefford 1995). NMS ordination has no assumptions of multivariate normality of the data, is able to handle large numbers of zeros, and yields the most accurate representation of data structure when data are non-normal or on discontinuous scales (Peterson and McCune 2001). To account for variable collection on different scales (ie. Julian days and centimeters) data were relativized using the standard deviates of each column variable. Prior to relativization, mean number of flowers was monotonically log transformed to compress high values and spread low values by expressing
values as orders of magnitude. Monotonic transformation of individual variables allows for independent changes to data point values without altering their rank. Individuals greater than 2 standard deviates from the mean Euclidian distance were defined as outliers and removed from the analysis (KP1113, OE1501, SP1711).

Since adaptive variation is important in designating seed transfer zones and is most directly related to variation within and among seed sources, we used individuals as our sampling units instead of mean population estimates. Thus, the main data matrix consisted of individual plants and traits measured at the common garden. To visually clarify the distribution of sampling units in ordination space, a second matrix with additional information (latitude, longitude, county, elevation, and climatic variables) was overlayed or jointly-biplotted (Table 1). NMS uses an iterative search for an ordination with low stress, a measure of the relationship between ranked distances in multidimensional space to the ranked distances in the reduced ordination (Peterson and McCune 2001). To ensure that the ordination was below an acceptable level of stress, we used a random seed with 250 runs of real data. Dimensionality was assessed visually using a scree plot. Monte Carlo simulations using 250 replicates were used to assess the probability that final stress could have been obtained by chance. A stability criterion of 0.0001 was used.

We compared the relative position of each population in ordination space by visual assessment. Due to the degree of difficulty assessing individual sampling units within a population, additional coding subgroup overlays such as county ( $\mathrm{n}=7$ ), Level 4 Ecoregion ( $\mathrm{n}=$ 3), 20 mile Eugene buffer ( $\mathrm{n}=2$ ) (Table 1). These subgroup overlays were based on geographicadministrative or habitat units that are either in practice in the Willamette Valley or logical potential seed transfer zone boundaries. To identify spatial clustering based on latitude and longitude we used hierarchical agglomerative clustering patterns calculated using Euclidean distances and nearest neighbor linkages to maximize distinctness of groups. The resulting clustering dendrogram was scaled using a log transformation and information was retained at the $75 \%$ level (Fig. 2). This allowed us to partition the dataset into more homogenous subsets based exclusively on geographic location. These subsets were then used as additional plot overlays. A subjective measure visually defining populations into reasonable zones (visual zones) was also used.

We quantitatively compared the relative position of populations in the ordination using Euclidian distances and unblocked Multi-Response Permutation Procedure (MRPP) analysis on weighted groups. MRPP is a nonparametric test used to examine whether populations on matrix plots occupy different regions of ordination space. Correlations between ordination axes and the environmental variables were calculated using Pearson Correlation coefficients. The percent of variation in the original ordination was also recorded. A Bonferroni correction was used when multiple comparisons were performed to maintain a low probability of relationships appearing significant when, in fact, they only appear significant by chance.

Univariate calculations were made for each variable by population. Traits were used to produce pairwise Pearson Correlation Coefficients for each variable with latitude,
longitude, elevation, and climatic variables using SAS 9.2 (SAS Institute 2008). Ordination axes were individually regressed on geographic and climatic variables in PcORD.

## Results

## Morphological and phenological patterns across populations

The Nonmetric Multidimensional Scaling (NMS) analysis provided a 3-dimensional ordination best solution to the data based with a final stress of 18.72940 , final instability of 0.00001 and 175 iterations Using Clarke's cutoff for acceptable instability, values between 10 and 20 represent a usable picture (McCune and Grace, 2002 Therefore, values at the upper end, such as our results, suggest a potential to be misleading and thus too much reliance on details of the plots should be discouraged. Together the axes explained $76.6 \%$ of the variance, $22.4 \%$, $24.8 \%$ and $29.5 \%$ for axes 1,2 , and 3 respectively. Axes 1 and 2 were weakly influenced by flower and seed set date, and axis 3 was moderately influenced by mean leaf length and width. Only leaf measurements show moderate correlations to ordination axes, explaining some of the distribution of individuals in ordination space (Fig. 3; Table 3). Since the majority of the variation is accounted for within axes 2 and 3 , they are used to visually describe the data.

NMS ordination provided little evidence of discrete clustering of populations in ordination space based on morphological and phenological characteristics. Post hoc analysis, however, using Multi-Response Permutation Procedure (MRPP) indicated significant differences between many individual populations (Table 4a). Much overlap between populations exists, but some populations seem to be slightly clustered in ordination space (Fig. 4). With 45 total comparisons 27 pairs of populations were significantly different representing $60 \%$ of the population level contrasts. Further, many $A$ values were close to zero suggesting high within group heterogeneity. $A$ values close to zero indicate little within population similarity (i.e., individuals were not necessarily more similar to other members of their populations than to individuals from other populations). The $A$ statistic is a descriptor of within-group homogeneity compared to random expectation (McCune and Grace 2002). If heterogeneity within groups equal chance expectation then $A=1$; however, if less agreement (heterogeneity) exists within groups than expected by chance, then $A=0$. In such cases where small $A$ values are statistically significant careful consideration of the ecological significance of the results is warranted. $A$ values of less than 0.3 represent substantial heterogeneity (variability) between contrasted groups.

Populations that dominate many of the population level comparisons have the potential to strongly influence subsequent overlays used in analysis. Using county as a subgroup overlay suggests that county is sufficient in visually defining some differences in plant variation based on the traits measured (Fig.5). Individuals within Washington, Lane, and Marion Counties appear to be somewhat clustered away from one another but all other counties greatly overlap visually. Post hoc MRPP analysis suggests all counties differ significantly from one another, which is expected since it reflects much of the population level contrasts (Table 4b).

No clustering appears in the Eugene overlay (Fig. 6). Yet significant differences were detected between populations as being either outside or within the Eugene 20 mile buffer ( $\mathrm{T}=$ 13.843156, A $=0.03987868, p<0.0001$ ) based on post hoc MRPP. This pattern matches many of the individual population and county level differences. However, $A$ values were close to zero. Bald Hill, Lupine Meadows, Kingston Prairie, Cooper Mountain, Bertheldorf, and Stayton were the populations that cluster outside the Eugene buffer and are geographically located throughout the Willamette Valley provide little information for zoning or movement distances.

The geographic visual overlay suggests little clustering based on our geographic groupings (Fig. 7). Clusters 1 and 4 overlap greatly, as do Cluster 2 and 3; whereas Cluster 1 appears slightly separated from Clusters 3 and 5. Clusters run from north to south, 1 through 5, respectively. No geographic structure is apparent based on the visual overlay.

Clustering of populations based on Hierachical Clustering at the 1.6E-01 (75\%) levels using Euclidean distances resulted in 4 geographic clusters (Fig. 2). At the $75 \%$ coarse clustering level, visual inspection of ordination revealed overlap with clusters 1 and 4 which are separated from clusters 2, and 3 (Fig. 8). MRPP suggests several differences in clusters comparable to population and county levels (Table 4). Thus, hierarchically defined clusters appear better than county and visual clusters for grouping our data for use as a meaningful ordination overlay. EPA defined ecoregions (level 4) overlapped between the two zone represented in this ordination (Fig. 9). These zones are almost identical to the Eugene buffer overlay due to the relatively low number of populations represented in the analysis. Post hoc MRPP analysis again indicates ecoregion level differences are significant yet not biologically meaningful ( $\mathrm{T}=-10.08$, $\mathrm{A}=0.01, \mathrm{p}<0.0001$ ). These results correspond to other overlays investigated in this analysis.

## Correlations of traits with geographic and climatic variables

Eleven traits were measured or calculated for each individual in each population. Ranges and means ( $\pm 1 \mathrm{SD}$ ) of populations indicate substantial variability both between and within populations (Appendices G and H). High levels of within population variability suggest high levels of polymorphism in populations. Population level variability is apparent in most traits.

Regression of ordination axes with climatic and geographic variables identified a few weak associations (Table 3), (Figs. 10 and 11). It is important to note that correlations with axes are difficult to ascertain since variables are not necessarily linearly correlated or parallel to axes.

Pearson's Correlation coefficients between univariate traits and geographic and climatic variables identified some significant but weak correlations. Longitude was positively correlated with flowering and seed set date, but these associations were very weak (Table 5). Latitude was negatively correlated with galea pubescence. Elevation was positively correlated with flowering and seed set date (Table 5). Fall frost date was positively associated galea pubescence, yet negatively associated with flowering date. The last day of spring frost was weakly negatively correlated with mean leaf width and positively with mean corolla length. Annual temperature and precipitation had the strongest correlations with galea pubescence. No correlation was stronger than 0.375 suggesting that measured morphological and phenological variables are poorly correlated with geographic and climatic variables.

## DISCUSSION

While statistically significant differences were detected among many populations of this taxon in the Willamette Valley, population level differences do not appear to follow a geographical pattern based on the measured characteristics in this study. The ordinations display considerable overlap among populations and individuals coded by different geographic, administrative, or ecological overlays. These results indicate that a large amount of differences in morphology and phenology exist within populations and while populations are sometimes different from one another, proximal populations are no more likely to be similar than distant populations.

Using county as a subgroup overlay suggests that county is marginally sufficient in visually defining differences in plant variation based on the traits measured based on the very limited number of populations sampled. Individuals within Washington, Lane, and Marion Counties appear to be somewhat clustered away from one another but all other counties greatly overlap. Visual clustering does not appear in the Eugene overlay but post hoc MRPP suggests otherwise yet again $A$ values are very small. Individuals outside the Eugene buffer, which do not overlap with those within are individuals from Bald Hill, Lupine Meadows, Kingston Prairie, Cooper Mountain, Berthelsdorf, and Stayton. Since these populations are geographically located throughout the Willamette Valley, this information provides little information for recommending transfer zones or movement distances within the Willamette Valley.

Geographically defined visual and hierarchical clusters yielded similar results as found at the population level. Hierarchically defined clusters appear marginally better than county and visual clusters for grouping these data for use as a meaningful ordination overlay.
Hierarchical clustering can be useful tool in defining spatial clusters when populations are on continuous scale or when elevation differences are included in the region wished to be defined. All defined areas in this analysis (county, visually, and hierarchically) are missing data from intervening populations, limiting the usefulness of these classification to inform seed zones. We strongly recommend addition of other populations and more individuals within all counties in future seed guideline studies. Too few populations make accurate inferences these areas of the valley slightly more complicated to ascertain.

## Correlations of traits with geographic and climatic variables

Morphological and phenological traits were poorly correlated with geographic and climatic variables. Pearson's Correlation Coefficients between univariate traits and geographic or climatic variables identified some significant although weak correlations. Western and higher elevation populations flowered and set seed later than eastern and low elevation populations. Earlier fall frosts and greater amounts of rainfall were correlated with more pubescence. Little correlation with climatic variables was anticipated since we do not expect to see major differences within the Willamette Valley climates based on coarse PRISM data. These results are
similar to other seed zones studies within the Willamette Valley (Erickson, Mandel, and Sorensen 2004; St. Clair, Mandel, and Vance-Borland 2005). Rather than highlighting mean climatic differences within the Willamette Valley, we suggest switching focus to biologically relevant characteristics such as emergence or flowering dates. Investigation of less coarse climatic variables to discern slight microclimate difference could prove useful.

Our findings suggest that populations of $P$. vulgaris ssp. lanceolata within the Willamette Valley are morphologically and climatically within a single ecotype up to 213 m in elevation; no collections were from higher elevations. Populations from a full range of environmental and climatic conditions in which this species occurs should be included in future seed guideline studies if a range larger than the Willamette Valley is in question. Thus, we do not recommend movement of seed from sources outside the Willamette Valley, or beyond within Valley distributions without further study. We also do not recommend using seed from populations where $P$. vulgaris ssp. vulgaris is present to reduce the chance of potential hybridization. Natural hybridization between these species has been documented in California (Nelson 1963) and it is unknown if this occurs within the Willamette Valley.

The strong overlap of measured characteristics across populations suggests movement of seed among populations with similar environments within the Willamette Valley would result in a high probability of plant establishment. Prunella vulgaris ssp. lanceolata is a widespread species ranging in distribution throughout the United States, proving its ability to adapt to a variety of environments.

## Agricultural production of native plant materials

The goal of acquiring locally adapted seeds is often difficult especially when populations are extirpated from restoration areas and wild collected seed in not abundant or is very expensive. These problems have encouraged agricultural production of many native species. While this aims to solve seed abundance matters it creates equally as difficult considerations. Planting collected seeds in production fields may not capture all the nonrandom mortality of a population. Agricultural production may influence seed dormancy and germination requirements, and survival/death of plants based on production management essentially selecting for good production plants but perhaps losing diversity in the process. Single year collection does not reflect changes in original population after seed collection (seed from seed bank germination or seeds/pollen arrival from another locations), or environmental/climatic changes (Rogers and Montalvo 2004). In addition, geographically associated pest, hybrids, and alternative genotypes can easily be increased and potentially transferred to new environments. For example, Prunella vulgaris ssp. vulgaris, a non-native weed with similar morphology can be mistaken; therefore, careful identification and clear labeling of sub varieties is essential. Since hybridization can occur collection should be limited to pure populations. Identification of ssp. vulgaris individuals within production field should be eliminated.

## RECOMMENDATIONS

In summary, our data indicate there is some minor morphological and phenological differentiation in populations of Prunella vulgaris ssp. lanceolata across the Willamette Valley. Populations overlapped considerably with each other in the measured traits, indicating high amounts of within population variation. A few weak correlations were present between morphological traits and geographic and environmental variables, but with very few populations being represented across the Willamette Valley the validity of these relationships is questionable. Because of the overlap among populations and the inconsistency of morphological similarity and spatial distance, we recommend a single seed transfer zone for Prunella vulgaris ssp. lanceolata within the Willamette Valley under 213 m and from within the area investigated here $\left(45.57^{\circ}\right.$ to $44.04^{\circ} \mathrm{N}$ ). We did not include plants from outside of this area and cannot assume their inclusion in this seed transfer zone. We suggest that using multiple populations in reintroductions will increase restoration success and assist in restoring more historic levels of gene flow. Last, additional studies are recommended to determine the presence and/or scale of local adaptation and the genetic basis for the adaptations if they are found.

Table 1. List of population names with corresponding abbrevations and number of individuals represented per population as sampling units. County: $1=$ Washington, $2=$ Yamhill, $3=$ Marion, $4=$ Linn, $5=$ Benton, $6=$ Lane. Eugene: $1=$ within 20 mile buffer, $0=$ not present in buffer. Visual Cluster is based on subjective map interpretation. Cluster is based on dendrogram interpretation at the 1.6E-01 level, retaining $75 \%$ of the original information. EPA defined Ecoregion level 4: $1=$ Valley Foothills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Gallery Forest.

| Population | $\begin{aligned} & \hline \text { Cod } \\ & \mathrm{e} \\ & \hline \end{aligned}$ | $\begin{gathered} \hline \text { Count } \\ y \\ \hline \end{gathered}$ | $\begin{gathered} \text { Eugen } \\ \text { e } \end{gathered}$ | $\begin{gathered} \text { Visua } \\ 1 \\ \hline \end{gathered}$ | Cluste $\mathbf{r}$ | $\begin{gathered} \text { Ecoregio } \\ \mathrm{n} \\ \hline \end{gathered}$ | Latitude | Longitude |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Bald Hill | 1 |  |  |  |  |  | 45.1563 |  |
|  | BH | 5 | 0 | 4 | 3 | 2 | 3 | -123.32490 |
| Berthelsdorf | 2 |  |  |  |  |  | 44.5674 |  |
|  | BF | 2 | 0 | 2 | 1 | 1 | 5 | -123.33220 |
| Cooper Mountain | 3 |  |  |  |  |  | 45.4492 |  |
|  | CM | 1 | 0 | 1 | 2 | 1 | 2 | -122.87190 |
| Coyote | 4 |  |  |  |  |  | 44.0252 |  |
|  | CY | 6 | 1 | 5 | 3 | 1 | 4 | -123.31520 |
| Kingston Prairie | 5 |  |  |  |  |  | 44.7753 |  |
|  | KP | 4 | 0 | 3 | 4 | 1 | 4 | -122.74430 |
| Lupine Meadows | 6 |  |  |  |  |  | 44.5510 |  |
|  | LM | 5 | 0 | 4 | 3 | 2 | 7 | -123.34880 |
| Oxbow East | 7 |  |  |  |  |  | 44.0565 |  |
|  | OE | 6 | 1 | 5 | 3 | 2 | 9 | -123.18420 |
| Sublimity Prairie | 8 |  |  |  |  |  | 44.8410 |  |
|  | SP | 3 | 0 | 3 | 4 | 1 | 1 | -122.74380 |
| Stayton/Sublimity | 9 |  |  |  |  |  | 44.8170 |  |
|  | SS | 3 | 0 | 3 | 4 | 1 | 0 | -122.79400 |
| Willow Creek | 1 |  |  |  |  |  | 44.0380 |  |
|  | WC 0 | 6 | 1 | 5 | 3 | 2 | 0 | -123.16990 |

Table 2. List of phenological and morphological traits measured on $P$. vulgaris plants in common garden study.

| Trait | Abbreviation | Description |
| :--- | :--- | :--- |
| Mean petal length | AVECORL | Length of the upper lip (longest <br> petal) measured on three flowers <br> haphazardly chosen for each plant <br> (mm). |
| Diameter | DIAM | Distance across broadest part of <br> plant, without pulling or stretching <br> stems (cm) |
| Emergence date | JULEMER | Date of first sign of cotyledons. <br> Monitored on a daily basis; when <br> cone-tainers contained more than one <br> seedling, germination was recorded <br> for the first seedling that emerged in <br> each cone-tainer (Julian) |
| Corolla color | CORCOLR | Categorically defined corolla color: <br> dark purple, medium purple, light <br> purple, or white |
| Mean flowers per inflorescence | AVEFLWR | All flowers were counted and <br> recorded in each inflorescence on <br> two haphazardly chosen racemes |
| Meed date | JULSEED | Date at first sign of flowering. <br> Monitored on transplanted <br> individuals three times a week until a <br> value was obtained for each study <br> plant (Julian) |
| Flowering date | AVEFLWR |  |

Table 3. Correlation of axes with measured trait variables and secondary geospatial and climatic variables.

|  | Axis 1 |  | Axis 2 |  | Axis 3 |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{R}^{2}$ | tau | $\mathrm{R}^{2}$ | tau | $\mathrm{R}^{2}$ | tau |
| Latitude | 0.077 | 0.187 | 0.031 | 0.094 | 0.01 | -0.154 |
| Longitude | 0.098 | 0.211 | 0.014 | -0.13 | 0.003 | -0.076 |
| Elevation | 0.084 | 0.224 | 0.075 | -0.225 | 0.048 | -0.12 |
| Flowering date | 0.158 | 0.286 | $\mathbf{0 . 4 3 2}$ | -0.491 | 0.093 | -0.212 |
| Height | 0.124 | 0.234 | 0.197 | -0.311 | 0.131 | 0.267 |
| Diameter | 0.001 | -0.011 | 0.137 | 0.28 | 0.018 | 0.107 |
| Mean flowers per |  |  |  |  |  |  |
| inflorescence | 0.005 | -0.006 | 0.098 | -0.202 | 0.237 | 0.386 |
| Mean corolla length | 0.107 | -0.213 | 0.3 | -0.375 | 0.001 | -0.004 |
| Seed set date | 0.12 | 0.244 | 0.223 | -0.373 | 0.139 | -0.284 |
| Mean leaf length | 0.166 | 0.271 | 0.045 | -0.157 | $\mathbf{0 . 5 6 9}$ | 0.566 |
| Mean leaf width | 0.183 | 0.306 | 0.016 | -0.075 | $\mathbf{0 . 4 9 8}$ | 0.479 |
| Emergence date | 0.087 | 0.138 | 0.079 | 0.113 | 0.057 | -0.126 |

Table 4. Significantly different MRPP multiple pair-wise comparisons with adjusted Bonferroni correction, test statistic T and agreement statistic A grouped by (a) population ( $p<0.001$ ) (b) county ( $p<0.003$ ) (c) visually defined cluster 75 ( $p<0.005$ ) (d) dendrogram defined cluster at the $75 \%$ level ( $p<0.007$ ).
(a)

| Populations |  |  | $\mathbf{T}$ | $\mathbf{A}$ |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | -9.105 | 0.051 |
| 1 | vs. | 3 | -13.833 | 0.065 |
| 1 | vs. | 4 | -9.002 | 0.043 |
| 1 | vs. | 5 | -11.394 | 0.055 |
| 1 | vs. | 7 | -9.371 | 0.049 |
| 1 | vs. | 8 | -9.811 | 0.046 |
| 2 | vs. | 3 | -10.539 | 0.056 |
| 2 | vs. | 7 | -9.374 | 0.062 |
| 2 | vs. | 8 | -12.935 | 0.078 |
| 2 | vs. | 9 | -7.785 | 0.043 |
| 2 | vs. | 10 | -4.802 | 0.048 |
| 3 | vs. | 4 | -12.096 | 0.056 |
| 3 | vs. | 5 | -10.827 | 0.054 |
| 3 | vs. | 6 | -8.417 | 0.047 |
| 3 | vs. | 7 | -17.409 | 0.100 |
| 3 | vs. | 8 | -18.427 | 0.093 |
| 3 | vs. | 9 | -16.628 | 0.075 |
| 3 | vs. | 10 | -6.850 | 0.052 |
| 4 | vs. | 5 | -5.700 | 0.026 |
| 4 | vs. | 7 | -6.930 | 0.038 |
| 4 | vs. | 8 | -14.435 | 0.072 |
| 4 | vs. | 9 | -7.797 | 0.036 |
| 5 | vs. | 7 | -9.491 | 0.053 |
| 5 | vs. | 8 | -8.138 | 0.039 |
| 6 | vs. | 8 | -8.171 | 0.049 |
| 7 | vs. | 8 | -12.212 | 0.070 |
| 7 | vs. | 9 | -7.073 | 0.038 |


| 5 | vs. | 1 | -12.5940 | 0.0491 |
| :---: | :---: | :---: | :---: | :---: |
| 5 | vs. | 3 | -14.0354 | 0.0421 |
| 5 | vs. | 4 | -3.7160 | 0.0150 |
| 5 | vs. | 6 | -6.2826 | 0.0167 |
| 6 | vs. | 3 | -18.9267 | 0.0436 |
| 6 | vs. | 4 | -9.8795 | 0.0287 |

(c)

| Visual |  |  | T | A |
| :--- | :--- | :--- | :---: | :---: |
| 1 | vs. | 3 | -20.212 | 0.050 |
| 1 | vs. | 5 | -19.710 | 0.059 |
| 2 | vs. | 1 | -13.833 | 0.065 |
| 2 | vs. | 3 | -11.786 | 0.028 |
| 2 | vs. | 4 | -7.856 | 0.031 |
| 2 | vs. | 5 | -8.833 | 0.026 |
| 4 | vs. | 1 | -12.594 | 0.049 |
| 4 | vs. | 3 | -10.696 | 0.024 |
| 4 | vs. | 5 | -6.283 | 0.017 |
| 5 | vs. | 3 | -18.287 | 0.033 |

(d)

| Cluster |  |  | $\mathbf{T}$ |
| :---: | :---: | :---: | :---: |
| 1 | vs. | 2 | -13.833 |
| 1 | vs. | 3 | -8.852 |
| 1 | vs. | 4 | -11.786 |
| 2 | vs. | 4 | -20.212 |
| 3 | vs. | 2 | -19.318 |
| 3 | vs. | 4 | -19.055 |

## (b)

| County |  |  | T | A |
| :---: | :---: | :---: | :---: | :---: |
| 1 | vs. | 3 | -22.2046 | 0.0740 |
| 1 | vs. | 4 | -10.8271 | 0.0543 |
| 1 | vs. | 6 | -19.7097 | 0.0592 |
| 2 | vs. | 1 | -13.8330 | 0.0655 |
| 2 | vs. | 3 | -10.1618 | 0.0328 |
| 2 | vs. | 4 | -11.3938 | 0.0550 |
| 2 | vs. | 5 | -7.8558 | 0.0311 |
| 2 | vs. | 6 | -8.8330 | 0.0262 |
| 4 | vs. | 3 | -6.5436 | 0.0213 |

Table 5. Univariate Pearson Correlation Coefficients of measured traits with geographic and climatic variables. Significant correlations are shown in bold.

|  | Latitude | Longitude | Elevation | First <br> day of <br> fall frost | Last day <br> of <br> spring <br> frost | Mean <br> annual <br> temp | Mean <br> annual <br> precip |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Emergence date |  |  |  |  |  |  |  |
| $p$-value | 0.097 | 0.098 | 0.03 | -0.061 | -0.072 | -0.093 | -0.027 |
| Flowering date |  |  |  |  |  |  |  |
| $p$-value | 0.146 | 0.139 | 0.656 | 0.358 | 0.277 | 0.162 | 0.683 |
| Height | 0.122 | $\mathbf{0 . 3 3 8}$ | $\mathbf{0 . 4 5 4}$ | $\mathbf{- 0 . 3 3 6}$ | -0.008 | -0.202 | 0.248 |
| $p$-value | 0.066 | $<.0001$ | $<.0001$ | $<.0001$ | 0.905 | 0.002 | 0 |
| Diameter | -0.086 | 0.162 | 0.17 | -0.121 | -0.08 | -0.236 | 0.188 |
| $p$-value | 0.196 | 0.015 | 0.011 | 0.069 | 0.23 | 0 | 0.004 |
| Mean flowers per | 0.036 | -0.24 | -0.196 | 0.079 | 0.127 | 0.062 | -0.077 |
| inflorescence | 0.591 | 0 | 0.003 | 0.238 | 0.057 | 0.356 | 0.247 |
| $p$-value | -0.095 | 0.176 | 0.062 | 0.091 | -0.225 | 0.005 | 0.039 |
| Corolla color | 0.155 | 0.008 | 0.351 | 0.172 | 0.001 | 0.939 | 0.561 |
| $p$-value | 0.199 | 0.06 | -0.211 | 0.127 | -0.205 | 0.166 | -0.207 |
| Mean petal length | 0.003 | 0.366 | 0.001 | 0.055 | 0.002 | 0.012 | 0.002 |
| $p$-value | -0.108 | -0.212 | -0.097 | -0.077 | $\mathbf{0 . 3 0 9}$ | 0.111 | -0.144 |
| Galea pubescence | 0.105 | 0.001 | 0.145 | 0.25 | $<.0001$ | 0.095 | 0.03 |
| $p$-value | $\mathbf{- 0 . 4 0 6}$ | -0.235 | -0.238 | $\mathbf{0 . 3 0 3}$ | 0.102 | $\mathbf{0 . 3 7 5}$ | $\mathbf{- 0 . 3 6 3}$ |
| Seed set date | $<.0001$ | 0 | 0 | $<.0001$ | 0.124 | $<.0001$ | $<.0001$ |
| $p$-value | 0.138 | $\mathbf{0 . 2 8 8}$ | $\mathbf{0 . 3 5 6}$ | -0.23 | -0.035 | -0.087 | 0.133 |
| Mean leaf length | 0.037 | $<.0001$ | $<.0001$ | 0.001 | 0.596 | 0.193 | 0.045 |
| -value | -0.153 | -0.041 | -0.099 | 0.167 | -0.103 | 0.05 | -0.016 |
| Mean leaf width | 0.022 | 0.54 | 0.139 | 0.012 | 0.123 | 0.453 | 0.815 |
| $p$-value | 0.237 | 0.126 | -0.077 | 0.05 | $\mathbf{- 0 . 2 7 7}$ | -0.095 | 0.099 |
|  | 0 | 0.059 | 0.248 | 0.455 | $<.0001$ | 0.152 | 0.138 |

Figure 1. Population distribution of $P$. vulgaris ssp. lanceolata seed collection locations within the Willamette Valley.


Figure 2. Hierarchical Dendrogram of populations to detect clustering defined by geographical variables latitude and longitude at the 1.6E-01 level ( $75 \%$ information remaining).
(a)


Figure 3. NMS 3-dimensional ordination; Population is used as the overlay. Each symbol represents an individual in multivariate space. Symbols that are closer spatially are more similar morphologically and phenologically. Vectors indicate variables with significant correlations to axes ( $\mathrm{R}^{2}($ or tau $\left.)<0.50\right)$.


Figure 4. NMS ordination of individuals within each population with variables with $>0.50 \mathrm{R}^{2}$ value: axis 2 vs. 3 . Vectors indicate variables with significant correlations to axes ( $\mathrm{R}^{2}<0.50$ ). Multiple individuals (sample units) are represented by a single color.


Axis 2

Figure 5. NMS ordination with County overlay: axis 2 vs. 3. . County: $1=$ Washington, $2=$ Yamhill, $3=$ Marion, $4=$ Linn, $5=$ Benton, $6=$ Lane.


Figure 6. NMS ordination with Eugene overlay: axis 2 vs. 3. Individuals within 20 miles of Eugene are shown in green, those outside of this seed transfer zone are shown in red.


Figure 7. NMS ordination with visually assessed clusters: axis 2 vs. 3 .


Figure 8. NMS ordination using Cluster overlay defined by hierarchical cluster analysis at $75 \%$ level: axis 3 and 2.


Axis 2

Figure 9. NMS ordination using Ecoregion Level 4 defined by EPA (axis 3 and 2): $1=$ Valley Foot hills, $2=$ Prairie Terraces, $3=$ Willamette River and Tributary Galley Forest.


Figure 10. NMS ordination axis 3 vs. 2 with climatic variable correlations jointly biplotted with population (a) annual precipitation, (b) annual temperature (c) first day of fall frost (d) last day of spring frost (e) number of frost free days (average growing season length). Red lines indicate regression lines (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes.
(a)

(b)

(c)

(d)

(e)


FRSTFREE
Axis 2
$r=.174$ tau $=.230$
Axis 3
$r=.265$ tau $=.191$


Figure 11. NMS ordination axis 3 vs. 2 with geographic variable correlations jointly biplotted with (a) latitude, (b) longitude, (c) elevation. Red lines indicate regression line (r), while the blue line represents Kendall' tau (a rank regression). Larger symbols represent larger magnitudes.
(a)


Latitude
Axis 2
$r=.176$ tau $=.094$
Axis 3
$r=-.099$ tau $=-.154$

(b)

(c)


## CONCLUSIONS

## Local adaptation in restoration

Obtaining seeds from locally adapted and ecologically similar environments is clearly a goal of most restorations. In some cases, use of non-local seed can have detrimental consequences for the success of restoration through the erosion of natural patterns of population genetic structuring and/or genetic swamping of locally significant genotypes (Krauss and He 2006). However, the degree of local adaptation for populations and species ranges considerably, from high local adaptation on very small spatial scales to no adaptation, and even cases of local populations that are maladaptive to their home site. Predicting where species fall out in this continuum is extremely difficult without extensive experimentation. Additionally, assuming that all populations are highly adapted ignores the fact that most populations display considerable within population diversity (clearly evident in these populations), where some individuals may be more locally adapted than others. The "maladaptive" genes present in populations may become adaptive to future conditions. Genetic diversity provides variation in species traits so that even as the environment changes some individuals within a population should be better suited to new conditions and may facilitate establishment in new habitats. Several studies suggest a highly significant correlation between species survival and genetic diversity (Reed and Frankham, 2003; Rogers and Montalvo, 2004).

Constraining restoration programs based on the probability that a few populations may experience a negative impact while the land continues to degrade and populations decline or become extinct is unwise (Broadhurst et al. 2008). Waiting for genetic or experimental data for each species used in restoration, although a noble conservation task, is logistically and economically unrealistic (McKay et al. 2005).

While the precautionary principle is prudent in light of the absence of information, it is important to understand that environmental degradation in the Willamette Valley is still occurring at an alarming rate. The human population of the Willamette Valley is expected to nearly double in the next 40 years (ODFW 2005) and greater land-use conversion and fragmentation is to be expected. Failure to act may imperil the populations we are trying to protect.

## Increasing future resiliency in restoration sites

Restoration efforts should reflect the current environmental limitations and new approaches should be incorporated in light of potential future changes. In the Willamette Valley habitats have become highly fragmented and degraded post Euro-American settlement. Habitat loss and fragmentation has reduced population sizes and almost certainly inhibited genetic movement among populations. This increases the chance of random genetic drift and, to a lesser extent selection, accelerates genetic differentiation among populations, particularly in rare populations or with plants that require pollination
for seed set (Johnson et al. 2004). The current and future consequences of global climate change and continued ecological changes to the habitats due to non-native species, pests, pollinators, etc. require altering our perception of adequate habitat and local adaptation. Restoration strategies need to include consideration of future changes to species distributions and should try to promote resiliency in existing populations. Increasing genetic diversity by using more populations across the range of the seed transfer zone would promote resiliency, and provide insurance towards species survival.

## Future research directions

Common garden studies measure a single component of local adaptation and not spatial or temporal autocorrelation. Spatial autocorrelation is the idea that populations that are closer together are more similar than those farther apart. Future work at a minimum should include investigation of whether spatial autocorrelation exists which may effectively identify movement guidelines, particularly the distance at which plants become dissimilar. Currently, IAE has these data for the species detailed in this report and pending further funding opportunities we plan performing these additional analyses. Additionally, reciprocal common garden studies can evaluate the relative amount of genetic and environmental effects influencing discrete characteristics and when individual fitness is measured, direct estimates of local adaptation are possible. Genetic variation within a population can be more accurately estimated when family relationships within a population are included in the study. This approach can also be used to determine the genetic architecture of the traits and how easily population can respond to selection. Further, without looking at a population over time it is impossible to know if a local or non-local population is better adapted to a location. Non-local sources may be better adapted to a location if the climate or environment has changed faster than the local population has evolved. A local population may actually be less optimal than other populations, especially when all the populations were once continuous and local fragmentation has occurred (Johnson et al. 2004, also see Lawrence and Kaye 2008). A strong need also exists to combine information from quantitative genetic studies and reciprocal transplants to investigations of selectively neutral and constrained regions of the genome. In this way, genetic markers associated with particular adaptive traits can be identified. Genetic and demographic risks must be estimated based on the biology of each species. Together this information will enhance our knowledge of how to best maximize restoration success, genetic diversity, and offers insight into determining seed transfer zones within the Willamette Valley.

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## APPENDICES

APPENDIX A: (SICA)

| POPULATION | AD |  |  | AL |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-9 | $6.00 \pm$ | $\pm 4.24$ | 3-10 | 7.10 | 3.13 |
| JULEMR | 2007056-2007084 | $2007070.00 \pm$ | $\pm 19.80$ | 2007056-2007106 | 2007077.10 | 16.52 |
| JULDATE | 2008164-2008168 | $2008166.00 \pm$ | $\pm 2.83$ | 2008161-2008175 | 2008169.00 | 5.35 |
| HT | 145-171 | $158.00 \pm$ | $\pm 18.38$ | 129-159 | 144.50 | 11.41 |
| AVEINFLL | 101.67-104.33 | $103.00 \pm$ | $\pm 1.89$ | 93-121 | 107.40 | 9.67 |
| AVEFLWR | 17.6-21.33 | 19.50 $\pm$ | $\pm 2.59$ | 15-26.33 | 20.09 | 4.31 |
| AVELEAFL | 40-48.53 | 44.27 $\pm$ | $\pm 6.03$ | 22.033-57.3 | 38.55 | 12.92 |
| AVELEAFW | 67.03-82.56 | $74.80 \pm$ | $\pm 10.98$ | 37.133-97.36 | 65.34 | 23.48 |
| AVEAL | 19.43-20.86 | $20.15 \pm$ | $\pm 1.01$ | 9.2-31.13 | 21.21 | 9.37 |
| AVBL | 14.4-21.63 | 18.02 $\pm$ | $\pm 5.11$ | 5.4-18.86 | 10.35 | 4.38 |
| LOBEDISS | 0.49-0.57 | $0.53 \pm$ | $\pm 0.06$ | 0.43-0.84 | 0.64 | 0.16 |
| AVELOBEW | 20.5-23.33 | $21.92 \pm$ | $\pm 2.00$ | 8.533-29.86 | 20.51 | 8.33 |
| AVESEPL | 8.5-10.36 | $9.43 \pm$ | $\pm 1.32$ | 7.3-10.23 | 8.90 | 1.02 |
| AVEPETL | 17.63-19.6 | 18.62 $\pm$ | $\pm 1.39$ | 12.066-16.36 | 14.30 | 1.58 |
| AVEPETW | 7.37-8.8 | $8.08 \pm$ | $\pm 1.01$ | 6.966-10.03 | 8.32 | 1.08 |
| TOTRACE | 14-15 | $14.50 \pm$ | $\pm 0.71$ | 15-27 | 17.57 | 4.31 |
| AVEBRANC | 7.67-11.33 | $9.50 \pm$ | $\pm 2.59$ | 6.333-12.33 | 8.52 | 2.44 |
| AVEINFLFL | 15.13-15.3 | 15.22 $\pm$ | $\pm 0.12$ | 9.6-19.3 | 14.50 | 3.17 |
| RFLOWCM | 1.17-1.39 | $1.28 \pm$ | $\pm 0.16$ | 1.075-1.56 | 1.39 | 0.15 |
|  |  |  |  |  |  |  |
| POPULATION | AS |  |  | BC |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 9-11 | $9.60 \pm$ | $\pm 1.15$ | 8-10 | 10 | 1 |
| JULEMR | 2007055-2007113 | $2007081.60 \pm$ | $\pm 29.28$ | 2007059-2007064 | $2007064 \pm$ | 2 |
| JULDATE | 2008156-2008168 | $2008160.00 \pm$ | $\pm 6.92$ | 2008149-2008170 | 2008170 | 7 |
| HT | 134-160 | $146.30 \pm$ | $\pm 13.05$ | 102-169 | 169 | 22 |
| AVEINFLL | 97.6-108.6 | 102.70 $\pm$ | $\pm 5.54$ | 89.6-110.3 | 110 | 8 |
| AVEFLWR | 18-24 | $21.44 \pm$ | $\pm 3.09$ | 11.66-29 | 29.00 | 7 |
| AVELEAFL | 44-50.8 | $48.31 \pm$ | $\pm 3.75$ | 39.03-60.23 | 60.23 | 8 |
| AVELEAFW | 80.13-92.5 | $86.36 \pm$ | $\pm 6.18$ | 67.76-93.03 | 93.03 | 10 |
| AVEAL | 20.66-24.46 | $22.75 \pm$ | $\pm 1.92$ | 12.73-21.4 | 21.40 | 4 |
| AVBL | 17.36-25.2 | $21.64 \pm$ | $\pm 3.96$ | 15.9-26.23 | 26.23 | 4 |
| LOBEDISS | 0.47-0.58 | $0.51 \pm$ | $\pm 0.06$ | 0.33-0.53 | 0.54 | 0 |
| AVELOBEW | 28.8-31.7 | $30.18 \pm$ | $\pm 1.45$ | 24.7-36.33 | 36.33 | 5 |
| AVESEPL | 7.96-10.06 | $9.10 \pm$ | $\pm 1.05$ | 8.33-10.73 | 10.73 | 1 |
| AVEPETL | 10.4-16.56 | $14.36 \pm$ | $\pm 3.44$ | 12.46-18.7 | 18.70 | 2 |
| AVEPETW | 6.06-8.23 | $7.32 \pm$ | $\pm 1.12$ | 5.53-10.16 | 10.17 | 2 |
| TOTRACE | 9-11 | $10.33 \pm$ | $\pm 1.15$ | 10-25 | 25.00 | 6 |
| AVEBRANC | 6-11.33 | $7.77 \pm$ | $\pm 3.07$ | 4-11.33 | 11.33 | 3 |
| AVEINFLFL | 16.23-21.2 | 19.11 | $\pm 2.57$ | 11.13-21.36 | 21.37 | 4 |
| RFLOWCM | 1.05-1.2 | 1.12 $\pm$ | $\pm 0.07$ | 0.74-1.65 | 1.65 | 0 |


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| POPULATION | BE |  |  | BL |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-9 | $7 \pm$ | $\pm 2$ | 7-9 | $8 \pm$ | 1 |
| JULEMR | 2007059-2007123 | $2007087 \pm$ | $\pm 26$ | 2007059-2007111 | 2007075 | 21 |
| JULDATE | 2008147-2008158 | $2008152 \pm$ | $\pm 4$ | 2008158-2008168 | $2008165 \pm$ | 5 |
| HT | 94-159 | $131 \pm$ | $\pm 21$ | 110-187 | 142 | 29 |
| AVEINFLL | 67.66-112.66 | $91 \pm$ | $\pm 17$ | 88-125 | 102 $\pm$ | 14 |
| AVEFLWR | 12-26.33 | 18.05 | $\pm 5.08$ | 16-23.33 | 19.60 | 2.85 |
| AVELEAFL | 27.73-58.26 | $45.46 \pm$ | $\pm 11.00$ | 30.46-67.06 | $48.75 \pm$ | 12.95 |
| AVELEAFW | 51.7-96.43 | $75.36 \pm$ | $\pm 16.06$ | 50.83-117.66 | 84.17 | 23.68 |
| AVEAL | 6.56-32.43 | $16.42 \pm$ | $\pm 10.01$ | 8.86-39.43 | $24.13 \pm$ | 10.93 |
| AVBL | 15-32.53 | $22.84 \pm$ | $\pm 5.96$ | 13-19.86 | $15.86 \pm$ | 2.87 |
| LOBEDISS | 0.24-0.68 | $0.39 \pm$ | $\pm 0.16$ | 0.34-0.75 | $0.58 \pm$ | 0.16 |
| AVELOBEW | 16.73-35.56 | $25.84 \pm$ | $\pm 5.75$ | 19.06-45.1 | 30.89 | 9.79 |
| AVESEPL | 7.43-10.9 | $9.44 \pm$ | $\pm 1.11$ | 9.13-9.5 | 9.32 | 0.15 |
| AVEPETL | 5.86-18.9 | 13.22 | $\pm 4.54$ | 14.13-19.86 | $16.49 \pm$ | 2.27 |
| AVEPETW | 5.93-9.43 | $7.20 \pm$ | $\pm 1.33$ | 7.56-9.8 | $8.53 \pm$ | 0.82 |
| TOTRACE | 8-19 | $16.71 \pm$ | $\pm 3.90$ | 9-20 | $15.00 \pm$ | 4.53 |
| AVEBRANC | 4-8 | $6.57 \pm$ | $\pm 1.47$ | 6.33-10 | $8.27 \pm$ | 1.44 |
| AVEINFLFL | 11.7-27 | $17.48 \pm$ | $\pm 5.34$ | 8-18.86 | $15.11 \pm$ | 4.18 |
| RFLOWCM | 0.61-1.29 | $1.06 \pm$ | $\pm 0.24$ | 1.02-2 | $1.38 \pm$ | 0.38 |
|  |  |  |  |  |  |  |
| POPULATION | CO |  |  | CS |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 9-10 | $9 \pm$ | $\pm 1$ | 5-10 | $8 \pm$ | 2 |
| JULEMR | 2007064-2007090 | $2007073 \pm$ | $\pm 14$ | 2007055-2007086 | $2007063 \pm$ | 11 |
| JULDATE | 2008161-2008168 | $2008164 \pm$ | $\pm 4$ | 2008156-2008172 | $2008163 \pm$ | 7 |
| HT | 99-163 | 137 $\pm$ | $\pm 34$ | 105-161 | $134 \pm$ | 18 |
| AVEINFLL | 73-102.33 | $85 \pm$ | $\pm 15$ | 64.66-112 | 88 $\pm$ | 17 |
| AVEFLWR | 10.66-16 | $12.67 \pm$ | $\pm 2.91$ | 11-21 | $17.24 \pm$ | 3.82 |
| AVELEAFL | 30.03-46.4 | $38.37 \pm$ | $\pm 8.19$ | 29.76-59 | $43.46 \pm$ | 12.38 |
| AVELEAFW | 56.5-80.53 | $67.50 \pm$ | $\pm 12.15$ | 53.03-105 | $75.19 \pm$ | 20.74 |
| AVEAL | 12.8-23.36 | $17.42 \pm$ | $\pm 5.41$ | 13.03-46 | $27.38 \pm$ | 11.52 |
| AVBL | 12.43-16.5 | 14.57 $\pm$ | $\pm 2.04$ | 3.93-17 | $9.85 \pm$ | 4.41 |
| LOBEDISS | 0.46-0.58 | $0.54 \pm$ | $\pm 0.06$ | 0.55-0 | $0.71 \pm$ | 0.13 |
| AVELOBEW | 11.13-27.83 | 18.07 $\pm$ | $\pm 8.70$ | 17-33 | 24.47 | 6.45 |
| AVESEPL | 9.96-42.63 | 20.88 $\pm$ | $\pm 18.84$ | 8.43-10 | $9.18 \pm$ | 0.86 |
| AVEPETL | 16.06-20.4 | 18.61 $\pm$ | $\pm 2.26$ | 10.4-21 | $16.18 \pm$ | 3.75 |
| AVEPETW | 7.06-9.23 | $8.24 \pm$ | $\pm 1.10$ | 5.86-8 | 7.10 | 0.84 |
| TOTRACE | 14-19 | 15.67 $\pm$ | $\pm 2.89$ | 11-29 | $19.00 \pm$ | 7.21 |
| AVEBRANC | 4-8.66 | $6.78 \pm$ | $\pm 2.46$ | 6.33-12 | $9.10 \pm$ | 2.46 |
| AVEINFLFL | 8-13.2 | $11.33 \pm$ | $\pm 2.89$ | 9.36-18 | $14.05 \pm$ | 3.32 |
| RFLOWCM | 0.8-1.41 | 1.16 $\pm$ | $\pm 0.31$ | 1.02-1 | $1.24 \pm$ | 0.19 |
|  |  |  |  |  |  |  |
| POPULATION | DL |  |  | DY |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |


| FLWRCLR | 10-10 |  | $\pm 0$ | 5-9 | $8 \pm \pm 2$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| JULEMR | 2007072-2007085 | $2007077 \pm$ | $\pm 7$ | 2007053-2007086 | $2007062 \pm$ | 10 |
| JULDATE | 2008149-2008161 | $2008155 \pm$ | $\pm 6$ | 2008156-2008170 | 2008165 $\pm$ | 6 |
| HT | 164-196 | $175 \pm$ | $\pm 18$ | 101-181 | 154 | 25 |
| AVEINFLL | 109.33-121 | $115 \pm$ | $\pm 6$ | 84-121 | $101 \pm$ | 14 |
| AVEFLWR | 14-20.66 | $16.89 \pm$ | $\pm 3.42$ | 13.66-20.33 | 17.29 | 2.77 |
| AVELEAFL | 23.06-58.1 | 41.52 $\pm$ | $\pm 17.59$ | 35.6-61.23 | 47.82 | 8.18 |
| AVELEAFW | 47.86-83.96 | $68.03 \pm$ | $\pm 18.42$ | 65.83-104.56 | 84.30 | 13.53 |
| AVEAL | 8.06-29.46 | $20.32 \pm$ | $\pm 11.03$ | 10.9-39.43 | $26.33 \pm$ | 10.14 |
| AVBL | 14.96-15.7 | $15.29 \pm$ | $\pm 0.37$ | 5.1-29.56 | 13.60 | 8.18 |
| LOBEDISS | 0.34-0.66 | $0.54 \pm$ | $\pm 0.17$ | 0.26-0.88 | 0.65 | 0.21 |
| AVELOBEW | 14.2-31.63 | $22.12 \pm$ | $\pm 8.82$ | 21.3-38.7 | 30.18 | 6.40 |
| AVESEPL | 7.33-9.2 | $8.40 \pm$ | $\pm 0.96$ | 7.63-11 | 9.03 | 1.36 |
| AVEPETL | 8.73-15.26 | 11.71 $\pm$ | $\pm 3.30$ | 10.46-18.66 | $15.53 \pm$ | 2.59 |
| AVEPETW | 4.43-6.93 | $5.91 \pm$ | $\pm 1.31$ | 5.9-8.4 | 6.81 | 1.00 |
| TOTRACE | 13-27 | 18.67 | $\pm 7.37$ | 9-26 | $17.63 \pm$ | 5.95 |
| AVEBRANC | 12.33-14.33 | $13.56 \pm$ | $\pm 1.07$ | 3.66-10 | $7.67 \pm$ | 1.94 |
| AVEINFLFL | 10.96-20.13 | 14.47 $\pm$ | $\pm 4.95$ | 8.36-19.1 | $15.16 \pm$ | 3.30 |
| RFLOWCM | 1.02-1.45 | $1.21 \pm$ | $\pm 0.22$ | 0.8-1.75 | 1.19 | 0.29 |
|  |  |  |  |  |  |  |
| POPULATION | EE |  |  | GP |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-10 | $8 \pm$ | $\pm 3$ | 4-10 |  | 2 |
| JULEMR | 2007056-2007113 | $2007076 \pm$ | $\pm 16$ | 2007059-2007117 | $2007074 \pm$ | 22 |
| JULDATE | 2008154-2008172 | $2008163 \pm$ | $\pm 6$ | 2008111-2008126 | $2008117 \pm$ | 7 |
| HT | 121-175 | $151 \pm$ | $\pm 19$ | 109-128 | $120 \pm$ | 7 |
| AVEINFLL | 81.66-148 | 108 $\pm$ | $\pm 18$ | 70-82.66 | $75 \pm$ | 5 |
| AVEFLWR | 11.66-22.66 | $18.23 \pm$ | $\pm 3.14$ | 12.33-24.66 | 18.11 $\pm$ | 4.22 |
| AVELEAFL | 27.43-59.4 | 38.97 | $\pm 9.84$ | 39.9-57.36 | $45.46 \pm$ | 6.33 |
| AVELEAFW | 45.23-97.23 | $66.61 \pm$ | $\pm 17.82$ | 66.5-97.93 | 75.86 | 12.83 |
| AVEAL | 9-27.33 | 17.11 $\pm$ | $\pm 6.32$ | 20.63-26.56 | $22.81 \pm$ | 2.13 |
| AVBL | 5.6-27.06 | 15.35 $\pm$ | $\pm 7.09$ | 7.76-21.36 | $15.19 \pm$ | 4.59 |
| LOBEDISS | 0.29-0.78 | $0.53 \pm$ | $\pm 0.17$ | 0.53-0.75 | $0.61 \pm$ | 0.08 |
| AVELOBEW | 8.03-34.53 | $22.45 \pm$ | $\pm 8.52$ | 21.5-31.6 | $24.46 \pm$ | 3.78 |
| AVESEPL | 7.56-33.23 | $11.08 \pm$ | $\pm 7.83$ | 7.3-10.66 | $9.21 \pm$ | 1.35 |
| AVEPETL | 8.86-19.56 | 14.76 | $\pm 4.39$ | 6.8-17.73 | $14.63 \pm$ | 3.95 |
| AVEPETW | 5.23-9.5 | $7.16 \pm$ | $\pm 1.33$ | 4.9-10.9 | 8.59 | 2.21 |
| TOTRACE | 10-60 | $23.10 \pm$ | $\pm 14.84$ | 5-27 | 17.67 | 7.37 |
| AVEBRANC | 5.33-20.66 | 10.67 $\pm$ | $\pm 4.80$ | 2-4 | 2.89 | 0.72 |
| AVEINFLFL | 7.86-21.56 | 16.44 | $\pm 3.96$ | 16.63-24.33 | 20.33 | 3.38 |
| RFLOWCM | 0.88-1.48 | $1.15 \pm$ | $\pm 0.22$ | 0.57-1.11 | 0.91 | 0.24 |
|  |  |  |  |  |  |  |
| POPULATION | HE |  |  | HI |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-10 |  | $\pm 3$ | 3-10 | $6 \pm$ | 3 |
| JULEMR | 2007055-2007082 | $2007072 \pm$ | $\pm 10$ | 2007055-2007096 | $2007074 \pm$ | 17 |
| JULDATE | 2008164-2008170 | 2008167 $\pm$ | $\pm 2$ | 2008149-2008177 | 2008168 $\pm$ | 9 |


| HT | 98-172 |  | $\pm 24$ | 1111-177 | 142 $\pm$ | 25 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AVEINFLL | 57-116.66 | $103 \pm$ | $\pm 19$ | 70.66-136.33 | 99 $\pm$ | 22 |
| AVEFLWR | 9-24.33 | $18.33 \pm$ | $\pm 4.84$ | 9-24 | $18.14 \pm$ | 5.28 |
| AVELEAFL | 27.36-61.43 | $42.06 \pm$ | $\pm 9.92$ | 25.96-62.7 | $42.46 \pm$ | 13.56 |
| AVELEAFW | 42.63-108.56 | 69.22 $\pm$ | $\pm 18.93$ | 40.43-111.6 | $72.54 \pm$ | 25.27 |
| AVEAL | 7.23-39.93 | 18.43 $\pm$ | $\pm 9.58$ | 6.73-23.86 | 16.80 | 6.11 |
| AVBL | 9.33-26.8 | 15.72 $\pm$ | $\pm 5.96$ | 11.93-31.73 | $18.75 \pm$ | 8.18 |
| LOBEDISS | 0.21-0.81 | $0.53 \pm$ | $\pm 0.18$ | 0.29-0.63 | 0.47 | 0.13 |
| AVELOBEW | 10.76-35.93 | $25.19 \pm$ | $\pm 7.83$ | 14.63-39.3 | 26.44 | 9.26 |
| AVESEPL | 7.03-9.76 | $8.55 \pm$ | $\pm 0.86$ | 6-10 | 7.99 | 1.33 |
| AVEPETL | 8.7-16.93 | 13.89 $\pm$ | $\pm 3.22$ | 8.26-16.76 | $11.27 \pm$ | 2.93 |
| AVEPETW | 4.2-8.36 | $6.45 \pm$ | $\pm 1.43$ | 4.2-9.56 | $6.20 \pm$ | 1.71 |
| TOTRACE | 12-29 | 19.38 | $\pm 6.55$ | 7-24 | $14.00 \pm$ | 5.83 |
| AVEBRANC | 2.66-16 | $9.46 \pm$ | $\pm 4.48$ | 6.66-19.33 | $11.67 \pm$ | 4.43 |
| AVEINFLFL | 4.7-17.13 | 14.08 | $\pm 4.31$ | 7.93-24 | $15.67 \pm$ | 5.29 |
| RFLOWCM | 1-1.91 | $1.37 \pm$ | $\pm 0.32$ | 0.83-1.51 | $1.20 \pm$ | 0.30 |
|  |  |  |  |  |  |  |
| POPULATION | JC |  |  | KN |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 9-11 | $10 \pm$ | $\pm 1$ | 3-11 | $7 \pm$ | 4 |
| JULEMR | 2007059-2007102 | $2007076 \pm$ | $\pm 18$ | 2007058-2007116 | $2007094 \pm$ | 20 |
| JULDATE | 2008156-2008179 | $2008170 \pm$ | $\pm 6$ | 2008149-2008170 | $2008163 \pm$ | 7 |
| HT | 71-148 | $124 \pm$ | $\pm 22$ | 119-148 | $136 \pm$ | 11 |
| AVEINFLL | 54-116.66 | $93 \pm$ | $\pm 18$ | 72-115.33 | 92 $\pm$ | 15 |
| AVEFLWR | 14.66-25.33 | 19.40 $\pm$ | $\pm 3.34$ | 11.33-26.66 | $16.75 \pm$ | 5.05 |
| AVELEAFL | 28.7-64.96 | $49.38 \pm$ | $\pm 10.42$ | 34.33-62.6 | $48.31 \pm$ | 10.61 |
| AVELEAFW | 50.7-98.2 | $77.81 \pm$ | $\pm 16.75$ | 62.43-114.86 | $81.57 \pm$ | 19.89 |
| AVEAL | 12.23-37.5 | 20.88 | $\pm 7.77$ | 23.1-35.63 | $27.83 \pm$ | 5.08 |
| AVBL | 8.76-27.43 | 19.48 | $\pm 5.86$ | 0.86-18.73 | 10.62 | 6.40 |
| LOBEDISS | 0.36-0.81 | $0.51 \pm$ | 0.13 | 0.57-0.96 | 0.74 | 0.13 |
| AVELOBEW | 16.76-35.13 | $29.53 \pm$ | $\pm 6.00$ | 23.33-32.13 | 27.80 | 2.98 |
| AVESEPL | 8.13-10.53 | $9.13 \pm$ | $\pm 0.80$ | 7.06-11.56 | $9.39 \pm$ | 1.66 |
| AVEPETL | 10.86-18.3 | $14.90 \pm$ | $\pm 2.09$ | 7.7-18.63 | $13.90 \pm$ | 3.55 |
| AVEPETW | 5.46-8.13 | $6.86 \pm$ | $\pm 0.92$ | 4.4-9.43 | $7.38 \pm$ | 1.59 |
| TOTRACE | 7-22 | $15.40 \pm$ | $\pm 4.14$ | 10-24 | $15.13 \pm$ | 5.00 |
| AVEBRANC | 3.66-10 | $8.03 \pm$ | $\pm 1.89$ | 6.33-12.33 | $9.04 \pm$ | 2.50 |
| AVEINFLFL | 7-22.66 | 13.61 $\pm$ | $\pm 4.59$ | 10.86-23.43 | 16.52 | 4.28 |
| RFLOWCM | 1.1-2.19 | $1.53 \pm$ | $\pm 0.42$ | 0.77-1.33 | $1.02 \pm$ | 0.20 |
|  |  |  |  |  |  |  |
| POPULATION | MC |  |  | MN |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-10 |  | $\pm 3$ | 3-10 | $6 \pm$ | 3 |
| JULEMR | 2007052-2007089 | $2007071 \pm$ | $\pm 13$ | 2007055-2007113 | $2007078 \pm$ | 19 |
| JULDATE | 2008154-2008177 | $2008167 \pm$ | $\pm 8$ | 2008156-2008168 | $2008164 \pm$ | 4 |
| HT | 62-150 | $123 \pm$ | $\pm 28$ | 132-187 | $154 \pm$ | 18 |
| AVEINFLL | 43.66-122.33 |  | $\pm 23$ | 92-121.33 | 105 $\pm$ | 8 |
| AVEFLWR | 8.33-23.33 | 15.87 | $\pm 4.65$ | 15-28.66 | 20.73 $\pm$ | 4.31 |


| AVELEAFL | \|23.83-47.63 | $35.79 \pm$ |  | 8.55 | 31.5-61.86 | 46.61 | $\pm \mid 11.19$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AVELEAFW | 44.76-92.6 | $63.74 \pm$ | $\pm$ | 17.18 | 53.43-105.73 | $80.58 \pm$ | $\pm 17.99$ |
| AVEAL | 8.4-28.93 | $15.51 \pm$ | $\pm$ | 6.29 | 11.76-30 | 18.30 $\pm$ | $\pm 5.79$ |
| AVBL | 10-26.36 | $15.80 \pm$ | $\pm$ | 5.33 | 14.1-32.1 | $21.34 \pm$ | 6.53 |
| LOBEDISS | 0.34-0.64 | $0.49 \pm$ | $\pm$ | 0.12 | 0.32-0.62 | $0.46 \pm$ | $\pm 0.11$ |
| AVELOBEW | 14.56-31.93 | $22.78 \pm$ | $\pm$ | 6.95 | 16.23-40.66 | $25.43 \pm$ | 7.14 |
| AVESEPL | 7.6-10.56 | $8.54 \pm$ | $\pm$ | 0.88 | 8.1-11.36 | 9.57 $\pm$ | $\pm 1.06$ |
| AVEPETL | 9.96-17.4 | $12.47 \pm$ | $\pm$ | 2.21 | 15.06-23.16 | 17.76 | 2.77 |
| AVEPETW | 6.43-9.63 | $7.91 \pm$ |  | 1.02 | 7.13-9.63 | $8.31 \pm$ | $\pm 0.88$ |
| TOTRACE | 10-43 | $20.20 \pm$ | $\pm$ | 9.10 | 14-34 | $20.50 \pm$ | $\pm 7.14$ |
| AVEBRANC | 4.33-20.66 | $8.97 \pm$ | $\pm$ | 4.68 | 6.33-12 | $9.17 \pm$ | 2.00 |
| AVEINFLFL | 7.93-18.83 | 13.59 $\pm$ | $\pm$ | 3.85 | 11.33-28.83 | $17.56 \pm$ | 5.23 |
| RFLOWCM | 0.6-1.71 | $1.20 \pm$ |  | 0.31 | 0.75-1.58 | $1.23 \pm$ | 0.24 |
|  |  |  |  |  |  |  |  |
| POPULATION | MO |  |  |  | MS |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN | STD |
| FLWRCLR | 3-9 |  |  |  | 3-10 |  | $\pm 3$ |
| JULEMR | 2007053-2007063 | $2007060 \pm$ | $\pm$ | 5 | 2007061-2007113 | 2007078 | $\pm 18$ |
| JULDATE | 2008161-2008179 | 2008169 |  | 7 | 2008142-2008182 | $2008165 \pm$ | 12 |
| HT | 62-159 | $128 \pm$ | $\pm$ | 44 | 75-150 | 116 | 24 |
| AVEINFLL | 24.66-99.66 | $76 \pm$ | $\pm$ | 35 | 54.33-106 | 87 | $\pm 16$ |
| AVEFLWR | 10-19.66 | 15.83 $\pm$ | $\pm$ | 4.38 | 14.66-39.66 | 20.17 | 7.47 |
| AVELEAFL | 33.5-62.23 | $43.91 \pm$ | $\pm$ | 13.55 | 22.3-57.16 | 39.71 | 11.95 |
| AVELEAFW | 46.2-86.76 | $64.50 \pm$ | $\pm$ | 17.57 | 39.76-100.83 | $67.51 \pm$ | $\pm 18.97$ |
| AVEAL | 11.43-26.33 | 16.98 $\pm$ | $\pm$ | 6.72 | 5.16-25.7 | $13.46 \pm$ | $\pm 6.86$ |
| AVBL | 18.06-23.9 | $20.27 \pm$ | $\pm$ | 2.63 | 15.86-28 | $21.32 \pm$ | $\pm 3.58$ |
| LOBEDISS | 0.38-0.56 | $0.44 \pm$ | $\pm$ | 0.08 | 0.21-0.61 | $0.37 \pm$ | $\pm 0.12$ |
| AVELOBEW | 21.6-32.43 | $24.84 \pm$ | 土 | 5.10 | 11.9-31.36 | $22.52 \pm$ | $\pm 6.12$ |
| AVESEPL | 8.73-9.8 | $9.46 \pm$ | $\pm$ | 0.49 | 7.56-9.26 | $8.45 \pm$ | $\pm 0.46$ |
| AVEPETL | 14.1-18.93 | $17.20 \pm$ | $\pm$ | 2.26 | 10.96-20.06 | 15.62 | $\pm 2.53$ |
| AVEPETW | 6.96-10 | $7.93 \pm$ | $\pm$ | 1.40 | 5.8-11.23 | $8.44 \pm$ | 2.01 |
| TOTRACE | 3-24 | $12.25 \pm$ |  | 9.03 | 2-22 | 10.70 | $\pm 6.04$ |
| AVEBRANC | 3.33-14 | $9.25 \pm$ | $\pm$ | 4.43 | 5.33-13 | $8.77 \pm$ | 2.60 |
| AVEINFLFL | 11.26-20.26 | 16.08 $\pm$ |  | 4.15 | 8.83-23.46 | 14.38 | $\pm 5.01$ |
| RFLOWCM | 0.88-1.06 | $0.98 \pm$ | $\pm$ | 0.09 | 0.9-1.84 | $1.45 \pm$ | $\pm 0.32$ |
|  |  |  |  |  |  |  |  |
| POPULATION | MT |  |  |  | MX |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN | STD |
| FLWRCLR | 1-9 |  | $\pm$ | 6 | 3-3 |  | $\pm 0$ |
| JULEMR | 2007081-2007083 | $2007082 \pm$ |  | 1 | 2007064-2007079 | $2007069 \pm$ | $\pm 9$ |
| JULDATE | 2008168-2008168 | 2008168 $\pm$ | $\pm$ | 0 | 2008154-2008175 | $2008164 \pm$ | $\pm 11$ |
| HT | 158-160 | $159 \pm$ | $\pm$ | 1 | 153-185 | 170 | $\pm 16$ |
| AVEINFLL | 101-104.66 | $103 \pm$ | $\pm$ | 3 | 103.66-129.33 | $117 \pm$ | $\pm 13$ |
| AVEFLWR | 16.33-18 | $17.17 \pm$ | $\pm$ | 1.18 | 16.66-25 | 19.78 | $\pm 4.55$ |
| AVELEAFL | 26.5-36.8 | $31.65 \pm$ |  | 7.28 | 45.7-49.83 | 47.30 | $\pm 2.22$ |
| AVELEAFW | 44.13-57.46 | 50.80 $\pm$ |  | 9.43 | 76.9-84.83 | $80.03 \pm$ | $\pm 4.22$ |
| AVEAL | 11.96-13.33 | 12.65 $\pm$ |  | 0.97 | 15.9-23.5 | 20.60 | $\pm 4.11$ |


| AVBL | 8.5-20.66 | 14.58 |  | 8.60 | 15.2-27.83 | 21.22 | $\pm$ \| 6.34 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LOBEDISS | 0.36-0.61 | $0.49 \pm$ | $\pm$ | 0.17 | 0.36-0.59 | 0.50 | $\pm 0.12$ |
| AVELOBEW | 13.6-23.8 | 18.70 | $\pm$ | 7.21 | 25.33-31.96 | 28.77 | $\pm 3.32$ |
| AVESEPL | 7.33-9.16 | $8.25 \pm$ | $\pm$ | 1.30 | 9.2-10.36 | 9.90 | $\pm 0.62$ |
| AVEPETL | 10.66-12.56 | 11.62 | $\pm$ | 1.34 | 15.8-20.3 | 17.46 | $\pm 2.47$ |
| AVEPETW | 6.03-7.56 | $6.80 \pm$ | $\pm$ | 1.08 | 8.93-10.33 | 9.80 | $\pm 0.76$ |
| TOTRACE | 22-31 | 26.50 | $\pm 6$ | 6.36 | 12-28 | 20.67 | $\pm 8.08$ |
| AVEBRANC | 9.33-13 | 11.17 $=$ | $\pm 2$ | 2.59 | 6.33-10 | 7.56 | $\pm 2.12$ |
| AVEINFLFL | 16.43-19.46 | 17.95 | $\pm$ | 2.14 | 11.5-22.8 | 16.28 | $\pm 5.85$ |
| RFLOWCM | 0.92-0.99 | 0.96 | $\pm 0$ | 0.05 | 1.09-1.44 | 1.25 | $\pm 0.18$ |
|  |  |  |  |  |  |  |  |
| POPULATION | PC |  |  |  | RF |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN | STD |
| FLWRCLR | 1-9 | 4 | $\pm$ | 4 | 1-7 |  | $\pm 2$ |
| JULEMR | 2007055-2007076 | $2007067 \pm$ | $\pm$ | 10 | 2007052-2007112 | 2007074 | $\pm 23$ |
| JULDATE | 2008154-2008175 | $2008162=$ | $\pm 9$ | 9 | 2008149-2008172 | 2008160 | $\pm 7$ |
| HT | 127-143 | 132 | $\pm$ | 6 | 108-170 | 139 | $\pm 22$ |
| AVEINFLL | 66-115.66 | 93 | $\pm$ | 17 | 70.66-122.66 | 97 | $\pm 20$ |
| AVEFLWR | 14.33-24 | 20.17 | $\pm$ | 3.69 | 10.33-28.33 | 16.71 | $\pm 5.53$ |
| AVELEAFL | 31.8-40.33 | 36.14 | $\pm$ | 3.27 | 22.53-48.66 | 36.65 | $\pm 8.24$ |
| AVELEAFW | 50.33-70.03 | 62.00 | $\pm$ | 7.45 | 38.63-78.56 | 65.87 | $\pm 15.16$ |
| AVEAL | 6.93-24.73 | $15.79=$ | $\pm$ | 7.27 | 5.1-19.26 | 11.88 | $\pm 4.32$ |
| AVBL | 7.33-26 | 15.26 | $\pm$ | 7.24 | 12.63-26.3 | 20.23 | $\pm 4.64$ |
| LOBEDISS | 0.25-0.77 | $0.51 \pm$ | $\pm$ | 0.22 | 0.25-0.6 | 0.36 | $\pm 0.11$ |
| AVELOBEW | 21.93-29.7 | $25.02 \pm$ | $\pm$ | 2.79 | 11-35.9 | 23.46 | $\pm 7.76$ |
| AVESEPL | 5.56-9.2 | 7.98 | $\pm$ | 1.33 | 5.83-8.76 | 7.46 | $\pm 0.92$ |
| AVEPETL | 6.76-16.3 | 13.90 | $\pm$ | 3.62 | 5.93-17.76 | 10.65 | $\pm 3.80$ |
| AVEPETW | 4.73-8.16 | $6.70 \pm$ | $\pm$ | 1.24 | 4.93-7.83 | 6.39 | $\pm 1.23$ |
| TOTRACE | 6-28 | 13.67 | $\pm$ | 7.87 | 17-53 | 31.25 | $\pm 15.62$ |
| AVEBRANC | 6.33-10 | 8.44 | $\pm$ | 1.39 | 6.33-12 | 9.58 | $\pm 2.11$ |
| AVEINFLFL | 7.86-17.13 | 13.57 | $\pm$ | 3.39 | 9.73-22.03 | 15.06 | $\pm 4.04$ |
| RFLOWCM | 1.25-1.82 | 1.53 | $\pm 0$ | 0.25 | 0.84-1.54 | 1.12 | $\pm 0.25$ |
|  |  |  |  |  |  |  |  |
| POPULATION | RP |  |  |  | SM |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN | STD |
| FLWRCLR | 1-10 | 5 | $\pm$ | 3 | 9-9 |  | $\pm 0$ |
| JULEMR | 2007056-2007070 | 2007065 | $\pm$ | 6 | 2007119-2007119 | 2007107 | $\pm 18$ |
| JULDATE | 2008158-2008172 | 2008167 | $\pm$ | 5 | 2008168-2008168 | 2008162 | $\pm 6$ |
| HT | 159-202 | 180 | $\pm$ | 16 | 149-149 | 120 | $\pm 31$ |
| AVEINFLL | 105.66-157 | 128 | $\pm$ | 21 | 109.66-109.66 | 91 | $\pm 25$ |
| AVEFLWR | 8-24.66 | $17.67 \pm$ | $\pm$ | 5.82 | 18.66-18.66 | 18.11 | $\pm 0.69$ |
| AVELEAFL | 40.9-51.2 | 46.26 + | $\pm$ | 4.37 | 34.06-34.06 | 28.41 | $\pm 6.51$ |
| AVELEAFW | 67.23-89.93 | 74.56 | $\pm$ | 9.11 | 55.4-55.4 | 48.36 | $\pm 8.51$ |
| AVEAL | 12.9-22.5 | 18.19 | $\pm$ | 2.99 | 17.46-17.46 | 12.16 | $\pm 6.39$ |
| AVBL | 15.1-31.8 | 21.10 | $\pm$ | 5.92 | 14.96-14.96 | 11.93 | $\pm 4.88$ |
| LOBEDISS | 0.28-0.57 | 0.47 + | $\pm$ | 0.10 | 0.73-0.73 | 0.49 | $\pm 0.24$ |
| AVELOBEW | 22.7-39.96 | 29.58 |  | 6.02 | 22.4-22.4 | 18.21 | $\pm 4.92$ |


| AVESEPL | 7.2-10.6 | $8.88 \pm$ | $\pm 1.05$ | 10.46-10.46 | $8.97 \pm$ | 1.41 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AVEPETL | 10.96-18.86 | $14.92 \pm$ | $\pm 2.65$ | 17.03-17.03 | $14.44 \pm$ | 2.80 |
| AVEPETW | 5.53-8.8 | $7.43 \pm$ | $\pm 1.29$ | 8.66-8.66 | $8.09 \pm$ | 0.76 |
| TOTRACE | 14-34 | $21.43 \pm$ | $\pm 6.78$ | 25-25 | $21.00 \pm$ | 4.58 |
| AVEBRANC | 6-13.33 | $8.86 \pm$ | $\pm 2.27$ | 6.66-6.66 | 6.22 | 0.51 |
| AVEINFLFL | 10.46-18.7 | $14.72 \pm$ | $\pm 2.70$ | 18-18 | $14.61 \pm$ | 3.14 |
| RFLOWCM | 0.76-1.55 | $1.18 \pm$ | $\pm 0.26$ | 1.58-1.58 | 1.28 | 0.31 |
|  |  |  |  |  |  |  |
| POPULATION | SN |  |  | SR |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 10-10 | $10 \pm$ | $\pm 0$ | 5-10 | $9 \pm 2$ |  |
| JULEMR | 2007065-2007108 | $2007079 \pm$ | $\pm 25$ | 2007056-2007077 | $2007067 \pm 9$ |  |
| JULDATE | 2008119-2008135 | $2008129 \pm$ | $\pm 9$ | 2008149-2008168 | $2008159 \pm 8$ |  |
| HT | 105-130 | $118 \pm$ | $\pm 13$ | 120-146 | 132 $\pm$ | 13 |
| AVEINFLL | 70.66-82.66 | $76 \pm$ | $\pm 6$ | 60.33-88 | $73 \pm$ | 11 |
| AVEFLWR | 13.33-22.33 | $18.33 \pm$ | $\pm 4.58$ | 14.33-23 | 19.92 $\pm$ | 3.82 |
| AVELEAFL | 30.56-57.26 | $44.56 \pm$ | $\pm 13.40$ | 32.2-57.66 | $46.81 \pm$ | 11.37 |
| AVELEAFW | 43.86-98.7 | $75.31 \pm$ | $\pm 28.29$ | 54.7-102.26 | $80.38 \pm$ | 21.07 |
| AVEAL | 15.46-34.26 | $23.90 \pm$ | $\pm 9.55$ | 13.26-35.33 | $20.68 \pm$ | 10.02 |
| AVBL | 6.26-16.7 | $11.80 \pm$ | $\pm 5.25$ | 6.93-23.9 | $16.58 \pm$ | 7.77 |
| LOBEDISS | 0.55-0.84 | $0.66 \pm$ | $\pm 0.16$ | 0.39-0.83 | $0.55 \pm$ | 0.20 |
| AVELOBEW | 12.6-29.2 | $21.83 \pm$ | $\pm 8.46$ | 20.3-39.5 | $29.55 \pm$ | 9.41 |
| AVESEPL | 6.4-8.63 | $7.82 \pm$ | $\pm 1.24$ | 8-11.56 | $9.77 \pm$ | 1.46 |
| AVEPETL | 8.93-16.23 | $11.63 \pm$ | $\pm 4.00$ | 7.43-17.16 | $13.88 \pm$ | 4.44 |
| AVEPETW | 7.16-9.36 | $8.11 \pm$ | $\pm 1.13$ | 4.36-8.46 | $6.95 \pm$ | 1.79 |
| TOTRACE | 12-16 | $14.67 \pm$ | $\pm 2.31$ | 8-21 | $12.00 \pm$ | 6.06 |
| AVEBRANC | 2.66-5 | $4.11 \pm$ | $\pm 1.26$ | 6-7 | $6.42 \pm$ | 0.42 |
| AVEINFLFL | 14.06-15.46 | $14.61 \pm$ | $\pm 0.75$ | 14.86-22.2 | $18.69 \pm$ | 3.07 |
| RFLOWCM | 0.94-1.44 | $1.25 \pm$ | $\pm 0.26$ | 0.96-1.18 | $1.06 \pm$ | 0.09 |
|  |  |  |  |  |  |  |
| POPULATION | ST |  |  | SU |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 9-10 | $9 \pm$ | $\pm$ | 3-10 | $7 \pm$ | 4 |
| JULEMR | 2007058-2007078 | $2007068 \pm$ | $\pm 10$ | 2007059-2007077 | $2007067 \pm$ | 8 |
| JULDATE | 2008156-2008161 | 2008158 | $\pm 3$ | 2008154-2008168 | $2008162 \pm$ | 7 |
| HT | 135-182 | 158 $\pm$ | $\pm 24$ | 107-186 | $145 \pm$ | 32 |
| AVEINFLL | 83.33-127 | $107 \pm$ | $\pm 22$ | 82.33-135 | $101 \pm$ | 23 |
| AVEFLWR | 16-18 | $16.89 \pm$ | $\pm 1.02$ | 11.33-21 | $16.83 \pm$ | 4.64 |
| AVELEAFL | 38.46-55.8 | $47.89 \pm$ | $\pm 8.76$ | 29.56-49.7 | $39.16 \pm$ | 8.51 |
| AVELEAFW | 63.2-95.76 | $81.14 \pm$ | $\pm 16.54$ | 52.4-89.96 | $69.09 \pm$ | 18.46 |
| AVEAL | 17.63-32.5 | $24.13 \pm$ | $\pm 7.61$ | 5.26-27.23 | $19.19 \pm$ | 9.59 |
| AVBL | 7.33-14.83 | $11.10 \pm$ | $\pm 3.75$ | 10.06-21.9 | $13.88 \pm$ | 5.44 |
| LOBEDISS | 0.54-0.81 | $0.68 \pm$ | $\pm 0.14$ | 0.19-0.69 | $0.56 \pm$ | 0.24 |
| AVELOBEW | 20.86-34.63 | $27.92 \pm$ | $\pm 6.89$ | 12.2-26.63 | $19.21 \pm$ |  |
| AVESEPL | 8.26-10.96 | $9.31 \pm$ | $\pm 1.45$ | 7.36-8.73 | $8.10 \pm$ | 0.70 |
| AVEPETL | 15.33-19.3 | $17.62 \pm$ | $\pm 2.05$ | 6.56-16.03 | $9.63 \pm$ | 4.32 |
| AVEPETW | 8.06-10.4 | $9.31 \pm$ | $\pm 1.17$ | 5.06-8.83 | $6.27 \pm$ | 1.77 |


| TOTRACE | 19-23 | 20.67 | $\pm \mid 2.08$ | 20-27 | $22.00 \pm$ | 3.37 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AVEBRANC | 6.66-11 | $9.44 \pm$ | $\pm 2.41$ | 4.33-11 | $7.50 \pm$ | 3.05 |
| AVEINFLFL | 12.73-19.83 | 17.11 $\pm$ | $\pm 3.83$ | 8.73-15.96 | $13.08 \pm$ | 3.16 |
| RFLOWCM | 0.88-1.25 | $1.02 \pm$ | $\pm 0.21$ | 1.13-1.38 | $1.28 \pm$ | 0.11 |
|  |  |  |  |  |  |  |
| POPULATION | TP |  |  | TU |  |  |
|  | RANGE | MEAN | STD | RANGE | MEAN | STD |
| FLWRCLR | 4-10 | $9 \pm$ | $\pm 2$ | 9-9 | $9 \pm$ | 0 |
| JULEMR | 2007054-2007111 | 2007074 | $\pm 16$ | 2007055-2007070 | $2007063 \pm$ | 11 |
| JULDATE | 2008158-2008179 | $2008169 \pm$ | $\pm 7$ | 2008158-2008161 | $2008160 \pm$ | 2 |
| HT | 127-180 | 157 $\pm$ | $\pm 16$ | 144-156 | $150 \pm$ | 8 |
| AVEINFLL | 97.66-131.66 | $117 \pm$ | $\pm 11$ | 72-93 | $83 \pm$ | 15 |
| AVEFLWR | 7-27.33 | 18.03 $\pm$ | $\pm 5.67$ | 17.66-26.66 | $22.17 \pm$ | 6.36 |
| AVELEAFL | 26.9-56.9 | $41.90 \pm$ | $\pm 11.57$ | 35.2-36.43 | $35.82 \pm$ | 0.87 |
| AVELEAFW | 45.23-102.73 | $70.88 \pm$ | $\pm 19.74$ | 60.53-61.73 | $61.13 \pm$ | 0.85 |
| AVEAL | 8.8-26.53 | $19.42 \pm$ | $\pm 5.78$ | 10.6-20.36 | $15.48 \pm$ | 6.91 |
| AVBL | 5.76-25.46 | $13.61 \pm$ | $\pm 6.12$ | 10.93-19.13 | $15.03 \pm$ | 5.80 |
| LOBEDISS | 0.25-0.81 | $0.59 \pm$ | $\pm 0.14$ | 0.35-0.65 | $0.50 \pm$ | 0.21 |
| AVELOBEW | 11.96-53.33 | $27.76 \pm$ | $\pm 13.24$ | 19.1-24.9 | $22.00 \pm$ | 4.10 |
| AVESEPL | 7.56-10.33 | $9.16 \pm$ | $\pm 0.92$ | 9.06-10.66 | $9.87 \pm$ | 1.13 |
| AVEPETL | 10.06-20.06 | 15.59 | $\pm 2.95$ | 17.5-17.5 | $17.50 \pm$ | 0.00 |
| AVEPETW | 4.73-7.73 | $6.48 \pm$ | $\pm 0.94$ | 8.96-8.96 | $8.97 \pm$ | 0.00 |
| TOTRACE | 12-27 | $20.60 \pm$ | $\pm 5.08$ | 10-22 | $16.00 \pm$ | 8.49 |
| AVEBRANC | 7.66-16.66 | 10.30 $\pm$ | $\pm 2.84$ | 6.33-13 | $9.67 \pm$ | 4.71 |
| AVEINFLFL | 6.43-33.03 | $13.83 \pm$ | $\pm 7.93$ | 12.3-19.93 | $16.12 \pm$ | 5.40 |
| RFLOWCM | 0.82-2.39 | 1.47 $\pm$ | $\pm 0.51$ | 0.88-2.16 | $1.53 \pm$ | 0.91 |

## APPENDIX B: (ERLA)

|  | RANGE | MEAN |  | STD |
| :--- | :--- | :--- | :--- | :--- |
| JULEMR | $2007008-2007113$ | 2007066.3 | $\pm$ | 21.01 |
| FLWRJUL | $2008128-2008161$ | 2008143.4 | $\pm$ | 5.82 |
| AVERAYL | $2.1-17.03$ | 10.714211 | $\pm$ | 2.17 |
| AVERAYW | $1.4-9.3$ | 5.3932079 | $\pm$ | 1.11 |
| AVEFHDIA | $3.96-12.3$ | 8.3260188 | $\pm$ | 1.10 |
| EXTOTRCM | $8-1704$ | 443.56113 | $\pm$ | 271.82 |
| HT | $11-79$ | 55.705329 | $\pm$ | 9.98 |
| DIA | $2-150$ | 95.780564 | $\pm$ | 21.05 |
| LARRGT | $1-2$ | 1.2633229 | $\pm$ | 0.44 |
| LEAFEDG | $1-5$ | 1.6206897 | $\pm$ | 0.52 |
| FLCR1 | $0-10.8$ | 3.5410658 | $\pm$ | 3.01 |
| FCPTL | $2.1-15.93$ | 10.347962 | $\pm$ | 2.14 |
| DIFFCOLR | $1.4-15.4$ | 6.8068966 | $\pm$ | 2.94 |
| FLCL2 | $1-5$ | 3.137931 | $\pm$ | 1.36 |
| AVEHDINFL | $1-18.33$ | 4.2685475 | $\pm$ | 2.22 |
| AVEPEDL | $2.66-53.66$ | 31.159875 | $\pm$ | 9.08 |
| AVERAY | $7-13.66$ | 9.7032497 | $\pm$ | 1.36 |


| LEAFCLR | $1-6$ | 2.7523511 | $\pm$ | 1.53 |
| :--- | :--- | ---: | :--- | :--- |
| AVELEAFL | $14.25-65.1$ | 37.982602 | $\pm$ | 7.53 |
| AVELOBE | $1-4.5$ | 2.6833856 | $\pm$ | 0.65 |
| AVELEAFTIP | $1-2$ | 1.30721 | $\pm$ | 0.43 |
| LEAFW | $1.9-19.4$ | 5.9200627 | $\pm$ | 2.59 |

APPENDIX C: (ERLA)

|  | CB |  |  |  | $\begin{aligned} & \text { CM } \\ & \hline \text { RANGE } \end{aligned}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | RANGE | MEAN |  | STD |  | MEAN | STD |  |
| JULEMR | 2007052-2007078 | 2007067.2 | $\pm$ | 6.4540229 | 2007059-2007082 | 2007072 | $\pm$ | 7.1657253 |
| FLWRJUL | 2008140-2008158 | 2008142.5 | $\pm$ | 4.3891578 | 2008140-2008154 | 2008146.5 | $\pm$ | 4.9458978 |
| AVERAYL | 2.1-13.56 | 10.092157 | $\pm$ | 3.2667842 | 7.23-12.6 | 10.161905 | $\pm$ | 1.3829231 |
| AVERAYW | 1.4-5.7 | 4.054902 | $\pm$ | 1.2918517 | 3.3-6.43 | 5.2825397 | $\pm$ | 0.7851836 |
| AVEFHDIA | 3.96-9.66 | 7.9352941 | $\pm$ | 1.411435 | 6.6-9.06 | 7.7873016 | $\pm$ | 0.5979108 |
| EXTOTRCM | 16-944 | 479.05882 | $\pm$ | 318.9468 | 200-1144 | 620.19048 | $\pm$ | 247.27143 |
| HT | 17-68 | 49.823529 | $\pm$ | 13.440774 | 48-72 | 58.761905 | $\pm$ | 6.8622501 |
| DIA | 32-124 | 85.176471 | $\pm$ | 27.461872 | 62-139 | 100.42857 | $\pm$ | 18.27723 |
| LARRGT | 1-2 | 1.3529412 | $\pm$ | 0.4925922 | 1-2 | 1.3333333 | $\pm$ | 0.4830459 |
| LEAFEDG | 1-2 | 1.5294118 | $\pm$ | 0.5144958 | 1-2 | 1.6190476 | $\pm$ | 0.4976134 |
| FLCR1 | 0-6.6 | 3.3764706 | $\pm$ | 2.4491145 | 0-8 | 4.9190476 | $\pm$ | 2.3340135 |
| FCPTL | 2.1-13.56 | 9.1333333 | $\pm$ | 3.0950004 | 7.23-12.6 | 10.115873 | $\pm$ | 1.5608623 |
| DIFFCOLR | 2.1-9 | 5.7568627 | $\pm$ | 1.78667 | 2.56-11.96 | 5.1968254 | $\pm$ | 2.6322763 |
| FLCL2 | 1-5 | 3.5882353 | $\pm$ | 1.4168108 | 1-4 | 3.5714286 | $\pm$ | 1.0757057 |
| AVEHDINFL | 1-6.66 | 3.5098039 | $\pm$ | 1.7123637 | 2-11 | 5.3650794 | $\pm$ | 2.1830274 |
| AVEPEDL | 4-39.66 | 25.45098 | $\pm$ | 10.743823 | 12-43 | 28.904762 | $\pm$ | 7.0175517 |
| AVERAY | 8-12.33 | 10.156863 | $\pm$ | 1.1311109 | 7.66-11.66 | 9.3968254 | $\pm$ | 1.1624641 |
| LEAFCLR | 2-5 | 3.4117647 | $\pm$ | 1.1213175 | 1-3 | 1.952381 | $\pm$ | 0.7400129 |
| AVELEAFL | 14.25-45.85 | 32.873529 | $\pm$ | 9.0423158 | 25.95-44.6 | 33.471429 | $\pm$ | 5.293689 |
| AVELOBE | 1.5-3.5 | 2.2352941 | $\pm$ | 0.5622957 | 2-4.5 | 3.2380952 | $\pm$ | 0.6248809 |
| AVELEAFTIP | 1-1 | 1 | $\pm$ | 0 | 1-2 | 1.452381 | $\pm$ | 0.4445436 |
| LEAFW | 1.9-9 | 4.7176471 | $\pm$ | 2.2517314 | 2.5-6.5 | 4.5904762 | $\pm$ | 1.1941125 |
|  | CR |  |  |  | CS |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007061-2007113 | 2007079.6 | $\pm$ | 12.586623 | 2007029-2007082 | 2007064.1 | $\pm$ | 17.709572 |
| FLWRJUL | 2008140-2008154 | 2008147.8 | $\pm$ | 4.3998834 | 2008137-2008151 | 2008142.1 | $\pm$ | 3.7402505 |
| AVERAYL | 7.76-12.26 | 10.266667 | $\pm$ | 1.2343839 | 6.06-9.33 | 7.9766667 | $\pm$ | 0.9501677 |
| AVERAYW | 5.13-7.76 | 6.5717949 | $\pm$ | 0.6395667 | 3.16-5.4 | 4.5383333 | $\pm$ | 0.6471959 |
| AVEFHDIA | 7.06-9.1 | 7.9974359 | $\pm$ | 0.6174112 | 7.73-10.6 | 8.96 | $\pm$ | 0.7773549 |
| EXTOTRCM | 80-472 | 232.61538 | $\pm$ | 118.99407 | 296-1208 | 668.4 | $\pm$ | 287.44361 |
| HT | 47-72 | 60.615385 | $\pm$ | 6.9106013 | 50-75 | 59.75 | $\pm$ | 5.8837421 |
| DIA | 49-111 | 82.692308 | $\pm$ | 19.567765 | 78-125 | 102 | $\pm$ | 13.062602 |
| LARRGT | 1-1 | 1 | $\pm$ | 0 | 1-2 | 1.45 | $\pm$ | 0.5104178 |
| LEAFEDG | 1-2 | 1.6923077 | $\pm$ | 0.4803845 | 1-2 | 1.65 | $\pm$ | 0.4893605 |
| FLCR1 | 0-9 | 6.3076923 | $\pm$ | 3.0666109 | 0-5.4 | 0.435 | $\pm$ | 1.3815609 |
| FCPTL | 7.3-13.8 | 10.251282 | $\pm$ | 1.8723928 | 5.6-10.1 | 8.2383333 | $\pm$ | 1.369222 |


| DIFFCOLR | 1.4-11 | 3.9435897 | $\pm$ | 2.601331 | 3.33-10.1 | 7.8033333 | $\pm$ | 1.9416909 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| FLCL2 | 1-4 | 3.6153846 | $\pm$ | 0.9607689 | 1-4 | 2.3 | $\pm$ | 0.8645047 |
| AVEHDINFL | 2.66-10.66 | 4.3846154 | $\pm$ | 2.1118982 | 1.66-5.33 | 3.6 | $\pm$ | 0.9403247 |
| AVEPEDL | 20-46.66 | 32.794872 | $\pm$ | 7.6805894 | 19.66-52.33 | 37.283333 | $\pm$ | 9.2497985 |
| AVERAY | 7.33-9 | 8.1794872 | $\pm$ | 0.4219747 | 8.33-12.66 | 11.333333 | $\pm$ | 1.2658354 |
| LEAFCLR | 1-3 | 1.7692308 | $\pm$ | 0.5991447 | 1-5 | 2.5 | $\pm$ | 0.8885233 |
| AVELEAFL | 31.15-45.5 | 38.007692 | $\pm$ | 4.4553267 | 24.35-44.7 | 35.165 | $\pm$ | 5.1688872 |
| AVELOBE | 2-3 | 2.4230769 | $\pm$ | 0.4003204 | 2-3.5 | 2.8 | $\pm$ | 0.5477226 |
| AVELEAFTIP | 1-2 | 1.5 | $\pm$ | 0.4082483 | 1-2 | 1.225 | $\pm$ | 0.3795773 |
| LEAFW | 3.4-12.6 | 6.3923077 | $\pm$ | 2.6417991 | 2.5-10.1 | 5.5 | $\pm$ | 2.0855392 |
|  | ER |  |  |  | FB |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007008-2007085 | 2007034.1 | $\pm$ | 23.882755 | 2007054-2007082 | 2007069.1 | $\pm$ | 7.2832514 |
| FLWRJUL | 2008130-2008154 | 2008140.2 | $\pm$ | 5.1344496 | 2008135-2008151 | 2008145.8 | $\pm$ | 4.5961052 |
| AVERAYL | 8.56-15.9 | 11.757895 | $\pm$ | 1.7604658 | 8.86-14.16 | 11.825926 | $\pm$ | 1.4633008 |
| AVERAYW | 4.66-7.36 | 5.5526316 | $\pm$ | 0.7520602 | 3.63-6.03 | 4.8074074 | $\pm$ | 0.5563132 |
| AVEFHDIA | 6.23-8.7 | 7.3701754 | $\pm$ | 0.753092 | 8.36-11.2 | 9.6851852 | $\pm$ | 0.8276451 |
| EXTOTRCM | 128-1704 | 522.10526 | $\pm$ | 376.64099 | 152-1088 | 517.33333 | $\pm$ | 249.11325 |
| HT | 11-67 | 51.157895 | $\pm$ | 12.383964 | 47-72 | 59 | $\pm$ | 7.0293502 |
| DIA | 73-120 | 93.736842 | $\pm$ | 10.613629 | 68-133 | 110.16667 | $\pm$ | 14.97547 |
| LARRGT | 1-2 | 1.2631579 | $\pm$ | 0.4524139 | 1-2 | 1.1666667 | $\pm$ | 0.3834825 |
| LEAFEDG | 1-2 | 1.5263158 | $\pm$ | 0.5129892 | 1-2 | 1.8333333 | $\pm$ | 0.3834825 |
| FLCR1 | 0-8.4 | 1.0315789 | $\pm$ | 2.5082204 | 0-8.8 | 4.6111111 | $\pm$ | 2.4726478 |
| FCPTL | 8.1-14.93 | 11.377193 | $\pm$ | 1.6331463 | 6-13.8 | 10.809259 | $\pm$ | 2.172821 |
| DIFFCOLR | 4.9-13 | 10.345614 | $\pm$ | 2.1834736 | 1.9-11 | 6.1981481 | $\pm$ | 2.2700227 |
| FLCL2 | 1-4 | 1.6842105 | $\pm$ | 1.1081833 | 1-4 | 3.6111111 | $\pm$ | 0.9164438 |
| AVEHDINFL | 2.33-13 | 4.4035088 | $\pm$ | 2.4635089 | 1-5.33 | 3.2407407 | $\pm$ | 1.0466601 |
| AVEPEDL | 12.66-39.33 | 22.421053 | $\pm$ | 6.3253772 | 21.66-53.66 | 34.87037 | $\pm$ | 8.5314787 |
| AVERAY | 7.33-13.33 | 9.1052632 | $\pm$ | 1.7215147 | 8.33-11.33 | 10.055556 | $\pm$ | 0.9305982 |
| LEAFCLR | 1-4 | 2.3684211 | $\pm$ | 1.4985373 | 3-5 | 4.2222222 | $\pm$ | 1.0032627 |
| AVELEAFL | 26.4-54.65 | 39.584211 | $\pm$ | 8.3117563 | 22.9-48.1 | 38.313889 | $\pm$ | 7.0594702 |
| AVELOBE | 1.5-4 | 2.8684211 | $\pm$ | 0.7039986 | 1.5-3.5 | 2.4722222 | $\pm$ | 0.605665 |
| AVELEAFTIP | 1-2 | 1.4210526 | $\pm$ | 0.4491708 | 1-1 | 1 | $\pm$ | 0 |
| LEAFW | 2.5-12.3 | 6.1 | $\pm$ | 2.2832725 | 2.8-12.1 | 6.2111111 | $\pm$ | 2.4354946 |
|  | GR |  |  |  | HA |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007064-2007089 | 2007081.5 | $\pm$ | 8.8132132 | 2007040-2007082 | 2007067.9 | $\pm$ | 11.656938 |
| FLWRJUL | 2008135-2008142 | 2008139 | $\pm$ | 2.6832816 | 2008140-2008154 | 2008147.9 | $\pm$ | 3.9323222 |
| AVERAYL | 8.73-12.3 | 10.354545 | $\pm$ | 1.2362374 | 5.66-14.43 | 9.545 | $\pm$ | 2.2140507 |
| AVERAYW | 3.86-5.83 | 5.1636364 | $\pm$ | 0.572801 | 2.8-7.5 | 5.8566667 | $\pm$ | 0.9479495 |
| AVEFHDIA | 7.66-10.86 | 8.6424242 | $\pm$ | 0.8712814 | 7.9-12.3 | 9.725 | $\pm$ | 1.1588901 |
| EXTOTRCM | 152-632 | 326.54545 | $\pm$ | 145.40727 | 8-376 | 163.2 | $\pm$ | 102.62738 |
| HT | 45-69 | 55 | $\pm$ | 6.8556546 | 33-68 | 54.25 | $\pm$ | 9.5359431 |
| DIA | 78-115 | 95.272727 | $\pm$ | 11.671255 | 60-125 | 95.85 | $\pm$ | 19.074025 |
| LARRGT | 1-2 | 1.1818182 | $\pm$ | 0.4045199 | 1-2 | 1.1 | $\pm$ | 0.3077935 |
| LEAFEDG | 1-2 | 1.5454545 | $\pm$ | 0.522233 | 1-2 | 1.65 | $\pm$ | 0.4893605 |
| FLCR1 | 0-5.4 | 3.9363636 | $\pm$ | 1.467837 | 0-10.8 | 1.935 | $\pm$ | 3.5440499 |
| FCPTL | 5.7-11.2 | 9.2363636 | $\pm$ | 1.5021802 | 5.66-14.4 | 8.5416667 | $\pm$ | 2.1964585 |


| DIFFCOLR | 2.6-11.2 | 5.3 | $\pm$ | 2.1927152 | 2.5-10.2 | 6.6066667 | $\pm$ | 2.1632659 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| FLCL2 | 2-5 | 3.9090909 | $\pm$ | 0.700649 | 1-4 | 1.75 | $\pm$ | 1.332785 |
| AVEHDINFL | 1-4 | 2.7878788 | $\pm$ | 0.9101004 | 1.66-6.66 | 3.6333333 | $\pm$ | 1.2182818 |
| AVEPEDL | 20-34 | 29.515152 | $\pm$ | 4.5737503 | 24-48.66 | 36.25 | $\pm$ | 7.2077565 |
| AVERAY | 9.66-12.33 | 10.787879 | $\pm$ | 0.8201995 | 8-11.66 | 9.3166667 | $\pm$ | 0.9761783 |
| LEAFCLR | 1-5 | 2.6363636 | $\pm$ | 1.0269106 | 1-2 | 1.55 | $\pm$ | 0.5104178 |
| AVELEAFL | 31.3-51.7 | 39.154545 | $\pm$ | 6.1084554 | 32.7-65.1 | 44.1025 | $\pm$ | 7.7433754 |
| AVELOBE | 1.5-3.5 | 2.7727273 | $\pm$ | 0.6067799 | 1.5-3.5 | 2.675 | $\pm$ | 0.5199949 |
| AVELEAFTIP | 1-1 | 1 | $\pm$ | 0 | 1-2 | 1.4 | $\pm$ | 0.4472136 |
| LEAFW | 3-8.6 | 5.2090909 | $\pm$ | 1.7506882 | 2.7-11.4 | 6.295 | $\pm$ | 2.6067675 |
|  | HR |  |  |  | HZ |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007008-2007089 | 2007051.9 | $\pm$ | 27.458887 | 2007043-2007087 | 2007074.5 | $\pm$ | 13.301497 |
| FLWRJUL | 2008130-2008149 | 2008140 | $\pm$ | 4.5439651 | 2008137-2008147 | 2008140.6 | $\pm$ | 1.9209525 |
| AVERAYL | 9.1-17.03 | 12.6 | $\pm$ | 2.6858684 | 9.86-15.6 | 12.826316 | $\pm$ | 1.4882079 |
| AVERAYW | 4.93-9.3 | 6.9174603 | $\pm$ | 1.2033573 | 5.66-7.7 | 6.8947368 | $\pm$ | 0.5867112 |
| AVEFHDIA | 6.86-9.83 | 8.3031746 | $\pm$ | 0.8117816 | 6.66-9.23 | 7.6894737 | $\pm$ | 0.7251269 |
| EXTOTRCM | 56-608 | 323.80952 | $\pm$ | 169.66073 | 72-624 | 345.68421 | $\pm$ | 152.38732 |
| HT | 19-65 | 53.857143 | $\pm$ | 9.9864193 | 39-63 | 53.736842 | $\pm$ | 6.479387 |
| DIA | 30-124 | 91.619048 | $\pm$ | 25.439096 | 49-102 | 85.789474 | $\pm$ | 12.686734 |
| LARRGT | 1-2 | 1.3333333 | $\pm$ | 0.4830459 | 1-2 | 1.0526316 | $\pm$ | 0.2294157 |
| LEAFEDG | 1-2 | 1.7142857 | $\pm$ | 0.46291 | 1-2 | 1.6315789 | $\pm$ | 0.4955946 |
| FLCR1 | 0-10.4 | 1.5666667 | $\pm$ | 3.3730303 | 0-8.5 | 4.9263158 | $\pm$ | 2.7684416 |
| FCPTL | 7.9-15.4 | 11.933333 | $\pm$ | 2.052424 | 10.3-15.2 | 12.001754 | $\pm$ | 1.31151 |
| DIFFCOLR | 2.53-15.4 | 10.366667 | $\pm$ | 3.5042514 | 4.6-13.4 | 7.0754386 | $\pm$ | 2.5339142 |
| FLCL2 | 1-4 | 1.6666667 | $\pm$ | 1.197219 | 1-4 | 3.3684211 | $\pm$ | 1.2565617 |
| AVEHDINFL | 1.66-9 | 5.1428571 | $\pm$ | 1.7875229 | 1.33-6 | 3.6666667 | $\pm$ | 1.0363755 |
| AVEPEDL | 4.33-40 | 27.698413 | $\pm$ | 8.8918844 | 18-41.66 | 31.45614 | $\pm$ | 5.5279431 |
| AVERAY | 7.66-11.33 | 9.3333333 | $\pm$ | 0.9486833 | 8-10 | 8.9298246 | $\pm$ | 0.6811297 |
| LEAFCLR | 1-4 | 2.9047619 | $\pm$ | 1.3380868 | 1-1 | 1 | $\pm$ | 0 |
| AVELEAFL | 25.65-63.6 | 37.519048 | $\pm$ | 9.8080385 | 28.15-52 | 39.123684 | $\pm$ | 6.3334291 |
| AVELOBE | 2-4 | 2.5714286 | $\pm$ | 0.5542047 | 1.5-4 | 2.2368421 | $\pm$ | 0.7143066 |
| AVELEAFTIP | 1-2 | 1.5714286 | $\pm$ | 0.4818121 | 1-2 | 1.9210526 | $\pm$ | 0.2507299 |
| LEAFW | 2.7-10.2 | 6.3666667 | $\pm$ | 1.8802482 | 6-14.5 | 9.1315789 | $\pm$ | 2.7129428 |
|  | KI |  |  |  | KN |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007052-2007085 | 2007068.8 | $\pm$ | 10.997752 | 2007043-2007096 | 2007065.2 | $\pm$ | 13.53704 |
| FLWRJUL | 2008137-2008154 | 2008146.8 | $\pm$ | 4.6769249 | 2008135-2008161 | 2008148.9 | $\pm$ | 7.2102915 |
| AVERAYL | 10.1-16.03 | 12.17381 | $\pm$ | 1.6805666 | 5.5-13 | 9.0263158 | $\pm$ | 1.8730901 |
| AVERAYW | 4.33-6.16 | 5.2261905 | $\pm$ | 0.566769 | 3.2-5.9 | 4.5614035 | $\pm$ | 0.7403088 |
| AVEFHDIA | 7.4-10.13 | 8.6928571 | $\pm$ | 0.7873116 | 6.63-10.33 | 8.5824561 | $\pm$ | 0.8810325 |
| EXTOTRCM | 176-832 | 456 | $\pm$ | 177.97493 | 112-1080 | 559.57895 | $\pm$ | 253.00292 |
| HT | 53-79 | 62.357143 | $\pm$ | 7.0011773 | 56-71 | 63.368421 | $\pm$ | 4.6572587 |
| DIA | 55-120 | 97.785714 | $\pm$ | 19.055298 | 39-145 | 106.31579 | $\pm$ | 24.702795 |
| LARRGT | 1-2 | 1.5 | $\pm$ | 0.5188745 | 1-2 | 1.6842105 | $\pm$ | 0.4775669 |
| LEAFEDG | 1-5 | 1.9285714 | $\pm$ | 0.997249 | 1-2 | 1.5789474 | $\pm$ | 0.5072573 |
| FLCR1 | 0-6.9 | 4.9785714 | $\pm$ | 2.2908442 | 0-3.9 | 1.3315789 | $\pm$ | 1.4678225 |
| FCPTL | 10.2-14.3 | 12.069048 | $\pm$ | 1.0596294 | 6-12.6 | 9.0526316 | $\pm$ | 1.8190798 |



| DIFFCOLR | 2.6-11.7 | 6.7166667 | $\pm$ | 2.5642429 | 2.6-9 | 5.3282051 | $\pm$ | 1.558896 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| FLCL2 | 1-5 | 3.6428571 | $\pm$ | 1.1507284 | 4-5 | 4.1538462 | $\pm$ | 0.3755338 |
| AVEHDINFL | 2-8.33 | 3.5952381 | $\pm$ | 1.7105933 | 1.66-5.33 | 3.2564103 | $\pm$ | 0.9921343 |
| AVEPEDL | 16.33-34.33 | 25.714286 | $\pm$ | 6.0423819 | 10.66-33.66 | 22.333333 | $\pm$ | 6.4132386 |
| AVERAY | 8.66-12.33 | 10.214286 | $\pm$ | 1.2513729 | 8.33-12 | 9.7179487 | $\pm$ | 1.1694107 |
| LEAFCLR | 1-6 | 2.9285714 | $\pm$ | 1.2688145 | 1-5 | 2.7692308 | $\pm$ | 1.2351684 |
| AVELEAFL | 28.75-45.2 | 35.017857 | $\pm$ | 5.4500038 | 22.6-41.3 | 33.915385 | $\pm$ | 5.3333614 |
| AVELOBE | 1-3.5 | 2.5714286 | $\pm$ | 0.7300459 | 2-3.5 | 2.6538462 | $\pm$ | 0.5157767 |
| AVELEAFTIP | 1-2 | 1.1071429 | $\pm$ | 0.2894671 | 1-1 | 1 | $\pm$ | 0 |
| LEAFW | 3.7-8.1 | 6.1642857 | $\pm$ | 1.3141872 | 2.7-7.7 | 4.7538462 | $\pm$ | 1.3042337 |
|  | RA |  |  |  | RO |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JULEMR | 2007052-2007082 | 2007078.1 | $\pm$ | 8.1230126 | 2007068-2007092 | 2007079.6 | $\pm$ | 6.8495742 |
| FLWRJUL | 2008135-2008147 | 2008140 | $\pm$ | 3.7237973 | 2008137-2008154 | 2008143.4 | $\pm$ | 4.7884409 |
| AVERAYL | 8.13-13.43 | 10.645833 | $\pm$ | 1.6817044 | 7.43-12.23 | 10.275 | $\pm$ | 1.154925 |
| AVERAYW | 3.83-6.03 | 4.8541667 | $\pm$ | 0.5898996 | 3.4-6.1 | 4.75625 | $\pm$ | 0.8528574 |
| AVEFHDIA | 6.93-9.9 | 8.2916667 | $\pm$ | 0.8094351 | 5.63-9.96 | 8.1416667 | $\pm$ | 0.9940192 |
| EXTOTRCM | 248-1080 | 608 | $\pm$ | 265.82801 | 160-1208 | 534.5 | $\pm$ | 322.00994 |
| HT | 40-62 | 50.4375 | $\pm$ | 7.1364674 | 18-66 | 47.9375 | $\pm$ | 13.517735 |
| DIA | 2-119 | 98.3125 | $\pm$ | 27.331834 | 33-123 | 88.5625 | $\pm$ | 23.366554 |
| LARRGT | 1-2 | 1.125 | $\pm$ | 0.341565 | 1-2 | 1.3125 | $\pm$ | 0.4787136 |
| LEAFEDG | 1-2 | 1.4375 | $\pm$ | 0.5123475 | 1-2 | 1.5625 | $\pm$ | 0.5123475 |
| FLCR1 | 0-6.7 | 4.61875 | $\pm$ | 1.8519247 | 0-6.9 | 4.36875 | $\pm$ | 2.324283 |
| FCPTL | 8.1-13.4 | 10.525 | $\pm$ | 1.6717478 | 6.4-13.5 | 9.6645833 | $\pm$ | 2.0178268 |
| DIFFCOLR | 1.73-10 | 5.90625 | $\pm$ | 2.020349 | 2.5-9.1 | 5.2958333 | $\pm$ | 1.692549 |
| FLCL2 | 2-4 | 3.875 | $\pm$ | 0.5 | 1-5 | 3.5625 | $\pm$ | 1.3149778 |
| AVEHDINFL | 1.33-5 | 3.2708333 | $\pm$ | 1.0626225 | 1-4.66 | 2.875 | $\pm$ | 1.1409872 |
| AVEPEDL | 19.33-41.66 | 30.6875 | $\pm$ | 5.9572163 | 2.66-35 | 25.041667 | $\pm$ | 8.4938976 |
| AVERAY | 9.33-13.66 | 10.395833 | $\pm$ | 1.1939275 | 8-11 | 9.6458333 | $\pm$ | 0.9620099 |
| LEAFCLR | 2-6 | 3.625 | $\pm$ | 1.3102163 | 1-5 | 3.4375 | $\pm$ | 1.3647344 |
| AVELEAFL | 29.5-42.8 | 34.84375 | $\pm$ | 4.0128076 | 25.9-46.05 | 35.453125 | $\pm$ | 6.1268662 |
| AVELOBE | 1.5-3.5 | 2.75 | $\pm$ | 0.5773503 | 1.5-3 | 2.21875 | $\pm$ | 0.5467708 |
| AVELEAFTIP | 1-1 | 1 | $\pm$ | 0 | 1-1 | 1 | $\pm$ | 0 |
| LEAFW | 3-6.9 | 4.9125 | $\pm$ | 1.0455461 | 2.3-7.7 | 5.09375 | $\pm$ | 1.6754974 |
|  | RU |  |  |  |  |  |  |  |
|  | RANGE | MEAN |  | STD |  |  |  |  |
| JULEMR | 2007010-2007085 | 2007072.5 | $\pm$ | 20.592254 |  |  |  |  |
| FLWRJUL | 2008137-2008156 | 2008143.6 | $\pm$ | 4.9239836 |  |  |  |  |
| AVERAYL | 6.96-15.93 | 11.584211 | $\pm$ | 2.0699904 |  |  |  |  |
| AVERAYW | 4.93-7.7 | 6.2491228 | $\pm$ | 0.8692532 |  |  |  |  |
| AVEFHDIA | 6.63-9.83 | 8.0526316 | $\pm$ | 0.7200236 |  |  |  |  |
| EXTOTRCM | 48-720 | 336.42105 | $\pm$ | 208.86102 |  |  |  |  |
| HT | 42-72 | 56.473684 | $\pm$ | 7.5008771 |  |  |  |  |
| DIA | 38-128 | 97.052632 | $\pm$ | 23.09515 |  |  |  |  |
| LARRGT | 1-2 | 1.1578947 | $\pm$ | 0.3746343 |  |  |  |  |
| LEAFEDG | 1-2 | 1.7368421 | $\pm$ | 0.4524139 |  |  |  |  |
| FLCR1 | 0-9.4 | 5.2631579 | $\pm$ | 3.8242226 |  |  |  |  |
| FCPTL | 8-15.93 | 11.663158 | $\pm$ | 1.882207 |  |  |  |  |


| DIFFCOLR | $1.6-13.8$ | 6.4 | $\pm$ | 3.8048539 |
| :--- | :--- | ---: | :--- | :--- |
| FLCL2 | $1-4$ | 3.1052632 | $\pm$ | 1.3701069 |
| AVEHDINFL | $2.33-6.66$ | 4.2807018 | $\pm$ | 1.2184951 |
| AVEPEDL | $13.66-46.33$ | 34.122807 | $\pm$ | 7.7922172 |
| AVERAY | $7-11.33$ | 8.5087719 | $\pm$ | 1.1672931 |
| LEAFCLR | $1-6$ | 2 | $\pm$ | 1.6996732 |
| AVELEAFL | $27.75-54.5$ | 39.352632 | $\pm$ | 7.3665813 |
| AVELOBE | $2-3.5$ | 2.4210526 | $\pm$ | 0.4491708 |
| AVELEAFTIP | $1-2$ | 1.3947368 | $\pm$ | 0.4588315 |
| LEAFW | $2.1-10.2$ | 5.2105263 | $\pm$ | 2.1170983 |

## APPENDIX D: (EPDE)

|  | AK <br> RANGE | MEAN | STD |  | BD <br> RANGE | MEAN | STD |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| JulianFlower | 2007194-2007211 | 2007204 | $\pm$ | 5.194319 | 2007190-2007197 | 2007194 | $\pm$ | 2.57645 |
| Ht | 60-81 | 68.93333 | $\pm$ | 6.584252 | 71-82 | 78 | $\pm$ | 3.683942 |
| Diameter | 45-73 | 59.86667 | $\pm$ | 7.347173 | 55-68 | 60.93333 | $\pm$ | 4.431489 |
| Pubescence | 1-1 | 1 | $\pm$ | 0 | 5-5 | 5 | $\pm$ | 0 |
| JulianEmerg | 2007060-2007063 | 2007061 | $\pm$ | 0.736788 | 2007060-2007063 | 2007061 | $\pm$ | 0.703732 |
| MeanPetalL | 4.6-6 | 5.533333 | $\pm$ | 0.373741 | 4.6-6.6 | 5.688889 | $\pm$ | 0.555873 |
| JulianSeed | 2007233-2007247 | 2007241 | $\pm$ | 4.780914 | 2007218-2007233 | 2007226 | $\pm$ | 6.204453 |
| MeanLeafL | 25.7-56.6 | 38.92 | $\pm$ | 9.268703 | 30.4-62.3 | 42.66 | $\pm$ | 8.118172 |
| MeanLeafW | 4-8.2 | 6.446667 | $\pm$ | 1.105581 | 4.8-7.7 | 5.876667 | $\pm$ | 0.767153 |
|  | BF RANGE | MEAN |  | STD | BH RANGE | MEAN |  | STD |
| JulianFlower | 2007187-2007206 | 2007194 | $\pm$ | 5.586548 | 2007178-2007201 | 2007192 | $\pm$ | 6.436503 |
| Ht | 66-105 | 80.33333 | $\pm$ | 10.1887 | 54-77 | 63.46667 | $\pm$ | 6.151268 |
| Diameter | 46-86 | 66.4 | $\pm$ | 11.74004 | 51-72 | 60.86667 | $\pm$ | 5.667787 |
| Pubescence | 1-2 | 1.066667 | $\pm$ | 0.258199 | 1-5 | 2.2 | $\pm$ | 1.567528 |
| JulianEmerg | 2007061-2007068 | 2007062 | $\pm$ | 1.75119 | 2007060-2007063 | 2007061 | $\pm$ | 0.676123 |
| MeanPetall | 4-6.6 | 5.355556 | $\pm$ | 0.695412 | 4-6 | 4.955556 | $\pm$ | 0.615497 |
| JulianSeed | 2007220-2007247 | 2007234 | $\pm$ | 7.620149 | 2007218-2007247 | 2007229 | $\pm$ | 9.718906 |
| MeanLeafL | 36.2-66.5 | 44.15667 | $\pm$ | 7.799997 | 16.3-54.4 | 37.18 | $\pm$ | 8.706951 |
| MeanLeafW | 5-8.8 | 6.813333 | $\pm$ | 1.072791 | 2.8-9 | 6.313333 | $\pm$ | 1.582546 |
|  | BS |  |  |  | CO |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JulianFlower | 2007190-2007207 | 2007200 | $\pm$ | 4.174825 | 2007192-2007219 | 2007212 | $\pm$ | 9.284908 |
| Ht | 70-88 | 79.5625 | $\pm$ | 4.992912 | 58-122 | 100.8667 | $\pm$ | 14.04517 |
| Diameter | 58-78 | 66.25 | $\pm$ | 5.662744 | 66-102 | 82.6 | $\pm$ | 8.304904 |
| Pubescence | 1-3 | 1.1875 | $\pm$ | 0.543906 | 1-5 | 1.8 | $\pm$ | 1.146423 |
| JulianEmerg | 2007060-2007063 | 2007061 | $\pm$ | 0.680074 | 2007060-2007062 | 2007061 | $\pm$ | 0.516398 |
| MeanPetall | 4.3-6.6 | 5.270833 | $\pm$ | 0.519526 | 4.6-6.6 | 5.377778 | $\pm$ | 0.628259 |
| JulianSeed | 2007233-2007247 | 2007243 | $\pm$ | 5.189733 | 2007222-2007253 | 2007246 | $\pm$ | 9.613978 |
| MeanLeafL | 34.6-58.7 | 45.41875 | $\pm$ | 6.427645 | 30.2-64.7 | 45.22333 | $\pm$ | 11.23631 |
| MeanLeafW | 4.6-8.9 | 6.534375 | $\pm$ | 1.199683 | 4.2-7.5 | 5.826667 | $\pm$ | 0.95204 |
|  | $\begin{aligned} & \hline \text { DC } \\ & \text { RANGE } \\ & \hline \end{aligned}$ | MEAN |  | STD | FI <br> RANGE | MEAN |  | STD |




## APPENDIX E: (POGR)

|  | Bald Hill |  |  |  | Fort Hill |  |  |  |  |  |  |
| :--- | :--- | ---: | :--- | :--- | :--- | ---: | :--- | :--- | :---: | :---: | :---: |
|  | RANGE | MEAN |  | STD |  | MEAN |  | STD |  |  |  |
| FLJUL | $2008140-2008170$ | 2008151 | $\pm$ | 7.96 | $2008168-2008168$ | 2008168 | $\pm$ | 0.00 |  |  |  |
| AVEBASHT | $16-35.33$ | 25.33 | $\pm$ | 4.94 | $27-40$ | 33.75 | $\pm$ | 4.38 |  |  |  |
| TOTHT | $52-103$ | 79.53 | $\pm$ | 14.14 | $78-92$ | 86.875 | $\pm$ | 4.79 |  |  |  |
| DIAFL | $48-156$ | 99.93 | $\pm$ | 29.74 | $37-82$ | 63.875 | $\pm$ | 14.46 |  |  |  |
| DIABAS | $42-80$ | 58.20 | $\pm$ | 11.74 | $46-68$ | 60.875 | $\pm$ | 7.62 |  |  |  |
| AVEPETL | $7.56-11$ | 9.24 | $\pm$ | 0.97 | $10.9-12.56$ | 11.77083 | $\pm$ | 0.60 |  |  |  |
| AVEPETH | $7.33-10.56$ | 8.86 | $\pm$ | 1.00 | $10.9-12.3$ | 11.65 | $\pm$ | 0.50 |  |  |  |


| TOTRAAC | 4-23 | 11.73 | $\pm$ | 4.86 | 4-11 | 6.375 | $\pm$ | 2.26 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| AVEBASLL | 4.23-9.96 | 6.86 | $\pm$ | 1.49 | 7.23-11.63 | 9.375 | $\pm$ | 1.57 |
| AVETCTV | 0.34-0.86 | 0.52 | $\pm$ | 0.13 | 1.07-1.52 | 1.31875 | $\pm$ | 0.13 |
| AVETVM | 0.83-1.51 | 1.09 | $\pm$ | 0.21 | 1.97-2.58 | 2.2475 | $\pm$ | 0.23 |
| STEMPUB | 0-0 | 0.00 | $\pm$ | 0.00 | 0-1 | 0.125 | $\pm$ | 0.35 |
| RACEBRAN | 4-48 | 19.67 | $\pm$ | 10.76 | 9-27 | 17.875 | $\pm$ | 6.24 |
| FLBRAN | 7-30 | 13.67 | $\pm$ | 6.01 | 7-19 | 11.875 | $\pm$ | 3.87 |
|  | Finely |  |  |  | Hazel Dell |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| FLJUL | 2008128-2008178 | 2008155 | $\pm$ | 8.68 | 2008158-2008172 | 2008164 | $\pm$ | 6.02 |
| AVEBASHT | 11.66-35.33 | 23.84444 | $\pm$ | 5.85 | 22.66-35.33 | 28.83 | $\pm$ | 5.43 |
| TOTHT | 60-110 | 86.1 | $\pm$ | 12.13 | 87-107 | 93.25 | $\pm$ | 9.32 |
| DIAFL | 37-154 | 103.3667 | $\pm$ | 28.43 | 63-107 | 80.50 | $\pm$ | 19.33 |
| DIABAS | 27-84 | 61 | $\pm$ | 13.32 | 48-71 | 60.00 | $\pm$ | 10.10 |
| AVEPETL | 7.23-11.1 | 9.271956 | $\pm$ | 1.00 | 9.63-11.56 | 10.93 | $\pm$ | 0.88 |
| AVEPETH | 6.76-11.9 | 9.403022 | $\pm$ | 1.31 | 10.36-12.46 | 11.47 | $\pm$ | 0.86 |
| TOTRAAC | 4-23 | 10.9 | $\pm$ | 4.88 | 3-7 | 4.75 | $\pm$ | 1.71 |
| AVEBASLL | 4.3-9.8 | 6.738 | $\pm$ | 1.26 | 8.6-12.23 | 10.50 | $\pm$ | 1.49 |
| AVETCTV | 0.25-0.95 | 0.497444 | $\pm$ | 0.17 | 0.77-1.22 | 0.94 | $\pm$ | 0.20 |
| AVETVM | 0.73-1.71 | 1.091256 | $\pm$ | 0.26 | 1.22-2.25 | 1.59 | $\pm$ | 0.45 |
| STEMPUB | 0-1 | 0.066667 | $\pm$ | 0.25 | 0-0 | 0.00 | $\pm$ | 0.00 |
| RACEBRAN | 7-40 | 17.1 | $\pm$ | 7.43 | 16-43 | 27.25 | $\pm$ | 11.44 |
| FLBRAN | 7-29 | 13.66667 | $\pm$ | 5.43 | 9-22 | 14.00 | $\pm$ | 5.60 |
|  | Junction City |  |  |  | Kingston Prairie |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| FLJUL | 2008154-2008164 | 2008160 | $\pm$ | 3.41 | 2008147-2008158 | 2008152 | $\pm$ | 3.11 |
| AVEBASHT | 17.66-31 | 24.46 | $\pm$ | 3.93 | 7.33-37 | 22.33 | $\pm$ | 8.02 |
| TOTHT | 62-123 | 92.00 | $\pm$ | 16.93 | 53-103 | 80.89 | $\pm$ | 12.04 |
| DIAFL | 55-115 | 79.25 | $\pm$ | 21.14 | 76-154 | 110.17 | $\pm$ | 21.41 |
| DIABAS | 34-77 | 59.75 | $\pm$ | 13.25 | 46-83 | 61.50 | $\pm$ | 9.45 |
| AVEPETL | 7.86-12.06 | 10.44 | $\pm$ | 1.30 | 7.1-10.06 | 8.73 | $\pm$ | 0.78 |
| AVEPETH | 8.16-13.86 | 11.44 | $\pm$ | 1.84 | 6.96-9.83 | 8.32 | $\pm$ | 0.81 |
| TOTRAAC | 3-9 | 5.25 | $\pm$ | 2.05 | 4-27 | 12.11 | $\pm$ | 5.86 |
| AVEBASLL | 4.13-10.33 | 7.54 | $\pm$ | 1.76 | 3.93-10.13 | 7.34 | $\pm$ | 1.70 |
| AVETCTV | 0.48-1.39 | 0.96 | $\pm$ | 0.31 | 0.25-0.58 | 0.41 | $\pm$ | 0.10 |
| AVETVM | 0.82-2.4 | 1.72 | $\pm$ | 0.49 | 0.7-1.23 | 0.95 | $\pm$ | 0.17 |
| STEMPUB | 0-0 | 0.00 | $\pm$ | 0.00 | 0-1 | 0.89 | $\pm$ | 0.32 |
| RACEBRAN | 9-39 | 16.63 | $\pm$ | 10.08 | 9-31 | 20.56 | $\pm$ | 5.90 |
| FLBRAN | 7-26 | 15.25 | $\pm$ | 7.63 | 7-16 | 10.33 | $\pm$ | 2.95 |
|  | Lupine Meadows |  |  |  | Oxbow East |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| FLJUL | 2008149-2008170 | 2008160 | $\pm$ | 4.73 | 2008161-2008170 | 2008166 | $\pm$ | 2.73 |
| AVEBASHT | 15.66-37 | 28.48 | $\pm$ | 4.99 | 7.33-39.33 | 24.94 | $\pm$ | 7.75 |
| TOTHT | 84-112 | 99.23 | $\pm$ | 7.81 | 64-100 | 83.45 | $\pm$ | 10.38 |
| DIAFL | 36-132 | 87.20 | $\pm$ | 25.80 | 45-107 | 76.09 | $\pm$ | 17.81 |
| DIABAS | 45-80 | 59.87 | $\pm$ | 8.40 | 34-68 | 52.27 | $\pm$ | 11.76 |
| AVEPETL | 8.23-12.46 | 10.11 | $\pm$ | 0.97 | 9.83-11.96 | 10.77 | $\pm$ | 0.58 |
| AVEPETH | 7.89-12.96 | 10.52 | $\pm$ | 1.07 | 10.56-13 | 11.58 | $\pm$ | 0.72 |



## APPENDIX F: (POGR)

|  | RANGE | MEAN |  | STD |  |
| :--- | :--- | ---: | ---: | :--- | :---: |
| FLJUL | $2008128-2008178$ | 2008160 | $\pm$ | 8.09 |  |
| AVEBASHT | $7.33-40$ | 26.03 | $\pm$ | 6.49 |  |
| TOTHT | $51-166$ | 88.35 | $\pm$ | 15.71 |  |


| DIAFL | $23-156$ | 93.43 | $\pm$ | 27.26 |
| :--- | :--- | ---: | :--- | :--- |
| DIABAS | $27-84$ | 59.56 | $\pm$ | 10.72 |
| AVEPETL | $6.65-12.96$ | 9.79 | $\pm$ | 1.35 |
| AVEPETH | $6.2-13.86$ | 9.86 | $\pm$ | 1.66 |
| TOTRAAC | $1-33$ | 10.33 | $\pm$ | 6.14 |
| AVEBASLL | $3.93-12.23$ | 7.65 | $\pm$ | 1.81 |
| AVETCTV | $0.22-1.6$ | 0.62 | $\pm$ | 0.30 |
| AVETVM | $0.53-2.82$ | 1.27 | $\pm$ | 0.43 |
| STEMPUB | $0-1$ | 0.30 | $\pm$ | 0.46 |
| RACEBRAN | $4-66$ | 18.48 | $\pm$ | 9.38 |
| FLBRAN | $5-30$ | 12.50 | $\pm$ | 4.83 |

## APPENDIX G: (PRVU)

|  | RANGE | MEAN |  | STD |
| :--- | :--- | ---: | :--- | :--- |
| JulEmer | $64-101$ | 67.69 | $\pm$ | 4.45 |
| Elevation | $93-213$ | 141.91 | $\pm$ | 31.45 |
| JulianFlower | $2007152-2007176$ | 2007162.87 | $\pm$ | 5.55 |
| Height | $25-92$ | 57.74 | $\pm$ | 14.51 |
| Diameter | $42-118$ | 84.24 | $\pm$ | 13.83 |
| MeanFlower | $46.5-117$ | 65.67 | $\pm$ | 9.70 |
| Corollacolor | $0-2$ | 0.85 | $\pm$ | 0.84 |
| MeanCorollaL | $17.1-22.86$ | 20.32 | $\pm$ | 1.03 |
| Galeapub | $0-1$ | 0.35 | $\pm$ | 0.48 |
| JulianSeed | $2007187-2007199$ | 2007191.23 | $\pm$ | 3.39 |
| MeanLeafL | $34.63-81.26$ | 59.31 | $\pm$ | 9.29 |
| MeanLeafW | $14.36-38.46$ | 25.73 | $\pm$ | 4.36 |

## APPENDIX H: (PRVU)

|  | BF <br> RANGE | MEAN | STD |  | $\begin{array}{\|l\|} \hline \text { BH } \\ \text { RANGE } \end{array}$ | MEAN | STD |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| POPULATION |  |  |  |  |  |  |  |  |
| JulEmer | 20070'64-2007073 | 2007067.18 | $\pm$ | 2.31 | 2007064-2007075 | 2007067.06 | $\pm$ | 3.19 |
| JulianFlower | 2007155-2007171 | 2007163.50 | $\pm$ | 4.19 | 2007155-2007164 | 2007157.06 | $\pm$ | 3.10 |
| Height | 34-78 | 53.21 | $\pm$ | 12.25 | 28-85 | 57.83 | $\pm$ | 16.63 |
| Diameter | 52-109 | 89.89 | $\pm$ | 13.83 | 61-115 | 88.22 | $\pm$ | 13.94 |
| MeanFlower | 49.5-67.5 | 59.07 | $\pm$ | 5.87 | 52-80 | 66.36 | $\pm$ | 8.20 |
| Corollacolor | 0-2 | 0.71 | $\pm$ | 0.90 | 0-2 | 0.61 | $\pm$ | 0.92 |
| MeanCorollaL | 19.36-22.5 | 21.01 | $\pm$ | 0.70 | 17.1-21.13 | 19.69 | $\pm$ | 0.99 |
| Galeapub | 0-1 | 0.21 | $\pm$ | 0.42 | 0-1 | 0.28 | $\pm$ | 0.46 |
| JulianSeed | 2007187-2007197 | 2007191.43 | $\pm$ | 3.44 | 2007187-2007194 | 2007188.22 | $\pm$ | 2.39 |
| MeanLeafL | 34.63-73.76 | 55.34 | $\pm$ | 10.18 | 38.86-74.23 | 61.64 | $\pm$ | 9.11 |
| MeanLeafW | 14.8-34.16 | 24.40 | $\pm$ | 4.00 | 17.03-34.93 | 26.73 | $\pm$ | 4.42 |
|  | CM |  |  |  | CY |  |  |  |
|  | RANGE | MEAN |  | STD | RANGE | MEAN |  | STD |
| JulEmer | 2007064-2007073 | 2007067.56 | $\pm$ | 2.68 | 2007064-2007070 | 2007065.43 | $\pm$ | 1.55 |
| JulianFlower | 2007155-2007167 | 2007161.52 | $\pm$ | 4.10 | 2007155-2007171 | 2007160.93 | $\pm$ | 5.69 |



## APPENDIX I: (Ecoregion level 4 map)



