Dynamics of Cold Hardiness Accumulation and Loss in the Great Basin Native Species *Eriogonum umbellatum*: Spatial and Temporal Changes in Populations

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by

Matthew R. Fisk

Major Professor: Anthony S. Davis, Ph.D.

Committee Members: R. Kasten Dumroese, Ph.D.; Tara W. Hudiburg, Ph.D.

Department Administrator: Randy Brooks, Ph.D.

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Authorization to Submit Thesis

This thesis of Matthew R. Fisk, submitted for the degree of Master of Science with a Major in Natural Resources and titled "Dynamics of Cold Hardiness Accumulation and Loss in the Great Basin Native Species *Eriogonum umbellatum*: Spatial and Temporal Changes in Populations" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date _____
Anthony S. Davis, Ph.D.

Committee Members: _____ Date _____
R. Kasten Dumroese, Ph.D.

_____ Date _____
Tara W. Hudiburg, Ph.D.

Department Administrator: _____ Date _____
Randy Brooks, Ph.D.

Abstract

Ecological restoration in the Great Basin is critical to repair damaged ecosystems. Progressive land managers strive to integrate new science and restoration techniques into their practices in order to achieve the highest level of landscape repair possible. However, many native species currently in demand have received little study. Building a network of information to help users understand the ecology of these species and selection of appropriate populations for planting site conditions is key to successful restoration work. The goal of this project was to develop useful physiological information for the desired restoration species, *Eriogonum umbellatum* (Polygonaceae: sulphur-flower buckwheat). One consideration for estimating the potential adaptation and resilience of a species is through cold hardiness assessment. We evaluated seasonal adjustments in cold hardiness in sulphur-flower buckwheat by calculating the temperature at which 50% of a plant's cells are damaged due to decreased temperature, described as the LT50. Five geographically distinct sulphur-flower buckwheat populations, represented as M, J, B, C, and W, were investigated on a six-week cycle across a complete year. These five populations represented an elevation range of 855 to 1856 meters, five Omernik level III and IV ecoregions, and 5 provisional seed zones. Using adjusted index of injury (IOI) values, non-linear regressions were performed to fit 3-paramater logistic sigmoidal functions to calculate the LT50 values. Repeated measures analysis of variance was used to identify differences indicated by the collection date*population interaction. Statistical significance was found in four of the 90 possible interactions (October 2013 W-J, $p = 0.0025790$, March 2014 C-B, $p = 0.0488780$, March 2014 W-B, $p = 0.0466285$, and April 2014 M-J, $p = 0.0229043$). Even though statistical significance was not found between the date*population interaction for the majority of the sample period, biological significance was detected through differences between populations LT50 values across sample dates. Tukey's honest significance (HSD) test ($\alpha = 0.05$) was deployed to separate means and help describe differences between the sample dates within populations. When individual populations were evaluated across sample dates, significance was detected within all five. One of the five populations was evaluated at the natural wildland site and a transplant location, to evaluate local adaptation effects and plasticity. Statistical significance was detected between the two locations for the March data, while other collection dates were similar. Sulphur-flower buckwheat was found to have a LT50 range of $-10^{o}C$ - $-58^{o}C$ across the calendar year. In the species' most vulnerable state (April-August), the average LT50 was $-13.8^{o}C$. In the species' most cold hardy state (November – February) the average LT50 value was $-56.4^{o}C$. Understanding sulphur-flower buckwheat population variation of cold hardiness vulnerabilities and strengths is a useful screening tool to improve the selection of populations adapted to conditions at the planting site.

Acknowledgements

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Introduction

Native plant producers and restoration specialists are integrating more science into their projects in an effort to overcome variable success in plant re-establishment on disturbed shrublands. Providing a basic understanding of plant growth and development for a broad range of important native plant species is crucial for the success of restoration projects. The key concept in achieving optimal, long term sustaining restoration success is through the methods of the target plant concept. This concept measures characteristics based on morphological and physiological traits critical for outplanting performance (survival and growth) rather than a set of basic standards set by a nursery or plant producer (Landis and Dumroese 2007). This concept includes eight basic considerations that have varying levels of application. These are: 1) reforestation or restoration objectives, 2) site evaluation (soil, climate, plants, etc.), 3) limiting factors, 4) mitigating measures, 5) genetics (species and source), 6) plant materials (seeds, cuttings, plants), 7) outplanting tools and techniques, and 8) outplanting windows (Landis 2011). For the purpose of this research, I will be focusing on creating tools to help guide consideration number five, which is making a selection on the sourcing of plant materials to be used in a restoration project using an evaluation of cold hardiness of the Great Basin native species sulphur-flower buckwheat (*Eriogonum* umbellatum).

Restoration project materials include seeds, cuttings, and plants. Regardless of the type of revegetation and soil stabilization material selected, the importance of matching the species, source, and type of project materials to planting site conditions is of major importance. All considerations of a plant material source must be evaluated and correctly paired with planting goals and project geographic location and conditions to ensure the materials selected are the most appropriate for the restoration site.

Habitat degradation within the Great Basin region of the Intermountain West of North America is a contemporary example of how disturbance is driving rapid changes in ecosystem structure and function across enormous spatial scales (Coates et al. 2015). Due to increasing frequency, size, and intensity of disturbance events, these changes often come with negative consequences. Many of these large disturbances have experienced habitat degradation that exceeds natural repair, and require human intervention to help restore healthy ecosystems. Available, locally adapted native plant seed is necessary to achieve successfully restored ecosystems. As seed requests are becoming more specific and needs more ecologically encompassing, seed producers are working through the process of filling gaps in the shifting and expanding seed market. Even with increased seed sources and quantities on the market each year, adequate supplies of material adapted to specific planting sites are often not available.

Federal agencies, non-governmental organizations (NGOs), and private companies all play a part in driving the seed market. Seed supplies are not meeting the demands. Requested species are often not available and sources purchased are frequently not the first or second choice of the buyer. The low volume of available native species on the market is due to unpredictable seed demands, high prices, poor seed production years, and a lack of appropriate cultural practices. Understanding the behavior of species population variation in adaptation is critical and little information is available for some species now entering the commercial seed market. Additional research and new restoration strategies are essential to increase the successful use of native species.

Non-native species, such as *Agropyron cristatum* (crested wheatgrass), have been introduced and selectively bred in the United States since the early part of the 20th century (Zlatnik 1999). With their ecological competitive edge and high forage value (Ogle 2001), these non-natives are commonly used in post-disturbance rehabilitation projects. However, their use comes at a cost to the native ecosystem's potential for natural recovery (Davies et al. 2013, Gunnell et al. 2010). It is critical to take into account species and source selection (origin, genetic diversity, competitive ability, interactions with other planted species, etc.) when selecting seeding or planting mixes when the goal is to maximize restoration success of native systems.

Historically, species have been planted without knowledge or concern regarding adaptation to local conditions. Introducing maladapted materials to planting sites can reduce the potential for long-term sustainability. To achieve restored ecosystems that are best adapted to current and future environmental change, locally adapted and regionally appropriate seed sources must be utilized (Johnson et al. 2010). Exotic materials can preclude success of ecological restoration, which is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (Society for Ecological Restoration International Science & Policy Working Group 2004). As we are becoming more aware of the consequences resulting from the use of intentionally introduced species such as crested wheatgrass (*Agropyron cristatum*) (Davies et al. 2013, Gunnell et al. 2010) and forage kochia (*Bassia prostrata*) (Gray and Muir 2013), scientists are conducting research to improve the establishment of native species. This will improve efforts to build a better understanding of functioning natural ecosystem and successful, self-regenerating, long-lasting communities. New tools developed through native plant research will provide guidelines for native plant materials development and incorporate the idea of the target plant concept for future restoration efforts.

As part of a national plan to conduct research and increase native plant material availability within the Intermountain Region, the Great Basin Native Plant Selection and Increase Project (GBNPSIP), recently changed to the Great Basin Native Plant Project (GBNPP) in

2014, was initiated meeting important objectives of the Great Basin Restoration Initiative (GBRI) in 2000 (Shaw 2003). The Interagency Native Plant Materials Development Program outlined in the 2002 United States Department of Agriculture (USDA) and United States Department of Interior (USDI) Report to Congress encouraged use of native plant materials for rangeland rehabilitation and restoration (Kilkenny et al. 2015). As part of these efforts, scientists and seed collecting teams were deployed throughout Oregon, Nevada, Utah, and Idaho. Seed collections from these efforts have been and are currently used for research and to an increasing extent as stock seed, providing the native seed industry with the seed needed to get new species into production. These collections have also contributed to research that provides strategies for future restoration efforts, seed production protocols, and development of plant materials on a seed zone basis. The focus on native plant material needs and required integration into federal and non-federal rehabilitation efforts has gained momentum with the recent release of the National Seed Strategy for Rehabilitation and Restoration whose goal is to provide a more coordinated approach for stabilization, rehabilitation, and restoration treatments using native plants (USDI BLM and PCA 2015).

One of the many native species selected for evaluation and increase in the Great Basin is sulphur-flower buckwheat (*Eriogonum umbellatum*). The species goes by many additional common names such as sulphur-flower, buckwheat bush, sulfur buckwheat, sulfur-flower buckwheat, sulphur wild buckwheat, and slender buckwheat (USDA NRCS 2015). Sulphur-flower buckwheat, is a native, low-growing, woody perennial in the buckwheat family (Polygonaceae) (Young-Mathews 2012). The species is highly variable and has been divided into 5, 25, 30, and 41 varieties (Dyer et al. 2011). Sulphur-flower buckwheat is native to the Great Basin and western North America at elevations ranging from 200 to 3700 meters (Dyer et al. 2011, USDA NRCS 2015). It is found from California to western Canada and east into Colorado and New Mexico (Dyer et al. 2011). Flower displays can color entire slopes starting in June at lower elevations and continuing into September or October at higher elevations (Dyer et al. 2011).

Sulphur-flower buckwheat is important for wildlife, livestock, pollinators, restoration, landscaping, Native American use (Young-Mathews 2012), and potential green roofs (Schneider et al. 2014). Within its natural range, the species is valuable for re-establishing native plant communities where the existing local seed bank has been lost (Parris et al. 2010). Recent studies have shown that the relative growth rate of sulphur-flower buckwheat was not reduced when it was grown with native grass neighbor Sandberg bluegrass (*Poa secunda*) and bottlebrush squirreltail (*Elymus elymoides*) (Parkinson et al. 2013).

Most of the Intermountain Region's (Figure 1) 3,487 described species are vastly understudied, specifically the native species. There are gaps in the literature that make it difficult to answer questions regarding ongoing ecological restoration efforts. This provides an avenue for new research, but it remains difficult for land managers when attempting to make time-sensitive critical decisions regarding efforts to restore damaged landscapes.

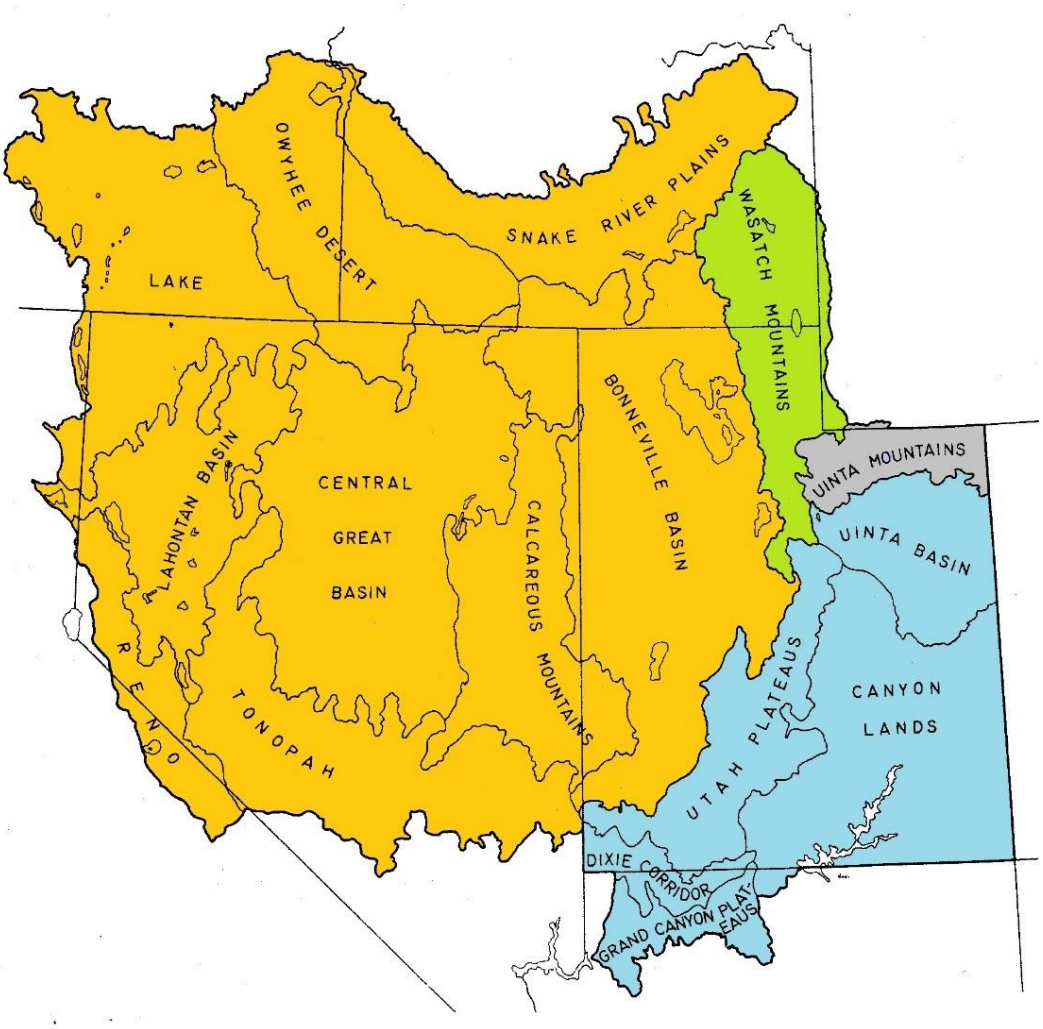

Figure 1. The Intermountain Region encompasses parts of Oregon, Idaho, Wyoming, Nevada, California, Utah, and Arizona. Colors denote four floristic divisions within the region: Great Basin (orange), Wasatch Mountains (green), Colorado Plateau (blue), and Uinta Mountains (grey) (Cronquist et al. 1972).

There are many important factors to consider when making the decision to select adapted materials for planting. One of the variables that determines the distribution of plant species and populations is cold hardiness. Cold hardiness refers to the capacity of a plant tissue to withstand exposure to freezing temperatures and can be induced by factors such

as photoperiod reduction, decreased soil moisture, or colder temperatures (Herriman and Davis 2012). Diurnal and seasonal temperature fluctuations in the Great Basin can be extreme; thus exposure of plants to very low temperatures may reduce establishment success in restoration projects. Elevation ranges within the Great Basin region vary over 3,700 m, exposing species to many different levels of extreme temperatures. Cold hardiness is variable across, and within, both species and geographic regions.

The main way hardiness is measured and rated is by resistance to cold injury. Cold hardiness provides a method for screening species and populations for adaptation and can be used as a factor in defining seed zones and guide selection of plant materials for restoration. Cold hardiness is a valuable trait for evaluating seedlings and is currently the second most common seedling test ordered by nurseries and reforestation experts (A.S. Davis, personal communication, September 10, 2014). Using cold hardiness as a potential indicator of a plant's overall resistance to low temperature stress is a powerful tool. When the maximum cold hardiness state of a plant is reached, it is also the most resistant to other environmental stressors (Haase 2011). As more cold hardiness work is conducted for additional species across the Great Basin, the compilation of such information will help provide the foundation needed for a streamlined strategy for plant producers and restoration specialists to target species and particular population sources for traits of interest when developing and testing seed zones. Cold hardiness is one of many known important traits that provide a greater understanding of the influence of changing climatic conditions on plant distributions and potentials for assisted migration.

Four main hypotheses were tested with this work:

1. *Eriogonum umbellatum* cold hardiness varies across geographically unique populations.
2. Localized adaptation of *Eriogonum umbellatum* will take place as populations are moved outside of their local zones to new locations.
3. *Eriogonum umbellatum* cold hardiness levels will vary across seed zones, ecoregions, and elevation.
4. *Eriogonum umbellatum* cold hardiness levels will change across the calendar year.

Understanding Cold Hardiness Evaluation

There are numerous methods by which one can assess plant cold hardiness. This is most commonly accomplished by exposing plant tissues to a range of temperatures and measuring their response by various means. Techniques involving freezing plant tissues include the freeze induced electrolyte leakage method (FIEL), whole plant freeze tests (WPFT), chlorophyll florescence (CF), or differential thermal analysis (DTA). Variability

exists phenologically throughout the year, across plant parts, and across testing methods. In order to effectively use cold hardiness data that can contribute to the development of effective seed transfer guidelines and provide information to restoration practitioners, a better understanding of the relative differences across tests and plant parts, as well as seasonal variability is needed. Many of these techniques are time sensitive and laborious, causing logistical difficulties in acquiring desired data. Burr et al. (1990) conducted a study comparing WPFT, FIEL, and DTA to measure cold hardiness. The results showed WPFT to be least precise, DTA intermediate, and FIEL the most precise. Consequently, we selected the FIEL method for our investigations.

The freeze induced electrolyte leakage test measures stress-induced ion leakage through damaged tissue cell membranes. This technique is a precise, sensitive, and objective predictor of changes or differences in tissue cold hardiness (Burr et al. 1990). Results are available in less than 50 hours (Burr et al. 1990). To determine actual cold hardiness of a plant, results can be calibrated to the response of the same tissue in the WPFT. FIEL measurements are expected to express the highest levels of cell damage when plants are actively growing, and lowest levels of damage when they are dormant, due to the ability of dormant plants to withstand intracellular freezing (Haase 2011).

Cold tolerance has been studied in depth in many crop plants such as barley (*Hordeum vulgare*) (Guy 1990, Rizza et al. 1994, Bravo et al. 1998, Mahfoozi et al. 2000), wheat (*Triticum aestivum*) (Pomeroy and Fowler 1973, Guy 1990, Limin et al. 1997, Mahfoozi et al. 2000, Kosová et al. 2012), grape vines (*Vitis vinifera*) (Stergios and Howell 1977, Hamman et al. 1996, Mills et al. 2006), and citrus and exotic fruits (Palonen and Buszard 1997, Li 2012) because they are often planted at their extreme limits of survival (Larcher 2003) and lack of tolerance may reduce or void economic benefits. There are some species that have been studied outside the agricultural world such as: rockcress (*Arabidopsis*) (Gilmour et al. 1988, Wanner and Junttila 1999), purple foxglove (*Digitalis purpurea*) (Bruelheide and Heinemeyer 2002), silver sagebrush (*Artemisia cana*) (Hou and Romo 1998), ponderosa pine (*Pinus ponderosa* var. *scopulorum*) (Burr et al. 1990), Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) (Burr et al. 1990, Balk et al. 2007, Rose and Haase 2002), Engelmann spruce (*Picea engelmannii*) (Burr et al. 1990), big sagebrush (*Artemesia tridentata*) (Loik and Redar 2003, Herriman and Davis 2012), winterfat (*Krascheninnikovia lanata*) (Hou and Romo 1997), Scots pine (*Pinus sylvestris*) (Repo 1992, Savolainen et al. 2004), Norway spruce (*Picea abies*) (Repo 1992), European beech (*Fagus sylvatica*) (Heide 1993), sugar sumac (*Rhus ovata*) (Boorse et al. 1998, Pratt et al. 2005), *laurel sumac* (*Malosma laurina*) (Boorse et a. 1998, Pratt et al. 2005), English walnut (*Juglans regia*) (Flint et al. 1967), *redosier dogwood* (*Cornus sericea* ssp. *sericiea*) (van Huystee et al. 1967), and loblolly pine (*Pinus palustris*) (Teskey et al. 1987).

A few Great Basin plants have been the focus of previous cold hardiness studies. Hou and Romo (1997) studied growth and freezing tolerance of *Krascheninnikovia lanata* seedlings by growing plants under different controlled temperatures for 7, 14, 21, and 28 days. Younger seedlings were more cold tolerant than older ones grown under the same conditions. Seedlings grown under lower temperatures were more cold tolerant than those grown under higher temperatures. Results also suggested that freezing temperatures may limit seedling establishment. Hou and Romo (1998) also studied the cold tolerance of *Artemisia cana* seedlings. Seedlings were grown from 1 week to 1 full growing season, exposed to freezing temperatures under controlled conditions, and lethal temperatures for 50% and 95% mortality (LT50 and LT95) were determined. Mortality was the method of cold hardiness evaluation. This study showed strong relationships between cold acclimation and de-acclimation and survivorship of seedlings.

Loik and Redar (2003) studied freezing tolerance and cold acclimation of *Artemisia tridentata* seedlings grown in a common garden by selecting plants from the garden, placing them in pots in growth chambers for 30 days to ensure transplant success and acclimation to the chamber conditions. Temperature treatments started at room air temperature and decreased / increased at a rate of 3^{o}/hr to the desired temperature at which it was held for 1 hr. Various temperature treatments were applied to the plants, followed by FIEL assessments to determine membrane damage. Also tested was the hypothesis that *Artemisia tridentata* seedlings from three different elevations within an 800 m gradient would exhibit ecotypic differentiation in freezing tolerance and ability to undergo cold acclimation. FIEL results showed no difference among seed sources. However, variation within populations for cold acclimation has been demonstrated in other species across broader elevations and latitudinal gradients greater than the 5 km distance and 800 m elevation range seen in their study. It is noteworthy that shifting the *Artemisia tridentata* populations between 25^{o}C/15^{o}C day temperatures to 15^{o}C/5^{o}C night temperatures initiated a 1.5^{o}C acclimation by plants from all three sources. There were however no differences across elevations.

Bruelheide and Heinemeyer (2002) studied frost tolerance in *Digitalis purpurea* in Germany. Frost sensitivity was investigated using the FIEL technique with increased electrical conductivity being the measure of tissue damage. They separated leaf, root, and bud tissue for examination, and obtained clearly different results. Leaves were the least cold hardy, suffering significant damage at -12^{o}C, followed by buds at -15^{o}C, and roots at -18^{o}C. Comparisons were made between FIEL results and field temperatures to determine the potential role of freezing in survivorship and distribution of the species.

No literature was found relating to the dynamics of cold hardiness accumulation and loss for Great Basin native forbs. Understanding this type of physiological information for

restoration species could contribute to improved plant materials selection and restoration efforts.

Cold acclimation research has entered a “golden age,” with tremendous advances being made in its understanding over the past two decades. There is reason to believe that the next 20 years will bring even more advances (Thomashow 2001). Rapid changes in climate across the globe combine elements effecting temperature fluxes, organismal distribution patterns, types of weather, and extreme interactions within and among these elements. With these current and future changes in climate, colder and warmer temperatures are becoming more unpredictable and extreme across landscapes. Thus necessitating a better understanding of the relationships between plant traits such as cold tolerance and climatic conditions.

Materials and Methods

In June 2006, the United States Department of Agriculture Forest Service established a *Eriogonum umbellatum* (sulphur-flower buckwheat) common garden in Boise, ID (elevation 845 m). The garden was installed in a random block design consisting of 5 blocks, each block containing 20 plants from each of the 17 geographically distinct populations of sulphur-flower buckwheat collected across the Great Basin.

For this study, I sampled five of the 17 common garden populations. These five populations were originally collected across an elevation range of 855 to 1856 meters, five distinct Omernik level III and IV ecoregions, five provisional seed zones (Figure 2), and four states (Table 1). For one population (ERUM01), I also sampled plants from its source location. Thus, I evaluated 6 sources.

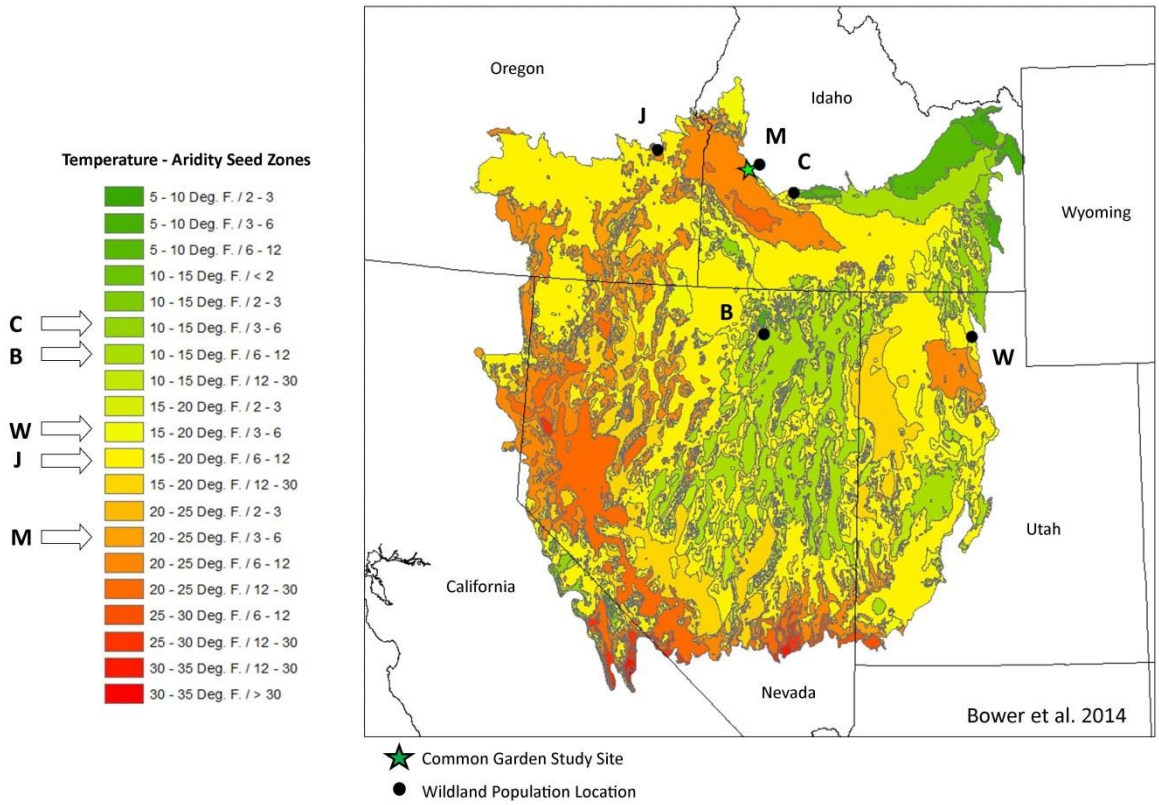

Figure 2. Five wildland seed collection sites (represented as M, J, B, C, W) and common garden location of *Eriogonum umbellatum* populations investigated for cold hardiness expressed on the Great Basin provisional seed zone map (Bower et al. 2014).

Accession	County	State	Latitude	Longitude	Elevation (meters)	Omernik Level III Ecoregion	Omernik Level IV Ecoregion	Provisional Seed Zone
ERUM 01 (M)	Boise	Idaho	43.665	-115.979	964	16 - Idaho Batholith	16k - Southern Forested Mountains	20-25 °F / 3-6
ERUM 13 (J)	Malheur	Oregon	43.799	-117.89	855	80 - Northern Basin & Range	80f - Owyhee Uplands and Canyons	15-20 °F / 6-12
ERUM 25 (B)	Elko	Nevada	41.392	-115.794	1856	13 - Central Basin & Range	13m - Upper Humboldt Plains	10-15 °F / 6-12
ERUM 36 (C)	Elmore	Idaho	43.296	-115.327	1607	12 - Snake River Plain	12f - Semiarid Foothills	10-15 °F / 3-6
ERUM 37 (W)*	Box Elder	Utah	41.3881	-112.025	1385	19 - Wasatch & Uinta Mountains	19f - Semiarid Foothills	15-20 °F / 3-6
Common Garden	Ada	Idaho	43.598	-116.162	847	12 - Snake River Plain	12a - Treasure Valley	20-25 °F / 6-12

Table 1. Collection sites for five *Eriogonum umbellatum* populations grown in Boise, ID common garden and evaluated for cold hardiness. *material from the site of origin also evaluated.

Plants were collected every 6 weeks for an entire 12 month cycle: 25 October 2013, 4 December 2013, 21 January 2014, 7 March 2014, 17 April 2014, 30 May 2014, 12 July 2014, 25 August 2014, and 16 October 2014. For the five common garden sources, one plant of each source from each block was sampled. When possible, tissue samples for each source were harvested from the same plants. When insufficient tissue was available for processing, the next closest plant within the same block was selected. For the wildland location, five plants separated by a minimum of 15 m were selected. Minimum and maximum daily actual and long-term average temperatures for the common garden location over the entire sampling period are shown in Figure 3 (National Climatic Data Center 2015). Temperature differentials are expressed in Figure 4 (National Climatic Data Center 2015).

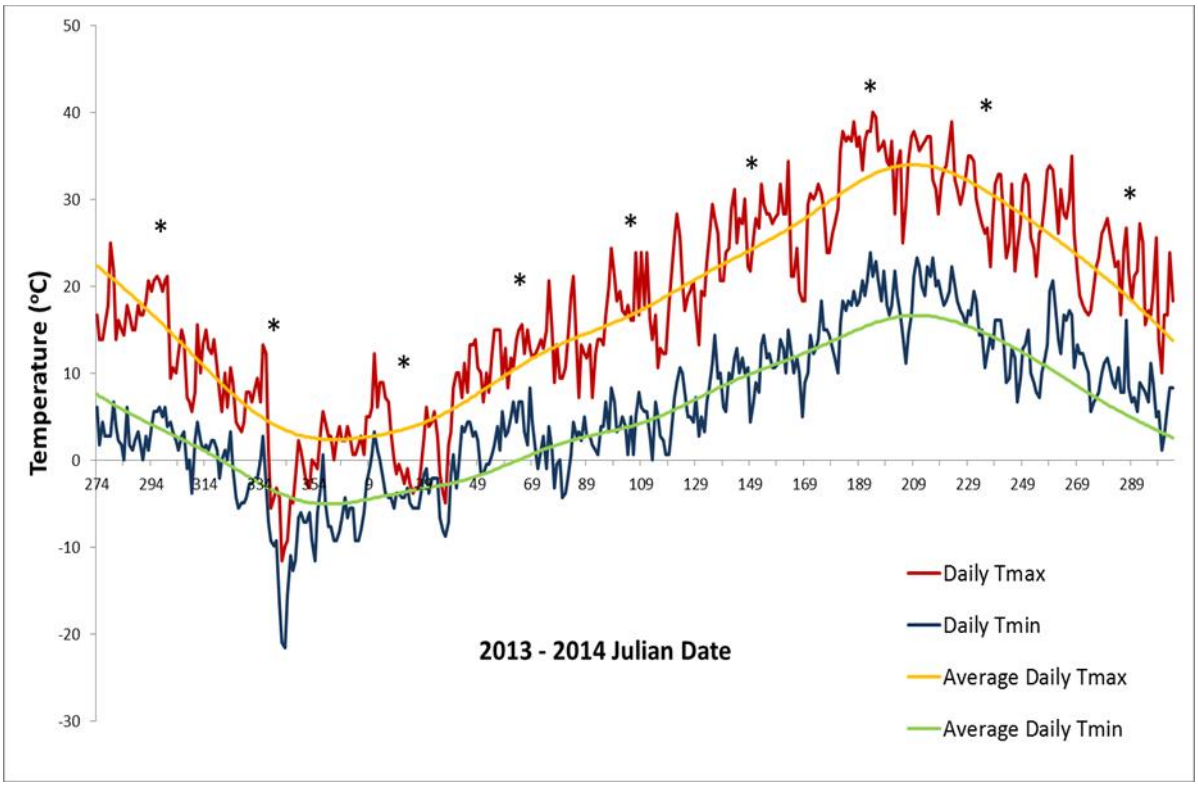

Figure 3. Boise, ID sulphur-flower buckwheat common garden daily temperature maximum (Daily Tmax) and minimum (Daily Tmin) expressed over 30 year daily averages (1981-2010). (National Climatic Data Center 2015). * Indicates field sample collection dates.

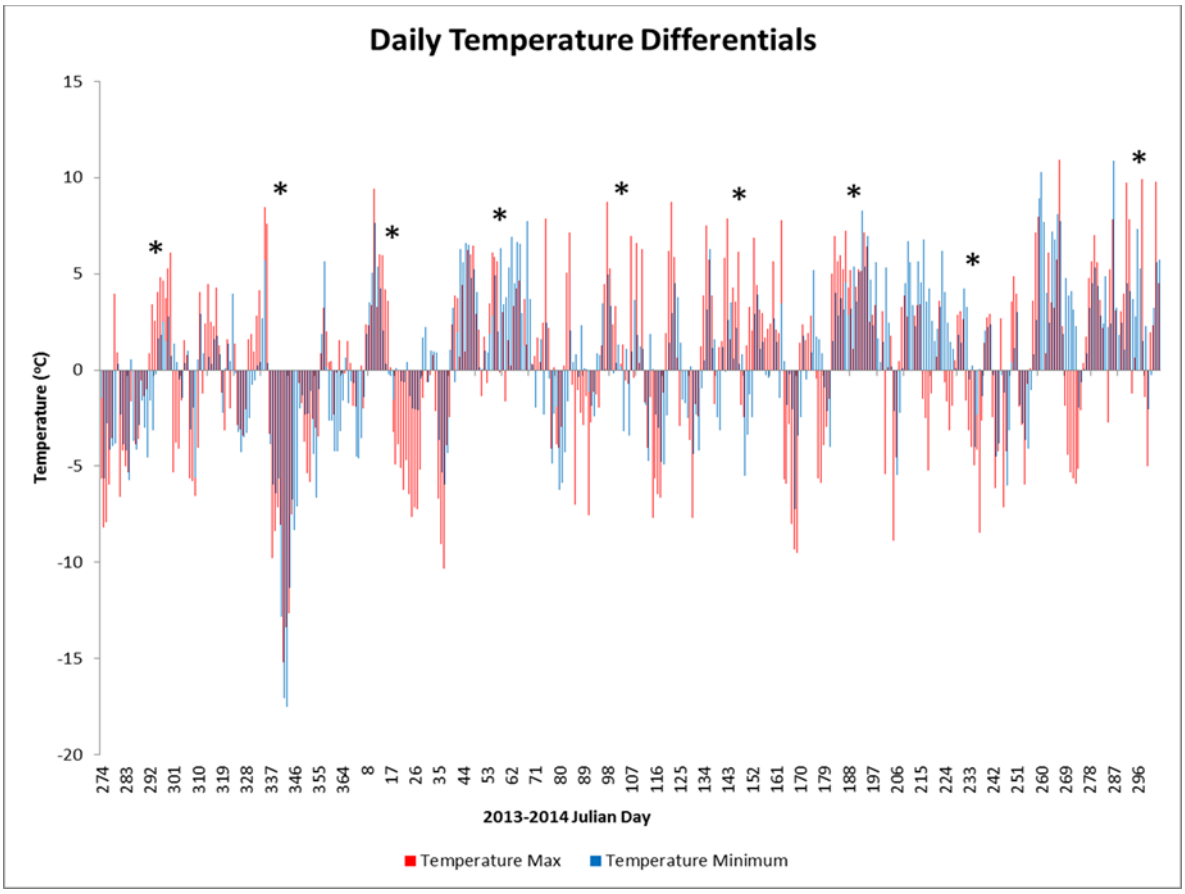

Figure 4. Boise, ID sulphur-flower buckwheat common garden site daily temperature maximum and minimum differentials based upon 30 year averages (1981-2010). (National Climatic Data Center 2015). * Indicates field sample collection dates.

Representative healthy leaves were sampled (Figure 5). Leaves were harvested with more than 5 cm of stem material attached to help preserve the integrity of the tissues for transport to University of Idaho Pitkin Nursery Laboratory. Bulk samples were clipped from plants, quickly wrapped in distilled water-soaked paper towels, placed in a 3/4 closed Ziploc® bag, and transported in a cooler. Once at the laboratory, samples were refrigerated at 2^{o}C until the next stage of processing. All samples were processed within 48 hours of collection.

Figure 5. Left photo shows variation in size of healthy, complete representative *Eriogonum umbellatum* leaf samples from five common garden populations and one wildland site used for cold hardiness investigation. Three photos on the right show sampling selection of a leaf and sampled discs used in freezing tolerance evaluation.

Collections were removed from the refrigerator in blocks (five plants at a time from the common garden, one plant from the wildland site), allowed to reach ambient temperature, and double rinsed in an ambient distilled water bath to remove any external ions and debris, blotted dry with paper towels, and allowed to air dry. Once completely dry, a standard hole punch (6.5 mm diameter) was used to create leaf tissue discs; leaf edges and main veins were avoided.

Each round of evaluation produced 30 replicates from 210 samples (7 temperatures x 5 plants x 6 sources), each sample consisting of five leaf discs. Leaf discs were placed in 20 ml wide-mouth scintillation copolymer plastic vials containing 2.5 ml of distilled water and a grain of sand to promote ice nucleation and decrease surface tension. Air-tight screw caps were placed on all vials and the vials were placed in the freezer at ambient temperature.

The seven investigated temperatures started with a non-freeze induced damage control treatment at 2^{o}C. The other 6 temperatures selected were: -7 ^{o}C, -14 ^{o}C, -21 ^{o}C, -28 ^{o}C, -35 ^{o}C, and -40 ^{o}C, using a ScienTemp Lo-Cold programmable freezer (Scientemp Corp., Adran, MI). The experimental design was set to examine tissue damage with a 5^{o}C /hour rate of decrease (ramp) and 1-hour soak for all samples. Soak time refers to exposing samples to a specific constant temperature. The last sample was removed from the freezer 14 hours and 24 minutes after freeze initiation. As each sample was removed from their designated cold environment, they were allowed a gradual thaw in the 2^{o}C refrigerator. They were then removed from the refrigerator and allowed to warm to room temperature at which time 7.5 ml of ambient temperature distilled water was added to the vials, bringing the sample liquid volume up to 10 ml. Samples were then placed on a shaker at a rate of 100 RPM for 1 hour. Solutions were measured for initial electrolyte (ion) leakage due to cell damage via electrical conductivity (EC) with a SevenEasy conductivity meter (Mettler Toledo, Columbus, OH). Samples were then covered with tinfoil and autoclaved (Maket Forge Sterilmatic, Vernon Hills, IL) at 121^{o}C for 20 minutes to achieve 100% cell damage. Once cooled, vials were shaken at 100 RPMs for 1 hour before final EC readings were taken.

Data Analysis

For each plant at each test temperature, initial EC values were divided by post autoclave EC values to calculate cell damage expressed through electrolyte leakage (%EL). Slight damage to plant cell tissue was unavoidable due to sample preparation. Using the calculated %EL readings of the control treatments (2^{o}C) for each plant, the Index of Injury (IOI) was calculated to account for non-cold induced tissue damage using equation 1:

Equation 1: ((%EL – AVE Control %EL) / (100 - AVE Control %EL)) * 100

Using the calculated IOIs, non-linear regressions were performed using R x64 3.1.2 (R Core Team 2015) statistical software, fitting 3-parameter logistic sigmoidal functions to each plant (Figure 6) to calculate the index of measure for cold hardiness expressed as LT50. The LT50 value is the temperature at which 50% of total electrolyte leakage occurs (Jacobs et al. 2008).

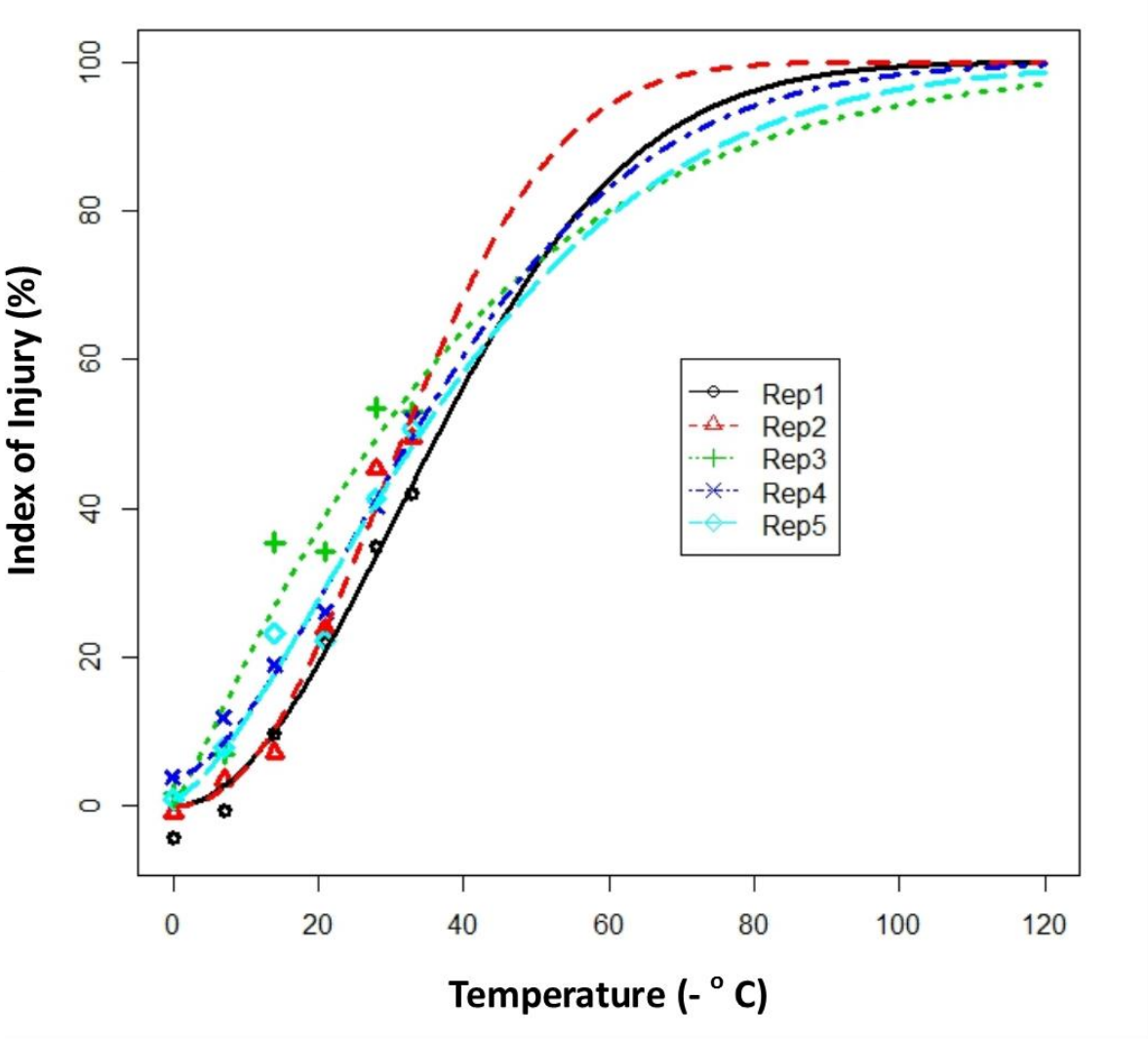

Figure 6. October 2013 population M (5 replications) fit plots generated in R to calculate LT50 values by fitting a logistic sigmoidal function based on index of injury values at each of the 6 test temperatures.

For the 5 populations in the common garden, repeated measures analysis of variance (SAS Institute Inc., version 9.4, Cary, NC) was used to examine the collection date x population interactions. Further investigation using Tukey's honest significance (HSD) test ($\alpha = 0.05$) to separate means helped to describe differences among the populations within sample dates. A two-way analysis of variance (JMP®, Version 8.0.1. SAS Institute Inc., Cary, NC, 1989-2007) was used to compare cold hardiness for population M growing in the common garden to the wildland site and also to detect differences between the duplicate October sample dates (2013 and 2014) in the common garden populations.

Results

Repeated measure analysis of variance showed a significant date*population interaction ($p = 0.0474$, Figure 7). Of 90 possible sets of interactions between population and sample date, four interactions were significant: October 2013 (W and J; $p = 0.0026$), March 2014 (C and B; $p = 0.0489$ & W and B; $p = 0.0466$), and April 2014 (M and J; $p = 0.0229$).

Investigation using Tukey's honest significance test (HSD, $\alpha = 0.05$) to separate means helped to describe differences between the populations within sample dates. The highest levels of variability between dates was seen in population M (Figure 8) and B (Figure 9), with four levels of statistical significance across collection dates. Population W (Figure 10) showed the least variability with only two levels of statistical significance. Populations J (Figure 11) and C (Figure 12) were intermediate with each having three levels of statistical significance.

A two-way ANOVA was used to examine differences between the sample dates of October 2013 and October 2014, one year apart. A statistical difference was detected for dates ($p < 0.0001$) and populations ($p = 0.0430$), however the interaction was insignificant.

Figure 13 expresses population M cold hardiness at its natural wildland site as it compares to the transplant location. Cold hardiness differed between the two sites only in March 2014 ($p < 0.05$).

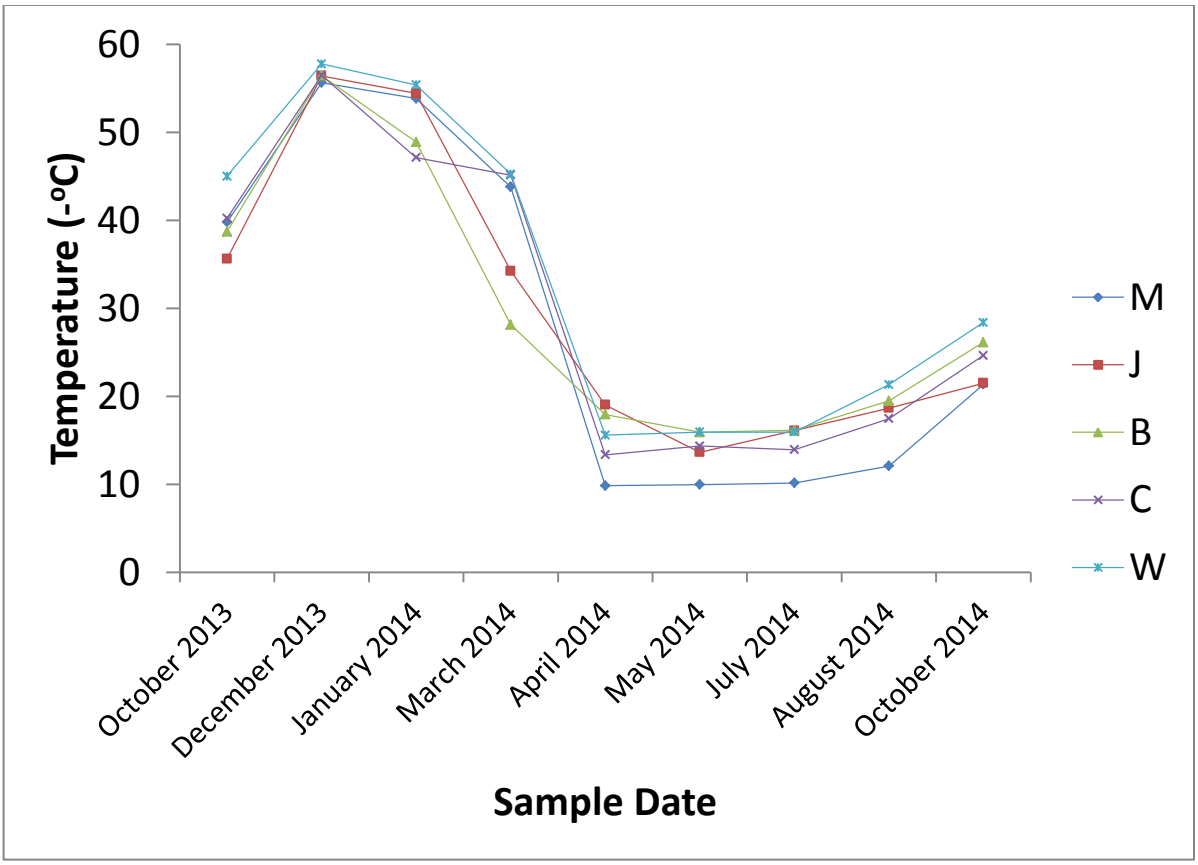

Figure 7. LT50 value for five populations of *Eriogonum umbellatum* for 9 collections dates.

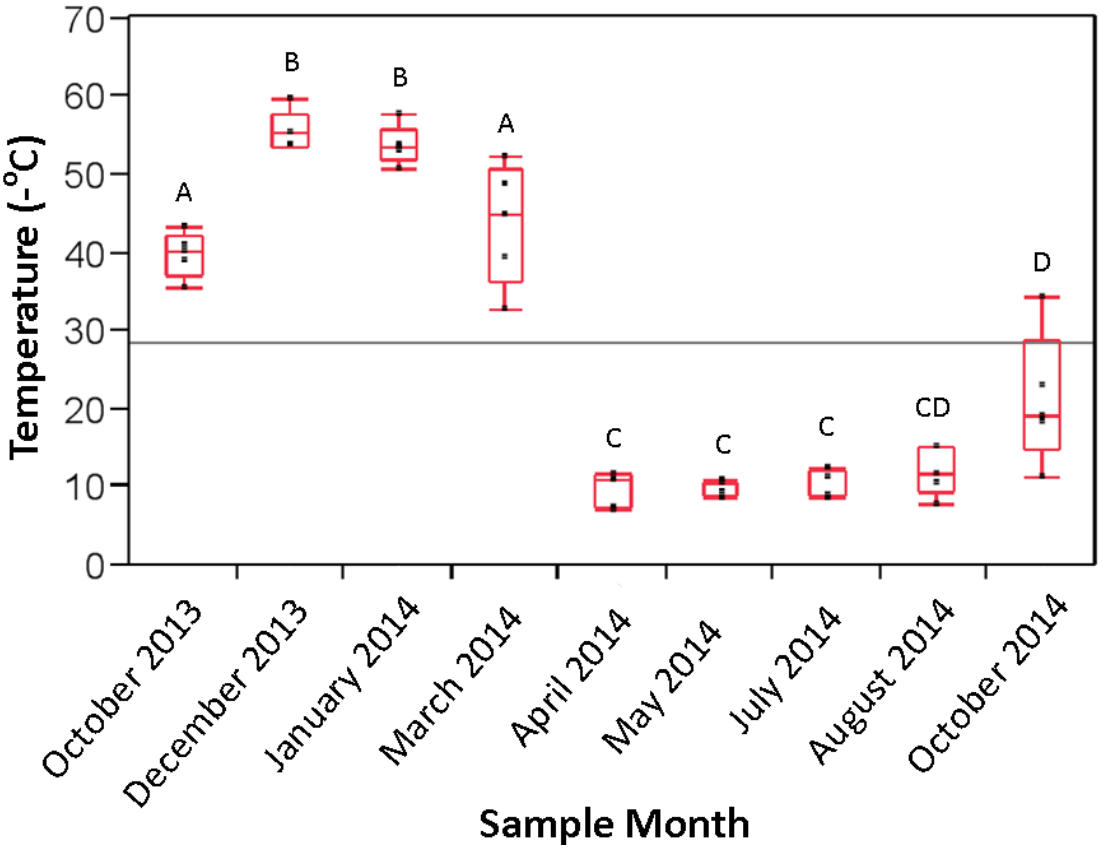

Figure 8. October 2013-October 2014 population M LT50 Tukey's honest significance (HSD) mean separation box and whisker plot for *Eriogonum umbellatum*. Mean values not sharing a letter differ significantly at P <0.05. Whiskers represent standard error of the mean (SEM). Line indicates the annual average temperature -28°C.

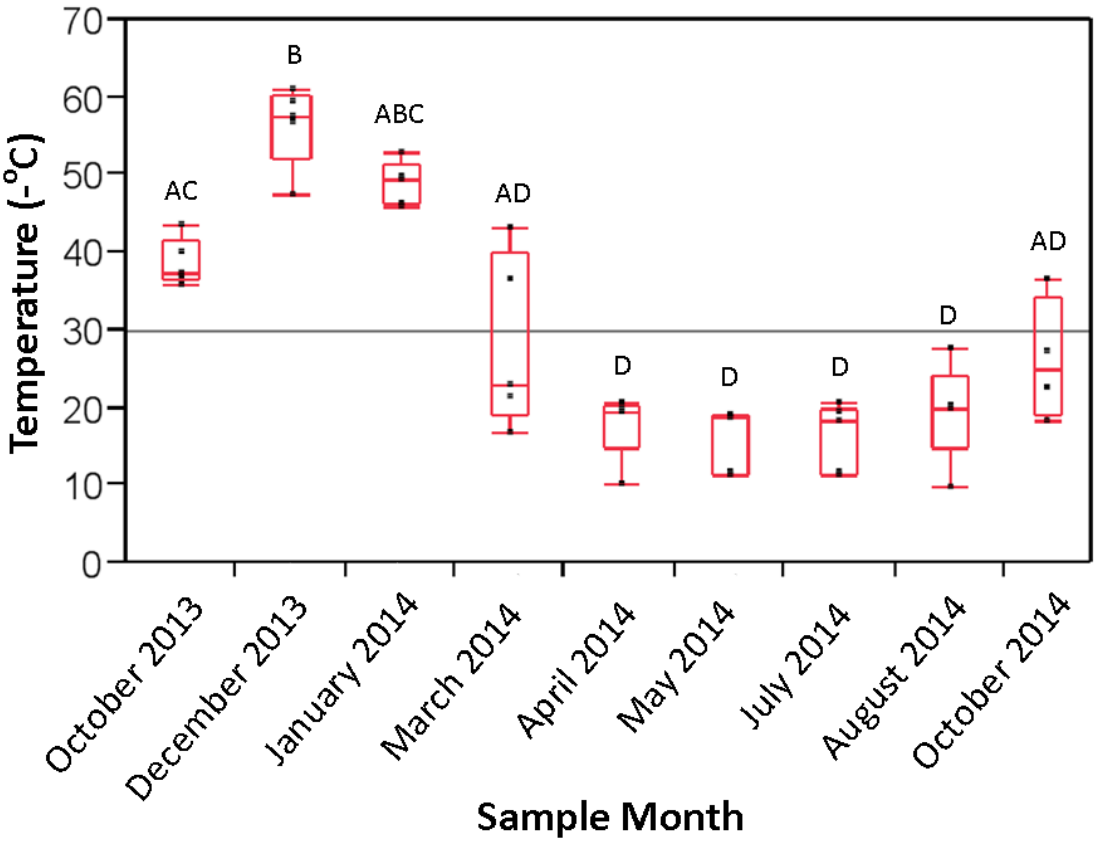

Figure 9. October 2013-October 2014 population B LT50 Tukey's honest significance (HSD) mean separation box and whisker plot for *Eriogonum umbellatum*. Mean values not sharing a letter differ significantly at P <0.05. Whiskers represent standard error of the mean (SEM). Line indicates the annual average temperature -30°C.

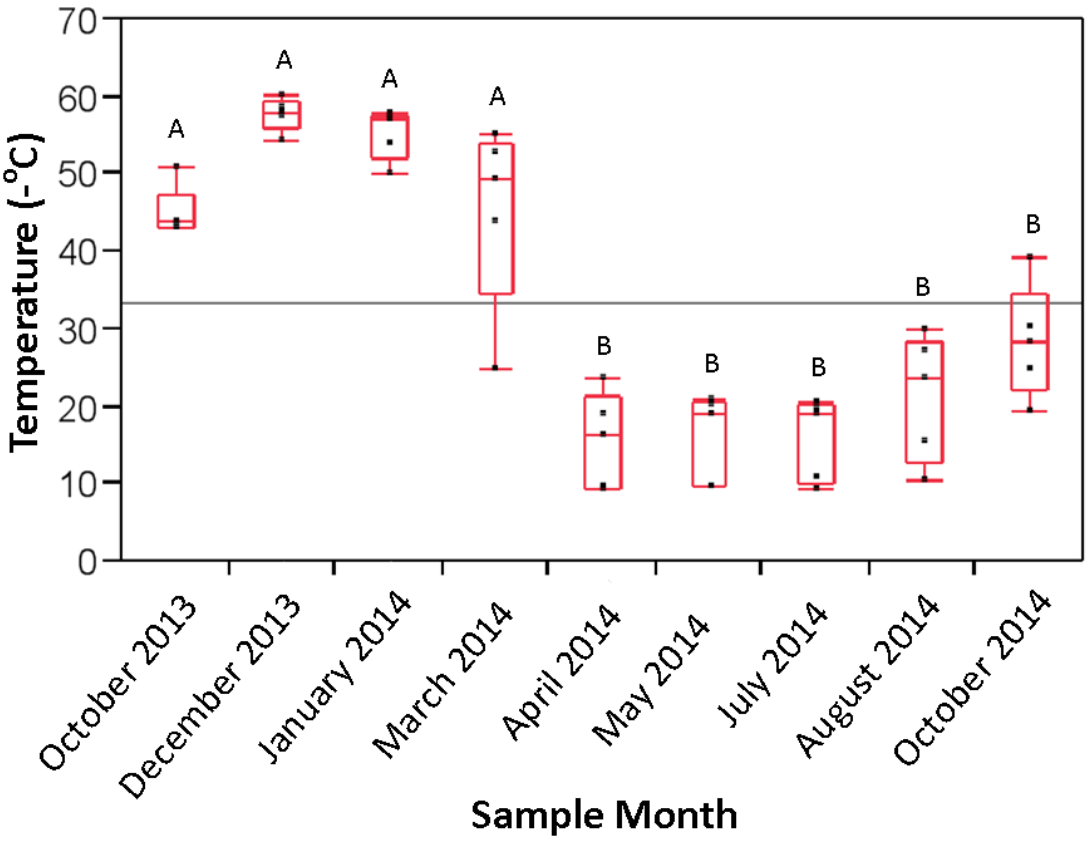

Figure 10. October 2013-October 2014 population W LT50 Tukey's honest significance (HSD) mean separation box and whisker plot for *Eriogonum umbellatum*. Mean values not sharing a letter differ significantly at P <0.05. Whiskers represent standard error of the mean (SEM). Line indicates the annual average temperature -33°C.

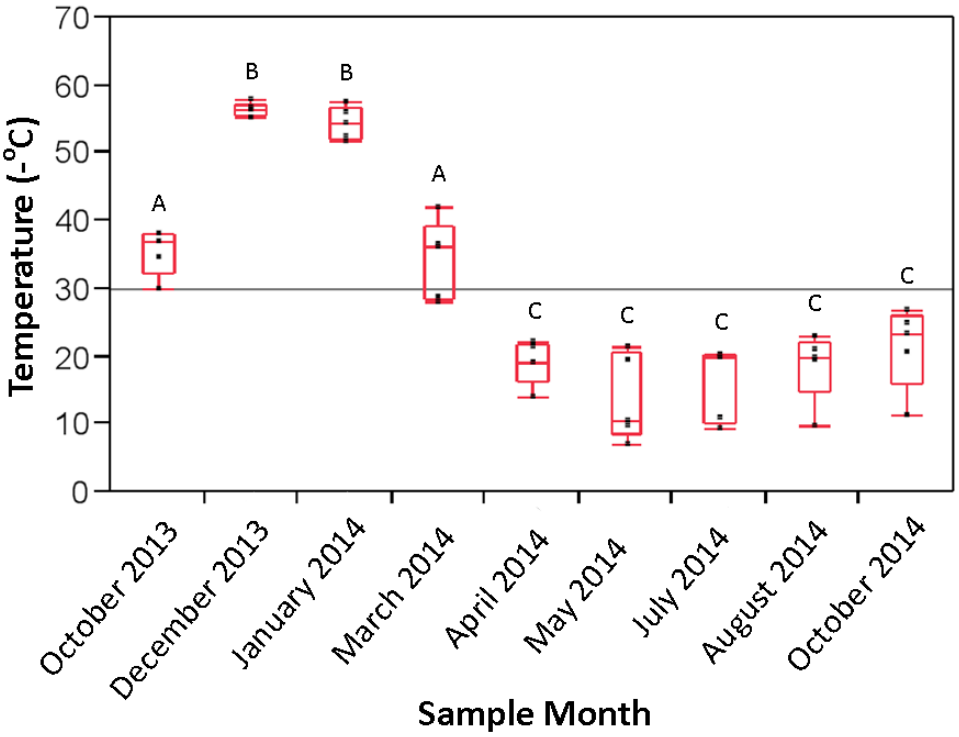

Figure 11. October 2013-October 2014 population J LT50 Tukey's honest significance (HSD) mean separation box and whisker plot for *Eriogonum umbellatum*. Mean values not sharing a letter differ significantly at P <0.05. Whiskers represent standard error of the mean (SEM). Line indicates the annual average temperature -30°C.

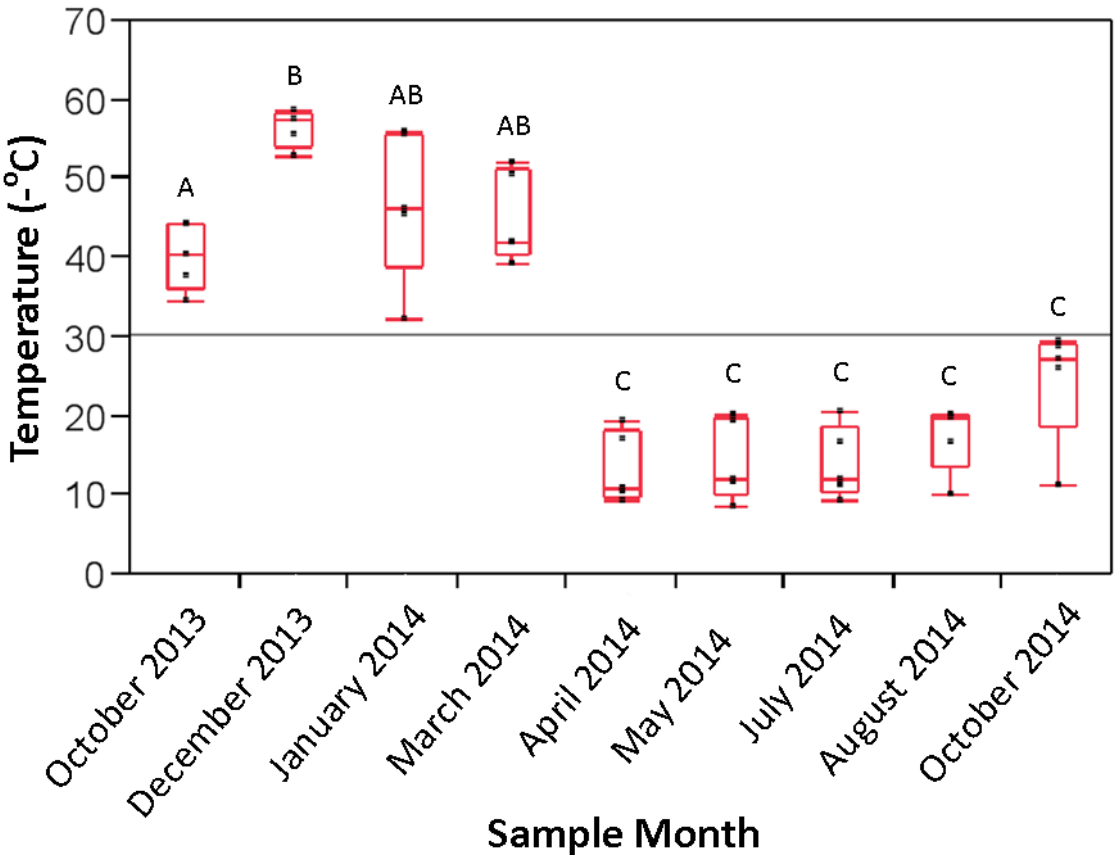

Figure 12. October 2013-October 2014 population C LT50 Tukey's honest significance (HSD) mean separation box and whisker plot for *Eriogonum umbellatum*. Mean values not sharing a letter differ significantly at P <0.05. Whiskers represent standard error of the mean (SEM). Line indicates the annual average temperature -30°C.

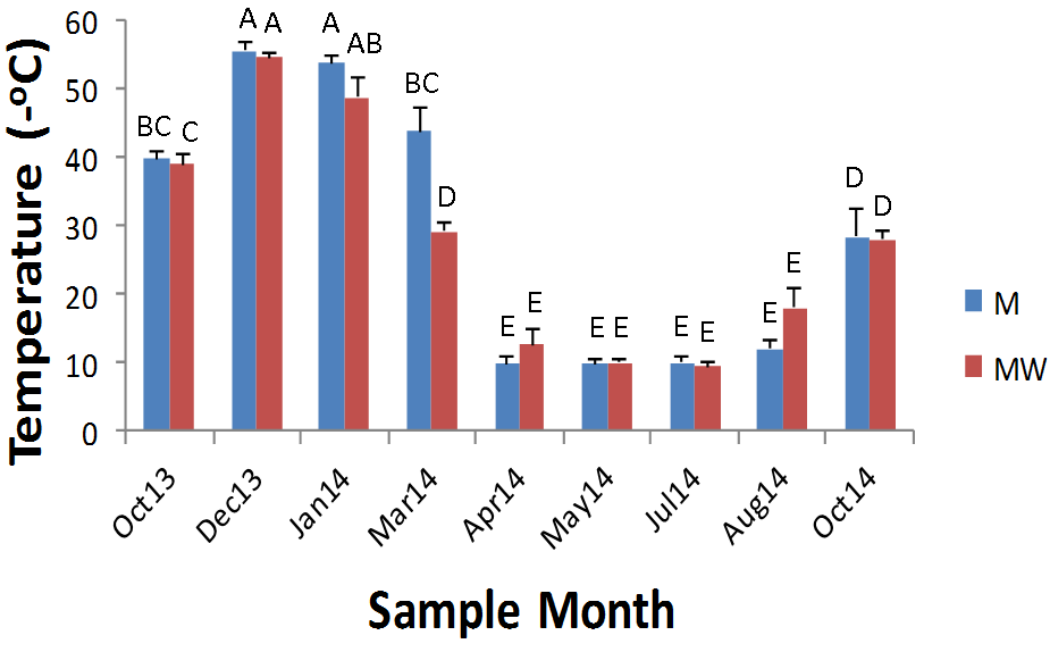

Figure 13. *Eriogonum umbellatum* population M LT50 value in the common garden study (M) compared to the natural wildland site (MW) analyzed using a two-way analysis of variance (ANOVA). Error bars represent the standard error of the mean. Mean values not sharing a letter differ significantly at P <0.05.

Discussion

Tracking five geographically distinct populations of *Eriogonum umbellatum* across the calendar year revealed important information to help explain ecophysiological species variation. As was hypothesized, seasonal changes were detected in LT50 value corresponding to the change of environmental temperatures. Four of the ninety different possible date*population interactions were found to be statistically significant. However, other non-statistically significant interactions were found to be biologically significant as was evident by the different temperatures at which the LT50 was reached between populations at specific sample dates. No correlation between elevation gradient and ability to tolerate cold was detected. This is important to consider when constructing seed zones as both elevation and cold hardiness are used as major contributors in transfer zone evaluation.

When sample dates were compared within each unique population, statistical significance was found. Some of the populations showed more variation among the sample dates, whereas others were more similar. The highest level of variability between dates was seen in population M (Figure 7) with four levels of statistical significance across collection dates. Population W (Figure 11) showed the least variability with only two levels of statistical significance. These results support the first hypothesis, showing that cold hardiness varies across geographically unique populations. The populations tested did not adjust to environmental conditions at the same rate. The results also conclude that when unique populations are grown in a similar environment, local adaptivity is not fully accounted for and each population still brings a set of unique qualities specific to its geographic origin. This makes sense ecologically as each population has evolved under a unique set of conditions. These adaptive traits have prepared the populations to thrive in their local environment. Population M's source of origin is the most local to the common garden and showed the most ability to adjust within the transplant site conditions. Its adjustment is more gradual and the triggers to climatic acclimation are less extreme and more gradual, enabling it to maximize growth potential while staying within the safety zone of experiencing damage from cold exposure. Results showed variation in the summer and winter months was less than in the spring and fall in all populations. Aitken and Adams (1996) found considerable family variation for cold injury scores in *Psuedotsuga menziesii* var. *menziesii* (Douglas-fir) tissues in early to midfall, but differences were smaller or nonsignificant in late fall to midwinter. These results were similar with those found in sulphur-flower buckwheat.

October sample dates were evaluated for 2013 and 2014, one year apart from one another. The results indicated statistical significance between the two sample dates and between populations, however the population*date interaction was not significant. The

significance between the October populations was lost in other analyses used in this study, but when teased out explains a vulnerability within the species. The two October sample dates experienced different weather patterns, which are expressed in figure 14 and table 2. The different weather between the two dates caused the plants to adjust to the surrounding conditions at different rates. The observed maximum low temperature for August –October of 2013 was 0.0 ^{o}C, a freezing event (days 284 & 291). In 2014 the observed maximum low temperature for August-October 2014 was 1.1 ^{o}C (day 300). The maximum low for a 30-year time frame (1981-2010) for that sample period is -11.1^{o}C (October 31, 2002). The LT50 range for the populations for October 2013 was -36 - -45^{o}C and -21 - -28^{o}C for October 2014. Table 2 shows how much colder October 2013 was than October 2014. Overall 2013 was more extreme year (figure 14). August was hotter and September and October were colder. Investigating these extreme low temperatures during the plants physiological acclimation state which enables them to tolerate cold tells us that if an extreme cold weather event occurs when the species is not prepared, then fatal cell damages could occur. The most local population that was also the slowest to adjust to the cold in this study, in its most vulnerable state, can only withstand temperatures down to -10^{o}C. This is important because within the last 30 years, cold events have been seen to exceed this temperature in the transition period. This supports the need for seedlings and plants to be well acclimated and hardened for the cold, as extreme events could be detrimental.

	Growing Season (March 1 – October 30)	**Summer (June 20 – September 21)**	**Winter (December 21 – March 19)**	**October 2013**	**October 2014**
Temperature Maximum (^{o}C)	243	84	36	-41	90
Temperature Minimum (^{o}C)	332	177	88	-51	94

Table 2. Daily maximum and minimum temperature cumulative divergence from 30-year average at the Boise, ID common garden.

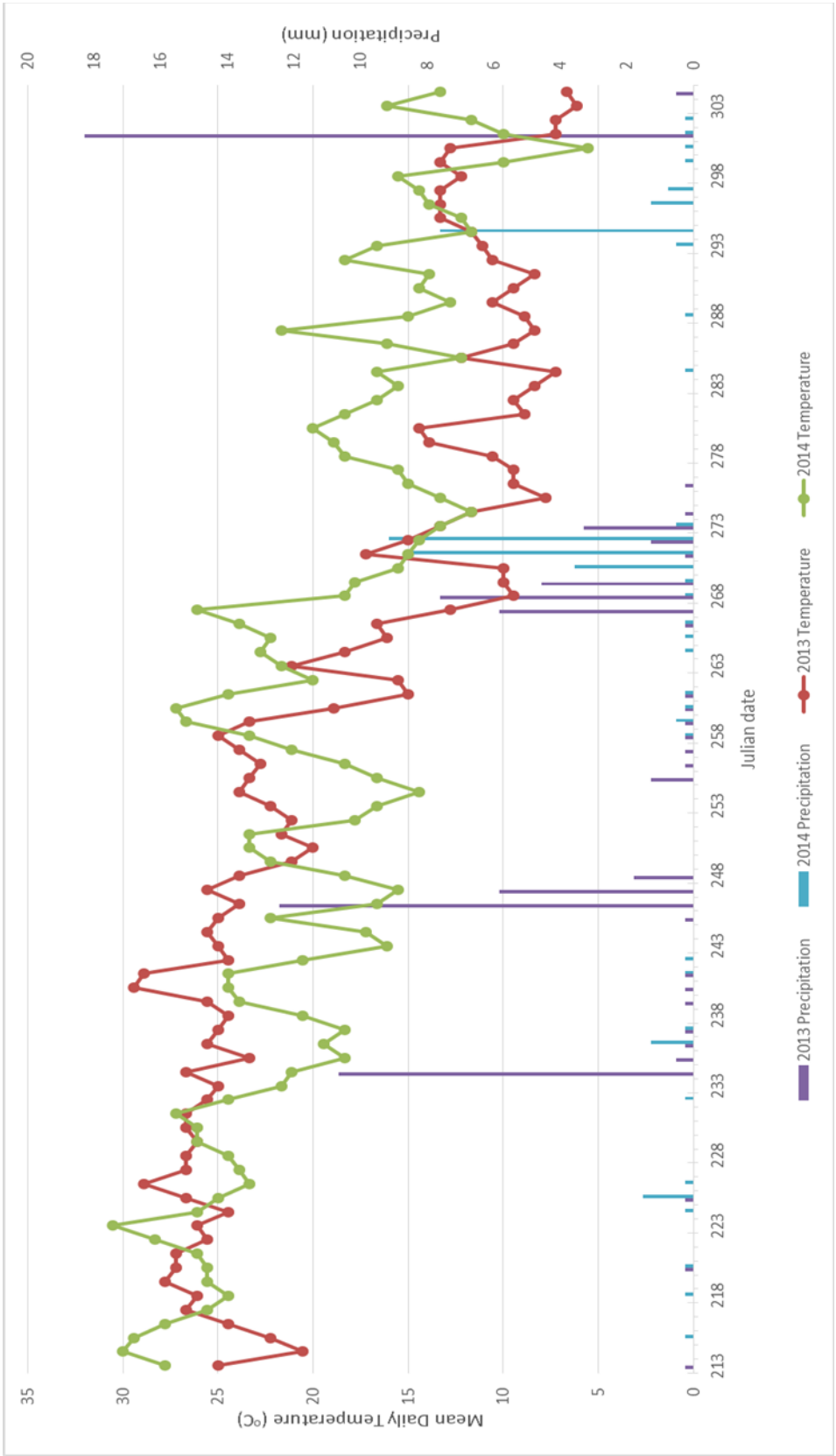

Figure 14. Weather graph comparing precipitation and mean daily temperature for common garden site location from October 2013 and October 2014 sample period from August 1 – October 31.

Looking at the local adaptive cold hardiness trait with population M, there was a statistical difference seen between the wildland (MW) and common garden (M) location during the collection date March 2014 (Figure 13). The two geographic locations are located ~10 miles apart, at elevations 965 meters (MW) and 847 meters (M). It appears as though the plants adjusted during the fall months at around the same rate, but as temperatures started to warm up in the spring, the wildland collection site started to become more metabolically active and came out of dormancy at an earlier date. Location MW never reached the cold hardiness level that the common garden site did, thus potentially letting the plants come out of dormancy quicker. It also may not have been exposed to as cold of temperatures over the winter due to snow pack at the wildland site. The common garden site did not receive the snow cover that the wildland site received for insulation during the winter months. To learn more about this, it would be necessary to look at more extreme comparisons to define the difference and local adaptation trait results.

Between the populations there was a low LT50 cold hardiness resistance level ranging from -10 to -16^{o}C during the spring collection months and a high LT50 cold hardiness resistance level ranging from -56 to -58^{o}C in the winter (Table 3). December was the sample collection that appeared to be the most cold resistant, whereas April – August months tended to all be similar in their lack of cold hardiness resistance. When the overall cold hardiness is averaged across all collection periods / population there was 5^{o}C variation between the lowest and highest LT50 level for the year.

	Population				
	M	**J**	**B**	**C**	**W**
	-^{o}C				
LT50 low	10	14	16	13	16
LT50 high	56	56	56	56	58
LT50 Average	28	30	30	30	33

Table 3. LT50 high, low and average values for nine sampling dates from October 25, 2015 to October 16, 2015 for five *Eriogonum umbellatum* populations at the Boise, ID common garden.

When evaluating the LT50 calculations for the species, as evaluated by the 5 distinct populations in this research, the figures can be roughly described as having a range from -10^{o}C to -58^{o}C, with an average high LT50 average of -56.4^{o}C and a low LT50 average of -13.8^{o}C. The yearly average LT50 for these populations of *Eriogonum umbellatum* is -30.4^{o}C.

The application of these LT50 values is directly tied to extreme events as a species is either in its least cold resistant stages (April – August), the transitional stages (March and October), or exposed to an environment that exceeds the species maximum cold hardy

level even in its least vulnerable state (November – February). If we were to plant outside the periods of maximum cold hardiness, then we would potentially expose the seedlings to damage as they may not be acclimated to environmental conditions. There is some variability by population in the slope of accumulation and loss of cold tolerance and this needs to be accounted for in outplanting success.

As climate change continues to garner momentum, as indicated by the ever growing list of supporting scientific literature proving time and time again to have a profound effect on future and existing species distribution and migration patterns, current and future extreme event variability is much more important than averages (Katz and Brown 1992). Whether these extreme events arrive in the form of heat, drought, type of precipitation, or cold, each could come with drastic consequences to existing distribution patterns. Plants have been moving across the landscape in response to changing climate for millennia; however, projections of contemporary climate change suggest that some species and populations will need to migrate faster than their natural ability (Williams and Dumroese 2013). With these proven needs in mind and human involvement, it has become crucial to make sure that the right plants get put in the right place, at the right time. Cold hardiness is one of the most important factors to consider when moving plants beyond their natural genetic and existing range.

In conclusion, investigating *Eriogonum umbellatum*'s ability to handle cold temperatures in an extreme, rapidly changing environment provides information to help describe woody perennial Great Basin plant resistance across the region. The species is found to express plasticity, where local adaptation to cold acclimation is present, but is limited. Specific geographic populations have unique abilities, levels, and rates of cold hardiness adjustment better suited for specific environmental conditions. Evaluating variation within a species' distribution, as well as between individuals within geographically unique populations is useful in determining effective restoration products and assisting in plant migration efforts where necessary. Further research into this area will help construct models to determine when a species is more biologically vulnerable to cold in the transition periods during spring (March) and fall (October). With additional cold hardiness investigation it is important to evaluate many tissue types, as the literature shows that single tissue examination is not adequate for assessing overall cold hardiness of genotypes (Aitken and Adams 1996). Cold hardiness is one of many areas in which Great Basin native species require more research to improve available tools used in developing seed zones and restoration strategies.

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