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Alpine ecology of a C4 grass, Muhlenbergia Richardsonis in the White Mountains of California

Archie Thomas Meyer

ALPINE ECOLOGY OF A C4 GRASS, *MUHLENBERGIA RICHARDSONIS* IN THE WHITE MOUNTAINS OF CALIFORNIA

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

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ABSTRACT

The C_4 grass *Muhlenbergia richardsonis* ((Trin.) Rydb.), grows as high as 3965 m in the alpine zone of California's White Mountains. C_4 plants are generally intolerant of low temperatures and rarely occur in alpine habitats. The central objective of this thesis was to understand how this unusual C_4 alpine grass, *Muhlenbergia richardsonis*, persists in the alpine zone along the western slope of California's White Mountains.

Stomatal density, leaf carbon isotope composition and nitrogen content were assessed in *M. richardsonis* and co-occurring C_3 species along an 900 m elevational gradient to determine whether the C_4 cycle provides C_4 species with any advantages over that of C_3 species in the low atmospheric CO_2 (pCO_2) conditions found at high elevations. Growing season development was assessed in *M. richardsonis* and co-occurring C_3 species to determine if this C_4 species exhibits a warm-season specialist type of phenology. Growth, reproduction, and survival of experimental plantings of *M. richardsonis* in selected alpine microsite treatments were assessed to see how microsite temperature

and soil moisture affected plant performance in the alpine zone.

Major findings are interpreted to indicate that *M. richardsonis* (a) has a relative advantage for photosynthesis under the low pCO_2 conditions of the alpine zone, (b) has a truncated but accelerated growing-season phenology compared to co-occurring C_3 species, and (c) exhibits enhanced plant performance at the warmest and moistest microsites near its current upper-elevation distribution limit. Data are also presented suggesting that water availability restricts the distribution of this species at its lower elevation limit in the arid White Mountain Range.

Consistent with ecophysiological theory, this work provides provisional evidence that C_4 species may become more frequent in CO_2-poor alpine plant communities as low-temperature limitations on C_4 photosynthesis are relaxed with warming climates.

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TABLE OF CONTENTS

CHAPTER ONE

BACKGROUND

Alpine Environments and Adaptive Traits in Alpine Plants

The alpine life zone is regarded as being among the most challenging habitat types on Earth for plant growth and survival (Körner, 2003). Although the alpine life zone only constitutes approximately 3% of all land surfaces, these inhospitable habitats are dispersed widely around the planet, occurring on every major continent other than Antarctica (Körner, 2003). Most terrestrial life zones are restricted to discrete latitudinal bands (e.g. boreal forests or warm deserts) but the presence of alpine habitats depends upon both latitude and elevation. Alpine habitats are, paradoxically, both cosmopolitan and insular. But, like other biomes, the alpine life zone is characterized primarily by its climate and its plant life. When compared to adjacent lowland ecosystems, the alpine life zone is characterized by the absence of trees (i.e., these zones occur above the timberline) and by having cool to cold summers and usually a short growing season (Bliss, 1966). Given their limited overall land area, their

scattered geography, and the extreme environmental conditions that characterize this life zone, it is interesting to consider how plants native to these habitats are able to make a living where they do.

There are several environmental factors that present challenges for plant growth in alpine zones. The abiotic environment becomes increasingly severe and plant diversity declines as the elevation increases in most mountain systems. Körner (2003) reports that in the Alps at 3000 meters there are over 200 plant species present but at 4000 meters that number drops down to fewer than 20 species. One environmental factor common to extratropical alpine zones is the abbreviated growing season. In addition, very cold temperatures not only damage vegetation directly but can also cause frost-heaving of the soil which can damage roots of young seedlings and shallow-rooted mature plants (Bliss, 1966). Furthermore, most alpine environments have high exposure and are subject to strong sustained winds that can desiccate and damage plant tissues. With increasing elevation there is also a decline in the partial pressure of atmospheric CO_2 (pCO_2) which may limit photosynthesis and plant growth (Körner, 2003). With these

extreme climatic conditions present at high elevations, it is not surprising that plants must exhibit specific traits to enable them to survive and reproduce in alpine zones.

Plants from most alpine ecosystems share several phenological and growth-form characteristics that are thought to reflect the short growing seasons of these habitats. Most alpine plant species are perennials, with annuals in most locations contributing little to high mountain flora (Körner, 2003). Among perennial alpine species, most have adopted herbaceous forms over woody forms. This appears to be a sensible strategy because all aboveground growth is dedicated to productive, short-lived photosynthetic tissues and none is allocated to long-lived but non-productive woody biomass (Billings and Mooney, 1968). Seedling establishment is rare due to the effects of short, cool, growing seasons and most alpine species rely heavily upon vegetative reproduction. The means of asexual reproduction commonly exhibited by alpine plants include spreading rhizomes, vegetative propagules such as bulbils, or stem layering. These diverse modes of vegetative reproduction are viewed as adaptive responses to the short growing seasons and low rates of seedling establishment in

cold alpine zones (Billings and Mooney, 1968). Alpine plants also have accelerated phenologies. This rapid development allows adequate growth each year to permit vegetative reproduction during the brief growing season and improves the probability of successful flowering, fertilization, and seed set during years when sexual reproduction is possible.

The cold and windy conditions commonly found at high altitudes have also contributed to shaping plant morphology in alpine plants. Most high-elevation plants have low statures to gain protection against these damaging winds. In fact, most alpine plant species exhibit low statures and grow in dense mats in the relatively calm air near the soil surface (Billings and Mooney, 1968). In a study by Bliss (1966), wind speed at 15 cm above the ground was 56% less than that at 60 cm above the ground at the same alpine site. The low stature and mat-forming characteristics of alpine vegetation also allows plant temperatures to rise above those of ambient air temperatures. Specific morphologies found among alpine plants include tussocks (mostly grasses and sedges), rosettes (mostly perennial forbs) and cushions and mats found in various growth forms

including grasses, forbs, and shrubs (Billings and Mooney, 1968). Alpine plants are also typically deep-rooted which is thought to partially compensate for frost heaving tendencies in soils at high elevation.

Elevational trends in leaf structure and function suggest that alpine plants employ several mechanisms to maintain adequate rates of photosynthesis in the face of cool daytime temperatures, the low pCO_2, and the short growing seasons. Table 1.1, drawn from Körner's recent synthesis of alpine plant ecology (2003), summarizes responses of key foliar traits to contrasting elevations. First, thicker alpine leaves contain more photosynthetic cells per unit of light-absorbing leaf area (Table 1.1). For the same investment in foliage support tissues (stems and petioles), a thicker leaf in a bright habitat can realize a greater net carbon gain for the plant than a thin leaf of the same area. The greater leaf thickness in these bright alpine habitats may partially compensate for temperature- and CO_2-limits on C_3 photosynthesis common to high elevations. Second, high-elevation plants often contain higher amounts of enzymatic proteins in their leaves which may help to improve photosynthetic performance

under these alpine conditions. Higher amounts of leaf protein translate into higher leaf nitrogen concentrations (%N) as shown in Table 1.1. Third, variation in stomatal density in C_3 plants is thought to reflect the atmospheric CO_2 concentrations under which the plant has developed. Woodward (1987) examined stomatal densities on herbarium specimens of several European tree species that had been collected at different times over the preceding 200 years. This study indicated that the average stomatal densities had declined by about 40% over this time period during which atmospheric CO_2 had increased from about 28 Pa to 34 Pa (Woodward, 1987). Körner (2003), surveyed 17 species in the Alps and found, with only one exception, all plants increased their stomatal density with increasing elevation and the corresponding decline in pCO_2. In general, a higher density of stomates improves diffusive transport of atmospheric CO_2 into the leaf and into the chloroplast for photosynthesis. Accordingly, C_3 plants growing at high elevations under low pCO_2 conditions usually have greater stomatal density than lowland plants (Table 1.1). In sum, the combined effect of these anatomical and biochemical responses to elevation permit higher rates of

photosynthesis in alpine C_3 plants compared to lowland C_3 plants when both are measured under similar conditions (Table 1.1).

Table 1.1 also highlights differences between alpine and lowland plants in the carbon isotope composition of leaf tissues. Carbon of atomic mass 12 and mass 13 are both stable (i.e., non-radioactive) isotopes. Relative measures of ^{13}C and ^{12}C abundances are quantified as; $\delta^{13}C$ (‰) $=(R_{sample}/R_{standard} -1)*1000$, where R is the ratio of carbon mass 13 to mass 12 (Taiz and Zeiger, 2002). C_3 plants discriminate strongly against ^{13}C -based CO_2 in favor of ^{12}C-based CO_2 during photosynthesis. However, when photosynthesis becomes increasingly diffusion limited, either because there is less CO_2 available in the atmosphere or because the stomata are more fully closed, C_3 plants will tend to fix relatively more of the intercellular ^{13}C -based CO_2. For example, shaded rainforest C_3 plants in Panama had foliar $\delta^{13}C$ values of -32‰ but, in full sun, these same plants had carbon isotope values of -27‰ (Skillman et al., 2005). The higher (less negative) isotope value in the sun-grown plants indicates they contained relatively more ^{13}C in their tissues. This was taken to indicate that these

plants had greater diffusion limitations on photosynthesis than the shade-grown plants, presumably because photosynthetic stomatal limitations were relatively more important under the hot tropical sun (Skillman et al, 2005). Alpine C_3 plants often accumulate relatively more ^{13}C in their tissues than lowland C_3 plants presumably reflecting the thin atmosphere at high elevations (Table 1.1). Reviewing several studies, Körner (2003) reports that the $\delta^{13}C$ value in C_3 plant tissues becomes less negative at an average rate of 1.2‰ per 1000 m gain in elevation. Although C_3 plants exhibit a number of anatomical and physiological responses to maintain high rates of photosynthesis in the mountains, the reduced discrimination against $^{13}CO_2$ suggests, nonetheless, that photosynthesis is still diffusion limited in these alpine plants.

The environmental conditions listed above (e.g. low temperatures, short growing seasons, reduced pCO_2) and the associated plant traits are consistently found in alpine zones around the world (Körner, 2003). This strong functional convergence among diverse alpine floras is consistent with the hypothesis that these traits are adaptive for life under these harsh alpine conditions. It

is noteworthy that there is a conspicuous absence of C_4 plants in most alpine floras (Sage and Wedin, 1999). *Muhlenbergia richardsonis* is a curious exception to this general observation. *M. richardsonis* is a broadly distributed North American C_4 grass species that can be found growing at 3965 m in the alpine zone of the White Mountains of eastern California. To my knowledge this is the highest recorded observation for a C_4 species in North America. *M. richardsonis* exhibits many of the previously described traits found in other alpine species such as low prostate growth, perennial herbaceous life-form, and deep roots. But it is unusual among alpine plants for its reliance on C_4 photosynthesis. Generally C_4 plants are restricted to warm climates, becoming poorly represented at high altitudes and/or high latitudes (Rundel, 1980; Sage and Sage, 2002). The presence of *M. richardsonis* in the alpine zone of White Mountains is an enigma.

C_4 Photosynthesis

For the purpose of this study, a review of the C_3 and C_4 photosynthesis syndromes is necessary. The more common and simplest photosynthetic pathway, C_3, is characterized by atmospheric CO_2 being fixed directly by the enzyme ribulose-

1, 5-bisphosphate carboxylase/oxygenase (Rubisco), the essential carboxylating enzyme of photosynthesis. Simple C_3 photosynthesis occurs in approximately 90% of the nearly 300,000 described species of terrestrial plants (Sage, 2004). Less common (but of greater importance for this study) is the C_4 photosynthetic pathway, found in an estimated 7,000 species worldwide (Sage, 2004). It should be pointed out that C_3 biochemistry underpins carbon fixation in all photosynthetic organisms but in C_4 plants this C_3 biochemistry is supplemented with additional 'upstream' biochemical and cellular transport processes. This additional 'upstream' metabolism serves to increase the concentration of CO_2 at Rubisco. As a result of this upstream C_4 metabolism, Rubisco and the entire C_3 cycle can operate more effectively at this higher cellular concentration of CO_2 (Sage and Monson, 1999) (See Figure 1.1)

Figure 1.1 illustrates the essential steps whereby the C_4 concentrating mechanism feeds CO_2 into the vicinity of Rubisco. Key to this process is the separation of different biochemical steps into different cell types within the leaf of the typical C_4 plant. The initial steps

of the C_4 cycle take place in mesophyll cells which dominate the tissues of C_4 leaves. The final Rubisco- mediated carbon fixation steps take place in a specific leaf tissue made up of bundle sheath cells (Figure 1.1). The concentrating of CO_2 within these bundle sheath cells begins with the synthesis of oxaloacetate (OAA), a four-carbon acid formed from bicarbonate (HCO_3^-) and the three carbon substrate phosphoenolpyruvate (PEP). This initial step is catalyzed by the enzyme phosphoenolpyruvate carboxylase (PEPcase) in the cytoplasm of leaf mesophyll cells. A four carbon derivative of the OAA product (malic acid or aspartic acid, depending upon the species) diffuses into the bundle sheath cells from the mesophyll cells via plasmodesmata which span the interface of the two cell types. In the bundle sheath, the four carbon acid is decarboxylated to yield CO_2 and pyruvate, the remaining three-carbon product. Pyruvate then diffuses back into the mesophyll cells where it may be converted back to PEP with the consumption of 2 ATPs per PEP produced (Kanai and Edwards, 1999). This consumption of 2 ATPs for a turn through the C_4 cycle represents an energetic cost of carbon fixation over and above that required in C_3 plants operating at maximum efficiency

(Skillman, 2008). However, this cycle can concentrate CO_2 in the bundle sheath cells near Rubisco up to 10 times over that found outside of the plant, increasing the effectiveness of the C_3 cycle in C_4 plants (Kanai and Edwards, 1999). Rubisco then uses the CO_2 in the carboxylation of RuBP in the same series of reactions as found in C_3 plants (Not shown. See, for example, Taiz and Zeiger, 2002). Interestingly, C_4 plants have considerably less of the costly enzyme Rubisco in their leaves than C_3 plants. As a result, C_4 plants often have lower leaf nitrogen requirements than C3 plants. In summary, C_4 photosynthesis can be more efficient than C_3 photosynthesis on the basis of CO_2 availability and leaf nitrogen concentration, but less efficient on the basis of energy required for carbon fixation.

One of the ways biologists can distinguish between C_4 derived plant matter and C_3 derived plant matter is by analyzing the relative amounts of the two stable C isotopes, ^{12}C and ^{13}C present in the material. The basis of this distinction is that the primary carboxylating enzymes (PEPCase in C_4 plants and Rubisco in C_3 plants) differ in their relative selectivity for these two isotopes in their

respective inorganic carbon substrates (HCO_3^- in C_4 plants and CO_2 in C_3 plants). As mentioned previously, during C_3 photosynthesis, Rubisco selects strongly for $^{12}CO_2$ over $^{13}CO_2$. During C_4 photosynthesis, PEPCase does not discriminate as strongly between $H^{13}CO_3^-$ and $H^{12}CO_3^-$. Consequently there is much stronger bias towards ^{12}C over ^{13}C in C_3 plant material than there is in C_4 plant material. Typical modal $\delta^{13}C$ values, as determined by ratio mass spectrometry for C_3 and C_4 plant tissues are -28‰ and -14‰, respectively. The more negative the carbon isotope value, the less ^{13}C present in the tissue relative to ^{12}C (Taiz and Zeiger, 2002).

Ecophysiological Implications of C_4 Photosynthesis

The fundamental difference between C_3 and C_4 plants in how CO_2 is captured from the atmosphere can give C_4 species advantages in some environmental conditions. First, because of the greater affinity of PEPCase for inorganic carbon over that of Rubisco, C_4 plants can sustain higher rates of photosynthesis at low concentrations of CO_2 (Pearcy and Ehleringer, 1984). In the absence of other limitations, under any conditions where atmospheric CO_2 is

potentially limiting for C_3 photosynthesis, including the alpine life zone, C_4 plants should have a relative advantage over C_3 plants. It has even been suggested that pCO_2 conditions of the past led to the diversification and spread of C_4 grasses (Ehleringer et al., 1997). Second, the carbon concentrating mechanism in C_4 plants allows for continued high rates of CO_2 assimilation even when the stomata are partially closed. This allows for savings in water due to reduced transpiration from plant tissues. These leaf-level effects can scale up to whole plant growth. Edwards and Walker (1983) reviewed data for several crop species and found that C_3 plants use approximately 700 grams of water for every gram of plant biomass produced but C_4 plants only used about 300 grams of water for every gram of plant biomass produced. Consequently, in the absence of other limitations, C_4 plants are expected to have an advantage over C_3 plants in habitats where water is limiting. Third, photorespiration is minimized in C_4 plants compared to C_3 plants. Photorespiration is an unavoidable inefficiency in C_3 photosynthesis which acts to lower the efficiency of C_3 photosynthesis particularly at higher temperatures

(Skillman, 2008). This C_3 vs. C_4 difference is the result of the ability of C_4 species to concentrate CO_2 high enough in the vicinity of Rubisco to minimize its oxygenase activity thus holding photorespiration in check. Photorespiration increases with increasing temperatures, lowering the energetic efficiency of photosynthesis in C_3 plants (Figure 1.2). At a leaf temperature of 15°C the effect of photorespiration on C_3 plants is modest and so the C_3 plant has a higher quantum yield than C_4 species at this same temperature. But at 40°C, the quantum yield of C_3 photosynthesis drops well below that of C_4 photosynthesis at the same temperature because of increasing photorespiration in the C_3 plant. Because C_4 plants undergo very little photorespiration their quantum yield is unaffected over these temperatures (see Figure 1.2) (Ehleringer and Björkman, 1977). Thus, in the absence of other limitations, C_4 plants should do better than C_3 plants at high temperatures but the reverse should be true in cooler climates. For the purposes of this study, and in the absence of other differential limitations on plant growth, C_4 plants would be expected to outperform C_3 plants under conditions of low pCO2 found at high elevations but C_3

plants would be expected to outperform C_4 plants at low temperatures typical of alpine habitats.

Biogeographic Patterns of C_4 Grasses

The distribution and abundance of C_4 plants appears to reflect some of these environmental factors that favor the C_4 photosynthesis syndrome. In particular, the relative abundance of C_4 grasses is strongly correlated with growing season temperatures (Long, 1983). C_4 species are more common at low latitudes and decrease with increasing latitudes. This pattern was first quantified for North America in a seminal study carried out by Teeri and Stowe (1976). Their work reveals that there is an overall decline of C_4 grass species as latitude increases. For example, in southern Florida, 80% of all grass species present were C_4 but in northern Maine only 12% of grass species were C_4 (Teeri and Stowe, 1976). This latitudinal pattern has since been documented for each of the major land continents including a recent re-analysis for the North American flora by Wan and Sage (2001).

Plants that rely upon C_4 photosynthesis also decline in diversity and importance with increasing elevation.

Chazdon's (1978) survey of grass species in the mountains of Costa Rica found that most C_4 grasses were restricted to warm lowland savannas while C_3 grasses were largely restricted to higher and cooler elevations. This same elevational trend for C_4 abundance has now been reported for numerous mountain ranges around the world (see figure 1.3)including Kenya (Tieszen et al., 1979), Hawaii (Rundel, 1980), Argentina (Cavagnaro, 1988) and Egypt (Sayed and Mohamed, 2000). Taken together these consistent cosmopolitan latitudinal and altitudinal trends make a strong case for the hypothesis that cold sensitivity of C_4 photosynthesis limits the ecological distributions of these plants.

Based upon these biogeographic patterns, plant physiological ecologists have identified what appear to be critical temperature thresholds for the ecological success of C_4 grasses. Figure 1.3 shows the relative number of C_3 and C_4 grass species as a function of elevation in the mountains of Hawaii from Rundel (1980). Rundel related the elevation where the dominance of C_4 gives way to the dominance of C_3 species to average temperatures at this point (the 'crossover point'). The 1400 m crossover point

in Hawaii corresponded to an average minimum growing season air temperature of 9°C and an average maximum growing season air temperature of 21°C. Rundel (1980) suggested that when temperatures drop below these thresholds C_4 grasses become rare or disappear altogether. Subsequently, Long (1983), reviewing several studies of latitudinal and altitudinal limits to C_4 distributions, reported a common average minimum mid-growing season air temperature of 8°C to 10°C, consistent with Rundel's initial suggestion. Ehleringer et al. (1997), also reviewing C_4 distributional patterns from around the world, reported a common average maximum mid-growing season air temperature of 20°C to 28°C, which is also consistent with Rundel's initial suggestion. Although the mechanism is not well understood, it is clear that for various C_4 grasses in various habitats, low temperatures, limit their ecological distributions (but see Edwards and Still, 2008).

The biology underlying the virtual absence of C_4 photosynthesis in cold habitats is poorly understood. Several hypothesis have been put forth to account for these distributional patterns. First, it has been suggested that there is some failure in the C_4 photosynthetic machinery at

low temperatures at one or more of the enzymatic steps in the C_4 cycle. For example, the enzymes pyruvate orthophosphate dikinase (PPDK) and phosphoenolpyruvate carboxylase (PEPCase) have been shown to dissociate in some C_4 species at temperatures of 8-12°C (Pittermann and Sage, 2000). These enzymes are involved in the regeneration of the C_3 acid pyruvate and fixation of CO_2 to form the C_4 acid, malate. If these enzymes are especially cold-labile it could help explain why there are relatively few C_4 plants found at higher and colder sites. Second, Ehleringer et al. (1997) argue that biogeography of C_3 vs. C_4 (Figure 1.3) is explained by the quantum yield differences at different temperatures (Figure 1.2). This largely is due to the effect of temperature on photorespiration in C_3 but not C_4 species (Ehleringer and Björkman, 1977). Third, it has been suggested that the restriction of C_4 plants to warmer climates may be connected to the reduced amount of Rubisco found in C_4 plant tissues. Kubien and Sage (2004) conducted diagnostic gas exchange studies on C_3 and C_4 plants that were grown at different temperatures and then measured activity levels of key photosynthetic enzymes from these plants grown at different temperatures. Their findings

suggest that at cool growing temperatures, CO_2 uptake in C_4 (but not C_3) plants is limited by Rubisco content, as opposed to other limitations such as the availability of PEPCase or ATP or NADPH. Therefore, the low amount of Rubisco found in C_4 plants may ultimately limit their distribution to warm places. But, this alone as a limit on C_4 ecology is difficult to reconcile with the fact that most plants have large potentials for morphological, physiological and biochemical plasticity in response to changing environmental conditions (Sage and McKown, 2005). The fourth idea to explain the limited distribution of C_4 plants is that C_4 plants may have limited plasticity at the leaf level. Sage and McKown (2005) have pointed out that the anatomy of C_4 photosynthesis restricts the amount of structural adjustments that can be made in leaves while still maintaining photosynthetic efficiency. Disruption of the mesophyll-bundle sheath complex could disrupt the shared metabolism across the two cell types, and increase CO_2 leakage from the bundle sheath cells. Ogle's (2003) literature survey shows convincingly that as the distance between adjacent vascular bundles (the IVD or interveinal distance) increases in C_4 grasses, the photosynthetic

energetic efficiency decreases, presumably because of increased leakage of CO_2 from the C_4 cycle. The carbon gain efficiency in C_3 grasses appears to be independent of the leaf IVD. This idea is intriguing because it would seem unite the Rubisco limitation suggested by Kubien and Sage (2004) and the carbon gain efficiency restrictions proposed by Ehleringer et al. (1997). It is too early to say how temperature, anatomical plasticity, and C_4 biogeography are or are not, related. At present, none of the four proposed mechanistic hypotheses (cold-labile C_4 enzymes, quantum yield differences between C_3 and C_4 plants, C_4-specific Rubisco limits on photosynthesis at cold temperatures, and the limits of C_4 anatomical plasticity) provides an unequivocal explanation for the distribution of C_4 grasses. A fifth hypothesis is that C_4 plants arose in warm habitats and have simply not had enough time to evolve tolerances to cold temperatures (Sage, 2003; Edwards and Still, 2008). It is generally held that C_4 plants arose and diversified in tropical and sub-tropical habitats relatively recently (Sage, 2004). Reviewing both molecular phylogenetic data and paleontological data, Sage (2004) suggested that C_4 grasses appeared as far back as the mid-Oligocene (~30

million years ago, long after the appearance of the grass lineage in terrestrial plants) and that C_4 dominated tropical grasslands only became common on the planet perhaps as recently as 10 million years ago. Thus, within the geological lifespan of higher terrestrial plants, which are thought to have first appeared as far back as the Silurian (438-408 million years ago), C_4 grasses appear to be 'newcomers'. As such, the ultimate explanation for the absence of C_4 in cooler climate species may be that they simply have not had enough time to radiate and adapt to cooler habitats found at higher elevation (Sage, 2004). Although uncommon, we know that today there are groups of C_4 species found growing in cooler places and among them is the curious exception in the White Mountains, the C_4 grass *M. richardsonis*.

White Mountains, California

The White Mountain-Inyo range, running roughly north to south in eastern California, is about 177 kilometers long and second only to the adjacent Sierras for height in the continental United States (Hall, 1991). The changes in climate and vegetation are striking as one moves from the

town of Bishop on the floor of the Owens Valley at 1220 meters up to a maximum elevation of 4345 meters at the peak of White Mountain (Figure 1.4). The range is more than 600 million years old and expresses good topographic and geologic diversity with representations of granitic rocks, basalt, metavolcanic rocks, metamorphosed sandstone, shale, limestone, and dolomite (Hall, 1991). The climatic conditons along the elevational gradient from Bishop up to the Alpine zone are as varied as its geology.

The climate of the range is mostly cold and dry with temperatures varying from a mean high of 21 °C at Bishop near the foot of the White Mountains (1252 m) to a mean high of 2 °C at the Barcroft, White Mountain Research Station (WMRS) (3780 m). With a rise in elevation the length of growing season (defined here as having monthly temperatures averaging over 0 °C) declines along the elevational gradient. For example, Bishop has average monthly temperatures over 0 °C all year long while Barcroft can, on average, expect only ~4 months above freezing (Figure 1.5). In addition, at higher elevations in the summer growing season, the temperature drops faster with elevation gain than it does in winter along the same

elevational gradient, further emphasizing that growing season temperatures at high-elevation sites are cool (Figure 1.6).

Annual precipitation in the White-Inyo range averages 102 mm per year at Bishop to 508 mm per year at higher elevations in the range (Figure 1.7). In Bishop, the precipitation mostly falls as rain and at higher elevations it falls up to 80% as snow (Powell and Klieforth, 1991). Most of the year's precipitation falls in the winter months but monsoonal storms moving from the south can be an important source of summer moisture in the White Mountains (Powell and Klieforth, 1991).

There is a steady drop in pressure as elevation increases. With the decreasing pressure less CO_2 is available for photosynthesis (Figure 1.8). In conclusion, conditions for plant growth within the range varies with local climatic conditions found along the elevational gradient in the White Mountains.

Vegetation Zones in the White Mountains

Several distinct vegetation zones along the White Mountain climatic gradient, from the town of Bishop at 1252

m to the peak of White Mountain at over 4000 m, have been described (see Table 1.4). Vegetation on the western slope of the White-Inyo range include the Desert Scrub zone, which is found between the elevations of approximately 1200-2000 meters and is dominated by its most common species shadscale, (*Atriplex confertifolia*) at lower elevations and great basin sagebrush (*Artemisia tridentata*) at higher elevations (Figure 1.9). Moving up from there in elevation, at approximately 2000-2900 meters, is the pinyon-juniper woodland which, as its name suggests, is characterized by the dominance of pinyon pine (*Pinus monophylla*) and Utah juniper (*Juniperus osteosperma*) (Figure 1.10). Above the pinyon-juniper woodland can be found the sub-alpine zone occurring at approximately 2900-3500 meters. Important plant species found here include bristlecone pine (*Pinus longaeva*), limber pine (*Pinus flexilis*), and great basin sagebrush (*Artemisia tridentata*), as well as the focal plant of this study, mat muhly (*Muhlenbergia richardsonis*). Within the sub-alpine zone, most authorities distinguish the so-called sagebrush steppe as a distinct vegetation type. Unlike the pine woodlands, trees are absent and the vegetation is dominated

by great basin sagebrush (Figure 1.12). Finally, the alpine zone is represented at elevation above approximately 3,500 meters, topping out at White Mountain peak (4,345 m), one of the tallest peaks in the Continental U.S. At these extreme elevations, trees are absent and shrubs are reduced in stature (Figure 1.13). Characteristic species found in the alpine zone of the White Mountains include raspberry buckwheat (*Eriogonum gracilipes*), fell-field buckwheat (*Eriogonum ovalatum*), June grass (*Koeleria macrantha*), dwarf sagebrush (*Artemisia arbuscula*) and again, mat muhly (*M. richardsonis*), the subject of this study.

Montane *Muhlenbergia richardsonis* and Climate Change

In light of the previous discussions of alpine plant ecology, C_4 ecophysiology, vegetation and climatic zones found in the White Mountains, I would like to focus now on the historical and current elevation range distribution of *M. richardsonis* in the mountains of eastern California. Now growing as high as 3965 meters in elevation, this may be the highest record for this species, and possibly a high altitude record for any C_4 plant in North America (personal observations and Sage and Sage, 2002). These recent

accounts are much higher than reported by published floras that describe the vegetation found within the current study area (see Table 1.5). The flora along the White Mountain elevational transect has been relatively well characterized because of the access and support provided to field biologists by the WMRS facilities since the early 1950's (Hall, 1991). Vegetation surveys by Mooney and others from the 1960's (see, for example, Mooney, 1973)indicate that *M. richardsonis* was not then present at Barcroft station (elevation 3780 m), consistent with range data in table 1.5. It is possible, but seems unlikely, that *M. richardsonis* was present at these elevations but was missed in these earlier surveys. An alternative and compelling possibility that might explain the current high-elevation distribution of *M. richardsonis* is that it has recently migrated up in response to climate change.

Although many environmental variables are sensitive to anthropogenic climate change, pCO_2 and air temperatures are key among these. These two variables are particularly notable for their contrasting effects on C_3 and C_4 physiology (Ehleringer et al., 1997). As discussed before, C_4 plants are generally more efficient at low CO_2 levels but

are more limited by cool temperatures when compared to C_3 plants. Consequently, the relative rates of change in pCO_2 and temperature associated with climate change in alpine zones could change the relative abundances of these photosynthetic types. For example, rapid increases in the partial pressure of atmospheric CO_2 in cold sites could favor C_3 productivity and expansion. Conversely, more rapid increases in growing season temperatures at high elevations with low CO_2 concentrations could favor C_4 productivity and expansion.

Fortunately, there is a wealth of relevant historical herbarium data and climate data for high altitude sites in North America from which to consider these possibilities. Figure 1.14 shows the average annual atmospheric CO_2 from 1958 to 2006 at an elevation of 4169m from Mauna Loa HI. This is the longest running atmospheric CO_2 record at high elevation in the Northern Hemisphere. Because the troposphere is well mixed, both Northern Hemisphere sites (Mauna Loa and Barcroft) are at similar elevations, and both sites are relatively isolated from strong industrial and geological CO_2 sources, the Mauna Loa CO_2 data are

believed to be representative of atmospheric CO_2 concentrations in the alpine zone of the White Mountains.

The temperature data from Barcroft date back to 1956, providing a similar historical window to that of the CO_2 dataset. Figure 1.15 shows growing season temperatures (averages of daily temperature readings for June, July and August for each year) from 1956 to 2006 at an elevation of 3780 m from the Barcroft station in the White Mountains of California. As expected from our current understanding of contemporary climate change, these data indicate a steady rise in both environmental variables over the last half-century (Fig. 1.14 and 1.15).

In order to compare long-term trends in both variables, average decadal values were calculated for the Mauna Loa CO_2 and the Barcroft temperature datasets. For both datasets, these decadal values were normalized relative to initial observations made in the late 1950's. This allows a quantification of the trends in both variables along the same relativized scale. The average annual CO_2 concentration at Mauna Loa relative to initial observations in the late 1950s and growing season temperatures at Barcroft relative to initial observations

in the late 1950's are plotted together in Figure 1.16. This graph indicates that the average atmospheric CO_2 concentration at high elevations in the Northern Hemisphere in the first decade of the 21st Century was about 20% higher than it had been in the mid-20th Century and average summer temperatures at high elevations in California's White Mountains were about 33% higher in the first decade of the 21st Century than they had been in the mid-20th Century. For both datasets, an exponential model gave the best fit to the relativized CO_2 and temperature data. The observation that the exponential rate of increase for temperature exceeds that of CO_2 is consistent with expectations from energy budget models, which predict that increasing CO_2 will have a particularly strong warming effect at high elevations where the atmosphere is dry and 'thin' (Houghton, 2004). This rapid warming trend in the mountains, where atmospheric pCO_2 continues to be potentially limiting for C_3 photosynthesis, suggests the possibility of rapid expansion of C_4 plants into higher elevation sites.

Historical herbarium data were compiled in an effort to examine the validity of this prediction. Figures 1.17,

1.18 and 1.19 show historical trends over the last ~60 years for 3 different C_3 grass species (*Koeleria macrantha, Achnatherum pinetorum* and *Elymus elymoides*). Figure 1.20 provides a similar analysis for *M. richardsonis*. These data come from our own voucher specimens from the Victor Valley Community College herbarium (Victorville, California) along with data accessed in 2006 from the Consortium of California Herbaria website (http://ucjeps.berkeley.edu/consortium/) which provides networked access to herbarium records from several herbaria located in California. Historical herbarium data were taken for six selected mountain counties from Eastern California (Alpine, Fresno, Inyo, Madera, Mono and Tuolumne), capturing a broad range of elevations within a narrow latitudinal belt, spanning portions of the western and eastern slopes of the Sierra Nevada and the west slope of the White-Inyo Mountain range. The herbarium data were lumped into 20-year increments to have as large a sample size as possible while still allowing an analysis of time dependent changes in distribution patterns. Data for the herbarium survey were only collected back to the 1940s because of the lack of reliable records available prior to

this date. Although outside the time scope of this analysis it is important to note that there were 2 observations of *M. richardsonis* at high elevation in the past. Both of these herbarium specimens were collected at a single location in Tuolumne Co. in 1937 in the Sierra Nevada by C. W. Sharsmith. These observations appear to be anomalies and are difficult to explain given the paucity of data from this earlier time period. This historical analysis of herbarium records suggests that among these C_3 species there is no discernable time-dependant trend in elevational distributions over this 60 year interval (Figure 1.17, 1.18 and 1.19). Interestingly, the historical data for *M. richardsonis* suggest that this species has been moving up in elevation in the last 10-20 years, losing territory at lower elevations and gaining ground at higher elevations.

This apparent movement to higher elevation in the C_4 species and the apparent absence of movement in the three C_3 species is qualitatively consistent with predictions based on knowledge of C_4 ecophysiology (Figure 1.2 and 1.3) and the observation that high-elevation temperatures are increasing faster than high-elevation pCO_2 (figure 1.16). There have been numerous observations of C_3 plants moving to

higher elevations in recent decades, apparently due to anthropogenic climate change (Walther et al., 2005; Parmesean, 2006). The apparent upward migration on *M. richardsonis* is novel in this regard because, to my knowledge, no one has documented climate change induced movement of a C_4 plant to higher elevations.

Focus of Study

With this background it should be clear that there is a great deal to be learned from having a better understanding of the ecology of this unusual high-elevation C_4 grass. I would like to orient the reader to what my work can contribute to this effort by outlining the central questions my thesis study has addressed.

(a) It is believed that low temperatures prevent the spread of C_4 grasses to cold alpine habitats. However, if these cold limitations are relaxed with warming climates we might expect C_4 plants to be pre-adapted to tolerating the low pCO_2 by virtue of their carbon concentrating C_4 cycle. I sought evidence in support of this proposal by doing a comparative study of leaf characteristics in *M.*

richardsonis and some co-occurring C_3 grasses along the White Mountain elevation gradient. This question was addressed by comparative analysis in *M. richardsonis* and co-occurring C_3 grasses of stomatal density, foliar nitrogen concentration, and the relative amounts of ^{12}C and ^{13}C in leaf tissue at different elevations. All of these foliar traits have been shown to change with elevation in C_3 species (see table 1.1) but this has not been studied previously in a montane C_4 species.

(b) Field observations suggest that *M. richardsonis*, at moderate elevations in the White Mountains, is able to grow in a variety of microsites but at higher elevations it is restricted to warm microsites, particularly on southerly facing slopes (Sage and Sage, 2002). I tested the proposal that microsite differences are an important determinant of ecological performance at higher elevations by planting out individual plants in different microsite treatments in the White Mountains and following their survival, growth, and reproduction over a two-year period. Regular growing season monitoring of air temperatures and soil moisture was also carried out in a subset of each of the experimental

sites. This study was designed to evaluate the microsite performance of *M. richardsonis* as it reaches its known upper elevational limits.

(c) Comparative studies of growing season phenology for *M. richardsonis* and co-occurring C_3 graminoid species were carried out at different elevational positions in the White Mountains. The central objective of this effort was to determine whether or not *M. richardsonis* exhibits an abbreviated growing season compared to reference C_3 species in the alpine zone.

CHAPTER TWO

ELEVATION EFFECTS ON LEAF CHARACTERS

Introduction

Muhlenbergia richardsonis ((Trin.) Rydb.), growing at nearly 4000 meters in California's White Mountains, is thought to hold the high-elevation record for any C_4 species in North America. Elevation effects on carbon gain characteristics have been repeatedly studied in leaves of C_3 plants (Woodward, 1987; Körner, 1989; Weih and Karlsson, 2000; Qiang et al., 2003) but not C_4 plants. A comparative leaf-level study was done along a 3000-3900 meter elevation gradient in the White Mountains with *M. richardsonis* and co-occurring C_3 graminoid species. The objectives were to examine, for the first time, elevational trends in foliar carbon gain characteristics in an alpine C_4 grass (*M. richardsonis*) referenced against co-occurring C_3 graminoid species to better understand how carbon gain physiology may be affected in C_4 plants by environmental factors along elevational gradients. In addition, a C_3 vs. C_4 comparison may help clarify proximal causes of the well-documented elevational changes in foliar traits that occur in C_3

species. It was hypothesized that the C_3 grasses would respond to increasing elevation in the White Mountains with increases in stomatal density (SD) and leaf nitrogen (%N), and with reduced photosynthetic discrimination against $^{13}CO_2$, as indicated by an increase in $\delta^{13}C$ values. These predictions were based upon the assumption that the elevation-dependent reduction in atmospheric CO_2 is the primary driver of elevation-dependent changes in SD, %N and $\delta^{13}C$ values frequently observed in C_3 species. In C_4 species, the carbon-concentrating C_4 cycle results in photosynthetic saturation at much lower partial pressures of atmospheric CO_2 (pCO2) than in C_3 species. Accordingly, it was hypothesized that these same foliar characters would not vary with elevation to the same degree in *M. richardsonis* as in the reference C_3 species. Results are interpreted in the context of how anthropogenic climate change may be expected to affect the C_3 vs. C_4 composition of high-elevation plant communities.

Materials and Methods

To address the hypothesis concerning foliar trends in the C_4 grass *M. richardsonis*, three co-occurring C_3 species

were selected that grow along the same elevational gradient in the White Mountains of California as reference species. These C_3 graminoid species were *Koeleria macrantha*, *Achnatherum pinetorum* and a common unidentified alpine sedge species referred to here as *Carex sp.* These three reference C_3 species were selected because they are commonly found growing in close proximity with *M. richardsonis* within each of selected elevation sites in the White Mountains. The White Mountain elevational gradient was chosen because of the abundance of the selected study species and because of the invaluable infrastructure support provided by the White Mountain Research Station (WMRS) system of field stations adjacent to the sampled plant populations.

The elevational gradient for all study species was determined by the lowest observed occurrence of *M. richardsonis* at 3060 meters and the highest known occurrence at 3965 meters along the White Mountain gradient. Sample intervals were determined by observation of all species being present in close proximity at any one elevation. For the 2005 growing season, sample intervals were 3060 m, 3515 m and 3780 m for all four study species.

For the 2006 growing season the number of sample elevations was expanded to include six sites at the following elevations; 3060 m, 3273 m, 3515 m, 3636 m, 3780 m and 3965 m for *M. richardsonis* (C_4) and *K. macrantha* (C_3) only.

Preliminary anatomical studies during the 2005 growing season revealed that among the four selected study species, only the target species *M. richardsonis* (C_4) and one of the reference C_3 species, *K. macrantha*, were readily amenable to accurate determinations of stomatal density. A complete survey of stomatal density for these two species was not possible during 2005 and so stomatal density data are only reported for the 2006 growing season. For both species in 2006, five random plants at each elevation site were selected at distances of at least 10 meters apart. These sampling distances for plants at each elevation were used to minimize the risk of pseudoreplication due to clonal spread and/or microsite effects. Fresh green material from fully enlarged leaves was clipped from selected plants and put into Zip-Lock bags to keep fresh for light microscopy studies. Sampling from both species for each of the six elevation sites took place over a two-day interval at mid-growing season in the summer of 2006. Individual leaf

samples were examined microscopically on the same day they were harvested. Individual leaves were mounted on a standard glass microscope slide with a drop of Biomeda Gel/Mount (Biomeda corporation, catalog number MU1) and cover slip and viewed at 400x on a compound light microscope (Olympus model CH30RF100). Five leaves per species from each elevation sampling site were examined. Five random areas of each leaf were viewed and the number of stomates within the field of view was tallied. The area of the field of view was determined with a calibrated stage micrometer. Stomatal density data were recorded as stomata number per square millimeter of leaf area. Stomatal density was assessed for both adaxial and abaxial leaf surfaces in both species.

Determinations of both nitrogen content and $\delta^{13}C$ values were made for dried leaf tissues from all four study species (*K. macrantha, A. pinetorum , Carex sp.* and *M. richardsonis*) sampled from plants at each of three elevational sample sites in 2005 (2005 sampling elevations listed above). For each species, five random plants were selected at a minimum separation distances of 10 meters apart at any given site. Five sampled plants from each of

three sites in 2005 yielded a total N of 15 per species for both %N and $\delta^{13}C$. In 2006, %N and $\delta^{13}C$ sampling focused on the same two study species that proved amenable to SD determinations, *M. richardsonis* and *K. macrantha*. The same sampling protocol was used but the elevation sampling intensity was increased to six elevation sites (2006 sampling elevations listed above). Five sampled plants from each of six sites in 2006 yielded a total N of 30 per species for both %N and $\delta^{13}C$. From the selected plants, leaf material was clipped from each plant and put into labeled envelopes for oven-drying at 60°C for 4 days. Sample processing involved grinding dry leaf material in a Wiley mill (Thomas Scientific, model 3383L10) until fine enough to pass though a number 40 sieve. Approximately 2.0 µg of powdered leaf material was weighed into 5 mm x 8 mm tin capsules (Elemental Microanalysis, number D1008). All packaged samples were sent to the Stable Isotope Facility at the University of California in Davis, California for determination of leaf %N and $\delta^{13}C$ values. Duplicate sub-samples from a subset of leaf samples from individual plants were included to assess measurement and/or instrument error. These effects proved to be minimal and

statistically indistinguishable. Consequently, leaf %N and $\delta^{13}C$ values from duplicate sub-samples were averaged for data analyses.

Linear correlation analyses were applied to all data sets using Data Desk statistical software (version 6.2.1, Date Description Inc., Ithaca, NY) where elevation was consistently treated as the independent variable. Linear correlations were considered significant at $p<0.05$.

Results

Stomatal Density

Across all elevations, *K. macrantha* proved to be consistently amphistomatous and *M. richardsonis* proved to be consistently hypostomatous. Average stomatal densities for *K. macrantha* across all plants at all elevations were 58.0±7.3 SD and 58.4±7.4 SD per square millimeter on adaxial and abaxial surfaces, respectively. Average stomatal densities for *M. richardsonis* across all plants at all elevations were 0 and 187.9±10.7 SD per square millimeter on adaxial and abaxial surfaces, respectively.

Figure 2.1 shows the stomatal densities summed across both surfaces for both species plotted against sampling

elevation. These results indicate that stomatal density varied with elevation in the C_3 grass *K. macrantha* but not in the C_4 grass *M. richardsonis* (Table 2.1).

Foliar Nitrogen Concentration

At each elevation for a given growing season (2005 or 2006), *M. richardsonis* consistently had lower leaf nitrogen concentrations on a dry mass basis than any other species (Fig 2.2). For example, in 2005 the average %N across all elevations for the C_4 species *M. richardsonis* was 1.3% whereas leaf %N averages for the C_3 species *K. macrantha*, *A. pinetorum*, and *Carex* sp. in 2005 were 1.7%, 1.5%, and 1.5%, respectively.

There was a significant positive correlation between elevation and leaf %N for all four study species, regardless of photosynthetic pathway, in 2005 (Fig 2.2a and Table 2.2). In 2006, the correlation between elevation and leaf %N was not significant for either species, regardless of photosynthetic pathway (Fig 2.2b and Table 2.3).

Carbon Isotope Values

At each elevation in both 2005 and 2006, foliar $\delta^{13}C$ values differed substantially between *M. richardsonis* and the other study species (compare Fig 2.3 with Fig 2.4). Observed foliar $\delta^{13}C$ values of approximately -16‰ in *M. richardsonis* fall within the range of expected values for C_4 plants (Fig 2.3). Observed foliar $\delta^{13}C$ values of approximately -27‰ in the other graminoid species are consistent with expected values for C_3 plants (Fig 2.4).

There was no significant correlation between elevation and $\delta^{13}C$ in 2005 or 2006 in *M. richardsonis* (Fig 2.3 and Tables 2.2 and 2.3). In general, for both 2005 and 2006, $\delta^{13}C$ values increased with elevation in each of the C_3 species although this trend was only marginally significant for *K. macrantha* during the 2005 growing season (Fig 2.4 and Tables 2.2 and 2.3).

Discussion

Trade-offs in photosynthetic efficiency between C_3 and C_4 plants will tend to play out differently depending upon interactions between daytime leaf temperatures and relative availability of atmospheric CO_2 (Ehleringer et al., 1997).

Key to this trade-off is the difference in carbon-fixation efficiencies between the two different primary carboxylating enzymes, ribulose bisphosphate (Rubisco) in C_3 plants, and phosphoenol pyruvate carboxylase (PEPCase) in C_4 plants. Competitive oxygenation reactions at the carboxylation site of Rubisco acts as the initial step in photorespiration. Photorespiration lowers the overall efficiency of C_3 photosynthesis but this loss in efficiency drops off with decreasing temperatures (Ehleringer and Björkman, 1977; Skillman, 2008). By contrast, PEPCase, the primary carboxylase in C_4 plants, does not undergo competitive oxygenation reactions and C_4 carboxylation efficiency is relatively insensitive to temperature. Additionally, compared to Rubisco, PEPCase has a high affinity for its inorganic C substrate HCO_3-. Consequently, C_4 plants do not suffer photorespiratory drains on photosynthetic efficiency to any appreciable extent and C_4 carbon fixation can continue to operate at maximum rates at a lower pCO_2 than C_3 plants. Thus, a relatively modest enzymological difference can make a large difference in the predicted ecologies of otherwise similar C_3 and C_4 plants. Assuming these differences scale up to affect long-term

competitive outcomes, C_3 plants should thrive where atmospheric CO_2 is abundant and/or where cool temperatures minimize photorespiration-based inefficiencies. C_4 plants should thrive where atmospheric CO_2 is limiting to C_3 plants and/or where high temperatures would lead to high photorespiratory losses in C_3 plants.

The significance of this temperature and CO_2 interaction for C_3 and C_4 ecology and biogeography has repeatedly been considered in the context of past and future climate change since temperature and CO_2 broadly co-vary over geological time scales (e.g., Ehleringer et al., 1991; Henderson et al., 1995; Sage and Kubien, 2003). There has been less consideration of how these factors may interact to affect the distribution of C_3 and C_4 plants along elevational gradients where temperature and CO_2 partial pressures also co-vary (Körner, 2007). My work is the first to evaluate these leaf level characteristics in a C_4 plant along an elevational gradient.

Stomatal Density

The stomatal density of a C_3 plant, in general, increases along with altitude as a species moves up elevational gradients. For instance, Woodward (1987)

working with *Vaccinium myrtillus* along an elevational gradient in central Scotland found that stomatal density was higher from populations at higher elevations than those sampled from lower elevations. Another study from the Qilian Mountains in China shows a similar trend (Qiang, et al., 2001). *Picea crassifolia*, growing along an elevational gradient up to 3000 m showed increases in stomatal density with increasing elevations (Qiang et al., 2001). This increase in stomatal density at higher elevations is thought to be a response to the relative decrease in available CO_2 for photosynthesis. The conventional explanation for this commonly observed trend in leaf anatomy is that C_3 plants increase the number of stomates on the leaf surface to help compensate for the reduced pCO_2 at high elevations. The present study provides evidence for the validity of this explanation because the C_4 carbon-concentrating mechanism will overcome any diffusion limitations that would otherwise be expected to hinder C_3 photosynthesis.

As expected, along the White Mountain elevation gradient, sample populations of *Koeleria macrantha* (C_3) increased their stomatal density as elevation increased.

On the other hand, sample populations of *M. richardsonis* (C_4) did not change stomatal density as elevation increased. These findings support what is currently known about C_3 species regarding increasing stomatal densities as pCO_2 decreases with increasing elevation. The findings in regard to the C_4 species, *M. richardsonis*, are new and support the notion that the C_4 carbon concentrating mechanism compensates for the reduction in available atmospheric CO_2 for photosynthesis at high elevations.

Foliar Nitrogen Concentration

The foliar nitrogen concentration of a C_3 plant, in general, increases along with altitude as a species moves up elevational gradients. Körner (1989), reviewed several studies documenting leaf nitrogen concentrations along elevation gradients from mountain zones around the globe. He found that in Sweden (subarctic zone), in the Austrian Alps (temperate zone) and in Papua New Guinea (tropical zone) that as elevation increases so does %N found in the plants growing at those higher elevations. The results for the 2005 growing season are consistent with this commonly observed pattern in that all C_3 species showed a significant increase in nitrogen concentration with elevation (Fig

2.2a, Table 2.2). In principle, an increase in %N might reflect a biochemical compensation to deal with either reduced pCO_2 or lower growing-season temperatures. My observation that *M. richardsonis* also showed increasing nitrogen concentration with elevation is strong evidence that this commonly observed pattern is a temperature effect rather than a pCO_2 effect. This interpretation is further supported by numerous controlled experimental studies on C_3 plants. For example, Tissue et al. (1995) grew *Abutilon theophrasti*, a C_3 species, under a broad range of atmospheric pCO_2 (15-70 Pa CO_2) and found leaf nitrogen concentration was essentially constant across these different treatments. However, under constant CO_2 levels, Weih and Karlsson (2000) showed that Mountain birch (*Betula pubescens*) plants grown at cold temperatures (9.5°C) had higher foliar nitrogen concentrations than in plants grown at warmer temperatures (13.6°C). Thus, whereas the contrasting stomatal density response to elevation between C_3 and C_4 plants appears to reflect differences in pCO_2, the parallel leaf nitrogen response to elevation in C_3 and C_4 plants in 2005 appears to reflect differences in air temperatures.

Interestingly, the elevation effect on %N differed between the two study years (cf Figure 2.2a and b, Tables 2.2 and 2.3). In contrast to observations for 2005, there was no significant effect of elevation on %N for either *K. macrantha* (C_3) or *M. richardsonis* (C_4) in 2006. It is likely the differences between years are due to other environmental factors that were not accounted for in this study. Long term (1970-2007) winter (Sept-May) precipitation for the Owens Valley is 115 (+/-57) mm each year (data accessed from Western Regional Climate Center, www.wrcc.dri.edu, on May 2008) (Table 2.4). Winter precipitation for 2004-2005 was above average at 226 mm but in 2005-2006 it was only 128 mm. Thus, it is possible that the abundance of water in the 2005 growing season allowed an expression of elevational effects on leaf nitrogen that was not possible under the more water limiting conditions of the 2006 growing season. Regardless of the explanation for the differences between the two years, the fact that there was no C_3 vs. C_4 contrasting nitrogen response to elevation in 2005 or 2006 supports the idea that temperature, rather than pCO2, has a strong impact on leaf

nitrogen budgets along elevational gradients, independent of differences in photosynthetic pathway.

Carbon Isotope Values

In C_3 plant species it has been shown that as elevation increases the relative content of ^{13}C increases in the leaf tissues of that plant. A global survey by Körner et al. (1988), found that among closely related C_3 species, $\delta^{13}C$ values for lowland plants tended to be around -30 to -27‰ whereas they tended to be around -27 to -24‰ at high elevations. The conventional explanation for this commonly observed trend in leaf isotopic composition is that C_3 plants remain diffusion limited for carbon fixation at high elevation despite any anatomical or biochemical compensatory responses to elevation dependent declines in atmospheric CO_2 (Körner et al., 1988). The present study provides evidence for the validity of this explanation because the C_4 carbon-concentrating mechanism overcomes any diffusion limitations that would otherwise be expected to affect the $\delta^{13}C$ of C_3 foliage.

As expected, the $\delta^{13}C$ value increased with elevation in all C_3 grasses in the 2005 growing season (Fig 2.4, Table 2.2). Similarly, a marginally significant trend for the C_3

grass *K. macrantha* increased $\delta^{13}C$ along the same elevational gradient was observed in 2006 (Fig 2.4, Table 2.3). By contrast, the C4 grass *M. richardsonis* surveyed along the same elevational gradient showed no significant increase in $\delta^{13}C$ for either growing season (Fig 2.3, Table 2.3). This new finding confirms the prediction that the C_4 photosynthetic pathway should largely compensate for the reduction of diffusible atmospheric CO_2 at high elevations.

In conclusion, this study is believed to be the first to show the effects of elevation on foliar traits in a C_3 and C_4 grass along the same elevational gradient. This study found that leaf stomatal density and $\delta^{13}C$ increased with elevation as expected in the C_3 plants but were unresponsive to elevation in *M. richardsonis*. We interpret the C_3 vs. C_4 contrast in stomatal density and $\delta^{13}C$ responses to mean that C_4 photosynthesis is not limited by the low pCO_2 present at high elevation and does not even bear any obvious plastic adjustments to the declining CO_2 conditions. This suggests that as temperatures warm with contemporary anthropogenic climate change, C_4 grasses may be expected to move readily into high-elevation ecosystems. These findings also support the proposal that declining pCO_2 is

the main environmental driver of these elevation-dependent changes often reported for C_3 species. This study also found that %N increased with elevation in foliage of all species during one study year but there was no elevation effect on %N during the other study year. Thus, there is year-to-year variation in the elevation effect on leaf nitrogen budgets but, in both cases, elevation effects on %N was independent of photosynthetic pathway differences. The similar trend in %N for C_3 and C_4 leaf tissues suggest this is a response to altitudinal variables other than pCO_2, most likely declining temperatures, which should affect C_3 and C_4 plants similarly.

CHAPTER THREE

MICROSITE EFFECTS ON PHENOLOGY AND PLANT PERFORMANCE

Introduction

The basis for the fact that C_4 plants are generally restricted to warm, low-elevations is not well understood. It has been suggested that the C_4 pathway arose among cold-intolerant tropical lineages that have not yet evolved the suite of traits required for cold-tolerance at high elevations (Sage, 2004; Edwards and Still, 2008). It is possible that *Muhlenbergia richardsonis* is among the first C_4 species to evolve these C_3-like cold-tolerances. If true, then there would be no clear reason to expect *M. richardsonis* to differ in growing season phenology from that of co-occurring C_3 species. Indeed, it is widely believed that the short montane growing seasons have selected for convergence in seasonal phenology among otherwise disparate groups of plants in various alpine habitats (Mooney and Billings, 1968).

An alternative proposal seeking to explain the paucity of C_4 species at high elevations holds that there is

something intrinsic to C_4 photosynthesis that unavoidably limits these plants to warmer habitats and microsites (Pittermann and Sage, 2000). The work of Sage and Sage (2002) is interesting in this regard. Their study was the first to document the presence of *M. richardsonis* at unexpectedly high elevations in California's White Mountains. At the same time, this report showed that this grass was restricted to warm, south-facing slopes at the highest study sites. In addition, they found that its short stature allowed it to achieve leaf temperatures substantially warmer than air temperatures or then leaf temperatures of taller C_3 species in the same sites. The restriction of *M. richardsonis* to these low-lying warm microsites in the alpine zone argues against the notion that this species shares similar levels of cold-tolerance with that of C_3 species found in the same communities.

With this background in mind, this chapter reports results of studies designed to test two relevant hypotheses. The first hypothesis is that *M. richardsonis* will exhibit a shorter growing season phenology than that of co-occurring C_3 species. Validation of this hypothesis would provide evidence suggesting that *M. richardsonis* is a

warm-season specialist as expected for a typical C_4 cold-intolerant plant. The second hypothesis is that *M. richardsonis* will exhibit greater ecological success when planted in warm, compared to cold, alpine microsites. This would provide evidence in support of a hypothesis put forth by Sage and Sage (2002) that the presence of this cold intolerant C_4 species can, in part, be explained by localization to warm microsites where temperatures favorable for C_4 photosynthesis are realized.

To examine the hypothesis that growing season phenology was affected by both photosynthetic pathway and elevation, phenological observations were made during 2005 and 2006 growing seasons for *M. richardsonis* and three common co-occurring C_3 graminoid species at three positions along the White Mountain elevational gradient. To address the hypothesis regarding microsite performance of *M. richardsonis* at high elevations, two experimental sites were selected; one high (3780 m) and one low (3060 m). At both elevations *M. richardsonis* was planted on either north-facing slopes or south-facing slopes. Within both slope treatments, *M. richardsonis* was planted in the open or sheltered among large rocks. These experimental

plantings were monitored for two growing seasons (2005 and 2006). Growing-season air temperatures and soil moisture status were monitored at representative plots from each of the microsite treatments as well.

Materials and Methods

Phenological Study

Three sites for the observation of seasonal development were selected using the following criteria: all four study species had to be present within close proximity of one another and the sites had to have reasonable access along the White Mountain elevational gradient. The elevations of the sites were 3060 m (near Crooked Creek Station), 3515 (Sheep Pass) and 3780 (near Barcroft Station). In 2005, observations began on June 9 of 2005 at the two lower sites and on July 11 at the highest (3780 m) site. This delay was due to blocked access from persistent snow banks. Observations were made at approximately two-week intervals though September 23rd of that year. In 2006 phenological data were recorded at approximately two-week intervals starting June 9 and finishing August 19. Species observed at each elevation were *M. richardsonis*, *Koeleria*

macrantha, Achnatherum pinetorum and a common unidentified *Carex* species. All individual plants for observation were selected haphazardly at each elevation with each individual of a given species located at least 10 m away from others at the sample site. This sampling regime was designed to minimize the possibility of pseudoreplication among ramets or among individuals in a common microsite. Nine or ten plants from each species were observed at each site on each census date for a total of 36-40 observations per sample site per census date and 108-120 observations across all sites per census date. Phenological growth stages were recorded for each individual plant on each census date. Identified phenological stages were based on prior observations of vegetative and reproductive characters for each species. All observed individuals, on each of the respective census dates, were classified into the following 7 ordinal phenological stages: initial (less than 50%) spring greening (stage 1), fully (over 90%) green (stage 2), initial (bud) flowering (stage 3); peak flowering (stage 4); seed set (stage 5); ripe seed/seed drop (stage 6); autumn browning/senescence (stage 7).

A seasonal rate of development was estimated from the slope of the best-fit-line of the phenological stage regressed against the day of the year. These estimates of the seasonal development rate were used in a second set of regression analyses to test the hypothesis that the growing season for a species is inversely related to elevation. In order to test for contrasting phenologies among species, seasonal development rates from each of the three elevation sites were treated as independent estimates for each species.

Microsite Study: Plant Propagation and Establishment

For the microsite study, *M. richardsonis* plant material was collected in the summer of 2003 and then propagated under greenhouse conditions prior to planting in the experimental sites. Collection of native *M. richardsonis* plant material was made near the Barcroft research station at an elevation of ~3780 m. Small plugs containing several tillers of *M. richardsonis* were removed from selected isolated populations and placed in plastic bags for transport to the California State University San Bernardino (CSUSB) greenhouse for propagation. Individual plugs were separated and tillers planted in commercial

potting soil (Supersoil) in Deep Pots (Steuwe and Sons, Inc, Oregon. model D40H) for a total of 260 individual plants. Plants were watered and fertilized as needed at the CSUSB greenhouse.

Early in the 2004 growing season these greenhouse-grown potted plants were transported to the White Mountains and were allowed to acclimate for several days outside at the Barcroft station before transplanting into field plots. Experimental growing sites were selected to test the effects of elevation, slope aspect, and rock sheltering on plant performance in the field. At each site, planting was done by using a large hand-held auger to make a planting hole in the rocky soil. Plants were immediately watered in after planting and clipped to within one centimeter of the soil surface. Clipping was done to minimize transplant shock and to delineate new growth following the establishment of the microsite treatments. To help facilitate establishment, plants were frequently watered during the remainder of the 2004 growing season.

These plantings were made in pre-selected sites. Two elevations were selected, one high, near the Barcroft field station (3780 m), and one low, near the Crooked Creek field

station (3060 m). At each elevation, five sites (experimental blocks) were selected that had both a north and south slope aspect, for a total of 10 blocks in the entire study. Each block was planted initially with 24 plants for a total of 240 plants in the entire study. On each of the two slopes, within a block, 12 individuals were planted. To establish rock sheltering treatments, large native rocks (granitic, approximately cuboidal, > 15 cm in mean diameter) were placed in a circle immediately adjacent to half of the new plants (rock-sheltered treatment). For the remaining individuals, all rocks and other materials were cleared from the immediate vicinity of the plants (no rock-sheltering treatment).

Microsite Study: Environmental Factors

At both elevations a representative planting block was selected to monitor growing season air temperature. Growing season temperatures were measured in 2005 (August 24th - September 9th) and 2006 (July 25th - August 7th) on both north- and south- facing slopes from one representative block at high (3780 m) and low (3060 m) elevation. At each elevation, a data logger (Campbell Scientific, Inc Logan, Utah, model CR23X micrologger) was

placed in the representative block for simultaneous temperature collection from each selected block. Thin wire thermocouples connected to the datalogger were used to record ambient air temperatures at 12 minute intervals in close proximity to selected experimental plants throughout the growing-season sampling intervals. The end of the thermocouple was shielded from direct sunlight by placing it in an open ended, 1-½ inch diameter, gray plastic tube, 3 inches long. Each slope aspect (north and south), had six thermocouples, three near plants with rock-sheltering and three near without rock-sheltering for a total of twelve thermocouples at each elevation.

Soil moisture was determined by collecting fresh soil samples from all planting sites in two representative blocks from each elevation. A soil corer was used to collect the upper 2 cm of soil from all 24 plots contained in each of the selected blocks. Soil samples were placed in labeled coin envelopes that were then placed in Zip-Lock plastic bags for storage until they could be weighed. The fresh soil samples were weighed within two hours after collection from each site. Soil samples were then placed in a drying oven at 60°C for 48 hours to remove all moisture

and weighed a second time to determine the soil dry weight. Soil moisture was calculated as fresh weight-dry weight/dry weight and is expressed as mg H_2O per g soil. A total of 48 samples were collected from each elevation.

Microsite Study: Statistical Analysis

Data for plants and planting blocks at the low-elevation site were not statistically analyzed (beyond basic descriptive statistics) because poor survivorship of these plants resulted in very low sample sizes. High survivorship among plants from the high-elevation site permitted statistical hypothesis testing. Analysis-of-Variance (ANOVA) models were run on data from plants and blocks from the high-elevation site to test effects of block, slope, and rock-sheltering on plant performance variables. In these ANOVAs, rock-shelter treatments were nested within slope-aspect treatments which, in turn, were nested within planting blocks. Response variables were log transformed where necessary to conform to ANOVA assumptions (Keppel et al. 1992). No block effects were detected. Therefore, all block data were pooled for the reported high-elevation results. Residual analyses were used to look for, and eliminate, outliers prior to running the

statistical models. ANOVA models were run again for mass, height, and inflorescence number (log transformed where necessary) where slope and rock-sheltering were treated as main factors. Rock-sheltering treatments were nested in slope-aspect treatments as before. Survivorship ANOVAs were based on percent survivorship within each block. Unless stated otherwise, all measures of variation are reported as Standard errors of the mean (+/- 1.0 S.E.M.).

Regression analyses were performed to look for any association between average maximum growing season air temperature and plant performance variables across microsite treatments. Similarly, regression analyses were performed to look for any association between available soil moisture and plant performance variables across microsite treatments.

Results

Phenological Study

Three of the four species (*Achnatherum pinetorum*, *Koeleria macrantha* and *Muhlenbergia richardsonis*) selected for the phenology study progressed through each of the growth stages in a regular sequence at each of the three

sites and for both 2005 and 2006 (Figure 3.1 and 3.2). The selected *Carex* species tends to be evergreen and only rare individuals ever flowered during both growing seasons (Figures 3.1 and 3.2). Consequently, data for this species are of limited use for comparison to the phenology of the other three species (Figures 3.1 and 3.2). Phenology data for *Carex* spp are included in Figures 3.1 and 3.2 but were omitted from statistical tests designed to look for elevation and/or species effects on seasonal development rates.

For the three species that proceeded through a regular developmental sequence, there is a strong linear association between the ordinal values assigned to each phenological stage and day of the year (Figures 3.1 and 3.2). This indicates that individuals of each of these species at a given elevation spend about the same amount of time in each of the respective phenological stages. Slopes from the regression analyses are interpreted as indices of growing-season development rates. There is no consistent trend for a faster rate of seasonal development with increasing elevation for either 2005 or 2006 data (Tables 3.1 and 3.2). Seasonal development rates for a species

from each of the three elevation sites were pooled in order to test for species differences in phenology.

Observations of plant phenology in 2005 suggest that there were differences among species in their seasonal rates of development that may be related to differences in photosynthetic pathway. Early in the 2005 growing season, *M. richardsonis* plants were at an earlier stage of development than co-occurring *K. macrantha*, *A. pineortorum*, or *Carex sp.* For example on day 192 (July 11, 2005; Figure 3.1) at each of the three elevations, the three C3 species were fully green (stage 2) and many individuals had already initiated flowering (stage 3). By contrast, *M. richardsonis* plants had not completed spring greening (stage 2) and none of the plants had yet begun to flower (stage 3). But, by the end of the 2005 growing season, *M. richardsonis* had, at all elevations, reached a more advanced phenological stage than any of the other species. This can be seen on day 266 (September 23, 2005; Figure 3.1) where most of the individuals of the C_3 species were still in a reproductive phase (phase 5 and 6) whereas many *M. richardsonis* plants had already dropped their seeds and were beginning to go into fall dormancy (stage 7). During the 2006 growing

season, observations began earlier in the year and again *M. richardsonis* exhibits a delayed onset of development at all elevations against all other species (Figure 3.2). The observations in 2006 ended much earlier than those during 2005, precluding year-to-year comparisons for late season development.

Microsite Study: Environmental Factors

During the 2005 growing season, the maximum daily air temperature was significantly influenced by sample day, elevation, and rock-sheltering (Figure 3.3 and Table 3.3). Some days were warmer than others. Lower microsites were warmer than those from high elevation. Microsites that had the rock-shelter treatment tended to be warmer than those without the rock treatment. Slope-aspect did not have a significant effect on temperature in 2005 (Table 3.3). Similar findings were made for the 2006 growing season (Figure 3.4 and Table 3.4).

Soil moisture sampled on August 23, 2005 was significantly influenced by elevation and rock treatment, but not slope-aspect (Figure 3.5a and Table 3.5). Higher elevation sites had greater amounts of soil moisture than those sites sampled from low-elevation sites. Rock-

sheltered sites had higher levels of soil moisture than those sites without rock-sheltering. Soil moisture sampled from each of the eight microsite treatments on September 10, 2005 yielded qualitatively similar results even though the overall moisture content was substantially reduced later in the year (Figure 3.5b and Table 3.6).

Microsite Study: Plant Performance

Poor survivorship of transplanted *M. richardsonis* at the low-elevation site (Crooked Creek 3060 m) resulted in small sample sizes unsuited for testing for treatment effects. Nevertheless, results presented in Table 3.7, suggests that plant performance measures (above ground biomass, plant height, and survivorship) are enhanced by the presence of the rock-sheltering on both north and south facing slopes. Survivorship at the high-elevation site (Barcroft 3780 m) was good and all measures of plant performance were suitable for testing for treatment effects.

Accumulated 2005 aboveground biomass per plant at the high-elevation site was significantly greater in rock-sheltered plots than in exposed plots (Figure 3.6 and Table 3.8). Plants on south-facing slopes tended to have

accumulated more aboveground biomass than those on north-facing slopes but this difference was not significant. Height of *M. richardsonis* plants at the high-elevation site was significantly greater in the rock-shelter plots than in the exposed plots (Figure 3.7 and Table 3.8). There was no detectable influence of slope-aspect or rock-sheltering on inflorescences per plant in *M. richardsonis* plants at the high-elevation site (Figure 3.8 and Table 3.8). Finally, overall survivorship of *M. richardsonis* plants at the high-elevation site was significantly greater in the rock-sheltered plot than in the exposed plots (Figure 3.9 and Table 3.8).

Regression analyses of plant performance from the four high-elevation microsite treatments against average observed maximum daily air temperature for the 2005 growing season were run to test for an association between temperature and phenological performance of this C_4 grass. Maximum temperature only explained 50-70% of the observed variation in plant performance characters at the high-elevation sites (Figure 3.10-3.13). Nevertheless, the trends were consistently positive wherein plant growth, survival, and reproduction increasing with temperature.

However, data from the low-elevation sites indicate factors other than temperatures are important determinants of plant performance too. Surprisingly, plants did poorly at these warm, high-elevation sites.

Regression analyses of available soil moisture, as measured on August 23, 2005, against these same measures of plant performance (biomass, height, reproductive effort and survivorship), averaged for each of the four high-elevation microsite treatments are presented in Figures 3.15-3.18. All measures of plant performance for the 2005 growing season were positively associated with this measure of soil water availability. Soil moisture, in most cases, explained approximately 90%, or more, of the observed variation in plant performance characters at the high-elevation sites (Figure 3.15-3.18). Although not included in the regression analyses, the scatter plots demonstrate the data from the low-elevation sites follow these same trends. Similar results were found when mean plant performance values were compared to soil moisture measured on September 10, 2005 (data not shown, but see Figure 3.5).

Discussion

Phenological Study

Alpine plants can avoid exposure to low temperature extremes though morphology (e.g., cushion plants), phenology (e.g., rapid development during the brief warm season), or microhabitat specialization (e.g., sheltered microsites). Because it is a C_4 species, *M. richardsonis* is assumed to be especially cold-intolerant compared to other graminoid species in the alpine zone of the White Mountains. This study was designed to look for the existence of either phenological specialization to the warm season in *M. richardsonis* or a warm microsite requirement of *M. richardsonis*. During both the 2005 and 2006 growing season, we were unable to detect any effect of altitude on phenological development along the White Mountain elevational gradient (Figure 3.1 and 3.2, Table 3.1 and 3.2). This is possibly due to all of the sample sites having a relatively short growing-season and all plants already exhibiting rapid rates of seasonal development typical of alpine plants. (Körner 2003; Billings and Mooney, 1968).

Focusing on the 2005 data, because it characterized the entire growing season, *M. richardsonis* at all elevations consistently exhibited the fastest rate of seasonal development, where it started growing later in the spring than the reference C_3 species and began senescing earlier in the fall than the reference C_3 species (Figure 3.1 and Table 3.1). It appears that *M. richardsonis* has a delayed start of development early in the season, but an accelerated development once rate conditions for C_4 growth improve. This is consistent with the general observation that C_4 grasses are warm-season specialists and implies that *M. richardsonis* will grow only where it is warm enough to achieve this temperature-dependent rate of development in the alpine zone (Monson and Williams, 1982; Ehleringer and Monson, 1987).

Microsite Study

As expected at low elevation we found warmer maximum daytime temperatures than those found at high elevation (Figure 3.3 and 3.4). Of the environmental characteristics surveyed, it is surprising that we did not detect an effect of slope-aspect on temperature at either elevation (Table 3.3 and 3.4). It is possible that the slopes selected for

the experimental plantings may have been too shallow to elicit the anticipated aspect effect on solar warming (Oke, 1987). Another possible explanation may be due to the windy conditions found at the high altitudes causing homogenous thermal mixing of the air. Rock-shelter plots were, on average, warmer than exposed plots (Tables 3.3 and 3.4). Dark native rocks used in the shelter plots could explain these enhanced daytime temperatures by absorbing and re-emitting solar radiation that would not have been possible in adjacent exposed plots. In addition, it is possible that the rock treatments enhanced pooling of warm air in the sheltered pockets. Based on these results and assuming that plants do better in warm compared to cool microsites, one might predict that the experimental plants would have performed better at low compared to high elevations and sheltered among rocks compared to the exposed treatment. Surprisingly, it was not consistently true that plants in warm microsites outperformed plants in cool microsites. Although, warmer plants did do better than cooler plants at the cool, high-elevation sites the effect of elevation ran counter to this trend. It is surprising that a C_4 grass did so poorly at warmer low-elevation sites. However, in the

arid mountains of eastern California, warmer sites are also drier sites. Thus, the counter intuitive elevation effect on plant performance shown in Figures 3.10-3.14 may actually represent the influence of soil moisture on *M. richardsonis*.

As for soil moisture, both sample days (August 23 and September 10, 2005) had the same qualitative treatment trends as observed for maximum temperatures, where rock-sheltering and elevation had significant effects but slope aspect did not (see Figure 3.5). Elevational effects on moisture were as predicted; it was wetter at cool high-elevation sites than at warm, low-elevation sites (Table 3.5 and 3.6). From an energy budget perspective, the absence of a slope-aspect effect on soil moisture is consistent with the absence of a slope-aspect effect on maximum daytime temperature. Plots sheltered by rocks tended to be wetter than those without. Those plots that were rock-sheltered may have had greater soil moisture due to reduced evaporation caused by the shading of the rocks and/or by rock-shelter enhancement of the aerodynamic boundary layer. Based on these results, and assuming that plants do better in moist compared to dry microsites, one

might predict that the experimental plants would have performed better at high compared to low elevations and sheltered among rocks compared to the exposed treatment.

Interestingly, this prediction was well supported. Regression analyses in Figure 3.15-3.18 provide strong corroborative evidence for the predicted relationship between water availability and plant performance. Growth, survival, and reproduction for the entire 2005 growing season were all strongly and positively associated with soil moisture as measured on a single day (August 23, 2005). It seems likely that more integrated, long-term measures of water availability at the different sites would only have reinforced this pattern.

Conclusion

This work sought to verify that *M. richardsonis* in the alpine zone behaves as a warm-season C_4 grass by comparing its summer phenology to that of co-occurring C_3 species. Unfortunately, one of the reference C_3 species (*Carex* spp.) did not serve well for phenological contrast. However, compared to *M. richardsonis*, the other two C_3 species did exhibit longer growing seasons and a slower rate of summer development. This result is consistent with the proposal

that *M. richardsonis* is comparatively cold-intolerant, as expected for a C_4 species, and that it is able to avoid the coldest part of the growing season by restricting its activity, to the mid-summer when temperatures are warm enough for C_4 photosynthesis.

This work also sought to evidence to support the proposition that *M. richardsonis* only persists in the alpine zone by growing in the warmest of microsites. Unexpectedly, experimental findings indicated that temperature *per se* was only a modest predictor of *M. richardsonis* growth, survivorship and reproduction. On the other hand, soil moisture was a strong predictor of plant performance. It appears that C_4 plants, often regarded as highly drought tolerant do have their limits. These findings suggest that *M. richardsonis* in the White Mountains of eastern California is at least as limited by H_2O availability as by low temperatures.

Climate change is expected to continue warming temperatures in the White Mountains. This will tend to extend the period of time each year when temperatures at high elevations are warm enough for C_4 photosynthesis and growth. However, increasing temperatures at high

elevations in the arid White Mountains will also result in greater evaporation and perhaps more frequent drought-limitations on alpine plant communities. With climate change, we speculate that the ecological distribution of the unusual C_4 alpine grass *M. richardsonis* will depend on the interactive effects of changing temperature and water availability in the microsite mosaic that characterizes the alpine landscape.

CHAPTER FOUR

SUMMARY AND SYNTHESIS

High or low, where to grow? This is the key question of alpine plant ecology. The central objective of this thesis was to help answer this question for the unusual C_4 alpine grass, *Muhlenbergia richardsonis,* along the western slope of California's White Mountains. Ecophysiological theory predicts that C_4 plants will have a relative advantage under conditions of low CO_2 and/or high temperatures. Because CO_2 and temperature decline in tandem as one moves up slope, it is not immediately obvious where along altitudinal gradients C_4 plants might thrive. In addition, CO_2 and temperature are changing in tandem worldwide as a result of anthropogenic climate change. This implies that distribution of montane C_4 grasses is, or will soon be changing too. My work seeks to better understand the physiological and environmental controls on the present and future distribution of this alpine C_4 grass.

Chapter Two focuses on the question of whether the C_4 carbon concentrating mechanism provides C_4 species with any advantages over that of C_3 species in the low pCO_2

conditions found in high-elevation habitats. Stomatal density increased with elevation in C_3 species *K. macrantha* but not with C_4 species *M. richardsonis* (Figure 2.1). The results for *K. macrantha* are similar to what has been observed many times before but comparable studies have not been carried out on C_4 species prior to this one. This contrasting C_3 vs. C_4 response indicates C_3 species make adjustments in leaf anatomy to compensate for changes in pCO_2 with elevation. These adjustments are presumably costly for C_3 plants and my work verifies these costs are avoided in high-elevation C_4 plants.

Results of carbon isotope analyses in Chapter Two verified that C_3 species were increasingly diffusion limited at higher elevations but the C_4 species *M. richardsonis* was not (Figure 2.3 and 2.4). It seems that the costly adjustments in stomatal density in C_3 species does not fully compensate for the decline in pCO_2 with elevation. However, there is no apparent diffusion limitation on photosynthetic carbon fixation in the C_4 species, even at the highest elevation. Thus, looking at stomatal density changes and carbon isotope changes together gives evidence in support

of the notion that C_4 plants have an advantage over C_3 plants in the pCO_2 conditions found in alpine habitats.

Chapter Two also reports comparative results of leaf nitrogen concentrations in this same set of species along the same elevation gradient (Figure 2.2). In general, leaf nitrogen concentration varied with elevation the same way in both photosynthetic types. This seems to indicate elevation-dependent changes in leaf nitrogen often reported for C_3 species reflect responses to changing temperature conditions rather than to changing pCO_2 conditions. Interestingly, leaf nitrogen in all species responded to elevation in the relatively high-precipitation year of 2005 but not during the relatively low-precipitation year of 2006.

Chapter Three first focuses on the question of whether C_4 species like *M. richardsonis* exhibit an accelerated growing-season phenology compared to co-occurring C_3 species when growing at cold, high-elevation sites. At all elevations, *M. richardsonis* had a later start of spring development than the reference C_3 species, and, at the end of the season, it began to enter dormancy earlier than the reference C_3 species (Figure 3.1). This contrasting C_3 vs.

C_4 phenology is consistent with the general observation of C_4 grasses being cold-intolerant and operating as warm-season specialists, as compared to C_3 plants.

Chapter Three next addresses whether *M. richardsonis* will exhibit greater ecological success when planted in warm compared to cold alpine sites. In general, there was no detectable difference in daytime temperature or plant success on north- compared to south-facing slopes (Table 3.3 and 3.8). Sites sheltered with rocks tended to have higher daytime temperatures than unsheltered sites and, as we would expect, *M. richardsonis* plants generally did better in these rock-sheltered sites (Table 3.3 and 3.8). High-elevation sites were substantially cooler than low-elevation sites but, unexpectedly, *M.richardsonis* plants generally did better at these cool, high-elevation sites than they did in warm, low-elevation sites (Table 3.3 and 3.8). This unexpected finding may reflect differences in water availability at the different sites. Indeed, overall plant success generally followed soil moisture availability across all of the eight microsite treatments (Figure 3.18).

Major findings from this work are that *M. richardsonis* appears to have a relative advantage under the low pCO_2

characteristic of the alpine zone and that it tends to avoid the coldest conditions of the alpine growing season though phenology and microsite selection. These results are consistent with ecophysiological theory and support the hypothesis put forth by Sage and Sage (2002) that the presence of this cold-intolerant C_4 species in the alpine zone can, in part, be explained by localization to warm microsites where temperatures are favorable for C_4 photosynthesis. But, this work also suggests that water availability is more important for *M. richardsonis* ecology than anticipated. First, the observation that this species appears to have recently disappeared from elevations below ~2000 m in the White Mountains (Figure 1.20) may reflect declining water availability associated with recent increases in temperature (Figure 1.15). Second, the observation that there was no elevation-dependent change in leaf nitrogen during the relatively dry 2006 growing season allows for the possibility that water was a more important limiting factor for leaf-level physiology than other factors (e.g. temperature) along this elevational gradient(Figure 2.2 and Table 2.4). Third, and most compellingly, the observation that growth, survival, and

reproduction of *M. richardsonis* scaled better with moisture than with temperature underscore the importance of water availability for this species. Despite the fact that C_4 plants are regarded as highly drought tolerant, these latter observations indicate the importance of water limitations for this species in the arid White Mountains.

Consistent with ecophysiological theory, this work provides provisional evidence that C_4 species may become more frequent in CO_2-poor alpine plant communities as low-temperature limitations on C_4 photosynthesis are relaxed with warming climates. However, climate warming can have profound and poorly understood effects on local ecosystem water budgets. The implied ecological importance of water availability, for *M. richardsonis* emerging from this study complicates our ability to make simple predictions of how the distribution of this species, or other montane C_4 species, may respond to anthropogenic climate change along elevational gradients.

APPENDIX A

FIGURES AND TABLES

Table 1.1. Representative values for several leaf characteristics associated with photosynthetic function in C_3 plant species taken from plants in different elevation classes. (Data adapted from Körner, 2003)

Leaf character	**Lowland plants (500-600 m)**	**Alpine plants (2500-3000 m)**
Leaf thickness (um)	229	334
Leaf nitrogen (% dry mass)	2.1	3.0
Stomatal density (mm^{-2})	80	101
Photosynthesis rate (umol m^{-2} s^{-1})	20	27
$\delta^{13}C$ (‰)	-29.0	-26.5

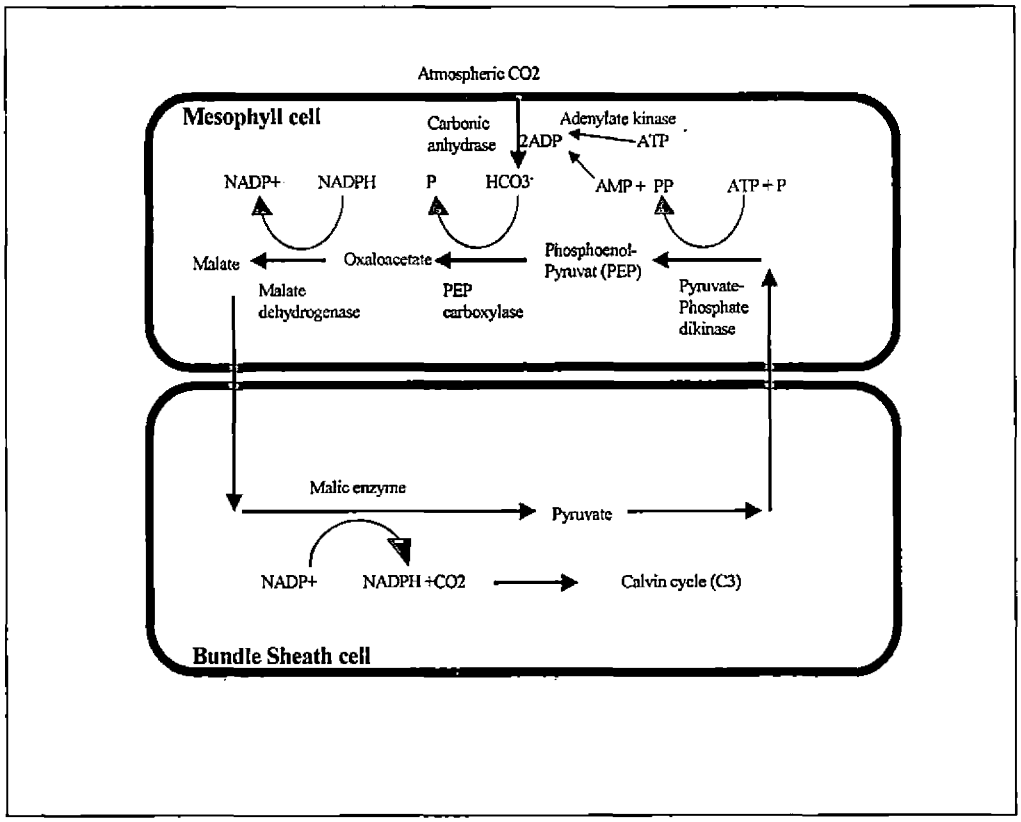

Figure 1.1. The C_4 photosynthetic pathway. The initial fixation of carbon takes place in the C_4 cycle in the mesophyll cells at a cost of two ATPs per C fixed. The fixed C is transported into the bundle sheath cells where it is reductively assimilated to sugar in the C_3 Calvin cycle. The bold black arrows form what is often referred to as the 'C_4 cycle', which feeds into the C_3 Calvin cycle. Adapted from Taiz and Zeiger (2002).

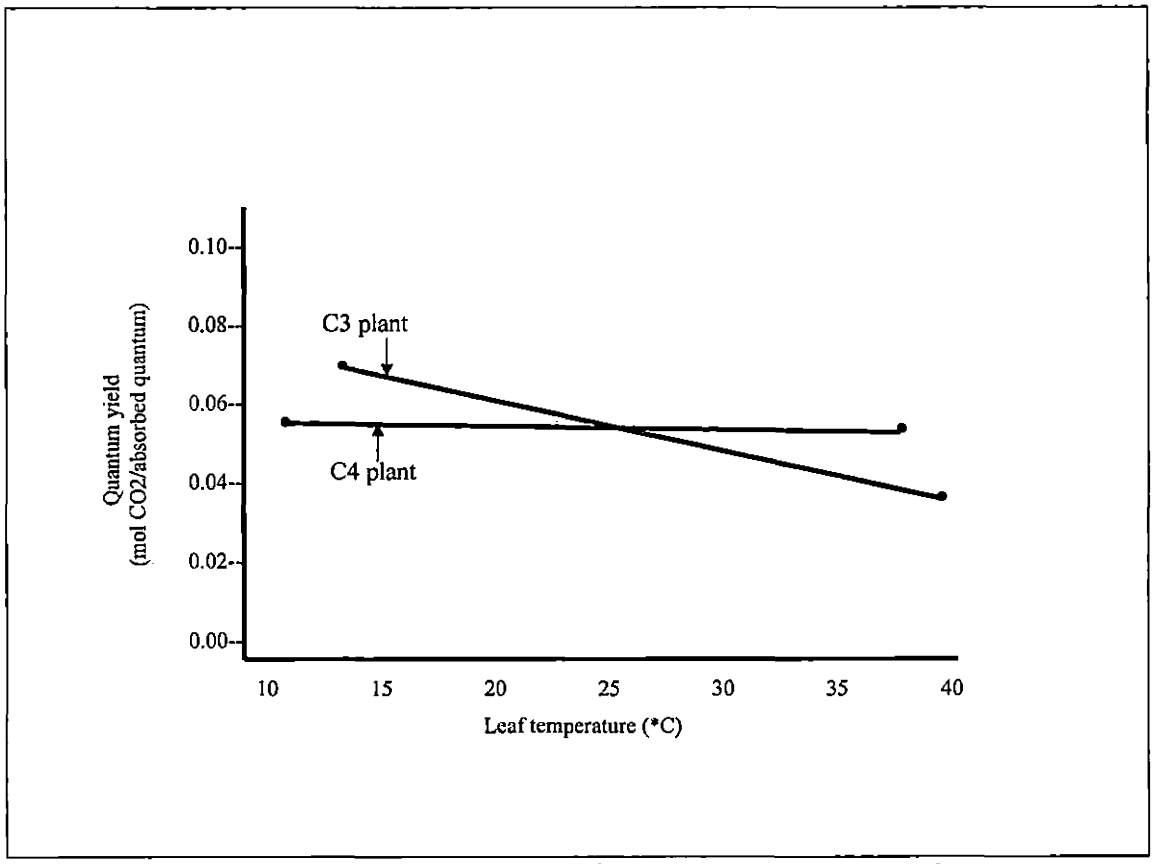

Figure 1.2. The effects of leaf temperature on the quantum yield of a C_3 plant and a C_4 plant. The quantum yield is a measure of the energetic efficiency of photosynthesis. Measurements of CO_2 fixation were made under ambient concentrations of CO_2 and under light limiting conditions over a range of temperatures to assess the influence of leaf temperature on maximum photosynthetic efficiency. Redrawn from Ehleringer and Björkman (1977).

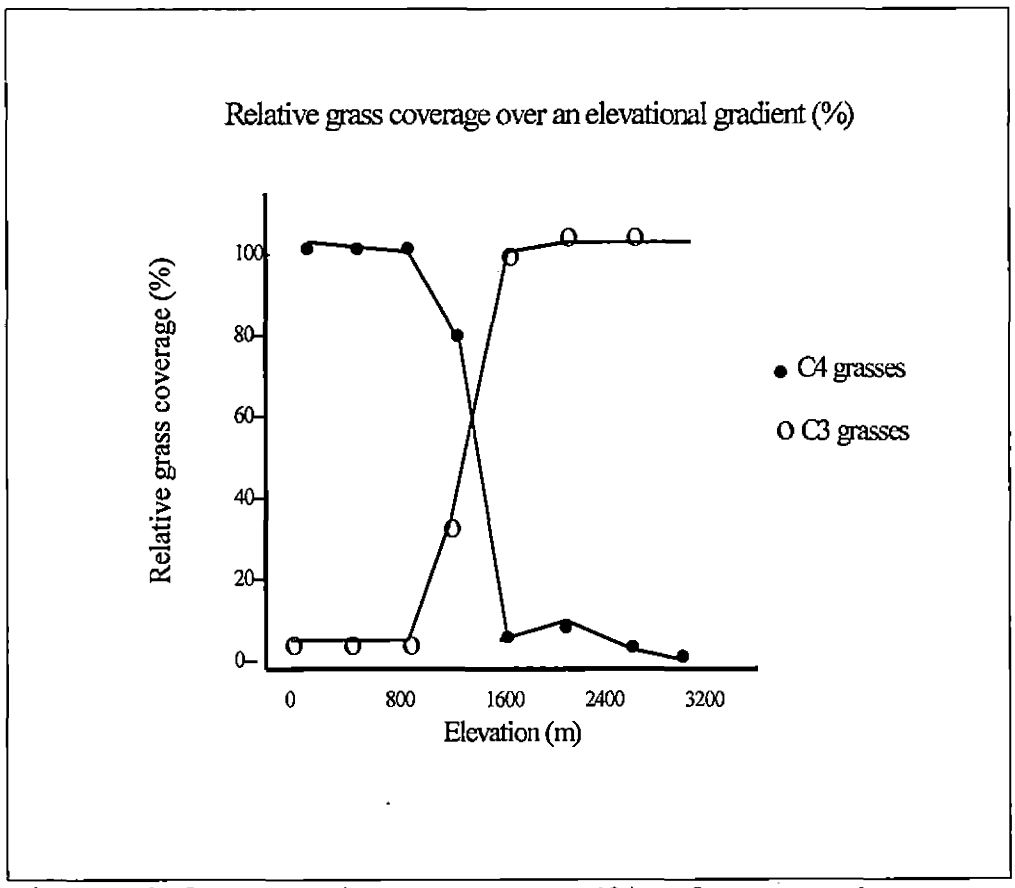

Figure 1.3. Relative coverage (%) of C_4 vs. C_3 grasses along an elevational gradient in mountains of Hawaii. As the elevation increases the percentage of C_4 grasses declines and the percentage of C_3 grasses increases. Adapted from Rundel (1980).

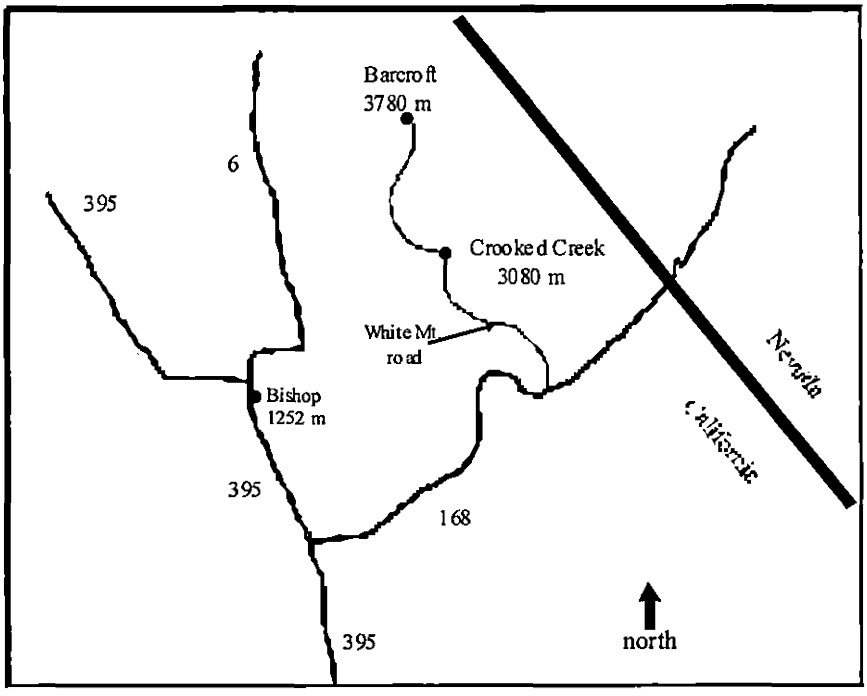

Figure 1.4. Map showing relative locations and elevations of field stations. (not to scale)

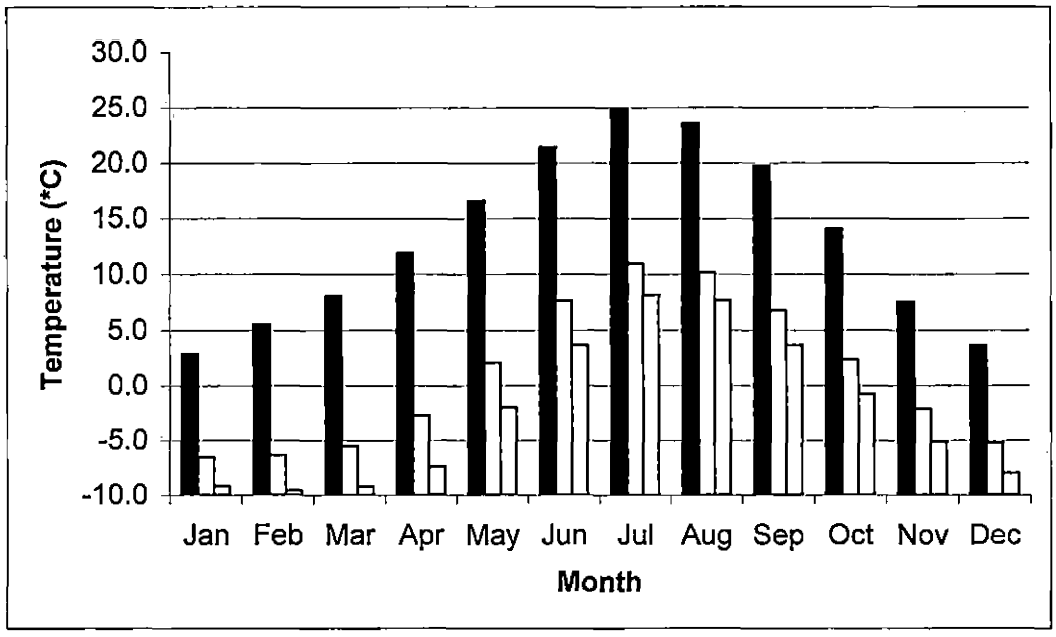

Figure 1.5. Seasonal trends for three locations in the White Mountain elevational gradient. Average monthly temperatures for Barcroft, 3780 m (grey), Crooked Creek, 3060 m (white) and Bishop, 1252 m (black). As elevation increases the growing season (i.e., average temperatures above 0 °C) decreases in duration. (Adapted from Powell and Klieforth, 1991)

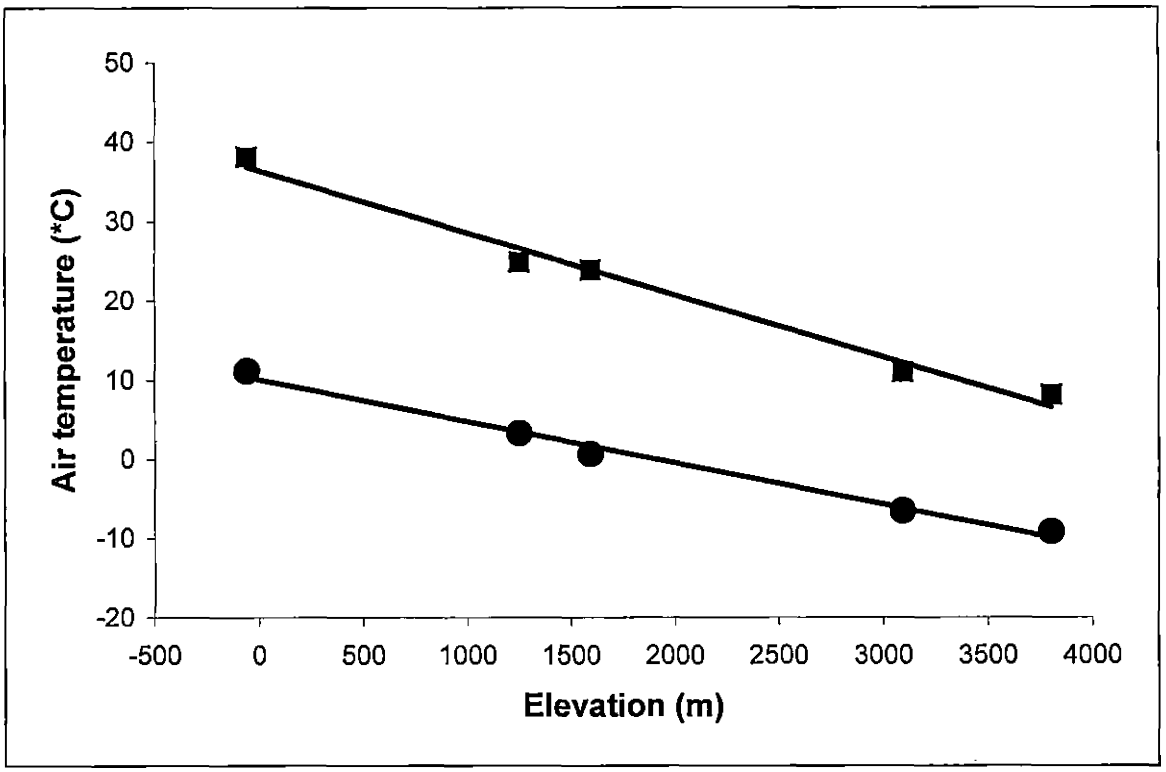

Figure 1.6. Average July (top line) and January (bottom line) air temperatures along an elevational gradient in eastern central California. The lines which best describe observed temperature lapse rates are given as; *air temperature = -0.0078*elevation + 36.335* (July) and *air temperature = -0.0053*elevation + 10.09* (January), where elevation is in meters and air temperature is in °C. Data for the Death Valley site (-86m) and the Deep Springs site(1581m) are from National Oceanic and Atmospheric Administration publication 'Climatography of the U.S. no 81. Monthly station normals of temperature, precipitation, and heating and cooling degree days 1971-2000.' The data for Bishop (1252 m), Crooked Creek (3060 m), and Barcroft (3780 m) are from Powell and Kleiforth (1991).

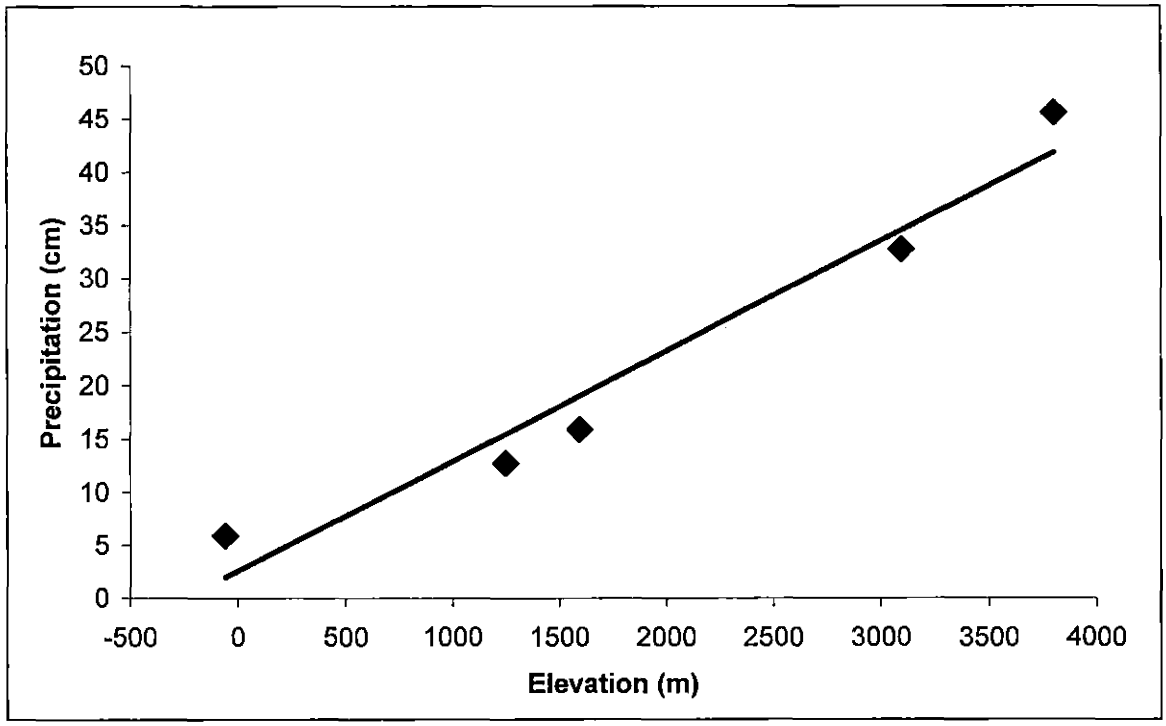

Figure 1.7. Average annual precipitation along the elevational gradient from Death Valley, Deep Springs, Bishop, Crooked Creek and Barcroft (sources of data as cited in fig 1.6).

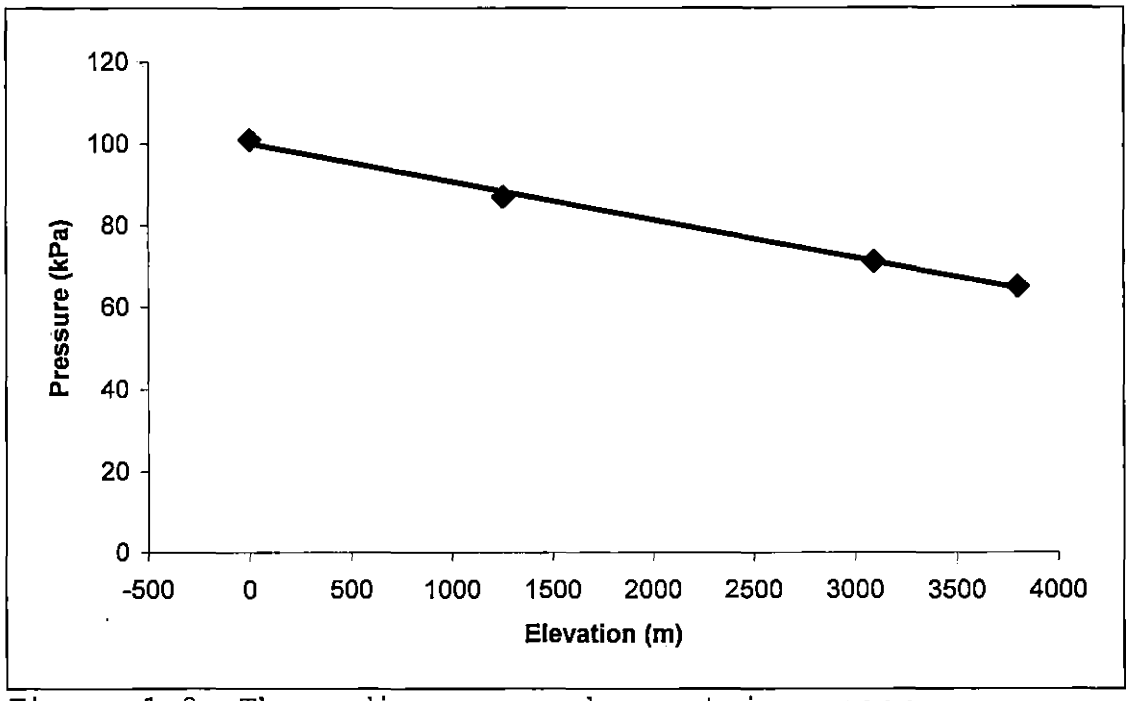

Figure 1.8. The median summer barometric pressures, over the range of elevations presently under consideration. Note that the partial pressure of atmospheric CO_2 declines with declining barometric pressure. (Calculations based upon Ideal Gas Law and the observed summer lapse rate reported in Figure 1.6. For further information on calculations, see Appendix C and http://en.wikipedia.org/wiki/Barometric formula (accessed December, 2007).

Table 1.4. Major vegetation zones of the White Mountains and selected plant species. (Data adapted from Spira, 1991)

Plant Zone	Representative species	
Desert Scrub Zone (~1,200-2,000 m)	shadescale	(*Atriplex confertifolia*)
	sagebrush	(*Artemisia tridentata*)
	rabbitbrush	(*Crysothamnus nauseosus*)
Pinyon-Juniper Zone (~2,000-2,900 m)	pinyon pine	(*Pinus monophylla*)
	Utah juniper	(*Juniperus osteosperma*)
	green ephedra	(*Ephedra viridis*)
Subalpine Zone (~2,900-3,500 m)	sagebrush	(*Artemisia tridentata*)
	bristlecone pine	(*Pinus longaeva*)
	limber pine	(*Pinus flexilis*)
Alpine Zone (~3,500-4,345 m)	raspberry buckwheat	(*Eriogonum gracilipes*)
	fell-field buckwheat	(*Eriogonum ovalatum*)
	june grass	(*Koeleria macrantha*)
	dwarf sagebrush	(*Artemisia arbuscula*)
	mat muhly	(*Muhlenbergia richardsonis*)

Figure 1.9. Typical summer view of Desert Scrub vegetation on the western slope of the White mountains (1,200-2,000 m)

Figure 1.10. Typical summer view of pinyon-juniper woodland on the western slope of the White Mountains (2,000-2,900 m).

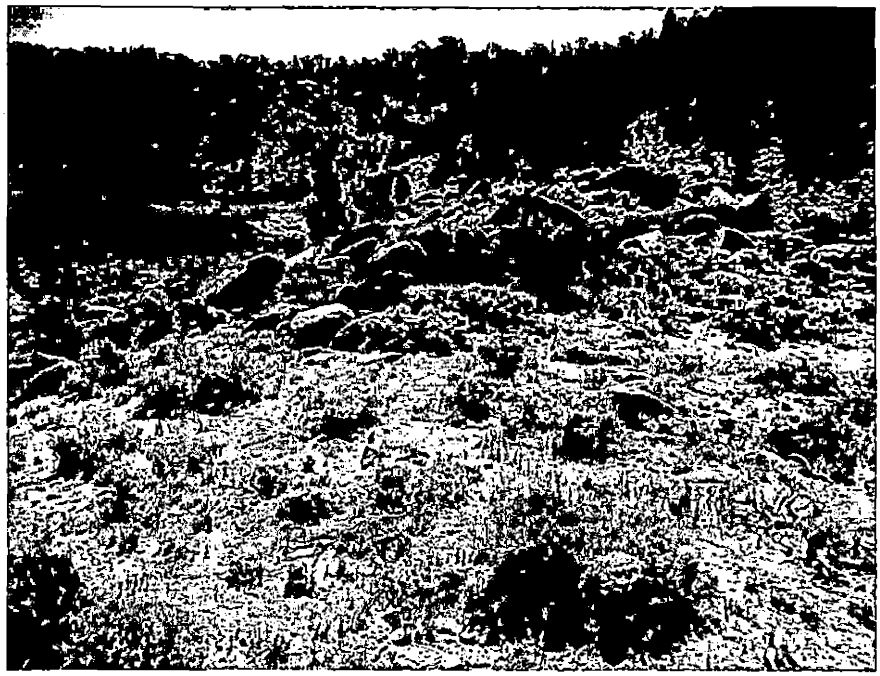

Figure 1.11. Typical summer view of the sub-alpine zone on the western slope of the White Mountains (2,900-3,500 meters). Note the open field of *Artemisia tridentata* on the left (Sagebrush steppe) and the open stands of *Pinus longaeva* on the right side of this scene (pine woodland).

Figure 1.12. Typical summer view of sagebrush steppe found within the sub-alpine zone on the western slope of the White Mountains (2,900-3,500 meters).

Figure 1.13. Typical summer view of the alpine zone on the western slope of the White Mountains (3,500-4,345 m). The peak of White Mountain itself is seen in the center of this photo.

Table 1.5. Published elevational ranges for *Muhlenbergia richardsonis* in California from several contemporary authoritative floristic treatments.

Reported Elevation	Source	Reference
2439-3384 m	A Flora of the White Mountains	Lloyd and Mitchell, 1973
1515-3333 m	A Flora of Southern California	Munz, 1974
2,134-3354 m	Natural History of the White-Inyo Range Eastern California	DeDecker, 1991
1220-3670 m	The Jepson Manual	Hickman, 1993

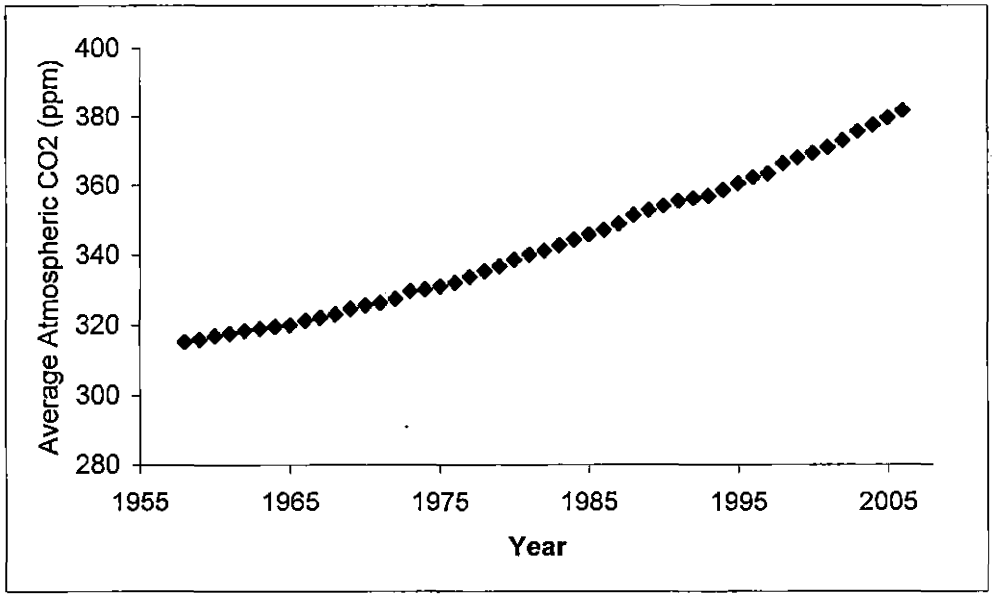

Figure 1.14. Average annual atmospheric CO_2 concentration (ppm) as recorded from Mauna Loa HI for the time period from 1958-2006 (http://www.esrl.noaa.gov/gmd/ccgg/trends/). Accessed September 2007.

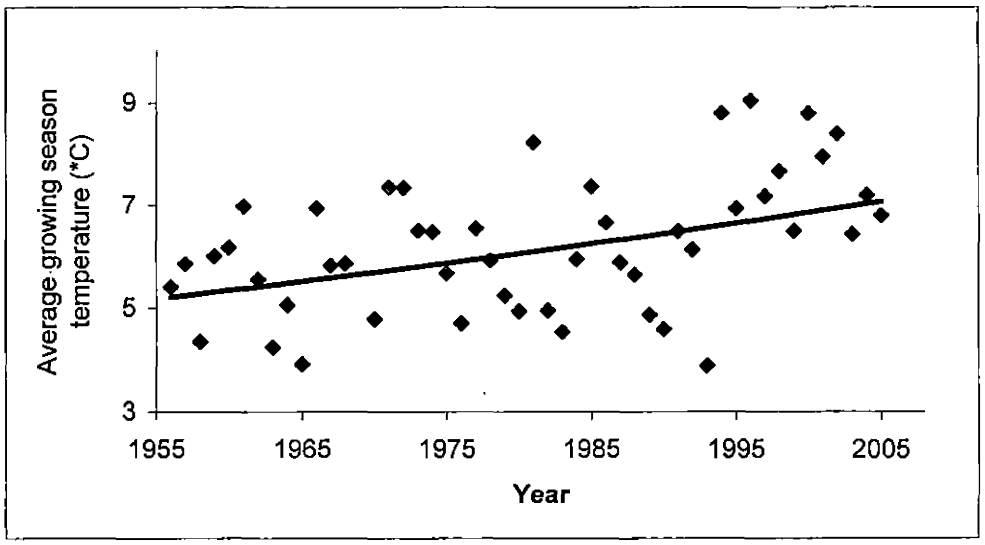

Figure 1.15. Average yearly growing season (June, July and August) air temperatures at Barcroft Station (3780 m) in the White Mountains of eastern California from 1956-2006 (Western Regional Climate Center, wrcc@dri.edu). Accessed September 2007.

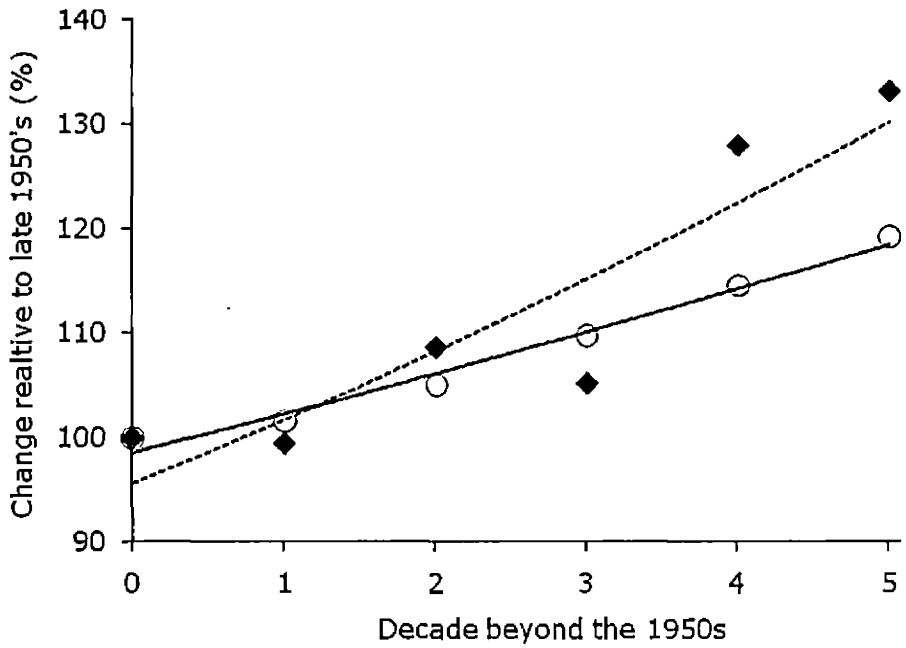

Figure 1.16. Average decadal atmospheric CO_2 (open circles with solid line) and temperature (solid diamonds with dashed line) relative to initial observations made during the late 1950s. Derived from data presented in Figure 1.14 and 1.15. Exponential models that gave the best fit to the data are; Relative Temperature = $95.7e^{0.062*time}$, $r^2=0.84$. Relative CO_2 = $98.7e^{0.037*time}$, $r^2=0.98$

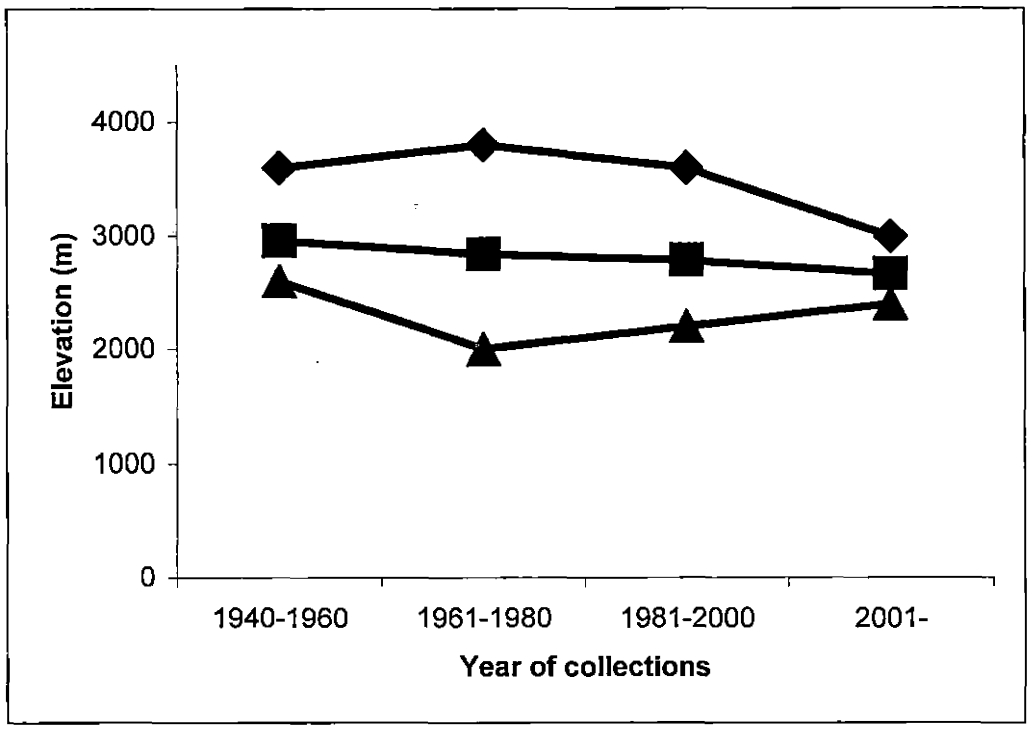

Figure 1.17. Historical altitudinal distribution for *Koeleria macrantha* (C_3) based upon herbarium records for the mountainous counties of eastern central California (1940-1960, 20 records, 1961-1980, 35 records, 1981-2000, 13 records and 2000-2007, 14 records). Squares = mean elevation for each time interval, diamond = maximum observed elevation for each time interval and triangles = minimum observed elevation for each time interval.

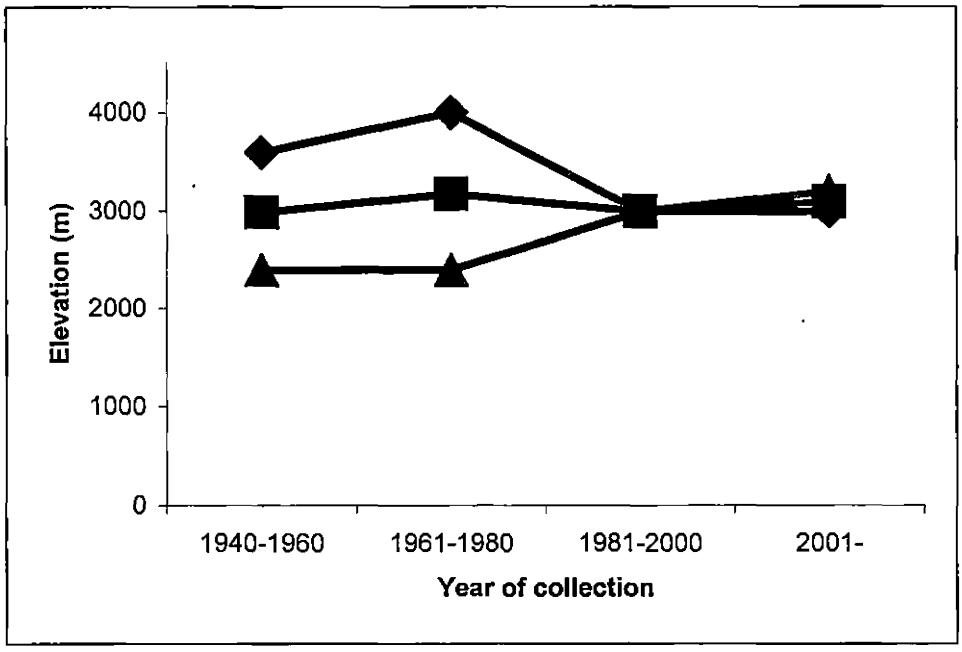

Figure 1.18. Historical altitudinal distribution for *Achnatherum pinetorum* (C_3) based upon herbarium records for the mountainous counties of eastern central California (1940-1960, 20 records, 1961-1980, 20 records, 1981-2000, 1 record and 2001-2007, 2 records). Symbols as described in figure 1.17.

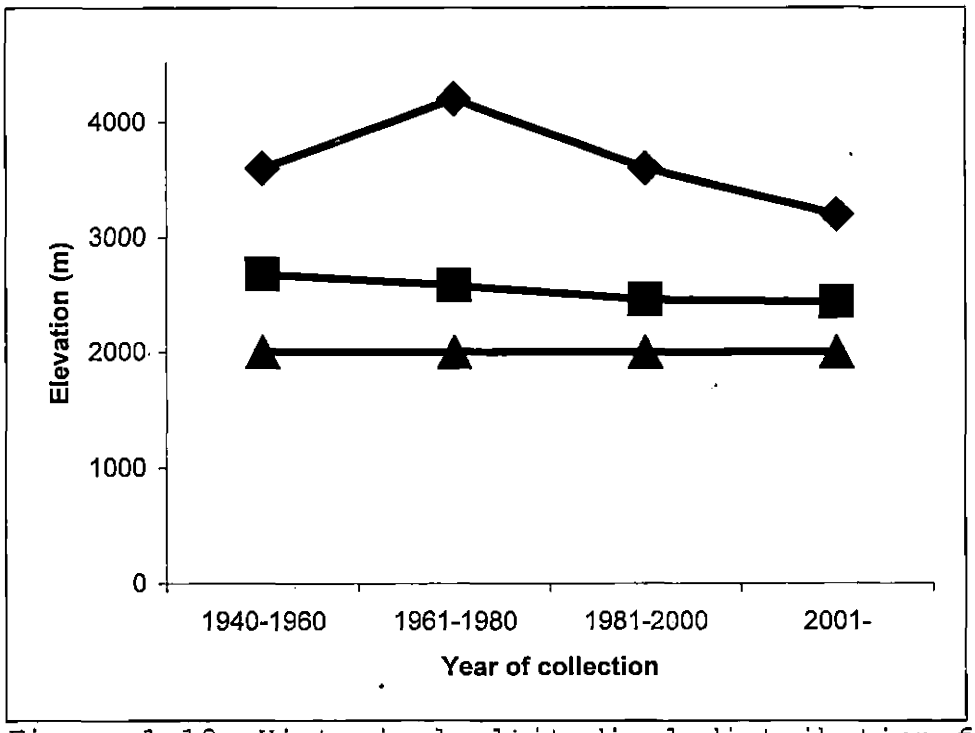

Figure 1.19. Historical altitudinal distribution for *Elymus elymoides* (C_3) based upon herbarium records for the mountainous counties of eastern central California (1940-1960, 39 records, 1961-1980, 40 records, 1981-2000, 26 records and 2001-2007, 22 records). Symbols as described in figure 1.17.

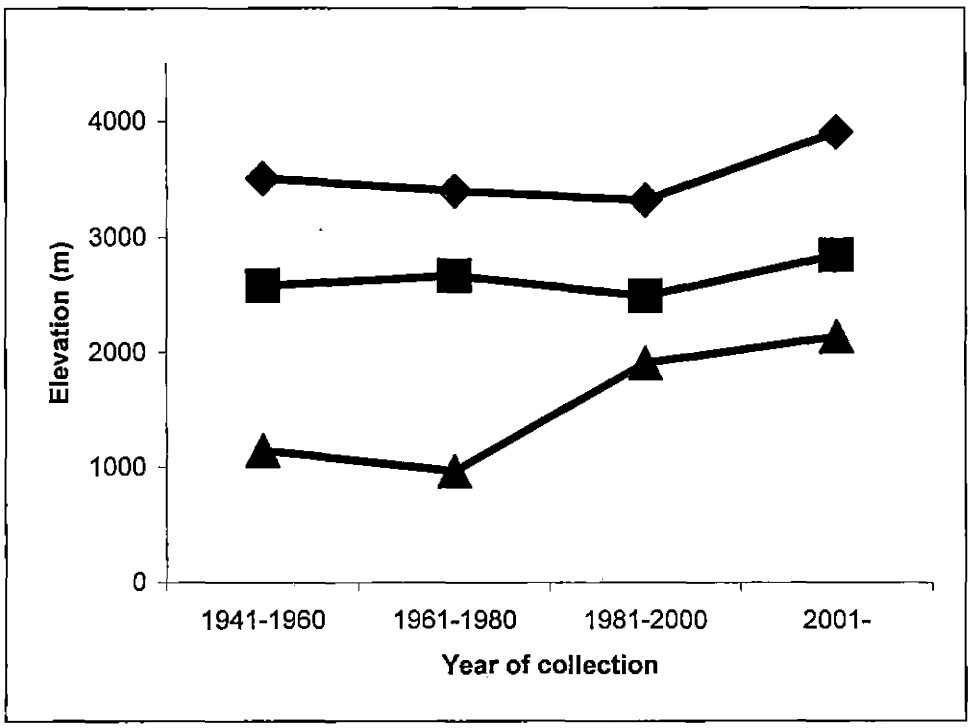

Figure 1.20. Historical altitudinal distribution for *Muhlenbergia richardsonis* (C_4) based upon herbarium records for the mountainous counties of eastern central California (1941-1960, 14 records, 1961-1980, 26 records, 1981-2000, 7 records and 2001-2007, 21 records). Symbols as described in figure 1.17.

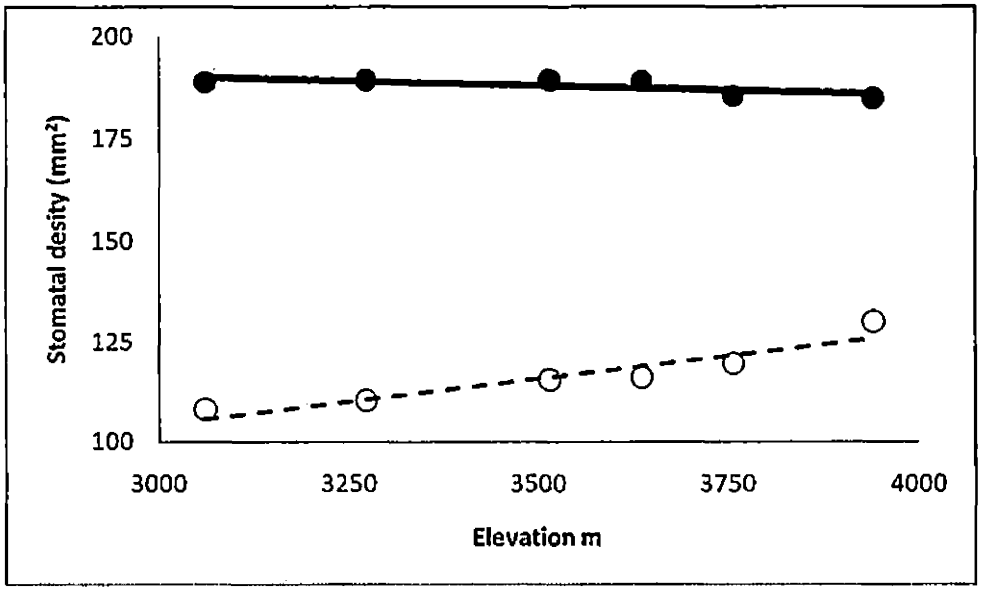

Figure 2.1. Stomatal density summed for both leaf surfaces in *Muhlenbergia richardsonis* (solid circle and solid trend-line) and *Koeleria macrantha* (open circle and dashed trend-line) from six sites, for the 2006 growing season. The line-of-best-fit for observed stomatal variation in *Koeleria macrantha* is given as; stomatal density = 0.0228*elevation + 35.914, R = 0.856. The correlation for *Muhlenbergia richardsonis* was not significant (see Table 2.1).

Table 2.1: Results of tests for significant correlations between stomatal density and elevation for *Muhlenbergia richardsonis* and *Koeleria macrantha* from six sample sites along the White Mountain elevational gradient during the 2006 growing season. Emboldened P-values highlight trends that were significant at the 0.05 level.

Dependant variable	Species	N	Correlation Coefficient [R]	P value
Stomatal density ($1/mm^2$)	*Muhlenbergia richardsonis*	30	0.316	0.0758
	Koeleria macrantha	30	0.856	**$\leq$ 0.0001**

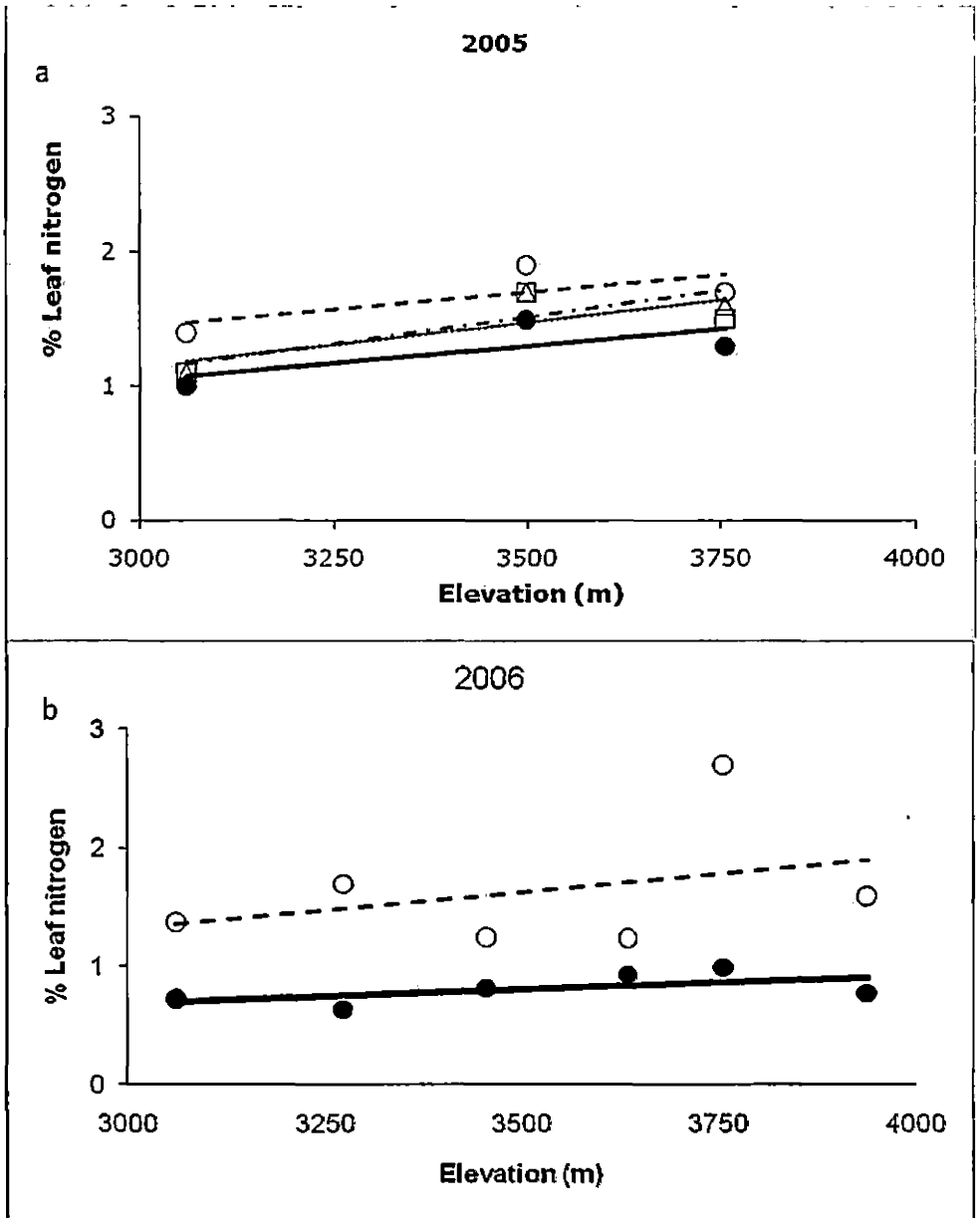

Figure 2.2. Leaf nitrogen concentration at different elevations in graminoid species from the White Mountains in 2005 (a) and 2006 (b). Sampled species in 2005 were C_3 plants *Achnatherum pinetorum* (open triangle and dot-dash trend-line), *Carex* sp. (open square and dotted trend-line) *Koeleria macrantha* (open circle and dashed trend-line), and the C_4 plant *Muhlenbergia richardsonis* (solid circle and solid trend-line). Sampled species in 2006 were *Koeleria macrantha* and *Muhlenbergia richardsonis*. All linear

fits were significant for 2005 (Table 2.2) but not for 2006 (Table 2.3). The lines that best describe the association between elevation and leaf nitrogen for 2005 are given as; leaf nitrogen = 0.0003*elevation - 1.123 (R = 0.631) for *A. pinetorum*, leaf nitrogen = 0.0003*elevation -1.686 (R = 0.817) for *Carex* sp., leaf nitrogen = 0.0002*elevation -0.129 (R = 0.587) for *K. macrantha*, and leaf nitrogen = 0.0001*elevation -0.294 (R = 0.613) for *M. richardsonis*. Regression equations for 2006 are not given since they were not statistically significant (see Table 2.2).

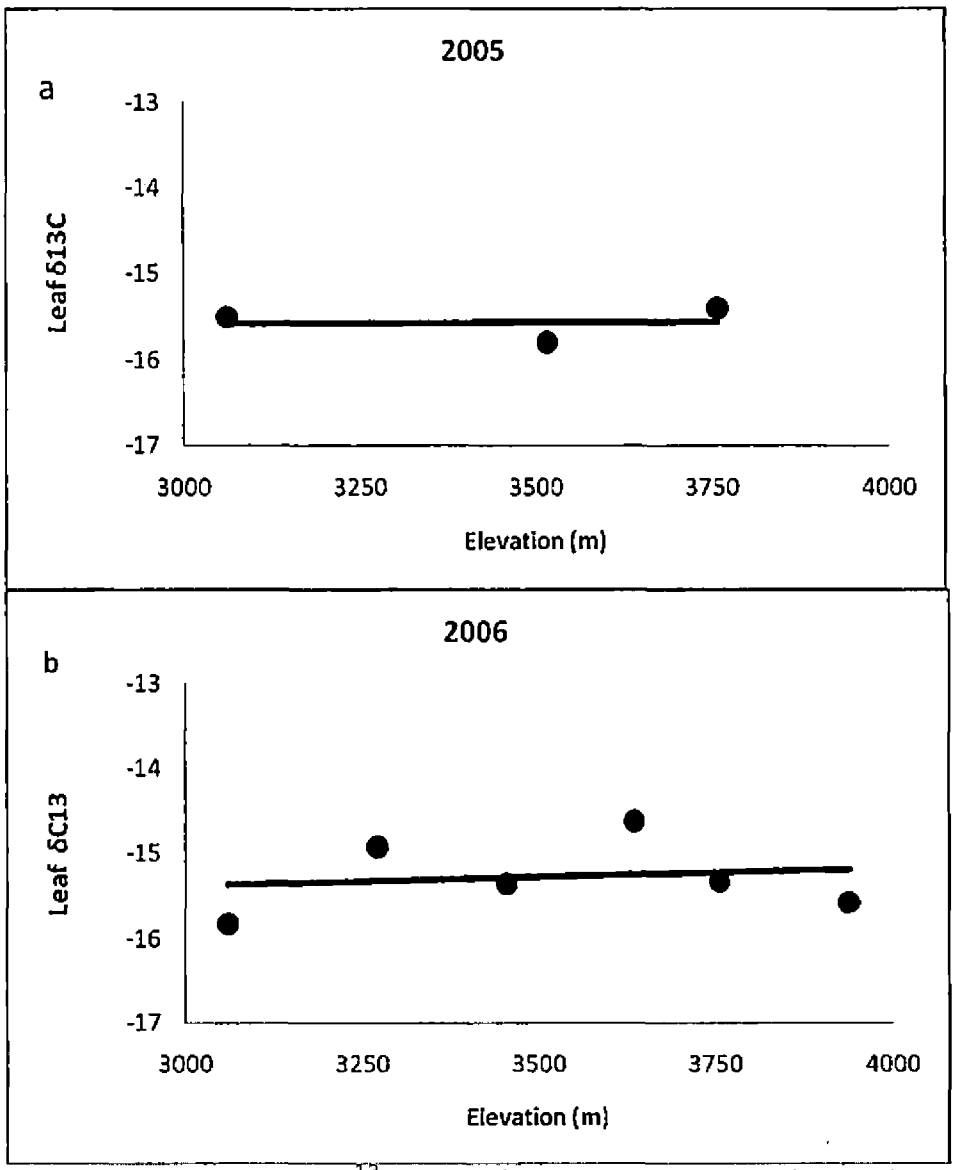

Figure 2.3. Leaf $\delta^{13}C$ at different elevations in *Muhlenbergia richardsonis* (C_4) from the White Mountains in 2005 (a) and 2006 (b). Linear fits were not significant for either 2005 or 2006 (see Table 2.2).

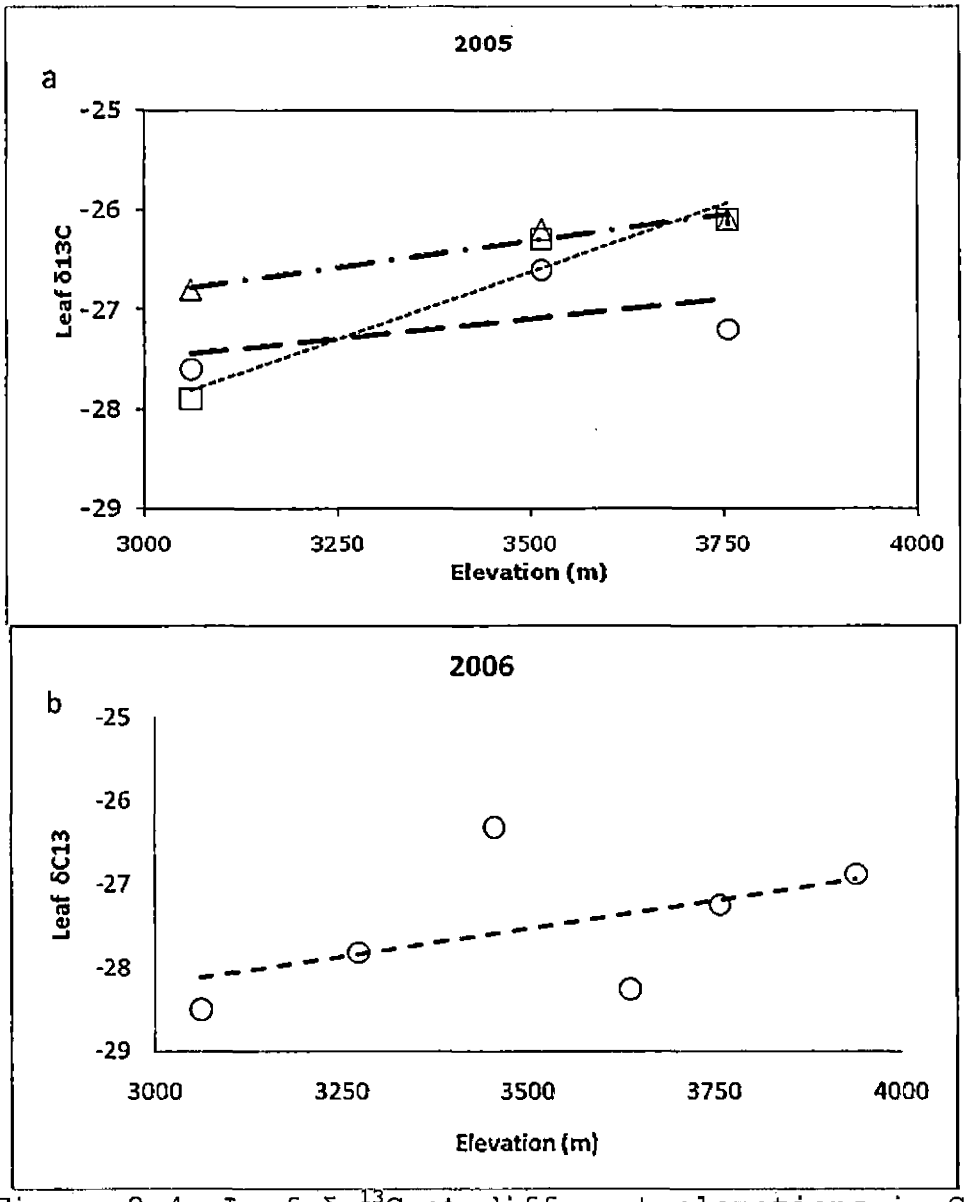

Figure 2.4. Leaf $\delta^{13}C$ at different elevations in C_3 graminoid species from the White Mountains in 2005 (a) and 2006 (b). Sampled species in 2005 were C_3 plants *Achnatherum pinetorum* (open triangle and dot-dash trend-line), *Carex* sp. (open square and dotted trend-line), and *Koeleria macrantha* (open circle and dashed trend-line). Among the C_3 species, only *Koeleria macrantha* was sampled in 2006. All linear fits were significant or marginally significant (*K. macrantha* in 2005) in 2005 (Table 2.2) and 2006 (Table 2.3). The lines that best describe the association between

elevation and leaf $\delta^{13}C$ for 2005 samples are given as; $\delta^{13}C$ = 0.00033*elevation -30.1 (R = 0.742) for *A. pinetorum*, $\delta^{13}C$ = 0.00076*elevation -35.4 (R = 0.932) for *Carex* sp., $\delta^{13}C$ = 0.00025*elevation -30.0 (R = 0.460) for *K. macrantha*. The line that best describe the association between elevation and leaf $\delta^{13}C$ for 2006 samples are given as; $\delta^{13}C$ = 0.00039*elevation - 32.063 (R=0.511) for *K. macrantha*.

Table 2.2. Results of tests for significant correlations between foliar nitrogen concentration or foliar $\delta^{13}C$ values and elevation for *Achnatherum pinetorum, Carex sp., Koeleria macrantha,* and *Muhlenbergia richardsonis* from three sample sites along the White Mountain elevational gradient during the 2005 growing season. Emboldened P-values highlight trends that were significant at the 0.05 level.

Dependent variable	Species	N	Correlation Coefficient [R]	P Value
Nitrogen (%)	*Achnatherum pinetorum*	15	0.631	**0.0050**
	Carex sp.	15	0.931	**≤ 0.0001**
	Koeleria macrantha	15	0.587	**0.0106**
	Muhlenbergia richardsonis	15	0.613	**0.0069**
$\delta^{13}C$	*Achnatherum pinetorum*	15	0.742	**0.0009**
	Carex sp.	15	0.932	**≤ 0.0001**
	Koeleria macrantha	15	0.460	0.0544
	Muhlenbergia richardsonis	15	0.032	0.6787

Table 2.3. Results of tests for significant correlations between foliar nitrogen concentration or foliar $\delta^{13}C$ values and elevation for *Koeleria macrantha* and *Muhlenbergia richardsonis* from six sample sites along the White Mountain elevational gradient during the 2006 growing season. Emboldened P-values highlight trends that were significant at the 0.05 level.

Dependent variable	Species	N	Correlation Coefficient [R]	P Value
Nitrogen (%)	*Koeleria macrantha*	30	0.077	0.1948
	Muhlenbergia richardsonis	30	0.100	0.0879
$\delta^{13}C$	*Koeleria macrantha*	30	0.511	**0.0039**
	Muhlenbergia richardsonis	30	0.056	0.3559

Table 2.4. Observed winter precipitation for Bishop California preceding sampling growing season of both 2005 and 2006 compared to the long-term average. Data from Western Regional Climate Center (www.wrcc.dri.edu). Accessed May 2008.

Time period	Winter precipitation (mm) (Sept-May)
Long term 1970 -2007	115 + 57 SD
2004-2005	226
2005-2006	128

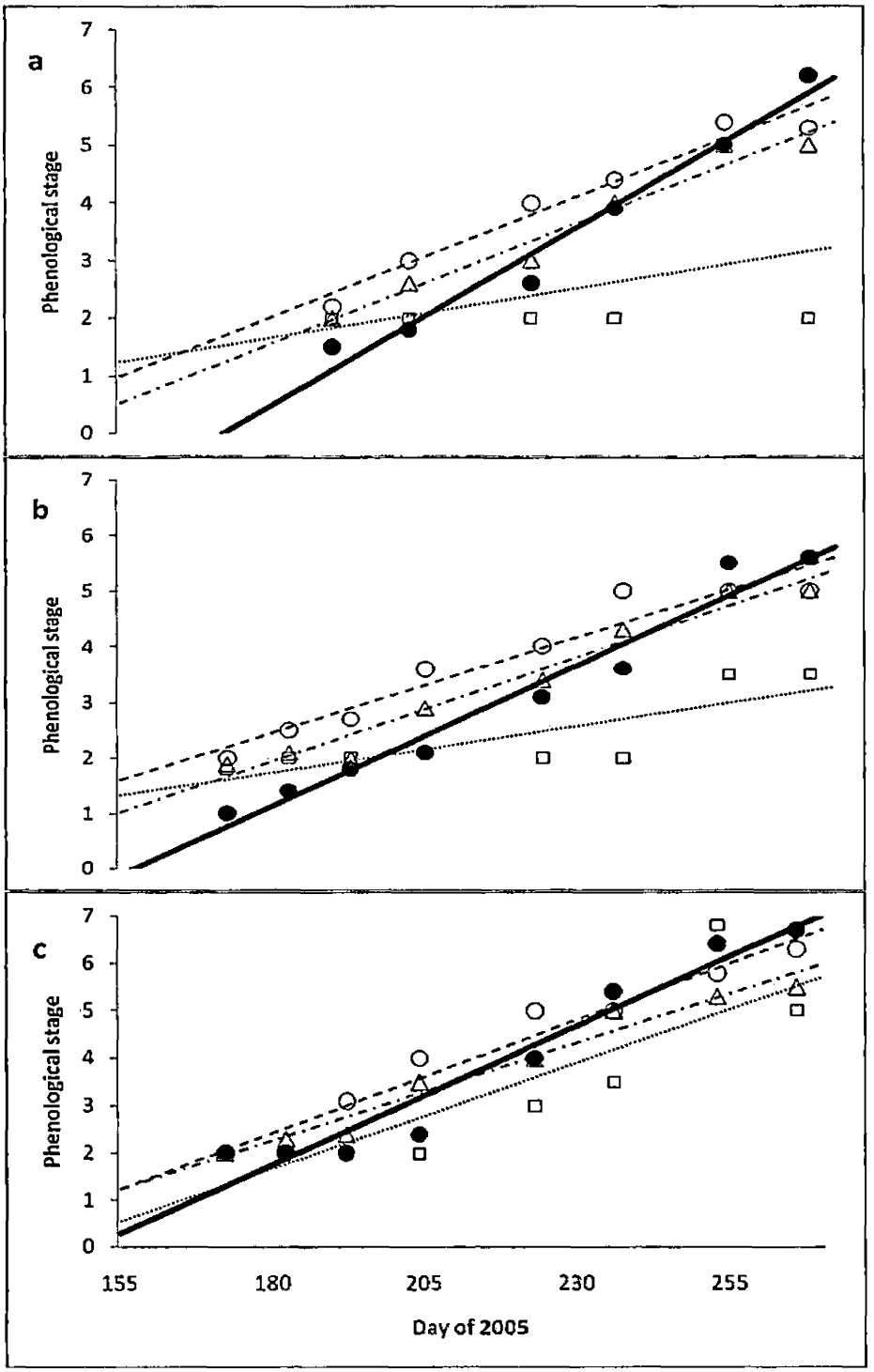

Figure 3.1. Phenological stage during the 2005 growing season (June 21-September 23) for *Muhlenbergia richardsonis* (solid circles and solid trend-line), *Koeleria macrantha* (open circles and dashed trend-

line), *Achnatherum pinetorum* (open triangles and dot-dash trend-line) and *Carex* spp. (open squares and dotted trend-line) at (a) Barcroft (3780 m), (b) Sheep Pass (3515 m) and (c) Crooked Creek (3060 m). Snow cover precluded sampling at Barcroft before July 11. Phenological stages designated as follows: initial greening (stage 1), fully (over 90%) green (stage 2), initial (bud) flowering (stage 3); peak flowering (stage 4); seed set (stage 5); seed drop (stage 6); autumn browning (stage 7). Each plot point is the mean for 9 observed individual plants. Error bars omitted for clarity. See Table 3.1 for trend-line results.

Table 3.1. Results of regression analyses of the 2005 phenology observations for each of the four study species from each of the three elevational sites along the White Mountain elevational gradient (Barcroft, 3780 m; Sheep Pass, 3515 m; Crooked Creek, 3060 m) during the growing season from June 21- September 23. For each species at each elevation, 9 individual plants were evaluated on each of 6-8 dates during the 2005 growing season. Product-Moment correlation tests were run to test for significant slopes ($p=0.05$, $df=4$ at 3780 m and $df=6$ at other sites). Significant slopes (seasonal rates of development) are emboldened. At each elevation *Muhlenbergia richardsonis* had the fastest rate of seasonal development of the species under observation.

Year	Elevation (m)	Species	Photosyn-thetic pathway	Slope $(day)^{-1}$	Y intercept (phenological stage number)	R
2005	3780	*Carex spp*	C3	0.0179	1.229	0.172
2005	3780	*Achnatherum pinetorum*	C3	**0.0437**	0.495	0.962
2005	3780	*Koeleria macrantha*	C3	**0.0438**	0.943	0.959
2005	3780	*Muhlenbergia richardonis*	C4	**0.0648**	-1.098	0.970
2005	3515	*Carex spp*	C3	0.0170	1.309	0.067
2005	3515	*Achnatherum pinetorum*	C3	**0.0378**	0.984	0.963
2005	3515	*Koeleria macrantha*	C3	**0.0347**	1.574	0.932
2005	3515	*Muhlenbergia richardonis*	C4	**0.0513**	-0.169	0.965
2005	3060	*Carex spp*	C3	0.0448	0.507	0.742
2005	3060	*Achnatherum pinetorum*	C3	**0.0410**	1.208	0.966
2005	3060	*Koeleria macrantha*	C3	**0.0475**	1.205	0.958
2005	3060	*Muhlenbergia richardonis*	C4	**0.0584**	0.240	0.943

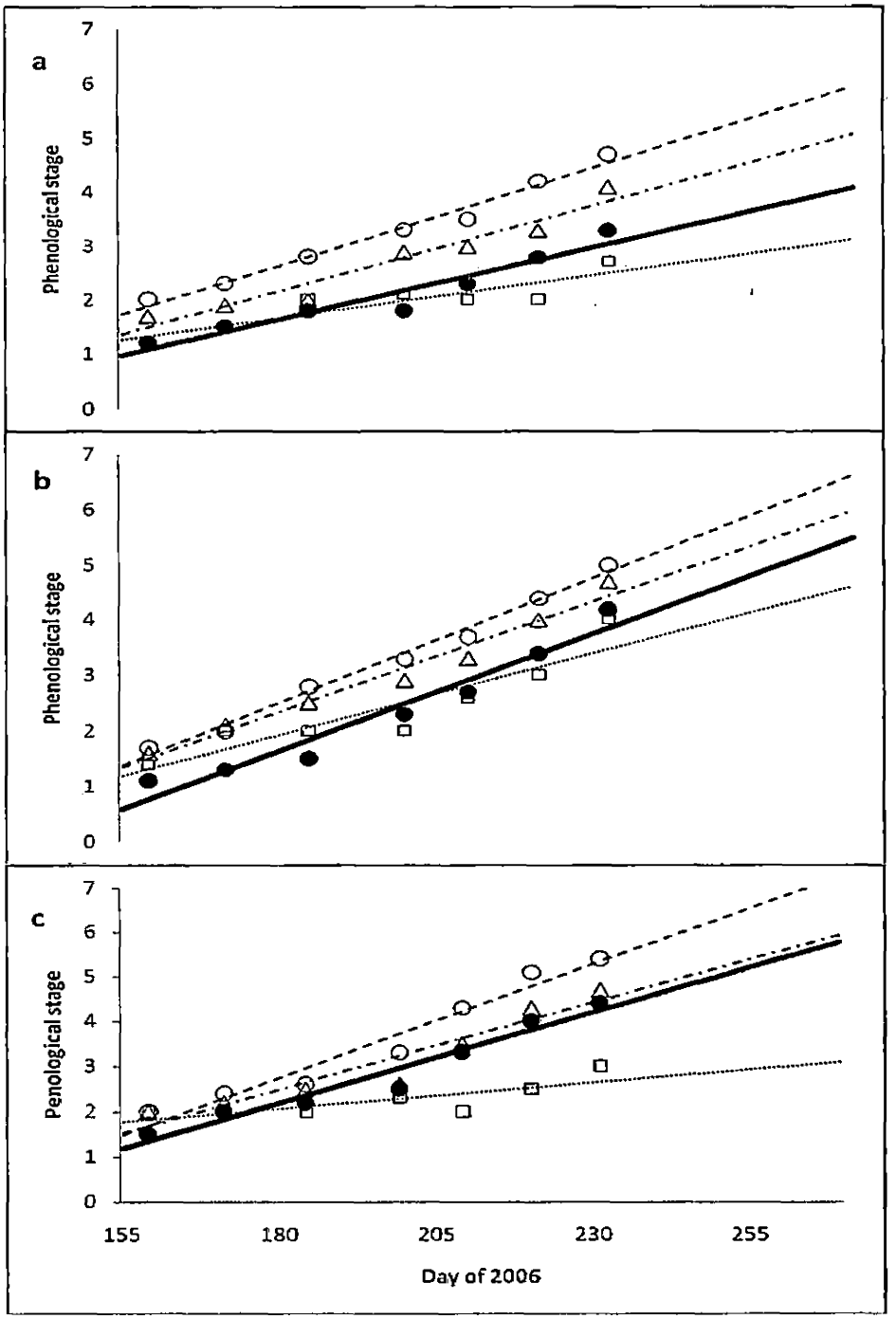

Figure 3.2. Phenological stage during the 2006 growing season (June 9-August 19) for *Muhlenbergia richardsonis* (solid circles and solid trend-line), *Koeleria macrantha* (open circles and dashed trend-

line), *Achnatherum pinetorum* (open triangles and dot-dash trend-line) and *Carex* spp. (open squares and dotted trend-line) at (a) Barcroft (3780 m), (b) Sheep Pass (3515 m) and (c) Crooked Creek (3060 m). Snow cover precluded sampling at Barcroft before July 11. Phenological stages designated as follows: initial greening (stage 1), fully (over 90%) green (stage 2), initial (bud) flowering (stage 3); peak flowering (stage 4); seed set (stage 5); seed drop (stage 6); autumn browning (stage 7). Each plot point is the mean for 10 observed individual plants. Error bars omitted for clarity. See Table 3.2 for trend-line results.

Table 3.2. Results of regression analyses of the 2006 phenology observations for each of the four study species from each of the three elevational sites along the White Mountain elevational gradient (Barcroft, 3780 m; Sheep Pass, 3515 m; Crooked Creek, 3060 m) during the growing season from June 9- August 19. For each species at each elevation, 10 individual plants were evaluated on each of 7 dates during the 2006 growing season. Product-Moment correlation tests were run to test for significant slopes (p=0.05, df=5). Significant slopes (seasonal rates of development) are emboldened.

Year	Eleva -tion (m)	Species	Photosyn -thetic pathway	Slope $(day)^{-1}$	Y intercept (phenological stage number)	R
2006	3780	*Carex spp*	C3	**0.0163**	1.243	0.805
2006	3780	*Achnatherum pinetorum*	C3	**0.0324**	1.336	0.942
2006	3780	*Koeleria macrantha*	C3	**0.0370**	1.698	0.983
2006	3780	*Muhlenbergia richardonis*	C4	**0.0273**	0.949	0.924
2006	3515	*Carex spp*	C3	**0.0300**	1.164	0.837
2006	3515	*Achnatherum pinetorum*	C3	**0.0407**	1.301	0.971
2006	3515	*Koeleria macrantha*	C3	**0.0460**	1.339	0.989
2006	3515	*Muhlenbergia richardonis*	C4	**0.0430**	0.545	0.947
2006	3060	*Carex spp*	C3	0.0116	1.769	0.629
2006	3060	*Achnatherum pinetorum*	C3	**0.0386**	1.488	0.903
2006	3060	*Koeleria macrantha*	C3	**0.0506**	1.452	0.953
2006	3060	*Muhlenbergia richardonis*	C4	**0.0402**	1.147	0.950

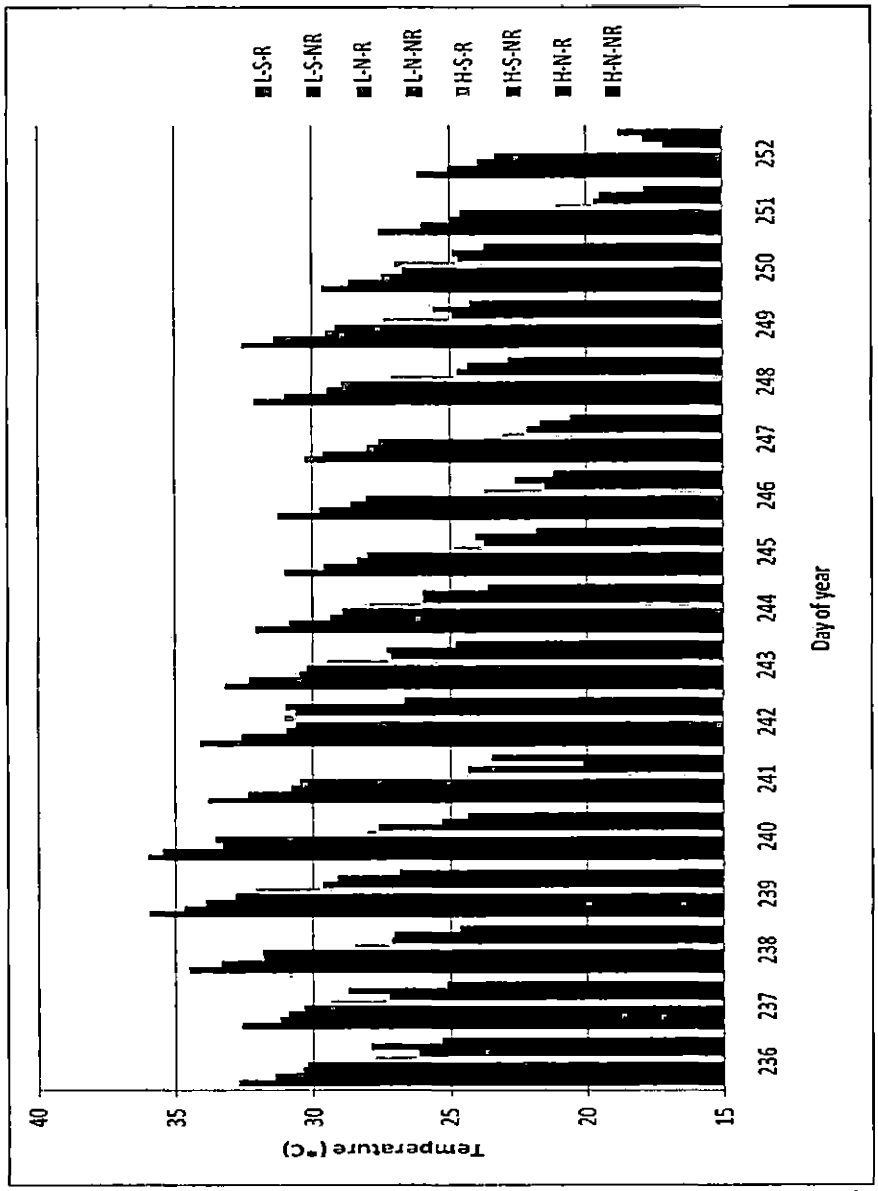

Fig 3.3. Average maximum daytime temperature by day for all eight microsite treatments recorded for 2005 (August 24 - September 9). L-S-R = Low elevation, south facing slope, rock shelter; L-S-NR = Low elevation, south facing slope, no rock shelter; L-N-R = Low elevation, north facing slope, rock shelter; L-N-NR = Low elevation, north facing slope, no rock sheltering; H-S-R = High elevation south facing slope, rock shelter; H-S-NR = High elevation, south facing slope, no rock shelter; H-N-R = High elevation, north facing slope, rock sheltering; H-N-NR = High elevation, north facing slope, no rock shelter. Each bar is the mean from 3 sensors. Error bars omitted for clarity.

Table 3.3. Repeated measures Analysis-of-variance for maximum daily temperatures in each of eight microsite treatments (high (3780 m) and low (3060 m) elevation, north- and south- facing slopes, and with and without rock-shelter) August 24 - September 9, 2005. P-values less than 0.05 are emboldened for emphasis.

Source of variation	df	Mean Squares	F ratio	P
Day of year	16	0.05	31.3	**<0.0001**
Elevation	1	0.08	21.3	**0.0438**
Slope aspect	2	0.04	4.63	0.0908
Rock shelter treatment	4	0.01	4.89	**0.0007**
Error	367	0.002		
Total	390			

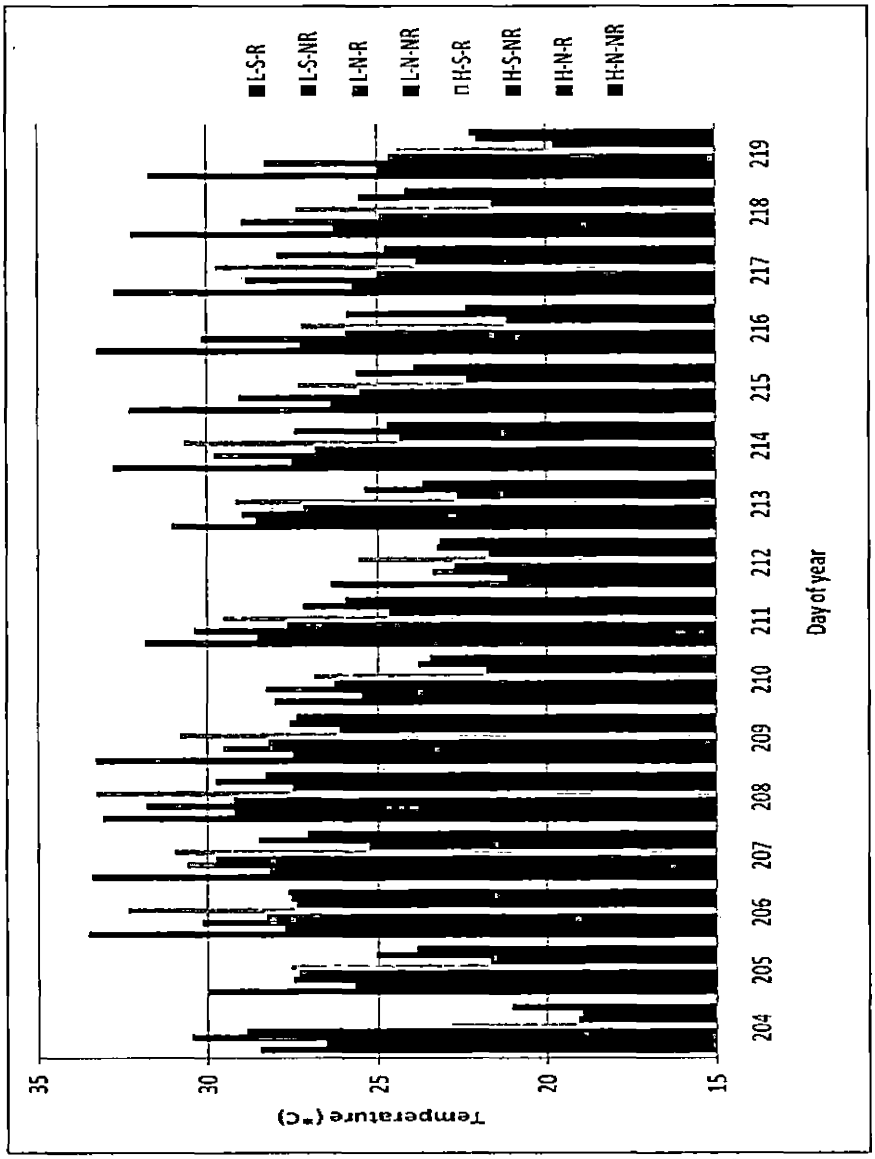

Fig 3.4. Average maximum daytime temperature by day for all eight microsite treatments recorded for 2006 (July 25 - August 7). L-S-R = Low elevation, south facing slope, rock shelter; L-S-NR = Low elevation, south facing slope, no rock shelter; L-N-R = Low elevation, north facing slope, rock shelter; L-N-NR = Low elevation, north facing slope, no rock sheltering; H-S-R = High elevation south facing slope, rock shelter; H-S-NR = High elevation, south facing slope, no rock shelter; H-N-R = High elevation, north facing slope, rock sheltering; H-N-NR = High elevation, north facing slope, no rock shelter. Each bar is the mean from 3 sensors. Error bars omitted for clarity.

Table 3.4. Repeated measures Analysis-of-variance for maximum daily temperatures in each of eight microsite treatments (high (3780 m) and low (3060 m) elevation, north- and south- facing slopes, and with and without rock-shelter), July 25 - August 7, 2006. P-values less than 0.05 are emboldened for emphasis.

Source of variation	df	Mean Squares	F ratio	P
Day of year	15	0.02	20.8	**<0.0001**
Elevation	1	0.407	27.7	**0.0343**
Slope aspect	2	0.03	0.35	0.7243
Rock shelter treatment	4	0.04	41.5	**<0.0001**
Error	345	0.001		
Total	367			

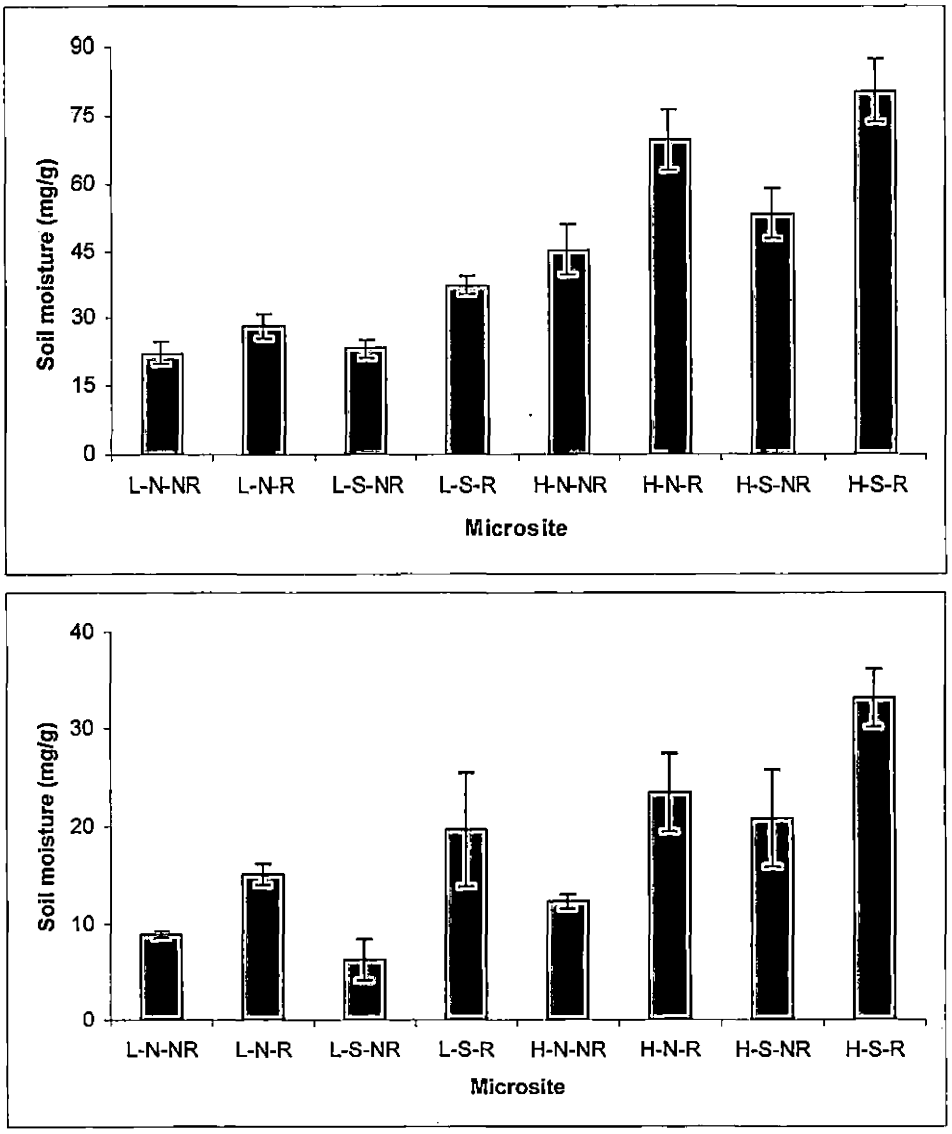

Figure 3.5. Gravimetric soil soil moisture (mean +/- SE) for all eight microsite treatments from (a) August 23, 2005 and (b) September 10, 2005. L-S-R = Low elevation, south facing slope, rock shelter; L-S-NR = Low elevation, south facing slope, no rock shelter; L-N-R = Low elevation, north facing slope, rock shelter; L-N-NR = Low elevation, north facing slope, no rock sheltering; H-S-R = High elevation south facing slope, rock shelter; H-S-NR = High elevation, south facing slope, no rock shelter; H-N-R = High elevation, north facing slope, rock sheltering; H-N-NR = High elevation, north facing slope, no rock shelter.

Table 3.5. Three-way Analysis-of-variance for soil moisture content in each of eight microsite treatments (high (3780 m) and low (3060 m) elevation, north- and south- facing slopes, and with and without rock-shelter), August 23, 2005. P-values less than 0.05 are emboldened for emphasis.

Source of variation	df	Mean Squares	F ratio	P
Elevation	1	42124.8	72.1	**0.0136**
Slope aspect	2	584.6	0.2	0.8520
Rock shelter treatment	4	3505.3	5.5	**0.0004**
Error	133	638.3		
Total	140			

Table 3.6. Three-way Analysis-of-variance for soil moisture content in each of eight microsite treatments (high (3780 m) and low (3060 m) elevation, north- and south- facing slopes, and with and without rock-shelter), September 10, 2005. P-values less than 0.05 are emboldened for emphasis.

Source of variation	df	Mean Squares	F ratio	P
Elevation	1	2529.34	8.02	**0.0053**
Slope aspect	2	735.20	2.33	0.1010
Rock shelter treatment	4	848.79	2.69	**0.0336**
Error	136	315.1		
Total	143			

Table 3.7. Mean values (+/- SE, when possible) for four measures of plant performance of *Muhlenbergia richardsonis* in the low elevation (Crooked Creek, 3060 m) plots. Sample size column refers to number of plants observed. Survivorship values are the average per cent plants surviving in each of five blocks per treatment. 'NA' means non-applicable. Low sample size due to poor survivorship precluded statistical hypothesis testing.

Aspect	Rock shelter treatment	Sample size	Above-ground biomass per plant (mg)	Plant height (cm)	Number of inflor-escene per plant	Plant survivors (%)
North	+ rock	5	47±13	4.3±0.7	1.7±0.7	17±8
North	-rock	0	NA	NA	NA	0
South	+rock	3	77±29	4.6±1.1	3.	10±7
South	-rock	1	49	3.8	1.0	3±3

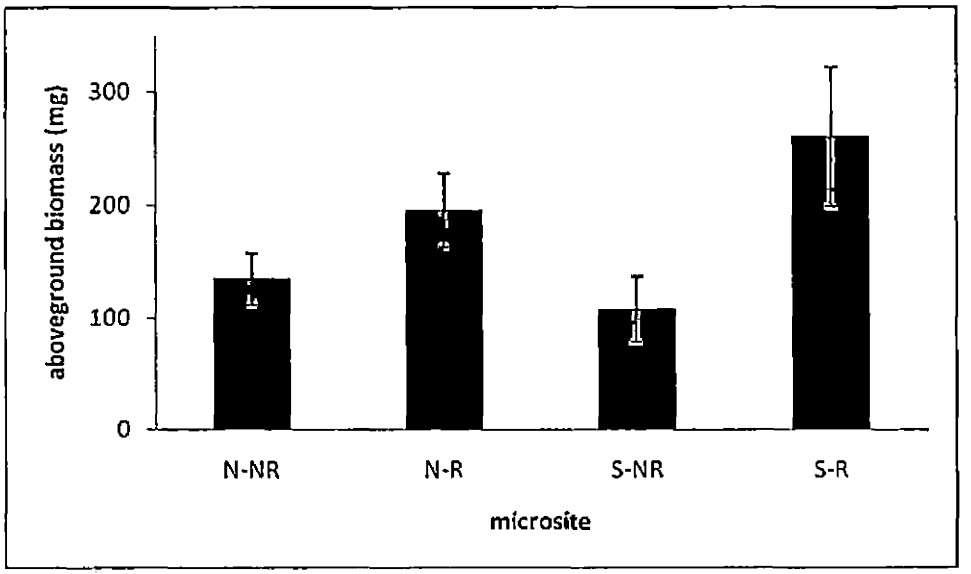

Figure 3.6. Above-ground dry biomass per plant (mean +/- SE) of *Muhlenbergia richardsonis* from each of four microsite treatments at the Barcroft site (3780 m). N-NR and S-NR refer to north- and south-facing slopes, respectivly, in the no rock-shelter treatment. N-R and S-R refer to north- and south-facing slopes, respectively, in the rock-shelter treatments. Sample sizes for each treatment (with statistical outliers removed): N-NR = 8, S-NR = 14, N-R =17, and S-R = 24

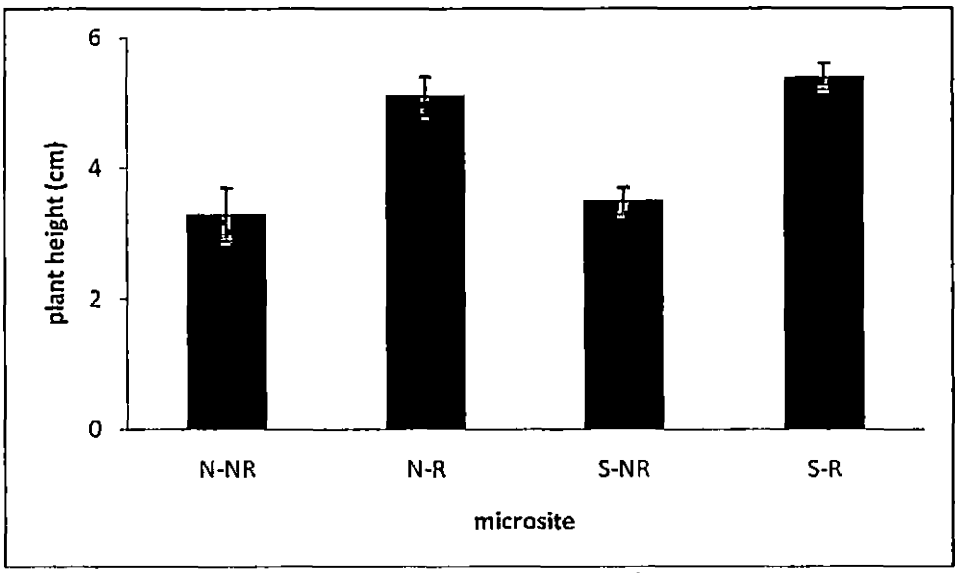

Figure 3.7. Plant height (mean +/- SE) of *Muhlenbergia richardsonis* from each of four microsite treatments at the Barcroft site (3780 m). Microsite symbols as defined in Figure 3.6. Sample sizes for each treatment (with statistical outliers removed): N-NR = 9, S-NR = 14, N-R =17, and S-R = 24

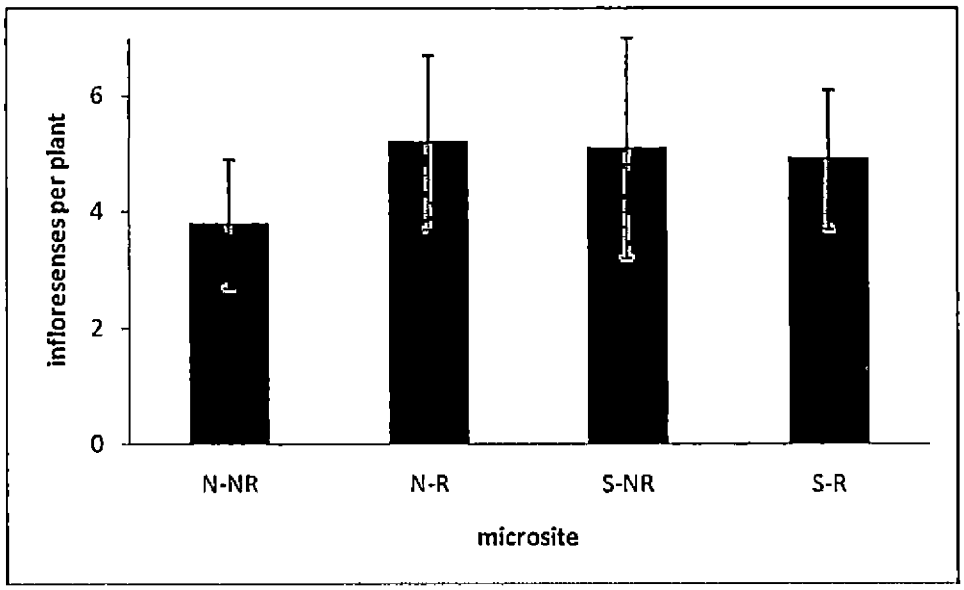

Figure 3.8. Number of infloresences per plant (mean +/- SE) of *Muhlenbergia richardsonis* from each of four microsite treatments at the Barcroft site (3780 m). Microsite symbols as defined in Figure 3.6. Sample sizes for each treatment (with statistical outliers removed): N-NR = 4, S-NR = 7, N-R =11, and S-R = 16.

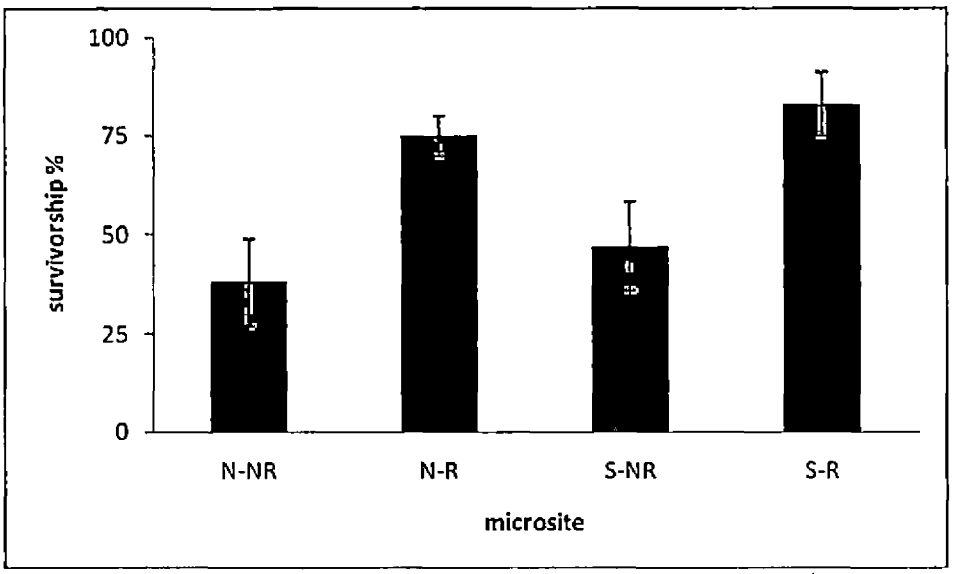

Figure 3.9. Plant survivorship (%; mean +/- SE) of *Muhlenbergia richardsonis* from each of four microsite treatments at the Barcroft site (3780 m). Microsite symbols as defined in Figure 3.6. Sample sizes for each treatment (with statistical outliers removed): N-NR = 4 blocks, S-NR = 5 blocks, N-R = 4 blocks, and S-R = 5 blocks.

Table 3.8. Two-way analysis-of-variance results for four measures of plant performance of *Muhlenbergia richardsonis* in the high-elevation (Barcroft 3780 m) plots. Survivorship data were analyzed by blocks. Rock-shelter treatments were nested within slope-aspect treatments. Response variables were log transformed where necessary for homogeneity of variance assumptions. Significant values are in bold print.

Response variable	Source of variation	df	Mean square	F ratio	P value
Above ground biomass per plant	Slope aspect	1	0.045	0.08	0.804
	Rock shelter	2	0.564	3.30	**0.044**
	Error	59	0.171		
	Total	62			
Plant height	Slope aspect	1	0.011	0.039	0.861
	Rock shelter	2	0.273	21.68	**<0.0001**
	Error	60	0.126		
	Total	63			
Inflorescences per plant	Slope aspect	1	0.004	0.400	0.592
	Rock shelter	2	0.009	0.072	0.931
	Error	34	0.131		
	Total	37			
% Plant survivorship	Slope aspect	1	340.278	0.110	0.772
	Rock shelter	2	3088.03	8.401	**0.004**
	Error	14	367.565		
	Total	17			

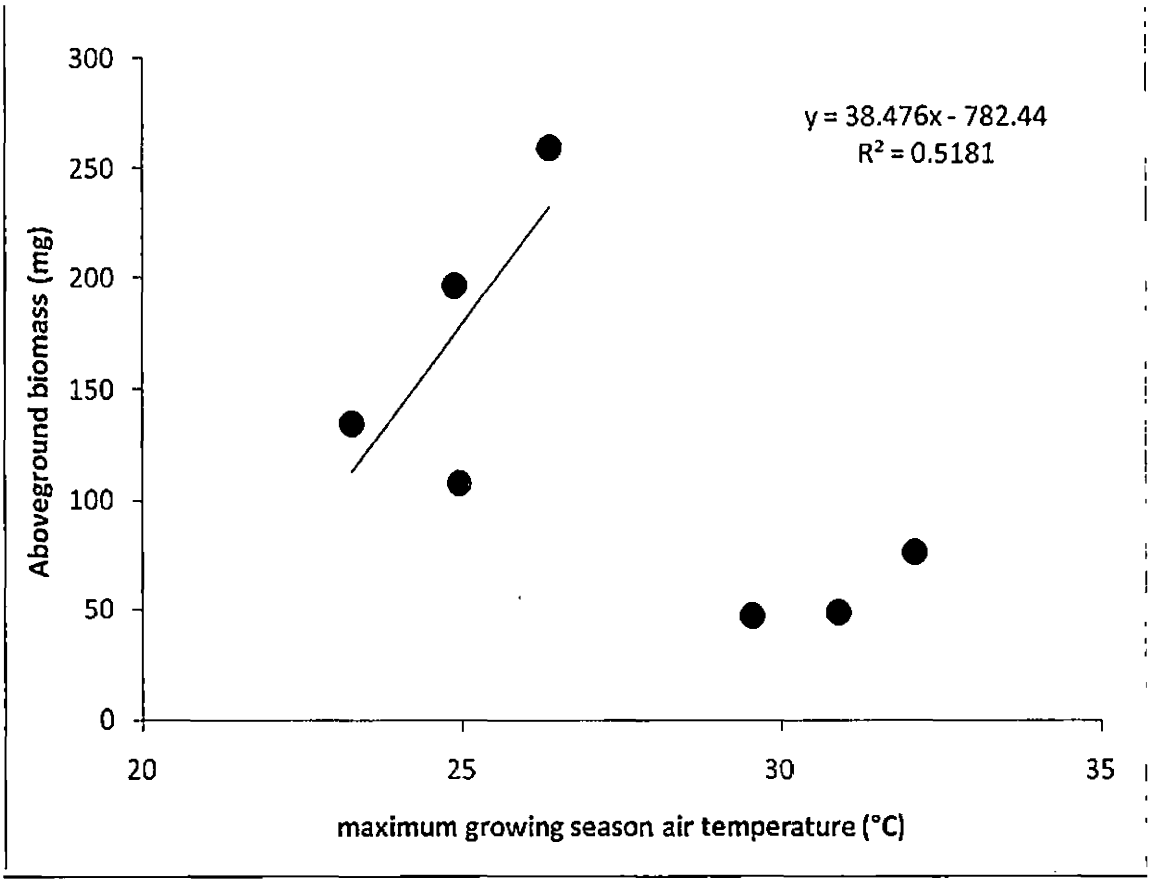

Figure 3.10. Relationship between aboveground biomass of *Muhlenbergia richardsonis* plants at the end of 2005 growing season and maximum daytime air temperature (as measured August 24-September 9, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

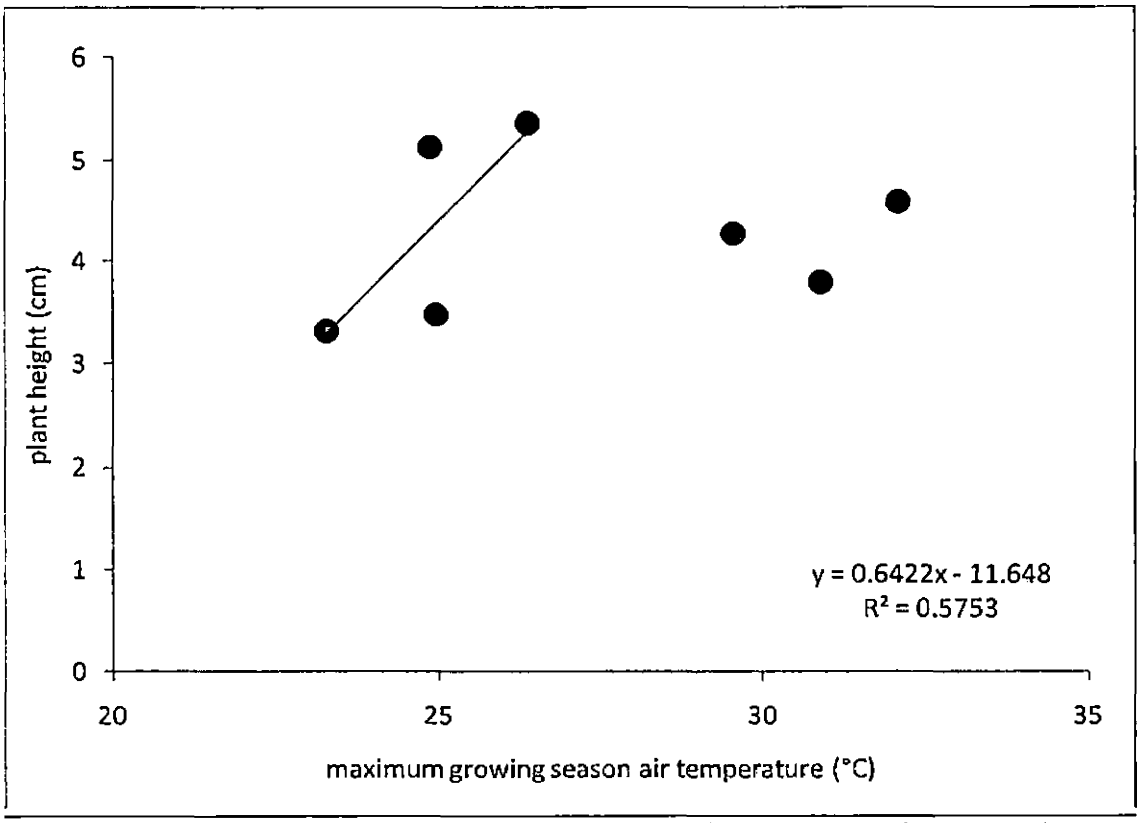

Figure 3.11. Relationship between height of *Mulenbergia richardsonis* plants at the end of 2005 growing season and maximum daytime air temperature (as measured August 24-September 9, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

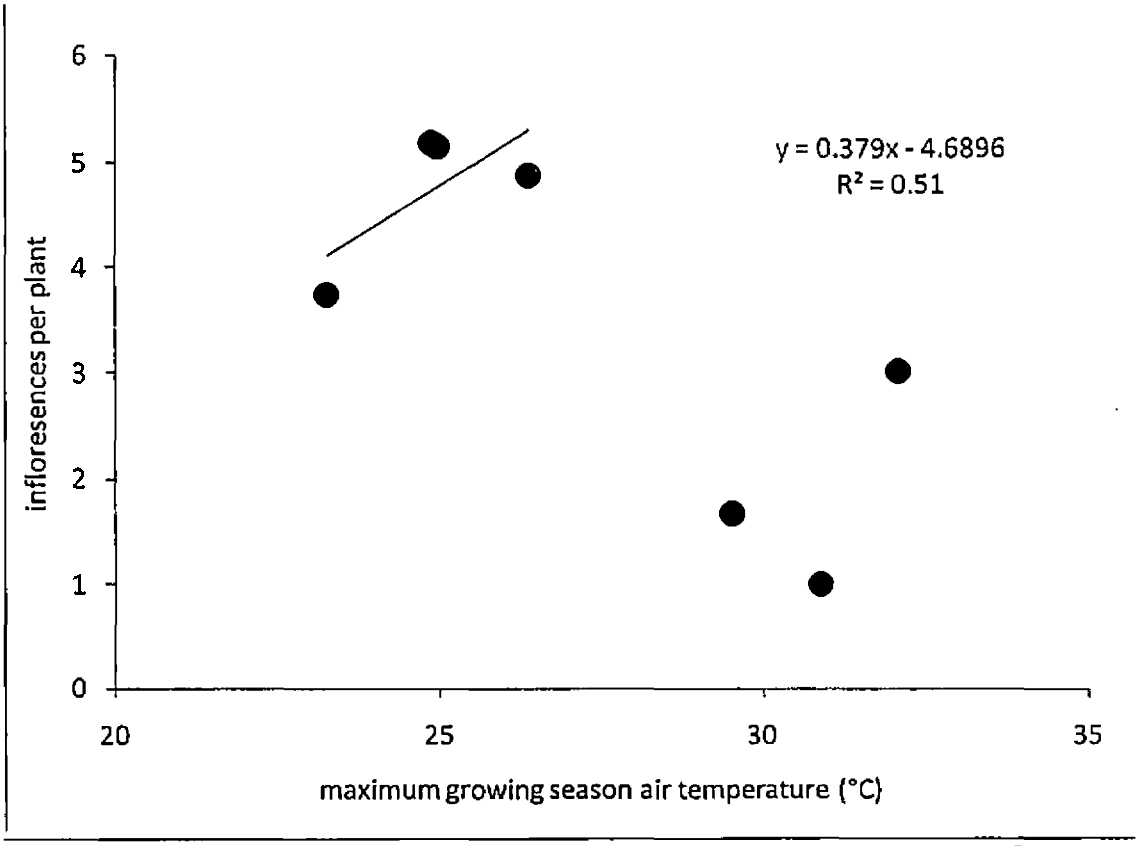

Figure 3.12. Relationship between infloresence per plant of *Mulenbergia richardsonis* at the end of 2005 growing season and maximum daytime air temperature (as measured August 24-September 9, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

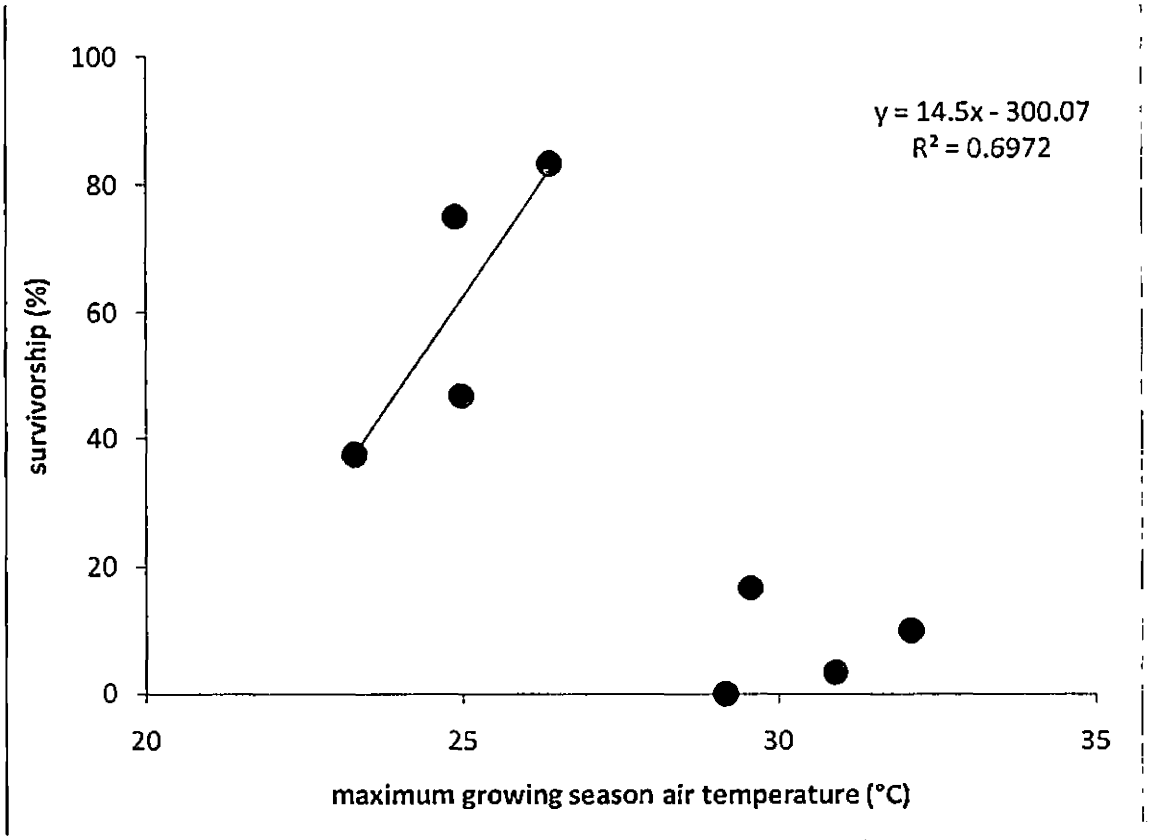

Figure 3.14. Relationship between survivorship of *Mulenbergia richardsonis* plants at the end of 2005 growing season and maximum daytime air temperature (as measured August 24-September 9, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

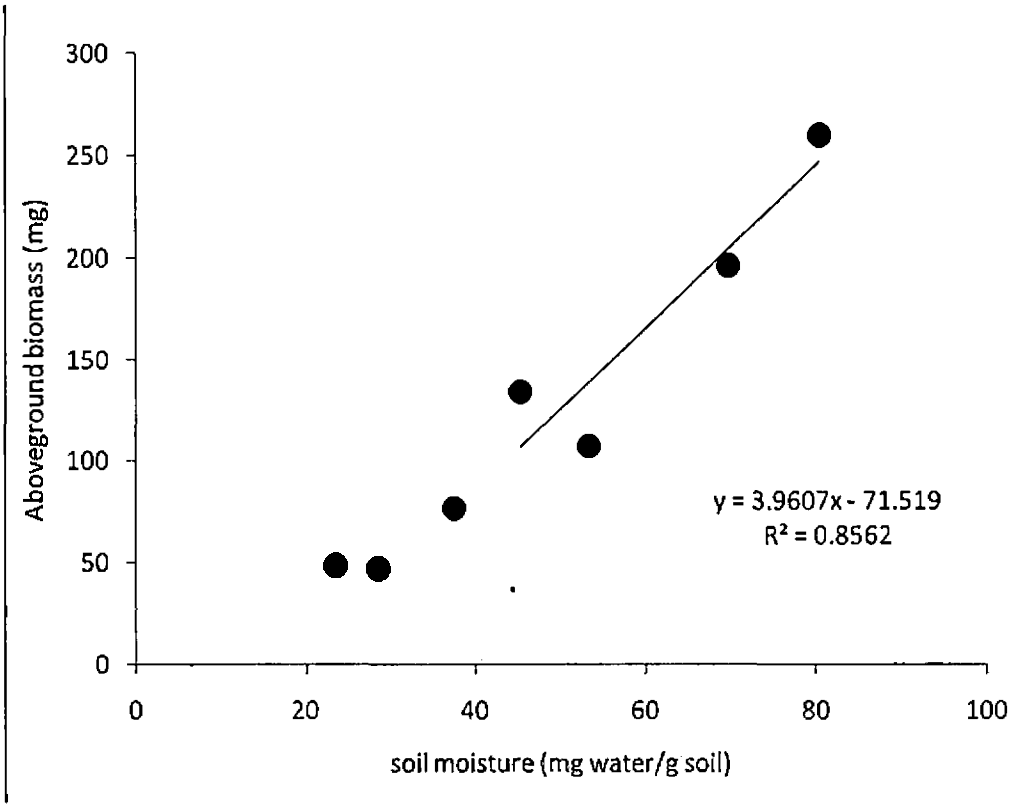

Figure 3.15. Relationship between aboveground biomass of *Muhlenbergia richardsonis* plants at end of 2005 growing season and soil moisture content (measured August 23, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

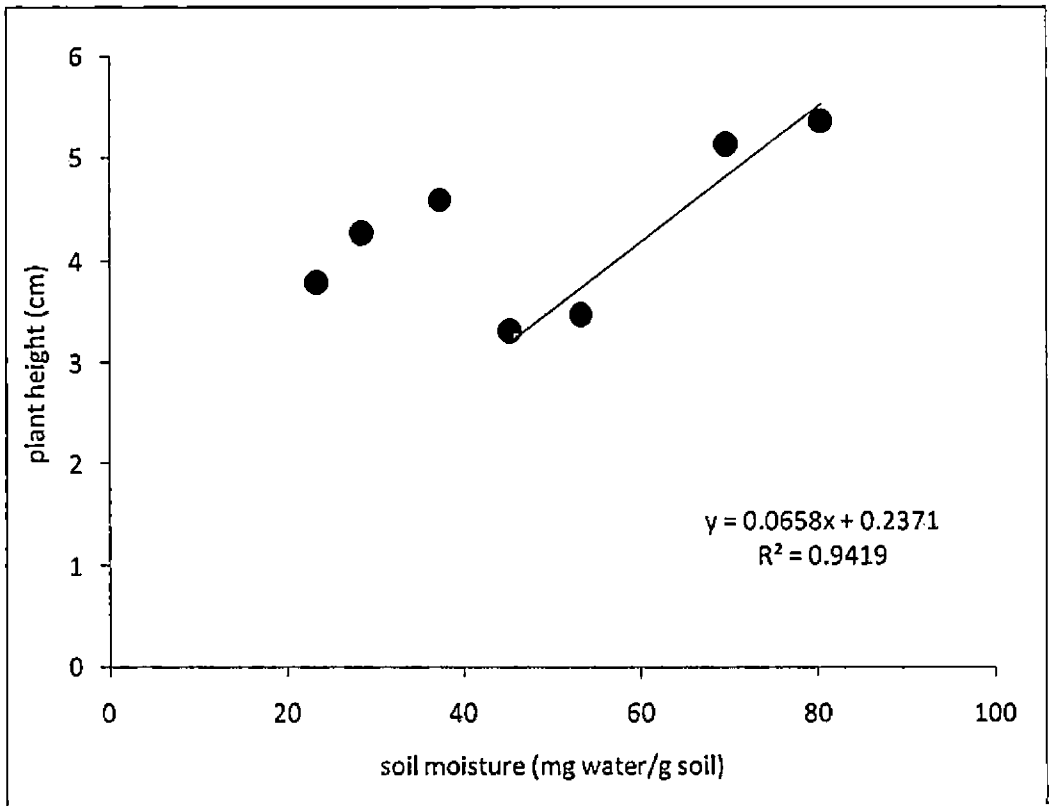

Figure 3.16. Relationship between height of *Mulenbergia richardsonis* plants at the end of 2005 growing season and soil moisture content (measured August 23, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

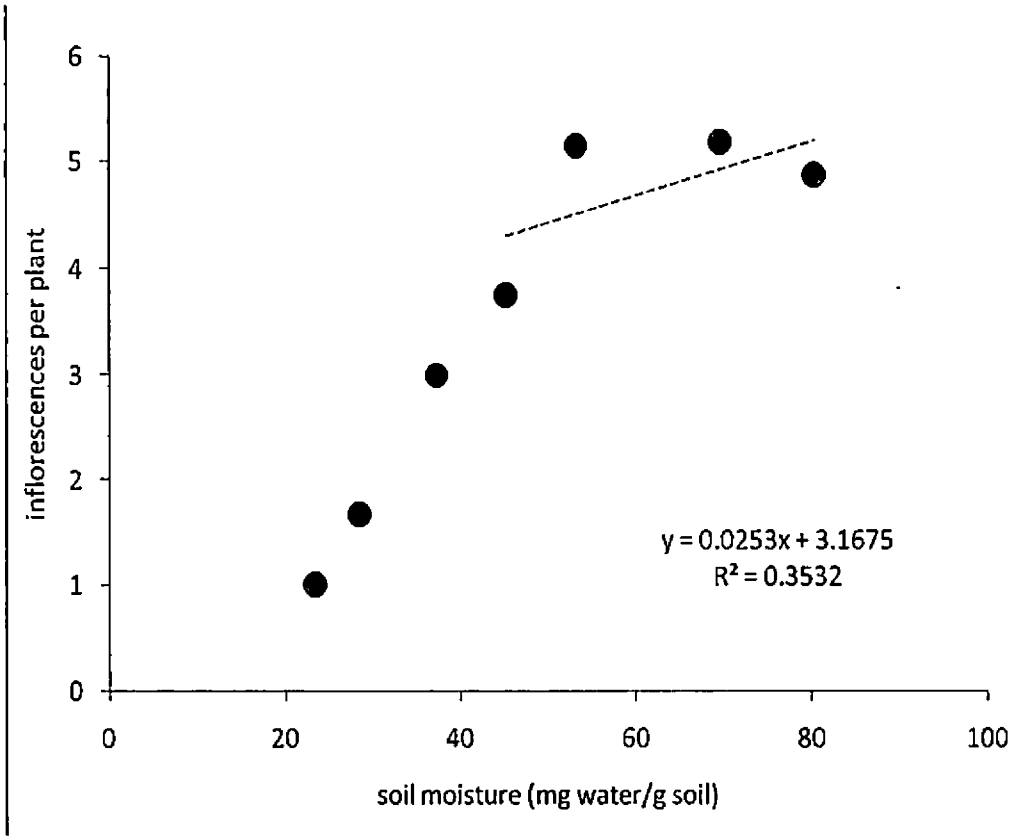

Figure 3.17. Relationship between infloresence per plant of *Mulenbergia richardsonis* at the end of 2005 growing season and soil moisture content (measured August 23, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

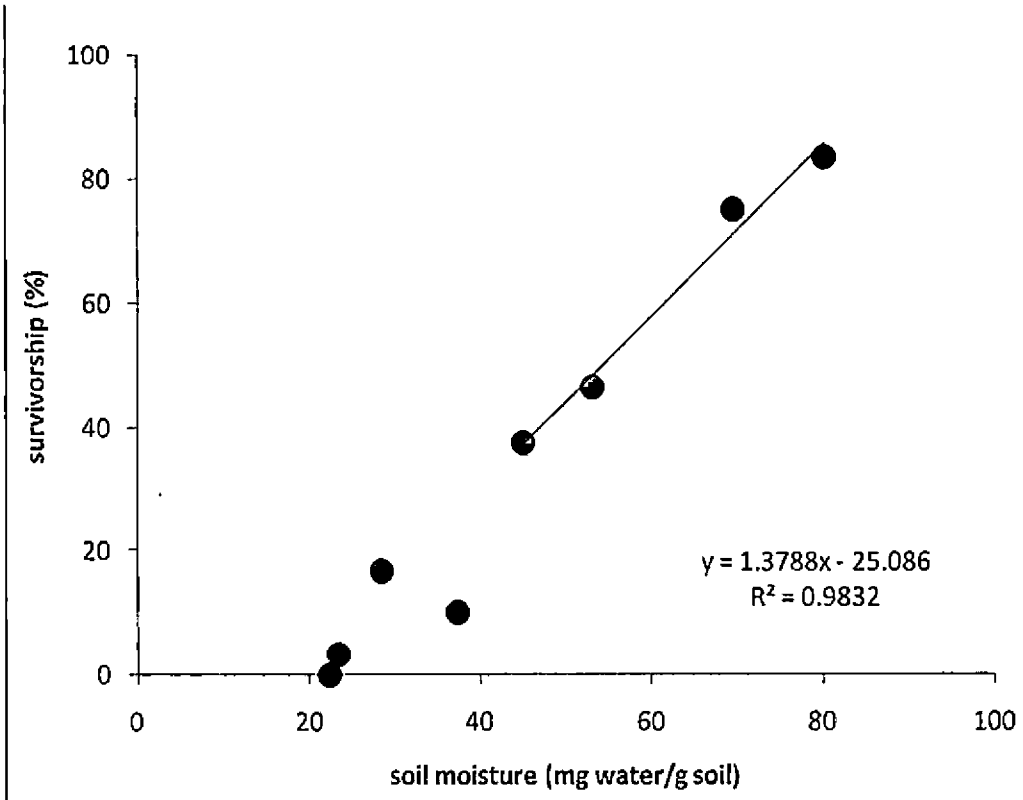

Figure 3.18. Relationship between survivorship of *Mulenbergia richardsonis* plants at the end of 2005 growing season and soil moisture content (measured August 23, 2005). Plot points are the averages from each of the eight microsite treatments. Mean values are plotted from all sites which had surviving plants at the time of harvest. Due to poor survivorship among low-elevation plants, the regression analysis was only applied to data from high-elevation plants. The results of the regression analysis are shown in the figure.

APPENDIX B

ECOTYPE STUDY

Ecotype Experiment Results

Because *M. richardsonis* has a fairly long flowering season, is wind pollinated, and grows in the very windy conditions of this montane habitat it seemed unlikely that there would be any discernible genetic differences between plants sampled from a sub-alpine population at ~3000 meters and plants sampled from an alpine population at ~3800 meters. At the same time, there are many examples of altitudinal ecotype differences in plants (Clausen et al,. 1948; Bowman and Turner, 1993; Jonas and Geber, 1999) and the possibility does exist that part of the solution to the enigmatic presence of *M. richardsonis* at the high elevations is that there has been strong selection among the alpine populations for traits adaptive for success in the high alpine zone. We explored these issues by carrying out a greenhouse 'common garden' study on plants collected from both the 3000 m and the 3800 m populations. The possibility of there being soil ecotypes was also explored in a greenhouse 'common garden' study where plants from both populations were raised in soils collected from each of the elevation sites. Finally, to look for genetic based difference in ecological performance at each

site, reciprocal transplant gardens were established at the 3000 m and the 3800 m site. These studies were carried out to look for the possibility of elevational ecotypes in *M. richardsonis*. Among these different studies, only the first greenhouse study yielded sufficient data to date from which to draw statistically reliable conclusions. Consequently, only these results are presented.

Plant trait	Mean		SE		P value
Mass (mg)	CC Bar	859 1269	CC Bar	.087 .131	.001
Leaf length (mm)	CC Bar	45.9 28.3	CC Bar	2.179 1.112	.001
Leaf width (mm)	CC Bar	1.71 1.5	CC Bar	.041 .045	.001
Plant height (cm)	CC Bar	16.97 13.83	CC Bar	.686 .520	.001
Inflorescence height (cm)	CC Bar	25.92 22.45	CC Bar	.826 .771	.005
Inflorescence number Per pot	CC Bar	6.32 19.47	CC Bar	.602 1.990	.001

Appendix B Table 1.Ecotype results from a common greenhouse study wherein plants were collected from low elevation (CC = Crooked Creek, 3060 m) and high elevation (Bar = Barcroft, 3780 m) and then grown side by side in individual pots in a controlled green house at CSUSB. Twenty plants

from each elevation were grown for six weeks and then plant traits measured.

Each of the six traits measured in these plants proved to differ significantly between plants from the two populations when grown together under the same conditions. This indicates strong genetic differences between these two populations of *M. richardsonis,* suggesting the possibility of ecotypic differention along the White Mountain gradient. This allows for the possibility that there exists strong selection for particular traits for the survival of this grass among plants at the most extreme high-elevation sites. Thus, evolution, in the form of ecotypic differentiation, as well as ecological cold-avoidance (through phenology and warm microsite preference) may help explain the success of this highest of all known North American C_4 species.

APPENDIX C

ESTIMATING THE ATMOSPHERIC PRESSURE LAPSE RATE

1. The atmospheric pressure was calculated as a function of altitude using a standard formula derived from the ideal gas law

$$P_a = P_r * \left[\frac{T_r}{T_r + L*(a - a_r)} \right]^{\frac{G*M}{R*L}}$$

where

P = Static atmospheric pressure (pascals)
T = Air Temperature (kelvins)
L = Lapse rate; -0.0078 kelvins per meter
(empirically derived for the White Mountains in the summer)
a = Altitude above mean sea level (meters)
R = Universal gas constant for air: 8.31432×10^3 N·m / (kmol·K)
G = Gravitational acceleration constant (9.80665 m/s²)
M = Molar mass of the Earth's atmosphere (28.9644 g/mol)

Subscript r refers to "reference values". These values selected were for altitudes below 11,000 meters and were originally from U.S. Standard Atmosphere, 1976, U.S. Government Printing Office, Washington, D.C., 1976 as used for the Barometric formula in http://en.wikipedia.org/wiki/Barometric_formula (2007).

P_r = 101,132.5 Pa
T_r = 288.15 K
a_r = 0 meters above mean sea level

(http://en.wikipedia.org/wiki/Barometric_formula) retrieved March 24, 2007

2. Mole fraction atmospheric CO2 as of Jan 2007 = 383 ppm or µmol/mol (http://www.esrl.noaa.gov/gmd/) retrieved March 24, 2007

3. Mole fraction is a conserved quantity and, as such, does not depend upon pressure or temperature of the air.

4. Photosynthesis depends upon the concentration of CO2 molecules in a volume of air. Even at a constant mole fraction of CO2, the concentration decreases with altitude as the overall atmospheric pressure declines.

REFERENCES

Billings, WD Mooney HA.1968. The ecology of arctic and alpine plants. *Biological Review* 43, 481-529.

Bliss, LC. 1966 Plant productivity in alpine microenvironments on Mt. Washington, New Hampshire. *Ecological Monographs* 36, 125-155.

Bowman WD, Turner L. 1993. Photosynthetic sensitivity to temperature in populations of two C_4 *Bouteloua* (Poaceae) species native to different altitudes. *American Journal of Botany* 80, 369-374.

Cavagnaro, JB. 1988. Distribution of C_3 and C_4 grasses at different altitudes in temperate arid region of Argentina. *Oecologia* 76, 273-277.

Chazdon RL. 1978. Ecological aspects of the distribution of C4 grasses in selected habitats of Costa Rica. *Biotropica* 10, 265-269.

Clausen J, Keck DD, Hiesey WM. 1948. Experimental studies on the nature of species. Environmental responses of climatic races of *Achillea*. *Carnegie Institution of Washington*. Puplication 581.

Edwards G, Walker DA. 1983. C_3, C_4: Mechanisms, and cellular and environmental regulation, of photosynthesis. *University of California Press*: Berkely.

Ehleringer JR, Björkman O. 1977. Quantum yields for CO_2 uptake in C_3 and C_4 plants. *Plant Physiology* 59, 86-90.

Ehleringer JR, Cerling TE, Helliker BR. 1997. C_4 photosynthesis, atmospheric CO_2, and climate. *Oecologia* 112, 285-299.

Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW. 1991. Climate change and the evolution of C_4 photosynthesis. *Trends in Ecology and Evolution*. 6, 95-99.

Hall, CA ed. 1991. Natural history of the white-inyo range, eastern California. *University of California Press*. Los Angeles.

Henderson S, Hattersley P, von Caemmerer S, Osmond CB. 1995. Are C_4 pathway plants threatened by global climatic change? In: Schulze ED, Caldwell MM, eds. *Ecophysiology of photosynthesis*. Berlin, Germany: Springer-Verlag, 529-549.

Jonas CS, Geber MA. 1999. Variation among populations of *Clarkia unguiculata* (Onagraceae) along altitudinal and

latitudinal gradients. *American Journal of Botany* 86, 333-343.

Kanai R, Edwards GE. 1999. The biochemistry of C_4 photosynthesis. In: Sage RF, Monson RK, eds. *C4 Plant Biology*. New York: Academic Press, 49-87.

Karlsson PS, Weih M. 2001. Soil temperatures near the distribution limit of the mountain birch (*Betula pubescens*): implications for seedling nitrogen economy and survival. *Arctic, Antarctic and Alpine Research* 33, 88-92.

Keppel G, Saufley WH, Tokunaga H. 1992. *Introduction to design and analysis*. New York: Freeman and Company

Körner C, Farquhar GD, Roksandic Z. 1988. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* 74, 623-623.

Körner C. 1989. The nutritional status of plants from high altitudes. A world wide comparison. *Oecologia* 81, 379-391.

Körner C. 2003. *Alpine plant life, functional plant ecology of high mountain ecosystems*. Springer, New York.

Körner C. 2007. The use of the 'altitude; in ecological research. *Trends in Ecology and Evolution*. 22, 569-574.

Kubien DS. et al 2003 C_4 photosynthesis at low temperature. A study using transgenic plants with reduced amounts of rubisco. *Plant Physiology* 132, 1577-1585.

Kubien DS, Sage RF. 2004. Low temperature photosynthetic performance of a C4 grass and co-occurring C3 grass native to high latitudes. *Plant, Cell and Environment* 27, 907-916.

Lloyd RM, Mitchell RS. 1973. A flora of the White Mountains, California and Nevada. *University of California Press*. Berkeley.

Long SP. 1983. C_4 Photosynthesis at low temperatures. *Plant, Cell and Environment* 6, 345-363.

Monson RK, Williams GJ. 1982. A correlation between photosynthetic temperature adaptation and seasonal phenology patterns in the shortgrass prairie. *Oecologia* 54, 58-62.

Mooney HA. 1973. Plant communities and vegetation. In: Lloyd RM, Mitchell RS, eds. *A flora of the White Mountains, California and Nevada*. Berkeley: University of California Press, 7-17.

Ogle K. 2003. Implications of interveinal distance for quantum yield in C_4 grasses: a modeling and meta-analysis. *Oecologia* 136, 532-542.

Oke TR, 1987. *Boundary layer climates*. New York: Methuen

Parmesean C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics*. 37, 637-669.

Pearcy RW, Ehleringer J. 1984. Comparative ecophysiologyof C_3 and C_4 plants. *Plant, Cell and Environment* 7, 1-13.

Pittermann J, Sage RF. 2000. Photosynthetic performance at low temperature of *Bouteloua gracilis* (Lag.), a high-altitude C_4 grass from the Rocky Mountains, USA. *Plant, Cell and Environment* 23, 811-823.

Pittermann J, Sage RF. 2000. The response of the high altitude C_4 grass *Muhlenbergia Montana* (Nutt.) (A.S. Hitchc.) To long and short-term chilling. *Journal of Experimental Botany* 52, 829-838.

Rundel, PW 1980. The ecological distribution of C_4 and C_3 grasses in the Hawaiian Islands. *Oecologia* 45, 354-359.

Sage RF. 2003. The evolution of C_4 photosynthesis. *New Phytologist* 161, 341-370.

Sage RF, Kubien DS. 2003. *Quo vadis* C_4? An ecophysiological perspective on global change and the future of C_4 plants. *Photosynthesis Research* 77,209-225.

Sage RF, McKown AD. 2005. Is C_4 photosynthesis less phenotypically plastic than C_3 photosynthesis? *Journal of Experimental Botany* 57, 303-317.

Sage RF, Monson RK. editors. 1999. *C_4 Plant biology*. Academic Press, San Diego.

Sage RF, Sage TL. 2002 Microsite characteristics of *Muhlenbergia richardsonis* (Trin.) Rydb., an alpine C_4 grass from the White Mountains, California. *Oecologia* 132, 501-508.

Sage RF, Wedin DA, Li M. 1999 *The biogeography of C_4 photosynthesis: patterns and controlling factors in C_4 plant biology*. Academic Press, San Diego.

Taiz L, Zeiger E. 2002. *Plant physiology*, Third edition. Sinauer, Sunderland.

Terri JA, Stowe LG. 1976. Climatic patterns and the distribution of C_4 grasses in North America. *Oecologia* 23, 1-12.

Tieszen LL, Senyimba MM, Imbamba SK, Troughto JH. 1979. The distributuin of C_3 and C_4 grasses and carbon isotope

discrimination along an altitudinal and moisture gradient in Kenya. Oecologia 37, 337-350.

Tissue DT, Wright SJ. 1995. Effect of seasonal availability on phenology and the annual shoot carbohydrate cycle of tropical forest shrubs. Functional Ecology 9, 518-552.

Walther GR, Beibner S, Pott, R. 2005. Cliamte change and high mountain vegetation shifts. In: Broll G, Keplin B. eds. *Mountain ecosystems: studies in treeline ecology*, Springer: Berlin, pp 77-96.

Wan CSM, Sage RF. (2001) Climate and the distribution of C_4 grasses along the Atlantic and Pacific coast of North America. *Canadian Journal of Botany* 79: 474-486.

Woodward FI, Williams BG. 1987. Climate and plant distribution at global and local scales. *Vegetation* 69, 189-197.