

Physiological and Morphological Changes in *Ipomopsis aggregata* Along an Elevational Gradient

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Abstract

Changes in the morphology and physiology of *Ipomopsis aggregata* and changes in environment were assessed along a roughly 1000 m elevation gradient at seven sites in the Rocky Mountains of central Colorado. Sites at the ends of the gradient had the lowest soil moisture, the lowest two being in sagebrush scrub land and the highest on rocky alpine talus. Soil moisture decreases for the highest five sites but is intermediate for the lowest two sites. Minimum temperatures and relative humidity decreased with altitude. *I. aggregata* showed significant changes in leaf structure, glutathione reductase activity and water use efficiency which correlate best with elevation. Plants at higher elevations had larger leaves with higher specific leaf area (SLA) and higher water content. Stomatal density declined at both ends of the gradient. Leaf hair density was very high at the low desert-like site but otherwise increased significantly with elevation. Glutathione reductase (GR) activity was very high at the second lowest site but low at the lowest, even though both sites should experience similar drought stress. GR activity increased significantly for the higher five sites. Photosynthetic rates were similar between all sites measured but transpiration was significantly lower at the lower and drier site compared to the higher sites. Water use efficiency (WUE) was significantly higher at the lower site compared to the higher sites. Overall, *I. aggregata* does not appear to be experiencing increasing environmental stress with increasing elevation and in fact seems to thrive at high elevation sites.

Introduction

Alpine plants show the ability to thrive at high elevations despite facing extreme variation in climate. The alpine environment is characterized by high light intensity and usually severe limits on growth (Williams *et al.* 2003). Under strong light, low temperatures or severe water shortage, alpine plants face the possibility that over stimulation of photosystems may cause photo-oxidative damage to overwhelm repair mechanisms. Alpine plants are well adapted to dissipating, or at least preventing damage from, excess photosynthetic energy gathered at the high irradiance and harsh conditions encountered in the mountains (Streb *et al.* 1998, Turunen and Latola 2005, Cui *et al.* 2003). Most alpine plants show low levels of photodepression (damage or downregulation of photosystems) (Cui *et al.* 2003, Streb *et al.* 1998) and do not seem to be limited by carbon gain (Diemer and Korner 1996). Species with wide altitudinal ranges also show their maximum rate of photosynthesis at temperatures near those normally experienced (Buchner and Neuner 2003, Wu and Campbell 2006, Korner and Diemer 1987).

Many physiological characters are known to change with elevation which are associated with energy dissipation. As the concentration of CO₂ decreases with altitude many species compensate by increasing their maximum rate of photosynthesis (A) through a variety of morphological and physiological changes (Korner and Diemer 1987, Friend *et al.* 1989). High rates of photosynthesis at low CO₂ partial pressures are associated with increased stomatal conductance in order to access the same amounts of carbon as at lower elevations, causing more extreme water stress (Friend *et al.* 1989). Changes in leaf morphology are also observed with increasing altitude. *Origanum vulgare* over a 1500 m elevation gradient showed increased leaf thickness, a 2-4 fold increase in stomatal density and a 3-fold increase in hair density on the top surface of leaves (Kofidis *et al.* 2003). Leaf orientation, affecting interception of light, also seems to play a significant role (Cui *et al.* 2003, Barker and Adams 1997). High elevation plants tend to have thicker leaves, higher leaf mass per area and higher nitrogen content per leaf area (Shi *et al.* 2004, Kofidis *et al.* 2003, Korner and Diemer 1987, Campbell *et al.* 2005). This allows them to collect less light and conserve water by reducing leaf surface area. Chlorophyll and carotenoid content has been shown to decrease with altitude, probably to prevent overwhelming electron acceptors and damaging cells (Kofidis *et al.* 2003, Lei *et al.* 2006, Bermadinger Stabentheiner 1996) but anthocyanins which attenuate light sometimes increase (Williams *et al.* 2003).

In high light environments where growth is severely constrained by the environment (water, nitrogen, cold) depletion of electron acceptors and formation of reactive oxygen species (ROS) is a serious concern. During photosynthesis, ROS are constantly being generated and detoxified by a battery of small molecule antioxidants and antioxidant enzymes. Increased levels of ROS generated under almost any stressful condition in turn can wreck havoc upon organic molecules, damaging membranes, DNA, RNA, proteins and the photosynthetic machinery itself, resulting in photoinhibition (Streb *et al.* 1998, Moller 2001). Besides morphological changes reducing light interception, plants have evolved many mechanisms to combat ROS or to prevent their formation in the first place. Alpine plants show either very efficient carbon metabolism or the ability to increase the expression or activity of antioxidants to rapidly scavenge ROS (Streb *et al.*

1998). Plants adapted to high altitude (Polle and Rennenberg 1992, Bermadinger Stabentheiner 1996, Ren *et al.* 1999) or drought conditions (Lei *et al.* 2006, Sairam *et al.* 1998, Schwanz and Polle 2001, Lei *et al.* 2006) tend to show increased activity of the antioxidant enzymes glutathione reductase (GR) and ascorbate peroxidase (APX) compared to lowland or drought avoiding species. As such, the activities of GR or APX are good indicators of both oxidative stress and a plant's ability to manage that stress. Excess energy may also be dissipated by non-photochemical processes via chlorophyll fluorescence and xanthophyll-cycle-dependent thermal energy dissipation (Barker and Adams 1997, Williams *et al.* 2003, Cui *et al.* 2003). Alternatively, photorespiration may account for a very significant portion of energy utilization (Cui *et al.* 2003).

The aim of this study is to examine how physiological and morphological traits change with altitude in *Ipomopsis aggregata* (Polemoniaceae) and to gather environmental data to identify the environmental variables that most correlate with these changes *I. aggregata*.

Hybrids between *I. aggregata* and closely related *Ipomopsis tenuituba* which lives at higher elevations show striking differences in photosynthetic gas-exchange traits (Wu and Campbell 2006) suggesting interesting physiological adaptations which vary with elevation. *Ipomopsis tenuituba* shows higher rates of photosynthesis optimized to higher temperatures and better water use efficiency (WUE) than *I. aggregata*. As elevation increases, growing conditions can be expected to become more difficult up to a point where upward range expansion is checked. At the site where *Ipomopsis* is forming hybrids, the higher elevation, more exposed hybrid site is warmer, drier, and has higher irradiances than the *I. aggregata* sites (Campbell *et al.* 2005). Thus, it seems that *I. tenuituba* and the success of hybrids over *I. aggregata* at the higher elevation (Campbell and Waser 2001) and more environmentally severe hybrid zone is at least partially due to WUE and enhanced rates of photosynthesis. Still, we do not know what the underlying physiological mechanisms are which contribute to this increased fitness at higher elevations.

Methods

Study sites

Seven sites were examined between 3,424 m on the steep southwest slopes of Mt. Bellview and proceeding directly South along Gothic Road down to around 2,400 m near Almont, Gunnison county, CO (Table 1). Sites are south or southwest facing and on relatively exposed, dry hillsides especially on the slopes of Mt. Bellview. When plants were abundant enough, sampling was performed using three, ten meter transects at each site, selecting the plant closest to each meter for a sample size of 28-30 vegetative rosettes of *Ipomopsis aggregata* at each site. Each plant was marked so that gas exchange, oxidative stress and vegetative characteristics could be analyzed by plant.

Measurement of environmental variables

Temperature and relative humidity or light (Table 1) were measured with Hobo data loggers (Onset corp.) for up to 22 days depending on the site ending August 7, 2006. Loggers were placed 4 cm above the ground, similar to the height of the small vegetative rosettes. At sites B,D, and E logging started on 7-17; Site E, 7-18; sites A and C, 7-27; site G, 7-31. Soil moisture was measured on July 20, Aug 9 and Aug 15, 2006 in the afternoon after it had been dry for at least one day. Daily thunderstorms typically saturated the ground from mid July to mid August. Measurements were made at each site near the base of each plant with a Hydrosense soil moisture probe (Campbell Scientific Australia) for a total of about 90 readings over 3 days per site.

Gas exchange measurements

Instantaneous rates of photosynthesis (A_{max}), transpiration (E), stomatal conductance (g) and internal CO₂ concentration (c_i) at 3 (Table 1) sites was performed with a LI-COR 6400 (LI-COR, Lincoln, NE, USA) portable infrared gas-analysis system (IRGA). Each leaf was measured twice, once controlling leaf temperature at 27C and once allowing the leaf chamber to vary with ambient temperature. Sample CO₂ concentration was 375 ppm and photosynthetic photon flux density (PPFD) was set to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Sampling was performed between 9 am and 1 pm when stomates were open on up to twenty vegetative plants. Instantaneous water use efficiency was calculated from A_{max}/E . All gas exchange measurements were corrected for leaf area by collecting each leaf sampled and determining its area using a scanner and ImageJ image analysis software (<http://rsb.info.nih.gov/ij/>).

Glutathione reductase activity

The ascorbate-glutathione pathway is one mechanism for scavenging H_2O_2 generated as a harmful biproduct of photosynthesis under stress and converting it to water. Glutathione is a

Table 1: Populations of *I. aggregata* along a South facing elevation gradient and sample sizes for vegetative, physiological and gas exchange measurements made at each site. Leaf = leaf length, number, specific leaf area, water content; Phys = stomatal density, hair density, glutathione reductase activity; Gas, gas exchange; Env, environmental variables.

Site	meters	Δ m	Area	Leaf	Phys	Gas	Env
A	3420	172	Mt. Bellview	30	10		temp/light
B	3248	36	Schofield Pass	24	10	20	temp/RH
C	3212	210	Mt. Bellview	30	10		temp/light
D	3002	127	Mt. Bellview	28	10		temp/RH
E	2875	205	Just South of Gothic	30	10	17	temp/RH
F	2670	235	Cement Creek Rd.	29	10	20	temp/RH
G	2435		Almont Campground	30	10		temp/light

sulfhydroxyl-containing tripeptide which is coupled to the regeneration of ascorbate for converting H_2O_2 (Dalton *et al.* 1986, Smith *et al.* 1989, Moller 2001). Reduced glutathione (GSH) is continuously converted to oxidized glutathione (GSSG) and must be continuously recycled back to GSH by the enzyme glutathione reductase in an NAD(P)H dependent reaction (Figure 1) (Dalton *et al.* 1986, Smith *et al.* 1989). Additionally, the oxidation of NAD(P)H increases the pool of available NADP⁺ to act as an electron acceptor, further helping to prevent the formation of ROS (Anderson *et al.* 1990).

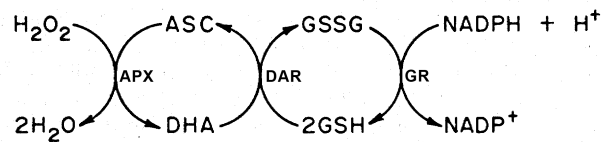


Figure 1: Oxidation-reduction reactions of the ascorbate-glutathione pathway for scavenging H_2O_2 in chloroplasts. ASC, Ascorbate; DHA, dehydroascorbate; GSSG, oxidized glutathione; GSH, reduced glutathione; APX, ascorbate peroxidase; DAR, dehydroascorbate reductase; GR, glutathione reductase). Figure from Dalton (1986).

Antioxidant defense in terms of glutathione reductase activity was determined at all sites for 10 plants each by following the GSSG-dependent oxidation of NAD(P)H at 340 nm (extinction coefficient $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) as modified from Lei *et al.* (2006) and Dalton *et al.* (1986). Leaves were collected in the morning before any mid-day stress, on July 31, 2006 for sites D thru G and August 1, 2006 for sites A thru C. Leaves were stored on ice and extracted within 6 hours of collection. Soluble proteins were extracted by macerating in a mortar and pestle about 400 mg of leaves in cold 50 mM potassium phosphate buffer (8 ml, pH 7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone. Extracts were centrifuged at high speed for 8 minutes at room temperature then stored at -20°C until use within 2 days. Assays were carried out in 2 mL-reaction mixtures containing 0.3 mM GSSG, 0.15 mM NAD(P)H in a buffer of 50mM potassium phosphate (pH 8) and 1.5 mM EDTA (Lei *et al.* 2006). To this 150 μL of extract was added. NAD(P)H was added last and immediately before monitoring absorbance at 340nm (12 readings at 340nm every 15 seconds with a 45 second recording delay at 25°C with a Milton Roy Spectronic 401 spectrophotometer). Specific activity was calculated for each extract as nmol NADPH oxidized per minute per mg protein. Protein content of the extracts was determined using the protein-dye binding assay of Bradford (1976) using BSA as the standard. A non-enzymatic treatment (no extract) and an assay to correct for NAD(P)H oxidase activity (no GSSG) were carried out as controls. NAD(P)H oxidase activity was determined for a subset of leaves ($n = 21$, 3 per site). A no intercept linear regression model with protein concentration as the independent variable and change in A340 as the dependent variable was used to generate a correction equation to determine background levels of NAD(P)H oxidase activity (linear regression $P = < .0001$, $r^2 = .89$). The calculated background change in A340 based on the protein concentration of each sample was then subtracted from the change in A340 measured during the assay to calculate the corrected glutathione reductase activity.

Morphological measurements

Length of the longest leaf and number of leaves were measured in the field for about 30 plants at all sites. Three leaves per plant per site were collected between July 7 and July 20th. Leaves were stored moist at 4C and weighed within a few hours of collection to determine wet weight. Leaf area was determined the same day or within 24 hours using a scanner and ImageJ. Leaves were then dried for 24 hours at 70C and weighed again (dry-weight) and specific leaf area (SLA) was calculated as Area/dry-weight; wet-weight – dry-weight/wet-weight was used for percent water content. Leaf hair density was estimated by scanning another leaf (600 dpi grey-scale) and enhancing leaf hair to leaf contrast using the GIMP (GNU image manipulation program, www.GIMP.org, freely distributed) and the unsharp mask filter (radius 12, amount 4, threshold 3) and then thresholding the image (lower limit 127, upper 255) to make it black and white for analysis in ImageJ. ImageJ was used to calculate the mean grey value (percent of leaf surface covered by hairs) for each leaf using the analyze particles function. Hairs were white and the leaf surface black resulting in higher mean grey values for hairier leaves. Stomatal density was determined by painting the lowest intact 2 or 3 leaflets with clear nail polish then drying, peeling off a print of the leaflet and mounting on a microscope slide. All stomates in three fields per leaf for 10 leaves per site were counted under a 25x lens and averaged.

Statistical analysis

Two-way analysis of variance was used to compare photosynthetic measures at the three sites and two temperature conditions tested. For traits where all 7 sites were measured, I used regression to examine the relationship of the trait to elevation.

Results

Environmental conditions

Overall, sites tended to become drier and cooler at night with increasing elevation (Figure 2). Soil moisture dropped with elevation for the highest 5 sites while the two lowest sites were generally drier. The five highest sites, E thru A, are all in the same valley and should experience the most similar weather while G and F are further south. Sites became more rocky with increasing elevation and the highest two (A and B) are on fast draining talus. The lowest two sites, F and G, are characterized by sagebrush scrubland including cactus at the lowest site. Most sites were still producing new buds on *I. aggregata* as of August 13, 2006. Average minimum temperatures also decreased with altitude ($P = .0145$, $r^2 = .674$). Minimum average relative humidity (RH) and average RH showed large variation because some days were always raining but overall RH decreases with altitude.

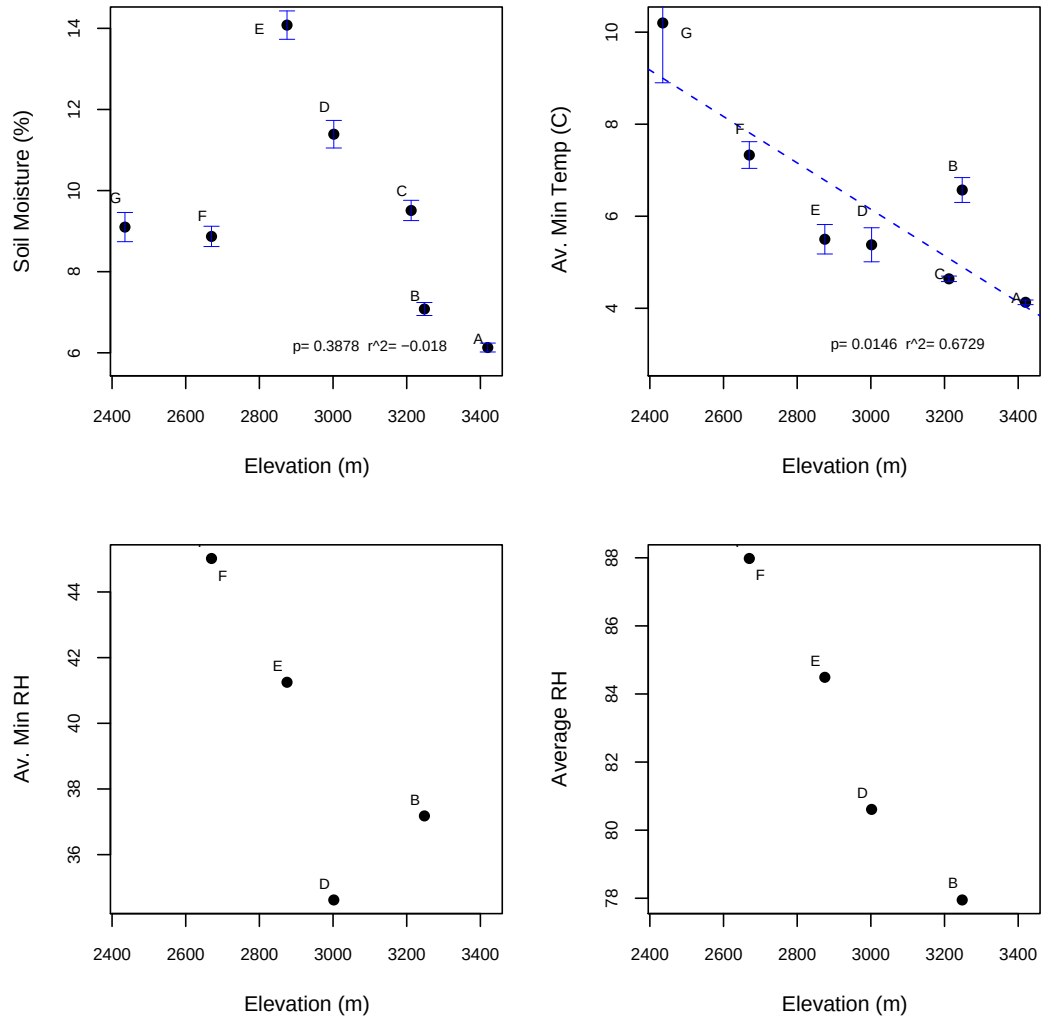


Figure 2: Environmental conditions at each study site. Temperature relative humidity (RH) were recorded for 7 to 22 days with Hobo data loggers. Average means are daily averages. Soil moisture was measured at each plant on 3 days. Sites increase in elevation from G to A. Error bars indicate $\pm$ SE, p -values and r^2 are from linear regression.

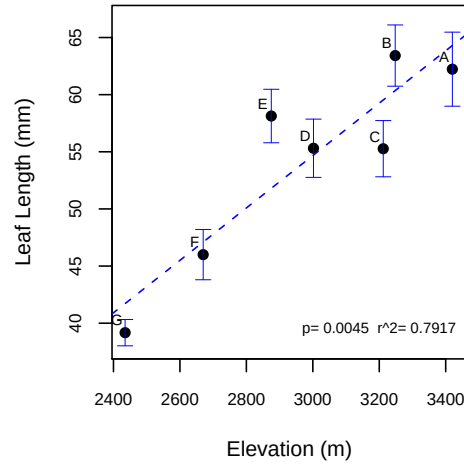


Figure 3: Leaf length of *I. aggregata* rosettes increases with elevation for each site A thru G. Total sample size = 202, 23 to 30 plants per site. Error bars indicate $\pm$ SE, p-values and r^2 are from linear regression.

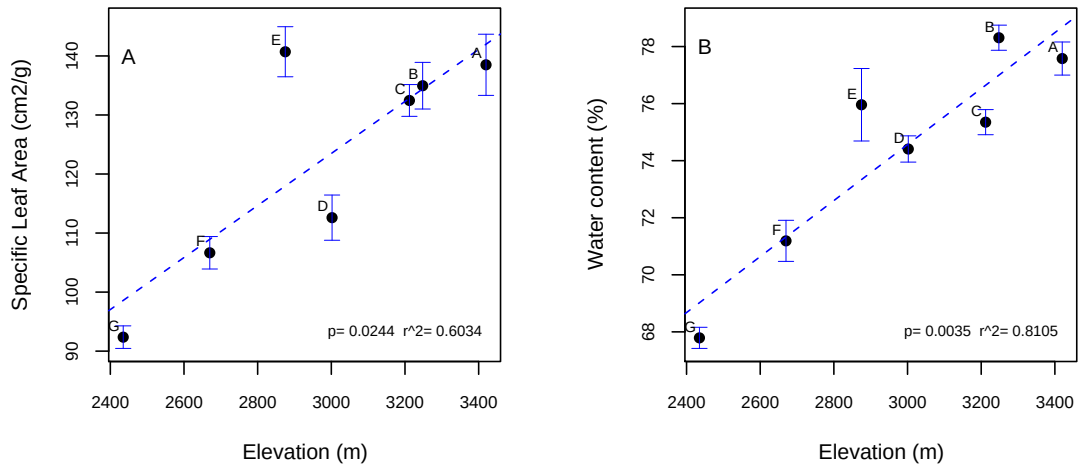


Figure 4: Specific leaf area and relative water content of leaves increases significantly with elevation. A) Mean specific leaf area (SLA) cm²/g dry weight and B) relative water content (%) for each site A thru G. Total sample size = 202, 23 to 30 plants per site. Error bars indicate $\pm$ SE, p-values and r^2 are from linear regression.

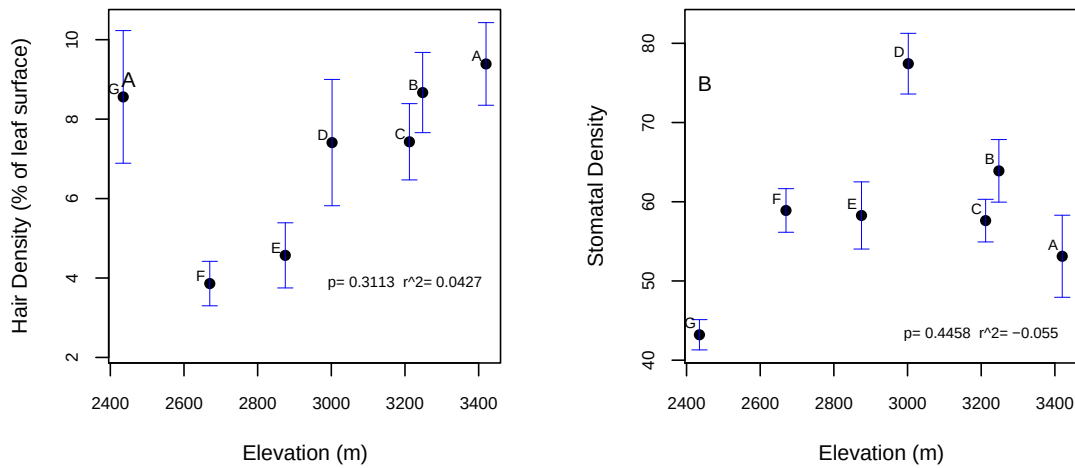


Figure 5: A) Mean hair density (% of leaf surface) and B) stomatal density for each site A thru G. $n = 10$ per site, Error bars indicate $\pm$ SE, p-values and r^2 are from linear regression.

Morphology

Ipomopsis aggregata rosettes were larger, retained more water and had more photosynthetic surface as elevation increased. Leaf length shows a remarkably strong linear increase from 40 to 60 mm ($P = .0045$, $r^2 = .79$) with elevation (Figure 3 A). Specific leaf area, a measure of leaf surface to dry weight that is usually indicative of photosynthetic area, increases strongly with elevation from 90 to 140 cm^2/g (Figure 4 A, from regression $P = .0244$, $r^2 = .604$). The wettest site (E) shows the largest deviation from a linear fit. Relative water content shows a similar increase from 68% – 78% (Figure 4 B, $P = .0035$, $r^2 = .812$) and again shows that the wettest site, E, has correspondingly high water content.

Leaf hairs and stomatal density present a slightly more complicated picture. Except for the lowest site, hair density increases beautifully with elevation (Figure 5 A). Linear regression on the upper 5 sites shows a significant increase in hair density with elevation ($P = .031$, $r^2 = .777$). Plants at the lowest elevation site (G) are noticeably furry with trichomes and show similar densities to high elevation, alpine sites. Stomatal density follows a nearly quadratic relationship with elevation, tapering off at low and high elevation sites (Figure 5 B, from quadratic regression $P = .098$). Stomatal density does not correlate significantly with soil moisture ($P = .549$, $r^2 = -.108$) but dry sites seem to show lower densities.

Glutathione Reductase Activity: oxidative stress

Glutathione reductase (GR) activity increases generally with elevation (Figure 6A; $P = .376$, $r^2 = -.009$) but is better correlated with soil moisture (Figure 6B; $P = .118$, $r^2 = -.298$), however neither is significant for all sites. When the linear model is restricted to the top 5 sites which are all in the same valley, GR activity correlates significantly with both elevation and soil moisture (elevation: $P = .011$, $r^2 = .887$; soil: $P = .017$, $r^2 = .85$). Soil and elevation

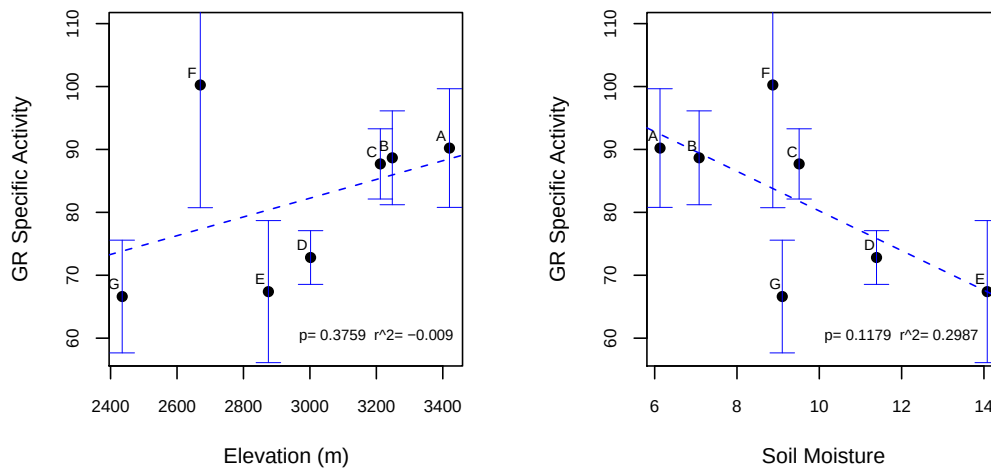


Figure 6: Glutathione reductase specific activity (nmol NADPH/min/mg protein) for all sites A thru G plotted against both A) elevation (m) and B) soil moisture (%), and determined spectrophotometrically by the NAD(P)H dependent reduction of GSSG. $n = 10$ per site. Error bars indicate $\pm$ SE, p -values and r^2 are from linear regression.

are highly correlated for the higher 5 sites ($P = .006$, $r^2 = .923$) which probably accounts for why GR is correlated with both measures similarly. Interestingly, the two lowest sites (F and G) show different activities. Based on vegetation and environmental data at site G, that site seems very dry and likely to experience constant drought stress but shows remarkably low GR activity. Contrastingly, site F which has similarly dry soil 2 shows remarkably GR activity for that elevation.

Gas exchange measurements

Maximum photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) did not differ significantly between sites (2-way ANOVA, site effect, $P = .20$) but seems to generally trend upward, especially when measured at ambient temperatures (Figure 7). At the lower sites ambient temperatures reached 40 C for F and 30 C for E, well above optimal temperatures for *I. aggregata* which hover around 22 C at nearby Poverty Gulch (Wu and Campbell 2006), potentially explaining their lower ambient photosynthetic rates. Photosynthetic rate actually decreased at ambient leaf temperature at site F, but not at the other sites. Transpiration is markedly lower at the lowest elevation site F which also has low soil moisture compared to site E, but is wetter than the highest site, B (Figure 8 A; ANOVA, site effect: $p < .001$, $n = 114$). Transpiration decreased when leaf temperature were allowed to fluctuate at ambient for site B. Instantaneous water use efficiency (WUE) is significantly higher at the dry low site (Figure 8 B; ANOVA, $p < .001$, $n = 114$) Interestingly here, when the leaf temperature conditions are switched at the high site (B) to ambient temperatures there is a significant increase in WUE leading to a significant site by condition interaction (ANOVA, $P = .014$, $n=114$, 57 per condition) for water use efficiency.

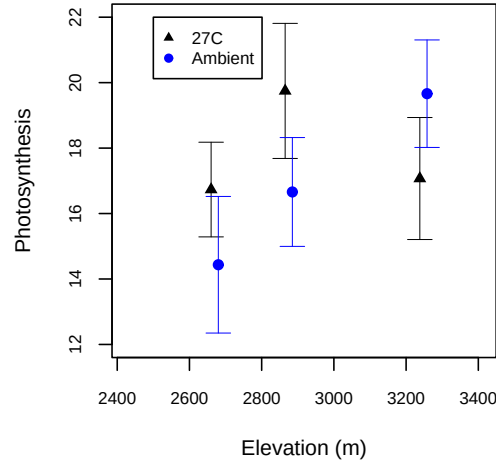


Figure 7: Maximum photosynthetic rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) for *I. aggregata* at three sites increasing in elevation: F, 2670m; E, 2875 m; B 3248 m. Two leaf chamber temperatures were used for measurements: constant 27 C (triangles), and the current ambient temperature (circles). $F_n = 20$, $E_n = 17$, $B_n = 20$. Error bars indicate $\pm$ SE

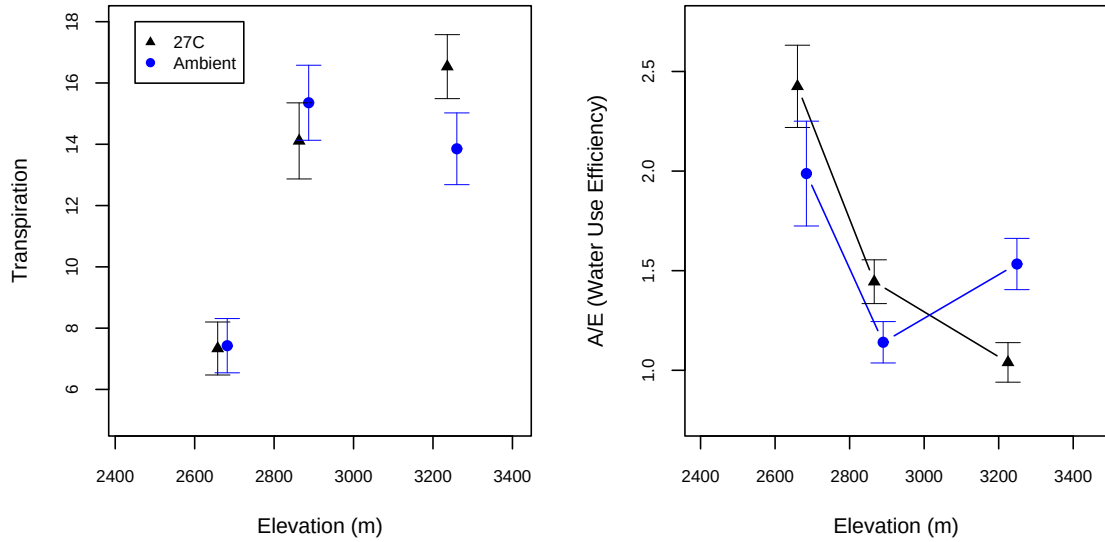


Figure 8: Transpiration (E , $\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$ and instantaneous water use efficiency (WUE, the photosynthetic rate divided by transpiration (A/E)) for three sites increasing in elevation: F, 2670m; E, 2875 m; B, 3248 m. Two leaf chamber temperatures were used for measurements: constant 27 C (triangles), and the current ambient temperature (circles). $F_n = 20$, $E_n = 17$, $B_n = 20$. Error bars indicate $\pm$ SE

Discussion

Ipomopsis aggregata shows large variations in morphology that seems to be best explained by change in elevation. *I. aggregata* is larger and has more photosynthetic area at higher elevations. Water content of leaves is high and photosynthetic rates are within the normal range for this species despite increasingly dry soil moisture conditions. This is generally in opposition to the range of responses seen as elevation increases. Alpine plants tend to have lower SLA and in turn less photosynthetic area but maintain consistent nitrogen content per unit dry-weight (Korner and Diemer 1987). Chloroplast size has also been shown to increase with elevation (Kofidis *et al.* 2003) possibly reflecting a similar allocation of protein to photosynthetic mechanisms despite decreased SLA. The large leaves of *I. aggregata* at high elevation also were much higher in water content accounting for the increased SLA because a smaller portion of leaf area was dry mass. This is an interesting observation given that normally alpine plants reduce light gathering capacity probably because of environmental constraints on carbon assimilation. For this species, it may be that the short growing season and frequent thunderstorms encountered at high elevation drives *I. aggregata* to get as much photosynthetic area out there as possible to capitalize on prime summer growing conditions. Decreased stomatal density and high water content at high elevation may reflect low soil moistures found on fast draining rocky slopes. The ability to rapidly uptake and store water in leaves may be essential to maintain rates of photosynthesis.

Limits on photosynthesis

Lower temperatures at high altitude may allow a longer period for photosynthesis each day. Photosynthesis at the lowest site measured (F) declined at ambient temperatures because they were on average well above the optimum for *I. aggregata*. By noon temperatures were over 30 C at site F and the site did not come into full sun until at least 8 am, providing a very short period for optimal photosynthesis. This may provide an explanation for the small growth form of lower elevation plants. All sites are covered in snow for a similar period each year, preventing growth in winter. By summer, high ground temperatures, though I don't yet have data to show this, may be the limiting growth factor for *I. aggregata*. It would be very interesting to test the photosynthetic responses of *I. aggregata* along this elevation gradient to see if there are differences in the optimal temperature for photosynthesis. Changes in leaf hair density seem to parallel increases in light intensity since the lowest site located in what is essentially a desert is very hairy and plants become more hairy as elevation increases. Hairs may also act to reduce leaf temperatures extending the photosynthetic period.

Transpiration was significantly lower and correspondingly water use efficiency was higher at the lowest site measured (F). This could be an artifact of higher ambient temperatures there decreasing overall transpiration or could indicate a change in physiology responsible for increased WUE. Since transpiration stays similarly low even when leaf temperature was lowered to 27 C it is suggested that the latter is true. Site F exhibits lower stomatal densities and a more conservative growth form because of lower SLA. Site B, which showed an increase in

transpiration and a decrease in WUE when leaf temperatures were raised to 27 °C shows the opposite result to F. Plants at B are larger with high SLA and slightly higher stomatal density than F suggesting that transpiration rates are probably highly dependent on temperature. These results are not surprising because site B is generally cooler than F and it is interesting to consider that the changes in leaf structure may produce the observed difference in WUE. Growing plants from both sites under common greenhouse conditions would help identify whether there was a genetic basis for the increase in WUE or if this is simply a response to warmer or more sunny growing conditions.

Oxidative stress

Rosettes within the same valley (the upper 5 sites) show consistently increasing glutathione reductase activity with elevation and with decreasing soil moisture. This is highly typical of alpine plants which show greater oxidative stress at higher altitudes or during drought stress (Bermadinger Stabentheiner 1996, Polle and Rennenberg 1992, Lei *et al.* 2006). Sites F and G deviate significantly from this pattern because they do not differ in altitude very significantly (235m) nor does soil moisture differ (about 9% at both sites, 2) but GR activity differs greatly, 66.6 and 100.3 nmoles NADPH/min/mg protein for F and G respectively 6. Leaves were sampled when environmental conditions should have been similar and after nearly a month of daily saturating thunderstorms. July and August 2006 have been unusually wet compared to the last decade. It is possible that the low sites, especially G, are usually much drier but because of daily thunderstorms do not have a chance to experience normal drying. If this is the case, under the same conditions site F would experience greater drought stress than G, potentially accounting for the increased GR activity at F. On the other hand, changes in leaf structure at G may reduce the incidence of oxidative stress requiring lower constitutive activities of GR while at site F plants may be responding more dynamically to the environment by changes in enzyme activity and may show higher constitutive GR activity as a result. Measurement of lipid peroxides, indicative of oxidative damage, would help to separate these possibilities.

The results of this study should provide insight into the phenotypic plasticity of *I. aggregata* to respond to stress at increasing altitudes. No characteristics were identified however, which would seem to become limiting to growth at high altitude. Quite the opposite, *I. aggregata* seems to thrive at high elevation and there does not seem to be any major limiting factor preventing it from growing higher. There are no *I. aggregata* above site A to the summit of Mt. Bellview though many other herbaceous species are found. Comparing the elevational trends observed here for *I. aggregata* with those in Poverty Gulch where *I. aggregata* forms hybrids at mid-altitudes with *I. tenuituba* would be an interesting follow up study. Differences in the two transects could point to specific adaptations in *I. tenuituba* which allow it to grow well at higher elevations but no *I. aggregata*. These may be prime candidates for gene transfer from *I. tenuituba* when forming hybrids which show greater environmental fitness at higher altitudes. More rigorous studies of those traits in common garden experiments and under specific stresses (irradiance, water, UVB, cold) could then be pursued. Molecular studies could eventually be designed to

identify specific genes or regulatory changes which are responsible for the phenotype of hybrids.

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