WARMING EFFECT ON THERMAL TOLERANCE AND PHOTOSYNTHETIC PERFORMANCE OF PARAMO PLANTS

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Warming effect on thermal tolerance and photosynthetic performance of paramo plants

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Abstract:

Paramos, as all tropical ecosystems are expected to have narrow thermal tolerance and limited acclimation capacity due to the constant climatic conditions of the tropics all year. Although this may be truth for tropical lowland species, high mountain tropical species are normally exposed to high diurnal variation in temperature, so they could have evolved broader thermal tolerance and higher acclimation potential than tropical lowland species. Given the lack of information on thermal tolerance and acclimation potential to increasing temperatures in paramo plants we can’t yet predict what their vulnerability to climate change will be. In this study we evaluated the thermal tolerance of 15 paramo species and the acclimation capacity to warming in an in situ warming experiment using open top chambers (OTC). Thermal tolerance was evaluated using chlorophyll a fluorescence, observing the decline on $F_v/F_m$ after heating or cooling leaf disks at different temperatures. The highest temperature causing 50% reduction of $F_v/F_m$ indicates the species critical thermal maximum ($CT_{max}$) and the lowest the critical thermal minimum ($CT_{min}$).

Thermal tolerance was then analyzed in terms of the species elevation distribution (restricted and widespread), growth form (rosettes, shrubs and forbs) and height (short and tall). To evaluate the acclimation capacity we registered thermal tolerance and a series of photosynthetic gas exchange parameters ($A_{max}$, $R_d$, ETR and $\varphi_{PSII}$), for 4 different species inside and outside 20 OTC chambers located in two paramo sites after 9 - 15 months of warming. We found that species with restricted distribution had the highest $CT_{max}$ values. As in other alpine ecosystems heat tolerance changed with growth form, with rosettes having the highest heat tolerance among the 3 growth forms.

$CT_{max}$ was not correlated with plant height; both tall and short species had high heat tolerance. In the in situ warming experiment, some of the species showed rapid changes in heat tolerance, indicating a short-term acclimation response, but after one year of warming all studied species maintained similar values of heat tolerance and photosynthetic performance indicating that a year of warming does not seems to affect negatively plant photosynthesis in this high mountain tropical ecosystem.

Key words: Climate change, $CT_{max}$, open top chamber, paramo, thermal tolerance, tropical alpine ecosystems, warming experiment.

Resumen: Los paramos, como ecosistemas tropicales, se dice tienen poca tolerancia térmica y una capacidad de aclimatación reducida dadas las condiciones climáticas estables en los trópicos. Pero a diferencia de las especies tropicales de tierras bajas, se hipotetiza que especies alpinas tropicales tienen gran capacidad de ajuste de su tolerancia térmica que surge por los cambios drásticos de temperatura diaria. Sin embargo, los estudios en la tolerancia térmica y su capacidad de aclimatación en plantas de paramo son escasos, por lo que no podemos predecir aún su vulnerabilidad al cambio climático. En este estudio, evaluamos la tolerancia térmica de 15 especies de paramo usando la fluorescencia de la clorofila a, además de su capacidad de aclimatación en 4 especies después de un experimento de calentamiento usando cámaras de cielo abierto (OTC). La
tolerancia térmica se evaluó usando la fluorescencia de la clorofila \( a \), evaluando la reducción de \( F_v/F_m \) después de calentar o enfriar discos de hojas a diferentes temperaturas, donde una reducción del 50% de \( F_v/F_m \) en el límite superior corresponde a la temperatura crítica máxima (CT\(_{\text{max}}\)) y el límite inferior a la temperatura crítica mínima (CT\(_{\text{min}}\)). La tolerancia térmica fue evaluada en términos de la altura, la distribución en la elevación y la forma de crecimiento de las 15 especies. Para evaluar la plasticidad fenotípica en la tolerancia térmica y en el desempeño fotosintético medimos la capacidad de aclimatación de CT\(_{\text{max}}\) y de una serie de parámetros fotosintéticos (\( A_{\text{max}} \), \( R_d \), ETR y \( \phi_{\text{PSII}} \)), para las 4 especies dentro y fuera de las cámaras de calentamiento a partir de 9 meses de tratamiento hasta un año. Encontramos que las especies de paramo de distribución restringida tienen los mayores valores de tolerancia al calor. Al igual que en estudios previos, se encontró que la tolerancia térmica está relacionada con la forma de crecimiento, siendo las rosetas la forma con los mayores valores de tolerancia al calor de las tres formas presentes en el estudio (rosetas, hierbas y arbustos). En el experimento de calentamiento, al cabo de un año, no se registraron diferencias en los valores de CT\(_{\text{max}}\) entre las plantas de las cámaras y las de los controles. Respecto a la capacidad de aclimatación de los parámetros del desempeño fotosintético, no registramos cambios al cabo de un año de experimento para ninguna de las especies. Nuestros resultados indican poca capacidad de aclimatación, tanto de la tolerancia térmica como del desempeño fotosintético, además de una alta tolerancia al calor en plantas de páramo, al cabo de un año de calentamiento. Sin embargo, estudios a largo plazo de la respuesta del paramo, no solo al calor sino también a otros factores estresantes, son necesarios para deducir su vulnerabilidad al cambio climático.

Introduction

One of this century’s main uncertainties is how climate change will affect tropical biodiversity. Tropical species, despite experiencing lower rate of warming compared to those in temperate zones (Williams et al., 2007; Deutsch et al., 2008), are supposed to be particularly vulnerable to climate change, because they have narrow thermal niches and limited acclimation ability due to the low yearly variability in climatic conditions they are exposed to (Ghalambor et al., 2006; Deutsch et al., 2008; Tewksbury et al., 2008). Janzen (1967) proposed that organisms should have evolved physiological adaptations that reflect the range of climatic variation they encounter during their lifetime, so that tropical species should have evolved narrow thermal tolerance and reduced acclimation reflecting the uniform climatic conditions of the tropics. However, some tropical ecosystems are not so uniform in their climatic conditions and contrary to Janzen’s expectations, in high altitude ecosystems in the tropics where diurnal variation in temperatures is high, species could have evolved wider thermal tolerances (Ghalambor et al., 2006). At high elevations in the tropics, organisms may experience summer and winter like conditions during a single day, so they might have needed to evolve broad thermal tolerances to cope with this drastic diurnal changes in temperature (Beck, 1994; Ghalambor et al., 2006). These broad thermal tolerances and acclimation capacity of tropical high elevation species have only been recorded in few taxonomic groups such as amphibians and reptiles (e.g., Navas, 2002; Navas, 2006; Luddecke & Sanchez, 2002) but never in tropical plants. In general, studies on thermal tolerance are scarce
and geographically and taxonomically biased (Perez et al., 2016), and a broad understanding of physiological responses in terms of climatic tolerances and acclimation capacity is urgently needed to improve predictions on the impact of climate change on tropical biodiversity (Calosi et al., 2008; Perez et al., 2016).

The sensitivity of a species to climate change can be at least partially predicted from its distribution and the characteristics of its niche, so that more vulnerable species are generally those with a narrow distribution, or narrow in their movement either by their limited dispersal abilities or by physical barriers such as mountains or coasts (Broennimann et al., 2006). Tropical high altitude Andean ecosystems, known as paramos, are said to be highly vulnerable to climate change due to the large percentage of endemic biota with narrow distribution, sometimes to a single mountain range (Kattan et al., 2004; Ruiz et al., 2008; Anderson et al., 2011; Ramirez-Villegas et al., 2014). This species with narrow distribution range (island-like), are expected to have narrow habitat tolerances, Therefore, high rates of species loss and turnover are expected in this ecosystem (Ramirez-Villegas et al., 2014) with somber expectation that 60% of plant species would be totally lost or be critically endangered by 2050 (Cuesta-Camacho et al., 2008). Even so, individual responses to climate change can differ between species; those with great migration capacities could be able to track the displacement of their niche, while those with broad thermal tolerances will be able to acclimate and deal with the new conditions (Broennimann et al., 2006), so understanding the response of paramo plants at a physiological level would help us start predicting their actual vulnerability to climate change and to envision the possible future of paramo plant communities.

Evaluating thermal tolerance has proven to be a handy tool for studying how plants will be affected by warmer climates (Smillie & Nott, 1979; Loik & Harte, 2000; Braun et al., 2002; Buchner et al., 2003; Krause et al., 2010; Krause et al., 2013), because it gives an insight into the plant’s ability to tolerate environmental stresses and into the damage these stresses have caused in the photosynthetic apparatus (Maxwell and Johnson, 2000; Ducruet et al., 2007). Photosynthesis has an optimum temperature with critical upper and lower limits. One of the most heat sensitive part of the photosynthetic apparatus is the photosystem II (PSII), the protein complex involved in the oxidation of water and the initiation of the electron transport using the energy of light (Braun et al., 2002). A widely used method to measure thermal tolerance in plants is to study how the efficiency of the PSII changes in response to warming by observing changes in chlorophyll a fluorescence as leaf disks are heated (Loik and Harte, 1996; Krause et al., 2010; Krause et al., 2013). The idea being that fluorescence emission will increase when the PSII is affected and is not able to use all the light energy it receives on photochemical reactions, and instead it emits as fluorescence the excess of energy it cannot handle.

Every species has an optimal temperature and two critical extremes. When the optimum temperature for the species is reached, any increase or reduction in temperature reduces the photosynthetic rate and therefore carbon uptake; this happens in response to a decline in Rubisco activation, which disrupts the electron transport process (Taiz, 2010) and to a reduction in the PSII efficiency. This temperature threshold is called the critical thermal temperature (the upper
threshold CT<sub>max</sub> and the lower threshold CT<sub>min</sub>) and is the temperature at which PSII damage is irreversible (Braun et al., 2002; Deutsch et al., 2007; Krause et al., 2010) and is highly correlated to 50% necrotic leaf damage and a decline in net carbon assimilation (Krause et al., 2010). The specie’s critical temperature is estimated by recording changes in chlorophyll fluorescence parameters while gradually changing the temperature. Two fluorescence parameters are commonly used and are considered reliable indicators of photoinhibition; the minimum fluorescence level or initial fluorescence (F<sub>o</sub>) and the maximum quantum yield of the PSII (F<sub>v</sub>/F<sub>m</sub>). F<sub>o</sub> is the fluorescence measured in the dark assuring all photochemical centers are open and increases drastically when thermal damage occurs in the PSII (Krause & Weis, 1984). F<sub>v</sub>/F<sub>m</sub> is calculated from the maximum fluorescence (F<sub>m</sub>), the fluorescence produced by the leaf when receiving a saturating light pulse assuring all reaction centers are closed (Ducruet et al., 2007), and F<sub>v</sub> which is the difference between the maximum and minimum fluorescence (F<sub>v</sub> = F<sub>m</sub> – F<sub>o</sub>). Values of F<sub>v</sub>/F<sub>m</sub> around 0.83 indicate a healthy non-stressed plant while any lower value indicates environmental stress (Björkman & Demmig, 1987; Maxwell and Johnson, 2000).

Thermal tolerance of tropical alpine species has been scarcely studied, in contrast to temperate alpine species where broad thermal tolerance and significant acclimation potential to warming have been reported (Smillie and Nott, 1979; Braun et al., 2002; Buchner et al., 2003; Buchner et al., 2017). A broad thermal tolerance and high acclimation potential could be favorable for species living in sites with drastic diurnal and seasonal changes in temperature, as in mountain tops, and may result from a greater tolerance to cold (Braun et al., 2002; Krause et al., 2013). Even though the photosynthetic machinery of temperate alpine plants can be vulnerable to high temperature stress, many studies indicate that they may be also very plastic and able to acclimate to short and long term exposure to moderately high temperatures (Brestic & Zivcak, 2013). Observational studies in the Austrian Alps showed that short-term high variation in leaf temperature resulted in rapid adjustments in the thermal tolerance of PSII (Braun et al. 2002). Other experimental studies also suggest that rapid acclimation of thermal tolerance in alpine plant species is common. In a long term warming experiment on the central European Alps, Buchner & Neuner (2003) found that all plant species increased their heat tolerance and could cope with the higher temperature of the warming plots, as long as the critical maximum was not exceeded and concluded that long term warming can lead to increased heat tolerance. The acquisition of thermal tolerance rises from the exposure to high temperatures below lethal values (Wahid et al., 2007) or from a gradual increase to lethal temperatures (Vierling, 1991). Mechanisms to acquire thermal tolerance involve the induction of heat shock proteins (HSP) mainly, but the abscisic acid (ABA) hormone, reactive oxygen species (ROS) and salicylic acid (SA) pathways have also been found to play a leading role in its acclimation capacity (Charng et al., 2006).

In order to determine the response of plants to climate change, the phenotypic plasticity could be more important than tolerance itself, since these values do not incorporate the capacity of species to adjust their thermal tolerance (Calosi et al., 2008; Simon et al., 2015). Most of the work on acclimation of thermal tolerance in tropical species comes from lowland species and they indicate little or no acclimation to warming. The work of Krause et al. (2013), with the tropical tree
species *Ficus insipid*, grown in the laboratory under augmented day and night temperatures, showed no acclimation capacity of their thermal tolerance. Slot and Winter (2017) grew seedlings of three tropical tree species over a range of temperature regimes and measured the temperature response of photosynthetic CO$_2$ uptake, and found that all species were able to acclimate to moderate warming, but that carbon gain decreased with more severe warming. In the tropical highlands the information is scarce. Most of the studies in paramo plant’s thermal tolerance evaluated the lower end of the thermal tolerance spectrum (Squeo et al., 1991; Squeo et al., 1996; Cavieres et al., 2000; Rada et al., 2009; Sklenar et al., 2010) but none have evaluated their tolerance to higher temperature and whether warming conditions will increase CO$_2$ assimilation rate and growth, or, will lead to heat damage and reduced CO$_2$ assimilation. Information on the ability to acclimate thermal tolerance to increasing temperatures in tropical alpine plants, and in particular in the paramos, is lacking, so that we can’t yet predict the fate of this ecosystems in a constantly warming climate.

In this project, we proposed to answer two questions: 1) what is the thermal tolerance of representative paramo plants species? and 2) how plastic are these paramo plants in their thermal tolerance and photosynthesis? For the first question, we hypothesize that species from tropical alpine paramos, with high diurnal variability in temperatures (-2 to 26 °C during a single day), will have broad thermal tolerance as other temperate alpine species (Ghalambor et al., 2006; Mani, 2013; Chan et al., 2016), a hypothesis that contrasts with the general notion that tropical species have reduced thermal tolerance since they have evolved under the stable and unseasonal climate of the tropics. Moreover, we wanted to identify some proxy for paramo plant’s thermal tolerance, from easily measured traits like plant height, growth form and species elevation distribution. In relation to this objective, two hypotheses were formulated; species with widespread distribution will have higher thermal tolerance than species with narrow distribution because they are exposed to different temperatures along the different elevations; or alternatively the elevation distribution does not explain the species thermal tolerance, because all species living in the paramo are equally exposed to drastic diurnal temperature variation during their lifetime. Regarding plant height we expect plants inhabiting warmer microhabitats, such as those experienced by short plants close to the soil, to have higher thermal tolerances than taller plants. And as to growth form, since rosettes can avoid overheating and sub-zero night time temperatures by leaf closure (Neuner et al., 1999), they will have lower heat tolerance than shrubs and forbs. For our second question, we are evaluating the plasticity of paramo plants by looking at changes in thermal tolerance, specifically in CT$_{max}$, and photosynthetic performance in response to warming in a field warming experiment using open top chambers (OTC). We propose that photosynthesis and respiration ($A_{max}$ and $R_d$) will increase in response to warming, because in paramo water is not a limiting factor and lower temperatures usually reduces plant performance (Hernandez-Fuentes et al., 2015); in which case warming will positively affect photosynthesis. Alternatively, warming may damage PSII, resulting in increased photoinhibition and reduced photosynthesis.
Methods

Study site

To evaluate the thermal tolerance of paramo plants (first question) leaves were collected from 15 species in the Parque Ecológico Matarredonda (4°33’38.1” N and 74°0’7.3” W) located in the oriental range of the Colombian Andes (Fig. 1), at elevations ranging from 3100 to 3600 m.a.s.l. Mean annual temperature in the study site is 12 °C, with a minimum of 0.4 °C and a maximum of 40 °C (data collected from August 2016 until June 2017), annual precipitation is 1178 mm and mean relative humidity 88% (Yuste et al., 2017; and Lasso personal communication).

![Figure 1. Map of Colombia showing the two study sites in the paramos in the oriental range of the Andes. Thermal tolerance was evaluated only in Matarredonda and its plasticity in an in situ warming experiment, at both sites.](image)

To evaluate how plastic are paramo plants in their thermal tolerance and photosynthesis (second question) open top chambers (OTC) were used to warm areas of vegetation in situ at two paramo sites (Fig. 1). The warming experiment was established in June/July 2016 at two paramo sites that are part of the Sumapaz-Cruz Verde paramo complex, located in the oriental range of the Colombian Andes (Fig. 1). The first paramo site was the same one used for the thermal tolerance experiment (see above description), and the second site was situated in Sumapaz near the Colombian Military High Mountain Battalion N°1 (Batallón de Alta Montaña N°1) (3°49’36.3” N and 74°24’47.1” W) at approximately 140 km from Bogotá. Elevations at this site ranged from 3200 to 3600 m.a.s.l. Mean annual temperature was 9.9 °C, with minimum of -4.5 °C and maximum of 43.5 °C (data collected from June 2016 until April 2017).


**Study species**

To evaluate the thermal tolerance of paramo plants (first question) 15 species were selected; representing two elevational distribution ranges (widespread and narrow), three growth forms (shrubs, forbs and rosettes) and two heights (near-ground [0-0.60 m] and tall species [> 0.60 m]) (Squeo et al., 1991). Six of the chosen species were rosettes, six shrubs and three forbs. Six of the total of species have widespread elevational distributions (from 600 to 4450 m.a.s.l.), while the other nine have narrow distributions (found from 2700 to 4700 m.a.s.l.). The elevational distribution of each species was obtained from occurrence data at the Global Biodiversity Information Facility (GBIF) database. We cleaned the dataset by clipping out data from unknown sources and records with wrong coordinates (i.e. in the ocean). Eight of the species were classified as tall, and seven as near-ground or short plants (Table 2). Plant heights were measured in 3 full grown individuals for each species in the Matarredonda paramo. Heights were measured from ground level to the tallest branch/leaf, considering only individuals in a vegetative stage.

To evaluate how plastic are paramo plants with respect to thermal tolerance and photosynthesis (second question) we selected four species in the field; *Espeletia argentea* and *Valeriana pilosa* in Matarredonda and *Espeletia grandiflora* and *Orthosanthus chimboracensis* in Sumapaz. Those species were selected for their dominance in the study sites and presence in at least eight of the warming chambers and their controls.

**Thermal tolerance – Critical thermal maximum (CT\textsubscript{max}) and critical thermal minimum (CT\textsubscript{min})**

To evaluate the thermal tolerance of paramo plants (first question) young mature leaves were collected from at least seven adult individuals per species from October 2016 until February 2017. The high thermal tolerance limit of the leaves is the temperature at which an irreversible damage occurs and is known as critical thermal maximum (CT\textsubscript{max}) (Krause et al., 2010). We estimated CT\textsubscript{max} evaluating the decline in maximum fluorescence of PSII (F\textsubscript{v}/F\textsubscript{m}) after heating leaf disks at 7 temperatures (34 °C, 38 °C, 42 °C, 48 °C, 52 °C, 56 °C, 58 °C, and at ambient temperature 20 °C (control); Krause et al., 2010). F\textsubscript{v}/F\textsubscript{m} was recorded from non-heated disks to obtain the initial value for each species and to be sure sampled leaves were healthy. A Sous Vide (Anova Precision Cooker) was used to heat the disks and keep constant bath water temperatures for fifteen minutes at each temperature. To prevent anaerobiosis, samples were put inside a tea cloth bag, with one cloth layer in the adaxial side and three layers in the abaxial side of the leaf disk (Krause et al., 2010). Disks enclosed in the tea cloth were put in a zipped bag and into another zipped bag containing a weight of 100 grams to ensure complete immersion of the leaf disks in the water bath without dipping them. Heated disks were then placed on petri dishes with wet paper towel and stored in the dark for 24h. After 24h had passed we recorded F\textsubscript{v}/F\textsubscript{m} with Li-COR 6400XT with the leaf chamber fluorometer installed. CT\textsubscript{max} values were obtained from a fitted curve of F\textsubscript{v}/F\textsubscript{m} and temperature from each individual to obtain the mean CT\textsubscript{max} for each species.

To record the whole range of thermal tolerance (CT\textsubscript{max} - CT\textsubscript{min}), we also evaluated the critical thermal minimum (CT\textsubscript{min}) or lower lethal temperature (Chown, 2000), measuring the same parameter (F\textsubscript{v}/F\textsubscript{m}) and following the same procedure as to determinate CT\textsubscript{max} but cooling leaf disks
at 5 temperatures (-3.5 °C, -8.0 °C, -9.5 °C, -15 °C, -20 °C and at ambient temperature 20 °C). To reach those temperatures and to be able to maintain it for 15 minutes we used a water bath with salt and alcohol 70% solutions and placed them in a -4 °C or a -20 °C freezer. The first 4 temperatures were achieved at the -4 °C freezer using different concentrations of NaCl salt as follow: 30g of salt for -3.5 °C, 50g of salt for -8.0 °C and -9.5 °C, and 150g of salt for -15 °C, each diluted in 500ml of tap water. For the last temperature we used the -20 °C freezer with a solution of 200g of salt and 100ml of alcohol 70% in 500ml of water. Then leaf disks were cooled for 15 minutes inside two zipped bags to avoid direct contact with water and with a 100g weight to ensure complete immersion in the water bath. $F_v/F_m$ values were recorded twenty four hours later in dark adapted leaves.

**Delta of temperatures**

The air temperature to which the plant is exposed and the actual temperature their leaves reach can be important variables in defining a plant’s critical thermal maximum. To evaluate how close the $CT_{\text{max}}$ is from air and leaf temperatures, we recorded with I-buttons, air temperatures at 5 cm above the ground during ten months every 30 minutes at Matarredonda and leaf temperatures with a Thermal Infrared Camera (Fluke TiS75 Infrared Camera). From the air temperature data, we obtained the maximum air temperature ($T_{\text{air max}}$) registered during this period at the site and subtracted the critical thermal maximum ($CT_{\text{max}}$) from the air temperature maximum to estimate how far these plants are from reaching a critical temperature. Plants have different strategies to regulate their leaf temperatures regardless of the air temperature they are exposed to, so we also calculated the delta between $CT_{\text{max}}$ to the species maximum leaf temperature ($T_{\text{leaf max}}$) which could be a more accurate predictor of the species vulnerability to warming. Leaf temperature was calculated from thermal photographs of three leaves from five individuals for each species between 9:00 and 10:30 in the morning during sunny days in March 2017.

**Plant plasticity**

To evaluate plasticity of paramo plants with respect to thermal tolerance and photosynthesis (second question) we used open top chambers (OTC) to warm the vegetation *in situ*. Open-top chambers are passive heating systems that work trapping the heat of the sun, elevating the temperature but diminishing the ecological effects that could arise from their installation (Robert D et al., 2000). They are also low cost and don’t require electric energy, which is why they are a great device to study the effects of warming on plants in remote locations (Marion et al., 1997). OTC consist of 6 fiberglass pieces made of Sun-Lite HP (0.040 inch thick) (Center et al., 1996), assembled in a 60° angle, reaching a diameter of 208 centimeters and covering an area of 3.87m$^2$.

In June 2016, we installed ten OTC in Matarredonda and ten in Sumapaz in a block design where each OTC was paired to a control plot of similar vegetation and characteristics. In Matarredonda, the 10 blocks were located along 1km and in Sumapaz 5 blocks were established in above the facilities of the Battalion No. 1, and 5 blocks below. All OTC and their control plots were established at similar elevations and in sites with vegetation that didn’t exceed the height of the chamber (0.60 m) and allowed proper anchor to the ground. To evaluate the performance of the
OTC as heating devices we recorded air temperature in and out a series of OTCs on each site using i-buttons at 5 cm above the ground. Additionally, to assess the effect of the OTC on water availability for the plants and the soil temperature we measured the soil temperature and water potential (KPa) at 20 cm underground with a MPS-6 Calibrated Water Potential Sensor (Decagon), in and out the OTC in four blocks in Matarredonda. Data were recorder every 30 minutes during March and September 2017, with at least one-month data for each block.

**Thermal tolerance plasticity**

To evaluate the thermal tolerance acclimation of paramo plants we estimated $CT_{max}$ as previously explained from plants growing in the control plots and plants subjected to warming in the OTCs. In Matarredonda leaves were collected from *E. argentea* and *V. pilosa* from 8 OTC plots and 8 control plots. In Sumapaz leaves were collected from *E. grandiflora* and *O. chimboracensis* from 10 OTC plots and 10 control plots. Data were collected after at least 9 months of warming. As previously mentioned, to obtain $CT_{max}$ values a $F_v/F_m$ and temperature curve was performed.

**Chlorophyll fluorescence and photosynthetic gas exchange**

To assess how warming was affecting the relationship between light use efficiency, CO$_2$ fixation and photoinhibition, we measured light response curves and chlorophyll fluorescence simultaneously with an Infrared gas analyzer (IRGA) Li-COR 6400XT with a leaf chamber fluorometer attached. Measures were taken on the field inside and outside the OTCs. Two species were measured in Matarredonda: *E. argentea* (n=8) and *V. pilosa* (n=8); and two in Sumapaz: *E. grandiflora* (n=10) and *O. chimboracensis* (n=10). The first set of data was collected on April 2017 (after 9/10 months of warming) and the second on September 2017 (after 14/15 months of warming). Maximum net carbon assimilation ($A_{max}$), dark respiration ($R_d$), quantum yield of PSII ($\phi_{PSII}$), yield of CO$_2$ fixation ($\phi_{CO2}$) and total rate of electron transport (ETR) were recorded. Quantum yield of PSII measures the proportion of absorbed light that is used in photochemistry, and it provides information on photosynthetic performance in the field, while yield of CO$_2$ measures the quantum yield calculated from CO$_2$ assimilation. We started with light adapted leaves and set the IRGA environmental control at: 420 μmol$^{-1}$ reference CO$_2$, 500 μmol s$^{-1}$ of flow and the desiccant almost completely bypassed (ambient humidity) and 2000 μmol mol$^{-1}$ of light. Fluorescence constants were left as default (BlueAbs: 0.92; RedAbs: 0.87; Adark: -1.0 μmol m$^{-2}$s$^{-1}$; PS2/1: 0.5) and we waited until stability was reached. Once this happened, flow control was changed to water mole fraction, and CO$_2$ was changed to target the sample chamber. We constructed a light curve using the auto program for ‘LightCurve2’, with 7 light points: 2000, 1500, 1000, 500, 250, 100, and 0 μmol mol$^{-1}$ and the previously established flow and CO$_2$ values.
Statistical analysis

Thermal tolerance – Critical thermal maximum (CT_{max}) and Critical Thermal minimum (CT_{min})

To evaluate whether elevation range, growth form and plant height could explain the thermal tolerance of the species, a multi-factor ANOVA was performed with elevation range, growth form and height, as the independent factors and CT_{max} as the dependant factor.

Thermal tolerance and photosynthetic response plasticity

The ANOVA helped us assess whether there is a difference between control and treatment in the critical thermal values and photosynthetic parameters, but in order to reveal the size of the effect of warming we used an effect size index. Cohen's d index is used to evaluate effect sizes between groups measured by the standardized difference of the means (Sullivan & Feinn, 2012). We used this index to measure the effect size of the warming treatment not only in thermal tolerance but also in all measured fluorescence and photosynthetic parameters. The effect size index we selected was calculated as follow:

\[
d = \frac{(X_e - X_c)}{s} \times J,
\]

where \(d\) is the the effect size, \(X_e\) is the mean found on the OTC plots (i.e. warmed plants), \(X_c\) the mean on the control plots, \(s\) the standard deviation for the data and \(J\), which is a correction for small sample size bias calculated as (Arft et al., 1999):

\[
J = 1 - \left(\frac{3}{4(N_e + N_c - 2) - 1}\right)
\]

where \(N_e\) is the sample size of the experimental group and \(N_c\) for the control group. According to Cohen, the effect can be classified in 3 categories: large effect when \(d \geq 0.8\), medium effect if \(d = 0.5\), and small effect when \(d = 0.2\). Furthermore, positive or negative values indicate positive or negative effects of the treatment (Rustad et al., 2001; Sullivan et al., 2012). The effect size and the 95% confidence interval were calculated with the R package compute.es, using the calculated \(d\), and the sample size of each treatment.

Additionally for the critical thermal maximum acclimation data, we performed a linear mixed model (LMM) with the treatment (OTC or control plot) as the fixed effect, and the time in treatment and the block as the random effects.

Light response curves and ETR and \(\Phi_{PSII}\) curves

In open top chambers, we compared \(A_{\text{max}}\) and \(R_d\) between heated and control plants, these values were obtained from fitted curves of seven different models (Michaelis-Menten based models, hyperbolic tangent based models, nonrectangular hyperbola-based model or an exponential based model) (Lobo et al., 2013). The best model per individual was selected by the minimal value of the sum of squared errors (SSE). We ran an ANOVA with warming as the dependent factor and \(A_{\text{max}}\) or \(R_d\) as the independent factors. For light response curves, the area below the curve was found in
order to test differences among treatments. We also tested for differences among periods of
warming in two of the 4 selected species for ETR and $\Phi_{PSII}$, paired t-test were performed with the
data recorded at two different moments and a two-sample t-test for the data with only one time
recording.

Results

Thermal tolerance – Critical thermal maximum ($CT_{max}$) and critical thermal minimum ($CT_{min}$)

**Table 1.** List of the 15 selected species with the corresponding categories of elevational range, growth form and height. Mean $CT_{max}$ values and standard deviation for 15 species of two elevation distributions, three growth forms and two
heights. The statistical dispersion measure is standard deviation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Elevation range (m)</th>
<th>Range</th>
<th>Height ± SD (m)</th>
<th>Height group</th>
<th>Growth form</th>
<th>$CT_{max}$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Macleania rupestris</em></td>
<td>600-4040</td>
<td>Widespread</td>
<td>1.33 ± 0.07</td>
<td>Tall</td>
<td>Shrub</td>
<td>48.3 °C ± 0.83 °C</td>
</tr>
<tr>
<td><em>Pernettya prostrata</em></td>
<td>640-4700</td>
<td>Widespread</td>
<td>0.68 ± 0.25</td>
<td>Tall</td>
<td>Shrub</td>
<td>49.7 °C ± 1.11 °C</td>
</tr>
<tr>
<td><em>Orthrosanthus chimboraensis</em></td>
<td>1450-4000</td>
<td>Widespread</td>
<td>0.60 ± 0.05</td>
<td>Short</td>
<td>Forbs</td>
<td>46.3 °C ± 2.69 °C</td>
</tr>
<tr>
<td><em>Eryngium humboldii</em></td>
<td>1600-5600</td>
<td>Widespread</td>
<td>0.40 ± 0.01</td>
<td>Short</td>
<td>Rosette</td>
<td>49.3 °C ± 0.59 °C</td>
</tr>
<tr>
<td><em>Bucquetia glutinosa</em></td>
<td>2066-4104</td>
<td>Widespread</td>
<td>0.99 ± 0.42</td>
<td>Tall</td>
<td>Shrub</td>
<td>45.6 °C ± 0.93 °C</td>
</tr>
<tr>
<td><em>Pentacalia vaccinioides</em></td>
<td>2500-4700</td>
<td>Widespread</td>
<td>1.91 ± 0.87</td>
<td>Tall</td>
<td>Shrub</td>
<td>48.6 °C ± 0.96 °C</td>
</tr>
<tr>
<td><em>Paepalanthus columbiensis</em></td>
<td>2600-3691</td>
<td>Restricted</td>
<td>0.27 ± 0.05</td>
<td>Short</td>
<td>Rosette</td>
<td>52.9 °C ± 2.32 °C</td>
</tr>
<tr>
<td><em>Espeletia corymbosa</em></td>
<td>2600-3724</td>
<td>Restricted</td>
<td>0.87 ± 0.08</td>
<td>Tall</td>
<td>Rosette</td>
<td>51.8 °C ± 3.07 °C</td>
</tr>
<tr>
<td><em>Valeriana pilosa</em></td>
<td>2600-3790</td>
<td>Restricted</td>
<td>0.29 ± 0.12</td>
<td>Short</td>
<td>Forbs</td>
<td>47.2 °C ± 2.37 °C</td>
</tr>
<tr>
<td><em>Espeletia argentea</em></td>
<td>2600-3800</td>
<td>Restricted</td>
<td>0.48 ± 0.16</td>
<td>Short</td>
<td>Rosette</td>
<td>52.4 °C ± 3.58 °C</td>
</tr>
<tr>
<td><em>Geranium multiceps</em></td>
<td>2640-3600</td>
<td>Restricted</td>
<td>0.17 ± 0.07</td>
<td>Short</td>
<td>Forbs</td>
<td>47.5 °C ± 1.99 °C</td>
</tr>
<tr>
<td><em>Berberis goudoti</em></td>
<td>2640-4000</td>
<td>Restricted</td>
<td>1.49 ± 0.20</td>
<td>Tall</td>
<td>Shrub</td>
<td>49.0 °C ± 2.12 °C</td>
</tr>
<tr>
<td><em>Espeletia grandiflora</em></td>
<td>2640-4100</td>
<td>Restricted</td>
<td>1.16 ± 0.51</td>
<td>Tall</td>
<td>Rosette</td>
<td>51.3 °C ± 4.02 °C</td>
</tr>
<tr>
<td><em>Oreopanax mutisianus</em></td>
<td>2850-3600</td>
<td>Restricted</td>
<td>1.09 ± 0.46</td>
<td>Tall</td>
<td>Shrub</td>
<td>49.0 °C ± 1.90 °C</td>
</tr>
<tr>
<td><em>Puya goudotiana</em></td>
<td>3095-3481</td>
<td>Restricted</td>
<td>0.63 ± 0.05</td>
<td>Tall</td>
<td>Rosette</td>
<td>47.9 °C ± 2.71 °C</td>
</tr>
</tbody>
</table>

The values for $CT_{max}$ all exceed the highest temperatures measured in the study site by almost 20
°C. The values ranged between 45.6 °C and 52.9 °C (Table 1). $CT_{max}$ values were significantly
different between the two categories of elevation distribution ($F = 14.33$; p-value 0.0008353).
Species with narrow distribution had higher values than species with widespread distribution,
contrary to our predictions. Mean value of $CT_{max}$ for species with widespread distribution, was 47.9
°C ± 1.6 °C, while the mean value species with narrow distribution was 49.9 °C ± 2.2 °C; that is 2 °C
higher (Fig. 2).
Figure 2. $CT_{\text{max}}$ for the 15 species (A) and for the species grouped by: growth form (B), elevational distribution (C), and height (D). * Significant differences. Error bars are the standard error.

We also found a difference in heat tolerance between growth forms (p-value = 1.723e-07), with rosettes having $CT_{\text{max}}$ mean values of 51.3 °C ± 2.07, shrubs of 47.9 °C ± 1.39 and forbs of 46.4 °C ± 0.95. Values from rosettes are statistically different from those found for shrubs and forbs (p < 0.0001), suggesting that critical temperature is related to growth form, with rosettes tolerating significantly higher temperatures (Fig. 2).

Since we noticed higher values of $CT_{\text{max}}$ for rosettes, we tested differences in the 3 growth forms and the two elevation distributions, and species with narrow distribution have always higher values of $CT_{\text{max}}$ even when grouped by growth form (supplementary material, Fig. 5; F = 26.03; p-value 1.31e-09). When evaluating the differences in $CT_{\text{max}}$ for species in different height categories, we found no significant difference among heights (F = 0.026; p-value = 0.8609) (Fig. 2). Short species had mean values of 49.7 °C ± 3.7 and tall species had mean values of 48.7 °C ± 1.4.
We compared maximum leaf temperature ($T_{\text{leaf max}}$) and maximum air temperatures ($T_{\text{air max}}$) to the $CT_{\text{max}}$ of each species, also called thermal-safety margin, to determine how close species are from
suffering drastic damage on their photosynthetic apparatus. CT\textsubscript{max} was always higher than maximum leaf temperature by 20 or more degrees (Fig. 3B). However, the difference between CT\textsubscript{max} and maximum air temperature are smaller, being closer to that maximum (Fig. 3A). Even so, as long as plants can control their leaf temperature and keep it below the critical thermal maximum, they are far from suffering irreversible damage.

In terms of species tolerance to colder conditions, the values of CT\textsubscript{min}, for two of the 15 studied species indicate differences in tolerance among them. E. grandiflora, a rosette, had mean values of -10.1 °C while B. glutonosa, a shrub, had a mean value of -5.6 °C (supplementary material, Fig. 6). Similar analysis are in process for the other 13 species to evaluate if species with higher thermal maximum will also show lower thermal minimum and therefore a broader thermal tolerance (CT\textsubscript{max} - CT\textsubscript{min}).

**Open-top chambers (OTC)**

Table 2. Air and soil temperatures and soil water potential registered for plants inside and outside OTC plots. Mean, minimum and maximum values are presented. Data recordings of one year in both paramo sites.

<table>
<thead>
<tr>
<th></th>
<th>Air Temperature (°C)</th>
<th>Soil Temperature (°C)</th>
<th>Soil Water potential (KPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>OTC</td>
<td>11.5 ± 0.03</td>
<td>-1.46</td>
<td>50.7</td>
</tr>
<tr>
<td>Control</td>
<td>10.7 ± 0.03</td>
<td>-4.52</td>
<td>40.9</td>
</tr>
</tbody>
</table>

We measured air and soil temperatures inside and outside treatments to verify that the OTC plots were effectively increasing air temperatures. We registered an increase in both air and soil temperatures of less than 1 °C, as seen in the mean values in Table 3. Even so OTC chambers are in fact increasing air and soil temperature (Table 2; air: F = 214.9; p-value < 0.0001 and soil: F = 750.3; P < 0.0001). The mean values between OTC and control plots may appear low, but air and soil temperature extremes are increased by several degrees (10 °C). On the other hand, the mean value for soil water potential in OTC chambers was -9.012 ± 0.008, while in control plots was -9.671 ± 0.009, meaning higher water content in soils inside warmed plots than outside (F = 2864.4; p < 0.0001).

We also found in Rojas & Lasso, (2016) (unpublished data), for E. grandiflora in Sumapaz and E. argentea in Matarredonda, higher leaf temperatures for plants inside OTC compared to those in control plots (supplementary material, Table 4). Estimate difference for E. grandiflora being 4.4 °C (p < 0.001) and for E. argentea of 3.7 °C (p-value 0.002). So not only the air temperature increases with the OTC but also the leaf temperatures in a higher magnitude.
**Thermal tolerance plasticity**

For the *in situ* warming experiment using OTC, we found the response of CT$_{\text{max}}$ to warming can be species specific. We found a short-term increase in CT$_{\text{max}}$ values but with time this difference disappeared, as it happened in *E. grandiflora*. After 4 months of warming plants inside the OTC showed an increase in the CT$_{\text{max}}$ of almost 3 degrees (F = 11.33; p-value 0.04354), but this large difference was reduced to 0.2 °C (F = 0.166; p-value 0.6928) when plants were measured again after 15 month of warming (supplementary material, Table 3 & Fig. 7A). So between the two warming periods, after 4 months and after 15 months of warming, there is a significant difference in CT$_{\text{max}}$ values (F = 47.00; p-value 1.76e-05) for *E. grandiflora*. Other two species; *E. argentea*, and *O. chimboracensis*, after 4 months and a year of warming (Fig. 4) showed a slight increase in CT$_{\text{max}}$ of 1.3 °C and 1.1 °C respectively (*E. argentea* F = 1.01; p-value: 0.343; *O. chimboracensis* F = 2.33; p-value: 0.1469; supplementary material, Fig. 8C and E). On the other hand *V. pilosa* (supplementary material, Fig. 7D) had similar CT$_{\text{max}}$ values (difference of 0.1 °C) for plants inside and outside the OTC (F = 0.268; p-value 0.6143).

How large is the effect size will give us an indication of the plasticity of the species to accommodate its photosynthetic apparatus to the new warmer conditions in the OTC. We found, that for CT$_{\text{max}}$ all the species fall into the small or medium effect category of Sullivan & Feinn, 2012 ($d = 0.2$ and $d = 0.5$). Even so, in all of the 4 species the confidence intervals include the $d = 0$, indicating no effect and no statistically significant difference in CT$_{\text{max}}$ between treatments. All effect values tend to be positive indicating that in some warmed plants CT$_{\text{max}}$ has increased (Fig. 4). For the photosynthetic performance parameters, we also found no effect of warming in any of the 4 species ($d = 0$ within the IC$_{95\%}$) in any of the parameters (A$_{\text{max}}$, Rd, fPSII and ETR; Fig.4 and supplementary material, Fig. 10-12). The strongest effect, though no significant, was in A$_{\text{max}}$ and fPSII for *E. argentea* at 9 months of warming, with $d$ values of 0.68 and 0.74 respectively (Fig. 4), which falls into the medium effect category in Sullivan & Feinn’s (2012) scale.
Figure 4. Effect size for CT$_{\text{max}}$, A$_{\text{max}}$, R$_{\text{d}}$ and $\varphi_{\text{PSII}}$ in the 4 species. In CT$_{\text{max}}$, both periods of time are graph for E. grandiflora since we found them statistically different, meanwhile for A$_{\text{max}}$, R$_{\text{d}}$ and $\varphi_{\text{PSII}}$ this was the case for E. argentea. Error bars are the confidence interval of the 95% for the Cohen’s $d$ index.

Chlorophyll fluorescence and photosynthetic gas exchange

The Light response curves for E. grandiflora in warmed plants were undistinguishable from the light response curve of control plants in Sumapaz after 10 months of warming (p-value = 0.941) and after 15 months of warming (p-value = 0.979). The same was true for E. argentea in Matarredonda, where no differences were found after 9 months of warming (p-value=0.1917), nor after 14 months of warming (p-value=0.823). And the same pattern was observed for V. pilosa and O. chimboracensis with no statistical difference between the curves, meaning no difference in the treatments in both species (V.pilosa; p=0.2136 and O. chimboracensis; p=0.4426; supplementary material, Fig. 10).

The maximum net photosynthetic rate ($A_{\text{max}}$) was the same in plants inside the OTC and in the controls plots for all species studied. $A_{\text{max}}$ for E. grandiflora was 12.99 ± 3.72 in the OTC plots and 13.26 ± 4.23 in the control plots and were not statistically different (p-value = 0.8445). For E.
argentea \(A_{\text{max}}\) was 18.35 ± 6.60 in the OTC plots and 16.34 ± 5.89 in the control plot and again these values were not statistically different (p-value = 0.4039), even though a tendency for a slight increase in maximum carbon assimilation was observed in warmed plots. Meanwhile for \(V.\ pilosa\) and \(O.\ chimboracensis\) control plots values of \(A_{\text{max}}\) were no different from those of warmed plots. For \(V.\ pilosa\) the mean value for control plots was 13.12 ± 3.21 while in warmed plots was 11.4 ± 2.17 (p-value = 0.2997), and for \(O.\ chimboracensis\) in control plots the mean value was 6.72 ± 3.25 and inside OTCs was 6.01 ± 2.49 (p-value = 0.6534). The effect size also shows no significant difference between \(A_{\text{max}}\) in OTC and control plots (Fig. 4).

When we compared the respiration in the dark (\(R_{\text{d}}\)) again we didn’t find a significant effect of warming in any of the 4 species (Fig. 16). For \(E.\ grandiflora\) dark respiration in plants inside the OTC was 2.13 ± 1.39 and in the control plots was 2.04 ± 0.85 (p-value = 0.8142). For \(E.\ argentea\) dark respiration in plants inside the OTC was 2.07 ± 0.80 and in control plots was 2.13 ± 0.82 (p-value = 0.8366). In the case of \(V.\ pilosa\) dark respiration in plants inside the OTC was 1.5 ± 0.80 and in control plots was 1.11 ± 1.14 (p-value = 0.4806). For \(O.\ chimboracensis\) dark respiration in plants inside the OTC was 1.35 ± 1.37 and in control plots was 1.62 ± 1.37 (p-value = 0.7186).

The electron transport rate (ETR) was not statistically different between plants growing in the OTC and the ones growing in the control plots for any of the species; \(V.\ pilosa\) (p = 0.5244), \(O.\ chimboracensis\) (p = 0.6151), \(E.\ argentea\) (p = 0.252) and \(E.\ grandiflora\) (p = 0.979) (supplementary material Fig. 11). These results are confirmed in the calculated effect size for ETR, where the largest \(d\) we found was for \(V.\ pilosa\) of 0.29, that accounts for a small, non significant effect of warming. Another fluorescence-based parameter we compared was the quantum efficiency of photosystem II that was also not significantly different between treatments at the different periods of warming for any of the species (supplementary material Fig. 12A-D). Among the warming periods there are no different between control and OTC plots in \(\phi_{\text{PSII}}\) as seen in the effect size index (Fig. 4), although we obtained a medium effect value for \(E.\ argentea\) after 9 months of warming (\(d = 0.72\)), every confidence interval contains 0, so the warming has no effect in \(\phi_{\text{PSII}}\) in any of the species.

**Discussion**

**Thermal tolerance – Critical thermal maximum (\(CT_{\text{max}}\)) and Critical Thermal minimum (\(CT_{\text{min}}\))**

We found a high heat tolerance in the 15 species of paramo studied (\(CT_{\text{max}} = 45.6 \text{ to } 52.9 \, ^{\circ}\text{C}\)) similar to what has been reported for temperate alpine species, where values of \(CT_{\text{max}}\) are between 47.4 and 58 \(^{\circ}\text{C}\) (Buchner et al., 2003; Buchner et al., 2017). It is expected for lowland tropical species to have high values of heat tolerance (46 \(^{\circ}\text{C}\) to 53.8 \(^{\circ}\text{C}\) Smillie and Nott, 1979), and higher values than those for temperate species (Smillie and Nott, 1979) due to the higher temperatures plants in the tropics are exposed all year long. In alpine ecosystems, temperature and irradiation changes drastically in a daily and yearly basis, so that it is of ecological importance for plants living in such conditions, to rapidly adjust and to possess high heat tolerance (Braun et al., 2002; Buchner et al., 2003). Broennimann et al. (2006) suggested that species vulnerability to climate change could be at least partially predicted \emph{a priori} from the species geographical
distribution and niche characteristics. Our results support the previous statement, since paramo plant’s heat tolerance and vulnerability to heat damage was dependent of species distribution in elevation, although we found that species with narrow evelational distribution, presented the highest values of $CT_{\text{max}}$ in comparison to those with widespread distribution. Of the 15 species studied belonging to 13 genera, seven are tropical paramo elements, which are genera that entered paramos from lower neotropical elevations, five are temperate elements, which have migrated from south and north temperate zones, and one genera has a worldwide distribution (Sklenar et al., 2011). Most of our restricted species belong to the group of genera with tropical geographic origin (3 species of Espeletia, Puya, Paepalanthus and Oreopanax) whereas those with widespread distribution belong to genera originated from temperate zones. This pattern is consistent with Smillie and Nott’s (1979) findings, in which tropical species have higher $CT_{\text{max}}$ values than temperate ones.

Growth forms and height are considered important functional traits in alpine plants, and can be related to the temperature a plant can experience during their lifetime. Here we found that rosettes had the higher values of $CT_{\text{max}}$. This growth form encloses genera such as Espeletia and Paepalanthus, which have densely pubescent leaves. Leaf pubescence together with leaf closure reduces cooling of the leaf, but a pubescent surface can also increase the boundary layer hindering transpiration on hot days, possibly increasing leaf temperature. Puya, the other rosette, lacks trichomes but has very thick leaves that can decrease damage caused by heat stress (Leight et al., 2012). In other alpine species, heat tolerance was found to be related to growth form and height, with shorter species, such as short shrubs, rosettes and cushions, tolerating higher temperatures than forbs and tall shrubs (Buchner and Neuner, 2003). As heat tolerance is usually a reflection of leaf temperatures, these prostrated forms have higher heat retention capacity than those on the upper microclimate conditions, so they can reach a higher heat tolerance (Buchner and Neuner, 2003). Therefore, growth forms such as rosettes and cushions are advantageous in alpine ecosystems, where plant temperature can become uncoupled from low air temperatures (Sklenar et al., 2016; Buchner et al., 2017).

The microclimate hypothesis was first proposed by Squeo in 1991, who found differences in paramo plant’s freezing resistance mechanisms in relation to their microclimate, so that tall or short plants will experience milder or stronger climatic conditions in the paramo, which would favor either freezing tolerate or avoidance. Even though, later findings contradicted this hypothesis in Ecuadorian paramos (Sklenar et al., 2010), the hypothesis hasn’t been tested with heat tolerance. In our case, we suggested shorter plants, which can decouple leaf temperatures from those of the air when temperatures are low, will also encounter overheating when wind and clear skies are present (Korner, 2003; Buchner et al., 2003), so that a higher heat tolerance will be advantageous. From our results, height does not correlate with $CT_{\text{max}}$, so both tall or short plants can have high or low $CT_{\text{max}}$ values. We attribute this to the diversity of growth forms within the height groups. Short group species include rosettes and forbs that have the highest difference in $CT_{\text{max}}$ values. The tall group encloses species of caulescent rosettes and shrubs, and in particular B. glutinosa, a shrub that presents the lowest values of $CT_{\text{max}}$ among the 15 species. This species lacks
heat damage preventing mechanisms such as thick leaves, present in other shrubs. Sklenar in 2010 found no correlation between height and freezing resistance mechanisms in Ecuadorian paramo pointing out anatomical characteristics as a possible explanation.

The air temperature to which the plant is exposed during its life is not necessarily the same temperature that their leaves can reach. We found that all the species are far from reaching or surpassing their critical thermal value. The difference between $CT_{\text{max}}$ values and maximum leaf temperatures was almost 20 degrees, while the differences between $CT_{\text{max}}$ and the maximum air temperature were smaller. Plants seem to control their leaf temperatures well below critical thermal maximum. However, we found great variation within and among species similar to what has been found in other alpine plants, since different growth forms experience differences in wind and radiation due to their contrasting morphologies and the different niches they inhabit (Braun et al., 2002; Korner 2003; Buchner et al., 2003). Leaf temperature is a better predictor of plant’s vulnerability to heat stress, since heat tolerance can increase rapidly as a result of an increase in leaf temperatures above a threshold below the critical maximum, but that causes leaf hardening (Alexandrov et al., 1969; Neuner et al., 2000; Buchner et al., 2003). Also, as leaf temperature rises, it causes changes in chlorophyll fluorescence and in heat tolerance, due to heat induced changes in the membrane where the chlorophyll is located (Smillie and Nott, 1979). So, tropical alpine plants not only have great tolerance to cold (Ghalambor et al., 2006) but also seem to have high tolerance to heat, which could point to broad thermal tolerance in tropical alpine species. High $CT_{\text{max}}$ values for the species evaluated could point towards a low vulnerability of paramo plants to warming, but in determining the responses to change, phenotypic plasticity may be more important than tolerance per se (Calosi et al., 2008; Simon et al., 2014), since the capacity to adjust thermal tolerance through acclimation can tell us the real ability of a species to cope with climate change.

Plant plasticity – Heat tolerance and photosynthetic response

We found that in the course of a year of warming the values of $CT_{\text{max}}$ for the four species growing in the OTC and the control plots increased only slightly but differences were not significant. The OTC chambers are increasing the mean daily temperature by one degree, but maximum and minimum by 10 and 3 degrees respectively. In the case of E. grandiflora, we found a significant increase of thermal tolerance after 4 months of warming but this difference disappeared when 15 months of warming had passed. This suggests a short-term response to warming that could induce rapid changes in heat tolerance, but then returns to its original state. Moderate heat stress in plants won’t result in an increase of the plant’s primary heat resistance, since they sustain their basal thermostability when temperatures don’t surpass the plant’s optimum; when that threshold is overcome heat hardening occurs and heat resistance increases (Alexandrov et al., 1970). Most of the studied species appear to be thermally stable, having only slight increases in $CT_{\text{max}}$ with almost a year of warming; this could mean a stronger temperature increase is required to elevate critical thermal maximum temperatures in these species or that the plasticity in this trait is low. The larger the thermostability of a species, the higher the temperatures should be in order to increase that primary obtained heat resistance (Alexandrov et al., 1970). Short-term adjustments of heat
tolerance have been reported for alpine plants and are considered a physiological adaptation in response to alpine’s constantly changing climate conditions (Neuner et al., 2000; Braun et al., 2002). The mechanism by which short-term increase in heat tolerance occurs is different to that for long-term heat tolerance acclimation. The mechanism for short term acclimation involves the division of xanthophylls between the light harvesting complex and the membrane liquid phase that decreases membrane fluidity and contributes to higher thermostability (Havaux, 1992). Also, this short-term heat hardening can be reversible; and usually takes 7 days to reverse. The rate of reversion depends on air temperatures, which could lead to heat tolerance values to return to those of non-heated plants (Alexandrov et al., 1970). This could have happened with *E. grandiflora*, as we observed a rapid response to heat during the first 4 months of warming, trend that disappears when measured upon a year of warming. Leaf temperature, leaf water potential and irradiance have been reported to induce this short term acclimation response (Weis and Berry 1988). Recordings of leaf temperature in *E. grandiflora* and *E. argentea* inside OTC chambers and in control plots showed that leaves of warmed plants have higher temperatures than those in the control plants (Rojas & Lasso, 2016, unpublished data), this increases in leaves temperatures being much higher than those in air temperatures (at least 2 °C more), even so long term acclimation response in heat tolerance was not recorded. Stillman (2003) found in crabs, that those with highest thermal limits have the lowest acclimation capacity, so that evolving great thermal tolerances is done at expense of the acclimatory ability of this tolerance; our findings of high heat tolerance in paramo plants and low acclimation of this trait, could also support this trade-off Stillman proposes, but further studies on the lower thermal limits (CT<sub>min</sub>) are needed to assess whether this trade-off also explains our findings.

In terms of acclimation of photosynthetic performance to warming, we found no pattern in the light response curves (LRC). LRC parameters also didn’t show a clear response to warming, since in some species they appeared to decrease while in others to increase. None of the species showed a trend to increase carbon assimilation in warmed plots. Since in paramos water is not a limiting factor, an increase in temperature will lead to an increase in photosynthesis as temperatures get closer to the optimum for this process (Sanfuentes et al., 2012). The amount of warming induced by OTC’s could be one of the factors contributing to the lack of differences among control and warmed plants in carbon assimilation. Increases in temperature in alpine ecosystems had lead to higher carbon assimilation values for plants inside OTC (Hernandez-Fuentes et al., 2015); they found rates of photosynthesis and respiration 6 times higher in plots where there was no water limitation. An increase in water availability inside warmed plots, due to water condensation in the OTC walls could have reinforced the positive effect in carbon assimilation and respiration, but our results show there was no such response. We recorded OTC maximum air temperatures of almost 10 degrees higher than those in control plots, which should induce changes in carbon uptake if they last long enough, but high soil water potential inside OTC chambers, could have helped cool leaves by transpiration and consequently no heat response. Since an increase in leaf temperatures and in light intensity can induce adjustments in PSII thermal tolerance, optimizing photosynthesis as long as critical limits are not surpass (Marchand et al., 2005), we suspect the warming treatment did not surpassed the thermostability threshold for the species (at least 2 °C increase in
leaves), hence no significant changes were recorded. Also, no changes in $\phi_{\text{PSII}}$, indicates that the level of warming tested does not affect photochemical reactions (Danby & Hik, 2007), and this is reflected in no changes in carbon assimilation either. Our results imply a short-term (1 year) increase in temperature of paramo plants would have no significant effect in the heat tolerance or the photosynthetic performance, as long as soil availability is not affected. In order to assess whether this could be because of low acclimation capacity or high thermostability in paramo plants, optimum temperature ranges of photosynthesis need to be recorded.

Conclusions

A strong correlation between $\text{CT}_{\text{max}}$ and growth forms and the elevational distribution was found for the 15 studied paramo species. Current elevational distribution of paramo plants can be used as a proxy of a plant’s thermostolerance and susceptibility. Warming did not affect $\text{CT}_{\text{max}}$ or photosynthetic performance in our experiment, but long term response to warming in these parameters in paramo plants needs to be study in order to predict their actual response to climate change. However, it is possible that higher temperature increases may cause an increase heat tolerance and photosynthetic performance. Climate change scenarios estimate an increase in temperature between 2.5 °C and 4.7 °C by the end of the century for tropical America (Cramer et al. 2004), along with an increase in the frequency of extreme events such as droughts and heat waves (Meehl et al. 2007). Heat stress combined with other types of abiotic stress that arise with climate change, can lead to the disappearance of tropical alpine ecosystems as species migration can’t cope with the rate of warming. The response to heat stress in plants involves transpiration cooling, which can only be achieved if drought doesn’t come as a consequence of warming, so in the synergic interaction of various abiotic stresses, such as heat and drought stress, paramo plants could surpass their thermal critical limit which would lead to lethal consequences. Our $\text{CT}_{\text{max}}$ values indicated, however, that paramo plants are still far away from reaching those lethal temperatures, but further in situ studies on the interaction of various abiotic and biotic stresses in paramo plants are desperately needed in order to model the impact of climate change on paramo ecosystems.

References


Chan, W., Chen, I., Colwell, R. K., Liu, W., Huang, C., & Shen, S. (2016). Seasonal and daily climate variation have opposite effects on species elevational range size, 351(6280).


Supplementary material

Thermal tolerance – Critical thermal maximum (CT\text{max}) and Critical Thermal minimum (CT\text{min})

Figure 5. CT\text{max} for the species grouped by growth form. Error bars are the standard error. Red points are restricted species and black points widespread species.

Figure 6. Critical thermal minimum (CT\text{min}) for two of the 15 studied species in the paramo of Matarredonda. Error bars are standard deviation.
Plant plasticity – Heat tolerance and photosynthetic response

Table 3. \(CT_{\text{max}}\) values for the four species in the OTC and control plots.

<table>
<thead>
<tr>
<th>Species</th>
<th>Time of warming</th>
<th>(CT_{\text{max}}-\text{OTC})</th>
<th>(n_{\text{OTC}})</th>
<th>(CT_{\text{max}}-\text{Control})</th>
<th>(n_{\text{Control}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. grandiflora</td>
<td>10 months</td>
<td>52.4 ± 1.0</td>
<td>3</td>
<td>49.4 ± 0.9</td>
<td>3</td>
</tr>
<tr>
<td>E. grandiflora</td>
<td>15 months</td>
<td>46.5 ± 1.3</td>
<td>6</td>
<td>46.8 ± 1.3</td>
<td>5</td>
</tr>
<tr>
<td>E. argentea</td>
<td>9 months</td>
<td>50.6 ± 1.4</td>
<td>5</td>
<td>49.3 ± 2.5</td>
<td>5</td>
</tr>
<tr>
<td>V. pilosa</td>
<td>11 months</td>
<td>44.8 ± 0.9</td>
<td>7</td>
<td>44.7 ± 0.3</td>
<td>7</td>
</tr>
<tr>
<td>O. chimboracensis</td>
<td>12 months</td>
<td>49.1 ± 1.4</td>
<td>9</td>
<td>48.0 ± 1.7</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 4. Leaf temperature for 2 species inside OTC and control plots.

<table>
<thead>
<tr>
<th>Species</th>
<th>Leaf temperature</th>
<th>Control</th>
<th>OTC</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. grandiflora</td>
<td>24.4 ± 6.2</td>
<td>28.8 ± 5.3</td>
<td>4.4 °C</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>E. argentea</td>
<td>19.1 ± 2.4</td>
<td>23.5 ± 3.3</td>
<td>3.7 °C</td>
<td>P = 0.002</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. \(Fv/Fm\) and temperature curve, values presented are the critical temperature for each curve.
Figure 8. Temperature and Fv/Fm curves for *E. grandiflora* after 4 months of warming (A) and after 15 months of warming (B). For *E. argentea* after 3 months of warming (C), for *V. pilosa* after 4 months of warming (D) and for *O. chimboracensis* after 4 months of warming (E). Error bars are standard deviation.
Figure 9. Effect size for ETR in the 4 species. Error bars are the confidence interval of the 95% for the Cohen’s $d$ index.

Figure 10. Light response curves for plants inside and outside the OTC for $E.~grandiflora$ after 15 months of warming (A) $E.~argentea$ after 14 months of warming (B), $V.~pilosa$ after 11 months of warming (C) and $O.~chimboracensis$ after 12 months of warming (D). Error bars are standard deviation.
Figure 11. ETR vs PAR for the 4 species. Curves for *E. grandiflora* and *E. argentea* are shown with the two warming periods (A and B). Curves for *V. pilosa* (C) were taken after 11 months of warming and for *O. chimboracensis* after a year of warming (D).
Figure 12. Quantum efficiency of photosystem II vs PAR for the 4 species. Upper curves shown the difference among periods of warming in OTC and Control plot’s φPSII (A-B). While for the lower two species only one warming period was recorded, 11 months for V. pilosa (C) and a year for O. chimboracensis (D).