

Stomatal responses of tree species from the cloud forest in central Veracruz, México

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Abstract

Stomatal conductance is considered as a key plant response because it plays an important role in plant physiology by controlling transpiration (water status) and CO₂ assimilation, regulating plant productivity. As stomatal conductance is affected by micro-environmental and physiological variables, changes in an altitudinal gradient will have a direct effect on stomatal conductance, which can explain their ecophysiological responses. In this work we used the envelope function method to assess the effect of three climate variables (air temperature, vapor pressure difference, photosynthetically active radiation), and two physiological (leaf water potential, transpiration) on the stomatal conductance response of four tree species (*Alnus acuminata*, *Liquidambar styraciflua*, *Pinus ayacahuite*, and *Quercus xalapensis*) from the central mountain region of Veracruz, Mexico. We found that all variables influenced stomatal conductance. We also found differential stomatal conductance responses among species, where *A. acuminata* had the highest stomatal conductance. We also estimated the optimal temperature when the highest stomatal conductance occurs, and among the species, optimal temperature varied from 26 to 29 °C. The most sensitive species to changes in photosynthetically active radiation, leaf water potential and transpiration was *L. styraciflua*, and for vapor pressure difference was *A. acuminata*. We also proposed that the stomatal conductance response could help to explain ecophysiological responses along the elevation gradient.

Key words: climate change, elevational gradient, leaf water potential, microclimate, transpiration

Respuestas estomáticas de especies arbóreas del bosque nublado del centro de Veracruz, México

Resumen

La conductancia estomática se considera como una respuesta vegetal clave porque juega un papel importante en la fisiología al controlar la transpiración (estado hídrico) y la asimilación de CO₂ regulando la productividad de la planta. Como la conductancia estomática es afectada por variables microambientales y fisiológicas, los cambios de éstas en un gradiente altitudinal tendrán un efecto directo en la conductancia estomática, con lo que se puede explicar sus respuestas ecofisiológicas. En este trabajo, utilizamos el método de las funciones envolventes para evaluar el efecto de tres variables climáticas (temperatura del aire, déficit de presión de vapor, radiación fotosintéticamente activa), y dos rasgos fisiológicos (potencial hídrico foliar, transpiración) en la conductancia estomática de cuatro especies arbóreas (*Alnus acuminata*, *Liquidambar styraciflua*, *Pinus ayacahuite* y *Quercus xalapensis*) de la región central montañosa de Veracruz, México. Encontramos que todas las variables microambientales y fisiológicas influyeron en la conductancia estomática. Se encontraron respuestas diferenciales de g_s entre especies, siendo la de *A. acuminata* la más alta. También se estimaron las temperaturas óptimas donde se registró la conductancia estomática más alta. La temperatura óptima varió de 26 a 29 °C. La especie más sensible a los cambios en radiación fotosintéticamente activa, potencial hídrico foliar y transpiración fue *L. styraciflua*, y a los de déficit de presión de vapor fue *A. acuminata*. También proponemos que la respuesta de la conductancia estomática podría ayudar a explicar las respuestas ecofisiológicas a lo largo del gradiente altitudinal.

Palabras clave: cambio climático, gradiente altitudinal, microclima, potencial hídrico foliar, transpiración

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Survival limits, distribution areas, and distribution of species and communities are defined along the altitudinal gradient by several environmental variables (Prentice *et al.*, 1992). Environmental variables in turn are influenced by altitude. Plant physiological responses at different altitudinal steps can be seen as an analogy for climate adaptation at different elevations (Hovenden and Brodribb, 2000). Elevation influences environmental variables such as photosynthetically active radiation, air temperature, vapor pressure deficit, and rainfall. Different micro- and macro-environmental conditions at different elevations can cause local plant acclimation and adaptation (Körner *et al.*, 1986; Friend *et al.*, 1989). Microenvironment factors such as photosynthetically active radiation, air humidity, air temperature, soil water availability, among other factors, affect stomatal movement and conductance (Jarvis, 1976; Fanjul and Barradas, 1985; Jones, 1992; Buckley, 2005; Buckley and Mott, 2013). Stomatal conductance (g_s) is an important physiological trait because stomatal function plays a key role in plant physiology (Jones, 1992; Meinzer *et al.*, 1997; Barradas *et al.*, 1994, 2004; Buckley, 2005; Esperón-Rodríguez and Barradas, 2014a). Also, stomatal behavior is considered a plant response to climate, as it controls transpiration (water status) and CO_2 assimilation, playing an important role in photosynthesis and plant productivity (Jones, 1992). Studying the physiological role of stomata helps to understand plant performance, g_s can help to explain ecophysiological responses along an altitudinal gradient. Previous studies focused on the effect of micro-environmental and physiological variables on g_s showed a broad diversity of g_s responses to different microclimatic and physiological factors (Fanjul and Barradas, 1985; Roberts *et al.*, 1990; Meinzer *et al.*, 1997; Beer *et al.*, 2007); and also it was discussed how altitudinal variations can affect the g_s response (Körner *et al.*, 1986; Carter *et al.*, 1988; Hovenden and Brodribb, 2000). The central mountain region of Veracruz, Mexico, holds the region of the Great Mountains, which possesses a very pronounced topography along the altitudinal gradient, going from the sea level up to 5,500 m asl, in a distance of 100 km (Barradas *et al.*, 2010). As a result, climate in this region is conformed as follows: (1) the complex interactions between the prevailing synoptic systems, such as the tropical systems at summer and mid-latitude at winter, (2) the mountainous topography, (3) the plant-atmosphere interaction, and (4) the proximity to the Gulf of Mexico (Barradas *et al.*, 2010). The physiographic and climatic characteristics of the region make it an interesting study area to assess the g_s response at different elevations.

We analyzed the g_s response and its relationship with three climate variables: air temperature, vapor pressure deficit and photosynthetically active radiation, and two physiological traits: leaf water potential and transpiration, and we compared these responses among four tree species from different elevation ranges from the central mountain region of Veracruz, Mexico.

Materials and methods

In order to compare ecophysiological responses among species measurements of physiological traits and climate variables must be taken as simultaneously as possible (Jones, 1992; Barradas *et al.*, 2004; Esperón-Rodríguez and Barradas, 2014a, b, 2015), hence, measurements of physiological traits and climate variables were conducted under greenhouse conditions.

Study Area. All plant specimens came from the central mountain region of Veracruz, Mexico (19° 54' 08" N, 96° 57' 19" W; Figure 1) which is part of TransMexican Volcanic Belt and the Sierra Madre Oriental. The region possesses several vegetation types that go from tropical montane cloud forest to semi-arid and arid communities (Gómez-Pompa, 1978; Barradas, 1983). Mean annual temperatures range between 10 and 29 °C, and annual precipitation ranges from 600 to 1,200 mm, with a maximum of 3,000 mm in wetter regions.

Plant material. We selected four tree species from different elevation ranges within the central mountain region of Veracruz, ranging from 400 to 3500 m asl: *Alnus acuminata* Kunth, *Liquidambar styraciflua* L., *Pinus ayacahuite* C. Ehrenb ex. Schltdl, and *Quercus xalapensis* Bonpl. (Table 1). Fifteen individuals of each species from 45 to 90 cm height were kept in the greenhouse. Individuals were transplanted in a mixture of peat moss after having been sterilized by autoclaving for 90 minutes. Individuals were kept at the humid greenhouse of the Institute of Ecology, Universidad Nacional Autónoma de México, under well-watered conditions.

Figure 1. Location of the region of the Great Mountains in Veracruz State, Mexico.

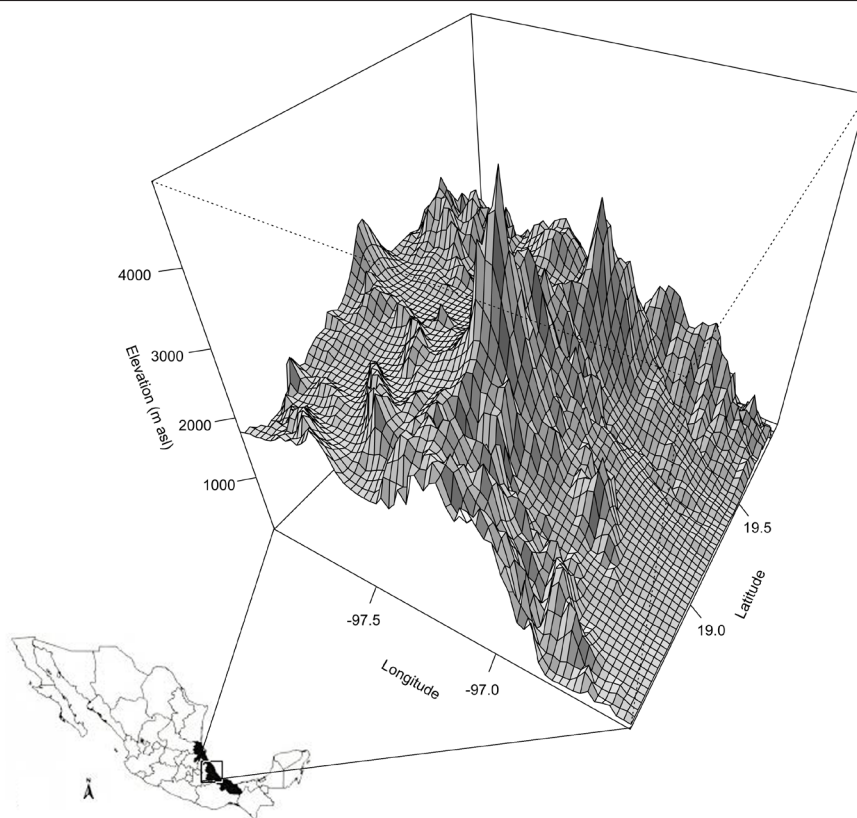


Table 1. Elevational distribution, precipitation range, optimal temperature (T_o), and optimal thermal range (T_R) for stomatal function for *Alnus acuminata*, *Quercus xalapensis*, *Liquidambar styraciflua* and *Pinus ayacahuite*.

Species	Distribution (m asl)	Precipitation range (mm)	T_o (°C)	T_R (°C)
<i>Alnus acuminata</i>	1,300 – 2,800	1,000 – 3,000	29.7 (0.5)	22.17–37.17
<i>Quercus xalapensis</i>	400 – 2,700	1,400 – 2,300	28.2 (0.4)	20.65–37.39
<i>Liquidambar styraciflua</i>	400 – 1,800	1,000 – 1,500	27.5 (0.3)	19.55–36.19
<i>Pinus ayacahuite</i>	2,000 – 3,500	800 – 1,500	26.2 (0.2)	18.26–34.56

Stomatal conductance and Leaf water potential. Stomatal conductance (g_s) and transpiration (E) were measured in all individuals of each species on at least four fully expanded leaves per plant, with a steady-state diffusion porometer (LI-1600, LI-COR, Lincoln, Nebraska, USA). Leaf water potential (Ψ_L) was measured in all individuals of each species on four fully expanded leaves per plant, with a pressure chamber (PMS, Corvallis, Oregon, USA) (Scholander *et al.*, 1964). Physiological measures were made daily from October 22 to December 7, 2012, at 0700 and from 1000 to 1800 hours (h, local time) at 2 h intervals.

Climatological measurements. Air temperature (T_A), photosynthetically active radiation (PAR), and relative humidity (RH) were determined next to each measured leaf with a quantum sensor (LI-190SB, LI-COR Ltd., Lincoln, Nebraska, USA), a fine wire thermocouple, and a humicap sensor (Vaisala, Helsinki, Finland). Leaf temperature (T_L) was also measured. Thermocouples were mounted in the porometer. The air vapor pressure difference (VPD) was calculated from T_A and RH measurements. VPD was calculated using the equation:

$$VPD = e_s - e \quad (1)$$

where e_s and e (kPa) are the saturation vapor pressure and actual. The saturation vapor pressure is derived as follows:

$$e_s = 0.6108 \exp\left(\frac{17.27T}{T+273.3}\right) \quad (2)$$

where T (°C) is the current air temperature, e is calculated with the following expression:

$$e = e_s(RH/100) \quad (3)$$

where RH (%) is the relative humidity (Barradas, 1994). Climate measurements were made daily from October 22 to December 7, 2012, at 0700 and from 1000 to 1800 hours (h, local time) at 2 h intervals.

The envelope function method. The effect that each climate variable has on g_s is determined from the envelope function method. This method consists of selecting data from the probable upper limit of the function, this function is represented by a cloud of points in each diagram produced by plotting g_s as a function of any variable (climatic and edaphic). This method has three theoretical assumptions: (1) the envelope function represents the optimal stomatal response to the selected parameter (i.e. PAR), (2) the points below the selected function are the result of changes in the other variables (e.g. VPD and T_A), and (3) there are not synergistic interactions among variables (Fanjul and Barradas, 1985; Ramos-Vázquez and Barradas, 1998; Barradas *et al.*, 2004).

The relationship of g_s in terms of air temperature (T_A) is given by the envelope values that fit a quadratic equation:

$$g_s = A + BT_A + CT_A^2 \quad (4)$$

where A, B and C are parameters of the parabola, being possible to determine the optimum temperature (T_o) at which g_{sMAX} occurs, and the cardinal temperatures (minimum and maximum).

Envelope values of g_s as a function of photosynthetically active radiation (PAR) are consistent with a hyperbolic function:

$$g_s = [aPAR/(b + PAR)] \quad (5)$$

where a is the asymptotic value of g_s or g_{sMAX} and b is g_s sensitivity to changes in PAR.

While the g_s function in relation to vapor pressure deficit (VPD) generates a simple linear equation.

$$g_s = a + bVPD \quad (6)$$

where b is g_s sensitivity to the VPD , and a is the zero drift.

Similarly, the stomatal response to water potential is also a simple linear equation:

$$g_s = a + b\Psi_L \quad (7)$$

where b is g_s sensitivity to Ψ_L , and a is the zero drift.

We also analyzed the relation between the g_s and the transpiration (E) by a simple linear equation.

$$g_s = a + bE \quad (8)$$

where b is g_s sensitivity to E , and a is the zero drift.

Statistical analysis. We used the non-parametrical test Kruskal-Wallis to evaluate our data whether there were significant differences of g_s , T_A , PAR, VPD , E and Ψ_L among species. Influence of each variable (T_A , PAR, VPD , Ψ_L , g_s) for each species was evaluated through a Principal Components Analysis (PCA). The PCA was based on the correlation matrix of variables (Jongman *et al.*, 1987) and was used to identify the principal sources of variability. We calculated the relative importance (RI) of each component by measuring the length of each vector (Legendre and Legendre, 1998). A linear regression analysis (Zar, 1984) was used to determine the effect of the micro-environmental variables on g_s . An analysis of variance

Table 2. Stomatal conductance (g_s), water leaf potential (Ψ_L), air temperature (T_A), vapor pressure difference (VPD), photosynthetically active radiation (PAR), transpiration (E) for *Alnus acuminata*, *Quercus xalapensis*, *Liquidambar styraciflua* and *Pinus ayacahuite*. Total averages are shown and \pm standard deviation (n=870).

Species	g_s (mmol m ⁻² s ⁻¹)	Ψ_L (Mpa)	T_A (°C)	VPD (kPa)	PAR (μmol m ⁻² s ⁻¹)	E (mmolH ₂ O m ⁻² s ⁻¹)
<i>Alnus acuminata</i>	433.9 ± 176.5	- 1.94 ± 0.9	25.9 ± 3.8	2.26 ± 0.5	151.25 ± 63.6	17.76 ± 8.8
<i>Quercus xalapensis</i>	320.8 ± 125.5	-1.65 ± 0.4	25.9 ± 3.7	2.28 ± 0.5	152.69 ± 62.7	13.13 ± 5.4
<i>Liquidambar styraciflua</i>	329.7 ± 138.2	-1.72 ± 0.6	25.9 ± 3.6	2.27 ± 0.5	144.93 ± 60.2	13.34 ± 6.3
<i>Pinus ayacahuite</i>	377.5 ± 138.2	- 0.57 ± 0.4	25.9 ± 3.5	2.21 ± 0.5	182.73 ± 84.9	14.07 ± 5.4

(ANOVA) was used to evaluate the performance of the regression. Statistical significance was considered at 95 % for all cases. All statistical analyzes were performed using the R (R Core Team, 2014) software.

Results

Greenhouse mean temperature was 24.09 ± 5.08 °C, maximum temperature was registered at 1400 hours (h, local time, 28.26 ± 1.78 °C) and minimum at 0800 h (17.33 ± 0.50 °C). Mean relative humidity (RH) was 36.56 ± 1.77 %, being maximum at 0800 h (38.37 ± 4.24 %), and minimum at 1600 h (33.38 ± 0.64 %). Photosynthetically active radiation averaged 133.57 ±

Table 3. Parameters of the calculated envelope functions for stomatal conductance (g_s) versus, air temperature (T_A), photosynthetically active radiation (PAR), vapor pressure difference (VPD), transpiration (E) and leaf water potential (Ψ_L) for *Alnus acuminata*, *Quercus xalapensis*, *Liquidambar styraciflua* and *Pinus ayacahuite* (r^2 is the coefficient of determination).

Species	A (°C)	T_A B	C (°C ⁻¹)	r^2
<i>Alnus acuminata</i>	- 4059.583	345.473	5.811	0.994
<i>Quercus xalapensis</i>	-1784.845	171.448	-2.948	0.949
<i>Liquidambar styraciflua</i>	-2017.468	198.647	-3.551	0.942
<i>Pinus ayacahuite</i>	-1580.765	177.339	-3.307	0.981
	PAR			
	a (mmol m ⁻² s ⁻¹)	b (mmol m ⁻² s ⁻¹)		r^2
<i>Alnus acuminata</i>	1007.364	6.752	-	0.929
<i>Quercus xalapensis</i>	671.906	8.675	-	0.884
<i>Liquidambar styraciflua</i>	723.639	9.087	-	0.964
<i>Pinus ayacahuite</i>	738.749	2.078	-	0.978
	VPD			
	a (mmol m ⁻² s ⁻¹)	b (mmol m ⁻² s ⁻¹ kPa ⁻¹)		r^2
<i>Alnus acuminata</i>	2381.825	466.341	-	0.971
<i>Quercus xalapensis</i>	1109.452	169.337	-	0.903
<i>Liquidambar styraciflua</i>	1577.411	304.068	-	0.947
<i>Pinus ayacahuite</i>	1129.183	175.242	-	0.979
	E			
	a (mmol m ⁻² s ⁻¹)	b (mmol m ⁻² s ⁻¹)		r^2
<i>Alnus acuminata</i>	87.968	30.093	-	0.93
<i>Quercus xalapensis</i>	70.366	25.632	-	0.978
<i>Liquidambar styraciflua</i>	66.313	30.312	-	0.963
<i>Pinus ayacahuite</i>	52.092	23.123	-	0.863
	Ψ_L			
	a (mmol m ⁻² s ⁻¹)	b (mmol m ⁻² s ⁻¹ MPa ⁻¹)		r^2
<i>Alnus acuminata</i>	1716.049	222.668	-	0.952
<i>Quercus xalapensis</i>	1754.504	247.865	-	0.985
<i>Liquidambar styraciflua</i>	1230.826	284.702	-	0.872
<i>Pinus ayacahuite</i>	1064.502	192.232	-	0.993

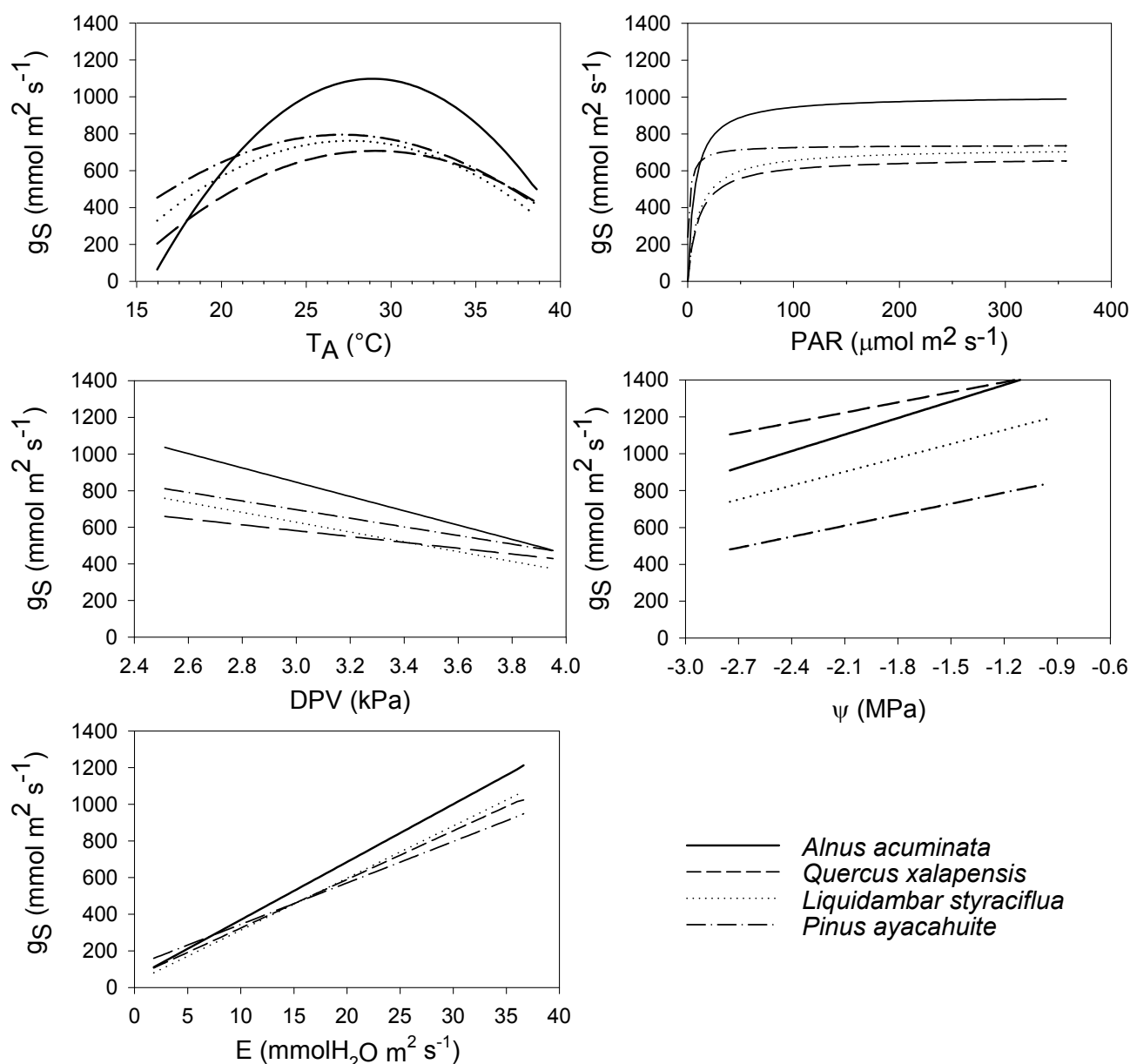
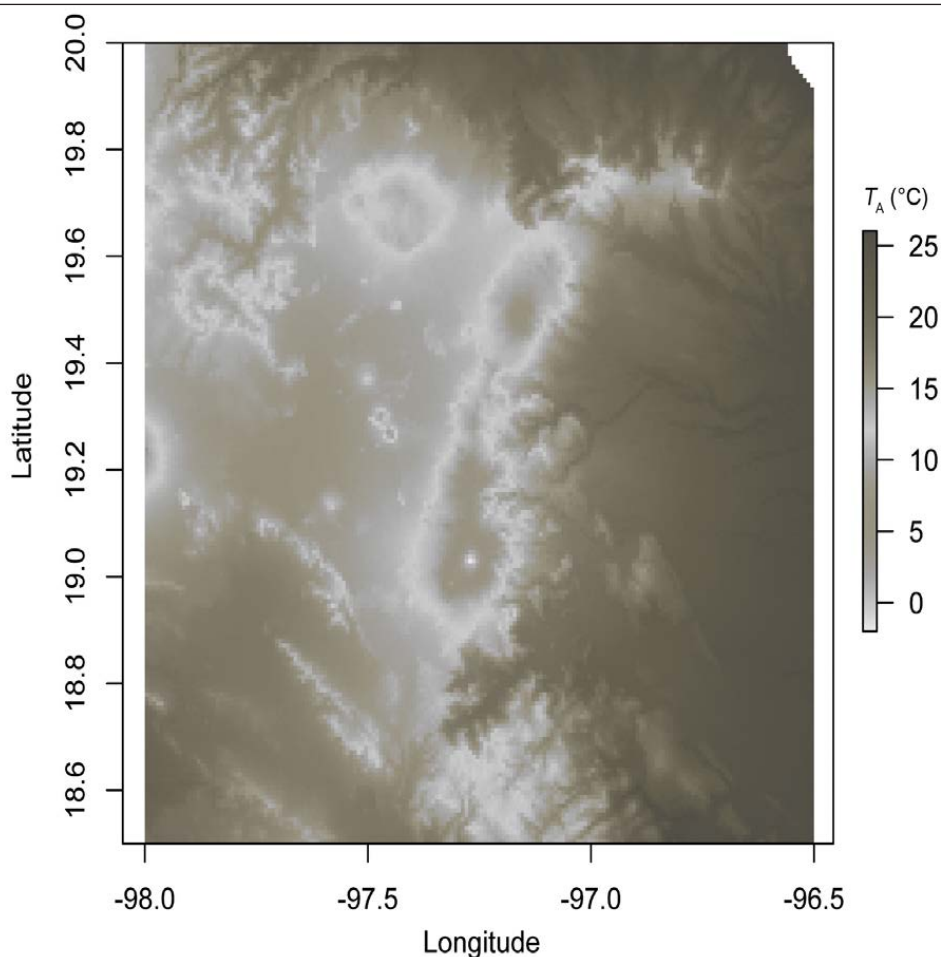


Figure 2. Diagram of the envelope function method for the parameters temperature (T_A), photosynthetically active radiation (PAR), vapor pressure difference (VPD), transpiration (E), and leaf water potential (Ψ_L) plotted against stomatal conductance (g_s) for *Alnus acuminata*, *Quercus xalapensis*, *Liquidambar styraciflua* and *Pinus ayacahuite*.

$96.0 \mu\text{mol m}^{-2} \text{s}^{-1}$, with maximum values at 1200 h ($289.06 \pm 65.90 \mu\text{mol m}^{-2} \text{s}^{-1}$) and minimum at 1800 h ($26.58 \pm 14 \mu\text{mol m}^{-2} \text{s}^{-1}$).

We found significant differences among all species when comparing g_s (Kruskal-Wallis $H = 209.174$, $P < 0.001$), Ψ (Kruskal-Wallis $H = 240.85$, $P < 0.001$), PAR (Kruskal-Wallis $H = 25.0318$, $P < 0.001$) and VPD (Kruskal-Wallis $H = 8.7461$, $P = 0.0329$). *Alnus acuminata* had the highest g_s and E . Concerning Ψ_L , the highest corresponded to *Pinus ayacahuite*. The highest temperature was registered for *A. acuminata*, VPD corresponded to *Quercus xalapensis* and PAR was associated to *P. ayacahuite* (Table 2). The most sensitive species to changes in PAR, E and Ψ_L was *Liquidambar styraciflua*, and for VPD was *A. acuminata* (Table 3). The comparison of the curves generated by the envelope function method among the four species performing the method for all the variables (T_A , PAR, VPD, E and Ψ_L) for each species is presented in Figure 2. From the g_s vs. T_A curve we obtained the optimal thermic range (T_R), and the optimal tem-

Figure 3. Mean temperature distribution in the region of the Great Mountains in the state of Veracruz, Mexico. Data from WorldClim-Global Climate Data (<http://www.worldclim.org/> Accessed November 2014).



perature (T_o) when the maximum stomatal conductance (g_{sMAX}) occurred. For *A. acuminata* $T_o = 29$ °C, for *Q. xalapensis* $T_o = 28$ °C, for *L. styraciflua* $T_o = 27$ °C, and for *P. ayacahuite* was $T_o = 26$ °C (Table 1).

After analyzing the PCA, we found differential influence and vector lengths for each variable (Table 4). We found that species response was differential; however, VPD and T_A had the highest RI for all species, except for *Alnus acuminata* where PAR had the highest RI (Table 4). As for the linear regression model, we found that all variables had an effect on g_s : 1) T_A : linear regression $t = 21.96$, $P < 0.001$; ANOVA $F = 1.72$, $P = 0.1903$; 2) PAR : linear regression $t = 4.48$, $P < 0.001$; ANOVA $F = 60.6078$, $P < 0.001$; 3) VPD : linear regression $t = -23.14$, $P < 0.001$; ANOVA $F = 526.71$, $P < 0.001$, and 4) Ψ_L : linear regression $t = -6.22$, $P < 0.001$; ANOVA $F = 38.66$, $P < 0.001$. For the model we found an adjusted R^2 of 0.5296 (F statistic: 156.9, $P < 0.001$).

When we analyzed Figure 2, we observed for T_A that *Quercus xalapensis*, *Liquidambar styraciflua*, and *Alnus acuminata* have analogous physiological behavior in temperatures between 27.1 and 31 °C, which is consistent with the T_o ranges of the three species (Table 1). When temperature is higher (31–34.2 °C) *Pinus ayacahuite* and *L. styraciflua* behaved similarly. And with highest temperatures reached (more than 34 and up to 40 °C), *A. acuminata* and *L. styraciflua* had the equal g_s behavior. We noticed that all the species had higher T_o and T_R (Table 1) than the mean temperatures of the region (Figure 3).

Stomatal behavior for PAR was similar for all four species (Figure 2), with a slight distinction of *Pinus ayacahuite*. We observed a greater affinity between *Quercus xalapensis* and *Liquidambar styraciflua*. These data can be corroborated with the equation values from the envelope function method (Table 3), where sensitivity values (b) were similar for *Alnus acuminata*, *Q. xalapensis*, and *L. styraciflua* (6.752, 8.675 and 9.087 respectively), against *P. ayacahuite* (2.078). As for VPD , we noted that at low VPD (2.51 – 2.85 kPa) all species behaved similarly. While

increasing VPD (3.15 - 3.72 kPa) *A. acuminata* and *L. styraciflua* behaved similarly and with higher VPD (3.7 - 4.0 kPa) *A. acuminata* and *P. ayacahuite* behaved similarly (Figure 2).

There was similar behavior among the species for E (Figure 2). We observed that *Quercus xalapensis*, *Alnus acuminata*, and *Pinus ayacahuite* behaved similarly in $E < 10 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Above $20 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, *Liquidambar styraciflua*, *Q. xalapensis* and *A. acuminata* had a similar behavior. Concerning Ψ_L , we observed that *L. styraciflua* and *P. ayacahuite* reached the lowest asymptotic g_s values (Table 3), with *L. styraciflua* being the most sensitive species to changes in Ψ_L .

Discussion

Stomatal conductance is an important physiological trait (see Introduction) that can help us to analyze ecophysiological responses related to changes in climate variables and other physiological traits. However, the g_s response also can help to understand the species' distribution along an elevation gradient, because g_s may be related to the altitude where plants grow, thus the physiological response in plants may be a heritable trait (Hovenden and Brodribb, 2000). In this work, we tried to make a first attempt to use g_s data taken in the greenhouse to explain the natural distribution of the species.

Studying the g_s response along an elevation gradient has the difficulty that g_s measurements must be taken simultaneously (see Materials and Methods), thus comparing species, in particular species that do not share the same distribution, in the field can be methodologically difficult. We found a possible solution to this problem by measuring species responses in the greenhouse, observing changes related to temperature. Studying tropical systems has the advantage that air temperature decreases when increasing elevation.

The adaptation of plants to different elevations is seen as an analogy for climate adaptation (Hovenden and Brodribb, 2000), where elevation influences the environmental variables T_A , PAR , VPD and rainfall by decreasing temperature with increasing altitude (Harper, 1977; Hikosaka *et al.*, 2002), and elevation influences the possibility of plants acclimation and adaptation to particular environmental conditions (Körner *et al.*, 1986, Friend *et al.*, 1989). In this work, we considered that high temperatures in the greenhouse could be related to low elevations in the elevation gradient and low temperatures to high elevations.

Our species selection was not arbitrary. Species are distributed along the elevational gradient of the central mountain region of Veracruz (Figure 1). Their distributions range from 400 to

Figure 4. Theoretical relationship among stomatal conductance (g_s), climate variables and species' abundance along an altitudinal gradient. Maximum stomatal conductance (g_{sMAX}) and maximum species' abundance (A_{spMAX}) is found where the elevation presents optimal temperature (T_o), vapor pressure deficit (VPD_o), photosynthetically active radiation (PAR_o) and precipitation; g_s decreases when temperature is minimal (T_{min}) and maximum (T_{MAX}).

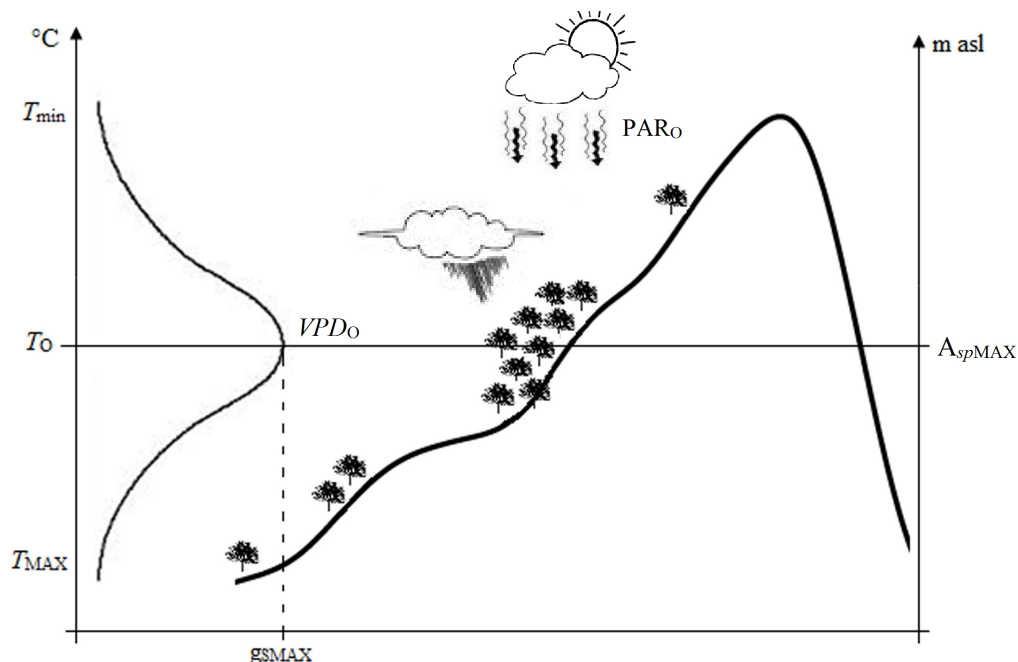


Table 4. Length of the vectors of the principal component analysis (PCA), their relative importance (RI), variance accounted for by each axis (%) and Eigenvector scores of the plant traits of air temperature (T_A), vapor pressure deficit (VPD), photosynthetically active radiation (PAR) and leaf water potential (Ψ_L) in the first three principal components analysis axes for *Alnus acuminata*, *Liquidambar styraciflua*, *Pinus ayacahuite* and *Quercus xalapensis*. Values are ranked in order of absolute magnitude along PCA axes for each species.

Species	Trait	Length	RI	PCA1	PCA2	PCA3
<i>Alnus acuminata</i>	Variance accounted (%)	-	-	55.5	24.55	19.49
	PAR	2.4845	1.000	-0.5691	2.41852	0.4995
	T_A	2.4407	0.9823	-2.4388	-0.09597	-0.6379
	VPD	2.4397	0.9819	-2.4385	-0.07887	-0.6414
	Ψ_L	1.5756	0.6341	1.4285	0.66500	-1.9849
<i>Liquidambar styraciflua</i>	Variance accounted (%)	-	-	52.23	25.17	22.17
	T_A	2.2877	1.0000	2.2826	-0.1542	-0.4164
	VPD	2.2816	0.9973	2.2714	-0.2160	-0.4494
	Ψ_L	1.1497	0.5025	1.0049	0.5587	2.0328
	PAR	2.2638	0.9895	0.1244	2.2605	-0.5738
<i>Pinus ayacahuite</i>	Variance accounted (%)	-	-	53.47	26.59	19.41
	T_A	2.2748	1.0000	2.2741	0.0585	0.4701
	VPD	2.2620	0.9943	2.2620	-0.0127	0.5293
	PAR	1.9377	0.8517	0.7555	1.78438	-1.3036
	Ψ_L	1.8493	0.8129	-0.8986	1.61643	1.4262
<i>Quercus xalapensis</i>	Variance accounted (%)	-	-	49.78	27.55	22.27
	VPD	2.4558	1.0000	2.44965	0.1740	-0.05134
	T_A	2.4533	0.9990	2.45121	0.1035	-0.12063
	Ψ_L	1.8661	0.7598	0.06434	-1.8650	-1.61259
	PAR	1.8116	0.7376	0.31383	-1.7842	1.67354

3,500 m asl. This means that the species are subject to different temperature and precipitation regimes (Table 1). Therefore, we found different g_s responses among species (Table 2). If we did not find differences, that would imply that the g_s response is similar for all species (Hubbell, 2001), but having a differential g_s response supports evidence of differences in the evolutionary history of each species and of particular adaptations to their respective niches along the elevation gradient.

Stomatal conductance helped us to understand species' ecophysiological responses along an elevation gradient. Stomatal responses to T_A for *Quercus xalapensis*, *Liquidambar styraciflua*, and *Alnus acuminata* in lower temperatures (Figure 2) were consistent with the T_o ranges and with their natural distribution (Table 1). We can find them from 1,300 to 1,800 m asl at lower altitudes where T_A increases. At higher temperature, *Pinus ayacahuite* and *L. styraciflua* behaved similarly although these species do not share the altitudinal distribution. This might indicate the pine plasticity to adapt to high temperatures, because it has the highest distribution (2,000-3,500 m asl) and the lower T_o (26.2 °C), and when temperatures were the highest (Figure 2), *A. acuminata* and *L. styraciflua* had the same g_s behavior, indicating that these two species have a greater heat tolerance. This tolerance is evident for *A. acuminata*, consistent with its T_o (29.7 °C), the envelope curve (Figure 2) and its altitudinal distribution (1,300 -2,800 m asl). For *L. styraciflua*, this result was also consistent with its distribution (the lowest of all species, 400-1,800 m asl).

The g_s response to E also showed evidence to support the altitudinal distribution. *Quercus xalapensis*, *Alnus acuminata*, and *Pinus ayacahuite* behaved similarly with low E (Figure 2), where all these species distribute at higher altitudes (above 2,000 m asl) where temperature is lower, and thus g_s and E are lower preventing water loss through stomata. But with higher E , *Liquidambar styraciflua*, *Q. xalapensis* and *A. acuminata* had a similar behavior, where high E can be related to lower elevational distribution (below 2,000 m asl, and outside the *P. ayacahuite* distribution) with high temperatures.

Stomatal conductance was influenced by all climate and physiological variables (Table 4). Finding T_A and VPD as the main sources of data variability indicates the importance of RH for all species (Table 4). We acknowledge the fact that conditions between the greenhouse and the field are different, where T_A , VPD , RH, water availability and other factors might influence the g_s response in the field. However, using the envelope function method allowed us to extrapolate the results beyond individuals. Using this method can help us to predict plant performance outside a species' native range (Sands *et al.*, 2000; Rodríguez *et al.*, 2002; Dye *et al.*, 2004) by increasing temperature or reducing water or VPD , trying to simulate climate change scenarios, environmental changes or climate conditions at different elevations.

In the field, the maximum g_s (g_{sMAX}) will be found in the elevation gradient where optimal precipitation, temperature (T_O), VPD (VPD_O) and PAR (PAR_O) occurs (Jones, 1992), depending on the characteristics of each plant (Mansfield, 1971). This g_{sMAX} can be translated to maximum photosynthesis and productivity rate; therefore, at this elevation we hypothesized that the maximum species' abundance might be found (A_{spMAX} , Figure 4). This relationship can be used as a tool for restoration and re-colonization plans of vulnerable species (Castellanos-Acuña *et al.*, 2015). It can also be a useful tool when studying the impacts of climate change. Species might migrate to higher altitudes where temperature is lower, reaching areas with more proper climates (Theurillat and Guisan, 2001), but always considering that factors such as spatial, nutrient and water availability, competition, and germination are determinant of the species' establishment and survival.

Finally, we must acknowledge that individuals might have acclimatized to the greenhouse conditions, affecting and differing their natural g_s response expected in the field. We also recognized that differences among species might also be caused by genetic differences. Nevertheless, as we mention before, this is a first attempt trying to correlate data obtained in the greenhouse to the natural distribution. And despite the consideration mentioned, we encourage future studies that can help to corroborate our results by measuring the g_s response in the field along the elevation gradient.

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