SHORT RESEARCH PAPER

The neotropical shrub *Lupinus elegans*, from temperate forests, may not adapt to climate change

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ABSTRACT

Considering that their distribution is limited to altitudinal gradients along mountains that are likely to become warmer and drier, climate change poses an increased threat to temperate forest species from tropical regions. We studied whether the understorey shrub *Lupinus elegans*, endemic to temperate forests of west-central Mexico, will be able to withstand the projected temperature increase under seven climate change scenarios. Seeds were collected along an altitudinal gradient and grown in a shade-house over 7 months before determining their temperature tolerance as electrolyte leakage. The plants from colder sites tolerated lower temperatures, *i.e.* the temperature at which half of the maximum electrolyte leakage occurred (LT_{50}), ranged from -6.4 ± 0.7 to -2.4 ± 0.3 °C. In contrast, no pattern was found for tolerance to high temperature (LT_{50} average 42.8 ± 0.3 °C). The climate change scenarios considered here consistently estimated an increase in air temperature during the present century that was higher for the maximum air temperature than for the mean or minimum. In particular, the anomaly from the normal maximum air temperature at the study region ranged from 2.8 °C by 2030 to 5.8 °C by 2090. In this respect, the inability of *L. elegans* to adapt to increasingly higher temperatures found here, in addition to a possible inhibition of reproduction caused by warmer winters, may limit its future distribution.

INTRODUCTION

Species of temperate origin that occur at high elevations within the tropics face an especially severe threat posed by climate change, considering that their temperate affinity and the sites adequate for their survival are only available along altitudinal gradients in mountains surrounded by a matrix of lowlands with higher mean temperatures and often lower precipitation (Rundel 1994; Lenoir *et al.* 2008; Sáenz-Romero *et al.* 2010). This is clearly the case for temperate forests in Mexico, where suitable sites are likely to disappear or at least have their areas greatly reduced as a consequence of climate change (Villers & Trejo 2004; Gómez-Mendoza & Arriaga 2007; Sáenz-Romero *et al.* 2010; Rehfeldt *et al.* 2012). Considering that the composition of plant communities greatly depends on the distributions of individual species, determining whether temperate forests will persist requires the study of responses by individual species to environmental conditions expected under climate change scenarios (Cody 1975; Ricklefs & Miller 1999; de la Barrera & Smith 2009).

Potential shifts in the distribution of plant species in response to climate change have been simulated with niche envelope models, also called bioclimatic models, that consider geo-referenced information about the distribution of a particular species (usually from collection records) and digital maps or layers of environmental information to identify sites with environmental similarities to those sites where a species is known to exist (Martínez-Meyer 2005; Elith *et al.* 2006). This approach, while useful as an initial assessment, does not consider the microenvironmental particularities that are determinants of the actual distribution of species (McMahon *et al.* 2011). For instance, while the mean air temperature is a good predictor of plant performance, extreme temperature events can actually limit a species' distribution (Nobel *et al.* 2002; Drennan 2009).

Species in the cosmopolitan genus *Lupinus* are nitrogen-fixing legumes, frequently found in temperate forests where they can act as nurse plants favouring the establishment of tree species (Blanco-García *et al.* 2011). In particular, *L. elegans* is a neotropical understorey shrub, endemic to temperate forests of west-central Mexico, which occurs at elevations ranging from 1800 to 3000 m a.s.l. (Dunn 2001). Here, the tolerance to extreme air temperatures was determined for *L. elegans* from various sites along an altitudinal gradient, in order to assess the potential ability of this species to adapt to climate change.

MATERIAL AND METHODS

From December 2008 to February 2009, seeds were collected from 12 individuals from each of five populations of *L. elegans* Kunth. (Fabaceae), occurring on southwest-facing slopes along an altitudinal gradient (2312–2885 m) in the west-central state of Michoacán, Mexico (Table 1; Soto-Correa *et al.* 2012). After submergence in H_2SO_4 (97% v/v) for 30 min for scarification

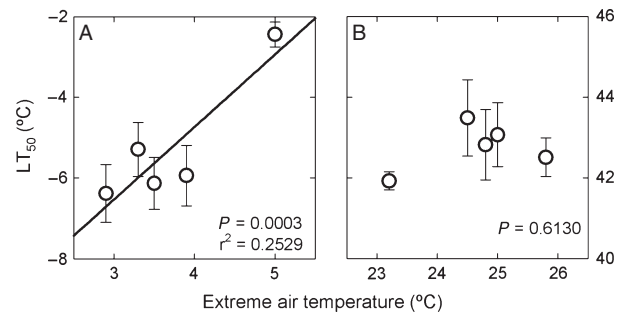**Table 1.** Localities where the seeds of *Lupinus elegans* were collected.

| site	latitude	longitude	altitude (m)	minimum temperature (ºC)	maximum temperature (ºC)
Puerto Garnica	19°39′58″	−100°49′25″	2885	2.9	23.2
Mil Cumbres la Cruz	19°37′20″	−100°45′59″	2650	3.3	24.5
San José de la Cumbre la Puerta	19°39′51″	−100°51′31″	2480	3.9	25.0
Mil Cumbres Caracol	19°36′57″	−100°46′25″	2480	3.5	24.8
San José de la Cumbre la Cienega	19°39′15″	−100°51′28″	2312	5.0	25.8

Fig. 1. Temperature tolerance to extreme temperatures for *Lupinus elegans* from west-central Mexico. The temperatures at which half electrolyte leakage occurs (LT_{50}) are shown as influenced by the prevailing low (A) or high (B) air temperatures at sites where seeds were collected. Data are shown as mean $\pm$ SE (n = 9 individuals per site).

(Medina-Sánchez & Lindig-Cisneros 2005), the seeds were planted in plastic containers (375 ml volume) with a 2:1 mix of a commercial potting mixture (CreciRoot, Sistemas Agrotec, Uruapan, Michoacán, Mexico) and washed silica sand. The experiment was established on 28 August 2009 in a shadehouse at the Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México (19°38′55.9″ N, 101°13′43.7″ W; 1967 m a.s.l.), where nine seeds from each population were planted and irrigated weekly to field capacity over 7 months, after which temperature tolerance measurements were conducted.

Tolerance to extreme temperatures was determined in April–May 2010 by measuring electrolyte leakage, an indicator of cell membrane integrity (Didden-Zopfy & Nobel 1982; Osmond *et al.* 1994; Drennan 2009). Leaf discs, 5 mm in diameter, were obtained with a core borer, surface-washed with deionised distilled water, and placed in 1.5-ml microtubes containing damp cotton to prevent desiccation. The leaf discs were incubated at a given temperature for 1 h, after which a disc was removed from the microtube, placed in a glass vial containing 15 ml deionised distilled water and shaken at 200 rpm for 40 min. A second disc was boiled for 5 min to disrupt cell membranes, leading to maximum electrolyte leakage, before being placed in the shaker as described above. Electrical conductivity was measured with an Orion-3 conductivity meter (Thermo Electron Corp., Marietta, OH, USA) and electrolyte leakage was expressed as a percentage of the maximum. For the remaining leaf discs, the temperature was changed for additional successive 1-h-long incubation periods and the process was repeated until maximum electrolyte leakage was attained.

The temperature at which half of maximum electrolyte leakage occurred (LT_{50}) for both the low and high incubation temperatures was identified (see Nobel *et al.* 2002 for a detailed description). These LT_{50} values have been found to be good predictors of extreme temperature tolerance in the field (Nobel *et al.* 2002; Drennan 2009). Differences among the resulting LT_{50} for plants from the different locations were evaluated with a one-way ANOVA.

In order to evaluate the vulnerability of *L. elegans* to climate change, the LT_{50} were contrasted with the temperature normals and seven scenarios of future climate for the region where the seeds had been collected (Table 1). Climate data and scenarios of future climate were obtained from a publicly available database (Rehfeldt *et al.* 2006; Crookston 2011; Sáenz-Romero *et al.* 2010). In particular, the study considered results of calculations performed with three general circulation models (gcm) for four carbon emission trajectories (Canadian gcm: A1B, A2, and B1; Geophysical Fluid Dynamics Laboratory gcm: A2 and B1; Hadley gcm: A2 and B2; Sáenz-Romero *et al.* 2010). Statistical comparisons among the climate change scenarios were conducted with two-way ANOVA's (factors were scenario and year). Statistical analyses were conducted with SAS v. 6.3 (SAS Institute, Raleigh, NC, USA).

RESULTS

The minimum air temperature for January, the coldest month ($R^2 = 0.792$ from a linear regression; Table 1), and maximum temperature for May, the warmest month ($R^2 = 0.972$; Table 1), were positively correlated with altitude of the collection sites. In turn, tolerance to low temperatures in *L. elegans* responded to the minimum air temperature (Fig. 1A; $P = 0.0003$). Plants from colder sites required a lower temperature to cause 50% electrolyte leakage (LT_{50}), ranging from -6.4 ± 0.7 °C for plants from a site where the minimum temperature was 2.9 °C, to -2.4 ± 0.3 °C for plants from a site where the minimum temperature was 5.0 °C. Thus, a shift of -1.27 °C occurred in the LT_{50} for each decrease of 1 °C in air temperature. In contrast, high temperature tolerance did not respond to the maximum air temperature (Fig. 1B; $P = 0.6130$); rather, the LT_{50} averaged 42.8 ± 0.3 °C. Therefore, these plants were not well adapted to increasing air temperatures occurring along the elevation gradient considered here.

The seven climate change scenarios considered in the present work consistently estimated that air temperature increments will occur within the present century for the region where the seeds of *L. elegans* were collected (Fig. 2). In particular, for the minimum air temperature of the coldest month, currently 2.9 ± 0.3 °C, the various scenarios estimated different air warming patterns (Table 2), which by the year 2090 could span from 4.2 ± 0.2 °C (from the B1 trajectory estimated in the GFDL general circulation model) to 7.5 ± 0.1 °C (A2, Hadley).
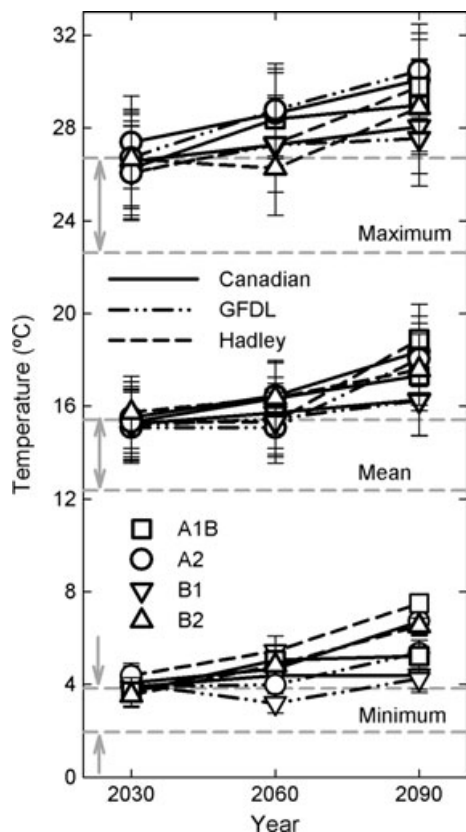
Fig. 2. Climate change scenarios for the region of Mil Cumbres, Michoacán, where seeds of *Lupinus elegans* were collected. Data represent average ± 95% confidence interval (n = 5 sites) for the estimated annual minimum, mean and maximum air temperature during the 21st century. The grey bands contained within dashed lines and marked with arrows indicate the span of the 95% confidence interval for the climate normals of the region. The Canadian (solid line), Geophysical Fluid Dynamics Laboratory (dashed and dotted line) and Hadley (dashed line) general circulation models were used to estimate scenarios for A1B (squares), A2 (circles), B1 (downward triangles) and B2 (upward triangles) trajectories of greenhouse gas emissions.

Table 2. Two-way ANOVA's for climate change scenarios from the region of Mil Cumbres, Michoacán, where seeds of *Lupinus elegans* were collected. Within a column, F-values with one asterisk (*) were significant at $P < 0.05$, while those with two asterisks (**) were significant at $P < 0.0001$.

factors	df	minimum air temperature F	mean air temperature F	maximum air temperature F
scenario	6	1.91	33.01 **	2.60 *
year	2	29.15 **	150.90 **	21.90 **
scenario × year	12	1.19	11.71 **	0.70

No differences were found among the scenarios for the future mean air temperature, which in combination predicted that it might reach 17.5 ± 0.2 °C in 2090, *i.e.* 3.6 °C warmer than the normal (Table 2, Fig. 2). With respect to the maximum air temperature that is currently 24.7 ± 0.7 °C, differences were found among the scenarios considered and

temperatures tended to increase with time (Table 2, Fig. 2). However, the fact that the interaction of scenario × year was not significant indicates that the models estimated different rate of change which may result in maximum air temperatures ranging from 27.5 ± 0.7 °C (B1, GFDL) to 30.5 ± 0.7 °C (A2, GFDL) in 2090.

DISCUSSION

Extreme temperatures can be more restrictive for the extent of a species' distribution than the prevailing mean air temperature (Drennan 2009; Rehfeldt *et al.* 2009; Vitasse *et al.* 2009). For instance, an altitudinal differentiation occurs for the distribution of Mexican pines as a result of the inability of this species from lower elevations to tolerate decreasing air temperatures. Indeed, *Pinus oocarpa* is found in warmer sites at lower elevations than *P. devoniana* (syn. *P. michoacana*), which occurs at lower elevations than *P. pseudostrobus* and *P. hartwegii* (syn. *P. montezumae* var. *rudis* (Endl.) Shaw; Sáenz-Romero *et al.* 2010).

In the present work, an adaptation to low air temperature was found for *L. elegans*. Individuals from colder sites were able to withstand lower temperatures than their counterparts from warmer sites. Indeed, a genetic differentiation has been confirmed among these and other populations of *L. elegans* within the study region (Lara-Cabrera *et al.* 2009; Soto-Correa *et al.* 2012). In contrast, no pattern was found in response to high temperatures, as the plants from different locations along the altitudinal gradient showed a similar response. Thus, natural history plays an important role in the tolerance of plant species to extreme temperatures. Arguably, the relatively mild high temperatures prevalent within the area of distribution did not exert selective pressure for high temperature-tolerant genotypes of *L. elegans*. A contrasting example can be illustrative: for plants in the Cactaceae native to environments with high temperatures, the high LT_{50} increases by 0.53 °C per each 1 °C increase in air temperature, while the low LT_{50} decreases by a mere 0.07 °C per each 1 °C decrease in air temperature (Drennan 2009).

The estimated anomalies in mean and maximum temperatures were higher than for the minimum temperature. Considering that the distribution of *L. elegans* did not appear to be limited by low temperatures, no changes in its geographic distribution can be expected. However, these increasingly warmer winters may lead to a reduction in the low temperature necessary for the onset of reproductive development (Rahman & Gladstone 1972; de la Barrera *et al.* 2009). For example, seedlings of *L. albus* in South Africa require temperatures below 5 °C at the time of emergence in a site where the annual minimum air temperature is close to 5 °C (Keeve *et al.* 2000); however, the cold requirements for *L. elegans* have not yet been determined.

The impending climate change in west-central Mexico poses special threats to the prevalence of species with temperate affinity, given their restricted altitudinal distribution. The capacity of individual species to adapt to higher temperatures, along with the severity of actual climate anomalies, will greatly influence the future composition of plant communities in the region. The physiological approach utilised in the present work can improve our ability to estimate the responses of other plant species to climate change.

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