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Effect of induced global warming on survival and performance of succulent species from Southern Chihuahuan Desert

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Dr. Joel David Flores Rivas

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%: Percentage

Φ_{PSII}: Effective quantum yield of photosystem II

µmol m^{-2} s^{-1}: Micromole per meter above minus two per second above minus one

µmol/mol: Micromole/mol

ANOVA: Variance analysis

ATP: Adenosine triphosphate

CI: Confidence Intervals

cm: Centimeters

CO_2: Carbon dioxide

DM: Dry mass

e.g.: For instance

ET: Daily mean evapotranspiration

ETR: Electron transport rate

FM: Fresh mass

F_v/F_m: Maximum quantum efficiency of photosystem II

GCCA: Generalized canonical correlation analysis

GSR: Global solar radiation

h: Hour

INIFAP: Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

IPCC: Intergovernmental Panel on Climate Change

IPICyT: Instituto Potosino de Investigación Científica y Tecnológica

IUCN: International Union for Conservation of Nature

m.a.s.l.: Meters above sea level

m: Meters

m^2: Square meters

mg: Milligrams

Mini-PAM: Pulse amplitude modulation fluorometer

mm: Millimeters

NADPH/H$^+$: Nicotinamide adenine dinucleotide phosphate

nm: Nanometers

NPQ: Non-photochemical quenching

°C: Celsius degree

OTC: Open-top chamber

PhD: Doctoral thesis

PPFD: Photosynthetic photon flux density

RH: Relative humidity

SAM: Shoot apical meristem

SEMARNAT: Secretaría de Medio Ambiente y Recursos Naturales

SSB: Soil seed bank

UV: Ultraviolet

VC: Vascular cylinder

VCC: Vascular cylinder contraction

WL: Water loss

Abstract

Numerous ecological studies have assessed the potential consequences of climate warming within several ecosystems worldwide. However, the impact of global warming on desert plants is still little studied. Most predictions of global change in desert species have been suggested for adult plants, neglecting other plant development phases, such as seeds, seedlings or young plants, which are often more susceptible to temperatures, drought and anthropogenic stresses than mature plants. The Chihuahuan Desert is largest warm desert of North America; it expands from southwestern United States to the Central Mexican Highlands. Global change predictions for this region indicate that summer temperatures (June–September) will increase by 1–2°C by 2030. However, the greatest temperature increase is predicted to occur during the winter months (January–March), when monthly average temperatures might increase by up to 6°C. This critical scenario has created strong uncertainty for conservation biologists because the southernmost section of the Chihuahuan Desert harbors an elevated richness of succulent plants, especially globose cacti, most of them protected by the Mexican environmental laws. Nevertheless, there is still little information about the thermal tolerance thresholds for plant species of this area. Passive open top-chambers (OTCs) are the most common and simplest tool to evaluate the responses of plants to climate warming in the field. The main goal of this research was to assess survival rate and tolerance mechanisms of several succulent species from the Cactaceae and Asparagaceae families in the earlier stages of their life cycles (seeds, seedlings and young plants) under a potential scenario of global warming in the Southern Chihuahuan Desert, within the state of San Luis Potosí, México. In our induced global

warming scenario using OTCs, the mean air temperature was higher and relative humidity was lower than in the control plots. These temperature increases and relative humidity decreases promoted the rise of the mean soil temperature within OTCs compared to open spaces. We hypothesize that this physical stress rises will have a severe detrimental effect in seeds, seedlings and young plants of the studied species. Differential responses were observed, which were in function of the development state and the analyzed species. *Agave striata* displayed high germination in both warming conditions (OTC and control plots) in spring (dry season); nevertheless, its seeds lost viability in summer and subsequently until the end of the experiment. *Yucca filifera* showed high germinability in both treatments in spring and summer, but in the former season, 100% germination in buried bags was found; thus, for this species no germination tests were performed in the next seasons. *Echinocactus platyacanthus* showed a positive feedback between mean soil temperature increase and seed germination; even, this soil temperature increment allowed a relative high germinability inside OTCs in summer. *Echinocactus platyacanthus* showed a transitory seed bank, which was promoted by cycles of dormancy. In a second experiment, after induced warming for the four months of the growing season, we found significantly lower seedling survival rate for *E. platyacanthus*, *Ferocactus histrix* and *Stenocactus coptonogonus* inside OTCs than in the control plots. As expected, this induced global warming approach structurally affected seedlings of the studied cacti, stressed seedlings showed lower both fresh and dry mass and higher water loss than non-stressed seedlings, and stressed seedlings displayed severe damage in chlorenchyma cells. Nevertheless, these changes did not affect the shoot apical meristem diameter, which was high for all species in both treatments. In addition, stressed seedlings from *E. platyacanthus* and *F. histrix* showed lower values of vascular cylinder length than those from *S. coptonogonus*, and they

suffered vascular cylinder contraction. Analyzing the photosynthetic performance on five years-old individuals of *E. platyacanthus* under our simulated global warming scenario during a 4-month period of drought-high temperature, this species displayed lower maximum quantum efficiency of photosystems II (F_v/F_m), effective quantum yield of photosystems II (Φ_{PSII}) and electron transport rate (ETR) values within OTCs than in the control plots; however, *E. platyacanthus* had 100% survival in both treatments. This extraordinary tolerance was attributed to high non-photochemical quenching (NPQ) values, a mechanism to avoid photoinhibition. Finally, although seasonal variations in photosynthetic performance were found (the Φ_{PSII} and ETR values were low in summer but high in autumn and winter), *E. platyacanthus* newly showed 100% survival in both, OTCs and control plots. Furthermore, using a generalized canonical correlation analysis (GCCA) we also found a strong correlation (0.71) between micro-environment (represented by OTC plots and open spaces) with ecophysiology of *E. platyacanthus*. This work is pioneer for Mexico as well as for the arid and semi-arid American deserts in that it examines the potential impact of global warming in the behavior and persistence of a threatened Mexican cacti species as well as two charismatic species from the Asparagaceae family; particularly, in their earlier stages of the life cycles in both, short and long-term. In addition, this study provides new insights about behavior and possible population changes in succulent species, which are social, cultural and economic key factors in American deserts.

Key words: Asparagaceae, Cactaceae, Chihuahuan Desert, Ecophysiology, Functional anatomy, GCCA, Global warming, Seed germination, Seedlings, Soil seed banks, Survival, Open-top chambers, Young plants.

Resumen

Numerosos estudios ecológicos han evaluado las potenciales consecuencias del calentamiento climático dentro de varios ecosistemas a nivel mundial. Sin embargo, el impacto del calentamiento global en las plantas desérticas es todavía poco conocido. La mayoría de las predicciones de cambio global en especies desérticas han sido realizadas para plantas adultas, descuidando otras fases de desarrollo de las plantas tales como semillas, plántulas o plantas jóvenes, las cuales son a menudo más susceptibles a estrés por temperatura, sequía o actividades humanas, que las plantas adultas. El desierto Chihuahuense es el desierto cálido más extenso de Norte América; este comprende desde el suroeste de los Estados Unidos de Norteamérica hasta el Altiplano central Mexicano. Las proyecciones de cambio global para esta región indican que la temperatura de verano (Junio-Septiembre) incrementará de 1-2ºC para el 2030. No obstante, el mayor incremento de temperatura está proyectado a ocurrir durante los meses de invierno (Enero-Marzo), cuando mensualmente la temperatura promedio podría aumentar hasta más de 6ºC durante el mismo periodo de tiempo. Este panorama critico ha creado fuerte incertidumbre en los biólogos conservacionistas debido que la sección sur del desierto Chihuahuense alberga una elevada riqueza de plantas suculentas a nivel mundial, especialmente cactus globosos y la mayoría de estos se encuentras protegidos por las leyes ambientales Mexicanas. Sin embargo, existe poca información todavía acerca de los umbrales de tolerancia térmica para estas especies en esta área. Las cámaras de techo abierto (OTCs por sus siglas en inglés) son la más común y simple herramienta para evaluar las respuestas de las plantas al calentamiento climático. Por consiguiente, el principal objetivo de esta investigación fue

evaluar la tasa de supervivencia y los mecanismos de tolerancia de varias especies suculentas de las familias Cactaceae y Asparagaceae en los estados iniciales de sus ciclos de vida (semillas, plántulas y plantas jóvenes) bajo un potencial escenario de calentamiento global en el sur del desierto Chihuahuense, en el estado de San Luis Potosí, México. En el escenario inducido de calentamiento global usando OTCs, la temperatura media del aire así como la humedad relativa siempre fueron mayor y menor, respectivamente, que en las parcelas control. Estos incrementos de temperatura y disminución de la humedad relativa promovieron el aumento de la temperatura superficial media del suelo en las OTCs comparada con los espacios abiertos. Se tiene la hipótesis que este aumento del estrés físico tendrá un efecto dañino severo en las semillas, plántulas y plantas jóvenes de las especies estudiadas. No obstante, fueron observadas respuestas diferenciales, las cuales estuvieron en función del estado de desarrollo y la especie analizada. *Agave striata* presentó alta germinación en ambas condiciones de calentamiento (OTC y parcelas control) en primavera (estación seca); sin embargo, sus semillas perdieron viabilidad en verano y subsecuentemente hasta el final del experimento. *Yucca filifera* mostró alta germinación en ambos tratamientos en primavera y verano, pero en esta última estación, se encontró 100% de germinación dentro de las bolsas enterradas; por consiguiente, no se realizaron pruebas de germinación para esta especie en las siguientes estaciones. *Echinocactus platyacanthus* mostró una respuesta positiva entre los incrementos de la temperatura de suelo y la germinación de sus semillas; incluso este aumento de la temperatura de suelo permitió una relativa alta germinación dentro de las OTCs en verano. Además, esta especie mostró un banco de semillas transitorio, el cual fue promovido por ciclos de latencia. En otro experimento, después de inducir calentamiento por cuatro meses en la estación de crecimiento, se encontró una menor tasa de supervivencia para *E. platyacanthus*, *Ferocactus histrix* y *Stenocactus coptonogonus* dentro de las OTCs que en las parcelas control. Como también se esperaba, este enfoque de calentamiento global inducido afectó estructuralmente a las plántulas de estos cactus. Estas especies tuvieron menor peso fresco y seco, y mayor pérdida de agua en plántulas estresadas que en las no estresadas; además, las plántulas estresadas presentaron un severo daño en las células de clorénquima. No obstante, estos cambios no afectaron el diámetro de los meristemos apicales, el cual fue alto para todas las especies. Además, las plántulas estresadas de *E. platyacanthus* y *F. histrix* tuvieron menores valores de longitud del cilindro vascular que las de *S. coptonogonus* y estos sufrieron una contracción del cilindro vascular. Analizando el desempeño fotosintético en individuos de cinco años de *E. platyacanthus* bajo nuestro escenario simulado de calentamiento global durante un periodo de cuatro meses de sequía-alta temperatura, esta especie mostró menores valores de eficiencia cuántica máxima del fotosistema II (F_v/F_m), cosecha cuántica efectiva del fotosistema II (Φ_{PSII}) y tasa de transporte de electrones (ETR) dentro de las OTCs comparados con las parcelas control, sin embargo *E. platyacanthus* tuvo 100% de supervivencia en ambos tratamientos. Esta extraordinaria tolerancia fue atribuida a altos valores de disipación no-fotoquímica (NPQ, por sus siglas en inglés), un mecanismo para evitar la fotoinhibición. Finalmente, aunque fueron encontradas variaciones en el desempeño fotosintético (los valores de Φ_{PSII} y ETR fueron bajos en verano pero altos en otoño e invierno), *E. platyacanthus* de nuevo mostró 100% de supervivencia tanto en OTCs como en las parcelas control. Además, usando un análisis generalizado de correlación canónica (GCCA) fue encontrada una fuerte correlación (0.71) entre el micro-ambiente (representado por las OTCs y los espacios abiertos) con la ecofisiología de *E. platyacanthus*. Este trabajo es pionero para México así

como para los ecosistemas áridos y semi-áridos americanos en examinar el potencial impacto del calentamiento global en el comportamiento y persistencia de especies de cactus mexicanos amenazados así como dos especies carismáticas de la familia Asparagaceae, particularmente, en los estados iniciales de sus ciclos de vida a corto y largo plazo. Esta investigación proporciona nuevos conocimientos acerca del comportamiento y posibles cambios poblacionales en especies suculentas, las cuales son social y económicamente clave en los desiertos americanos.

Palabras clave: Anatomía funcional, Asparagaceae, Banco de semillas del suelo, Cactaceae, Calentamiento global, Cámaras de techo abierto, Desierto Chihuahuense, Ecofisiología, GCCA, Germinación de semillas, Juveniles, Plántulas, Supervivencia.

Thesis Outline

I addressed the following scope and thesis structure:

Chapter 1 presents a general review about concept, implications and drivers of global environmental change. In addition, possible consequences of these human-induced changes on the structure, function and composition of ecosystems, as well as the actual research framework of global warming within natural systems are showed in this chapter. Finally, projections to imminent global warming from Chihuahuan Desert species, as well as the most useful tools to assess the responses of plants to climate warming on the field studies are presented.

Chapter 2 reports the photosynthetic performance as well ecophysiological mechanisms to avoid photoinhibition in young plants of *Echinocactus platyacanthus* f. *visnaga* Link & Otto, an endangered cacti species from the Chihuahuan Desert under simulated warming conditions.

Chapter 3 shows the results about the seedling survival rate of three protected cacti of Chihuahuan Desert endemic to México, namely: *Echinocactus platyacanthus*, *Ferocactus histrix* (DC) G.E. Lindsay, and *Stenocactus coptonogonus* (Lem.) A. Berger ex A.W. Hill under induced climate warming. Furthermore, based on these findings, the persistence of natural recruitment of the studied species is discussed in this chapter.

Chapter 4 describe both, qualitatively and quantitatively the structural changes in vascular cylinder length, shoot apical meristems diameters, fresh and dry mass, and water loss in seedlings of *Echinocactus platyacanthus*, *Ferocactus histrix* and *Stenocactus* *coptonogonus* under high temperature conditions. Moreover, how these anatomical-ecophysiological shifts can be interpreted as mechanisms to allow an efficient water conduction through the stems of these cacti seedlings to face future climatic scenarios in arid and semi-arid environments.

Chapter 5 presents findings about the effects of some environmental variables such as: daily mean air temperature, daily mean air relative humidity (RH), photosynthetic photon flux density (PPFD), daily mean evapotranspiration (ET), daily rainfall, global solar radiation (GSR) and daily rainfall. It is also described the intensity of relations as well as their importance in the seasonal variations of photosynthetic responses [effective quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR)], and survival (survival rate) to long-term impact of global warming in young individuals of *E. platyacanthus*.

Chapter 6 reports the effect of induced climate warming on the seasonal dynamic of soil seed bank (SSB) in three succulent species from Chihuahuan Desert: *Echinocactus platyacanthus* (Cactaceae), *Yucca filifera* Chabaud (Asparagaceae), and *Agave striata* Zuccarini (Asparagaceae).

Finally, the final remarks and general conclusions of this research are presented in **chapter 7** and **chapter 8,** respectively. The references consulted for this study are listed at the end of each chapter.

Chapter 1

Introduction

Global Environmental Change: concept and implications

Global change is a complex response to several human-induced disturbances (e.g., increased fossil fuel burning, industrial processes, excessive use of removable natural resources, and land use changes) and the natural climatic variability of the planet across time (Vitousek, 1994; Sala et al., 2000; IPCC, 2014), which results in an accelerated increase of CO_2 concentration in the troposphere. Global environmental change implies both, biophysical and socioeconomic changes, which alter the structure and functioning of the Earth Systems (Steffen et al., 2007; Bellard et al., 2012). This includes shifts in a wide range of global-scale phenomena, such as atmospheric composition, riverine flow, nutrients cycle (like nitrogen and carbon), physical climate, marine food chains, biological diversity, population, economy, etc. (Steffen et al., 2007). Thus, global change has become in one of the most important scientific and political challenges of our time (Bulkeley and Newell, 2015).

Drivers of Global Environmental Change

The current global change is driven by the rapidly and explosive growth of human population and the associated increased rates of resources consumption (Vitousek, 1994). The interactions and linkages among these factors are as important as the individual changes themselves within global environmental change (Steffen et al., 2007). Therefore, it is clear that it is only not restricted to changes in climate but rather to the interaction to several drivers, which exert also severe pressure within the ecosystems (Walker and Steffen, 1997; Thomas et al., 2006; Matesanz et al., 2009). These drivers comprises: land-use/land cover change (Foley et al., 2005), atmospheric carbon dioxide (CO_2) concentration increases (Körner, 2003), anthropogenic climate change (Parmesan, 2006), biogeochemical shifts and alterations in anthropogenic nitrogen (Stevens et al., 2004) and sulfur dioxide (Charlson et al., 1992) depositions, increasing biotic exchange/non-native species (Facon et al., 2006), and widespread distribution of synthetic organic compounds (Brown, 1990).

Anthropogenic climate change is the main driver of biodiversity loss at regional and global scales (Bellard et al., 2012). Particularly, the increased concentration of atmospheric CO_2 is responsible for more than half of global warming (Rodhe, 1990). The concentration of CO_2 is projected to rise up to 550 µmol/mol by 2050 (Solomon, 2007), under the emissions scenario A1B of the IPCC (see Carter et al., 2007). This increase could trigger an average increase in the global air temperature by 0.2ºC per decade, reaching 1.8-4.0ºC by 2100 (Solomon et al., 2007). This global warming could bring key changes on the structure, function and composition of ecosystems (Chapin III et al., 1997; Tilman et al., 1997), which threats the global biodiversity because it increases the extinction risk of species with low tolerances to raising temperatures (Malcolm et al., 2006; Hannah, 2012).

Global warming research framework in natural systems

Many studies have assessed the potential impact of climate warming on long-term persistence of species, populations and communities in tropical (e.g., Cleland et al., 2007; Colwell et al., 2008; Costion et al., 2015; Prieto-Torres et al., 2016), temperate (Svenning and Skov, 2006; De Frenne et al., 2010; Camarero et al., 2015), arctic, sub-arctic and alpine (Totland and Nyléhn, 1998; Hollister and Webber, 2000; Aerts et al., 2004; Bokhorst et al., 2013; Gauthier et al., 2015) ecosystems. However, the effect of global warming on desert plants and drylands is yet little know and much information is still being required (Tielbörger and Salguero-Gómez, 2014; Golodets et al., 2015).

Because drylands are expected to be less affected ecosystems by climate change, as compared to other ecosystems (Sala et al., 2000; Maestre et al., 2012), there is the overall expectation this vegetation type will expand (11-23% by 2100; Huang et al., 2015) as a result of climate change (Prentice et al., 1992). For instance, it has been documented that desert vegetation (an important component of drylands) can rapidly adapt to water shortage and high temperatures because they evolved in stressful habitats with highly variable and unpredictable climatic conditions (Vicente-Serrano et al., 2013; Tielbörger and Salguero-Gómez, 2014). Further, most predictions of the effects of global warming on succulent plant species have been conducted just considering adult plants (e.g., Martorell et al., 2015), while studies taking into account other plant stages such as seeds, seedlings or young plants, are still being scarce (Jiménez-Alfaro et al., 2016). These life cycle stages are perhaps more important because they are more sensitive to changing temperatures (Flores et al., 2004; Drennan, 2009), drought stress (Delgado-Sánchez et al., 2013) and anthropogenic disturbances (Godínez-Álvarez et al., 2003) than adult plants. Thus, the impacts of climate warming on seeds, seedlings and young plant may have strong detrimental effect on the recruitment rates (Loik et al., 2004) and population dynamics (Ooi et al., 2009) of these species.

Chihuahuan Desert and global warming forecasts

The most extensive warm-arid ecosystem of North America is the Chihuahuan Desert (Archer and Predick, 2008). It ranges from southwestern United States to the Central

Mexican Highlands. Climate change projections for this region indicate that summer temperatures (June–September) will increase by 1–2ºC by 2030 (Tejeda-Martínez et al., 2008). Nevertheless, the greatest temperature increase is predicted for winter months (January–March), when the monthly average temperature might increase up to 6ºC (Tejeda-Martínez et al., 2008) during this period. This has created strong concerns in conservation biologists because the southernmost section of this desert harbors the highest richness of succulent plants worldwide, especially for globose cacti (Rzedowski, 1991), and most of them are protected by Mexican environmental laws (SEMARNAT, 2010). However, there are little information about the thermal tolerance thresholds in these species, especially in the earlier stages of their life cycles such as seeds, seedlings and young plants.

Devices to simulate global warming on the field

Several temperature manipulation devices have been used to assess the effect of climate warming on plant species (e.g., Harte and Shaw, 1995; Kimball, 2005; Flury and Gessner, 2011). However, open top-chambers (OTCs) are the most common and simplest tool to evaluate the responses of plants to climate warming on the field (Aerts et al., 2004; Bokhorst et al., 2013). OTCs have the advantage of causing passive warming within them, and this no other technological support is required (Hollister and Webber, 2000). The OTC design is not sealed to the ground and allow free air and gas exchange; thus, minimizes undesirable chamber effects, like low light and humidity levels, natural precipitation blocking, as well as access of pollinators and herbivories (Marion, 1996).

OTCs are useful in relatively inaccessible areas, such as tundra, alpine and artic (Hollister and Webber, 2000). Nevertheless, some recent researches have also used these devices in others systems to assess plant responses to future temperature increases. Musil et al. (2005; 2009), found an increase in the mean daily air temperature by 2-5.5°C using OTCs in the succulent karoo biome of semi-arid regions of South African. These air temperatures induced massive mortalities, as well as several metabolic impairments, in succulent plant species on this ecosystem. Nevertheless, we still have a gap in our understanding about potential impact of climate change in the persistence and tolerance mechanisms of succulent species, especially in earlier stages of their life cycles.

Hypothesis

The survival and ecophysiological responses of earlier life-cycle stages of succulent plant species from the Chihuahuan Desert will be adversely affected by induced global warming.

Research aims

Main goal

Determining whether the survival rates and tolerance mechanisms of earlier life-cycle stages (seeds, seedlings and young plants) of succulent plants of the families Cactaceae and Asparagaceae are affected under global warming scenarios.

Specific goals

- To assess the photosynthetic performance -to short and large term- of young plants of *Echinocactus platyacanthus* f. *visnaga* Link & Otto, an endangered cactus species from the Chihuahuan Desert, under simulated warming conditions.

- To evaluate the survival of seedlings of three protected cacti of Chihuahuan Desert that are endemic to México, including *Echinocactus platyacanthus*, *Ferocactus histrix* (DC) G.E. Lindsay, and *Stenocactus coptonogonus* (Lem.) A. Berger ex A.W. Hill, under induced climate warming.
- To describe qualitatively and quantitatively the structural changes (vascular cylinder length and shoot apical meristems diameters), the fresh and dry mass, and the water loss in seedlings of *E. platyacanthus*, *F. histrix* and *S. coptonogonus* under the projected temperature conditions for the next 20 years in the Chihuahuan Desert.
- To assess the variations in the environmental variables, including daily mean air temperature, daily mean air relative humidity (RH), photosynthetic photon flux density (PPFD), daily mean evapotranspiration (ET), daily rainfall, and global solar radiation (GSR), and their impact on the photosynthetic performance and survival on young plants of *E. platyacanthus* under a global warming scenario.
- To evaluate the seasonal dynamics of the soil seed bank (SSB) under simulated air and soil temperature increases in three succulent species from the Chihuahuan Desert, namely: *Echinocactus platyacanthus* (Cactaceae), *Yucca filifera* Chabaud (Asparagaceae), and *Agave striata* Zuccarini (Asparagaceae).

Study area

Because several of protected cacti inhabit the Southern Chihuahuan Desert, our study was addressed in the state of San Luis Potosí, México. The vegetation of the study area is dominated by sclerophyllous shrubs, cacti and succulent monocots scattered among these woody species. Mean annual temperature is 20.8^{o}C, but it can surpass 40^{o}C in summer and fall below -1^{o}C in winter (Medina et al., 2005). Average annual precipitation is 341 mm and rainfall events are concentrated in the summer months (Medina et al., 2005).

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Chapter 2 (Published in Flora, 2014, 209: 499–503)

Induced climate change impairs photosynthetic performance in *Echinocactus platyacanthus*, an especially protected Mexican cactus species

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Abstract

The responses of desert plants to climate warming have been poorly assessed, perhaps due to the overall expectation that desert vegetation will expand as a consequence of this component of climate change. However, determining what plant species will tolerate the expected increases in temperatures is a question that remains unanswered. The Chihuahuan Desert is the largest warm desert of North America, and predictive models of climate change indicate that summer temperatures in this desert will increase by 1-2°C in the next decade. This study experimentally assessed the performance of an endangered cacti species from the Chihuahuan Desert under simulated warming conditions. Hexagonal open top chambers (OTCs) were used to simulate the effects of global warming on five-years-old individuals of the specially protected species *Echinocactus platyacanthus*. Temperature was 1.9^{o}C higher in open top-chambers than in control plots. In contrast, relative humidity was 3.1% higher in control plots than in open top-chambers. *Echinocactus platyacanthus* showed 100% survival for 14 weeks in both OTC and control plots. However, induced warming negatively affected the photosynthetic performance of this species. Cacti located within OTCs displayed lower maximum quantum efficiency of photosystem II (F_v/F_m), effective quantum yield of photosystem II (Φ_{PSII}), and electron transport rate (ETR) values, but higher non-photochemical quenching (NPQ) values, than cacti from control plots. This is the first study focused on the potential impact of climate warming on survival and photosynthetic performance of young individuals of a succulent species from American deserts. Induced warming negatively affected the photosynthetic performance of young *E.*

platyacanthus, but it also increased non-photochemical quenching, a mechanism for avoiding photoinhibition.

Key words: Cactaceae, Global warming, Chlorophyll fluorescence, Stress tolerance.

Highlights

- Global warming is expected to affect physiological performance also of desert plant
- We therefore assessed the effects of induced warming in a protected cactus species
- We found 100% survival in young plants with induced warming
- Increased temperature affected the photosynthetic performance of young cacti
- Induced warming caused high non-photochemical quenching, to avoid photoinhibition

Introduction

Changes to the Earth's climate system during the last century have arisen from the accumulation of greenhouse gases in the atmosphere (IPCC, 2013: Watson et al., 1990). Together with other disturbances that humans have induced on natural ecosystems, including extensive deforestation and changes in albedo of ice or snow covered surface, such an accumulation of greenhouse gases is strongly influencing global temperatures (Le Treut et al., 2007). This human-induced climate change increases the extinction risk of plant species with low tolerances to raising temperatures (Malcolm et al., 2006). Nevertheless, the responses of plants to climate warming have been mainly assessed in tropical (e.g., Colwell et al., 2008) and temperate forests (De Frenne et al., 2010) or in artic and alpine ecosystems (Bokhorst et al., 2013), while little is yet known about the effects of warming on desert plants. This situation is perhaps due to the overall expectation that desert vegetation will expand as consequence of climate change (Prentice et al., 1992), but determining what plant species will tolerate the expected increases in temperatures is a question that remains unanswered.

The Chihuahuan Desert is the largest warm desert of North America, ranging from southwestern United States to the Central Mexican Highlands (Archer and Predick, 2008). Predictive models of climate change indicate that summer temperatures (June-September) in this desert will increase by 1-2°C in the 2020 decade (Magaña et al., 2004). However, the major temperature changes by 2030 are predicted to occur during winter months (January-March), when monthly average temperatures might increase up to 6°C (Magaña et al., 2004). These predicted climate changes have triggered strong concerns in conservation biologists because the Chihuahuan Desert harbors an elevated richness of succulent plants (Rzedowski, 1991). Nevertheless, there is little information about how these plants will respond when facing increased temperatures, especially in their regeneration phase (Pérez-Sánchez et al., 2011). The expected increase in temperatures might surpass the thermal tolerance thresholds of native species and this could affect their recruitment and survival. Therefore, climate change could lead to local species extinctions across the Chihuahuan Desert during the next two decades. Thus, the work hypothesis of this study was that increased temperature stress due to induced climate change would negatively impact on the specially protected *Echinocactus platyacanthus* Link & Otto (Cactaceae), causing lower photosynthetic performances than those observed in conspecific individuals subjected to current climate conditions. This is the first study focused on the potential impact of simulated climatic warming (using open-top-chambers) on survival and photosynthetic performance of young individuals of a succulent species, common in arid and semiarid areas of México.

Materials and methods

Study species

Echinocactus platyacanthus, is a barrel-like cactus that can reach 2 m tall and 80 cm in diameter (Jiménez-Sierra et al., 2007). This cactus is endemic to México, but overexploitation for ornamental proposals has heavily endangered the natural populations of this species (Jiménez-Sierra et al., 2007). For this reason, *E. platyacanthus* has been included as protected species in the framework of the environmental laws and regulations of México (SEMARNAT, 2010). According to Jiménez- Sierra et al. (2007), mean annual mortality in natural populations is highest for seedlings (19.7%) and lowest for adults (2.5%).

Study area

This study was conducted in the southernmost section of the Chihuahuan Desert, within the state of San Luis Potosí (México). The experimental site was located at an abandoned agricultural field (22° 14’ 11’’ N, 100° 51’ 46’’ W, 1844 m.a.s.l.) where current vegetation is composed by sclerophyllous shrubs, cacti and other succulent plants. Mean annual temperature at the study site is 17.8°C, but it can surpass 35°C in summer and fall down below -1°C in winter (Medina et al., 2005). Average annual precipitation is 341 mm and rainfall events are concentrated in summer months, June-September (Medina et al., 2005).

Seed collection

The effects of climate warming were assessed on young individuals of *E. platyacanthus* that were developed from seeds in the greenhouses of Instituto Potosino de Investigación Científica y Tecnológica (San Luis Potosí, México). For this, mature fruits were harvested in the field between summer and autumn 2007. Fruits were taken to the laboratory and cleaned to recover the seeds, which were germinated on peat moss trays within growth chambers (25°C, 80% relative humidity, and photoperiod 12 h light/dark). Seedlings were later moved to the greenhouse and transplanted into individual plastic pots (one seedling per pot). These posts had a capacitance of 2 L and were filled with a mixture of gravel (10%), sand (30%) and clay (60%). The plants were grown for five years in the greenhouse prior to be used in the experiment described below. Environmental conditions in the greenhouse were 33ºC, 80–90% of total daily photosynthetic photon flux density (PPFD, 1300 µmol m^{-2} s^{-1}), and 60% relative humidity. The size of the plants after five years was 4 cm in height and 6 cm in diameter.

Open top-chambers design

Several manipulative systems have been proposed to simulate the influence of climate change on plants (e.g., Bokhorst et al., 2013). Nevertheless, open-top chambers (OTCs) are still being the most common and simplest approach for assessing the responses of plants to climate warming in the field (De Frenne et al., 2010). In this study, hexagonal OTCs were used to simulate the effects of global warming on *E. platyacanthus*. These structures were built with UV-resistant transparent acrylic (3 mm thick; wavelength transmission < 280 nm) by following the design proposed by Marion (1996). The resulting OTCs were 50 cm tall, 150 cm width in the open-top, and 208 cm width in the base attached to ground. This OTC design allows daytime passive heating by increasing air temperature by 2-5°C, as compared to the external environment (Musil et al., 2005; 2009).

Ambient and open top-chambers microenvironments

Because the larger increases in temperatures for the Chihuahuan Desert are expected in winter months, coinciding with the dry seasons, the experimental assessment of the responses of *E. platyacanthus* to induced warming was started in January 2013. The experiment was conducted within a 25 x 25 m exclosure previously established at the study site. This exclosure was fenced with woven wire (2 m height) to avoid the access of cattle and people to the experiment. On January 7th 2013, twelve plots (5 x 5 m = 25 m^2 each) were drawn within the exclosure by following a rectangular arrangement (3 plots width x 4 plots long). Six plots were randomly selected within the exclosure and an OTC was established at the center of each of these plots. The other six plots were maintained as controls. Temperature and relative humidity were continuously recorded within and outside OTCs to determine whether OTCs effectively modify microclimate. For this, we used microclimatic data-loggers (HOBO Pro v2, Onset Computer Corporation, MA, USA) programmed to record temperature and relative humidity every 1 h. A data-logger was located 20 cm above the ground at the center of each experimental plot, at 3–5 cm above plants, resulting in six data-loggers in plots containing open top chambers (OTCs) and six data-loggers in plots without OTCs. The incident photosynthetic photon flux density (PPFD) 20 mm above the saplings was measured at mid-day with a quantum sensor (LI250-A, Li-Cor, Inc., Lincoln, Nebraska, USA).

Chlorophyll fluorescence measures

One week after mounting OTCs in the field (January 14th 2013), all experimental plots received three pots with young individuals of *E. platyacanthus* (one individual per pot). The pots were placed on the soil. In those plots subjected to warming conditions, plants were placed at the center of OTCs directly below the open-top, to avoid overwarming due to proximity to the acrylic walls of these structures. All these cacti were watered every week to field capacity until the beginning of the experiment. Field capacity was determined in pots containing overwatered mixture and allowed to drain overnight. To reach field capacity, 200 mL water per pot was required. Nevertheless, the plants did not receive further watering during the experiment.

Three months after beginning the experiment (April 23rd 2013), we counted the number of *E. platyacanthus* individuals that survived within each plot. After that, two rounds of chlorophyll fluorescence measures were taken on all living cacti by using a portable pulse amplitude modulation fluorometer (Mini-PAM; H. Walz, Effeltrich, Germany). These data were used to estimate a series of variables related to the photosynthetic performance of plants located within and outside OTCs. The first round of chlorophyll fluorescence measures was conducted on dark-adapted cacti at the predawn (between 05:00 and 06:00 h) in order to assess the maximum quantum efficiency of photosystem II. This variable was estimated as $F_v/F_m = (F_m - F_0)/F_m$, where F_v = variable fluorescence determined in darkness, F_m = maximal level of fluorescence measured in darkness, and F_0 = minimal level of fluorescence measured in darkness (Maxwell and Johnson, 2000). The values for this ratio oscillate between 0.80 and 0.83 if environmental stress is negligible for plants, but these values decrease with increasing environmental stress (Maxwell and Johnson, 2000). In our case, lower F_v/F_m values were expected for *E. platyacanthus* individuals located within OTCs.

The second round of chlorophyll fluorescence measures was conducted at the noon (between 13:00 and 14:00 h), when plants faced the maximum daily temperature. These data were used to estimate the effective quantum yield of photosystem II (Φ_{PSII}). This variable was computed as $\Phi_{PSII} = (F'_m - F_t)/F'_m$, where F_t is the chlorophyll fluorescence emitted by plants under steady-state illumination (i.e., light conditions in the field) and F'_m is the maximum fluorescence emitted by chlorophyll when a saturating pulse of actinic light is superimposed to environmental levels of light (Genty et al., 1989). Similarly to the ratio F_v/F_m, the values of Φ_{PSII} should decrease as thermal stress increases and, thus, lower Φ_{PSII} were expected for cacti located within OTCs.

Because the fluorometer we used also measures the photon flux density (PPFD) in the environment surrounding plants, we also calculated the electron transport rate (ETR) across the electron chain of chloroplasts. This variable was then estimated as ETR = Φ_{PSII} x PPFD x 0.84 x 0.5, where PPFD is the photosynthetic photon flux density recorded by the sensor in the leaf clip, 0.84 is the estimated mean proportion of incident light absorbed by the photosystems (Ehleringer, 1981) and 0.5 is the required factor for both photosystems to account for absorb photons (Roberts et al., 1996).

Because ETR is directly and positively related to the generation of chemical energy (ATP and NADPH/H^+) that will be later used in the Calvin cycle, lower values of this variable are indicative of reduced photosynthetic performance in plants (Ritchie and Bunthawin, 2010). Therefore, if induced warming negatively affects the performance of *E. platyacanthus*, cacti located within OTCs should display lower ETR values than cacti from control plots.

Finally, because chlorophyll fluorescence was measured at both predawn and noon, we also calculated the non-photochemical quenching efficiency (NPQ) of cacti. This variable was calculated as NPQ = $(F_o - F'_m)/F'_m$, where F_o is the basal chlorophyll fluorescence emitted by cacti at the predawn (dark-adapted plants), and F'_m is the maximum fluorescence emitted by chlorophyll after imposing a saturating pulse of actinic light at noon. NPQ specifically refers to the mechanism used by plants to dissipate the excess of light energy captured by chlorophylls as heat. This mechanism of energy dissipation is linked to the xanthophyll cycle, and higher NPQ values are expected with increasing levels of environmental stress (Maxwell and Johnson, 2000). Therefore, if thermal stress induced by warming negatively affects *E. platyacanthus*, cacti located within OTCs should display higher NPQ values than cacti in control plots.

Statistical analyses

Biotic and abiotic measures were based on six replicates (from six OTC and six controls). Each replicate had three plants, and the value for a replicate was based on their average. The biotic (F_v/F_m, Φ_{PSII}, NPQ, and ETR) and abiotic variables (air temperature and relative humidity and PPFD) were analyzed through one-way ANOVA. Most data fulfilled the requirements of variance homogeneity and homoscedasticity, except abiotic variables, thus these data were analyzed through non-parametric ANOVA (Kruskal-Wallis One Way ANOVA on Ranks).

Results

Mean air temperature (Figure 1a) and relative humidity (Figure 1b) significantly differed ($P < 0.001$) between open top-chambers and control plots. Temperature was on average 1.9°C higher in open top-chambers than in control plots. In contrast, relative humidity in control plots was 3.1% higher than in open top-chambers. Maximum and minimum mean temperatures were 41.7°C and 4.8°C in the OTCs, as well as 34.2°C and 4.7°C in the control plots. The light intensity received at mid-day by plants inside OTCs (1922.1 ± 47.5 μmol m^{-2} s^{-1}) was similar to that received by plants in the control plots (1941.9 ± 9.4 μmol m^{-2} s^{-1}), without significant differences between treatments ($P = 0.825$).

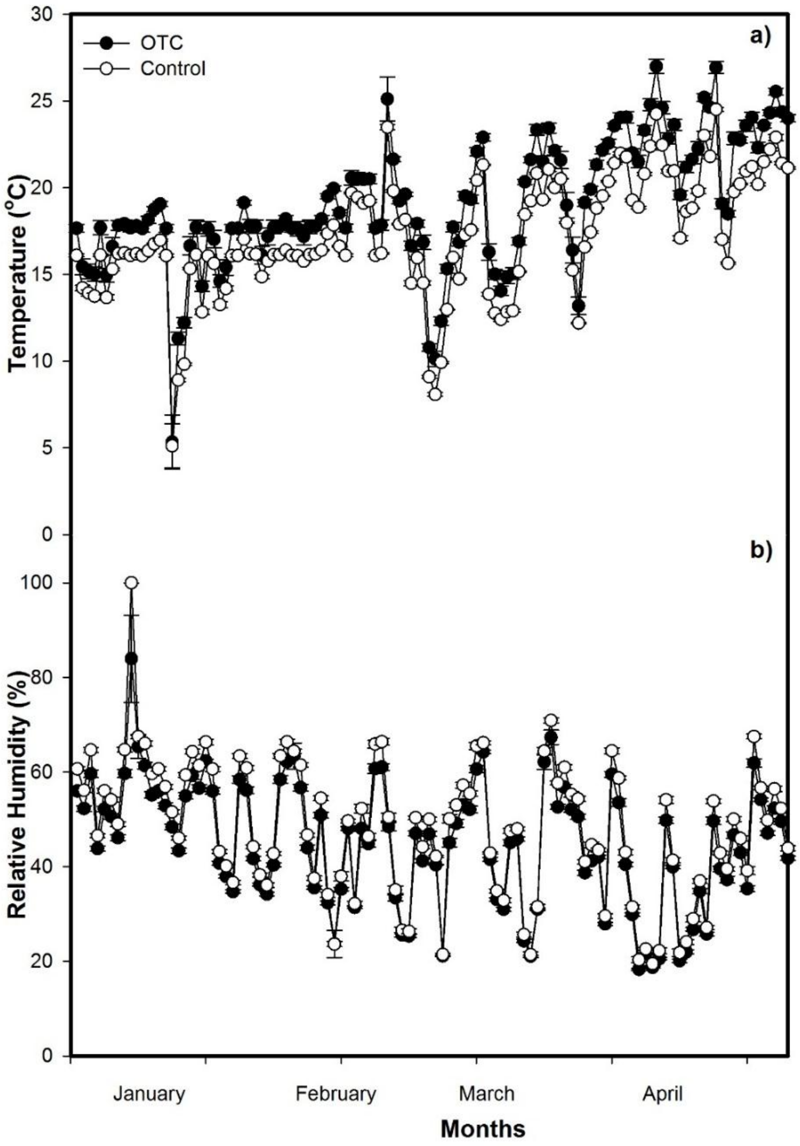

Figure 1: Daily changes (±SE) in mean air temperature (a) and mean relative humidity (b) in open top-chambers (black circles) and control plots (white circles), over 120 days. Significant differences between treatments were found ($P < 0.05$).

Echinocactus platyacanthus showed 100% survival in both OTC and control plots. However, induced warming negatively affected the photosynthetic performance of this species. The F_v/F_m values were significantly higher in the control plots than in the open top-chambers ($F_{1,10} = 22.427$, $P < 0.001$; Table 1). The quantum yield of photosystem II (Φ_{PSII}) was higher in the control than in the OTC plots ($F_{1,10} = 23.001$, $P < 0.001$; Table 1). ETR values were also higher in the control than in the OTC plots ($F_{1,10} = 18.539$, $P < 0.005$; Table 1), and NPQ values were higher in the OTC than in the control plots ($F_{1,10} = 20.150$, $P < 0.005$; Table 1).

Table 1. Effect of induced warming on photosynthetic performance of young plants of *Echinocactus platyacanthus*.

Variable	**OTC Mean (± S.E.)**	**Control Mean (± S.E.)**
F_v/F_m	0.630 ± 0.025^{a}	0.760 ± 0.01^{a}
Φ_{PSII}	0.199 ± 0.02^{b}	0.327 ± 0.02^{a}
ETR μmol m^{-2} s^{-1}	142.0 ± 18.3^{b}	266.0 ± 16.1^{a}
NPQ	0.755 ± 0.08^{a}	0.484 ± 0.06^{b}

Different letters indicate significant differences between treatments ($P < 0.05$).

Discussion

The recorded 1.9°C increase in mean daily air temperature in the OTCs over the treatment period fitted to 1-2°C increase predicted for the Chihuahuan Desert under summer in the next decade, although did not fit for winter months for which predictive models suggest increase up to 6°C (Magaña et al., 2004). OTC plots had lower relative humidity than control plots, which was also expected because the warming inside and because the semiarid climate. Low relative humidity diminishes the atmospheric water vapor and therefore the water condensation in the soil; affecting the water uptake by the superficial roots of some succulents (Von Willert et al., 1992; Martorell and Ezcurra, 2002; Matimati et al., 2012). Thus, increased temperature and decreased relative humidity inside OTCs were expected to affect the *E. platyacanthus* performance. We hypothesize that the lower Φ_{PSII} values found in OTC plots than in controls were a consequence of the lower relative humidity inside OTCs which could have reduced water uptake.

OTC effects on spectral composition of global radiation were ignored, and radiation from the chamber walls was partly neglected (Jetten, 1992; Nussbaum and Fuhrer, 2000). Thus, the effects of the acrylic walls on spectral composition and its possible effects on chlorophyll concentration and chlorophyll fluorescence measurements remain to be tested.

The Φ_{PSII} values were lower than the F_v/F_m ratios in both OTC and control plots. This decrease in the values of the ratio between variable and maximum fluorescence during the day is related to increase in the emission of fluorescence when chlorophylls are exposed to more light that can be handled by the electron transport chain in the thylakoid membrane (Duan et al., 2005). However, cacti inside OTCs always displayed lower Φ_{PSII} and ETR values than those in control plots. This suggests that plants under induced warming were exposed to intense physical stress, as compared to plants in control plots. Our findings are in agreement with Musil et al. (2009), who found a decline in both photochemical efficiency and ETR for the succulent *Cephalopyllum spissum*, a South African succulent species, following short (2 h) exposures to heat of increasing intensity in the temperature range 42-54^{o}C.

In addition, because ETR is positively related to the ability of plants to assimilate CO_2 (Kitao et al., 2003; Kakani et al., 2008), these results suggest diminished photosynthetic performance of cacti under induced warming. In a contrary way, induced warming caused high NPQ values, which indicates that cacti within OTCs required dissipation of quantities of light energy in excess of those required for photochemistry or of those re-emitted to the environment as fluorescence (Müller et al., 2001; Krause and Jahns, 2004).

We found 100% survival for *E. platyacanthus* in both OTC and control plots, which is contrary to findings for southern African quartz-field succulents (Musil et al., 2005), which after 4-months summer treatment, displayed between 2.1 and 4.9 times greater plant and canopy mortalities in the open top chambers than in the control plots. It is possible that high survivorship for *E. platyacanthus* plants is related with high non-photochemical quenching values, because high NPQ is a mechanism for avoiding photoinhibition (Barker et al., 2002). Non-photochemical quenching was not investigated for African quartz-field succulents, but we hypothesized that it was low.

Our hypotheses that cacti located within OTCs displayed lower F_v/F_m, Φ_{PSII}, and ETR, as well as higher NPQ values, than cacti from control plots were corroborated. Nobel (2010), suggests that desert succulents have high tolerance to water and temperature stress; however, predictions of global changes studies propose that succulents will decrease in species range due to increased temperature and diminished precipitation (Butler et al., 2012; Dávila et al., 2013). Ureta et al. (2012), studied two threatened *Mammillaria* species (Cactaceae) with contrasting distribution ranges under climate change scenarios, and suggested that the most widespread species would be less affected by climate change, proposing that past selection on plasticity allows it to survive under variable conditions. Interestingly, *E. platyacanthus* is one of the most widespread Mexican cactus species (Jiménez-Sierra et al., 2007) and young plants of this species showed 100% survivorship under simulated warming. In addition, it is possible that adult individuals of *E.*

platyacanthus are less sensitive to temperature increases than young plants, because adult *E. platyacanthus* preferentially tilt towards the south, which reduces temperatures on the apical meristems during the hot season (Herce et al., 2014).

Most predictions of global changes have been suggested for adult plants, but there are no studies taking into account other plant development phases, such as: seeds, seedlings or young plants, which are more susceptible to extreme temperatures (Drennan, 2009) or drought (Delgado-Sánchez et al., 2013). The seasonal variation of *E. platyacanthus* performance inside OTCs to evaluate the hypothesis on 6°C increase in the next 20 years remains to be studied. Nevertheless, after induced warming by 1.9ºC for four months, we found 100% survival of young *E. platyacanthus*. However, this simulated global warming reduced photosynthetic efficiency and also resulted in high non-photochemical quenching to avoid photoinhibition. The long-term consequences of these physiological responses for plant fitness have still to be investigated.

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Chapter 3 (Accepted in Plant Species Biology, 2016)

Seedling survival of three endemic and threatened Mexican cacti under induced climate change

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Abstract

Human-induced warming may increase the risk of local extinction for plant species with low tolerance of elevated temperatures. The Chihuahuan Desert harbors the highest diversity of globose cacti in the world and most of them are at risk of extinction. Predictive models of climate change indicate an increase in summer temperature of 1-2^{o}C by 2030 for this desert. Nevertheless, studies on the vulnerability of cacti species in early development phases to future climate change are scarce. We assessed the survival of three threatened cacti species from the Chihuahuan Desert under induced warming. Open top-chambers (OTCs) were used to simulate the effect of global warming on 2 year seedlings of *Echinocactus platyacanthus* f. *visnaga*, *Ferocactus histrix* and *Stenocactus coptonogonus*. OTCs had higher temperature and lower humidity than control plots, and these elevated temperatures reduced seedling survival. Within the OTCs, no living individuals of any species were found after 105 days. Conversely, in the control plots, the three cacti species showed variable numbers of survivors after this period. Therefore, the predicted global warming scenarios will greatly limit plant recruitment and the long-term persistence of natural populations of Mexican endemic cacti species.

Key words: Cactaceae, Chihuahuan Desert, Global warming, Seedling survival

Introduction

Long-term reconstructions of climate over the past millennium indicate that the average temperature of the Earth increased by almost 1°C during the 20th century, and this has been mainly the result of enhanced emission of greenhouse gases that occurred after 1950 due to human activities (Le Treut et al., 2007; IPCC, 2014). Climate models developed by the Intergovernmental Panel on Climate Change indicate that temperature will continue increasing during this century, reaching 3-5°C above the mean temperature of the 1990 decade by 2090 (IPCC, 2014). This warming may seriously impair vegetation processes (Hughes, 2000; McLaughlin et al., 2002; Sih et al., 2011; Hänel and Tielbörger, 2015), increasing the risk of local extinction for those plant species with low tolerance of elevated temperatures (Malcolm et al., 2006; Martorell et al., 2015).

Arid ecosystems are at the forefront of climate change, but much information about the potential effects of warming on desert plants is still required (Tielbörger and Salguero-Gómez, 2014; Golodets et al., 2015). There is the overall expectation that desert vegetation will expand as a result of climate change. This relies on the assumption that desert plants can rapidly adapt to water shortage and high temperatures because they evolved in stressful habitats with highly variable climatic conditions (Vicente-Serrano et al., 2013; Tielbörger and Salguero-Gómez, 2014). Nevertheless, some studies have already indicated the negative effects of warming on the physiological performance of desert plants (Aragón-Gastélum et al., 2014; Tielbörger and Salguero-Gómez, 2014; Martorell et al., 2015), which may preclude the persistence of their populations in future climate scenarios.

The Chihuahuan Desert is the most extensive warm-arid ecosystem of North America (Archer and Predick, 2008) and the southernmost section of this desert, located in central México, harbors the highest diversity of globose cacti in the world (Rzedowski, 1991). Because these cacti have slow recruitment dynamics and growth, as well as narrow distributions, and require highly specialized habitats (Godínez-Álvarez et al., 2003; Martorell et al., 2015), most of them are protected by Mexican environmental laws (SEMARNAT, 2010). The growing season for plants in the southern Chihuahuan Desert occurs in summer (June-September), coinciding with the rainfall period (Medina et al., 2005). Although rainfall regimes are not expected to change in semi-arid México, different climatic models predict that the average temperature will increase by 1–2°C by end of 2030, as compared with current temperatures(Tejeda-Martínez et al., 2008). Despite this expected increase in temperatures, the thermal tolerance thresholds of native cacti of central México have been seldom evaluated (Aragón-Gastélum et al., 2014) and there is no information on how climate warming may affect the earlier stages of the life cycles of these species.

Seedlings of cacti species are often more sensitive to abiotic (Flores et al., 2004) and anthropogenic (Godínez-Álvarez et al., 2003) stressful environmental conditions than adult plants. Thus, the response of seedlings to elevated temperatures is crucial for their establishment success (Nobel, 1984; Loik et al., 2004). This study assesses the potential consequences of climate warming for the survival of seedlings of three protected cacti of Chihuahuan Desert that are endemic to México, namely *Echinocactus platyacanthus* f. *visnaga* Link & Otto, *Ferocactus histrix* (DC) G.E. Lindsay, and *Stenocactus coptonogonus* (Lem.) A. Berger ex A.W. Hill.

México has the highest richness (660 species) and endemisms (518 species, 78%) of cacti species (Ortega-Baes and Godínez-Álvarez, 2006); furthermore, Mexican cacti have a high social, economic and cultural value (Anderson, 2001). Thus, conservation efforts should focus on these species in order to preserve their diversity (Ortega-Baes and Godínez-Álvarez, 2006). Given the protection status of the three studied species, understanding the implications of loss of biodiversity of these cacti is crucial for developing effective conservation, propagation and management strategies for these species, which is becoming increasingly urgent under a climate change scenario (Goodman et al., 2012).

We hypothesized that warming will negatively affect the seedling survival of the three cacti species. Seedlings are expected to be negatively affected by elevated temperatures, as they are highly sensitive to changes in soil water deficits and the low relative humidity conditions caused by increased temperatures (Flores et al., 2004). To test this hypothesis, we conducted a warming experiment in the field, where seedlings of these species were subjected to the temperature conditions expected for summer months in the next decade.

Materials and methods

Study species and plant material

Echinocactus platyacanthus and *Ferocactus histrix* are barrel-like cactus that can surpass 1 m height and 80 cm in diameter (Bravo-Hollis and Sánchez-Mejorada, 1991). *Stenocactus coptonogonus*, on the other hand, is a small globose cactus that can reach up to 10 cm height and 11 cm in diameter (Arredondo-Gómez and Sotomayor, 2009). These cacti are endemic to México, but overexploitation and looting for food and ornamental purposes have heavily endangered their natural populations (Jiménez-Sierra et al., 2007; Arredondo-Gómez and Sotomayor, 2009), although there is no information about natural population sizes in the study area. According to Jiménez-Sierra et al. (2007), the mean annual mortality of natural populations of *E. platyacanthus* is highest for seedlings (19.7%) and lowest for adults (2.5%). There are no available data on these parameters for the other two species. For this reason, the environmental regulations of México consider them as specially protected species (SEMARNAT, 2010).

Additionally, *E. platyacanthus* and *F. histrix* are included as near threatened species in the Red List of the International Union for Conservation of Nature (IUCN, 2014). *E. platyacanthus* is one of the most widespread Mexican cacti (Jiménez-Sierra et al., 2007); its range extends over 18°-25° N latitude and 97°-107° W longitude (Trujillo-Argueta, 1984). The other two species studied are more narrowly distributed (Guzmán et al., 2003). The range of *F. histrix* lies between 20°-24° N latitude and 98°-104° W longitude (del Castillo, 1983), and for *S. coptonogonus*, the range lies between 21°-24° N latitude and 100°-102° W longitude (Salas de León et al., 1999). *Ferocactus histrix* fruits mature during the spring and early summer (del Castillo, 1983). *Echinocactus platyacanthus* fruits are present on the plant all year long, with a peak in August (Trujillo-Argueta, 1984). The fruits of both species remain on the plants when ripe, until they are eaten by animals (Trujillo-Argueta, 1984; del Castillo, 1983). Seed mass is 2.62 mg for *E. platyacanthus* and 0.296 mg for *F. histrix* (Flores et al., 2011). There is no information about dispersal time and agents or seed mass for *S. coptonogonus*.

For this study, seedlings of these three cacti were developed from seeds collected in the surroundings of the city San Luis Potosí (central México) during summer 2011. Seeds of three species were collected from at least 10 mother plants each.

Seeds were sown on peat moss using trays (19.7 cm x 15.5 cm x 4.5 cm) and placed in growth chambers (25°C, 80% relative humidity, photoperiod 12 h dark/light). Emerged seedlings were later transplanted into plastic pots (5 cm diameter x 10 cm deep) filled with a mixture of gravel (10%), sand (40%) and clay (50%) and grown in the glasshouse (temperature, 25-30°C; relative humidity, 40%) during 2 years. Within each pot we transplanted four seedlings of the same species; we decided to plant four seedlings instead of one because in cacti seedlings, soil water evaporation is significantly reduced when the population density increases (Bacilio et al., 2011). All pots were watered every week to their field capacity. Field capacity was determined by overwatering the pots and allowing them to drain overnight, which indicated that 20 mL of water were required for watering the pots. After 2 years in the glasshouse, the sizes of the three species fluctuated between 1.46-2.16 cm height and 1.52-1.92 cm diameter. The very small size of the seedlings before 2 years was the reason we used 2-year-old seedlings. We consider that our plants were seedlings because they did not show differentiated ribs (Loza–Cornejo and Terrazas, 2011).

Experiment design

These 2-year-old individuals were subjected to an induced warming treatment in the field to assess how climate change could affect their survival. This experiment was conducted in an abandoned agricultural field located in the southernmost section of the Chihuahuan Desert (22°14’11’’ N, 100°51’46’’ W, 1844 m), central, México. Annual precipitation in the study site averages 341 mm and rainfall events are concentrated in the summer months. Mean annual temperature is 20.8°C and mean temperature in the summer is 23.0°C but it can surpass 40.0°C and be as low as 5.5°C during this time (Medina et al., 2005). Vegetation in the study site is dominated by sclerophyllous shrubs, with some cacti and succulent monocots scattered among these woody species. In this study site, vegetation was cleared on a surface area of 25 x 25 m that was fenced with woven wire (2 m height) to avoid the access of cattle and people. Within this exclosure, 12 experimental plots of 5 x 5 m were drawn by following a rectangular arrangement (three plots width x four plots long). Six of these plots were randomly assigned to the induced warming treatment, while the other six plots were used as controls under the current environmental conditions.

Hexagonal open-top chambers (OTCs) were used to simulate warming conditions. We used these structures because they allow passive heating and are the simplest approach for assessing the responses of plants to climate warming in the field (Musil et al., 2005; 2009; De Frenne et al., 2010; Bokhorst et al., 2013; Aragón-Gastélum et al., 2014). OTCs were built with UV-resistant transparent acrylic (3 mm thick; wavelength transmission 110 < 280 nm) by following the design proposed by Marion (1996). The resulting structures were 0.50 m tall, 1.5 m wide at the open-top and 2.08 m wide at the base attached to the ground. This OTC design allows daytime passive heating by increasing the air temperature by 1.9-5°C with respect to the external environment in a dry-lands ecosystem (Musil et al., 2005; 2009; Aragón-Gastélum et al., 2014).

To assess the magnitude by which OTCs modified microclimate, temperature and relative humidity were continuously monitored within and outside these structures throughout the entire experiment. For this, data-loggers (HOBO Pro v2, Onset Computer Corporation, Bourne, MA, USA) were programmed to record temperature and relative humidity every hour and were installed 10 cm above the ground at the center of six randomly selected OTCs and six control plots. These measures were conducted from 1 June to September 27th 2013, during the rainfall period, which corresponds to the growing season, and data recorded by data-loggers were used to calculate the daily average temperature, minimum and maximum temperatures, and the average daily relative humidity of the air both within and outside OTCs.

The pots containing 2-year old cacti were arranged within this experimental design a week after mounting the OTCs in the field. For each native species, five pots containing four seedlings each were placed at the center of each experimental plot, resulting in 20 seedlings per species per plot, giving 120 seedlings per species per treatment. Watering was not applied to the seedlings during the experiment.

In plots with OTCs, the pots were located directly below the open-top to avoid overwarming and reduce rainfall interception by the acrylic walls. Survival of all these individuals was monitored every seven days until 27 September 2013, when all plants within the OTCs were recorded as dead.

Statistical analysis

The climatic variables mentioned above were summarized for each data-logger (*n* = six per treatment) and compared between OTCs and control plots with repeated measures ANOVAS. To assess differences in survival rates of cacti between these two treatments, we used failure-time-analyses. In these analysis, the individuals have a value of 1 (one) at the beginning of the experiment because all of them are alive. Their death across time are the “failure events” that change these values to 0 (zero). Thus, each individual is considered as a replicate in these analyses (*n* = 120 per species at each treatment), where the failure time is the date at which the death of each individual was recorded (Aalen et al., 2008). With these data we computed the survival rates of each species across time with the Kaplan-Meier method (Kaplan and Meier, 1958) and these estimations were used to construct survival curves for the two treatments (warming vs. control). Cox-Mantel's two-sample test (Lee et al., 1975) was used to compare the survival rates of each species between these treatments. All analyses described in this section were conducted in R 3.0 (R Core Team, 2013).

Results

Climatic variables

The mean daily temperature during this study was 21.5 °C (± 0.3, standard error) in the control plots and 23.4°C (± 0.4°C) within the OTCs. This variable (Figure 1a) significantly differed between these two treatments ($F_{(1,6)} = 89.35$, $P < 0.0001$) and, although these values decreases from the beginning to the end of the experiment ($F_{(118,708)} = 619.76$, $P < 0.0001$), OTCs always maintained higher average temperatures than control plots ($F_{(118,708)} = 18.02$, $P < 0.0001$).

During the study period, mean maximum temperatures averaged 33.5°C (± 0.7) in control plots and 39.3°C (± 1.0) within OTCs, whereas minimum temperatures averaged 12.5°C (± 0.4) and 12.8 °C (± 0.4) in control plots and OTCs, respectively. Maximum temperature (Figure 1a) significantly differed between treatments ($F_{(1,6)} = 60.3$, $P = 0.0002$) and, although the values of this variable decreased with time ($F_{(118,708)} = 223.87$, $P < 0.0001$), maximum temperatures within OTCs were higher throughout the entire experimental period ($F_{(118,708)} = 223.87$, $P < 0.0001$). Minimum temperatures (Figure 1a) did not differ between control plots and OTCs ($F_{(1,6)} = 2.83$, $P = 0.143$), but their values increased with time ($F_{(118,708)} = 1050.4681$, $P < 0.0001$). No interactive effect was found between treatment and time for minimum temperatures ($F_{(118,708)} = 1.05$, $P = 0.35$).

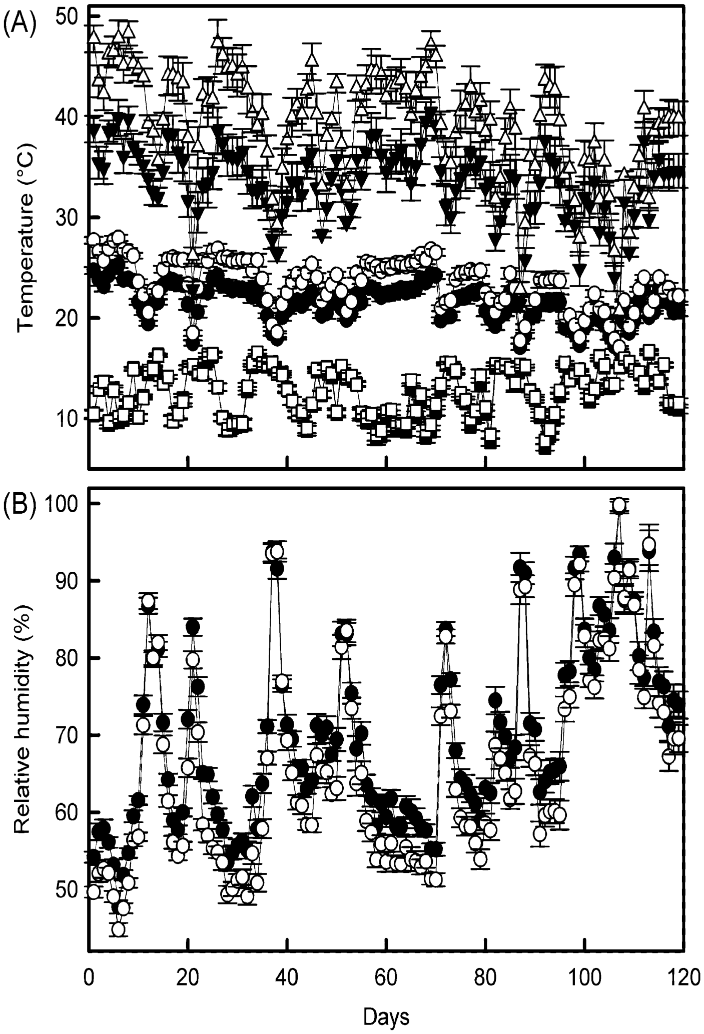

Figure 1. Climatic variables measured in control plots (solid symbols) and within open-top chambers (OTCs, empty symbols) used in the field experiment. The upper panel (a) shows the average values (± standard error) of daily temperature (circles), maximum temperatures (triangles) and minimum temperatures (squares). The lower panel (b) shows the average values (± standard error) of relative humidity.

Total rainfall during the study period was 126.4 mm in the study area. Average daily relative humidity of the air in the control plots was 69.6% (± 2.1) during the study period, while this variable averaged 65.9% (± 2.4) within the OTCs. Significant differences in relative humidity (Figure 1b) were found between OTCs and control plots ($F_{(1,6)}$ = 84988.75, $P < 0.0001$) and these values also differed across time ($F_{(118,708)}$ = 1895.32, $P < 0.0001$). Nevertheless, the interaction between treatments and time ($F_{(118,708)}$ = 12.04, $P < 0.0001$) indicated that OTCs displayed lower values of relative humidity than control plots only on those days where maximum temperatures surpassed 40°C within OTCs (Figure 1b).

Survival rates of cacti

Survival rates of the three cacti were significantly lower within OTCs than in control plots (Cox-Mantel test $P < 0.0001$ in all cases). Within the OTCs, no living individuals of any species were found after 105 days (7 June to 27 September 2013). Conversely, in the control plots, the three cacti species showed variable numbers of survivors after this period.

Within the OTCs, survival rates *E. platyacanthus* markedly decreased during the first 20 day of the experiment, and all plants died after 65 days (Figure 2a). In the control plots, this cactus showed a slight decrease in its survival throughout the entire experiment, but more than 80% of the individuals were alive after 105 days (Figure 2a).

Survival rates of *F. histrix* monotonically decreased within the OTCs until day 55 of the experiment, when all individuals were dead (Figure 2b). Survival of this cactus species also decreased in the control plots until day 65 but, after this day, survival stabilized and more than 15% of the individuals reached 105 days in the experiment (Figure 2b).

In the case of *S. coptonogonus*, survival rates decreased throughout the entire experiment in both, OTCs and control plots (Figure 2c). Nevertheless, although all individuals of this species had died on day 105 of the experiment, more than 22% of individuals located in the control plots were still alive (Figure 2c).

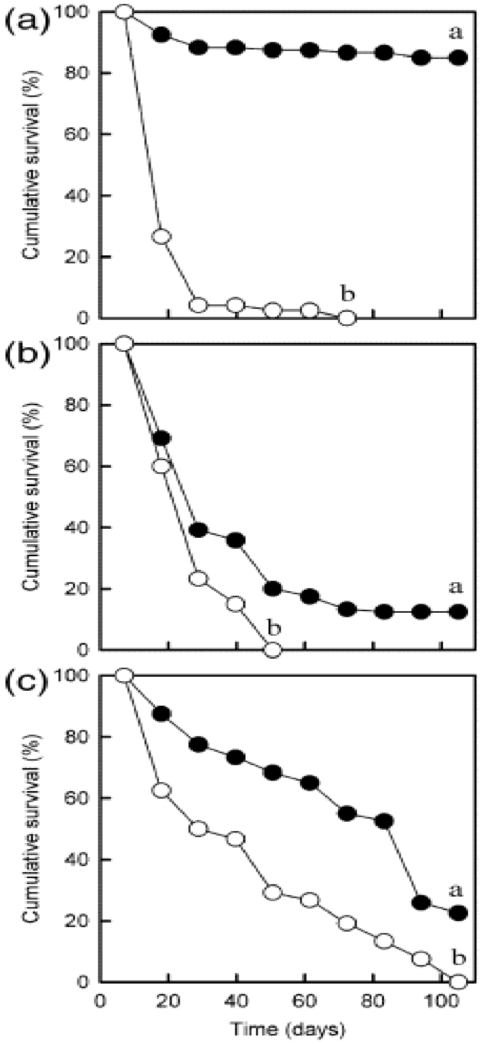

Figure 2. Kaplan-Meyer survival curves estimated for (a) *Echinocactus platyacanthus*, (b) *Ferocactus histrix*, and (c) *Stenocactus coptonogonus* in control plots (solid symbols) and within OTCs (empty symbols). Different letters indicate significant differences ($P < 0.001$).

Discussion

Predictive models of climate change suggest that summer temperatures in the Chihuahuan Desert will increase by 1-2°C by 2030 (Tejeda-Martínez et al., 2008). In agreement with this prediction, we increased 1.9°C in the OTCs over our study period. These conditions are also similar to findings by Aragón-Gastélum et al. (2014) in the same place but during four-month period of drought. As a result of the higher temperature inside OTC plots also had lower relative humidity (3.7%) than control plots. Low relative humidity reduces the atmospheric water vapor and therefore the water condensation in the soil; affecting the water uptake by the superficial roots of some succulent species (Von Willert et al., 1992; Martorell and Ezcurra, 2002; Matimati et al., 2012). Thus, increased temperature and decreased relative humidity inside OTCs were expected to affect the survival of the three species studied by increasing soil water deficits (Flores et al., 2004).

The effects of OTCs on spectral composition of global radiation were unmeasured and radiation from the chamber walls was not taken into account (Jetten, 1992; Nussbaum and Fuhrer, 2000; Aragón-Gastélum et al., 2014). Thus, the effects of the acrylic walls on spectral composition and its possible effect on seedling survival remain to be tested.

Under future climate-change conditions, plants could be vulnerable to extinction because they are unlikely to migrate fast enough to high elevation habitats and because their dispersal is usually blocked by mountain barriers (Ren et al., 2014). Adult (Smith et al., 1984) and seedling (Nobel, 1984) cacti have been shown to be rather tolerant of high temperatures. However, *S. coptonogonus, F. histrix* and *E. platyacanthus* seedlings seem to poorly tolerate elevated temperatures because they suffered an extremely negative impact on their survival rate in a scenario of induced climate change. High seedling mortality values during the growing season might impair the long-term persistence of natural populations of these species under increasing global warming scenarios.

The seedlings used in our experiment were 2 years old and they were not tolerant of high-temperature. However, Aragón-Gastélum et al. (2014) evaluated the effect of simulated warming on 5-year-old *E. platyacanthus* during a drought season and found 100 % survival. Our study implies that seedling tolerance of elevated temperatures may increase with age, although further studies are needed to test this.

Our findings are similar to those of Musil et al. (2005) for southern African quartz-field succulents, which after a 4-month summer treatment, had up to 4.9 times greater mortality in the open top-chambers than in the control plots. However, our results are in contrast to those of Aragón-Gastélum et al. (2014), who found 100% survival for young (5 years) individuals of *E. platyacanthus* f. *visnaga* in both OTC and control plots. The possible explanation is that although young plants are susceptible to extreme temperatures (Drennan, 2009), earlier seedling stages are at higher risk (Valiente-Banuet and Ezcurra, 1991; Flores et al., 2004). Our results agree with those of Shryock et al. (2014), who investigated climatic drivers of population growth using a 22-year demographic model for *Pediocactus bradyi*, an endangered cactus in northern Arizona. These authors suggested that climatic extremes beyond historical ranges of variability may threaten rare desert species with low seedling establishment under climate change.

Cacti species may be highly vulnerable to global warming because they are extremely specialized in terms of their environmental requirements (Hernández and Gómez-Hinostrosa, 2011). Ureta et al. (2012), studied two threatened *Mammillaria* species (Cactaceae) with contrasting distribution ranges under climate change scenarios and suggested that the most widespread species would be less affected by climate change, proposing that past selection on plasticity might allow them to survive under variable conditions. However, *E. platyacanthus* has a wider distribution than the other species but was severely affected by induced warming.

It has been suggested that desert succulents have a high tolerance of water and temperature stress (Nobel, 2010); however, models of global change predict that some cacti species will decrease their distribution range due to increased temperature and diminished rainfall (Butler et al., 2012; Cortes et al., 2013; Dávila et al., 2013; Martorell et al., 2015), although these predictions were proposed for adult individuals. Our hypothesis that cacti seedlings located within OTCs will experience a higher mortality rate in field conditions than in control plots was corroborated and supports these predictions for adult plants.

The effect of elevated temperatures on seedlings of the studied species is different to the effect on their seeds. Pérez-Sánchez et al. (2011) found that *E. platyacanthus* seeds had increased germination after heat exposure, but *F. histrix* seeds were not affected by heat exposure. The effect of increased temperatures on *S. coptonogonus* seed germination remains to be tested.

The effect of elevated temperatures on seedlings from other environments has been evaluated and there are contrasting results. At many locations in maritime Antarctica, several species have shown dramatic increases in numbers as a result of greater seed germination and seedling survival after a series of warm summers (Smith, 1994). However, whole-season heating reduced germination and establishment, significantly in four out of 10 species from subarctic tundra (Shevtsova et al., 2009).

Conclusions

After induced warming for the 4 months of the growing season, we found significantly less seedling survival for *E. platyacanthus* f. *visnaga, F. histrix* and *S. coptonogonus* seedlings inside OTCs. Most predictions of global changes have been suggested for adult plants, and there are very few studies taking into account seedlings or young plants (Shryock et al., 2014), which are perhaps more susceptible to extreme temperatures (Drennan, 2009) and drought (Delgado-Sánchez et al., 2013; Pérez-Sánchez et al., 2015). This study is the first focused on experimentally evaluating the potential impact of climate warming on seedling survival of endangered cacti species.

Our findings provide new insights about changes in seedling survival under global warming scenarios. Our study suggests that predicted elevated temperatures threaten the persistence of natural populations of Mexican endemic cacti species and therefore conservation and management practices are highly needed.

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Chapter 4

Anatomical mechanisms to tolerate global warming in seedlings of threatened Mexican cactus species

Abstract

Cactus have been shown to be thermal stress-tolerant because their exceptional adaptations; thus, it is expected that cacti will expand their distribution range as consequence of climate change. Nevertheless, thermal stress tolerance has been found mostly in adult cacti. We evaluated thermal stress tolerance in two-year cactus seedlings of the threatened *Echinocactus platyacanthus* f. *visnaga*, *Ferocactus histrix* and *Stenocactus coptonogonus*, using forecast temperatures for next decade from southernmost of the Chihuahuan Desert. The response variables evaluated are shoot apical meristem (SAM) diameter, vascular cylinder length, changes on fresh (FM) and dry mass (DM), and water loss (WL). We found differences in FM, DM and WL between non stressed and stressed seedlings and they displayed severe damage in chlorenchyma cells. However, these changes did not affect the SAM diameter, which was high in all species. Stressed seedlings from *E. platyacanthus* and *F. histrix* showed lower values of vascular cylinder length than those from *S. coptonogonus*, and they suffered vascular cylinder contraction. Modifications on SAM and vascular cylinder can be interpreted as mechanisms to allow an efficient water conduction through of the stems of these cacti seedlings to face future climatic scenarios in arid and semi-arid environment.

Key words: Cactaceae, Chihuahuan Desert, Endangered species, Functional anatomy, Global warming, Shoot apical meristems, Vascular cylinder.

Introduction

Current and future anthropogenic climate change is one of the main threats to loss biodiversity worldwide (Thuiller et al., 2008; IPCC, 2014). In arid and semi-arid ecosystems, global climate models indicate a mean temperature increase of 1-3^{o}C by the late twenty-first century (IPCC, 2014). These could increase the risk of local extinction in these environments (Martorell et al., 2015). However, there is the overall expectation that this vegetation will expand in a scenario of climate change, and this relies on the assumption that desert plants can rapidly adapt to water shortage and high temperatures because they evolved in stressful habitats with unstable weather conditions (Vicente-Serrano et al., 2013; Tielbörger and Salguero-Gómez, 2014). Nevertheless, the thermal tolerance thresholds under global warming in these species is an issue that has received little attention.

Recent studies have been focused on the photosynthetic performance of cactus species under induced warming (Aragón-Gastélum et al., 2014; 2016). However, the anatomical mechanisms that lead to exceptional tolerance in these species under high temperature remains almost unknown. Thus, we hypothesized that the protection of the apical meristems (Zutta et al., 2011) as well as possible structural modifications of the vascular cylinder (Ponce-Bautista, 2012) in these species could help to tolerate the expected temperature increases for arid and semi-arid environment.

Most plants have shoot apical meristem (SAM) quite uniform in size (Mauseth, 1988). However, some cacti show exceptional modifications in width (Anderson, 2001) and diameter (Mauseth, 1978b) of SAM. In addition, this structure is well protected by surrounding tissue, which is an envelope that covers the apex with trichomes and spines (Thomas, 1991). Thus, whether the apex is killed or otherwise damaged by high temperature, the SAM of cactus may possibly continue alive and healthy under this great shield and its high diameter. Furthermore, cactus species have the ability to store large amounts of water (Ehleringer and Monson, 1993), and the vascular cylinder play a crucial role to have an efficient water conduction through the stems, because the movement of water occurs from the central vascular cylinder to epidermis by diffusion (Barcikowski and Nobel, 1984).

There is evidence of changes on the root diameter of some succulents (North et al., 2008), in response to water scarcity. Even for cactus species, these structural modifications in both, drought (Ponce-Bautista, 2012), and extreme temperature (Garrett et al., 2010) conditions have been found. Nevertheless, it has been poorly studied whether these changes affect structurally at the vascular cylinder. Ponce-Bautista (2012), showed a contraction of the vascular cylinder in *Leuchtenbergia principis* (Cactaceae), after a severe drought treatment. Thus, it is possible that these conditions (greater SAM protection and vascular cylinder contraction) can be also observed in cactus species under the projected temperature increases to global warming.

The Chihuahuan Desert is the largest warm ecosystem of North America, ranging from southwestern United States to the Central Mexican Highlands (Archer and Predick, 2008). In terms of biodiversity, this desert harbors an elevated richness of succulent plants, especially globose cacti (Rzedowski, 1991) and most of them are protected by Mexican environmental laws (SEMARNAT, 2010). In a pilot study inducing climatic change, Aragón-Gastélum et al. (2014), found a mean temperature increase by 1.9^{o}C and mean maximum temperature up 40^{o}C in the southernmost of Chihuahuan Desert. These temperature increases are similar to the climatic forecasts to global warming for this region by 2030 (Tejeda-Martínez et al., 2008).

Cactus seedlings are often more sensitive to abiotic (Flores et al., 2004) and anthropogenic (Godínez-Álvarez et al., 2003) stresses than adult plants. Thus, the impacts to the warming on tolerance of seedlings may have an important detrimental effect in its recruitment rates (Loik et al., 2004). Consequently, because of the structural (in vascular cylinder and higher SAM diameters) modifications in cacti species, we hypothesized that the *Echinocactus platyacanthus* Link and Otto, *Ferocactus histrix* (DC) G.E. Lindsay and *Stenocactus coptonogonus* (Lem.)A.Berger ex A.W.Hill (Cactaceae) seedlings will show greater protection on their SAM, as well as will display a vascular cylinder contraction (VCC). These changes are expected as anatomical mechanisms to tolerate the projected increases due to global warming. Our aim was to describe these structural changes both, qualitatively and quantitatively under the projected temperature conditions for the next 20 years in the Chihuahuan Desert.

Materials and methods

Study Species

Stenocactus coptonogonus is a globose cactus that can reach 10 cm height and 11 cm in diameter (Arredondo and Sotomayor, 2009). *Echinocactus platyacanthus* and *Ferocactus histrix* are barrel-like cactus that can surpass 1 m height and 80 cm in diameter (Bravo-Hollis and Sánchez-Mejorada, 1991); According to Jiménez-Sierra et al. (2007), the mean annual mortality in wild populations of *E. platyacanthus* is highest for the seedlings

(19.7%) and lowest for adults (2.5%). There are no available data on these parameters for the other two species.

These cactus are endemic to México, but overexploitation and looting for food or ornamental purposes has heavily endangered the natural populations of these species (Jiménez-Sierra et al., 2007; Arredondo and Sotomayor, 2009). Thus, these three cacti species have been included as “specially protected species” in the framework of the environmental regulations of México (SEMARNAT, 2010). Furthermore, *E. platyacanthus* and *F. histrix* are included as near threatened species in the Red List of the International Union for Conservation of Nature (IUCN, 2014). *E. platyacanthus* is one of the most widespread Mexican cacti (Jiménez-Sierra et al., 2007); its range extends over 18°–25°N latitude and 97°–107°W longitude (Trujillo-Argueta, 1984). The other two species studied are more narrowly distributed (Guzmán et al., 2003). The range of *F. histrix* lies between 20°–24°N latitude and 98°–104°W longitude (del Castillo, 1983), and for *S. coptonogonus* the range lies between 21°–24°N latitude and 100°–102°W longitude (Salas de León et al., 1999). *Ferocactus histrix* fruits mature during the spring and early summer (del Castillo, 1983). *E. platyacanthus* fruits are present on the plant all year long, with a peak in August (Trujillo-Argueta, 1984). The fruits of both species remain on the plants when ripe, until they are eaten by animals (Trujillo-Argueta, 1984; del Castillo, 1983). Seed mass is 2.62 mg for *E. platyacanthus* and 0.296 mg for *F. histrix* (Flores et al., 2011). There is no information about dispersal time and agents or seed mass for *S. coptonogonus*.

Seed Collection

Seedlings of *E. platyacanthus*, *F. histrix* and *S. coptonogonus* were developed from seeds, which were harvested in the surroundings of the city San Luis Potosí, México during summer 2011. Seeds of three species were collected from at least 10 mother plants each. They were germinated on peat moss trays within growth chambers at 25°C ± 2°C, photoperiod 12 h light/dark and 30-40 µmol m^{-2} s^{-1} PPFD (photosynthetic photon flux density), and 80% relative humidity (RH). Seedlings were later moved to the greenhouse and transplanted in individual 150 ml plastic pots (four seedlings per pot, per species). We planted four seedlings per pot in place of one because in cactus seedlings, soil water evaporation is significantly reduced when the population density increase (Bacilio et al., 2011). These pots were filled with a mixture of gravel (10%), sand (30%) and clay (60%). These seedlings were grown for two years in the greenhouse prior to be used in the experiment described below. Environmental conditions in the greenhouse were: 33°C, 40% RH and 1300 µmol m^2 s^{-1} PPFD. For the three species, the seedling size after two years fluctuated between 1.46-2.16 cm in height and 1.52-1.92 cm in diameter.

Seedling Anatomy

Ten seedlings of each species were randomly chosen and taken to the laboratory. The seedlings were removed from pots, washed with distilled water and weighed to obtain fresh mass (FM). These seedlings were placed on petri dishes (one seedling per dish). Then, five seedlings of each species were placed within growth chambers at 42^{o}C ± 2^{o}C, 80% RH, photoperiod 12h light/dark and 30-40 µmol m^{-2} s^{-1} PPFD. These temperature and relative humidity values were according to the mean maximum of these variables under induced climatic change for southernmost of Chihuahuan Desert (Aragón-Gastélum et al., 2014). The remaining five seedlings were placed within another growth chambers at 25°C ± 2°C, photoperiod 12 h light/dark and 30-40 µmol m^{-2} s^{-1} PPFD and 80% RH as control treatment.

In order to avoid that seedlings were immediately exposed to the high temperature treatment described above, which could trigger their accelerated death; the seedlings were subjected to thermal pre-treatments (25ºC ± 2 ºC, 30 ºC ± 2ºC and 35ºC; ± 2ºC, for 24 h each one with the values of RH, photoperiod and PPDF described above). Once that final treatment temperature (42ºC ± 2ºC, 80% RH) was reached, we hypothesize an accelerated and severe damage on the parenchymatous tissue in seedlings. Thus, to ensure that it was due to the temperature increase only, each seedling in both treatments (high temperature and control) were continuously irrigated during the entire experiment (3 ml during morning and 3 ml in afternoon).

The seedlings were maintained within the growth chambers until damage was evident on the parenchyma and cell necrosis was shown at least in one seedling of the three species within of the high temperature treatment. This cell necrosis was evident after three weeks. Then, seedlings were removed from growth chambers to obtain their biomass, estimating the dry mass (DM) and the water loss (WL) as the difference between fresh and dry weight. Finally, seedlings in both treatments were sectioned longitudinally from apices to assess the SAM diameter with a stereomicroscope (Leica EZ4D). After of this, we determined whether their apical meristems remained intact as well as possible longitudinal changes on vascular cylinder, using a Light microscope (DM 2000, Leica Microsystems, DE).

Statistically Analysis

All our measurements and comparisons were conducted between species as well as between stressed and non-stressed seedlings. The effect of high temperatures in the apical meristems persistence of seedlings was descriptively analyzed. The SAM diameter, vascular cylinder length, fresh and dry mass as well as water loss were subjected to one-way ANOVA after verifying data normality. These analyses were conducted in Statistica (Stat-Soft 8.0, 2007).

Results

Anatomical differences among species

Differences on the SAM diameter (*F* = 4.178, *P* = 0.042) were found. Seedlings of *E. platyacanthus* (7298.34µm ± 931.47) and *S. coptonogonus* (6549.40µm ± 676.83) exhibited similar high SAM diameters (*F* = 0.941, *P* = 0.787). In contrast, *F. histrix* had the smallest (4103.59µm ± 447.93) SAM diameter, which was statically different of *E. platyacanthus* (F= 3.916, P = 0.042), but not of *S. coptonogonus* (*F* = 2.974, *P* = 0.131) (Figure 1).

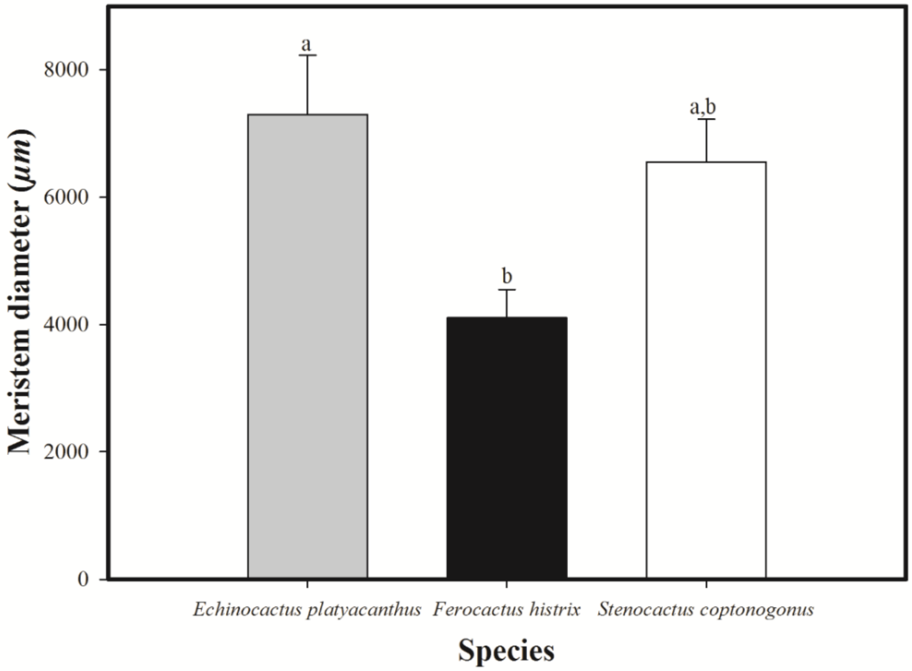

Figure 1. Shoot apical meristem (SAM) diameter (mean ± standard error) in seedlings (n=5) of three threatened cactus species. Different letters indicate statistically significant differences (P < 0.005).

Anatomical differences between stressed and non-stressed seedlings

There were no differences on SAM diameter between stressed (*E. platyacanthus* = 6816.68µm ± 802.07; *F. histrix* = 4303.73µm ± 201.23, and *S. coptonogonus* = 6212.56µm ± 742.73), and non-stressed (*E. platyacanthus* = 7298.34µm ± 931.47, *F* = 0.155, *P* = 0.705; *F. histrix* = 4103. 59µm ± 447.93, *F* = 0.194, *P* = 0.673, and *S. coptonogonus* = 6549.40µm ± 676.83, *F* = 0.107, *P* = 0.753) seedlings (Figure 2). There was null mortality for *F. histrix* and *S. coptonogonus*, while in *E. platyacanthus* it was 20% (n = 5).

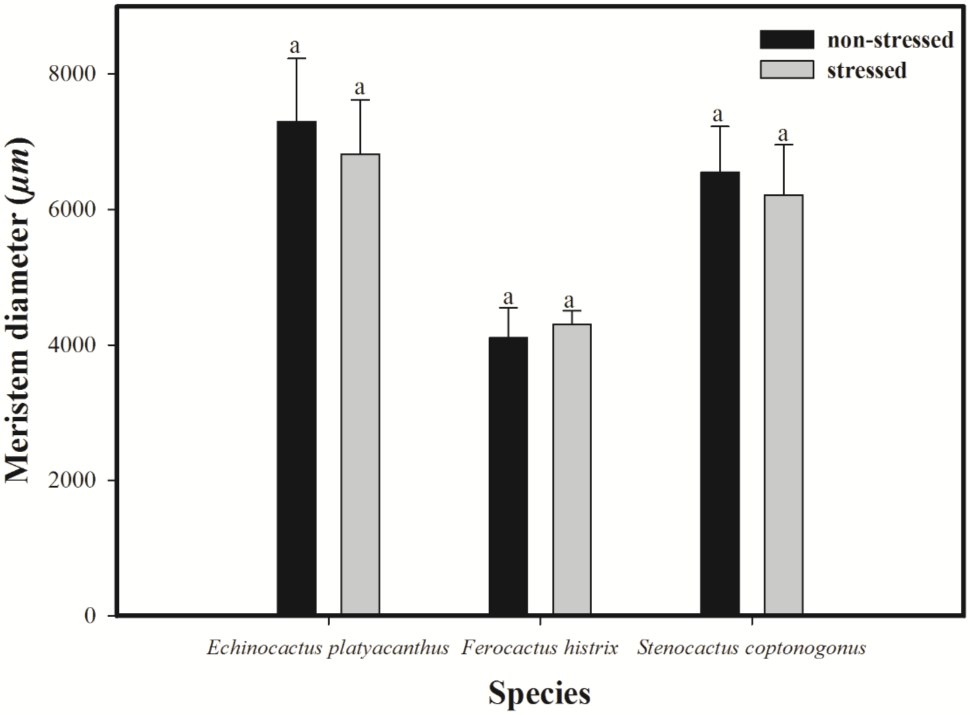

Figure 2. Shoot apical meristem (SAM) diameter (mean ± standard error) in both, stressed and non-stressed seedlings (n=5) of three threatened cactus species. Different letters indicate statistically significant differences (P < 0.005).

We found significant differences on vascular cylinder length values in non-stressed than in the stressed seedlings. These values were lower in the *E. platyacanthus* (653.35µm ± 35.25; *F* = 294. 498, *P*<0.001), and *F. histrix* (320.44µm ± 40.34; *F* = 378. 676, *P*<0.001) stressed than in non-stressed (*E. platyacanthus* = 1275.76µm ± 8.53, and *F. histrix* = 1276.09µm ± 28.00) seedlings, but not for *S. coptonogonus* (918.63µm ± 26.91 in stressed and 860.82µm ± 13.33, in not-stressed seedlings; *F* = 3.705, *P* = 0.103) (Figures 3 and 4).

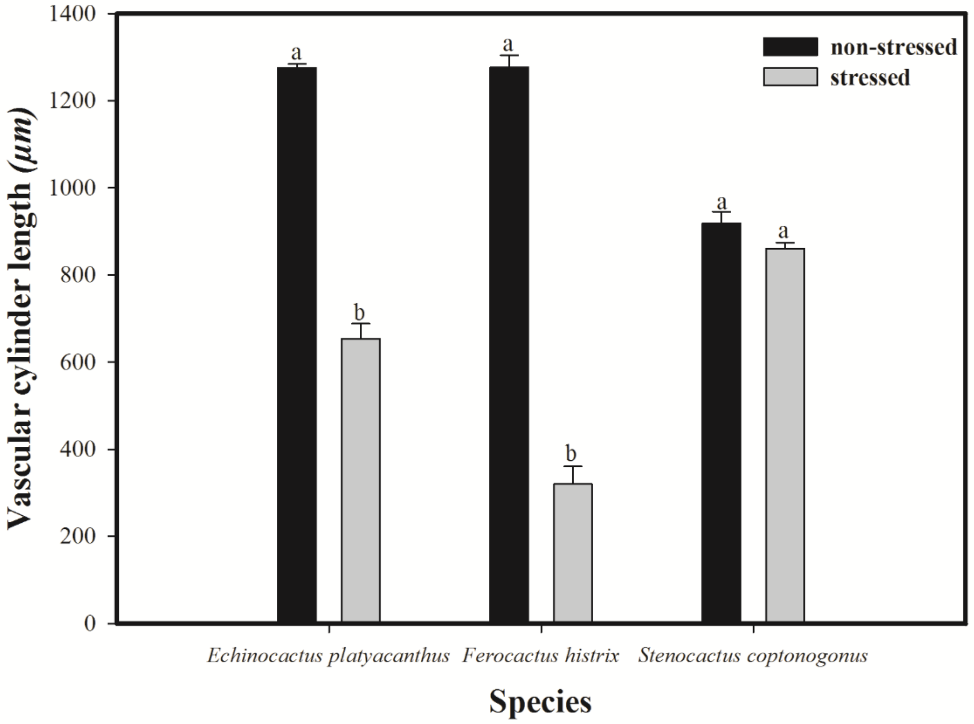

Figure 3. Vascular cylinder changes (mean ± standard error) in stressed and non-stressed seedlings (n=5) of three threatened cacti species. Different letters indicate statistically significant differences (P < 0.005).

We also found differences among species for fresh (*F* = 36.948, *P* <0.001), and dry (F = 19.408, P <0.001) mass, as well as water loss (*F* = 48.174, *P* <0.001). *Ferocactus histrix* showed higher dry mass and lower water loss than *E. platyacanthus* but not compared with *S. coptonogonus* (Table 1). In addition, due to increased thermal stress, the three species displayed severe damage in chlorenchyma cells. Nevertheless, meristematic cells tolerated these conditions and consequently remained turgid, and also exhibited presence of chloroplasts (Figure 4).

Table 1 Changes in fresh (FM), and dry (DM) mass as well as water loss (WL) in seedlings of three cactus species after three weeks under high temperature treatment. Comparisons were made between variables.

	mg		**ml**
Species	**FM mean (±S.E.)**	**DM means (±S.E.)**	**WL means (±S.E.)**
E. platyacanthus	4223.93± 378.94^{a}	219.46± 60.79^{b}	4.004± 0.32^{a}
F. histrix	1414.10± 291.14^{b}	818.34± 56.23^{a}	0.596± 0.30^{b}
S. coptonogonus	789.53± 208.26^{b}	382.84± 89.19^{b}	0.407± 0.23^{b}

Different letters indicate significant differences between variables ($P < 0.005$).

Discussion

The SAM diameter values were high in both, not stressed and stressed seedlings in the three cacti species analyzed, which was expected. Mauseth (2004a) found a wide variation on

SAM diameter of 183 cacti species; even up to 2565µm for *E. platyacanthus*; these results are similar with our findings. Despite the fact that *F. histrix* had the SAM diameter smallest (4303.73 µm) than the other two cacti, this value is considerably higher compared to the SAMs diameter of other succulent (e.g., *Euphorbia fortuita* = 412µm, *E. horrida* = 525µm, *Caralluma burchardii* = 112µm, *Hoodia gordonii* = 427µm, *H. ruschii* = 375µm, *Larryleachia cactiformis* = 337µm, *Pseudolithops migiurtinus* = 187µm, and *Stapelia gettleffei* = 120µm*)* species (Mauseth, 2004b).

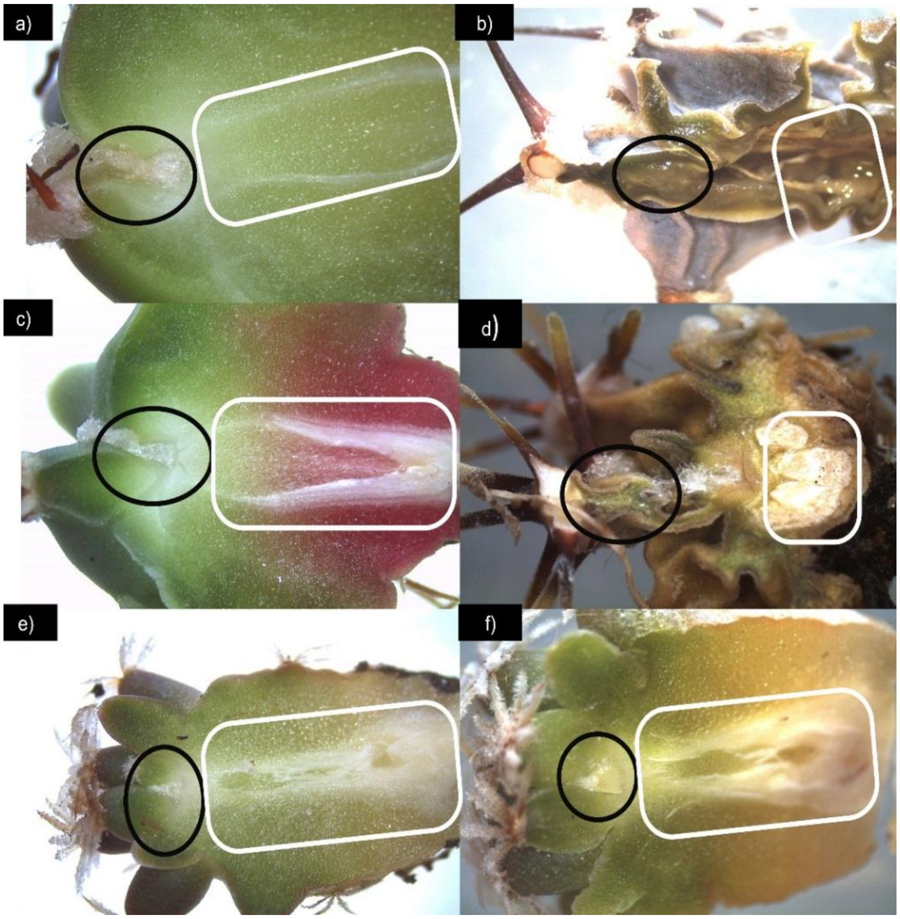

Figure 4. Longitudinal sections (scale 2mm) in non-stressed and stressed seedlings (n=5) views with light microscope. *E. platyacanthus* (a & b), *F. histrix* (c & d) and *S. coptonogonus* (e & f). Black circle show the shoot apical meristem (SAM), and white rectangle indicate vascular cylinder (VC).

There is evidence about high SAM diameter in widespread than in narrow cactus species (Mauseth, 2004a), which is consistent with our results because *E. platyacanthus* had larger SAM diameter than *F. histrix* and *S. coptonogonus* and the former have most widespread distribution (Jiménez-Sierra et al., 2007) than the other two species (Guzmán et al., 2003). Our findings showed that in spite of the high temperature, the SAM on the three cactus species remained intact. High temperature tolerance on adult cactus tissue have been widely studied (Didden-Zopfy and Nobel, 1982; Nobel, 1984; Smith et al., 1984; Nobel and De la Barrera, 2002; Nobel, 2010; Zutta et al., 2011). Our results are similar to those of Zutta et al. (2011), who found a great tolerance of meristematic cells under extreme temperature (45^{o}C day/night air temperatures) in *Nopalea cochenillifera*, *Opuntia robusta*, and *Selenicereus megalanthus*, three cultivated cacti.

Seedlings of *E. platyacanthus* and *F. histrix* had higher water loss than *S. coptonogonus*. Due to this and their concomitant dry mass loss in these species, we expected an accelerated cell death on their tissues. However, the three cacti species exhibited great tolerance to high temperatures. This result indicates that the highest SAM diameter in *S. coptonogonus*, *E. platyacanthus*, and *F. histrix* derived in an extraordinary protection of meristematic cells, which allowed the seedling persistence. Therefore, our hypothesis about the seedlings of these species will show greater protection on their SAM was corroborated.

Nobel and De la Barrera (2002), found a dry mass gain of 1900 mg in *Hylocereus undatus* plants, a hemiepiphytic cactus, after they were subjected at 40^{o}C/30^{o}C day night air temperature for 31 weeks. This condition probably indicates the need of cacti to obtain water and nutrients before investing in new shoot growth, because this dry mass gain was mainly partitioned in roots. Although the stems and roots resource allocation was not considered in our study, *F. histrix* was the only species that exhibited a high dry mass, and this could better explain its persistence. However, our findings in *E. platyacanthus* and *S. coptonogonus* suggest an opposite pattern; because they did not exhibit this gain. Seedlings of *E. platyacanthus* showed a great sensibility to high temperature and they apparently give priority to their persistence investing all the resources at this time to protect the meristematic zone, which may give them a new chance to re-growth once the conditions are suitable. The dry mass gain in *S. coptonogonus* (described above) was expected because this species had the smallest water loss than the other two species.

The extreme SAM diameter can be interpreted as a trait of evolution in the Cactaceae and also could explain their current successful persistence (at least partly) within arid and semi-arid environments. There is evidence that this exceptional protection of the apical meristem is an effective mechanism to tolerate the effect of high temperature on wild populations of cacti. In this sense, Thomas and Goodson (1992), found high survival of 17 mature succulents species (including some cacti) to naturally occurring fires in desert grasslands in Arizona, USA, because these species showed a high percentage of apical re-growth and offsets one month after burning. In the three cacti species studied the SAMs remained intact after intense physical stress conditions and although the cactus seedlings are often more sensitive to abiotic stress than adult plants (Flores et al., 2004; Godínez-Álvarez et al., 2003), it is possible these seedlings could have the ability for apical re-growth as a mechanism to tolerate the expected increases temperature for global warming and thus, increase the chances to their persistence.

We also hypothesized that seedlings of the three cacti species would show a vascular cylinder contraction (VCC). Nevertheless, we found a VCC in seedlings of *E.*

platyacanthus and *F. histrix*. For *S. coptonogonus* the presence of a large amount of spines in the cuticle was evident and they showed an apparent greater thickness; although these parameters were not measured; we believe that these conditions could be the explanation why this species did not exhibit VCC. Diameter fluctuations in roots (North et al., 2008; Ponce-Bautista, 2012) and stems (Kozlowski, 1967; Nonami and Boyer, 1990; Zweifel et al., 2000) have been focused on monitoring changes in water potentials in plants as a only stress factor, and the effect of heat stress as well as anatomical and structural changes on vascular cylinder in desert plants had not been assessed. Therefore, this research is pioneer to evaluate these variations in cacti species under the expected high temperature for the next decade in the Chihuahuan Desert.

Garrett et al. (2010), found that the root contraction in *Ariocarpus fissuratus* help to protect this species from lethal high temperatures when is growing in rocky soil. Our results shows that VCC appears to be also an adaptive response to environmental stress conditions such as extreme temperature events and represents a possible efficient mechanism that could enhance the efficiency of the flow water movement through of stems in *E. platyacanthus* and *F. histrix* because this contraction. Nevertheless, this premise must also be corroborated.

Under future climate change conditions, plants could be vulnerable to extinction because they are unlikely to migrate fast enough to high elevation habitats and because their dispersal is usually blocked by mountain barriers (Ren et al. 2014). Thus, the plants are forced to adopt several tolerance mechanisms. Aragón-Gastélum et al. (2014) evaluated the effect of simulated warming on 5-year-old *E. platyacanthus* during a drought season and found 100 % survival. This extraordinary tolerance was attributed to high non-photochemical quenching (NPQ) values, a mechanism to avoid photoinhibition. Therefore, modifications on SAM diameter and vascular cylinder found in the three cactus species studied can be interpreted as adaptive traits, as well as also a mechanism which would allow the persistence of these cacti species in their earlier stages of life cycles face to future climatic scenarios in arid and semi-arid environments.

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Chapter 5

Seasonal variation in photosynthetic performance and survival under simulated warming of *Echinocactus platyacanthus*, an endangered Mexican cactus species

Abstract

Climate change is one of the principal threats to biodiversity loss, as well as to shift on structure and ecosystem functioning. The Chihuahuan Desert harbors the highest diversity of globose cacti at worldwide and climate change models by this desert indicate an increase in summer by 1-2^{o}C and winter by 6^{o}C mean temperature by 2030. However, studies focused on the impact of global warming in desert plants are still scarce. We assessed the effect of induced warming in the photosynthetic performance and survival on five-year individuals of *Echinocactus platyacanthus* through the seasons during one year. Hexagonal open-top chambers (OTCs) were used to simulate the effect of global warming; furthermore, a canonical discriminant analysis was used to identify a subset of ecophysiological variables, which allows the separation treatments at the maximum. Subsequently, a generalized canonical correlation analysis (GCCA) was used to measure the intensity of the relationship among environmental (mean air temperature, evapotranspiration, global solar radiation and rainfall), micro-environmental (mean temperature, mean relative humidity and photosynthetic photon flux density) and ecophysiological [effective quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR), both within and outside OTCs] variables. OTCs had higher temperature than control plots through the seasons. *Echinocactus platyacanthus* showed 100% survival in both OTCs and control plots; nevertheless, higher seasonal variations in photosynthetic performance were found inside OTCs than in the control. The Φ_{PSII} and ETR values decreased in summer but increased in autumn and winter, especially inside OTCs. The GCCA allowed determination of a general model of relationships between environmental and micro-environmental variables with the ecophysiological ones. We found a strong influence (0.71) of micro-environment with ecophysiology in *E. platyacanthus*. This is the first study evaluating the potential long-term impact of global warming on the survival and photosynthetic performance of a cactus species.

Key words: Global warming, Chlorophyll fluorescence, Survival, GCCA, Chihuahuan Desert, Cactaceae, Open-top Chambers.

Introduction

Increases in emissions of greenhouse gases in the atmosphere and concomitant enhance in mean temperature in the world are the main indicators of climatic change (Walther, 2010; IPCC, 2013; 2014), which has caused an accelerated ecosystemic modification in different biomes on earth (Zhang et al., 2004; Lawrence et al., 2007; Reynolds et al., 2007), and represent a severe threats to the biodiversity loss, as well as to shifts in the structure and ecosystem functioning in our planet (Malcolm, et al., 2006; IPCC, 2013; 2014). Particularly, deserts are one of the most diverse biomes worldwide, because they host about 20 per cent of plant diversity worldwide (White and Nackoney, 2003). Despite to harsh environmental (e.g., high temperatures, salinity and drought) conditions in the deserts (Nobel, 2010), it has been suggested that the wild vegetation into these ecosystems will expand their distribution range as result of climate change (Prentice et al., 1992); because these plant species can rapidly adapt to water shortage and high temperatures (Vicente-Serrano et al., 2013; Tielbörger and Salguero-Gómez, 2014). However, climate change models indicate that deserts are highly sensitive to global climatic change (Sala et al., 2000). Thus, defining what plant species could tolerate the imminent increases in temperatures acquires crucial importance.

The most widespread warm desert of North America is the Chihuahuan Desert (Archer and Predick, 2008); it is ranging from southwestern United States to the Central Mexican Highlands. Global change projections for this region indicate that summer temperatures (June–September) will increase by 1–2^{o}C by 2030 (Tejeda-Martínez et al., 2008). Nevertheless, the greatest temperature increase is predicted to occur during winter months (January–March), when monthly average temperatures might increase by up to 6^{o}C

(Tejeda-Martínez et al., 2008) during this same time period. This critical outlook has created strong concerns in conservation biologists because the southernmost section of Chihuahuan Desert harbors an elevated richness of succulent plants worldwide, especially globose cacti (Rzedowski, 1991) and most of them are protected by Mexican environmental laws (SEMARNAT, 2010). However, still there is little information about the thermal tolerance thresholds for plant species of this desert.

In recent studies, differential responses of some cacti species from Chihuahuan Desert under simulated warming conditions were found. For instance, a decrease of photosynthetic performance, but 100% survival in young individuals of *Echinocactus platyacanthus* Link & Otto (Cactaceae), an especially protected Mexican cactus species during a drought period (January-April), were found (Aragón-Gastélum et al., 2014). Nevertheless, a severe decline on seedling survival rate of three endemic and threatened cacti species during the growing season (June-September), was also found (Aragón-Gastélum et al., 2016). Thus, because these results were found in a short-term experiment, long-term studies to assess the effect of the two projections suggested by Tejeda-Martínez et al. (2008) on survival and ecophysiological performance of succulent species are crucial. In this paper, seasonal variations in daily men air temperature, daily mean air relative humidity (RH), photosynthetic photon flux density (PPFD), daily mean evapotranspiration (ET), daily rainfall and global solar radiation (GSR), as well as their impact in photosynthetic performance and survival on young plants of *E. platyacanthus* were evaluated. We hypothesized that photosynthetic responses and survival would be adversely affected due to the increased temperature stress by induced climatic warming through the most critical seasons of the year. To confirm this premise, we assessed two types of response variables: ecophysiological, [(survival rate, effective quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR)], and environmental [(mean air temperature, RH, PPFD, GSR, ET, and daily rainfall). Finally, we also examined the effects of environmental variables in order to describe the intensity of relations as well as their importance in photosynthetic responses and survival of young individuals of *E. platyacanthus* under induced climatic warming.

Materials and methods

Study species

Echinocactus platyacanthus f. *visnaga* is a barrel-like cactus that can reach 2 m height and 80 cm in diameter (Jiménez-Sierra et al., 2007). It is endemic to México; nevertheless, overexploitation for food and ornamental proposals has seriously endangered the natural populations of this species (Jiménez-Sierra et al., 2007). This species has been included as specially protected species in according of the environmental laws of México (SEMARNAT, 2010). Additionally, *E. platyacanthus* is also included as *near threatened species* in the Red List of the International Union for Conservation of Nature (IUCN, 2014). *Echinocactus platyacanthus* is one of the most widespread Mexican cacti, its range extends over 18º - 25º N latitude and 97 º - 107 º W longitude (Trujillo-Argueta, 1984). The mean annual mortality in natural populations of 19.7% for seedlings and 2.5% for adults individual (Jiménez- Sierra et al., 2007).

Study area

Our experimental site was located at an abandoned agricultural field (22º 14´11´´N, 100º 51´46´´W, 1844 m a.s.l.), in the southernmost section of the Chihuahuan Desert, within the state of San Luis Potosí, México. The vegetation is dominated by sclerophyllous shrubs, cacti and succulent monocots scattered among these woody species. Mean annual temperature is 20.8ºC, however, it can surpass 40ºC in summer and be as low as -1ºC in winter (Medina et al., 2005). Average annual precipitation is 341 mm and rainfall events are concentrated in the summer months (Medina et al., 2005).

Seed collection

Young individuals of *E. platyacanthus* were developed from seeds harvested in the surroundings of the city San Luis Potosí, México, during summer and autumn 2007. Seeds were collected from at least 10 mother plants each. Fruits were taken to the laboratory and cleaned to recover the seeds, which were germinated on peat moss trays within growth chambers (25ºC, 80% RH, photoperiod 12 h light/dark and 30-40 µmol m^{-2} s^{-1} PPFD.

Seedlings were later moved to the greenhouse and transplanted into individual plastic pots (one seedling per pot). These pots had a volume of 2 L and were filled with a mixture of gravel (10%), sand (30%) and clay (60%). The plants were grown for five years in the greenhouse prior to be used in the experiment described below. The greenhouse conditions were 33ºC, 1300 µmol m^{-2} s^{-1} PPFD, and 60% RH. The size of the plants after five years was 4 cm in height and 6 cm in diameter.

Open top-chambers design

Passive open top-chambers (OTCs) are the most common and simplest tool to assess the responses of plants to climate warming in the field (Bokhorst et al., 2013). These have the advantage of being passive warming devices that do not require technological support (Hollister and Webber, 2000). These structures are built with UV–resistant transparent acrylic (3 mm thick; wavelength transmission <280 nm) by following the design proposed by Marion (1996). Thus, we obtained OTCs with 50 cm tall, 150 cm wide in the open-top, and 208 cm wide at the base attached to ground. This OTC design allows daytime passive heating by increasing air temperature by 1.9-5^{o}C, respect to the external environment in dryland ecosystems (Musil et al., 2005; 2009; Aragón-Gastélum et al., 2014; 2016). In our experiment, hexagonal OTCs were used to simulate the effects of global warming.

Environment and open top-chamber microenvironments

To evaluate the seasonal variations in photosynthetic performances and survival under induced warming in five-year individuals of *E. platyacanthus*, we started in June 2013. The experiment was conducted within a 25 m x 25 m exclosure previously established at the study site. This exclosure was fenced with woven wire (2 m height) to avoid the access of cattle and people to the experiment. On June 1st 2013, twelve plots (5 m x 5 m = 25 m^2 each) were drawn within the exclosure by following a rectangular arrangement (3 plots width x 4 plots long). Six plots were randomly selected within the exclosure and an OTC was established at the center of each of these plots. The other six plots were maintained as controls; the distance between plots was five meters (Aragón-Gastélum et al., 2014; 2016).

Temperature and relative humidity were steadily recorded within and outside OTCs to determine whether OTCs effectively modify microclimate. Thus, we used microclimatic data-loggers (HOBO Pro v2, Onset Computer Corporation, Bourne, Massachusetts, USA), which were programmed to record temperature and RH every 1 h during all experiment. A data-logger was installed 10 cm above the ground at the center of each experimental plot, at 3–5 cm above plants, resulting in six randomly selected OTCs and six control plots. Additionally, data of daily rainfall (mm), daily mean evapotranspiration = ET (mm) and global solar radiation = GSR (μmol m^{-2} s^{-1}) were obtained from the meteorological station of National Institute of Forestry, Agriculture and Livestock (INIFAP San Luis Potosí, México), which is adjacent to the study area described above.

Chlorophyll fluorescence measures

In June 7th 2013, all experimental plots received five pots with young individuals of *E. platyacanthus* (one individual per pot). The pots were placed on the soil. In these plots subjected to warming conditions, the plants were placed at the center of OTCs directly below the open-top, to avoid overwarming due to proximity to the acrylic walls of these structures and plants received the same amount of rainfall than control plots. All these cacti were watered every week to field capacity before the beginning of the experiment. Field capacity was determined in pots containing overwatered mixture and allowed to drain overnight. To reach field capacity, 200 mL water per pot was required. However, the plants did not receive further watering during the experiment.

Subsequently, in June 14th, we conducted the first chlorophyll fluorescence measures on all cacti by using a portable pulse amplitude modulation fluorometer (Mini-

PAM; H. Walz, Effeltrich, Germany). These data were used to estimate a series of variables related to the photosynthetic performance of plants located within as well as outside OTCs. These chlorophyll fluorescence measures were taken at noon (between 13:00 and 14:00 h), when plants faced the maximum daily temperature. These data were used to estimate the effective quantum yield of photosystem II (Φ_{PSII}). This variable was calculated $\Phi_{PSII} = (F'_m - F_t)/F'_m$, where F_t is the chlorophyll fluorescence emitted by plants under steady-state illumination (e.g., light conditions on the field) and F'_m is the maximum fluorescence emitted by chlorophyll when a saturating pulse of actinic light is superimposed to environmental levels of light (Genty et al., 1989). Therefore, the values of Φ_{PSII} should decrease as thermal stress increases and, thus, lower Φ_{PSII} were expected for cacti located within OTCs.

The fluorometer was also used to measures PPFD into environment surrounding plants, then, we also calculated the electron transport rate (ETR) across the electron chain of chloroplasts. This variable was then estimated as ETR = Φ_{PSII} x PPFD x 0.84 x 0.5, where PPFD is the photosynthetic photon flux density recorded by the sensor in the leaf clip, 0.84 is the estimated mean proportion of incident light absorbed by the photosystems (Ehleringer, 1981) and 0.5 is the required reflection factor for both photosystems to absorb photons (Roberts et al., 1996).

Because ETR is directly and positively related to the generation of chemical energy (ATP and NADPH/H^+) that will be later used in the Calvin cycle; lower values of this variable are indicative of reduced photosynthetic performance in plants (Ritchie and Bunthawin, 2010; Aragón-Gastélum et al., 2014). Consequently, if induced warming negatively affects the performance of *E. platyacanthus*, cacti located within OTCs should display lower ETR values than cacti from control plots.

We also counted the number of alive individuals of *E. platyacanthus* in OTCs and control plots. The Φ_{PSII} and ETR values were every three weeks registered in both treatments, whereas survival was weekly monitored from June 14th 2013 to May 31st 2014 in order to evaluate the seasonal variations in photosynthetic performance as well as persistence in *E. platyacanthus* young plants.

Statistical analyses

Response variable measured were based on six replicates (six OTCs and six controls) and the value for a replicate was based on their average. Thus, to evaluate the seasonal variation of photosynthetic performance in *E. platyacanthus*, the ecophysiological (Φ_{PSII} and ETR) and micro-environmental (PPFD) variables in both treatments (OTC and controls plots) were grouped by seasons (five measurements by season) and subjected to repeated measures one-way ANOVA after that requirements of variance homogeneity and homoscedasticity were corroborated. The daily average of air temperature and RH (within and outside OTCs) as well as GSR, ET and rainfall data were analyzed by one-way ANOVA through seasons. In addition, failure time analyses were used to determine mortality rates between treatments (OTC and control). In these analysis, the individuals had a value of 1 (one) at the beginning of the experiment because all of them are alive. Nevertheless, their death across time are the "failure events" that become these values into 0 (zero). Thus, each individual is considered as a replicate in these analyses (n = 5 plants x

6 replicates = 30 at each treatment), where the failure time is the date in which the death of each individual was recorded (Aalen et al., 2008; Aragón-Gastélum et al., 2016).

Furthermore, we conducted a generalized canonical correlation. The goal of this analysis is to examine the relationships between more than two sets of variables, to measure the intensity of the relationships and to delineate strategies of simple canonical correlation by pairs of canonical variates (Ortega-Alcalá et al., 1992; Yáñez-Espinosa et al., 2006; Delgado-Sánchez et al., 2013). Furthermore, there are original variables and canonical variates, but the first canonical variates of each set of variables generate a canonical vector of canonical variates; in contrast to simple canonical correlation where there are pairs of canonical variates, and the correlation matrix of the canonical variates is designated the first canonical correlation.

The first vector of canonical variates is composed of canonical variates of each set of variables, optimizing a function of their correlation matrix. In the method of maximum variance, the first vector of canonical variates presents the maximum variance of the first eigenvector, corresponding to maximization of the first eigenvalue of the vector of the canonical variates correlation matrix. Therefore, the optimum vector of canonical variates for this method is the one whose first eigenvector maximizes their variance (Ortega-Alcalá et al., 1992). A redundancy analysis was also performed to calculate the variance in a set of original variables explained by a canonical variable of another set (McGarigal et al., 2000).

Finally, we conducted a canonical discriminant analysis to know the complex relationship between three groups of original variables (ecophysiological, environmental and micro-environment) and the relative contribution of these variables (within each group) to explain the effect of our experimental design, describing the linear combination of the original variable coefficients (canonical variables) that maximally discriminates between groups.

The aim of the canonical correlation is to analyze the relationships between two sets of variables, and thus, explain the relationship between the two sets of variables. One set of variables consists of response variables and the other set consists of explanatory variables, similar in a multiple regression, except that there are several variables in both sides of the equation. Sets of variables on each side are combined to produce, for each side, a predicted value that has the highest correlation with the predicted value on the other side. First, there are variables (variables measured in research), then, there are canonical variates. These canonical variates are linear combinations of variables, one combination on the 'response' variable side and a second combination on the 'explanatory' variable side, and finally there are pairs of canonical variates (the combination of the canonical variates) (Yáñez-Espinosa et al., 2006).

Results

Environmental and micro-environmental variables

We found statistically significant differences between treatments in mean daily air temperature ($F_{(3, 2052)}$ =22.28, P<0.001), but not in mean air relative humidity ($F_{(3, 2052)}$ = 2.00, P = 0.1573) nor PPFD ($F_{(1, 238)}$ = 0.008, P = 0.9293). Between June 1st 2013 to May 31st 2014 the average temperature was 1.6°C higher inside OTCs (19.3°C ± 0.2, 95% Confidence Intervals) than in control plots (17.7°C ± 0.08); while relative humidity was 1.4% higher (although not significantly) in control plots (67.4% ± 0.7) than in open top-chambers (66.0% ± 0.9). The PPFD mean was 1465.6 µmol m^{-2} s^{-1} (± 26.0 µmol m^{-2} s^{-1}) in OTCs and 1460.6 µmol m^{-2} s^{-1} (± 18.3 µmol m^{-2} s^{-1}) in control plots. Maximum ($F_{(3, 2052)}$ = 151.93, P<0.001) and minimum ($F_{(3, 2052)}$ = 0.472, P = 0.4924) average temperatures during this period were 36.8ºC (± 0.6ºC) and 7.6ºC (± 0.1ºC) inside OTCs, as well as 31.0ºC (± 0.6ºC) and 7.4ºC (± 0.1ºC) in control plots.

Mean ($F_{(3, 2052)}$ = 167.74, P<0.001) and maximum ($F_{(3, 2052)}$ = 148.61, P<0.001) daily temperature also statistically differed with time. Also, we found statistically significant interactions between treatment and time (mean: $F_{(3, 2052)}$ = 82.32, P<0.001 and maximum $F_{(3, 2052)}$ = 164.61, P<0.001) through the year seasons. Although these variables decreased from the beginning of the experiment, they increase to the end of the study period, and always were higher in OTCs than in control plots. Average daily temperature in summer was higher inside OTCs (23.0ºC ± 0.1) than in control plots (21.2ºC ± 0.07; $F_{(1,188)}$ = 10.39, P<0.001) (Figure 1a); also in autumn (OTC = 17.7ºC ± 0.1 and control plots = 16.1ºC ± 0.2; $F_{(1, 176)}$ = 143.214, P<0.001) (Figure 1b); winter, 1.6ºC (OTC = 14.8ºC ± 0.2 and control plots = 13.5ºC ± 0.04; $F_{(1, 178)}$ = 57.906, P<0.001) (Figure 1c); and spring, 2.5ºC (OTC = 23.0ºC ± 0.3 and control plots = 21.1ºC ± 0.1; $F_{(1, 180)}$ = 23.18, P<0.001) (Figure 1d).

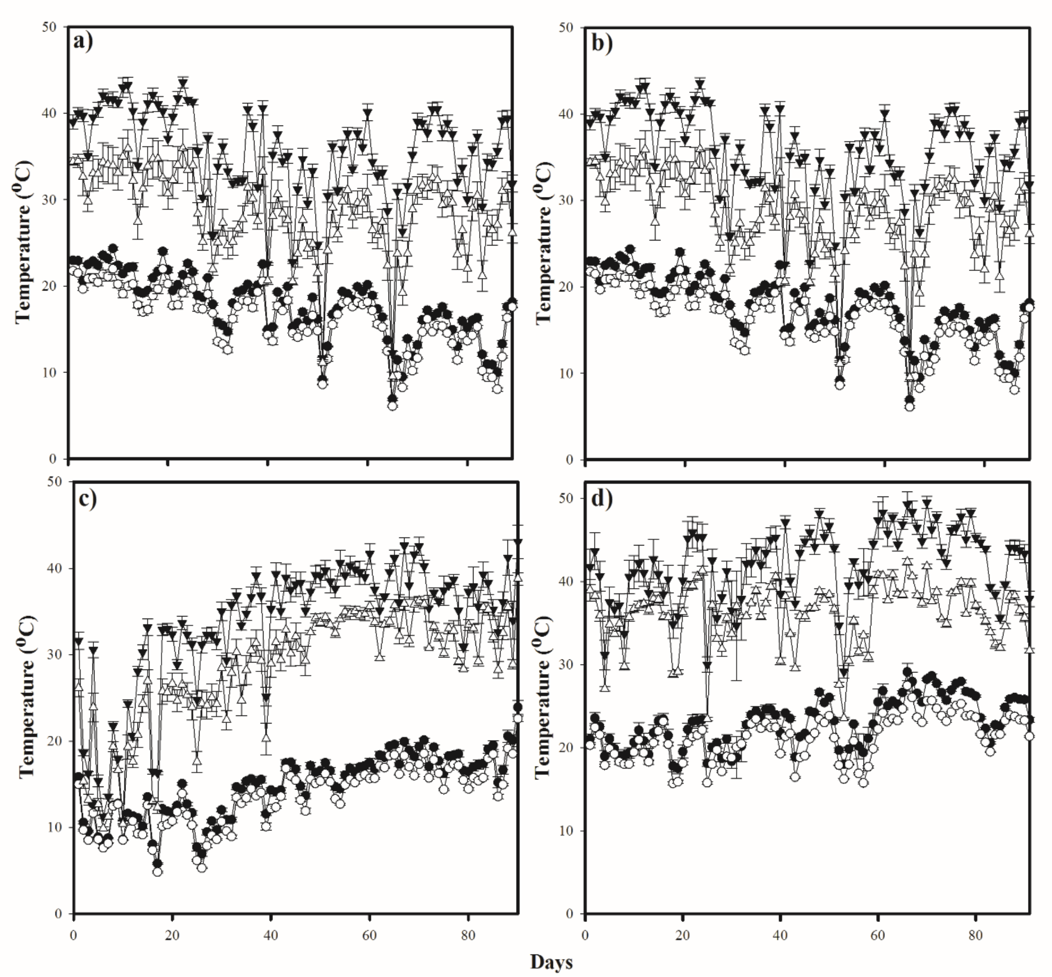

Figure 1. Mean and maximum daily temperature measured in control plots (empty symbols) and within OTCs (solid symbols) used in the field experiment across of the seasons (± 95% CI). Summer (a), autumn (b), winter (c) and spring (d). Daily temperature (circles) and maximum temperatures (triangles).

Maximum temperatures in summer were lower in control plots (32.8°C ± 0.3) than inside OTCs (38.4°C ± 0.6) ($F_{(1, 188)}$ = 16.57, P<0.001; Figure 1a). In autumn, we registered 28.5°C ± 1.5 ($F_{(1, 176)}$ = 471.884, P<0.001) in control plots and 35.3°C (± 0.5) within OTCs (Figure 1b); in winter, 28.1°C ± 0.7 ($F_{(1, 178)}$ = 81.716, P<0.001) in control plots and 33.4°C (± 0.6°C) within OTCs (Figure 1c) and in spring, 35.9°C ± 0.2 ($F_{(1, 180)}$ = 32.31, P<0.001) in control plots and 42.0°C (± 0.9) within OTCs (Figure 1d). Minimum temperature did not differ between control plots and OTCs across seasons; it was: summer ($F_{(1, 188)}$ = 0.716, P = 0.398), autumn ($F_{(1, 176)}$ = 0.0059, P = 0.938), winter ($F_{(1, 178)}$ = 0.563, P = 0.453) and spring ($F_{(1, 180)}$ = 0.265, P = 0.606). However, this variable decreased in autumn and winter but increased in spring.

Average daily relative humidity of the air in the control plots in summer was 70.8% (± 0.3%) and 67.3% (± 0.4%) within OTCs (Figure 2a); autumn, 74.8% (± 1.4%) and 73.9% (± 0.6%) within OTCs (Figure 2b); winter, 65.8% (± 1.2%) and 66.7% (± 1.3%) within OTCs (Figure 2c) and spring, 56.5% (± 0.6%) and 53.9% (± 1.4%) within OTCs (Figure 2d).

Despite we found a significant interaction among the treatment and time over the relative humidity, there were statistically significant differences in this variable between treatments only in summer ($F_{(1, 188)}$ = 3.898, P = 0.049); nevertheless, control plots always displayed higher relative humidity values than open-top chambers during the entire study. Although PPFD values did not differ between OTC and control plots (as described above); significant differences were found in these values across seasons ($F_{(3, 236)}$ = 9.094, $P<0.001$) regardless of the treatments. The PPFD values in summer were 1339.1 µmol m^{-2} s^{-1} (± 28.5 m^{-2} s^{-1}); autumn, 1370.0 µmol m^{-2} s^{-1} (± 24.4 m^{-2} s^{-1}); winter, 1547.3 µmol m^{-2} s^{-1} (± 36.1 m^{-2} s^{-1}) and spring, 1688.9 µmol m^{-2} s^{-1} (± 24.9 m^{-2} s^{-1}). Nevertheless, the interaction between season and time ($F_{(3, 236)}$ = 3.516, $P<0.001$) indicated that winter and spring displayed higher PPFD values than summer and autumn (Figure 3).

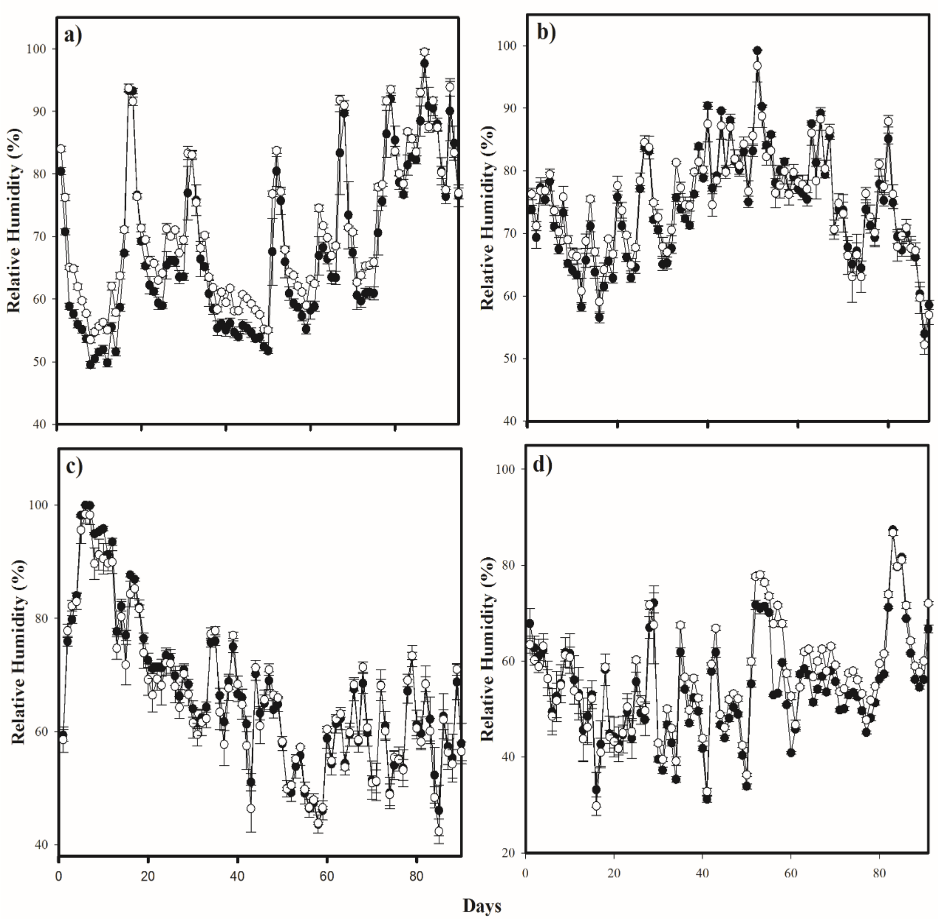

Figure 2. Average daily relative humidity of the air measured in control plots (empty symbols) and within OTCs (solid symbols) used in the field experiment across of the seasons (± 95% CI). Summer (a), autumn (b), winter (c) and spring (d).

The total rainfall during our study period was 382.6 mm, ET and GSR mean were 4.3 mm ± 0.09 and 1576. 5 µmol m^{-2} s^{-1} ± 21.9, respectively. Seasonal variations in these variables were found; in all cases, higher values were recorded during spring and summer (ET: $F_{(3, 361)} = 30.462$, $P<0.001$; GSR: $F_{(3, 361)} = 11.012$, $P<0.001$ and rainfall, $F_{(3, 361)} = 10.401$, $P = 0.015$) than in autumn and winter (Table 1).

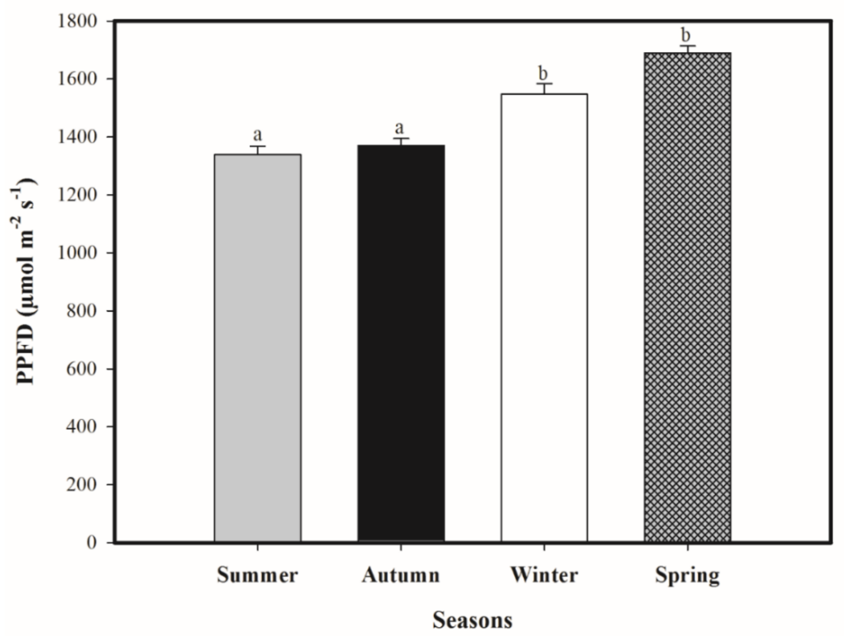

Figure 3. PPFD (photosynthetic photon flux density) values in the field experiment across of the seasons (mean ± standard error) Different letters indicate significant differences ($P<0.005$).

Ecophysiological variables

The photosynthetic performance of *E. platyacanthus* showed variations throughout of different seasons of year. Nevertheless, these fluctuations did not affect the survival rate in this species, which was 100% in both OTCs and control plots. However, the quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR) values were lower during summer and spring in the OTCs than in control plots, although significant differences were only found for summer (Φ_{PSII}, $F_{(3, 894)} = 5.189$, $P<0.001$ and ETR, $F_{(3, 894)} = 4.735$, $P = 0.002$). In contrast, an opposite pattern was observed in autumn and winter (without statistical differences between both seasons). In general, the Φ_{PSII} and ETR values were higher in OTCs than in control plots (Figures 4a and 4b).

Table 1. Seasonal changes in abiotic variables registered by meteorological station of National Institute of Forestry, Agriculture and Livestock (INIFAP) Between June 1st 2013 and May 31st 2014.

Variables/Seasons	***GSR (μmol m^{-2} s^{-1}) mean ± S.E**	***ET (mm) mean ± S.E.**	**Rainfall (mm) total ± S.E**
Summer	1597.44 ± 45.95^{b}	4.73 ± 0.17^{b}	103.4 ± 0.30^{a}
Autumn	1427.96 ± 37.72^{c}	3.36 ± 0.11^{c}	87.9 ± 0.31^{b}
Winter	$1515.49 \pm 49.09^{b,c}$	3.81 ± 0.17^{c}	83.6 ± 0.43^{b}
Spring	1757.90 ± 34.09^{a}	5.42 ± 0.18^{a}	107.7 ± 0.41^{a}

Different letters indicate significant differences between treatments ($P<0.005$)
*ET = mean daily evapotranspiration.
*GSR = global solar radiation.

Generalized canonical correlation

We had three components within of set of variables: environmental [daily rainfall (mm), daily mean evapotranspiration (ET), and global solar radiation (GSR)], micro-environmental [mean air temperature, mean air relative humidity (RH), and photosynthetic photon flux density (PPFD)] and ecophysiological [effective quantum yield of photosystem II (Φ_{PSII}), electron transport rate (ETR) and survival rate]. However, this latter did not vary (as was above described) during the study period, by which this variable was removed for this analysis.

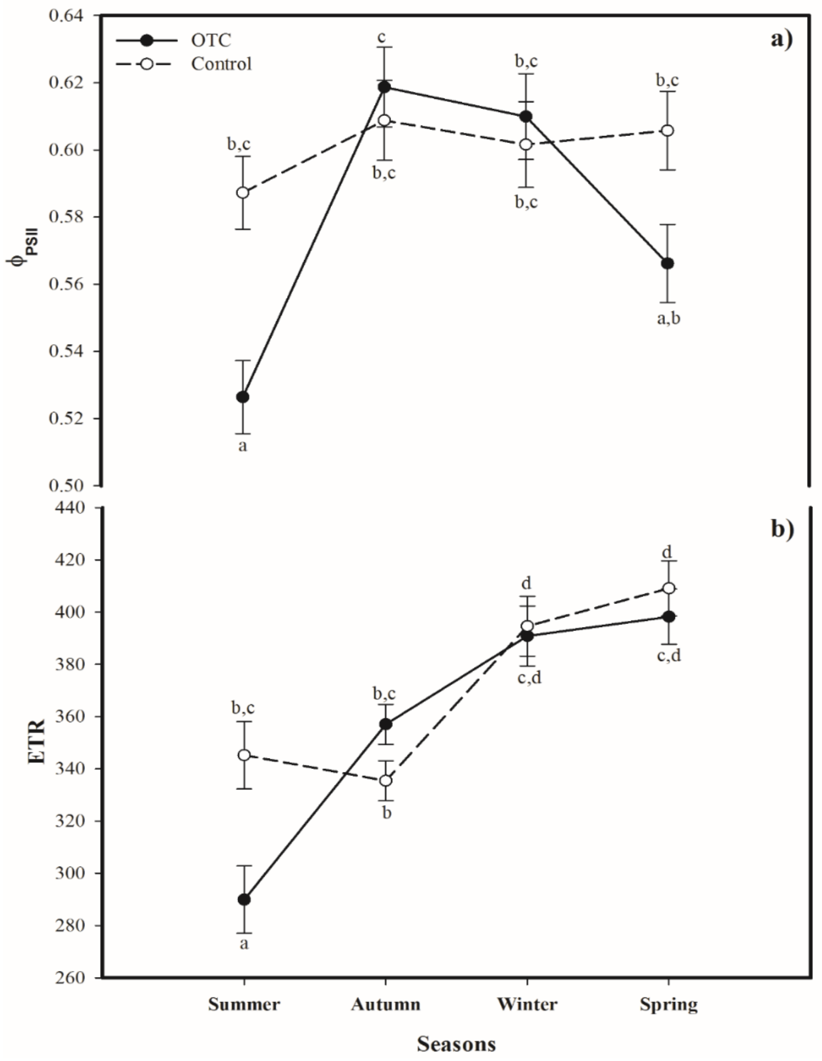

Figure 4. Seasonal variations of induced warming on photosynthetic performance of young plants of *Echinocactus platyacanthus*, in open top-cambers (OTC; black line) and control plots (dotted line). a), effective quantum yield of photosystem II (Φ_{PSII}) and b) electron transport rate (ETR). The mean comparison was made within the dates only. Different letters indicate significant differences between treatments (P<0.005).

The results indicated that there was only one canonical variable. The eigenvalue (2.17; Wilks' ƛ= 1.35; P <0.0001, n = 1440) represents most of the variance of the first canonical variable (Table 2). In addition, this eigenvalue showed that two of three of set of variables are highly correlated, where is found a strong influence between the micro-environment and cacti ecophysiology (0.71) (Figure 5). The eigenvector elements showed that the environment component had the lowest value (e_1 = 0.538 $_{\text{environment;}}$ 0.515 $_{\text{ecophysiology}}$; 0.666$_{\text{micro-environment}}$). However, all sets have the same importance within the canonical correlation.

Table 2. Results from canonical variables derived from generalized canonical correlation.

Sets	Original variables	Canonical variates
Ecophysiology	Effective quantum yield of photosystem II (Φ_{PSII})	-0.625
	Electron transport rate (ETR)	0.548
Environment	Rainfall	-0.245
	Evapotranspiration (ET)	0.989
	Global solar radiation (GSR)	0.547
Micro-environment	Temperature	0.565
	Relative humidity (RH)	-0.773
	Photosynthetic photon flux density (PPFD)	0.747

The correlation coefficients between the original variables and the canonical variates within sets confirmed a high correlation between Φ_{PSII} and ETR inside ecophysiological variables. In addition, a higher association of ET with environmental variables was found. Finally, the PPFD and RH values had the highest correlations with micro-environmental variables (Table 3).

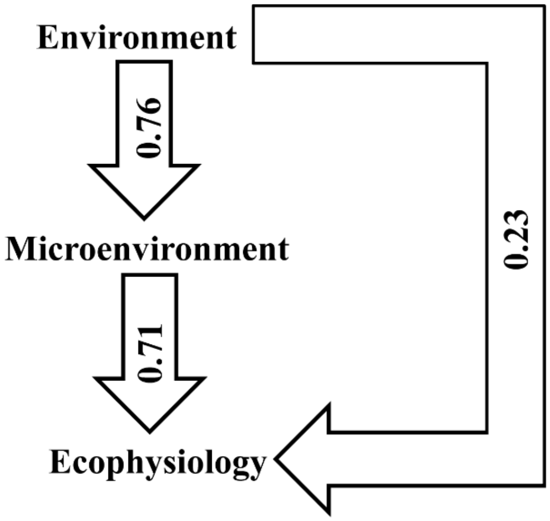

Figure 5. Proposed model derived from the correlation matrix between the canonical variables. It indicates a high correlation between induced warming (micro-environment) and ecophysiology responses in young plants of *E. platyacanthus*.

Canonical discriminant analysis

Because EP was correlated with environment variates, this variable was included for this analysis within of set of variables. In addition, three micro-environmental (mean temperature, PPFD and RH) as well as two ecophysiological (Φ_{PSII} and ETR) variables (both within OTCs and control plots) were analyzed, in order to determinate their significance in photosynthetic responses of young individuals of *E. platyacanthus* under induced climatic warming.

Our two discriminant functions accounted for 100% of the data set total variation, contributing significantly to the separation between treatments (Wilks' ƛ= 0.042; P <0.0001, n = 1440). The first function (eigenvalue of 0.955; P <0.0001) explained 94.1% of the total variation (Table 4) and the second (eigenvalue of 0.060; P <0.0001) explained 5.8 %. Finally, the redundancy value was 0.23.

Table 3. Correlation between original and canonical variables within groups.

Sets	Original variables	Canonical variates
Ecophysiology	Effective quantum yield of photosystem II (Φ_{PSII})	-0.879
	Electron transport rate (ETR)	0.820
Environment	Rainfall	0.157
	Evapotranspiration (ET)	1.039
	Global solar radiation (GSR)	0.017
Micro-environment	Temperature	0.329
	Relative humidity (RH)	-0.452
	Photosynthetic photon flux density (PPFD)	0.619

The classificatory discriminant analysis showed that centroids of each group were significantly different ($P < 0.0001$). ETR, $\Phi_{PSII,}$ PPFD, ET and temperature were the variables that most contributed to centroid separation between treatments (Figure 6). All observations were correctly classified for both treatments (Figure 7).

Discussion

Despite the fact that wide variation observed due to the interaction of several sets of variables, the canonical correlation analysis showed a strongest influence (0.71) between environmental and micro-environmental, as well as micro-environmental and ecophysiological variables. These correlations between climate and micro-climate variables and their influence in ecophysiological aspects have been reported for tropical forest (Yáñez-Espinosa et al., 2006) and arid environment (Delgado-Sánchez et al., 2013). However, our study is the first focusing on the potential long-term impact of climate warming in performance photosynthetic of young individual in cacti species, which simultaneously were analyzing several sets of variables.

Table 4. Results from the first canonical variates vector.

Set	Original Variables	Canonical Variates
Ecophysiology	Effective quantum yield of photosystem II (Φ_{PSII})	-0.643
	Electron transport rate (ETR)	0.990
Environment	Temperature	-0.038
	Relative Humidity (RH)	-0.030
	Photosynthetic photon flux density (PPFD)	0.996
	Evapotranspiration (ET)	-0.016

Our findings allowed to develop a general model for best comprehension of the ecophysiological behavior of *E. platyacanthus* based in environmental and micro-environmental (represented by OTCs) variables. This model provides a greater understanding of the potential photosynthetic responses of cacti species under simulated climatic warming, as well as the possible intensity which global change might impairs the ecophysiology in this species.

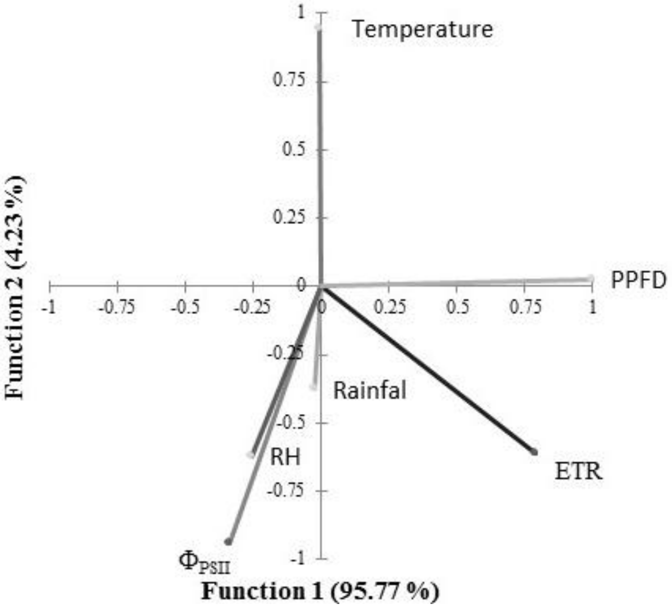

Figure 6. Correlations between the variables and their canonical variates. PPFD = photosynthetic photon flux density, ETR = electron transport rate, Φ_{PSII} = effective quantum yield of photosystem II, and RH = relative humidity.

Most climate models indicate that mean temperature for drylands ecosystems worldwide will increase by 1-3°C by the late twenty-first century (IPCC, 2013; 2014). The recorded 1.6°C increases in mean daily air temperature during our study period fit into these predictions. Particularly, global change projections for Chihuahuan Desert indicate an increase by 1–2°C during summer (June–September) by 2030; and up to 6°C during winter months (January–March) (Tejeda-Martínez et al., 2008). Our results showed a similar temperature increase by summer (1.8°C), but not for winter temperature (1.6°C). Thus, we believe it is necessary to carry out more rigorous field studies in order to assess these forecasts in cold seasons for this desert.

Although not statistically significant, control plots had higher relative humidity than OTCs, which was anticipated because the semiarid climate and warming within these structures. Low relative humidity diminishes the atmospheric water vapor and consequently the water condensation in the soil. This condition modifies the water uptake by the superficial roots of some succulent species (e.g., Matimati et al., 2012; Aragón-Gastélum et al., 2014; 2016). Furthermore, OTC effects on spectral composition of global radiation were unmeasured, and radiation from the chamber walls was not taken into account (Aragón-Gastélum et al., 2014; 2016). In addition, the differences found in PPFD values among seasons may be associated with the amount of light naturally absorbed by the cactus into those. Thus, the effects of the acrylic walls on spectral composition and its possible effects on seedling survival remain to be tested.

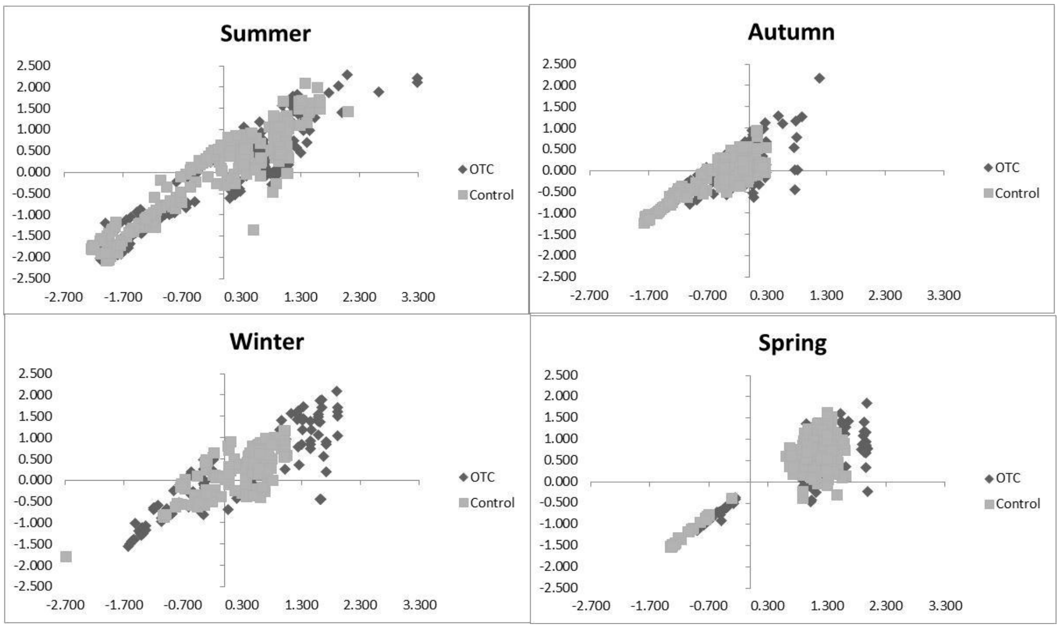

Figure 7. Scatterplot showing the canonical correlation between centroids of the first pair of canonical variates and lineal tendency line between treatments [open top-chambers plots (OTC) and control plots] and seasons.

Increased temperature as well as decreased relative humidity inside OTCs were expected to affect survival of the *E. platyacanthus* young individuals. Nevertheless, we found 100% survival in both OTCs and control plots, which agree with findings for young cacti of *E. platyacanthus* in a four-month period under drought/high temperature conditions (Aragón-Gastélum et al., 2014), but is contrary to findings for southern African quartz-field succulents, which after 4-months summer treatment, displayed between 2.1 and 4.9 times greater plant and canopy mortalities in the open top-chambers than in the control plots (Musil et al., 2005).

Under future climate change conditions, plants could be vulnerable to extinction because their dispersal is usually blocked by mountain barriers and because they are unlikely to migrate fast enough (Ren et al., 2014). Such adult (Smith et al., 1984), young (Aragón-Gastélum et al., 2014), and seedling (Nobel, 1984) cacti have been shown to be rather tolerant of high temperatures. Our results for young individuals of *E. platyacanthus* support the high tolerance for young cacti (Aragón-Gastélum et al., 2014). However, *Stenocactus coptonogonus, Ferocactus histrix* and *E. platyacanthus* two years old seedlings appear be not tolerant because they suffered an extremely negative impact in their survival rate in a scenario of induced climate change (Aragón-Gastélum et al., 2016). Thus, it is possible that seedling cacti tolerance to high temperatures increase with age, as suggested by Nobel (1984) and which is also supported by our results.

Nobel (2010), suggests that desert succulents have high tolerance to water and temperature stress. However, predictions of global change studies propose that some succulent species will decrease their species range due to increased temperature and diminished precipitation (Butler et al., 2012; Cortes et al., 2013; Dávila et al., 2013). The high survival rate found in young *E. platyacanthus* individuals confirms an extraordinary tolerance of this species under extreme temperature in field and therefore do not support these predictions. This amazing tolerance in *E. platyacanthus* might be explained due it is the most widespread cacti species (Jiménez- Sierra et al., 2007). It has been suggested that the most widespread species would be less affected by climate change, proposing that past selection on plasticity might allow them to survive under variable conditions (Ureta et al., 2012).

In addition, seasonal variations in photosynthetic responses were registered. We found decreased Φ_{PSII} and ETR values during summer and spring but increased in autumn and winter inside OTCs. Thus, our hypothesis that photosynthetic responses and survival would be adversely affected due to the increase temperature stress by induced climatic warming through the different seasons of the year was confirmed.

The induced warming increases the abiotic stress on succulent plants (Musil et al., 2005; 2009; Aragón-Gastélum et al., 2014; 2016). However, physiologically these species have diverse adaptive, avoidance, or acclimation mechanisms to cope stress caused by high temperatures (Kozlowski and Pallardy, 2002; Hasanuzzaman et al., 2013), and they can trigger change in both physiological and molecular responses in order to facilitate the plant persistence (Chen et al., 2006). These mechanism includes the synthesis of heat-shock proteins (HSP), which have crucial importance in the plants because it act as molecular chaperones to avoid denaturation or aggregation of vital proteins and facilitating protein refolding (Sarkar et al., 2009). HSP has been linked with maintenance of electron transport through photosystem II during heat stress (Heckathorn et al., 1998). Consequently, a high HSP synthesis is expected to occur in *E. platyacanthus* during summer and spring when heat stress is possibly more detrimental by cactus.

Furthermore, increases (although not statistically significant) in Φ_{PSII} and ETR values in autumn and winter might indicate a buffer to low temperature (with lower PPFD values) by micro-climate (OTC plots), which improve the photosynthetic response of *E. platyacanthus*, and possibly this micro-environment allows the maintenance of the thermal threshold as well as tolerate the effect of low temperature in this species. It can also be explained by the accumulation of one specific protein known as dehydrin (Close, 1997). Dehydrins are proteins involved in formation of plant protective reactions in response to low temperature (Close, 1997; Allagulova et al., 2003). There is evidence of the responses in cacti species at low temperatures from -7 to -20^{o}C (Nobel, 1982; Nobel, 1996; Nobel and De la Barrera, 2003). Our results suggest a possible higher synthesis of HSP during the warm season as well as higher synthesis of dehydrins in cold season, which would elucidate better the extraordinary tolerance mechanisms of *E. platyacanthus*; however, both premises, about high HSP as well as dehydrins synthesis in cacti species remains also to be tested.

Conclusions

We found a strongest correlation between environmental and micro-environmental, as well as micro-environmental and ecophysiological variables. Besides, after induced warming *E. platyacanthus* showed a decrease in the photosynthetic performance during the warmer seasons (summer and spring), but an increase in autumn and winter. However, this species showed 100% survival within OTCs and control plots. These results reflect the extraordinary tolerance of *E. platyacanthus* to high temperatures under an induced climate change scenario. Thus, the potential effects to future global warming in young individual for this species possibly not be as severe.

Most predictions of global changes have been suggested for adult plants (e.g., Musil et al., 2005; 2009; Ureta et al., 2012). However, there are few studies taking into account other plant development phases, such as seeds, seedlings or young plants (Aragón-Gastélum et al., 2014; 2016), which are also susceptible to extreme temperatures (Drennan, 2009). In future research, we believe necessary to simultaneously assess the behavior of cacti species in these development phases in order to identify the most critical thermal tolerance thresholds within each of those under simulated global warming.

Our study acquires substantial importance because it provides novel data to better understanding about the potential impact of global warming in desert plants, specifically cacti species, in early stages of development. These results can help to refine population persistence models for these or related species.

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Chapter 6

Seasonal dynamics of seed soil bank and dormancy of three succulent species under a climate change scenario

Abstract

Soil seed banks (SSB) are crucial in the vegetation dynamics in arid environments. However, the long-term effect of climate change in plant regeneration mechanisms has been poorly studied. The Chihuahuan Desert harbors a high diversity of succulent plants. We experimentally assessed the seasonal dynamics and SSB persistence in three succulent species from this area under simulated soil warming conditions. Hexagonal open top-chambers (OTCs) were used to simulate the effect of global warming in buried seeds of *Echinocactus platyacanthus*, *Yucca filifera* and *Agave striata* during one year. Fresh seeds were buried within and inside OTCs in February 2013 and exhumed to test germinability at the end of each season. OTCs had higher soil temperature than in control plots. *Yucca filifera* was not sensitive to temperature increases and showed high germination buried in the soil; *A. striata* exhibited high germinability in spring, and varied across time, being significantly high within OTCs than in control plots in autumn. Seeds of *A. striata* lost viability in summer and subsequently until the end of the experiment. *Echinocactus platyacanthus* showed dormant cycling, which lead to high germinability in spring and low germinability in summer and autumn; nevertheless, it had significantly higher germinability within OTCs in summer, but exponentially increased in both treatments at end of winter.

Having non-dormant (*Y. filifera*) and dormant cycling (*E. platyacanthus*) seeds, might help these species to achieve persistence under expected temperature increments. Seed viability decline in *A. striata* might increase the risk of local extinctions of this species. This is the first report of SSB persistence under induced soil warming in succulent species for American deserts.

Key words: Asparagaceae, Cactaceae, Chihuahuan Desert, Dormancy cycling, Germination, Global warming, Soil seed bank.

Introduction

Soil seed banks (SSB) are crucial for population dynamics in many plant communities (Baskin and Baskin, 1998; Leishman et al., 2000a). SSB guarantees the natural regeneration and persistence of ecosystems because it provides a reserve of viable seeds either on the surface or buried in the soil (Barbour et al., 1999; Sánchez Salas et al., 2015); mainly in habitats subjected to variable disturbance regimens and unpredictable environmental conditions (Fenner and Thompson, 2005; Venable, 2007), such as arid environment (Montiel and Montaña, 2003; Flores and Jurado, 2005; Álvarez-Espino et al., 2014; Sánchez Salas et al., 2015). Global climate models forecast that arid environment are expected to experience both, increased temperatures and increased variability in the frequency and magnitude of rainfall events (IPCC, 2013). Thus, in order to elucidate the long-term effects of climate change in plant species, it is necessary to link future environmental changes (e.g., increased temperature and decreased rainfall) to mechanisms that control plant population processes such as seed germination (Ooi et al., 2009; Walck et al., 2011).

Despite the ecological dominance of succulent species in American arid and semi-arid environments (Reyes- Agüero et al., 2000; Ortega-Baes and Godínez-Alvarez, 2006); population and demographics studies (like SSB) in these taxa are still scarce (Bowers, 2000; 2005; Montiel and Montaña, 2003; Cheib and Garcia, 2012; Álvarez-Espino et al., 2014). The latter authors determined a high germination on dormant seeds of *Stenocereus stellatus* (Cactaceae) after 6 months buried in the soil. However, the potential impact of climatic warming on seed germination and SSB persistence of succulent species has been poorly assessed (Pérez-Sánchez et al., 2011).

A key factor in the demographic patterns of plants is seed dormancy (Rojas-Aréchiga and Vázquez-Yanes, 2000). It is a process where physiological activities cease in a reversible form, in order to help maximize the probability of seedling establishment and spread the risk of recruitment failure (bet-hedging strategy) across time (Baskin and Baskin, 1998; Flores and Jurado, 2005) until conditions for seed germination are suitable (e.g., suitable moisture, light or temperature); thus, this mechanism is linked with the formation of SSB (Baker, 1989). Some recent research about global warming effect in succulent species have considered physiological (Aragón-Gastélum et al., 2014), ecological (Aragón-Gastélum et al., 2016), biochemical (Musil et al., 2005: 2009) and human (Martorell et al., 2015) aspects; neglecting some functional traits in population and community aspects such as: the key role of seed germination (Jiménez-Alfaro et al., 2016) and the SSB dynamics in these species under temperature increases expected for arid environments. Therefore, experimental studies focusing in demographic responses of plant populations to climate changes projections are crucial in order to predict recruitment patterns, population dynamics as well as future species distributions (Thomas et al., 2004), particularly in arid and semi-arid environments.

The largest warm desert of North America is The Chihuahuan Desert (Archer and Predick, 2008); ranging from southwestern United States to the Central Mexican Highlands. Forecasts of global change for this region indicate an increase in summer temperatures (June–September) of 1–2^{o}C by 2030 (Tejeda-Martinez et al., 2008). However, the greatest temperature increment is predicted to occur during the winter months (January–March), when monthly average temperatures might increase by up to 6^{o}C (Tejeda-Martinez et al., 2008). This critical outlook has created strong concerns in ecologists today because this desert harbors a high richness of succulent plants (Rzedowski, 1991), and many of them protected by Mexican environmental laws (SEMARNAT, 2010).

Aragón-Gastélum et al. (2014; 2016), using OTC chambers found a mean air temperature increase by 1.9^{o}C in the southernmost section of the Chihuahuan Desert. These findings could indicate a potential increase also in soil temperature; nevertheless, this premise must be assessed. Thus, because of the crucial role that SSB and dormancy might have in population dynamics in arid and semi-arid environments; our aims in this research were: 1) to determinate whether induced increment in mean air temperature in field might increase the mean soil temperature, and 2) to assess the seasonal dynamics and persistence of SSB in three succulent species from Chihuahuan Desert, namely: *Echinocactus platyacanthus* Link & Otto form *visnaga* (Cactaceae), *Yucca filifera* Chabaud (Asparagaceae), and *Agave striata* Zuccarini (Asparagaceae) during one year under simulated soil warming conditions. We hypothesized that induced increments in mean air temperature will increase the mean soil temperature and this will shift the seasonal dynamics and persistence of SSB in the three succulent species studied, decreasing their germinability across time.

Materials and methods

Study species and plant material

The three target species are endemic to México and have a wide distribution (Irish and Irish, 2000; Jiménez-Sierra et al., 2007). *Echinocactus platyacanthus* f. *visnaga* is a barrel-like cactus that can reach 2 m height and 80 cm in diameter (Jiménez-Sierra et al., 2007).

Nevertheless, overexploitation for food and ornamental purposes has seriously endangered the natural populations of this species (Jiménez-Sierra et al., 2007). Accordingly, it has been included as a specially protected species in the environmental laws of México (SEMARNAT, 2010). *Echinocactus platyacanthus* is included as *near threatened species* in the Red List of the International Union for Conservation of Nature (IUCN, 2014). This species has a mean annual mortality in natural populations of 19.7% for seedlings and 2.5% for adults. (Jiménez-Sierra et al., 2007).

Yucca filifera is one of the largest and most common yucca species; it can reach 9 m in height and 1.5 m in diameter (Irish and Irish, 2000). Because of its large size, the main use of *Y. filifera* is ornamental (Irish and Irish, 2000). *Agave striata* is a short rosette plant with that can reach 100 cm in height and 119 cm in diameter (Irish and Irish, 2000). This species forms dense and extensive colonies even in dry and very dry environments; thus, it is often used as a garden plant in northeastern México (Irish and Irish, 2000). *Yucca filifera* and *A. striata* are not included under any protection status (SEMARNAT, 2010), although they are widely distributed, their population dynamics have been poorly studied.

In San Luis Potosí (México), *E. platyacanthus* shows maximum flowering periods during summer (June-September), produced fruits from late summer to mid-winter (January-February), which coincides with increasing photoperiod (Trujillo-Argueta, 1982). For *A. striata* and *Y. filifera*, both flowering and fruiting periods are similar to that for *E. platyacanthus* (Rentería and Cantú, 2003; Trejo-Salazar et al., 2015). Ripe fruits from the three studied species were collected in the surroundings of the city San Luis Potosí during late January 2013, from at least 10 mother plants for each species.

Initial viability and germination trails

The harvested fruits from each species were transferred to the ecology lab from the Instituto Potosino de Investigación Científica y Tecnológica (San Luis Potosí, México); where they were opened and their seeds removed. Fifty batches of seeds for species were separated; in a lot, 30 seeds for species were separated in three groups of 10 seeds in order to evaluate initial viability. These seed groups were imbibed in distilled water for 24 hr; after this, a tetrazolium solution (at 1%) was applied to each group; then, each seed was transversely cut to expose the embryos in the stereoscope after 48 hours. The seeds showing no apparent damaged embryos were considered viable (Baskin and Baskin, 1998).

In a second seed lot, 100 seeds of each species were randomly selected; thus, initial germinability was assessed in a completely randomized experiment considering ten replicates in experimental units represented by a petri dish with 10 seeds using cotton as a substrate. Petri dishes were placed in a growth chamber with 80% relative humidity, a photoperiod of 12 /12, under a constant temperature of 25°C. Germination records were made daily until 30 days after the beginning of the experiment. The remaining 48 batches containing 55 seeds (2640 seeds) for species were separated randomly. Each seed lot was placed inside translucent cloth bags of 50 cm by 50 cm and they were sewn with thread to prevent seed loss. These seed batches were subjected to an induced warming treatment in the field in order to assess how climate change could affect the seasonal dynamics and persistence of SSB in these species.

Experimental design

This experiment was conducted in an abandoned agricultural field located in the southernmost section of the Chihuahuan Desert (22°14’11’’ N, 100°51’46’’ W, 1844 m), central, México. Annual precipitation in the study site averages 341 mm and rainfall events are concentrated in the summer months. Mean annual temperature is 17.8°C, but it can be higher than 35°C in summer (Medina et al., 2005). Vegetation in the study site is dominated by sclerophyllous shrubs, with some cacti and succulent monocots scattered among these woody species. In this study site, vegetation was cleared on a surface area of 25 x 25 m that was fenced with wire fence (2 m height) to avoid the access of cattle and people. Within this exclosure, twelve experimental plots of 5 x 5 m were drawn by following a rectangular arrangement (3 plots width x 4 plots long). Six of these plots were randomly assigned to the induced warming treatment, while the other six plots were used as control under the current environmental conditions (Aragón-Gastélum et al., 2014; 2016).

Hexagonal open-top chambers (OTCs) were used to simulate warming conditions. We used these structures because they allow passive heating and are a simple approach for assessing the responses of plants to climate warming in the field (Musil et al., 2005, 2009; De Frenne et al., 2010; Bokhorst et al., 2013; Aragón-Gastélum et al., 2014; 2016). OTCs were built with UV-resistant transparent acrylic (3 mm thick; wavelength transmission 110 <280 nm) by following the design proposed by Marion (1996). The resulting structures were 0.50 m tall, 1.5 m wide in the open-top, and 2.08 m wide in the base attached to the ground. This OTC design allows daytime passive heating by increasing air temperature by 1.9-5.0ºC, respect to the external environment (Musil et al., 2005; 2009; Aragón-Gastélum et al., 2014; 2016).

To assess the magnitude with which OTCs modified microclimate, air and soil temperature as well as relative humidity were continuously monitored within and outside these structures during the entire experiment. For this, data-loggers (HOBO Pro v2, Onset Computer Corporation, MA, USA and HOBO Pendant® Onset Computer Corporation, MA, USA) were programmed to record these variables every hour. The first devices were fixed 10 cm above the ground at the center, while the second were buried <0.5 cm under the soil surface, both inside and outside of each OTC. These measures were conducted from March 1st 2013 to February 29th 2014 and data recorded by data-loggers were used to calculate the daily mean air and soil temperatures, maximum and minimum soil temperature as well as average daily mean air relative humidity in both treatments (OTC and control). The maximum mean soil temperature was estimated considering a temperature range by 13:00-16:00 h during day, when temperatures reach their higher values (Medina et al., 2005). Additionally, data of daily rainfall (mm) were obtained of meteorological station of National Institute of Forestry, Agriculture and Livestock (INIFAP San Luis Potosí, México), adjacent to the study area.

The batches containing the seeds of three succulent species were arranged within this experimental design a week after mounting the OTCs in the field. Four lots for species were buried <0.5 cm under the soil surface, at the center of each experimental plot. In plots with OTCs, the seed lots were located directly below the open-top to avoid overwarming and reduced rainfall interception by the acrylic walls. One bag for species and treatment (six in OTC and six in controls) was exhumed at the end of each season during the study; then; germinability was assessed in a completely randomized experiment considering twelve replicates in experimental units represented by a petri dish (5 petri dishes by replicate) with 10 seeds each (50 seeds by bag). Petri dishes were placed in a growth chamber with 80% relative humidity and a photoperiod of 12 /12h, under a constant temperature of 25°C. Germination records were made daily for 30 days. The remaining five seeds x replicate and treatment (60 seeds for each species) were transversely cut in order to assess viability across seasons by exposing of the embryos in the stereoscope.

Statistical analysis

Abiotic variables mentioned above were summarized for each data-logger (n = six per treatment) and compared between OTCs and control plots with repeated measures ANOVAS. Viability and germinability were analyzed by one-way ANOVAS for each species. Germinability across seasons was analyzed by two-way ANOVAS for each species with burial time and treatment (OTCs and control plots) as predictor factors.

Results

Abiotic variables

Between March 1st 2013 and February 28st 2014 average daily air temperature during the entire study was 19.8ºC (± 0.16ºC, 95% Confidence Intervals) inside OTCs and 18.1ºC (± 0.02ºC) in control plots. This variable significantly differed between these two treatments ($F_{(1,6)}$ = 23.94, P< 0.0001), as well as with burial time ($F_{(3,2184)}$ = 156.61, P< 0.0001), where OTCs always showed higher mean temperatures than control plots ($F_{(3,2184)}$ = 92.17, P< 0.0001). In addition, we found significant differences in mean temperature across seasons, maintaining a trend similar to that explained above. During spring, mean daily air temperature was higher in OTCs (23.6ºC ± 0.16ºC) than in control plots (21.3ºC ± 0.04ºC;

$F_{(3,552)}$ = 31.80, P< 0.0001; Figure 1a). In summer, OTCs recorded higher temperature (23.0^{o}C ± 0.19^{o}C) than in control plots (21.2^{o}C ± 0.07^{o}C; $F_{(3,558)}$ = 38.27, P< 0.0001; Figure 1b). During autumn, it was also higher temperature inside OTCs (17.7^{o}C ± 0.16^{o}C) than in control plots (16.4^{o}C ± 0.01^{o}C; $F_{(3,528)}$ = 5.799, P< 0.0001; Figure 1c), and in winter it was also higher temperature inside OTCs (14.5^{o}C ± 0.2^{o}C) than in the control plots (13.2^{o}C ± 0.01^{o}C); $F_{(3,528)}$ = 6.045, P = 0.0149; Figure 1d).

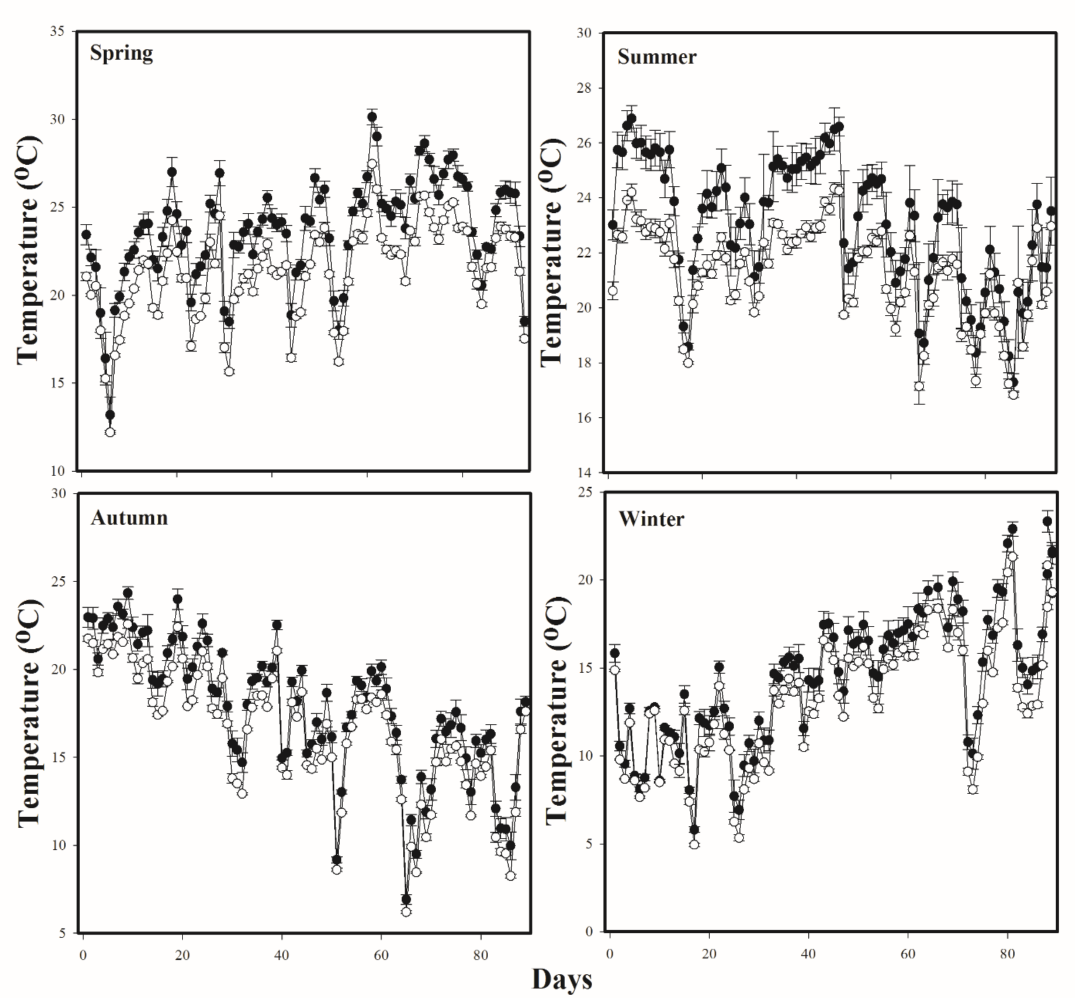

Figure 1. Daily air mean temperature measured in control plots (empty symbols) and within OTCs (solid symbols) used in the field experiment across of the seasons (± 95% CI).

Total rainfall during the study period was 296.2 mm, it was distributed mainly in summer, autumn, and winter (Table 1). Average daily relative humidity of the air in the control plots was 65.8% (± 0.33%) during the study period, while this variable averaged 63.3% (± 1.12%) within the OTCs. We found significant effects of the treatment ($F_{(3,2184)}$ = 4.36, P = 0.0371) and the burial time ($F_{(3,2184)}$ = 86.92, P< 0.0001); aslo the interaction between treatment and burial time was statistically significant ($F_{(3,2184)}$ = 99.29, P< 0.0001), findings higher values of relative humidity in the control than in the OTCs. However, daily relative humidity was not affected by seasons (P> 0.005).

Table 1. Seasonal changes in precipitation registered by meteorological station of National Institute of Forestry, Agriculture and Livestock (INIFAP) Between March 1st 2013 and February 28st 2014.

Season	**Precipitation (mm) ± S.E.**
Spring	21.7 ± 0.12
Summer	103 ± 0.30
Autumn	87.9 ± 0.31
Winter	83.6 ± 0.43
Total	**296.2 ± 17.93**

Average daily mean soil temperature was 23.1°C (± 0.21°C) in OTCs and 22.1°C (± 0.15°C) in control plots. Thus, mean soil temperature was affected by treatments ($F_{(1,6)}$ = 4.78, P = 0.0290) and burial time ($F_{(3,2184)}$ = 16.09, P< 0.0001), and by the treatment x burial time interaction ($F_{(3,2184)}$ = 115.70, P< 0.0001). In spring, we found higher daily mean soil temperature inside OTCs (29.0°C ± 0.21°C) than in control plots (27.0°C ± 0.10°C; $F_{(3,552)}$ = 31.01, P< 0.0001; Figure 2a). We found no differences between treatments in the other seasons: summer ($F_{(3,558)}$ = 3.45, P = 0.0647; Figure 2b); autumn ($F_{(3,528)}$ = 0.099, P = 0.7529; Figure 2c), and winter ($F_{(3,528)}$ = 2.209, P = 0.1390; Figure 2d).

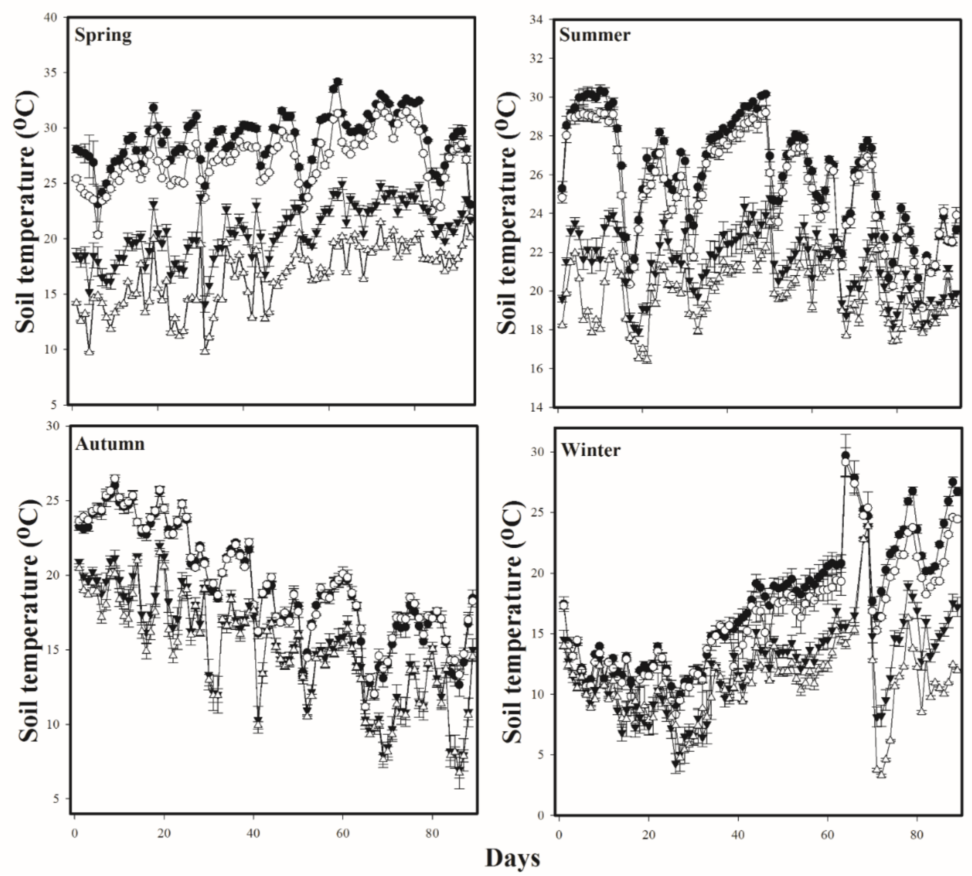

Figure 2. Mean and minimum daily soil temperature measured in control plots (empty symbols) and within OTCs (solid symbols) used in the field experiment across of the seasons (± 95% CI). Daily temperature (circles) and minimum temperatures (triangles).

Minimum daily soil temperature differed between treatments ($F_{(1,6)} = 30.092$, $P < 0.0001$), burial time ($F_{(3,2184)} = 123.989$, $P < 0.0001$) and the interaction between time and treatments ($F_{(3,2184)} = 57.358$, $P < 0.0001$). It was higher inside OTCs (17.4ºC ± 0.30ºC) than in the control plots (15.5ºC ± 0.08ºC). Minimum daily soil temperature also was affected by season. In spring, it was lower in the control plots (16.5ºC ± 0.11ºC) than within OTCs (20.6ºC ± 0.52ºC; $F_{(3,552)} = 107.534$, $P < 0.0001$; Figure 2a). Similar results were found for summer (control plots recorded 19.8ºC ± 0.11ºC) and OTCs had 21.3ºC ± 0.31ºC); $F_{(3,558)} = 36.63$, $P < 0.0001$; Figure 2b); autumn (control plots was 14.7ºC ± 0.25ºC) and OTCs had 15.1ºC ± 0.5ºC; $F_{(3,528)} = 0.740$, $P = 0.3909$; Figure 2c) and winter (10.8ºC ± 0.22ºC) in the control plots vs. 12.2ºC ± 0.33ºC in OTCs ($F_{(3,528)} = 7.364$, $P = 0.0073$; Figure 2d).

Maximum daily soil temperature was affected by burial time ($F_{(3,2184)} = 37.43$, $P < 0.0001$) and by the treatment x burial time interaction ($F_{(3,2184)} = 147.53$, $P < 0.0001$), but not by treatment ($F_{(1,6)} = 0.11$, $P = 0.7417$). The average value for this variable in OTCs was 31.5ºC (± 0.52 ºC), while in control plots it was 31.3ºC (± 0.35ºC). In addition, maximum daily soil temperature did not differ by treatment across seasons: spring ($F_{(3,552)} = 0.37$, $P = 0.5417$; summer ($F_{(3,558)} = 1.382$, $P = 0.2412$); autumn ($F_{(3,528)} = 0.075$, $P = 0.7845$), and winter ($F_{(3,528)} = 0.049$, $P = 0.8259$). The hottest days were at the end of April (mid spring) and this variable recorded 47.1ºC ± 0.81 in OTCs and 46.9ºC ± 0.17 in control plots, without significant differences between both treatments ($F_{(1,4)} = 0.064$, $P = 0.8119$).

Seed viability and initial seed germination

Initial viability of fresh seeds was high ($F = 1.00$, $P = 0.4218$) for the three studied species, which was 100% for *Y. filifera* and *E. platyacanthus* as well as 90 % (± 10%) for *A. striata*

(Figure 3a). There were no changes in seed viability across seasons in these species, except for *A. striata*, which showed less viability in autumn (30%) and winter (15%). Significant differences (F = 16.20, P = 0.0002) between species in germination of fresh seeds were found. For *Y. filifera* the initial germination reached 83% ± 9.4%, while for *A. striata* and *E. platyacanthus* was 30% ± 6.1% and 49% ± 4.4%, respectively (Figure 3b).

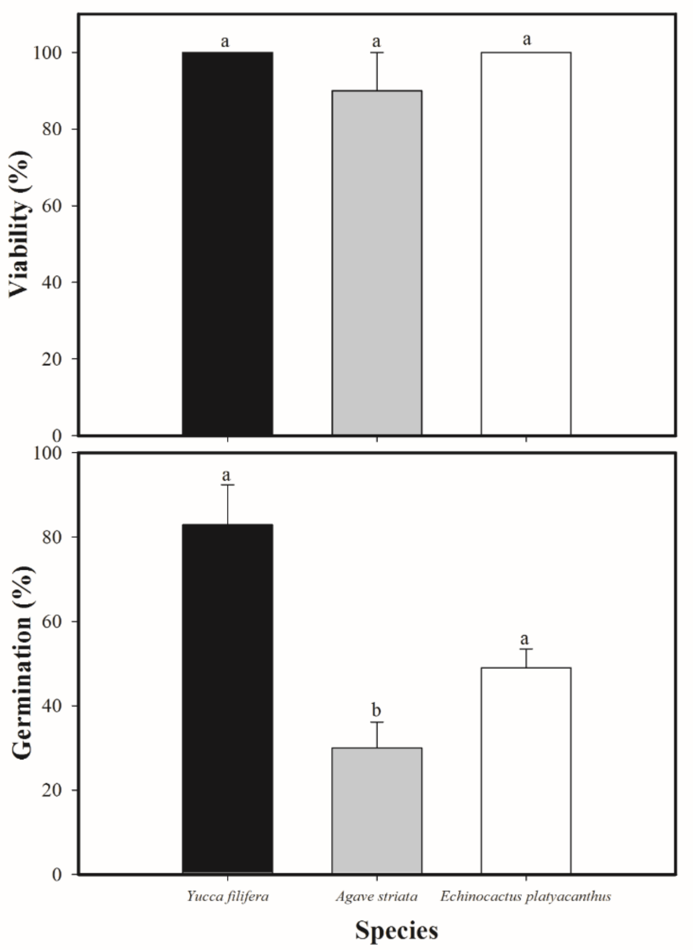

Figure 3. Initial viability and germination (mean ± standard error) in fresh seeds of three succulent species. Different letters indicate significant differences (P < 0.005).

Germination under warming treatments across seasons

We found variation in germinability responses for the three studied species; *Y. filifera* seeds always had high germination; which was similar between treatments ($F_{(1,116)} = 1.411$, P = 0.2372) and seasons ($F_{(1,116)} = 3.920$, P = 0.0500). In spring and in summer, germinability was high ≥ 87% in both treatments (Figure 4). Seeds exhibited high germination (up 90% in both OTC and control plots) within the bags buried on soil in autumn. Thus, the remaining bags of *Y. filifera* seeds were exhumed and excluded for rest of experiment.

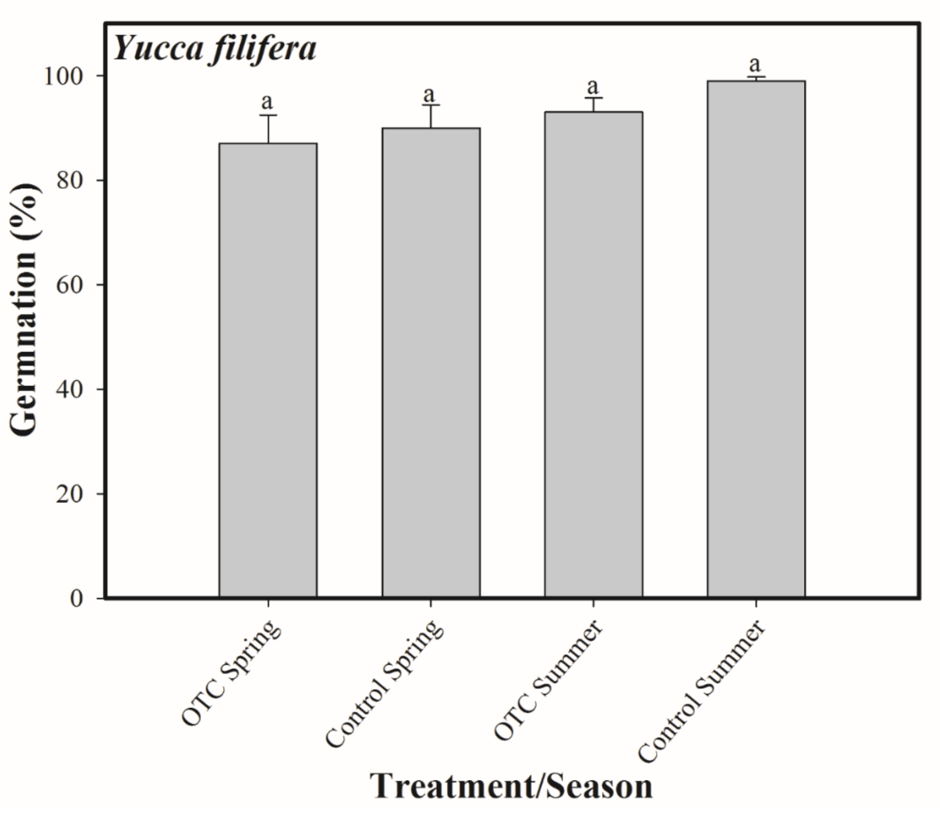

Figure 4. *Yucca filifera* seed germination in simulated warming conditions (OTC) and in control plots across the seasons (mean ± standard error). Different letters indicate significant differences (P < 0.005).

In contrast, *A. striata* exhibited significant differences between treatments ($F_{(3,208)}$ = 17.1867, P< 0.0001), seasons ($F_{(3,208)}$ = 89.0053, P< 0.0001) and the interaction treatment/seasons ($F_{(3,208)}$ = 9.2934, P< 0.0001). In spring, *A. striata* seeds had high germination (> 85%) in both treatments. In summer, the germinability within OTCs was similar than in the control plots; but in autumn, germinability was higher inside OTCs than in the control plots, and in winter germinability was low although similar within OTCs and in control plots (Figure 5a).

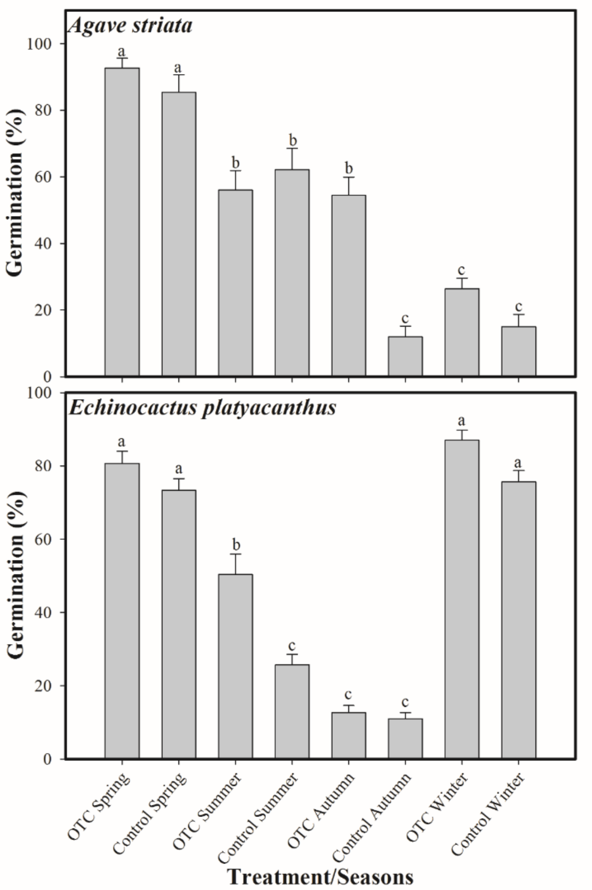

Figure 5. *Agave striata* and *Echinocactus platyacanthus* seed germination in induced warming conditions (OTC) and in control plots across the seasons (mean ± standard error). Different letters indicate significant differences (P < 0.005).

E. platyacanthus germination also was affected by treatment ($F_{(3,227)} = 23.693$, $P < 0.0001$), season ($F_{(3,227)} = 204.915$, $P < 0.0001$), and the treatment x season interaction ($F_{(3,227)} = 4.579$, $P = 0.0039$). In spring, this species had high germination (>70%) in both treatments, but in summer germinability was higher inside OTCs than in the control plots although germinability was low in general. However, in winter germinability was higher again in both treatments (Figure 5b).

Discussion

Forecast of climate change indicate a 1-3°C increase of air temperatures for arid zones by the late twenty-first century (IPCC, 2013; 2014). The high mean air temperature recorded in a global warming scenario (OTC = 1.7°C) between March 1st 2013 and February 28st 2014 is fitted for this projection as expected. In addition, we found a decreased in mean relative humidity within OTCs (2.5%) than in control plots.

The low relative humidity inside OTC diminishes the atmospheric water vapor and consequently the water condensation in the soil (Aragón-Gastélum et al., 2014; 2016). These conditions promoted the increase in mean air temperature above explained and consequently, an increase of mean soil temperature (1°C) was registered during the study period. Our hypothesis that higher mean air temperature inside OTCs will induce an increase in the soil mean temperature was corroborated and support previous findings for annuals and short-lived perennials species from arid Australia region (Ooi et al., 2009; 2012; Ooi, 2012). In the Southern Chihuahuan Desert, soil temperature can within short time periods reach 70ºC in the hottest time of the day (Nobel et al., 1986). During the study period, we found the higher maximum temperature registered values at mid spring (48.0ºC) in the warming treatment. Our maximum temperature records are lower than those above. It is possible that this variation be provoked for rainfall variations across time; in our experiment, it was relatively high (nearly 300 mm). Nobel et al. (1986) do not indicate the rainfall values.

In alpine areas, there are expectation because these soil warming modifies their properties and it can constrain regeneration of plant via seeds (Brown and Vellend, 2014). Thus, it is possible that the mean soil temperature increase could also shift the physic, chemical, biochemical and biological structure of the soil in arid and semi-arid ecosystems; thus, elucidate these hypothetical changes as well as the potential effect in the population dynamics of plant species in these areas, remain to be evaluated.

We also hypothesized that an increase in mean soil temperature will change the seasonal dynamics and persistence of SSB in the three target species and, subsequently, this mean soil temperature will decrease their germinative capacity across time. Our data shows differential responses in germinability for the studied species; *Agave striata* displayed high germination in both treatments during spring (dry season); however, it lost viability in summer and subsequently until the end of the experiment. This species had shown high germination response over a wide range of temperature, from 15 to 30ºC (Ramírez-Tobías et al., 2012). Then, it is possible that this viability lost can be associated with attack of fungi and pathogens because soil moisture increased with rainfall in summer.

In contrast, the seeds of *Y. filifera* displayed high germinability in both treatment (OTC and control plots) during spring and summer, but because 100% seeds germinated in the buried bags in summer, we can establish that this species do not form persistent SSB, although seeds of this species can maintain their viability after four years stored (Cambrón-Sandoval et al., 2013). Our results in germinability support evidence of no seed dormancy for *Agave striata* and *Y. filifera* (Jiménez-Aguilar and Flores, 2010).

Low seedling emergence (1-20%) of *Agave macroacantha* (Arizaga and Ezcurra, 2002) and *Yucca brevifolia* (Reynolds et al., 2012) have been found under and outside nurse plants after two years; moreover, this low seedling emergence is also consistent for *Banksia* species from Mediterranean ecosystems under temperature soil increases and rainfall variations using OTCs (Cochrane et al., 2015). However, Pérez-Sánchez et al. (2011), found high seed germination (75%) in *Agave lechugilla* after exposure for 2 h at 70ºC and then at room temperature every day for 14 d. These results are contrary for germination recorded in *A. striata*; thus, it is possible that future temperature increases could put at risk sexual reproduction of this species. Furthermore, we found a high tolerance to physic stress in *Y. filifera* even inside OTCs, which was reflected in a high germinability; however this result do not agree with low germinability (48%) after exposure for 2 h at 70ºC and then at room temperature every day for 14 d by Pérez-Sánchez et al. (2011) for *Y. decipiens*. Our findings indicate that *Y. filifera* and *A. striata* do not form SSB. Therefore, our hypothesis about that the increments in mean soil temperature will shift the seasonal dynamics and persistence of SSB, as well as germinability across time, was only confirmed for *A. striata*.

The germinative results for *E. platyacanthus* showed a positive feedback between mean soil temperature increases and seed germination; even, these soil temperature enhances allowed a relative high germinability within OTCs in summer. Our findings agree with Ordoñez-Salanueva et al. (2015), which found that projected future temperature increases do not have detrimental effect in germinability in *Polaskia chende* and *P.*

chichipe, two Mexican cacti species. However, do not agree with Ooi et al. (2009; 2012), and Ooi (2012), which established that because of the close relationship between climatic variables, seed dormancy and seed germination, it is expected that the forecast climatic change will affect plant recruitment.

The buried seeds of *E. platyacanthus* acquired secondary dormancy during the rainy season (autumn), which was alleviated during the end of the subsequent dry season (spring). For *E. platyacanthus*, seed germination increased after exposure for 2 h at 70ºC and then at room temperature every day for 14 d, although seed dormancy was not broken (Pérez-Sánchez et al., 2011), probably because its seeds need seasonal temperature changes to break seed dormancy. Our results are similar to findings for *Comanthera bisulcata*, *Syngonanthus anthemidiflorus*, and *Syngonanthus verticillatus* (Eriocaulaceae), which form seed banks of the persistent type and have consecutive cycles of germination/dormancy, accompanying annual seasonal changes (Garcia et al., 2014).

The germination inter-seasonal data of *E. platyacanthus* indicated that this species has a transitory seed bank, which was promoted by dormancy cycles. Cycling/or enforced dormancy is regulated by environmental conditions such as light and/or temperature (Rojas-Aréchiga and Vázquez-Yanes, 2000); thus, seeds are ready to germinate once the environmental restrictions are eliminated; therefore, it represent a mechanism that help regulate both, germination and emergence timing (Baskin and Baskin, 1985; 1996), providing a favorable window to increase the chances of seedlings survival in seasonally varying environments. Our results shows that, dormancy cycling in *E. platyacanthus* leads to the formation of SSB with ungerminated and viable seeds, which are capable of replacing parent plants.

Fluctuating temperatures have been found to influence the timing of *in situ* seed cycling/or enforced dormancy germination in alpine communities (Mondoni et al., 2012; Jaganathan et al., 2015; Rosbakh and Poschlod, 2015), and cold deserts (Cao et al., 2014) in order to contribute to seedling establishment and to maintenance of a persistent SSB in these species. The exponential germinability increase showed in *E. platyacanthus* during warm season (spring) as well as induction of cycles of seed dormancy during wet/cold seasons (autumn) support these results. According to the seasonal germinative behavior showed by *E. platyacanthus*, the first step to a successful population dynamic (such as seedlings emergence) does not appear to be compromised under simulated expected temperature increments. Thus, these results are contrary to our germinative hypothesis.

Recent studies on ecophysiology of *E. platyacanthus* under a scenario of induced climate change in this area were carried out. Aragón-Gastélum et al. (2014), found 100% survival on five year old plants, which is agreement with our findings; but not with Aragón-Gastélum et al. (2016), who registered high mortality on two year old plants. Thus, under future global warming this species might have an exponential emergence that could ensure its persistence; it considering that tolerance to elevated temperature may increase with age (Nobel, 1984; Aragón-Gastélum et al., 2014; 2016). Nevertheless, this premise needs to be tested considering an integral approach considering other biotic factors such as: seed production, seed predation, and dispersal in cacti species and more succulent plants.

The environmental, ecological and anatomical implications of dormancy have been widely studied in Cactaceae (e.g., Olvera-Carrillo et al., 2003; Mandujano et al., 2005; Orozco-Segovia et al., 2007; Flores et al., 2008; Delgado-Sánchez et al., 2010; 2011); however, studies about dynamics of SSB are scarce (see Bowers, 2000; 2005; Álvarez-Espino et al., 2014). In Asparagaceae, both issues are still unexplored; therefore, our work acquires substantial importance because it is pioneer in assessing these two key aspects of seed ecology under expected climate conditions in the southernmost section of the Chihuahuan Desert by 2030. The germination cycles are well known in annual (e.g., Baskin and Baskin, 1998; Carter and Ungar, 2003) species, even within cold desert (Cao et al., 2014). Nevertheless, it is the first study founding dormancy cycling for a cactus species, and the first in projecting mean soil temperature under a future scenario of global warming from American deserts.

Conclusions

Coupled mean air increases (1.7ºC) with decreases in mean air relative humidity (2.5%) promoted a significant mean soil temperature increase (1ºC) within our induced climatic change after one year. This soil temperature enhance changed the dynamics and persistence of soil seed bank (SSB) as well as dormancy in the species studied, which was reflected in differential responses in germinability across time.

Demographic patterns are expected to shift both, spatially and temporally in response to environmental variability, restricting recruitment rates of plant species and that it is already evident inside and between wild populations (Reynolds et al., 2012). Thus, conservation effort must focus in assessing this approach in order to increase the accuracy of projections expected to future species distributions in drylands.

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Chapter 7

Final Remarks

This research is pioneer for the arid and semi-arid American ecosystems in examining the potential impact of global warming on the persistence of endemic, threatened and endangered (SEMARNAT, 2010) cactus species (*Echinocactus platyacanthus* f. *visnaga*, *Ferocactus histrix* and *Stenocactus coptonogonus*), and charismatic (Irish and Irish, 2000) species of the family Asparagaceae (*Agave striata*, and *Yucca filifera*). In particular, this study is the first in assessing the impacts of climate warming on the earlier stages of their life cycles. Within our simulated global warming scenarios, using OTCs, we found mean air temperature increases by 1.6-1.9ºC and decreases in air mean relative humidity by 1.4-3.7 %, as compared to the external environment (control plots). It is similar to summer climate change forecasts (1-2ºC) by end 2030 from Chihuahuan Desert (Tejada-Martínez et al., 2008). Musil et al. (2005; 2009) found temperature enhances by 2.5-5.5ºC in a semi-arid region within the South African succulent Karoo biome using these devises. Thus, the use of OTCs to assess responses of American desert plants in face to climate change projections was validated. Additionally, these temperature increases promoted the rise of the soil mean temperature within OTCs (1ºC) during the period analyzed (March 1st 2013 to February 29st 2014).

Because the physical stress increase above explained, we hypothesize that these increases in temperature may have a severe detrimental effect on seeds, seedlings and young plants of the studied species. Differential responses were observed, which were in function of the development state and the analyzed species. *Agave striata* displayed high germination in both climatic conditions (OTC and control plots) in spring (dry season); nevertheless, their seeds lost viability in summer. On the another hand, *Y. filifera* showed high germinability in both treatments in spring and summer, but in the former season up to 100% of germination was found into buried bags. Thus, for this species, no germination tests were performed in the next season. These results confirm the high tolerance to physic stress (temperature increase and decrease of relative humidity) for *Y. filifera*, which was reflected in their high germination rates. Thus, *Y. filifera* and *A. striata* do not form SSB, and do not have seed dormancy.

Echinocactus platyacanthus showed a positive feedback between the increase of mean soil temperature and seed germination; indeed, this temperature increase allowed a relative high germinability inside OTCs in summer. Furthermore, it showed a transitory seed bank, which was promoted by cycles of dormancy. Cycling or enforced dormancy is regulated by environmental conditions such as light and/or temperature (Rojas-Aréchiga and Vázquez-Yanes, 2000). Thus, seeds are ready to germinate once the environmental restriction is eliminated and this represent a mechanism that may help to regulate both, germination and emergence time (Baskin and Baskin, 1985; 1996), also providing a favorable window to increase the chance of seedlings to survive in seasonally varying environmnts such as arid and semi-arid ecosystems. Cycling dormancy in *E. platyacanthus* may lead to the formation of SSB with viable seeds, which could be capable to replace the parent plant after its dead. Within global warming scenarios, the non-dormancy of *Yucca filifera* and cycling dormancy of *E. platyacanthus* seem to be efficient mechanisms to achieve a successful seedling emergence in these species, but *A. striata* seeds could be affected by global warming.

This outlook was completely opposite when we analyzed the seedling survival under the same temperature increases. After induced warming during 4 months in the growing season, we found significantly lower seedling survival for *E. platyacanthus, F. histrix* and *S. coptonogonus* inside OTCs than in the control plots. Our findings are similar to those of Musil et al. (2005) for southern African quartz-field succulents, which had up to 4.9 times greater mortality in the open-top chambers than in the control plots after a 4 months of warming in summer. Cacti belonging to different growth stages, such adults (Smith et al., 1984) as seedlings (Nobel, 1984), have been shown to be rather tolerant of high temperatures. However, the seedlings included in this study seem to be poorly tolerant to elevated temperatures because they suffered extreme negative impacts on their survival rates under induced climate change. Thus, high seedling mortality during the growing season might impair the long-term persistence of natural populations of these species under global warming scenarios.

As expected, this induced global warming also affected the structural organization of seedlings of these cacti. We found higher fresh mass (FM), dry mass (DM), and water loss (WL) in stressed than in non-stressed seedlings, and stressed seedlings displayed severe damage in chlorenchyma cells. Nevertheless, these changes did not affect the shoot apical meristems (SAM) diameter. In addition, stressed seedlings of *E. platyacanthus* and *F. histrix* showed lower length of the vascular cylinder than seedlings of *S. coptonogonus*, which suffered vascular cylinder contraction (VCC).

The high SAM diameter found in these species reflects an extraordinary mechanism of protection in its meristematic zone (Thomas, 1991), which may give them a new opportunity to re-growth as the environmental conditions are suitable. Furthermore, VVC appears to be an adaptive response to environmental stress conditions such as extreme temperature events, and represents an efficient mechanism that could enhance the efficiency of the flow water movement through stems in *E. platyacanthus* and *F. histrix* because this contraction. For *S. coptonogonus*, the presence of a large amount of spines in the cuticle was evident and they showed a greater thickness (although these parameters were not measured in this research); we believe that these traits could explain why it did not exhibit VCC. Thus, modifications on SAM diameter and vascular cylinder found in the three cacti species can be interpreted as adaptive traits. Furthermore, these two mechanisms could allow the persistence of earlier life-cycles stages of these cacti species in face to future climatic scenarios in arid and semi-arid environments.

Nobel (1984; 2010), suggests that desert succulent plants have high tolerance to water and temperature stress. This assumption was corroborated analyzing the photosynthetic performance on five years-old individuals of *E. platyacanthus* under our simulated global warming scenarios during the 4-month period of drought. Although this species displayed lower maximum quantum efficiency of photosystem II (F_v/F_m), the effective quantum yield of photosystem II (Φ_{PSII}) and the electron transport rate (ETR) within OTCs than control plots, it had 100% survival in both climatic treatments. This extraordinary tolerance was attributed to the higher no-photochemical quenching (NPQ) values of this species, which is a mechanism to avoid photoinhibition (Maxwell and Johnson, 2000).

Considering that these results were obtained in a short-term experiment, we decided to assess the high temperature stress tolerance of *E. platyacanthus* using the same cohort of plants in a new long-term experiment across seasons during one year. Thus, although seasonal variations in photosynthetic performance were found (the Φ_{PSII} and ETR values were low in summer but high in autumn and winter), this cactus newly showed 100% survival in both OTCs and control plots.

Moreover, using a generalized canonical correlation analysis (GCCA) we also found a strong correlation (0.71) between micro-environment (represented by OTC plots and open spaces) with ecophysiology of *E. platyacanthus*. This result can explain the high Φ_{PSII} and ETR values in cold seasons (autumn and winter) because is probable that the increase of temperature inside OTCs help to improve the photosynthetic response in *E. platyacanthus*. It has been suggested that the most widespread species would be less affected by climate change, proposing that past selection on plasticity might allow them to survive under variable conditions (Ureta et al., 2012). *E. platyacanthus* is one the most widespread cacti species (Jiménez- Sierra et al., 2007) and our findings in both, short and long-term experiments confirm this premise to expected temperature increases.

Chapter 8

General Conclusions

Human-induced climate change is the main components of the global environmental change (Bellard et al., 2012) and these shifts could severally impair the wild ecosystems (Chapin III et al., 1997; Tilman et al., 1997). Thus, defining what plant species could tolerate the imminent increase in temperatures acquires crucial importance. This PhD thesis emphasizes the essential need to assess some ecophysiological and anatomical aspects of some succulent species in initial phases of their life cycles from southern Chihuahuan Desert, to simulated warming.

We found surprising results about behavior of these species under these warming conditions. It seems global warming promote a high germination and seedling emergence in most analyzed species. However, this extreme temperatures had a severe impact in survival rate of succulent seedlings. Finally, through physiological tolerance mechanisms and intrinsic characteristics, *E. platyacanthus* showed extraordinary tolerance to future high temperatures.

The pathways that human societies follow in population growth and resource use will make an enormous difference to the humanity future and the Earth systems (Vitousek, 1994). Thus, we need to recognize, anticipate and work with the change in order to minimize their consequences. Our work provide new insights about behavior and possible population changes in succulent species; according these results, we believe it is very important linking many more species within this systems in future research to refine population persistence models for these or related species, as well as implement effective conservation and management practices focusing in seedling phase in this species in order to preserve these social, cultural and economic key American plant species.

Future work and perspectives

México has the higher richness of cacti (Ortega-Baes and Godínez-Álvarez, 2006), agaves (García-Mendoza, 2002) and yuccas (Irish and Irish, 2000) species. Thus, the results showed in this research open the door to new studies to evaluate the potential effect of human-induced climate change on the initial development phases (seeds, seedlings and young individuals) which are crucial to get a successful persistence (Kitajima and Fenner, 2000), in arid and semi-arid regions.

For this, under the expected temperature increases and in order to strengthen the conservation and management programs above explained, the following key points are essential:

- To link both, temperature and CO_2 increases, as well as rainfall decreases and its effect in behavior of succulent species in laboratory as well as at the field conditions using OTCs.
- To assess possible changes in the physic, chemical, biochemical and biological structure of soil and the differential effect in seed germination, seedling emergence and seedling survival *in situ* of these species.
- To determine biochemical (chlorophylls *a* and *b*, chlorophyll *a*/*b* ratios, carotenoids, proline, titratable acidity), molecular (dehydrins and heat shock proteins), anatomical

(chloroplast and stomatal dynamics) shifts as tolerance mechanisms to high temperature in these species.

- To compare survival and photosynthetic performance in species with wide and narrow distributions range in order to evaluate its tolerance under future extreme temperature conditions.
- To evaluate the possible ability for apical re-growth as a mechanism to tolerate the expected increases temperature in these species.
- To assess potential changes in the reproductive biology (flowering, fructification and dispersal timing, as well as dynamics of pollination) under global warming conditions for these succulent species.

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