Effects of spine-shading on aspects of photosynthesis for three cactus species

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Abstract

We evaluated the effect of spine removal on three cactus species varying in spine-shading of stems. *Turbinicarpus schmiedickeanus* showed the greater spine shading (*c*. 61 %), followed by *Mammillaria zephyranthoides* (43 %), and *Echinocactus platyacanthus* (17 %). We evaluated photosynthetic activity [titratable acidity, electron transport rate, potential quantum yield of Photosystem II, effective quantum yield of Photosystem II, and non-photochemical quenching], as well as pigment content (chlorophylls *a* and *b*, total chlorophyll, chlorophyll *a*/*b*, carotenoids, and total chlorophyll/carotenoids ratio). For *T. schmiedickeanus*, the species having the higher spine shading, spine removal increased the CAM activity (acidity level) and the effective quantum yield of Photosystem II and diminished both electron transport rate and non-photochemical quenching at 15:00 hours. For *M. zephyranthoides*, the species having intermediate spine shading, spine removal also increased the acidity level but diminished effective quantum yield of Photosystem II at midday, as well as chlorophyll *a*, total chlorophyll, and carotenoids, although there was higher non-photochemical quenching (heat quenching) as an adaptation to avoid photosynthetic damage. Spine removal did not affect acidity level or pigment content from *E. platyacanthus*, the species having the lower spine shading; however, at midday this species without spines showed a decrease of effective quantum yield of Photosystem II, as well as an increase of non-photochemical quenching and electron transport rate. We suggest that a main function of cactus spines is reduction of sun exposure to avoid damage to the photosynthetic apparatus.

Keywords: Cactaceae, CAM activity, plant ecophysiology, spine removal.

Efectos del sombreado de las espinas en aspectos de fotosíntesis para tres especies de cactáceas

Resumen

Se evaluó el efecto de la remoción de espinas en tres especies de cactos que varían en el sombreado de sus tallos por las espinas. *Turbinicarpus schmiedickeanus* mostró el mayor sombreado (*c*. 61 %), seguido por *Mammillaria zephyranthoides* (43 %) y *Echinocactus platyacanthus* (17 %). Se evaluó la actividad fotosintética [acidez titulable, tasa de transporte de electrones, rendimiento cuántico potencial del Fotosistema II, rendimiento cuántico efectivo del Fotosistema II y disipación no fotoquímica], así como el contenido de pigmentos (clorofilas *a* y *b*, clorofila total, clorofila *a*/*b*, carotenoides y proporción clorofila total /carotenoides). Para *T. schmiedickeanus*, la especie con el mayor sombreado de espinas, la remoción de espinas incrementó la actividad MAC (nivel de acidez) y el rendimiento cuántico efectivo del fotosistema y disminuyó la tasa de transporte de electrones y la disipación no fotoquímica (disipación de calor) a las 15:00 h. Para *M. zephyranthoides*, la especie con el sombreado de espinas intermedio, la remoción de espinas incrementó el nivel de acidez pero disminuyó el rendimiento cuántico efectivo del fotosistema al mediodía, así como también la clorofila *a*, la clorofila total y los carotenoides, aunque hubo mayor disipación no fotoquímica (disipación de calor) como una adaptación para evitar el daño fotosintético. La remoción de espinas no afectó el nivel de acidez ni los pigmentos de *E. platyacanthus*, la especie con el menor sombreado de espinas. Sin embargo, sin espinas esta especie mostró disminución del rendimiento cuántico efectivo del fotosistema al mediodía, así como también incremento en disipación no fotoquímica y tasa de transporte de electrones. Se sugiere que una función principal de las espinas es la reducción de la exposición del sol para evitar daños en el aparato fotosintético.

Palabras clave: Actividad CAM, Cactaceae, ecofisiología vegetal, remoción de espinas.

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Within the Cactaceae family, spines are modified leaves without guard cells, stomata, hypodermis, chlorenchyma, and vascular tissue (Mauseth, 2006). Several functions are described for cactus spines: (i) water absorption (Schill and Barthlott, 1973); (ii) reduction of the impact of extreme temperatures (Nobel, 1980; Nobel *et al*., 1991; Drezner, 2011); (iii) reflection and absorption of the environmental energy load (Gibbs and Patten, 1970); (iv) dissemination of shoots and fruits (Gibson and Nobel, 1986); (v) camouflage or recognition by pollinators and seed dispersers (Mauseth, 2006); (vi) herbivory reduction (Gibson and Nobel, 1986; Rebollo *et al*., 2002); and (vii) parasitism prevention (Medel, 2000).

Spine characteristics differ within and among taxa (Anderson, 2001). Spines show wide variation in color, size, number, shape and texture, which could provide different shade levels (Gibson and Nobel, 1986; Mauseth, 2006); thus, the mean shading of the stem by spines can vary, depending of the taxa (Nobel, 1980, 1983; Loik, 2008). When abundant, cactus spines shade photosynthetic cortex from intense solar radiation and UV radiation; thus, they can alter the microclimate of the cactus surface reducing incident photosynthetic photon flux density (PPFD) (Nobel, 1983; Geller and Nobel, 1986; Nobel *et al*., 1991), even diminishing photosynthesis and growth (Nobel, 1983; Norman and Martin, 1986; Loik, 2008).

Although spines can reflect some PPFD toward the stem surface of a cactus, spines typically decreases the incident PPFD on that surface, which can reduce photosynthetic activity if light quantity is lower than light saturation point of the photosynthesis (Nobel, 1983; Norman and Martin, 1986). However, the effect of the spine-shading on the photosynthesis can vary among cactus species, depending on the spine density and the light transmittance by spine. For instance, for *Opuntia erinacea*, whose stem is highly shaded because it is densely covered with spines, Loik (2008) found that effective quantum yield of Photosystem II, photochemical quenching, and electron flux within PSII were lower, and non-photochemical quenching was higher, in comparison to *O. basilaris* and *O. phaeacantha*, species with fewer spines. Thus, shade reduced photosynthetic activity. These results were found in species belonging to the same genus and growth form, but comparative studies on cactus species differing in lineage and growth form have not been performed.

Strong light effects on plants include changes in content of photosynthetic pigments, *e.g*., reduced chlorophyll content and decreased chlorophyll:carotenoid ratio (Hendry and Price, 1993; Maxwell *et al*., 1994), as well as increased carotenoid content (Adams III *et al*., 1987). However, the potential effects of spine-shading on cactus stems for pigments have been little evaluated (but see Loik, 2008).

In order to obtain better knowledge about spine function in cactus, we evaluated the effect of spine removal on plants from three cactus species: *Turbinicarpus schmiedickeanus* subsp. *macrochele* (Werderm.) Glass & R. A. Foster, *Mammillaria zephyranthoides* Scheidw., and *Echinocactus platyacanthus* Link & Otto. The first two species are depressed-globose and the latter is columnar (Vázquez-Sánchez *et al*., 2012). We chose these cactus species because they differ in habitat, which can affect spination greatly in terms of both selection and acclimation. *Turbinicarpus schmiedickeanus* occurs in rosetophyllous and microphyllous shrubland; it grows in cracks or cavities of limestone rock outcrops or under nurse plants such as *Hechtia* sp., as well as in sandy soils or on very low hills (Sotomayor *et al*., 2004), *M. zephyranthoides* grows in semiarid grasslands, in red soils of volcanic origin (Arredondo and Sotomayor, 2009), and *E. platyacanthus* grows in calcareous soils in semiarid grasslands, as well as in slopes of low hills in rosetophyllous and microphyllous shrubland (Arredondo and Sotomayor, 2009). They also differ in lineage; *E. platyacanthus* belongs to the *Ferocactus* clade, *T. schmiedickeanus* belongs to the *Turbinicarpus clade*, and *M. zephyranthoides* belongs to the *Mammillaria* clade (Vázquez-Sánchez *et al*., 2013).

Due to illegal extraction, land-use change and overgrazing (Flores *et al*., 2006), two species (*Turbinicarpus schmiedickeanus* and *Mammillaria zephyranthoides*) are threatened, and *Echinocactus platyacanthus* is under special protection status following the Mexican Official Norm (SEMARNAT, 2010). Thus, the physiological and ecological studies in these species are necessary to help their conservation.

In the present study, three hypotheses were tested: (1) bigger spine shading will reduce incident PPFD and temperature at the stem surface (*i.e*. under spines); (2) for species showing

bigger spine shading, shade will decrease photosynthetic activity in general, because less energy is flowing towards both C fixation and the xanthophyll cycle and other energy dissipation processes; (3) the species with the lowest spine shading will exhibit lower reductions in photosynthetic activity at high PPFD than species having more spine shading. Hypotheses were tested by comparing for the three species titratable acidity, chlorophyll fluorescence [electron transport rate (ETR), potential quantum yield of Photosystem II (F_v/F_m), effective quantum yield of Photosystem II (Φ_{PSII}), and non-photochemical quenching (NPQ)], as well as pigment content (chlorophylls *a* and *b*, total chlorophyll, chlorophyll *a/b*, carotenoids, and total chlorophyll/carotenoids ratio). Evaluations were done in individuals from whom the spines had been removed and were compared with those of individuals with spines left intact.

Materials and methods

Study species. *Echinocactus platyacanthus* is a short-columnar plant with 60-80 cm in diameter and 250 cm in height, having a single spherical to cylindrical stem with an average of 5-8 ribs in juvenile (Bravo-Hollis and Sánchez-Mejorada, 1991a) and 30-40 ribs in the adult stage (Del Castillo and Trujillo, 1991). *Mammillaria zephyranthoides* is a solitary plant having a depressed-globose form, with 8 cm height and 10 cm diameter, and big tubercles in the adult stage (Bravo-Hollis and Sánchez-Mejorada, 1991b). *Turbinicarpus schmiedickeanus* during the juvenile phase has depressed-globose form similar to the adult one, but it is less contracted (Donati and Zanovello, 2005). When adult, it has 1.5-5 cm height and 1.5-4 cm diameter (Bravo-Hollis and Sánchez-Mejorada, 1991a). Juvenile *E. platyacanthus* show a few spines. In contrast, in *T. schmiedickeanus* spines cover almost all the stem and in *M. zephyranthoides* there is a central spine and several radial spines covering the areoles (structures carrying the spines).

The three cactus species were obtained from the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) campus San Luis Potosí, México, they were grown from seed in conditions similar to those found in their native habitat and under shade, because they grow under the shade of nurse plants when juveniles, as most cacti (Flores and Jurado, 2003). Individuals of *Mammillaria zephyranthoide*, *Turbinicarpus schmiedickeanus* and *Echinocactus platyacanthus* were juvenile having a diameter of 4.8 ± 0.28, 1.68 ± 0.10, and 4.08 ± 0.13 cm, respectively.

Experimental design. All plants were placed in a greenhouse, with 23.7 mol m^{-2} day^{-1} of photosynthetic photon flux density (PPFD; 400-700 nm) during the experiment and watered at field capacity, which was determined in pots containing overwatered mixture, and allowed to drain overnight. We used soil from the region for the three species; we did not evaluate soil composition in the pots but it was the same for all three species. To reach field capacity, 50 mL water per pot was required. After of this, we evaluated F_v/F_m during two weeks, time in which the values were close to 0.83, indicating a healthy value for any plant (Björkman and Demmig, 1987; Maxwell and Johnson, 2000). After this acclimation, we removed the spines of three replicates of individuals belonging to the same cohort; thus, there were three replicates by species with spines and three replicates without spines. We used a nail-clipper to remove the spines. We used three replicates, the minimum recommended number for photosynthetic analyses (Pérez-Harguindeguy *et al.*, 2013). Two months after spines were removed; we evaluated chlorophyll fluorescence components, photosynthetic pigment contents, and acidity content in plants with and without spines. Measurements were always made on the top of the shoot, where spines are located.

Chlorophyll fluorescence evaluations. Two types of chlorophyll fluorescence measures were taken on all cacti by using a portable Photosynthesis Yield Analyzer (Mini-PAM; H. Walz, Effeltrich, Germany). The Mini-PAM was equipped with a leaf-clip holder (2030-B; Walz), where the optic fiber was inserted; the distance between the optic fiber and the surface stem was approximately 12 mm, with an angle of 60° relative to the upper surface of stem. The PPFD was measured by a micro-quantum sensor (0.5 mm diameter), and the temperature was evaluated with the aid of a NiCr-Ni thermocouple, both measurements were done at the photosynthetic

surface of stem (Hernández-González and Briones-Villarreal, 2007). In cactus with spines, we placed the sensors under the spines to measure the PPFD and temperature of stem.

Chlorophyll fluorescence data were used to estimate a series of variables related to the photosynthetic performance of plants with and without spines. The first type of chlorophyll fluorescence measures was conducted on dark-adapted cacti at 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).

The second type of chlorophyll fluorescence measures was conducted each three hours during the day (09:00, 12:00, 15:00, and 18:00 h), when plants faced higher temperature than at predawn. 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'_m is the maximum fluorescence emitted by chlorophyll when a saturating pulse of actinic light is superimposed to environmental levels of light and F_t is is the chlorophyll fluorescence emitted by plants under steady-state illumination (Genty *et al*., 1989).

Because the Mini-PAM also measures the photosynthetic 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} × PPFD × 0.5 × 0.84, where 0.5 mean two photons are used for exciting one electron, as we have assumed an equal distribution of excitation between photosystems II and I, and 0.84 is considered the most common leaf absorbance coefficient for plants under a wide range of environmental conditions, including cacti (Hernández-González and Briones-Villarreal, 2007).

Finally, because chlorophyll fluorescence was measured at both predawn and during the day, we also calculated the non-photochemical quenching efficiency (NPQ) of cacti. This variable was calculated as $(F_m - F_m')/F_m'$, which is linearly related to heat dissipation and lies on a scale of 0 - infinity (Maxwell and Johnson, 2000). We used the F_m (maximal level of fluorescence measured in darkness) at predawn to calculate NPQ during the day in place of the dark adapted F_m during the day, because F_v/F_m predawn values were close to 0.83 for the three species. NPQ values could be underestimated when F_v/F_m predawn values did not totally recover during the night (Demmig-Adams and Adams III, 1996), but this is not our case because the F_v/F_m predawn values did recover.

Photosynthetic pigments. Higher plants utilize chlorophylls *a* and *b* and a variety of carotenoids to capture light for photosynthesis. Chlorophyls *a* and *b* absorb light for maximum utilization under non-saturating conditions, but they can act as protective pigments under over-saturating light conditions, when absorbed light is dissipated as heat (Nishio, 2000). Carotenoids are involved in photoprotection and photorepair, transfering energy to the photosynthetic reaction centers as accessory pigments to chlorophylls (Takahashi and Badger, 2011; Lichtenthaler, 2015).

To evaluate the content of photosynthetic pigments (chlorophylls and carotenoids) we collected shoot samples using a cork borer of 0.2 cm diameter at 7:00 h, the samples were placed in plastic bags and kept on ice in a cooler for transport to the laboratory. This process was made in darkness to avoid pigments degradation. All samples were stored at -20 ℃ before analysis. Chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and carotenoids were determined by measuring absorbance at 480, 645 and 663 nm wavelengths on a spectrophotometer (Thermo Scientific®, Spectronic Genesys 10 Bio) and computed following the method of Hendry and Price (1993). Then, total chlorophyll (Chl), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a*/chlorophyll *b* ratio (Chl *a/b*), carotenoids (Carot) and chlorophyll/carotenoid ratio (Chl/Carot) were calculated. The contents of photosynthetic pigments were expressed as μmol g^{-1} FW.

Titratable acidity. Acidity levels, mainly malic acid, but also oxaloacetic acid and citric acid in cacti were determined from titratable acidity, which was determined at 6:00 h because acid concentrations in plants performing CAM are typically highest just before dawn (Delgado-Sánchez *et al*., 2013). We did not estimated titratable acidity at night; thus, we cannot be absolutely certain

that our juvenile plants are exclusively CAM, but we can explore differences with and without spines. Transverse samples of plant tissue were obtained from cactus shoots using a steel borer of cross section 0.2 cm^2. The plant material was sectioned and preserved in ethanol (80 %) in 1.5 mL Eppendorf tubes. Titration was carried to neutrality with a 0.01 N NaOH solution to determine the acidity level (mmol equiv. H$^+$m^{-2}) per tissue according to (Ricalde *et al.*, 2010).

Spine shading (%). Photographs of the plants with and without spines were taken in three plants by species at the end of the experiment, in order to estimate the percentage of spine-shading on stem as an estimation of the coverage by spines. Photographs of the plants were scanned with an HP 2400 ScanJet scanner. The images were analyzed with the software ImageJ 1.40g (Wayne Rasband National Institutes of Health, USA). This software has been used to determine the photosynthetic area in other cactus (Delgado-Sánchez *et al.*, 2013).

Statistical analyses. In order to evaluate the effect of spines removal on chlorophyll fluorescence variables (Φ_{PSII}, NPQ and ETR) for each species, we performed one-way ANOVA considering presence or absence of spines as a factors at each specific hour (9:00, 12:00, 15:00 or 18:00 hours). Also for each species, the differences in F_v/F_m, pigments as well as acidity levels among treatments were tested for statistical significance using one-way ANOVA. The differences in temperature among species were tested using also one-way ANOVA for each specific hour. To detected possible differences in PPFD between plants with spines and without them, we performed a one-way ANOVA at 15:00 h, because at this time the cactus species showed lower Φ_{PSII} and higher ETR and NPQ. All statistical analyses were carried out using STATISTICA 7.0 with a significance level equal to 0.05.

Results

Spine shading and temperature of the stems. The spine-shading of stem (percentage of coverage by spines) was different among species in intact plants and those with spines removed ($P < 0.05$; Table 1). *Echinocactus platyacanthus* is the species having lesser coverage by spines (17 %), followed by *Mammillaria zephyranthoides* (43 %) and *Turbinicarpus schmiedickeanus* (61 %). We did not find significant differences in temperatures between plants with and without spines ($P > 0.05$; Figure 1A, E, I).

Photosynthetic photon flux density. The amount of PPFD that the three cacti species received was significantly higher in individuals without spines than with them at 15:00 h ($P > 0.05$; Figure 1A, E, I), although the differences between treatments, in terms of absolute values, were

Table 1. Spine-shading, maximum quantum efficiency of photosystem II (F_v/F_m), titratable acidity, and pigment contents of stems of three cacti species after eight weeks intact and without spines. Each value is the mean ± 1 SE; ns = non-significant; asterisks indicate significant differences between treatments ($P < 0.05$).

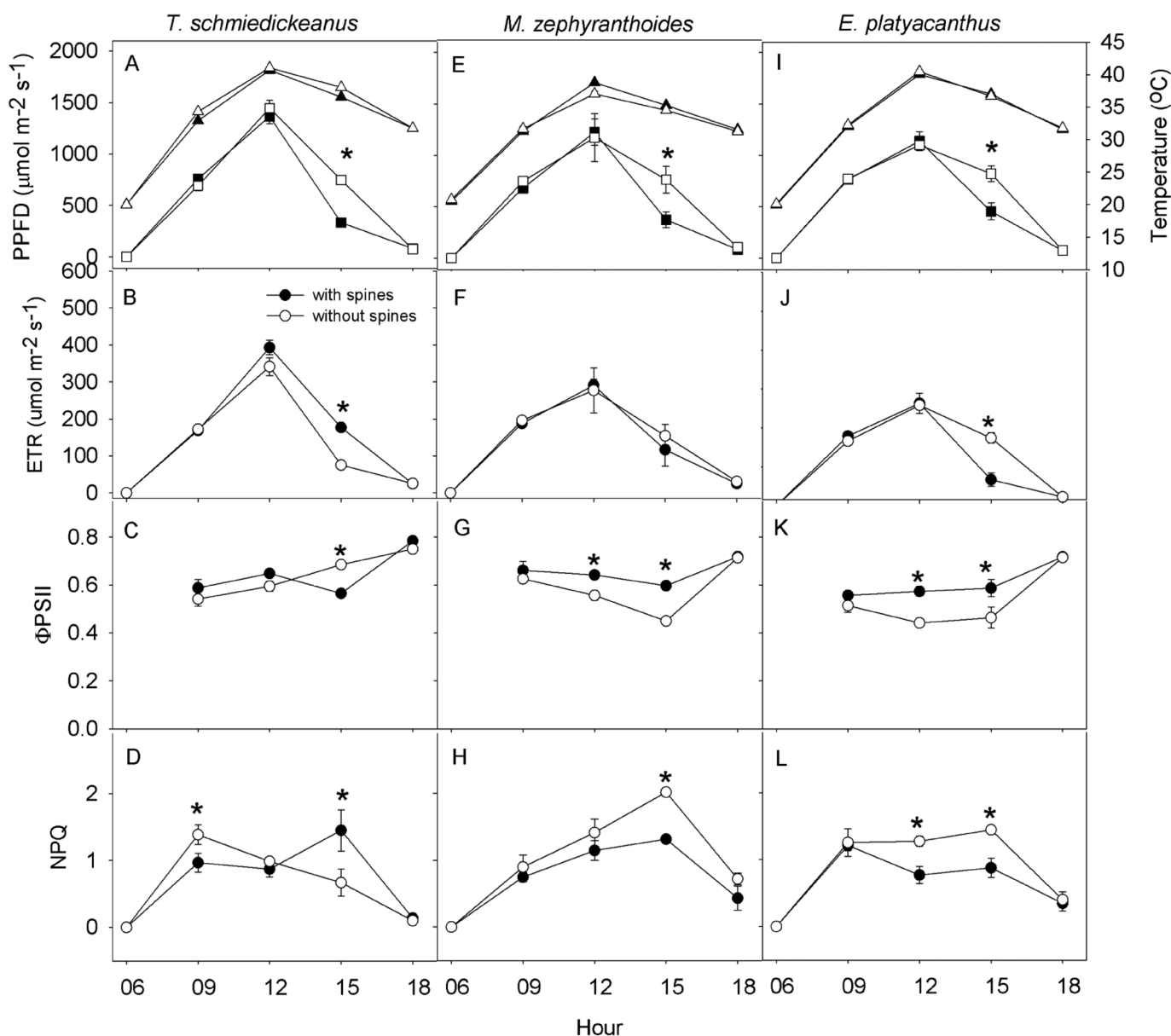|	*Turbinicarpus schmiedickeanus*		*Mammillaria zephyranthoides*		*Echinocactus platyacanthus*	
	Intact	Without spines	Intact	Without spines	Intact	Without spines
Spine-shading on stem (%)	60.98 ± 0.1	0 *	43.00 ± 1.5	0 *	17.08 ± 0.03	0 *
Fv/Fm	0.79 ± 0.02	0.78 ± 0.01 ns	0.81 ± 0.01	0.80 ± 0.01 ns	0.79 ± 0.01	0.80 ± 0.01 ns
Titratable acidity (mmol H$^+$ m^{-2})	74.1 ± 5.7	115.4 ± 9.4 *	171.2 ± 6.2	224.8 ± 6.2 *	81.4 ± 3.5	90.7 ± 4.3 ns
Chlorophyll *a* (µmol.m^{-2})	13.47 ± 1.37	4.87 ± 1.46 *	29.67 ± 2.13	19.57 ± 0.81*	8.68 ± 0.64	9.05 ± 1.48 ns
Chlorophyll *b* (µmol.m^{-2})	2.62 ± 1.42	2.94 ± 0.56 ns	11.47 ± 0.98	11.46 ± 1.73 ns	5.29 ± 0.88	5.87 ± 2.1 ns
Total chlorophyll (µmol.m^{-2})	16.10 ± 0.63	7.82 ± 1.52 *	41.14 ± 1.17	31.04 ± 1.07 *	14.02 ± 1.47	14.96 ± 3.6 ns
Carotenoids (µmol.m^{-2})	8.10 ± 2.5	8.11 ± 1.99 ns	44.55 ± 5	25.5 ± 1.61 *	7.09 ± 0.69	8.64 ± 1.28 ns
Chlorophyll *a/b* (µmol.m^{-2})	3.68 ± 0.82	1.77 ± 0.67 ns	2.66 ± 0.45	1.79 ± 0.3 ns	1.94 ± 0.28	1.41 ± 0.28 ns
Total chlorophyll / carotenoids (µmol.m^{-2})	2.87 ± 1.22	0.99 ± 0.06 ns	0.93 ± 0.07	1.22 ± 0.09 ns	1.93 ± 0.02	1.95 ± 0.09 ns

Figure 1. Diurnal changes in photosynthetic photon flux density (PPFD), temperature of the surface, electron transport rate (ETR), effective quantum yield of Photosystem II (Φ_{PSII}) and non-photchemical quenching (NPQ), of *Turbinicarpus schmiedickeanus*, *Mammillaria zephyrantoides* and *Echinocactus platyacanthus* intact and eight weeks without spines. Each value is the mean ± 1 SE. The filled triangle represents temperature on cactus surface with spines, empty triangle without spines; filled square represents PPFD with spines and empty square without spines. Asterisks indicate significant differences between treatments ($P < 0.05$).

lower in *Echinocactus platyacanthus* (295 μmol m^{-2} s^{-1}) compared to *Turbinicarpus schmiedickeanus* (413 μmol m^{-2} s^{-1}) and *Mammillaria zephyranthoides* (387 μmol m^{-2} s^{-1}) at this hour.

Chlorophyll fluorescence variables (F_v/F_m, Φ_{PSII}, ETR and NPQ). The three cactus species showed F_v/F_m values close to 0.83, without significant differences between plants with and without spines ($P > 0.05$; Table 1).

Spine removal had a significant effect on Φ_{PSII} and NPQ ($P > 0.05$, Figure 1G, H) of *Mammillaria zephyranthoides* and on Φ_{PSII} and NPQ ($P < 0.05$, Figure 1K, L) of *Echinocactus platyacanthus*; both species had diminishing Φ_{PSII} at higher solar radiation (12:00 and 15:00 h) and increasing NPQ at this time. *M. zephyranthoides* had similar ETR at higher solar radiation hours

(12:00 and 15:00 h) with and without spines ($P > 0.05$; Figure 1F). *E. platyacanthus* had higher ETR in plants without spines than with them at 15:00 h ($P < 0.05$; Figure 1J). For *Turbinicarpus schmiedickeanus*, spine removal increased Φ_{PSII} ($P < 0.05$; Figure 1C) and diminished both ETR and NPQ ($P < 0.05$; Figure 1B and D) at 15:00 h.

Pigment content (Chlorophylls a and b, total chlorophyll, chlorophyll a/b, carotenoids, and total chlorophyll/carotenoids ratio). *Turbinicarpus schmiedickeanus* had higher contents of chlorophyll a ($P < 0.05$) and total chlorophyll ($P < 0.05$) with spines than without them (Table 1). This species had no differences with and without spines in the rest of the pigments ($P > 0.05$). *Mammillaria zephyranthoides* had higher contents of chlorophyll a ($P < 0.05$), total chlorophyll ($P < 0.05$), and carotenoids ($P < 0.05$) with spines than without them (Table 1). This species had no differences with and without spines in chlorophyll *b*, chlorophyll *a/b*, and total chlorophyll/carotenoids ratio ($P > 0.05$ in all cases). *Echinocactus platyacanthus* was the only species that had no significant differences in pigment contents with and without spines ($P > 0.05$; Table 1).

Titratable acidity levels. Acidity was higher without spines than with them for both *Turbinicarpus schmiedickeanus* and *Mammillaria zephyranthoides* ($P < 0.05$ in both species; Table 1). In contrast, *Echinocactus platyacanthus* did not show differences in acidity content between treatments ($P > 0.05$; Table 1).

Discussion

Cactaceae has more than 1,800 species, most of them with spines (Anderson, 2001), however, there is only one previous experimental study about spine-shading on the photosynthetic capacity of a cactus species, *Opuntia erinacea* (Loik, 2008). Norman and Martin (1986) evaluated the effect of spine removal on the cactus *Coryphantha vivipara* in Central Kansas, but they only studied the acid accumulation in the stems. In our research, we evaluated the effect of the spine-shading on the photosynthetic capacity of other three cacti species, more research is necessary on this topic in other cacti.

The three cactus species here studied were predawn F_v/F_m close to 0.83, the typical value found in healthy plants (Björkman and Demmig, 1987; Romo-Campos *et al.*, 2013; Aragón-Gastélum *et al.*, 2014), having no differences between plants with and without spines, which indicate that they can recover after receiving high PPFD.

The spine-shading on stem was different among species, *Echinocactus platyacanthus* is the species less shaded by spines, followed by *Mammillaria zephyranthoides* and *Turbinicarpus schmiedickeanus*. Although juvenile plants of *E. platyacanthus* have the lower spine density than the other studied species, they could face higher light in arid environments due to several morpho-physiological traits such as thick epidermis, water tissues stored, sunken stomata and crassulacean acid metabolism (Gibson and Nobel, 1986), and to stem tilting (Herce *et al.*, 2014). In *T. schmiedickeanus* spines cover almost all the stem (61% of shade), whereas in *M. zephyranthoides* there is a central spine and several radial spines covering the areoles (structures carrying the spines); in both species, the shade by spines and areoles could help them to reduce the stress by light incident on stem surface (Drezner, 2011). The spine shading on stem (%) of the three species did not coincide with the PPFD values between 6:00 and 18:00 hours in plants with spines and without them, perhaps the difference could be partially explained by the way in which we estimated the spine shading, by scanning photographs.

We hypothesized that greater coverage by spines, such as for *Turbinicarpus schmiedickeanus* and *Mammillaria zephyranthoides* in comparison to *Echinocactus platyacanthus*, reduces incident PPFD and temperature at the stem surface. This hypothesis was partially confirmed in that *E. platyacanthus* is the species having lesser coverage by spines and thus higher PPFD, followed by *M. zephyranthoides* and *T. schmiedickeanus*. However, it was not corroborated for temperature, because we did not find significantly differences in temperatures between plants with and without spines, which is similar to Loik (2008) who found that stem temperatures were not affected by spine coverage for three species of sympatric cacti in the Mojave Desert. The lack of greater stem

heating after spine removal may be related to increased levels of convective cooling as result of the morphology of the studied species (Norman and Martin, 1986; Drennan, 2009).

The second hypothesis, that for species showing greater coverage by spines, spine-shading of stems decreases photosynthetic activity [titratable acidity, electron transport rate (ETR), potential quantum yield of Photosystem II (F_v/F_m), and effective quantum yield of Photosystem II (Φ_{PSII})], was also corroborated. For *Turbinicarpus schmiedickeanus*, the species having the higher coverage by spines, we found both lower acidity and Φ_{PSII}, and both higher ETR and NPQ at 15:00 h in plants with spines than without them, which means that shading of stems by spines limits the photosynthetic efficiency for this species although there are higher non-photochemical quenching (heat quenching) as an adaptation to avoid photosynthetic damage. Higher Φ_{PSII} provoked by shading by spines was also found for *Opuntia erinacea* (Loik, 2008). In addition, lower acidity content occasioned by shaded by spines was also found for cactus like *O. bigelovii* and *Ferocactus acanthodes* (Nobel, 1983), as well as *Coryphantha vivipara* (Norman and Martin, 1986). For *T. schmiedickeanus*, most pigments were not affected by spine removal, but chlorophyll *a* and total chlorophyll had higher contents with spines than without them. These pigments were lower without spines because strong light provokes an inability of cells to synthesize and insert the same amount of new chlorophyll into the PSII chlorophyll protein binding complexes in the thylakoid membranes (Lambers *et al*., 1988). Thus, for *T. schmiedickeanus* the stems were somewhat pale (overall loss of chlorophyll) as a result of the higher PPFD when the spines were removed.

For *Mammillaria zephyranthoides*, the species having intermediate coverage by spines, the ETR at higher solar radiation hours (12:00 and 15:00 h) was similar with and without spines, but lower acidity and higher contents of chlorophyll *a*, total chlorophyll, and carotenoids were found in juvenile plants with spines than without them. The lower predawn acidity for this species, similar to *Turbinicarpus schmiedickeanus*, suggests that shading of stems by spines for this species also limits its CAM activity; in addition, the chlorophyll and carotenoids loss in plants without spines reduces the amount of photons absorbed by photosynthetic stems, avoiding damage in the photosynthetic apparatus (Adams III *et al*., 1987; Demmig-Adams and Adams III, 1996). However, for this species, spine removal diminished Φ_{PSII} at higher solar radiation hours (12:00 and 15:00 h), as well as increased NPQ at the same time. Thus, highlight after spine removal diminish the Φ_{PSII} at midday and increase the NPQ (heat dissipation), which is an adaptation to avoid damage in the photosynthetic apparatus (Demmig-Adams and Adams III, 1996).

Echinocactus platyacanthus had higher ETR in plants without spines than with them at 15:00 h, but did not show differences in acidity content nor pigment contents with and without spines, which could be related to its lower spine density. However, spine removal had a significant effect on Φ_{PSII} and NPQ, showing diminishing Φ_{PSII} at higher solar radiation hours (12:00 and 15:00 h), but increasing NPQ at the same time. Thus, the third hypothesis, that *E. platyacanthus*, the species with the lowest spine coverage, exhibit lower reductions in photosynthetic activity at high PPFD, in comparison to the two species with considerably more coverage by spines (*Turbinicarpus schmiedickeanus* and *Mammillaria zephyranthoides*), was also corroborated.

Other interesting results found in our study are the similar carotenoids between plants with spines and those without them for *Turbinicarpus schmiedickeanus* and *Echinocactus platyacanthus*, and the unexpected greater concentration of carotenoids in plants with spines than in those without spines for *Mammillaria zephyranthoides*. The acclimation of the carotenoid composition of photosynthetic organs to shade environments, with a high demand for efficient light collection, versus fully sun-exposed sites, with a high demand for photoprotection, has been described for a variety of species of higher plants, finding that the total number of carotenoid molecules per chlorophyll molecule is typically greater under direct sun compared with shaded photosynthetic organs (Demmig-Adams and Adams III, 1996). Perhaps our studied species have the relatively rare carotenoids of higher plants, α-carotene and lactucaxanthin, which follow this pattern of a greater accumulation in shade photosynthetic organs (Demmig-Adams *et al*., 1996).

In conclusion, shading of stems by spines can limit photosynthetic productivity for species having high spine density, such as *Turbinicarpus schmiedickeanus*, but in species having less coverage by spines such as *Echinocactus platyacanthus* the spines did not have much effect in

photosynthetic activity. We suggest that a major function of spines is limiting sun exposure and thus avoid damage in the photosynthetic apparatus. Several functions have been described for cactus spines; spine-shading of stems is one of them and studies about the importance of each described function of the spines are necessaries. Our results are of great importance to understand the adaptations of the succulent plants to tolerate high solar radiation levels in deserts.

Acknowledgements

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