Does cladode inclination restrict microhabitat distribution for *Opuntia puberula* (Cactaceae)?

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In contrast with other *Opuntia* species, most of the cladodes of *Opuntia puberula* have a horizontal position. This study explores whether the horizontal cladodes are an adaptive trait to increase light interception in the understory or are a neutral trait, and if this characteristic may prevent its distribution in full sun habitats. Cladode inclination angle and its effect on light interception, cladode temperature, and carbon gain are characterized, and anatomical and physiological traits of upper and lower cladode surfaces are described. Inclination angle was under 50° for 95% of the cladodes, and the frequency of low inclination angles increases as light availability decreases. Nocturnal acid accumulation increases with total daily PPFD intercepted, but no significant differences were detected between typical horizontal cladodes and the few vertical cladodes. Chlorophyll content differed in the upper and lower surfaces of horizontal cladodes; however, chlorenchyma thickness, stomatal conductance, and nocturnal acid accumulation were similar between surfaces. The horizontal position of *O. puberula* cladodes, which is anatomically determined, restricts it to shaded habitats, where the plants do not overheat, but seems to have no effect on carbon gain.

Key words: Cactaceae; carbon gain; cladode position; light interception; *Opuntia puberula*; plant architecture; Tehuacán Valley; temperature.

The exposed area and orientation of photosynthetic surfaces determine light interception and carbon gain. Many angiosperms maximize light interception by having a high surface area to volume (SA/V) ratio. Cacti, however, possess a low SA/V ratio presumably because of their requirements for water economy (Nobel, 1988; Maushet, 2000). A low SA/V ratio decreases transpirational water loss and increases water storage capacity. However, this trait and the vertical position of the photosynthetic stems in many cacti constrain light interception, limiting CO₂ fixation during the growing season, even in arid zones where light is not considered a limiting factor (Solbrig and Orians, 1977; Harris and Campbell, 1981; Gibson and Nobel, 1986; Nobel, 1988). For plants with crassulacean acid metabolism (CAM) like cacti, nocturnal CO₂ fixation depends on the total photosynthetically active radiation intercepted during the day. Thus, under nonlimiting temperature and water availability conditions, morphological and architectural features (number, orientation, angle, and arrangement of photosynthetic stems) that increase light interception can increase CO₂ uptake (Nobel, 1981, 1988; Geller and Nobel, 1987).

The effect of photosynthetic surface orientation on light interception and its consequences on carbon gain have been widely studied in platyopuntias (Becerra-Rodríguez et al., 1976; Nobel, 1980a, 1981; García de Cortazar et al., 1985; García de Cortazar and Nobel, 1986; Geller and Nobel, 1987). In platyopuntias, the photosynthetic surface is represented by its flattened stems, known as cladodes, which in most species exhibit a vertical position and nonrandom orientation. Terminal cladodes are often oriented for maximum light interception and, consequently, carbon gain and growth during the growing season (Nobel, 1988). In intertropical latitudes, cladodes often face east–west, intercepting more radiation during the year than north–south oriented cladodes (Becerra-Rodríguez et al., 1976; Nobel, 1980a, 1981, 1988; Cano-Santana et al., 1992).

At higher latitudes, the trend is to face north–south in areas where the favorable growing season is during winter (Nobel, 1988).

The ecophysiological consequences of the inclination angle of cladodes, however, have been less studied (Nobel, 1980a, 1988; Gibson and Nobel, 1986; Cano-Santana et al., 1992). Most species have vertical cladodes. The vertical configuration has been interpreted as an architectural design that avoids high tissue temperature at midday, when ambient temperature and light interception would be maximum for a horizontal surface (Nobel, 1978, 1980b). For these CAM plants, stomatal opening and CO₂ fixation occur mostly at night, when vapor pressure deficit is low, thereby decreasing water loss by transpiration. During the day, stomata are closed and cooling by latent heat loss does not occur; therefore, the vertical position of cladodes minimizes overheating by decreasing light interception at midday. Therefore, the canopy architecture of *Opuntia* spp. may be determined by a compromise between light interception maximization and temperature regulation during the growing season (Solbrig and Orians, 1977; Harris and Campbell, 1981; Cano-Santana et al., 1992).

In contrast with most *Opuntia* species, *Opuntia puberula* possesses cladodes with a horizontal position (Fig. 1). This feature makes *O. puberula* an interesting system to study the functional meaning of cladode inclination and provides an opportunity to explore the acclimation response of cladode surfaces to different light environments. *Opuntia puberula* is
restricted to the understory of tropical deciduous forests and thorn forests dominated by evergreen species such as *Prosopis laevigata* (Hum. & Bonpl. ex Willd.) M.C. Johnston in the Tehuacán Valley (Bravo-Hollis, 1978; Valiente-Banuet et al., 2000). It is not clear however, whether the horizontal cladodes are an adaptive trait to increase light interception or are a neutral trait. We explore the hypotheses that (1) in shaded habitats where light is a limiting factor, horizontal cladodes might be advantageous for increasing light interception, and that (2) this architectural design prevents *O. puberula* from growing in fully exposed habitats where it would experience high light interception and temperature at midday, affecting its performance. Considering that the upper and lower faces of the horizontal cladodes are exposed to contrasting light conditions, we predict that the upper face would have more chlorophyll content, stomatal density, and chlorenchyma than the lower surface. If surfaces were differentiated, we would expect that the upper surface had a major role in carbon gain.

In this study, we characterize cladode inclination angle in two populations of *O. puberula* and determine its consequences for light interception, cladode temperature, and carbon gain. Furthermore, we measure seasonal changes in light availability, carbon gain, and water content, and describe the anatomical and physiological traits of the upper and lower cladode surfaces.

**MATERIALS AND METHODS**

**Study area**—The study was carried out in the Tehuacán-Cuicatlán Valley in southern Mexico, which lies in the states of Puebla and Oaxaca. The climate is arid to semi-arid with maximum precipitation in June and September (Rzedowski, 1978). Two areas were selected on the basis of their differences in vegetation and canopy structure. One of the areas, the Jardín Botánico Helia Bravo, is located 1.8 km east of Zapotitlán de las Salinas, at 1407 m a.s.l. (18°20' N, 97°28' W). The vegetation is a tropical thorn forest dominated by evergreen arboreal species such as *Prosopis laevigata*, *Celtis pallida* Torr., *Castella tortuosa* Liebm., *Myrtillocactus geometricus* (C. Mart.) Console, and *Maytenus phyllanthoides* Benth. (Osorio-Beristain et al., 1996; Valiente-Banuet et al., 2000). The average annual mean temperature is 21°C, and the mean annual precipitation is 376.4 mm, with the maximum precipitation in June (96.5 mm; Valiente, 1991). In this area, *O. puberula* grows on the edges along an ephemeral stream as colonies under the crown of *P. laevigata*.

The second area, San Rafael, Municipio Coxcatlán, is located at 18°16' N and 97°09' W (1010 m a.s.l.). The vegetation is a tropical deciduous forest with *Ceiba parvifolia* Rose, *Celtis pallida*, *Cercidium praecox* (Ruiz y Pavón) Harms, *Bursera morelensis* Ramírez, *B. fagaroides* (Kunth) Eng., *B. arida* (Rose) Standl., *Ziziphus amole* (Sessé & Moc.) M.C. Johnst., *Castella tortuosa*, *Pachycereus weberi* (J.M. Coult.) Backeb., *Stenocereus stellatus* (Pfeiffer) Riccob., and *S. pruinosa* (Otto) Buxb. as dominants of the arboreal strata, and *Mimosa laisana* Brandegee and *Acacia cochliacantha* Humb. & Bonpl. ex Willd. as dominants of the understory where *O. puberula* grows (Fernández, 1999; Valiente-Banuet et al., 2000).

**Cladode inclination angle and light environment**—To quantify cladode inclination angle and to explore the relationship between this angle and the light environment, we measured the angle of 50 cladodes (selected at random) of each of 30 individuals of *O. puberula* using a clinometer (Suunto, model PM5/360PCB). Inclination angle was defined as the angle with respect to a horizontal plane. The light environment for each individual and its seasonal variation was characterized using hemispherical photographs (Rich, 1988, 1990; Pearcy, 1989). The photographs were taken from the same point above each individual at three different dates: in May 1997 when the angles were measured, in July 1997 during the rainy season, and in March 1998 during the dry season. Photos where taken with an 8-mm fish-eye lens (SIGMA) and a 35-mm semiautomatic Pentax camera model ME; the lens was set to 5.6 and focused to infinity in a self-leveling mount-borne tripod.

Photographs were analyzed with the CANOPY program (Rich, 1988). This program provides two factors of light, the direct site factor (DSF) and the indirect site factor (ISF), which represent the proportion of direct and diffuse light intercepted.
radiation under the canopy relative to the levels outside the canopy (Rich, 1990). The relationship between DSF and cladode angle was analyzed by taking the average cladode angle per individual and considering four angle categories: 1 = angles <17°, 2 = from 18° to 23°, 3 = from 24° to 29° and 4 = angles >30°. Data were analyzed with a multinomial logistic regression with the average DSF as the independent variable using JMP version 3.1 (Sall and Lehman, SAS Institute, 1996). The null hypothesis was that cladode angles do not change upon increasing light (DSF). The mean cladode angle of each population was compared with a t test for circular data (Zar, 1999) using circular statistics Oriana for Windows, version 1.03 (Kovach, KCS, 1994). The null hypothesis was that the mean cladode angle is equal between populations.

**Effect of inclination angle on temperature, light interception, and carbon gain**—Diurnal courses of cladode temperature, air temperature, and photosynthetic photon flux density (PPFD) intercepted by cladodes were measured in both populations. Measurements were made on clear days in July 1997 (rainy season) and in March 1998 (dry season). These parameters were measured in typical horizontal cladodes and in cladodes explicitly searched with vertical posture (angles greater than 60°) under the canopy. In addition, measurements were taken of detached cladodes moved to fully open sites where they do not grow and oriented east–west both horizontally and vertically. These cladodes were supported by thin pieces of wood to keep them about 10 cm above the soil, the same height as the cladodes growing in the understory. Cladode and ambient temperatures were measured with copper-constantan thermocouples 0.51 mm in diameter. For ambient temperatures, sensors with radiation shelters were placed 1 m above the ground both beneath the canopy and in fully exposed sites. For cladode temperature, the sensor was inserted 2 mm below the surface of the cladode. The PPFD received by the two sides of the cladode was measured with small gallium-arsenide photodiode (GaAsP) photodiodes (Hanamatsu Model G1118). GaAsP photodiodes were calibrated against a LICOR quantum sensor 190SB (Pearcy, 1989). PPFD received by a horizontal plane inside and outside the canopy was also measured. Both PPFD and temperature measurements were taken every 5 s, and 5-min averages were stored in Campbell 21X data loggers.

Nocturnal accumulation of titratable acidity (NAA) was determined as an index of carbon gain (Nobel and Harstock, 1983). Two samples were collected, late in the afternoon and early in the morning, with a 2.0-cm cork borer. The samples were cut in small pieces and placed in vials with 80% ethanol. Tissue acids were extracted by boiling samples in distilled H₂O; acid equivalents were determined by titration with 0.01 N NaOH to an endpoint of pH 7.0 (Olivares and Medina, 1984; Barker and Adams, 1997). Nocturnal accumulation of acid was measured as the difference between the malic acid values measured in the morning and in the afternoon. Titratable acidity was determined for horizontal and vertical cladodes whose PPFD was measured (N = 24). The effect of cladode position, site, and season on daily PPFD interception, maximum temperature, and nocturnal acid accumulation was explored through ANOVA using JMP software, version 3.1 (Sall and Lehman, SAS Institute, 1996).

To explore whether site and season influenced nocturnal acid accumulation, we sampled five cladodes for each of six individuals in each site and season. Seasonal variation in relative water content (RWC) of cladodes was measured according to Beadle et al. (1987) and Lerdau et al. (1992). RWC was measured in 10 cladodes for each of six individuals. The effect of site and season on titratable acidity accumulation and RWC was explored through ANOVA using JMP software, version 3.1 (Sall and Lehman, SAS Institute, 1996).

**Anatomical and physiological traits of the upper and lower cladode surfaces**—Chlorophyll content, stomatal density, cuticle and chlorenchyma thickness, NAA, and stomatal conductance (gₛ) were measured in upper and lower surfaces of horizontal cladodes. For these measurements, we selected five cladodes from each of three individuals growing in the shadiest and sunniest conditions within their natural distribution; therefore, note that “sunniest” does not mean exposed to full sun. For chlorophyll content, samples were taken with a 2.0-cm cork borer and extracted with 80% acetone; absorbance was measured at 663 and 645 nm, and chlorophyll calculated according to Arnon (1949). Stomatal density was determined in five cladodes of each of three shadiest individuals and the three sunniest individuals. Epidermal strips from the central part of the cladodes were dissected and dehydrated with 70% ethanol for 24 h and 96% ethanol for 1 h. Afterward the epidermal strips were stained with safranin for 1 h and with pure green for 5 s, and then mounted with Canada balsam (Pinienta-Barriéros et al., 1993). Stomatal density was determined in 10 fields selected at random (4 ×) per sample with an Olympus (B201) optical microscope. Chlorenchyma, cuticle, and hypodermis thicknesses were determined in cross sections of samples taken at the middle part of the cladodes, using Media Cybernetics version 3.1 (1997). Cross sections were 3 mm thick and were dehydrated and stained as described before for epidermal strips. Daily courses of stomatal conductance were measured in the upper and the lower surfaces with a steady-state porometer (LI-1600, LICOR Inc.) during the dry season. The effect of light environment (“sunniest” and “shadiest”) and position (upper and lower surfaces) on chlorophyll content, stomatal density, cuticle and chlorenchyma thickness, and nocturnal acid accumulation were analyzed through ANOVA with light and surface declared as random effects (JMP software, version 3.1; Sall and Lehman, SAS Institute, 1996).

**RESULTS**

**Inclination angle and light environment**—Most cladodes of O. puberula in both populations showed a horizontal position. No significant differences were found between populations (t = 0.96, P = 0.33); for Coxcatlán, the average angle was 19.89° ± 0.90° and for Zapotitlán, 18.54° ± 0.98°. Average inclination angle ± 1 SE in both populations was 19.15° ± 0.94°. The pooled data of the frequency of cladode inclination angle for both populations is shown in Fig. 2; 95% of the cladodes (N = 2850) had angles between 0 and 50°, and the remaining 5% were at angles >50° (N = 150). The characterization of the light environment of 30 individuals in each population indicated that O. puberula grows under low light conditions. The analysis of hemispherical photographs indicated that ISF and DSF were highly correlated (r = 0.99, P = 0.0001); therefore, only the DSF results are presented here. The DSF for all the individuals ranged between 0.043 and 0.371, with 81% of the individuals falling between 0.04 and 0.20. DSF between populations showed significant differences (t = 5.124, P = 0.001). Within this range of light, the fre-
frequency of individuals with higher average cladode angle increased with light availability ($\chi^2 = 11.962, P = 0.0075$; Fig. 3). The frequency of cladode angles smaller than $17^\circ$ was higher in low light, and decreased as DSF increased.

**Effect of cladode angle on light interception, temperature, and nocturnal acid accumulation**—Under fully exposed conditions, horizontal cladodes intercepted more PPFD than vertical cladodes (Table 1, Fig. 4A, B). A vertical cladode intercepted 45% less total daily PPFD than a horizontal cladode. The diurnal course of PPFD for a vertical cladode showed the maximum interception early in the morning for the east-oriented face and late in the afternoon for the west-oriented face (Fig. 4A). The lowest values of PPFD were observed at mid-day, as sunlight was parallel to the cladode surface. For horizontal cladodes, the maximum PPFD interception was at mid-day. Under the canopy, no significant differences were detected in total daily PPFD intercepted by vertical and horizontal cladodes ($F = 6.39, P = 0.091$). Significant differences were detected between sites ($F = 14.57, P = 0.0009$)—the cladodes in Coxcatlán received more light than those in Zapotitlán—but no difference was found between seasons ($F = 0.38, P = 0.54$). Differences between horizontal and vertical cladodes were greater in Zapotitlán than in Coxcatlán, especially during the wet season (Table 1). In general, horizontal cladodes had greater PPFD interception when fully exposed and under the canopy; however, differences among individuals under the canopy were less pronounced (Table 1, Fig. 4A, B).

As a consequence of the horizontal position, cladode surfaces received different amounts of PPFD (Table 1). In an exposed site, the lower surface intercepted 93–96% less PPFD than the upper surface, whereas under the canopy, the lower surface intercepted 88.0–96.9% less total daily PPFD than the upper surface. For vertical cladodes, differences in PPFD interception between surfaces were smaller in exposed and understorey sites (Table 1).

Under exposed conditions, temperature was higher in horizontal than vertical cladodes (Table 1). On a clear day in Coxcatlán, horizontal cladode temperature was above $45^\circ$C for about 6 h, reaching a maximum of $12.9^\circ$C above air temperature, whereas a vertical cladode reached a maximum of $6.2^\circ$C above maximum air temperature (Table 1, Fig. 4C). Under the canopy, differences between cladode and air temperature were smaller, and no significant differences were found between horizontal and vertical cladodes ($F = 2.73, P = 0.11$) or between sites ($F = 1.67, P = 0.20$). However, differences between seasons were significant ($F = 18.08, P = 0.0003$), with the highest temperatures observed in the dry season (Table 1, Fig. 4D).

No significant differences were detected in NAA between vertical and horizontal cladodes ($F = 0.17, P = 0.68$); the average $\pm$SE was $16.5 \pm 1.9$ and $15.0 \pm 2.1 \, \mu\text{eq} \cdot \text{cm}^{-2}$, respectively. Significant differences in NAA were found between populations ($F = 34.8, P = 0.0001$); however, no significant differences were detected between seasons ($F = 0.464, P = 0.496$; Table 2).

Nocturnal acid accumulation increased with total daily PPFD intercepted; Fig. 5A shows this relationship, including

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**Table 1.** Effects of *Opuntia puberula* cladode angle. Total daily photosynthetic photon flux density (PPFD) intercepted by both sides of horizontal cladodes (lower and upper) and vertical cladodes (east and west). Maximum diurnal cladode temperature ($T_{c\max}$) for horizontal and vertical cladodes, and maximum air temperature ($T_{a\max}$). Measurements were made for one clear day for each site and season, under exposed conditions (*) and under the canopy. The average $\pm$ SE ($N = 3$) is shown.

<table>
<thead>
<tr>
<th>Site and season</th>
<th>Cladode position</th>
<th>Lower or east</th>
<th>Upper or west</th>
<th>Total</th>
<th>$T_{c\max}$ (°C)</th>
<th>$T_{a\max}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zapotitlán wet</td>
<td>Horizontal1,*</td>
<td>1.4</td>
<td>44.3</td>
<td>45.7</td>
<td>41.9</td>
<td>37.4</td>
</tr>
<tr>
<td></td>
<td>Vertical1,*</td>
<td>13.23</td>
<td>11.91</td>
<td>25.1</td>
<td>33.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horizontal</td>
<td>0.84 ± 0.31</td>
<td>13.5 ± 4.8</td>
<td>14.3 ± 5.0</td>
<td>38.6 ± 2.7</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Vertical</td>
<td>1.73 ± 0.20</td>
<td>0.73 ± 0.2</td>
<td>4.7 ± 0.6</td>
<td>34.8 ± 1.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Zapotitlán dry</td>
<td>Horizontal</td>
<td>0.73 ± 0.2</td>
<td>12.99 ± 2.26</td>
<td>13.9 ± 2.4</td>
<td>52.8 ± 2.7</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>Vertical</td>
<td>3.70 ± 2.19</td>
<td>7.76 ± 2.60</td>
<td>11.5 ± 1.8</td>
<td>48.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horizontal1,*</td>
<td>2.95 ± 0.5</td>
<td>44.42 ± 3.1</td>
<td>47.4 ± 4.1</td>
<td>49.4</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>Vertical1,*</td>
<td>10.75 ± 2.5</td>
<td>17.04 ± 2.6</td>
<td>27.8 ± 3.2</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horizontal</td>
<td>1.063 ± 0.51</td>
<td>20.6 ± 5.4</td>
<td>21.7 ± 6.0</td>
<td>48.4 ± 5.5</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>Vertical</td>
<td>2.92 ± 0.84</td>
<td>16.45 ± 5.38</td>
<td>19.4 ± 6.2</td>
<td>45.3 ± 4.4</td>
<td>42.2</td>
</tr>
<tr>
<td></td>
<td>Horizontal</td>
<td>1.8 ± 0.28</td>
<td>21.75 ± 0.84</td>
<td>23.5 ± 3.5</td>
<td>49.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vertical</td>
<td>8.14 ± 3.24</td>
<td>7.84 ± 2.57</td>
<td>16.0 ± 2.4</td>
<td>47.7 ± 1.9</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4. Diurnal courses of photosynthetic photon flux density (PPFD) intercepted by the upper and lower surfaces of a horizontal cladode and for west- and east-oriented faces of a vertical cladode of Opuntia puberula in a fully exposed site (A) and in the understory (B). Temperatures of the same horizontal and vertical cladodes, as well as air temperature, are also shown for the fully exposed site (C) and the understory (D).

horizonal and vertical cladodes of both sites and seasons. A simple regression analysis showed a significant effect of PPFD on NAA ($F = 9.326, P = 0.0072$). However, the effect of DSF on NAA was not statistically significant ($F = 2.193, P = 0.154$; Fig. 5B).

Cladode water content—The relative water content (RWC) of cladodes varied seasonally and between populations. RWC was higher in Zapotitlán than Coxcatlán ($F = 21.2, P = 0.0003$). A significant decrease in RWC during the dry season was observed ($F = 11.0, P = 0.001$; Table 2).

Anatomical and physiological traits of the upper and lower cladode surfaces—No differences were found in cuticle and chlorenchyma thickness and stomatal density between sunniest and shadiest habitats or between upper and lower cladode surfaces (Table 3). Stomatal density did not show significant differences between sun and shade ($F = 1.952, P = 0.395$), nor between surfaces ($F = 0.209, P = 0.72$). Clear differences were found, however, in chlorophyll content between upper and lower surfaces ($F = 868.4, P = 0.021$); the upper surface had higher chlorophyll content than the lower one (Table 3). NAA only shows differences between sunniest

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**Table 2. Site and seasonal effects on Opuntia puberula cladodes.**

Nocturnal acid accumulation (NAA) and relative water content (RWC) of cladodes from both study sites and seasons. The average ±SE (N = 5–6) is shown.

<table>
<thead>
<tr>
<th>Site</th>
<th>Season</th>
<th>NAA (μeq·cm⁻²)</th>
<th>RWC (g water/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zapotitlán</td>
<td>wet</td>
<td>10.1 ± 1.7</td>
<td>11.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>dry</td>
<td>12.4 ± 1.8</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Coxcatlán</td>
<td>wet</td>
<td>24.3 ± 1.7</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>dry</td>
<td>19.5 ± 1.9</td>
<td>6.3 ± 0.5</td>
</tr>
</tbody>
</table>
Fig. 5. (A) Nocturnal malic acid accumulation (NAA) in cladodes of Opuntia puberula as a function of the total daily photosynthetic photon flux density (PPFD) intercepted. (B) Average individual NAA in cladodes as a function of direct site factor (DSF). The line represents the linear fit.

Daily courses of stomatal conductance were typical of a CAM plant, with maximum values reached at night and early in the morning, but no differences were found between upper and lower surfaces of the cladodes (Fig. 6). The diurnal pattern and the maximum $g_s$ reached were different between sites (Fig. 6). In Zapotitlán, maximum $g_s$ was 55 mmol·m$^{-2}$·s$^{-1}$, and because stomata did not close completely during the day, midday values remained at 25 mmol·m$^{-2}$·s$^{-1}$. In Coxcatlán, maximum $g_s$ was 150 mmol·m$^{-2}$·s$^{-1}$, and stomata remained partially open early in the morning and late in the afternoon.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Surface</th>
<th>Sun</th>
<th>Lower surface</th>
<th>Shade</th>
<th>Lower surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (mg·L$^{-1}$)</td>
<td>4.60 ± 0.98</td>
<td>2.03 ± 0.17</td>
<td>5.45 ± 0.35</td>
<td>2.70 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>Cuticle thickness (μm)</td>
<td>136.2 ± 3.7</td>
<td>129.2 ± 3.8</td>
<td>122.4 ± 3.9</td>
<td>108.6 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Chlorenchyma thickness (mm)</td>
<td>1.62 ± 0.10</td>
<td>1.62 ± 0.12</td>
<td>1.69 ± 0.17</td>
<td>1.59 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>Stomata density (number of stomata · mm$^{-2}$)</td>
<td>42.34 ± 1.17</td>
<td>34.42 ± 1.72</td>
<td>29.31 ± 0.89</td>
<td>32.26 ± 1.64</td>
<td></td>
</tr>
<tr>
<td>NAA (μeq·cm$^{-2}$)</td>
<td>18.61 ± 3.22</td>
<td>14.01 ± 2.32</td>
<td>8.17 ± 3.15</td>
<td>5.27 ± 2.27</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

Cladodes of *O. puberula* are nearly horizontal and grow preferentially in shaded habitats. Cladode angle changes little in response to the range of light environments where it naturally occurs, increasing only slightly in the sunniest conditions. We suggest that in shade habitats where light is a limiting factor, horizontal cladodes might be advantageous for increasing light interception. Our results showed that under shaded conditions, the horizontal position apparently seems to confer an advantage over the vertical position on PPFD interception. However, because the horizontal position under the canopy does not necessarily lead to greater carbon gain, the adaptive or neutral significance of this design remains to be demonstrated.

In shaded habitats where *O. puberula* grows, light availability might be a limiting factor, as shown by the relationship between PPFD and nocturnal acid accumulation. The daily PPFD measured in the understory of the study sites was sometimes close to the light compensation point reported for cacti (4 mol · m⁻²; Nobel, 1988). The light response curve for several cacti shows a linear increase in carbon fixation as total daily PPFD increases above the compensation point up to 15 mol · m⁻²; after this value, increases in carbon gain are smaller as total daily PPFD increases, until the light saturation point is reached at 20–25 mol · m⁻² (Gibson and Nobel, 1986).

Under shaded conditions, the horizontal cladodes might intercept more light than the vertical ones at midday, when direct sunlight passes through the canopy and reaches the understory. However, no differences in carbon gain were found between horizontal and vertical cladodes. In horizontal cladodes, the side facing the soil is generally below the light compensation point, whereas the upper side received most of the PPFD. It has been shown that the two surfaces of *Opuntia* cladodes act independently in terms of light absorption (Nobel et al., 1994). In *O. puberula*, we found higher chlorophyll content in the upper side, which might contribute to a better light-capture efficiency for the more exposed side. No differences in chlorenchyma thickness or nocturnal acid accumulation were detected between surfaces. Other studies have found considerable malate accumulation and photosynthetic enzyme activity in deep layers of the chlorenchyma of *O. ficus-indica* (L.) Miller, despite much lower light and chlorophyll content in comparison with surface layers (Nobel et al., 1994). A possible explanation of these results is that high-energy compounds generated during the day in more exposed layers could move to deeper regions of the chlorenchyma, allowing CO₂ fixation at night (Nobel et al., 1994). In *O. puberula*, high stomatal density and stomatal conductance in the lower side of cladodes indicate that this face might also play an important role in carbon fixation.

The horizontal position of *O. puberula* cladodes might be an important factor that prevents this species for growing under full sun conditions. Horizontal cladodes experimentally exposed to full sun conditions intercepted more light than vertical cladodes. Total daily PPFD intercepted by horizontal cladodes is twice the amount reported for light saturation in cacti (Nobel, 1988); more PPFD interception by horizontal cladodes does not represent an advantage over the vertical ones, because the light is received mostly in one surface that becomes saturated at a lower PPFD. On the other hand, the lesser interception of light in vertical cladodes might be compensated by its more uniform distribution in both surfaces, making vertical cladodes more efficient in light utilization than horizontal ones (Nobel, 1982). The highest light interception occurs at midday, when ambient temperature reaches the maximum value and when stomata are generally closed, preventing energy dissipation by latent heat loss (Gibson and Nobel, 1986).

In exposed sites and in the understory in the dry season, horizontal cladodes can reach high temperatures (around 50°C) for more than an hour. These temperatures are similar to those that some cacti can tolerate for short periods (Smith et al., 1984; Nobel, 1988; Nobel and De la Barrera, 2003). However, under full sun, prolonged exposure to high temperature, and light interception well above the saturation point may induce photoinhibition in *O. puberula*. Photoinhibition has been observed in several CAM species, including platypuntias (Adams et al., 1988; Demming-Adams and Adams, 1992; Wintz and Lesh, 1992; Barker and Adams, 1997). Exposure of *O. ficus-indica* cladodes to a PPFD of 1150 μmol · m⁻² · s⁻¹ for more than 6 h (36 mol · m⁻² · d⁻¹) decreased acid accumulation, and after 1 wk chlorophyll content also decreased (Nobel and Hartsock, 1983). For *O. basilaris* Engelm. & Bigel growing in natural conditions, photoinhibition was observed in the side of the cladode exposed to higher PPFD (Adams et al., 1987). The combination of high temperature and/or water stress conditions with high light interception increases the susceptibility to photoinhibition in CAM plants (Adams et al., 1987; Lu et al., 2003). Although we did not measure photoinhibition, field observations indicate that when the shade is modified by cutting the trees around the *O. puberula* plants, most of the cladodes developed a red coloration (Munsell 5R 5/8, 1977) with small parts of the cladodes with a pale-green coloration (Munsell 2.5GY 7/8, 1977) and many died. Therefore, we suggest that photoinhibition of horizontal cladodes may be an important factor restricting the distribution of this species to the understory. However, future studies are needed to test this hypothesis. Experimental studies on inclination angle, photosynthetic responses, and mechanical and anatomical characteristics of cladode joints of plants in response to different light and water availability conditions will provide a better understanding of the role of photoinhibition on the habitat restriction of *O. puberula*.

The limited response of *O. puberula* to modify cladode angle may have a structural basis. Several authors have found that the structure of secondary xylem in the stem and joints of platypuntias is closely correlated with growth habit (Gibson, 1973, 1977, 1978; Bobich and Nobel, 2001). Anatomical studies of *O. puberula* showed that stem wood does not have fibers, and vessel elements are short and narrow. The wood of this species also has a ground tissue with wide-band tracheids and un lignified rays. These anatomical characteristics indicate weak structural support and may determine the horizontal position of *O. puberula*. Studies of the biomechanic properties and anatomical characteristics of cladode joints are needed to understand the mechanical basis of cladode horizontal position.

*Opuntia puberula* is a widespread species distributed mainly along the Pacific coast of Mexico from Sinaloa to Oaxaca and along the Gulf of Mexico from Tamaulipas to Veracruz. It is an important element in the understory of the tropical deciduous forests. It produces bright red fruits with many seeds that are dispersed by birds; therefore, it has the potential to reach other habitats. This species might have evolved in the forest understory, and its presence at the Zapotitlán site might have
resulted from seed dispersal. At this site, the species distribution is completely restricted along the edges of ephemeral streams and beneath the canopies of evergreen species in conditions of high water availability and shade. These conditions contrast with the more exposed conditions present in the deciduous shrublands which dominate the landscape. Whether this distribution is the result of the horizontal cladodes character or whether it evolved after O. pubera was restricted to shaded habitats is unknown. Unfortunately, our knowledge about the distribution of the horizontal cladode character in Opuntia spp. is limited, and given our poor knowledge of Opuntia phylogeny, it is difficult to infer whether this character is derived or ancestral.

LITERATURE CITED


FERNÁNDEZ, N. 1999. Análisis de la dinámica de comunidades vegetales con relación a la orientación del paisaje, en la zona semiárida de Coxcatlán, Puebla. Tesis de Maestría, Universidad Nacional Autónoma de México, Mexico City, Mexico.


MUNSELL, A. H. 1977. Munsell color charts for plant tissues, Munsell color, 2nd ed. Macbeth Division of Kollmorgan Corporation, Baltimore, Maryland, USA.


VALIENTE, L. 1991. Patrones de precipitación en el valle semiárido de Te-
