

Seasonal patterns of acid fluctuations and resource storage in the arborescent cactus *Opuntia excelsa* in relation to light availability and size

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Summary. We investigated relationships between light availability, diel acid fluctuation, and resource storage in the arborescent cactus *Opuntia excelsa* growing in western Mexico. We compared canopy and understory individuals from a deciduous forest as well as open-grown plants of the same approximate size as those in the understory. During the wet season light availability and daily fluctuations in titratable acidity (an index of carbon uptake) were lower in the understory than in unshaded habitats. In the dry season all plants had reduced levels of acid fluctuation, with the smallest individuals, regardless of habitat, showing the greatest reduction. These data suggest that light availability in the forest understory constrains carbon assimilation during the wet season, but that a factor associated with plant size, possibly water status, limits carbon gain during the dry season. Plants in all habitats remained physiologically active for at least five months into the dry season. We suggest that this was possible due to the maintenance of constant concentrations of water and nitrogen in the photosynthetically active chlorenchyma. Parenchyma in terminal cladodes showed a different seasonal pattern of resource storage; water content and nitrogen concentration were reduced from the wet to the dry season in the parenchyma. Using the parenchyma to supply photosynthetic tissues during times of reduced resource availability allows *O. excelsa* to assimilate carbon during times of the year when most other trees in the forest are leafless.

Key words: Crassulacean acid metabolism – Light availability – *Opuntia excelsa* – Resource storage – Tropical deciduous forest

Patterns of resource acquisition have been well characterized for many cactus species growing in open habitats

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(Hanscom and Ting 1977; Luttge et al. 1989; Nobel 1977; Szarek and Ting 1974, 1975; Szarek and Troughton 1976). Cacti acquire and store resources from the soil when they are readily available and then utilize them in a conservative manner during periods of drought (Cockburn 1983; Neales et al. 1968; Kluge and Ting 1978; Luttge et al. 1989; Teeri 1984). Cacti are able to store large quantities of the resources necessary for photosynthesis because they possess specialized tissues immediately adjacent to photosynthetically active cells (MacDougal 1912 in Nobel 1988; Tissue et al. 1991). Cacti take advantage of these stored resources by using Crassulacean acid metabolism (CAM) to assimilate CO₂ in a manner that minimizes water loss (Kluge and Ting 1978; Luttge 1987; Osmond 1978). Although these adaptations allow cacti to grow in dry environments, they are energetically costly. High maintenance respiration costs are associated with large quantities of water storage tissue (Luttge and Ball 1987), and CAM is energetically less efficient than C₃ photosynthesis (Luttge 1987; Osmond 1978, 1984).

In the high light environments where many cacti are found high water-use efficiency more than compensates for increased energetic demands. Some cacti, however, grow in forest environments where carbon assimilation may be constrained by low light availability in the understory (Adams 1988; Backeberg 1976; Cody 1986; Nobel 1988). Previous studies on growth and photosynthesis in shade-tolerant CAM plants have focused on small, non-arborescent species (Adams 1988; Adams et al. 1987; Martin et al. 1981, 1985; Medina et al. 1977, 1986; Pfitsch and Smith 1988; Winter et al. 1986). Patterns of resource acquisition and storage enabling a cactus that is reproductively mature in the forest canopy to grow through the understory are unknown.

In this study we examined the distribution of individuals of the arborescent cactus *Opuntia excelsa* Sanchez-Mejorada growing in the understory of a deciduous forest in western Mexico in relation to light availability. We measured seasonal changes in carbon gain, water storage, and nutrient concentrations in *O. excelsa* individuals growing within the forest (both canopy and understory individuals) and in two open areas (salt marsh and open

field). Comparisons among forest plants and those in open habitats were designed to contrast plants of similar stature but growing in seasonally shaded (understory) or full sun environments. We investigated two questions: 1) Do plants growing in the forest understory occur preferentially in high-light microsites; and 2) What patterns of resource acquisition and storage enable this forest cactus to compensate for its energy inefficient morphology and metabolism?

Materials and methods

Species, site, and dates

Opuntia excelsa occurs throughout western Mexico, where it grows in diverse habitats ranging from upland deciduous forest to open salt marsh (Backeberg 1976). Although *O. excelsa* lacks leaves and instead supports a crown of flattened, photosynthetic cladodes (morphologically branches), it is tree-like. Large individuals grow to 14 m in height and have woody trunks up to 40 cm in diameter with the lowest branch typically occurring at about 8 m above the ground.

We conducted this study at and near the Chamela Biological Station of the Universidad Nacional Autónoma de México, located on the Pacific coast of the state of Jalisco (19° 30' N, 105° 03' W). This region receives an average rainfall of approximately 70 cm/yr, 80% of which falls from June through November (see Bullock 1986 for details on topography, soils, and rainfall). The upland vegetation in which this study was conducted consists of dense, closed canopy forest (4–12 m tall), with a well-developed shrub and vine understory (Lott et al. 1987). In the dry season, more than 95% of the trees found in the upland forest are leafless (Bullock and Solis-Magallanes 1990).

Field measurements were made during the latter third of the wet season (September 1990) and toward the end of the dry season (March 1991). The forest plants were all growing in undisturbed forest within 10 m of a trail. The plants in full sun habitats (hereafter referred to as sun and salt plants) were growing near the station in an open field and adjacent salt marsh. Canopy individuals were large, all at least 8 m in height, whereas the understory individuals ranged from 1.6 to 6 m in height, with most individuals between 2 and 5 m. Sun and salt individuals were between 1 and 3 m in height and lacked the single "trunk" of the forest plants.

Sampling

Stomatal aperture. Stomatal aperture was measured directly by making stomatal impressions, at approximately 2 hour intervals from mid-afternoon until mid-morning of the following day. Dental impression paste (Xantopren Blue™, Unitek™) was spread onto attached cladodes, allowed to harden, and then removed with forceps. Clear nail polish was then painted onto the paste, dried, removed, and viewed under a stereo microscope. Stomata were scored as either open or shut. Stomatal aperture was not measured on the canopy plants because the height of the trees (no branches lower than 6 m) made it impossible to access attached cladodes.

Light availability. Light availability in the forest understory and above individual understory cacti was estimated using hemispherical photography (Rich 1989 and 1990). Hemispherical photographs were taken pointed upwards using a Nikkor 8 mm fisheye lens and a Nikon FM2 body supported on a self-leveling mount at the end of a monopod. To characterize general light availability in the forest, photographs were taken at 20 randomly chosen locations along a 200 m transect at heights of 1, 2, 4, and 6 m for each location. To characterize the light environment of understory cacti as a function of plant size, photographs were taken just above the tops of 26 understory cacti of known height. All photographs were taken

during both the wet and dry seasons. Photographs were digitized and the proportion of direct and indirect (diffuse) light calculated using the image analysis program CANOPY (Rich 1989). We corrected for minor lens distortion, assumed a uniform distribution of potential indirect light as a function of sky direction, equal contributions of direct and indirect solar radiation, and a clear atmospheric correction for direct sunlight penetration. Maximum photosynthetic photon flux density (PPFD) was calculated as the proportion of total solar radiation (both direct and indirect) beneath the canopy relative to the total radiation above the canopy. Dry season measurements were calculated by integrating over December-May and wet season by integrating over June-November.

Tissue analyses. Titratable acidity, water content, and concentrations of nitrogen and phosphorus were measured on 10 canopy, 10 understory, 5 salt, and 5 sun plants for each season. All material was collected from cladodes with a cork-borer. The samples were immediately wrapped in Parafilm™ and returned to the lab where the chlorenchyma were cut away from the parenchyma with a razor blade and each tissue type analyzed separately. The chlorenchyma layers were distinguished from the parenchyma primarily by color; the chlorenchyma was bright green and the parenchyma off-white. Additionally, numerous mucilage canals anastomosing at the parenchyma/chlorenchyma interface helped demarcate the tissue types (Metcalf and Chalk 1950). Samples for analyses that required repeated measures of the same individual (e.g., titratable acidity) were taken from the same cladode whenever possible. Because canopy plant cladodes were accessible only using a pole pruner, a different cladode was sampled each time.

Titratable acidity. Titratable acidity was determined for chlorenchyma and parenchyma by collecting two samples, one just before dawn and one at dusk, from each cactus studied. The fresh tissue was ground in a sand/distilled water mixture and titrated to pH 7.0 using 10 mM NaOH (Nobel and Hartsock 1983). We calculated the difference between pre-dawn and dusk acidity levels on a two-sided area basis as an index of daily carbon gain.

Water content. Tissue water content was determined by collecting samples just before dawn and measuring the thickness of the chlorenchyma and the parenchyma with calipers. The two tissue types were then separated, weighed individually, and dried to a constant weight at 80°C. Water content was calculated as the difference of wet and dry measures normalized by dry weight.

Nitrogen and phosphorus analysis. Tissue samples for total nitrogen and total phosphorus analysis were collected just before dawn and dried to a constant weight at 80°C. We used a sulfuric acid digestion mixture heated to 400°C with a vanadium catalyst to convert all N to ammonia and P to phosphate. Samples were then analyzed in an Alpkem RFA/2™ autoanalyzer.

Results

Stomatal aperture

During both the wet and dry seasons all the stomata of *O. excelsa* were closed during the day; at night 90% were open in the dry season and 95% in the wet season. No variation was observed between plants growing in different habitats during either season.

Light availability

Calculated maximum PPFD increased both with height during a given season and from the wet to dry season at any height (Fig. 1a). Seasonal changes in light availability

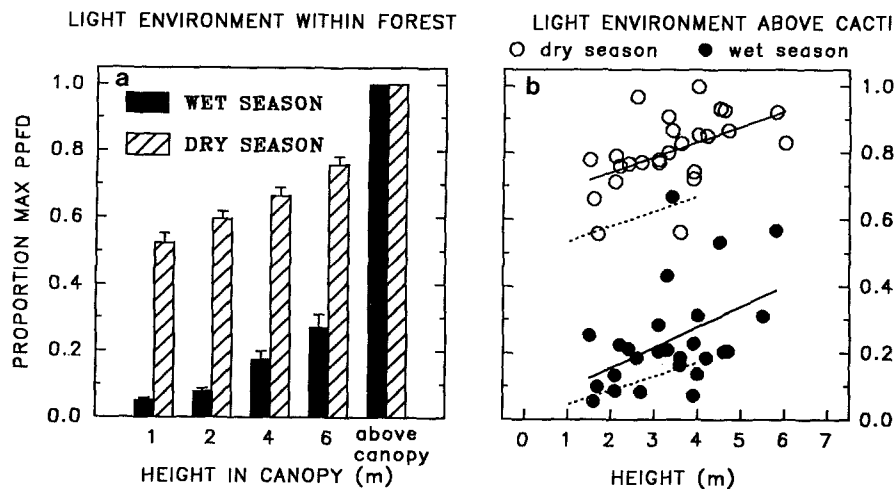


Fig. 1a, b. Maximum photosynthetic photon flux density (PPFD) calculated from hemispherical photographs taken in both the wet and dry seasons (a) along a forest transect at heights of 1, 2, 4, and 6 m ($n=20$ for each height and season), and (b) above individual cacti growing in the same forest ($n=26$). PPFD below the canopy is expressed as proportion of above-canopy PPFD. For the light environment of the forest transect, error bars represent one standard error of the mean. For the light environment above cacti, the solid lines are the least-square regressions for the dry season (above) and the wet season (below). Dashed lines are the least square regressions of the forest transect PPFD calculations

were pronounced; in the understory (1 m height) calculated maximum PPFD increased seasonally more than ten-fold, from approximately 5% to near 50% of the above-canopy solar radiation. Close to the top of the canopy (6 m height), calculated maximum PPFD increased seasonally almost three-fold, from approximately 25% to near 75% of the above-canopy solar radiation.

During the wet season, the light environment measured above understory cacti did not differ from the average for that same height in the forest transect (Fig. 1b). During the dry season, however, the calculated maximum PPFD above understory cacti was significantly higher than that measured at the corresponding heights in the forest transect. The least squares regression line for dry season light availability above each cactus vs. height has a similar slope to the least squares regression line for calculated maximum PPFD vs. height for the forest transect ($p > .05$) but a larger intercept ($p < .05$).

Tissue analyses

Titrateable acidity. Diel changes in titrateable acidity are presented on a unit area basis (calculated for a two-sided area), with the values for the parenchyma and chlorenchyma combined. By adding the values for the two tissue types and expressing them on a unit area basis, we show total cladode photosynthetic performance rather than the properties of individual tissues.

During the wet season, plants growing in full light (canopy, salt, and sun) showed similar diel changes in titrateable acidity, while the understory plants had much lower fluctuations (Fig. 2). In contrast, during the dry season the smaller plants (understory, salt, and sun) had similar diel changes in titrateable acidity, while the larger (canopy) plants showed titrateable acidity fluctuations more than twice that of the smaller plants.

Because diel fluctuations in titrateable acidity are an index of daily carbon uptake when the stomata are open at night and closed during the day (Nobel 1988), one can use the seasonal differences of fluctuations as an indication of seasonal changes in carbon assimilation. Understory plants have dry season daily assimilation rates that are

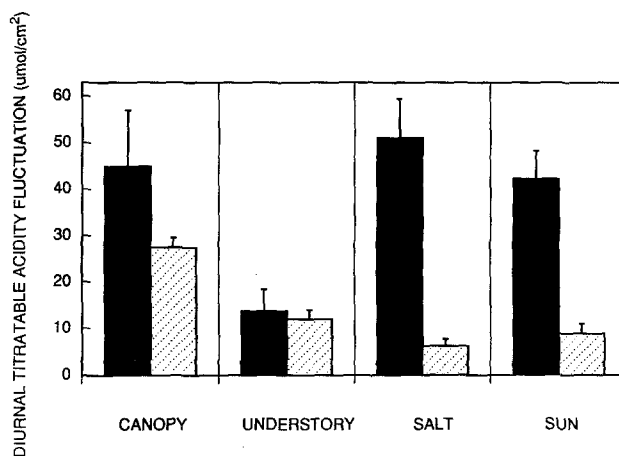


Fig. 2. Diurnal titrateable acidity fluctuations (pre-dawn - dusk values) for plants from the four habitats during two seasons (wet = dark cross-hatching, dry = light diagonal stippling). Data are presented on an area basis with results from parenchyma and chlorenchyma combined (see text for details). Error bars are one standard error of the mean

almost equal to their wet season rates. If this relationship were maintained throughout the entire year, then the understory plants would perform nearly half of their carbon assimilation during the dry season. For the plants in full sun habitats, dry season photosynthesis would account for a smaller fraction of total carbon assimilation.

Water content. *Opuntia excelsa* cladodes were significantly thicker during the wet season than in the dry season (Fig. 3). Within each season canopy and understory cladodes had similar thicknesses, although they were thinner than those of salt and sun plants (which did not differ from each other). Most of the seasonal change in cladode thickness was due to changes in the parenchyma (Fig. 3). In contrast, relative parenchyma layer thickness was constant among plants from all sites (56% of total thickness in the wet season; 33% in the dry season).

Variations in cladode water content (g water/g dry mass) reflected changes in thickness. Whereas the water content of the chlorenchyma was relatively constant

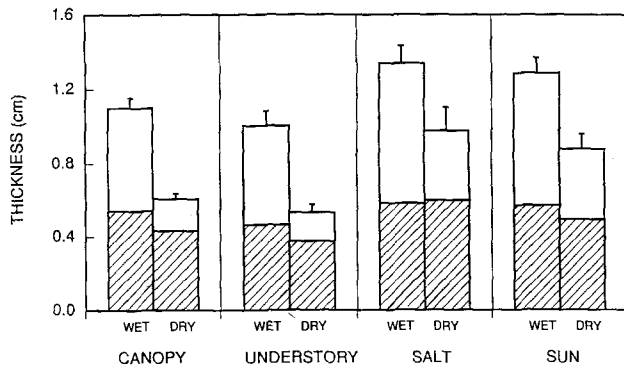


Fig. 3. Cladode thickness in cm for tissue samples from plants of the four habitats during two seasons (*wet* = left-hand bar, *dry* = right-hand bar). The *upper* and *lower* chlorenchyma are combined to one measure (*diagonal striped*), and the *parenchyma* tissue is presented above (*white*). Error bars are one standard error of the mean

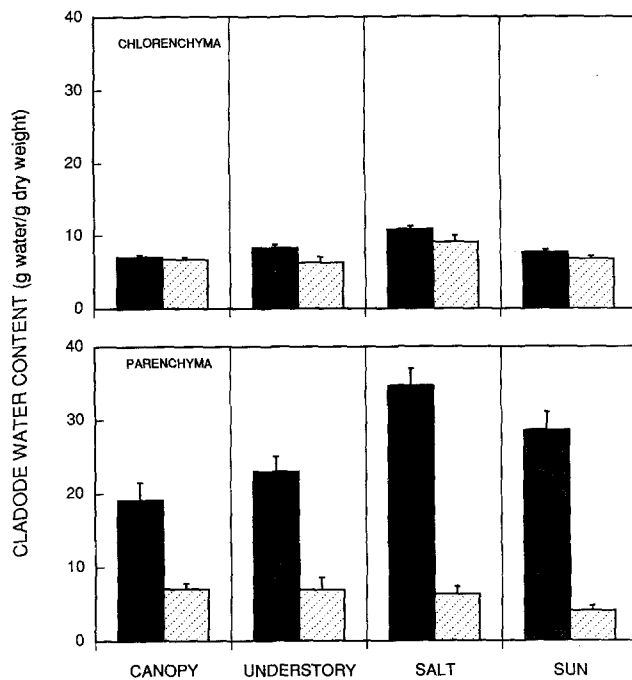


Fig. 4. Water content as a fraction of tissue dry weight for chlorenchyma (*upper panel*) and parenchyma (*lower panel*) for plants from the four habitats during two seasons (*wet* = dark cross-hatching, *dry* = light diagonal stippling). Error bars are one standard error of the mean

across both habitat and season, parenchyma water content was up to six times lower in the dry season than the wet (Fig. 4). Wet season parenchyma water contents were higher for the salt and sun plants than for the canopy and understory plants. There were no differences among habitats in the dry season.

Nitrogen. Chlorenchyma of all plants showed small and inconsistent seasonal variations in nitrogen concentrations. Parenchyma tissue nitrogen concentrations, however, dropped sharply from the wet to the dry season (Fig. 5). The magnitudes of these declines did not differ significantly among habitats.

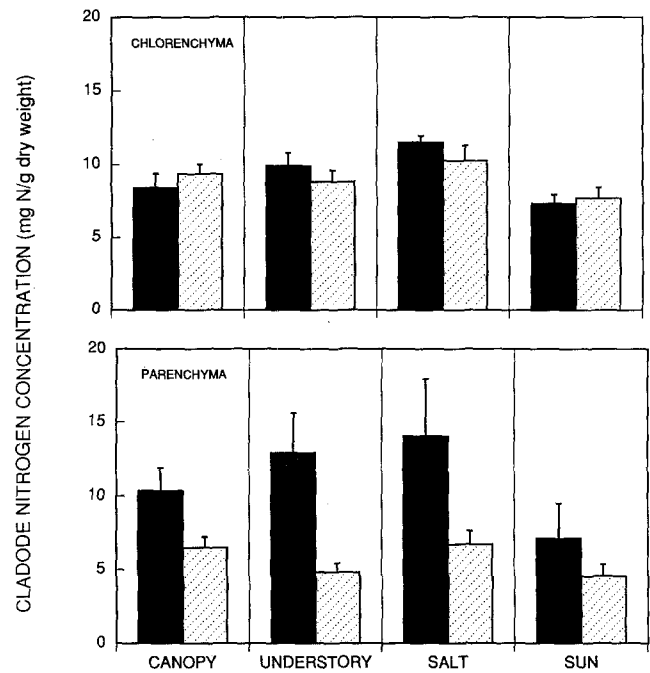


Fig. 5. Nitrogen concentration as a percentage of tissue dry weight for chlorenchyma (*upper panel*) and parenchyma (*lower panel*) for plants from the four habitats during two seasons (*wet* = dark cross-hatching, *dry* = light diagonal stippling). Error bars are one standard error of the mean

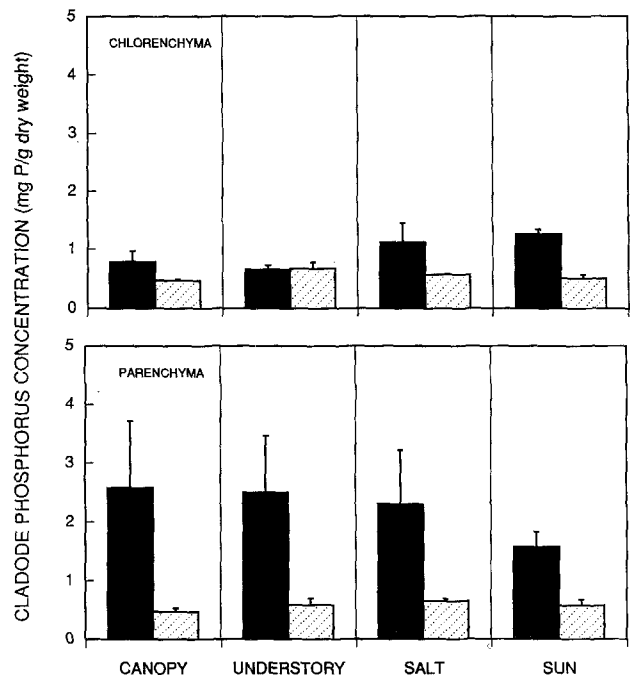


Fig. 6. Phosphorus concentration as a percentage of tissue dry weight for chlorenchyma (*upper panel*) and parenchyma (*lower panel*) for plants from the four habitats during two seasons (*wet* = dark cross-hatching, *dry* = light diagonal stippling). Error bars are one standard error of the mean

Phosphorus. There were no differences among habitats for either chlorenchyma or parenchyma tissue phosphorus concentrations. The chlorenchyma of all plants, except those in the understory, showed a decline of approximately 30% in phosphorus concentration from the wet to the dry season. Parenchyma phosphorus concentrations declined seasonally by approximately 70% in all habitats (Fig. 6).

Discussion

The distribution of understory *O. excelsa* appeared to be independent of light availability. The understory cacti did not occur preferentially in gap or high-light microsites. During the wet season, the estimated maximum PPFD immediately above understory plants did not differ from the average of measurements made at the same height at randomly chosen points along the forest transect and was significantly lower than what occurred above the canopy individuals. During the dry season the estimated maximum PPFD above understory plants was higher than along the transect. However, similarities in titratable acidity among understory, salt, and sun plants make it unlikely that this increase in light availability was physiologically significant. Increased maximum PPFD above understory *O. excelsa* during the dry season probably results from the influence of these plants on canopy space filling by the branches of adjacent trees and vines.

Understory *O. excelsa* had lower diel changes in titratable acidity during the wet season (when their light availability is reduced) than did those growing in the canopy, open field, or salt marsh, supporting the idea that carbon assimilation in understory CAM plants is constrained by light availability. In contrast, during the dry season, diel changes in titratable acidity of small individuals were significantly lower than those of large individuals. The correlation between plant size and dry season fluctuations in titratable acidity suggests that larger individuals were able to utilize water stored in their trunks and main branches. Studies of other CAM plants have also shown that water stressed individuals do not respond to increases in light intensity by increasing carbon uptake (Medina et al. 1986; Virzo de Santo et al. 1980).

Despite differences in carbon assimilation rates, individuals from all four sites showed similar seasonal patterns of resource storage. In the photosynthetically active chlorenchyma, all plants maintained constant concentrations of the two resources most closely linked to photosynthetic capacity, water and nitrogen. The parenchyma, which has been shown to act as a daily supplier of water to photosynthetically active tissue in other cacti (Tissue et al. 1991), appeared to supply water and nitrogen to the chlorenchyma when soil resources were unavailable. Phosphorus, which is only loosely linked to photosynthetic capacity in cacti (Nobel 1983), varied seasonally in both chlorenchyma and parenchyma.

These patterns of resource storage, similar to those found in cacti growing in open habitats, allowed carbon assimilation by *O. excelsa* five months into the dry season. Photosynthetic activity during the dry season may be an important mechanism by which *O. excelsa* can compete

with co-occurring deciduous C_3 trees that have energetically more efficient photosynthesis but are unable to maintain leaves for many months of the year.

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