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Seasonal Trends of Chlorophylls a and b and Carotenoids_(x + c) in Native Trees and Shrubs of Northeastern Mexico

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Abstract

*During two consecutive years, the content of chlorophylls **a** and **b** and carotenoids_(x + c) was determined, seasonally, in foliar tissue of native trees (T) and shrubs (S) from northeastern Mexico such as *Acacia rigidula* (S), *Bumelia celastrina* (T), *Castela texana* (S), *Celtis pallida* (S), *Croton cortesianus* (S), *Forestiera angustifolia* (S), *Karwinskia humboldtiana* (S), *Lantana macropoda* (S), *Leucophyllum frutescens* (S), *Prosopis laevigata* (T), *Zanthoxylum fagara* (T). Pigment determinations were carried out in a region of the state of Nuevo Leon, Mexico at three county (Los Ramones, China, Linares) sites, which are grouped under a similar climatic pattern. Measurements were quantified spectrophotometrically. With exception of the interaction year*plant of carotenoids content at Los Ramones site, all pigments were significantly different between years, seasons and between plants within years and seasons. All plants had marginal higher chlorophyll **a** content at Linares (overall mean = 0.79 mg g⁻¹ fw) than China (0.71) or Los Ramones (0.66) site. Chlorophyll **b** content followed a similar trend as chlorophyll **a** (0.29, 0.25 and 0.23, respectively). Marginal differences in carotenoids content, in all plants, were found among sites being the overall mean of 0.2 mg carotenoids g⁻¹ fw. Yearly and seasonal variations in plant pigments might have been related to seasonal water deficits, excessive irradiance levels during summer and extreme low temperatures in winter that could have affected leaf development and senescence.*

Introduction

Chlorophylls and carotenoids are essential pigments of higher plant assimilatory tissues and responsible for variations of color from dark-green to yellow. Moreover, they play important roles in photosynthesis capturing light energy which is converted into chemical energy (Bauernfeind, 1981; Young and Britton, 1993). Through the process of photosynthesis, chlorophylls are capable of channeling the radiant energy of sunlight into the chemical energy of organic carbon compounds in the cell (Nichiporovich, 1974). Carotenoids are a class of natural

fat-soluble pigments found mainly in plants, algae, and photosynthetic bacteria, where they also play a critical role in the photosynthetic process. In some non-photosynthetic bacteria, yeasts, and molds, they may carry out a protective function against damage by light and oxygen (Biswall, 1995; Gitelson *et al.*, 2002). Although animals appear to be unable of synthesizing carotenoids, many incorporate carotenoids from their diet. Within animals, carotenoids provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity (Britton *et al.*, 1995). Moreover, carotenoids develop important functions in plant reproduction, through their role in attracting pollinators and in seed dispersal (Yeum and Russell, 2002). Native shrubs and trees that grow in the semiarid regions of northeastern Mexico are important feed resources for range ruminants and white-tiled deer. They also provide high quality fuelwood and timber for fencing and construction, and are widely distributed in combination with other species (scattered), or are found in pure stands (Reid *et al.*, 1990; Fulbright *et al.*, 1991); however, are affected by climatic conditions and probably causing differences in the concentrations of photosynthetic pigments (chlorophyll **a** and **b** and carotenoids) when considering effects in space (sites) and weather (seasonality). To our knowledge, this is the first study of pigment profile estimation in native woody species and leaf structures growing in northeastern Mexico. Thus, the study was carried out with aims to quantify and compare, seasonally during two consecutive years, the content of photosynthetic pigments in trees and shrubs that grow under a similar climatic pattern in northeastern Mexico.

Materials and Methods

Study Site

This study was carried out at three sampling sites located in the state of Nuevo Leon, Mexico. The first site was located at “El Abuelo” Ranch in Los Ramones county (25°40' N; 99°27' W) with an elevation of 200 m. The climate is semiarid with warm summer. Annual mean air temperature is about 22° C. Average annual rainfall is approximately 700 mm. The second site was located at “Zaragoza” ranch in China county (25°31' N and 99°16' W). It has an elevation of 200 m. The climate is dry and warm throughout the year. Average total annual rainfall ranges from 400 to 600 mm with an annual mean air temperature of 22°C. The third site was located at the Experimental Station of Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León (24°47' N; 99°32' W; elevation of 350 m) located at Linares county. The climate is subtropical and semiarid with warm summer. Monthly mean air temperature ranges from 14.7° C in January to 22.3° C in August, although daily high temperatures of 45° C are common during summer. Average annual precipitation is about 805 mm with a bimodal distribution. The three sites are grouped under a similar climatic pattern with peak rainfall during May, June and September. The main type of vegetation is known as the Tamaulipan Thornscrub or Subtropical Thornscrub Woodlands (SPP-INEGI, 1986). The dominant soils are deep, dark-gray, lime-gray, lime-clay Vertisols, with montmorillonite, which shrink and swell noticeably in response to changes in soil moisture content.

Plant Material

Plant species such as *Acacia rigidula* Benth. (Fabaceae, shrub), *Bumelia celastrina* H. B. K. (Sapotaceae; tree), *Castela texana* Torr & Gray (Verbenaceae; shrub), *Celtis pallida* Torr. (Ulmaceae; shrub), *Croton cortesianus* Kunt. (Euphorbiaceae; shrub), *Forestiera angustifolia* Torr. (Oleaceae; tree), *Karwinskia humboldtiana* Roem et Schult. (Rhamnaceae; shrub), *Lantana macropoda* Torr., (Simaroubaceae; shrub), *Leucophyllum frutescens* Berl. (Scrophulariaceae; shrub), *Prosopis laevigata* (Willd) M.C. Johnst. (Fabaceae; tree) and *Zanthoxylum fagara* L. (Rutaceae; tree), that are the most representative of the native vegetation of the northeastern Mexico and the subtropical savanna ecosystems of southern Texas, USA (Vines, 1984; Reid *et al.*, 1990), were selected for pigment analysis.

Plant Tissue Sampling Procedures, Pigment Extraction and Analyses

Terminal shoots with fully expanded leaves from different plants per species were randomly chosen from a 50 m x 50 m representative and undisturbed thornscrub plot located in each site. Collections were undertaken, seasonally during two consecutive years: in summer, 2004 (August 28); fall, 2004 (November 28); winter, 2005 (February 28); spring, 2005 (May 28); summer, 2005 (August 28); fall, 2005 (November 28); winter, 2006 (February 28) and spring, 2006 (May 28). Shoots were excised and sampled from the middle side of four plants (replications) of each species. Leaves were placed into plastic bags and stored on ice under dark condition using a chest insulator. Cooled samples were then transported to laboratory for pigment analyses which were performed within 12 h after collections. Quadruplicate samples (1.0 g of leaf tissue) of each plant species were used for analyses. The chlorophylls **a** and **b** and carotenoids_(x + c) were extracted in 80% (v/v) aqueous acetone and vacuum filtered through a Whatman No. 1 filter paper. Pigment measurements were quantified spectrophotometrically using a Perkin-Elmer Spectrophotometer (Model Lambda 1A). Absorbances of chlorophylls **a** and **b** and carotenoids_(x + c) extracts were determined at wavelengths of 663, 645 and 470 nm, respectively. Concentrations (mg g⁻¹ fw) of pigments were calculated by equations of Lichtenthaler and Wellburn (1983).

Statistical Analysis

Data of chlorophylls and carotenoids were statistically analyzed using one-way analysis of variance with a multi-factorial arrangement being years (2), seasons (4), plant species (11) the factors. Where the F-test was significant ($P < 0.05$), differences were validated using the Tukey's honestly significant difference. Assumptions of normality of data were tested using the Kolmogorov-Smirnov test (Steel and Torrie, 1980). All applied statistical methods were computed using the SPSS package (Version 9).

Results and Discussion

Chlorophyll **a** content was significantly different between years, seasons and between plants within years and seasons (Table 1). During the first year, at Los Ramones site, chlorophyll **a** content in shrub *A. rigidula* (summer 2004) was lower (0.3 mg g⁻¹ fw) and in the tree *P. laevigata* (spring 2005; Figure 1a) was higher (1.1 mg g⁻¹ fw). Conversely, in second year, lower values (0.3 mg g⁻¹ fw) were found in *L. macropoda* in winter 2006 and higher (0.9 mg g⁻¹ fw) in *C. pallida* during summer 2005. At China site (Figure 1b) *C. cortesianus* (fall 2004) and *L. macropoda* (spring 2005) achieved lower (0.4) and higher (1.8) chlorophyll **a** content, respectively. During the second year, *C. cortesianus* (winter 2006) and *A. rigidula* (summer 2005) acquired lower (0.3) and higher (1.2) content, respectively. In Linares (Figure 1c), during the first year, higher (0.3) and lower (1.2) contents were registered in *P. laevigata* during fall 2004 and *B. celastrina* in winter 2005, respectively. However, during the second year, *L. macropoda* and *P. laevigata* resulted with lower (0.4) and higher (1.4) contents of chlorophyll **a** during winter 2006 and spring 2006, respectively. At Los Ramones and China sites, chlorophyll **a** content, in all plants, resulted higher in the first year than the second year. Conversely, at Linares, the second year was higher. It appears, that all plants had marginal higher chlorophyll **a** content at Linares (overall mean = 0.79 mg g⁻¹ fw) than China (0.71) or Los Ramones (0.66).

Chlorophyll **b** content was significantly different between years, seasons and between plants within years and seasons (Table 1). During the first year, at Los Ramones site (Figure 2a), chlorophyll **b** content in the shrub *A. rigidula* (summer) was lower (0.2 mg g⁻¹ fw) and in *L. frutescens* (summer) was higher (0.4). Moreover, in second year *A. rigidula* (winter) was lower (0.03) and *C. cortesianus* (spring) was higher (0.4). At China (Figure 2b) lower values (0.1) were detected in *C. texana* in spring and higher (0.6) in *L. macropoda* in spring too. During the second year, chlorophyll **b** content followed a similar pattern as the first year. At Linares (Figure 2c), during the first year, lower (0.1) and higher (0.6) values were observed in *F. angustifolia* and *L.*

Table 1. Calculated mean square values from the statistical analysis corresponding to data collected between summer 2004 and spring 2006 of eleven plant species at northeastern Mexico.

Sites	Sources of Variation	Chlorophyll a			Chlorophyll b			Carotenoids		
		MS	F value	Sig	MS	F value	Sig	MS	F value	Sig
Ramones	Years (Y)	0.3	25	***	0.2	152	***	0.02	21	***
	Seasons (S)	0.1	6	***	0.1	63	***	0.1	96	***
	Plant Species (PS)	0.1	11	***	0.01	5	***	0.03	34	***
	Y*S	0.3	28	***	0.1	35	***	0.02	20	***
	Y*PS	0.04	4	***	0.01	5	***	0.001	1	**
	S*PS	0.1	7	***	0.01	8	***	0.01	5	***
	Y*S*PS	0.1	8	***	0.01	4	***	0.01	5	***
	Error	0.01			0.002			0.001		
China	Years (Y)	0.2	15	***	0.2	53	***	0.01	9	***
	Seasons (S)	0.03	3	*	0.2	61	***	0.1	79	***
	Plant Species (PS)	0.4	33	***	0.1	14	***	0.1	108	***
	Y*S	0.1	11	***	0.02	8	***	0.01	22	***
	Y*PS	0.1	10	***	0.01	5	***	0.01	8	ns
	S*PS	0.1	8	***	0.01	3	***	0.01	11	***
	Y*S*PS	0.1	11	***	0.01	4	***	0.01	6	***
	Error	0.01			0.003			0.001		
Linares	Years (Y)	0.6	38	***	0.02	6	**	0.04	31	***
	Seasons (S)	0.1	6	***	0.2	51	***	0.1	70	***
	Plant Species (PS)	0.2	13	***	0.1	18	***	0.04	36	***
	Y*S	1.0	47	***	0.1	44	***	0.04	32	***
	Y*PS	0.1	9	***	0.01	4	***	0.01	5	***
	S*PS	0.1	9	***	0.02	7	***	0.01	6	***
	Y*S*PS	0.1	6	***	0.02	7	***	0.01	4	***
	Error	0.01			0.003			0.001		

MS = mean square; Y = years; S = seasons PS = plant species; Sig = significant level; *($P < 0.05$); **($P < 0.01$); ***($P < 0.001$); ns = not significant.

frutescens during summer 2004, respectively. Conversely, in the second year, *C. pallida* in winter 2005 was higher (0.1) and *K. humboldtiana* in autumn was lower (0.6). In all plants, annual and seasonal contents of chlorophyll **b** followed a similar trend as chlorophyll **a**; Linares was marginal high (overall mean = 0.29 mg g⁻¹ fw) than Los Ramones (0.25) or China (0.23).

With exception of interaction year*plant species at Los Ramones site, carotenoids content was significantly different between years, seasons and between plants within years and seasons (Table 1). At Los Ramones site (Figure 3a), during the first year, lower (0.1 mg g⁻¹ fw) and higher (0.3 mg g⁻¹ fw) values were detected in *P. laevigata* (summer) and *K. humboldtiana* (fall), respectively. In second year, lower (0.1) and higher (0.4) values were observed in *A. rigidula* (spring) and *K. humboldtiana* (winter), respectively. During first year, at China (Figure 3b), *C. texana* (summer) and *K. humboldtiana* (winter) were lower (0.1) and higher (0.5), respectively. Moreover, in second year, *C. texana* (spring) was lower (0.03) and *K. humboldtiana* (winter) higher (0.5). In Linares (Figure 3c), during the first year, minimum (0.03) and maximum (0.4) concentrations were registered in *C. cortesianus* (summer) and *K. humboldtiana* (winter). During second year, *C. texana* (spring) was lower (0.1) and *K. humboldtiana* (fall-2005) was higher (0.5). Low marginal differences were found in all plants among research sites being the overall mean of 0.2 mg carotenoids g⁻¹ fw. Chlorophylls and carotenoids absorb light energy and transfer

it into the photosynthetic apparatus of leaves; therefore, determinations of leaf pigments content can provide a valuable tool to integrate and understand the physiological and biochemical function of leaves (Sims and Gamon, 2000).

Chlorophylls and carotenoids absorb light energy and transfer it into the photosynthetic apparatus of leaves; therefore, determinations of leaf pigments content can provide a valuable tool to integrate and understand the physiological and biochemical function of leaves (Sims and Gamon, 2000). Results of this study suggest that chlorophylls **a** and **b** and carotenoids content of plant species varied between years, seasons and between plants within years and seasons. This finding confirm the importance to address research on these native plants, with ecological and forage potential value, in order to understand the function that, plant pigments, may play in ecosystem productivity, and the influence of drought and extreme temperatures that prevails during winter and summer seasons in this region (González *et al.*, 2000).

It has been established that productivity of higher plants is mediated by leaves and adaptations of plants to the environment involve leaf traits (Valladares *et al.*, 2000). Native vegetation in northeastern Mexico, composed mainly by shrubs and small trees, is characterized by low biomass productivity (about 3.2 kg DM ha⁻¹ year⁻¹; Villalón, 1989) because during summer, native plants have to deal with soil water deficits, high temperatures and high irradiance levels (González *et al.*, 2004). Furthermore, these plants have low tissue water potential because are exposed to low temperatures (-3 to 5° C) during winter and dry and warm whether in spring and summer seasons (González *et al.*, 2000, 2004). Under such environmental conditions, photosynthesis may be limited by temperature, stomatal control and light energy damage. In addition, chlorophylls content and the chlorophyll **a/b** ratio, in some plants, are affected by temperature (Ottander *et al.*, 1995) and prevailing shade characteristics (Castrillo *et al.*, 2001).

Seasonality of pigments in plants evaluated in this study was also reported by Schlerf *et al.* (2003) in spruce needles; however, they found higher values. It has been suggested that variation of pigments could be related to phenological phases of plant species such a flowering and production of sprouts (Arthur *et al.*, 1987). Other studies have revealed that loss of pigment content might have been associated with reduction in nitrogen flux into leaf tissue, alterations in the activity of enzyme systems such as nitrate reductase (Morilla *et al.*, 1973) or nitrogenase in legumes (Engin and Sprent, 1973). In this study, Fabaceae species showed higher content in chlorophyll **a** than non Fabaceae species. However, contents of chlorophyll **b** and carotenoids remained the same across Families. In this study, chlorophylls in all plants were higher than carotenoids. Moreover, a significant positive linear relationship was found between carotenoids and chlorophyll **a** content. Chlorophyll **a** content explained about 32% (China; $P < 0.001$), 39% (Los Ramones; $P < 0.001$), and 45% (Linares; $P < 0.001$) of the total seasonality of carotenoids. These finding are in agreement with Sims and Gamon (2002), who studied the relationships between leaf pigment content and spectral reflectance across a wide range of plant species. Relationships between carotenoids and chlorophyll **a** content included a range of healthy leaves and stressed leaf tissue due to drought and extreme temperatures (Table 1). During winter of 2006 a temperature of -5° C was registered in the region causing a reduction of chlorophyll **b** content at the three sites. However, the seasonal pattern of chlorophyll **a** content was more variable than chlorophyll **b** or carotenoids in all plants. Evaluated plants are characterized by a wide range of taxonomic groups exhibiting differences in growth patterns, leaf life spans, textures, growth dynamics, and phenological development (Reid *et al.*, 1990; McMurtry *et al.*, 1996; Northup *et al.*, 1996). In general, tree species had higher contents of chlorophyll **a** than shrubs. In contrast, contents of chlorophyll **b** and carotenoids did not differ among growth forms. Perennial plants such as *Bumelia celastrina*, *Celtis pallida*, *Karwinskia humboldtiana* and *Zanthoxylum fagara* had higher (mean = 0.7 mg g⁻¹ fw) chlorophyll **a** than deciduous species such as *Forestiera angustifolia*, *Castela texana* and *Croton cortesianus* (0.6).

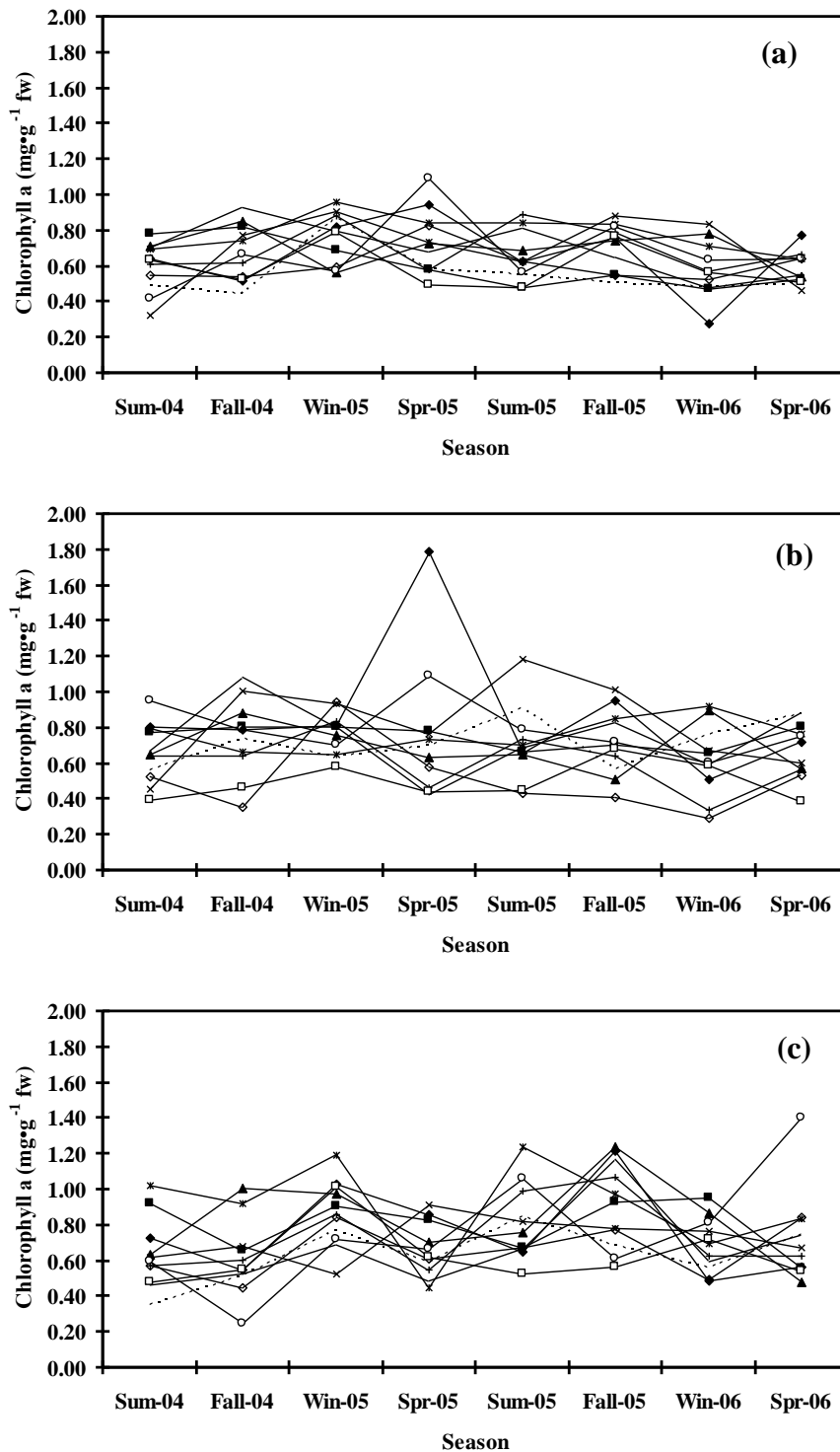


Figure 1. Seasonal contents of chlorophyll *a* at Los Ramones (a), China (b) and Linares (c) sites in eleven native trees and shrubs. Plotted data points are means from four independent measurements per plant species. Sum-04 = Summer 2004; Win-05 = Winter 2005; Spr-05 = Spring 2005; Sum-05 = Summer 2005; Win-06 = Winter 2006; Spr-06 = Spring 2006. *C. cortesianus* (\diamond); *L. frutescens* (\blacksquare); *K. humboldtiana* (\blacktriangle); *A. rigidula* (\times); *B. celastrina* (*); *P. laevigata* (\circ); *C. pallida* (+); *Z. fagara* (-); *F. angustifolia* (-); *L. macropoda* (\blacklozenge); *C. texana* (\square).

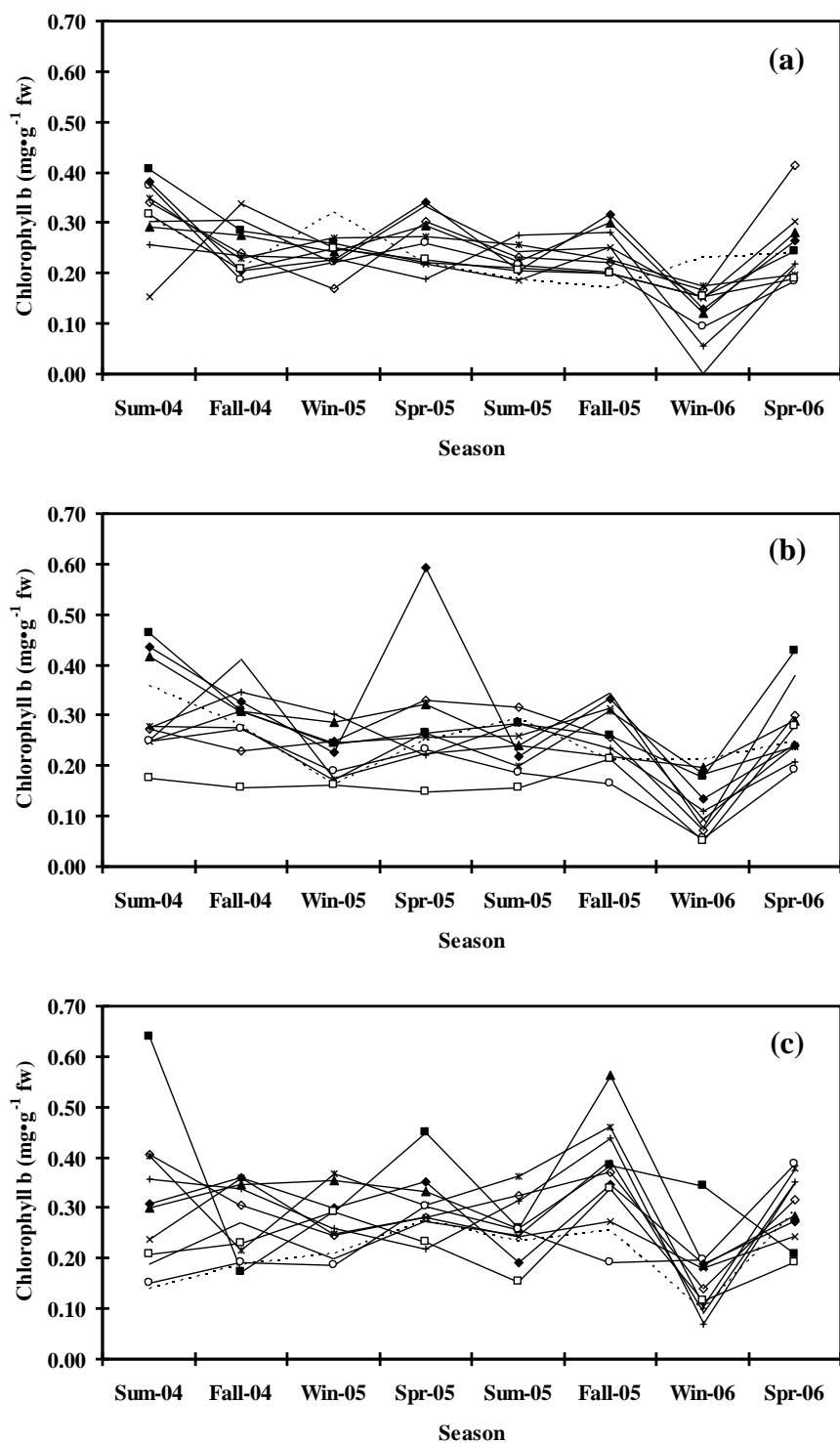


Figure 2. Seasonal contents of chlorophyll **b** at Los Ramones (a), China (b) and Linares (c) sites in eleven native trees and shrubs. Plotted data points are means from four independent measurements per plant species. Sum-04 = Summer 2004; Win-05 = Winter 2005; Spr-05 = Spring 2005; Sum-05 = Summer 2005; Win-06= Winter 2006; Spr-06 = Spring 2006. *C. cortesianus* (\diamond); *L. frutescens* (\blacksquare); *K. humboldtiana* (\blacktriangle); *A. rigidula* (x); *B. celastrina* (*); *P. laevigata* (\circ); *C. pallida* (+); *Z. fagara* (-); *F. angustifolia* (-); *L. macropoda* (\blacklozenge); *C. texana* (\square).

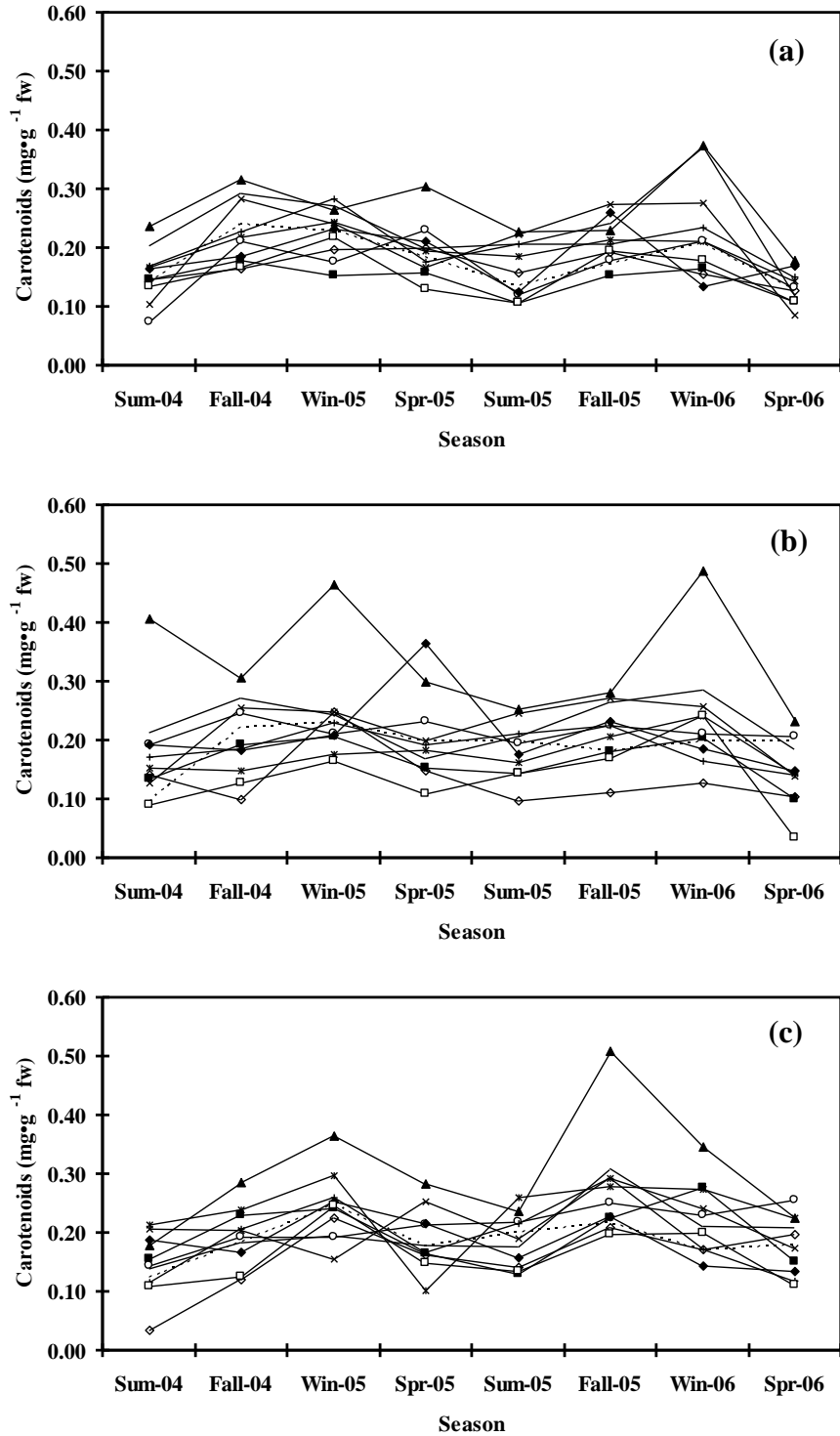


Figure 3. Seasonal contents of carotenoids_(x+c) at Los Ramones (a), China (b) and Linares (c) sites in eleven native trees and shrubs. Plotted data points are means from four independent measurements per plant species. Sum-04 = Summer 2004; Win-05 = Winter 2005; Spr-05 = Spring 2005; Sum-05 = Summer 2005; Win-06= Winter 2006; Spr-06 = Spring 2006. *C. cortesianus* (\diamond); *L. frutescens* (\blacksquare); *K. humboldtiana* (\blacktriangle); *A. rigidula* (\times); *B. celastrina* ($*$); *P. laevigata* (\circ); *C. pallida* ($+$); *Z. fagara* ($-$); *F. angustifolia* ($-$); *L. macropoda* (\blacklozenge); *C. texana* (\square).

Variations in chlorophyll content between plants have been related to leaf development and senescence (Gitelson and Merzlyak, 1994; Gamon and Surfus, 1999; Carter and Knapp, 2001). Furthermore, Valladares *et al.* (2000) reported that chlorophyll content was higher in shade leaves than sun leaves, whereas carotenoids content and nonphotochemical quenching increased with light.

Studies on carotenoids composition of sun leaves of plants with different life forms have revealed that sun leaves contained greater amounts of the components of the xanthophyll cycle violaxanthin, antheraxanthin and zeaxanthin as well as of β -carotene than the shade leaves (Demmig-Adams and Adams III, 1992). However, in the present study, it remains unclear whether lower or higher chlorophyll content at a given season is related to shade or sun leaves, since a pooled leaf sample was taken from each individual species. Kyparissis *et al.* (1995) have indicated that reduction of chlorophylls does not result from severe photoinhibitory damage, instead, it may be an adaptive response against the adverse conditions of the Mediterranean summer. This rationale could be extended to northeastern Mexico ecosystems, since water availability, as in Mediterranean field conditions (Kyparissis *et al.*, 2000; Valladares *et al.*, 2000; Oliveira and Peñuelas, 2001), is the most limiting factor controlling plant growth, survival and distribution in dry climates (Kramer, 1983; Newton and Goodin, 1989).

The great diversity of native plants in northeastern Mexico reflects the plasticity of how trees and shrubs species deal with seasonal water deficits, extreme temperatures (frost or heat) and excessive irradiance levels as main multiple stresses that may co-occur either during the winter or summer seasons.

Implications

Results of the present study suggest that, even though, all plants differed in pigment content and followed a seasonal pattern, during adequate or adverse conditions such as extreme temperatures and water shortages, they still could play important roles in maintaining the productivity of dry rangeland ecosystems. However, studies on leaf tissue at morphological, anatomical, biophysical, biochemical, physiological, and molecular level should be addressed to elucidate the underlying mechanisms employed by these trees and shrubs to adapt to this ecosystem and to deal with prolonged drought periods, high temperatures and high irradiance levels, with the purpose to identify fundamental mechanisms that increase or reduce pigment concentration, and how they are related to photochemical efficiency, photoinhibition and tissue water relations. These questions could be focused at both leaf and chloroplast (thylakoid) level. Thus, thornscrub ecosystems in northeastern Mexico provide a good opportunity to investigate the ecophysiology and photoprotective capacity of native trees and shrubs that traditionally have been used as a forage source for domestic livestock and wildlife.

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