SEASONAL VARIATION IN SPECIFIC LEAF AREA, EPICUTICULAR WAX AND PIGMENTS IN 15 WOODY SPECIES FROM NORTHEASTERN MEXICO DURING SUMMER AND WINTER

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Abstract

The present study has been undertaken on the variability in specific leaf area, epicuticular wax and pigment content of 15 native woody species in northeastern Mexico. The species showed considerable variability in responses of these leaf traits. Majority of the species showed a decline in specific leaf area and epicuticular wax content. With respect to pigments, only few species showed a decrease, but some species showed an increase in pigments (chlorophyll a, b and total chlorophyll (a+b)) showing mechanism of adaptation to winter season. However, in few species there was a decline in pigment contents showing susceptibility to winter.

Key words: Woody species, Leaf aea, Epicuticular wax, Leaf pigments, Seasonal variation, Summer, Winter.

Introduction

Native shrubs and trees in semiarid region of Northeastern Mexico serve as important resources for a wide range ruminants and white tailed deer (Ramirez, 1998) and also provide high quality fuel and timber for fencing and construction (Reid *et al.*, 1990; Fulbright *et al.*, 1991).

Leaves contributes greatly to the productivity of trees and shrubs through the process of photosynthesis. In this respect, leaf area and pigments contribute greatly. Epicuticular wax helps in the reflection of solar radiation thereby reducing leaf temperature and impart drought resistance (reference).

The vegetation in a large area of northeastern Mexico is mainly composed of dominant shrubs and trees with a wide spectrum of species (Foroughbakhch *et al.*, 1989, 2005). The vegetation prevailing in this region is exposed to extreme temperature, infertile and saline soil and irregular low precipitation (Villanueva, 1993; López, 2006; Alvarado *et al.*, 2008). These species are largely used for various purposes such as construction of fences, various agricultural implements, and also for firewood, charcoaland other uses in crops and pastures (Reid *et al.*, 1990).

Various studies have been undertaken on the dimensions and structures of leaves which vary greatly under different environmental conditions such as altitude (Francisca & Torres, 2003), latitude (Rico & Palacios, 1996; King & Mandonald, 1999), precipitation (Klich, 2000), temperature (Reich *et al.*, 2004), edaphic conditions (Mallarino *et al.*, 2001), quality and quantity of light (Pearcy &Yang, 1998; Valladares *et al.*, 2000; Balaguer *et al.*, 2001; Gamage *et al.*, 2003; Kikuzawa, 2003; Reich *et al.*, 2004), moisture availability (McDonald *et al.*, 2003) and leaf positions (Mitchell & Bolstad, 1999; Valladares *et al.*, 2000; González, 2001).

González (2001) assessed the qualitative and quantitative characterization of leaf morphology.Meade & Parnell, (2003) studied the leaf and floral characters of *Quercus* for taxonomic purpose and multivariate analysis was done using these characters.

Various researches have studied the patterns of foliar morphology in relation to adaptation to environmental conditions with respect to the variations in foliar patterns in relation to the phenological variation of the leaves at gradients of altitudes, latitudes and edaphic conditions (Tang & Ohsawa, 1999). The variation in morphological pattern is dependent on the availability of water, wind velocity, light intensity, intra and interspecific variations (Futuyma, 1998). Besides, foliar morphology is determined on genetic basis as it is exposed to intense selection pressure in the environment, thereby expressing different forms and foliar size (Aguiar *et al.*, 2002).

The phenotypic plasticity contributes to leaf morphology and its size. The species with a wide range of distribution have greater phenotypic plasticity compared to those with limited distribution (Futuyma & Moreno, 1988). The environmental changes contribute to phenotypic plasticity and physiological function. Bradshaw (1965), Sultan (1995) and Schlichting (2002), considered the phenotypic plasticity as one of the mechanism of the plant for its survival to adverse environmental conditions. It is stated that the phenotypic plasticity is frequently represented as one form of reaction of individual plants to the environment (De Kroon *et al.*, 2005). Therefore, all these factors determine the leaf area of a plant.

The role of leaf area is envisaged as a fundamental aspect of research on plant physiology in agriculture and dendrology (Broadhead *et al.*, 2003). The leaf area contributes to several processes in agronomy, biology, environment and physiology which are involved in the analysis of growth, photosynthesis, transpiration, light interception, biomass estimation and water balance (Kucharik *et al.*, 1998). The plant physiologists, biologists, and agronomists have documented well the importance of leaf area in the growth analysis, estimation of potential biological and agronomic yield, basis of the efficient use of solar radiation and mineral nutrition (Sonnentag *et al.*, 2008).

The leaf area of a species at a particular stage is defined as the capacity of the plant cover for interception of photosynthetically active radiation (PAR) nedded for elaboration of tissues and organic matter. Therefore, the growth and the productivity of a crop is the function of a genotype and its interaction with its environments (Rincón et al., 2007). Growth analysis is an methodology used for crop improvement, crop physiology and crop ecology (Poorter & Garnier, 1996). The leaf characteristics are exposed to continuous process of selection under environmental changes and its capacity to adaptation to these changes. Shipley (1995) investigated the effects of specific leaf area (SLA) on a wide range of 34 herbaceous species and stated that all species having large leaves had lower SLA, although the same pattern was not detected in the interspecific level. However, recent studies reported that the production of dry matter per unit of leaf area increase with an increase with leaf size and yield inversion (light capture) and decrease with an increase of leaf size (Milla & Reich, 2007; Niklas et al., 2007). This is found both among and within the species through the production of biomass and growth forms (Milla & Reich, 2007; Niklas et al., 2007). This is probably attributed to the differences in biomass among productive tissues compared to the small leaves (Niinemets et al., 2006; 2007). However, differences of these traits are observed among species (Shipley, 1995; Niklas et al., 2007) or revealed intra-specific tendency (Milla & Reich, 2007).

Alvarez, (2006) studied variation in leaf morphology in Quercus crassifolia at three different stages of canopy. Zúñiga et al. (2009) studied the leaf morphological variation of Quercus laeta, en el Parque Nacional Los Mármoles measuring 17 morphological characters in 470 leaves. The analysis of variance in morphological traits had significant differences in the leaves of Quercus laeta among localities in some morphological traits only. Gonzalez Rodriguez et al. (2015) studied large variability in leaf traits and its correlation of 34 trees and shrubs in summer season in northeastern, Mexico.Leaf area showed highly significant and positive correlations with leaf dry weight (r= 0.94), leaf length (r = 0.88), leaf breath (r = (0.81), and petiole length (r = (0.71)) while, leaf dry weight showed highly significant correlation with leaf length (r =0.88), leaf breadth (r = 0.77), and petiole length (r = 0.73). Specific leaf area did not show any significant correlations with any of the variables studied. Therefore, leaf area, leaf length, petiole length have significant roles in plant productivity and reveals that there exists interspecific diversity among distinct leaf characteristics which in turn determine distinct functional trends among the plant community studied.

Epicuticular wax:Leaves contain waxy coating called epicuticular wax. Environmental conditions may influence greatly the quantity, composition and morphology of the waxy coverings of leaf surfaces. Epicuticular wax increases the reflectance of visible and near infrared radiation from leaf surface thereby reducing net radiation and cuticular transpiration, and seems to contribute to drought resistance of plants (Kurtz, 1950; Ebercon *et al.*, 1977; Hull & Bleckmann, 1977). These waxes also contributes to

resistance to plants in the absorption and penetration of foliar-herbicides (Sharma & Born, 1970; Wilkinson, 1980). It has beendocumented that leaves of mesquite (*Prosopis* spp.) produce a thick waxy cuticle (Hull, 1970; Meyer *et al.*, 1971). Kurtz (1950) foundan increase in wax with leaf maturity in velvet mesquite (*P. velutinu*), while Mayeux *et al.* (1984) observed most rapid wax accumulation on honey mesquite (*P. glandulosa*) at early leaf development and expansion.

Some researches reported that the production of epicuticular wax under controlled condition is affected by various factors such as light level (Juniper, 1960; Reid & Tukey, 1982) photoperiod (Wilkinson, 1972), temperature (Reid & Tukey, 1982) and water stress (Skoss, 1955; Bengtson et al., 1978; Baker & Procopiou, 1980) influencing the wax contents in leaves. Variations in wax properties can affect the cuticular functions such as regulation of gas exchange and transpiration, protection against pathogens and absorption of foliar-applied chemicals as herbicides in agriculture. It has been reported by Rao & Reddy,(1980)that the composition and quantity of epicuticular waxes of shrubs in a semiarid environment showed seasonal variations in temperature and rainfall, and both cuticular and total transpiration appeared to be affected with changes in wax composition. Ecotypic variation was observed in the quantity and composition of waxes on leaves of salt cedar (Tamarix pentandra Pall.) and was considered as the basis of differences among populations in sensitivity to herbicide sprays (Wilkinson, 1980). Leaves of a cabbage cultivar (Brassica oleracea L. var. capitata), tolerant to foliar applications of the herbicide nitrofen (2,4-dichlorophenylp-nitrophenyl ether) were more heavily waxed than an intolerant cultivar (Pereira et al., 1971). The heavier wax deposit on leaves of the tolerant cultivar substantially decreased the rate and extent of herbicide penetration of the cuticle. Hull et al. (1975) investigated the development of leaf cuticle of velvet mesquite and detected well defined crystalline wax structures on even the youngest leaves. The amount of wax appeared to increase with leaf maturity and a dendritic-shaped wax plate was formed in July in addition to the small, linear structure already present.

It has been documented by Maiti *et al.* (2016) that there exist large variations in the contents of epicuticular wax with respect to the adaptation of these species to semiarid environment. The species selected with high epicuticular wax contents were *Forestiera angustifolia* (702.04 µg/cm²), *Diospyros texana* (607.65 µg/cm²), *Bernardia myricifolia* (437.53 µg/cm²), *Leucophylum frutescens* (388.50 µg/cm²)and may be well adapted to the semi arid conditions. Future research needs to be directed on these selected species with special reference to their physiological function and adaptation to water stress.

Pigment: The role of plant pigments in plant metabolism is well known. Various leaf pigments such as chlorophyll, carotenoids, xanthophylls and flavonoids can play important roles in the physiological performance of the plant. The leaf pigment content can be related with varying leaf structural characteristics indices for protection. Chlorophyll and carotenoids play an important role in photosynthetic process in higher plants. They play a vital role in capturing light energy, which is converted to chemical energy. Chlorophyll has capacity in absorbing radiant energy of sunlight into chemical energy of organic carbon through the process of photosynthesis. Carotenoids are natural fat-soluble pigments found in plants, algae and photosynthetic bacteria, where they also playa role in photosynthesis. In some non-photosynthetic bacteria, they may help in protective function against damage by light and oxygen radicals (Biswel, 1995, Giuffrida et al., 2006). Animals appear not to have capacity to synthesize carotenoids and may incorporate carotenoids from their diets. In animals, carotenoids impart bright coloration and serve as antioxidants and a source for vitamin A activity (Brotton, 1995). Besides, carotenoids in plants play an important role in plant reproduction by attracting pollinators and also in seed dispersal (Yeun & Ruseel, 2002). In a recent study carried out by González Rodríguez et al. (2017) repoted large variation in leaf pigments (chlorophyll and carotenoid) of 37 woody species in northeastern Mexico.

Materials and Methods

Study area: This study was carried out at the Forest Experimental Research Station of Facultad de Ciencias Forestales, UniversidadAutónoma deNuevoLeón (24° 47' N; 99° 32' W; elevation 350 m) in Linares municipality, Nuevo Leon, Mexico. Climate is subtropical and semi-arid with a warm summer. Mean monthly air temperature ranges from 14.7 °C in January to 23°C in August. Average annual precipitation is about 800 mm.Themain type of vegetation is known as the Tamaulipan Thornscrub or Subtropical Thornscrub Woodlands (González Rodríguez *et al.*, 2017). The dominant soils are deep, dark-gray, limeclay vertisols, with montmorillonite, which shrink and swell noticeably as soil moisture content varies.

Plant material: The present studies were undertaken to determine a comparative estimation of specific leaf area, epicuticular wax, and pigment content (chlorophyll a, chlorophyll b and carotenoids) of 15 native woody species in summer 2015 and winter 2016. Plant species were randomly chosen from a representative and undisturbed thronscrub plot located at the research site.Studied plant species were Helietta parvifolia (A. Gray) Benth (Rutaceae), Fraxinus greggii A. Gray (Oleaceae), *Amyris texana* (Buckley) P. Wilson (Rutaceae), *Condalia hookeri* M. C. Johnst (Rhamnaceae), Acacia rigidula Benth. (Fabaceae), Sargentia greggii S. Wats (Rutaceae), Diospyros texana Scheele (Ebenaceae), Zanthoxylum fagara (L.) Sarg. (Rutaceae), Ebenopsis ebano (Berland.) Barneby & J.W. Grimes (Fabaceae), Celtis pallida Torr (Ulmaceae), Acacia farnesiana (L) Willd (Fabaceae), Bernardia myricifolia (Scheele) Benth. & Hook. F. (Euphrobiaceae), Berberis chococo Schlecht (Berberidaceae), Salix lasiolepis Benth. (Salicaceae), and Havardia pallens (Benth.) Britton & Rose (Fabaceae).

Specific leaf area: Fifty leaves from each species were taken at random from five plants (10 leaves from each plant). The leaf area (cm²) was quantified using a leaf area analyser (LI-COR, model LI-3100, Lincoln, NE, USA). Leaf dry mass(g) was taken after drying in an oven at

 60° C for 72 hours. Specific leaf area (cm² g⁻¹) was calculated as theratio of leaf area to leaf dry mass (Cornelissen *et al.*, 2003).

Epicuticular wax: Fresh leaves from different woody plant species were collected from the forest plot. Then leaflets were separated individually to complete a sub sample having an approximate surface area of 100 cm² which is determined by a leaf area meter. Sub-samples were gently rinsed in distilled water to remove foreign material, then air dried, and then placed in a 200 ml beaker with 40 mL of analytical grade chloroform (99% pure) and heated to 45°C. After 30s, the chloroform was poured into preweighed foil pans which were then placed in a well ventilated laboratory and evaporated to dryness for 24 hours. Foil pans were then reweighed to quantify the amount of residual wax. Amount of epicuticular wax for a field sample was detrmined as an average of five replications. Epicuticular wax content (µg cm⁻²) was calculated as reported (Jacoby et al., 1990) on a weight per area basis.

Determinations of chlorophylls and carotenoids: Determination of pigments was done in two seasons, winter (February, 2015) and summer (June, 2015) for this study. Four samples of leaf tissue (1.0 g of fresh weight) of each plant species were used for analysis. The chlorophyll a and b and carotenoids were extracted in 80% (v/v) aqueous acetone and vacuum filtered through a Whatman No.1 filter paper. Pigment measurements were determined spectrophotometrically using a Perkin-Elmer UV/VIS Spectrophotometer (Model Lambda 25). Absorbance of Chlorophyll a and b and carotenoids extracts were determined at wavelengths of 663, 645 and 470 nm, respectively. Carotenoid (mg/g dry weight) of pigments were calculated according to the equations given by Lichtenthaler & Wellburn (1983). Results are reported on afresh weight basis (mg of plant pigment per g fresh weight). Total chlorophyll (a+b) was caluclated by adding chlorophyll a and chlorophyll b.

Results

Specific leaf area: It was observed that majority of the species decrease specific leaf area drastically in winter as compare to summer except few species such as *Salix lasiolepis,Bernardia myricifolia, Fraxinus greggii, Acacia farnesiana* where specific leaf area maintain more or less equal relating tolerance to cold (Fig. 1). In summer season,*Condalia hookeri* had the highest leaf area (199.19 cm²g⁻¹) followed by *Celtis pallida* (171.34 cm²g⁻¹) and *Ebenopsis ebano* (158.78 cm²g⁻¹). *Fraxinus greggii* had minimum specific leaf area (59.55 cm²g⁻¹). The large variability in specific leaf area help in the co-existence and adaptation to the environment.

Epicuticular wax: It is observed that in most of the species epicuticular wax content is reduced drastically in winter except in *Acacia rigidula* and *Condalia hookeri* where epicuticular wax remained almost equal in both seasons. During summer season, *Diospyros texana* contained maximum epicuticular wax (698.26 µgcm⁻²) followed by *Acacia farnesiana* (441.85 µgcm⁻²) and *Bernardia myricifolia* (126.34 µgcm⁻²) while *Amyris texana*, *Acacia rigidula* and *Sargentia greggii* contained minimum epicuticular wax (Fig. 2).



Fig. 1. Specific leaf area in 15 native woody species in northeastern Mexico during summer and winter season.



Fig. 2. Epicuticular wax contentin 15 native woody species in northeastern Mexico during summer and winter seasons.

Leaf pigments: It is observed that the species showed considerable variability in Chlorophyll *a*content both in summer and winter season. It is interesting to note that some species showed an increase in Chlorophyll *aviz.*, *Berberis chococo* 1.56 mg g⁻¹ fw followed by *Bernardia myricifolia* 1.37 mg g⁻¹ fw, *Havardia pallens* 1.37 mg g⁻¹ fw and *Zanthoxylum fagara* 1.18 mg g⁻¹ fw whereas,only few species showed reduction in Chlorophyll *a* content, probably showing an adaptation to winter (Fig. 3).

With respect to Chlorophyll *b*content, the species showed variable responses in summer and winter seasons similar to that observed in Chlorophyll *a*; some showing increase and few decrease in Chlorophyll *b*, probably showing mechanism of adaptation to winter season (Fig. 4).

With respect to total Chlorophyll (*a+b*), species *Berberis chococo* followed by*Bernardia myricifolia*, *Amyris texana*, and *Havardia pallens* probably facedan adaptation to cool temperature, since the species showed variable responses. Most of the species showed a decrease in total Chlorophyll in winter, but only few showed an increase in total Chlorophyll such as *Ebenopsis ebano*, *Salix lasiolepis*, *Sargentia greggii*, and*Acacia farnesiana* (Fig. 5).

With respect to carotenoidscontent, the species showed variable responses in summer and winterseasons; some showed anincreasing trend and the few showed a decreasing trend.In few species, carotenoids content remained equal in both the seasons (Fig. 6).

Discussion

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The present study was intended to investigate the seasonal variation of specific leaf area, epicuticular wax and leaf pigments (chlorophylls and carotenoids) of 15 woody

species, northeastern Mexico, showing specific variations during summer and winter.In this respect, the roles of specific leaf area in the productivity of plant and crop are well documented (Rincón et al., 2007; Milla & Reich 2007; Niklas et al., 2007). On the other hand, the role of epicuticular wax in the reflectance of visible and near infrared radiation from leaf surface thereby its contribution to drought resistance of plants was reported by some authors (Kurtz, 1950; Ebercon et al., 1977; Hull & Bleckmann, 1977) and herbicide resistance (Sharma & Born, 1970; Wilkinson, 1980). Studies have been undertaken on the deposition of epicuticular wax in mesquite (Prosopis spp.) which developed a thick waxy cuticle (Bleckmann & Hull, 1975; Meyer et al., 1971). Variability in epicuticular wax in woody species in northeastern Mexico has been reported by (Maiti et al., 2016). On the other hand, variation in leaf pigments of trees and shrubs have been reported recently by González Rodríguez et al. (2017).

As evidence from the results that all the species showed considerable variability in their responses of pigments (chlorophyll *a* and *b*, and total) in both summer and winter. It is interesting to note and point out that unlike the variable specific leaf area and epicuticular wax these pigments were not reduced in majority of the species. Some species such as *Berberis chococo*, *Bernardiamyricifolia*, *Celtis pallida*, *Amyris texana*, and *Havardia pallens* showed an increase in chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a+b*)during the winter season showing an adaptation to winter season. On the other hand, species such as *Ebenopsis ebano*, *Helietta parvifolia*, *Salix lasiolepis*, *Sargentia greggii*, and *Acacia farnesiana* showed a trend to decrease in pigment content and thuscould be related to temperature susceptiblity during the winter season.



Fig. 3. Chlorophyll a content in 15 native woody species in northeastern Mexico during summer and winter seasons.



Fig. 4. Chlorophyll bcontent in 15 native woody species in northeastern Mexico during summer and winter seasons.



Fig. 5. Total Chlorophyll (a+b) content in 15 native woody species in northeastern Mexico during summer and winter seasons.



Fig. 6. Carotenoidscontentin 15 native woody species in northeastern Mexico during summer and winter seasons.

Acknowledgements

Valuable technical assistance provided by Elsa Dolores González Serna and Manuel Hernández Charles is gratefully recognized. This research was funded by Consejo Nacional de Ciencia y Tecnología (CONACYT, grant 250732, Convocatoria de Investigación Ciencia Básica 2015). Useful suggestions from two anonymous reviewers helped to improve the manuscript.

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(Received for publication 17 April 2016)