Effect of expansion time and sunlight radiation on the functional and anatomical traits of mango tree leaves

Roger Matheus Alves de Azeredo[1]

Marcela André Mendes[2]

Ciro Yoshio Joko[3]

Marina Neves Delgado[4]

Abstract

The mango tree (*Mangifera indica* L.) is cultivated on a large scale in Brazil for economic purposes. Since the leaves, as the main photosynthetic organs, play an essential role in fruit production, the primary objective of the present study was to analyze comparatively the morphological characteristics of leaves at different stages of expansion and of leaves exposed to different levels of solar radiation. Leaves were collected at the beginning of expansion, during intermediate expansion and when completely expanded, and sun and shade leaves were compared. The individuals were adult plants without flowers and fruits located along the South Lake, Brasília. The leaves were analyzed for area, specific leaf area (SLA), thickness, water content and anatomical traits. Data were analyzed by ANOVA post hoc Tukey test to test the influence of leaf expansion time and by the T-test to determine the effect of radiation ($\alpha = 5\%$). Greater scleromorphy was observed in the completely expanded leaves compared to the leaves at the beginning of expansion, with lower water content and SLA in completely expanded leaves. Higher sclerophyll content was observed in sun leaves than in shade leaves, with lower SLA, greater leaf thickness and greater thickness of adaxial epidermis, palisade parenchyma and mesophyll in sun leaves. Based on these results, greater care is indicated regarding leaf management at the beginning of expansion and the management of shade leaves, since they are more susceptible to damage from herbivores. The objective of this management is to maximize fruit production.

Keywords: Anatomy. Fruits. Scleromorphy. Specific Leaf Area.

Introduction

Mangifera indica L. (mango tree) is native to South Asia and is currently cultivated in tropical and subtropical countries (DONADIO; FERREIRA, 2002; FERREIRA et al. 2003). This species produces tasty, sweet and fleshy fruits with considerable amounts of vitamins A and C and a lower quantity of vitamins B (CARDELLO; CARDELLO, 1998).

In Brazil, the mango tree was introduced in the Northeast during the 16th century by the Portugueses and later spread to the other regions (SANTOS et al., 2009), being one of the main products responsible for the Northeastern economy (AGRIANUAL, 2008). Mango trees develop very well in most

1 Centro Universitário do Distrito Federal, graduado em Ciências Biológicas. noticabode@hotmail.com. Setor de Edifícios Públicos Sul, Eq 704/904, Conj. A, Asa Sul, Brasília - DF, 70390-045.

2 Centro Universitário do Distrito Federal, graduado em Ciências Biológicas. marcela.andre@msn.com.

3 Centro Universitário do Distrito Federal, professor pesquisador. cjokos@gmail.com.

4 Instituto Federal de Brasília, *Campus* Planaltina, professora pesquisadora. mnevesdelgado@gmail.com.

of Brazilian territory since they need a hot temperature and dry periods to produce fruits of good quality (SILVA, 2006). Brazil, where mango trees are cultivated on a large scale, is among the seven largest producers and exporters of mangoes in the world (FRANCO; RODRIGUEZ-AMAYA; LANÇAS, 2004).

In productive terms, it is known that mango trees have a vigorous crown with many leaves which may be subjected to direct or indirect solar radiation, being classified as sun or shade leaves. This radiation can influence leaf morphology and physiology, with a greater leaf size and photosynthetic rate being generally observed in sun leaves in relation to their area (ROSSATTO et al. 2010). Therefore, plant development may be affected (ROSSATTO; KOLB, 2010) because leaves are responsible for carbohydrate production, which in turn will compromise the production of fruits (TAIZ; ZEIGER, 2010).

The age of leaf expansion also influences fruit production since the photosynthetic rate is lower in younger leaves and maximum in completely expanded leaves (PAULA et al., 2015). The younger leaves that are still draining organs (TAIZ; ZEIGER, 2010) also undergo greater herbivory pressure than mature leaves due to the smaller amount of support structures, which makes the younger leaves softer and more palatable to herbivores than completely expanded (mature) leaves. In addition, younger leaves are more eaten by herbivores because of their greater amount of nitrogen (BARÔNIO, 2012). Leaves of different coloring are also observed in mango trees. For example, at the beginning of expansion the leaf is red, presenting high levels of anthocyanin and low photosynthetic activity, while the mature leaf is dark green, with a low amount of anthocyanin and high photosynthetic activity (NII et al., 1995). To sum up, these adaptive responses to the environment, called phenotypic plasticity, may occur due to herbivory, nutrient availability in the soil, humidity, degree of light incidence and organ age (ROSSATTO; KOLB, 2010; DELGADO et al., 2013; DELGADO et al., 2014; DELGADO et al., 2017), with leaves being the organs with greater plasticity within the plants (DICKISON, 2000).

A study about the leaf morphology of mango trees is important because morphological observation provides important data about the phenotypic plasticity of the species, which is fundamental when it comes to fruit production, since the leaves are directly related to photosynthesis that is essential for agronomic production (TAIZ; ZEIGER, 2010).

Thus, the objectives of the present study were: (1) to compare the macroscopic and microscopic morphology of leaves at different stages of expansion and of leaves exposed to different levels of solar incidence, (2) to determine the existence of a variation in structural defense in different leaf types, and (3) to suggest a new management technique for mango tree leaves.

Material and methods

Analysis of functional and macroscopic-morphological traits of leaves

Seventeen trees of *M. indica* L. located in the region of Lago Sul, Distrito Federal, were selected. In September 2011, three mature and sun leaves, mature and shade leaves, leaves at the beginning of expansion and leaves in intermediate expansion were collected randomly from each plant (Figure 1). All individuals were without flowers and fruits.

According to NII et al. (1995), mature leaves of mango trees are those with a dark green coloration. Therefore, in the present study, mature leaves were those that were dark green in color, with the consistency of leather, rigid and hard to the touch, and inserted in nodes more distant from the apex of the branch. The difference between sun leaves and shade leaves was the degree of solar radiation to which they were subjected during development: the sun leaves developed under direct sunlight and the shade leaves were those that developed under diffused sunlight (shadow). The leaves

at the beginning of expansion were those collected from the first nodes counting from the apex of the branch. In the subsequent nodes, between the nodes where the leaves were at the beginning of expansion and the mature leaves, the leaves at intermediate expansion were collected. The criterion used to define mature leaves were coloration, consistency and positioning of the node in the branch; the criterion used to define leaves at the beginning of expansion and intermediate expansion was the position of the node in the branch; and the criterion used to define sun leaf and shade leaf was the incidence of direct sun or diffused sunlight on the leaves.

The collected leaves were analyzed with a micrometer to determine leaf thickness and were then processed to obtain specific leaf area (SLA) (leaf area/dry weight) according to CORNELISSEN et al. (2003). For this procedure, the leaves were scanned for area analysis with Image J software, dried in an oven for 72 hours at 60 ^{o}C and then weighed on a precision scale with three decimal places. For the measurement of water content, a leaf disc of the same area from one leaf per individual was weighed before and after oven drying, and percent water content was calculated: ((fresh weight - dry weight)/fresh weight) *100.

Analysis of anatomical and microscopic-morphological traits of leaves

For anatomical analysis, four trees were randomly chosen from the 17 selected trees. In these four trees, four mature and sun leaves, mature and shade leaves, leaves at the beginning of expansion and leaves at intermediate expansion (Figure 1) were collected at random. Following the procedures of KRAUS and ARDUIN (1997), a small fragment in the median portion was cut into each leaf and fixed for 24 hours in FAA 70, a solution consisting of 90 ml 70 % ethanol, 5 ml acetic acid and 5 ml formaldehyde. After this time, the fragments were stored in 70 % ethanol for a few months and then again washed in 70 % alcohol and dehydrated with an alcohol series up to 95 % every two hours. The material was pre-infiltrated with a resin solution (Technovit 7100) plus 100 % alcohol (V/V) for 12 hours and then infiltrated with a pure resin solution (Technovit 7100) for 72 hours. Both pre-infiltration and infiltration processes were carried out under refrigeration. The fragments were embedded in pure resin plus a hardener in Leica blocks and dried in an oven at 40 °C for another seven days. Finally, 5 μm thick slices were cut with a rotary microtome (Leica RM2235) with six sections being obtained per block.

The sections were stained with Toluidine Blue in phosphate buffer, pH 6.8, for five minutes and the slides were mounted in water. Each cut was documented using an Axiophot microscope (Zeizz - Germany) coupled to a computer with AxioVision LE software. The median rib and mesophyll of each leaf fragment were photographed and the following anatomical traits were then measured using Image J software: thickness of the epidermis from adaxial and abaxial leaf surfaces, thickness of palisade and spongy parenchyma, total leaf thickness, and width (w) and height (h) of the midrib. The midrib area was obtained by the formula (((w/2) * (h / 2)) * 3.14)) and its value was transformed from μm^2 to mm^2. The difference in percent thickness of the foliar tissues analyzed was obtained using the following formula: (thicker tissue thickness - less thicker tissue thickness) / less thicker tissue thickness.

Statistical analysis

Using the Statistica 7.0 software, the normality of the data was determined by the Shapiro-Wilk test. ANOVA for dependent samples was then used to compare the influence of ontogeny on the development of leaf traits. The post hoc Tukey test (a = 0.05) was used to test for differences among

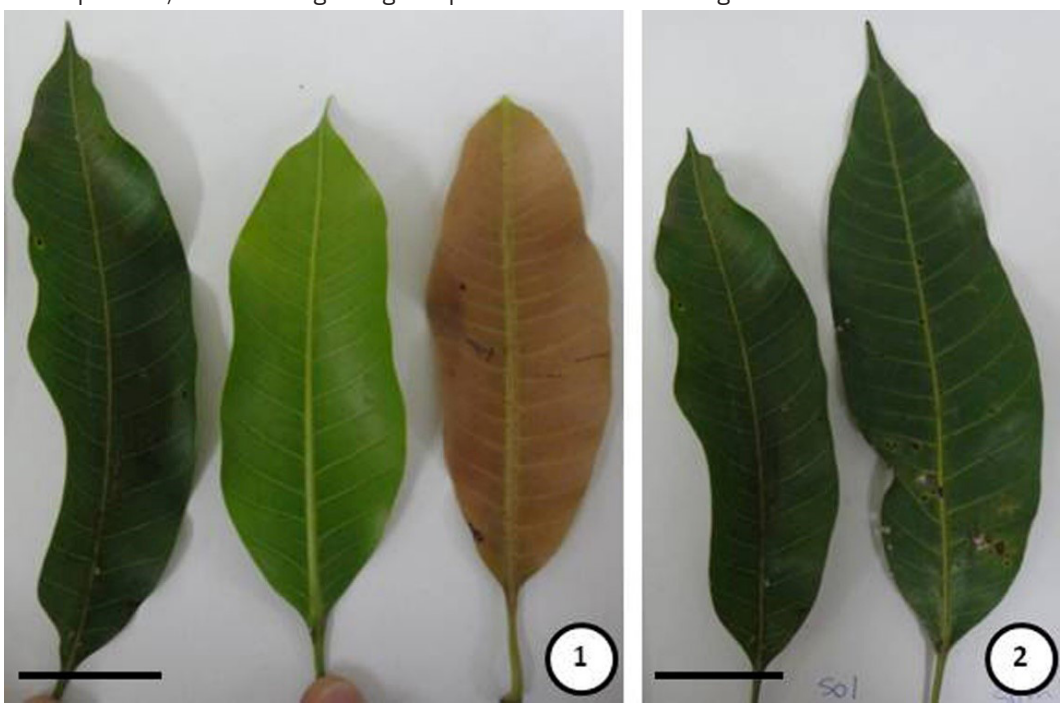
treatments. The t-test for dependent samples was applied to compare the influence of direct sunlight and diffused sunlight on the development of leaf traits (mature sun leaves X mature shade leaves, respectively). Significance was considered to be an α value of 5 %.

Results and discussion

The leaves collected from *Mangifera indica* (Figure 1), regardless of the age of expansion, are petiolate, lanceolate and green, except for the leaves at the beginning of expansion that are red. According to NII et al. (1995), the younger leaves of *M. indica* contain a high concentration of anthocyanin that disappears during leaf expansion while cellulose concentration increases concurrently. Therefore, they can be easily distinguished by coloration.

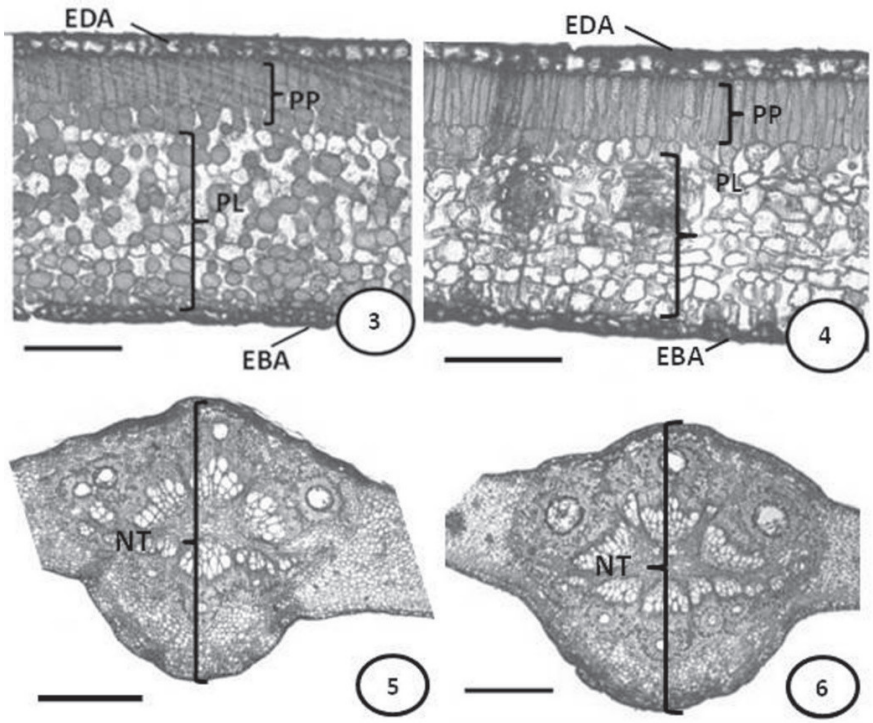
Regarding anatomy, the leaves are hypostomatic and have dorsiventral mesophyll. The palisade parenchyma is composed of one to two cell layers and the spongy parenchyma consists of six to seven cell layers (Figures 3-4, 7-8). The epidermis has one cell layer, with cells thicker on the adaxial surface than on the abaxial surface. The vascular system has a bicolateral bundle with a medullary region consisting of parenchyma. In addition, the midrib shows channels with secondary metabolites (Figures 5-6, 9-10). Leaf channels are a typical feature of the Anacardiaceae family (METCALFE; CHALK, 1957) where secondary metabolism compounds are stored (REIS et al., 2014).

Figures 1-2: Leaves of *Mangifera indica*. 1. From left to right: completely expanded leaf (mature leaf), leaf at intermediate expansion, leaf at the beginning of expansion. 2. From left to right: sun leaf and shade leaf. Scale: 4 cm.



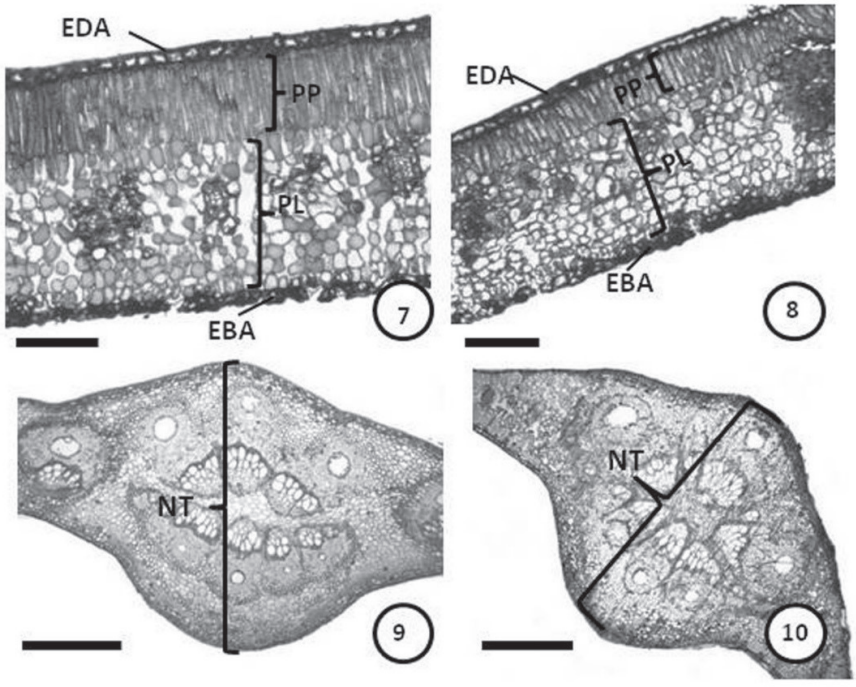
Source: Elaborated by the authors.

Figures 3-6: Transverse sections of *Mangifera indica* leaves. 3. Leaf blade at the beginning of expansion. 4. Leaf blade at intermediate expansion. 5. Midvein of a leaf at the beginning of expansion. 6. Midvein of a leaf at intermediate of expansion. PP- palisade parenchyma; PL- spongy parenchyma; EAD- adaxial epidermis; EAB- abaxial epidermis; NT- midvein. Scales: 100 μm (3, 4) e 500 μm (5, 6).



Source: Elaborated by the authors.

Figures 7-10. Transverse sections of *Mangifera indica* leaves. 7. Leaf blade of a sun leaf. 8. Leaf blade of a shade leaf. 9. Midvein of a mature sun leaf. 10. Midvein of a shade leaf. PP- palisade parenchyma; PL- spongy parenchyma; EAD- adaxial epidermis; EAB- abaxial epidermis; NT- midvein. Scales: 100 μm (7-8); 500 μm (9-10).



Source: Elaborated by the authors.

The leaves at different levels of expansion (leaves at the beginning of expansion, at intermediate expansion and completely expanded) showed both macroscopic (Figure 1, Table 1) and microscopic (Figures 3-6, Figure 7 and Figure 9, Table 1) differences.

Table 1. Functional and anatomical traits of *Mangifera indica* leaves at different levels of leaf expansion.

Leaf traits	Average	SD	F	p
Area of leaf at the beginning of expansion (cm^2)	47.610 A	24.933		
Area of leaf at intermediate expansion	50.517 A	18.069		
Area of leaf completely expanded	63.352 A	21.426	6.37	> 0.05
Water content of leaf at the beginning of expansion (g)	62.787 A	19.028		
Water content of leaf at intermediate expansion	46.933 B	16.622		
Water content of leaf completely expanded	42.578 B	11.503	6.37	< 0.05
Total thickness of leaf at the beginning of expansion (mm)	0.232 A	0.035		
Total thickness of leaf at intermediate expansion	0.260 A	0.050		
Total leaf thickness of leaf completely expanded	0.267 A	0.064	6.37	> 0.05
SLA of leaf at the beginning of expansion	112.554 A	24.729		
SLA of leaf at intermediate expansion	69.527 B	22.689		
SLA of leaf completely expanded	56.324 B	18.602	6.37	< 0.05
PP of leaf at the beginning of expansion (μm)	51.372 B	11.308		
PP of leaf at intermediate expansion	55.113 B	6.743		
PP of leaf completely expanded	96.072 A	31.285	4.54	< 0.05
PS of leaf at the beginning of expansion (μm)	147.245 A	23.031		
PS of leaf at intermediate expansion	152.206 A	27.085		
PS of leaf completely expanded	159.406 A	17.438	4.54	> 0.05
EAD of leaf at the beginning of expansion (μm)	22.707 A	0.768		
EAD of leaf at intermediate expansion	23.766 A	2.467		
EAD of leaf completely expanded	24.354 A	1.931	4.54	> 0.05
EAB of leaf at the beginning of expansion (μm)	14.244 A	1.610		
EAB of leaf at intermediate expansion	15.869 A	1.721		
EAB of leaf completely expanded	15.832 A	0.941	4.54	> 0.05
EM of leaf at the beginning of expansion (μm)	200.513 A	32.897		
EM of leaf at intermediate expansion	208.460 A	31.254		
EM of leaf completely expanded	255.652 A	36.078	4.54	> 0.05
Midvein area of leaf at the beginning of expansion (mm^2)	1.450 A	0.342		
Midvein area of leaf at intermediate expansion	1.987 A	0.591		
Midvein area of leaf completely expanded	1.724 A	0.519	4.54	> 0.05

Numbers with different letters differ significantly. F = ANOVA. SD = standard deviation. SLA - specific leaf area; PP- palisade parenchyma; PS- spongy parenchyma; EAD- adaxial epidermis; EAB- abaxial epidermis; EM- mesophyll thickness.
Source: Elaborated by the authors.

There was a morphological difference between the leaves at intermediate expansion and completely expanded compared to those at the beginning of expansion regarding leaf percent water content and SLA. In addition, there was differentiation in the staining pattern (Figure 1) because leaves at the beginning of expansion were red, leaves at intermediate expansion were greenish and mature leaves were dark green. Leaves at the beginning of expansion had functional attributes that classified them as potential targets for attacks from herbivores. Both SLA and percent water content can be directly proportional indicators of leaf quality: the larger the SLA and water content, the higher the leaf quality for herbivorous insects. In addition, a larger SLA means less investment in structural defenses (SILVA; BATALHA, 2011). Therefore, the present study demonstrated that leaves at the beginning of expansion are more attractive to herbivorous insects and exhibited fewer structural defenses compared to intermediate expanded and completely expanded leaves.

Greater scleromorphy was observed in completely expanded leaves than in leaves at the beginning of expansion. Scleromorphism can be considered to be a type of structural defense. In mango trees, scleromorphism in completely expanded leaves may be related to the greater amount of palisade parenchyma than to spongy parenchyma (DELGADO et al., 2013), lower SLA (SILVA; BATALHA, 2011) or greater leaf thickening, because the tissues become more compact, thus also becoming more rigid (CUTLER; BOTHA; STEVENSON, 2011). These data highlight structural defense as a strong adaptive attribute. Probably the trigger factor for the greater scleromorphism of mature leaves was the longer time of exposure of these leaves to the sun, since they were exposed to solar radiation more than leaves at the beginning of expansion. According to Vogelmann et al. (1996), the palisade parenchyma is more developed in areas of the leaves that are more illuminated.

However, despite the low structural defense of younger leaves, chemical defense was observed in leaves at the beginning of expansion due to the presence of anthocyanins, which give the red color to the leaves. Anthocyanins inhibit the growth of insect larvae on the leaves (ZUANAZZI; MONTANHA, 2003). Besides, the red color determined by anthocyanin can be considered to be an adaptive trait of younger leaves because this coloration makes herbivorous insects less camouflaged, facilitating the hunting process of visually oriented predators such as birds and some flying insects. Thus, natural enemies are able to predate herbivorous insects more easily on red leaves. Therefore, we can confirm the existence of a trade-off between structural and chemical defenses in the leaves of *M. indica* at different levels of expansion.

We observed macro- (Figure 1, Table 2) and microscopic differences (Figure 7-10 and Table 2) between leaves submitted to different levels of sunlight radiation. The morphological variation between sun and shade leaves in response to different levels of sunlight radiation is quite common in species that have the capacity of acclimatization (BORKORKMAN, 1981); however, such foliar variations may differ between species (MCMAHON; KELLY, 1995).

Table 2. Functional and anatomical traits of *Mangifera indica* leaves under different light conditions.

Leaf traits	Average	SD	T	p
Area of mature sun leaves (cm^2)	63.352 A	21.426		
Area of mature shade leaves	77.567 A	34.665	1.315	> 0.05
Water content of mature sun leaves (g)	42.578 A	18.602		
Water content of mature shade leaves (g)	46.071 A	9.246	0.634	> 0.05
Total thickness of mature sun leaves (mm)	0.267 A	0.064		
Total thickness of mature shade leaves	0.219 B	0.022	-3.271	< 0.05
SLA of mature sun leaves	56.324 B	18.602		
SLA of mature shade leaves	67.571 A	9.246	-3.172	< 0.05
PP of mature sun leaves (μm)	102.059 A	24.390		
PP of mature shade leaves	56.315 B	5.533	3.299	< 0.05
PS of mature sun leaves (μm)	156.964 A	20.950		
PS of mature shade leaves	139.327 A	26.600	1.809	> 0.05
EAD of mature sun leaves (μm)	24.829 A	1.195		
EAD of mature shade leaves	18.281 B	1.436	6.572	< 0.05
EAB of mature sun leaves (μm)	15.774 A	1.042		
EAD of mature shade leaves	18.281 A	1.436	-2.318	> 0.05
EAB of mature sun leaves (μm)	15.774 A	1.042		
EAB of mature shade leaves	13.214 A	1.316	2.614	> 0.05
EM of mature sun leaves (μm)	266.843 A	16.326		
EM of mature shade leaves	195.886 B	31.324	3.974	< 0.05
Midvein area of mature sun leaves (mm^2)	1.724 A	0.519		
Midvein area of mature shade leaves	1.783 A	0.607	-0.164	> 0.05

Numbers with different letters differ significantly. T = t-test. SD = standard deviation. SLA - specific leaf area; PP- palisade parenchyma; PS- spongy parenchyma; EAD- adaxial epidermis; EAB- abaxial epidermis; EM- mesophyll thickness.
Source: Elaborated by the authors.

The thickness of sun leaves is greater than that of shade leaves, as observed in leaves of *Coffea arabica* (VOLTAN; FAHL; CARELLI, 1992) and *Ocimum gratissimum* (MARTINS et al., 2008) submitted to different levels of luminosity. Greater thickness of mesophyll and adaxial epidermis have also been observed in sun leaves of *M. indica*. The greater leaf and mesophyll thickness in sun leaves is due to greater thickness of adaxial epidermis and palisade parenchyma, which were 35.82 % and 82.17 % thicker in mature sun leaves than in mature shade leaves, respectively. It is known that strong illumination is a triggering factor for the formation of palisade parenchyma (VOGELMANN; JOHN; SMITH, 1996; LARCHER, 2004). In addition, leaves under direct sunlight radiation tend to have a thicker adaxial epidermis, since this tissue protects the photosynthetic apparatus from excessive luminosity (ROSSATTO; KOLB, 2010).

The difference in SLA between mature sun leaves and mature shade leaves is an important indicator of scleromorphy (SILVA; BATALHA, 2011). A smaller SLA was observed in sun leaves, determining a greater degree of structural defense and scleromorphy, because these leaves are thick

and rigid, showing a greater thickness of palisade parenchyma and adaxial epidermis (DICKISON, 2000). This fact is also proven by the greater thickness of the leaves. It is known that the sun is a factor directly related to a greater investment in the cell wall (DICKISON, 2000; CUTLER, 2011).

It is known that the photosynthetic rate is lower in leaves at the beginning of expansion and maximum in completely expanded leaves (NII et al., 1995; PAULA et al., 2015) and higher in mature sun leaves than in mature shade leaves in terms of area (ROSSATTO et al., 2010). On this basis and considering the results obtained in the present study, new management techniques of mango trees can be suggested. The pruning of shade leaves is encouraged, increasing productivity, since these leaves are less defended, being likely to suffer more damage from herbivores and also having a lower photosynthetic rate. Greater care should also be devoted to the management of leaves at the beginning of expansion since they are more susceptible to herbivory, which may decrease their leaf areas. This occurrence will decrease the photosynthetic capacity of the plant when such leaves have completely expanded, a situation that could compromise the agronomic production of mangoes.

Conclusions

Phenotypic plasticity was observed in leaves of *Mangifera indica*. However, scleromorphy was only observed in mature sun leaves. Leaves at the beginning of expansion had reddish coloration, higher water content and SLA than leaves at intermediate expansion and completely expanded. Leaves in the process of expansion showed a smaller thickness of the palisade parenchyma than mature leaves. Mature sun leaves showed greater mesophyll, palisade parenchyma and adaxial epidermis thickness and lower SLA than mature shade leaves. There was variation in the structural defense between the different types of leaves. Leaves at the beginning of expansion had less structural defense, being also more attractive to herbivorous insects than leaves at intermediate expansion and completely expanded (mature) leaves. On the other hand, mature shade leaves were less scleromorphic and had less structural defenses than mature sun leaves. The presented results can be used to support a new method of leaf pruning in order to reach maximum mango production.

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Efeito do tempo de expansão e da radiação solar na morfoanatomia de folhas de Mangueira

Resumo

A mangueira (*Mangifera indica* L.) é cultivada em larga escala no Brasil com finalidade econômica. Como as folhas têm papel imprescindível quando se trata de produção de frutos, visto que são os principais órgãos fotossintéticos, o objetivo principal deste trabalho foi analisar comparativamente características morfológicas em folhas em diferentes estágios de expansão e em folhas expostas a diferentes níveis de radiação solar. Foram coletadas folhas em início de expansão, expansão intermediária e completamente expandida e folhas de sol e de sombra. Todos os indivíduos

eram plantas adultas, sem flores e frutos e estavam no Lago Sul, Brasília. Nas folhas foram feitas análises para área, área foliar específica (SLA), espessura, conteúdo de água e atributos anatômicos. Os dados foram analisados por ANOVA para testar a influência do tempo de expansão foliar e teste t para averiguar o efeito da radiação (α = 5%). Observou-se maior escleromorfia nas folhas completamente expandidas em relação às folhas em início de expansão, com menor conteúdo de água e SLA nas folhas maduras. Constatou-se maior escleromorfia nas folhas de sol em relação às folhas de sombra, com menor SLA, maior espessura foliar e maior espessura da epiderme adaxial, do parênquima paliçádico e do mesofilo nas folhas de sol. A partir desses resultados, é indicado maior cuidado no manejo das folhas em início de expansão e nas folhas de sombra, pois elas estão mais susceptíveis aos danos por herbivoria. O objetivo desse manejo é maximizar a produção de frutos.
Palavras-chave: Anatomia. Área foliar específica. Escleromorfia. Frutos.

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