Australian Journal of Crop Science

AJCS 13(05):792-797 (2019) doi: 10.21475/ajcs.19.13.05.p1663 AJCS ISSN:1835-2707

Leaf characterization of Spermacoce verticillata at three stages of development

Dauri Aparecido Fadin, Patricia Andrea Monquero*

Program in Agriculture and Environment (PPGAA), São Carlos University, Araras, São Paulo State, Brazil

*Corresponding author: pamonque@ufscar.br

Abstract

Weed control is an essential practice in crop management. However, the use of herbicides may not be effective in certain situations, such as when problems are encountered in the application technology and when tolerant individuals and resistant biotypes are observed. In the cotton producing areas of the state of Bahia, Brazil, *Spermacoce verticillata* (shrubby false buttonweed) is not effectively controlled during burndown applications. During different developmental stages, plants can modify their leaves' anatomical structures, which may influence herbicide control by modifying the retention, absorption, translocation and final effect of the chemical. This study assessed the morphological and histological differences in *Spermacoce verticillata* leaves at different stages of development. Leaves were collected from plants at different phenological stages (2-4 leaves, 4-6 leaves and flowering) and subjected to histological and scanning electron microscopy analyses. In the histological analysis, four leaves were collected from the upper nodes. The evaluated characteristics were the total leaf blade thickness, palisade and spongy parenchymal thicknesses, cuticle and epidermal cell thicknesses on the abaxial and adaxial surfaces and midrib height and width. Surface analyses were performed by observing the leaves under a scanning electron microscope. As the developmental stages of *Spermacoce verticillata* advanced, the plants began to show reduced leaf thickness due to the decreased abaxial epidermal thickness, transport vessel size and parenchymal thickness. At more advanced stages of development (4-6 leaves and flowering), the adaxial leaf surfaces showed fewer stomata and more trichomes.

Keywords: Cuticle, epidermis, stomata, trichomes.

Introduction

The *Spermacoce* genus represents more than 150 species distributed among the tropical and subtropical regions of the American continents. Among these species, the shrubby false buttonweed (*Spermacoce verticillata* [L.]) is considered a rustic plant because it develops in acidic and poor soils (Kissmann and Groth, 2000).

This plant is native to Brazil, where it is widely distributed and infests pastures, unoccupied areas and perennial crops, and it was recently reported as a problem in Luiz Eduardo Magalhães, Bahia because it escaped herbicide control during crop desiccation operations (MCT, 2002; Toni and Mariath, 2004). Per Caldeira et al. (2014), the best chemical control option is 2,4-dichlorophenoxyacetic acid (2,4-D) + picloram. Another alternative to the herbicide glyphosate is to apply paraquat and paraquat + diuron to plants at full vegetative stages (Fontes, 2007).

Herbicides can be absorbed through shoots and underground structures, such as leaves, stems, roots and rhizomes. However, the herbicide's main route of entry into the plant is through the leaf (Silva et al., 2007). The leaf presents structures that can create barriers to absorption, such as epicuticular waxes and trichomes, as well as anatomical modifications that can alter the herbicide's translocation (Vidal, 2002).

The leaf morphology regulates herbicide retention, while the leaf anatomy regulates the absorption rate of the active

ingredient (Procópio et al., 2003). Thus, leaf modifications can affect plant sensitivity to the herbicide (Ferreira et al., 2006).

Some plants, such as *Lolium multiflorum* (ryegrass), show leaf differences that modify glyphosate sensitivity, including changes in the mesophyll cell compaction and phloem/xylem ratio (Galvan et al., 2012). Foliar absorption is a resistance mechanism to glyphosate in *Sorghum halepense* (Johnson grass) because it leads to higher leaf retention and lower absorption rates in less sensitive plants (Vila-Aiub et al., 2011).

Differences in herbicide absorption are also characteristic of tolerant plants (Cruz-Hipolito et al., 2011). Certain characteristics, such as the presence of trichomes and epicuticular wax, can influence herbicide absorption (Hess and Falk, 1990; Baker, 1982). The wax surface type influences the herbicide solution's wettability. Smooth wax-free surfaces are easily wet, whereas those with wax crystals are more difficult to wet (Hess, 1997). *Abutilon theophrasti* plants subjected to increasing luminosity showed decreased total epicuticular wax content in the leaves and a subsequent increase in the absorption of herbicides, such as bentazon (Hatterman-Valenti et al., 2011). The environment also modified the total fluazifop absorption by *Setaria faberi* plants. Plants subjected to low luminosity and water deficits presented lower rates of herbicide absorption mainly due to

modifications in the epicuticular wax composition (Hatterman-Valenti et al., 2006).

The plant's developmental stage and leaf shape, orientation and specific area may also alter herbicide efficacy. The priorities for transporting photoassimilates in the plant also differ at different stages, which affect herbicide translocation (Hetherington et al., 1998; Johnson and Hoverstad, 2002; Silva et al., 2002). In general, applications performed at the early stages of development result in greater control efficacy (Marques et al., 2012).

Studies of *Urochloa decumbens* and *Urochloa plantaginea* anatomy showed that adult plants presented higher percentages of sclerenchyma and parenchyma, which increase the difficulty of absorbing and transporting herbicides (Marques et al. 2012). In the case of *Spermacoce verticillata*, the causes of the control failures are unknown. To our knowledge, the literature does not include reports on the basic biology of the species, such as its leaf characterization, which may support targeted management (Passos and Mendonça, 2006).

This work analyzes the leaf anatomy of *Spermacoce verticillata* at different stages of vegetative development and relates the leaf anatomy to the plant's susceptibility to herbicide application.

Results and Discussion

Anatomical analysis

In Spermacoce verticillata, greater plant development was accompanied by smaller leaf thickness. An analysis of the cuticle on the adaxial leaf surface showed no differences in thickness at the stages evaluated; however, the abaxial face cuticle was thicker in the more advanced stages, in contrast to the observation for the total leaf thickness (Table 1). The cuticle thickness results showed little effect on the total leaf thickness (adaxial + abaxial) and accounted for an average of 0.06% of the total value (Table 1). The adaxial epidermis presented no significant differences in thickness during the developmental stages; however, the abaxial epidermis showed decreased thickness with increased development, with reduced thickness observed in flowering plants compared with plants with 2-4 leaves. The epidermis in this case represented 31% of the total leaf thickness (Table 1). The adaxial surface cuticle maintained the same thickness throughout the plant's development; therefore, this factor may not be involved in the differences observed with herbicide application. However, to negate the cuticle's effect on species differences in herbicide susceptibility, the chemical components must be analyzed since these can change with the plant's developmental stage (Silva et al., 2002). Importantly, differences in the cuticle and epidermal thicknesses on the plant's abaxial surfaces would have little impact on the herbicide's effects since herbicide retention in eudicot leaves generally occurs on the adaxial (upper) plant surface; in grasses, however, more herbicide can be retained on the abaxial surface because of their leaf orientation (Schott et al., 1991). Physiologically, the leaf thickness is inversely proportional to the leaf area and proportional to the stomatal density. The observed increases in leaf area and decreases in stomatal density with advancing plant age may be related to mechanisms that increase transpiration (Boeger and Wisniewski, 2003). However, differences in the

abaxial surface cuticle (in this case, increased thickness) can prevent transpiration and balance the effect (Larcher, 2000). A reduced leaf epidermal thickness can increase the plant's vulnerability to environmental factors, but *Spermacoce verticillata* presented greater leaf thicknesses at all stages analyzed compared with other weeds, including *Galinsoga parviflora*, *Conyza bonariensis* and *Ipomoea cairica* (Procópio et al., 2003).

Data on the transport vessels showed that in later stages of development, *Spermacoce verticillata* presented lower vessel width and height, thus resulting in transport vessels with smaller diameters as the plant's development advanced. This decrease is corroborated by the lower thicknesses of the palisade, spongy parenchyma and lower leaf. Lower-diameter vessels translocate less herbicide (Table 2).

The palisade parenchyma consists of several cell extracts that respond to changes in light intensity. Fewer palisade parenchymal extracts in the more advanced developmental stages accompanied increases in leaf area, thus contributing to a reduction of leaf thickness. This compensatory mechanism may explain the reductions in the spongy parenchyma and the possible greater plant transpiration since the reduction in intercellular space reduces the CO₂ available for photosynthesis (Fahn, 1990). Compaction of cell spaces may decrease the herbicide translocation rate, as previously observed in Lolium multiflorum and Brassica juncea with the use of the glyphosate herbicide (Galvan et al., 2012; Huangfu et al. 2009). With advancing stages of development, Spermacoce verticillata showed fewer stomata on both leaf surfaces (adaxial and abaxial), which indicates that this plant is an amphistomatic species because it has stomata on both sides of the leaf surface, as is common among weed species (Machado et al., 2008; Procópio et al., 2003; Ferreira et al., 2002). The stomatal index for both leaf surfaces was reduced in the developmental stages when the plant had 4-6 leaves and in flowering plants. The number of trichomes on the adaxial surface increased as the developmental stages advanced and varied at the abaxial surface, and it was highest in the intermediate stage of development and lowest in the initial stage (Table 3). The stomata density is related to the leaves' photosynthetic capacity since a greater number of stomata per area correspond to a lower resistance to gas diffusion. Thus, the lower stomatal density observed in both Spermacoce verticillata leaf surfaces with increasing plant age may lead to lower photosynthetic rates. This difference can be compensated for by a higher number of leaves per plant as well as a greater leaf size (Lima Junior et al., 2006). The stomatal index is generally higher when the plant is under higher light intensity (Lima Junior et al., 2006). Because Spermacoce verticillata is a weed, it commonly adapts by displaying greater stomatal density in its initial stages to compete with other species during its development, whereas it will show a reduced number of stomata in the later stages because it is subjected to lower light intensity. This strategy is corroborated by the fact that plants that are more adapted to stress would theoretically have a higher sap transport capacity and a reduced transport vessel size in more advanced stages (Alves and Andyalossy-Affonso, 2000). Trichomes can impair the control effect of herbicides by intercepting the spray droplets and causing them to adhere (Hess and Falk, 1990; Procópio et al., 2003; Galvani et al.

Table 1. Thicknesses of the leaf blade, cuticles and epidermis of *Spermacoce verticillata* leaves at three stages of vegetative development.

Stage/Leaf Surface	Leaf Blade	Cuticle (µm)		Epidermis (µm)	
	Thickness (µm)	Adaxial	Abaxial	Adaxial	Abaxial
2-4 leaves	252.12 a	0.76 a	0.69 ab	40.68 a	36.88 a
4-6 leaves	248.33 ab	0.79 a	0.67 ab	42.67 a	35.25 ab
Flowering	235.65 b	0.80 a	0.74 a	42.94 a	32.86 b
F	3.61	1.64 ^{NS}	4.27	1.63 ^{NS}	4.24
LSD ¹	15.19	0.05	0.05	3.23	3.28
CV ² (%)	13.06	15.41	17.4	16.21	19.82

¹Least significant difference. ²Coefficient of variation. Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability. *Significant at 5% probability. ^{NS}Not significant.



Fig 1. General appearance of the adaxial leaf surface of *Spermacoce verticillata* at the 4-6-leaf stage under scanning electron microscopy. T, trichomes; S, stomata; C, subsidiary cells; P, papillae.

Table 2. Midrib widths and heigh	nts and parenchymal h	eights in <i>Spermacoce</i>	verticillata leaves at thre	e stages of development.
		- 0		

Stage/Leaf Surface	Midrib (µm)		Parenchymal he	Parenchymal heights (µm)		
	Widths	Heights	Palisade	Spongy		
2-4 leaves	16.96 a	45.36 a	0.16 a	0.37 a		
4-6 leaves	17.8 a	43.24 a	0.13 b	0.34 b		
Flowering	13.12 b	35.98 b	0.13 b	0.34 b		
F	20.3	** 16.53	** 16.73	** 13.72	**	
LSD ¹	1.85	4.005	0.011	0.016		
CV ² (%)	24.53	20.6	17.2	9.62		

¹Least significant difference. ²Coefficient of variation. Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability. ** Significant at 1% probability



Fig 2. General appearance of the abaxial leaf surface of *Spermacoce verticillata* under scanning electron microscopy detailing the trichrome distribution in plants with 4-6 leaves. T, trichomes.

Stage/Leaf Surface	Number of stomata		Stomatal ir	Stomatal index		Number of trichomes	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	
2-4 leaves	16.96 a	45.36 a	0.16 a	0.37 a	36.84 c	22.04 c	
4-6 leaves	17.8 a	43.24 a	0.13 b	0.34 b	43.56 b	43.56 a	
Flowering	13.12 b	35.98 b	0.13 b	0.34 b	49.12 a	31.5 b	
F	20.3	16.53	* 16.73 *	* 13.72	** 15.24	** 61.41 **	
LSD ¹	1.85	4.005	0.011	0.016	5.27	4.61	
CV^{2} (%)	24.53	20.6	17.2	9.62	25.79	30.07	

Table 3. Number of stomata, stomatal index and number of trichomes of *Spermacoce verticillata* leaves at three stages of development.

¹Least significant difference. ²Coefficient of variation. Means followed by the same letter in the column do not differ from each other by Tukey's test at 5% probability. ** Significant at 1% probability



Fig 3. General appearance of the adaxial leaf surface of *Spermacoce verticillata* under scanning electron microscopy detailing the reduced number of stomata in later stages of development. A: plants with 2-4 leaves; B: flowering plants.



Fig 4. General appearance of the adaxial leaf surface of *Spermacoce verticillata* under scanning electron microscopy detailing the increased number of stomata in later stages of development. A: plants with 2-4 leaves; B: flowering plants.

2012). The relationship between the number of stomata and trichomes and the ease of absorbing herbicides is poorly understood. Certain authors have argued that more stomata may increase the product's efficacy; however, herbicide absorption is conducted not by these structures but rather in the intercellular spaces of these structures (Greene and Bulkovac, 1974; Santos et al., 2009; Barroso et al., 2015). For the herbicide to penetrate the stomata, it must break the surface tension of the droplet, in which case, adding organosilicon surfactants to the spray solution is recommended (Ferreira et al., 2012; Procópio et al., 2003). Moreover, a reduced amount of cuticle exists in the guard cells of the stomata, which may facilitate herbicide absorption (Schonherr, 2006).

The leaf's anatomical structures can influence the deposition, retention, absorption and translocation of solutions applied to the leaves and function as barriers, thus leading to increased or decreased susceptibility to herbicide control. However, as the stages of development advance in *Spermacoce verticillata*, the plant showed reduced leaf thickness, which reduced the thickness of its abaxial epidermis, transport vessels and photosynthetic cells as well as the number of stomata, and these characteristics are likely unrelated to control failures.

Moreover, plants in more advanced developmental stages presented more trichomes on their adaxial surfaces, which is the surface with the most contact with the herbicide spray droplets, and these structures may represent a barrier to absorbing certain products, as previously observed by other authors (Procópio et al., 2003).

Scanning electron microscopy

The foliar absorption rates and, hence, the biological efficacy of pesticide applications depend largely on the structures found in leaves and the permeability of crop and weed cuticles (Baker, 1982).

Spermacoce verticillata leaves present low epicuticular wax thicknesses, as seen in Figure 1. The stomata of this species, which are amphistomatic, are of the paracytic type, with two subsidiary cells parallel to the guard cells, and they have a reticulated distribution pattern common in eudicots. The epidermal cells contain papillae that may converge light rays into the mesophyll, which represents an adaptive mechanism to aid photosynthesis and is a common feature in the Rubiaceae family (Pereira et al., 2003).

The unicellular, tector-type trichomes were spread over the abaxial and adaxial leaf blade surfaces, with a row of trichomes on both sides of the midrib (Figure 2). Ricotta and Masiunas (1992) evaluated 14 tomato genotypes and obtained an inverse correlation between the acifluorfen activity in the genotype and the number of trichomes (with a higher number of trichomes corresponding to a lower activity).

Histological analyses showed that as the plant development stages advanced, the number of stomata decreased (Figure 3) while the number of trichomes increased (Figure 4). Procópio et al. (2003) observed that the main leaf barrier to herbicide penetration in *Galinsoga parviflora* was the low stomatal density on the adaxial surface, while for *Crotalaria incana*, the main barrier to herbicide penetration was the large amount of epicuticular wax. For *Conyza bonariensis*, the high trichome density, high cuticle thickness on the adaxial surface and low stomatal density were the main barriers to herbicide penetration. In *Ipomoea cairica*, the high cuticle thickness on the adaxial surface and the low stomatal density were possible obstacles to herbicide penetration applied postemergence.

Materials and Methods

Plant material

Spermacoce verticillata (shrubby false buttonweed) seeds were collected in producing regions in Luís Eduardo Magalhães, Bahia State (BA), Brazil and placed in plastic germination boxes (Gerbox) containing two sheets of paper. The boxes were placed in a germination chamber with a 12 h/12 h light-dark photoperiod at 26°C. Seedlings in the initial phase (first pair of true leaves) were transplanted into 3-L pots containing a mix of Tropstrato[®] Brazilian commercial substrate (pine bark, peat and expanded vermiculite, enriched with macro- and micronutrients) + soil in a 1:1 ratio. The pots were kept in the greenhouse until the experiment began.

Three phenological stages were evaluated: a) plants with 2-4 fully expanded leaves, b) plants with 4-6 fully expanded leaves and c) flowering plants. For each stage, four leaves were collected from the two most apical nodes. For each pair of leaves, one was used to prepare slides for counting stomata and normal epidermal cells and scanning electron microscopy analyses, and the second leaf was used to prepare histological slides to measure the other parameters. For each individual plant, two leaves were analyzed from different regions.

Anatomical analyses

All collected leaves were fixed in Karnovsky's solution (Karnovsky 1965, modified with the use of phosphate buffer, pH 7.2) and vacuum pumped to remove the air in the tissues for later structural analyses. To prepare the permanent histological slides, certain samples were dehydrated in an ethanol series and embedded in historesin (Leica Historesin[®]) per the manufacturer's instructions. The obtained blocks were sectioned at a thickness of 5 μ m on a manual rotary microtome (Leica[®]) with type C blades. Sections were stained with 0.05% toluidine blue (Sakai, 1973) in phosphate and citrate buffer (pH 4.5) and mounted on Entellan[®] synthetic resin (Merck[®]).

To characterize the epidermal cells in the frontal view and count the stomata, normal epidermal cells and trichomes, the epidermis was broken down in a solution of ethanol, glacial acetic acid and glycerin (at a concentration of 3:1:1) (Johansen, 1940). The fragments were stained with 1% safranin in water (Bukatsch, 1972) and mounted on glycerin for subsequent image capture.

The following parameters were obtained from cross-sections of the leaf blade: cuticle thickness of the adaxial and abaxial surfaces, epidermal cell thickness on both surfaces, total leaf blade thickness, palisade and spongy parenchymal thicknesses and midrib height and width. For each parameter, five measures/counts were performed. ImageJ (Rasband, 2006) and Image-Pro Plus software were used for the measurements and counts.

The results were documented by capturing images of the slides using a Leica® DFC310Fx video camera coupled to a Leica® DM LB microscope using LAS 4.0 software. Observations of each parameter were repeated five times on two leaves for every five individuals, totaling 50 replicates per stage. The data were subjected to an analysis of variance, and when significant, the means were compared by Tukey's test at 5% probability.

Scanning electron microscopy

Leaf surface analyses were performed with samples previously fixed in Karnovsky's solution (Karnovsky, 1965 modified using phosphate buffer, pH 7.2), dehydrated in a graded ethanol series to absolute ethanol, subjected to CO₂ critical-point drying (Horridge and Tamm 1969) in a CPD 030 Balzers apparatus, mounted on aluminum stubs and coated with a gold layer of 30 to 40 nm using an SCD 050 Balzers sputter coater. Electromicrographs with the scales directly printed on them were taken using an LEO scanning electron microscope (model VP 435 operated at 20 kV) at the Research Center in Electronic Microscopy Applied to Agriculture (NAP/MEPA) of the Luiz de Queiroz College of Agriculture (Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ) at the University of São Paulo (USP) and evaluated by observing the electromicrographs.

Conclusion

As Spermacoce verticillata's developmental stages advanced, the plants showed reduced leaf thickness due to reduced

abaxial epidermal thickness, transport vessel size and parenchymal thickness. In more advanced stages of development, the leaves' adaxial surfaces showed fewer stomata and more trichomes.

Acknowledgements

The authors thank Corteva for the *Spermacoce verticillata* seeds.

References

- Alves ES, Angyalossy-Alfonso V (2000) Ecological trends in the wood of some Brazilian species 1: growth rings and vessels. lawa Journal. 21:3-30.
- Baker EA (1982) Chemistry and morphology of plant epicuticular waxes. In: Cuttle DF, Alvin KL, Price CE (ed) The plant cuticle, Academic Press, London.
- Barroso AAM, Galeano E, Albrecht AJP, Reis FC (2015) Does sourgrass leaf anatomy influence glyphosate resistance? Comunicata Scientiae. 6:445-453.
- Boeger MR, Wiskiewski C (2003) Comparação da morfologia foliar de espécies arbóreas de três estágios sucessionais distintos de floresta ombrófila densa (floresta atlântica) no sul do brasil. Revista Brasileira de Botânica. 26:61-72.
- Caldeira DAS, Amaral VN, Casadei RA, Barros LV, Figueiredo ZN (2014) Controle de plantas daninhas em pastagem usando doses e misturas de herbicidas. Enciclopédia Biosfera. 10:1.052-1.060.
- Cerqueira FB, Erasmo EAL, Silva JIC, Nunes TV, Carvalho GP, Silva AA (2013) Competição inicial entre cultivares de arroz de terras altas tolerantes à seca e plantas daninhas sob condições de estresse hídrico. Planta Daninha. 31:291-302.
- Cruz-Hipolito H, Rojano-Delgado A, Domínguez-Valenzuela JÁ, Heredia A, Castro MDL, De Prado R (2011) Glyphosate tolerance by *Clitoria ternatea* and *Neonotonia wightii* plants involves differential absorption and translocation of the herbicide. Plant Soil. 347:221-230.
- Fahn A (1990) Plant Anatomy. Pergamon press, Oxford.
- Ferreira EA, Santos JB, Silva AA, Oliveira JA, Vargas (2006) Translocação do glyphosate em biótipos de azevém (*Lolium multiflrum*). Planta Daninha. 24:365-370.
- Ferreira EA, Procópio SO, Silva EAM, Silva AA, Rufino RJN (2003) Estudos anatômicos de folhas de espécies de plantas daninhas de grande ocorrência no Brasil. IV Amaranthus deflexus, Amaranthus spinosus, Alternanthera tenella e Euphorbia heteroplylla. Planta Daninha. 21:263-271.
- Fontes JRA (2007) Manejo de plantas daninhas em seringais de cultivo na Amazônia. Manaus: Embrapa-CPAA. 6p.
- Galvani J, Rizzardi MA, Carneiro CM, Bianchi MA (2012) Leaf anatomy of *Lolium multiflorum* sensitive and resistant to glyphosate. Planta Daninha. 30:407-413.
- Greene DW, Bukovac MJ (1974) Stomatal penetration: effect of surfactants and role in foliar absorption. A J Bot. 61:101-106.
- Hatterman-Valenti H, Pitty A, Owen M (2011) Environmental Effects on Velvetleaf (*Abutilon theophrasti*) Epicuticular Wax Deposition and Herbicide Absorption.Weed Sci. 59:14-21.
- Hatterman-Valenti H, Pitty A, Owen MDK (2006) Effect of environment on giant foxtail (*Setaria faberi*) leaf wax and fluazifop-P absorption. Weed Sci. 54:607-614.
- Hetherington P, Reynolds, TL, Marshall G, Kirkwood RC (1998) The absorption, translocation and distribution of the herbicide glyphosate in maize expressing the CP-4 transgene. J Exp Bot. 50:1.567-1.576.
- Hess FD (1997) Absorption. In: Weller SC. Herbicide action course. West Lafayette: Purdue University, 2:15-37.

- Hess FD, Falk RH (1990) Herbicide deposition on leaf surfaces. Weed Science. 38:280-288.
- Huangfu C, Song X, Qiang S (2009) Morphological disparities in the epidermal and anatomical features of the leaf among wild *Brassica juncea* populations. Weed Bio Manag. 9:232-242.
- Johnson GA, Hoverstad TR (2002) Effect of row spacing and herbicide application timing on weed control and grain yield in corn (*Zea mays*). Weed Tech. 16:548-553.
- Kissmann KG, Groth D (2000) Plantas infestantes e nocivas: Tomo III, 2nd edn. São Paulo, Basf. 722p.
- Larcher W (2000) Ecofisiologia vegetal. Rima artes e textos, São Carlos.
- Lima-Junior EC, Alvarenga AA, Castro EM, Vieira CV, Barbosa JPRAD (2006) Aspectos fisioanatômicos de plantas jovens de *Cupania vernalis* camb. submetidas a diferentes níveis de sombreamento. Revista Árvore. 30:33-41.
- Machado AFL, Meira RMS, Ferreira LR, Ferreira FA, Tuffi-Santos LD, Fialho CMT, Machado MS (2008) Caracterização anatômica de folha, colmo e rizoma de *Digitaria insularis*. Planta Daninha 26:1-8.
- Marques RP, Rodella RA, Martins D (2012) Characteristics of the leaf anatomy of Surinam grass and Alexandergrass related to sensitivity to herbicides. Planta Daninha. 30:809-816.
- Ministério da Ciência e Tecnologia, MCT (2002) Recuperação de áreas de pastagens abandonadas e degradadas através de sistemas agroflorestais na Amazônia Ocidental. Disponível em: https://qprocura.com.br/dp/15637/recuperacao-de-areas-depastagens-abandonadas-e-degradadas-atraves-de-sistemasagroflorestais na Amazonia. Ocidental. Acorso. em: 20. agro

agroflorestais-na-Amazonia Ocidental. Acesso em: 20 ago. 2009.

- Passos MAB, Mendonça MS (2006) Epiderme dos segmentos foliares de *Mauritia flexuosa* L. f. (Arecaceae) em três fases de desenvolvimento. Acta Amazonica. 36:431-436.
- Pereira ZV, Meira RMSA, Azevedo AA (2003) Morfoanatomia foliar de *Palicourea longepedunculata* Gardiner (Rubiaceae). Revista Árvore. 27:759-767.
- Procópio SO, Ferreira EA, Silva EAM, Silva AA, Rufino RJN, Santos JB (2003) Leaf anatomical studies in weed species widely common in Brazil. III - Galinsoga parviflora, Crotalaria incana, Conyza bonariensis and Ipomoea cairica. Planta Daninha. 21:1-9.
- Ricotta JA, Masiunas JB (1992) Relantionship of leaf surface characteristics to acifluorfen tolerance in tomato (*Lycopersicon esculentum*) and related species. Weed Sci. 40:402-407.
- Schott JJ, Dufour JL, Gauvrit C (1991) Effects of adjuvants on herbicidal action. III Effects of petroleum and rapeseed oils on diclofopmethylaction on ryegrass. Agronomie. 11:27–34.
- Schonherr J (2006). Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. J Exp Bot. 57:2.471-2.491.
- Silva AA (2007) Herbicidas: absorção, translocação, metabolismo, formulação e misturas. In: Silva AA (Orgs.). Tópicos em manejo de plantas daninhas, Viçosa:, UFV.
- Toni KLG, Mariath JEA (2004) Desenvolvimento do rudimento seminal em *Borreria verticillata* (L). G. Mey. (Rubiaceae – Rubioideae – Spermacocaea). Revista Brasileira de Botânica. 27:185-192.
- Tuffi-Santos LD, Sant'Anna-Santos BF, Meira RMSA, Ferreira FA, Tiburcio RAS, Machado AFL (2009) Leaf anatomy and morphometry in three eucalypt clones treated with glyphosate. Brazilian Journal of Biology. 69:129-136.
- Vila-Aiub MM, Balbi MC, Distéfano AJ, Fernández L, Hopp E, Powles SB (2011). Glyphosate resistance in perennial *Sorghum halepense*, endowed by reduced glyphosate translocation and leaf uptake. Pest Manag. Sci. 68:430-436.