

# Leaf area and epicuticular wax content of *Sida* spp.<sup>1</sup>

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## ABSTRACT

Morpho-physiological characteristics and chemical composition are directly related to superior competitive ability of crops. This study intended to make a comparative analysis of dry matter production, leaf area and amount of epicuticular wax of three species of *Sida* spp: *S. urens* L., *S. rhombifolia* L. and *S. spinosa* L. Plants were collected at three growth stages: V1: stage described as up to 10 fully expanded leaves; V2: between 11 leaves and flowering; and R: after flowering. At stages V<sub>2</sub> and R, the highest number of leaves was recorded for *S. rhombifolia*, followed by *S. spinosa* at V<sub>2</sub> and *S. urens* at R. These results were relatively proportional to leaf area for all species. *S. spinosa* at the vegetative stage produced the highest values of specific leaf area (SLA), with no significant differences between species at the stage R. The amount of wax per unit of leaf area between species at the same developmental stage was significantly different only at the reproductive stage, where *S. spinosa* produced 23.18 and 6.23 fold more wax than *S. urens* and *S. rhombifolia* respectively. Between the growth stages of each species, there was decrease in the amount of wax with plant age and increase in leaf area (AFE), number of leaves and dry matter. The leaves of the *Sida* species exhibit different characteristics and this information can be used to optimize the use of herbicides in the control of these weeds.

**Key words:** *Sida rhombifolia* L., *Sida spinosa* L., *Sida urens* L., weeds.

## RESUMO

### Área foliar e conteúdo de cera epicuticular de *Sida* spp.

Características morfofisiológicas e composições químicas estão diretamente relacionadas com a maior habilidade competitiva das culturas. O objetivo deste trabalho foi realizar análise comparativa da produção de matéria seca, de área foliar e de quantidade de cera epicuticular de três espécies de *Sida* spp.: *Sida urens* L., *Sida rhombifolia* L. e *Sida spinosa* L. Essas espécies foram coletadas em três estádios fenológicos, caracterizados como V1: formação de até dez folhas completamente expandidas; V2: entre 11 folhas e antes do florescimento, e R: após florescimento. Verificou-se, para os estádios V2 e R, maior número de folhas para a espécie *Sida rhombifolia*, seguida por *S. spinosa* em V2 e *S. urens* em R. Esses resultados foram relativamente proporcionais à área foliar para todas as espécies. *S. spinosa*, na fase vegetativa, produziu os maiores valores de área foliar específica (AFE), não se observando diferenças entre as espécies avaliadas no estágio caracterizado por R. Para a quantidade de cera por unidade de área foliar, entre

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as espécies, num mesmo estágio, verificou-se diferença somente na fase reprodutiva, em que *S. spinosa* produziu valores 23,18 e 6,23 vezes maiores que os encontrados para *S. urens* e *S. rhombifolia*, respectivamente. Entre os estádios para cada espécie, observou-se decréscimo na quantidade de cera com a idade das plantas e um aumento da área foliar, AFE, do número de folhas e da massa seca. A estrutura foliar das espécies de *Sida* sp. avaliadas apresenta diferentes características e essas informações podem ser utilizadas para otimizar o uso dos herbicidas no controle dessas plantas daninhas.

**Palavras-chave:** *Sida rhombifolia* L., *Sida spinosa* L., *Sida urens* L., plantas daninhas.

## INTRODUCTION

The genus *Sida* comprises more than 170 species and most of them are considered as potential weeds in pastures and annual crops. These species are widely distributed, hard to be controlled and adapted to weak and acid soils, although they develop better in fertile clay soils (Kissmann & Groth, 2000).

Although botanically similar, species of *Sida* differ in their susceptibility to herbicides, especially in post-emergence application (Albert & Victoria Filho, 2002 a). The efficiency of herbicides applied to leaves depends on the morphology and physicochemical characteristics of the leaf surface and the presence of adjuvants in the herbicidal solution (Constantin *et al.*, 2007).

Several authors reported that morphological and physiological characteristics such as shoot, root and total dry mass (Wortmann, 1993; Olesen *et al.*, 2004; Bianchi *et al.*, 2006), plant height (Fleck, 1980; McDonald, 2003), leaf area (Wortmann, 1993; Lemerle *et al.*, 1996) and leaf area index (Gibson *et al.* 2003; Haefele *et al.*, 2004) are directly related to superior competitive ability of crops. Over the past few years, research topics have also been directed to the integrated weed management (Bianco *et al.*, 2008) and providing relevant information to the evaluation of characteristics within a species that may interfere with the dynamics of control.

Morphology of plants, especially leaves, influences the amount of herbicide intercepted and retained (Hess & Falk, 1990), and explains the distinct responses of plant species to several products (Chun *et al.*, 1997). However, plant anatomical and micro-morphological characteristics do not determine how easily these products will be absorbed (Hess & Falk, 1990). The cuticle or cuticular membrane is the first barrier to the absorption of chemicals applied to the leaves and has been the subject of several studies. Some studies have emphasized its nature as well as the factors influencing its permeability (Holloway, 1982, Bukovac *et al.*, 1990, Albert & Victoria Filho, 2002 b). Cuticle is the major route of herbicide absorption, which makes the knowledge of this structure of fundamental importance in studies of absorption of these compounds (Procópio *et al.*, 2003).

The deposition of epicuticular wax is continuous until full morphological development of the leaf. The deposition rate depends directly on temperature and light, being inversely proportional to water content in the plant (Skoss, 1955). Water, from atmospheric precipitation, besides causing removal of epicuticular wax, reduces cuticle thickness and consistency, influencing the oxidation and condensation of fatty acids, which are processes involved in cuticle formation (Evans, 1984).

The importance of the genus *Sida* in agricultural ecosystems highlights the need for basic studies aiming to optimize the effectiveness of practices of weed management. In this way, this work aimed to comparatively analyze the dry mass, leaf area and amount of epicuticular wax in three species of the genus *Sida*, at three developmental stages.

## MATERIAL AND METHODS

A phytosociological study in areas of pasture held at the Experimental Station of the University of the Jequitinhonha and Mucuri (UFVJM), Curvelo Moura, Minas Gerais, demonstrated that species of the genus *Sida* have a high importance value. Thus, on May 2009, samples of three species of this genus were collected (*Sida urens*, *Sida rhombifolia* and *Sida spinosa*) at three developmental stages (V1: up to 10 fully expanded leaves; V2: between 11 leaves and flowering and R: after flowering). Plants from the spontaneous vegetation were collected randomly and subjected to 9 treatments resulted from a combination of the three species and three sampling stages, each one with six replications.

Plants were cut close to the ground and brought to the Chemistry Lab at UFVJM for evaluation of leaf area, shoot dry mass and extraction and quantification of wax. Plants were kept in a cooler with ice to avoid dehydration and weight loss during transportation between field and laboratory.

Leaf area estimation was performed by scanning detached leaves and digitalizing into the software Digital Determinator Areas (DAA). Leaf dry mass was weighed in a digital scale with 0.0001 g precision, withdrawing a

sample for quantification of epicuticular wax. For that purpose, in a petri dish, samples from each treatment were dried (65° C for 72 hours) and immersed in 100 mL of chloroform for 30 seconds (Hamilton, 1995). The solution (chloroform, wax and impurities) was filtered through Isofar filter paper (12.5 cm diameter) and transferred to a flask attached to a Rotavapor, for evaporation and recovery of most of the chloroform. The remaining solution containing the wax was transferred to previously weighed test tubes. After complete chloroform evaporation, the tubes were weighted again to obtain the content of epicuticular wax expressed in mg/cm<sup>2</sup>.

The remaining leaves were also measured using the same procedure described for the samples used for the determination of leaf area. Based on these data, the specific leaf area (SLA) and wax content per unit area (mg/cm<sup>2</sup>) were determined.

Data was subjected to tests of normality and homogeneity of variances, and then to analysis of variance and the means, when significant, were compared by Duncan's multiple range test at 5% probability.

## RESULTS AND DISCUSSION

The highest leaf number was recorded at the vegetative stage V2 for *Sida spinosa* and reproductive stage R for the other species (Table 1). The influence of this variable in leaf area was evaluated at the final stage and produced similar results, e.g. more leaf area for *S. rhombifolia*, greater than 200 cm<sup>2</sup>, and 171.49 cm<sup>2</sup> for *S. urens*. *S. spinosa* showed lower leaf area, around 20 cm<sup>2</sup>. The highest leaf number for *S. spinosa* can be explained by the relatively smaller leaf size of this species during V2 than *S. urens* (Lorenzi, 2006). Leaf number of *S. urens* and *S. rhombifolia* increased 8.46 and 3.48 fold between the stages V2 and R, providing additional photosynthates for fruit formation (Streck *et al.*, 2005).

Pierce *et al.* (1994) observed that the specific leaf area (SLA - ratio between leaf area and leaf dry mass) is an important ecophysiological characteristic for the understanding of plant carbon-water relations. It is also

an important factor from a physiological standpoint because it describes the allocation of leaf biomass per unit area, reflecting the capacity and efficiency in conserving nutrients by fast biomass accumulation (Poorter & Garnier, 1999). The highest SLA value for *S. spinosa* was recorded at the vegetative stage (Table 2). At the reproductive stage (R), no difference was found for SLA between species, with mean equal to 113.24 cm<sup>2</sup>/g (Table 2). Thus, results could help in understanding the aggressive competitive ability of *S. spinosa* in crops like cotton (Chandler, 1977) and soybean (Payne and Oliver, 2000).

Differences in the amount of wax per unit of leaf area between species at the same stage only occurred at the reproductive stage, with *S. spinosa* having more wax than the other species (Table 2). The amount of wax in *S. spinosa* at the reproductive stage was 6-23 times larger than for those found for *S. urens* and *S. rhombifolia* respectively. In general, the amount of wax decreased with plant age. This result is contrary to expectations, since it was believed that the difficulty in controlling the *Sida* sp., in more advanced stages of development, was due to increased layers of wax. However, as observed by Monqueiro *et al.* (2004), the composition of the epicuticular wax layer itself is longer related to this difficulty in the chemical control of some weeds, than its quantity.

*Sida urens* and *S. rhombifolia* had a reduction in the amount of wax as plants advanced from the developmental stage V2 to R (Table 2). Several other studies show evidences that older leaves are less capable of controlling water loss than young leaves, as observed in banana (*Musa* spp.) by Costa *et al.* (2009). Alves *et al.* (1990), studied the effect of simulated acid rain on the amount of epicuticular wax of soybean plants and found that regardless of irrigation there was a decrease in epicuticular wax content with elapsed days. In this study, we found that the decrease in wax accumulation was significantly lower in *S. spinosa*, which is likely the cause for the high aggressive competitive ability of this species in cotton and soybean crops, as previously reported. In studies with sugarcane varieties, higher amounts of epicuticular wax were found in varieties classified as more tolerant to

**Table 1.** Total number of leaves and leaf area of three species of the Genus *Sida*, sampled at three distinct phenological stages

Species	Phenological Stages <sup>1/</sup>					
	N° of Leaves			Leaf Area (cm <sup>2</sup> )		
	V1	V2	R	V1	V2	R
<i>Sida urens</i>	8 Ba	15 Bc	82 Ab	5.80 Ba	20.25 Ba	171.49 Ab
<i>Sida spinosa</i>	4 Ba	51 Ab	28 Bc	1.71 Aa	13.45 Aa	20.01 Ac
<i>Sida rhombifolia</i>	3 Ca	103 Ba	308 Aa	1.13 Ca	58.67 Ba	204.23 Aa
CV (%)	24.00	32.80				

<sup>1/</sup>V1: From plant formation until up to 10 completely expanded leaves; V2: between 10 leaves and before flowering; R: after flowering. Means followed by the same letter in the rows (capital letters) and columns (small letters), for each parameter, are not significantly different by the DMRT test at 5% probability.

herbicides (Ferreira *et al.*, 2005). It is therefore possible that the results of lower susceptibility of *S. spinosa* to some herbicides can be explained by a high wax content compared to other species of the genus *Sida*. Beyers *et al.* (2002), evaluating the effectiveness of the herbicide flumiclorac-pentyl, reported that its addition to ammonium glufosinate did not significantly improve the control of *S. spinosa*.

Constantin *et al.* (2007) found that among several herbicides tested for control of four species of *Sida* (*S. rhombifolia*, *S. spinosa*, *S. cordifolia* and *S. glaziovii*), fomesafen and lactofen, even with adjuvant, showed the lowest control rates for *S. spinosa*. However, the same authors found that the herbicide flumiclorac-pentyl was an excellent option, successfully controlling this species.

Studies (Albert & Victoria Filho, 2002a; 2002b) evaluating the leaf surface of three species of *Sida* (*S. rhombifolia*, *S. cordifolia* and *S. glaziovii*) found that *S. rhombifolia* had higher amounts of randomly orientated epicuticular striate wax and that this trait was found more abundantly at the adaxial side, suggesting further studies on this characteristic to allow a correlation with herbicide tolerance.

For Schmidt *et al.* (1981), the epicuticular layer of young leaves presents higher polarity, which allows the

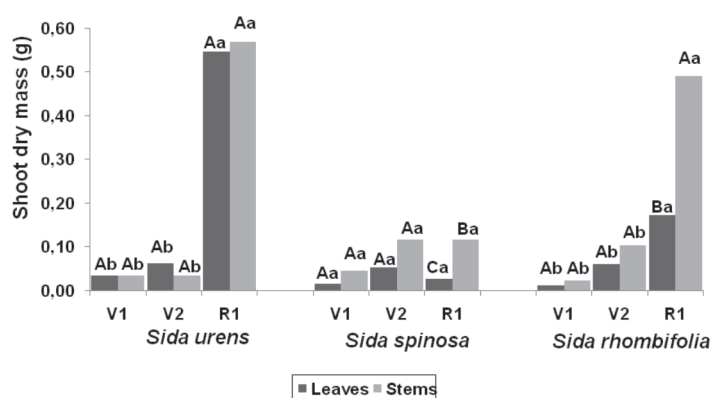
passage of large amounts of hydrophilic compounds such as some herbicides. Grover and Cessna (1991) reported evidences that penetration of herbicides decreases as leaf age increases. For Silva & Silva (2007), the absorption of herbicides is not necessarily related to the thickness or weight of the cuticle, but to its degree of impermeability which can be attributed to changes in composition or increases in formation of waxes.

It is possible to infer that the competitive ability of *S. spinosa* is associated to the production of epicuticular wax, instead of mass accumulation. Thus, the higher proportion of wax in relation to the rest of the dry mass of leaves would be an important factor in tolerance to the penetration of herbicides. Chun *et al.* (2001), evaluating the absorption of oxyfluorfen by two species of wheat, found that in average 65% of the herbicide was retained at the epicuticular wax, even after 120 hours post-application. Regarding the infestation of pastures, assuming intensive use of 2,4-D alone or mixed with picloram, the lower wax content in *S. urens* would help elucidating the best results of control for this species relatively to *S. spinosa* (Lorenzi, 2006).

**Table 2.** Specific leaf area (SLA) and epicuticular wax content of three species of the Genus *Sida*, sampled at three distinct phenological stages

Species	Phenological Stages <sup>1/</sup>					
	SLA (cm <sup>2</sup> /g)			Wax (mg/cm <sup>2</sup> )		
	V1	V2	R	V1	V2	R
<i>Sida urens</i>	87.47 Bb	94.97 Bb	107.04 Aa	3.28 Aa	1.31 Ba	0.11 Cb
<i>Sida spinosa</i>	111.57 Aa	130.68 Aa	130.01 Aa	4.92 Aa	2.72 Ba	2.55 Ba
<i>Sida rhombifolia</i>	97.40 Aab	91.32 Ab	102.66 Aa	4.16 Aa	4.08 Ba	0.41 Bb
CV (%)	17.09	32.11				

<sup>1/</sup>V1: From plant formation until up 10 completely expanded leaves; V2: between 10 leaves and before flowering; R: after flowering. Means followed by the same letter in the rows (capital letters) and columns (small letters), for each parameter, are not significantly different by the DMRT test ant 5% probability.



**Figure 1.** Shoot dry mass (leaves and stems) of three *Sida* species (*Sida urens*, *S. spinosa* and *S. rhombifolia*).

\*Means followed by the same capital letters indicate that the species of *Sida* did not differ in the amount of shoot dry mass in different vegetative stages and by the same lower small letters indicate that different stages do not differ in the accumulation of shoot dry mass by DMRT test ant 5 % probability



*S. urens* and *S. rhombifolia* showed higher shoot dry mass mainly due to the accumulation of biomass in stems (Figure 1). Among the stages evaluated for each species, no difference was observed for biomass accumulation in *S. spinosa*. However, for *S. urens*, the accumulation of biomass was five times higher in the reproductive stage compared to the vegetative.

## CONCLUSIONS

The number of leaves, leaf area, quantity of epicuticular wax and dry matter vary with the stage of development of each species of *Sida* spp. evaluated.

*Sida spinosa* presented greater values than *Sida rhombifolia* and *Sida urens* of specific leaf area at the vegetative stage and of wax at the reproductive stage, which can be related to its competitive ability and lower susceptibility to some herbicides.

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