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Article

# Effect of Sethoxydim Herbicide in the Leaf Anatomy and Physiology of Brachiaria Grass under Water Stress

*Efeito do Herbicida Sethoxydim na Anatomia Foliar e Fisiologia de Plantas de Capim-Braquiária Sob Estresse Hídrico*

PEREIRA, M.R.R.[1]*
MARTINS, A.R.[2]
MARTINS, D.[3]
SASSO, G.[3]
SILVA JR, A.C.[3]

ABSTRACT - The goal of this study was to analyze the leaf anatomy and physiological behavior of Brachiaria grass plants (*Urochloa decumbens*) under different water conditions and with the application of sethoxydim herbicide. The used experimental design was the completely randomized one, with four replications, consisting of a 3 x 2 factorial scheme, with the combination of three water managements (-0.03, -0.07 and -1.5 MPa) with and without the application of sethoxydim herbicide + Assist mineral oil, at the recommended dose for the species (184 g a.i. $ha^{-1}$). The assessed physiological and anatomicalal parameters were photosynthetic rate, stomatal conductance, transpiration, difference between leaf and room temperature, dry mass of plants, thickness of bulliform cells, adaxial and total epidermis. Under the conditions in which the experiment was conducted, it appears that Brachiaria grass leaves showed uniseriate epidermis, homogenous mesophyll, with radiated distribution of parenchymal cells around the vascular bundles. The adaxial epidermis presented bulliform cells; the vascular bundles are collateral and are present in different sizes. Water stress had a negative influence on herbicide effectiveness and decreased all physiological parameters. The application of the herbicide caused anatomical changes in plants with no water stress (-0.03 MPa), such as limitations in the growth of epidermic and bulliform cells, and in the total leaf thickness. However, in treatments with stress (-0.07 and -1.5 MPa), there were no differences in leaf anatomy, but an increase in the total thickness of leaves, probably as a result of the water stress conditions to which plants were submitted..

**Keywords:** chemical control, effectiveness, *Urochloa decumbens*.

*RESUMO - O objetivo do presente trabalho foi analisar a anatomia foliar e o comportamento fisiológico de plantas de capim-braquiária (**Urochloa decumbens**) submetidas a diferentes condições hídricas e com a aplicação do herbicida sethoxydim. O delineamento experimental utilizado foi o inteiramente casualizado com quatro repetições, constituído de um fatorial 3 x 2, sendo a combinação de três manejos hídricos (-0,03, -0,07 e -1,5 MPa) com e sem a aplicação do herbicida sethoxydim + óleo mineral Assist, na dose recomendada para a espécie (184 g i.a. $ha^{-1}$). Os parâmetros fisiológicos e anatômicos avaliados foram: taxa fotossintética, condutância estomática, transpiração, diferença entre temperatura ambiente e foliar, massa seca das plantas, espessura das células buliformes, da epiderme adaxial e epiderme total. Nas condições em que o experimento foi realizado, verifica-se que as folhas de capim-braquiária apresentaram epiderme unisseriada e mesofilo homogêneo, com distribuição radiada das células parenquimáticas ao redor dos feixes vasculares. A epiderme adaxial apresentou células buliformes, e os feixes*

* **Corresponding author:**
<mariarenatarp@hotmail.com>

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[1] Faculdade de Tecnologia de Capão Bonito, Capão Bonito-SP, Brasil; [2] Universidade Estadual Paulista "Júlio de Mesquita Filho", Ilha Solteira-SP, Brasil; [3] Universidade Estadual Paulista "Júlio de Mesquita Filho" Jaboticabal-SP, Brasil.

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*vasculares são do tipo colateral e estão presentes em diversos tamanhos. O estresse hídrico influenciou negativamente na eficiência do herbicida, bem como diminuiu todos os parâmetros fisiológicos. A aplicação do herbicida ocasionou modificações anatômicas em plantas sem estresse hídrico (-0,03 MPa), como limitação no crescimento das células da epiderme e das células buliformes e na espessura total das folhas. No entanto, nos tratamentos com estresse (-0,07 e -1,5 MPa) não houve diferenças na anatomia foliar; apenas ocorreu aumento na espessura total da folha, provavelmente reflexo das condições de estresse hídrico ao qual as plantas foram submetidas.*

**Palavras-chave:** controle químico, eficiência, *Urochloa decumbens*.

## INTRODUCTION

The signal grass species (*Urochloa decumbens* Stapf) is a tropical grass belonging to the Poaceae family, native to the African cerrado area, with a narrow natural distribution, introduced in Brazil in the 1960's. It is considered as a weed because of important characteristics, such as extremely quick dissemination ability in pastures and a high capacity of seed production and dispersion, providing a constant and abundant flow of new individuals, even under extremely diverse environmental conditions (Kissmann, 1997).

Various authors report that water stress on plants affects many growth aspects, causing anatomical, morphological, physiological and biochemical changes, which may influence the water potential of leaves, the stomatal resistance and conductance, the transpiration, photosynthesis, temperature and withering of leaves (Bezerra et al., 2003; Marenco and Lopes, 2005; Mattos et al., 2005).

According to Roman et al. (2005), environmental stresses such as the increase of light intensity and low soil moisture may induce a change in the composition and structure of leaves, and these changes may influence the penetration, absorption and translocation of herbicides, since they tend to induce the increase of hairiness, stomata sunk in the epidermis, increase in the number of bulliform cells involved in the mechanism of leaf rolling and unrolling and cuticle synthesis, with the subsequent increase of the leaf surface lipophilic character. Air relative humidity plays an important role in the cuticle hydration and in the re-dissolution of herbicide salts on the leaf surface.

Plants under water stress may have dehydrated cuticles, which may reduce herbicide absorption, thus resulting in a possible lower phytotoxicity for the culture and lower product effectiveness in controlling weeds (Peregory et al., 1990).

The anatomical structures that may be influenced by water stress and hinder the effectiveness of herbicides are: epidermis, vascular bundle, vascular bundle sheath, parenchyma, sclerenchyma, stomata, trichomes and leaf thickness, which may function as barriers, limiting the deposition, retention, absorption and translocations of solutions applied on leaves (Costa et al., 2010).

In order to better understand the relation of the water that is available for plants in the soil with the performance of the studied herbicide, it is necessary to know about foliar anatomy and plant physiological processes, as well as the determination on what is the water potential in the soil which may damage the action of the herbicide in controlling this species.

Thus, the goal of this work was to analyze the foliar anatomy and physiology, as well as the control, of *Urochloa decumbens* plants submitted to different water conditions and with the application of sethoxydim.

## MATERIAL AND METHODS

The experiment was installed and conducted in a greenhouse with the following internal climate characterizations of the period (averages): 19.8 $^{o}$C minimum temperature, 29.8 $^{o}$C maximum temperature, 77.8% relative air humidity and 3.1 mm month$^{-1}$ evapotranspiration, which were monitored daily through a Class A Tank.

The used species was *Urochloa decumbens*, cultivated in 2 L plastic planters, containing soil whose texture was classified as medium by the granulometric analysis (65.6% sand, 6.7% silt and 27.7% clay).

Plants were submitted to three minimum water potentials in soil (Ψs), which were established starting from the results of the water retention curve of the used soil, obtained through Richards' pressure plate (Klar, 1984); they were: -0.03 (no water stress), 0.07 (moderate water stress) and -1.5 MPa (severe water stress). Soil moisture was 13%, 10% and 8% respectively, composing the water managements, evaluated by weighing the planters. When getting close to the potential defined for each treatment, the evapotranspired water was restored, until reaching the mass of the maximum water potential of soil water retention (-0.01 MPa/14% soil moisture). Water managements started during the development stage of two leaves in each plant.

The used herbicide was sethoxydim (184 g a.i. $ha^{-1}$) + Assist mineral oil, and the application was made when plants had 4-6 leaves, on an average, 30 days after emergence, depending on the adopted management. A backpack sprayer was used, equipped with an application bar containing four XR11002VS flat spray nozzles, with 200 L $ha^{-1}$ mixture consumption.

The used experimental design was the completely randomized one with four replications, constituted by a 3 x 2 factor scheme, that is, three water managements (-0.03, -0.07 and 1.5 MPa) and two herbicide doses (0 and 184 g a.i. $ha^{-1}$).

The effects of the treatments on plants were visually evaluated on day 14 and 28 DAA, by a percentage scale of grades, where "0" consists in the absence of injuries and "100" consists in plant death (SBCPD, 1995). At the end of the evaluations, plants were collected and dried in a forced air ventilation oven at 60 °C until reaching constant weight; after that, the dry mass of the samples was determined.

The collection of plant material for the anatomical analyses occurred 14 days after the application of the herbicide (DAA), as well as the physiologic evaluations. The evaluated physiologic characteristics were: photosynthetic rate, stomatal conductance, transpiration and difference between room and foliar temperature. To do so, an infrared gas analyzer was used (IRGA Li-6400 Licor). Evaluations were made between 8 and 10 o'clock in the morning.

As for the anatomical analyses, leaf samples were fixed at FAA 50 (Johansen, 1940). For a better fixation, samples were taken to a vacuum pump to remove the air contained in tissues.

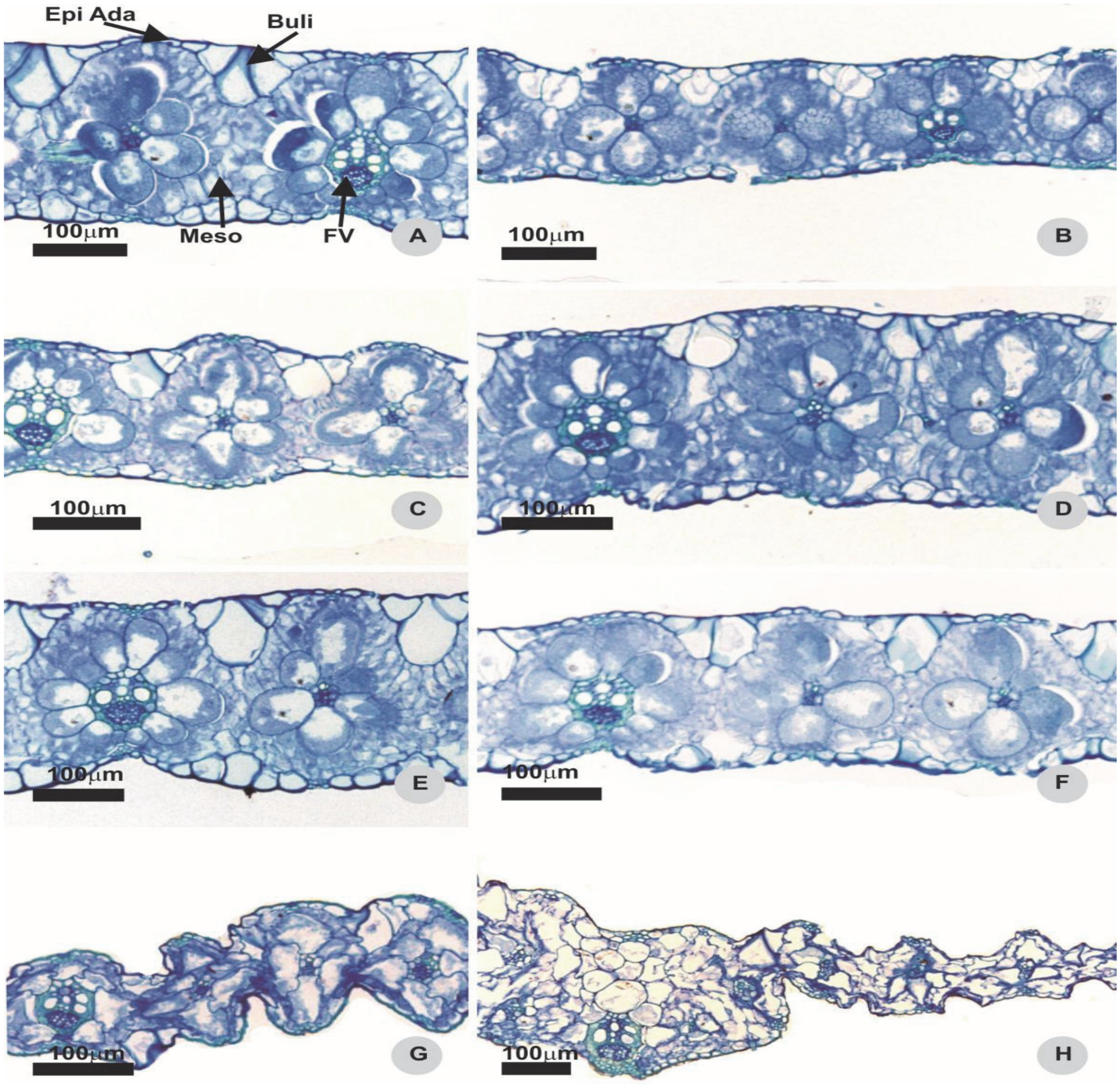
As for the anatomical analyses, leaf samples were fixed at FAA 50 (Johansen, 1940). For a better fixation, samples were taken to a vacuum pump to remove the air contained in tissues. After fixation, samples were dehydrated in an ethylic series and included in hydroxy-ethylmethacrylate (Leica Historesin), and the obtained blocks were sectioned at 8-10 mm thickness. The material was stained with 0.05% toluidine blue in a pH 4.5 phosphate and citric acid buffer (Sakai, 1973); the slides were sealed in Entellan synthetic resin.

Photomicrographs of the materials prepared on slides were taken with a Leica DM LB trinocular microscope, combined with a Leica DC 300 F video-camera; images and micro-metric scales were captured on the micro-computer, under the same optical conditions. In order to analyze tissues, 10 pictures were obtained for each analyzed treatment; they were submitted to the image analysis program Image Tool 3.0, which enabled to measure in micrometers the thickness of epidermis, bulliform cells and the total leaf thickness.

The obtained results were submitted to analysis of variance by F test; treatment averages were compared by Tukey's test at 5% probability ($p<0.05$).

## RESULTS AND DISCUSSION

*Urochloa decumbens* leaves coming from the used treatments presented uniseriate epidermis, homogeneous mesophyll, with radiated distribution of the parenchymal cells around the vascular bundles (Figure 1). This kind of organization is called Kranz Anatomy, and it is typical of C4 plants. The adaxial epidermis presents bulliform cells, or motor cells, which are responsible for the closing of leaves during the hottest periods of the day. Vascular bundles are collateral and are present in different sizes (Figure 1).




Buli = bulliform epidermic cells; Epi Ada = adaxial epidermis cells; Meso = homogeneous mesophyll; VB = vascular bundle.

***Figure 1*** - A-H – Cross sections of *Urochloa decumbens* leaves submitted to water potentials of -1.5 MPa (A-B), -0.07 MPa (C-D) e -0.03 MPa (E-H), with sethoxydim treatment (B, D, F, H) and without sethoxydim treatment (A, C, E, G). G H - leaves that presented high anatomical damage rate.

It is possible to notice that the thickness of the epidermis and bulliform cells and the total thickness of the leaves from plants that were submitted to the -0.03 MPa minimum water potential in soil were influenced by the applied herbicide, since they presented a limitation in the growth of epidermis and bulliform cells and in the total thickness of the leaves (Figure 1 and Table 1), compared to treatments with the same water potential, but with no herbicide application. Probably, these alterations may be due to the herbicide action, since plants were not submitted to stress; thus it was possible to observe the effectiveness of the product with the alteration of leaf development. This herbicide acts on the ACCase enzyme (Vidal and Merotto Jr., 2001), reducing plants' capacity to produce malonyl-coA; this results in impeding the formation of fat acids and, consequently, of lipids (Gronwald, 1991), which are essential constituents of cells' and organelles' plasma membranes (Ovejero et al., 2008).

On the other hand, in treatments where plants were submitted to lower potentials of groundwater (-0.07 MPa and -1.5 MPa) with the application of herbicides, there was no significant difference in relation to the size change in the epidermis and in the total leaf thickness, compared

***Table 1*** **-** Average thickness values (mm) of epidermis, bulliform cells of the epidermis and total thickness of leaves from *Urochloa decumbens* plants submitted to water potentials of -0.03, 0.07 and -1.5 MPa (13%, 10% and 8% of soil moisture) with no herbicide application and after 21 days from sethoxydim application

| Water Management (MPa) | Herbicide application | | | | | |
|---|---|---|---|---|---|---|
| | No | Yes | No | Yes | No | Yes |
| | Bulliform cell thickness | | Epidermis thickness | | Total leaf thickness | |
| -0.03 | 66.51 a A | 37.84 b B | 7.81 a A | 4.94 b B | 176.16 b A | 119.85 b B |
| -0.07 | 58.67 a A | 64.10 a A | 7.42 a A | 8.58 a A | 185.49 b B | 223.31 a A |
| -1.5 | 73.00 a A | 65.41 a A | 8.96 a A | 7.76 a A | 224.35 a A | 203.36 a A |
| $F_{\text{Water Management}}$ (M) | 7.011** | | 7.339** | | 19.906** | |
| $F_{\text{herbicide}}$ (H) | 7.656** | | 4.620* | | $2.041^{ns}$ | |
| F (M) x (H) | 7.152** | | 6.754** | | 8.877** | |
| VC (%) | 28.9 | | 28.2 | | 23.2 | |

Averages followed by the same letter, lowercase in the column and capital letter on the line, do not statistically differ among themselves by Tukey's test (P<0.05); ** Significant at 1% probability; * Significant at 5% probability; ns - not significant.

with treatments with the same water potential but with no herbicide application (Table 1). Also, it is possible to observe that the total leaf thickness submitted to a 0.07 MPa potential with herbicide application increased, in relation to the total leaf thickness with no application under the same water potential (Table 1).

Probably, in these treatments the herbicide action was not the main cause of anatomical changes, since, according to Taiz and Zeiger (2004), high levels of water stress may stimulate natural defense mechanisms, such as stomata closing, cell wall adjustment, production of smaller leaves and reduction of the leaf area, thus hindering the absorption of herbicides by the plant.

It is possible to notice that the total thickness of the epidermis was 27.4% bigger in plants submitted to severe water stress (-1.5 MPa), in relation to those without stress (-0.03 MPa) and with no herbicide application. However, the epidermis and bulliform cell thickness was, on average, 65.4% and 71.2% higher in plants with water stress and herbicide application, respectively. Santos et al. (2013), also verified anatomical differences in *Urochloa ruziziensis* plants during the rainy and dry season, and the area of bulliform cells was 17% higher on plant leaves during the dry season.

In treatments with no water stress (-0.03 MPa), bulliform cell and epidermis thickness was reduced with the herbicide application, supporting Costa et al. (2011), who verified that the thickness of *Eichhornia crassipes* (water hyacinth) leaves was reduced with the application of 2.4-D herbicide; Ferreira et al. (2007), also observed less thick epidermis in sugarcane clones more sensitive to the application of herbicides, demonstrating that a similar behavior is verified even with the application of products with different action mechanisms.

In Table 2, the results of day 14 and 28 after herbicide application are presented. On day 14 DAA, higher phytotoxicity was observed in the treatment with no water stress (-0.03 MPa of soil moisture). With -0.07 and -1.5 MPa managements, control effectiveness was 55% on average, not differing between the managements. On day 28 DAA, with the application of the recommended dose, sethoxydim obtained more than 90% control on plants with no water stress. In the -0.07 MPa water management, control was satisfactory: 84% on average. There was less control on plants kept under severe water stress (-1.5 MPa), with a maximum 78% control, damaging the herbicide effectiveness and making the application ineffective.

The application of sethoxydim was also not effective in applications on southern sandbur plants (*Cenchrus echinatus*) under water stress, reaching an average control of 66%, as reported by Pereira et al. (2015a) and Klar et al. (2015), regardless of the stage (4-6 leaves and 2-3 tillers). The Indian goosegrass species (*Eleusine indica*), when submitted to a high level of water stress, also had less herbicide effectiveness, as demonstrated in a study conducted by Pereira et al. (2015b), as well as the morning glory species (*Ipomoea grandifolia*), who had its control damaged with the application of chlorimuron-ethyl and imazethapyr, when under water deficit conditions (Vitorino and Martins, 2012).

***Table 2*** - Phytotoxicity (%) of *Urochloa decumbens* plants submitted to different water managements with and without sethoxydim on day 14 and 28 after application and dry mass (g)

| Water Management (MPa) | Herbicide application | | | | | |
|---|---|---|---|---|---|---|
| | No | Yes | No | Yes | No | Yes |
| | Phytotoxicity 14 DAA | | Phytotoxicity 28 DAA | | Dry mass (g) | |
| -0.03 | 0.00 B | 40.00 b A | 0.00 B | 93.50 a A | 0.81 a A | 0.12 ab B |
| -0.07 | 0.00 B | 56.30 a A | 0.00 B | 84.00 b A | 0.58 b A | 0.16 a B |
| -1.5 | 0.00 B | 54.50 a A | 0.00 B | 78.30 b A | 0.17 c A | 0.08 b B |
| $F_{\text{Water Management}}$ (M) | $2.672^{ns}$ | | 4.665** | | 133.634** | |
| $F_{\text{herbicide}}$ (H) | 254.450** | | 1714.684** | | 539.482** | |
| F (M) x (H) | $2.672^{ns}$ | | 4.665** | | 98.404** | |
| VC (%) | 30.7 | | 11.8 | | 13.2 | |

Averages followed by the same letter, lowercase in the column and capital letter on the line, do not statistically differ among themselves by Tukey's test (P<0.05); ** Significant at 1% probability; * Significant at 5% probability; ns - not significant.

Considering the obtained results, it is possible to deduce that *U. decumbens* plants under 1.5 MPa water stress suffered less injuries with the application of the herbicide, resulting in ineffective control. On the other hand, the control provided by the herbicide on plants kept in soil with -0.03 and -0.07 MPa minimum tensions was similar and higher. This fact may be related to plants' responses to the water deficit, which, according to Taiz and Zeiger (2004), consist in a decrease of the leaf area production, stomata closing, senescence acceleration and leaf abscission, damaging the absorption of the applied products.

According to the results of the anatomical analyses, the higher total thickness of leaves, epidermis and bulliform cells was observed on plants submitted to water stress (Table 1), which may have prevented the herbicide absorption, which was one of the responsible causes for the lower phytotoxicity (Sinoit and Kramer, 1976; Muzik, 1976).

Plants with no herbicide application, submitted to a soil with minimum tension of 0.03 MPa, obtained the best dry mass results, according to Table 2. There was a 28% and 85% reduction, compared to the mass of plants submitted to a 0.07 MPa and -1.5 Mpa water management, respectively. This result may be one of the responsible factors for the higher phytotoxicity observed in plants with water restriction, due, for example, to the larger leaf area, which may have provided higher contact of the product with them. With the application of sethoxydim, it was possible to notice a 33.3% reduction in the dry mass of plants with severe water stress (-1.5 MPa), when compared to the one of plants with no stress (-0.03 MPa). However, on plants submitted to -0.07 MPa water management, there was no reduction, but a 30% mass increase, on average.

These results are supported by a study conducted by Pereira et al. (2015a), who observed that sethoxydim applied on southern sandbur plants caused a 90% dry mass reduction on plants kept with no water stress, in relation to the dry mass of plants with no herbicide application. These authors also affirm that the lowest dry masses obtained in plants under severe water stress are not related to the effect of the applied herbicides, but to the plants' morphological conditions, since they develop mechanisms of drought resistance, according to Taiz and Zeiger (2004).

According to Appezzato-da-Glória and Carmello-Guerreiro (2006), the increase in the epidermis and leaf blade thickness is related to the increase in the plant physical protection, since this characteristic is associated to a response to different kinds of stress. This response was also observed in this study, since an increase in thickness of the epidermis, bulliform cells and total leaf thickness when compared to treatments with no water stress (-0.03 MPa) and higher water stress (1.5 MPa), regardless of the herbicide application (Table 1).

On Table 3, it is possible to observe a reduction in all studied parameters with the application of the herbicide, when plants are not submitted to water stress. The stomatal conductance was negatively influenced by the stress increase; on an average, it was 58% lower in relation to plants with no stress, regardless of the herbicide application.

Transpiration and the photosynthetic rate also followed these results, decreasing as stress increased. These factors caused an increase in the leaf temperature, mainly transpiration,

***Table 3*** - Stomatal conductance (mmol $m^{-2} s^{-1}$), transpiration (mol H2O $m^{-2} s^{-1}$), photosynthetic rate (mmol $m^{-2} s^{-1}$) and difference between foliar and room temperature ($^{o}$C) of *Urochloa decumbens* plants submitted to different water managements with and without sethoxydim, on day 14 after application

| Water Management (MPa) | Herbicide application | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No | Yes | No | Yes | No | Yes | No | Yes |
| | Stomatal Conductance | | Transpiration | | Photosynthetic Rate | | Leaf T - room T | |
| -0.03 | 0.059 a A | 0.034 a B | 2.47 b | 2.32 a | 3.51 a A | 1.36 a B | 4.56 a | 5.32 a |
| -0.07 | 0.039 b A | 0.018 b B | 1.69 b | 1.18 b | 2.16 b A | 1.24 ab B | 6.27 ab | 7.67 a |
| -1.5 | 0.025 b A | 0.014 b A | 1.05 a | 0.77 b | 1.50 b A | 0.59 b B | 8.21 b | 7.80 a |
| $F_{\text{Water Management}}$ (M) | 26.403** | | 36.650** | | 31.076** | | 9.401** | |
| $F_{\text{herbicide}}$ (H) | 38.333** | | 4.781$^{ns}$ | | 85.361** | | 1.000$^{ns}$ | |
| F (M) x (H) | 1.846$^{ns}$ | | 0.505$^{ns}$ | | 8.171* | | 0.820$^{ns}$ | |
| VC (%) | 16.8 | | 15.7 | | 14.4 | | 15.3 | |

Averages followed by the same letter, lowercase in the column and capital letter on the line, do not statistically differ among themselves by Tukey's test (P<0.05); ** Significant at 1% probability; * Significant at 5% probability; ns - not significant.

since it works as a "cooling" effect on the plant (Taiz and Zeiger, 2004). In plants under water stress, it is possible to notice bigger differences between leaf and room temperature; it even gets to be 80% higher in plants at -1.5 MPa with no herbicide application and up to 46.6% with the application, in relation to plants kept at -0.03 MPa (Table 3). It is possible to deduce that the application of the herbicide causes reductions in the physiological parameters, regardless of the water management.

The same behavior was observed in other species, as reported by Pereira et al. (2015b), who observed higher rates in Indian goosegrass plants with no water stress and higher temperatures in plants under stress.

Water deficiency in fodder plants intervenes in the morphology and physiology, causing a decrease in their survival (Mattos et al., 2005). According to these authors, water stress occurs when the transpiration is higher than the absorption and transportation of the water in the plant; the low water availability causes dehydration, reducing growth because it reduces photosynthesis and leaf expansion rates.

These physiological factors also interfere in the anatomical parameters, since stressed plants develop structures of drought adaptation, such as stomata closing, cuticle thickening, adjustment of the cell wall, production of smaller leaves, reduction of the leaf area and increase in the root density; some of these factors are important in the herbicide absorption and the control effectiveness (Taiz and Zeiger, 2004).

In light of this, it is possible to conclude that the water stress increase caused an increase in the thickness of the epidermis, bulliform cells, and the leaf thickness with no herbicide application. In treatments with the application of sethoxydim, the observed alterations were: limitation in the growth of the epidermis cells, bulliform cells and total leaf thickness. Effectiveness in controlling plants was damaged by the water stress, as well as the physiological parameters.

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