Advances in Weed Science
formerly Planta Daninha

RESEARCH ARTICLE

Morphoanatomic characterization of *Gomphrena perennis* and *Gomphrena pulchella* leaves

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Abstract: Background: *Gomphrena perennis* (Gpe) and *G. pulchella* (Gpu) are perennial glyphosate-tolerant amaranthaceous weeds of central and northern Argentina where glyphosate-resistant soybeans are grown with no-till. The study of weeds leaves morphoanatomy is important to understand environmental adaptation and could be used to explain herbicide absorption. Leaf surfaces of *Gomphrena* species were previously described for phylogenetic studies, and morphoanatomy of two *Gomphrena perennis* populations was characterized for glyphosate tolerance. There are no reports on comparative studies of Gpe and Gpu. We hypothesize that these two species differ in their morpho anatomical characteristics.

Objective: The aim of this research was to characterize and compare their leaf morphoanatomy throughout their life cycle.

Methods: Fully developed leaves of the upper middle third were extracted from plants grown in pots in a greenhouse at three phenological stages: eight true leaves (S1), beginning of branching (S2), and full flowering (S3). In both leaf sides, densities of epidermal cells, stomata and trichomes, and ostiole lengths were quantified. In main and secondary bundle anatomic parameters were measured: thickness of total blade, upper and lower epidermis, and mesophyll, and sheath length.

Results: Both species are amphiestomatic. Gpu had higher stomata densities in lower epidermis than Gpe at S1. Gpu had higher trichome densities than Gpe, whereas the latter presented higher values in anatomic variables mainly in secondary bundle (total blade and total mesophyll) at all stages.

Conclusions: There were no marked differences in the morphoanatomy between the two species throughout their life cycle.

Keywords: Lámina total; Epidermis; Mesófilo; Estomas; Tricomas

Journal Information:
ISSN - 2675-9462
Website: http://awsjournal.org
Journal of the Brazilian Weed Science Society

How to cite: Ochoa MC, Catan A, Targa MG, Fraño A, Caro R, Chaila S. Morphoanatomic characterization of *Gomphrena perennis* and *Gomphrena pulchella* leaves. Adv Weed Sci. 2023;41:e020202203. https://doi.org/10.51694/AdvWeedSci/2023;41:00023

Approved by:
Editor in Chief: Anderson Luis Nunes
Associate Editor: Luis Antonio de Avila

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Received: June 18, 2020
Approved: September 20, 2022

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1. Introduction

The genus *Gomphrena* is one of the largest in the family Amaranthaceae, including approximately 120 species (Bena, 2017), distributed mainly in South America (Mussury et al., 2006), Australia and the Indo-Malayan region (Oliveira, 2018) with predominance in the Central Andes of Argentina and Bolivia, and in the Cerrado habitats in Brazil (Bena, 2017). According to Borsch et al. (2001) cit. by Carvalho (2005), the Amaranthaceae family is dominant in arid and semiarid ecosystems due to morphological and anatomical characteristics that allow them to survive in unfavorable environments: C4 photosynthetic metabolisms (Oliveira, 2018; Carvalho et al., 2015), specialized roots systems like xylopodium, dense pubescence in aerial parts, stems and leaves senescence in dry seasons, and thick cuticles (Carvalho, 2015).

In Argentina, 22 species of *Gomphrena* grow in temperate and warm subtropical areas, ranging from the northern part of the country to northern Patagonia, with a great diversity of habitats (Acosta, 2012). *Gomphrena perennis* L. var. *perennis* and *G. pulchella* Mart. ssp. *albisericea* (E. Holzh.) Pedersen, are two perennial species, considered glyphosate tolerant weeds whose increase in central and northern Argentina is attributed to the use of glyphosate resistant soybean cultivars in direct sowing (Rainero, 2008; Olea, 2013). *G. perennis* L. is an erect plant up to 1 m high. It has cylindrical stems somewhat thickened at the nodes, striated, and pubescent. Leaves are opposite, lanceolate, ovate-lanceolate, with acute apex and short petiole, few hairs in upper surface and very pubescent on the underside. It has globose inflorescences, single or branched, with small, yellowish-white hermaphrodite flowers. The fruit is a small utricle containing one seed that serves as a propagation organ (Burkart, 1987; Acosta et al., 2018).

G. pulchella Mart. is an erect plant, with deep taproot, stems 30-50 cm high, hairy, and with a woody base. Leaves are opposite, lanceolate, mucronate, with short petiole, and densely pubescent at the back. Inflorescences in dense round clusters of pink-purple colored flowers on long peduncles. The fruit is a one-seeded utricle (Carrizo e Isasmendi, 1998).

Leaves anatomical characteristics are useful to infer adaptations to a specific environment, due to their relationship with functional parameters like photosynthesis

(Carvalho et al., 2015). Presence of stomata within a species can be modified by light intensity, leaf thickness, photosynthetic capacity, and stomatal conductance. Stomata in abaxial leaf side favor plants growing in habitats with low relative humidity and high temperatures, by reducing water vapor losses, because of lower temperatures. On the other hand, stomata in both sides allow easier CO_2 diffusion, and this is characteristic of plants with high photosynthetic capacity growing in environments with elevated luminosity (Carvalho et al., 2015). Furthermore, morphological and anatomical characteristics of leaves can affect herbicide absorption, by exclusion from the leaf area, depending on number and size of stomata and trichomes in the leaves (Carbone, 2015). Under water deficit conditions, an increase in cuticle thickness can difficult herbicides absorption (Dall´Armellina, Zimdahl, 1989).

Morphoanatomical characteristics of epidermis, wax deposits, and trichome and stomata densities behave as physical or mechanical barriers for the absorption and penetration of glyphosate (Acosta et al., 2018). Hence, knowledge of morpho anatomical characteristics of leaves is a useful tool to evaluate plant adaptability to environments and for weed management in crops.

The aim of this research was to characterize the morpho anatomy of the leaves of these two weed species at three phenological stages.

2. Materials and Methods

The trial was conducted in the locality of El Zanjón (27°46'60''S and 64°16'00'' W). Seeds *G. pulchella* were collected at Quimili, *Departamento Moreno* (27°38'46'' S 62°24'47'' W), and seeds of *G. perennis* were collected at Bandera, *Departamento Belgrano* (28°54'10'' S and 62°16'00'' W). Seeds of both species were collected in 2015. Seeds were sown in multicell trays and at the stage of two true leaves were transplanted to pots with soil (sandy loam textured soil, pH: 7.1; %MO: 2.2; CE 0.28 dSm^{-1}). Twenty pots were established for each sampling of the following phenological stages: S1 (early vegetative-eight true leaves), S2 (late vegetative-beginning branching), and S3 (reproductive-full flowering). The minimum and maximum air temperatures were registered with data logger. For photoperiod, data from National Institute of Agricultural Technology – INTA nearest experiment station were used. At each stage, a pool of fully developed leaves from the middle third of the plant was sampled (Catan et al., 2017). After collection, samples were fixed with a solution of ethanol and acetic acid in the ratio 3:1 (Carnoy). Dates of sowing and sample collection, data on temperatures and daylength are included in Table 1.

To obtain upper and lower epidermis, leaf blades were digested in aqueous 50% sodium hypochlorite, then washed repeatedly with distilled water, and clarified with chloral hydrate 5% aqueous solution (Johansen, 1940). A total of 5 samples of each upper and lower epidermis of individual leaves were mounted with a solution of gelatin-glycerin in slides and coverslips (Johansen, 1940). From each holder glass, 6 random fields were selected for observations, readings, and counting. A total of 30 microscopic fields were read for each epidermis and treatment. The following parameters were observed and quantified: epidermal cell densities (EpCed), stomatal densities (Sd), trichomes densities (Td), and ostioles lengths (OL). All observations and measurements were made with a microscope with image digitizer Motic BA210 Digital (Motic China Group., Ltd. (China).

To observe cross-sections of Blades, the Dizeo de Strittmatter (2000) technique was followed, to prepare 5 slides, 5 paraffin blocks from each species and stage were obtained. Cuttings were made with a rotary microtome at 12 mm thickness. Sections were stained with safranine-fast green. A total of 30 fields were read for each species and stage. Observations and quantifications were made on the following parameters: thickness of total blade (BT), upper and lower epidermis (UEp and LEp), mesophyll (MT), sheath length of secondary bundle (SB2), and collenchyma (C). All data were analyzed with a t-test for independent samples (Di Rienzo et al., 2017).

3. Results and Discussion

a. Epidermic variables

Values of morphological characteristics in adaxial and abaxial leaf sides at different phenological stages, can be seen in Table 2. The only difference in epidermal cell densities, was found in upper epidermis at S1, where Gpe had higher values than Gpu (359.51 and 295.80 cells mm^{-2}).

Table 1 - Dates of sowing, emergence, and leaves collection in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Temperatures and photoperiod at sampling dates

	Sowing	Emergence	S1: 8 true leaves	S2: beginning branching	S3: full flowering
	01/15/2018	01/17/2018	02/06/2018	02/20/2018	03/28/2018
T° max	31.8	28.8	40	36.4	31.9
T° min	16.3	20.7	19.4	18.3	10.6
T° mean	24.1	24.8	29.7	27.4	21.3
Photoperiod	13.59	13.56	13.17	12.83	11.82

Table 2 - Upper and lower epidermis (UEp, LEp) epidermal cells densities (Ced), stomatal densities (Sd), ostiole lengths (OL), and trichome densities (Td) evaluated at stages S1, S2, and S3 in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Densities and length values expressed in mm^{-2} and µm, respectively

Variable	S1		S2		S3	
	Gpe	Gpu	Gpe	Gpu	Gpe	Gpu
UEp-Ced	359.51a	295.80b	237.53a	246.42a	279.01a	317.04a
LEp-Ced	208.89a	380.74a	232.59a	246.91a	275.56a	289.88a
UEp-Sd	106.67a	105.68a	75.56a	73.09a	97.78a	101.73a
LEp-Sd	64.20a	112.59b	66.17a	72.59a	76.54a	80.00a
UEpOL	11.71a	12.30a	14.17a	15.53b	13.08a	12.77a
LEpOL	12.71a	12.50a	14.67a	15.70b	12.55a	12.37a
UEpTd	5.05a	9.27b	5.66a	7.03b	9.08a	9.33a
LEp-Td	14.86a	15.08a	8.84a	9.14a	5.96a	10.95b

For each variable and phenological stages, means followed by different letters, differ by the t-test at p≤0.05.

Gpu had higher stomata densities in lower epidermis than Gpe at S1 (112.59 and 64.20 stomata mm^{-2}, respectively). Both species are amphiestomatic (having stomata in the two leaf sides), like other species of *Gomphrena* such as *G. arborescens* (Carvalho, Ribeiro, 2005), *G. pohlii, G. virgate,* and *G. globosa* (Carvalho et al., 2010).

Stomatal densities (stomata mm^{-2}) in both epidermises to Gpu and Gpe were higher than those reported by Carvalho and Ribeiro (2005) in *G. arborescens* (44.47 and 40.31 in upper and lower epidermis, respectively). However, Frank de Carvalho et al. (2010) studying *Gomphrena* adult species from Cerrado in Brazil determined higher stomatal densities in *G. arborescens, G. pohlii* and *G. globosa* in upper epidermis (138, 136, and 137 stomata mm^{-2}) and lower epidermis (140, 126 and 113 stomata mm^{-2}). In *G. virgata* they found lower values for upper epidermis (32 stomata mm^{-2}) and lower epidermis (45 stomata mm^{-2}), due to higher stomata sizes. Large stomata in low densities is usually associated to adaptation to drought and high luminosity conditions (Carvalho, Ribeiro, 2005).

Species from Cerrado have amphiestomatic leaves, intermediate leaf thickness (100-500 µm), and similar stomatal densities in both leaf surfaces. These are considered an adaptation to increase stomatal conductance, associated to C4 species with high photosynthetic rates (Carvalho et al., 2010). In our trials, where plants were grown without water restrictions, stomatal densities were higher (Table 2). Stomatal density values were similar for both species, and did not show differences in UEp-Sd in the stages evaluated; and were generally lower in LEp, except for Gpu in S1. Values are intermediate between those determined by Carvalho et al. (2010) and those of Carbone (2015), who, studying stomatal densities of both leaf sides in biotypes of Gpe growing in field conditions, found lower values in plants in Santiago del Estero (40 and 38 stomata mm^{-2}) as compared to those in Córdoba (54 and 36 stomata mm^{-2}). Carbone (2015) concluded that this was an adaptive response to avoid water losses.

Acosta et al. (2018) state that lower stomatal density in adaxial side determines the low sensitivity of *G. perennis* to glyphosate. Likewide, Procopio et al. (2003) in weed species like *Galinsoga parviflora*, *Conyza bonaeriensis* and *Ipomoea cairica*, considered a potential barrier to penetration of herbicides applied to the canopy, the low stomatal densities in the adaxial side, that were of (48, 42 and 47 stomata mm^{-2}) in the adaxial side and of (156, 162 and 143 stomata mm^{-2}) in the abaxial side.

Ulguin et al. (2017), in studies on *Eleusine indica*, found that characteristics of surface and leaf anatomy may affect the effectiveness of glyphosate absorption by weeds. Features such as composition of the cuticle, number of stomata, and trichomes interfere with the foliar absorption of herbicides and may indicate auxiliary resistance mechanisms. Thus, weeds anatomy characteristics may influence resistance to herbicides, and therefore their determination is important.

Weeds in general are amphiestomatic and, in most of them, higher stomatal densities occur in the abaxial leaf side (Ferreira et al., 2002). Amphiestomatic leaves are considered an adaptation to increase stomatal conductance, which associated with high photosynthetic rates favor accumulation of CO_2 in periods of water availability. Besides, it allows them to successfully establish in environments with high solar radiation (Carvalho, Ribeiro, 2005).

Gpu had higher ostioles lengths than Gpe in both leaf sides at S2 (15.53 µm UEpOL, and 15.70 µm LEpOL).

Also, the two species under study had trichomes at each leaf side, and this could be a barrier to herbicide penetration, according to Carbone (2015). Trichome densities were higher than Gpe in upper epidermis for Gpu at S1 and S2 (9.27 and 7.03 trichomes mm^{-2}, respectively), and higher in lower epidermis at S3 (10.95 trichomes mm^{-2}) (Figure 1a and 1b). Presence of trichomes

in both leaf sides would indicate adaptation to dry environments and may prevent herbicides from reaching epidermis (Ferreira et al., 2002). Trichome densities (trichomes mm^{-2}) found at S3 in Gpe (9.08 and 5.96 trichomes mm^{-2}) and Gpu (9.33 and 10.95 trichomes mm^{-2}), were similar to densities in *G. arborescens* (5 and 5 trichomes mm^{-2}) and *G. pohlii* (5.9 and 6.22 trichomes mm^{-2}), lower than densities in *G. globosa* (16.5 and 28.05 trichomes mm^{-2}), and higher than those of *G. virgata* (2.6 and 1.4 trichomes mm^{-2}), in adaxial and abaxial sides, respectively (Carvalho et al., 2010). Carbone (2015), working with *G. perennis* biotypes, determined densities in upper and lower epidermis of 23.9-31.9 and 40-50 trichomes mm^{-2}, respectively, in plants collected in rainfed in Santiago del Estero, whereas in those collected in Córdoba, values were of 7.5-11.5 and 8.3-11 trichomes mm^{-2}. Acosta et al. (2018) and Carbone et al. (2021), state that in Gpe, presence in abundance of trichomes on both leaf sides associated to teicodes, constitute a barrier to chemical control of the species. There are no data on trichome density on Gpu under field conditions, where growing environment could modify trichome number, and thus affect the efficacy of its control.

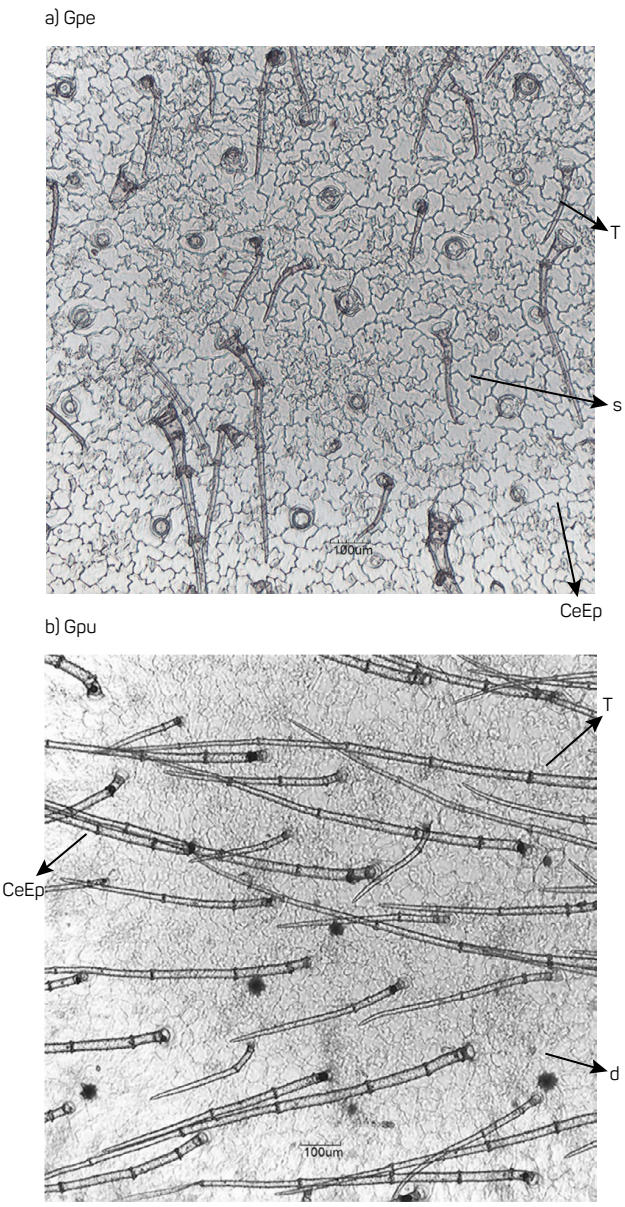

Figure 1 - Lower epidermis 4X of a) Gpe and b) Gpu at S3, CeEp (epidermal cells), T (trichomes), s (stomata) and d (druses).

b. Anatomic variables in main bundle

Thickness values of anatomic variables in main bundle are shown in Table 3. There were significant differences in upper epidermis, with higher values for Gpu at S2 (20.56 µm), and for Gpe at S3 (28.83 µm). Lower epidermis values were higher for Gpu at S2 (18.61 µm). Gpe showed higher values for leaf blade at S1 (572.75 µm) and S2 (642.30 µm). Mean total blade thickness in Amaranthaceae ranges between 100-500 µm. However, Gpe at S1 and S2 was above this range. Both, Gpe and Gpu showed higher thickness than other weed species of the family, such as *Amaranthus deflexus* (149.50 µm), *A. spinosus* (264.34 µm), *Alternanthera tenella* (252.39 µm) (Ferreira et al., 2003), *Amaranthus hybridus* (220 µm) (Silva et al., 2018), and weeds of other families (Ferreira et al., 2002a; 2002b). Upper and Lower Epidermis thickness values in general were similar in the two species studied, as it was observed in *A. spinosus*. However, in *A. deflexus* and *A. tenella* upper epidermis thickness values were higher (17.93 µm and 18.80 µm) than lower epidermis values (13.91 µm and 7.92 µm) (Ferreira et al., 2003).

c. Anatomic variables in secondary bundle

Thickness values of anatomic variables in SB2 are presented in Table 4. Gpe had higher values than Gpu, for lower epidermis at S2 (27.35 µm), for BT (190.22 to

Table 3 - Upper and lower epidermis (UEp and LEp) and thickness of total blade (BT), at stages S1, S2, and S3 in *G. perennis* (Gpe) y *G. pulchella* (Gpu) in main bundle. Values expressed in µm

Variable	S1		S2		S3	
	Gpe	Gpu	Gpe	Gpu	Gpe	Gpu
UEp	15.36a	16.91a	14.75a	20.56b	28.83a	16.68b
LEp	15.31a	13.62a	15.12a	18.61b	16.13a	14.29a
BT	572.75a	454.72b	642.30a	484.54b	417.51a	408.65a

For each variable and phenological stages, means followed by different letters, differ by the t-test at p≤0.05

Table 4 - Upper and lower epidermis (UEp and LEp), thickness of total blade (BT), sheath of secondary bundle (SB2) and total mesophyll (MT) evaluated at stages S1, S2 and S3 in *G. perennis* (Gpe) and *G. pulchella* (Gpu). Values expressed in µm

Variable	S1		S2		S3	
	Gpe	Gpu	Gpe	Gpu	Gpe	Gpu
UEp	31.49a	30.27a	26.93a	24.80a	24.25a	24.40a
LEp	29.33a	25.50a	27.35a	22.41b	21.66a	23.94a
BT	245.94a	198.71b	264.27a	177.29b	190.22a	181.55b
SB2	90.19a	82.74a	110.27a	72.07b	88.85a	77.79b
MT	230.97a	143.38b	208.72a	132.95b	145.23a	134.76b

For each variable and phenological stages, means followed by different letters, differ by the t-test at p≤0.05

264.27 µm) MT (145.23 to 230.97 µm) at all stages, and for leaf sheath at S2 (110.27 µm) and S3 (88.85 µm) (Figure 2a). All values of MT were lower than those of *G. arborescens* (384.40 µm) (Carvalho et al., 2005). MT values of Gpe at S1 (230.97 µm) and S2 (208.72 µm) were higher than those determined by Silva et al. (2018) in *A. hybridus* (160 µm). Gpu had lower values than Gpe at all stages (Table 4 and Figure 2b).

The study of weeds leaf anatomy is useful to identify and discover structures that could affect herbicide absorption (Procopio et al., 2003). Weed management in arid regions poses higher difficulties due to thickening of epidermis, which confers a barrier to herbicide penetration (Dall´Armellina, Zimdahl, 1989). Santos et al. (2014) postulated that higher thickness of anatomic variables in adaxial leaf side, coupled with higher trichome densities and lower stomatal densities, would be barriers to herbicide absorption.

4. Conclusions

Under the conditions of the experiment (without water stress) we found no marked differences in the morphoanatomy of both species, except for higher trichome densities in upper epidermis in G.pulchella at S1 and S2. Gpu had higher stomata densities in lower epidermis than Gpe at S1. Also Gpu had higher ostioles lengths in both leaf sides at S2.

Thickness of mesophyll and total blade were significantly higher in *G. perennis*.

Hairiness in Gpu at early stages could mean a barrier to herbicides

Abscence of marked differences between both species would suggest similar behavior in the efficacy of foliar herbicides applications.

It would be necessary to study morphoanatomical characteristics of the two species under field conditions to determine implications on their management.

Authors' contributions

MCO, RC and SC: conceptualization of the manuscript and development of the methodology. MCO, AC, GT, and AF: data collection and data analysis. MCO and SC: data interpretation. MCO, RC, and SC: writing the original

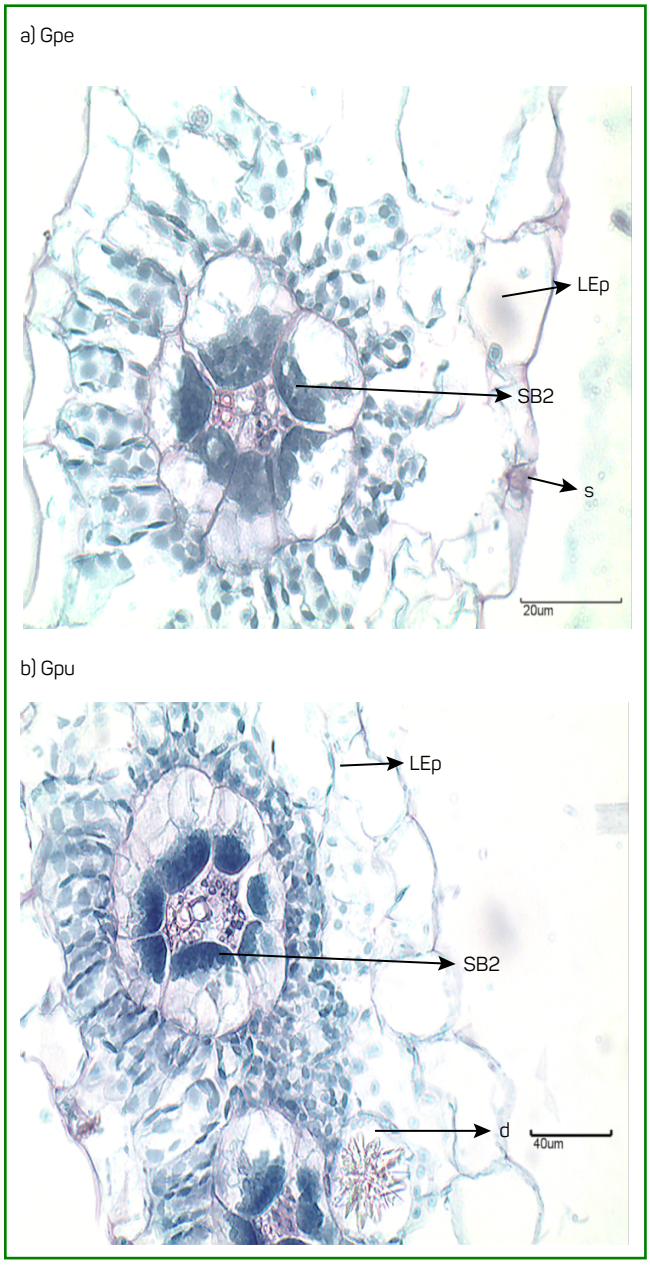

Figure 2 - cross-sections 40X of a) Gpe and b) Gpu at S2, LEp (lower epidermis), s (stomata), d (druse), SB2 (sheath of secondary bundle)

draft of the manuscript. MCO, RC, and GT: writing, review and editing. AC: reading and measurement of anatomic variables in *G. pulchella*. MGT: reading and measurement of anatomic variables in *G. perennis*. AF: preparation and cuttings for laboratory measurements.

Acknowledgements

To Erica Raña for statistical analysis of results, Claudia Degano for reviewing the English translation, and to INDEAS CICyT-UNSE for funding the research.

References

Acosta JM. [Architecture and growth models of glyphosate-tolerant weeds: characterization of possible escape strategies to herbicide action] [tesis]. Santa Fe: Universidad Nacional del Litoral; 2012[access Mar 13, 2020]. Spanish. Available from: http://hdl.handle.net/11185/732

Acosta JM, Carbone A, Perreta MG. [*Gomphrena perennis L*]. In: Fernández OA, Leguizamón ES, Acciaresi HA, Villamil CB, editors. [Weeds and invaders of Argentina: volume III: history and biology]. Bahia Blanca: Universidad Nacional del Sur; 2018[access July 16, 2020]. Spanish. Available from: https://aulavirtual.agro.unlp.edu.ar.

Bena MJ. Typification and nomenclatural notes on twenty-seven names of *Gomphrena* L, (Amaranthaceae) linked to the South American flora. Phytotaxa. 2017;296(2):131-46. Available from: https://doi.org/10.11646/phytotaxa.296.2.3

Burkart A. [Illustrated flora of Entre Rios (Argentina) volume III]. Buenos Aires: Instituto Nacional de Tecnología Agropecuaria; 1987. Spanish.

Carbone A. [Morpho-anatomical characterization of two populations of Gomphrena perennis L. and its possible relationship with sensitivity to the herbicide glyphosate] [thesis]. La Plata: Universidad Nacional de La Plata; 2015[access Jan 22, 2021]. Spanish. Available from: https://doi.org/10.13140/RG.2.1.3408.9689

Carbone AV, Hernández MP, Scardino M. [Efficacy of different phytosanitary products for the control of *Gomphrena perennis* L. (Amaranthaceae)]. Malezas. 2021;5:18-30. Spanish.

Carvalho SMF, Gomes MRA, Silva PIT, Bao SN. Leaf surfaces of *Gomphrena spp,* (Amaranthaceae) from Cerrado biome. Biocell. 2010;34(1):23-35. Available from: https://doi.org/10.32604/biocell.2010.34.023

Carvalho SMF, Graciano-Ribeiro D. [Architecture, anatomy and histochemistry of the leaves of *Gomphrena arborescens* L.f. (Amaranthaceae)]. Acta Bot Bras. 2005;19(2):377-90. Available from: https://doi.org/10.1590/S0102-33062005000200021

Carvalho SMF, Somavilla NS, Marchioretto MS, Bao SN. Linking structure and function. In: Lo YH, Blanco JA, Roy S, editors. Biodiversity in ecosystems. London: IntechOpen; 2015. p. 425-60. Available from: https://doi.org/10.5772/59066

Catan A, Targa Villalba MG, Fraño A, Degano C, Medina Cortez V, La Vega D. [Xeromorphic characters present in the herbaceous vegetation of the semiarid]. In: Albanesi A, Nazareno MA, Domínguez-Núñez JA, Helman S, editors. [Contributions of the Faya for the agricultural and agroindustrial development of the NOA Volume 1]. Santiago del Estero: Universidade Nacional de Santiago del Estero; 2017. p. 1-13. Spanish.

Dall´Armellina A, Zimdahl R. Effect of watering frequency, drought, and glyphosate on growth of field bindweed (*Convolvulus arvensis*). Weed Sci. 1989;37(3):314-8. Available from: https://doi.org/10.1017/S0043174500071988

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. [Infostat version 2017]. Córdova: Universidade Nacional de Córdoba; 2017. Spanish. Available from: htpp: //www,infostat.com.ar

Ferreira EA, Procopio SO, Silva EAM, Silva AA, Rufino RJN. [Anatomical studies of weed leaves: I - *Nicandra physaloides*, *Solanum viarum*, *Solanum americanum* and *Raphanus raphanistrum*]. Planta Daninha. 2002a;20(2):159-67. Portuguese. Available from: https://doi.org/10.1590/S0100-83582002000200001

Ferreira EA, Procopio SO, Silva, EAM, Silva AA, Rufino RJN. [Leaf anatomical studies in weed species widely common in Brazil: IV- *Amaranthus deflexus*, *Amaranthus spinosus*, *Alternanthera tenella* and *Euphorbia heterophylla*]. Planta Daninha. 2003;21(2):263-71. Portuguese. Available from: https://doi.org/10.1590/S0100-83582003000200012

Ferreira EA., Procopio SO, Silva EAM, Silva AA, Rufino RJN. [Leaf anatomical studies in weed species: II - *Bidens pilosa*, *Emilia sonchifolia*, *Ageratum conyzoides* and *Sonchus asper*]. Planta Daninha. 2002b;20(3):327-35. Portuguese. Available from: https://doi.org/10.1590/S0100-83582002000300001

Johansen DA. Plant microtechnique. New York: McGraw-Hill; 1940.

Mussury RM, Scalon SPQ, Gomes AA, Barros SSU. [Characterization of seedlings' morphology and anatomy of *Gomphrena elegans* Mart. (Amaranthaceae)]. Acta Sci Biol Sci. 2006;28(2):87-93. Portuguese. Available from: https://doi.org/10.4025/actascibiolsci.v28i2.1004

Olea I. [Glyphosate-resistant weeds in northwestern Argentina: current situation and management]. In: Ríos A, editor. [Viability of glyphosate in sustainable production systems]. Montevideo: INIA; 2013[access Jan 22, 2021]. p. 51-8. Spanish. Available from: http://www.ainfo.inia.uy/digital/bitstream/item/2551/1/18429080413103109.pdf

Oliveira JE. [Metabolism and localization of fructans in *Gomphrena marginata* Seub. (Amaranthaceae), endemic to Campos Rupestres] [thesis]. São Paulo: Instituto de Botânica; 2018. Portuguese.

Procopio SO, Ferreira EA, Silva EAM, Silva AA, Rufino RJN, Santos JB. [Leaf anatomical studies in weed species widely common in Brazil: III *Galinsoga parviflora*, *Crotalaria incana*, *Conyza bonariensis* and *Ipomoea cairica*]. Planta Daninha. 2003;21(1):1-9. Portuguese. Available from: https://doi.org/10.1590/S0100-83582003000100001

Rainero HP. [Problems of weed management in current production systems]. Manfredi: Instituto Nacional de Tecnología Agropecuaria; 2008. Spanish. Available from: https://www.agroconsultasonline.com.ar/documento.html?op=d&documento_id=221.

Santos FM, Vargas L, Christoffoleti PJ, Agostinetto D., Martin TN, Ruchel Q et al. [Developmental stage and leaf surface reduce the efficiency of chlorimuron-ethyl and glyphosate in *Conyza sumatrensis*]. Planta Daninha. 2014;32(2):361-75. Portuguese. Available from: https://doi.org/10.1590/S0100-83582014000200014

Silva DV, Cabral CM, Ferreira EA, Carvalho FP, Santos JB, Dallabona JLD. Anatomical adaptations to different soil moisture contents in palisade grass and smooth pigweed. Rev Ceres. 2018;65(4):306-13. Available from: https://doi.org/10.1590/0034-737X201865040002

Strittmatter CD. [Johansen's paraffin inclusion technique modification]. Domiguezia. 2000;16(1):55-8. Spanish.

Ulguin AR, Franco JJ, Silva JDG, Agostinetto D, Vargas L. Evaluation of the mechanism responsible for the low-level resistance to glyphosate in goosegrass. Planta Daninha. 2017;35(1):1-11. Available from: https://doi.org/10.1590/S0100-83582017350100005