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Morphoanatomical characterization to differentiate two biotypes of *Paspalum virgatum*

Alana Nascimento Vasco[1], Ricardo Fagundes Marques[2]*, Cristina Filomena Justo[1], Dagoberto Martins[2], Sidnei Roberto de Marchi[1]

[1] Universidade Federal de Mato Grosso, Campus Universitário do Araguaia, Barra do Garças, MT, Brasil E-mail: alananascimentov@hotmail.com; cfjusto23@gmail.com; sidneimarchi.ufmt@gmail.com
[2] Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, SP, Brasil. E-mail: rfmarques94@gmail.com; dagoberto.martins@unesp.br

ABSTRACT: The species *Paspalum virgatum* L. is considered to be a pasture weed that causes great damage to livestock. There are two empirically identified biotypes that differ in leaf blade width. Thus, the objective of this work was to differentiate two biotypes of *P. virgatum* based on the main aspects of leaf morphology and anatomy. Morphological characterization used the leaves of the third node of each biotype with five replications for both of them. For anatomical characterization, the median part of the leaf of the third node was collected. The evaluations consisted in the main leaf morphoanatomical parameters. The biotypes differ in leaf area, leaf blade width, sheath length and diameter, and inflorescence and canopy height. The "narrow" leaf biotype showed lower stomatal density on both sides of the blade. Therefore, leaf blade width is valid as the basis for empirical observation of the difference between biotypes.

Key words: razor grass; stomatal complex; vascular bundle sheath

Caracterização morfoanatômica foliar para diferenciar dois biótipos de *Paspalum virgatum*

RESUMO: A espécie *Paspalum virgatum* L. é considerada uma planta daninha de pastagem que tem causado grandes prejuízos à pecuária. Existem dois biótipos identificados empiricamente, que diferem na largura do limbo foliar. Assim, o objetivo deste trabalho foi diferenciar dois biótipos de *P. virgatum* com base nos principais aspectos da morfologia e anatomia foliar. Para a caracterização morfológica foram utilizadas as folhas do terceiro nó de cada biótipo com cinco repetições para ambos. Para a caracterização anatômica coletou-se a parte mediana da folha do terceiro nó. As avaliações se consistiram nos principais parâmetros morfoanatômicos das folhas. Os biótipos diferem na área foliar, largura do limbo foliar, comprimento e diâmetro da bainha e na altura da inflorescência e do dossel. O biótipo de folha "estreita" apresentou menor densidade estomática em ambas as faces do limbo. Portanto, a observação empírica da diferença entre os biótipos baseada na largura do limbo foliar é válida.

Palavras-chave: capim-navalha; complexo estomático; bainha do feixe vascular

* Ricardo Fagundes Marques - E-mail: rfmarques94@gmail.com (Corresponding author)
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Introduction

Paspalum L. is a large genus of grasses with about 400 native species from predominantly tropical and subtropical regions, whose center of diversity lies mainly in South American countries. Most species of this genus are considered weeds, and few of them have been commercially developed for use as fodder or pasture (Williams et al., 2011).

Invasive grasses are the most complex weeds to be effectively controlled in cultivated pastures because they are morphologically, physiologically and biochemically similar to forage species (Araújo et al., 2020; Martins et al., 2022). For example, the razor grass (*Paspalum virgatum* L.) is considered one of the main invasive pasture plants as a result of its high multiplication capacity and competition with forage grasses (Silva et al., 2017).

The main features that distinguish this species from others of the genus *Paspalum* L. are height and vigorous stalks. The adult plant is cespitose and rhizomatous, and it forms clumps that can reach 1.7 m in height (Maciel et al., 2009). There are reports of its occurrence in pastures in Central and South America, from Mexico and the Caribbean to Argentina and Bolivia (Maciel et al., 2009). In Brazil, it is found in all states of the North and Midwest, as well as in Maranhão, Pernambuco, Minas Gerais, São Paulo, and Paraná (Oliveira et al., 2015).

In the field, the species has two biotypes with visually noticeable differences: one with a "narrow" and one with a "broad" leaf blade. This observation led to the hypothesis that differences in leaf morphology and anatomy between biotypes may be related to a number of variables, such as leaf blade length and width, sheath thickness, leaf orientation, deposition and composition of epicuticular waxes, cuticle thickening, stomatal density and their disposition on leaf surfaces and other cell types, such as bulliform cells (Ruchel et al., 2015).

It is known that the place of origin of weeds can alter the process of plant formation and, thus, influence their growth and development (Mendonça et al., 2014). Some authors have argued that biotypes may originate from variations in the environment in which they survive, resulting in phenotypic changes that ensure individuals' evolutionary and ecological success and, consequently, greater ability to invade and settle in an area (Melo et al., 2017).

Because of the great competition posed by weeds, several studies have been developed in order to provide further insights into the behavior of these biotypes (Marques et al., 2019; Marchi et al., 2020). Given the above, the objective of this research was to differentiate two biotypes of *P. virgatum* based on the main aspects of leaf morphology and anatomy.

Materials and Methods

Panicles of *P. virgatum* containing physiologically mature spikelets were manually collected from pasture areas located in Rondonópolis, MT, Brazil ("Broad" leaf biotype) (16° 44' 53.5" S and 54° 28' 19.3" W Gr), and Cacoal, RO, Brazil ("Narrow" leaf biotype) (11° 05' 53.8" S and 61° 26' 05.1" W Gr). The climate conditions of the sites of origin are shown in Table 1.

The point of physiological maturity was considered the moment when there was natural threshing of spikelets from the inflorescences (Lopes & Franke, 2011). Approximately 1.5 kg of seeds were collected and stored in paper bags. The seeds from the different origins were cleaned using a set of soil sieves with dimensions 8" × 2" to obtain only the dispersal units of the species. Each lot of clean seed was then subjected to a uniform ventilation blower previously regulated with an adequate opening to separate empty seeds from those considered pure.

The experimental phase of this research was conducted in the geographical coordinates of 15° 52' 30" S and 52° 18' 34" W GR, with an altitude of 325 m. The type of climate of the region, according to the Köppen classification, is Aw, characterized as having average temperatures above 27 °C in the warmer months (November to February), average temperatures above 18 °C in the colder months (June to August), and average annual rainfall between 1,000 and 1,500 mm, distributed over two well-defined periods in terms of rainfall: a heavy rain period from October to March, and a marked drought period from April to September (Marchi et al., 2021; Marchi et al., 2022).

The germination and initial growth of seedlings were conducted in a greenhouse. The seeds were sown in trays filled with soil collected in the arable layer of a Humic Dystroferric Red-Yellow Latosol. The chemical and physical characteristics of this soil were: pH in $CaCl_2$ of 4.4; 70.0 g dm^{-3} of organic matter; non-significant values of P resin; V of 9.5%; and K, Ca, Mg and H + AL contents of 0.21, 0.63, 0.22 and 10.0 $cmol_c$ dm^{-3}, respectively; 695 g dm^{-3} sand, 125 g dm^{-3} silt, and 180 g dm^{-3} clay. No amendments were made for soil acidity or fertility.

The soil of the trays remained moist during the experimental period by means of automatic irrigation programmed to dispense an amount of water close to the field capacity of the soil. The average maximum and minimum daily temperatures inside the greenhouse during the experiment period were 36 and 23 °C, respectively.

The plants were transferred to the field 15 days after sowing, in an enclosed area of approximately 30 m^2 in a Dystrophic Red-Yellow Latosol, with the following chemical

Table 1. Climate data [1] of the sites of origin of Paspalum virgatum seeds.

Origin	Climate [2]	Annual mean temperature (°C)			Solar radiation [3]	Annual preciptation (mm)
		Minimum	Mean	Maximum		
Rondonópolis, MT	AW	20.4	26.8	33.2	16.49	1,313
Cacoal, RO	AW	22.4	27.6	32.9	18.92	1,982

[1] Average for the last 10 years. [2] Köppen classification. [3] Solar radiation in W m^{-2} day^{-1}. Source: Agritempo - Sistema de Monitoramento Agrometereológico.

and physical characteristics: pH value in $CaCl_2$ of 4.8; 22.0 g dm^{-3} organic matter; 4.0 mg dm^{-3} P resin; 44.6% V; and K, Ca, Mg and H+AL contents of 0.31, 1.8, 0.6 and 3.4 $cmol_c$ dm^{-3}, respectively; 706 g dm^{-3} sand, 85 g dm^{-3} silt, and 209 g dm^{-3} clay. No corrections were made for soil fertility and acidity.

The seedlings of the biotypes were transplanted at a spacing of 20 cm in rows three meters long, totaling 15 seedlings per biotype. The rows were arranged below ground level in order for the irrigation system to result in slight soaking, providing a favorable environment for growth and development of seedlings. Thinning of other invasive plants in the area was performed manually with the aid of a hoe and a rake as soon as necessary.

The morphological study was performed on 150 days after transplant, a period when the plants were starting the reproductive phase (inflorescence appearance), in order to avoid the assimilates from being destined for seed filling and maturation.

Five plants of each biotype were randomly selected for the analysis. The median leaf of the tiller, located at the third node from the apex to the base, was collected for evaluation of leaf blade length and width and sheath length, width, and diameter. Leaf blade length was measured with a graduated ruler from the apex to the base of the blade, and leaf blade width reading was performed from the middle third of the leaf.

To determine sheath length, measurements were taken from the apex to the base. Because this species has a slightly oval sheath (ellipse), sheath diameter was evaluated in two directions, thus obtaining the values for diameter of largest sheath axis (DBSA) and diameter of smallest sheath axis (DSSA), with the use of a digital caliper.

Total leaf length was determined by summing leaf blade length and sheath length. Inflorescence height was measured from the ground to the height of the last seeds of the spikelet, and plant height was measured from the ground to the last fully expanded or most erect leaves.

Leaf area was estimated using the model proposed by Sousa et al. (2015), in which the triangle area value is summed with the trapezium area value, obtaining according to Equations 1, 2 and 3:

$$A_{\Delta} = \frac{\frac{L_T}{2} \times L_M}{2} \quad (1)$$

$$A_{TRA} = \frac{L_M + L_B}{2} \times \frac{L_T}{2} \quad (2)$$

$$LA = A_{\Delta} + A_{TRA} \quad (3)$$

where: A_{Δ} - Triangle area; A_{TRA} - Trapeze area; LA - Leaf area; L_T - Total leaf blade length; L_M - Median leaf blade width; and, L_B - Leaf blade base width.

The total number of leaves was determined by counting fully expanded leaf units per tiller, and the total number of nodes was determined by counting the tiers of each tiller. The total number of tillers was determined by removing the plants from the soil and counting the tillers that were using the same root system as the main tiller. Internode diameter was selected from the middle part of the tiller and measured with the aid of a caliper.

The anatomical study was developed using a microscope in the laboratory where five repetitions of the broad leaf biotype and five repetitions of the narrow leaf biotype were selected. The third leaf was collected from the apex to the base (vegetative leaf) of the tiller - followed by removal of the middle portion of the leaf blade and the sheath - and stored in a tube with 70% alcohol for later analysis in the laboratory. All sections were manually performed.

Paradermal sections were made on the adaxial and abaxial surfaces of each repetition; five sections were selected for mounting the slides of each surface and each biotype, in a total of 100 slides. For the preparation of such slides, the material obtained was clarified using 2.5 mL of 2.5% commercial sodium hypochlorite and then washed with distilled water. For staining, astra blue and safranin were used in a 9:1 ratio, and 2.0 mL was applied to each watch glass. At the end of the procedure, a drop of glycerine was applied to the slide to place the sections on glycerin, and a cover slip was placed on top of it, sealing the slide with enamel, in order to make them semipermanent.

Paradermal sections were evaluated with an Olympus CX41 optical microscope for number of stomata, stomatal density, number of rows of bulliform cells, number of rows of suberose and silica cells, with a 400-fold magnification. Photographs of these structures were taken using a Nikon Eclipse microscope. In the cross sections, the evaluation consisted of describing the structures; the photographic records were made with a camera.

A micrometer ruler was used in the microscope to obtain the field-of-view radius at 400 X magnification, which was 240 µm, converted to 0.24 mm. Stomatal density was expressed as the number of stomata per mm^2, according to the model of Laboureau et al. (1961). For statistical analysis, data underwent analysis of variance at the level of 5% of significance by the F-test, using the AgroEstat statistical software (Barbosa & Maldonado Jr., 2015).

Results and Discussion

Morphological characteristics

The morphological characteristics of the *P. virgatum* "narrow leaf" biotype showed an erect linear-lanceolate leaf blade with a very sharp, serrated margin, with an average length of 68.6 cm, an average width of 1.28 cm. The "broad" leaf biotype showed a slightly arched linear-lanceolate leaf blade, with a very sharp serrated margin, average length of 76.0 cm, average width of 1.94 cm (Table 2).

No significant contrast was found for leaf blade length (LBW). However, there was significance for the leaf blade

Table 2. Summary of the analysis of variance of the biometric characterization of two biotypes of razor grass (*P. virgatum*).

Biotype	LBL	LBW	SL	DBSA	DSSA	IH	CH
				(cm)			
"Narrow"	68.6	1.28	29.6	0.70	0.39	170.0	123.4
"Broad"	76.0	1.94	35.9	0.75	0.51	122.2	101.8
F (Biotype)	1.87^{NS}	35.10**	5.39*	1.17^{NS}	14.66**	53.33**	5.64*
C.V. (%)	11.8	12.6	13.1	8.4	11.3	7.1	12.8

NS - Non-significant; ** Significant at 1% probability; * Significant at 5% probability. C.V. (%) = coefficient of variation. (LBL) leaf blade length, (LLW) leaf blade width, (SL) sheath length, (DBSA) diameter of smallest sheath axis, (DSSA) diameter of smallest sheath axis, (IH) inflorescence height, (CH) canopy height.

width (LBW) variable (Table 2), thus proving that the visual character as an assumption for differentiating biotypes is valid. It is assumed that this characteristic is directly associated with higher amounts of chlorophyll parenchyma that make up the mesophyll, interfering with the number of photosynthetic pigments and, consequently, resulting in better photosynthetic rates (Lucena et al., 2017).

There was a significant difference for sheath length (SL): an average of 29.6 cm for the "narrow" leaf biotype and 35.9 cm for the "broad" leaf biotype. The diameter of the largest sheath axis (DBSA) was 0.70 cm for the narrow leaf biotype and 0.75 cm for the broad leaf biotype, with no significant difference based on the analysis of variance. There was a significant difference in the diameter of the smallest sheath axis (DSSA) variable, with the narrow leaf biotype averaging 0.39 cm and the broad leaf biotype, 0.51 cm (Table 2).

Inflorescence height (IH) for the narrow leaf biotype averaged 170.0 cm, with a statistical difference from the broad leaf biotype, which averaged 122.2 cm for this variable. There is a significant contrast between the narrow and broad leaf biotypes for the variable plant canopy height (CH), with 123.4 cm and 101.8 cm, respectively (Table 2). For this reason, it is assumed that the "narrow' leaf biotype has the most upright canopy while the leaves of the "broad" leaf biotype have a slight curvature. The fact that the leaves are more erect indicates greater tissue support, which is related to the presence of sclerenchyma fibers and their degree of lignification (Valente et al., 2011).

Leaf area showed that the "narrow" leaf biotype is smaller than the "broad" leaf biotype, with an average of 75.3 and 134.2 cm^2, respectively, with a significant difference. The analysis of variance showed that there was no significant difference between the biotypes for the variables total number of leaves per tiller (NL), total number of nodes (NN), total number of tillers (NT), internode length (IL) and internode diameter (ID) (Table 3).

Anatomical characteristics

Both biotypes are amphistomatic, that is, they present stomata on the abaxial and adaxial surfaces of the leaf blades. The stomata are paracytic and typical of grasses, because they have the shape of dumbbells arranged into two or more rows, with triangular subsidiary cells (Santos et al., 2013). The "broad" leaf biotype has apparently smaller stomata and some deformation in comparison to the narrow "leaf" biotype, a qualitative characteristic shown in Figure 1.

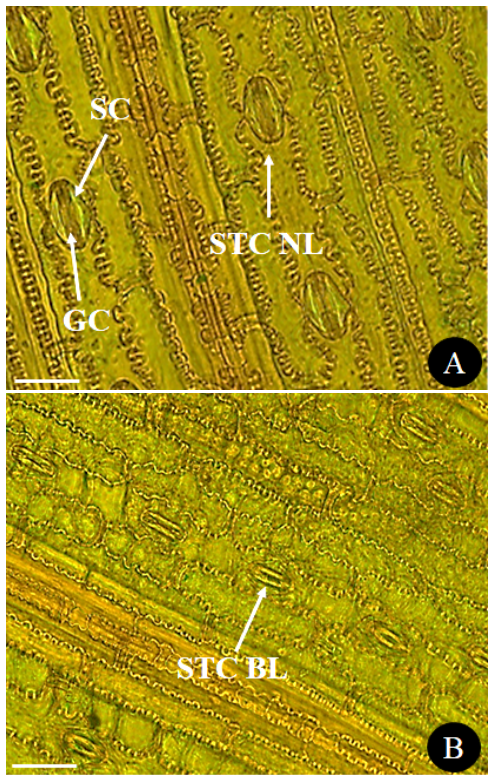

Figure 1. Paradermal sections of the abaxial surface of the leaf blade of *P. virgatum*. A. STC NL: stomatal complex of the "narrow" leaf; GC: guard cell, SC: subsidiary cell. B. STC BL: stomatal complex of the "broad" leaf. 400x magnification. Staining: Astra blue and safranin (9:1). Bars: 50 µm.

Table 3. Summary of analysis of variance of morphological characteristics of two biotypes of razorgrass (*P. virgatum*).

Biotype	LA (cm^2)	NL	NN	NT	IL	ID
					(cm)	
"Narrow"	75.3	8.2	5.8	3.4	15.0	0.31
"Broad"	134.2	7.4	5.4	2.8	16.0	0.33
F (Biotype)	2.45*	1.28^{NS}	0.53^{NS}	1.80^{NS}	3.33^{NS}	0.20^{NS}
C.V. (%)	3.55	14.3	15.5	22.8	5.6	27.2

NS - Non-significant; * Significant at 5% probability. C.V. (%) = coefficient of variation. (LA) leaf area, (NL) number of leaves, (NN) number of nodes, (NT) number of tillers, (IL) internode length, (ID) internode diameter.

The presence of small stomata with some deformation may be directly related to the formation environment of the "broad" leaf biotype, as this behavior is an important event in the regulation of gas exchange, since leaves with smaller stomata have higher water use efficiency, since they have smaller stomatal pores, resulting in lesser water loss of water through the transpiration (Schmidt et al., 2017).

In both biotypes, the stomatal rows are arranged into two or more rows between the intercostal zone and the arrangement that has more than one stomatal row, and they are separated by more than one row of long cells (Figure 1).

In the narrow-leaf biotype, the silica cells have a short dumbbell shape and the suberose cells are larger than the silica cells and irregularly shaped. In the broad-leaf biotype, the silica cells are dumbbell-shaped, relatively larger than the suberose cells, while the suberose cells have a shorter, enclosed shape (Figure 2).

In an anatomical description of species of genus *Paspalum* L., Reis et al. (2015) reported that suberose cells may appear solitary or paired, with width greater or similar to length, isolated from one another in all regions or grouped in pairs or more, in costal zones. Silica cells are generally associated with suberose cells, and they are present in the costal and intermediate regions. They have a varied shape, ranging from dumbbell-shaped or nodular, to short, broad and crenated or smooth in the intermediate regions, or other formats, solitary paired or in large rows.

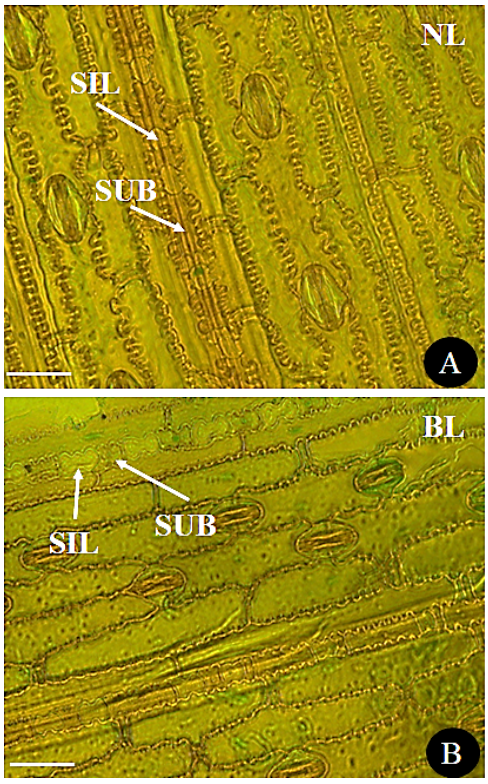

Figure 2. Paradermal sections of the abaxial surface of the leaf blade of *P. virgatum*. A. NL: "narrow" leaf; B. BL: "broad" leaf; SIL: silica cells; SUB: suberose cells; Staining: Astra blue and safranin (9:1). Bars: 50 µm.

The comparison of the number of stomata between the biotypes shows that the "broad" leaf showed a significant difference in comparison to the "narrow" leaf on both leaf surfaces (Table 4). Stomatal density was calculated as the number of stomata per mm^2. The significance of the analysis of variance shows that the "broad" leaf biotype also has higher stomatal density than the "narrow" leaf biotype on both leaf surfaces. Variations in stomatal behavior, both in terms of density and number of stomata, are a very variable characteristics in plants, depending on the environment where they are found (Melo et al., 2007).

Stomata can have a very variable presence and distribution in leaves depending on the environment in which the biotypes exist. The number of stomata per unit area, as well as the positional level of guard cells with respect to the other cells, are such variables that they have little taxonomic value. These variations can occur between biotypes and within each biotype (Galvani et al., 2012). However, this factor is of paramount importance to plants because, according to Lima Júnior et al. (2006), the increase in stomatal density may allow the plant to increase gas exchange and, thus avoiding limiting, photosynthesis under different environmental conditions.

The number of rows of bulliform cells (NCB) ranged from 3 to 4 rows, with a greater number in the narrow leaf biotype; however, the difference between the biotypes was not significant for this variable. The silica and suberose cells occur in rows in the costal zone, between the stomatal rows. The average number of rows of silica and suberose cells on the adaxial (CSSAD) and abaxial (CSSAB) surfaces did not differ statistically between the "narrow" and "broad" leaf biotypes (Table 5).

Table 4. Summary of the analysis of variance of stomatal count of two biotypes of razorgrass (*P. virgatum*).

Biotype	Number of stomata (field observation)		Stomatal density (stomata mm^{-2})	
	Adaxial	Abaxial	Adaxial	Abaxial
"Narrow"	16.3	20.9	90.19	115.82
"Broad"	22.8	24.7	126.21	136.61
F (Biotype)	6.22*	8.46*	6.22*	8.46*
C.V. (%)	21.1	8.9	21.1	8.9

* Significant at 5% probability. C.V. (%) = coefficient of variation.

Table 5. Summary of analysis of variance of the anatomical characteristics of two biotypes of razorgrass (*P. virgatum*).

Biotype	Anatomical features		
	NBC	CSSAB	CSSAD
"Narrow"	4.2	2.2	2.2
"Broad"	3.2	2.2	2.0
F (Biotype)	1.14^{NS}	0.01^{NS}	0.29^{NS}
C.V. (%)	40.1	44.3	28.2

NS - Non-significant. C.V. (%) = coefficient of variation. (NBC) number of rows of bulliform cells, (CSSAB) silica and suberose cells on the abaxial surface, (CSSAD) silica and suberose cells on the adaxial surface.

Conclusions

The species *P. virgatum* presents morphologically different biotypes. The measurements performed confirm the empirical observation for leaf blade width, and it is concluded that this variable is valid for the identification of biotypes.

The Biotypes also differ in the following morphological variables: sheath length and diameter, inflorescence and canopy height, and leaf area.

The broad leaf biotype has less tissue support.

The biotypes differ in stomatal density of both leaf surfaces, with lower values for the "narrow" leaf biotype.

The number of rows of bulliform cells and the average number of rows of silica and suberose cells of the adaxial and abaxial surfaces are similar between the narrow-leaf and broad-leaf biotypes.

Compliance with Ethical Standards

Author contributions: Conceptualization: ANV, CFJ, DG; Data curation: ANV, RFM, CFJ, SRM, DG; Formal analysis: ANV, RFM, CFJ, SRM, DG; Investigation: RFM, SRM; Project administration: ANV, CFJ, DG; Supervision: DG; Validation: DG; Writing - original draft: RFM, SRM; Writing - review & editing: DG.

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