

Resistance of forage grasses to *Blissus pulchellus* Montandon (Hemiptera: Blissidae)¹

Resistência de poáceas forrageiras ao *Blissus pulchellus* Montandon (Hemiptera: Blissidae)

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HIGHLIGHTS:

Chinch bugs cause large production losses that affect agribusiness worldwide.

Compaction and lignification of the parenchyma and sclerenchyma cells of leaf sheaths can confer resistance to bedbugs.

Pest resistant materials are a solution to overcome economic losses.

ABSTRACT: Chinch bugs [*Blissus pulchellus* Montandon (Hemiptera: Blissidae)] suck the phloem from susceptible forage grasses, injecting toxins that destroy plant vessels and compromise the flow of water and nutrients, leading to plant death. Thus, the aim of this study was to assess eight forage grasses for antixenosis resistance to *B. pulchellus* and compare the anatomical characteristics of leaf sheath tissue from resistant and susceptible species/cultivars. Experiments were conducted in a laboratory and greenhouse, using choice and no-choice tests with the following forage grasses: *Urochloa ruziziensis*, *U. humidicola*, *U. brizantha* 'Piatã', *U. brizantha* 'Paiaguás', *U. brizantha* 'Marandu', *Panicum maximum* 'Mombaça', *P. maximum* 'Zuri', and *Andropogon gayanus*. The oviposition results demonstrated that in the choice test there was a change in the stink bug's behavior in relation to grasses four hours after infestation, with *U. humidicola*, *P. maximum* 'Mombaça' and 'Zuri', *U. brizantha* 'Marandu' and *A. gayanus*, proving to be less attractive to insects. It is concluded that *U. humidicola*, *P. maximum* 'Mombaça' and 'Zuri', *U. brizantha* 'Marandu', and *A. gayanus* are less attractive to *B. pulchellus*. *A. gayanus* and *P. maximum* 'Mombaça' and 'Zuri' showed non-preference resistance (antixenosis) to oviposition by *B. pulchellus*. The resistance of *U. humidicola*, *P. maximum* 'Zuri', and *A. gayanus* to *B. pulchellus* may be associated with greater compaction and lignification of the parenchyma and sclerenchyma cells of leaf sheaths.

Key words: antixenosis, damage rating, forage pests, insect pest

RESUMO: Os percevejos-das-gramíneas [*Blissus pulchellus* Montandon (Hemiptera: Blissidae)] sugam a seiva de espécies forrageiras suscetíveis e injetam toxinas que destroem os vasos condutores e dificultam o transporte de água e nutrientes, levando as plantas a morte. Assim, o objetivo deste estudo foi avaliar a resistência por antixenose e tolerância em oito poáceas forrageiras à *B. pulchellus* e comparar as características anatômicas dos tecidos das bainhas foliares de espécies/cultivares resistentes e suscetíveis. Os ensaios foram conduzidos em laboratório e casa de vegetação, em testes com e sem chance de escolha, utilizando-se as forrageiras: *Urochloa ruziziensis*, *U. humidicola*, *U. brizantha* 'Piatã', *U. brizantha* 'Paiaguás', *U. brizantha* 'Marandu', *Panicum maximum* 'Mombaça', *P. maximum* 'Zuri', e *Andropogon gayanus*. Os resultados de oviposição demonstraram que no teste de escolha houve uma mudança no comportamento do percevejo em relação às gramíneas quatro horas após infestação, sendo *U. humidicola*, *P. maximum* 'Mombaça' e 'Zuri', *U. brizantha* 'Marandu' e *A. gayanus* menos atrativos para os insetos. Conclui-se que *U. humidicola*, *P. maximum* 'Mombaça' e 'Zuri', *U. brizantha* 'Marandu', e *A. gayanus* são menos atrativas a *B. pulchellus*. *A. gayanus*, e *P. maximum* 'Mombaça' e 'Zuri' apresentam resistência do tipo não-preferência para oviposição a *B. pulchellus*. A resistência de *U. humidicola*, *P. maximum* 'Zuri', e *A. gayanus* a *B. pulchellus* pode estar associada a maior compactação e lignificação das células dos tecidos parenquimático e esclerenquimático das bainhas foliares.

Palavras-chave: antixenose, nível de dano, pragas de pastagens, inseto-praga

INTRODUCTION

In Brazil, the genera most used for pasture formation are *Urochloa* and *Panicum*. In Roraima it is no different, however, in addition to these, *Andropogon gayanus* stands out, a species widely cultivated in the Savannah areas of the State (Jank et al., 2014). Notably, the lack of information on the management of pests, diseases, and weeds that affect these pastures are factors that contribute to their degradation (Dias-Filho & Andrade, 2019; Feltran-Barbieri & Féres, 2021; Costa et al., 2023). Locally, there are few studies on pest insects associated with pastures. However, many cattle ranchers have reported severe damage caused by these organisms in the region, with negative impacts on forage production. The first report of the occurrence of the Chinch bug (*Blissus pulchellus* - Montandon (Hemiptera: Blissidae)) in Roraima and Brazil was made in 2021 associated with pastures (Fidelis et al., 2021).

Chinch bugs from the genus *Blissus* are known to primarily attack species of the Poaceae family, including several economically important plants such as maize, wheat, sorghum, forage grasses, and turfgrass (Milla-Lewis et al., 2017). To circumvent the economic losses caused by pests, studies have been carried out to find resistant material and understand the resistance mechanisms involved (Vashisth et al., 2022; Zhang et al., 2022).

The selection of plants/varieties resistant to insects by antixenosis and/or antibiosis, can be interpreted by means of insect responses to them. However, studies have been developed seeking the identification and recommendation of materials resistant to the wireworm bug (Simon et al., 2021). Milla-Lewis et al. (2017), cited possible inhibitory morphological characteristics in St. Augustinegrass genotypes resistant to the southern chinch bug. The study aimed to assess eight forage grasses for antixenosis resistance to *Blissus pulchellus* and compare the anatomical characteristics of leaf sheath tissue from resistant and susceptible species/cultivars.

MATERIAL AND METHODS

The study was conducted from June to September 2019, at the headquarters of Embrapa Roraima, in Boa Vista, Roraima state, Brazil (2° 45' 27" N, 60° 43' 52" O). According to Köppen's classification, climate in the region is categorized as wet tropical (Aw), with average annual rainfall, relative humidity and temperature of 1,667 mm, 70% and 27.4 °C, respectively (Araújo et al., 2001).

The initial population of *B. pulchellus* in the laboratory was obtained from insect pairs collected in the field from infested *Urochloa ruziziensis* plants. The insects were individually placed in acrylic Petri dishes (6.0 cm in diameter × 2.0 cm high) lined with moist paper and fed with segments of stem and leaves + sheath from the relevant forage grass species. The plant material was replaced daily with freshly collected plant parts and eggs laid by the females were removed with tweezers, washed in distilled water under voile fabric, counted, dated in spreadsheets and then transferred to different Petri dishes until nymph emergence. The individual nymphs were placed in Petri dishes, fed and monitored until adulthood,

and then sexed to form pairs for use in the experiments. All the Petri dishes were placed in a biochemical oxygen demand (BOD) incubator, with controlled temperature (27 ± 5 °C) and relative humidity ($60 \pm 10\%$), and a 12-hour photoperiod (Cohen, 2018).

Eight forage grass species/cultivars were used, namely *Urochloa ruziziensis* Germain e Evrard, *U. humidicola* (Rendle) Morrone e Zuloaga, *U. brizantha* (Hochst. ex A. Rich.) Stapf 'Piatã', *U. brizantha* 'Paiaguás', *U. brizantha* cv. 'Marandu', *Panicum maximum* Jacq. 'Mombaça', *P. maximum* 'Zuri', and *Andropogon gayanus* Kunth. The seeds were planted in plastic pots (30 dm³) containing soil, sand, and cattle manure as substrate, at a ratio of 1:1:1 (v/v), supplemented with 10 g of N-P-K (10-12-10) and watered daily. Thirty seeds were planted per pot and thinning was performed 10 days after emergence (DAE), leaving only five plants per pot. From planting and throughout testing, all the pots were protected with voile netting fixed to the ceiling of the greenhouse and attached to the floor, to prevent possible natural infestation by chinch bugs and/or other insects.

The choice test was carried out in a BOD incubator under conditions like those used to rear the insects, with the eight previously mentioned grass species arranged in a randomized block design with six replications (arenas). A transparent plastic container with eight tubes inside (one for each forage grass) was placed in each test arena, containing three 25-day-old plants (25 DAE). Infestation was achieved by releasing 50 (25 males and 25 females) recently emerged adult *B. pulchellus* in the center of each of the six arenas. Assessments were performed 2, 4, 8, 16, 32, and 64 hours after infestation (HAI), whereby the number of individuals present on each grass species was counted and their location on the different plant parts (base, sheath, and leaf) recorded. Oviposition was recorded once, at 64 HAI, by counting the number of eggs on the plant base, sheath, and leaves of three plants from each tube.

The no-choice test was conducted in a greenhouse. The eight grasses were arranged in a randomized block design, with four replications. The pots were infested (previously mentioned for forage grass cultivation) at 25 DAE, using eight pairs of recently emerged *B. pulchellus* per plant, totaling 80 insects per pot since each pot contained five plants. This population density was maintained until testing was complete. To that end, insects that died before completion were immediately replaced without considering their sex (due to the difficulty of obtaining the same sex to replace and also the greater difficulty of discovering the sex of insects after they are dead). Each pot was covered with voile netting to prevent *B. pulchellus* individuals from escaping during testing and possible natural infestation by chinch bugs and/or other insects.

Damage was assessed 6, 12, 18, and 24 days after infestation (DAI), based on a visual damage scale adapted from Heng-Moss et al. (2002), who evaluated damages from 1 to 5, whereby 1 = light green coloring on up to 10% of the leaf area, 2 = 11 to 25% of the leaf area light green to yellow, 3 = yellowing on 26 to 50% of the leaf area, 4 = 51 to 75% of the leaf area exhibiting yellowing and necrosis, 5 =

necrosis on more than 75% of the leaf area. Thus, the damage of the grasses was tested based on the visual damage rating scale, whereby R – resistant (damage ≤ 1), MR – moderately resistant ($1 > \text{damage} < 3$), MS – moderately susceptible ($3 \geq \text{damage} < 4$) and S – susceptible (damage ≥ 4) (Baldin et al., 2019).

To verify anatomy of leaf sheath tissue, plants grown under the same conditions used in the no-choice test were evaluated at 50 DAE, without infestation. Sheath segments from fully expanded permanent leaves were cut 1.0 cm below the ligule and 10 cm above the plant base. The leaf segments were cut into cross-sections approximately 10 μm thick and bleached for approximately 4 hours in 50% NaClO solution. For tissue identification, 1% safranin stain solution, which reacts with lignin and turns lignified cells a reddish pink, was used for 5 min. The leaf fragments were washed in distilled water to remove the excess dye, followed by 30s-staining with 1% Alcian blue stain (5:1), which reacts with cellulose and turns cellulose-rich cells blue (Luque et al., 1996). The double stained sections were mounted on slides and analyzed using an optical microscope equipped with a micrometer eyepiece (200 \times magnification) coupled to a digital camera (48 megapixels) to capture the images.

The data were subjected to normality (Shapiro-Wilk) and homogeneity of variance tests (Hartley), and normally distributed homogeneous data were analyzed by the F-test ($p \leq 0.05$). The means of the different forage grass species were compared by the Skott-Knott and Tukey test for plant parts at 0.05 of probability. All statistical analyses were performed in the R program version 4.2.3 (R Core Team, 2023).

RESULTS AND DISCUSSION

In the choice test a change in chinch bug behavior toward the grasses was observed at 4 HAI, with *U. humidicola*, *P. maximum* ‘Mombaça’ and ‘Zuri’, *U. brizantha* ‘Marandu’ and *A. gayanus* proving less attractive to the insects when compared to *U. ruziziensis*, *U. brizantha* ‘Paiaguás’, and *U. brizantha* ‘Piatã’ (Table 1).

Assessments conducted at 8, 16, and 32 HAI also indicated variations in *B. pulchellus* preference between the grasses tested, stabilizing at 64 HAI, where a clear preference was observed for feeding and/or oviposition in *U. brizantha* ‘Paiaguás’, *U. ruziziensis*, *U. brizantha* ‘Piatã’, with an average of two to four times the number of bugs that *P. maximum* ‘Mombaça’, *U. humidicola*, *A. gayanus*, *P. maximum* ‘Zuri’, and *U. brizantha* ‘Marandu’ (Table 1).

Analysis of the oviposition data 64 HAI, (Table 1) demonstrated that *B. pulchellus* were least attracted to *A. gayanus* (0.35 ± 0.16), *P. maximum* ‘Mombaça’ (0.42 ± 0.07), *P. maximum* ‘Zuri’ (0.55 ± 0.08), and *U. humidicola* (0.79 ± 0.49) eggs plant⁻¹, respectively, when compared to *U. brizantha* ‘Paiaguás’, *U. brizantha* ‘Piatã’, and *U. ruziziensis*, which exhibited ≥ 1 egg plant⁻¹ and were therefore preferred.

Youngs et al. (2014) also observed antixenosis or non-preference resistance to chinch bugs in grasses, corroborating the results obtained here. The lesser appeal of *A. gayanus* and *P. maximum* cv. ‘Mombaça’ and ‘Zuri’ for oviposition may be associated with the plant’s morphological characteristics, such as an indumentum, the presence of trichomes, wax or compounds that inhibit egg-laying behavior in *B. pulchellus* females. Milla-Lewis et al. (2017), studied the resistance of St. Augustinegrass genotypes to *B. insularis* and found that cv. ‘FX10’ exhibited significantly lower oviposition when compared with Seville, attributing this response to possible inhibitory morphological characteristics in the former.

With regard to chinch bug distribution, regardless of the number of hours since infestation, there were always more insects on the sheath, followed by the plant base and leaf (Table 1). The number of eggs also declined from the sheath to the leaf, with *B. pulchellus* demonstrating a clear preference for feeding and oviposition on the sheath.

Antixenosis or non-preference factors, such as a trichomes, wax, and leaf length and width, may have an inverse effect on insect behavior, prompting them to seek more attractive plant parts that ensure less exposure to predators, such as the sheath (Smith, 2005). This behavior is characteristic of chinch bug females, which often lay eggs in protected locations such

Table 1. Number (mean \pm standard deviation) of *Blissus pulchellus* adults at 2, 4, 8, 16, 32, and 64 hours after infestation (HAI) and number of eggs 64 HAI on each forage grass species and the different plant parts (leaf, base, and sheath)

Forage grasses and plant parts	Chinch bugs per plant (hours after infestation)						Average number of eggs (64 HAI)
	2	4	8	16	32	64	
<i>U. brizantha</i> ‘Paiaguás’	0.75 \pm 0.44 a	0.77 \pm 0.37 a	0.70 \pm 0.37 a	1.03 \pm 0.47 a	0.98 \pm 0.76 a	1.26 \pm 0.59 a	1.01 \pm 0.39 a
<i>U. ruziziensis</i>	0.72 \pm 0.35 a	0.90 \pm 0.46 a	0.85 \pm 0.46 a	0.98 \pm 0.43 a	1.14 \pm 0.62 a	1.24 \pm 0.61 a	1.03 \pm 0.42 a
<i>U. brizantha</i> ‘Piatã’	0.72 \pm 0.42 a	0.79 \pm 0.38 a	0.74 \pm 0.50 a	0.75 \pm 0.53 a	0.72 \pm 0.66 b	0.76 \pm 0.57 b	1.07 \pm 0.33 a
<i>P. maximum</i> ‘Mombaça’	0.57 \pm 0.38 a	0.46 \pm 0.28 b	0.53 \pm 0.39 b	0.40 \pm 0.29 b	0.42 \pm 0.35 b	0.40 \pm 0.31 b	0.42 \pm 0.27 b
<i>U. humidicola</i>	0.55 \pm 0.32 a	0.42 \pm 0.19 b	0.49 \pm 0.38 b	0.46 \pm 0.28 b	0.51 \pm 0.43 b	0.46 \pm 0.34 b	0.79 \pm 0.49 b
<i>A. gayanus</i>	0.55 \pm 0.40 a	0.62 \pm 0.30 b	0.59 \pm 0.38 b	0.46 \pm 0.38 b	0.53 \pm 0.41 b	0.44 \pm 0.32 b	0.35 \pm 0.16 b
<i>P. maximum</i> ‘Zuri’	0.53 \pm 0.31 a	0.53 \pm 0.26 b	0.44 \pm 0.36 b	0.35 \pm 0.29 b	0.40 \pm 0.33 b	0.31 \pm 0.28 b	0.55 \pm 0.28 b
<i>U. brizantha</i> ‘Marandu’	0.53 \pm 0.40 a	0.53 \pm 0.28 b	0.51 \pm 0.38 b	0.46 \pm 0.28 b	0.44 \pm 0.37 b	0.50 \pm 0.32 b	0.96 \pm 0.39 a
F	1.147 ^{ns}	5.081 ^{***}	2.099 [*]	8.998 ^{***}	5.168 ^{***}	12.91 ^{***}	9.429 ^{**}
CV (%)	14.23	22.34	26.58	18.9	18.78	27.41	24.54
Leaf	0.31 \pm 0.24 c	0.42 \pm 0.13 b	0.20 \pm 0.13 c	0.26 \pm 0.13 c	0.13 \pm 0.09 c	0.24 \pm 0.14 c	0.49 \pm 0.15 c
Base	0.63 \pm 0.23 b	0.56 \pm 0.24 a	0.60 \pm 0.24 b	0.65 \pm 0.25 b	0.67 \pm 0.27 b	0.75 \pm 0.37 b	0.75 \pm 0.26 b
Sheath	0.91 \pm 0.34 a	0.90 \pm 0.34 a	1.02 \pm 0.43 a	0.92 \pm 0.35 a	1.13 \pm 0.25 a	1.02 \pm 0.47 a	1.09 \pm 0.37 a
F	50.484 ^{**}	25.879 ^{**}	115.753 ^{**}	36.996 ^{**}	74.625 ^{**}	33.324 ^{**}	22.959 ^{**}
CV (%)	22.34	15.89	19.45	27.02	22.46	21.45	25.76

^{ns}, ^{**}, ^{*}, and ^{***} - Not significant, significant at 0.001, 0.01, and 0.05 of probability, respectively, according to the F-test. Means followed by the same letter in the columns do not differ according to the Skott-Knott for forage grasses and Tukey test for plant parts at 0.05 of probability

as crevices at the nodes or between leaf blades where they come together at the base (sheath).

The damage caused by *B. pulchellus* was evident in some of the grasses at six DAI. Less damage was evident in *U. humidicola* and *P. maximum* 'Zuri', differing significantly from two forages (Table 2), with light green coloring (damage score 1) on a large leaf area, particularly younger leaves. According to Kaur et al. (2016), this change in color occurs because chinch bug attack compromises water balance and exhausts the supply of soluble carbohydrates used in plant growth.

Symptoms were more evident 12 DAI and evolved in *U. brizantha* 'Piatã' and 'Paiaguás'. These cultivars stood out as the least resistant among all the grasses studied, with 11 to 25% of the leaf area varying from light green to yellow (damage score 2) and the remainder rated 1 on the visual damage scale, with up to 10% of the surface light green.

At 18 DAI, *U. brizantha* 'Marandu', *U. humidicola*, *P. maximum* 'Zuri' and *A. gayanus* remained at a damage rating of 1 (up to 10 % of the leaf surface light green), whereas *U. brizantha* 'Piatã' reached a rating of 3, with 26 to 50% yellow leaves. Symptoms on *P. maximum* 'Mombaça', *U. ruziziensis*, and *U. brizantha* 'Paiaguás' reached a score of 2, with leaf color varying from light green to yellow.

At the final assessment (24 DAI), damage had progressed slightly in *U. humidicola*, *P. maximum* 'Zuri', *U. brizantha* 'Marandu' and *A. gayanus*, which evolved from light green and yellow, particularly on basal leaves, to a damage rating of 2, albeit without interfering in plant resistance, characterizing them as moderately resistant (MR). Valverde et al. (2018), found that a more resistant plant can offset insect damage to prevent it from compromising growth and thereby delay or decrease symptom expression, as occurred in the abovementioned grasses.

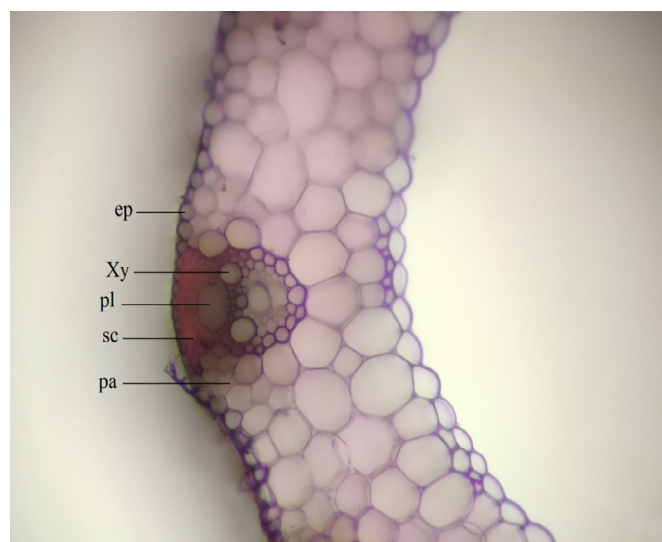
Urocloa ruziziensis, *P. maximum* 'Mombaça', and *U. brizantha* 'Marandu', whose average damage scores did not differ statistically from a rating of 3, were classified as moderately susceptible (MS), while *U. brizantha* 'Paiaguás', and 'Piatã', which exhibited yellowing and necrosis on 51 to 75% of the leaf surface (damage score of 4), were classified as susceptible (S). According to Rangasamy et al. (2015), the speed of symptom development in grasses is due, among other factors, to the longer duration of the insect bite, resulting in greater sap suction because of the absence of harmful compounds or structures on the plant.

Huang et al. (2013) found that damage caused by mealybugs directly affects the growth rate and performance of the host plant because the insects reduce its net photosynthesis. This feeding mechanism decreases carbohydrate transport from the leaves to other plant organs, as well as water and mineral salts from the stem to leaves, resulting in small yellow leaves that become necrotic, confirming the responses obtained in the present study for *U. brizantha* 'Paiaguás' and 'Piatã'.

Huang et al. (2022), found that sorghum genotype T x 2783 proved to be resistant and was able to defend against virulent sugarcane aphid. The authors report that resistant plants have a variety of built-in mechanisms to prevent unexpected attack, including a sophisticated molecular defense system such as phytohormone-mediated defense.

Double staining (safranin and Alcian blue) of leaf sheath cross-sections from the forage grasses studied made it possible to detect cell wall lignification of the phloem, xylem, sclerenchyma, and parenchyma, indicated by reddish pink coloring, and cellulose-rich cells (blue) in epidermal and bundle-sheath cells (Figure 1).

Advanced cell wall lignification was observed in the xylem, sclerenchyma, and parenchyma, particularly in *U. humidicola*, *P. maximum* 'Zuri', and *A. guayanus* (Figures 2A, B, and C). This



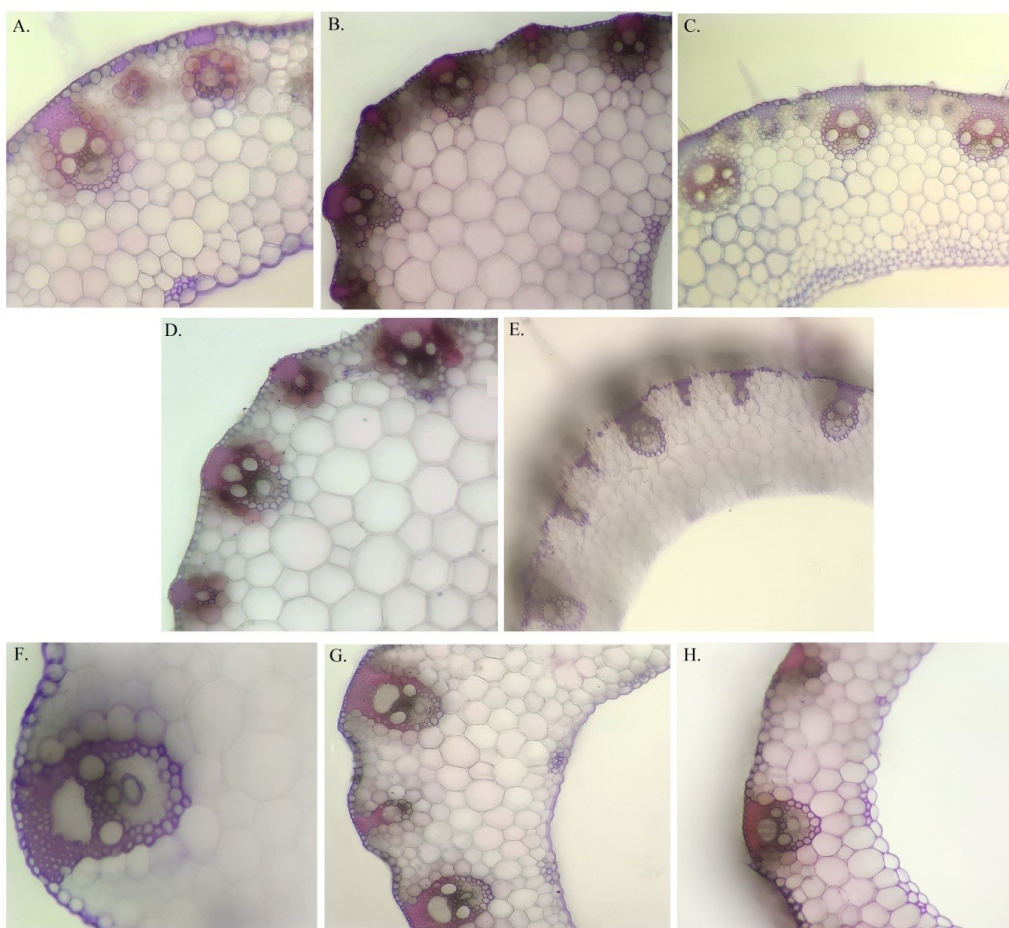
ep - epidermis, Xy - xylem, pl - phloem, sc - sclerenchyma, and pa - parenchyma

Figure 1. Cross-section of bundle-sheath cells of the leaf sheath, with reddish pink indicating the presence of lignin and blue cellulose

Table 2. Damage (mean \pm standard deviation) in eight forage grasses caused by the chinch bug *Blissus pulchellus* at 6, 12, 18, and 24 days after infestation (DAI)

Forage grasses	Damage rating (days after infestation)			
	6	12	18	24
<i>U. brizantha</i> 'Paiaguás'	1.40 \pm 0.00 a	2.20 \pm 0.16 a	2.90 \pm 0.25 b	4.00 \pm 0.28 a
<i>U. brizantha</i> 'Piatã'	1.35 \pm 0.10 a	2.25 \pm 0.10 a	3.17 \pm 0.10 a	3.95 \pm 0.19 a
<i>U. brizantha</i> 'Marandu'	1.30 \pm 0.11 a	1.45 \pm 0.10 b	1.60 \pm 0.00 d	2.70 \pm 0.38 b
<i>A. gayanus</i>	1.25 \pm 1.10 a	1.55 \pm 0.30 b	1.80 \pm 0.28 d	2.05 \pm 0.41 c
<i>P. maximum</i> 'Mombaça'	1.25 \pm 0.10 a	1.60 \pm 0.23 b	2.25 \pm 0.19 c	2.90 \pm 0.35 b
<i>U. ruziziensis</i>	1.20 \pm 0.00 a	1.70 \pm 0.11 b	2.27 \pm 0.10 c	3.07 \pm 0.15 b
<i>P. maximum</i> 'Zuri'	1.10 \pm 0.11 b	1.40 \pm 0.00 b	1.60 \pm 0.16 d	2.10 \pm 0.26 c
<i>U. humidicola</i>	1.05 \pm 0.10 b	1.40 \pm 0.16 b	1.60 \pm 0.16 d	1.90 \pm 0.26 c
F	6.771**	16.441**	38.742**	24.503**
CV (%)	7.38	10.01	8.73	11.71

** and * - Significant at 0.01 and 0.05 probability, respectively, according to the F- test. Means followed by the same letter in the columns do not differ according to the Skott-Knott test for forage grasses at 0.05 probability



A, B, and C- *Urocloa humidicola*, *Panicum maximum* cv. 'Zuri' and *Andropogon guayanus*. D, E- *Urocloa brizantha* cv. 'Marandu', and *Panicum maximum* cv. 'Mombaça'. F, G, and H- *Urocloa ruziziensis*, *Urocloa brizantha* cv. 'Paiaguás', and *Urocloa brizantha* cv. 'Piatã'

Figure 2. Leaf sheath cross-sections of the forage grasses studied

fact was not identified in *U. brizantha* cv. Marandu, *P. maximum* cv. Mombaça. *U. ruziziensis*, *U. brizantha* cv. Paiaguás, and *U. brizantha* cv. Piatã (Figures 2D, E, F, G, and H). Additionally, there was greater compaction and lignification in parenchyma and sclerenchyma cells, respectively, indicated by reddish pink coloring. Lignified tissue could potentially have prevented the mouthpiece from reaching the chinch bug's preferred tissue. Herbivorous insects actively select their feeding sites and secrete saliva to facilitate nutrient acquisition from host plants during feeding (Jiang et al., 2019).

Andropogon guayanus was unique in that it exhibited smaller and more compact parenchyma cells and more trichomes than the remaining forage grasses studied (Figure 2C). In turn, *P. maximum* cv. 'Zuri' displayed greater lignin deposition in the bundle-sheath cells and along the epidermis of the leaf sheath (Figure 2B). Finally, the forage grasses that experienced less damage tended to have a smaller phloem cylinder diameter and shorter distance between bundle-sheath cells in the leaf sheath, conferring better resistance by making these fractions more difficult for insects to pierce (Belete, 2018).

According to Rangasamy et al. (2009), the susceptibility of lawn grass to chinch bug attack is directly related to less parenchyma cell compaction and the distribution of compounds such as lignin in plant tissue. The authors reported that greater compaction between parenchyma cells reduces intercellular spaces, making cell walls more rigid

and hampering or preventing the insect mouthpiece from piercing plant tissue. However, in the present study, both the parenchyma and sclerenchyma cells performed this function.

U. ruziziensis and *U. brizantha* cv. 'Paiaguás', and 'Piatã' exhibited lower compaction and lignin deposition in parenchyma and sclerenchyma cells as well as higher cellulose concentrations, especially in *U. ruziziensis*, which had the lowest lignin deposition of all the forage grasses analyzed. These findings corroborate the results obtained by Ribeiro-Júnior et al. (2017), who evaluated tissue changes in *Urocloa* cultivars. Another characteristic observed was the presence of small aerenchymae in the cortex of the most damaged grass species, which may have acted as facilitating agents for the insect mouthpiece to reach the plant's vascular cylinder, since the lignin layer is the final barrier to the cortex (Rajhi & Mhadhbi, 2019).

Cells with thick lignified walls hamper insect access to the vascular tissue, making it difficult for their mouthpiece to penetrate. As a result, nutrients cannot be fully absorbed while the mouthpiece remains inside the tissue. Rangasamy et al. (2006) investigated categories of resistance to chinch bugs in St. Augustinegrass lines FX-10 and NUF-76 and found high levels of antixenosis or non-preference in both. Electrical penetration graphs (EPG) revealed that the insect took longer to reach the sieve elements of the phloem and spent less time feeding on FX-10 and NUF-76 when compared to the susceptible controls 'Floritam' and 'Palmetto'.

Molecular studies of mostly leaf mesophyll cell-infecting pathogens and chewing insects have advanced our understanding of many aspects of the plant immune system (Jones et al., 2016). In particular, there is now a quite advanced understanding of plant interactions with leaf mesophyll cell-infecting pathogens. It appears that individual plant leaf cells can broadly respond to microbial signals to initiate 2 branches of immunity, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). For plant defense against phloem-feeding insects, both PTI and ETI seem to be required for resistance (Jiang et al., 2019).

A comprehensive understanding of phloem defense against pathogens and insects is crucial for the development of innovative long-term control measures. However, current knowledge on phloem-based defense is limited. There are many questions that need to be addressed as follows. What aspects of leaf mesophyll cell-based plant immunity are applicable to phloem-inhibiting pathogens and insects? Are there unique phloem defense responses? Can new technologies be developed to advance the study of phloem defense responses?

Future studies of phloem–insect/pathogen interactions will rely increasingly on cell type-specific technologies in order to achieve a next-level understanding of how various phloem cell types respond to phloem-associated pathogens and insects. In addition to developing phloem cell type-specific biosensor lines that could monitor immune responses in situ, as mentioned earlier, alternative techniques need to be developed to advance the study of phloem defense responses.

CONCLUSIONS

1. *Urocloa humidicola*, *Panicum maximum* ‘Mombaça’ and ‘Zuri’, *Urocloa brizantha* ‘Marandu’ and *Andropogon gayanus* are less attractive to *Blissus pulchellus*.

2. *Andropogon gayanus* and *Panicum maximum* ‘Mombaça’ and ‘Zuri’ show non-preference resistance to oviposition by *Blissus pulchellus*.

3. The resistance of *U. humidicola*, *P. maximum* ‘Zuri’, and *A. gayanus* to *Blissus pulchellus* may be associated with greater compaction and lignification of the parenchyma and sclerenchyma cells of leaf sheaths.

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