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ORIGINAL ARTICLE

Behavior of *Megathyrsus maximus* cv. BRS Tamani to brown leaf spot, caused by *Bipolaris yamadae*, as a function of nitrogen fertilization doses

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Abstract
One of the diseases threatening pasture health is brown leaf spot, caused by *Bipolaris yamadae*, recently reported in Brazil. The objective was to assess the effect of increasing nitrogen (N) doses on the behavior and severity of brown spot in *Megathyrsus maximus* cv. BRS Tamani. The experiment followed a randomized block design with four replicates. Treatments included five N doses (0, 50, 100, 150, and 200 mg N dm^{-3}) in the form of urea (45% N) diluted in water. Disease severity was estimated on 15 randomly selected leaves per pot at 7, 14, and 21 days after inoculation (DAI). At 14 DAI, the plants were cut 20 cm above the ground to simulate grazing effects, and the final disease severity assessment was done at 21 DAI. Chlorophyll content in 15 fully expanded leaves per pot was estimated at 7 and 14 DAI. Yield traits were determined through cutting at 14 DAI. Fertilization increased growth, yield, chlorophyll index and brown leaf spot severity. The highest nitrogen dose at 7 and 14 DAI resulted in the highest brown spot severity while the dose of 168 mg N dm-3 caused the highest severity at 21 DAI. Simulated grazing removed infected tissue and prevented an increase in severity at 21 DAI.

Keywords Forage · *Panicum maximum* · Integrated management · Fungi

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Introduction

Pastures are the primary food source for extensively raised ruminant animals worldwide (Michalk et al. 2019), constituting the largest land use on a global scale (Mottet et al. 2017). Out of this vast expanse of land, 1.3 billion hectares cannot be converted into cultivated areas due to edaphoclimatic limitations for human food production (Puche et al. 2019).

In the tropical regions of Brazil, pastures form the foundation for beef cattle production (Arcanjo et al. 2022). Within the Cerrado biome, forage species of *Megathyrsus maximus* (formerly *Panicum maximum*) are extensively utilized due to their high yield and adaptability to tropical climates (Euclides et al. 2019; Véras et al. 2020). These grasses reproduce through facultative apomixis, giving rise to clonal individuals through seeds (De et al. 2019), resulting in the establishment of extensive monospecific pastures with limited genetic variability (Liu et al. 2020).

These associated factors facilitate the occurrence and spread of diseases in pastures (Dignam et al. 2016), which can disrupt pastoral ecosystems (Marcos et al. 2015; Liu et al. 2020). Forage plants of the species *M. maximus* are susceptible to fungal diseases such as smut (*Tilletia ayresii*), rust (*Uromyces setariae-italicae*), and *Bipolaris maydis* leaf spot (Avelino et al. 2019).

The first report of *Bipolaris yamadae* in forage grasses in the Americas was registered by Adhikari et al. (2021) and Alves et al. (2023). This pathogen was recently identified as the causal agent of brown leaf spot in genotypes of *M. maximus* in Brazil (Sanches et al. 2024) and the lack of information raises significant concerns, as it threatens other forage species and annual grasses used in crop-livestock integration systems (Chávez-Valdez et al. 2023). In *M. maximus*, the symptoms of this disease are characterized by small necrotic lesions on leaf blades, which can lead to plant death (Adhikari et al. 2021). It is assumed that some aspects of this disease are similar to those caused by *B. maydis*, potentially spreading through infected seeds, wind, and rainwater, surviving in crop debris (Marchi et al. 2010).

Some research suggests that mineral nutrition can potentially affect the progression of fungal diseases in plants (Asghar et al. 2019; Liu et al. 2020; Castellanos González et al. 2020). Fertilization can induce changes in plant structure to enhance adaptability to the environment. However, higher nitrogen inputs can result in increased severity of fungal diseases in various plant species (Huber et al. 2012; Wu et al. 2015; Liu et al. 2017).

Nitrogen is an essential component of proteins, promoting cellular synthesis and enhancing regrowth vigor and mass production in forage species (Bernardi et al. 2018; Galindo et al. 2019). On the other hand, there are reports that high nitrogen concentrations in leaf tissue of various plant species predispose them to fungal disease development (Veresoglou et al. 2013; Gupta et al. 2017).

There is a scarcity of information regarding the effects of nitrogen fertilization on the behavior of forage plants towards various etiological diseases. Therefore, the objective of this study is to assess the effect of increasing nitrogen doses on the behavior and severity of brown spot caused by *Bipolaris yamadae* in *Megathyrsus maximus* cv. BRS Tamani.

Materials and methods

The experiment was carried out in a greenhouse at the Faculty of Veterinary Medicine and Animal Science (FAMEZ) of the Federal University of Mato Grosso do Sul (UFMS), in Campo Grande, Mato Grosso do Sul, Brazil (coordinates 20°26’17’’S and 54°51’24’’W, at an altitude of 548 m).

The soil used was classified as Dystrophic Red Latosol, collected from the experimental area of FAMEZ, with the following characteristics: pH: 5.6; OM (g kg^{-1}): 30.22; Ca ($cmol_c$ dm^{-3}): 2.5; Mg ($cmol_c$ dm^{-3}): 2.1; K ($cmol_c$ dm^{-3}): 0,26; P (mg dm^{-3}): 3.0, H + Al ($cmol_c$ dm^{-3}): 6.3; sand (g kg^{-1}): 130; silt (g kg^{-1}):100; clay (g kg^{-1}): 770.

The soil was sieved through 5 mm mesh screens, amended with dolomitic limestone (1.1 g dm^{-3}) and phosphorus (277.8 mg dm^{-3} of single superphosphate), and placed in plastic pots with a capacity of 7 dm^{-3}. It was incubated for 30 days with frequent irrigations to maintain field capacity.

Subsequently, 32 seeds of *M. maximus* cv. BRS Tamani were sown per pot. Fifteen days after sowing, selective thinning was carried out, retaining five plants per pot. In May 2023, a uniform cutting of the plants to a height of 20 cm above the ground was performed, followed by the fertilization with nitrogen at different rates (0, 50, 100, 150, and 200 mg N dm^{-3}) in the form of urea (45% N) diluted in water.

The experimental unit consisted of the pot, and a randomized complete block design was adopted with five treatments represented by N doses: 0, 50, 100, 150, and 200 mg N dm^{-3}, with four replications. Throughout the experimental period, the plants were irrigated daily to maintain soil moisture close to field capacity. Fifteen days after applying the treatments, inoculation with a *B. yamadae* isolate, the causal agent of brown spot, was performed.

The inoculum used was previously isolated from BRS Tamani grass plants in the experimental field of Embrapa Gado de Corte, Campo Grande, MS. After isolation, it was cultured on Potato Dextrose Agar (PDA) and maintained in a BOD-type incubator at 27 °C. The mycelium and conidia on the culture medium were scraped off with sterile distilled water containing Tween 20 (0.05%) and filtered through gauze. The concentration of the obtained suspension was estimated by counting the conidia in a Neubauer chamber under an optical microscope.

Inoculation was carried out by spraying the plants in the pots with a concentration of 6×10^4 conidia ml^{-1}. The plants were sprayed using a manual air compressor, and the conidia suspension was continuously dispersed over the leaves until droplets formed on the leaf blade (dew point). After spraying, the plants were kept in a humid chamber for 48 h (Fig. 1A) to provide the infection stage and typical symptoms of brown spot (Fig. 1B).

The disease severity was estimated in percentage on 15 randomly selected leaves per pot. Evaluations were conducted at 7, 14, and 21 days after inoculation (DAI). At 14 DAI, the plants were also cut to a height of 20 cm above the ground to simulate the effect of grazing. After seven days of cutting (21 DAI), the final disease severity assessment was carried out.

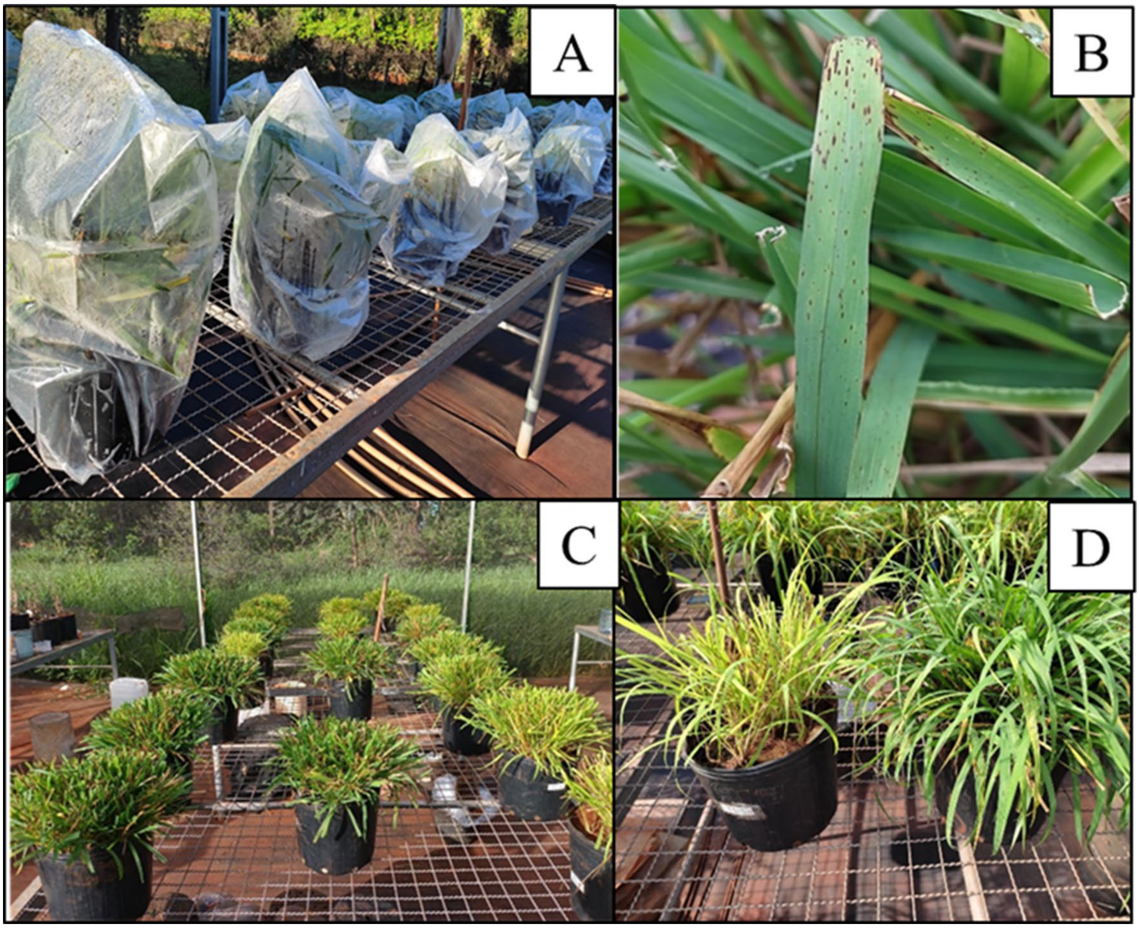

Fig. 1 Humid chamber (**A**), typical symptoms of brown spot at 7 DAI (**B**), cut at 14 DAI (**C**) and the effect of nitrogen fertilization in the cutting moment (**D**) in *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae*

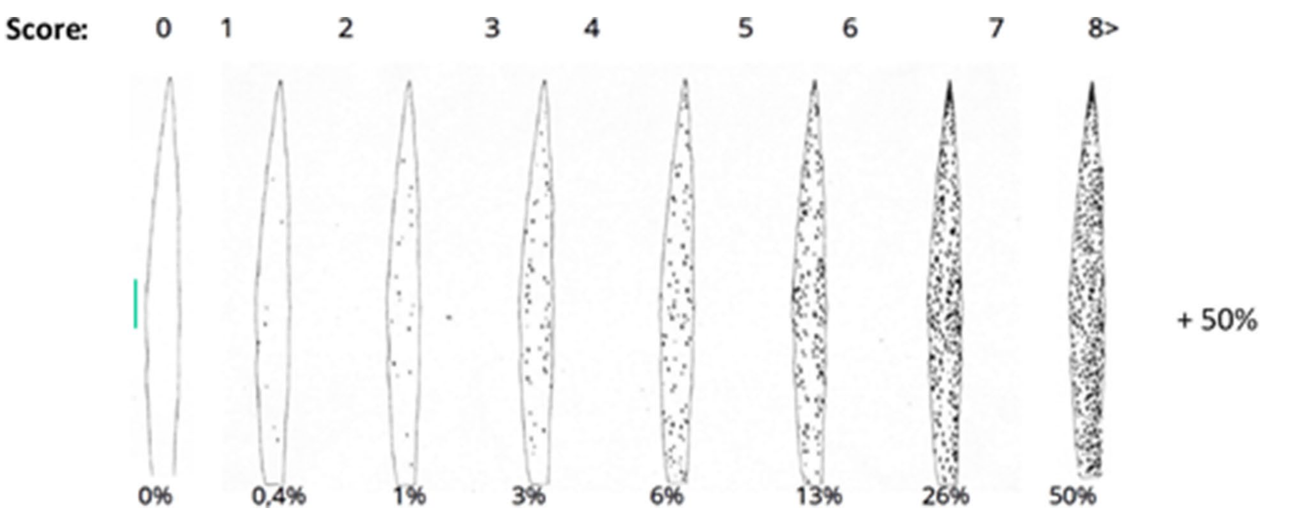

Fig. 2 Diagrammatic scale for evaluating leaf spot caused by *Bipolaris maydis* on *Megathyrsus maximus*, proposed by Martinez (2006) and modified by Fernandes et al. (2011)

The estimation of chlorophyll content was performed at 7 and 14 DAI on 15 fully expanded leaves per pot, using a portable digital chlorophyll meter, model at LEAF CHL BLUE according to (Chaves et al. 2023).

The productive characteristics of *M. maximus* cv. BRS Tamani plants were assessed by cutting the plants at a height of 20 cm above the ground, 14 days after the uniform cutting (Fig. 1C), as follows: fresh mass (FM), dry mass (DM), height of the tiller from the ground to the first fully expanded ligule (TH), height of the extended tiller (HET), and the number of tillers per pot (NTP). The NTP was determined by counting the total tillers in the pots, following the methodology described by (Martuscello et al. 2018).

The FM was assessed when the plant canopy reached an average height of 40 cm. The cutting was done at 20 cm above ground level, and the collected material was weighed to determine the forage mass per pot (Fig. 1D). After weighing the FM samples, they were placed in paper bags and dried in a forced air oven at 55 °C for 72 h. Subsequently, the dried material was weighed again to obtain the DM.

The height from the soil to the first fully expanded ligule and the height of the extended tiller were determined by selecting three representative tillers in each pot, using a graduated ruler in centimeters.

Severity values and the area under the disease progress curve (AUDPC) were transformed using the square root of (x+0.01). From the transformed data, severity over time and the AUDPC was calculated following the methodology used by Souza et al. (2020).

The data on the characteristics evaluated were submitted to regression analysis as a function of nitrogen doses. Equations were selected based on the coefficient of determination (R^2) and the significance level of 5% for equation parameters according to the Duncan test.

Results

The characteristics FM, DM, NTP and TH of Tamani grass responded quadratically to increasing doses of N (Figs. 3 and 4). The HET did not exhibit a significant effect from the N doses.

Based on the regression analysis, the highest responses of the production variables in *M. maximus* cv. Tamani to nitrogen fertilization were estimated as follows: MV was estimated at 48.54 g pot^{-1} (293.80 mg N dm^{-3}); MS at 15.32 g pot^{-1} (261.50 mg N dm^{-3}); DPP at 177.91 tillers pot^{-1} (160.80 mg N dm^{-3}); and TH at 11.14 cm (315.00 mg N dm^{-3}).

The chlorophyll index assessed at 7 days exhibited a quadratic effect, with the highest response estimated at 41.54 (175.80 mg N dm^{-3}). By the 14 days, it demonstrated a linear response to rising nitrogen doses (Fig. 5).

Regarding the effect of nitrogen fertilization on brown spot disease, the severity data at 7 and 14 DAI were fitted to a linear equation, whereas at 21 DAI, the fit was better with a quadratic equation. The highest nitrogen dose at 7 and 14 DAI resulted in the highest brown spot severity (Fig. 6A) while the dose of 168 mg N dm^{-3} caused the highest severity (3.71%) at 21 DAI.

Considering the effect of N doses on the AUDPC, the data were better fitted to a quadratic equation, however, the highest severity was estimated only at a dose of 383 N dm^{-3} (Fig. 6B).

There was no interaction between evaluation days and nitrogen doses ($P=0.504$) regarding brown spot severity. However, there was a significant isolated effect for evaluation days and nitrogen doses, as shown in Table 1. The regression analysis models for nitrogen doses and disease severity can be found in (Fig. 6).

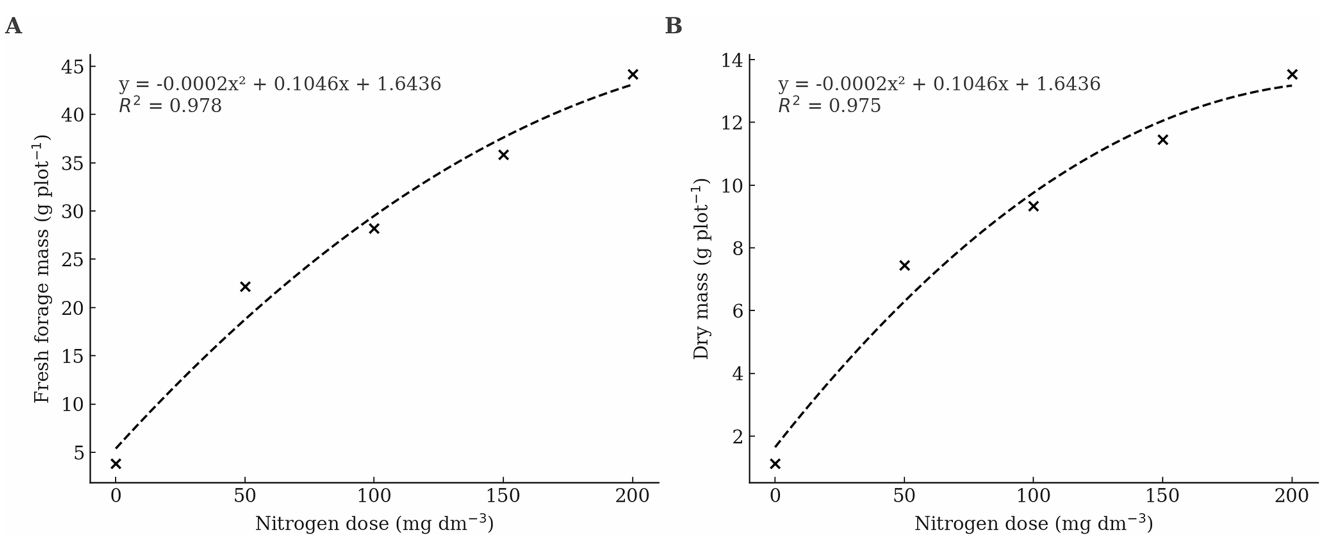

Fig. 3 Fresh (**A**) and dry mass (**B**) of *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae* and fertilized with different nitrogen doses

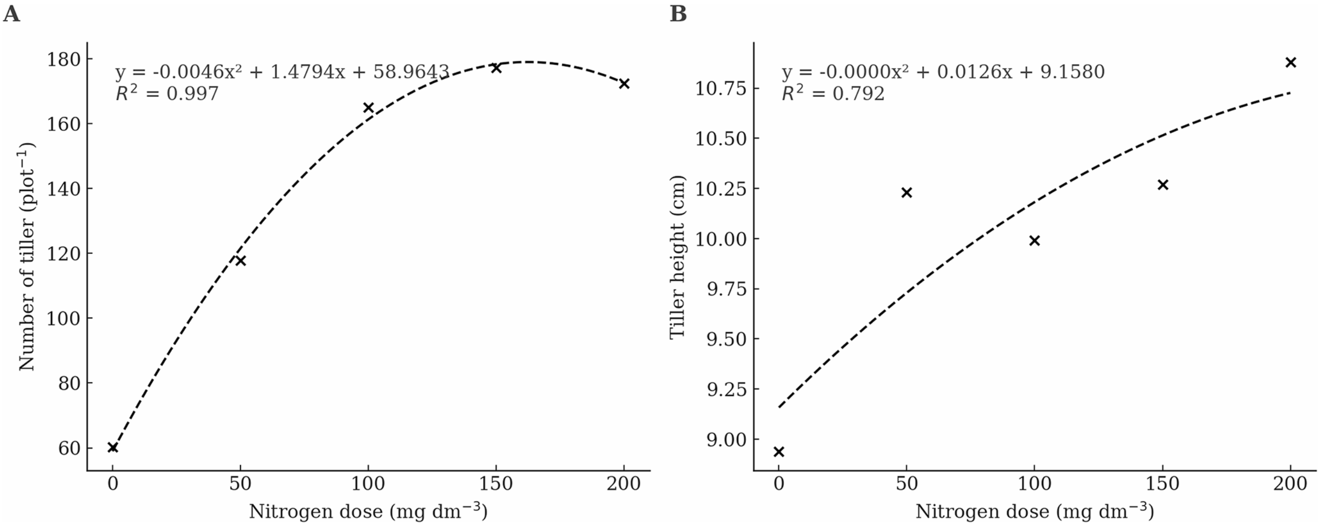

Fig. 4 Number of tiller per pot (**A**) and tiller height (**B**) of *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae* and fertilized with different nitrogen doses

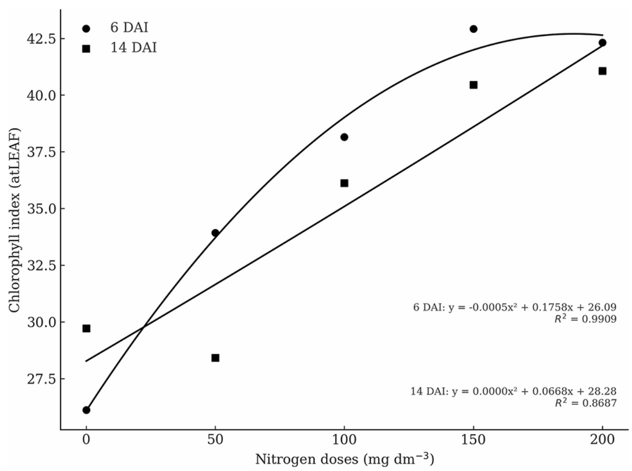

Fig. 5 Chlorophyll index (atLeaf) at 6 and 14 DAI in *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae* and fertilized with different nitrogen doses

Discussion

The quadratic behavior observed in the productive characteristics of Tamani grass in this study is due to the fact that forage plants of the species *M. maximus* are highly responsive to increases in soil fertility (Vasconcelos et al. 2020), especially nitrogen fertilization (Martuscello et al. 2019) as shown in Fig. 1D.

In greenhouse study, Abreu et al. (2020) observed a quadratic effect of increasing N doses on leaf length, tiller number, and dry mass in the BRS Tamani cultivar. Doses between 280 and 380 mg N dm^{-3} impacted canopy structure and grass biomass. Almeida et al. (2023) found quadratic responses in green and dry forage mass for *M. maximus*

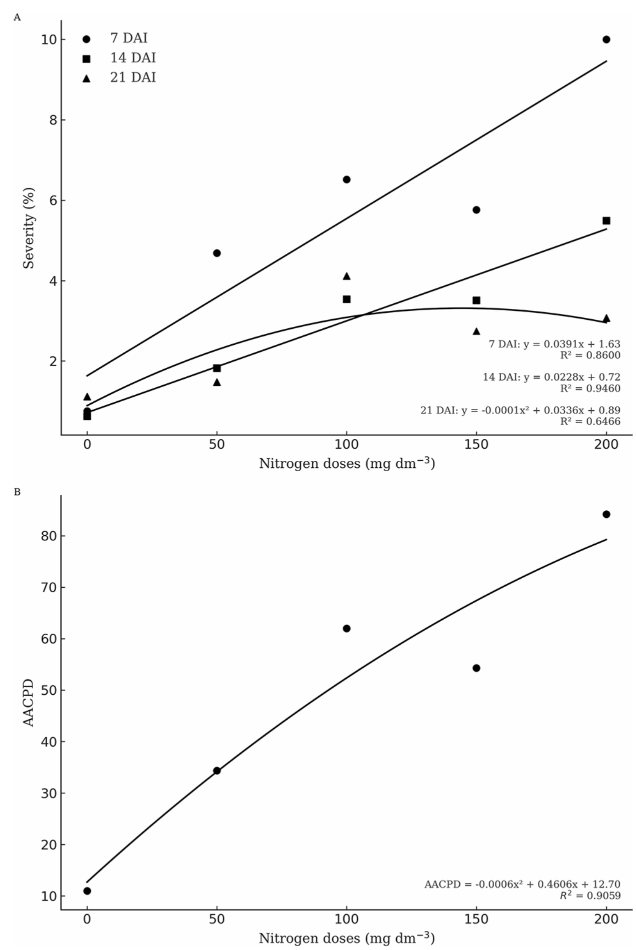

Fig. 6 Severity (**A**) and AUDPC (**B**) of brown spot (*Bipolaris yamadae*) in *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae* and fertilized with different nitrogen doses

Table 1 Interaction between evaluation day after inoculation (DAI) and nitrogen doses (mg dm^{-3}) in *Megathyrsus maximus* Cv. BRS Tamani plants inoculated with *Bipolaris Yamadae*

CV (%)	p-Value			DAI	N (mg dm^{-3})					Mean
	DAI	N	DAI*N		0	50	100	150	200	
31.85	0.0012	< 0.001	0.504	7	0.86	2.02	2.53	2.29	2.94	2.13 a
				14	0.78	1.34	1.81	1.84	2.32	1,61 b
				21	1.00	1.18	1.89	1.49	1.72	1.46 b
				Mean	0.88	1.51	2.08	1.87	2.32	1.73

Means followed by letters within the same row differ from each other according to the Duncan test at a 5% probability level. P-value: probability of a significant effect (> 0.05). CV: coefficient of variation

cultivars, BRS Quênia and BRS Tamani, peaking at 200 mg dm^{-3} of N.

Nitrogen fertilization accelerates tissue growth in plants (Martuscello et al. 2015), which, in turn, affects leaf and tiller production. Numerous studies have shown linear increases in the number of tillers in *M. maximus* cultivars under the influence of nitrogen rates (Martuscello et al. 2015, 2018, 2019; Abreu et al. 2020).

NTP exhibited quadratic behavior in response to nitrogen doses, a condition also observed by Costa et al. (2016) when evaluating the effect of increasing N doses on production and morphogenesis of Massai grass. These authors found that the highest tillering occurred at a dose of 150.9 mg dm^{-3} of N. In this study, the highest tillering occurred at a dose of 160.80 mg dm^{-3} of N, with an average of 177.91 tillers per plant. According to the same authors, tillering is strongly correlated with the yield of morphological components of grasses, making the adequate supply of this nutrient important for optimizing forage production.

The quadratic effect of the variable TH may be related to morphophysiological factors and the availability of growth factors. Increasing doses of nitrogen have an impact on the apical meristem, contributing to an increase in the number of plant cells as well as cell division (Schnyder et al. 2000). Nitrogen fertilization also induces the production of hormonal metabolites, such as auxins and gibberellins, which are associated with tissue growth. However, for the full development of plants, other factors such as water availability and the presence of other nutrients play crucial roles in this process of plant growth (Véras et al. 2020; Oliveira et al. 2020; Prajapati et al. 2023).

An important factor to consider regarding the behavior of the variables such as FM, DM, NTP, and TH may be due to the limitation imposed by the disease on the full development of the forage plant. Martinez et al. (2010) assessed the damage caused by *B. maydis* in *M. maximus* cv. Tanzania and observed that as disease severity increased, there was a decrease in FM, DM, and plant tillering. Anjos et al. (2004), reporting the occurrence of *B. maydis* in *Paspalum atratum* cv. Pojuca grasses, also mentioned a reduction in photosynthetic area and green mass production losses in plants infected by the fungus.

The progression of the disease, as evidenced by the variable AUDPC in response to increasing nitrogen doses, exhibited a quadratic behavior. This may be associated with a higher number of leaves and tillers, especially at higher N doses, resulting in the highest estimated disease severity at the dose of 383 mg N dm^{-3}. According to González et al. (2020) although high N doses accelerate plant metabolism and the speed of new leaf and tiller emergence processes, they can cause metabolic imbalances, leading the plant to reduce the absorption rate of certain nutrients, including silicon, which play a role in tissue protection against pathogens (Santos et al. 2014; Guazina et al. 2020).

The quadratic behavior of chlorophyll content at 6 DAI may be associated with higher disease severity seven days after pathogen inoculation (Fig. 7). Under stress conditions, plants reduce photosynthetic activity in diseased leaves, which can lead to tissue yellowing (Dallagnol et al. 2011; Victoria Arellano et al. 2022). Chlorophyll in the leaf is closely related to the concentration of N and, therefore, serves as an indicator of the nutritional status with respect to this important nutrient (Mandal and Dutta 2020). Since there was an increase in the N dose, it is likely that there was also an increase in its concentration in the plant tissue.

Forage plants of the species *M. maximus* exhibit varied responses in terms of resistance to leaf spots caused by *B. maydis*, ranging from high susceptibility, as seen in Tanzânia and Mombaça grasses (Martinez et al. 2010) to low susceptibility, as observed in Massai grass (Santos et al. 2015).

The linear increase in disease severity in response to N doses, as evidenced at 7 and 14 DAI, is associated with the pathogen's behavior and the rate of leaf emergence in forage plants. The highest brown spot severity was observed in plants that received the highest N dose, linked to a greater quantity of senescent leaves. Since this disease has been recently reported in this forage grass, it is worth noting that it established itself on the older leaves of the plants, a behavior also observed in the field.

This phenomenon is believed to be explained by the nutritional relationship of *B. yamadae* and the effects of nitrogen fertilization on forage plants. Although it is a species recently associated with *M. maximus* (Adhikari et al. 2021; Sanches et al. 2024), we believe this pathogen

Fig. 7 Condition of *Megathyrsus maximus* cv. BRS Tamani plants inoculated with *Bipolaris yamadae* and fertilized with different nitrogen doses at 7 DAI

possibly exhibits a hemi-biotrophic feeding habit, meaning that while it establishes itself in live plant tissues, it prefers senescent leaves because they contain tissues that are more easily degraded by enzymes that break down plant cell walls (Aich et al. 2017). As forage plants fertilized with increasing doses of nitrogen have a higher rate of new leaf emergence but a genetically limited total leaf count, a higher rate of leaf senescence is also observed (Roma et al. 2012; Lopes et al. 2013).

Analyzing the effect of evaluation dates on disease severity (Table 1), it was observed that disease severity decreased at 14 DAI. This suggests that evaluations under controlled conditions should preferably be conducted 7 DAI if the leaves used in this process are chosen randomly.

Since the disease remained more restricted to older leaves and there was a greater presence of new leaves, recently emerged, elongated, and expanded after nitrogen fertilization (Martuscello et al. 2019) at 14 DAI, the randomness in choosing leaves emitted at any height of the plant stem contributed to the reduction in the average severity per pot. In future experiments, in addition to the random selection of leaves distributed throughout the plant, stratification of the plant should be employed during brown spot severity assessment, as done in other pathosystems (Souza et al. 2020; Guazina et al. 2020).

At 21 DAI, brown spot severity remained similar ($P > 0.05$) to that observed at 14 DAI and was fitted to the quadratic model. Since there was a plant cutting at 14 DAI to simulate the grazing effect on disease progression at 21 DAI, it is hypothesized that the removal of tissue infected by *B. yamadae* contributed to the maintenance of disease severity level in the plants. Future studies should be conducted to elucidate the effect of grazing on the progression of foliar diseases in pastures.

Some indications suggest that grazing can be a tool to preserve plant health and has already been reported as a cultural control method for diseases such as some leaf spots in *M. maximus* cultivars (Santos et al. 2015). According to Victoria Arellano et al. (2022), cutting, combined with measures like biological seed treatment and silicon fertilization, has the potential to reduce leaf spot severity in pastures by 40 to 80%.

The data obtained demonstrate that the BRS Tamani cultivar, despite showing an increase in brown spot severity with increasing N doses, did not exhibit a negative effect on its productive characteristics with nitrogen fertilization. This behavior is believed to be attributed to the prevalence of symptoms on older leaves, especially in the lower canopy stratum, a condition also observed by (Martinez et al. 2010) when studying the damage caused by *B. maydis* in *M. maximus* plants.

In this study, nitrogen fertilization increased growth, yield, chlorophyll index and brown spot severity in *M. maximus* cv. Tamani. The severity data at 7 and 14 DAI were fitted to a linear equation, whereas at 21 DAI, the fit was better with a quadratic equation. The highest nitrogen dose at 7 and 14 DAI resulted in the highest brown spot severity while the dose of 168 mg N dm-3 caused the highest severity (3.71%) at 21 DAI. Considering the effect of N doses on the AUDPC, the highest severity was estimated only at a dose of 383 mg N dm^{-3}. The cutting removed infected tissue and prevented an increase in severity at 21 DAI, which could be a way of controlling the disease.

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Declarations

Conflict of interest The authors have no conflict of interest to declare that are relevant to this article.

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