Scientific Note

Bipolaris yamadae as a causal agent of brown leaf spot in *Megathyrsus maximus* in Brazil[1]

Márcio Martinello Sanches[2], Celso Dornelas Fernandes[2], Margareth Vieira Batista[2],
Jaqueline Rosemeire Verzignassi[2], Gustavo de Faria Theodoro[3], Diego Vaz Gutoski[4]

ABSTRACT

Guinea grass (*Megathyrsus maximus*) is widely used as a forage crop in Brazil, but its production faces disease threats. Recently, new symptoms, such as oblong to irregular brown lesions on leaves, have emerged in *M. maximus* across the country. Particularly severe symptoms were observed in the BRS Tamani cultivar, and some other genotypes showed lesion coalescence and intense leaf necrosis. This study aimed to identify the cause of these symptoms. A fungus was isolated from the lesions and, using morphological measurements and molecular analysis of the ITS, *GPDH* and *TEF-1α* genes, it was identified as *Bipolaris yamadae.* Pathogenicity tests confirmed its ability to cause disease in *M. maximus* cv. Tamani. A field survey of 205 *M. maximus* genotypes showed severe symptoms in 8.35 % of the materials.

KEYWORDS: *Panicum maximum*, forage diseases, molecular phylogeny.

RESUMO

Bipolaris yamadae como agente causal da mancha marrom em *Megathyrsus maximus* no Brasil

O capim-colonião (*Megathyrsus maximus*) é amplamente utilizado como cultura forrageira no Brasil, mas sua produção enfrenta ameaças de doenças. Recentemente, novos sintomas, como lesões marrons oblongas a irregulares nas folhas, surgiram em *M. maximus* em todo o país. Sintomas particularmente severos foram observados na cultivar BRS Tamani, e alguns outros genótipos apresentaram coalescência de lesões e intensa necrose foliar. Objetivou-se identificar a causa desses sintomas. Um fungo foi isolado das lesões e, por meio de medidas morfológicas e análise molecular dos genes ITS, *GPDH* e *TEF-1α*, identificado como *Bipolaris yamadae*. Testes de patogenicidade confirmaram sua capacidade de causar doença em *M. maximus* cv. Tamani. Um levantamento de campo com 205 genótipos de *M. maximus* revelou sintomas severos em 8,35 % dos materiais.

PALAVRAS-CHAVE: *Panicum maximum*, doenças de forrageiras, filogenia molecular.

Megathyrsus maximus (Jacq.) B. K. Simon & S.W. L. Jacobs (syn. *Panicum maximum* Jacq.), also known as Guinea grass, belongs to the Poaceae family and is widely used as a forage crop in Brazil (Jank 2019). *M. maximus* cultivars are estimated to cover 20 million ha across various biomes in the country, serving as essential livestock feed consumption and as cover crop in integrated systems (Jank 2019).

One of the primary concerns for farmers is the incidence of diseases caused by fungi, viruses and nematodes (Marchi et al. 2011), which have the potential to render pastures economically unviable. Leaf blight, caused by *Bipolaris maydis*, is the most severe disease reported for *M. maximus* in Brazil to date, particularly affecting the Tanzania-1 cultivar, inhibiting its growth and altering forage quality (Martinez et al. 2010).

During experiments conducted at the Embrapa Gado de Corte, in Campo Grande, Mato Grosso do Sul state, Brazil, unusual foliar symptoms were observed in the BRS Tamani cultivar, distinct from typical *B. maydis* lesions. These symptoms manifest as small necrotic lesions of 1-2 mm in size, with brown color and oblong to irregular shape. Under conditions of high humidity and elevated temperatures, the symptoms worsen, leading to lesion coalescence, intense leaf necrosis and blight, primarily affecting the lower parts of the plants due to microclimate

conditions. Similar symptoms have been reported by farmers in the southeast, mid-western and northern regions of Brazil (Sanches et al. 2023), and the disease has been termed brown leaf spot.

To elucidate the etiology of the disease, a series of experiments was conducted in laboratory, greenhouse and field settings.

Leaves displaying typical symptoms of brown leaf spot from BRS Tamani plants were collected at the Embrapa Gado de Corte (20º26'44''S; 54º43'18''W), and small pieces of symptomatic tissues were surface-sterilized with 1 % NaOCl and plated on water-agar medium plates. The plates were then incubated in a BOD chamber at 28 ºC, with a 12-hour photoperiod, until mycelial growth was observed. Gray to black mycelium and typical *Bipolaris* sp. conidia were observed on the plates. Individual conidia were transferred using a sterile needle to potato-dextrose-agar (PDA) medium plates to obtain monosporic pure cultures. These plates were incubated under the same conditions for 7 days. The colony displayed a fast growing rate (70 mm diameter in 7 days). Morphological observations, measurements and photographic documentation were conducted using an O500R microscope with a digital camera ToupCam2.0 and the OPTHD software (Opticam).

The observed morphology revealed cylindrical conidiophores, with dimensions ranging from 74.8 to 215.5 µm in length and 3.4 to 7.5 µm in width (average = 134.3 x 5.3 µm), displaying a brown hue, smooth surface and occurring either individually or clustered in groups. They appeared simple, septate, straight or occasionally geniculate and swollen at the upper part, with a swollen, darker brown basal cell (n = 30). The conidia exhibited dimensions ranging from 40.2 to 90.0 × 9.5 to 14.4 µm (average = 70.8 x 11.8 µm), displaying colors ranging from brown to black, and varying in shape from curved to straight, elliptical, cylindrical or obclavate, with 3 to 10 distoseptations (n = 45) (Figure 1).

The observed morphology is typical of fungi in the *Bipolaris*/*Curvularia* complex, closely resembling that described for *Bipolaris yamadae* (Manamgoda et al. 2014), and the isolate was designated as MM_CG01. Genomic DNA from MM_CG01 was extracted from 7-day-old cultures grown on PDA. Mycelium and conidia fragments (0.1 g) were scraped from the surface of colonized agar plates and transferred to 2.0 mL microtubes containing a sterilized stainless-steel bead. The microtubes were then frozen in liquid nitrogen, and the fungal fragments were ground in a laboratory mixer mill (Retsch MM400). Subsequently, DNA purification was performed using the Easy Pure Plant genomic DNA kit (Transgen), following the manufacturer's protocol. For molecular identification,

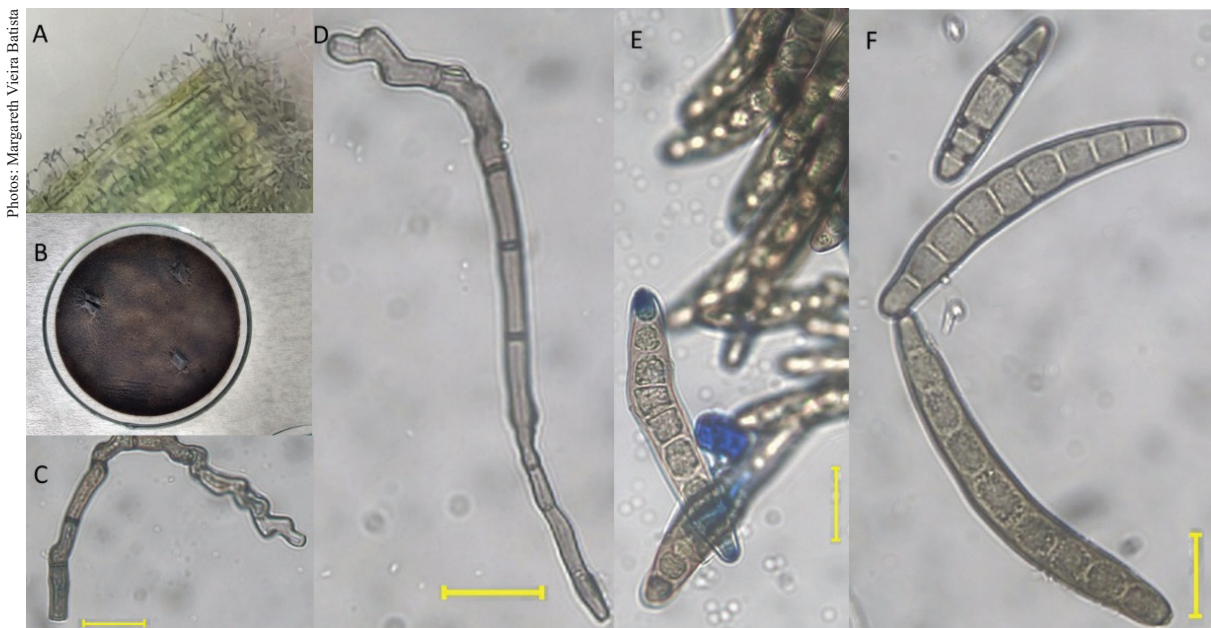

Photos: Margareth Vieira Batista

Figure 1. *Bipolaris yamadae*. A: conidiophores and conidia on the leaf spot; B: top view of the colony on PDA medium; C and D: conidiophores; E and F: conidia. Scale bar: 20 µm.

the primers ITS1/ITS4 (White et al. 1990), EF1-983F/ EF1-1567R (Rehner & Buckley 2005) and gpd1/gpd2 (Berbee et al. 1999) were used to amplify the internal transcribed spacer (ITS) regions of rDNA, part of the translation elongation factor (*TEF-1α*) and part of the glyceraldehyde-3-phosphate dehydrogenase (*GPDH*) genes, respectively. The PCR products were sequenced in both directions at Macrogen (Korea). The consensus sequences were deposited in the GenBank: OR509540 (ITS), OR509454 (*TEF-1α*) and PP273438 (*GPDH*). BLAST analysis in NCBI (Altschul et al. 1990) showed that the sequences showed similarity of 99.91, 99.77 and 99.80 %, respectively, to the type isolate of *B. yamadae* (CBS 202.29). The sequences were aligned using CLUSTALW and the alignment was trimmed for length. Maximum-likelihood trees were constructed with the combined ITS region, *GPDH* and *TEF-1α* genes using 1,000 bootstrap samples based on the K2+G substitution model, selected by Bayesian information criterion using MEGA 11 (Tamura et al. 2021). The analyses placed MM_CG01 in a clade with *B. yamadae* isolates (Figure 2).

To fulfill the Koch's postulates, the isolate was inoculated into BRS Tamani plants. Ten 30-day-old plants were inoculated with a conidial suspension in 0.5 % Tween water adjusted to 10^4 conidia mL^{-1}, using a Neubauer chamber. The suspension was sprayed onto the leaves with an airbrush sprayer until runoff. Ten control plants were inoculated only with 0.5 % Tween water. The plants were kept in a dew chamber for two days and then transferred to a greenhouse for 15 days. The Koch's postulates were replicated three times. Typical symptoms of brown leaf spot were observed (Figure 3) in the plants inoculated with conidia, whereas the control plants did not develop any symptoms.

The isolations performed from the lesions of inoculated plants confirmed the presence of *B. yamadae* structures. Based on molecular and morphological identification, the isolate MM_CG01 associated with brown leaf spot in *M. maximus* was identified as *B. yamadae*. A field experiment was conducted with 205 genotypes of the breeding program of *M. maximus* at the Embrapa Gado de Corte over two years to assess the natural incidence and severity of brown leaf spot. The seedlings were cultivated in Dystrophic Red Latosol (Santos et al. 2018), equivalent to Ferralsols (FAO 2014), 30 % of clay, along four rows of five plants spaced 0.5 m apart.
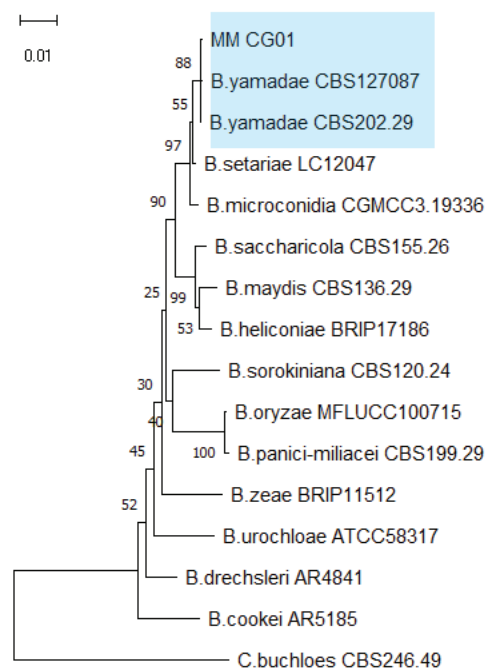

Figure 2. Multilocus (ITS region, *GPDH* and *TEF-1α* sequences) maximum-likelihood tree constructed with 1,000 bootstrap samples based on the K2+G substitution model, selected by Bayesian information criterion. The isolates belong to the *Bipolaris* species and the isolate CBS246.49 of *Curvularia buchloes* was used as outgroup. The isolate from this study (MM_CG01) is highlighted in the branch with other *Bipolaris yamadae* isolates.

The disease severity evaluations began after the first cutting, and subsequent evaluations were performed during the rainy season. A modified version of the diagrammatic scale developed for *B. maydis* severity evaluation (Martinez 2006), as adapted by Fernandes et al. (2011), was employed. The disease was considered severe (lesions covering more than 13 % of the leaf area) in 8.35 % of the analyzed genotypes, causing considerable damage to these plants (Figure 4). To our knowledge, this is the first report of *B. yamadae* infecting *M. maximus* in Brazil.

Bipolaris yamadae was previously reported causing leaf spot on Guinea grass in the United States (Adhikari et al. 2021). The symptoms were similar to those reported in *M. maximus* in Brazil (Wang 2019, Adhikari et al. 2021). It has also been reported to infect other hosts of the *Panicum* genus, such as *P. capillare*, *P. miliaceum* and *P. implicatum*, and in other members of the Poaceae family, such as *Oryza* sp., *Saccharum* sp., *Setaria plicata* and *Zea mays* (Manamgoda et al. 2014, Wang 2019). In Brazil,

Figure 3. Symptoms of brown leaf spot in *Megathyrsus maximus* BRS Tamani. A: natural infection in the field; B and C: symptoms in inoculated plants after 7 and 12 days, respectively.

Figure 4. Severe symptoms of brown leaf spot in a highly susceptible *Megathyrsus maximus* genotype, under natural infection conditions.

it has recently been reported infecting the *Digitaria insularis* weed (Alves et al. 2023). Due to its potential to cause severe symptoms in certain *M. maximus* genotypes and to infect invasive weeds commonly found in pastures, the disease has been prioritized for genotype phenotyping, aiming at resistance in breeding programs.

Further researches on brown leaf spot management need to be performed due to the capacity of *B. yamadae* to infect maize and sorghum (Chávez-Valdez et al. 2023). These two crops hold significant importance in livestock farming and integrated systems with *M. maximus*.

REFERENCES

ADHIKARI, A.; WANG, X.; LANE, B.; HARMON, P. F.; GOSS, E. M. First report of *Bipolaris yamadae* leaf spot disease on Guinea grass (*Panicum maximum*) in Florida. *Plant Disease*, v. 105, n. 4, p. 1208-1208, 2021.

ALTSCHUL, S. F.; GISH, W.; MILLER, W.; MYERS, E. W.; LIPMAN, D. J. Basic local alignment search tool. *Journal of Molecular Biology*, v. 215, n. 3, p. 403-410, 1990.

ALVES, J. F.; FABBRIS, C.; INOKUTI, E. M.; NOBREGA, T. F.; BARRETO, R. W.; VIEIRA, B. S. *Bipolaris yamadae* causes foliage blight of *Digitaria insularis* in Brazil. *Australasian Plant Disease Notes*, v. 18, n. 1, e21, 2023.

BERBEE, M. L.; PIRSEYEDI, M.; HUBBARD, S. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. *Mycologia*, v. 91, n. 6, p. 964-977, 1999.

CHÁVEZ-VALDEZ, M. J.; FONSECA-CHÁVEZ, E.; SÁNCHEZ-KEN, J. G.; MORA-ROMERO, G. A.;

FÉLIX-GASTÉLUM, R.; SÁNCHEZ-SOTO, B. H.; LEYVA-MADRIGAL, K. Y. First report of *Bipolaris yamadae* causing leaf blight on giant reed (*Arundo donax* L.) in Mexico and its pathogenicity on sorghum and maize. *Journal of Plant Diseases and Protection*, v. 130, n. 1, p. 169-175, 2023.

FERNANDES, C. D.; CHERMOUTH, K. S.; JANK, L.; MALLMANN, G.; FERNANDES, E. T.; QUEIROZ, C. A.; CARVALHO, C.; QUETEZ, F. A.; SILVA, M. J.; BATISTA, M. V. Reação de híbridos de *Panicum maximum* à mancha das folhas em condições de infecção natural. *In*: INTERNATIONAL SYMPOSIUM ON FORAGE BREEDING, 3., 2011, Bonito. *Anais...* Campo Grande: Embrapa Gado de Corte, 2011. p. 59-61.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). *World reference base for soil resources*: international soil classification system for naming soils and creating legends for soil maps. Rome: FAO, 2014.

JANK, L. *Desenvolvimento de cultivares de Panicum maximum para a diversificação, sustentabilidade e intensificação das pastagens*. 2019. Available at: https://www.embrapa.br/busca-de-projetos/-/projeto/208929/desenvolvimento-de-cultivares-de-panicum-maximum-para-a-diversificacao-sustentabilidade-e-intensificacao-das-pastagens. Access on: Sep. 4, 2023.

MANAMGODA, D. S.; ROSSMAN, A. Y.; CASTLEBURY, L. A.; CROUS, P. W.; MADRID, H.; CHUKEATIROTE, E.; HYDE, K. D. The genus *Bipolaris*. *Studies in Mycology*, v. 79, n. 1, p. 221-288, 2014.

MARCHI, C.; FERNANDES, C.; VERZIGNASSI, J. *Doenças em plantas forrageiras*. 2011. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/54746/1/Doencas-em-plantas-forrageiras-DOC187.pdf. Access on: Sep. 4, 2023.

MARTINEZ, A. S. *Avaliação do dano provocado por Bipolaris maydis em Panicum maximum cv. Tanzânia-1*. 2006. Dissertação (Mestrado em Agronomia) - Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, 2006.

MARTINEZ, A. D. S.; FRANZENER, G.; STANGARLIN, J. R. Dano causado por *Bipolaris maydis* em *Panicum maximum* cv. Tanzânia. *Semina: Ciências Agrárias*, v. 31, n. 4, p. 863-870, 2010.

REHNER, S. A.; BUCKLEY, E. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps teleomorphs*. *Mycologia*, v. 97, n. 1, p. 84-98, 2005.

SANCHES, M. M.; FERNANDES, C. D.; VERZIGNASSI, J. R.; SANTOS, M. F.; JANK, L.; BATISTA, M. V.; GONÇALVES, D. R.; GUTOSKI, D. V. Etiologia da mancha marrom, uma nova doença foliar em *Panicum maximum* no Brasil. *In*: CONGRESSO BRASILEIRO DE FITOPATOLOGIA, 53., 2023, Brasília. *Anais...* Brasília, DF: Sociedade Brasileira de Fitopatologia, 2023. p. 538.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. A.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; ARAUJO FILHO, J. C.; OLIVEIRA, J. B.; CUNHA, T. J. F. *Sistema brasileiro de classificação de solos*. 5. ed. Brasília, DF: Embrapa, 2018.

TAMURA, K.; STECHER, G.; KUMAR, S. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, v. 38, n. 7, p. 3022-3027, 2021.

WANG, X. *First report of Bipolaris yamadae leaf spot disease on Guinea grass in Florida*. 2019. Dissertação (Mestrado em Plant Science) - University of Florida, Gainesville, 2019.

WHITE, T.; BRUNS, T.; LEE, S.; TAYLOR, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: INNIS, M. A.; GELFAND, D. H.; SNINSKY, J. J.; WHITE, T. J. (ed.). *PCR protocols*: a guide to methods and applications. New York: Academic Press, 1990. p. 315-322.