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Occurrence of *Pyricularia oryzae* Triticum in plants of the genus *Urochloa* in Brazil

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ABSTRACT: *In this study Pyricularia spp., P. oryzae and the P. oryzae pathotype Triticum (PoT) were detected and identified in leaf segments of forage and invasive grasses located in or next to wheat fields. In 2018 and 2019, 66 samples of lesion leaf segments of Urochloa and other grasses were collected in Londrina (PR), Patos de Minas (MG), and Uberaba (MG). The detection and/or identification of the pathogens on the samples was conducted using moist chamber procedures and with the primers MoT3 and Pot2 by PCR. There were DNA amplification with the primer MoT3 (specific for PoT) for 13 (19.69%) of the samples, all of them from Urochloa. The finding that Urochloa hosts PoT at a relatively high rate raises concerns about the importance which these plants may have on the wheat blast cycle as an alternative host for the pathogen and/or source of inoculum for the disease.*
Key words: *wheat blast, primer MoT3, source of inoculum.*

Ocorrência de *Pyricularia oryzae* Triticum em plantas do gênero *Urochloa* no Brasil

RESUMO: *Neste estudo Pyricularia spp., P. oryzae e o patótipo Triticum (PoT) de P. oryzae foram detectados e identificados em segmentos foliares de forrageiras e gramíneas invasoras de lavouras de trigo. Em 2018 e 2019, foram coletadas 66 amostras de segmentos foliares lesionados de Urochloa e outras gramíneas em Londrina (PR), Patos de Minas (MG) e Uberaba (MG). A detecção e/ou identificação dos patógenos nas amostras foi realizada por meio de procedimentos de câmara úmida e com os iniciadores MoT3 e Pot2 por PCR. Houve amplificações de DNA com o primer MoT3 (específico para PoT) em 13 (19,69%) das amostras, todas provenientes da Urochloa. O resultado de que Urochloa hospeda PoT em uma taxa relativamente alta levanta preocupações sobre a importância que essas plantas podem ter no ciclo de brusone do trigo como hospedeiro intermediário para o patógeno e / ou fonte de inóculo para a doença.*
Palavras-chave: *brusone do trigo, primer de PCR MoT3, fonte de inóculo.*

Pyricularia oryzae (syn. *Magnaporthe oryzae*) is a fungus that causes diseases known as rice blast (VALENT & CHUMLEY, 1991), gray leaf spot in perennial ryegrass (FARMAN, 2002) and wheat blast (IGARASHI et al., 1986). According to the host specialization of *P. oryzae*, the fungus is classified in groups of pathotypes (OU, 1980) or lineages (phylogenetically distinct group) (TALBOT et al., 1993). Some of these specializations are the following: *P. oryzae Oryzae* pathotype (PoO; rice blast), *Lolium* pathotype (PoL; gray leaf spot) and *Triticum* pathotype (PoT; wheat blast).

The occurrence of the wheat blast is more common in the tropic regions where the yield losses caused by this disease may reach 100% (MACIEL, 2018) and its presence is especially associated with wheat head blast (CRUZ & VALENT, 2017). The condition of *P. oryzae* with a wide range of hosts including forage and weeds plants (MACIEL et al., 2014; TOSA & CHUMA, 2014; CRUZ & VALENT, 2017) raises concerns about the role and importance that these hosts play in the wheat blast cycle, both as a source of primary inoculum and as part of the process of generating genetic variability in this species of fungus (CASTROAGUDÍN et al., 2016).

In Brazil, plants of the genus *Urochloa* (syn. *Brachiaria*) have received greater attention regarding their possible interference in the wheat blast cycle because they are widely disseminated forages in the Brazilian agricultural system (CASTROAGUDÍN

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et al., 2016; REGES et al., 2016; REGES et al., 2019). An estimate made in 2012 indicated that the participation of species of the genus *Urochloa* as cultivated pasture in Brazil was 70% of a total area of about 117 million hectares (ZIMMER et al., 2012). Furthermore, the record made by URASHIMA et al. (1993) that PoT isolates were able to recombine sexually, producing perithecia with viable ascospores when crossed under controlled conditions with *P. oryzae* from *Urochloa plantaginea,* adds more worries about the possible influence of *Urochloa* on the development of wheat blast. Accordingly, other grasses may play an important role on wheat blast epidemiology such as *Panicum maximum, P. miliaceum, Eleusine coracana, Lolium perenne*, *Stenotaphrum secundatum, Rhynchelytrum roseum* (URASHIMA et al., 1993; FARMAN, 2002; COUCH et al., 2005; TOSA & CHUMA, 2014; CRUZ & VALENT, 2017; PAK et al.; 2021).

To know more about the role of alternative hosts in the wheat blast cycle is important to confirm if PoT is really infecting these hosts in the natural environmental, especially in the wheat fields. This can be accomplished by accurate methods of diagnosis and identification of the pathogen. Conventionally used, the cultural diagnosis of *P. oryzae* based on "the moist chamber procedures" requires a minimum incubation period for the fungus (KRUG, 2004) and the use of a microscope to find the reproductive structures of the pathogen. The diagnostic technique using molecular methods based on the polymerase chain reaction (PCR) in plant tissues is a precise, fast, and sensitive option for several pathosystems (LAU & BOTELLA, 2017).

Some PCR primers for the detection of *P. oryzae* infecting plants have been reported. Two of them are specific to PoT, MoT3 (PIECK et al. 2017) and C17 (THIERRY et al., 2020), which amplify DNA fragments with 361 and 500 base pairs (bp), respectively. In addition to these, the PCR primer Pot2 has also been used; although, it is not specific for any pathotype of *P. oryzae*, that is, it amplifies 410 bp fragments in any positive detection of *P. oryzae* (KACHROO et al., 1994).

The objective of this study was to detect and identify *Pyricularia* spp., *P. oryzae,* and its pathotype PoT in leaf segments of forage and invasive grasses located into or next to wheat fields

Leaf segments with symptoms of lesions were collected from forage and invasive grass plants in agricultural areas of three Brazilian municipalities: Londrina, PR; Patos de Minas, MG; and Uberaba, MG. These samples were sent to Embrapa Trigo, Passo Fundo, RS, where, in the Phytopathology and Biotechnology laboratories, they were subjected to evaluation for detection and identification of *P. oryzae* and PoT. Initially, the samples were registered and subjected to drying at 40-50 ºC for 30 min, and stored at -20 ºC. From each sample and using a scissors, two very homogeneous sub-samples were formed in relation to the symptoms they presented; A-sub-samples and B-sub-samples. Depending on the sub-sample, A or B, each sub-sample was subjected to one of the following methods of detection and identification of plant pathogen fungi; (A) cultural, subjected to moist chamber procedures, and (B) molecular, by PCR technique.

Moist chambers were conducted in Petri dishes. Three sheets of blotting paper were placed inside each plate and moistened with distilled and autoclaved water. Two leaf segments of each A-subsample were allocated per plate, in four replications. The plates were randomly placed under continuous fluorescent illumination (25 µm m^{2-1} s^{-1}) at 25 ºC +/-2, and photoperiod of 12 h, for 120 h. After 7 days, the leaf segments were carefully examined with the aid of stereoscopic magnifiers and compound light microscopes. Positive detections of *Pyricularia* spp. were confirmed based on the visualization of conidiophores and conidia of the fungus.

The molecular detection and identification of *P. oryzae* and PoT was carried out in the DNA extracted from the B-sub-samples. Each one of them provided material to conduct two DNA extractions and each DNA extraction was done in five lesioned leaf segments of approximately 2 x 2 cm. The PCR primers used were MoT3 (PIECK et al., 2017) and Pot2 (KACHROO et al., 1994). DNA amplification was done in a capillary electrophoresis system. Data were evaluated using the GENEMAPPER®ID software – Version 3.7, generating the electropherograms.

Cultural detection via moist chamber allowed us to identify reproductive structures of *Pyricularia* spp. in 32 of the 66 sub-samples evaluated, equivalent to 48.48% of the sub-samples (Table 1). From the 2018 and 2019 sub-samples, out of 32 and 34, 14 and 18 of them showed sporulation of *Pyricularia* spp., respectively. These results represent;respectively, 43.75% and 52.94% of the subsamples with positive detection for *Pyricularia*. There was abundant production of conidia in all samples with positive detection of the fungus by the cultural method. *Pyricularia* sporulation in symptomatic lesions of plants of the genus *Urochloa* occurred in typical and characteristic blast lesions, with elliptical shape and gray center.

Table 1 - Detection and identification of *Pyricularia* spp., *P. oryzae* and *P. oryzae* Triticum on symptomatic segments of poaceae plants collected in three Brazilian municipalities.

Year of sampling	Sample	Local	Host plant	Cultural detection	---------PCR primer[2]--------	
					MoT3	PoT2
2018	1	Patos de Minas - MG	*Urochloa brizantha*	+[1]	+	+
	2	Patos de Minas - MG	*Urochloa brizantha*	+	-	+
	3	Patos de Minas - MG	*Urochloa brizantha*	+	-	+
	4	Patos de Minas - MG	*Urochloa brizantha*	+	-	+
	5	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	6	Patos de Minas - MG	*Urochloa brizantha*	+	+	+
	7	Patos de Minas - MG	*Urochloa brizantha*	+	+	+
	8	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	9	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	10	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	11	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	12	Patos de Minas - MG	*Urochloa decumbens*	-	-	-
	13	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	14	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	15	Patos de Minas - MG	*Urochloa decumbens*	-	-	-
	16	Patos de Minas - MG	*Urochloa decumbens*	+	+	+
	17	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	18	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	19	Londrina - PR	*Urochloa brizantha*	+	+	+
	20	Londrina - PR	*Urochloa brizantha*	-	+	+
	21	Londrina - PR	*Urochloa brizantha*	+	+	+
	22	Londrina - PR	*Urochloa brizantha*	-	-	-
	23	Londrina - PR	*Urochloa brizantha*	-	-	-
	24	Londrina - PR	*Urochloa brizantha*	-	-	-
	25	Londrina - PR	*Urochloa ruziziensis*	-	-	-
	26	Londrina - PR	*Urochloa ruziensis*	-	-	-
	27	Londrina - PR	*Panicum maximum*	-	-	-
	28	Londrina - PR	*Urochloa mutica*	-	-	-
	29	Londrina - PR	*Urochloa plantaginea*	-	-	-
	30	Londrina - PR	*Urochloa ruziensis*	-	-	-
	31	Londrina - PR	*Urochloa ruziensis*	-	-	-
	32	Londrina - PR	*Urochloa ruziensis*	-	-	-
2019	33	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	34	Patos de Minas - MG	*Urochloa brizantha*	+	+	+
	35	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	36	Patos de Minas - MG	*Urochloa brizantha*	-	-	-
	37	Patos de Minas - MG	*Urochloa decumbens*	-	-	-
	38	Patos de Minas - MG	*Urochloa decumbens*	+	+	+
	39	Patos de Minas - MG	*Urochloa decumbens*	+	-	+
	40	Patos de Minas - MG	*Urochloa decumbens*	-	-	-
	41	Patos de Minas - MG	*Urochloa humidicola*	+	-	+
	42	Patos de Minas - MG	*Urochloa humidicola*	-	-	-
	43	Patos de Minas - MG	*Urochloa humidicola*	-	-	-
	44	Patos de Minas - MG	*Urochloa humidicola*	-	-	-
	45	Uberaba - MG	*Andropogon gayanus*	-	-	-
	46	Uberaba - MG	*Urochloa brizantha*	+	+	+
	47	Uberaba - MG	*Urochloa brizantha*	+	+	+
	48	Uberaba - MG	*Urochloa brizantha*	-	-	-
	49	Uberaba - MG	*Urochloa brizantha*	+	-	+
	50	Uberaba - MG	*Urochloa brizantha*	+	-	+
	51	Uberaba - MG	*Urochloa brizantha*	+	-	+
	52	Uberaba - MG	*Urochloa brizantha*	-	-	-
	53	Uberaba - MG	*Urochloa brizantha*	+	-	+
	54	Uberaba - MG	*Urochloa brizantha*	+	-	+
	55	Uberaba - MG	*Urochloa decumbens*	+	+	+
	56	Uberaba - MG	*Urochloa decumbens*	+	-	+
	57	Uberaba - MG	*Urochloa decumbens*	+	+	+
	58	Uberaba - MG	*Urochloa hibrida*	+	-	+
	59	Uberaba - MG	*Urochloa hibrida*	+	-	+
	60	Uberaba - MG	*Urochloa mutica*	-	-	-
	61	Uberaba - MG	*Cynodon dactylon*	-	-	-
	62	Uberaba - MG	*Cynodon datylon*	-	-	-
	63	Uberaba - MG	*Cynodon plectostachyus*	+	-	+
	64	Uberaba - MG	*Melinis minutiflora*	+	-	+
	65	Uberaba - MG	*Panicum maximum*	-	-	-
	66	Uberaba - MG	*Setaria anceps*	-	-	-

[1]"+" and "–" mean presence or absence, respectively, of *Pyricularia* spp., *P. oryzae* Triticum, *P. oryzae* depending on the evaluation.
[2]Primers PCRs PoT2 and MoT3 were used considering their specificity for *P. oryzae* and *P. oryzae* Triticum, respectively.

All samples that showed conidia sporulation of *Pyricularia* spp. in the cultural diagnosis also had positive detection when PCR primer Pot2 was used. Out of 66 samples, in 23 there was amplification for the Pot2 marker (34.85%), and in 13 for the MoT3 marker (19.69%). From the 2018 sub-samples, out of 32, 15 amplified for Pot2 (46.87%) and 7 for MoT3 (21.87%). From the 2019 sub-samples, out of 34, 18 amplified for Pot2 (52.94%) and 6 for MoT3 (17.65%). The positive control resulted in PCR amplification for the two primers used in the study. The DNA used for that was from samples of wheat plants, which had been subjected to artificial inoculation with PoT conidia. The results obtained demonstrated PCR efficiency and sensitivity, as amplification was observed in all wheat samples, except for the negative control (without DNA) and in one sample in which was used DNA of *Fusarium graminearum*. It was also detected the presence of *P. oryzae* in two forage species not belonging to the genus *Urochloa*, which were the following ones, *Cynodum plectostachyus* and *Melinis minutiflora*.

As the identification of PoT in the subsamples was based on the DNA amplification promoted by the PCR primer MoT3, it is important to consider some observations already registered regarding the specificity of this diagnostic system for the causal agent of wheat blast. PIECK et al. (2017) testing PCR primer MoT3 observed no amplification for a wheat-infecting isolate and presence of amplification for a grass weed isolate (*Bromus tectorum*) collected into a wheat field. GUPTA et al., 2019 also verified that MoT3 did not distinguish between rice and wheat isolates from Bangladesh. According to the authors of the MoT3 diagnostic primer, different PCR conditions, reagents, and thermocycler machines could be the reasons for the unexpected results. After that, the authors retested the specificity of the MoT3 using the same isolates and it was reconfirmed the previous results (YASUHARA-BELL et al., 2019).

The finding about the occurrence of PoT in *Urochloa* raises a lot of concern about wheat blast in Brazil, especially in places where this disease occurs more frequently. These concerns refer to potential consequences of this biological event. One of them may be the possibility of increasing of the genetic variability of the pathogen under natural conditions. This possibility has relation with the records done by Urashima et al (1993) about the occurrence of sexual recombination between isolates of PoT and of *P. oryzae* from *Urochloa plantaginea*. This situation may mean greater difficulties for the grower, since new variants of the pathogen, eventually, may present (a) ability to break any resistance to wheat blast present in wheat cultivars; or (b) have less sensitivity to fungicides used to control this disease. It is also important to consider the particularities of the wheat crop in Brazil, where the fields are established in sequence with summer crops (soybean and corn), providing the return of wheat plants to the field after a period that normally lasts from 5 to 8 months. As PoT does not survive in wheat straw for more than 5 months (PIZOLOTTO et al., 2019), plants such as those of the genus *Urochloa* can play very important key role in the wheat blast cycle as an intermediate host for the pathogen and source of inoculum for the disease.

The findings that *Urochloa* plants host PoT at relatively high rate raises concerns about their importance on wheat blast cycle and based on that we recommend further studies on the subject. Concerns come from the condition that Urochloa is widely used as forage in Brazil, normally established in locations relatively close to wheat fields, and some plant species of this genus are weeds on wheat crop.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

JLNM, ALVB and IFDC conceived and designed experiments. ANS and MK carried out the lab and statistical analyses of experimental data. JLNM prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

CASTROAGUDÍN, V. L., et al. *Pyricularia graminis-tritici*, a new *Pyricularia* species causing wheat blast. **Persoonia: Molecular Phylogeny and Evolution of Fungi**, v.37, p.199-216, 2016. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5315288/>. Accessed: Oct. 03, 2020. doi: 10.3767/003158516X692149.

COUCH, B. C., et al. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. **Genetics**, 170:613–630, 2005. Available from: <https://pubmed.ncbi.nlm.nih.gov/15802503/>. Accessed: Sep. 16, 2021. doi: 10.1534/genetics.105.041780.

CRUZ, C. D.; VALENT, B. Wheat blast disease: danger on the move. **Tropical Plant Pathology** 42:210–222, 2017. Available from: <https://link.springer.com/ article/10. 1007/s40858-017-0159-z>. Accessed: Oct. 24, 2021. doi: 10.1007/s40858-017-0159-z.

FARMAN, M. L. *Pyricularia grisea* isolates causing gray leaf spot on perennial ryegrass (*Lolium perenne*) in the United States: Relationship to *P. grisea* isolates from other host /lants. **Phytopathology**, 92:245–254, 2002. Available from: <https://pubmed. ncbi.nlm.nih.gov/18943995. Accessed: Oct. 10, 2021. doi: 10.10 94/PHYTO.2002.92.3.245.

GUPTA, D. R., et al. Cautionary notes on use of the MoT3 diagnostic assay for *Magnaporthe oryzae* wheat and rice blast isolates. **Phytopathology**, 109:504-508, 2019. Available from: <https://apsjournals.apsnet.org/doi/full/10.1094/PHYTO-06-18-0199-LE?mobileUi=0>. Accessed: Oct. 16, 2021. doi: 10.1094/PHYTO-06-18-0199-LE.

IGARASHI, S., et al. Occurrence of *Pyrcularia* sp. in wheat (*Triticum aestivum* L.) in the State of Paraná, Brazil. **Fitopatologia Brasileira**, 11:351–352, 1986.

KACHROO, P., et al. Pot2, an inverted repeat transposon from the rice blast fungus *Magnaporthe grisea*. **Molecular General Genetics**, v.245, p.39-48, 1994. Available from: <https://pubmed.ncbi.nlm.nih.gov/7816044>. Accessed: Oct. 14, 2021. doi: 10.1007/bf00290114.

KRUG, J. C. Moist chambers for the development of fungi. In: MUELLER, G. M.; BILLS, G. F.; FOSTER, M. S. **Biodiversity of Fungi: Inventory and Monitoring Methods**, Eds. New York, Academic Press, pp.569–574, 2004.

LAU, Y. H.; BOTELLA, J. R. Advanced DNA-Based point-of-care diagnostic methods for plant diseases detection. **Frontiers in Plant Science**. 2017. Available from: <https://www.frontiersin.org/articles/10.3389/fpls.2017.02016/full>. Accessed: Sep. 30, 2021. doi: 10.3389/fpls.2017.02016.

OU, S. H. Pathogen variability and host resistance in rice blast disease. **Annual Reviews Phytopathology**, 18:167–187, 1980. Available from: <https://www.annualreviews.org/doi/abs/10.1146/annurev.py.18.090180.001123>. Accessed: Oct. 14, 2021. doi: 10.1146/annurev.py.18.090180.001123.

MACIEL, J. L. N. Diseases affecting wheat: wheat blast. In: OLIVER, R. (Org.), **Integrated disease management of wheat and barley**. Cambridge: Burleigh Dodds Science Publishing, p.155-169, 2018.

MACIEL, J. L. N. et al. Population structure and pathotype diversity of the wheat blast pathogen *Pyricularia oryzae* 25 years after its emergence in Brazil. **Phytopathology**, v.104, n.1, p.95-107, 2014. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/23901831>. Accessed: Oct. 19, 2020. doi: 10.1094/PHYTO-11-12-0294-R.

PAK, D. et al. Management of rice blast (*Pyricularia oryzae*): implications of alternative hosts. **European Journal of Plant Pathology**. 2021. Available from: <https://research-repository.uwa.edu.au/en/publications/management-of-rice-blast-pyricularia-oryzae-implications-of-alter>. Accessed: Oct. 24, 2021. doi: 10.1007/s10658-021-02326-4.

PIECK, M. L. et al. Genomics-based marker discovery and diagnostic assay development for wheat blast. **Plant Disease**, 101:103–109, 2017. Available from: <https://apsjournals.apsnet.org/doi/10.1094/PDIS-04-16-0500-RE>. Accessed: Aug. 20, 2021. doi: 10.1094/PDIS-04-16-0500-RE.

PIZOLOTTO, C. A. et al. Saprotrophic survival of *Magnaporthe oryzae* in infested wheat residues. **European Journal of Plant Pathology**, 153:327–339, 2019. Available from: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/195860/1/ID44558-2019v153p327EurJPlantPathol.pdf>. Accessed: Oct. 22, 2021. doi: 10.10 07/s10658-018-1578-5.

REGES, J. T. de A. et al. *Pyricularia pennisetigena* and *P. zingibericola* from invasive grasses infect signal grass, barley and wheat. **Pesquisa Agropecuária Tropical**, 46:206–214, 2016. Available from: <https://www.scielo.br/j/pat/a/h6rNLyD58RWN4Wvs6m5gFZp/?lang=en>. Accessed: Oct. 16, 2021. doi: 10.1590/1983-40632016v4641335.

REGES, J. T. de A. et al. Teste de patogenicidade dos isolados de *Pyricularia oryzae* nos hospedeiros de trigo, cevada, arroz e braquiária. **Revista Cultura Agronômica**, 28:19–28, 2019. Available from: <https://ojs.unesp.br/index.php/rculturaagronomica/article/view/2446-8355.2019v28n1p19-28>. Accessed: Oct. 21, 2021. doi: 10.32929/2446-8355.2019v28n1p19-28.

TALBOT, N. J. et al. Karyotypic variation within clonal lineages of the rice blast fungus, *Magnaporthe grisea*. **Applied and Environmental Microbiology**, 59:585–593, 1993. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC202148/>. Accessed: Aug. 26, 2021. doi: 10.1128/aem.59.2.585-593.1993.

THIERRY, M. et al. A genomic approach to develop a new qPCR test enabling detection of the *Pyricularia oryzae* lineage causing wheat blast. **Plant Disease**, 104:60–70, 2020. Available from: <https://doi.org/10.1094/PDIS-04-19-0685-RE>. Accessed: Oct. 24, 2021. doi: 10.1094/PDIS-04-19-0685-RE.

TOSA, Y.; CHUMA, I. Classification and parasitic specialization of blast fungi. **Journal of General Plant Pathology**, 80:202–209, 2014. Available from: <https://www.researchgate.net/publication/271639900_Classification_and_parasitic_specialization_of_blast_fungi>. Accessed: Oct. 19, 2021. doi: 10.1007/S10 327-014-0513-7.

URASHIMA, A. S. et al. Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. **Plant Disease**, 77:1211–1216, 1993. Available from: <https://www.apsnet.org/publications/PlantDisease/BackIssues/Documents/1993Abstracts/PD_77_1211.htm>. Accessed: Oct. 3, 2021. doi: 10.1094/PD-77-1211.

VALENT, B.; CHUMLEY, F. G. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. **Annual Reviews Phytopathology**, 29:443–467, 1991. Available from: <https://www.annualreviews.org/doi/abs/10.1146/annurev.py.29.090191.002303>. Accessed: Sep. 29, 2021. doi: 10.1146/annurev.py. 29.090191.002303.

YASUHARA-BELL, J. et al. A response to GUPTA et al. (2019) regarding the MoT3 wheat blast diagnostic assay. **Phytopathology**, 109:509–511, 2019. Available from: <https://apsjournals.apsnet.org/doi/pdf/10.1094/PHYTO-10-18-0397-LE>. Accessed: Oct. 14, 2021. doi: 10.1094/PHYTO-10-18-0397-LE.

ZIMMER, A. H. et al. **Degradação, recuperação e renovação de pastagens**. Campo Grande, MS: Embrapa Gado de Corte, 2012. 42 p.: 21 cm. – (Documentos / Embrapa Gado de Corte, ISSN 1983-974X; 189). Available from: <http://www.cnpgc.embrapa.br/publicacoes/doc/DOC189.pdf>. Accessed: Oct. 25, 2021.