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Control of *Magnaporthe oryzae* Pathotype *Lolium* on Ryegrass (*Lolium multiflorum* Lam.) Seeds Using Fungicides

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Abstract

Blast, caused by the fungus *Magnaporthe oryzae* pathotype *Lolium* (MoL; anamorph *Pyricularia oryzae*), is a foliar disease capable of inducing severe yield losses and impairing both the sanitary and physiological quality of Italian ryegrass (*Lolium multiflorum* Lam.) seeds. Although the seed is not considered the primary source of inoculum for epidemic development, MoL can be transmitted to the coleoptile, leading to seedling mortality, compromising pasture establishment, and facilitating pathogen dissemination to new fields and production areas. Information regarding seed-to-coleoptile transmission and the control of this pathogen through seed-applied fungicides in ryegrass remains limited. The objectives of this study were to determine the transmission rate of MoL from seed to coleoptile, assess its impact on seedlings, and evaluate the efficacy of six fungicide seed treatments, as well as their effects on germination and early seedling vigor under laboratory conditions. The experiment was conducted at the Seed Laboratory of INIA La Estanzuela using a seed lot with 27% MoL incidence, as determined by the blotter test according to ISTA rules. In untreated seeds, MoL transmission from seed to coleoptile reached 55.6%, resulting in 11.7% seedling mortality. Treatments T3 (experimental), T4 (sedaxane + difenoconazole + fludioxonil), and T7 (carbendazim + thiram + iprodione) resulted in no detectable MoL infection in coleoptiles under the experimental conditions. No phytotoxic effects on seed germination or seedling vigor were detected in any treatments.

Keywords: germination, gray leaf spot, seed pathology, seed treatment

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Control de *Magnaporthe oryzae* patotipo *Lolium* en semillas de raigrás (*Lolium multiflorum* Lam.) con fungicidas

Resumen

El brusone, causado por el hongo *Magnaporthe oryzae* patotipo *Lolium* (MoL, anamorfo *Pyricularia oryzae*), es una enfermedad foliar que puede ocasionar pérdidas severas de rendimiento y deteriorar la calidad sanitaria y fisiológica de las semillas de raigrás (*Lolium multiflorum* Lam.). Si bien la semilla no constituye la principal fuente de inóculo para epidemias, MoL puede transmitirse al coleóptilo, provocando mortalidad de plántulas, comprometiendo la implantación de la pastura y facilitando la diseminación del patógeno hacia nuevos lotes y áreas de producción. La información disponible sobre la transmisión semilla-coleóptilo y el control del patógeno mediante fungicidas aplicados a semillas de raigrás es limitada. El objetivo de este trabajo fue determinar la tasa de transmisión de MoL desde la semilla al coleóptilo, evaluar su efecto sobre las plántulas y analizar la eficacia de seis tratamientos fungicidas aplicados a la semilla, así como su impacto sobre la germinación y el vigor inicial, en condiciones de laboratorio. El ensayo se realizó en el Laboratorio de Semillas de INIA La Estanzuela; se utilizaron semillas con una incidencia del patógeno de 27 %, determinada mediante blotter test según normas ISTA. En las semillas sin tratar, la transmisión de MoL desde la semilla al coleóptilo fue de 55,6 %, lo que resultó en una mortalidad de plántulas de 11,7 %. En los tratamientos T3 (experimental), T4 (sedaxane + difenoconazole + fludioxonil) y T7 (carbendazim + thiram + iprodione) no se detectó infección por MoL en los coleóptilos bajo las condiciones evaluadas. No se detectaron efectos fitotóxicos sobre la germinación ni el vigor de las plántulas en ninguno de los tratamientos.

Palabras clave: germinación, mancha foliar gris, sanidad de semillas, tratamiento de semillas

Controle de *Magnaporthe oryzae* patotipo Lolium em sementes de azevém (*Lolium multiflorum* Lam.) com fungicidas

Resumo

O brusone, causado pelo fungo *Magnaporthe oryzae* patótipo *Lolium* (MoL, anamorfo *Pyricularia oryzae*), é uma doença foliar que pode ocasionar perdas severas de rendimento e comprometer a qualidade sanitária e fisiológica das sementes de azevém (*Lolium multiflorum* Lam.). Embora a semente não constitua a principal fonte de inóculo para epidemias, MoL pode ser transmitido ao coleóptilo, provocando mortalidade de plântulas, prejudicando o estabelecimento da pastagem e favorecendo a disseminação do patógeno para novos lotes e áreas de produção. As informações disponíveis sobre a transmissão semente–coleóptilo e o controle do patógeno por meio de fungicidas aplicados às sementes de azevém ainda são limitadas. O objetivo deste trabalho foi determinar a taxa de transmissão de MoL da semente ao coleóptilo, avaliar seus efeitos sobre as plântulas e analisar a eficácia de seis tratamentos fungicidas aplicados às sementes, bem como seu impacto sobre a germinação e o vigor inicial, em condições de laboratório. O ensaio foi conduzido no Laboratório de Sementes do INIA La Estanzuela, utilizando sementes com incidência do patógeno de 27 %, determinada pelo teste de papel de filtro (blotter test), de acordo com as normas da ISTA. Nas sementes não tratadas, a transmissão de MoL da semente ao coleóptilo foi de 55,6 %, resultando em mortalidade de plântulas de 11,7 %. Os tratamentos T3 (experimental), T4 (sedaxano + difenoconazol + fludioxonil) e T7 (carbendazim + tiram + iprodiona) não apresentaram infecção detectável por MoL nos coleóptilos nas condições experimentais. Não foram detectados efeitos fitotóxicos sobre a germinação nem sobre o vigor das plântulas em nenhum dos tratamentos.

Palavras-chave: germinação, mancha cinza na folha, sanidade de sementes, tratamento de sementes

1. Introduction

Ryegrass blast, caused by *Magnaporthe oryzae* pathotype *Lolium* (MoL), is a disease widely distributed in forage grass-producing areas worldwide. In Uruguay, an increase in the incidence and severity of the disease has been observed in recent years, particularly in northern and eastern regions of the country. Early infections have been recorded in these areas, causing seedling mortality and compromising the correct implantation of the pasture.

Foliar symptoms are characterized by elongated, grayish to brown necrotic lesions (Rossi & Pereyra, 2018). The disease can also affect the rachis of the spikes, causing total or partial abortion of the spikelets and significant reductions in seed production (Martínez & Escalante, 2016).

The primary inoculum of the pathogen comes mainly from infected stubble from nearby crops or grasslands, as well as from volunteer plants, where MoL can survive between harvests. Under favorable environmental conditions, temperatures between 25 and 28 °C, relative humidity above 90% and prolonged periods of leaf wetting, especially during the night, conidia are formed and dispersed by the wind, giving rise to epidemics (Martínez & Escalante, 2016).

Worldwide, there are no ryegrass cultivars with genetic resistance to MoL, so the chemical management of blast through foliar applications is a fundamental control strategy. However, this control is essentially preventive and highly dependent on the occurrence of favorable environmental conditions for infection.

In Uruguay, Martínez and Escalante (2016) detected the presence of MoL in seeds from ryegrass seedbeds with a history of blast, although at incidences below 1%. Even though the seed is not the main source of inoculum in the crop, it can play a relevant role in the introduction and dispersion of the pathogen to new productive areas. In this context, chemical seed treatment is presented as a potentially effective strategy to reduce seed-seedling transmission of MoL.

Goulart and Paiva (1991) and Lasca et al. (2001) evaluated different chemical seed treatments for the control of MoL in wheat, reporting complete infection control through the use of iprodione, thiophanate-methyl, and carbendazim. Likewise, the use of azoxystrobin as a seed treatment has been evaluated in systems with high inoculum pressure from stubble and environmental conditions favorable to the development of blast. A reduction of 80% in the foliar severity of the disease was observed 30 days after sowing (Lobo, 2004).

In Uruguay, previous studies evaluating fungicides applied to seeds for the control of MoL are scarce and have been limited to rice cultivation. In this regard, Ávila et al. (personal communication, 2010) evaluated the efficacy of different fungicides applied as seed treatment for the control of blast, with artificial inoculation of seedlings. Tricyclazole, applied at a dose of 100 g of active ingredient per 100 kg of seed, showed the highest control efficiency at 39 days after inoculation, although the level of control was moderate (25%) and it was associated with a reduction in germination compared to the untreated control. However, there is limited information on the transmission capacity of MoL from infected seeds to ryegrass seedlings and on the efficacy of fungicide seed treatments as a strategy to reduce this process.

The objective of this study was to analyze the transmission of MoL from ryegrass seeds to the coleoptile, its impact on seedlings, and the efficacy of different fungicide treatments applied to the seeds for pathogen control. The working hypothesis was that MoL is transmitted from the seed to the coleoptile in ryegrass and that the application of fungicides as a seed treatment can reduce this transmission without compromising germination or initial seedling vigor.

2. Materials and Methods

2.1 Selection of Experimental Material

Thirteen commercial seed lots of annual ryegrass (*Lolium multiflorum* Lam.) were analyzed, corresponding to the La Estanzuela 284 variety, from seedbeds located in western coastal and eastern Uruguay, sent by different seed production companies.

MoL diagnosis was performed using the blotter test method, following the recommendations of the International Seed Testing Association (ISTA, 2025). The seeds were placed on filter paper moistened with sterile distilled water in 90-mm-diameter Petri dishes and incubated at 25 ± 2 °C, under a 12-h light/12-h dark regime, for seven days.

At the end of the incubation period, the seeds were examined under a stereoscopic microscope and a light microscope for pathogen identification, considering the typical morphological characteristics of MoL conidia (ISTA, 2025). The incidence of the pathogen was expressed as the percentage of infected seeds.

Based on the health diagnosis, the seed lot with the highest incidence of MoL was selected for the evaluation of seed-to-coleoptile transmission and the efficacy of fungicide treatments.

2.2 Treatments

2.2.1 Description of Treatments

Commercially available fungicides and an experimental product were evaluated, applied as seed treatments, without specific registration for the control of MoL in ryegrass. The treatments evaluated, their active ingredients, and the doses applied, expressed as grams of active ingredient per 100 kg of seed, are detailed in Table 1. The T1 treatment corresponded to the control, with no seed treatment applied.

Table 1. Treatments evaluated, active ingredients, and doses applied in seed treatment for the control of MoL in ryegrass

Treatment code	Active ingredient (a.i.)	Dose applied (g a.i. 100 kg^{-1} seed)
T1	—	—
T2	iprodione + fluopyram	122.5 + 49
T3	thiophanate-methyl + metalaxyl-M + penflufen	315 + 17.5 + 17.5
T4	sedaxane + difenoconazole + fludioxonil	20 + 10 + 10
T5	fluxapyroxad + triticonazole	24.9 + 2.5
T6	azoxystrobin + thiamethoxam	60 + 35
T7	carbendazim + thiram + iprodione	50 + 50 + 25

2.2.2 Application of Treatments to Seeds

For each treatment, 50 g of seeds were used, to which a suspension of the corresponding fungicide in distilled water was applied, with a total volume equivalent to 1.5% of the sample weight. The application was carried out in a rotary drum (Batch Lab Treater, model BLT; Gustafson Inc., United States), with continuous rotation for 1.5 min. After the application, the seeds were dried at 25 °C for 24 h before being used in the trials. The equipment was thoroughly washed between treatments to prevent cross-contamination.

2.3 Efficacy of Treatments on Seed-to-Coleoptile Transmission of MoL

The efficacy of fungicide treatments was determined from the reduction of pathogen transmission from seed to coleoptile, compared to the untreated control (T1). The transmission rate was calculated as the ratio of infected coleoptiles to the incidence of MoL in the seeds of the selected lot. This procedure represents a population estimate of seed-to-coleoptile transmission and did not involve individual monitoring of each infected seed. This approach was adopted because fungicide treatments can inhibit the superficial development of the pathogen on the seed without necessarily eliminating the internal infection, making it difficult to reliably verify the continuity of infection between an individual seed and its corresponding coleoptile.

For this evaluation, seedlings obtained from seeds incubated using the blotter test technique were used (ISTA, 2025). At 15 days of incubation, the number of coleoptiles showing symptoms consistent with blast was

recorded. Subsequently, each sample was examined under a stereoscopic microscope and a light microscope to confirm the presence of MoL by observing its characteristic conidia. Only coleoptiles with morphological confirmation of the pathogen were considered infected (Figure 1).

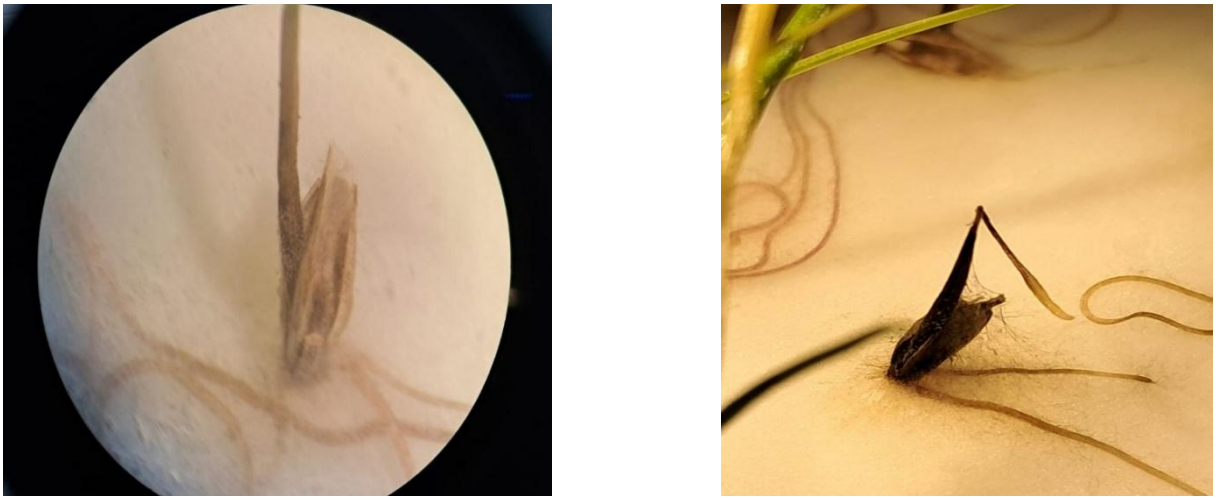

Figure 1. Development of MoL infection in coleoptiles of *Lolium multiflorum*

Left: presence of MoL mycelium colonizing the coleoptile, corresponding to the tissue colonization stage. Right: death of the seedling associated with the infection of the coleoptile.

2.4 Germination and Vigor

For this evaluation, a seed lot free of pathogenic fungi was selected to ensure that the effects observed could be exclusively attributed to the treatments. Germination was evaluated using the paper-based sowing method in accordance with ISTA rules (ISTA, 2025). Seeds were incubated at alternating temperatures of 20-30 °C, with a photoperiod of 16 h darkness/8 h light. The germination percentage was determined 14 days after sowing.

Vigor was estimated from the combined dry weight of 30 seedlings randomly selected by treatment at 14 days after sowing, dried in a forced-air oven at 60 °C for 48 h (Undersander et al., 1993).

2.5 Experimental Design

The trials were conducted under a randomized complete block design, in which replicates corresponded to different sowing dates, considered as the blocking factor. For the seed health and seed-to-coleoptile transmission trials, the experimental unit consisted of a plate, in which 20 seeds were sown per treatment, with eight replicates. For the evaluation of seed germination and vigor, the experimental unit was the germination roll, in which 50 seeds were sown per treatment, with eight replicates.

2.6 Statistical Analysis

The variables incidence of MoL in seeds and seed-to-coleoptile transmission were analyzed using generalized linear mixed models, assuming a binomial distribution and a logit link function. Given that some treatments showed complete absence of infection (0% incidence) and generated an excess of zeros not attributable to chance but to the effect of fungicide treatments, zero-inflated binomial models were used.

Germination was analyzed assuming a binomial distribution with a logit link function. The dry weight of seedlings, used as an estimator of vigor, was analyzed using general linear models, assuming a normal distribution and homogeneity of variances. The assumptions of residual normality and homoscedasticity were verified prior to the analysis.

Treatments were considered as fixed effects, while sowing dates (replicates) were incorporated as blocks in the model, according to a randomized complete block design. Multiple comparisons of means between treatments were performed using Tukey's procedure ($\alpha = 0.05$) on the model-estimated means. Statistical analyses were performed using the InfoStat software with the integrated console of the R environment.

3. Results and Discussion

3.1 Selection of the Experimental Material

Of the 13 commercial ryegrass seed lots analyzed, only two presented MoL, with incidences of 5 and 27%. Although Martínez and Escalante (2016) reported incidences close to 1% in commercial lots, the results obtained indicate that, in certain situations, the infection can reach considerably higher values, representing a risk to crop establishment and a high inoculum potential for pasture.

For the trials, the seed lot that presented the highest incidence of the pathogen (27%) was selected to evaluate the efficacy of fungicide treatments under conditions of higher inoculum pressure.

3.2 Efficacy of Fungicide Treatments

In the control treatment, the transmission of MoL from the seed to the coleoptile was 55.6% (Table 2) and was associated with high seedling mortality, indicating that infected seeds may play an important role in the spread of the pathogen and in the reduction of pasture establishment. These results are consistent with those reported by Rossi and Pereyra (2018), who described early infections with severe effects on the emergence and survival of plants.

Table 2. Coleoptiles infected with MoL pathotype *Lolium* and their relationship with seedling survival

Treatment code	Infected coleoptiles (%)	Dead seedlings (%)
T1	15.0 ± 3.3 b	11.7 ± 2.9 b
T2	13.3 ± 3.1 B	10.0 ± 2.7 b
T3	0.0 ± 0.0 A	0.0 ± 0.0 a
T4	0.0 ± 0.0 A	0.0 ± 0.0 a
T5	15.8 ± 3.3 B	12.5 ± 3.0 b
T6	10.8 ± 2.8 B	9.2 ± 2.6 b
T7	0.8 ± 0.8 A	0.0 ± 0.0 a

Values expressed as estimated mean ± standard error. Values followed by different letters within each column differ significantly according to Tukey's test ($p \leq 0.05$).

The treatments T3 (experimental), T4, and T7 reduced pathogen transmission to the coleoptiles by an average of 98.2% compared to the untreated control. In particular, the behavior of T7 is consistent with that reported by Goulart and Paiva (1991), who observed complete control of MoL through seed treatments with thiophanate-methyl and carbendazim, attributable to their systemic action. The high level of control observed with treatment T4 could be explained by the complementary action of its active ingredients, which combine contact and systemic fungicides with different mechanisms of action, effectively limiting the transmission of the pathogen from the seed to the coleoptile.

In contrast, treatments T2, T5, and T6 did not show significant differences against the control, indicating limited efficacy in suppressing the initial transmission of the pathogen. Although azoxystrobin (T6) has demonstrated

efficacy against foliar infections originating from environmental inoculum, its performance as a seed treatment against internal seed infections could be limited.

Table 3. Effect of treatments on germination (%) and seedling dry weight (mg)

Treatment	Germination (%)	Dry weight (mg)
T1	83.3 ± 1.7	66.2 ± 1.7
T2	82.9 ± 1.7	66.1 ± 1.1
T3	83.5 ± 1.7	68.9 ± 0.8
T4	77.9 ± 1.9	67.9 ± 0.4
T5	79.2 ± 1.8	68.3 ± 0.6
T6	80.9 ± 1.8	68.7 ± 2.3
T7	84.3 ± 1.6	70.4 ± 1.9
p-value	0.09	0.35

3.3 Germination and Vigor

Fungicide treatments did not significantly affect seed germination or vigor, indicating the absence of phytotoxic effects under the evaluated conditions (Table 3). Although these results provide evidence on the efficacy of the treatments evaluated, their scope is limited to controlled laboratory conditions and the seed lot used. Therefore, their validation across different seed lots and under field conditions is a relevant aspect for future studies.

4. Conclusions

The results of the present study confirm that MoL can be efficiently transmitted from seed to coleoptile in ryegrass, causing early infections and seedling mortality that compromise crop establishment. Although the incidence of the pathogen in commercial seeds was low in most of the lots analyzed, two seed lots with high levels of infection were identified, which highlights the importance of conducting a sanitary diagnosis prior to seed use.

The most efficient fungicide treatments did not negatively affect germination or initial seedling vigor, indicating that the use of appropriate seed treatments may improve seed health without compromising crop establishment. Overall, these results provide relevant information for the sanitary management of ryegrass seeds in Uruguay and highlight the need to continue evaluating seed treatment strategies as a complement to integrated blast management.

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Transparency of Data

Data not available: The data set that supports the results of this study is not publicly available.

Author Contribution Statement

	S González	A Fáber	V Pazos	S Pereyra	P Silva
Conceptualization	■				
Formal analysis	■				
Investigation	■		■		
Methodology	■				
Project administration	■				
Resources		■			
Writing – original draft	■				
Writing – review and editing				■	■

References

Goulart, A. C. P., & Paiva, F. A. (1991). Control of *Pyricularia oryzae* and *Helminthosporium sativum* through wheat seed treatment with fungicides. *Pesquisa Agropecuária Brasileira, 26*(11-12), 1983-1988. https://doi.org/10.1590/S1678-3921.pab1991.v26.3552

International Seed Testing Association. (2025). 7-011: Detection of *Pyricularia oryzae* in *Oryza sativa* (rice) seed. In *International rules for seed testing 2025: Validated seed health testing methods*. https://www.seedtest.org/api/rm/AF6XKGH8X898255/ista-rules-2025-shmethods-7-011-final.pdf

Martínez, S., & Escalante, F. (2016). La piricularia o brusone en semilleros de raigrás. *Revista INIA*, (46), 16-19.

Lasca, C. C., Kruppa, P. C., Barros, B. C., Schmidt, J. R., & Chiba, S. (2001). Controle de *Pyricularia grisea* e *Bipolaris sorokiniana* em sementes de trigo mediante tratamento com fungicidas. *Arquivos do Instituto Biológico,* 68(1), 55-63. https://doi.org/10.1590/1808-1657v68n1p0552001

Lobo, V. L. da S. (2004). *Tratamento de sementes para o controle da brusone nas folhas em arroz*. Embrapa Arroz e Feijão. http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/213607

Rossi, C., & Pereyra, S. (2018). *Pyricularia en semilleros de raigrás*. INIA. https://inia.uy/noticias/pyricularia-en-semilleros-de-raigras

Undersander, D., Mertens, D. R., & Thiex, N. (1993). *Forage analysis procedures*. National Forage Testing Association. https://www.foragetesting.org/_files/ugd/24f64f_4502c4143ec34a6ba1c4940c6147fd07.pdf