

## Resistance of Annual Ryegrass Germplasm to a Highly Aggressive New Strain of Blast (Gray Leaf Spot)

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### ABSTRACT

Annual ryegrass (*Lolium multiflorum* Lam.) is a highly nutritive, fast-growing, C3 cool-season annual forage. Blast or gray leaf spot is a fungal disease of ryegrass caused by *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*). The disease kills seedlings as well as adult plants. Blast-resistant annual ryegrass cultivars are not available at present. Therefore, identifying sources of resistance and developing blast-resistant germplasm are priorities to circumvent the devastating disease. Incorporation of germplasm screening in a breeding program requires an efficient, low-cost, and high-throughput evaluation system. Screening methods reported in literature lack both efficiency and throughput capabilities suitable for breeding. The aims in this work were to develop a low-cost, high-throughput phenotyping system, and to screen the annual ryegrass National Plant Germplasm System (NPGS) collection with a highly aggressive, newly isolated *M. oryzae* strain from naturally infected annual ryegrass plants, which was named MoGA1. Host specificity was tested on four species in addition to annual ryegrass. Extensive damage was caused to perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Lolium arundinaceum* (Schreb.) Darbysh), whereas rice (*Oryza sativa* L.) and orchard grass (*Dactylis glomerata* L.) exhibited a hypersensitivity response and resisted the infection, suggesting that pathogenicity of the strain was limited to the *Lolium* genus. A portable low-cost, high-throughput screening system capable of screening 1,536 plants per unit was developed and used to screen 138 ryegrass accessions with MoGA1. Two accessions showed resistance to the pathogen and produced seed. These two entries can be used to incorporate blast resistance in annual ryegrass germplasm.

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## Introduction

Annual ryegrass (*Lolium multiflorum* Lam.), also called Italian ryegrass, is a fast-growing, C3 cool-season annual grass species with high forage yield and high nutritive value (Ball, Hoveland, and Lacefield 2002). It is a member of the Pooideae subfamily within the Poaceae family that includes the major staple food cereals and the model grass *Brachypodium distachyon*. Annual ryegrass is currently used in the southeastern United States for the beef stocker industry and grazing dairies. Animal performance studies have shown that gains from this forage exceed those from winter forage cereals (Coffey et al. 1999). Extending the seasonal distribution of annual ryegrass into early fall and later into summer would benefit producers enormously. One major challenge to the early fall establishment of annual ryegrass in the southeastern United States is the potential outbreaks of ryegrass blast (also known as gray leaf spot), caused by the fungal pathogen *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*) (Couch and Kohn 2002).

Ryegrass blast is a disease of significant economic importance in the United States and worldwide. In the southern United States, sporadic and severe epidemics of blast in annual ryegrass can result in significant loss of crop (Vincelli, Dixon, and Farman 2008). The blast fungus kills young plants, thus reducing the time producers have for winter grazing. In addition, a close relative of the annual ryegrass pathogen causes gray leaf spot of perennial ryegrass and fescue (Farman 2002). *M. oryzae* is pathogenic to more than 50 grass species and remains potentially the most damaging disease of many economically important grass species (Viji, Wu, and Uddin 2001). Perennial ryegrass blast can cause significant economic loss to forage producers and the turfgrass industry. Outbreaks of blast disease in ryegrass pastures in the southeastern United States date back to 1972 in Louisiana and Mississippi (Bain, Patel, and Patel 1972; Rush, Lindberg, and Carver 1972).

The blast fungus can remain latent in the infested plant material as dormant mycelia and resume growth and spore production when environmental conditions become favorable for growth. Spores can be spread by wind, grazing animals, or harvest equipment. In the southeastern United States, gray leaf spot usually occurs in late summer to early fall when humidity and temperature are favorable for spore germination and infection of plant tissue (Lemus and Thomas-Peterson 2010). To avoid blast threats, growers resort to management practices to help reduce the impact of the disease. These practices are centered on keeping humidity low by reducing the frequency of irrigation, increasing the frequency of defoliation to remove dead plant material, allowing aeration and diffusion of sunlight through the canopy, and minimizing the application of nitrogen fertilizer. Another widely adopted practice is delaying planting to late in the fall when humidity and temperatures have decreased. Currently, disease-resistant cultivars of annual

ryegrass are not available to farmers. Incorporation of genetic resistance to blast into ryegrass cultivars should provide a durable solution to this devastating disease and increase producers' ability to extend the grazing season of their pastures, leading to improved long-term sustainability and profitability of livestock operations. Inoculation methods have been previously reported by Kusaba et al. (2006) and Vincelli, Dixon, and Farman (2008). However, these disease-screening methods lack rapid and high-throughput capabilities suitable for use in selection and breeding programs. A rapid and efficient screening method is a necessary tool for high-throughput evaluation for blast resistance in annual ryegrass. In this study, we describe (i) the isolation of a highly aggressive new strain of *M. oryzae* from field-infected ryegrass in Georgia, (ii) the development of a portable, low-cost, high-throughput inoculation and phenotyping system for annual ryegrass, and (iii) the screening of the annual ryegrass NPGS collection with the isolated *M. oryzae* strain designated MoGA1.

## Materials and methods

### *Fungal material and inoculation*

An *M. oryzae* strain was isolated from naturally infected annual ryegrass and was stored frozen dried at  $-20^{\circ}\text{C}$  to maintain full pathogenicity (Valent, Farrall, and Chumley 1991). The strain was assigned the code name MoGA1. To produce inoculum, the strain was grown on oatmeal agar plates in an incubator (Percival, Perry, IA) at  $24^{\circ}\text{C}$  and constant light for 14–21 days. The inoculation method described by Valent, Farrall, and Chumley (1991) was followed with some modifications. *M. oryzae* conidia were harvested from fungal cultures by adding 3 ml of 0.25% aqueous gelatin (Gelatin from bovine skin, Sigma-Aldrich, St. Louis, MO). Spores were released into solution by gently rubbing the surface of the culture with a sterile microcentrifuge tube. The spore suspension was then strained through miracloth to filter out any mycelia. A hemocytometer was used to determine the concentration of the spore harvest. The spores collected were diluted to a final concentration of  $1 \times 10^5$  spores  $\text{ml}^{-1}$ . Three-week-old seedlings were inoculated using an artist spray brush to apply 6 ml of conidial suspension per tray.

### *Plant materials*

A set of annual ryegrass genotypes consisting of 138 accessions from the National Plant Germplasm System (NPGS) collection were planted in the fall of 2013 in a greenhouse at the University of Georgia (UGA), Athens. Three-week-old seedlings were spray-inoculated with a conidial suspension of MoGA1, and plant responses were scored after three weeks. The ryegrass

accessions included 19 tetraploids and 119 diploids, as determined by flow cytometry. Each accession was represented by six plants. The growing media contained soil:sand:Fafard mix (3:1:6, v:v:v). Fafard superfine germination mix (Fafard, MA) contained Canadian sphagnum peat, vermiculite, and perlite. The average daily temperature inside the greenhouse was 25°C and relative humidity was maintained near 90%. Four tall fescue cultivars, two perennial ryegrass cultivars, and two orchard grass cultivars were also planted in the greenhouse to test the pathogenicity of the newly isolated ryegrass blast strain on closely related grass species. Rice (*Oryza sativa*) accession YT16 was also grown under long-day conditions (14/10 hours, day/night) in a Conviron PW36 growth chamber, with a day-time temperature of 28°C and a night-time temperature of 24°C. Plants were grown in 10 cm pots with Fafard 3B soil mix. Iron chelate solution (3.25% iron chelate in water) was added at the time of planting, then 20-10-20 Peat Lite fertilizer (J.R. Peters, Inc., PA) was applied once a week.

### ***Disease scoring in annual ryegrass***

We recorded mortality (M) and disease severity (DS) by counting the number of affected leaves per plant. Disease severity can be defined from different perspectives (Seem 1984). In this study, we defined disease severity as the percentage of leaves with disease lesions (0–100%) on the plants that were not killed by the fungus. Trevathan (1982) used a similar disease index based on the total number of lesions on an inoculated plant that survived. In our study, we established a disease index (DX) based on the number of dead plants (mortality) and the disease severity score; such that  $DX = (M \times DS) / 100$ . The disease index comprised 11 classes (0–10), with 0 indicating no disease (0%), 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100%.

## **Results and discussion**

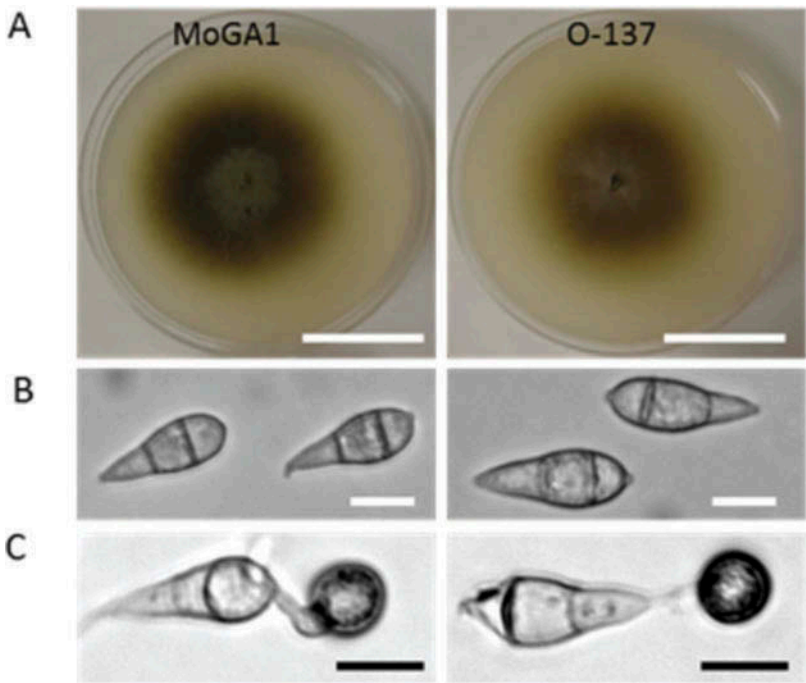
### ***Isolation of a new *M. oryzae* strain from infected annual ryegrass***

During greenhouse propagation of annual ryegrass plants obtained from field plots in Georgia, we noticed oval-shaped lesions on some of the plants that first appeared brown and later turned gray in the center. The lesions were the typical symptoms of ryegrass blast, also called gray leaf spot (GLS), caused by *M. oryzae* (Figure 1; left). The lesions continued to expand, covering most of the foliar portions of the plants and quickly spreading to neighboring annual ryegrass plants in the greenhouse (Figure 1; middle). The infection was so severe that plants were completely killed and leaves appeared twisted and gray, seemingly a result of sporulation on the leaf surface (Figure 1; right).



**Figure 1.** Annual ryegrass plants infected by the *Magnaporthe oryzae* strain MoGA1 that was isolated from naturally infected ryegrass plants. This strain killed even adult plants that had been well established.

From these naturally infected leaves, we isolated one *M. oryzae* strain, which we named MoGA1 (*Magnaporthe oryzae* isolated in Georgia). MoGA1 exhibited typical morphological and developmental characteristics of *M. oryzae* strains (Ou 1985). Nine-day-old culture of MoGA1 on oatmeal agar (OMA) grew at a comparable rate in a similar appearance as *M. oryzae* strain O-137, a highly pathogenic strain on rice (*Oryza sativa*) (Orbach et al. 2000) (Figure 2A). Both MoGA1 and O-137 produced conidia that were obclavate,



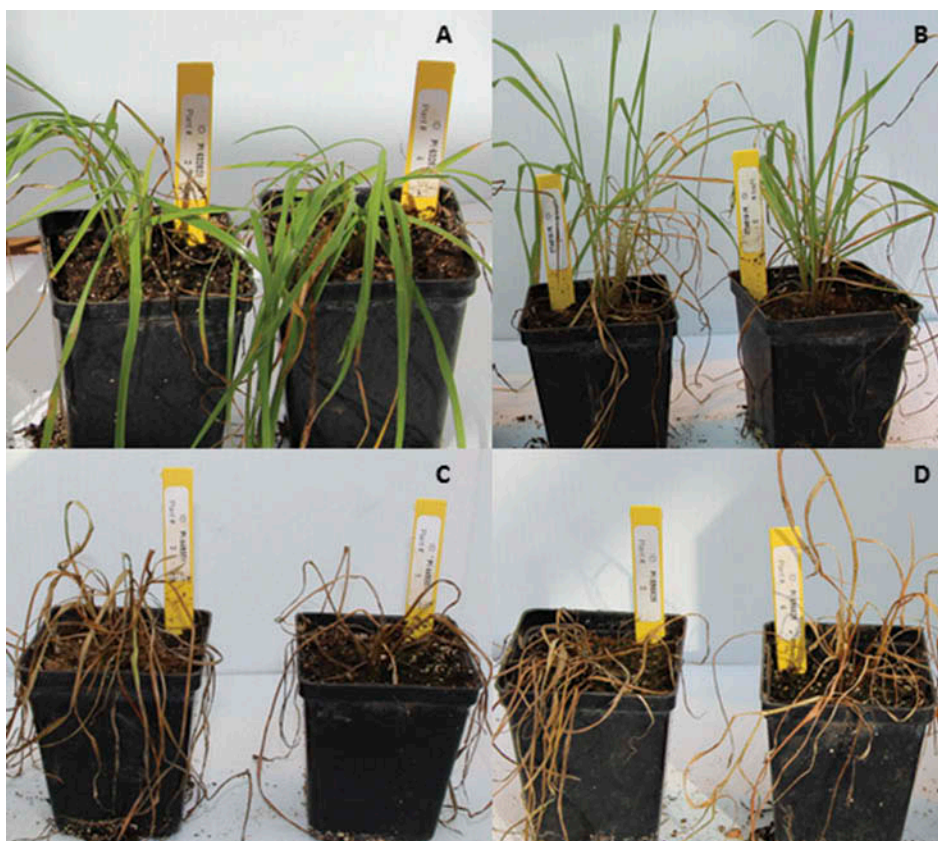
**Figure 2.** A) Nine-day-old cultures of MoGA1 (left) and O-137 (right) on oatmeal agar (OMA). Bars = 2 cm. B) Bright-field images of conidia from MoGA1 (left) and O-137 (right). Bars = 10  $\mu$ m. C) Bright-field images of appressoria formed on a coverglass 22 hours after germination of conidia from MoGA1 (left) and O-137 (right). Bars = 10  $\mu$ m.



2-septate, and approximately  $22 \times 8 \mu\text{m}$  (length  $\times$  width) (Figure 2B). These conidia germinated and formed melanized appressoria on a hydrophobic surface (Figure 2C).

### *Initial screening for blast resistance in annual ryegrass*

Of the 138 accessions screened, we identified two accessions that showed resistance to MoGA1 (PI 632528 and PI 619473) (Figure 3A and B). PI 449301 (Figure 3C) and PI 596625 (Figure 3D) were among 136 susceptible accessions that were either killed or showed extensive damage caused by the blast infection. The collection we screened included three plant introductions (PIs) that were previously reported as partially resistant to an *M. oryzae* strain isolated from naturally infected ryegrass at Starkville, MS (Trevathan 1982). However, these three PIs were highly susceptible to MoGA1. The

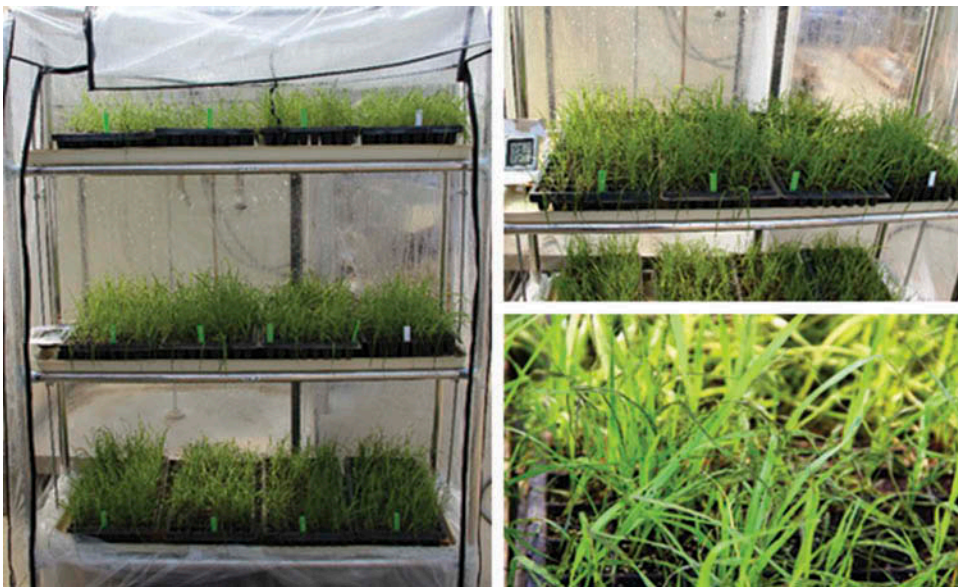


**Figure 3.** Annual ryegrass accession infected with MoGA1. The plants were infected at the seedling stage (three weeks), and then allowed to grow for eight weeks. PI 632528 (A) and PI 619473 (B) showed resistance to the infection. PI 449301 (C) and PI 596625 (D) were among 136 susceptible accessions that were killed by the blast infection.

resistant accessions are currently being used in a recurrent selection program to incorporate blast resistance into elite annual ryegrass cultivars. Two pseudo testcross mapping populations were developed from these accessions to identify genomic regions associated with resistance and develop molecular markers to facilitate the incorporation of the trait into various backgrounds.

### ***Development of a portable high-throughput, low-cost plant-screening system***

To facilitate the screening process for blast resistance in annual ryegrass, we developed a portable, low-cost, whole-plant screening system that can be easily set up in a greenhouse or a laboratory. The system consisted of a 3-Tray Flora Cart (191 cm H  $\times$  135 cm L  $\times$  51 cm W) (Growers Supply, South Windsor, CT) (Figure 4). The cart had three adjustable shelves and was fitted with a clear plastic tent to maintain humidity to the desired level. Each shelf had a large 125 cm  $\times$  51 cm fiberglass tray and was equipped with 2  $\times$  40 watt Verilux fluorescent light tubes. Each shelf could hold 4 flats with each flat containing 8  $\times$  16 cells. Therefore, each cart had a capacity of screening 1,536 plants (3 shelves  $\times$  4 flats  $\times$  128 cells) (Figure 4). Water evaporation from the fiberglass trays maintained the high humidity conducive to the development and spread of the blast fungus. This system was used to screen the NPGS annual ryegrass collections. Results of the screening are presented in Table 1.



**Figure 4.** Portable whole plant system for blast screening of annual ryegrass seedlings (left); middle rack on the cart with thermometer-cum-hygrometer (top right); and seedlings showing blast symptoms after four days of inoculation (bottom right).

**Table 1.** Response (disease index) of annual ryegrass plant introductions (PIs) to *Magnaporthe oryzae* isolated in Georgia (MoGA1) via artificial inoculation under greenhouse conditions<sup>†</sup>.

Disease Index <sup>‡</sup>	Plant Introductions
1	376876, 611144, 610798, 610801, 611145, 619473, W6-9271
2	189151, 197975, 235157, 240632, 240731, 283609, 286466, 422477, 577242, 595075, 600748, 610794, 610797, 610800, 610827, 610831, 619470, 619471, 634251, 636509, 639776, 655110
3	187220, 235038, 239486, 251555, 255172, 255174, 265337, 272118, 274638, 286467, 306691, 324711, 339701, 370676, 376875, 420020, 517948, 600782, 610796, 610799, 632528, 632563, 655108
4	188732, 189150, 204878, 240732, 241586, 241912, 250023, 251826, 255882, 274636, 276665, 370675, 371951, 376877, 577243, 577248, 593651, 611146, 619468, 619469, 619472, 632481, 632537, 639777, 655109
5	189390, 238937, 240698, 255173, 266111, 272119, 283612, 410154, 578754, 632557, 636508, W6-20321
6	251556, 255881, 265338, 577245, 577247
7	189389
10	162455, 162678, 170519, 179358, 189152, 193145, 199251, 200344, 201980, 202509, 211828, 222526, 223568, 225726, 238885, 238886, 238939, 239804, 239805, 241913, 267057, 283610, 343155, 343156, 370674, 376874, 449301, 449302, 545668, 545671, 577241, 577244, 577246, 577249, 578752, 595076, 596625, 598939, 600749, 655111, W6-9267, W6-16134, W6-20362, /tb>

<sup>†</sup>Three-week-old seedlings of annual ryegrass inoculated with conidial suspension (6 ml of a  $1 \times 10^{-5}$  per ml suspension per tray); humidity maintained by water in the trays; and the plants maintained in the greenhouse under natural light condition, 25°C temperature, and 90% humidity level.

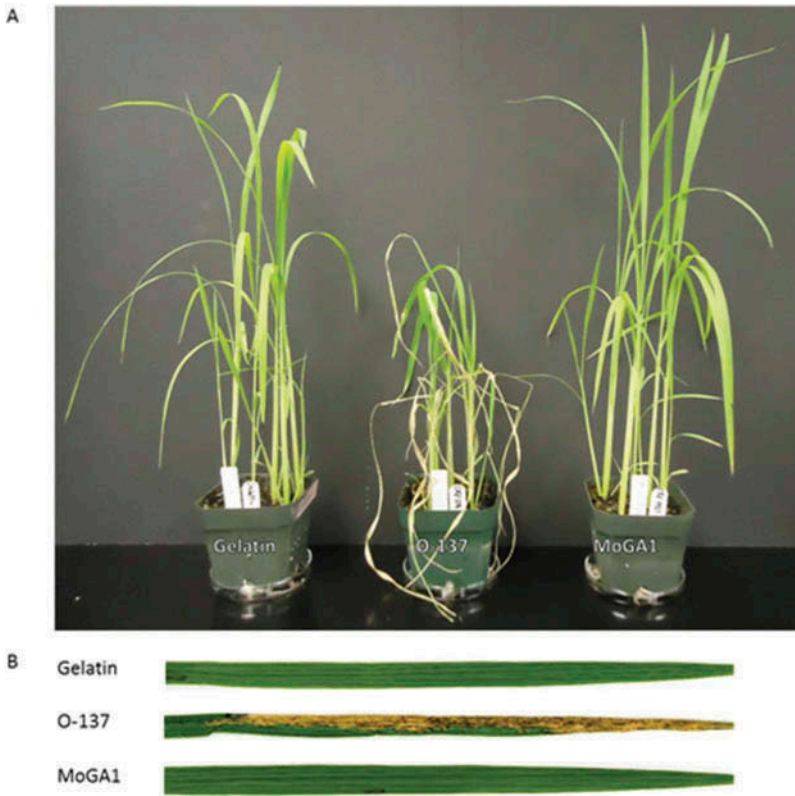
<sup>‡</sup>Disease index based on mortality and percent leaves affected by MoGA1 in each accession. Disease index levels from 0 to 10, with 0 as “no disease” and 10 as “extremely affected” by the pathogen (see complete rating scale in Materials and Methods).

Based on disease index values, seven accessions had a score of 1. But when allowed to grow beyond three weeks, additional lesions developed and all the plants with disease index 4 and above eventually died. After 6 weeks of growth, most of the remaining plants developed further damage and were quite weak, except for two accessions (PI 632528 and PI 619473), which grew vigorously and were able to produce seed. These observations suggested that to reach definitive conclusions regarding resistance or susceptibility, the plants would need to be grown for at least 4 weeks.

**Host specificity of MoGA1**

Despite the wide range of grass species that *M. oryzae* strains collectively infect, individual strains exhibit host specificity (Khang and Valent 2010). To investigate host specificity of MoGA1, we inoculated rice plants with a conidial suspension of MoGA1. Although MoGA1 was highly aggressive to most annual ryegrass germplasm (Figure 3; Table 1), the strain was avirulent to rice. There was no visible indication of infection but barely visible dark brown specks on MoGA1-inoculated rice leaves (Figure 5A and B), indicative of hypersensitive response (Valent, Farrall, and Chumley 1991). This

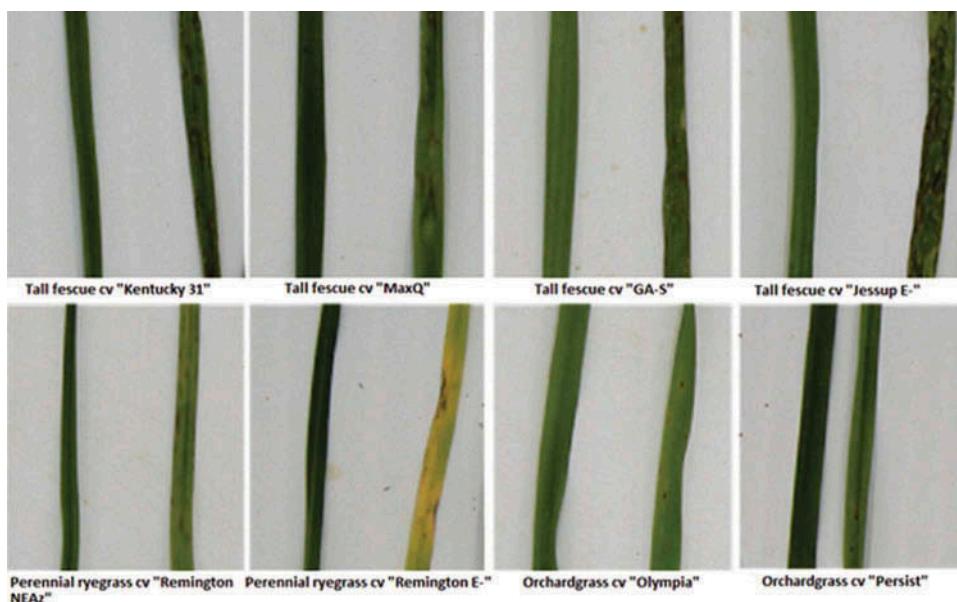




**Figure 5.** Whole plants (A) and leaves (B) of rice strain YT16 one week after spray inoculation with 0.25% gelatin control, rice pathogenic *M. oryzae* (O-137), and annual ryegrass pathogenic *M. oryzae* (MoGA1). O-137 was highly virulent, while MoGA1 failed to cause disease on rice.

contrasted sharply with severe lesions produced on rice plants when inoculated with rice pathogenic strain O-137.

Host specificity of MoGA1 was also tested on cool season grasses closely related to annual ryegrass. Inoculation of two perennial ryegrass cultivars, one harboring the endophyte (*Neotyphodium lolii*) and the other without, resulted in extensive damage to both cultivars (Figure 6). Both orchardgrass (*Dactylis glomerata* L.) cultivars inoculated with the strain did not show the typical lesions of gray leaf spot, instead some small brown spots were present, a possible indication of hypersensitivity response (Figure 6). The overall health of the plants did not differ from that of the control. Inoculation of four different tall fescue (*Lolium arundinaceum* Shreb.) cultivars (two harboring endophytes and two endophyte-free) resulted in extensive damage to all four cultivars (Figure 6). The two tall fescue cultivars (Kentucky 31 and MaxQ) harbored the fungal endophytes (*Neotyphodium coenophialum*) that are known to confer on the host plants tolerance to various stresses and also deter insects and diseases. These endophytes did not appear to confer any protection from the blast strain



**Figure 6.** Images showing results of inoculation of three-week old seedlings of tall fescue, perennial ryegrass, and orchardgrass cultivars with the blast strain MoGA1. The left leaf of each panel represents control plants that were sprayed with 0.25% gelatin. The leaf on the right represents plants that were inoculated with the blast strain.

MoGA1. These results confirm previous reports suggesting that *M. oryzae* isolates obtained from annual ryegrass might be pathogenic to perennial ryegrass and fescue species (Vincelli, Dixon, and Farman 2008). DNA fingerprinting tests indicated that the *M. oryzae* isolates causing disease on annual ryegrass appeared to be from the same pathogen population responsible for gray leaf spot infection on perennial ryegrass (Vincelli, Dixon, and Farman 2008).

Phylogenetic analysis based on Pot2 transposon fingerprints of 20 blast strains identified from 16 Poaceae species, showed that perennial ryegrass isolates were similar to those of wheat (*Triticum aestivum*) and triticale ( $\times$  Triticosecale) isolates (Viji, Wu, and Uddin 2001). A polymerase chain reaction (PCR)-based profiling of the transposon Pot2, present only in the *M. oryzae* genome, was developed for the detection and identification of *M. oryzae* in infected perennial ryegrass (Harmon, Dunkle, and Latin 2003). Characterization of 89 blast isolates from annual ryegrass in Japan showed that these isolates were closely related to those from *Eleusine* but distinct from those from *Triticum* (Kusaba et al. 2006). Blast disease on annual ryegrass in Japan is caused by blast isolates that are relatives of the finger millet (*Eleusine coracana*) isolates; nevertheless, they are host-specific to annual ryegrass as they do not produce susceptible reactions on finger millet (Kusaba et al. 2006).

This work described a highly aggressive *M. oryzae* strain (MoGA1) isolated for the first time from infected annual ryegrass in Georgia. Using this strain, it was shown that previously reported blast-resistant ryegrass PIs (Trevathan 1982) were susceptible to blast, pointing to the possible presence of multiple races. The study also describes an efficient high-throughput inoculation method, suitable for disease screening in crop breeding programs, and the development of a practical rating system for annual ryegrass reaction to blast. Using this method, together with MoGA1, we have screened 138 annual ryegrass accessions and have successfully identified two blast-resistant accessions that are currently used to introgress blast resistance into annual ryegrass elite germplasm. The results of this study showed that the pathogenicity of the MoGA1 blast strain was specific to species belonging to the genus *Lolium* since it failed to infect rice and orchardgrass. The results also showed that the presence of endophytes in tall fescue and perennial ryegrass did not confer tolerance to blast.

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## References

- Bain, D. C., B. N. Patel, and M. V. Patel. 1972. Blast of ryegrass in Mississippi. *Plant Diseases Reports* 56:210.
- Ball, D. M., C. S. Hoveland, and G. D. Lacefield. 2002. *Southern forages*, 3rd ed. Norcross, GA: Potash and Phosphate Institute, Foundation for Agronomic Research.
- Coffey, K., D. Shockey, W. Coblenz, C. Rosenkrans Jr., S. Gunter, and G. Montgomery. 1999. Performance of stocker calves backgrounded on winter annuals or hay and grain. *AAES Research Series* 470:77–79.
- Couch, B. C., and L. M. Kohn. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *magnaporthe oryzae*, from *M. grisea*. *Mycologia* 94:683–93. doi:10.2307/3761719.
- Farman, M. L. 2002. Isolates of *Pyricularia grisea* causing gray leaf spot of perennial ryegrass in the US: Relationship to *P. grisea* isolates from other grasses. *Phytopathology* 92:245–54. doi:10.1094/PHYTO.2002.92.3.245.
- Harmon, P. F., L. D. Dunkle, and R. Latin. 2003. A rapid PCR-based method for the detection of *Magnaporthe oryzae* from infected perennial ryegrass. *Plant Disease* 87:1072–76. doi:10.1094/PDIS.2003.87.9.1072.
- Khang, C. H., and B. Valent. 2010. *Magnaporthe oryzae* and rice blast disease. In *Cellular and molecular biology of filamentous fungi*, eds. K. A. Borkovich, and D. J. Ebbole, 593–606. Washington, DC: ASM Press.

- Kusaba, M., K. Hirata, Y. Sumida, A. Yamagashira, H. Konagai-Urata, and H. Yaegashi. 2006. Molecule genetic characterization and host specificity of *Pyricularia* isolates from annual ryegrass in Japan. *Plant Pathology Journal* 5 (1):72–79. doi:[10.3923/ppj.2006.72.79](https://doi.org/10.3923/ppj.2006.72.79).
- Lemus, R., and M. Thomas-Peterson. 2010. *Gray leaf spot (Blast) in annual ryegrass*. Publication 2633. Starkville, MS: Extension Service of Mississippi State University, cooperating with U.S. Department of Agriculture.
- Orbach, J. M., L. Farrell, A. J. Sweigard, G. F. Chumley, and B. Valent. 2000. A telomeric avirulence gene determines efficacy for the rice blast resistance gene Pi-ta. *The Plant Cell* 12:2019–32. doi:[10.1105/tpc.12.11.2019](https://doi.org/10.1105/tpc.12.11.2019).
- Ou, S. H. 1985. Fungus diseases-foilage diseases. In *Rice diseases*, 2nd ed., 109–201. Kew, UK: Commonwealth Mycological Institute.
- Rush, M. C., G. D. Lindberg, and R. B. Carver. 1972. Blast: A serious new disease of forage grasses in Louisiana. *Phytopathology* 62:806.
- Seem, R. C. 1984. Disease incidence and severity relationships. *Annual Review of Phytopathology* 22:133–50. doi:[10.1146/annurev.py.22.090184.001025](https://doi.org/10.1146/annurev.py.22.090184.001025).
- Trevathan, L. E. 1982. Response of ryegrass plant introductions to artificial inoculation with *Pyricularia grisea* under greenhouse conditions. *Plant Diseases* 66:696–97. doi:[10.1094/PD-66-696](https://doi.org/10.1094/PD-66-696).
- Valent, B., L. Farrall, and F. G. Chumley. 1991. *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. *Genetics* 127:87–101.
- Viji, G., B. Wu, and W. Uddin. 2001. *Pyricularia grisea* isolates causing gray leaf spot of perennial ryegrass turf: Population structure and host specificity. *Plant Disease* 85:817–26. doi:[10.1094/PDIS.2001.85.8.817](https://doi.org/10.1094/PDIS.2001.85.8.817).
- Vincelli, P., E. Dixon, and M. Farman. 2008. Susceptibility of selected cultivars of forage grasses to *Magnaporthe Oryzae* isolates from annual ryegrass and relatedness of the pathogen to strains from other grasses. *Forage and Grazinglands*. doi:[10.1094/FG-2008-0226-01-RS](https://doi.org/10.1094/FG-2008-0226-01-RS).